BACTERIOLOGICAL INVESTIGATIONS OF URINARY INFECTIONS.

THESIS SUBMITTED FOR THE DEGREE OF

DOCTOR OF MEDICINE

OF THE UNIVERSITY OF GLASGOW.

BY

JAMES McGEACHIE, M.B., Ch.B.

SUMMARY.

1. A quantitative stroke-plate method for bacterial counting was developed which compared closely with the more time-consuming pour-plate method. A comparison with a quantitative leucocyte counting method showed good correlation between significant bacteriuria and pyuria. Gram-film methods for assessing the levels of bacteriuria and pyuria were found to correlate well with the quantitative methods, and the inaccuracy of the routine wet-film estimation was clearly shown.

2. Biochemical typing of the isolates from different bacterial count levels revealed that the Escherichia coli was the most frequently isolated species from patients with significant bacteriuria. It was shown that the presence of urinary tract abnormality decisively altered the frequency of the species isolated.

3. Serological grouping, with twelve specific antisera, was used to investigate the strains of Escherichia coli. Nearly two-thirds of the strains isolated from patients with symptomatic, significant bacteriuria were grouped serologically. No significant difference could be demonstrated in the frequency of the serological groups of the strains from high and low bacterial count specimens.

4. The haemolytic reaction of the Escherichia coli strains was predominantly associated with the serological groups: '01', '04', '06', '018' and '075', which accounted for more than ninety per cent of the haemolytic strains. There was no statistical difference shown in the frequency of the serological groups, or of the haemolyti reaction, in the strains isolated from males and females.

5. A method for colicine typing was developed which classified more than seventy per cent of the strains. There was no significant difference noted in the frequency of total colicinicity of the strai from the high and low bacterial count specimens, an increased frequency of one colicine sensitivity pattern was noted in the low count specimens. The colicine extraction method revealed that

different media could affect both the production and diffusion of colicine.

7. The lack of significant difference in the frequencies of the serological groups and the colicinogenic patterns, between the strains from the high and low bacterial count specimens, was suggested as indicating that the infecting strain originates in the lower urinary tract.

8. The evidence of the typing markers, used to investigate the strains of Escherichia coli isolated from the recurrent infections, indicated that recurrence is due to reinfection by a new strain, in the majority of instances. Comparisons between the recurrences of patients with and without urinary tract abnormalities did not reveal any significant differences in the frequencies of recurrences, the time intervals or the change of strains.

VOLUME ONE

INDEX.

VOLUME ONE.

VOLUME TWO.

INTRODUCTION.

Urinary tract infections are now among the commonest bacterial infections, the prevalence of pyelonephritis has been estimated at ten per cent of all autopsies performed, the majority of these lesions having been unsuspected during life. Similarly, the frequency of unsuspected infection during life, asymptomatic bacteriuria, has been found to be equally high, about sixteen per cent of female patients admitted to the hospital wards.

The intentions of this thesis were threefold, firstly, to find simple, but accurate methods for the diagnosis of urinary infections; secondly, to investigate the frequency of species isolated from the urine and to type the most common of these, Escherichia coli, by precise methods; and thirdly, to consider the problem of recurrent urinary infection by the Escherichia coli in the light of the typing methods employed.

One of the difficulties presented to the clinician and to the bacteriologist has been the interpretation of the significance of the urine culture. Although it had been shown that the bacterial count levels of the urine were of significance, many laboratories appeared to find difficulty implementing these findings. The first part of this thesis is concerned with finding and correlating simplified, accurate methods for investigating the bacterial and leucocyte content of the urine.

The second part is concerned with the typing of Escherichia coli. Since the reported frequencies of species isolated from the urine showed wide variation it was considered necessary to confirm the frequency of Escherichia coli by biochemical methods before proceeding to more precise typing methods. Particular emphasis was to be given to the bacterial count grouping of the isolates and to the presence or absence of urinary tract abnormalities, since it was felt that both

these factors might influence the incidence of species.

Although the enteropathogenic Escherichia coli had been frequently investigated serologically, less interest had been shown in the serology of the urinary strains. Using the bacterial count levels as a guide for comparisons, it was proposed to assess the frequency of the more common serological groups. The frequency of haemolytic strains of Escherichia coli, in the isolates from males and females with significant bacteriuria, was another feature requiring reassessment.

For the more precise method of typing the isolates, the colicine reaction was employed. Although colicine had been used as a typing marker for the enteropathogenic Enterobacteriaceae, it had not received the same consideration for the typing of urinary strains. Using methods one had developed for investigating colicine production and sensitivity, it was hoped to find more precise indicators of specific types which could be used for the epidemiological study of urinary infections.

Finally, the problem of recurrent infection required study, now as much as ever, in spite of the availability of a wide range of anti-bacterial drugs. It was hoped that the methods of typing would decide whether the recurrent infection was a reinfection by a new strain or the recrudescence of an inadequately treated infection.

CHAPTER I

METHODS FOR INDICATING BACTERIURIA AND PYURIA

Introduction

The incidence of significant pyelonephritis at autopsy has been estimated by various authors from 6.18 per cent (Brod, 1956) to 20 per cent (Rhoads, Billings and O'Connor, 1952), the average being approximately 10 per cent of all necropsies. Many of these cases have gone undiagnosed in life, probably due to the absence of obvious symptoms referrable to the urinary tract. Kleeman, Hewitt and Guze (1960) reported that only 16 per cent of 629 cases of pyelonephritis had been diagnosed before death and similar figures have been noted by Macdonald, Levitin, Mallory and Kass (1957). The incidence of significant bacteriuria, that is more than 100,000 organisms per millilitre urine, in a series of one hundred and thirty-nine female patients admitted to a medical ward of the Glasgow Royal Infirmary is shown in Figure I. These patients did not have symptoms referrable to the urinary tract and yet some 16.5 per cent had a urinary tract infection indicated by the bacterial count. This figure is similar to that of Kaitz and Williams (1960) who found 17 per cent of hospital

cases had significant bacteriuria and that 75 per cent of these were unsuspected.

The first part of this thesis is concerned with the development of simplified techniques which would enable all patients admitted to hospital to be screened easily but accurately.

Material and Methods

The seven hundred and nine patients investigated were either in-patients at Glasgow Royal Infirmary or attending an out-patient clinic for medical renal diseases which I attend. The age and sex incidence is shown in Figure 2 and Table I. Of the five hundred and fifty-nine female patients, seventy-four (13.2 per cent) had known structural renal tract lesion e.g. acute and chronic glomerulo-nephritis, acute tubular necrosis or polycystic kidneys; ninety-six (64 per cent) of the one hundred and fifty males had similar lesions. More than two thousand mid-stream specimens of urine were examined. All specimens were examined within one hour of micturition or, where this was impracticable, stored at 4^{0}C. until it was convenient. Although an investigation revealed that refrigeration up to forty-eight hours did not affect the bacterial counts, none of the specimens were left for more than twenty-four hours and the large majority were dealt with in less than three hours, an important factor in the counting of leucocytes which tended to degenerate, even in the refrigerator, in alkaline urine. The well-mixed sample of urine was divided into two parts one of which was used for quantitative cell counts and the other for bacteriological studies. The results of each part were compared after the completion of the batch of samples.

Methods of bacteriological study.

Bacterial counts were performed initially by a pour-plate method using a 1 in 10,000 dilution of urine in sterile water. One millilitre of the diluted urine was placed on a sterile petri dish and mixed with nutrient agar, a similar volume was mixed with

MacConkey No. 2 agar (Oxoid). Both plates were incubated at 37°C. and examined after twenty-four, forty-eight and seventy-two hours. Each colony represented 10,000 organisms per millilitre of the uncentrifuged urine. In practice it was found that the equipment (Figure 3A) and the time required for this method made it unsuitable for convenient use in a busy bacteriological laboratory. For similar reasons other dilution methods such as those described by Miles, Misra and Irwin (1938) and recently Bradley & Little (1963) were rejected. Accordingly the following stroke-plate method, using a bacteriological loop delivering a known volume, was developed. The essential equipment required is shown in Figure 3B.

1. Preparation and calibration of the loop.

A tungsten wire of approximately 21 S.W.G. (Standard wire gauge) was bent to form a loop around a No. 15 knitting needle (S.W.G. 16). This was found to be the diameter which approximated most closely a volume of 1/300th of a millilitre. When a batch of loops had been prepared they were separately calibrated using the volume of distilled water removed in each loopful. A capped Universal container was filled with distilled water and weighed. Ten loopeful of water were removed, drying on blotting paper between each loopful. The Universal was reweighed, the loss of weight giving the weight of the ten loopeful. This was repeated twice and the average volume of water calculated. With the wire and needle used this was 0.00336 ($\pm$ 0.0003) millilitre. A variation of more than 10 per cent in the three readings excluded the loop from use. At first this calculation was correlated with accurate plate counts and found to agree completely. There was no significant alteration up to three hundred urines tested with the same loop, between three hundred and six hundred the volume delivered increased, on the average, by 10 per cent and after one thousand urines the volume delivered had increased by 25 per cent. As a routine, therefore, the loops were not used for more than three hundred urines.

2. Inoculation with the calibrated loop.

A loopful of the well mixed urine was inoculated directly on to a blood agar plate and on to a MacConkey plate. The loopful was plated across one edge of the blood agar plate to a width of one centimetre, using twenty strokes. Using a second large sterile loop an area one centimetre wide was plated two centimetres from the edge of, and at right angles to, the initial inoculum. This procedure was repeated twice to give a box-like inoculated plate with each side one centimetre thick (Figure 4). After incubation the number of colonies was counted and multiplied by the reciprocal of the loop's volume to give the number of organisms per millilitre of urine. Growth was also noted as +, ++, +++ or ++++, depending on whether one, two, three or four sides of the inoculated area showed colonies.

3. Gram-film estimation of bacteriuria

A measured loopful of uncentrifuged urine was placed on a glass slide and stained by Gram's method. Fifty fields were examined under the oil-immersion objective.

Method of cell counting.

1. The quantitative cell count

The cellular excretion using the Addis technique (Lippman 1957), and the modification of it described by Houghton and Peers (1957), utilised a specimen of urine passed after a measured period of time, and both Lippman (1957) and Houghton and Peers (1957) recommended that in women the urine be collected by catheter. For reasons which have been cogently expressed by Beeson (1958), regarding routine catheterisation, a mid-stream specimen of urine was used and the technique of cell counting was modified as follows.

A standard 10 millilitre volume of the mid-stream specimen was centrifuged in a graduated tube at 3,000 r.p.m. for three minutes, 9.5 millilitres of the supernatant urine were pipetted off and tested

for protein, and the sediment was resuspended in the remaining 0.5 millilitre of urine by vigorous mixing with a Pasteur pipette. A drop of the suspension was used to fill a Neubauer counting chamber and the white cells in the area shown in black in figure 5 were counted. If the count was low, three or four such areas were counted and the mean estimated. In order to keep the procedure as simple as possible, the multiplication factor (x833) required to express the result as cells per millilitre was not employed and the white cell count is referred to simply as cells per measured area.

2. The non-quantitative wet film cell estimation

Two loopsful of a centrifuged deposit were covered with a standard 7/8 x 7/8 inch coverslip and the number of white cells per twenty high power fields (H.P.F.) estimated by examination under the 1/6th inch objective. All centrifuged deposits were from 10 millilitre of urine spun at 2,500 r.p.m. for five minutes, the supernatant was decanted and the deposit mixed using a bacteriological loop.

3. Gram film pus cell estimation

This was performed on the Gram stained film of the measured loopful of uncentrifuged urine. The number of pus cells per fifty fields examined under the oil-immersion objective was estimated.

Results and Interpretation

Following the work of Kass (1956) on the significance of bacterial counts, the urine samples were divided into high, intermediate and low bacterial counts as follows:-

1. Bacterial count over 100,000 organisms per millilitre meant infection.

2. Bacterial count 10,000 to 100,000 organisms per millilitre meant infection doubtful, developing or suppressed.

3. Bacterial count below 10,000 organisms per millilitre meant infection absent.

Of the two thousand and eight-five specimens, examined three hundred and forty-six (16 per cent) were in the high group or positive, one hundred and eighty-one (8.7 per cent) in the intermediate or doubtful group and one thousand five hundred and fifty-eight (74.7 per cent) in the low or negative group (figure 6).

Reproduction of bacterial counts using mid-stream specimens of urine.

The statistical interpretation of bacterial counts was confirmed by Kass (1956) on catheter specimens of urine and he compared two specimens of urine from each patient at an unspecified time interval. He found that 96 per cent of the two specimens with high and low bacterial counts were in agreement and suggested that mid-stream specimens might show a much lower correlation down to 80 per cent. (Kass 1962). To consider the constancy of the results of mid-stream specimens it was decided to be more strict than Kass and compare three mid-stream specimens of urine obtained at three-hourly intervals. Table II gives the results of this investigation on fifty patients, 84 per cent of the one hundred and fifty consecutive specimens showed complete correlation in all three specimens. In the high bacterial count group the patient with disagreement had two successive counts of more than 100,000 organisms per millilitre and the third fell below 10,000. In the low bacterial count group the patient with disagreement had a count of 23,200 organisms per millilitre in the second specimen, the third was again below 10,000. In the intermediate group the second and third specimens from all the patients fell below 10,000 organisms per millilitre.

The reproducibility in the high and low bacterial groups combined was, therefore, 95 per cent in all three specimens and 97 per cent when the first two specimens from each patient are compared.

Comparison between pour-plate and stroke-plate counts.

In one hundred and sixty-five specimens a direct comparison was made between the pour-plate and stroke plate methods (Tables III & IV). There was very good correlation between the two techniques in the high and low counts. Of the nineteen specimens classified as 'intermediate' by the pour-plate methods, four were in the high group by the stroke-plate method; three of these, however, had a bacterial count of more than 50,000 organisms per millilitre by the pour plate method, while the other was from a patient on tetracycline therapy. In the low bacterial count group, by the pour-plate method, eight per cent of the specimens had higher counts by the stroke method. The two specimens with the count of more than 100,000 organisms by the stroke-plate contained almost a pure growth of Doderlein's bacillus due to contamination of the mid-stream specimen. In the intermediate count group the discrepancies were all below 30,000 organisms on the stroke-plate. (Table III).

The correlation between the bacterial count and the number of sides of the stroke-plate showing growth is given in Table V. It will be seen that 94.2 per cent of the specimens with counts more than 100,000 organisms per millilitre had growth on three or four sides of the plate; conversely 80.6 per cent of the plates with growth on three or four sides had counts of more than 100,000 organisms per millilitre. Indeed if one excludes the non-pathogenic organisms, mainly Doderlein's bacillus, 90 per cent of plates with growth on three or four sides had counts of more than 100,000 organisms per millilitre.

Correlation between bacterial counts and quantitative cell counts.

A direct comparison was made between these two counts in one thousand five hundred and twenty-five specimens. Quantitative cell and bacterial counts were done in a further five hundred and sixty samples of urine from patients with glomerulonephritis and tubular

necrosis, but these are excluded from this comparison since it was felt that the cell count would be affected by the glomerular or tubular lesion. In Figure 7 the specimens are divided into negative, doubtful or positive according to the bacterial count. In the bacteriologically negative group the cell count was low, 90.1 per cent of these specimens had a cell count of ten or less cells per measured area. In the doubtful group the pattern was similar, but, less marked, while in the positive group the pattern was largely reversed. Table VI shows the correlation between pyuria and bacteriuria using ten cells per measured area as a level of possible significance. Of the high bacterial count specimens 84.7 per cent had cell counts of more than ten cells per measured area, while in the low bacterial count group only 9.9 per cent had a cell count of more than ten cells per measured area; of the small intermediate bacterial count group 49.3 per cent had a cell count of more than ten cells per measured area.

Of the twenty-three patients with asymptomatic significant bacteriuria, twenty-one had a quantitative leucocyte count of more than ten cells per measured area. (Figure I)

Correlation between quantitative cell counts and wet-film estimation.

A direct comparison was made of these two techniques in one thousand and two urine samples (Table VII). As might be expected, both techniques revealed the gross degrees of pyuria. If, however, the usual criterion of five or more cells perhhigh power field, that is, one hundred or more per twenty high power fields, on wet-film examination is taken as indicative of infection it will be seen that 65 per cent of specimens with lesser degrees of pyuria had in fact white cell chamber counts of more than ten cells per measured area, which is the level found to have a high correlation with infection (Table VI). Even if the wet film level of significance is lowered to include specimens with ten cells or more per twenty high power

fields the discrepancy between the two techniques is still considerable (30 per cent). There is good overall agreement (83 per cent) if ten or fewer cells for the chamber count value is correlated with a value of below ten cells per twenty high power fields on the wet film.

Correlation between the quantitative cell count and the estimation of pus cells by a Gram-stained film.

The assessment of cells in the Gram-stained film was made on one thousand eight hundred and seventy-eight specimens. (Table VIII). Eighty-six per cent of the one thousand one hundred and ninety-six chamber cell counts of ten or less per measured area did not reveal any pus cells in the fifty oil-immersion fields of the Gram-stained film. On the other hand 79 per cent of the quantitative cell counts of more than ten cells per measured area showed one or more cells per fifty oil-immersion fields. If one takes more than ten cells per fifty oil-immersion fields as equivalent to pyuria the overall agreement is 79.3 per cent, however, 66 per cent of the counts of more than ten cells per measured area would be missed.

When one reduces the level of pyuria by the Gram-film estimation to one to ten cells per fifty oil-immersion fields the overall agreement is 83.4 per cent and only 20 per cent of the significant pyuria would be missed. Indeed, although the level of one to ten cells was taken for convenience (Table VIII) the majority of these estimations contained between five and ten cells per fifty fields. The error of 20 per cent at this level occurred in specimens from patients with red cells and protein in the urine.

Correlation between viable bacterial count and bacterial estimation by Gram-stained film.

The comparison between viable bacterial count and the estimation of organisms in the uncentrifuged specimen of urine was performed on two thousand and five specimens (Table IX). Of the high bacterial

counts, 73.5 per cent revealed more than fifty organisms in the fifty oil-immersion fields; on the other hand none of the one thousand five hundred and fifty-eight low bacterial count specimens showed more than fifty organisms in the area examined. Indeed 90.2 per cent of the high bacterial count specimens showed eleven or more organisms in the area examined, whereas only 0.83 per cent of the low bacterial counts showed more than ten organisms in the fifty oil-immersion fields. Thus if one takes the presence of one organism per five oil-immersion fields (ten organisms per fifty oil-immersion fields) as being a significant indication of bacteriuria we find a correlation, between the high and low bacterial counts and Gram-film estimation, of 97.8 per cent.

Discussion

Mid-stream technique

The relationship between catheterisation and urinary tract infection has been confirmed by many authors (Marple, 1941; Kass, 1955; Beeson, 1955; Kaitz and Williams, 1960; Kleeman, Hewitt and Guze, 1960; Paterson, Barr and Macdonald, 1960; Slade and Linton, 1960; Brumfitt, Davies and Rowsen, 1961; Gillespie, Lennon, Linton and Slade, 1962; Oseasohn, Quilligan, Porsky and Rosenblum, 1962). Turck, Goffe and Petersdorf (1962), however, have stated that in the physically well catheterisation is a negligible hazard. Nevertheless a risk still exists and at the introduction of the study it was considered that the evidence of the correlation between catheter specimen and mid-stream specimens of urine, (Monzon, Ory, Dobson, Carter and Yow 1958; Riley 1958; Frylos and Steg 1959, and later Clarke 1960), was consistent enough to use mid-stream specimens for this study. The mid-stream technique which I advised, and has been used throughout the study, relies on the simple mechanical toilet of the vulva using sterile saline. Antiseptics were

excluded for three main reasons, firstly antiseptics require time to act on bacteria particularly in the presence of desquamated cells. Secondly, the liberal use of antiseptics may result in the introduction of the antiseptic into the specimen with the resultant partial or complete sterilisation. Lastly, a small proportion of patients develop sensitisation to antiseptics.

From the bacterial count aspect the correlation between three mid-stream specimens, taken at three-hourly intervals, was very good, 95 per cent, a factor which Kass (1962) suggested might be less well correlated.

Bacterial counting method

The incidence of asymptomatic bacteriuria has been estimated to lie between 6 per cent (Kass, 1956) and 26 per cent (Nuvos and Rocha, 1959) of female patients. One of the investigations in this study revealed that some 16 per cent of one hundred and thirty-nine asymptomatic female patients had significant bacteriuria, that is a bacterial count of more than 100,000 organisms per millilitre urine. Similar incidences have been reported recently (Rengarts, 1960; Kaitz and Williams, 1960; Cattell and Lefford, 1963). Since the only way to demonstrate the presence and significance of bacteriuria is by quantitative culture methods, a simplified, but accurate, quantitative culture method is required for widespread use.

The method which I have described, using a loop delivering a known volume of urine, was shown to be accurate when compared to pour-plate techniques. Similar loop methods have been described by Hooprich (1960) and O'Sullivan, Fitzgerald, Moynell & Malins (1960). The important difference in my method was the use of the box-like method of inoculation which accelerates the reading of the count in the high bacterial counts where 90 per cent of the plates with growth on three or four sides of the "box" had counts of more than 100,000 pathogenic organisms per millilitre of urine.

It has recently been suggested (Cattell and Lofford 1963) that routine techniques of bacteriological culture of the urine are adequate for indicating significant bacteriuria, but they admit that "the use of bacterial counts halved the number of equivocal results". By the use of quantitative bacteriology I have found that the interpretation of the significance of the culture results is clearer to both the bacteriologist and the clinician. This results in a more satisfactory appraisal of the necessity for, and efficacy of, therapy.

It has been my practice to recommend the examination of two specimens of urine from the patients in the wards. This is particularly necessary in the intermediate bacterial count group where the diagnosis of infection may be in doubt. In the series summarised in Table II the count dropped below the 10,000 organisms per millilitre level in all cases. However, in another part of this study two patients developing infection demonstrated that the counts can rise from an intermediate level to a significant level of bacteriuria within twenty-four hours. (Page 77).

Measurement of pyuria

Several authors have considered that pyuria is a poor indication of bacteriuria (Loopuyt, 1946a; Barnard, Story & Root, 1953; Anderson, 1956; Brod, 1956; McCrea and Anderson, 1956; Kass, 1957; Macdonald, Levitin, Mallory and Kass, 1957; Jackson, Grieble & Knudson, 1958; Muvos and Radha, 1959; Thomson, 1959; Clarke, 1960; Kaits and Williams, 1960; Rengarts, 1960; Pinkerton, Wood, William & Calman, 1961). This is not surprising since they used the relatively inaccurate wet-film technique and their levels of significance varied from two to three cells per high power field up to more than ten cells per high power field. The inadequacy of this technique was clearly demonstrated when it was compared to a quantitative chamber count method (Table VII). The levels of pyuria quoted by the authors above would miss from 30 per cent to

65 per cent of the specimens with more than ten cells per measured area in the chamber count, a level of pyuria which correlates closely with the bacterial count.

Similar results of pus cell counting by slightly different chamber methods have been reported by Drumfitt, Davies & Rossen (1961), Stansfeld (1962), Little (1962) and Bradley and Little (1963), confirming the need for an accurate estimation for pyuria.

The Gram-stained film in the diagnosis of urinary tract infection.

Freedman (1958) found good agreement between the frequency of bacteria in the film and significant bacteriuria using methylene blue stained film of uncentrifuged urine and Kass (1955) found 80 per cent correlation using a Gram-stained film. In my study, using a Gram-stained film of uncentrifuged urine, there was 97.8 per cent agreement between the bacterial counts and film estimation when the level of ten organisms in the fifty oil immersion field was considered as significant. The advantage with the Gram film is that it confirms the presence of Gram-negative bacilli and differentiates these from the diphtheroids of Doderlein's bacillis which may occur in the mid-stream specimens of urine.

The usefulness of the same film for indicating pyuria was also considered. There was 83 per cent agreement between quantitative cell count and the film pus cell estimation when one or more pus cells were noted in the fifty oil immersion fields. There was a disagreement of 20 per cent in the significant pyuria by this method which was confined to specimens with protein and red cells.

Thus the use of a stained film of the uncentrifuged urine for estimating pyuria and bacilluria is superior to the wet-film technique and is to be preferred when there is some urgency in the diagnosis, for example when there is some difficulty in the differential diagnosis of pyelonephritis and acute appendicitis.

The incidence of infection in this study.

Significant bacteriuria was present in 29.9 per cent of all the patients, 31.5 per cent of those without urinary tract abnormalities and 24.7 per cent of those with urinary tract abnormalities. The abnormalities were glomerulonephritis and tubular necrosis in the main. Of the female patients, 32 per cent had bacterial counts of more than 100,000 organisms per millilitre, 35.1 per cent of those with urinary tract abnormalities and 31.5 per cent of those without such lesions. Of the male patients 22 per cent had this significant bacteriuria; 16.7 per cent of those with, and 31.4 per cent of those without, urinary tract abnormalities.

Summary.

In an investigation of seven hundred and nine patients, from whom two thousand and eighty-five mid-stream specimens of urine were examined, sixteen per cent of the specimens had a significant bacterial count of more than 100,000 organisms per millilitre of uncentrifuged urine.

Of one hundred and thirty-nine patients in a medical ward, who had no symptoms referrable to the urinary tract, 16.5 per cent had a significant bacteriuria.

The quantitative method of bacterial counting on the uncentrifuged urine using a bacteriological loop, delivering a known volume, was found to correlate very closely to a pour-plate technique. The loop method used a box-like inoculum which accelerated the bacterial count by noting the number of the sides of the "box" on which growth appeared.

A comparison between the quantitative bacterial counts and a quantitative leucocyte count showed good correlation using a level of more than 10 cells per measured area as an indication of pyuria.

The comparison between the quantitative leucocyte count and

the estimation by wet-film technique showed the inaccuracy of the routine wet-film.

A Gram-stained film of a measured loopful of uncentrifuged urine was shown to be successful for the estimation of both pyuria and bacteriuria when fifty oil-immersion fields are examined and it is suggested as a useful method when employed with the quantitative leucocyte counts when there is urgency in the differential diagnosis.

It was also shown that the use of a mid-stream urine in the diagnosis of urinary infection was satisfactory when tested for reproducibility.

Significant bacteriuria was present in nearly thirty per cent of the patients.

CHAPTER II

THE IDENTIFICATION AND INCIDENCE OF ORGANISMS ISOLATED.

Introduction

Although the incidence of organisms isolated from the urinary tract has often been studied biochemically these studies, until recently, have not been related to bacterial counts. Since the renewed interest in urinary infections following the work of Kass (1955) the reported incidences of various organisms isolated have not differed markedly from pre-quantitative reports. This is particularly so in the United States where studies by Macdonald, Levitin, Mallory and Kass (1957), Kaitz and Williams (1960) and Osoasohn, Quilligan, Persky and Rosenblum (1962) still show a relatively low incidence of the Escherichia coli. It has been suggested by Jackson (in Quinn and Kass, 1960) that the biochemical identification may be at fault and he quotes the example of "urease-positive Escherichia coli" being reported in one study. Since the thorough classification of the Enterobacteriaceae by Kauffman and his colleagues (Kauffman 1954) and Edwards and Ewing (1955, 1962) have been available to bacteriologists these grosser errors in identification should be infrequent. The biochemical

classification of the organisms in this study has been performed after Edwards and Ewing (1962) and related to the bacterial counts of the urine.

Material and Methods

The organisms were isolated from the urine of patients described in the previous chapter and the initial isolation was performed using the quantitative methods detailed there. Initial identification was made by means of colonial appearance on blood agar and on MacConkey's No. 2 (Oxoid) medium. This divided the Enterobacteriaceae into lactose + and non-lactose fermenters.

Identification

Staphylococcus. The staphylococci were identified by their cultural characteristics, Gram+ staining and by coagulase-testing.

Streptococcus. The streptococci were identified by their cultural characteristics, Gram+ staining and heating to 60°C for thirty minutes.

Pseudomonas aeruginosa.

The pseudomonads were identified by their cultural characteristics, pigment formation, characteristic odour and by biochemical testing.

The Enterobacteriaceae.

The carbohydrate fermentations were performed using one per cent fermentable substance in peptone water containing one per cent Andrade's indicator and consisted of glucose, lactose, sucrose, mannitol, dulcitol, maltose and salicin. Where non-lactose fermenters were encountered xylose, arabinose, adonitol, rhamnose and inositol were incorporated in the series of tests.

Indole production was tested in one per cent peptone water, after twenty-four hours incubation its presence was confirmed by the reaction with Ehrlich's rosindole reagent (Mackie and

MacCartney 1960).

Urease production was tested using Christensen's urea agar (Christensen 1946). The urea slopes were examined after six hours and again after twenty-four hours incubation.

The Methyl red and Voges-Proskauer reactions were performed in a glucose-phosphate medium (Mackie and MacCartney 1960) and incubated for five days.

Citrate utilisation was tested in Koser's citrate medium (Oxoid) (Mackie and MacCartney 1960) and incubated for seven days.

Motility was tested for on semi-solid agar medium (test No. 16, Report 1958).

All the tests were performed at 37°C and the substrates were inoculated from young agar cultures. The citrate medium was inoculated with a needle point to avoid the objection of Talbot, Cunliffe and Gower (1957), concerning the fallacy of a large inoculum in liquid citrate media.

Results and interpretation

The main biochemical differences of the Enterobacteriaceae encountered in this study are shown in Table X, abstracted from Edwards and Ewing (1962).

The incidence of organisms first isolated from the patients is shown in Table XI. All these organisms were present in the urine at more than 100,000 organisms per millilitre. Only those cases where bacterial counts of both organisms were more than 100,000 per millilitre are included in the mixed cultures at that level. Where two organisms were present one of which had a count less than 100,000 organisms per millilitre only the one which was above the significant level was included in Table XI and the other included in Table XII in the appropriate column.

Table XI is also divided into females and males and further sub-divided into those patients without urinary tract abnormalities (columns 1 and 3) and those with urinary tract abnormalities (columns

2 and 4).

Table XII gives the overall incidence of organisms isolated in the various bacterial count groups. It includes recurrent isolates from the same patient and strains isolated from patients under treatment.

Incidence of infecting organisms on primary isolation.

Single species were present in the primary isolates from two hundred and one (94.8 per cent) of the two hundred and twelve patients with significant bacteriuria. Two species were present in eleven specimens but only two of these were obtained from patients without underlying urinary tract lesions. Both of these patients had a long history of recurrent infection occurring before they were investigated.

Escherichia coli

Escherichia coli was solely isolated from one hundred and sixty-nine of the two hundred and twelve patients and associated with other organisms in six cases. Statistically there was no significant difference in the frequency of Escherichia coli isolated from the female and male patients with normal urinary tracts. There was, however, a significantly higher frequency of Escherichia coli isolated from the patients with normal urinary tracts compared to those with abnormalities, Chi square 43.48, P - greater than 0.001.

Proteus mirabilis

This organism was isolated solely in seventeen patients and associated with other organisms in a further six cases. There was a higher frequency of this organism isolated from patients with abnormalities of the urinary tract than those without such lesions, (Chi square 33.3906, P - greater than 0.001). Most of the patients with Proteus infections had a history of instrumentation for investigation of the urinary tract and consequently these infections may be nosocomial.

The Gram-positive cocci

All the patients from whom Staphylococci (4) or Streptococcus faecalis (6) were isolated gave a history of instrumentation, usually catheterisation, operation on the urinary tract or recurrent treatment.

Pseudomonas aeruginosa (Pseudomonas pyocyanea).

The isolations of Pseudomonas aeruginosa were from two patients, one of whom had diabetes mellitus and the other acute tubular necrosis. The double isolation of Pseudomonas aeruginosa and Proteus mirabilis was from a patient with acute renal failure following abdomino-perineal resection.

Citrobacter freundii (Escherichia freundii).

This species was isolated on three occasions on its own and once accompanying Streptococcus faecalis. In the last case the patient had a nephrotic lesion.

Klebsiella species

This species was isolated from two patients, one of whom had pyonephrosis and the other nephrocalcinosis.

Recurrent infections

Seventy patients in this study had one hundred and forty recurrent infections. There was a change of species in twenty-one cases and of the others, five patients had forty-six incidents of the same species isolated at various times. These five patients had underlying disease of the urinary tract comprising of pyonephrosis, chronic glomerulonephritis, acute renal failure and post-operative infections, following prostatectomy and nephrectomy. From the patient with pyonephrosis Klebsiella was isolated on eighteen occasions over four years. Proteus mirabilis was isolated from the patients with the nephrectomy and post-prostatectomy infection five and six times, respectively, over two years. The patient with the glomerulonephritis and infection had Escherichia coli isolated on seven occasions, and the patient with acute renal failure had

Escherichia coli isolated on ten occasions over two years.

Discussion

Escherichia coli

By careful quantitative techniques for the isolation of organisms from the urine and their subsequent identification, a high incidence of Escherichia coli has been demonstrated. In the primary isolation 79.2 per cent of the two hundred and twelve patients had Escherichia coli as the sole infecting organism at a significant level of more than 100,000 organisms per millilitre urine. Where these patients were classified into normal and abnormal urinary tracts (Table XI) the incidence of Escherichia coli was even more marked, 93.8 per cent, in those without abnormalities. These figures are higher than most reports, particularly those of the United States where the incidence of Escherichia coli tends to be about 50 per cent of the isolates from patients with significant bacteriuria. Macdonald, Levitin, Mallory and Kass (1957) found only 38 per cent of their cases had Escherichia coli whereas 54 per cent had Klebsiella species isolated. Kaitz and Williams (1960) found 45 per cent of specimens had Escherichia coli isolated and 28 per cent contained either B. proteus or Klebsiella. Osseasohn, Cuilligan, Persky and Rosenblum (1962) found 50 per cent of isolates had Escherichia coli present. In urines from diabetic patients Nuvos and Rocha (1959) reported 46 per cent, and Rengarts (1960) only 26 per cent, with Escherichia coli. In all these patients there was a history of previous treatment and, usually, of instrumentation. Recent United Kingdom reports, however, are nearer my figures, Drumfitt, Davies, Rossen, (1961) found 84.6 per cent, and Turner (1961) 87.9 per cent, of specimens had Escherichia coli isolated. Both of these investigations were carried out in ante-natal clinics. Brod (1956) found 45 per cent of cases, with chronic pyelonephritis had Escherichia coli and some 25 per cent had Staphylococcus aureus isolated.

When the organisms isolated from recurrent infections are added

to the primary isolates from significant bacteriuria, Table XII, the frequency of Escherichia coli drops to 65.6 per cent. The inclusion of the isolates from all bacterial count levels brings this frequency still lower to 50.7 per cent. It may be that the inclusion of recurrent infections, particularly those of Proteus and Klebsiella species, which are notoriously difficult to treat successfully, accounts for the high incidence of non-Escherichia, coliform bacilli in the American series. Of course, the error in biochemical identification due to lack of purity of the culture may be present in some series (Jackson, 1960). It is more likely, however, that the infections in patients with unrecognised associated urinary tract abnormalities are included. When one compares the frequencies of isolates from patients with underlying surgical or medical lesions associated with the urinary tract the figures approach those of the American studies. In one study I compared the isolates from one hundred and twenty-seven patients in surgical, gynaecological and urological wards and the incidence of Escherichia coli was 44.8, 55.2 and 50 per cent respectively and the frequency of Proteus species was 10.3, 24.1 and 16 per cent respectively from these wards. (unpublished investigation)

This present investigation has revealed that, although there was no statistically significant difference in the isolation of Escherichia coli from the female and male patients, there was a significant reduction in the frequency of Escherichia coli in the patients with underlying urinary tract pathology, mainly renal parenchymatous damage, compared to those with apparently normal urinary tracts.

Garrod, Shooter and Curwen (1954) found that their isolates of Escherichia coli occurred more frequently in females than males and the converse applied to the Proteus and Pseudomonas species. However, their figures include recurrent infections mainly in patients with urinary tract abnormalities and they fail to associate

such abnormalities with the sex and frequency of isolation of organisms. Since the majority of patients with abnormalities were males, it is thus likely that the organism isolated would be other than Escherichia coli.

Proteus species

In contradistinction to the Escherichia coli there was a statistically increased frequency of Proteus species isolated from patients with underlying urinary tract abnormalities. As is to be expected, most of these patients had a history of urethral instrumentation, an exception to this, however, was the isolation of Proteus mirabilis from a boy of six, with a history of recurrent infection, in whom no demonstrable lesion was noted in the routine radiological investigations. The investigations have not, as yet, included a micturating cystogram to exclude ureteric reflux, which is recognised as being associated with recurrent urinary infections in children and adults (Hodson and Edwards, 1960; Edwards, 1961; Garrett, Rhamy, Carr, 1961; Winman and Hutch, 1962; Williams, 1962).

All the Proteus strains isolated were biochemically Proteus mirabilis with one exception of an isolate of Proteus vulgaris from a patient with a nephrotic lesion. Similar findings of high frequency of Proteus mirabilis have been noted by Middleton (1957), who found fifty-one of fifty-eight strains, and Whitby and Muir (1961), who found fifty-one of fifty-five strains of Proteus, to be Proteus mirabilis.

The patients from whom Proteus species were isolated frequently had recurrence of the same species. Twenty-six such recurrences occurred, seventeen in five patients. Although the bacterial count fell to below 10,000 organisms per millilitre whilst on treatment it soon rose to more than 100,000 organisms per millilitre on the cessation of therapy. It is possible the antibacterial agent used, usually nitrofurantoin, failed to penetrate to the nidus of infection although inhibiting the proliferation of the organism in the urine.

Brado and Wiesinski (1960) demonstrated that the Proteus species had the ability to invade the renal tubular cells and this may be the reason for the failure of treatment. Certainly the use of an antibiotic which penetrates the cell wall resulted in the eradication of the infection in the majority of cases.

Other Gram-negative bacilli

Pseudomonas aeruginosa (pyocyanea) was primarily isolated, as a sole cause of infection, in the specimens from two patients, one of whom had acute renal failure and the other diabetes mellitus. A third patient had Pseudomonas aeruginosa and Proteus mirabilis isolated together, this patient had acute renal failure following an abdomino-perineal resection for rectal carcinoma. Recurrent isolations of Pseudomonas occurred in these two patients with acute renal failure on four occasions and once from a diabetic patient who had been catheterised. Pyrah, Goldie, Parsons and Raper (1959), Dutton and Ralston (1957) and Kohod (1958) in their investigations of cross-infection in a urological ward demonstrated the ubiquity of Pseudomonas aeruginosa on the fomites, in irrigating fluids and in dettol solutions. Again the risk of infection during continuous catheterisation has been demonstrated by several investigators, for example, Gillespie, Linton, Miller and Slade (1960); Patterson, Kass and McDonald (1960); Vasauteln (1960). Kass and Schneiderman (1957) demonstrated that Serratia marcescens, spotted on the glans penis or vulva of three patients on continuous catheter drainage, entered a previously sterile bladder within seventy-two hours. Thus it seems likely that these patients with acute renal failure, which necessitated continuous catheterisation, developed a nosocomial infection in the urological ward.

Klebsiella species were isolated from two male patients both of whom had underlying renal pathology, one had pyonephrosis and the other nephrocalcinosis. The patient with pyonephrosis had a constricted ureter on one side which accounted for the failure of appropriate

antibacterial therapy and continued excretion of the organisms on eighteen occasions. The other patient responded satisfactorily to treatment.

Citrobacter freundii was primarily isolated from three patients in pure culture and in one it was associated with Streptococcus faecalis. The three patients from whom it was isolated in pure culture had acute leukaemia, recurrent pyelonephritis and a nephrotic lesion. The mixed culture was also from a patient with nephrosis. Recurrent isolations were obtained from four patients with associated renal lesions.

The Gram-positive cocci.

Staphylococci occurred as the primary isolates from four patients. Two of these, who were free from urinary tract lesions but had a history of catheterisation, had a coagulase-negative Staphylococcus albus isolated in pure culture, the associated Gram-stained film showed pyuria and clusters of Gram-positive cocci. The other two patients had Staphylococcus aureus in their specimens, both of these had had instrumentation in a ward with an endemic Staphylococcus aureus, 'phage-type 'A', which was the same type as the isolated organisms. A further five isolates of a coagulase-negative Staphylococcus albus were observed and three more isolations of the endemic Staphylococcus aureus were noted from patients who had had their original infections, by coliform organisms, eradicated before operation or instrumentation.

The importance of Staphylococcus aureus as a pathogen is well recognised, particularly in cross-infection, and in this study all the Staphylococcus aureus strains were directly associated with cross-infection in a ward with a "resident" strain, 'phage type 'A'.

Coagulase-negative strains of Staphylococcus albus have also been noted recently as a cause of urinary infection by Pereira (1962) and by Mitchell (1964). In Pereira's cases the isolates were from primary infections without a history of instrumentation

and without recognised underlying pathology. He noted that nine per cent of urinary infections were due to staphylococci, fifty-seven per cent of these were Staphylococcus aureus and forty-three per cent were coagulase-negative Staphylococcus albus. Mitchell's cases were mainly patients who had undergone urethral catheterisation. He did not give the frequency of staphylococci isolated but states that seventy-five per cent of cases who had instrumentation developed infection due to a coagulase-negative Staphylococcus albus. Seven of his cases from whom this organism was isolated, however, had no history of instrumentation.

Streptococcus faecalis was primarily isolated, in pure culture, from six patients, all of whom had underlying lesions of the urinary tract. It was also isolated in mixed culture from four patients, two of whom did not have underlying lesions but had a long history of treatment for recurrent infections. It occurred in a further nineteen specimens, only one of which was from a patient without an underlying lesion.

An alpha-haemolytic streptococcus was isolated from a patient with chronic glomerulo-nephritis who had a previous infection with Escherichia coli. There was no pyuria associated with the specimen but the patient excreted a similar organism on three further occasions at an intermediate bacterial count level.

Thus the majority of infections due to organisms other than Escherichia coli were associated with urinary tract abnormalities, instrumentation or recurrent treatment. This confirms the findings of Coleman and Taylor (1949) and the view of Kass (1955) that the presence of infection by organisms other than Escherichia coli is probably an indication of underlying renal pathology or recurrent treatment.

Summary

A high frequency of Escherichia coli was noted in this investigation. Of the primary isolates from two hundred and twelve patients with bacterial counts of more than 100,000 organisms per millilitre urine, one hundred and sixty-nine (79.7 per cent) had a pure culture of Escherichia coli. When the isolates from patients with urinary tract abnormalities were excluded, Escherichia coli was solely isolated from one hundred and fifty of one hundred and sixty specimens (93.8 per cent).

Although there was no significant difference in the frequency of Escherichia coli isolated from the sexes there was a highly significant reduced frequency of isolation of the organism in those patients with urinary tract abnormality.

Conversely Proteus species, obtained from seventeen patients on primary isolation, was significantly more frequently isolated from those patients with a urinary tract abnormality.

Similarly, with the other non-Escherichia, coliform organisms and the Gram-positive cocci, there was a real association between urinary tract abnormalities or instrumentation and their isolation from patients.

The presence of organisms other than Escherichia coli should suggest that the patient has some abnormality of the urinary tract associated with infection.

CHAPTER III

THE SEROLOGICAL TYPING OF ESCHERICHIA COLI.

Introduction.

The incidence of Escherichia coli infections of the urinary tract is high, this has been confirmed in the previous chapter. Where Escherichia coli has been found epidemiologically associated with disease, as in infantile gastro-enteritis, the pathogenic significance of certain serological types has been noted. Kauffmann(1947) suggested that certain serological groups of Escherichia coli were isolated more frequently from the urine than from the faeces. Vahlne (1945) and Sjöstedt (1946) considered that although certain serological groups were found more commonly in human infections these were not limited to the urinary tract infections. More recently Rantz (1962) suggested that certain serological groups were more invasive for the urinary tract than other types and that these caused clinically evident pyelonephritis more frequently than other serological groups. Turck and Peterdorf (1962) confirmed that certain groups were associated with non-enteric infections but suggested that this may be due to an increased prevalance in the environment. The majority of patients studied in this thesis were not exposed to the risk of

nosocomial infection, this was particularly so with those patients who had Escherichia coli isolated from the urine, thus there is little possibility of the incidence of serological groups being biased by the inclusion of environmentally prevalent strains.

When this study started there were no antisera available for grouping non-enteropathogenic Escherichia coli, consequently antisera had to be made. The choice of prevalent serotypes was suggested by the incidence of these groups in the Scandanavian studies and obtained from Dr. Franz Ørskov.

Materials and methods.

Five hundred and thirty-four strains of Escherichia coli were investigated, three hundred and sixteen were from urines with bacterial counts more than 100,000 per millilitre, eighty-six from the intermediate count group and one hundred and thirty-two from counts below 10,000 organisms per millilitre. They were all identified biochemically as Escherichia coli by the methods in the previous chapter.

Preparation of specific 'O' antisera.

The following strains of Escherichia coli were obtained from Dr. Franz Ørskov of the Statens Serum-Institut, Copenhagen.

01	U5/41	01:K1:H7
02	U9/41	02:K1:H4
04	U4/41	04:K3:H5
05	U1/41	05:K4:H4
06	B17458/41	06:K2a,2c:H1
07	B17509/41	07:K1:H-
08	G3404/41	08:K8:H4
09	B1316/42	09:K9:H12
011	B1623/42	011:K10:H10
018	F10018/41	018:K76:H14
039	H7	039:K?:H-
075	E3b	075:K74:H5

These strains were grown on digest nutrient agar for 18 hours and the cultures emulsified in saline and placed in a Koch steamer for two hours to destroy the 'K' antigen. (Vahlne, 1945). The deposits were alcohol-treated and acetone-dried, then reconstituted

in twenty millilitres of saline. Rabbits were inoculated twice weekly with graded doses of the suspension, starting with 0.5 millilitres (1/40th of the culture) intramuscularly for the first week, then two one-millilitre amounts intraperitoneally in the second week. For the next fortnight one-millilitre amounts were inoculated intramuscularly. Blood samples were removed from an ear vein for antibody level. When the titre was greater than 1 in 2000 the animal was exsanguinated by carotid section.

Preparation of Escherichia coli strains for typing.

The organisms to be tested were grown on digest nutrient agar for eighteen hours and a smooth colony was removed and inoculated into nutrient broth for six to eight hours. The suspension was then placed in the Koch steamer for two hours to remove the 'K' antigen. If the suspension appeared rough two further attempts were made to obtain smooth suspensions by subculturing on to blood agar plates. If these failed to produce smooth suspensions the organism was termed 'rough' and designated 'non-typable rough (N.T.R.), this occurred with thirty-five strains. Where no agglutination occurred with the antisera the strain was designated non-typable smooth (N.T.S.), one hundred and ninety-six smooth strains were untypable.

Typing procedure

The strains were screened using three pools of antisera:-

'A' contained antisera 01, 02, 04, 07.

'B': 05, 06, 08, 09.

'C': 011, 018, 039, and 075.

The antiserum dilution for each component was 1 : 150. Where agglutination occurred the strain was tested against monovalent antisera of the pool at a dilution of 1 : 300. The tubes were incubated at 50°C for eighteen to twenty-four hours and examined at four and again at eighteen to twenty-four hours. The four-hour examination of the pooled antisera screen often enabled the specific

antisera to be used the same day.

Reliability of Serological typing from Stroke plate.

To consider the validity of serological typing of one colony from the original diagnostic plate it was decided to test the serology of five colonies from each plate. These colonies were removed from different sides of the box-like inoculum of the original urine culture, (Figure 4), two samples were taken from the first side. In the low counts the samples were removed from colonies as separate as possible. Seventy culture plates were examined in this way, sixty-three in the high bacterial count group, three in the intermediate group and four in the low count group.

Results

Cross agglutination with unabsorbed antisera

Using unabsorbed antisera at a dilution of 1 : 150, forty-two strains showed cross agglutination with two antisera. These cross reactions were predominantly between strains of 04 and 018 or 01 and 075 serological groups. By diluting the antisera to 1 : 2,000 or by using absorbed antisera the serological identity of the strain was confirmed. Similar cross-reactions have been noted by Knipschildt (1945), Vahlne (1945) and Kauffmann(1947).

Reproducibility of serological typing

Three hundred and fifty colonies from the initial isolation plates of seventy specimens of urine were examined. (Table XXXI). With three hundred and twenty-five smooth strains there was complete agreement, with the remaining twenty-five precise typing was not possible due to roughness of the strains. There was no difference noted in the various bacterial count groups.

Prevalence of 'O' groups.

Of the five hundred and thirty-four strains, three hundred and two (56.6 per cent) were typable by the twelve antisera and thirty-five (6.5 per cent) were not typable due to the roughness of the

strains. (Table XIII). The strains most frequently isolated were O1, O2, O4, O6, O18, O75 which accounted for 47.4 per cent of all the strains. Forty-four per cent were typable in the first eight serological groups, namely O1, O2, O4, O5, O6, O7, O8, O9.

Of the one hundred and eighty-two primary isolates, one hundred and nineteen were typable (65.4 per cent) and nine (4.9 per cent) were rough and untypable. (Table XIV). The most frequently isolated serological groups were O1, O2, O4, O6 which accounted for 47.8 per cent of the isolates. In the first eight serological groups 54.4 per cent of the strains were found. The majority, one hundred and forty, of the primary isolates were from patients with symptomatic infections without any associated urinary tract lesions, ninety-two (65.7 per cent) of whom had strains isolated which were typable and six had strains which were untypable due to roughness of the colonies. The prevalent groups were O1, O2, O4 and O6 which accounted for 48.6 per cent of the total strains within these four serological groups.

Of the recurrent isolates, Table XV, sixty-seven (50 per cent) were typable. There is, of course, an increase in frequency of serological groups O18 and O75 which were persistently isolated from two patients. The serological group O2 does not appear frequently as a recurrent isolate although it does account for nearly ten per cent of the typable strains from the symptomatic patients without renal lesions. (Table XIV).

In the bacterial count of less than 10,000 organisms per millilitre urine the distribution of serological groups was similar to that of the high bacterial count group, groups O1, O4, O6 and O18 were the most frequently isolated, accounting for 70.6 per cent of the typable strains (Table XIII). As with the recurrent isolates in the high bacterial counts, serological groups O2 does not appear as a frequent isolate.

Comparisons of frequency of serological groups in the bacterial count groups.

There was no significant difference in the frequency of typable strains from the counts of more than 100,000 organisms per millilitre of urine and those below 10,000 organisms per millilitre. (Table XIII). No significant difference in the frequency of typable strains was detected between the isolates from patients with symptoms, but without urinary tract abnormalities, and the isolates from patients with such lesions. (Table XIV). There was a possibly significant, increased frequency of typable strains in the primary isolates compared to the recurrent isolates from the patients with significant bacteriuria. (Chi square= 6.9217, P - between 0.01 and 0.001). (Table XV).

Overall there was no significant difference in the frequency of typable strains in the primary symptomatic group compared to the low bacterial count group. With the individual serological groups there was no significant difference in the frequency of 01, 02, 04, 05, 06, or 075. The numbers in the other groups were too small for adequate comparison.

Comparison of the serological groups from females and males.

There was no significant difference in the frequency of serological groups occurring in the sexes in the total, primary and primary symptomatic groups of isolates of more than 100,000 organisms per millilitre urine (Tables XVI and XVII and XVIII).

Discussion

Although there are now one hundred and forty-five recognised serological 'O' groups of Escherichia coli, relatively few are associated with urinary infections. In this study more than two thirds of the primary isolated strains from patients with significant bacteriuria were typable using twelve antisera. Vahlne (1945), using twenty-five antisera, typed sixty-one per cent of his strains and Rantz (1962), using one hundred and twenty-six antisera, found

three quarters of his coliform infections to have typable isolates, although these fell into only twenty-eight serological groups. Turck and Petersdorf (1962), using one hundred and thirty-seven antisera, typed 76.8 per cent which occurred in only forty-nine of the serological groups. Indeed the majority of pathological strains occur in the first ten of Kauffmann's serological groups (Kauffmann 1947). Vahlne (1945) found 47.2 per cent, Ujvary (1958) 48 per cent and Rantz (1962) 49.2 per cent of their strains occurred in these first ten groups and I have found that 54.4 per cent of the primary isolates in eight of these ten groups, I did not use antisera for groups 03 and 010.

Prevalence of serological groups

It is clear that the majority of isolates fall within a narrow spectrum of serological groups; which in my series consisted of 01, 02, 04 and 06. These types accounted for seventy-three per cent of the typable strains. Vahlne (1945) found 01, 02, 04, 07, 018 occurred in eighty-four per cent of his typable strains, Rantz (1962) had a spectrum of 02, 04, 06, 075 (60.7 per cent), Turck and Petersdorf (1962) had 02 replaced by 01 resulting in 01, 04, 06, 075 being found in fifty-seven per cent of their significant bacteriurias.

The most frequently encountered serological groups in my study have been 04 and 06, these accounted for one third of all the strains, and nearly fifty per cent of the typable strains, found in the symptomatic significant bacteriurias. Similar figures were obtained by Rantz (1962) and Turck and Petersdorf (1962).

Reproducibility of serological grouping

The validity of using one colony for serological typing was evaluated and confirmed. If one excludes the difference in typing due to roughness of the strain then there was complete agreement in the typing of five widely dispersed colonies for the plates inoculated initially from the urine. Indeed in one instance thirty colonies were examined from a patient over a period of five days and the

serological type remained constant. This finding would suggest the homogeneity of infective organism in urinary infection, that is, one serotype being responsible for each infection. Turck and Petersdorf (1962) found a single serotype in the urine but noted that faecal isolates tended to be multigroupable. Vahlne (1945) found that there was only one serotype present in ninety-five per cent of his urinary isolates, similar figures were obtained by Vosti, Monto and Rantz(1962).

Comparison of the serological groups in the bacterial count groups

In this present study the frequency of serological groups isolated from patients in the high and low bacterial count groups have not shown any demonstrable statistically significant difference. Since the presence of organisms in concentrations of less than 10,000 per millilitre of urine is generally accepted as indicating urethral contamination, this lack of difference would suggest that the origin of the urinary infection is from the urethral flora. It has been shown by Holmholtz (1950) and by Shelman and Nossent (1954) that Escherichia coli are commonly present in the urethra and it may be that these assume an invasive role, the mechanism of which is still open to speculation.

An interesting feature noted in my study was the lack of demonstrable statistical difference in the frequency of the serological groups between the sexes. This fact is correlated elsewhere in this thesis (Chapter VI) and it would suggest that the routes of infection by Escherichia coli in the sexes may not be different.

Summary

Using only twelve antisera it has been possible to group 56.6 per cent of all strains. Forty-four per cent of all strains were typable in the first eight of the serological groups used. Of the primary isolates from symptomatic infections 65.7 per cent were typable; 48.6 per cent of all strains of the serological groups 01,

02, 04 and 06 occurred in these primary symptomatic isolates.

There was a good correlation in the serological typing of five colonies isolated from the initial isolation plates. There was complete agreement in all the typable strains, three hundred and twenty-five of the three hundred and fifty colonies. Twenty-five strains were not typable due to roughness. It is considered that this reproducibility confirms the valididy of using one colony for serotyping of urinary isolates.

Statistically, there was no significant difference in the number of typable strains from the high bacterial count specimens and the low bacterial count specimens. Nor was there any difference in frequency of the typability of strains in patients with primary symptomatic infection compared to those with associated urinary tract abnormalities. There was a possibly significant, increased frequency of typable strains in the primary infection compared to the recurrent infection. There was no significant difference in the frequency of specific serological groups in the high and low bacterial count groups.

There was no statistical difference in the frequency of individual serological groups isolated from the two sexes.

CHAPTER IV

HAEMOLYSIS OF ESCHERICHIA COLI

Introduction

Before the serological typing of Escherichia coli was established one of the differentiating features of strains was the production of haemolysin. Dudgeon, Wordley and Dawtree (1921) were the first to comment on the relationship between haemolysis and urinary tract infections. Kauffmannand his co-workers (Kauffmann, 1947) also investigated the incidence of haemolysis of the Escherichia coli with particular reference to pathogenicity and association with serological grouping. None of these workers had used bacterial counts in the isolation of their strains and it was considered relevant to this study to compare the haemolytic reaction to the serological grouping.

Materials and methods.

The strains of Escherichia coli were those described in the previous chapter. Five hundred and thirty-four strains were examined.

Method.

Sandwich blood-agar plates were used for the demonstration of

haemolysis. These were made by pouring ten millilitres of ten per cent horse-blood agar over a solidified layer of digest nutrient agar (Mackie and McCartney, 1960). The strains to be tested were grown on digest nutrient agar slopes and spot inoculated through the blood agar layer by a needle-point. The plate was incubated at 37°C for twenty-four hours then examined for haemolysis. Haemolysis was noted as a thin zone of beta-haemolysis around the colony.

Results

The frequency of haemolytic strains in the bacterial count groups

Of the five hundred and thirty-four strains, one hundred and fifty-seven (29.4 per cent) demonstrated haemolysins after twenty-four hours incubation (Table XIX). There was no significant difference in the frequency of haemolytic strains in specimens from the high and low bacterial counts (Chi square = 0.7562, P - between 0.5 and 0.3). (Table XX).

Comparison of the frequency of haemolytic strains from females and males

Table XXI shows the frequency of the haemolytic strains in the specimens with high bacterial counts in the two sexes. There was no significant difference in the frequency of haemolytic strains in the males compared to that in the females. (Chi square = 1.257, P - between 0.3 and 0.2).

Comparison of serological grouping and haemolytic strains.

There was a highly significant, increased frequency of haemolysis in the five serological groups O1, O4, O6, O18 and O75 in comparison with the other strains isolated. (Chi square = 42.17, P - more than 0.001). (Table XIX).

These five serological groups, O1, O4, O6, O18, O75, accounted for 91.9 per cent of the haemolytic strains which were serologically grouped and 72 per cent of all the haemolytic strains isolated. The greatest percentage of haemolytic strains occurred in group O6, in which 63.5 per cent of the strains were haemolytic, groups O4 and O75 were next in frequency with 57.6 and 55.0 per cent respectively.

Frequency of haemolytic strains related to primary and recurrent infections in patients with, and without, urinary tract abnormalities

In the significant bacteriuria, excluding the asymptomatic group, there was no significant difference in the frequency of haemolysis in the primary isolates from those patients with and without an associated urinary tract lesion, (Table XXII). (Chi square = 1.897, P = between 0.2 and 0.1).

With the recurrent isolates, the frequency of haemolytic strains was possibly higher in the specimens from patients with normal tracts compared to those from patients with abnormalities. (Chi square = 6.990, P = between 0.01 and 0.001). This was due to the repeated isolation of a group O18 strain on several occasions from one patient with a normal tract.

Discussion

Dudgeon, Wordley and Bawtree (1921) considered that the difference in the frequency of haemolytic strains isolated from the sexes was more than fortuitous. They reported that the frequency of isolation of haemolytic strains was higher from the male than from the female, seventy-four per cent of the strains from twenty-seven males and only twenty-six per cent of the strains from forty-two females were haemolytic. They postulated that this difference was due to the mode of infection, suggesting that the male patient developed urinary infection by the blood-borne route from the bowel. However, in a later paper (Dudgeon, Wordley and Bawtree, 1922) they failed to correlate the faecal organism with the urinary isolate. In my series, I have been unable to demonstrate a significant difference in frequency of haemolytic strains between the sexes, thirty-one per cent of the strains from females, and forty-four per cent of strains from males, with significant bacteriuria, were haemolytic.

Incidence of haemolytic strains.

The total incidence of haemolytic strains from all bacterial count levels was 29.4 per cent; of the serologically groupable strains 40.7 per cent were haemolytic. Vahlne (1945) noted 26 per cent of his urinary strains to be haemolytic. Although the ability to produce haemolysis is not attached exclusively to strains within a few 'O'-groups, it may be observed more frequently in certain serological groups, (Knipshildt, 1945). Vahlne (1945), Knipschildt (1945), Ewertsen (1946) and Sjöstedt (1946) noted that haemolytic strains of Escherichia coli occurred most frequently in groups 02, 04, 06, and 018. In my strains, I have found that there was a significantly increased frequency of haemolysis in the serological groups 01, 04, 06, 018 and 075 compared to the other serological groups. These five groups accounted for 91.9 per cent of the haemolytic strains occurring in the serologically groupable isolates. There was no significant difference in the frequency of these strains isolated from females and males.

There was no significant difference in the frequency of haemolytic strains in the primary isolates, of more than 100,000 organisms per millilitre urine, compared to the isolates in the low bacterial group. No difference was noted in the frequency of haemolytic strains in primary isolates, with high bacterial counts, from those patients with and without associated urinary tract abnormalities. In the recurrent isolates there was a possible increased frequency of haemolytic strains in the patients with normal urinary tract. This may be accounted for by the fact that serological group 018 occurred as a recurrent isolate on sixteen occasions and nearly fifty per cent of these strains were haemolytic.

Summary.

Using a sandwich-blood-agar plate five hundred and thirty-four strains of Escherichia coli isolated from the urine were examined for

the ability to produce haemolysis. One hundred and fifty-seven strains (29.4 per cent) were haemolytic.

There was a close correlation between haemolysis and certain serological groups. Groups '01', '04', '06', '018' and '075' accounted for 91.9 per cent of the haemolytic strains which were serologically groupable.

There was no statistically significant difference noted either in the frequency of haemolytic strains in the high and low bacterial count groups or in the frequency of haemolytic strains from the primary isolates from patients with and without urinary tract abnormalities.

Thirty-one per cent of the strains from females, forty-four per cent from males, with significant bacteriuria, were haemolytic. There was, however, no significant difference in the frequency of haemolytic strains between the sexes.

CHAPTER V

COLICINE TYPING

Introduction

The first description of bacterial antagonism was noted by Pasteur and Joubert (1877) in a paper describing experiments with the Bacillus anthracis. They wrote on page 107"... mais si, au moment de déposer dans l'urine les bactéridies à titre de semence, ou même en outre un organisme aérobie, par example une des bactéries communes, la bactéridie charbonneuse ne se développe pas ou très-peu, et elle périt entièrement après un temps plus ou moins long." In a later paper, Pasteur (1880) accurately described the phenomenon of bacterial inhibition, theoretically, but failed to confirm his hypothesis experimentally with the organism of fowl cholera.

Probably the first experiment using a coliform bacillus, in culture, was described by Levek in 1889. In a series of experiments on microbial antagonism he noted the inhibition of Bacillus anthracis by an intestinal, Gram-negative bacillus. Grimbert (1894) demonstrated that, when a coliform bacillus and a typhoid bacillus were present in water, only the coliform bacillus survived and Bienstock, in two papers (1900, 1906), considered that Escherichia coli could inhibit

the growth of B. putrificus, an organism which he had previously described.

The investigations of Eijkman (1904) and Frost (1904) are probably the most important of the early studies on colicines. Eijkman (1904) demonstrated the antagonism on solid media, and considered that the inhibitory substances were thermolabile and diffusible. He had noted that, although Vibrio cholerae inhibited the growth of Escherichia coli, the converse was notethe case and he suggested that this antagonism could be usefully employed to obtain pure cultures of Vibrio cholerae by a 'coli-gelatin' plate, which would inhibit the other coliform bacilli in the mixed culture from the faeces. Frost (1904) performed and illustrated a series of experiments on bacterial antagonism using both liquid and solid media, and including collodion sacs to demonstrate the diffusion of the substances. He stated that the antagonism was not due to the exhaustion of nutrients but was due to "Antibionts" which were thermostable and which were lethal, not merely inhibitory.

Nissle (1916) described the effects of the coliform bacilli on the intestinal pathogens and suggested the term "antagonistic index" to describe the efficiency of the inhibition of the pathogens by the strain of Escherichia coli. The "index" was a calculation of the relative number of viable organisms of both species following the simultaneous culture of both organisms in a broth culture for fourteen hours. The "index" was the number of pathogenic colonies to one hundred Escherichia coli colonies, a high "index" was one where the pathogen count was low, for example, 100 : 3.

McLeod and Govenlock (1921) noted that broth filtrates from Escherichia coli would not support the further growth of Escherichia coli or of other species, but if the filtrate was diluted with water, growth did occur, thus confirming that the inhibition was not due to exhaustion of the medium.

It was Gratia (1925) who firmly established the basis for

investigation of colicines. In this paper he gave a preliminary report of the inhibition of a strain of Escherichia coli, coli 'phi', by another strain, coli 'V', and in a fuller discourse on his experiments (Gratia, 1932), he showed that the substance produced by coli 'V' was freely diffusible through cellophane, was stable, withstanding boiling for one hour or 120°C for half an hour, and that it was precipitable by acetone. He also noted that, although coli 'phi' was sensitive to the substance, resistant variants developed and if this inhibition-experiment was performed in broth culture, although there was an initial retardation of growth, eventually growth occurred due to these resistant variants.

Guelin (1943) described a thermolabile substance, produced by a strain of Escherichia coli, which could lyse young cultures of Shigella flexneri and other strains of Escherichia coli. Wielding (1945) showed that Penicillium notatum could be inhibited by a similar thermolabile substance and Goblentz and Levine (1947) recorded the production of a substance by one strain of Escherichia coli which inhibited fifty other strains and, to a lesser extent, strains of Klebsiella.

In 1946, Gratia and Fredericq named the substances 'colicines' and suggested that the colicine was distinct and characteristic for the particular strain which produced it. Fredericq (1946a) also demonstrated that several colicines may be produced by the one strain and that colicinogenic strains may themselves by sensitive to other colicines. Most of the investigations of colicinic properties have been related to the enteropathogenic Enterobacteriaceae and it was suggested by Roland and Stuart (1951) that the bacteriocins of the Enterobacteriaceae should be termed 'enterins'.

Since then similar substances have been described in other families for example, Pseudomonas pyocyanea, named pyocines, (Jacob, Siminovitch and Wollman, 1953); Gram-positive bacilli named megacines (Ivanovics and Alfoldi, 1954) and Pasteurella pestis, named pesticins

(Ben-Gurion and Hertman, 1958). Corynebacteria diphtheriae (Thibaut and Fredericq, 1956) and Mycobacteria (Mora and Eisenstark, 1958) have also been shown to produce inhibition of allied species. Consequently, it has been proposed that the general term 'bacteriocins' be used to include those antibiotics of a protein nature whose biosynthesis is associated with the death of the producing organism (Jacob, Lwow, Siminovitch and Wollman, 1953) and whose action is conditioned by the presence of specific receptors on the surface of the susceptible cell. (Bordet, 1948, Bordet and Beumer, 1948). Their action is bactericidal, not bacteriolytic. The range of susceptible species may be restricted to the same species, that is, species specific, or may have a much wider spectrum of genera. Cook, Blackford, Robbins and Parr (1953) and Robbins, Somer and Parr (1957) have demonstrated examples of a wide spectrum antagonism by some strains of coliform bacilli against Mycobacterium smegmatis, Vibrio metschnikovii, Neisseria catarrhalis, Corynebacterium diphtheriae and Staphylococcus aureus.

Resistant mutants may arise spontaneously during bacterial growth and the classification of colicines by Fredericq (1948) depends on the patterns of resistance using variants developed from an Escherichia coli, strain 'phi', used by Gratia and by Fredericq. The seventeen colicines, named alphabetically A, B, C, etc., can be distinguished from one another by the specific action on natural strains, specificity of resistant variants, characteristic diffusion in agar, thermostability and the stability to proteolytic enzymes.

Many colicines are destroyed by proteolytic enzymes (Heatley and Florey, 1947; Fredericq, 1948; Halbert and Magnusson, 1948; Gardner, 1950) and so were considered proteins or peptides. More recent observation suggests that they may be lipo-carbohydrate-protein complexes (Goebel, Barry, Jesaitis and Miller, 1955, Hutton and Goebel, 1962) but that the antibacterial activity is in the protein moiety (Goebel and Barry, 1958).

Since the investigation in this chapter concern Escherichia coli the term colicine will be retained for the bacteriocins investigated.

Methods of colicine typing.

The streak method of Fredericq, Thibaut and Gratia (1946), with slight modifications, has been the routine procedure for investigating colicine production. It consists of a diametric streak of the test organism on the surface of a nutrient agar plate, preferably containing a catalase in the form of blood to prevent the non-specific inhibition of hydrogen peroxide (Gardner, 1950). The plate is incubated for twenty-four to forty-eight hours at 37°C, following which the culture is killed by chloroform vapour, which does not affect colicine (Gratia 1925). The chloroform vapour is allowed to evaporate and indicator strains are streaked at right angles to the test streak, the plate is then reincubated overnight. The inhibition of the growth of the indicators is noted. (Figure 8). Shannon (1957) modified the method by removing the initial streak with a glass slide before inoculating the indicator organisms. The use of a disc of cellophane on the surface of the medium, with the streak on the disc, obviated the need for scraping the test organism off the surface of the medium (Gratia, 1944; Heatley and Florey, 1947). The disadvantage of this method is that not all colicines can diffuse through cellophane.

The routine method for testing the sensitivity of the test organism to colicines is that described by Fredericq (1948). Briefly, this consists of spot inoculation of known colicine producers on the surface of a nutrient agar plate. The plate is then incubated for forty-eight hours following which the cultures are killed by chloroform vapour. A seeded agar culture of the test organism is then poured over the plate and incubated overnight. The colicine produced by the initial cultures diffuses on to the seeded layer and inhibition of the test organism may be noted.

Other techniques employing liquid media for colicine production

have had variable success, mainly due to the poor production of colicine in stationary cultures. (Gratia, 1925, Heatley and Florey, 1947; Halbert and Magnusson, 1948; Gardner, 1950; Depoux and Chabbert, 1953; Papavassiliou, 1963). Budford and Lederer (1953) and Chapple (1962) have described electrophoretic techniques, using an agar gel, which are technically rather laborious.

The method which I have developed depends on the extraction of the water soluble colicine from a nutrient agar culture by a freeze-thaw procedure. The extract of the test organism is used to produce inhibitory patterns on indicator organisms (colicinogenicity) and the extracts of known colicine producers are used to give inhibitory patterns on the test organism (colicine sensitivity).

TYPING PROCEDURE.

1. Extraction of colicine

The strain to be tested for colicinogenicity was inoculated on to the surface of digest nutrient agar and incubated at 37°C for twenty-four hours. The plate was then exposed to chloroform for one hour to kill the culture, uncovered for a further hour to allow the chloroform to evaporate and then frozen at -15°C in a deep-freeze cabinet overnight. The plates were thawed at room temperature and the fluid expressed was removed by Pasteur pipette, spun at 2,000 r.p.m. for fifteen minutes to remove the organisms and the supernatant stored at -15°C until required. (Figure 9).

2. Indicators of colicine production

Fifteen indicators of colicine activity were used. These consisted of fourteen Shigella sonnei strains: 2, 56, 17, 2M, 38, 56/56, 56/98, R1, R6, 2/7, 2/64, 2/15, R5, a Shigella schmitzi, M19 and an Escheriohia coli - coli 'Row'. The Shigella strains were those used by Abbott and Shannon (1958).

3. Demonstration of Production of colicine.

The indicator plates were inoculated either by surface-seeding or by pour-plate seeding, there was no significant difference in the zones of inhibition produced by these methods (Figure 10). Using a number 3 cork-borer, holes, five millimetres in diameter, were bored in the seeded plates. The holes were then filled with the extracts obtained by the freeze-thaw method, it required approximately 0.15 millilitres of extract to fill each hole. Thirteen or fourteen extracts could be tested on each indicator plate. The plates were then incubated overnight and the patterns of inhibition read. (Figure 10).

4. Demonstration of sensitivity to colicine.

The colicine sensitivity of the test organism was performed using extracts from eight colicinogenic strains which produced different colicinogenic patterns. The strain to be tested was surface-seeded on to peptone-water agar plates and eight holes bored in the surface using the number three cork-borer. The eight known colicine extracts were placed in the holes and the plate incubated at 37^{o}C overnight and the zones of inhibition noted. (Figure 11).

5. Colicine extracts used for sensitivity

The eight colicine extracts were designated 146, 208, 252, 264, 01, 07, 29, 234. They were produced by the freeze-thaw method in bulk, tested for potency against a known sensitive organism then stored in five millilitre amounts in the frozen state. The extracts remained potent in the frozen state for at least eighteen months. A control organism '43' was sensitive to all the extracts and was incorporated in every batch of tests.

At a later stage in the investigation these strains were typed by Professor P. Fredericq by his method and were considered by him to produce the following colicines.

Strains 01, 234 and 264 produced Colicine V
07 produced Colicine E and V
29 " " E
146 " " E and B
208 " " V and K
252 " " V, B, and E

However, as reference to the colicine sensitivity patterns in the appendix (Volume II) will show, the isolates of Escherichia coli did not always confirm these findings. If strains 01, 234 and 264 produced identical colicine 'V' then one would have expected that all the patterns showing sensitivity to one of these extracts would show similar inhibition to the others. For example, in pattern '1', which contains forty-four strains, only 234 has produced inhibition. Similar discrepancies are apparent in other patterns of sensitivity. Consequently it was considered permissible to continue to use these extracts for typing the sensitivity.

6. The effects on colicine production and diffusion produced by different media.

During an early part of this investigation it was noted that there was a discrepancy in the colicine production in one batch of strains. The only variation in technique had been the use of a different nutrient medium. This suggested that there may be a variation in production by different media and this was further investigated.

Media investigated.

Petri dishes containing twenty millilitre amounts of the following media were used.

Digest nutrient agar This was prepared from Hartley's broth as described in Mackie and McCartney (1960) with 1.2 per cent agar (May and Baker) added.

Peptone water agar This was prepared as described in Mackie and

McCartney (1960) with 1.2 per cent agar (May and Baker) added.
Blood agar base number 2 (Oxoid) which contained 1.2 per cent agar Number 3 (Oxoid).
Sensitivity test agar (Oxoid) which contained 1.2 per cent Ionagar number 2.
Tryptone soya agar (Oxoid) which contained 1.5 per cent agar number 3 (Oxoid). The last three media were prepared according to the manufacturers' specifications.

Several batches of these media were compared to ensure that batch variation did not occur. Only one medium, Tryptone soya agar (Oxoid) showed batch variation and this caused variation in both production and sensitivity (Table XXIII).

The organism used for colicine production was a strain of Escherichia coli isolated from a patient with significant bacteriuria and produced colicine V.

The methods for extraction and indication were those already discussed. The indicator of colicine activity was coli 'Row' and it was surface seeded.

Results.

The effect of the medium on colicine production

The comparison of colicine production on different media is shown in Table XXIII. It will be noted that digest nutrient agar extract (E1) gave the largest zones of inhibition on all diffusion media tested. Peptone water agar (E2) did not produce recordable amounts of colicine.

The effect of medium on diffusion

The comparison of colicine diffusion in different media is also shown in Table XXIII. Diffusion occurred best on peptone water agar. On sensitivity agar (Oxoid) diffusion was poor. Resistant variants developed within the zones of inhibition on sensitivity test agar, blood agar base number 2, and in both batches of tryptone soya agar.

Time for adequate colicine production

Table XXIV and Figure 12 demonstrate the colicine produced on

digest nutrient agar at various time intervals. Peptone water agar was used for diffusion. Adequate colicine was produced after six hours at 37°C and there was little difference in the amount of colicine produced after fourteen hours' incubation.

The effect of peptone on the production

Bacteriological peptone (Oxoid), in concentrations of 0.0625, 0.125, 0.25, 0.5 and one per cent, was added to digest nutrient agar. Control plates of digest nutrient agar and one per cent peptone water agar were also inoculated. The zones of inhibition were decreased from 19 millimetres by the "peptone-free", nutrient agar extract to 18 millimetres by the extracts of the peptone at concentrations of 0.625pper cent to 0.5 per cent, 15 millimetres by the one per cent and 10 millimetres by the extract of the peptone water control.

A comparison of other commercial peptones was made. Table XXV shows the zones of inhibition produced by the extracts of digest nutrient agar to which one per cent of the various peptones had been added.

The effect of dextrose on colicine diffusion.

Dextrose, in concentrations of 0.0625, 0.125, 0.25, 0.5, one and two per cent, was added to one per cent peptone water agar. The diffusion was reduced from 23 millimetres in the dextrose-free, control peptone water agar plates to 21 millimetres in the 0.0625 per cent, 17 millimetres in the 0.125 per cent and to 14 millimetres in the 0.25, 0.5, one and two per cent dextrose-peptone water agar plates.

THE TYPING OF THE URINARY ISOLATES.

Materials and methods.

Five hundred and thirty-four strains of Escherichia coli, isolated from the urine of patients attending the Medical renal clinic or of in-patients at the Glasgow Royal Infirmary, were examined. Three hundred and sixteen specimens were isolated in bacterial counts of

more than 100,000 organisms per millilitre urine; eighty-six were from counts of between 10,000 and 100,000 organisms and one hundred and thirty-two were isolated in counts of less than 10,000 organisms per millilitre urine.

The isolates were tested for colicine production and colicine sensitivity by the methods described above.

Results

Five hundred and thirty-four strains were investigated for colicine properties, one hundred and ninety-three, 36.1 per cent, were producers; two hundred and sixty-six, 49.8 per cent, were sensitive to the colicine extracts and seventy-five, 14.1 per cent, were both producers and sensitive. There was an overall typability of 71.9 per cent (Table XXVI).

Incidence of colicine production

Eighty-one colicine production patterns were noted using the Shigellae and Coli Row as indicators. These are tabulated in the appendix, Volume II. Six of these patterns are excluded from this series of comparisons, two, "47" and "73", because they were produced by two strains of Citrobacter freundii and four, '1', '13', '21', '62' were produced by strains of Escherichia coli not included in this investigation due to loss of the strains before complete comparison of serology, biochemistry or colicine sensitivity typing could be performed. The one hundred and ninety-four strains were contained, therefore, in seventy-five pattern types. One hundred and four strains were found to be in seven patterns, 53.6 per cent of the typable strains, and 72.3 per cent were present in twenty-one patterns (Table XXVII). There was no significant difference in the incidence of colicine production in the various bacterial count groups. (Table XXVIII).

The most frequently isolated patterns are shown in Table XXIX. Pattern '26' accounted for 21.1 per cent of the typable strains, '79' for 8.2 per cent and '66' and '7' occurred in 7.2 per cent and 6.7

per cent, respectively, of the typable strains. In the primary isolates from significant bacteriuria '26' accounted for 23 per cent of the typable strains, but only for 10.9 per cent of the low bacterial count specimens. However the differences in the frequencies of patterns"26" in both the total primary significant bacteriuria and the primary symptomatic bacteriuria are not highly significant when compared to that of the low bacterial count group. (Chi square = 2.309, P between 0.2 and 0.1 for the total primary and 2.8314, P between 0.1 and 0.05 for the symptomatic primary.)

Incidence of colicine sensitivity

Two hundred and sixty-four strains were sensitive to colicine and occurred in seventy-two patterns, tabulated in the appendix, Volume II. One hundred and seventy-two, 65.2 per cent, occurred in sixteen groups and 71.2 per cent occurred in twenty groups (Table XXVII). There was no significant difference in the frequency of sensitivity in the various bacterial count groups. (Table XXX).

The most frequently isolated type (Table XXX) was pattern '1' which accounted for 16.7 per cent of the sensitive strains, and patterns '39' and '64' were next in frequency occurring in 7.2 per cent and 6.8 per cent of strains, respectively. The primary isolates showed no particularly outstanding pattern although 45.9 per cent of the strains were present in seven patterns (Table XXX). In the low bacterial count group, however, pattern '1' was present in 26.5 per cent of the strains. There was a highly significant difference in this frequency and that of the high bacterial count. (Chi square = 18.522, P greater than 0.001).

Incidence of both producers and sensitive strains.

Seventy-two strains both produced colicine and were sensitive to colicine. There was no correlation between the patterns of production and sensitivity. Production pattern '26' occurred with sensitivity pattern '64' in five instances, and production and sensitivity patterns '43':'26'; '71':'26'; '14':'66'; '66':'54' and '79':'64' each occurred with two strains.

Reproducibility of colicine typing.

Five widely-separated colonies were removed from the original diagnostic plates of seventy urines and tested for colicine production and colicine sensitivity. Forty-five specimens were from patients, not on treatment, with high bacterial counts of more than 100,000 organisms per millilitre urine; eighteen were from patients, on treatment, with high bacterial counts; three were from intermediate bacterial counts between 10,000 and 100,000 organisms per millilitre urine, one of which was from a patient on treatment; four were from low counts of less than 10,000 organisms per millilitre urine, two of which were from patients on treatment. (Table XXXI).

Sixteen per cent of the specimens were colicine producers only; forty-nine per cent were colicine sensitive only; twenty-three per cent were both producers and sensitive and fourteen per cent were neither producers nor sensitive.

Agreement in colicine production.

Forty-three of the forty-five cultures, in the high count group from patients not on treatment, showed complete agreement in all five colonies, and in two hundred and twenty-one of the two hundred and twenty-five colonies there was agreement (98.2 per cent).

With the eighteen specimens in the high count group, from patients on treatment, agreement was shown by all five colonies of seventeen, and by eighty-nine of the ninety colonies (98.9 per cent).

There was complete agreement in all the colonies from the remaining specimens in the intermediate and low bacterial count groups from the patients both receiving, and not receiving, treatment.

Thus sixty-seven of the seventy culture plates examined showed complete agreement in all five colonies removed and, overall, there was agreement in three hundred and forty-five of the three hundred and fifty colonies tested (98.6 per cent).

The variation in production by five colonies was due, in four instances, to a loss of a single indicator inhibition and in the fifth

to the absence of colicine production.

Agreement in colicine sensitivity.

Of the forty-five high bacterial count specimens from patients without treatment, agreement, in all five colonies, was shown by forty cultures, and by two hundred and fifteen of the two hundred and twenty-five colonies (95.6 per cent).

Thirteen of the eighteen high count specimens, from patients on treatment, showed agreement in all the five colonies and there was agreement shown by seventy-eight of the ninety colonies (86.7 per cent).

In the intermediate bacterial count range, there was complete agreement shown by all the colonies from the two specimens from patients not on treatment and by four of the five colonies from the patient on treatment.

There was complete agreement in eight of the ten colonies examined from the low bacterial count specimens, both in the treatment and non-treatment patients.

For the colicine sensitivity there was an overall agreement by fifty-seven of the seventy cultures and with three hundred and twenty-three of the three hundred and fifty colonies (92.3 per cent).

If one excludes the twenty-one specimens from patients on treatment, there was complete correlation shown by two hundred and thirty-three of the two hundred and forty-five colonies from forty-nine specimens (95.1 per cent). The differences in the colicine sensitivity were, in the main, associated with specimens from patients on treatment with alkali mixtures and were accompanied by the development of serologically 'rough' variants.

Discussion

The agar extraction method.

Most of the investigations on colicine production have been

performed using the streak method of Fredericq, Thibaut and Gratia (1946). The method which I have described depends on the extraction of the water-soluble colicine which has diffused into the medium. It was not until the method was well established that it was noted that Halbert and Magnusson (1948) had described a somewhat similar method of extraction using solid carbon dioxide to freeze the plate. One of the advantages of the freeze-thaw method of extraction is that ample extract is available, not only for the initial testing of production against the indicator strains, but also for repeated confirmation, if necessary. The colicine extract remains potent in the frozen state for more than anyear and can be used as a control for the indicators which may produce resistant variants, particularly if they are frequently sub-cultured in broth.

The testing of the sensitivity of the isolates to colicine is also simplified and controlled by the bulk production of extracts of known potency, which can be stored in aliquots and used for testing strains at later dates. This avoids the frequent use of sub-cultures of the known colicine producers which may vary in their potency, a feature noted by Hutton and Goebel (1962) in their experiments on the purification of colicine. The possibility of variation in production due to media is also lessened.

The method is also more reliable for the comparison of several isolates from the same patient which can be tested on the one indicator plate, thus reducing the technical error which may occur if several plates are used, as in the streak method where the cross-streaking of the diametric inoculum requires a separate plate for each strain.

<u>The effects of media on colicine production and diffusion.</u>

One feature which the freeze-thaw method revealed was the effect of media on the production and diffusion of colicine. Previously, when the cross-streak method was used, it was difficult to dissociate variation in production from that in diffusion since both these effects

were studied on the same plate. By using extracts of colicine produced on the various media one could compare the production and diffusion on the different media and it became clear that inhibition of production occurred quite separately from the reduction of diffusion. The best medium for production was found to be digest nutrient agar, the worst medium for production was bacteriological peptone. The addition of various peptones to the digest nutrient agar demonstrated that bacteriological peptone, both Oxoid and Evans products, and mycological peptone (Oxoid) reduced the production of colicine. The other peptones, Proteose Number 3 (Difco) and Neopeptone (Difco) did not cause a marked reduction in production. Examination of typical analyses of these peptones, (Difco Manual 1953, Oxoid Manual 1961), did not suggest any factor responsible for the inhibition of production, however, the manufacturers consider that proteose peptone and 'Neopeptone' are superior to other peptones for the growth of the more fastidious organisms and for diphtheria toxin production (Difco Manual, page 259; Oxoid Manual, page 238). Thus is may be that the purification of these two peptones removes the factor or factors which inhibit the production of colicine.

The most suitable medium for testing diffusion was found to be bacteriological peptone water agar. The addition of dextrose to peptone water agar reduced the zones of inhibition of the colicine. The critical level of concentration was at 0.25 per cent dextrose, increased concentrations did not reduce the diffusion any further. This confirms the suggestion of Blackford, Parr and Robbins (1951) that dextrose might reduce the diffusion of colicine. The poor diffusion noted with Oxoid 'sensitivity agar' is probably due to the one per cent dextrose which it contains.

<u>The colicine sensitivity indicators.</u>

The results of the sensitivity of the isolated strains of Escherichia coli to the colicine extracts obtained from the eight colicine producers, "146", "208", "252", "264", "01", "07", "29"

and "234", demonstrated the variation in colicine typing by Fredericq's streak method and the agar extraction method. Although the strains "01", "234" and "264" were considered by Professor Fredericq to produce colicine 'V' solely, the sensitivity patterns of the urinary isolates, in the appendix, Volume II, do not always confirm this. For example, sensitivity pattern "1", which was observed in forty-four isolates, shows inhibition by "234" extract but no inhibition is produced by the extracts from strains "01" or "264". The colicine production patterns of the three strains, "01", "234" and "264", using as indicators the Shigella strains and Coli Row, differ slightly in their inhibition of 2M and R5 indicators. Extract of "01" did not inhibit 2M or R5; "234" extract inhibited both 2M and R5; "264" extract inhibited 2M but not R5.

It may be that the freeze-thaw extraction method reveals colicines which are not assayable by the streak method. However, Stocker, during studies on bacterial genetics, has noted a similar discrepancy between two colicines apparently identical by Fredericq's method of classification. (personal communication).

The indicators which Fredericq used consist of resistant variants derived from Escherichia coli 'phi'. Since the development of resistance is associated with the loss of receptor points, (Fredericq 1948) the maximum number of these receptors is present on Coli 'phi' and thus should a colicine be present in an extract for which corresponding receptors are absent on the Coli 'phi' none of the indicators will be inhibited. This could explain the ability of the relatively large numbers of "indicators" of the "wild strains" of urinary isolates to indicate these differences in colicine production which may not be apparent with a few indicators, particularly those developed from a single strain.

It is considered that the colicine sensitivity patterns justified the use of the eight extracts to type the isolates.

Colicine typing of the urinary isolates.

Although colicine typing has been used for investigations of the Shigellae (Fredericq and Levine, 1947; Halbert, 1948; Halbert and Gravatt, 1949; Ludford and Lederer, 1953; Levine and Tanimoto, 1954; Robbins, Parr and Hann, 1958; Abbott and Shannon, 1958; Huet, Papavassiliou and Bonnefous, 1961; Papavassiliou and Huet, 1962; Gillies, 1964), it has not received the same application to the Escherichia coli. Fredericq, Betz-Bareau and Nicolle (1956) and Shannon (1957) investigated the colicinogenic properties of the enteropathogenic Escherichia coli and Fredericq (1946b) tested urinary strains as part of a general investigation on colicine activity. When my study of the colicine activity of isolates from the urinary tract infections started there had been no comparable report, since then Linton (1960) has used colicine typing in an investigation of cross-infection in a urological ward. Fredericq (1946b) found that 74.4 per cent of ninety-four coliform strains were colicine active; 17 per cent were solely producers, 47.9 per cent were colicine sensitive and 9.5 per cent were both producers and sensitive. Linton (1960) noted that 87 per cent of his two hundred and thirty-five strains were active; 13.6 per cent were solely producers, 45.5 per cent solely sensitive and 27.6 per cent were both producers and sensitive. My findings have been similar with 72.0 per cent of five hundred and thirty-four strains active; 22.1 per cent solely producers, 35.8 per cent sensitive only and 14.1 per cent both producers and sensitive.

Although seventy-five colicinogenic patterns occurred, 72.3 per cent of the typable strains were present in twenty-one patterns. Colicinogenic pattern "26" occurred frequently and was found in 25 per cent of the colicinogenic isolates from patients with significant bacteriuria. In the bacterial counts of less than 10,000 organisms per millilitre urine it was present in 10.4 per cent of the isolates. Although there was no significant overall difference in colicine

production between the high and low bacterial count groups, pattern "26" was possibly significantly more frequent in the high bacterial count groups.

In the typing by colicine sensitivity 71 per cent of the typable strains occurred in twenty-one patterns. As with the production there was no significant difference in the overall colicine sensitivity of the strains in the high and low bacterial count groups. However, the sensitivity pattern "1", which occurred in 16.7 per cent of the colicine sensitive isolates, was significantly more frequent in the low bacterial count group than in the high.

There was no significant correlation between the production patterns and the sensitivity patterns in the strains which demonstrated both these activities.

The reproducibility of colicine typing.

The capacity for producing colicine is a stable character and is rarely lost (Frederiq, 1948; Jacob, Schaeffer, and Wollman, 1960), thus the use of colicine production as a method of typing isolates should prove reliable. This has been confirmed by the investigation on the reproducibility of colicine markers taking five widely-separated colonies from seventy isolation plates. There was 98.6 per cent correlation with the colicine production of the three hundred and fifty colonies. Where there was disagreement, in five colonies, it was due to the loss of one indicator-inhibition in four cases and in only one instance did marked variation occur.

The reproducibility of the colicine sensitivity patterns for the same colonies was 92.3 per cent. The differences were mainly attributable to the loss of "smoothness" of the strains from patients on therapy, where the reproducibility was reduced to 86.7 per cent. Indeed if one excludes the strains from such patients, the agreement was much closer at 95.1 per cent. A similar loss of colicine sensitivity has been reported by Ikari, Robbins and Parr (1958) who noted that the smooth cultures of Shigella boydii were less sensitive

to colicine than non-'S' variants. They considered that the somatic antigen was responsible for the characteristic pattern of the organism. Similar reproducible results for colicinicity have been noted in the faeces (Robbins, Somer and Parr, 1957; Abbott and Shannon, 1958) where only one type of antibiotic producer was found at any one time, although Robbins and her colleagues noted that the pattern may change from day to day in normal faeces. This daily variation was not observed in the infected urines in my study where repeated isolations were found to remain the same, in one patient for five days and in six others for two or three successive days.

This part of the investigation has suggested that there is uniform colicinicity of the organism responsible for the urinary infection.

Summary.

A method for the extraction of colicine by a "freeze-thaw" technique has been described. The colicine production of the Escherichia coli was tested against indicator strains of fifteen Shigella sonnei, one strain of Shigella schmitzi and one strain of Escherichia coli - Coli Row. The sensitivity to eight different colicine extracts was also tested.

The effects of different media on the production and diffusion of colicine was examined. It was noted that bacteriological peptone inhibited the production and that dextrose affected the diffusion.

Five hundred and thirty-four strains of Escherichia coli, isolated from urine, were examined for colicine production and sensitivity. There was an overall typability of 71.9 per cent. Eighty-one colicine production patterns and seventy-two colicine sensitivity patterns were noted.

There was no significant difference in the overall colicine typability of the strains from the high and the low bacterial count groups. However, colicine sensitivity pattern '1' was significantly more frequently isolated from the low bacterial counts.

The reproducibility of the colicine typing methods was shown to be good on examining five colonies from the original isolation plates and the results suggest homogeneity of the infecting organism in urinary infections.

CHAPTER VI

THE TYPING OF ESCHERICHIA COLI

General comparisons and discussion

In an early part of this investigation it was demonstrated that Escherichia coli was the organism most frequently isolated from the patients with urinary tract infections and the typing methods of serology, haemolysis and colicine activity were applied. Five hundred and thirty-four strains of Escherichia coli were tested, 89.1 per cent of these could be classified by the serological or colicine methods (Table XXXII). Serologically, 56.5 per cent could be typed; by colicine production, 36.5 per cent and by colicine sensitivity, 49.1 per cent could be classified.

Of the strains from specimens with significant bacteriuria of more than 100,000 organisms per millilitre urine, 88.3 per cent were typable by one or more of the methods employed (Table XXXII). Serologically 58.9 per cent, by colicine production 38.0 per cent, and by colicine sensitivity 47.2 per cent, were typable. In the primary symptomatic infections (Table XXXIII), 90.7 per cent were typable. Serologically 65.7 per cent, by colicine production

35.0 per cent and by colicine sensitivity 45.0 per cent, were typable.

There was no significant difference in the frequency of typable strains in the high and low bacterial count groups. The intermediate count group of isolates, that is, those strains isolated in the bacterial count range between ten thousand and one hundred thousand organisms per millilitre urine, were excluded from the statistical comparisons since they were of doubtful significance in the concentrations associated with infection or non-infection.

Serological grouping.

There was no significant difference in the frequency of the serological groups in the high and low bacterial counts, nor was there any significant difference in the frequency in the sexes.

Haemolytic reaction.

There was no evidence of any difference in the frequencies of the haemolytic strains isolated from the sexes. This was not unexpected since there was no significant difference in the frequency of isolation of the serological groups which were shown to be particularly associated with haemolysis.

Colicine typing.

The only significant difference in the frequency of colicine activity, between the high and low bacterial counts, was noted in the higher frequency of sensitivity pattern '1' in the low count group compared to the high bacterial count group. This is not due to a bias in the incidence of patterns in either group, that is, there was no significant difference in the number of patterns for the number of strains showing those patterns, Chi square = 0.8894, P between 0.5 and 0.3.

Comparison of typing markers.

1. Colicine patterns and serological groups.

There was no correlation observed between a specific colicine pattern, either production or sensitivity, and a specific

serological group. (Table XXXIII). Similarly, there was no correlation between the colicine production patterns and the colicine sensitivity patterns.

2. Serology and haemolysis.

There was a high frequency of haemolytic strains associated with several serological groups, in particular, with groups O1, O4, O6, O18 and O75.

3. Pyuria and the typing markers.

As already described in Chapter I, pyuria has been accepted as the presence of more than ten leucocytes per measured area of the Neubauer counting chamber. Since it was also shown that there is not always pyuria present with significant bacteriuria, page 7, comparisons of the various markers and pyuria were made to see if the discrepancy between significant bacteriuria and pyuria could be associated with any factor.

Of the three hundred and sixteen strains from the specimens with significant bacteriuria, fifty-four had ten or fewer cells per measured area, seventeen per cent. Only twenty-three of the one hundred and eighty-two isolates from the primary infections were found to have insignificant pus cell counts.

There was no significant correlation shown between the presence of pyuria and the typability or non-typability by the serological method, or the specific serological groups, (Table XXXV).

Similarly, with the colicine typing methods, no relationship was demonstrated between the colicinogenicity and pyuria, (Table XXXVI); or between the colicine sensitivity and pyuria, (Table XXXVII).

Discussion.

It has been found in these investigations on the typing of the Escherichia coli that the organism may be grouped by serological methods and by its colicine properties. Since there was no significant correlation found between the serological grouping and

the colicine typing it would appear that there exists a large spectrum of specifically separate strains associated with urinary tract infections.

Correlation between pyuria and the typing markers.

The correlation between high toxicity to mice, necrotising ability and haemolysis of the Escherichia coli was noted by Sjöstedt (1946) and this was confirmed by Ewertsen (1946), both showed that the haemolytic and necrotising strains had a high frequency of toxicity compared to the non-haemolytic strains. Furthermore Sjöstedt (1946) considered that Escherichia coli strains of the same serological type possess the same toxicity. It was attempted in my study to see if these findings could be correlated using the presence of pyuria as the indication of toxicity. It was demonstrated in Chapter I that high bacterial counts were associated with increased white cell excretion as shown by quantitative leucocyte counts, nevertheless, in this series of investigations, seventeen per cent of all the specimens from patients with significant bacteriuria did not have a raised pus cell count in the urine. This comparison, however, failed to distinguish any association between the various typing markers and pyuria. The numbers of asymptomatic patients investigated was too small for comparison of the different typing methods, but it was noted that those patients who did not present with frank urinary tract symptoms, but who had significant bacteriuria, also had the same frequency of pyuria as the patients with symptomatic infections (Figure I) thus it would be unlikely to find any statistical difference in the typing markers and pyuria between this group and the symptomatic group.

The reproducibility of the typing markers.

From seventy initial isolation plates five widely-separated colonies were removed to compare the constancy of the typing methods. The results have been noted in the separate chapters on typing and it was shown that there was a good degree of reproducibility by the various markers, particularly in the isolates from patients not on

therapy. (Table XXXI). Serologically, there was complete agreement with all the smooth, typable strains. The colicine typing showed some variability in constancy, colicine production was good with 98.6 per cent agreement; colicine sensitivity was less good with 92.3 per cent correlation. Where variation occurred it was associated, in the majority of instances, with an alteration in the physical character of the strain, demonstrated by the development of "roughness". All the differences in the serological grouping, and the bulk of the variations in the colicine sensitivity, were due to this feature. These strains were, in the main, obtained from patients who were receiving alkali mixtures.

The colicine production was the most stable marker and was not demonstrably affected by the 'smooth-rough' 'variation of the organism, a feature also noted by Fredericq (1946c).

These findings would suggest that methods which have been described, for typing the Escheriohia coli, are reliable for epidemiological purposes and, furthermore, suggest that the infecting organism in urinary tract infection is homogeneous.

Genetics of colicine production

The investigations on colicinogenic properties with particular reference to the transduction of colicinogenic activity have revealed interesting facets of genetics. Fredericq (1954, 1955) and Fredericq and Betz-Bareau (1952 and 1956) demonstrated that colicinogenic strains could transfer the colicinogenic property to non-colicinogenic strains by conjugation of the parent strains with a resultant recombinant which was colicinogenic, the transfer being independent of other classifying features. It did not appear possible to transfer non-colicinogenic properties. Furthermore, Fredericq (1956) noted that the ability to transduce colicines varied with the colicine type, colicine type 'I' was frequently transmissible, colicines, 'B', 'E' and 'K' less so and 'V' was rarely transmissible. Alfoldi, Jacob and Wollman (1957) had similar results and they used the term

"zygose letale" to describe the death of the non-colicinogenic recombinant resulting from the combination of Hfr colicine$^-$ and F$^-$ colicine$^+$. The transfer of the colicinogenic property was shown by Stocker (1960) to be achieved by cell contact for some colicines but not all. From these findings it is likely that, should an Escherichia coli responsible for urinary infection possess transferable colicinogenic properties, all the isolates would be uniform, since there is close contact within a restricted area in the urinary tract. Furthermore if the infection were due to two colicinogenic types then one or other or both colicines would be noted in the isolates. My results on the reproducibility of colicine typing have confirmed the uniformity of colicine patterns in the urine, not only for the one day but up to five days from one patient. This uniformity is confirmed still further in the next chapter, and is an important factor in the study of recurrent infections.

The origin of the Escherichia coli in urinary infection

The various routes of infection of the kidney have their proponents, most of the corroborating evidence relying on animal experimentation. The work of Winsbury-White (1933), using particles of indian-ink and living and dead tubercle bacilli, suggested that the organisms may spread from the lower urinary tract and genital tract via the periureteral lymphatics to the interstitial tissues of the kidney. Gorrill (1958) considered that the haematogenous route is most likely and support from this has come from De Navasquez (1950, 1956, 1958), Hepinstall and Brumfitt (1960) and Gorrill and De Navasquez (1964). However, Talbot (1958); Vivaldi, Cotran, Zangwill and Kass (1959), and Anderson and Jackson (1961) have given equally convincing evidence that the ascending route is the most likely and, indeed, Anderson and Jackson (1961) consider that by retrograde inoculation of the various species it is much easier to produce infection that by haematogenous methods. If one supports

the ascending route of infection then one would expect the organisms responsible for causing urinary infection could be normally present in the lower urinary tract. The application of the various typing methods in this study have suggested that, with the exception of colicine sensitivity pattern '1', there was no statistically significant difference in the frequency of typable strains of Escherichia coli in the bacterial count groups which are associated with urinary infection, more than 100,000 organisms per millilitre urine, and those associated with contamination, less than 10,000 organisms per millilitre urine.

It may be argued that, since many of the patients from whom low bacterial counts were obtained had a history suggestive of urinary infection, they may harbour organisms in the urethra from previous attacks which would account for the relative frequency of typable strains being the same from both sources. However, the large majority of patients from whom my low bacterial count isolations were obtained had no bacteriological evidence of infection in the recent past, most were investigated to exclude the possibility of renal disease, or had renal parenchymatous disease. Furthermore where the isolates were obtained from patients with recently proven infection there was an interval, sufficiently long enough and with intervening cultures free from any Escherichia coli, to avoid this possible fallacy. Indeed where this last possibility was noted adjustment to the figures did not affect the comparative frequencies.

It is difficult to explain the increased frequency of colicine sensitivity pattern '1' in the low bacterial count group. This was the only marker to give a demonstrably significant difference in the bacterial count groups and one is tempted to consider this as a possible indication of the lack of pathogenicity. However, there are two points against this suggestion, firstly, although pattern '1' occurred more frequently in the low count group, it was, nevertheless, associated with infection in the high bacterial counts. Of the thirty-two strains with sensitivity pattern '1' in the high and low

count specimens, fourteen were associated with infection. Secondly, if this pattern was associated with a lack of pathogenicity it might be expected to find a lower frequency of pyuria in the urine specimens from which this pattern was isolated in significant bacteriuria, this was not demonstrated.

In conclusion, it seems likely that although a large number of Escherichia coli, serologically distinct, occur in the intestines, there is a particular association with relatively few serological groups and urinary infection. The use of the more precise methods of colicine typing methods has revealed that a much wider spectrum of specific strains may be concerned in infection. Since it is generally accepted that the primary origin of the Escherichia coli in the urinary tract is the bowel, the occurrence of such a variety of colicinogenic types can be explained by transduction. Transduction can also be the reason for the variation in the patterns of the faecal isolates which has been noted by Robbins, Somer and Parr (1957).

The establishment of the faecal strains in, or near, the external urethral meatus may be a simple matter of hygiene mechanics, Leishman (1939) has suggested that it may be related to the cleansing of the perineum after defaecation; in urinary infection of the infant the soiled diaper has been incriminated (Engel, 1955). Loopuyt (1946a) considered that there was a relationship between the frequency of urinary infection and the social group, being more frequent in the lower economic groups, where a high standard of hygiene may not always be possible or maintained.

That certain serological groups, found in the faeces, are more common than others as a cause of infection was shown by Kauffmann and his associates (Kauffmann, 1947). In my investigations, I have confirmed that these serological groups are associated with a high proportion of the strains from significant bacteriuria. Nevertheless, there was no significant difference in the frequency of these specific groups, isolated from the specimens with high bacterial counts, associated with infection, and low bacterial counts, associated with

contamination. These findings suggest that, although these "pathogenic" groups are commonly responsible for urinary infections, they can also be present in the low concentrations compatible with urethral contamination. The failure to demonstrate any significant difference in the frequencies of the serological groups or colicinogenic patterns, between the isolates from the specimens with significant bacteriuria and low bacterial count specimens, suggests that the urinary infection arises from an organism in the lower urinary tract. It is likely, therefore, that some factor, or factors, is required to permit these strains to proliferate in the urinary tract, and that this is other than the somatic antigen. It may be an inherent property of the organism or may be dependant on the urine.

It has been suggested that the inhibition or reduction of natural inhibitors is a factor permitting the proliferation of the organism in the urine, Beeson and Rowley, (1959) have given experimental evidence that complement is adversely affected by ammonia formation by the renal tissue. Freedman (in Quinn and Kass, 1960) noted that the addition of an acidifying salt, ammonium chloride, to the diet of rats increased their susceptibility to coliform infection of the kidney and that this was associated with the stimulation of renal glutaminase activity, which was consistent with the theory of complement-inhibition by ammonia.

Kass (1960) has postulated that the hormonal changes in pregnancy may account for the increased frequency of significant bacteriuria in the first trimester. He suggests that this susceptibility may be due to the striking increase in the chorionic gonadotrophins and corticosteroid which occurs in the early weeks of pregnancy.

Thus it may be that the higher incidence of non-obstructive, urinary infection in women, compared to men, is due to variation in hormones rather than the simple anatomical association of the shorter urethra. It is felt, however, that the establishment of urinary infection and its variation in frequency in the sexes, is not due to a single factor

but depends on several factors, of which the pathogenic property of the organism, the biochemistry of the urine and the integrity of the urinary tract have separate roles.

Summary:

The methods for typing the five hundred and thirty-four isolates from the urine have been compared. There was an overall typability of 89.1 per cent of the strains, using the typing markers of serological grouping, colicine production and colicine sensitivity.

In the comparisons of the various typing markers, the only real correlation noted was between haemolysis and the serological groups 01, 04, 06, 018 and 075. There was no demonstrable correlation between the serological groups and colicine production or sensitivity patterns.

Using the frequency of pyuria as a possible indication of virulence, there was no significant association noted between pyuria and the serological groups, colicine production patterns or colicine sensitivity patterns.

The reproducibility of the typing markers was examined and it was found that colicine production was the most stable, with 98.6 per cent reliability. The disagreements in both serology and colicine sensitivity were mainly associated with the "smooth-rough" variation of the strain. It is suggested that the reproducibility of the typing markers indicate the homogeneity of the infecting organism in urinary tract infection.

The failure to demonstrate significant difference, in the serological and colicine production typing of the Escherichia coli, between the isolates from the high and low bacterial count groups is suggested as evidence that urinary infection originates from the lower urinary tract and the possible reasons for proliferation are considered.

CHAPTER VII

RECURRENT INFECTIONS: RECRUDESCENCE OR REINFECTION?

Introduction

Although urinary tract infections may respond to modern antibacterial therapy, the frequency of recurrent urinary infections has altered little from the pre-antibiotic era (Rhoads, Billings and O'Conor, 1952). The frequency of recurrence depends to a great extent on the pathogenesis of the infection, obviously, where some obstructive factor is present then the probability of recurrence is high without the necessary correction of the underlying defect. Even where no associated urinary tract lesion is apparent the rate of recurrence is still frequent (Garrod, Shooter and Curven, 1954; Jackson, Poirier and Gitchle 1957). Indeed it is probably true to say that, although the bacteriological cure of urinary infection has been accelerated with the use of modern therapy, the recurrence rate following short-term therapy is no lower than formerly. The problem with recurrence may be expressed by the question: is recurrence a recrudescence of an inadequately eradicated infection or is it an infection by a new organism? Where the recurrence is associated with a different species, assuming that this species was absent from

the initial infection, then little doubt can exist that this is a reinfection. However, where the species isolated is the same in both episodes then the differentiation requires further classification other than biochemical. It was hoped that by employing the typing methods described in the previous chapters that some clarification of this problem might be obtained.

Material and methods.

Forty-nine patients, attending the Medical renal clinic, or in-patients in medical wards of the Glasgow Royal Infirmary, were investigated for one hundred and eight recurrent infections with Escherichia coli over four years. Infection was accepted by the presence of more than 100,000 organisms per millilitre urine demonstrated by the quantitative bacterial count techniques described in Chapter I. Where possible two or more pre-treatment, mid-stream specimens of urine were obtained to confirm the presence of significant bacteriuria. Of the forty-nine patients with recurrent infections, thirty-seven females and the sole male had no demonstrable urinary tract abnormality. The remaining eleven patients, all females, had urinary tract abnormalities as follows: three had glomerulonephritis, three had had acute tubular necrosis, and one patient each with nephrosis, renal calculus, Kimmelstiel-Wilson kidneys, duplication of the renal pelvis, and one patient had disseminated sclerosis. These eleven patients had thirty-nine recurrent episodes.

The patients were prescribed a course of appropriate antibacterial therapy. The course of treatment varied from five to ten days short-term therapy to continuous therapy for up to six months. The proof of eradication of infection was taken by the continued sterility of urine, associated with the absence of symptoms and pyuria, during treatment; for at least two days following the cessation of therapy and again at ten days or longer. Indication of recurrence was considered when there was a bacterial count of more than 100,000 organisms per millilitre urine following the period of sterile urine

cultures. Further mid-stream specimens were obtained at the Medical renal clinic at two weeks, one month and then monthly intervals for six months to one year and at three monthly intervals for a further year and, should no further episodes of infection occur, at six monthly intervals of the next two years.

It is not intended to discuss the therapeutic management of urinary infection in detail, however, it will be sufficient to state that short-term therapy was only used for comparison of antibacterial substances and as a rule long-term therapy was the method of choice.

Methods of Investigation

The colonies of Escherichia coli were isolated on the diagnostic culture plates and sub-cultured on to nutrient agar slopes, incubated for eighteen hours at 37°C, allowed to cool to room temperature and stored at 4°C. If it was not possible to investigate the subcultures within a few days, they were lyophilised.

The cultures were typed by serological methods and by colicine production and colicine sensitivity methods previously described. (Chapters III and V).

Results

Reproducibility of pre-treatment isolates

Sixty-five pre-treatment specimens were obtained from twenty-three patients, sixty-three (97 per cent) agreed for colicine production and for colicine sensitivity, sixty-two (95.4 per cent) agreed serologically, with their companion specimens. Two of the specimens were serologically non-typable due to roughness, if one excludes these from comparison, the serological agreement was 98.4 per cent.

The recurrent infections

One hundred and eight recurrences occurred, seventeen (15.8 per cent) had the same strain of Escherichia coli and ninety-one (84.2 per cent) had a strain of Escherichia coli which differed from the infecting strain previously isolated. (Table XXXVIII).

Distribution of the time interval in recurrences.

Recurrences occurred with thirty patients in one month or less since the last infection, forty-six between one and three months, twenty-one between three and six months, six between six and twelve months and five, more than twelve months. (Table XXXVIII).

Of the thirty patients who had recurrences within one month of the previous infection, fifteen had them in two or three weeks. Thirteen of these fifteen incidents were due to a different strain of Escherichia coli, the other two were due to similar strains as shown by the typing markers.

The changes in the typing markers of the recurrent isolates.

Fifty-one (56 per cent) of the variations in the isolates were due to a change in the colicine production patterns, sixty-one (67 per cent) were due to a change in serology and sixty-five (71.4 per cent) were due to a change in the colicine sensitivity pattern. (Table XXXIX). These changes are more specifically considered in Table XL. The commonest difference was shown by a change in all the markers, this occurred in nineteen of the ninety-one incidents, nearly twenty per cent. A change in both the colicine sensitivity and the serology occurred in eighteen incidents, nearly twelve per cent. In fourteen incidents both the colicine production and sensitivity changed and in a similar number the colicine sensitivity only changed. The remaining incidents showed changes in colicine production and serology (eleven), serology only (eight) and colicine production only (seven).

Urinary tract abnormalities and recurrences.

Eleven patients with urinary tract abnormalities had thirty-nine recurrences, there was a change in the strains in thirty-seven. The difference in frequency of the change of marker between the patients with abnormalities and those without was not highly significant. (Chi square = 4.007, P between 0.05 and 0.02).

The number of recurrences of Escherichia coli in those patients

with abnormalities was not significantly higher than those without such abnormalities. (Chi square = 2.300, P between 0.2 and 0.1).

There was no significant difference in the time intervals of the recurrences between the patients with normal urinary tracts and those with abnormalities.

The rise and fall of the bacterial counts.

Two patients, who were under close surveillance following recent attacks of urinary infection, demonstrated that the bacterial count may rise from the intermediate count to the high count overnight. One of the patients was in the wards and was having daily bacterial counts and the other, a medical student, was having bi-weekly counts. The in-patient, with previous sterile cultures, produced a count of 47,100 organisms per millilitre urine which was followed next day, by a count of more than 100,000 organisms per millilitre. The other patient had a count of 51,000 organisms per millilitre which also rose overnight to the significant level. Both had repeated confirmatory cultures over the next twenty-four hours and the strains of Escherichia coli isolated were identical with the intermediate and the high counts.

Similarly, identical isolates have been noted as the bacterial counts dropped following a response to treatment, particularly with sulphonamide therapy, where the bacterial counts tended to fall less abruptly than with other antibacterial drugs.

Discussion

The relapse rate of urinary tract infections has been little influenced by the use of the newer antimicrobials. (Rhoads, Billings and O'Conor, 1952). Looptyt (1946b) found that thirty per cent of his patients had relapses when 'followed-up' for one to three years; Rhoads, Billings and O'Conor (1952) considered that a relapse rate of up to eighty-five per cent occurred over several months, even in the absence of urinary tract abnormalities. Garrod, Shooter and Curwen (1954) had a relapse rate in patients without abnormalities, over several months, of forty per cent in males and eighteen per cent in

females. Kass (1955) stated that ninety per cent of patients with chronic pyelonephritis may have recurrence of infection, and Jackson, Poirier and Crieble (1957) found relapses in fifty per cent of patients with acute pyelonephritis and seventy-five per cent of those with chronic pyelonephritis. In children, Forsythe and Wallace (1958) noted recurrent infections in thirty per cent of those without abnormalities, Dunn, Hine and MacGregor (1964) had similar results. These relapses occurred in patients who had had short-term therapeutic regimes with a wide variety of antimicrobial substances and suggest that long-term therapy would be more appropriate, particularly in the patient with a history of recurrence.

In my investigations of the typing methods of the Escherichia coli, one hundred and eight incidents have been recorded. Nearly twenty-eight per cent occurred within a month of the last infection; 42.6 per cent within one to three months, 19.4 per cent within three to six months, 5.6 per cent in six to twelve months and 4.6 per cent after twelve months. Thus ninety per cent of the recurrences had occurred up to six months since the last infection. Of these recurrences, eighty-four per cent of the strains were different from the strain isolated at the previous infection. Nearly twenty per cent of these differences were shown by a complete change in the typing markers and sixty-eight per cent demonstrated two or more changes in the markers.

There was no significant difference in the frequency of, or the time interval between, recurrences in the patients with urinary tract abnormalities and those without. Of course, this comparison deals solely with recurrences in patients from whom Escherichia coli was isolated and so may not be a true reflection on the recurrence rate of the patients with urinary tract abnormalities. As has been noted in Chapter II, the patients abnormalities tend to have a higher frequency of infection by the non-Escherichia organisms. (Page 19)

The reproducibility of the typing methods was shown to be good by the correlation between the pre-treatment samples from the patients

and this supplements the reliability of the isolates from the diagnostic plates described in the previous chapter. The reproducibility was further confirmed by the identical strains in the two developing infections and also in the regressing infections.

These investigations on the recurrent infection of the urinary tract with Escherichia coli would suggest that the recurrence is due to a strain which differs from the previous isolate in the majority of patients, that is, recurrence is re-infection rather than recrudescence in the majority of patients.

This poses a further question, that of therapeutic regimes, if the recurrent infection is due to another organism, is there any justification for long-term therapy? Studies on renal biopsy may be of assistance in answering this. Although the difficulties, arising from the focal and patchy nature of the disease, make the routine use of renal biopsy of doubtful value in the diagnosis of pyelonephritis, several reports on renal biopsy in patients with pyelonephritis have appeared. In particular, those of Hutt and de Wardener (1961) and Brun and Raaschou (1961), both of which appeared in the Ciba Symposium on renal biopsy (editors: Wolstenholme and Cameron, 1961), are germane to this discussion. Hutt and de Wardener found evidence of persisting pathological changes, consistent with acute pyelonephritis, from five to seventy days following the onset of symptoms and after response to treatment. Brun and Raaschou commented on one patient, who had post-partum, acute renal failure due to acute pyelonephritis, where histological changes of acute pyelonephritis persisted at three weeks and were still noted, at a further biopsy, four and a half years later, however, they fail to state whether or not she had had further infections during this period. Nevertheless, both these reports suggest that the kidney may remain damaged long after the clinical and bacteriological cures may be pronounced.

Experimentally, it has been shown that the damaged kidney is more

readily infected than the normal; Braude, Shapiro and Siemienski (1955) and McCabe and Jackson (in Quinn and Kass, 1960) used renal 'massage' to render the kidney more susceptible. De Navasquez (1956) used staphylococci to produce a scarred kidney in which coliform bacilli subsequently produced infection and Rocha, Guze, Freedman and Beeson (1958) scarred the kidneys by electrocautery.

Thus it is likely that, following an attack of pyelonephritis, the kidney is more liable to reinfection for a period of time, as yet unknown for certainty, but probably to be measured in months. During this period of healing it seems advisable to supply antimicrobial 'cover'. The length of time for which this must be continued is at present being studied at various centres, including the group with which I am working, and will of necessity need some further time before analysis of the results is complete. The evidence of the recurrent infections which I have presented suggest that it is not unlikely that the minimum period of six months is required, since ninety per cent of the incidents occurred within this time.

Summary.

Forty-nine patients were investigated for one hundred and eight recurrent attacks of urinary infection with Escherichia coli. Thirty-eight patients, without demonstrable abnormalities of the urinary tract, had sixty-nine incidents; eleven patients, with urinary tract abnormalities, had thirty-nine recurrences. There was no significant difference noted in the frequency of recurrent attacks in these two groups.

Sixty-five pre-treatment specimens, obtained from twenty-three patients, confirmed that the reproducibility of the typing markers was good. There was an agreement of 97 per cent in both the colicine production and sensitivity, and with the serologically 'smooth' strains there was agreement in 98.4 per cent.

Of the one hundred and eight recurrences, ninety-one (84.2 per cent) were associated with different strains of Escherichia coli as shown by the typing markers.

Ninety per cent of the recurrences had occurred within six months of the previous infection. There was no significant difference in the time intervals of recurrence between the patients with and without abnormalities.

It is suggested that recurrent infections with Escherichia coli are due, in the majority of incidents, to a reinfection rather than a recrudescence. The importance of this suggestion on the antibacterial management of the patient is discussed.

ACKNOWLEDGEMENTS.

I should like to express my indebtedness to Dr. A. C. Kennedy, not only for performing many of the quantitative leucocyte counts compared in the first chapter, but also for his close co-operation which has made the clinical and bacteriological comparisons in this thesis possible.

I am grateful to Dr. J. C. J. Ives for his encouragement and advice and to Professor E. M. McGirr for permitting me access to patients and records.

I wish to express my appreciation to Dr. Franz Ørskov, for supplying the strains of Escherichia coli used in the preparation of the antisera, and to Professor P. Fredericq, for typing the strains used for colicine sensitivity, and to both for the helpful criticism of my results.

For technical assistance at various times, I have to thank Messrs. W. McCormick and J. Forrester. The photography was performed by Messrs. T. Parker and I. MacGregor, and the diagrams were produced by Miss Avril McIntosh.

Dr. A. Fazekas was most helpful in translating the references from German sources. Mr. M. W. Birch, of the Department of Mathematics, University of Glasgow, was good enough to confirm my statistical interpretations.

Parts of this thesis have already been published; "Simplified methods for bacteriuria and pyuria." (1963). J. clin. Path., 16, 32., McGeachie, J. and Kennedy, A.C.; and "Comparison of colicine production and diffusion on different solid media." (1963). J. clin. Path., 16, 278., McGeachie, J. and McCormick, W.

Finally, I wish to thank Miss Aileen Clark for typing this thesis.

VOLUME TWO

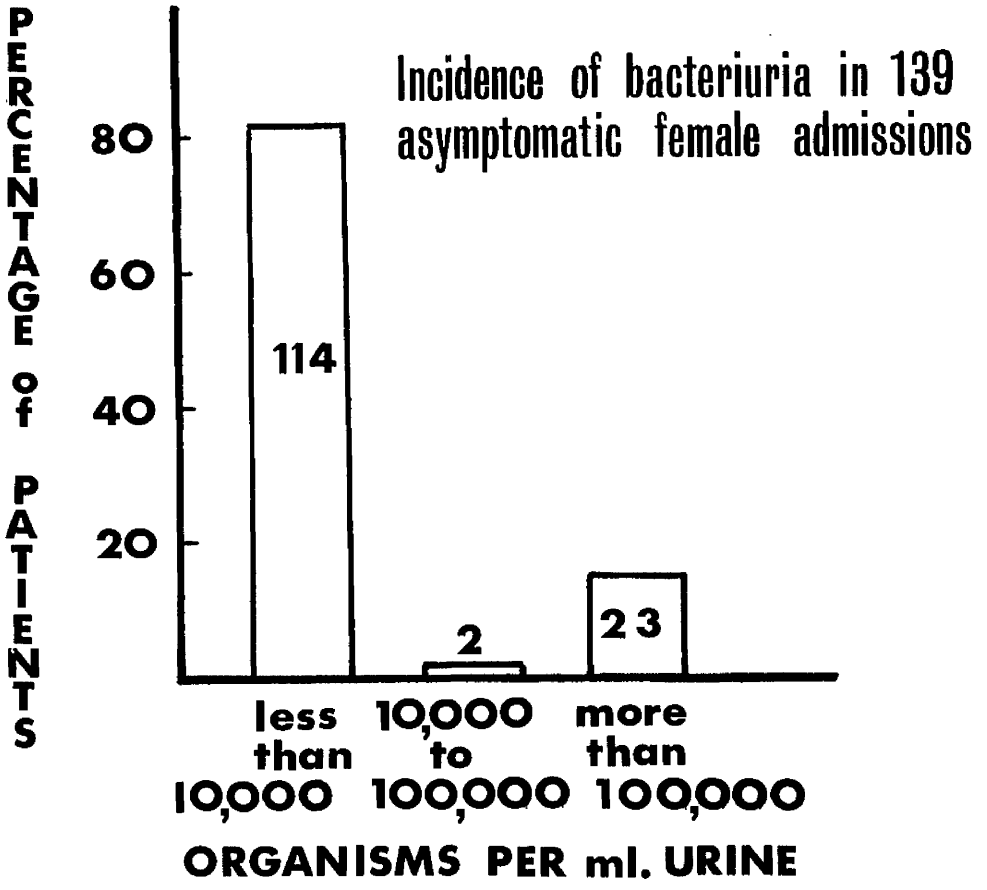
APPENDICES

VOLUME TWO.

INDEX TO FIGURES AND TABLES.

FIGURES.

Figure I



Twenty-one of the twenty-three patients with counts of more than 100,000 organisms per millilitre urine had quantitive leucocyte counts of more than ten cells per measured area of the Neubauer chamber (Figure 5).

Figure 2

THE AGE AND SEX INCIDENCE OF THE PATIENTS.

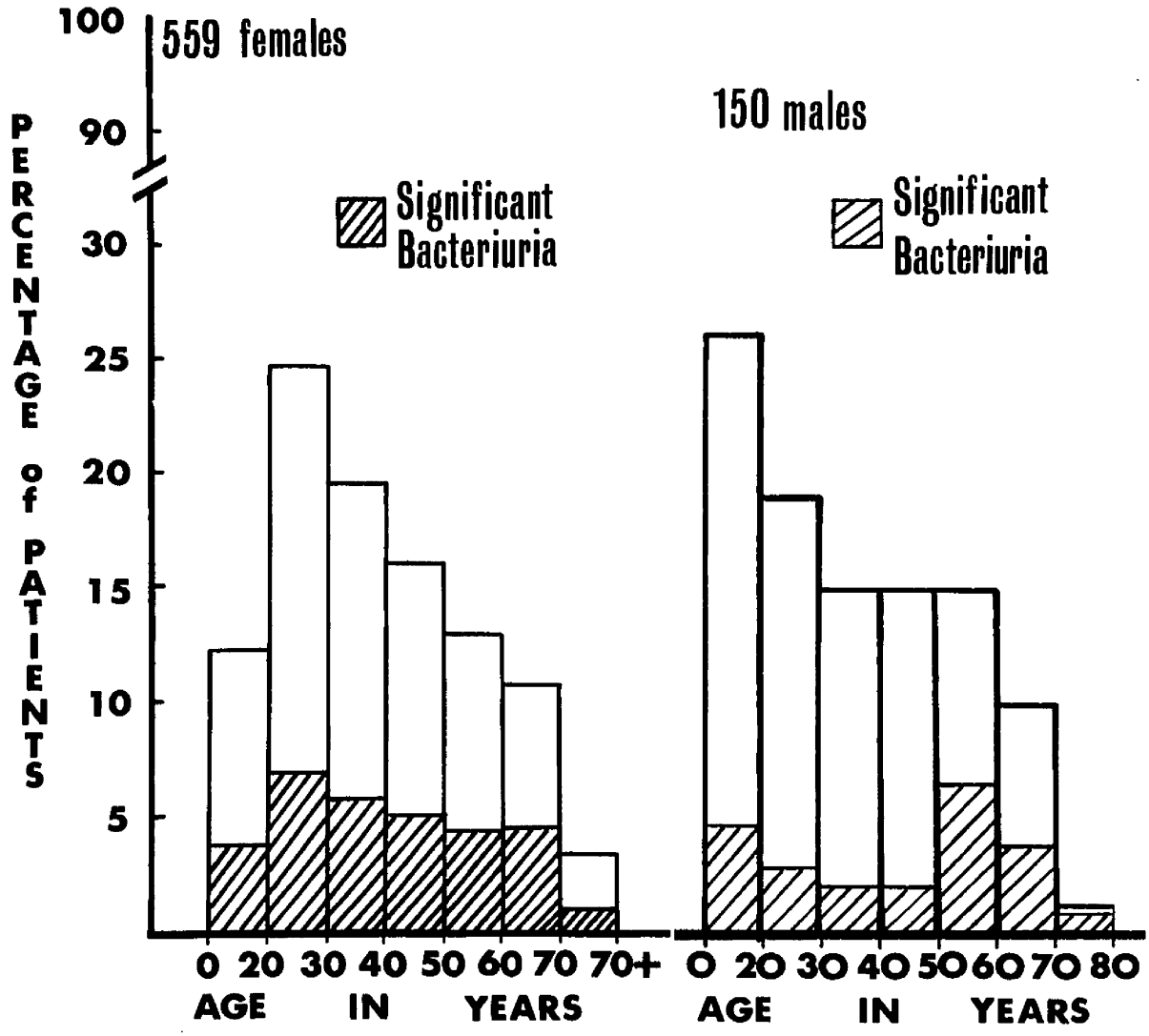
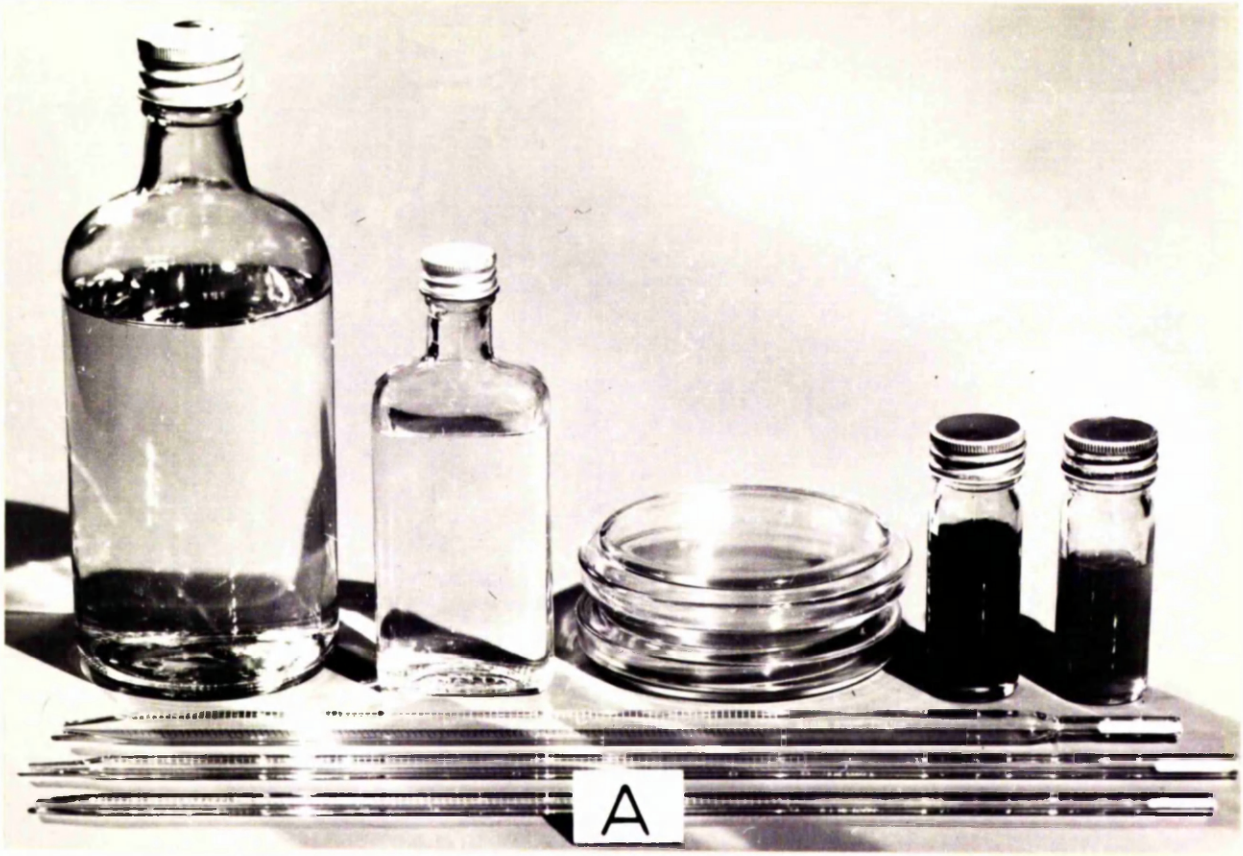
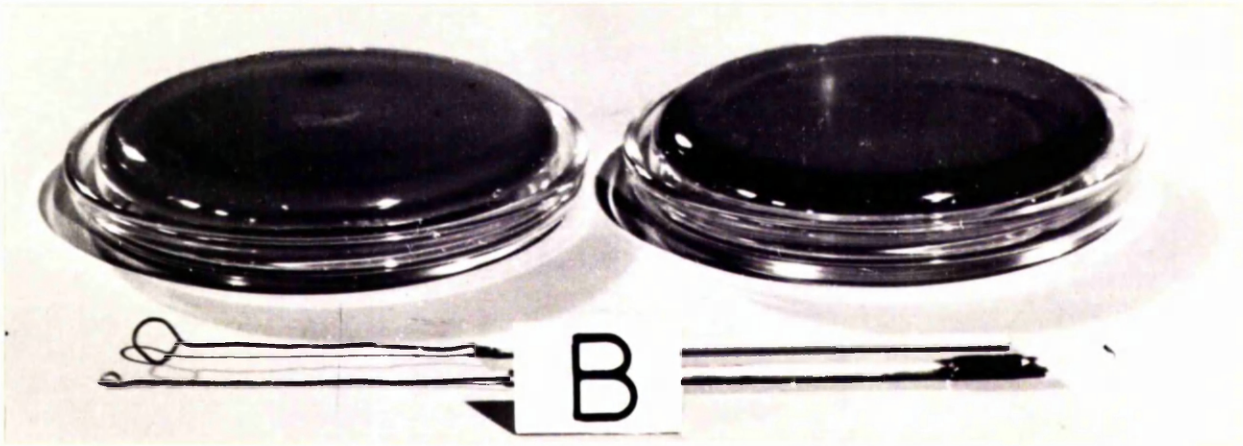


Figure 3

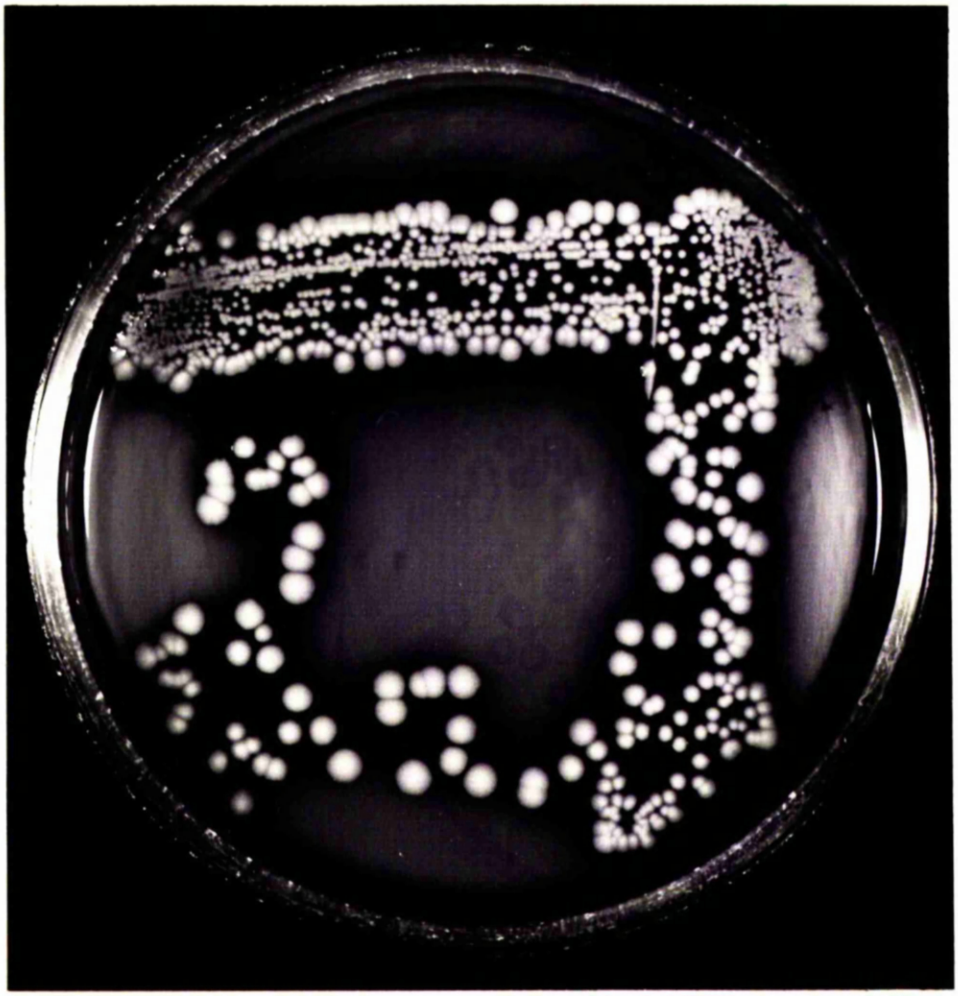




Equipment required for the pour-plate technique (A) and that required for the stroke-plate (B).

Figure 4

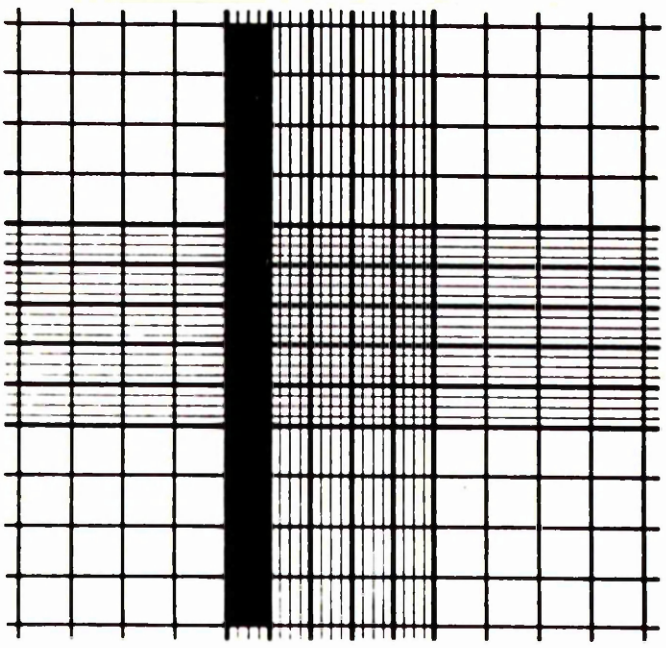
THE METHOD OF INOCULATION OF THE STROKE - PLATE



This plate has been inoculated with a loopful of urine from a specimen containing more than 100,000 organisms per millilitre.

Figure 5

THE QUANTITATIVE LEUCOCYTE COUNT.



The shaded area is examined for cells over it lie 0.00006 millilitres of urine. This is equivalent to 0.0012 millilitres of uncentrifuged urine.

Figure 6

THE DISTRIBUTION OF THE 2,085 SPECIMENS OF URINE IN THE BACTERIAL COUNT GROUPS.

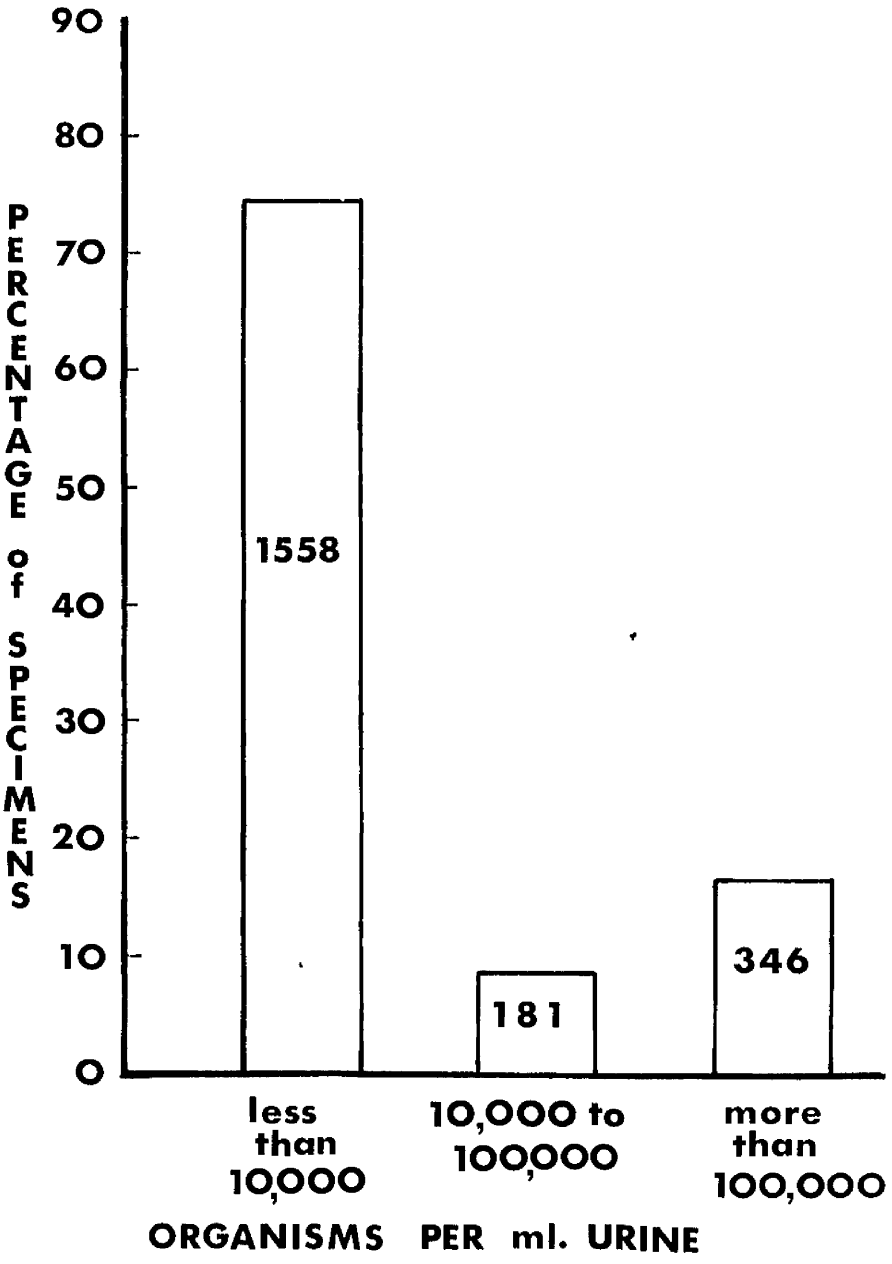


Figure 7

THE COMPARATIVE DISTRIBUTION OF THE QUANTITATIVE LEUCOCYTE COUNTS AND BACTERIAL COUNTS.

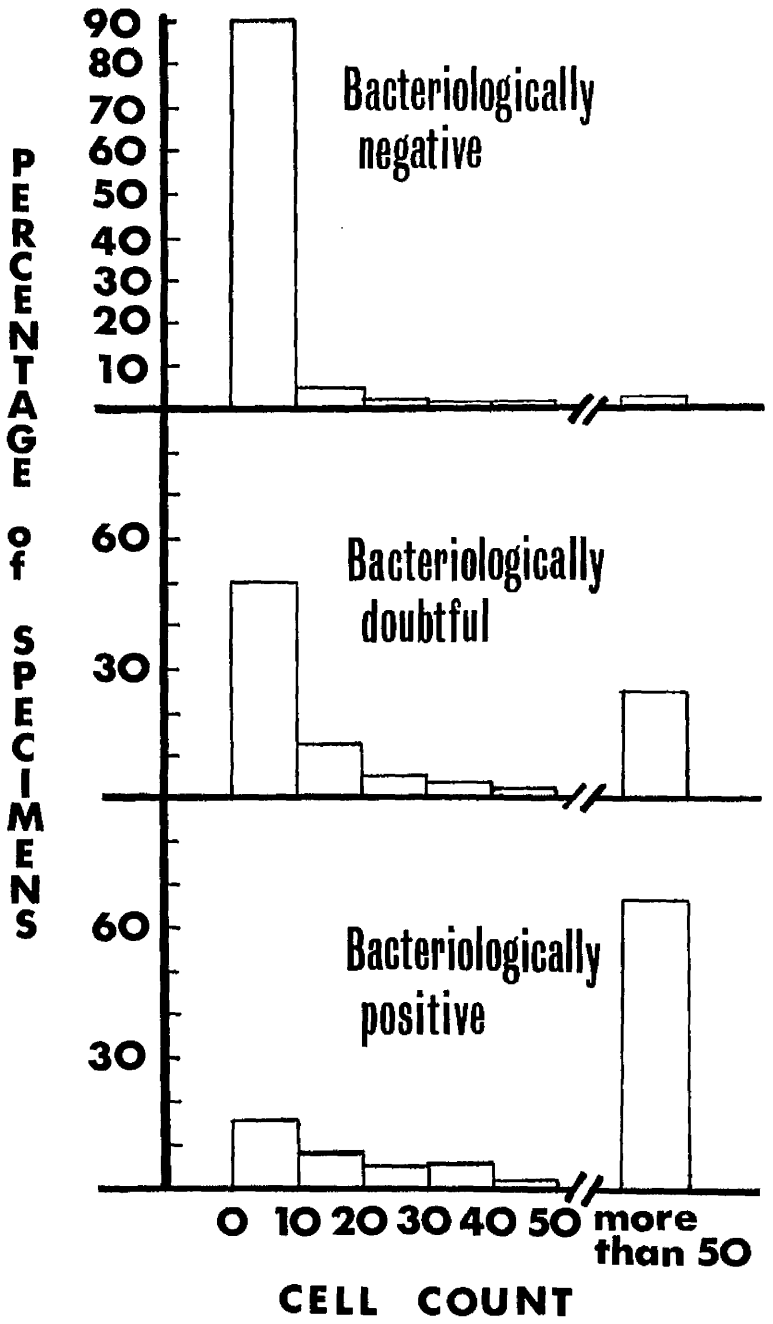
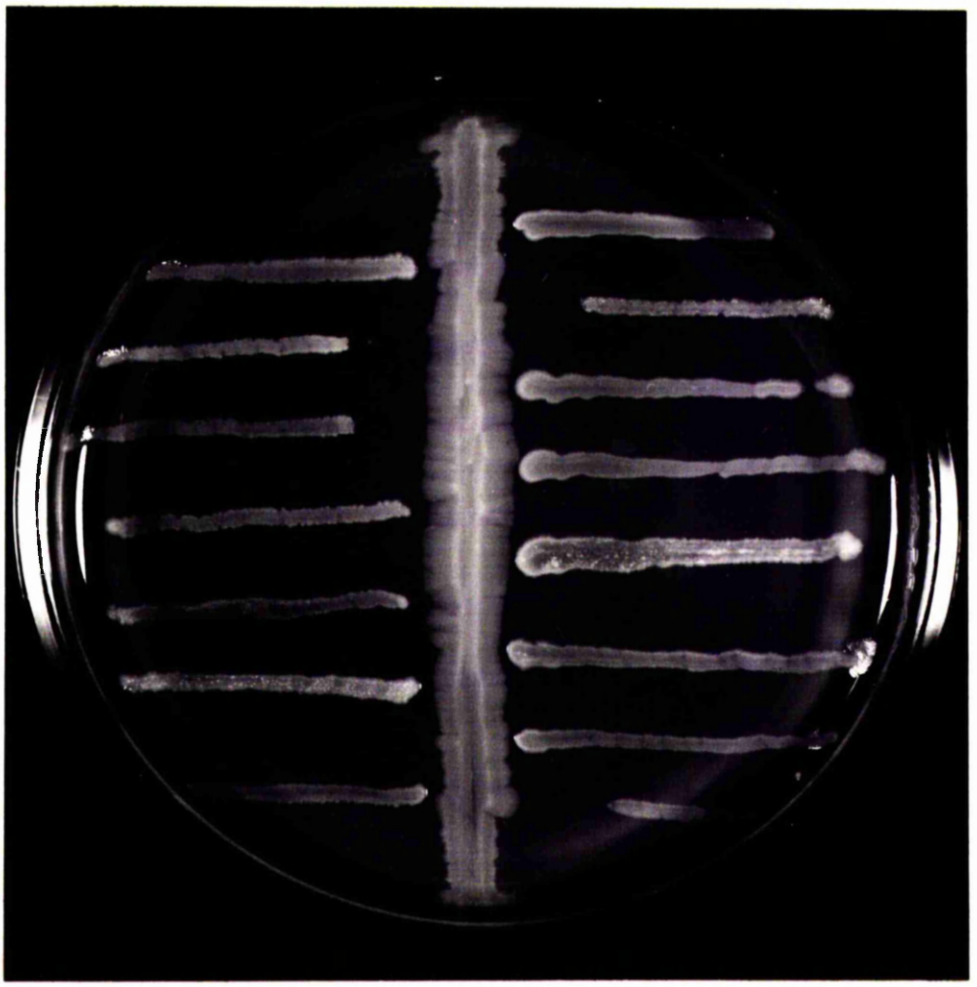


Figure 8

THE DIAMETRIC STREAK METHOD OF COLICINE TYPING.



The central stroke is the test organism and the lateral strokes are the indicators.

Inhibition of the indicators 2 and 3, on the left, and 9 and 15, on the right has occurred.

Figure 9

THE PROCEDURE FOR COLICINE EXTRACTION
BY THE FREEZE-THAW METHOD

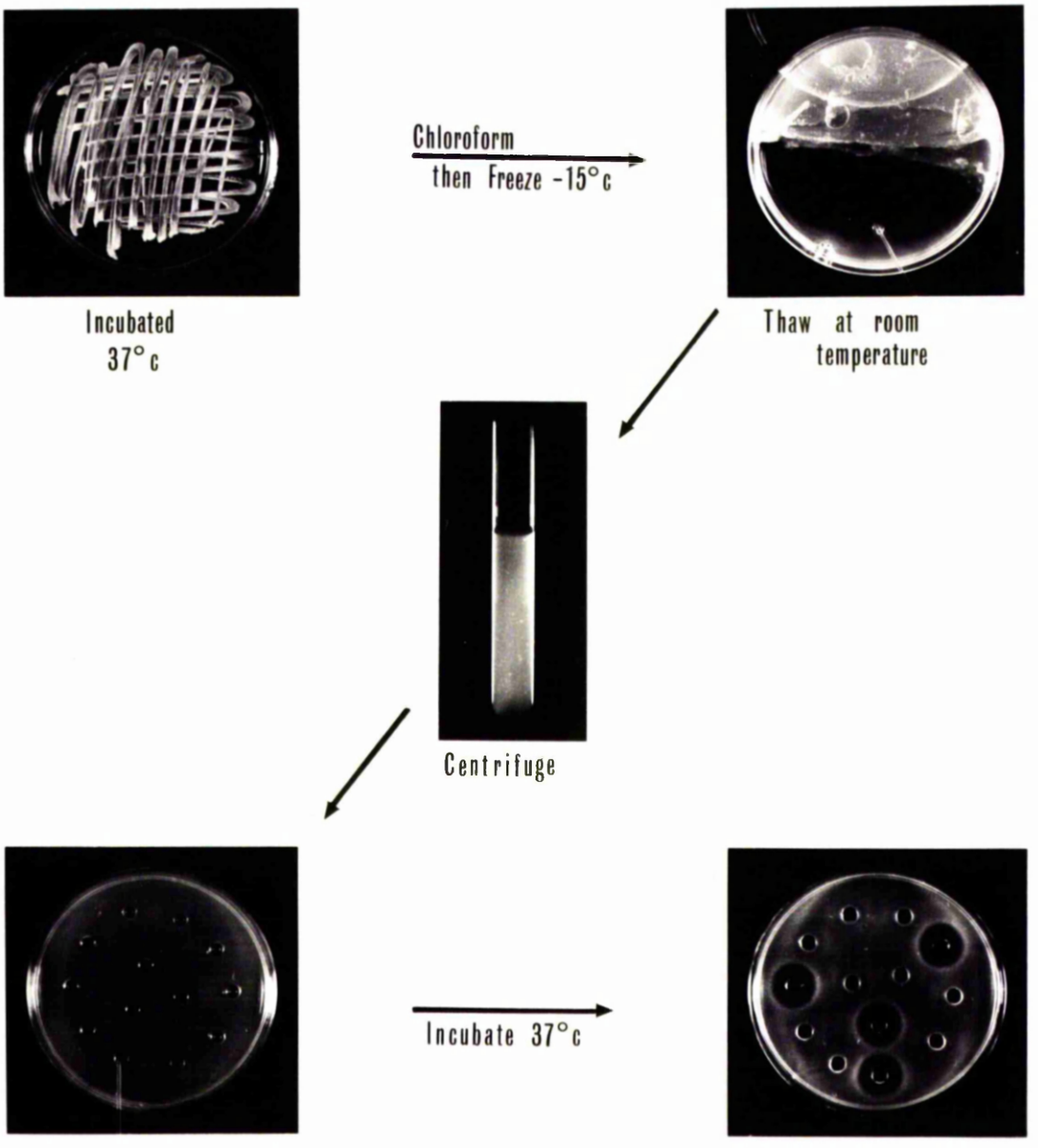
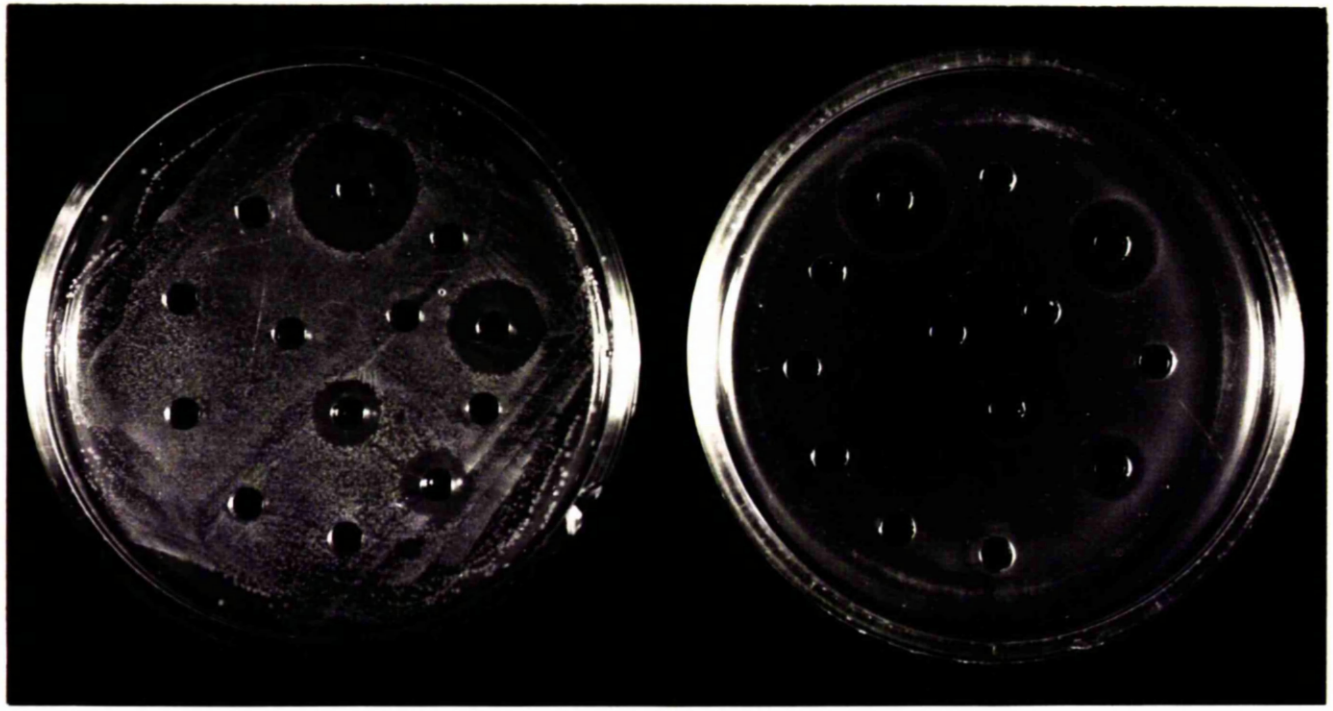


Figure 10

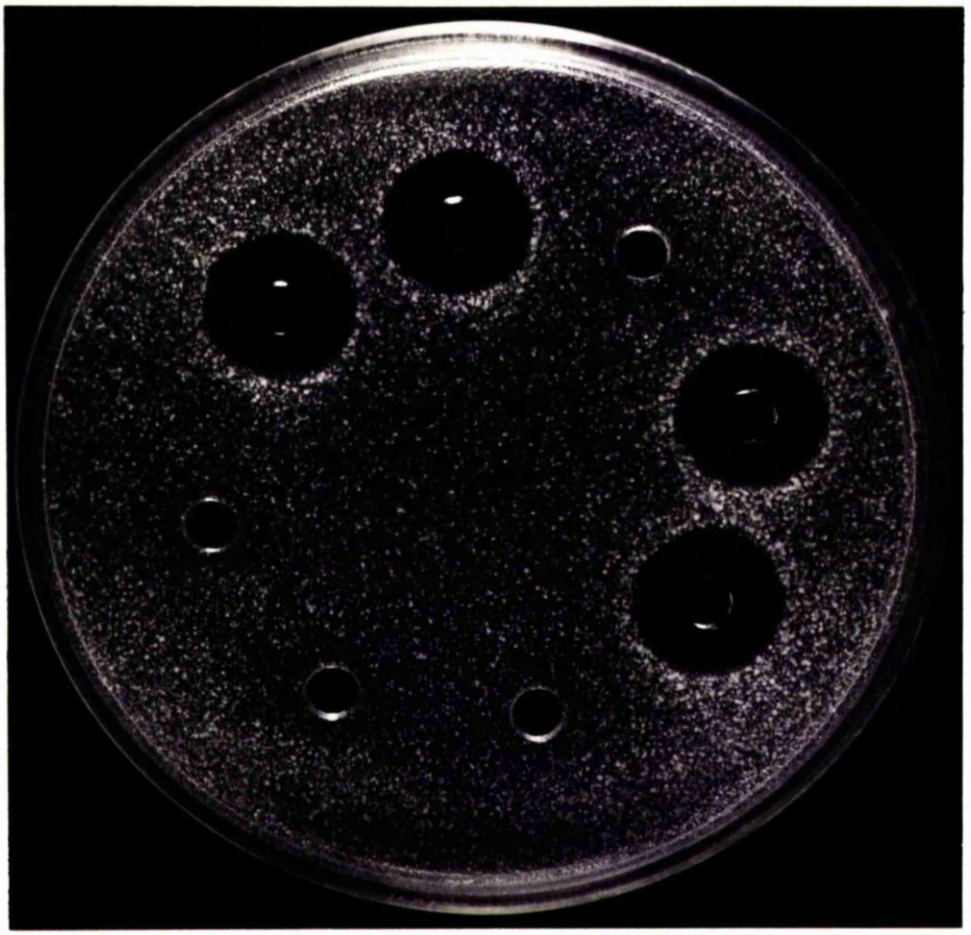
THE EXTRACTION METHOD OF COLICINE TYPING.



The plate on the right has been pour-plate seeded, that on the left has been surface-seeded, with an indicator organism. The effects of thirteen colicine extracts are shown.

Figure 11

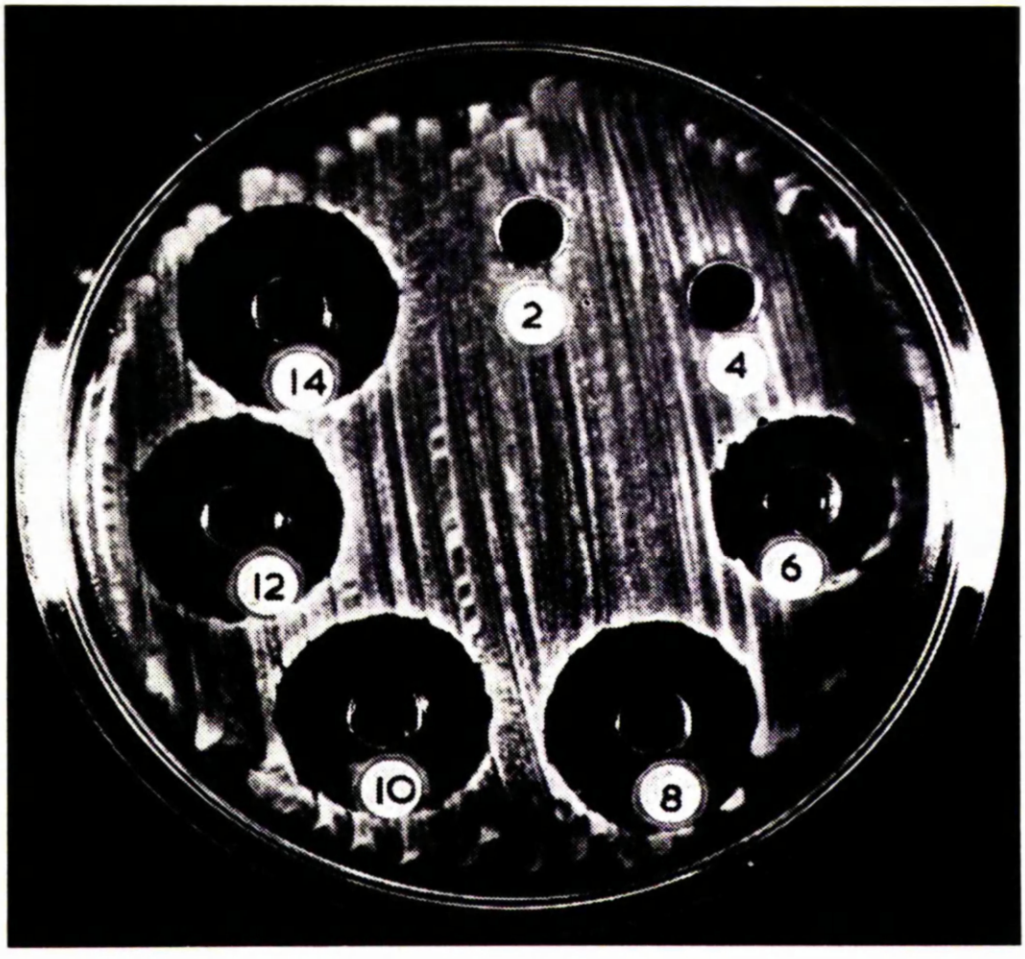
TESTING OF COLICINE SENSITIVITY.



The plate has been pour-plate seeded with the test organism and the sensitivity to the eight colicine extracts is shown.

Figure 12

TIME REQUIRED TO DEMONSTRATE COLICINE PRODUCTION.



The plate has been seeded with an indicator organism. The extracts from cultures of the colicinogenic strain, incubated for two to fourteen hours before freezing, have been tested.

TABLES.

Table I

DISTRIBUTION OF PATIENTS BY, SEX, AGE, INFECTION AND URINARY TRACT STATE.

Age	Infection present		No infection present		Total
Females	(1)	(2)	(3)	(4)	
0-10	2	0	5	0	7
11-20	15	3	38	7	63
21-30	39	5	81	13	138
31-40	23	9	68	9	109
41-50	26	2	53	8	89
51-60	20	4	40	7	71
61-70	22	3	35	3	63
71-80	6	0	10	1	17
81-90	0	0	2	0	2
	153	26	332	48	559
Males					
0-10	1	0	1	1	3
11-20	3	3	12	18	36
21-30	4	0	7	17	28
31-40	1	2	3	16	22
41-50	0	3	6	13	22
51-60	5	5	3	9	22
61-70	2	3	5	5	15
71-80	1	0	0	1	2
80-	0	0	0	0	0
	17	16	37	80	150
Total	170	42	369	128	709

Columns (1) and (3) : patients without associated structural urinary tract lesions.

Columns (2) and (4) : patients with associated structural urinary tract lesions, e.g. acute and chronic glomerulo-nephritis; polycystic kidney; acute tubular necrosis, post-prostatectomy infection.

Table II

REPRODUCIBILITY OF BACTERIAL COUNTS ON MID-STREAM SPECIMENS OF URINE.

Bacterial count	Number of Patients: Agreement in all specimens	Disagreement with first specimen	Total
More than 100,000 organisms per ml.	8 (24)	1 (1)	9 (27)
Less than 10,000 organisms per ml.	34 (102)	1 (1)	35 (105)
Between 10,000 & 100,000 organisms per ml.	0 (0)	6 (12)	6 (18)
	42 (126)	8 (14)	50 (150)

Number of specimens examined shown in parenthesis.

Table III

COMPARISON OF BACTERIAL COUNTS BY THE POUR-PLATE AND STROKE-PLATE METHODS.

Pour-plate count: organisms per millilitre	Stroke-plate count: organisms per millilitre urine								Total
	more than 100,000	80,000	50,000	40,000	30,000	20,000	15,000	less than 10,000	
More than 100,000	37								37
80,000		1							1
60,000	1	1							2
50,000	2		1						3
40,000				2		1			3
30,000					1	3			4
20,000						1	1		2
10,000	1		1		1		1		4
Less than 10,000	2				1	2	4	100	109
Total	43	2	2	2	3	7	6	100	165

Table IV

CORRELATION BETWEEN THE BACTERIAL COUNTS BY THE POUR-PLATE AND STROKE-PLATE METHODS.

Pour plate organisms/ml	Stroke plate organisms/ml			Number of Specimens
	More than 100,000	10,000 to 100,000	Less than 10,000	
More than 100,000	100%	-	-	37
10,000 to 100,000	21%	79%	-	19
Less than 10,000	2%	6%	92%	109

Table V

CORRELATION BETWEEN BACTERIAL COUNTS AND STROKE-PLATE GROWTH.

	Sides of "box" showing growth					
Bacterial count	0	+	++	+++	++++	Total
Less than 10,000	802	214	23	-	-	1039
Non-pathogens	-	348	127	40	4	519
10,000 to 100,000	-	31	116	31	3	181
More than 100,000	-	-	20	93	233	345
Totals	802	593	286	164	240	2085

Table VI

CORRELATION BETWEEN BACTERIAL COUNTS AND QUANTITATIVE LEUCOCYTE COUNTS.

Bacterial counts.

Cells per measured area	less than 10,000	10,000 to 100,000	more than 100,000	Totals
10 and less	993	39	53	1085
more than 10	109	38	293	440
Total	1102	77	346	1525
Percentage more than 10	9.9	49.3	84.7	31.5

Patients on treatment and with glomerular lesions excluded.

Table VII

CORRELATION BETWEEN THE ASSESSMENT OF PYURIA BY THE QUANTITATIVE CELL COUNT METHOD AND THE WET-FILM ESTIMATION.

Quantitative white cell count	Wet film estimation White cells per 20 H.P.F.					Number of specimens
	0	1-9	10-99	100-250	> 250	
10 and less	314 49.6%	258 40.7%	59 9.3%	2 0.3%	-	633
More than 10	22 5.9%	92 24.9%	128 34.7%	78 21.1%	49 13.3%	369

30% error

65% error

Table VIII

CORRELATION BETWEEN QUANTITATIVE LEUCOCYTE COUNTS AND ESTIMATION OF PUS CELLS BY A GRAM-STAINED FILM.

	Pus cells per 50 oil immersion fields			
Cells per measured area	0	1-10	More than 10	Totals
10 and less	1029	160	7	1196
more than 10	144	237	301	682
Totals	1173	397	308	1878
Percentage more than 10	12.3	59.7	97.7	36.4

Table IX

CORRELATION BETWEEN BACTERIAL COUNT AND BACTERIAL ESTIMATION BY A GRAM-STAINED FILM.

Bacterial Count	Organisms per 50 oil immersion fields			
	0-10	11-50	more than 50	Total
more than 100,000	28	48	211	287
10,000 to 100,000	121	29	10	160
less than 10,000	1545	13	0	1558
Totals	1694	90	221	2005

Table X

MAIN BIOCHEMICAL REACTIONS OF THE ENTEROBACTERIACEAE

	Escherichia	Citrobacter	Klebsiella	Proteus mirabilis
Gas from Glucose	+	+	+	+
Lactose	+ or x	+ or x	+	-
Sucrose	d	d	+	x
Mannitol	+	+	+	-
Dulcitol	d	d	d	-
Salicin	d	d	+	+
Adonitol	-	- or x	+	-
Inositol	-	-	+	-
Indole	+	-	-	-
Methyl Red	+	+	-	+
Voges-Proskauer	-	-	+	d
Citrase	-	+	+	d
Urease	-	-	(+)	+

+ : Positive at 24 or 48 hours

x : Late and irregularly positive

- : Negative

d : Differs with biotypes

(+): Hydrolysed at 24 hours but not at 6 hours

Table XI

INCIDENCE OF ORGANISMS ON PRIMARY ISOLATION FROM PATIENTS WITH SIGNIFICANT BACTERIURIA

	Female		Male		Total
Organism isolated	(1)	(2)	(3)	(4)	
Escherichia coli	140	14	10	5	169
Proteus mirabilis	2	6	1	8	17
Citrobacter freundii	2	1	0	0	3
Pseudomonas aeruginosa	1	1	0	0	2
Staphylococcus albus (coagulase negative)	2	0	0	0	2
Staphylococcus aureus (coagulase positive)	0	2	0	0	2
Streptococcus faecalis	0	2	0	4	6
Klebsiella species	0	0	0	2	2
E. coli and Strep. faecalis	2	0	0	0	2
E. coli and Proteus mirabilis	0	4	0	0	4
Proteus mirabilis and Strep. faecalis	0	0	0	1	1
Proteus mirabilis and Ps. aeruginosa	0	0	0	1	1
Citrobacter freundii and Strep. faecalis	0	0	0	1	1
Total	149	30	11	22	212

Columns (1) and (3) no urinary tract abnormality.

Columns (2) and (4) associated urinary tract abnormality

Table XII

FREQUENCY OF ALL ORGANISMS ISOLATED IN THE BACTERIAL COUNT GROUPS.

Species	Bacterial count : Organisms per millilitre urine.			
	More than 100,000	10,000 to 100,000	Less than 10,000	Total
Escherichia coli	232	71	91	394
Proteus species	43	33	42	118
Staphylococcus aureus and albus	12	9	52	73
Streptococcus faecalis	19	19	18	56
Klebsiella species	20	0	0	20
Citrobacter freundii	6	2	1	9
Pseudomonas aeruginosa	6	2	0	8
alpha-haemolytic streptococcus	1	3	0	4
Non-haemolytic streptococcus	0	1	1	2
Hafnia species	0	1	1	2
Enterobacter cloacae	0	1	1	2

Table XII (continued)

Species	More than 100,000	10,000 to 100,000	Less than 10,000	Total
E. coli and Proteus	4	10	6	20
E. coli and Strep. faecalis	4	15	13	32
E. coli and Staph. albus	0	3	3	6
E. coli and Ps. aeruginosa	1	0	0	1
Proteus and Strep. faecalis	1	8	5	14
Proteus and Ps. aeruginosa	1	1	0	2
Proteus and Staph. albus	0	0	2	2
Citrobacter and Strep. faecalis	2	1	0	3
Citrobacter and Staph. albus	0	0	1	1
Ps. aeruginosa and Strep. faecalis	0	1	0	1
Staph. albus and Strep. faecalis	0	0	1	1
E. coli, Strep. faecalis and Proteus	0	4	0	4
Totals	352	185	238	775

Table XIII

FREQUENCY OF SEROLOGICAL GROUPS OF ESCHERICHIA COLI IN THE BACTERIAL COUNT GROUPS OF ISOLATION FROM URINE

Serological type	Bacterial count:- Organisms per millilitre urine			
	More than 100,000	10,000 to 100,000	less than 10,000	Total
01	32	5	17	54
02	19	2	3	24
04	38	12	16	66
05	7	4	4	15
06	35	7	13	55
07	6	-	4	10
08	2	3	2	7
09	1	-	4	5
011	4	1	3	8
018	21	6	7	34
039	4	-	-	4
075	17	1	2	20
N.T.R.	22	4	9	35
N.T.S.	108	41	48	197
Total	316	86	132	534

N.T.R.:- Not typable due to roughness of strain

N.T.S.:- Smooth but not typable.

Table XIV

FREQUENCY OF SEROLOGICAL GROUPS OF ESCHERICHIA COLI STRAINS PRIMARILY ISOLATED FROM PATIENTS WITH SIGNIFICANT BACTERIURIA.

Serological type	Symptomatic infection	Asymptomatic infection	Renal lesion	Total
01	17	3	2	22
02	9	1	5	15
04	20	1	5	26
05	4	-	1	5
06	22	1	1	24
07	4	1	-	5
08	-	-	1	1
09	1	-	-	1
011	3	-	-	3
018	2	1	2	5
039	2	-	1	3
075	8	-	1	9
N.T.R.	6	3	-	9
N.T.S.	42	3	9	54
Total	140	14	28	182

Table XV.

FREQUENCY OF SEROLOGICAL GROUP IN PRIMARY AND RECURRENT ISOLATES FROM PATIENTS WITH SIGNIFICANT BACTERIURIA.

Serological type	Primary isolates	Recurrent isolates	Total
01	22	10	32
02	15	4	19
04	26	12	38
05	5	2	7
06	24	11	35
07	5	1	6
08	1	1	2
09	1	-	1
011	3	1	4
018	5	16	21
039	3	1	4
075	9	8	17
N.T.R.	9	13	22
N.T.S.	54	54	108
Total	182	134	316

Table XVI

FREQUENCY OF SEROLOGICAL GROUP AND HAEMOLYTIC REACTION RELATED TO THE SEX OF THE PATIENTS WITH SIGNIFICANT BACTERIURIA.

Serological type	Female		Male		Total
	+	-	+	-	
01	9	22	1	0	32
02	3	13	1	2	19
04	18	15	4	1	38
05	2	5	0	0	7
06	19	13	3	0	35
07	0	5	0	1	6
08	0	2	0	0	2
09	0	1	0	0	1
011	0	4	0	0	4
018	10	11	0	0	21
039	0	4	0	0	4
075	8	7	1	1	17
Not typable	21	99	1	9	130
Total	90	201	11	14	316

\+ =haemolytic strains
\- =non-haemolytic strains

Table XVII

FREQUENCY OF SEROLOGICAL GROUPS OF THE PRIMARY ISOLATES FROM FEMALES AND MALES WITH SIGNIFICANT BACTERIURIA.

	Female	Male	Totals
01	21	1	22
02	13	2	15
04	22	4	26
05	5	0	5
06	22	2	24
07	4	1	5
08	1	-	1
09	1	-	1
011	3	-	3
018	5	-	5
039	3	-	3
075	7	2	9
Totals	107	12	119

Table XVIII.

FREQUENCY OF SEROLOGICAL GROUPS AND HAEMOLYTIC REACTION OF THE PRIMARY SYMPTOMATIC ISOLATES FROM FEMALES AND MALES.

Serological group	Female		Male		
	Haemolysis	No haemolysis	Haemolysis	No haemolysis	
01	6	11	-	-	17
02	1	7	-	1	9
04	9	10	1	-	20
05	2	2	-	-	4
06	12	8	2	-	22
07	-	3	-	1	4
08	-	-	-	-	-
09	-	1	-	-	1
011	-	3	-	-	3
018	2	-	-	-	2
039	-	2	-	-	2
075	4	3	-	1	8
Total typable	36	50	3	3	92
Non-typable	7	40	-	1	48
Total	43	90	3	4	140

Table XIX

COMPARISON OF SEROLOGICAL GROUPING AND HAEMOLYSIS OF THE TOTAL ISOLATES.

Serological group	Haemolysis	No Haemolysis	Total
01	16	38	54
02	4	20	24
04	38	28	66
05	4	11	15
06	35	20	55
07	-	10	10
08	1	6	7
09	1	4	5
011	-	8	8
018	13	21	34
039	-	4	4
075	11	9	20
Total typable	123	179	302
Not typable	34	198	232
Total	157	377	534

Table XX

FREQUENCY OF HAEMOLYTIC STRAINS IN THE BACTERIAL COUNT GROUPS.

	Bacterial counts organisms per millilitre urine				
	More than 100,000 Primary	Recurrent	10,000 to 100,000	Less than 10,000	Total
Haemolytic	62	39	22	36	159
Non haemolytic	120	95	64	96	375
Total	182	134	86	132	534

There is no significant difference in the frequency of haemolysis between the strains from the counts of more than 100,000 and less than 10,000 organisms per millilitre.

Table XXI

FREQUENCY OF HAEMOLYTIC STRAINS RELATED TO THE SEX OF THE PATIENTS WITH SIGNIFICANT BACTERIURIA

	Female	Male	Total
Haemolytic	90	11	101
Non-haemolytic	201	14	215
Total	291	25	316

The difference in the frequency of haemolytic strains between males and females is not significant (Chi square = 1.2578).

Table XXII

FREQUENCY OF HAEMOLYTIC STRAINS IN SIGNIFICANT BACTERIURIA RELATED TO PRIMARY AND RECURRENT ISOLATES FROM PATIENTS WITH AND WITHOUT ASSOCIATED URINARY TRACT ABNORMALITIES.

	Normal tracts		Associated lesions	
	+	-	+	-
Primary isolates	47	92	10	18
Recurrent isolates	31	53	5	42

\+ = haemolytic strains

\- = non-haemolytic strains

The seventeen patients with asymptomatic bacteriuria are excluded. There is no significant difference in the frequency of haemolytic strains in the primary isolates. With the recurrent isolates, there is possibly a significantly higher frequency of haemolytic strains in the normal tracts than the abnormal tracts. (Chi square, 6.990, P between 0.01 and 0.001.)

Table XXlll

THE COMPARISON OF THE EFFECTS OF VARIOUS MEDIA ON THE PRODUCTION AND THE DIFFUSION OF COLICINE.

	Diffusion media					
Colicine Extract	Digest	Peptone water	"Sensitivity Test"	Blood agar base No 2	Tryptone soya (1)	Tryptone soya (2)
E1	21 mm.	21 mm.	15 mm. (R)	20 mm. (R)	20 mm. (R)	20 mm. (R)
E2	-	-	-	-	-	-
E3	10 mm.	16 mm.	-	9 mm.	10 mm. (R)	15 mm. (R)
E4	10 mm.	16 mm.	-	-	10 mm.	14 mm. (R)
E5	-	11 mm.	-	-	-	10 mm.
E6	-	9 mm.	-	-	-	-

E1, E2, E3, E4, E5, E6, are extracts from digest nutrient agar, peptone water agar, "Sensitivity test" agar, blood agar base No. 2, batch I and II tryptone soya agar respectively.

(R) = resistant variants present in the zones of inhibition.

10 mm. = the diameter of the zone of inhibition in millimetres.

- = no inhibition recorded.

Table XXIV

VARIATION IN ZONES OF INHIBITION WITH TIMES OF INCUBATION BEFORE EXTRACTION.

	Incubation time in hours											
	2	4	6	8	10	12	14	16	18	20	22	24
Diameter of inhibition in millimetres	0	0	18	20	20	20	21	21	21	21	21	21

Table XXV

COMPARISON OF THE EFFECTS OF VARIOUS PEPTONES ON COLICINE PRODUCTION.

Medium	Zones of inhibition (millimetres)
'Peptone-free' control	21
Bacteriological peptone (Oxoid) 1%	9
Bacteriological peptone (Evans) 1%	No inhibition
Mycological peptone (Oxoid) 1%	No inhibition
Proteose no. 3 peptone (Difco) 1%	20
Neopeptone (Difco) 1%	20

Table XXVI

TOTAL COLICINICITY OF THE URINARY ISOLATES

Colicine	Bacterial count: organisms per millilitre urine			Total (percentage)
	More than 100,000	10,000 to 100,000	Less than 10,000	
Production only	71	16	31	118 (22.1)
Sensitivity only	100	30	53	191 (35.8)
Both Production and Sensitivity	49	11	15	75 (14.1)
Total typable by Colicine. (Percentage)	220 (69.6)	69 (75.6)	99 (74.9)	384 (71.9)
Total strains examined	316	86	132	534

There is no significant difference in the frequency of typable strains between the high and low bacterial counts
(chi square 1.0549, P = between 0.50 and 0.30)
There is no significant difference in the frequency of colicine producing strains and colicine sensitive strains in the high and low bacterial count groups.

Table XXVII

COLICINE TYPING OF THE ESCHERICHIA COLI.

Colicine production patterns

75 patterns for 194 strains

7 patterns contained 104 strains = 53.6 per cent of strains

21 patterns contained 140 strains = 72.2 per cent of strains

Main patterns:-

7	(13)	30	(5)
8	(9)	66	(14)
26	(41)	79	(16)

Colicine Sensitivity patterns

72 different patterns for 264 strains

7 patterns contained 123 strains = 46.6 per cent of strains

20 patterns contained 188 strains = 71.2. per cent of strains

Main patterns:-

1	(44)	9	(11)	43	(5)	59	(5)
3	(8)	14	(5)	48	(5)	63	(6)
5	(12)	38	(11)	50	(6)	64	(18)
7	(5)	39	(19)	57	(6)	72	(6)

Figures in parenthesis give the number of strains in the group.

Table XXVIII

FREQUENCY OF COLICINICITY IN THE BACTERIAL COUNT GROUPS

Colicine	Bacterial count: organisms per millilitre urine			
	More than 100,000		10,000 to 100,000	Less than 10,000
	Primary isolates	Recurrent isolates		
Production	35.7%	41.0%	31.4%	36.4%
Sensitivity	46.7%	47.7%	57.0%	50.0%
Both production and sensitivity	13.7%	17.9%	12.8%	11.4%
Total typable	68.7%	70.9%	75.6%	75.0%
Number of strains	182	134	86	132

Table XXIX

FREQUENCY OF COMMON PRODUCTION PATTERNS IN THE BACTERIAL COUNT GROUPS

Production patterns	Bacterial counts: organisms per millilitre urine					Total
	Primary	Recurrent	Total	10,000 to 100,000	Less than 10,000	
7	5	2	7	1	5	13
8	4	2	6	2	1	9
26	15	15	30	6	5	41
66	4	6	10	2	2	14
79	2	5	7	3	6	16
Total	30	30	60	14	19	93
Total number of typable strains	65	55	120	27	48	195

There is no significant difference in the frequency of each production pattern in the high and low bacterial count groups.

Pattern 7 (Chi square 1.4012)
8 (Chi square 2.888)
26 (Chi square 2.3909)
66 (Chi square 0.0802)
79 (Chi square 2.8392)

Table XXX

FREQUENCY OF COMMON SENSITIVITY PATTERNS IN THE BACTERIAL COUNT GROUPS

Sensitivity pattern	Bacterial counts: organisms per millilitre urine					Total
	More than 100,000			10,000 to 100,000	Less than 10,000	
	Primary	Recurrent	Total			
1	7	7	14	12	18	44
5	4	3	7	4	1	12
9	7	2	9	2	-	11
38	5	3	8	2	1	11
39	6	7	13	4	2	19
63	4	1	5	1	-	6
64	6	9	15	1	2	18
Total	39	32	71	26	24	121
Total number of typable strains	85	64	149	49	66	264

There is a significant difference in the frequency of sensitivity pattern "1" in the low bacterial count group and high count group. (Chi square 18.522, P more than 0.001.)

Table XXI

REPRODUCIBILITY OF THE STRAIN MARKERS

Bacterial count: Organisms per millilitre urine.	Number of Specimens Agreement in all five colonies			Total	Number of colonies. Agreement.			
	CP	CS	Ser		CP	CS	Ser	Total
More than 100,000								
No treatment	43	40	43	45	221	215	216	225
On treatment	17	13	15	18	89	78	79	90
10,000 to 100,000								
No treatment	2	2	2	2	10	10	10	10
On treatment	1	0	1	1	5	4	5	5
Less than 10,000								
No treatment	2	1	1	2	10	8	5	10
On treatment	2	1	2	2	10	8	10	10
Total	67	57	64	70	345	323	325	350

CP = Colicine production. CS = Colicine sensitivity.

Ser = Serology.

Table XXXII

FREQUENCY OF TYPING METHODS OF THE ESCHERICHIA COLI

Total isolates

Method of typing.	Bacterial count groups: Organisms per millilitre urine			
	More than 100,000	10,000 to 100,000	Less than 10,000	Total
Colicine production only	17	8	13	38
Colicine sensitivity only	50	24	22	96
Serology only	59	12	21	92
Production and sensitivity	26	4	10	40
Production and serology	54	8	18	80
Sensitivity and serology	50	14	31	95
Production, sensitivity and serology	23	7	5	35
Not typable	37	9	12	58
Total	316	86	132	534

Table XXXIII

FREQUENCY OF TYPING METHODS OF THE ESCHERICHIA COLI

Primary isolates from the patients with significant bacteriuria

Method of typing	Clinical presentation.			
	Symptomatic	Asymptomatic	Renal Lesion	Total
Colicine production only	8	1	-	9
Colicine sensitivity only	19	2	3	24
Serology only	31	2	5	38
Production and sensitivity	8	2	1	11
Production and serology	25	2	4	31
Sensitivity and serology	26	4	6	36
Production, sensitivity and serology	10	-	4	14
Not typable	13	1	5	19
Total	140	14	28	182

Table XXXIV

THE CORRELATION BETWEEN THE PREDOMINANT COLICINE PATTERNS AND THE SEROLOGICAL GROUPS

Serological group	Production pattern						Sensitivity pattern							
	7	8	26	66	79	Other	1	5	9	38	39	63	64	Other
	Number of strains						Number of strains							
01	1	1	2		5	12	6		2	1	1	1	1	9
02	2		2	1	1	5	2	1				1	2	4
04	1		5	1		7	4	1	1				2	12
05			1			1	2						4	4
06				1		8	2	2	1	1	1			15
07						2	1							1
08						1	2							3
09	1					1								3
011														2
018	1	2	4	1		3	1	2	1	1	1			1
039	1					2				1				1
075						5		1						1
Totals	7	3	14	4	6	47	20	7	5	4	3	2	9	56

There was no obvious correlation between the serological groups and the colicine patterns.

Table XXXV

CORRELATION BETWEEN SEROLOGICAL GROUPING

AND PYURIA IN PRIMARY SIGNIFICANT BACTERIURIA

	Pyuria	No pyuria	Total
01	16	1	17
02	8	1	9
04	17	3	20
05	4	0	4
06	18	4	22
07	4	0	4
08	0	0	0
09	1	0	1
011	2	1	3
018	1	1	2
039	2	0	2
075	7	1	8
Not typable	45	5	49

No significant difference, was noted, in the frequency of pyuria associated with those strains which are serologically groupable and those which were ungroupable. (Chi square 0.0321, P is between 0.8 and 0.7). There was no significant difference in any specific serological group.

Table XXXVI

CORRELATION BETWEEN COLICINE PRODUCTION AND PYURIA

Production pattern	Pyuria	No pyuria	Total
7	5	0	5
8	3	1	4
26	15	0	15
66	4	0	4
79	2	0	2
Other patterns	32	3	35
Non-colicinogenic strains	98	19	117
Total	159	23	182

The difference in the frequency of pyuria associated with the colicinogenic strains and that with the non-colicinogenic strains is not significant. (Chi square = 3.000, P is between 0.1 and 0.2).
There is no significant difference in the frequency of pyuria associated with the five common colicinogenic patterns and that with the remaining patterns. (Chi square = 1.524, P is between 0.3 and 0.2).

Table XXXVII

CORRELATION BETWEEN PYURIA AND COLICINE SENSITIVITY IN SIGNIFICANT BACTERIURIA

Colicine sensitivity pattern	Primary isolates		Total isolates	
	Pyuria	No pyuria	Pyuria	No pyuria
1	7	0	10	4
5	3	1	5	2
9	5	2	7	2
38	4	1	7	1
39	5	1	11	2
63	3	1	4	1
64	6	0	14	1
Other patterns	38	8	59	9
Insensitive	89	8	144	23
Total	160	22	261	55

There was no significant difference in the frequency of pyuria between the predominant patterns and the other patterns, or between the colicine sensitive and insensitive strains, in the primary and total isolates.

Table XXXVIII

THE INCIDENCE OF RECURRENT INFECTIONS AT THE VARIOUS TIME INTERVALS.

Time since previous infection	Change of strain	Strain identical	Total
One month or less	25	5	30
One to three months	35	11	46
Three to six months	21	-	21
Six to twelve months	5	1	6
More than twelve months	5	-	5
Total	91	17	108

Table XXXIX

THE VARIATION IN THE TYPING MARKERS IN THE RECURRENT INFECTIONS DUE TO DIFFERENT STRAINS.

Time since previous infection	Change of marker from previous specimen.			Total incidents
	Production	Sensitivity	Serology	
One month or less	12	18	15	25
One to three months	23	26	19	35
Three to six months	10	14	19	21
Six to twelve months	4	3	4	5
More than twelve months	2	4	4	5
Total	51	65	61	91

Table XL

THE VARIATIONS IN MARKERS OF THE ISOLATES FROM THE RECURRENT INFECTIONS.

Time since previous infection	Difference from previous isolate.							
	P	S	Ser	P/S	P/Ser	S/Ser	P/S/Ser	
One month or less	3	4	2	3	2	7	4	25
One to three months	1	5	3	10	5	4	7	35
Three to six months	2	5	2	1	3	4	4	21
Six to twelve months	1		1				3	5
More than twelve months					1	3	1	5
Total	7	14	8	14	11	18	19	91

P = Colicine production only.
S = Colicine sensitivity only.
Ser = Serology only.
P/S = Both colicine production and sensitivity.
P/Ser = Both colicine production and serology.
S/Ser = Both colicine sensitivity and serology.
P/S/Ser = Colicine production, sensitivity and serology.

COLICINE PRODUCTION

PATTERNS.

COLICINE PRODUCTION PATTERNS

Pattern type	2	56	17	2M	38	56/56	56/98	R1	R6	M19	2/7	2/64	2/15	R5	Coli Row.
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
3	+	+	+	+	+	+	+	+	+	+	+	+	+		
4	+	+	+	+	+	+	+	+	+	+		+	+	+	+
5	+	+	+	+	+	+	+	+		+	+	+	+	+	+
6	+	+	+	+	+			+	+		+	+	+	+	+
7	+	+	+	+	+			+	+			+	+	+	+
8	+	+	+	+	+			+	+			+	+		+
9	+	+	+		+	+	+	+	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+	+	+	+	+		+	

COLICINE PRODUCTION PATTERNS

Pattern type	2	56	17	2M	38	56/56	56/98	R1	R6	M19	2/7	2/64	2/15	R5	Coli Row.
11	+	+	+		+	+	+	+	+	+	+	+	+	+	
12	+	+	+		+	+	+	+	+	+	+	+	+		+
13	+	+	+		+	+	+	+	+	+	+	+		+	+
14	+	+	+		+	+	+	+	+	+		+	+	+	+
15	+	+	+		+	+	+	+	+	+		+	+	+	
16	+	+	+		+	+	+	+	+	+		+	+		+
17	+	+	+		+	+	+	+		+	+	+		+	+
18	+	+	+		+	+	+	+			+	+	+	+	+
19	+	+	+		+	+	+			+	+	+		+	+
20	+	+	+		+	+		+	+			+	+		+

COLICINE PRODUCTION PATTERNS

Pattern type	2	56	17	2M	38	56/56	56/98	R1	R6	M19	2/7	2/64	2/15	R5	Coli Row.
21	+	+	+	+	+		+	+	+	+	+	+			
22	+	+	+		+		+	+	+	+	+			+	+
23	+	+	+		+		+	+	+			+	+		+
24	+	+	+		+		+	+	+						+
25	+	+	+		+			+	+			+	+	+	+
26	+	+	+		+			+	+			+	+		+
27	+	+	+		+			+				+	+		+
28	+	+	+			+	+		+	+	+	+			+
29	+	+	+			+	+		+	+		+	+		+
30	+	+	+			+	+			+	+	+		+	+

COLICINE PRODUCTION PATTERNS

Pattern type	2	56	17	2M	38	56/56	56/98	R1	R6	M19	2/7	2/64	2/15	R5	Coli Row.
31	+	+	+			+	+			+	+	+			+
32	+	+						+		+		+			+
33	+	+							+				+		+
34	+	+				+	+	+	+	+	+	+	+	+	+
35	+	+				+	+	+	+	+	+	+	+		+
36	+	+				+	+	+	+	+	+	+			+
37	+	+				+	+		+	+	+	+			+
38	+	+				+		+	+	+	+	+			+
39	+	+				+		+				+	+		+
40	+	+						+	+	+	+				+

COLICINE PRODUCTION PATTERNS

Pattern type	2	56	17	2M	38	56/56	56/98	R1	R6	M19	2/7	2/64	2/15	R5	Coli Row.
41	+	+						+			+	+			+
42	+	+						+				+			+
43	+	+							+	+	+	+			+
44	+	+							+				+		+
45	+	+									+	+			+
46	+		+	+	+	+	+	+	+	+	+	+	+	+	
47	+		+		+	+	+	+			+		+	+	+
48	+		+		+	+	+			+			+	+	+
49		+	+	+	+								+	+	+
50	+		+		+			+	+			+	+		+

COLICINE PRODUCTION PATTERNS

Pattern type	2	56	17	2M	38	56/56	56/98	R1	R6	M19	2/7	2/64	2/15	R5	Coli Row.
51	+		+		+			+				+	+	+	+
52	+		+		+				+			+			+
53	+		+			+		+		+	+		+		
54	+							+				+	+		+
55	+									+					+
56	+											+			+
57	+												+	+	+
58	+												+		
59		+	+		+	+	+		+	+	+		+	+	
60		+	+		+			+	+			+	+		+

COLICINE PRODUCTION PATTERNS

Pattern type	2	56	17	2M	38	56/56	56/98	R1	R6	M19	2/7	2/64	2/15	R5	Coli Row.
61		+	+		+	+			+						+
62		+	+		+				+			+	+		+
63		+	+		+				+						+
64		+	+			+		+		+	+	+			+
65		+	+					+	+						+
66		+	+						+						+
67		+			+				+			+			+
68		+						+				+	+		
69		+							+				+	+	
70		+							+					+	

Pattern type	2	56	17	2M	38	56/56	56/98	R1	R6	M19	2/7	2/64	2/15	R5	Coli Row.
71		+													
72		+									+		+		+
73		+	+			+	+		+	+			+		+
74						+				+					+
75								+				+	+		+
76									+						
77												+			+
78													+		
79															+
80										+					
81	+							+					+		

COLICINE SENSITIVITY

PATTERNS.

COLICINE SENSITIVITY PATTERNS

Pattern type	146	208	252	264	01	07	29	234
1								+
2							+	
3						+		+
4						+	+	
5						+		
6					+			+
7				+		+		+
8				+		+	+	+
9				+				+
10			+					+
11			+	+	+	+		+
12			+		+	+		+
13			+	+		+		+
14			+	+				+
15			+	+		+		

Pattern type	146	208	252	264	01	07	29	234
16			+	+				
17		+	+	+	+	+	+	+
18		+	+		+	+	+	+
19		+	+	+		+	+	+
20		+		+		+	+	+
21		+				+	+	+
22		+	+	+			+	+
23		+		+			+	+
24		+					+	+
25		+		+	+	+		+
26		+	+	+		+		+
27		+				+		+
28		+	+	+	+			+
29		+		+	+			+
30		+	+	+				+

Pattern type	146	208	252	264	01	07	29	234
31		+		+				+
32		+						+
33		+					+	
34		+				+	+	
35		+	+	+		+		
36		+		+		+		
37		+		+	+			
38		+						
39	+	+	+	+	+	+	+	+
40	+	+		+	+	+	+	+
41	+	+			+	+	+	+
42	+	+	+	+		+	+	+
43	+	+		+		+	+	+
44	+			+		+	+	+
45	+	+	+	+	+		+	+

Pattern type	146	208	252	264	01	07	29	234
46	+	+	+	+			+	+
47	+	+					+	+
48	+	+	+	+	+	+		+
49	+			+	+	+		+
50	+		+		+	+		+
51	+	+			+	+		+
52	+		+	+		+		+
53	+					+		+
54	+		+	+	+			+
55	+		+		+			+
56	+	+	+	+				+
57	+		+	+				+
58	+			+				+
59	+							+
60	+	+	+	+	+	+	+	

Pattern type	146	208	252	264	01	07	29	234
61	+	+		+	+	+	+	
62	+	+	+	+		+	+	
63	+	+	+			+	+	
64	+	+				+	+	
65	+					+	+	
66	+	+					+	
67	+						+	
68	+	+	+	+		+		
69	+	+				+		
70	+					+		
71	+	+						
72	+							

REFERENCES.

REFERENCES.

ABBOTT, J.D. and SHANNON, R. (1958). J. clin. Path., 11, 71.

ALFOLDI, L., JACOB, F. and WOLLMAN, E.L. (1957). C.R. Acad. Sci. (Paris), 244, 2974.

ANDERSON, T.G. (1956). Penn. med. J., 59, 1475.

ANDERSON, B.R. and JACKSON, G.G. (1961). J. exp. Med., 114, 375.

BARNARD, R.M., STORY, R.D, and ROOT, H.F. (1953). New Engl. J. Med., 248, 136.

BEESON, P.B. (1955). Yale J. Biol. Med., 28, 81.

BEESON, P.B. (1958). Amer. J. Med., 1, 1.

BEESON, P.B. and ROWLEY, D. (1959). J. exp. Med., 110, 685.

BEN-GURION, R. and HERTMAN, I. (1958). J. gen. Microbiol., 19, 289.

BIENSTOCK. (1900). Ann. Inst. Pasteur, 14, 750.

BIENSTOCK. (1906). Ann. Inst. Pasteur, 20, 407.

BLACKFORD, V.L., PARR, L.W., and ROBBINS, M.L. (1951). Antibiot. and Chemother., 1, 392.

BORDET, P. (1948). C.R. Soc. Biol., 142, 257.

BORDET, P. and BEUMER, J. (1948). C.R. Soc. Biol., 142, 259.

BRADLEY, J.M. and LITTLE, P.J. (1963). Brit. med. J., 2, 361.

BRAUDE, A.I., SHAPIRO, A.P. and SIEMIENSKI, J. (1955). J. clin. Invest., 34, 1489.

BRAUDE, A.I., and SIEMIENSKI, J. (1960). J. Bact., 80, 171.

BROD, J. (1956). Lancet, 1, 973.

BRUMFITT, W., DAVIES, B.I. and ROSSEN, E.I. (1961). Lancet, 2, 1059.

BRUN, C. and RAASCHOU, F. (1961). Page 245, Ciba Foundation Symposium on Renal Biopsy, Eds, Wolstenholme, G.E.W., Cameron, M.P., Churchill, London.

CATTELL, W.R. and LEFFORD, M.J. (1963). Brit. med. J., 1, 97.

CHAPPLE, P.J. (1962). J. clin. Path., 15, 484.

CHRISTENSEN, W.B. (1946). J. Bact., 52, 461.

CLARKE, S.H.C. (1960). Brit. med. J., 2, 1491.

COBLENTZ, J.M., and LEVINE, M. (1947). J. Bact., 53, 455.

COLEMAN, P.N. and TAYLOR, S. (1949). J. clin. Path., 2, 134.

COOK, M.K., BLACKFORD, V., ROBBINS, M.L. and PARR, L.W. (1953). Antibiot. and Chemother. 3, 195.

DE NAVASQUEZ, S. (1950). J. Path. Bact., 62, 429.

DE NAVASQUEZ, S. (1956). J. Path. Bact., 71, 27.

DE NAVASQUEZ, S. (1958). Guy's Hosp. Rep. 107, 399.

DEPOUX, R. and CHABBERT, Y. (1953). Ann. Inst. Pasteur, 84, 798.

DESAUTELS, R.E. (1960). New Engl. J. Med., 263, 189.

DIFCO MANUAL. (1953). Ninth edition, Difco Laboratories Inc., Detroit, Michigan.

DUDGEON, L.S., WORDLEY, E. and BAWTREE, F. (1921). J. Hyg. (Cambridge), 20, 137.

DUDGEON, L.S., WORDLEY, E. and BAWTREE, F. (1922). J. Hyg. (Cambridge) 21, 168.

DUNN, P.M., HINE, L.C. and MACGREGOR, M.E. (1964). Brit. med. J., 1, 1081.

DUTTON, A.A.C. and RALSTON, M. (1957). Lancet, 1, 115.

EDWARDS, D. (1961). Proc. roy. Soc. Med., 54, 1096.

EDWARDS, P.R. and EWING, W.H. (1955). First edition, (1962, second edition), "Identification of Enterobacteriaceae", Burgers Pub. Co., Minnesota.

EIJKMAN, C. (1904). Zbl. Bakt., 1 Abt. Orig., 37, 436.

ENGEL, W.J. (1955). Med. clin. N. Amer., 39, part 2, 965.

EVERTSEN, H.W. (1946). Nyt Nordisk Forlag, Arnold Busck, Copenhagen, (with English summary.)

FORSYTHE, W.I. and WALLACE, I.R. (1958). Brit. J. Urol., 30, 297.

FREDERICQ, P. (1946a). C.R. Soc. Biol., 140, 1055.

FREDERICQ, P. (1946b). C.R. Soc. Biol., 140, 1033.

FREDERICQ, P. (1946c). C.R. Soc. Biol., 140, 1057.

FREDERICQ, P. (1948). Rev. belge Path., 19, Suppl. 4.

FREDERICQ, P. (1954). C.R. Soc. Biol., 148, 399.

FREDERICQ, P. (1955). C.R. Soc. Biol., 149, 2028.

FREDERICQ, P. (1956). C.R. Soc. Biol., 150, 1036.

FREDERICQ, P., THIBAUT, J. and GRATIA, A. (1946). C.R. Soc. Biol., 140, 1036.

FREDERICQ, P. and LEVINE, M. (1947). J. Bact., 54, 785.

FREDERICQ, P. and BETZ-BAREAU, M. (1952). Ann. Inst. Pasteur, 83, 283.

FREDERICQ, P. and BETZ-BAREAU, M. (1956). C.R. Soc. Biol., 150, 615.

FREDERICQ, P., BETZ-BAREAU, M. and NICOLLE, P. (1956). C.R. Soc. Biol., 150, 2039.

FREEDMAN, L.R. (1958). Conn. Med. J., 22, 705.

FREEDMAN, L.R. (1960). Page 433, "Biology of pyelonephritis", Eds. Quinn, E.L. and Kass, E.H., Churchill, London.

FROST, W.D. (1904). J. infect. Dis., 1, 599.

GARDNER, J.F. (1950). Brit. J. exp. Path., 31, 102.

GARRETT, R.A., RHAMY, R.K. and CARR, J.R. (1961). Trans. Amer. Ass. gen.-urin. Surg., 53, 120.

GARROD, L.P., SHOOTER, R.A. and CURWEN, M.P. (1954). Brit. med. J., 2, 1003.

GILLESPIE, W.A., LINTON, K.B., MILLER, A. and SLADE, N. (1960). J. clin. Path., 13, 187.

GILLESPIE, W.A., LENNON, G.G., LINTON, K.B. and SLADE, N. (1962). Brit. med. J., 2, 13.

GILLIES, R.R. (1964). J. Hyg., (Cambridge), 62, 1.

GOEBEL, W.F., BARRY, G.T., JESAITIS, M.A. and MILLER, E.M. (1955). Nature, 176, 700.

GOEBEL, W.F. and BARRY, G.T. (1958). J. exp. Med., 107, 185.

GORRILL, R.H. (1958). Guy's Hosp. Rep., 107, 405.

GORRILL, R.H. and DE NAVASQUEZ, S.J. (1964). J. Path. Bact., 87, 79.

GRATIA, A. (1925). C.R. Soc. Biol., 93, 1040.

GRATIA, A. (1932). Ann. Inst. Pasteur, 48, 413.

GRATIA, A. (1944). C.R. Soc. Biol., 138, 893.

GRATIA, A. and FREDERICQ, P. (1946). C.R. Soc. Biol., 140, 1032.

GRIMBERT, M. (1894). C.R. Soc. Biol., 46, 399.

GUELIN, A. (1943). Ann. Inst. Pasteur, 69, 382.

HALBERT, S.P. (1948). J. Immunol., 58, 153.

HALBERT, S.P. and MAGNUSSON, H.J. (1948). J. Immunol., 58, 397.

HALBERT, S.P. and GRAVATT, M. (1949). J. Immunol., 61, 271.

HEATLEY, N.G. and FLOREY, H.W. (1947). Brit. J. exp. Path., 27, 378.

HELMHOLTZ, H.F. (1950). J. Urol., 64, 158.

HEPINSTALL, R.H. and BRUMFITT, W. (1960). Brit. J. Exp. Path., 41, 381.

HINMAN, F. and HUTCH, J.A. (1962). J. Urol., 87, 230.

HODSON, C.J. and EDWARDS, D. (1960). Clin. Radiol., 11, 219.

HOEPRICH, P.D. (1960). J. Lab. clin. Med., 56, 899.

HOUGHTON, B.J. and PEARS, M.A. (1957). Brit. med. J., 1, 622.

HUET, M., PAPAVASSILIOU, J. and BONNEFOUS, S. (1961). Arch. Inst. Pasteur, (Tunis), 38, 109.

HUTT, M.S.R. and de WARDENER, H.E. (1961). Page 262, Ciba Foundation Symposium on Renal Biopsy, Eds., Wolstenholme, G.E.W. and Cameron, M.P., Churchill, London.

HUTTON, J.J. and GOEBEL, W.F. (1962). J. gen. Physiol., 45, 125.

HUVOS, A. and ROCHA, H. (1959). New Engl. J. Med., 261, 1213.

IKARI, N.S., ROBBINS, M.L. and PARR, L.W. (1958). Proc. Soc. exp. Biol., 98, 142.

IVANOVICS, G. and ALFOLDI, L. (1954). Nature, 174, 465.

JACKSON, G.G. (1960). Page 479, "Biology of Pyelonephritis", Eds. Quinn, E.L. and Kass, E.H., Churchill, London.

JACKSON, G.G., POIRIER, K.P. and GRIEBLE, H.G. (1957). Arch. intern. Med., 47, 6.

JACKSON, G.G., GRIEBLE, H.G. and KNUDSEN, K.B. (1958). J. Amer. med. Ass., 166, 14.

JACOB, F., LWOW, A., SIMINOVITCH, L. and WOLLMAN, E.L. (1953). Ann. Inst. Pasteur, 84, 222.

JACOB, F., SIMINOVITCH, L. and WOLLMAN, E.L. (1953). Ann. Inst. Pasteur, 84, 314.

JACOB, F., SCHAEFFER, P. and WOLLMAN, E.L. (1960). Tenth Symposium Soc. Gen. Microbiol., 10, 67.

KAITZ, A.L. and WILLIAMS, E.J. (1960). New Engl. J. Med., 262, 424.

KASS, E.H. (1955). Amer. J. Med., 18, 764.

KASS, E.H. (1956). Trans. Ass. Amer. Phycns. , 69, 56.

KASS, E.H. (1957). Arch. intern. Med., 100, 709.

KASS, E.H. (1960). Bact. Rev., 24, 177.

KASS, E.H. (1962). Ann. intern. Med., 56, 1.

KASS, E.H. and SCHNEIDERMAN, L.J. (1957). New Engl. J. Med., 256, 2.

KAUFFMANN, F. (1947). J. Immunol., 57, 71.

KAUFFMANN, F. (1954). "Enterobacteriaceae", Second edition, Munksgaard, Copenhagen.

KLEEMAN, C.R., HEWITT, W.L. and GUZE, L.B. (1960). Medicine, 39, 3.

KNIPSCHILDT, H.E. (1945). Nyt Nordisk Forlag, Arnold Busck, Copenhagen (with English summary).

LEISHMAN, A.W.D. (1939). Lancet, 2, 971.

LEVINE, M. and TANIMOTO, R.H. (1953). J. Bact., 67, 537.

LEWEK, T. (1889). Beitr. path. Anat., 6, 277.

LINTON, K.B. (1960). J. clin. Path., 13, 168.

LIPPMAN, R.W. (1957). "Urine and the Urinary Sediment", Second edition, Thomas, Springfield, Illinois.

LITTLE, P.J. (1962). Lancet, 1, 1149.

LOOPUYT, L. (1946a). Acta med. scand., 125, 245.

LOOPUYT, L. (1946b). Acta med. scand., 125, 357.

LUDFORD, C.G. and LEDERER, M. (1953). Aust. J. exp. Biol. med. Sci., 31, 553.

McCABE, W.R. and JACKSON, G.G. (1960). Page 39, "Biology of Pyelonephritis", Eds. Quinn, E.L. and Kass, E.H., Churchill, London.

McCREA, L.E. and ANDERSON, T. (1956). West Va. med. J., 52, 293.

MacDONALD, R.A., LEVITIN, H. MALLORY, G.K. and KASS, E.H. (1957). New Engl. J. Med., 256, 915.

MACKIE, J. and MCCARTNEY, J.E. (1960). "Handbook of Bacteriology", Ed. Cruickshank, R., Tenth edition, Livingston, Edinburgh.

McLEOD, J.W. (1958). Lancet, 1, 394.

McLEOD, J.W. and GOVENLOCK, P. (1921). Lancet, 1, 900.

MARPLE, C.D. (1941). Ann. intern. Med., 14, 2220.

MIDDLETON, J.E. (1957). Brit. med. J., 2, 497.

MILES, A.A., MISRA, S.S. and IRWIN, J.D. (1938). J. Hyg. (Cambridge), 38, 732.

MITCHELL, R.G. (1964). J. clin. Path., 17, 105.

MONZON, O.T., ORY, E.M., DORSON, H.L. CARTER, E. and YOW, E.M. (1958). New Engl. J. Med., 259, 16.

MORA, E.C. and EISENSTARK, A. (1958). Bacteriol. Proc., 81.

NISSLE, A. (1916). Dtsch. med. Wschr., 42, 1181.

OSEASOHN, R., QUILLIGAN, E.J., PERSKY, L. and ROSENBLUM, J.M. (1962). J. Lab. clin. Med., 60, 451.

O'SULLIVAN, J.D., FITZGERALD, M.G., MEYNELL, M.J. and MALINS, J.M. (1960). J. clin. Path., 13, 527.

OXOID MANUAL. (1961). Second edition, Oxoid Division of Oxo Ltd., London.

PAPAVASSILIOU, J. (1963). Pathologia et Microbiol., Basle, 26, 74.

PAPAVASSILIOU, J. and HUET, M. (1962). Arch. Inst. Pasteur, (Tunis), 39, 327.

PASTEUR, L. (1880). C.R. Acad. Sci., Paris, 90, 952.

PASTEUR, L. and JOUBERT, J.F. (1877). C.R. Acad. Sci., Paris, 85, 101.

PATTERSON, M.L., BARR, W. and MACDONALD, S. (1960). J. Obstet. Gynaec. Brit. Emp., 67, 394.

PEREIRA, A.T. (1962). J. clin. Path., 15, 252.

PINKERTON, J.H.M., WOOD, C., WILLIAMS, E.R. and CALMAN, R.M. (1961). Brit. med. J., 2, 539.

PRYLES, C.V. and STEN, N.L. (1959). Pediatrics, 23, 441.

PYRAH, L.N., GOLDIE, W., PARSONS, F.M. and RAPER, F.P. (1955). Lancet, 2, 314.

RANTZ, L.A. (1962). Arch. intern. Med., 109, 37.

RENGARTS, R.T. (1960). Amer. J. med. Sci., 239, 159.

REPORT. (1958). Int. Bull. bact. Nomenol., 8, 25.

RHOADS, P.S., BILLINGS, C.E. and O'CONOR, V.J. (1952). J. Amer. med. Ass., 148, 165.

RILEY, H.D. (1958). J. Lab. clin. Med., 52, 840.

ROBBINS, M.L., SOMER, A.M. and FARR, L.W. (1957). J. Bact., 74, 377.

ROBBINS, M.L., PARR, L.W. and HANN, W.D. (1958). Amer. J. Hyg. 68, 6.

ROCHA, H., GUZE, L.B., FREEDMAN, L.R. and BENSON, P.B. (1958). Yale J. Biol. Med., 30, 341.

ROLAND, F. and STUART, C.A. (1951). Antibiot. and Chemother., 1, 530.

SHAKMAN, R. and MESSENT, D. (1954). Brit. med. J., 2, 1009.

SHANNON, R. (1957). J. med.Lab. Technol., 14, 199.

SJÖSTEDT, S. (1946). Acta path. microbiol. scand., Supplement 63, 1.

SLADE, N. and LINTON, K.B. (1960). Brit. J. Urol., 32, 416.

STANSFELD, J.M. (1962). Arch. Dis. Childh., 37, 257.

STOCKER, B.A.D. (1960). Tenth Symposium Soc. Gen. Microbiol., 10, 1.

TALBOT, J.M., CUNLIFFE, A.C. and GOWER, N.D. (1957). J. clin. Path., 10, 222.

TALBOT, H.S. (1958). J. Amer. med. Ass., 168, 1595.

THIBAUT, J. and FREDERICQ, P. (1956). C.R. Soc. Biol., 150, 1512.

THOMSON, E.F. (1959). Med. J. Aust., 46, 330.

TURCK, M. and PETERSDORF, R.G. (1962). J. Urol., 88, 834.

TURNER, G.C. (1961). Lancet, 2, 1062.

UJVARY, G. (1958). Zbl. Bakt. (Orig), 170, 394.

VAHLNE, G. (1945). Acta path. microbiol. scand., Supplement 62, 1.

VIVALDI, E., COTRAN, R., ZANGWILL, D.P. and KASS, E.H. (1959). Proc. soc. exp. Biol. (N.Y.), 102, 242.

VOSTI, K.L., MONTO, A.S. and RANTZ, L.A. (1962). Clin. Res. Proc., 10, 113.

WHITBY, J.L. and MUIR, G.G. (1961). Brit. J. Urol., 33, 130.

WIELDING, S. (1945). Nature, 156, 204.

WILLIAMS, D.I. (1962). Postgrad. med. J., 38, 520.

WINSBURY-WHITE, H.P. (1933). Brit. J. Urol., 5, 249.