A STUDY OF ASYMPTOMATIC BACTERIURIA IN GENERAL PRACTICE

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INTRODUCTION

This study of certain aspects of asymptomatic bacteriuria was initiated by a growing awareness of the prevalence of hypertension in the middle-aged and elderly patients in the Practice, and, particularly, in female patients. Although a rise in blood-pressure is frequently an accompaniment of increasing age, and although in most cases of hypertension no renal cause can be demonstrated, chronic pyelonephritis can be accompanied by hypertension and it seemed that this was a condition whose developement might be prevented by the early recognition and treatment of urinary tract infection.

The study was planned to give an overall picture of the prevalence of symptomless bacteriuria by examining urine specimens from as many patients as was possible, and relating this to various factors such as age, parity, sex, presenting diagnosis, and a history of previous urinary tract infection. It was also decided to assess the value of a single course of treatment in erradicating a bacteriuria, and to follow-up the effects of such a course of treatment over a period of three months. It was hoped that, by this means, the size and extent of the problem of asymptomatic bacteriuria could be identified, and steps could be taken to try to prevent the future developement of chronic pyelonephritis in the younger patient.

In planning the study, a major factor was the difficulty in ensuring that urine specimens reached the hospital bacteriology department in a fresh condition. In this rural practice, specimens have to be sent to the nearest hospital by post, a distance of seventeen miles, and there is therefore a time-lag of at least twenty-four hours between the specimen being voided and being received at the laboratory, during which time it would assume the prevaling climatic temperature. As this time-lag could render bacteriological results inaccurate a method would have to be used whereby the identification of a positive or negative bacteriuria could be accurately done by the General Practitioner in his own surgery. At first, attempts were made to count bacteria microscopically after staining, but this method was soon abandoned as being too difficult, time-consuming

and inaccurate. The chemical methods of identifying bacteria in urine were considered in relation to their accuracy and ease of usage, and the method eventually chosen was Sleigh's modification of the Greiss-Ilosvay nitrite test.

The study occupied a period of six months and was divided into several stages. The first stage, which lasted a little over three months, involved obtaining a mid-stream urine specimen from all adult patients presenting at the Surgery, and these were tested with the modified nitrite test. The second stage involved re-testing the urine of all patients who had shown a true positive test, and also the re-testing of a similar number of patients whose urine had been negative, these latter patients being selected at random. The patients whose urine had now given two positive results were divided into two groups, one of which was given a course of treatment, the other being used as a control group. The third stage was to re-test urine samples from all patients who had originally shown a positive nitrite test, re-testing being done on three occasions at intervals of one month, commencing one month after the group treated had started their treatment.

THE FEATURES OF ACUTE AND CHRONIC URINARY TRACT INFECTIONS

Infections of the urinary tract are a common occurrence in General Practice, and the vast majority of such infections are diagnosed and treated by the General Practitioner without recourse to hospital investigations other than the culture and identification of the infecting organism. In the acute infection there is a tendency to put them into compartments labelled 'Cystitis' and 'Pyelonephritis' depending on the presenting symptoms, but this is dangerous and misleading as there is no easy and accurate method of differentiating between 'pure' cystitis and cystitis with renal involvement (Kass, E.H. 1955). It is therefore necessary to think of all infections in relation to the urinary tract as a whole, and to consider that in all cases, there may be involvement of the kidney.

The Incidence of Urinary Tract Infection.

No age group of the population is exempt from the possibility of contracting urinary infections, and evidence of infection has been found in 0.8% of the admissions to a general paediatric hospital, with a peak incidence between the ages of twelve and eighteen months (Campbell, M., 1951). In infancy both sexes tend to be equally affected, but from childhood onwards, the incidence becomes greater in females. Among schoolgirls, the number with symptomless infection has been found to be 1.2% (Kunin et al. 1964), and the incidence rises still further in adult life. Married women are more liable to develope urinary infections than are those who are unmarried, and this is thought to relate more to sexual intercourse than to the effects of pregnancy.

Much of the information on the prevalence of symptomless infection in the adult female comes from the many studies that have been made on patients attending ante-natal, post-natal and infertility clinics and, in these studies, the incidence has been found to lie between 3.5% and 11% with the majority at or about 7% (Kass, E.H. 1957; Turner, G.C. 1961 Boshell, B.R. et al. 1962; Monzon, O.T. et al. 1963; Sleigh, J.D. et al. 1964; Layton, R. 1964; Kincaid-Smith, P. 1964; Stuart, K.L. et al. 1965). In most of these studies further information has been sought on the effects of the age and parity of the patient on the incidence of symptomless infection, and on these matters opinions differ. Turner (1961) and Wilson (1966) state that there is no variation in incidence with

age, a finding confirmed by Williams et al. (1965) and Layton (1964), but the opposite view was taken by Kass (1956), Boshell et al. (1962) and Stuart et al. (1965). Turner (1961) also found that the incidence of asymptomatic infection did not increase with parity, a finding supported by Williams et al. (1965), but in three other studies the frequency of infection was found to be directly related to the parity of the patient (Boshell et al. 1962; Layton, R. 1964; Stuart, K.L. et al. 1965).

The incidence of asymptomatic bacteriuria was studied by Kass (1957) in several other groups of patients attending hospital out-patient clinics. Bacteriuria was found in 6% of women attending the medical out-patient department, and in 5% of males attending the same clinic. In diabetic patients, 18% of female and 5% of male out-patients showed a positive bacteriuria, and in in-patients at the hospital, an infected urine was found in 11% of pregnant patients coming to term, 23% of women in gynaecological wards for repair of cystocoele, 30% of women and 12% of men in the general medical wards, and 70% of men in the genito-urinary wards. In one final group of patients, those who had had an in-dwelling catheter for more than 96 hours, the incidence of infected urine was 98%.

Several studies have been made on the prevalence of acute urinary tract infections in General Practice. In three such studies the sole criterion of a diagnosis of infection was the presence of 100,000 organisms per millilitre of urine in patients presenting with symptoms of a urinary tract infection, and an incidence of 1.2% was reported by Fry et al. (1962) and Loudon and Greenhalgh (1962), and of 0.83% by Mond et al. (1965). Fry et al. (1962) noticed a high incidence of urinary infection in women between the ages of 20 and 30 years, and in males over the age of forty when prostatic obstruction could be an important predisposing factor. Loudon and Greenhalgh found their peak incidence in females between the ages of 20 and 49, but were unable to prove whether this was directly related to pregnancy. Mond et al. (1965) found that only 45% of those who presented with symptoms of a urinary tract infection gave a count of more than 100,000 organisms per millilitre in the urine, a figure which compares with that of 59% found in a comparable study by Gallagher et al. (1965). Mestitz et al. (1965) studied the results of urine bacterial counts from certain selected patients who presented to General Practitioners with symptoms referable to the urinary

tract. The patients in this study fell into one of three groups : patients with symptoms of urinary tract infection, patients with or without urinary symptoms who had been treated within the previous three months for proved or presumed urinary infection, and patients with an above average risk of having a symptomless bacteriuria, such as those with hypertension, diabetes, pregnancy, and repeated previous urinary infections. In this group of selected patients less than one-third of those with symptoms of infection such as dysuria and frequency gave a positive bacterial growth, 15% of male patients and 25% of female patients gave a positive urine culture, and there was a high incidence of asymptomatic bacteriuria in those patients with a history of repeated urinary infections.

Apart from the studies of hospital patients made by Kass (1957) there is no information of the prevalence of asymptomatic bacteriuria in the population as a whole, and the frequency of chronic pyelonephritis is likewise a matter for conjecture. A proportion of patients with this disease are diagnosed when the results of renal damage become manifest, but, in many, chronic pyelonephritis is first discovered, unexpectedly, at autopsy. In a series of unselected post-mortem studies, Macdonald et al. (1957) found that a total of 33% of the patients showed evidence of active or healed pyelonephritis, and stated that 'in many of those in which the diagnosis was not suspected, extensive pyelonephritis, sufficient to be a major cause of death, was found'.

Aetiology of Urinary Tract Infection

Infection can reach the kidney either by a haematogenous route or as an ascending infection via the urethra, bladder, and ureters, the latter route sometimes being associated with an element of obstruction to the free downward passage of urine.

In infants, the equality of the sex incidence favours the haematogenous route (Wilson, C. 1966), but as infants up to the age of 18 months are frequently encased in napkins, which may remain sodden with urine and faeces for hours at a time, the ascending route from the perianal area to the kidney may be a more likely mode of entry for the infecting organisms, (Murdoch, J.McC. 1963). Congenital malformations of the renal tract, such as mega-ureter, ureteral valves, bladder diverticulae, and urethral valves, lead to obstruction with consequent stasis of urine and infection. Defects of the ureteric-vesicular region may be associated

with reflux of urine from the bladder into the ureter during micturition, and this may lead eventually to chronic pyelonephritis. Once infection has reached the kidney and renal scarring has been produced, the area of scarring can become a focus for infection by a haematogenous route. Hodson and Wilson (1965), in a survey extending over eight years, studied the prevalence and progress of chronic pyelonephritic scarring radiographically, and showed that severe renal damage can occur in childhood, and can progress rapidly or remain quiescent until adult life.

In the absence of an anatomically abnormal urinary tract, three factors are of importance in causing infection in the adult female patient. The shortness of the urethra and its approximation to the infected anal region are important factors in the aetiology of renal tract infection. Thus a lowered degree of personal hygeine can be a contributary factor in the introduction of infection. After marriage, the act of sexual intercourse can facilitate the passage of organisms from the urethra to the bladder, and, if vesicular- ureteric reflux exists, thence to the kidneys (Loudon, I.S.L. and Greenhalgh, G.P. 1962; Murdoch, J.McC. 1963b; Gallagher et al. 1965). Pregnancy and child-birth form the next period of increased risk to the female patient. During pregnancy the compression of the ureteric-vesicular junction by the gravid uterus, and the hormonal induced uereteric dilatation which occurs, provide an element of obstruction which leads to stasis of urine and consequent infection. After child-birth, the trauma to the pelvic floor and the presence of an alkaline lochia increase the risk of infection gaining access to the urinary tract. At all ages, obstruction of the urinary tract may occur from renal, vesical, and ureteric calculi, from urethral strictures, and from neoplasms within or outwith the renal tract, all of which will lead to infection.

In the adult male patient the incidence of urinary infection is much less than in the female. Obstruction may occur from calculi and strictures, but the most common cause of infection is prostatic hypertrophy, with or without prostatitis. Studies of the incidence of acute urinary infection in General Practice show that male patients under the age of forty years are seldom seen on this account, and the numbers rise markedly in those who would be expected to have prostatic hypertrophy (Fry, J. et al. 1962; Loudon, I.S.L. and Greenhalgh G.P. 1962; Gallagher, D.J.A. et al. 1965).

In general, in the case of an ascending infection, chronic pyelonephritis may result from a single instance of an acute infection, or from the persistence of a sub-clinical infection, which, in its turn, may result from an inadequately treated acute infection, or from repeated low-grade re-infections.

Pathology of Pyelonephritis

The term 'chronic pyelonephritis', as Kleeman et al. (1960) suggest, represents a broad spectrum of renal disease, and includes both the granular contracted kidney and the more irregularly scarred kidney. One or both kidneys may be affected, and active infection may or may not be present. The surface of the organ may show broad depressed scars, and arteries may be prominant and show evidence of obliterative endarteritis. Microscopically, the glomeruli may be normal in non-affected areas, and those that are affected show a variety of lesions including capsular fibrosis and dilatation, ischaemic atrophy, and focal necrosis with capsular adhesions. The tubules show focal zones of atrophy or dilatation. This destructive process in the kidney, which may be symptomless, leads to a reduction in renal function and the patients may present with the complications of chronic pyelonephritis.

The list of organisms that have been found to be associated with urinary tract infection is long, but most studies agree that the commonest pathogens are E. Coli, B. Proteus, A. Aerogenes, Staph. Albus, and the Enterococcus, with the vast majority of infections being caused by Escherichia Coli. Pure cultures are most frequently found in the acute uncomplicated infection, while mixed cultures become more common in chronic infections complicated by structural abnormalities (Coleman, P.N., and Taylor S., 1949). The type of infecting organism varies also with previous instrumentation and anti-bacterial therapy, and while E. Coli is most frequently found in acute infections, the percentages of Staphylococci, Enterococci, and Proteus increase in chronic and complicated infections. (Kass, E.H. 1955).

The Symptoms of Urinary Tract Infection

In the acute infection the most usual features are of increased frequency of micturition with dysuria and urgency, suprapubic discomfort, renal angle pain, and systemic symptoms of fever, malaise and anorexia.

It must be stressed that only infrequently are all of these features present in any one case, and conversely, many of these symptoms may be present without an infecting organism being identifiable in the urine. The dangers of differentiating between cystitis and pyelonephritis has already been stressed, and all cases, however they present, must be considered to be infections of the whole urinary tract until proved to the contrary. In the infant and young child none of the above symptoms may be present and an acute infection may present as failure to thrive, vague ill-health, or with unexplained episodes of diarrhoea or fever.

As with acute urinary infection, chronic pyelonephritis may present with a variety of symptoms. The most common presenting features are persistent or occasional nocturia, vague bachache and loin pain, and malaise and lassitude with or without anaemia. Anaemia, if caused by renal disease is normochromic and unaffected by iron therapy, and is thought to be caused by depression of erythropoetic activity, but the precise mechanism of this depression is not known. The incidence of asymptomatic infection is higher in diabetic women than in non-diabetic controls (Kass, E.H. 1957), and the first sign of an exacerbation of the infection in such patients may be an unexpected deterioration in their diabetic control. In some patients chronic pyelonephritis may be completely symptomless, and its presence may only come to light after the investigation of repeated, acute, exacerbations of the infection.

The most serious consequencies of chronic pyelonephritis are renal failure and hypertension, and the disease may be symptomless until these complications become apparent. Hypertension does not invariably develop, but there appears to be a direct link between chronic pyelonephritis and malignant hypertension (Manfield, J.S. et al. 1943; Saphir, O. and Taylor B. 1952), and to the development in pregnancy of pre-eclampsia (Peters, J.P. et al. 1936; Finnerty, F.A. 1956; Stuart, K.L. et al. 1965). Owing to the preponderance of medullary and tubular damage, impairment of urine concentration and polyuria are more marked than is urea retention, but as the disease process in the kidney progresses, this feature becomes more important and leads to renal failure.

Diagnosis of Urinary Tract Infection

The diagnosis of both acute and chronic urinary tract infections

is made from the history and general examination, backed up by bacteriological, radiographic and pathological procedures. The work of Gallagher et al. (1965) showed that in many patients with symptoms of an infection, no organism was identified in the urine, and the bacteriological examination of the urine is an essential step in formulating the diagnosis. Once established, the extent of renal damage, and the presence of structural abnormalities in the renal tract can be assessed by radiographic procedures. Anatomical abnormalities in the urinary tract which have predisposed to infection can be identified by the straight X-Ray of the abdomen, by intravenous and retrograde pyelography, and by aortograms, and the presence of vesicular-uereteric reflux can be demonstrated by the micturating cystogram. Biochemical abnormalities in the blood are seldom of help unless the degree of renal damage is such that the blood urea is markedly raised, and the creatinine clearance only gives an estimate of the numbers of functioning glomeruli without indicating the cause of the damage. Renal biopsy has the limitation that, in the case of early chronic pyelonephritis, the needle may not transgress an affected area of the kidney, but it is of more value when performed in the course of the operation of nephropexy on an apparently normal kidney (Murdoch, J. McC. 1963).

Bacteriological examination of the urine is an essential step in the diagnosis of urinary tract infections, and the four constituents that are of importance are protein, pus cells, lymphocytes and bacteria. Proteinuria may be present in conditions other than pyelonephritis, and studies confirm that its presence or absence is a poor guide to the presence of infection. (MacDonald, R.A. et al. 1957; Mond, N.C. 1964; Williams, J.D. et al. 1965). Much work has been done in assessing the importance of pus cells and an increased lymphocyte count, but, although the presence of both encourages the diagnosis of infection, their absence does not mean that infection is absent (MacDonald, R.A. et al. 1957; Kass E.H. 1957; Loudon, I.S.L. and Greenhalgh, G.P. 1962; Mond, N.C. 1964; Layton, R. 1964). This leaves bacteriuria as the only accurate reflection of the presence of pyelonephritis, and it is now the accepted means by which urinary tract infection can be diagnosed from the examination of the urine.

The Prevention of Urinary Tract Infection

Many women believe that a 'chill on the bladder' is an unavoidable accompaniment of being female, and they therefore fail to seek medical advice unless their symptoms become severe. The importance of acute

urinary infections, not as isolated episodes but as part of a progressive renal disease, has only been appreciated by the Medical Profession in recent years, and much can be done to prevent the occurrence of chronic pyelonephritis.

A rise in the standard of personal hygeine, with improvement in social and domestic circumstances, will help to reduce the incidence of acute urinary infections, but much more can be done by the General Practitioner in preventing these acute infections from becoming chronic. The management of acute infections is difficult in that symptoms disappear fairly rapidly after the start of treatment, and always before the infecting organism has been erradicated. The course of treatment given, although of an appropriate antibiotic, may not be of sufficient length to erradicate the infection unless urine samples are investigated bacteriologically at the end of treatment, and, even if a sufficient course is prescribed, patients may fail to complete their treatment due to the early disappearance of their symptoms. These factors tend to encourage the persistence of a low-grade, subclinical infection, and a chronic pyelonephritis, which at this stage would be symptomless, may develop.

The management of the acute infection should therefore include the identification of the organism responsible, at the start of the illness, and an antibiotic to which the organism is sensitive should be given for a course of at least ten days. After the course of treatment, a week should be allowed to elapse before further urine samples are sent for bacteriological examination, and treatment should be continued until two consecutive urine samples are found to be sterile. Urine specimens should always be obtained by the clean-catch method, and, whenever possible, use of the catheter should be avoided. Kass (1957) showed that the likelihood of introducing infection with a single catheterisation was of the order of 2 to 4%, and this is a quite unwarranted risk. Further investigation is always required in cases of repeated urinary tract infection, and in those cases which fail to respond to treatment. Patients with an increased likelihood of harbouring urinary bacteria, such as diabetics and pregnant women, should be screened as often as possible, remembering that such an infection may be completely symptomless.

THE DETECTION OF BACTERIURIA

Before giving an account of the methods available for the detection of bacteriuria, it is necessary to consider the method by which the specimen is collected, and the level of the bacterial count that is to be considered as significant.

Collection of the Specimen

The dangers of the use of the catheter in obtaining urine specimens has been stressed and, except for a few studies which have used this method (Loudon, I.S.L. and Greenhalgh, G.P. 1962; Fry, J. et al. 1962; Gallagher, D.J.A. et al. 1965), the collection of a mid-stream urine from both men and women is the method of choice. Controversy exists as to whether cleansing of the vulvar area should preceed the collection of the specimen. A complicated and time-consuming proceedure was described by Boshell et al. (1962) in which the patient sat in a Sitz chair which had previously been filled with a green soap solution, and washed the labia, perineum, and inguinal regions carefully for about five minutes. The urethral orifice and surrounding area were then washed with benzalkonium chloride sponges either by the nurse or the patient, and the urine specimen was collected with the patient sitting on a comode. The labia were separated and two specimens were collected from the flow, the first of which was discarded and the second kept for examination and culture. Such methods are time-consuming for both patient and staff, and rely on considerable co-operation from the patient, and are therefore unsuitable when large numbers of urines have to be collected and tested. Mond et al. (1965), used mid-stream specimens after the vulva had been swabbed with a dilute solution of cetrimide and chlorhexidine, and in twelve other papers instructions have been given to patients to clean the vulvar area prior to passing the specimen (Wood, E.C. et al. 1960; Cattell, W.R. and Lefford, M.J. 1963; Murdoch, J.McC. 1963b; Monzon, O.T. et al. 1963; Dunn, P.M. et al. 1964; Mond, N.C. 1964; Editorial, B.M.J. 1964; Williams, J.D. et al. 1965; Kunin, C.M. 1965; Stuart, K.L. et al. 1965; Williams D.I. 1965; Seneca, H. 1966). Turner (1961) questioned the necessity for any prior cleansing of the vulvar area and, by comparing the bacteriological findings in urines from 200 patients who were prepared in the recognised manner with those from a similar number of patients who were not prepared, showed that

contamination was not significantly less in specimens from prepared patients. This was confirmed by Sleigh et al. (1964), but three other studies since that date have advised that cleansing with soap and water should be done prior to the collection of the sample (Layton, R. 1964; Kincaid-Smith, P. 1964; Sleigh, J.D. 1965). The impression gained from these various studies is that, while more elaborate procedures are unnecessary, it is advisable that simple washing of the vulva should precede the collection of the specimen.

Significant Bacteriuria

The concept of a level of bacteriuria above which it is significant of urinary tract infection, was initiated by Kass (1957) and has now become generally accepted. The reason for the development of this concept was the necessity to differentiate between true bacteriuria, or actual residence of bacteria within the renal tract, and contamination, or the adventitious entry of organisms into the urine during the collection of the specimen. Kass (1957) advanced the following observations in support of his concept;

1. Urine is an excellent culture medium for the common pathogens of the urinary tract, and if small numbers of bacteria are released from a focus of infection within the urinary tract, they will multiply rapidly to give high bacterial counts in the urine. Urine obtained from the renal pelvis in cases of infection have been found to contain less than 10,000 organisms per millilitre, but, in urine obtained at the same time from the bladder, a count of 100,000,000 organisms per millilitre has been found (Kass, E.H. 1955).

2. If bacterial counts are done on urine from patients with a clinical diagnosis of pyelonephritis, in 95% of cases a count of more than 100,000 organism per millilitre is obtained.

3. About 100,000 organisms per millilitre must be present in a urine sample before stained slides can be consistently read as positive in cases of symptomatic infection.

4. If patients exhibit such features as fever, loin pain, dysuria and pyuria, more than 100,000 organisms per millilitre of urine are almost always found.

Most authorities agree that a level of 100,000 organisms per millilitre of urine represents 'significant bacteriuria', and some go further in stating that levels of up to 10,000 organisms per milli-

litre represent contamination of the specimen, that in those cases where the count is between 10,000 and 100,000 further specimens should be tested, and that above the figure of 100,000 organisms per millilitre infection is proved (Monzon, O.T. et al. 1963; Bulger, R.J. and Kirby, W.M.M. 1963; Deutch, M. and Jespersen, H.G. 1964; Williams, J.D. et al. 1965). Kass (1955) gave several reasons why the bacterial count might fall below 100,000 organisms per millilitre, in the presence of active pyelonephritis:

1. A bacteriostatic agent is present in the urine.

2. If the rate of urine flow is high, small numbers of organisms released from an infective focus would not remain in the bladder long enough for multiplication to occur. To avoid this possibility, urine samples for bacteriological examination should be collected from the urine first passed after rising in the morning.

3. The rate of multiplication of the common urinary pathogens is reduced in an acid urine of pH below 5.5, and in an alkaline urine of pH above 8.5.

4. Unusual pathogens such as Group A Streptococci, some Streptococcus viridans strains, occasional enterococci and staphylococci, and anaerobic streptococci may grow poorly in urine, and low counts will be obtained from what is in effect an infected urine.

The Methods of Estimating the Bacterial Count

The numbers of bacteria present in the urine can be estimated by visual counting of the organisms, by counting the number of colonies obtained after incubation on a suitable medium, and by chemical methods.

In the acute infection, diagnosis from examinations of the urine seldom presents much difficulty, as the numerous pus cells and organisms are easily seen. In the stained slide of the centrifuged urine, a qualitative estimation of the degree of infection can be made by assessing the numbers of Gram-negative rods present. In the chronic infection, and in the investigation of asymptomatic bacteriuria, more sophisticated methods are required. A dried slide can be made from a known quantity of uncentrifuged urine, and is stained with Gram's stain. The organisms are counted by direct microscopy, and the numbers per millilitre of urine are thus estimated.

Quantitative bacterial counts can also be made after the causative organism is cultured on appropriate media. Tenfold

dilutions are made of the uncentrifuged urine, and 0.1 millilitre is spread on a well dried MacConkey agar plate. After incubation the bacteria present are counted by microscopic examination, and, by reference to the actual dilution used, an accurate bacterial count is made. A variation of this method is by use of the 'pour-plate' technique. Tenfold dilutions of the uncentrifuged urine are made, and a quantity of this, either 1.0 millilitre or 0.1 millilitre, is mixed with agar at a temperature of 46°C. This is incubated for twenty-four hours and a bacterial count is made.

The disadvantage of bacterial culture counts is that all bacteria, whether pathogenic or contaminant, are included in the count, and colony counts, by differentiating between the different types of bacteria, are therefore to be preferred. Colony counts are made after twenty-four to forty-eight hours incubation of a known quantity of diluted urine on suitable media, such as blood agar, MacConkey agar, and thioglycolate broth (Cruickshank, R., 1965; Monzon, O.T. et al. 1963). Using 0.01 millilitres of urine and overnight incubation at 37°C. on blood agar and MacConkey plates, a colony count of 1,000 represents a bacterial count of 100,000 organisms per millilitre (Turner, G.C. 1961). A modification of the culture methods is to use a loop of standard diameter to plate out uncentrifuged urine onto solid media, the plate being read after overnight incubation, in accordance with a pre-arranged scheme (Cruickshank, R., 1965).

These methods are the most accurate available by which the numbers of bacteria in urine can be counted, but they have the disadvantage that they are time-consuming, they use considerable amounts of laboratory materials, and they require experienced technicians both to set up and to read the culture plates. They are ideally suited when only a small number of urines have to be tested, but they become impractical if large numbers of urines have to be screened for the presence of significant bacteriuria. In recent years attention has been focused on various chemical methods which have the advantage that they are relatively simple to set up and read, but are on the whole less accurate in their results, and much work has been done to perfect these methods so that they might compare favourably with the accuracy of quantitative bacteriological proceedures.

The basis of all chemical methods of detecting bacteriuria is that certain groups of organisms possess the ability to reduce one chemical substance to another, and the presence of the altered chemical can be shown by a colour change on the addition of certain reagents. The three chemical tests which will be considered are the Potassium-Iodide starch test, the Greiss-Ilosvay test, and the triphenyl tetrazolium chloride test. In the case of the first two tests, the chemical reduction is from a nitrate to a nitrite, and the ability to effect this reduction is possessed by all Gram negative bacilli belonging to the genera Escherichia, Klebsiella, Citrobacter, Proteus, Salmonella and Shigella, Pseudomonas aeruginosa, Staphylococcus aureus and albus, and by the Providencia group of paracolons (Sleigh, J.D. 1965). In the triphenyl tetrazolium chloride test, actively respiring bacteria are able to reduce 2,3,5, triphenyl tetrazolium chloride to red insoluble triphenyl formazan (Simmons, N.A. and Williams, J.D. 1962).

The Potassium-Iodide Starch Test

This test is mainly of historical interest, and is not in general use. A few drops of 10 per cent. Potassium iodide solution and 5 millilitres of dilute boiled starch solution are added to the urine to be tested, and a few drops of dilute sulphuric acid added. In the presence of nitrite, iodine is liberated, and combines with the starch to give a blue compound (Cruickshank, J. and Moyes, J.M. 1914).

The Greiss-Ilosvay Test.

The first description of the connection between the presence of nitrites in the urine and infection, and of the use of the Greiss-Ilosvay test to detect nitrite, was given by Cruickshank and Moyes (1914). During an investigation of urines from hospital in-patients, it was discovered that the occurrence of nitrituria was not uncommon, and it was decided to investigate this finding in some detail. Some 600 urine samples were examined, 150 specimens from healthy subjects and the remainder from patients with 'mental and other diseases'. In the control group, only two were found to contain nitrites, and subsequent examinations showed that these were caused by contamination. In the other group of 350 patients, nitrites were found in about 12%, a larger number of positives being obtained

from female subjects than from male, and there seemed to be no connection between the presence of nitrituria and the patients' illness, diets, or the medicines they were taking. When the positive urines were examined bacteriologically, all were found to contain Gram-negative bacilli in varying numbers, and, although no organism common to all cases was found, it was concluded that the presence of organisms was responsible for the finding of nitrites in the urine. In support of this conclusion samples of fresh nitrite-free urine were sterilised, and incubated after innoculation with a series of organisms including those that had been isolated from the nitrite containing urines. It was found that the urinary organisms were much more active in the production of nitrites than were the other control organisms. The paper written on these investigations concluded that 'the most common cause of nitrituria is an associated urinary infection, and the presence of bacilluria should be sought for in all cases' (Cruickshank, J. and Moyes, J.M. 1914).

The details of the Greiss-Ilosvay Test are as follows. Two solutions are made up, one containing 1.25 Gms. of Sulphonilic acid in 500 millilitres of 30% Acetic acid, and the other containing 2.5 Gms. of alpha-naphthylamine in a similar quantity of 30% Acetic acid. The two solutions are kept separate until just before use as the mixture tends to absorb nitrites rapidly from the air. When testing urine for the presence of nitrites, an equal quantity of each solution is mixed together, and the test is read as positive if a red or pink colour developes on adding 1.0 ml. of the resulting mixture to 1.0 ml. of urine. This reaction is highly sensitive, and results in the formation of azo-alpha-aminonaphthylamine-parabenzine-sulphonic acid, which produces the red colouration. The reaction is unaffected by moderate amounts of protein, and the pH of the urine is unimportant as an excess of acid is added with the Greiss reagents. Cloudiness of the urine, if caused by the presence of phosphates, disappears on the addition of the strongly acid reagent, and decolourisation of the urine before reading the test is not usually necessary (Schaus, R. 1956).

As the figure of 100,000 organisms per millilitre of urine is accepted as being the borderline between significant and non-significant bacteriuria, the value of any chemical test in screening patients

for the presence of asymptomatic bacteriuria rests on its being able to approach in accuracy the quantitative bacteriological methods which it is attempting to replace. In published reports, the ability of the nitrite test to detect significant bacteriuria has varied considerably, and various modifications have been proposed to increase the accuracy of the test. Schaus (1956) strongly advocated the routine use of the nitrite test because of 'its simplicity and reliability', but gave no figures in support of the latter attribute. The possible causes of a negative nitrite reaction, in addition to the more obvious causes of a sterile urine and a urine infected with organisms which lacked the ability to reduce nitrate, were discussed by Schaus (1956) and the following reasons were given. The urine may contain nitrate reducing bacteria, but one of the following conditions may exist:

1. The reagent may have deteriorated by being formed from its two constituent solutions some days before the test is done.

2. Although most individuals excrete nitrates in their urine, instances occur when no nitrate is present to be reduced by the bacteria.

3. The patient's micturition may be so frequent as to allow insufficient time for reduction of the nitrate to occur.

4. Conversely, in cases of urinary retention, nitrate may be reduced, and the nitrite so formed may be further decomposed by the bacteria before the test is performed.

Schaus (1956) proposed that these causes of a false negative could be eliminated by the addition of a few drops of a 10% solution of sodium nitrate to the voided urine, which should be allowed to stand undisturbed at a temperature of 37°C. for two hours before testing.

The quantitative aspects of the Greiss Nitrite reaction were studied by Kahler and Guze (1957) using strains of E. Coli, A. Aerogenes, B. Proteus and Paracolon isolated from patients with proved pyelonephritis, and testing the time necessary for the bacteria to reduce the nitrate. Their results showed that, although the test was positive when bacterial counts of more than 1,000,000 per millilitre were present, lower concentrations required a much longer time to produce detectable nitrite. They considered that counts of 10,000 or more organisms per millilitre were significant of infection, and at this level, the nitrite test was negative even in the presence of

an excess of nitrate. A large clinical study of infective urines by Bechgaard and Jansen was mentioned in which the nitrite test was positive in only 69% of cases, and it was concluded that the test was too unreliable for routine use.

Smith et al. (1961) stated that a positive Greiss test almost invariably indicated significant bacteriuria, but in their series, 50% of the urines with a bacterial count of more than 100,000 per millilitre gave a negative nitrite test. The addition of nitrate to the urine and incubation of the specimen at room temperature for one hour was found to increase the number of positive tests by 25%, and it was suggested that the accuracy might be further improved if the urine specimen was concentrated ten to twenty times before testing. Sleigh et al. (1964) found that the test was positive in only 40% of urines with more than 100,000 organisms per millilitre, but the chance of a single test being positive was increased when the bacterial count was still higher. They considered that the test was of more value if repeated specimens from the same patient were tested, and attributed this to certain variable factors such as the amount of nitrate present, and the length of time the urine had been in the bladder before being voided. In a series by Deutch and Jespersen (1963) the nitrite test detected only 60% of urines with significant bacteria, and, again, it was noticed that the accuracy of the test improved with higher bacterial counts. The nitrite test, as modified by Smith et al. (1961), was used in a survey of the frequency of asymptomatic bacteriuria in pregnancy, but as it was accurate in only 50% of cases, it was discarded as a reliable screening method (Kincaid-Smith, P. et al. 1964). Only two studies have so far credited the nitrite test with greater accuracy. In a survey of asymptomatic infection among pregnant women, Turner (1961) found that the nitrite test identified 80% of infective urines, and that there were no recorded instances of false positive results. Using a further modification of the Greiss test, Sleigh (1965) found an accuracy of 97% with an incidence of false-positives of less than 2%.

The modification of the nitrite test, as described by Sleigh (1965), involved both the addition of substrate and the incubation of the urine before testing. Substrate, in a quantity of 0.04 millilitre of a 5% solution of potassium nitrate in distilled water, was added to

1.0 millilitre of urine which was then incubated in a water-bath at a temperature of 37°C. for four hours, before testing in the usual way. Sleigh evaluated this modification in a study of over 1,500 urines obtained from women attending ante-natal and infertility clinics, and compared the results with those obtained with the triphenyl tetrazolium chloride test, and his results indicate that this modification provides the most accurate nitrite test available.

The Triphenyl Tetrazolium Chloride Test

The triphenyl tetrazolium chloride (T.T.C.) test was described and tested by Simmons and Williams (1962) in an attempt to find a chemical method which was more accurate than the Greiss test in the identification of significant bacteriuria. The reduction of T.T.C. takes place in an alkaline pH, and the reagent was therefore made by dissolving 750 mgs. of T.T.C. in 100 millilitres of a saturated solution of disodium hydrogen phosphate. A working solution was prepared by taking four millilitres of this solution and diluting them to 100 millilites with saturated disodium hydrogen phosphate. Both the stock and working solutions were sterilised by Seitz filtration and stored in the dark, and the stability of the stock and working solutions was of the order of two months and two weeks respectively. The test was performed by adding 0.5 millilitre of the working solution to 2.0 millilitres of urine in a clean test-tube, and incubating for four hours at a temperature of 37°C. A positive result was seen by the appearance of a red precipitate, and a negative result by the absence of it. The effect of the presence of various common abnormal constituents of urine, such as red cells, bilirubin, glucose, and ketones was assessed in relation to the interpretation of the result. Red Cells can form a deposit similar to that of a positive test, and when they were present in excess after the period of incubation, the deposit was removed and examined. On microscopic examination, the reduced triphenyl formazan was easily seen and the red cells were no longer seen. Urine containing bilirubin, which was thus highly coloured, was centrifuged after incubation, and the result of the test was apparent when the urine was replaced by water. It was found that the presence of the other abnormal constituents mentioned above had no effect on the accuracy of the test, and further, that urine specimens which had been refrigerated overnight gave as accurate

results as did fresh samples.

The organisms which have the ability to give a positive T.T.C. result are of the Gram-negative group, and the list of bacteria corresponds to those organisms most commonly found in urinary tract infections, and to those which have the ability to reduce nitrates to nitrites. This test was designed to give a positive result in cases where there was a concentration of 100,000 or more organisms per millilitre, and it was stated that its accuracy was of the order of 94%, with a 2% incidence of false positives (Simmons, N.A. and Williams, J.D. 1962).

Results from other surveys of the accuracy of this test have, however, not fulfilled the belief that the T.T.C. test could rival bacteriological methods. Steers and Jackson (1963) reported that, in their series of 112 specimens of urine examined by the T.T.C. test and by quantitative bacteriological methods, the T.T.C. test identified only 60% of urines containing organisms of more than 100,000 per millilitre, and that, therefore, there was an incidence of false negatives of 40%. Williams and Simmons (1963)supported their test with the observation that in a series of more than 4000 urine specimens, the T.T.C. test identified 96% of those with significant bacteriuria. Two observations were made on technical aspects of the test: in the preparation of the reagent, it was necessary to Seitz filter the solutions immediately they were made, otherwise auto-reduction may occur, and in the reading of the test result, a concave mirror should be used to assist in spotting the slight red deposit obtained in marginally positive results. Evaluation of the T.T.C. test by its comparison with bacterial counts was made by Chard and Cole (1963) in a series of 400 urine specimens, and an accuracy of 97% was reported, but an accuracy of only 75% was claimed by Bulger and Kirby (1963), who thought that a combination of the Gram stain of urinary sediment with microscopic examination of a single drop of unspun urine afforded a more accurate test. In this last mentioned study, the use of the concave mirror in reading the result was not thought to increase the accuracy, and mention was made of the technical difficulties in the making and storing of the solutions.

Three further studies have been made of the comparison in

accuracy between the T.T.C. test and quantitative bacteriological methods. Guze and Kalmanson (1963) found the test accurate in only 65% of cases, and, although they were unable to suggest the reasons for the wide variations previously reported, concluded that the test was not a reliable screening method. The opposite view was taken by Deutch and Jespersen (1964), who found the T.T.C. test positive in 93% of cases with significant bacteriuria, and if it were to be combined with qualitative bacteriological examination the accuracy rose to to 97%. In a screening programme designed to detect bacteriuria in pregnant women, Williams et al. (1965) used the T.T.C. test and found it to be accurate in 87% of cases, the results being checked by quantitative bacterial counts.

The causes of these wide fluctuations in results may be related to several factors. The individual batches of T.T.C. reagent may vary in quality, and the red precipitate formed in a positive test may be so slight as to be unobserved by a less experienced eye. As the reaction will only take place in an alkaline pH, a more stable buffer may be required to maintain this pH during the period of incubation (Lancet, 1964).

From the accounts written above of the various methods available to detect significant bacteriuria, the choice appears to rest between quantitative bacteriological methods on the one hand, and either the T.T.C. test or Sleigh's modification of the nitrite test on the other. Quantitative bacteriological methods because of the time involved in doing them, are unsuitable for use as a screening method for large numbers of patients, although they are unquestionably the most accurate methods available. The T.T.C. test has the practical disadvantages that the solution requires careful handling in preparation and in use, and the interpretation of the result is sometimes difficult, but more important is the fact that so many varying degrees of accuracy have been attributed to it. Sleigh's modified Greiss nitrite test is simple to perform and easy to read, and although only one report of its use has as yet been made, it appears to be accurate to a high degree.

Several factors governed the selection of the method to be used in this study. Accurate results could have been obtained by sending all urine specimens to the hospital bacteriology department for bacterial counts and culture, but this would have placed an unjustifiably high load on a department not equipped in facilities and man-power to deal with it. It would also have meant that some means would have to be found to transport the urine samples to the hospital in a refrigerated state. Elliot and Sleigh (1963) described the use of refrigerated containers for the transport of urine samples at a temperature of 10°C., but this possible method was rejected in view of the cost of the flasks, and the difficulty in transporting them to a laboratory eighteen miles away. Sleigh's modification of the nitrite test seemed to be the ideal method for use by the General Practitioner, in that the initial screening of the urines could be done in the surgery with the minimum interference with the normal day's work. Urine samples could be incubated within one hour of being voided, and the transport of the few positive urines to the hospital laboratory did not pose the same problem as it would have done in larger quantities. The fact that the initial screening of the urines was being done in the surgery gave a considerable added interest to the study, and showed that this particular aspect of preventive medicine could be studied by a General Practitioner without excessive recourse to hospital facilities.

PART 2

A DETAILED DESCRIPTION OF THE STUDY

A DETAILED DESCRIPTION OF THE STUDY

The preparation for this study started in March 1965, with the practical work being started in June of that year and finishing in January, 1966.

The Organisation of the Practice.

The Practice is centred on a small town of approximately 1,600 inhabitants, and extends to twenty-five miles north, five miles east, ten miles south, and twenty-five miles west of this town. The organisation of the Practice is that of two single-handed Practices working in a loose alliance, as each doctor has his own list of patients and consults on three days of each week, the other doctor consulting on the alternate days. There is only one Surgery in the Practice, two outlying village areas being visited each week, the calls for the area being collected from a house in the village. The exception to this arrangement is an Eventide Home, in which a 'Surgery' is held each week, and which was included as a Surgery for the purposes of this study. This study is the work of one of these two doctors, whose list of patients numbers 1,217, this figure being the total number of patients in both the town and the surrounding country area.

For the purposes of this study, the Practice was divided into two parts, those patients living in the town and within one mile of the outskirts, and those living beyond this limit. As one of the main features of this study was that urine samples could be collected and incubated within a short time of being voided, only patients living in the town and within one mile of it were included, it being impossible to collect urines from patients living further away. As this study was concerned with the

overall picture of asymptomatic urinary infection in adult patients, it was decided to fix an arbitary age of twenty years as the lower age limit of 'adult' patients. From a study of the N.H.S. medical record cards, an accurate breakdown of the age incidence of the whole Practice has been made, and this gives a total of 933 patients in the town and immediate surrounding area, and 284 patients who lived beyond this limit. A tabulation of the Practice population in age decades is given in Figures 1 and 2, below.

	Under 20	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90+
Male	140	53	41	52	61	42	25	11	2
Female	138	64	60	57	71	58	35	18	5

Figure 1

(Patients in town and immediate area).

	Under 20	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90+
Male	40	8	22	27	26	14	8	2	1
Female	37	12	21	18	22	15	7	4	-

Figure 2

(Patients living outwith pre-defined area).

In the area from which patients would be included in the study, the Practice population totalled 933 patients, of whom 427 were male and 506 were female. As only those over the age of twenty years were to be included in the study, the total possible number of patients available was less than the figure of 933

quoted above. As will be seen from Figures 1 and 2, the total number of male and female patients available to the study was 287 and 368 respectively.

The Organisation of the Study.

It was decided that all patients over the age of twenty years who presented at the Surgery and who lived within the pre-determined geographical limits would be included in the study, and only those in whom a diagnosis an acute urinary tract infection was made on clinical grounds, would be excluded. It was necessary to define the symptoms and signs that would justify this clinical diagnosis being made, and it was decided that if dysuria and frequency, or frequency and renal angle tenderness, were present and complained of by the patient, a diagnosis of urinary tract infection would be made, and the patient excluded from the study.

After their consultation, the patients were asked if they would co-operate in a study being done on the incidence of asymptomatic urinary tract infection, and all patients thus approached agreed to do so. A special study record card was then completed for each patient (Figures 3 and 4), giving details of name, address, and age, previous medical and renal history, presenting diagnosis, blood pressure, and, in the case of female patients, parity. The patients were given a pre-sterilised Universal Container with a card of instructions telling them how to obtain a mid-stream urine sample (Figure 5) and they were asked to pass the urine sample at or about 8 a.m. on the following day, from the first urine passed after rising. They were told that the sample would be collectdd from their home before 9 a.m. on the same day.

Several headings on the patients record cards require

explanation. In the case of female patients, parity was included as it was hoped that the incidence of bacteriuria could be related to the parity of the patient and the results compared with those obtained in other studies. For the same reason, details of the patient's previous medical and renal history were included in order to relate the incidence of bacteriuria to a history of previous urinary tract infection, and particular care was taken to note the number of times a patient had had symptoms of infection and whether or not treatment had been given on each occasion. In all instances the diagnosis of the patient's presenting complaints was noted both to ensure that those in whom a diagnosis of urinary tract infection was made were not included, and to see whether, in retrospect, patients with positive urines had presented with vague symptoms which might after all have been referable to the urinary tract. It must be stressed that not in all cases did a patient's consultation result in a firm diagnosis. In this practice, all ante-natal, child welfare and innoculation clinics are conducted by the doctor, and many patients, particularly young mothers, came to the Surgery for advice or reassurance rather than with an organic disease.

Three readings of the blood pressure were taken and an average result noted. At the onset of the study it was hoped to be able to relate the presence of bacteriuria to a raised blood pressure, but as it would be too difficult to standardise the blood pressure readings obtained with relation to the patient's weight, thickness of arm, age, and after obtaining true resting conditions, this idea was abandoned.

The collecting bottle used was a pre-sterilised plastic container, one inch in diameter by two and a half inches deep, and it was labelled beforehand with the patient's name, address, and the date of collection. As Surgeries were held on three days of each week, namely

on Monday, Wednesday, and Friday, urine samples were collected on the mornings of Tuesday, Thursday, and Saturday. Three separate surgeries were held on each surgery day, and, as there were usually between ten and fifteen patients included in the study from each day, the collection of the samples from the patients' homes was usually completed before 9 a.m. The delay which inevitably resulted between the time the urine was voided and the time at which it was brought to the surgery was thus never more than one and a half hours.

After the urine specimens had been collected they were taken to the surgery. One millilitre of each urine was put into a cleaned test-tube, and 0.04 millilitres of a 5% solution of potassium nitrate was added by means of an 0.02 millilitre pipette. The urines with the added substrate were then incubated for four hours at a temperature of 37°C. in an incubator manufactured by Charles Heatson and CO. Ltd. in which a steady temperature was maintained by a capsule. Immediately after the one millilitre had been withdrawn from the urine sample bottle, the bottles were sealed with elastoplast tape and stored in a domestic refrigerator at a temperature of 4°C. After the four hour incubation period, the nitrite reagent was prepared by mixing together equal quantities of the two Greiss-Ilosvay solutions, and one millilitre of this prepared solution was added to each test-tube containing one millilitre of urine with added substrate. The result of the test was determined by examining each test-tube against a white background in a good light, and the test was noted as positive if a pink or red colour was produced in the urine immediately after the nitrite reagent was added. The patient's study record card was then marked with this result.

FEMALE

NAME AGE DATE

ADDRESS PARITY

PREVIOUS MEDICAL HISTORY

PREVIOUS RENAL HISTORY

PRESENTING DIAGNOSIS

	1	2	3	Average
B.P.				
NITRITE TEST				
DATE OF TEST				

Fig. 3

MALE

NAME AGE DATE

ADDRESS

PREVIOUS MEDICAL HISTORY

PREVIOUS RENAL HISTORY

PRESENTING DIAGNOSIS

	1	2	3	Average
B.P.				
NITRITE TEST				
DATE OF TEST				

Fig. 4

MID-STREAM SPECIMEN OF URINE

The object is to obtain a fresh, clean and uncontaminated specimen of your urine.

1) After a bath or throrough wash of the front passage, dry yourself well with a clean towel.

2) Remove the cap of the sterile container. Put it down rim upwards and be careful not to touch the inside of the bottle or the cap. Take the bottle in one hand.

3) Separate the lips (labia) of the front passage with the fingers of the other hand.

4) Start passing urine, allowing some to fall into the pan, then pass the container into the flow filling it if possible.

5) Replace the cap carefully, dry the outside of the container and label it with your name and date.

Stale specimens are useless. The specimen should be collected not more than two hours before bringing it to the surgery or laboratory.

Fig. 5

All urine specimens which showed a positive nitrite result were sent to the nearest hospital bacteriological department for further investigation, and despite the fact that the laboratory was seventeen miles away, this was managed with the minimum of delay. Occasionally urines were delivered personally or by ambulance on the afternoon of the day of collection, and when this was not possible, as was more frequently the case, a different method was used. The sample bottles were kept in the refrigerator from the time incubation started until the following morning, when they were collected by one of the laboratory technicians who lived in the town, and taken to the hospital, a journey lasting about thirty minutes. They were thus only exposed to climatic conditions for a period of up to one and a half hours before refrigeration, and for about forty-five minutes after refrigeration before being examined in the laboratory.

Ideally, a bacterial count should have been done on each positive urine, but as the laboratory was not able to devote time and manpower to this, a modified form of examination was done. The urines were examined microscopically for the presence of pus cells, red blood corpuscles, and lymphocytes, and a culture was made of the uncentrifuged specimen. Particular note was made of the presence or absence of contamination in the culture, the dominant organism or organisms, and their sensitivity to various antibiotics. A report was sent by the laboratory of each specimen submitted with details of the results of these examinations, and these reports were attached to the patients' study record cards. The laboratory was fully informed of the aims and purpose of this study, and as they were unable to do bacterial counts on the specimens, it was agreed that an organism would only be reported following culture, if it had been obtained as a pure growth. The modified Greiss-Ilosvay test, as described by Sleigh (1965), was evolved so that it would become positive at a bacterial count of 100,000 organisms per millilitre or above, and in a large series of urine specimens, Sleigh found that it identified 97% of urines containing this level of bacterial count. In this study, a pure culture from a chemically positive urine was taken to represent a bacterial count of 100,000 organisms per millilitre or above, a pure culture from a chemically negative urine represented a count of below 100,000 organisms per millilitre, and the absence of a pure culture from a urine which had showed a positive nitrite reaction was considered a false positive.

The study can be subdivided into three stages. Stage 1 involved

the screening of as many of the available practice population as possible, and, by the end of three and a half months, 375 patients had been included in the study. At this stage, so very few new patients were being seen in the surgery that it was decided to conclude the initial screening at that point. During this stage all positive urines were forwarded for bacteriological examination as described above, and, in addition, 70 urines which had given a negative nitrite reaction were also sent to the laboratory. This last was done both to check the bacteriology of a series of unselected negative urines, and to see whether any variation of the culture or nitrite reaction occurred when they were retested at a later date.

Stage 2 started immediately after the conclusion of the initial screening programme, and involved the re-testing of two groups of patients. The first group consisted of 87 patients whose initial nitrites reaction had been positive, and in whom a pure culture had been obtained, and of these 87 patients, only 77 were available or willing to have further urine samples tested. The collection of the urine samples occupied about two weeks, and a time interval, which varied between three and twelve weeks, therefore elapsed between the initial test in Stage 1 and this further test. The second group consisted of the 70 patients whose initial negative urines had been sent for laboratory examination, and the collection of specimens from these 70 patients was staggered so that the time intervals between their two tests were similar to the time intervals for the other group.

The re-testing of the 77 patients whose initial test had been positive, resulted in 50 positive and 27 negative reactions, and thus identified a group of patients with two consecutive positive tests. Unfortunately a further two patients dropped out of the study, leaving 48 as the 'hard-core' of patients with two positive tests. Stage 3 involved giving a course of treatment to half of these patients, and assessing the result by monthly urine testing over a follow-up period of three months. The 48 patients were subdivided into two groups according to age, those between the ages of twenty and forty-nine, and those over the age of fifty. This gave a total of fourteen patients in the former group, and thirty-four in the latter. By a completely random method of selection, each age group was further sub-divided into two sub-groups, one of which was to receive a course of treatment and the other to act as a control. In numbers this meant that seven patients in the under fifty age group and seventeen in the over fifty age group

received treatment, while an exactly similar number did not.

Two antibiotics were used in the treatment of these twenty four patients. Cycloserine was to be the drug of choice and all urines which had been sent to the laboratory had been examined to check the sensitivity of the causative organism to this drug as well as to a standard list of antibiotics. Cultures were tested for sensitivity to Chloramphenicol, Streptomycin, Tetracycline, Furadantin, Penbriten, Negram, and Cycloserine. Where sensitivity to Cycloserine had been established, patients were given this drug in a dosage of 250 mgms. twice daily for fourteen days, and in cases where the organism was shown to be resistant to it, Tetracycline, in a dosage of 250 mgms. four times daily for ten days, was given.

Two weeks were allowed to elapse after the treatment before the first urine specimen of the follow-up period was collected. This delay was necessary to ensure that, in those who had had treatment, no trace of the antibiotic was present in the urine to inhibit any bacterial growth that might be present. Urine samples were obtained, not only from those patients who had received treatment, but from all patients who had shown an initial positive nitrite test, and who were still available to the study. For ease of recognition, three Groups have been described whose composition is as follows,

Group A	Patients given treatment
Group B	Remainder of those patients showing two consecutive positive tests, and who were not given treatment.
Group C	Those patients who gave a positive test in Stage 1 and a negative test in Stage 2.

At the time of the second follow-up test, one patient from Group A and Group B, and two patients from Group C were unavailable for testing, and, at the time of the third and final follow-up test, two patients in Group A and Group B, and one from Group C were unavailable. The time interval that elapsed between each of the follow-up tests was four weeks. The three months that elapsed between the start of treatment and the final follow-up test was considered an adequate length of time over which to assess the value of the treatment, and the study was therefore concluded at that time.

ORGANISATION OF THE STUDY

Stage 1. Initial screening of available practice population, and testing of 375 urine samples.
Positive urines sent to laboratory for examination.
Bacteriological examination of 70 negative urines selected at random.

Stage 2. Re-testing of urines which were positive at Stage 1.
Re-testing of the 70 negative urines sent for bacteriological examination.

Stage 3. Identification of patients showing two consecutive positive tests, half of whom given treatment.
Three follow-up tests done on patients in
Group A those given treatment.
Group B remainder of those showing two positive tests and who were not given treatment.
Group C Those who were positive in Stage 1 and negative in Stage 2.

Figure 6

PART 3

THE RESULTS OF THE STUDY

THE RESULTS OF THE STUDY

Patients Participating in the Study

During the three and a half months of the first stage of this study a total of 452 patients were seen at the surgery, and from these, 375 patients were included in the study. Of the 77 patients who were not included, 39 were males and 38 females, and they were excluded on one of three grounds: that they lived outside the limit of one mile from the town, that they were under the age of twenty years, or that they presented with a diagnosis of urinary tract infection according to the criteria mentioned above. Among the 39 male patients, 17 lived outside the mileage limit, 22 were under the age of 20 years, and none fell into the urinary infection category. In the 38 female patients, 13 lived outside the mileage limit, 22 were under age, and 3 were included in the urinary infection category.

Relationship to Age and Sex

As the minimum age at which a patient could be included in the study had been pre-determined as 20 years, the ages of the patients ranged from this age to 92 in the female group, and from 20 to 84 years in the male group. For ease in tabulation patients were grouped into the decade in which their actual age fell, and the numbers in each decade are shown in Figure 7. In the case of females, the greatest percentage of the total number seen was in the twenty to twenty-nine age group, with a steady reduction in numbers thereafter, while with male patients, greater numbers were seen from the fifty to fifty-nine age group.

Relationship of Female Patients to their Marital Status

Patients were grouped into two classes, those who were unmarried, and those who were married or widowed. Of the 375 patients included in the study, 269 were female and, of those, 44 were unmarried, a percentage of 16.3.

Relationship of Female Patients to Parity

The parity of the patient was expressed as the number of live births the person had had, and this was tabulated as 0,1,2,3,4, and

5 or more children. The numbers of patients in each parity group, who were included in the study, is shown in the table below.

	Unmarried	0	1	2	3	4	5+
Total Number	44	35	49	73	28	17	23
Average age in years	55.4	51	48.4	41.8	42.1	45.7	53.7
Percentage of total	16.3	13	18.2	27.2	10.3	6.3	8.5

Figures are correct to the first decimal point.

Incidence of Previous Urinary Infection

Particular care had been taken, when patients were first seen, to note on their study record cards any history of previous urinary tract infections, but no attempt was made to try to differentiate between 'cystitis' and 'pyelonephritis'. Out of the 269 female patients, 133 gave such a previous history, a percentage of 49.4%. A previous history of urinary tract infection was related to the parity of the patient, and the results are shown in the table below.

		0	1	2	3	4	5+
Total number	44	35	49	73	28	17	23
No. with history of infection	14	13	30	38	20	11	7
Percentage	31.9	37.4	61.2	52	71.4	64.7	30.4

Percentages are correct to the first decimal point.

In the 106 male patients, a total of 14 gave a history of previous urinary tract infection, a percentage of 13.2, and considerably lower than the overall figure for female patients of 49.4%

High Risk Groups

Patients attending surgery for ante-natal consultations, and who had an established diagnosis of diabetes were considered to be 'high risk' patients as they had an increased likelihood of harbouring an asymptomatic urinary infection. In the 269 female patient, fifteen were attending the ante-natal clinic, and one was a diabetic on oral hypoglycaemic agents only. In the male patients, two out of the total of 106 who were included in the study, were diabetics receiving insulin as their treatment.

Age Decades	20-29.	30-39.	40-49.	50-59.	60-69.	70-79.	80-89.	90 +
No. of female patients	58	50	40	38	34	30	18	1
Percentage of total	21.5	18.5	14.8	14.1	12.6	11.1	6.6	0.3
No. of male patients	15	15	20	23	16	13	4	0
Percentage of total	14.1	14.1	18.8	21.6	15	12.2	3.7	0

Fig. 7

(Percentages calculated to first decimal point)

Age Decades	20-29.	30-39.	40-49.	50-59.	60-69.	70-79.	80-89.	90+
No. of females showing +ve test	9	8	10	7	12	16	16	1
Percentage in each age group	15.5	16	25	18.4	35.2	53.3	88.8	100
No. of males showing +ve test	0	0	1	1	4	2	0	0
Percentage in each age group	0	0	5	4.3	25	15.3	0	0

Fig. 8

(Percentages calculated to first decimal point)

Stage 1

Results of the first Nitrite Tests

In this first stage of the study, a total of 375 urines were examined with the modified nitrite test, and 91 positive and 284 negative results were obtained. Of the 91 positive urines, 82 were from female patients and 9 from males, the incidence of positive tests among those included in the first stage of the study being 30.4% of the female patients, and 8.4% of the male.

All of the 91 positive urines were sent for bacteriological examination to the hospital laboratory, where the urine sediment was examined and the causative organism was identified by culture. Pus cells were found in 29 urines, that is in 31.8% and this figure, when divided into sexes, gave an incidence of 22.2% in the male positive urines, and 32.6% in the female positive urines. On culture, one male and one female urine was found to be grossly contaminated which was presumably the reason why they had given positive nitrite results, and two female urines were reported as giving no growth. The organisms found on culture in the remaining 87 urines were as is shown in the table below.

	Female		Male	
E. Coli	62	78.4%	6	75.0%
Proteus	4	5.0%	0	
Staph. Albus	2	2.5%	1	12.5%
Staph. Aureus	2	2.5%	0	
B. Lactis	1	1.2%	0	
E. Coli and Proteus	2	2.5%	0	
E. Coli and Staph. Albus	2	2.5%	0	
E. Coli and Strept. Faecalis	3	3.7%	1	12.5%
Proteus and Strept. Faecalis	1	1.2%	0	

Percentages are correct to the first decimal point.

The two patients whose urine had been grossly contaminated, and the two from whom no growth had been recorded, have not been included in the group of positive urines resulting from this initial test, which only contains the 87 urines from which a pure culture was obtained.

Positive tests in relation to age

The percentage of patients, both male and female, in each age decade, who showed a positive nitrite test is shown in Figure 8. This gives, in the case of female patients, the lowest percentage (13.7%) in the twenty to twenty-nine age group, with a steady rise in incidence with age, except for a slight drop in the decade fifty to fifty-nine years. In the case of male patients, the highest incidence is in the sixty to sixty-nine and seventy to seventy-nine age groups with no positives being recorded below the age of forty.

Relation of the results of initial tests to parity

Figure 9 shows the details of the relationship between parity and a positive nitrite test.

	Unmarried	0	1	2	3	4	5+
Number of patients	44	35	49	73	28	17	23
Numberwith +ve Test	20	11	14	13	5	5	11
Percentage	45.9	21.4	28.5	17.8	17.8	29.4	47.8

Figure 9

It will be noticed that the two highest incidences are in those who are unmarried, and in those who have had five or more children. The lowest level (17.8%) is found in those who have had two or three children, and thereafter there is a rise with increasing parity.

Relationship of the result of the initial test to a history of previous urinary tract infection

The relationship of a previous history of infection to the presence of bacteriuria is shown in Figure 10. In the case of the female patients, between the age groups of twenty to twenty-nine years and sixty to sixty-nine years there is a markedly consistent figure of just above 50% with a history of a previous urinary infection, but where this would be expected to stay at, or rise above, the 50% mark, in the seventy and above age groups, there is a drop. This can perhaps be explained by a lack of memory in those elderly patients of episodes of infection in the past, and these results are probably very inaccurate. Similarly, in considering the percentage of those who admit to a previous urinary infection and who show a positive nitrite test, the results for the age groups above the age of seventy are probably inaccurate as they are deduced from figures that are themselves likely to be inaccurate.

Age Decades	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90+
Number of patients	58	50	40	38	34	30	18	1
% with history of previous infection	56.8	52	52.5	52.6	52.9	33.3	22.2	100
% of those, with +ve test	18	11.5	38	20	38.8	60	100	100

Figure 10
(Female Patients)

Age Decades	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90+
Number of patients	15	15	20	23	16	13	4	0
% with history of previous infection	20	13.3	15	4.3	12.4	30.7	0	0
% of those, with +ve test	0	0	0	0	50	50	0	0

Figure 11
(Male Patients)

As can be seen from Figure 10, there is not a high incidence of positive nitrite tests in those under the age of sixty who have a history of previous urinary tract infection, but between the ages of sixty and ninety there is a progressive rise in incidence. In the case of male patients there is no clear-cut connection between a history of previous infection and a positive nitrite test. In the age groups sixty to sixty-nine years and seventy to seventy-nine years there were only two patients who gave such a previous history, and in each group, one gave a positive nitrite result. The numbers are therefore too small for any conclusion to be based on them. (Figure 11).

High Risk Groups

Fifteen patients attending surgery for ante-natal care were included in the study, and of the fifteen, four gave positive nitrite results. Of the three diabetic patients, two men and one woman, all gave negative nitrite results. The numbers of patients in these two groups are too small for the above figures to be significant.

Results of the initial negative urines

Out of the 375 urines tested in this first stage 284 gave negative nitrite results, and of these, seventy were selected at random and sent to the laboratory for bacteriological examination. The method of

selection was that every fourth urine specimen that gave a negative nitrite result was sent, with those that were positive, to the laboratory, and of these seventy urines, eight were from male patients and sixty-two from female.

On examination of the female urines, pus cells were not found in any of the specimens, and no growth was recorded, after culture, in thirty-nine urines, a percentage of 62.9%. The results of culture of the remaining 23 urines was as follows:

E. Coli	16	69.5%
E. Coli and Proteus	2	8.6%
E. Coli and Strept.Faecalis	5	21.7%

In the case of the five urines giving cultures of E. Coli and Strept. Faecalis, the first named organism was reported as the dominant organism, and the Strept. Faecalis is likely to be a contaminant.

Urines from eight male patients were also sent for bacteriological examination. All eight gave no growth on culture, and pus cells were not found in any case.

The breakdown of these seventy initial negative urines into their age decades is shown in Figure 12.

ge Decades	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90+
o. of female patients	16	19	9	7	6	4	1	0
ercentage of total	23.3	30.6	14.5	11.2	9.6	6.4	1.6	0
o. of male patients	1	3	2	0	1	0	1	0
ercentage of total	12.5	37.5	25	0	12.5	0	12.5	0

Figure 12

The percentages of female patients compares in all age groups except that of 30 to 39 years, with the figures given in Figure 7 for the total number of 269 female patients included in the study. This group of female patients, whose urine had been negative to the nitrite test, can thus be considered to be a cross section of the total female patients tested. The small numbers of male patients, as shown in Figure 12 make it inaccurate to compare with the total number of male patients tested.

Relationship to a history of previous urinary infection

Those patients whose urine, although giving a negative nitrite test, gave a growth on culture were tabulated in relation to a previous history of urinary tract infection, and the results are shown in Figure 13.

Age Decades	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90+
Number of patients	16	19	9	7	6	4	1	0
% with history of previous infection	56.2	42.1	55.5	71.4	50	50	0	0
% of those, with a growth on culture	44.4	50	40	20	33.3	100	0	0

Figure 13
(Female Patients)

In the eight male patients, none had given any growth on culture, and one had given a history of previous urinary tract infection. The numbers of males are, however, too small to afford accurate conclusions.

High Risk Groups

In the 62 female patients, three were attending surgery for ante-natal care, and in all three cases, no growth was obtained on culture. No diabetics were included in either the male or female patients of this group.

Stage 2

After the initial screening of the 375 patients had been completed, a second mid-stream urine specimen was obtained from all those whose first test had been positive with a positive culture when examined bacteriologically, and from the 70 negative urines which had been examined for a bacterial growth. In both the positive and the negative urines, a time interval elapsed between the first and second testing, and this varied from three to twelve weeks. Figures 14 and 15 show the relation between the numbers re-tested and the varying time intervals.

Time interval between tests in weeks	3	4	5	6	7	8	9	10	11	12
No. of urines re-tested	5	3	19	1	5	6	10	9	10	9
% of total urines re-tested	6.5	3.8	24	1.2	6.5	7.7	13	11.6	13	11.6
	. 10.3% .			39.4%		.		49.2%		

Figure 14
(Urines originally positive)

Time interval between tests in weeks	3	4	5	6	7	8	9	10	11	12
No. of urines re-tested	2	6	12	6	6	4	8	10	8	8
% of total urines re-tested	2.8	8.5	17	8.5	8.5	5.6	11.4	14.3	11.4	11.4
	. 11.3% .			39.6%		.		48.5%		.

Figure 15
(Urines originally negative)

Results of the second testing of positive urines.

Of the 87 patients whose urines had given both a positive nitrite result and a positive culture, only 77 were still available, the remaining ten either having left the area temporarily or permanently and they were therefore excluded from the study from that point. Of the seventy-seven patients whose urine was thus retested, seven were males and seventy were females. The nitrite test resulted in 46 positive and 24 negative results in the female group, and four positive and three negative in the male group. All urines, whether positive or negative were sent to the laboratory for bacteriological examination. In the female group, eight were found to have pus cells, of which one had given a negative nitrite result, and in the male group, pus cells were found in one urine, which gave a positive result. The results of the bacterial culture of all seventy-seven urines is shown in table below.

Organisms	Nitrite Test			
	Female		Male	
	+ve	-ve	+ve	-ve
E. Coli.	42	9	4	2
B. Proteus	1	1	-	-
Staph. Albus	1	-	-	-
E. Coli and Staph. Albus	1	1	-	-
E. Coli and Strept. Faecalis	1	-	-	-
B. Lactis Aerogenes	-	1	-	-
No growth	-	12	-	1

Of the fifty urines which gave a positive nitrite result, all but three showed the same organism on culture on this second testing as they had done on the first test. Of the three in which the organism was different, one showed a growth of E. Coli where Staph. Albus had been grown before, and two gave a growth of

E. Coli where Proteus had been found before. In the 14 urines giving a negative nitrite reaction in which a culture was obtained, twelve gave the same organism as had been found on the previous test. Of the remaining two, one gave a growth of E. Coli where E.Coli and Proteus had been found before, and one grew E. Coli where there had been E. Coli and Staph. Albus in the previous specimen.

This second testing of patients whose urine had initially given a positive reaction, gave a 'hard-core' of fifty patients with two consecutive positive results, and it is necessary to look at these fifty patients in relation to their age, parity, marital status, previous urinary history, and the time interval that had elapsed between their two tests.

1. Relationship to age - the number of female patients in each age decade, who were available for the second test, and who had one and two positive results, is shown in Figure 16.

ge.Decades	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90+
o. of patients	58	50	40	38	34	30	18	1
o. with +ve test n Stage 1, available or Stage 2	9	8	10	6	10	12	14	1
o. with +ve test in tages 1 and 2	7	4	4	3	5	10	12	1
with +ve test in tage 1	15.5	16	25	18.4	35.2	53.3	88.8	100
with +ve test in tages 1 and 2	12	8	10	8.1	15.6	38.4	75	100
of those +ve in tage 1 who were ve in Stage 2	77.7	50	40	50	50	83.3	85	100

Figure 16

The four male patients whose urine showed two positive results were limited to the age decades sixty to sixty-nine and seventy to seventy-nine years, there being three in the former and one in the latter age groups. Figure 17 shows the numbers in each age group with one and two positive results.

ge Decades	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90+
o. of patients	15	15	20	23	16	13	4	0
o. with +ve test n Stage 1	0	0	1	1	4	2	0	0
o. with +ve tests n Stages 1 and 2	0	0	0	0	3	1	0	0

Figure 17

2. Relationship to parity - The female patients who had shown one, and two positive tests are shown related to their parity and marital status in Figure 18.

	Unmarried	0	1	2	3	4	5+
o. of patients	44	35	49	73	28	17	23
o. with +ve test in tage 1, available for tage 2	18	9	13	13	5	5	7
o. with +ve test in tages 1 and 2	12	7	11	7	2	3	4
with +ve test in tage 1	45.9	21.4	28.5	17.8	17.8	29.4	47.8
with +ve test in tages 1 and 2	28.5	21.2	22.8	9.6	7.1	17.6	21
of those +ve in tage 1 who were +ve Stage 2	66.6	75.5	84.6	53.8	40	60	57.1

Figure 18

3. Relationship to Previous Urinary Infection - Figure 19 sets out the percentage incidence of a history of previous infection in those female patients who were positive in the first test, and those who were positive in both tests.

Age Decages	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90+
No. +ve in Stage 1	9	8	10	7	12	16	16	1
No. +ve with history of infection	6	3	8	4	7	5	4	1
Expressed as %	66	37	80	57	58	31	25	100
No. +ve in Stages 1 and 2	7	4	4	3	5	9	12	1
No. +ve with history of infection	6	1	4	2	4	2	3	1
Expressed as %	85	25	100	60	80	22	25	100

Figure 19

Apart from the age decades thirty to thirty-nine and seventy to seventy-nine years, this shows that a higher percentage of patients had a history of infection in those who showed two positive results, as compared with those who gave a positive test in Stage 1.

4. Relationship to the time interval that elapsed between the two tests - The number of patients tabulated under the time interval between the two tests are shown in Figure 14. In order to see whether the time interval had influenced the numbers of positive tests in Stage 2, patients were classified into three groups: those in whom the time interval was between 0 and 4 weeks, those in whom the interval was 5 to 8 weeks inclusive, and those in whom the interval was from 9 to 12 weeks inclusive.

There were no patients in whom the interval exceeded twelve weeks. The age incidence of the patients is also of importance, as there is an increase in the incidence of positive tests with increase in age (Figure 16).

	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90+
0 to 4 week interval	1	1	1	2	1	2	0	0
No. with +ve test(Stage 2)	1	1	1	1	1	1	0	0
5 to 8 week interval	2	1	2	2	4	9	10	1
No. with +ve test(Stage 2)	2	1	1	1	1	7	9	1
9 to 12 week interval	6	6	8	3	8	3	4	0
No. with +ve test(Stage 2)	4	2	2	1	6	3	3	0

Figure 20
(Male and Female patients)

The effect of the time interval between tests seems to be that while an interval of up to eight weeks does not affect the numbers of patients showing a positive repeat test, if the interval exceeds eight weeks, the number of patients under the age of fifty years who show a positive repeat test falls considerably.

Results of the re-testing of the seventy initially negative urines

The seventy negative urines that had been sent for bacteriological examination in Stage 1 of this study, were re-tested with the nitrite test, and, regardless of its result, were sent again for laboratory examination. Of the seventy patients, one had left the district before the second testing was done, and

was therefore excluded from the study. Mid-stream urine specimens were obtained from eight male and 61 female patients. The nitrite test gave 10 positive and 59 negative results, all the male urines being negative, and the ten positive and 51 negative results being from female patients.

On examination of the female urines, no growth was obtained in 38 of the negative urines, and pus cells were found in four cases, two of which had a negative reaction and a growth of E. Coli, one of which had a negative reaction and no growth on culture, and the remaining one gave a positive nitrite reaction, and a growth of E. Coli. In the thirteen negative urines which gave a growth on culture, eight grew the same organism as had been found previously, three grew E. Coli when in the previous test no growth had been obtained, one grew Proteus when there had been no growth previously, and one Proteus where E. Coli had been found before. In the 38 urines from which no growth was obtained, 32 had also given no growth on the previous test and six had given negative nitrite tests but positive cultures on the first testing.

Ten positive reactions were obtained from female patients on this second testing. In one of the positives, the laboratory reported a negative culture and absence of contaminants, and this was therefore a false positive. In the other nine, seven gave cultures of the same organism as had been present previously, one gave a growth of E. Coli when previously there had been no growth, and one gave a culture of Proteus from a urine which had previously grown E. Coli and Proteus.

In the eight male patients, all gave negative results, and, while all eight had given no growth on culture on the previous test, this test gave

six with no growth, one with a growth of E. Coli, and one with a growth of Strept. Faecalis which was likely to be a contaminant.

The ages of the patients who gave a culture with a negative nitrite test on this occasion are shown in Figure 21.

	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90+
No. of patients tested	17	22	11	7	7	4	2	0
No. with culture in first test	6	8	2	2	2	3	0	0
No. with culture in second test	2(2)	6(3)	2(1)	1(1)	2(1)	0(1)	0	0
No. with culture in both tests	2(2)	4(3)	1	1(1)	1(1)	0(1)	0	0

Figure 21
(Numbers in brackets refer to those giving +ve test)

From the figures stated above, there is no variation in the age incidences of those patients showing cultures in the first, second, or both tests.

Importance of history of previous infection - The overall incidence of a history of previous urinary tract infection in the 62 female patients whose urine had given negative reactions and which had been sent for laboratory examination, was 51.6%. Nine females of this group gave positive reactions on the second testing, and of these, six, or 66% gave a history of previous infection. Out of the eight patients who gave a culture in the first test and a positive nitrite

reaction in the second test, five gave a history of previous infection. This gives a percentage of 62.5%, which is slightly higher than in the group as a whole, but the numbers are too small to draw the conclusion that the finding of a culture is related to this previous history.

Importance of the time interval between tests - Eight female patients gave a culture in their first test, and a positive nitrite test in the second, and of these, the time interval in the case of two was 7 weeks, and in the remaining six the interval was over 9 weeks. The ages of these eight patients is given in the table below, and it can be seen that they are evenly distributed between the ages of 20 and 79 years.

e Decades	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90+
to 4 week terval	0	0	0	0	0	0	0	0
to 8 week terval	0	2	0	0	0	0	0	0
to 12 week terval	2	0	1	1	1	1	0	0

Stage 3

Having identified a group of patients who had given two positive nitrite tests, Stage 3 was concerned with giving one half a course of treatment, and with the follow-up tests on all patients who had given positive tests in Stage 1. In the group of fifty patients who had shown two positive tests, two became unavailable and the remaining 48 were divided into two groups, those below the age of fifty and those above this age. It had been hoped to subdivide patients into three groups representing in females, those of child-bearing age, those in the menopausal period, and those above the age of sixty, but as the total number of patients was small (48) it was decided to divide them into two groups only. This subdivision gave 14 patients in the lower age group and 34 in the upper age group, and half of each group was given a course of treatment while the other half acted as controls. The patients who were to receive treatment were selected at random by allocating to each a number, and drawing half of the numbers out of a hat.

Two antibiotics were given to the 24 patients who received treatment. Cycloserine was given to 21 patients in a dosage of 250 mgms. twice daily for a course of fourteen days, and three patients whose organisms were resistant to cycloserine were given a ten day course of tetracycline in a dosage of 250 mgms. four times daily. Although none of the patients had any clinical evidence of impaired renal function, they were all warned of the possible side effects of cycloserine, and were told to report any occurrence of symptoms such as vertigo, drowsiness, or confusion, which would imply that the blood concentration of the drug was rising due to impaired renal excretion. In the younger age group of seven patients, six were given cycloserine and one tetracycline, and of those receiving the former drug,

one stopped the course after nine days due to a gastro-intestinal upset. No other side effects were reported from this group. In the older age group of seventeen patients, two were given tetracycline and fifteen cycloserine. All of those given the former drug completed their course of treatment with no reported side effects, but only nine of those receiving cycloserine were able to complete the course. In the six patients who failed to complete the course, side effects of confusion, vertigo, and headache appeared after four days in the case of two patients, after seven days in one case, and after nine days in the other three. All of these patients were over the age of 75 years (Figure 22).

Four weeks after the start of treatment, further mid-stream urine specimens were obtained, not only from those who had received treatment, but from all patients who had originally shown a positive nitrite test. These patients are designated as follows,

Group A	Patients who received treatment.
Group B	Patients with two positive tests but who were not treated.
Group C	Patients whose second test had been negative.

Results of Group A

1. First follow-up test - Of the seven patients below the age of fifty, six gave a negative nitrite reaction and one a positive result, and all urines were sent for laboratory examination. The one which gave a positive result grew a pure culture of E. Coli, as it had done on the previous two tests, and was therefore considered a 'failed treatment'. Of the six which gave a negative nitrite result, four gave no growth on culture and were therefore considered as 'cured', and two gave cultures of the same organisms as had been found in previous tests, and in these it was considered that

Patients treated with Cycloserine

Patient number	Age	Days of treatment	Reasons for stopping treatment.
1	26	14	None
2	49	14	None
3	20	9	Gastro-intestinal upset
4	32	14	None
5	22	Completed a 10 day course of tetracycline	
6	26	14	None
7	29	14	None
1	83	4	Vertigo, headache
2	86	9	Vertigo
3	84	9	Vertigo, confusion
4	81	14	None
5	87	Completed a 10 day course of tetraycline	
6	67	14	None
7	54	14	None
8	60	14	None
9	61	14	None
10	71	Completed a 10 day course of tetraycline	
11	75	14	None
12	74	14	None
13	68	14	None
14	76	9	Vertigo, confusion
15	80	4	Vertigo
16	81	7	Vertigo
17	76	14	None

Fig. 22

the bacterial count had been lowered by the course of treatment.

In the group of older patients, ten gave a positive nitrite result, and seven a negative result, and all urines were sent for laboratory examination. Of those giving a negative result, two gave no growth on culture and were considered as 'cures', four gave a growth similar to that obtained previously, and one gave a growth of Proteus where E. Coli had been found in the previous tests. In the ten urines which again showed a positive nitrite test, all grew pure cultures of E. Coli which had been the organism present in the previous two tests.

Thus in the lower age patients, four out of seven (57%) had their urine rendered sterile after the course of treatment, two (28%) had the bacterial count lowered, and one (14%) showed no response to treatment. In the older patients, two (12%) had sterile urine following treatment, five (29%) had their bacterial count lowered, and ten (59%) showed no response to treatment.

2. Second follow-up test - four weeks after the first follow-up test, further urine specimens were obtained from all patients in the younger age group, and from sixteen out of the seventeen in the older age group. In the younger age group, four positive and three negative nitrite results were obtained. Of the four positives, one had been positive following treatment and on this second test grew a pure culture of the same organism. Of the other three, two had had a negative nitrite reaction following treatment, but had given a growth on culture of the same organism as had been present before treatment, and they again grew the same organism on this further test. The remaining one positive had grown E. Coli on the first testing, Proteus on the second, and no growth on the test following the course of treatment when

a negative nitrite result was obtained, but a growth of E.Coli occurred with a positive nitrite test on this second follow-up.

In the older group of patients, eight positive and eight negative results were obtained. Of the eight positives, six had given positive results following the course of treatment, and the organisms grown on this second follow-up were the same as had been obtained previously. One had given a negative nitrite result following treatment but had given a growth of the same organism as had been found previously, and this patient now gave a positive nitrite test with a culture of this same organism. The one remaining positive urine was reported as giving no growth, and was therefore a false positive.

Eight patients gave negative nitrite tests on this second follow-up, and of these, five had been negative following treatment and three had been positive. In the five that had been negative, two had given no culture after treatment and again on this further testing, and three gave a growth on this follow-up test similar to that found both before and after treatment. Three patients had given positive results following treatment, and of these, two now gave no growth and one gave a growth of the same organism as had been found previously. The one positive urine with absence of growth on culture had given a negative nitrite test with a positive culture following treatment.

3. Third follow-up test - In the younger group of seven patients, the nitrite test resulted in four positive and three negative results. Three of the four positives were all patients who had given a positive result on the previous test, and now gave cultures of the same organisms as had been grown on previous examinations. The remaining one positive

gave no growth on culture, but had, on the previous test, given a positive nitrite test and positive culture.

Of the three negatives, two gave no growth on culture and were from patients whose urine had become sterile following treatment and had remained so over the follow-up period, while one, which had been sterile following treatment and at the second follow-up test, now gave a growth of the same organism as had been found previously.

In the older age group of patients, only fifteen were available for this follow-up test, and from these, six negative and nine positive reactions were obtained. Of those giving a positive result, five had been positive on all previous occasions despite treatment, and they grew the same organism as had been previously cultured, while three had all given a negative result on the previous test. One of these last three had given a negative result following treatment but had grown the same organism as had been present before therapy, one had given a positive result following treatment, and the remaining one had shown a sterile uirne following treatment and at the second follow-up, but now gave a growth of E. Coli which had been the infecting organism before the course of treatment. The one remaining positive urine gave no growth on culture, as it had done after treatment and at the second follow-up, and had also given a false positive result to the nitrite test on the second follow-up.

Six of these older patients gave negative reactions to the nitrite test, and four of them had also been negative on the previous occasion. Two had been sterile on the previous test, but now gave a growth culture. One had shown no growth on the previous test and again on this third follow-up, and the fourth had given a negative nitrite with a positive culture on all of the post-treatment tests. The remaining two

chemically negative urines had given positive results on the preceeding test, and, although one had been chemically positive and one chemically negative following treatment, both had given a positive culture on that occasion.

Figure 23 shows the patients who received treatment, in alphabetical order, with their ages, the number of days of treatment, and the results of their follow-up tests. In this Figure a negative nitrite test with no growth on culture is represented by the sign -ve (S), and a negative nitrite result with a positive culture by the sign -ve (C). The three positive nitrite results in which no growth was obtained have been marked as false positives.

Results of Group B

Group B consisted of seven patients below the age of fifty, and seventeen above that age, to whom no treatment had been given despite the fact that they had all shown persistently positive nitrite reactions.

1. First follow-up test - In the younger age group, when urines were tested, five positive and two negative results were obtained, and in those that were positive, all grew a pure culture of the same organism as had been found previously. In the two that were negative, one gave a growth of E. Coli, which had been present in previous tests, and one gave no growth on culture.

In the group of older patients, the nitrite test resulted in eleven positive and six negative results. Of the eleven positives nine grew the same organism as on previous occasions, one grew a culture of Proteus when the first and second tests had grown Proteus and E. Coli respectively, and one gave a grow th of E. Coli and Proteus where E. Coli alone had been grown previously. Five negative urines gave cultures of the same organism as had been found in their previous two tests, and one gave no growth on culture.

Patient number	Age	No. of days treated	Follow-up tests 1st.	2nd.	3rd.
1	26	14	+	+	+
2	49	14	- S	+	+
3	20	9	- C	+	False +
4	32	14	- S	- S	- C
5	22	10 (Tet/cycline)	- C	+	+
6	26	14	- S	- S	- S
7	29	14	- S	- S	- S
1	83	4	+	+	+
2	86	9	+	+	+
3	84	9	+	+	+
4	81	14	+	- S	- C
5	87	10 (Tet/cycline)	+	+	+
6	67	14	- C False +		False +
7	54	14	- C	- C	- C
8	60	14	+	- C	+
9	61	14	- S	- S	+
10	77	10 (Tet/cycline)	- C	- C	No sample
11	75	14	- S	- S	- S
12	74	14	- C	- C	+
13	68	14	+	+	- C
14	76	9	+	- S	- C
15	80	4	- C	+	- C
16	81	7	+	No further samples	
17	76	14	+	+	+

Figure 23.

Comparison of age of patient, number of days of treatment, and results of the follow-up tests.

- C refers to a negative nitrite test with a pure growth on culture.

- S refers to a negative nitrite test with no growth on culture.

False + refers to a positive nitrite test with absence of growth on culture.

In the control group of younger patients, therefore, one out of seven (14%) showed a sterile urine without having had treatment, five (71%) showed no change, and in the remaining one the bacterial count had dropped to below the 100,000 per millilitre mark. In the older group of controls, one showed no growth on culture, five (29%) showed a reduction in the bacterial count, and eleven (64%) showed no change from previous tests.

2. Second follow-up test - In the younger group of seven patients the nitrite test resulted in five positive and two negative results. The five positive urines were those that had been positive in the previous test, and the cultures obtained from them were the same as in the first follow-up test. In the two negative urines, the results of culture were the same as previously.

The older group of seventeen patients had, on the first follow-up test, given eleven positive and six negative results, and on this follow-up gave eleven positive and five negative results, one patient being unavailable for further specimens. Of the eleven that had been positive in the first follow-up, nine were found to be still positive and two had become negative, but gave cultures of the same organism as had been found previously. Of the six that had been negative, one was from the patient that had become unavailable, three remained negative and two became positive, and both the latter gave a culture of the same organism as had been previously found.

3. Third follow-up test - The younger group of patients gave, on this third follow-up, four negative and three positive nitrite results. All three patients who remained positive grew the same organism as they had done previously, and the two who had been persistently negative gave the same bacteriological results as they had given on the previous occasions.

This leaves two patients who had been positive in all previous tests but who now gave negative results, and, while one gave a growth of the same organism as had been present before, the other gave a growth of Proteus on this occasion where E. Coli had been persistently found previously.

The group of older patients was depleted by two who were unavailable for further urine samples, and of the remaining fifteen patients, five gave a negative result and ten were positive. One of the positives gave a grossly contaminated culture in which no dominant organism was found, and the other nine gave cultures of the same organism as had been found previously. Of the five negative nitrite results, two gave no growth on this occasion, a result which was the same as had been obtained on the previous testing, while the remaining three gave cultures of the same organism as had been found before.

The results obtained from all urine tests from patients in Groups A and B, that is in the twenty-four patients who received treatment and the twenty-four patients who did not, have been presented diagrammatically in Figures 23 and 24. In these Figures, patients have been numbered in strict alphabetical order, and there is not intended to be any comparison between patients of each group having the same number.

In each Figure the numbers 1 to 7 refer to the younger group of patients in Groups A and B, and the numbers 1 to 17 to the older patients of each Group. The results of the nitrite tests, with the results of culture in the case of negative urines, is shown, and the variation in results obtained in individuals over the five tests is apparent.

Results of Group C

1. First follow-up test - Twenty-seven urine specimens were tested and of these 23 gave a negative result and four were positive. Of the four positive

urines, two were considered to be false positives as the laboratory reported the growth of contaminants only with no dominant organism, and two gave pure cultures of the same organism as had been found at the first test. As some limitation had to be made on the numbers of urine samples sent to the laboratory, it was decided that the 23 chemically negative urines from this test would not be examined and cultured, so it is not known whether these showed a negative result to the nitrite test because they were sterile or because the bacterial count was below 100,000 organisms per millilitre. It was hoped that, at the final test, all negative urines from this Group would be cultured to assess how many would give a growth on culture.

2. Second follow-up test - At the time of this test two patients became unavailable for further samples, and the 25 patients who were tested gave nine positive and sixteen negative results. Three of the positives gave no growth on culture, and were therefore false positives, and of the other six, three had previously given a negative nitrite result, two had been false positives in the first follow-up test, and one had also been positive on the previous occasion. All gave cultures of the same organism as had been present before. As only the positive urines were sent for laboratory examination, it is not known whether the sixteen negative urines represented sterile urines, or whether a bacterial growth would have been obtained on culture.

3. Third follow-up test - The number of patients from whom urine specimens were obtained on this occasion was 26, and the nitrite test resulted in six positive and twenty negative reactions. Three positive urines were reported as giving no growth, and were thus false positives; of the other three, two had been negative on the previous test when, unfortunately, they could not be examined for bacterial growth, and one had been

Patient Number	Age	Initial tests 1st.	Initial tests 2nd.	Follow-up tests 1st.	Follow-up tests 2nd.	Follow-up tests 3rd.
1	32	+	+	+	+	+
2	45	+	+	- C	- C	- C
3	24	+	+	+	+	+
4	42	+	+	- S	- S	- S
5	26	+	+	+	+	- C
6	36	+	+	+	+	+
7	44	+	+	+	+	- C
1	62	+	+	+	+	+
2	60	+	+	- C	- S	- S
3	70	+	+	+	+	+
4	72	+	+	- S	+	+
5	87	+	+	+	+	- C
6	71	+	+	+	+	+
7	87	+	+	+	+	+
8	67	+	+	- C	- S	- S
9	56	+	+	+	- C	- C
10	64	+	+	+	+	Unavailable
11	85	+	+	- C	Unavailable	"
12	90	+	+	- C	- S	False +ve
13	71	+	+	+	- C	+
14	78	+	+	+	+	+
15	85	+	+	+	+	+
16	74	+	+	- C	+	- C
17	87	+	+	+	+	+

Figure 24

Male and female patients of Group B (Untreated). 'C' refers to a pure culture obtained, 'S' refers to a negative urine from which no culture was obtained.

a false positive. Of the twenty negative urines five had been positive on the previous occasion, and four of them gave a growth similar to that obtained then, and the remaining one gave a culture of E. Coli where B. Lactis Aerogenes had been found on previous tests. Of the remaining negatives, thirteen had been negative on the previous occasion, one had given a false positive result in the second follow-up test, and the other was from a patient who had not been available for the previous test, but who had given a positive result at the first follow-up. Of the twenty negative tests, eight were reported as giving no growth on culture, while the other twelve were bacteriologically positive.

The twenty-seven patients in the Group are tabulated in Figure 25. Patients are numbered in alphabetical order with their ages and the results of both the nitrite and bacteriological tests. A negative nitrite test with a pure growth on culture is shown as -ve (C), and a negative nitrite test with no growth on culture is shown as -ve (S). The letter 'M' after the last three patients signifies that they are males.

Patient Number		Age	Initial tests 1st.	Initial tests 2nd.	Follow-up tests 1st.	Follow-up tests 2nd.	Follow-up tests 3rd.
1		26	+	- S	-	-	+
2		65	+	- C	-	+	- C
3		66	+	- C	-	False +	False +
4		58	+	- C	-	-	- C
5		43	+	- S	-	-	- S
6		43	+	- C	-	-	+
7		34	+	- S	-	+	- C
8		26	+	- S	-	-	- S
9		44	+	- C	-	-	- S
10		55	+	- S	-	False +	- S
11		60	+	- C	+	Unavailable	- C
12		70	+	- C	-	-	- C
13		85	+	- S	-	-	- C
14		83	+	- C	+	+	- C
15		43	+	- C	-	+	- C
16		30	+	- S	False +	+	False +
17		72	+	- C	-	-	- S
18		31	+	- S	-	Not available	
19		62	+	- S	-	-	- C
20		66	+	- C	-	-	- C
21		48	+	- C	-	-	- C
22		53	+	- C	-	-	- S
23		42	+	- S	False +	+	- C
24		30	+	- S	-	-	- S
25	M	54	+	- C	-	-	False +
26	M	70	+	- C	-	False +	+
27	M	41	+	- S	-	-	- S

Figure 25

Male and female patients of Group C. A negative nitrite test with a pure culture is represented by - C, a negative nitrite test with no growth on culture as - S.

Part 4

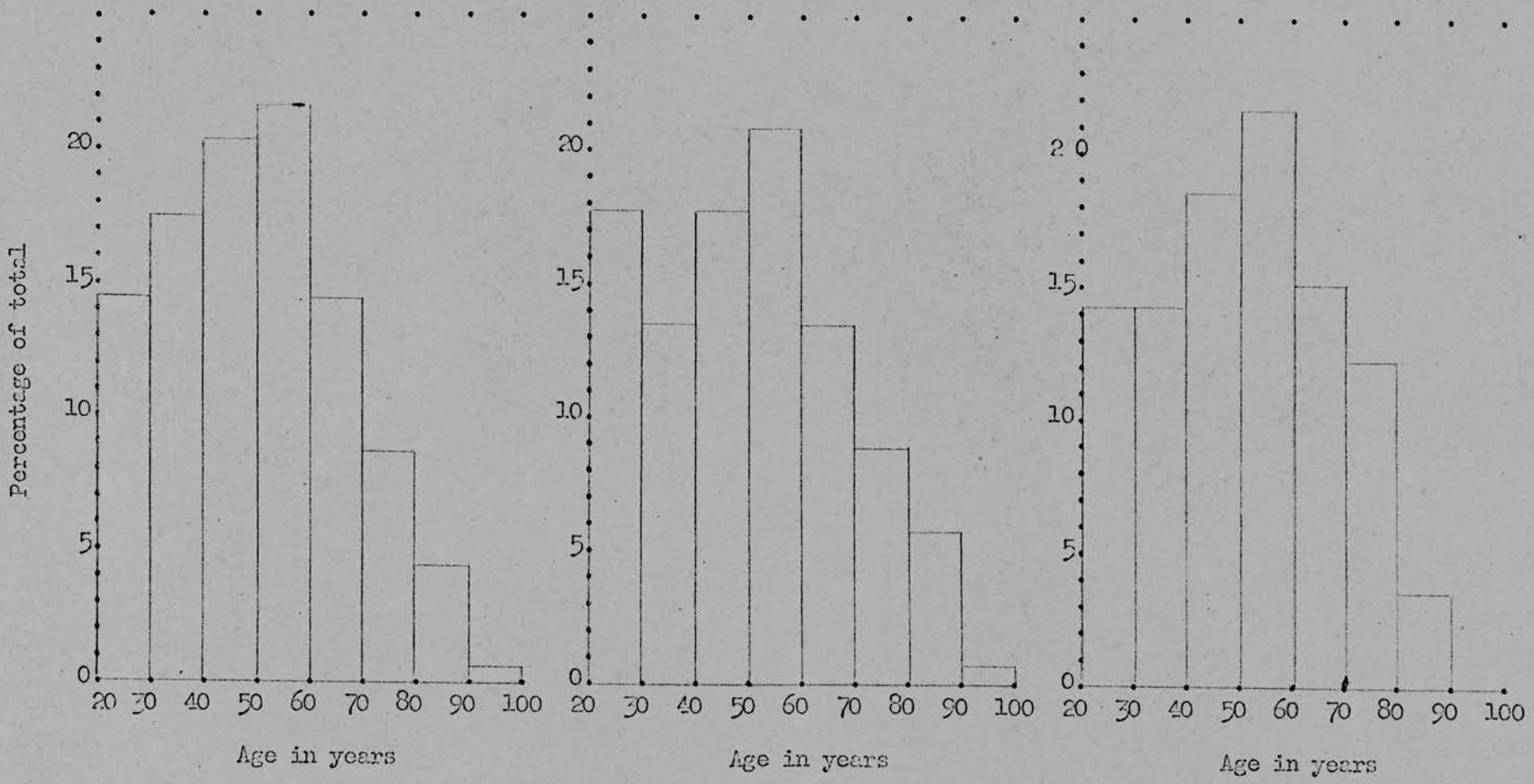
DISCUSSION OF RESULTS

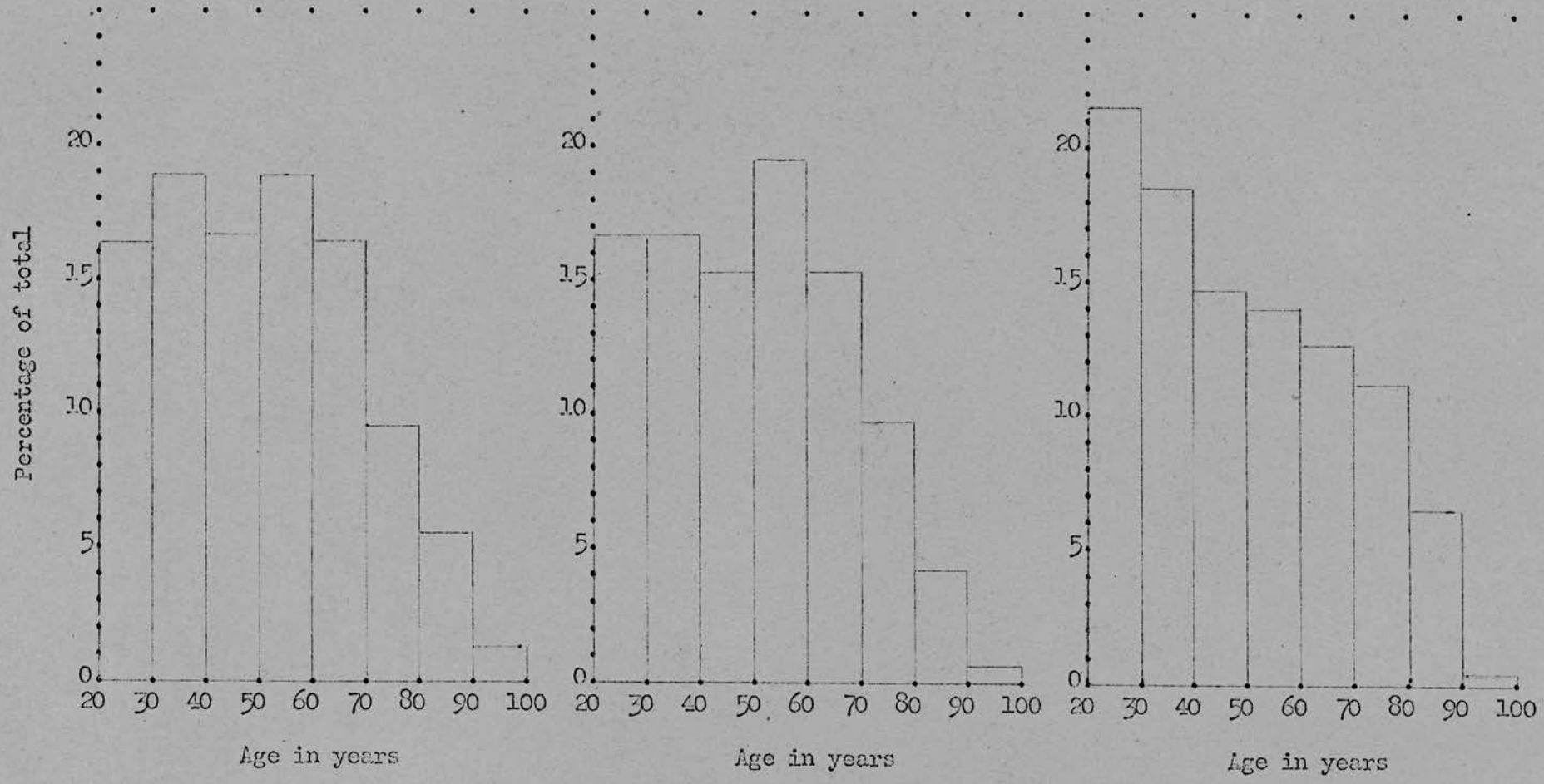
DISCUSSION OF THE RESULTS OF THE STUDY

Patients participating in the Study.

The numbers of male and female patients in the Practice as a whole, in the pre-determined study area, and those who were included in the study, expressed in age decades as a percentage of the total numbers of patients, are shown in the histograms on pages 71 and 72. In the case of male patients, all three histograms are similar for the age decades above forty years, but differences occur for the decades 20 to 30 years and 30 to 40 years. Taken overall, the histogram for the patients included in the study bears a closer resemblance to that of the total patients in the Practice than does the histogram for the patients in the study area. The histograms for female patients, on page 72, show that there is considerable similarity between the one for the patients in the Practice and the one for patients in the study area, with the histogram for those patients included in the study varying considerably from the other two.

The patients included in the study were all self-selected in that they presented themselves at the Surgery for advice or treatment. At their initial consultation, the diagnoses reached had been entered on the patients study record cards, and it had been hoped to subdivide patients into three groups, representing those with a specific illness, those in whom the resulting diagnosis was vague or purely symptomatic, and those who had come to the Surgery without any symptoms. It would then be of interest to examine the frequency of bacteriuria in each of these groups, since, as the symptoms of a low grade urinary infection may be purely those of vague ill-health, the group of patients with vague or symptomatic diagnoses might show a greater incidence of bacteriuria than would the other two groups. It was found to be too inaccurate to try to differentiate between patients with a specific diagnosis and those in whom the diagnosis was vague or symptomatic, and, therefore, only two groups were formed, comprising those who


Percentage of total
0
5
10
15
20
20 30 40 50 60 70 80 90 100
Age in years
Male patients in Practice
0
5
10
15
20
20 30 40 50 60 70 80 90 100
Age in years
Male patients available to Study
0
5
10
15
20
20 30 40 50 60 70 80 90 100
Age in years
Male patients included in Study


Percentage of total
20
15
10
5
0
20 30 40 50 60 70 80 90 100
Age in years
Female patients in Practice
20
15
10
5
0
20 30 40 50 60 70 80 90 100
Age in years
Female patients available to Study
20
15
10
5
0
20 30 40 50 60 70 80 90 100
Age in years
Female patients included in Study

complained of symptoms of ill-health, and those who came to the Surgery for advice about relatives, accompaning their children, or for routine medical examinations or innoculations. The latter group comprised a total of 8 male and 35 female patients, while the remainder, 98 males and 234 females, were allocated to the former group. Patients attending for ante- or post-natal care were included in the former group, as they were considered to have an above average chance of harbouring an asymptomatic bacteriuria.

The one factor differentiating between the patients included in the study and those not included, was that the former presented at the Surgery. Of those who came to the Surgery, 11.4% fell into the 'no diagnosis' group, and the remainder presented with some symptom of ill-health; and the former are clearly closer to the population not seen than are the latter. The table below sets out the numbers in each age decade in whom no diagnosis was made with the percentages of the total number, but the numbers are too small, and the trend too different for these patients to be considered as representitive of the whole practice.

Age Decades	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90+
No. of male patients	2	3	1	1	-	1	-	-
No. of female patients	15	7	4	4	4	-	1	-
%	42.8	20.0	11.4	11.4	11.4	-	2.8	-

The histograms of the patients included in the study can, to some extent, be compared with the histograms of the patients in the Practice as a whole. The male patients included in the study differ from those in the Practice by containing a smaller percentage of those in the 30 to 40 year decade, and a larger percentage of those in the 70 to 80 age group. This is to be expected, as the morbidity among those

of 30 to 40 years of age is likely to be low, and to be high in the 70 to 80 decade. Apart from this small difference, the male patients included in the study can be considered to be a representitive sample of the Practice as a whole.

The histogram of female patients included in the study varies from that of the female population of the Practice in containing a higher percentage of those between 20 and 30 years, and a lower percentage of the 40 to 70 year age group. The higher numbers of young women seen can be explained by the frequency with which young mothers attend Surgery with their children and for advice about their family, as well as with personal symptoms of ill-health. From the lower percentage of patients in the age group of 40 to 70 years who were included in the study it must be assumed that the morbidity of female patients is low in this age group. These figures apply to a three month period in the summer, in a town where during the holiday season most middle-aged women have a part-time or full-time job, and the figures might be considerably different if they were taken over the whole year, or at another season.

Results of the initial screening tests

In the initial screening programme, mid-stream urine specimens were tested from 269 female and 106 male patients, and the nitrite test resulted in 91 positive results. Four of these positives were discounted as the bacteriological report was of no growth or of heavy contamination, and of the remaining 87 urines, eight were from male patients and 79 from females. The percentage values for positive tests in the total male and female population included in the study are therefore, in males, 7.5% (2 x S.E. = 5.64) and in females, 29.3% (2 x S.E. = 5.28). The table below gives the incidence of positive tests in the various age decades, and this

table shows that there is a significant trend in the increase in the incidence with increasing age of the patient ($P < 0.0005$).

Age Decades	20-29	30-39	40-49	50-59	60-69	70-79	80-89 90+	
No. of patients	58	50	40	38	34	30	19	269
No. with +ve test	8	9	9	7	13	16	17	79
% with +ve test	13.7	18.0	22.2	18.4	36.5	53.3	89.4	
2 x S.E.	9.16	10.8	12.1	12.4	16.4	18.2	13.6	

Female patients

As there was only one patient in the age decade 90 to 99 years this decade has been combined with the previous one, and the combined totals have been used to estimate the percentage of positive tests and the standard error for the age group 80 to 100 years.

The increasing incidence of bacteriuria with increasing age agrees with the findings of several other studies (Kass, E.H., 1959; Boshell, B.R. et al., 1962; Stuart, K.L. et al. 1965), as does the smaller incidence in male patients (Kass, E.H., 1957), but the overall incidence in female patients of 29.3% requires some discussion. When the female patients are subdivided into those of child-bearing age, under 50 years, and those above this age, the numbers of patients and the percentages showing a positive urine are as shown in the table below.

	Below 50 yrs.	Above 50 yrs.	Totals
Number of patients	148	121	269
No. of +ve tests	26	53	79
expressed as a %	18.7	43.8	

S.E. of difference in percentages = 5.56, $P < 0.00009$

The observed difference between the proportions showing

positive tests in these two age groups is thus highly significant.

In the second stage of the study, urine samples were obtained from 70 of the female patients who had initially shown a positive nitrite test with a pure growth on culture, and on re-testing with the nitrite test, 46 positive and 24 negative results were obtained. The ages of these 46 patients are shown in the table below.

Age Decades	20-29	30-39	40-49	50-59	60-69	70-79	80-100	
No. of patients available	58	50	40	37	32	26	17	260
No. with two +ve tests	7	4	4	3	5	10	13	46
Expressed as %	12.0	8.0	10.0	8.1	15.6	38.4	76.4	

There appears to be rise in the incidence of two positive tests with increasing age after the age of 50 years, but the numbers are too small to afford accurate assessment. When these patients are grouped into those below the age of fifty years and those above this age, the percentages showing two positive tests are as follows,

	Below 50 yrs.	Above 50 yrs.	
Number of patients	148	112	260
No. with two +ve tests	15	31	46
expressed as a %	10.1	27.6	

S.E. of difference between percentages = 5.0 $P<0.00009$

The difference in incidence between the two groups is thus significant.

There is therefore a drop in the incidence of bacteriuria in both the age groups when urines are retested, the difference in incidence in the younger age group being 8.6% ($P= 0.045$), and in the older group 16.2% ($P = 0.007$).

Two explanations for the lower incidence obtained when

the results of the second test are related to the female patients as a whole, can be considered. Those urines which became negative on the second test might have been positive on the initial test due to contamination, and when patients were asked to repeat the collection of a mid-stream sample, the technique of collection had improved and some of the urines became negative. The 46 female patients who gave positive urines on the repeat test could then be considered to be the true bacteriurics of the study population, and the lower incidences of 10.1% for the younger group, and 27.6% for the older group, would then be the true incidence of bacteriuria in the patients included in the study. If this were the case, the figure of 10.1% for patients of child-bearing age would compare favourably with the range of 4% to 11% which has been found in other studies of patients attending ante-natal and infertility clinics. A recent study of the prevalence of bacteriuria in elderly patients gave an incidence of 26% for females and 23% for males (Dontas, A.S. et al., 1966), and this would compare with the figure of 27.6% found in this study for patients over the age of fifty years.

The other explanation is that the excretion of bacteria from an infective focus within the renal tract varies from time to time, and the result of the nitrite test will therefore vary between positive and negative. In the first phase of this study 70 urines, negative to the nitrite test, were examined bacteriologically and then re-tested and re-examined after an interval of between three and twelve weeks. In the re-test of 61 female patients, ten, or 16.4%, became positive and gave a pure growth of bacteria on culture. Considerable variation in the nitrite test reaction also occurred in the 75 urines which gave a positive test in the initial screening, and which were then re-tested on up to four further occasions. In the twenty-four patients whose urines gave positive nitrite reactions in the first and second tests, and who were not given treatment, fourteen, or 58.3%, gave one or more negative reactions in the three follow-up tests, and of the 27 patients

whose urine was positive on the first test and negative on the second, seven, or 25.9%, subsequently gave one or more positive results. Thus, of the 51 patients whose urines gave positive reactions on the first test, who were not given treatment, and who were available for further tests, 41.1% gave one or more variations in nitrite result before the conclusion of the study.

The question of whether recurrent urinary tract infections are re-infections or represent the recrudescence of a dormant infection was studied by McGeachie (1966), in a study of 49 patients over a period of four years. Infection was accepted if a bacterial count in the urine of 100,000 or more organisms per millilitre was present, and two confirmatory urine samples were obtained, where possible, at the start of the study. By the identification of different strains of E. Coli, it was shown that 84.2% of the recurrences of infection were caused by different strains of E. Coli, and it was suggested that recurrent episodes of infection were due to re-infection rather than recrudescence. In this, no difference was noted between 38 patients with no demonstrable abnormality of the renal tract, and 11 patients in whom an abnormality was present. Of the 108 incidents of infection investigated, 27.8% occurred within one month of the previous infection, and 42.6% within one to three months, and in two cases under close surveillance the bacterial count was found to rise from about 50,000 organisms per millilitre of urine to above 100,000 organisms per millilitre overnight, with the same strain of organism present on both occasions.

From the evidence available, it would seem that the bacterial count in urine can rise and fall to either side of the figure of 100,000 organisms per millilitre which is at present accepted as the significant level, and the position is further complicated by the finding that re-infections of the urinary tract can occur within one to three months of a previous infection. In this study, the results of the

initial screening tests are considered to be indicative of the numbers of patients who show a single instance of bacteriuria, but that those whose urine was positive on two consecutive tests are more likely to have an established focus of infection and to run the risk of having, or developing, some degree of renal damage.

The relationship between the presence of bacteriuria and the presenting diagnosis.

The table below sets out the numbers of male patients who showed positive nitrite tests in the initial screening, related to the presence or absence of symptoms of ill-health.

	Total No.	No. of +ve tests	%
Male patients with symptoms	98	7	7.1
Male patients without symptoms	8	1	12.5

S.E. of difference in percentages = 11.7 P = 0.64 , the difference between the two percentages is not significant.

In the 269 female patients included in the study, 35 presented without symptoms, and the numbers showing positive nitrite reactions are detailed in the tables below.

	Total No.	No. with +ve test	%
Females, 20 to 50 yrs. with symptoms	122	20	16.4
Females, 20 to 50 yrs without symptoms	26	6	23.0

S.E. = 8.8 P = 0.45, the difference in percentages is not significant.

	Total No.	No. with +ve test	%
Females over 50 yrs. with symptoms	112	49	43.7
Females over 50 yrs. without symptoms	9	4	44.7

S.E. = 17.1 P = 0.96, the difference in percentages

is not significant.

There is no difference, therefore, in the numbers of female patients in the two age groups who show positive tests, with regard to whether they present with or without symptoms of ill-health.

Significance of the time interval between the first and second test.

In order to examine the effect of the time interval which elapsed between the first two tests of those patients who initially showed a positive result, patients have been grouped into those below the age of fifty years and those above this age. As the numbers for each different time interval are small, the time intervals have been grouped into those between three and eight weeks, and those between nine and twelve weeks, and the results are set out in the table below.

		20-49 yrs.	50-99 yrs.
3 to 8 week interval	Number tested	8	31
	Number +ve	7	23
	as %	87.5	74.1

S.E. of difference = 13.9 P = 0.33

9 to 12 week interval	Number tested	20	18
	Number +ve	8	13
	as %	40.0	72.2

S.E. of difference = 15.3 P =0.035

In those patients in whom the time interval was below eight weeks, the difference in the percentage in each age group showing positive tests is not significant. In those with a time interval of over eight weeks, 72.2% of patients in the older group and 40.0% of the younger patients show positive tests, and this difference is significant. In the older group of patients there is no significant difference in the percentages showing positive tests in the two time-

interval groups, but the difference in the percentages in the younger age group is significant (S.E. = 15.8 P = 0.002).

The deductions drawn from these figures are that, in the group of patients retested, the age of the patient had no effect on the proportions showing positive tests with a time interval between the tests of up to eight weeks, and the time interval between the tests is of no importance in patients over 50 years of age. In the younger group of patients, however, a smaller proportion show positive repeat tests if the time interval is greater than eight weeks. This is in keeping with the idea that, in younger patients, infection is erradicated or the bacterial count lowered spontaneously, but that in older patients, who are more likely to have an established chronic pyelonephritis, the bacteriuria is persistent.

Relationship of positive tests to parity.

In considering the effect of parity on the incidence of positive nitrite tests, attention has also to be paid to the age of the patient, as it has been shown that the incidence varies with age. When the numbers of positive tests are related to both the age decade and the parity of the patient, the numbers in each age-parity box are too small to afford accurate deductions, and patients have therefore been grouped into those below 50 years and those above this age. The details of this relationship are set out in the table below.

	Parity						
	Unmarried	0	1	2	3	4	5+
Number of patients below 50 years	19	16	22	51	19	12	10
Number with +ve tests	2	3	2	8	5	4	3
Expressed as a %	10.5	18.7	9.0	15.6	26.3	33.3	30.0

	Parity						
	Unmarried	0	1	2	3	4	5+
Number of patients above 50 years	26	18	28	22	9	5	13
Number with +ve tests	18	8	12	5	-	1	8
Expressed as a %	69.2	44.4	42.8	22.7	-	20.0	61.5
S.E. of difference between % +ve in both age groups	11.5	15.2	11.1	10.2	-	22.4	19.8
P =	<0.00009	0.09	0.002	0.49	-	0.56	o.13

In the group of patients below the age of fifty years, there appears to be an increasing incidence of positive nitrite tests with an increase in parity over para 1 , but the variability of the percentages are such that they might occur once in ten trials purely by chance (P = 0.1) , and as such the increasing incidence observed is not significant. In considering the percentages of positive tests for both groups of patients at each parity level, the difference in percentage is considered to be significant if the P factor is 0.05 or less, and this condition is fullfilled in the case of patients in the Unmarried and Parity 1 groups.

These figures suggest that there is a tendency for the frequency of bacteriuria to increase with increasing parity, but this is not fully supported by statistical analysis, and is only evident in patients under the age of fifty years. No such pattern is evident in patients over this age, but a significantly higher percentage of older patients show positive tests in parity group 1 and in those who are unmarried. In the higher parity groups, there is no significant difference in the percentage of positive tests with age.

Bacteriological results.

During the course of this study, 252 nitrite positive urines and 233 nitrite negative urines were examined by the

bacteriology laboratory, and the presence or absence of pus cells and bacterial growth was noted.

Of the total of 375 patients screened, 42 showed the presence of pus cells in one or more of their urine samples, a percentage of 11.2 . Pus cells were found to be associated with a positive nitrite result in 34 cases, and with a negative nitrite test in 4 cases. Four other patients had pus cells associated with both positive and negative nitrite results during the course of the study. The presence of pus cells is a poor guide to the presence of infection as they were only associated with a significant bacterial growth on 38 out of 234 occasions (11.9%) , and, conversely, were associated with a negative nitrite result on 8 occasions.

A pure bacterial growth was obtained from 307 out of the 485 urine samples sent for bacteriological examination, and the details of the organisms found and their frequency are shown in the table below.

			+ve Nitrite		-ve Nitrite		
Organism	Total no.	% of total	Total	% of total	Total	% of total	'P'
E. Coli	260	84.6%	176	87.5%	84	79.2%	0.057
E. Coli & Proteus	8	2.6%	2	0.9%	6	5.6%	0.057
E. Coli & St. Albus	11	3.5%	6	2.9%	5	4.7%	0.42
E. Coli & Str. faecalis	5	1.6%	5	2.4%	-	-	-
Proteus	20	6.5%	10	4.9%	10	9.4%	0.16
B. Lactis	3	0.9%	2	0.9%	1	0.9%	-

Taking a 'P' factor of 0.05 for the difference in percentages between the positive and negative nitrite groups as being significant, the above table shows that the frequency with which the various organisms are found does not vary with the result of the nitrite test.

The different types of organisms found and the frequency of their occurrence compares favourably with the results of other studies, in particular with regard to the high percentage of E. Coli found. It will be seen that a mixed growth occurred in 24 urine samples, eleven of which gave a negative nitrite result. Although the laboratory undertook to report a culture only if it was obtained in a pure form, there is some doubt as to whether one of the organisms was in fact a pathogen, as in each instance of a culture of mixed organisms, only E. Coli was found on the next test.

During the course of the study eighteen false positives were recorded, a percentage of 7.1 , and in seven of these false positives the laboratory reported heavy growths of contaminants. A heavy mixed growth with no dominant organism present was regarded by the laboratory as contamination. In eleven of the false positives no growth was reported by the laboratory, and the only explanation for this is that there was sufficient nitrite present in the test-tube used for incubation to produce a false positive result. The test-tubes used for incubating urine samples were changed after each incubation, and then cleaned and sterilised by boiling before being used again. This was not a wholly satisfactory proceedure as , of the 241 urines giving a positive nitrite test with a growth of an organism capable of reducing nitrate to nitrite, eleven, or 4.5%, gave a false positive result in the absence of bacterial growth. The number of false positives caused by contamination of the sample was small (2.9%), and showed that it is possible to obtain true midstream urine samples from patients of all ages in General Practice by the 'clean-catch' method. The other possible cause of false positives, namely the presence of nitrite in the test-tube prior to incubation, could have been avoided if a new test-tube had been used for each new incubation.

Bacteriuria in male patients.

Of the 375 patients tested in the first stage of the study, 106 were males and, from these patients, the nitrite test resulted in nine positive and 97 negative results. One of the positive results proved to be due to contamination of the urine sample, and the relationship of the other eight to the age of the patient is shown in the table below.

Age Decade	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90+
Number of patients	15	15	20	23	16	13	4	-
Number with +ve tests	-	-	1	1	4	2	-	-
Number with two +ve tests	-	-	-	-	3	1	-	-

It will be noted that all the male positive urines were from patients between the ages of 40 and 70 years, with the highest numbers occurring in the 60 to 69 age decade, in an age group when prostatitis would be a likely cause of infected urine.

The numbers of male positive urines are too small for accurate conclusions to be reached regarding the relationship of bacteriuria to age or to previous urinary infection. If the percentages of male patients and of female patients who showed a positive test in the first phase of the study are compared, the difference is statistically significant (% of females - 29, % of males - 7, S.E. of difference = 3.6 , $P < 0.00009$). Similarly, the difference in the percentage incidence of bacteriuria in male and female patients who gave two consecutive positive results is significant (% of females - 17 , % of males - 3 , S.E. of difference = 2.6 , $P < 0.00009$).

The value of treatment.

Twenty-four patients, each of whom had given two consecutive positive nitrite tests with positive cultures, were given a course of treatment, and the results of treatment were assessed by nitrite tests and bacteriological examination two

weeks after the end of the course of treatment, and again after six weeks and ten weeks. The drug of choice was cycloserine but, where the organism identified was resistant to this drug, tetracycline was given instead.

Cycloserine is an antibiotic produced by a strain of Streptomyces Orchidaceous, and was first described in 1955. The drug is administered orally, is well absorbed from the intestine, and gives high blood and tissue concentrations. It is excreted in the urine, and as it is bactericidal to a wide range of both Gram positive and Gram negative bacteria, and as its activity is unaffected by the pH of the urine, it appears to be well suited for use in urinary tract infections. The drug is toxic if the kidney is unable to excrete it and thus allows high blood levels to accumulate, and the manifestations of this high blood level are drowsiness, vertigo, and headache, which, if treatment is not stopped and the blood level rises further, may lead to confusion and epileptiform convulsions. The ideal drug for use in urinary tract infections has been described as '...capable of oral administration, easily tolerated, well absorbed from the intestine, giving high blood and tissue levels especially in the kidney, and bactericidal to the E. Coli species', (Murdoch, J.McC. 1963a.), and cycloserine appears to be close to this theoretical ideal.

In this study twenty-one patients were given a two week course of cycloserine in a dosage of 250 mgs. twice daily, and seven of these patients were unable to complete the course due to the appearance of side-effects. Except for one patient of 21 years of age, all the patients who were unable to complete their course were over the age of 70 years, and in the case of the young patient, it is uncertain whether the gastro-intestinal upset which caused her to stop her treatment was in fact due to the cycloserine she was having. None of the six elderly patients who failed to complete the course had clinical evidence of impaired renal function, but the side-effects which caused them to stop treatment were the same as the recorded effects of a high blood level of cycloserine. Of the ten

patients over the age of seventy years who were given cycloserine, only four were able to complete their course, and this emphasises both the impaired renal function that is present in this age group, and the care that must be exercised in using this drug in elderly patients.

In this study the success of treatment is assessed in relation to the results of urine testing two weeks after the cessation of treatment, and again at ten weeks after treatment. The numbers of urines which gave both a negative nitrite test with an absence of growth on culture on the first follow-up test, and those that remained so until the third follow-up test are listed in the table below.

Patients below 50 years	1st Follow-up	3rd. Follow-up
Number treated	7	7
Number with sterile urine	4	2
Number untreated	7	7
Number with sterile urine	1	1
Patients over 50 years.		
Number treated	17	15
Number with sterile urine	2	1
Number untreated	17	15
Number with sterile urine	1	0

Male and female patients

In the younger group of patients, four out of the seven who were treated (57%) gave no growth on culture when tested two weeks after the end of treatment, as opposed to one out of seven in the corresponding untreated group (14%) . It would appear that, in the younger group of patients, treatment is of value when assessed in the short term, but statistically this is not conclusively proved ($P = 0.25$). In the longer term there is no significant difference in the numbers with sterile urine in the treated and untreated groups. The

numbers of patients in the treated and untreated groups were small, and more value would have been obtained if larger numbers of patients had formed these groups.

In the older group of seventeen patients who received treatment, and the seventeen who acted as controls, urine samples were found to be sterile in 11.8% of the treated group and in 5.8% of the untreated group, two weeks after the end of treatment. This difference in percentage is not significant (S.E. = 9.7 , P = 0.54), and it may be said that, in this group of seventeen elderly patients, no benefit was obtained from a single course of treatment. In the treated group only one patient still showed a sterile urine at the third follow-up test, and no sterile urines were recorded at this test from the untreated group. Due to the small numbers involved, little significance can be placed on these figures, but it would appear that in the older patient, little value is obtained from a single short course of treatment.

More encouraging results in both the short and long term treatment of established pyelonephritis with cycloserine have been reported by several authors. Hughes et al. (1958) reported on the results of treatment of thirty-seven patients with chronic pyelonephritis, all of whom had previously been unsuccessfully treated with most of the antibiotic drugs then available. With a dosage of 250 mgs. twice daily for one to three weeks, five patients showed a bacteriological cure (13%), twelve patients had a bacteriological cure immediately following treatment but relapsed within four weeks, two patients with two organisms present prior to treatment showed elimination of one of the organisms, and eighteen patients had positive urines following treatment (48%). In this paper, no mention was made of the ages of the patients concerned. The response to treatment of E. Coli and A. Aerogenes was found to be greater than that of Proteus or Pseudomonas, and the authors remarked on the low incidence of purely minor toxic effects, a feature which was attributed to

the low dosage used. In a paper describing the treatment of five female patients with severe and repeated attacks of pyelonephritis, also treated previously with other antibiotics, Murdoch et al. (1959) found a rapid and lasting remission of symptoms with negligable toxic effects. They showed that the levels of the drug in the plasma during treatment were considerably lower than those required for in-vitro inhibition, and they attributed the effectiveness of cycloserine to its concentration in the renal tubules. This variation between the effective in-vivo and in-vitro drug levels was also noted by Landes et al. (1960) in a paper describing the treatment of acute urinary tract infection with cycloserine, although no explanation was offered. They found that, out of 111 patients treated with 250 mgs. of the drug twice daily for two to six weeks, 85% became asymptomatic and apyuric, 62% of these obtained a bacteriological cure, while 25% had a positive urine throughout, and were thus considered to be failures. Again, the low incidence of side effects was noted.

The long term result of treatment with cycloserine was studied by Syme et al. (1961) when forty patients, of ages ranging from thirteen to seventy-eight years, with clinical evidence of chronic urinary tract infection were given 250 mgs. of the drug eight hourly for fourteen days. All patients were followed-up with urine culture 72 hours after the end of treatment, and a proportion after one, three, and six months. At each follow-up examination, 9% to 17% showed evidence of urinary infection although 90% of the forty patients had shown a sterile urine immediately after treatment. Toxic effects, sufficient to stop therapy, were noted in only two patients, but the ages of these two patients were not noted. Kubik and Datta (1961) treated 150 patients, of whom 85% were over the age of sixty years and 73% were symptomless, with cycloserine in a dosage of between 500 mgs. and 750 mgs. daily for one week, and reported the results of treatment as assessed by urine examination soon after the one week course was

completed. The urine was sterile in 50% of cases, and in 28% no improvement was noted, but the longer term results were not investigated.

Due to the small number of patients who were given treatment in this study, it is not possible to compare the results with those obtained in these various papers, but it would appear that more benefit is to be obtained from treatment of the younger patient both in the short and long term, and that both recurrence and spontaneous erradication of the bacteriuria is liable to occur in all age groups.

Relationship of bacteriuria to a history of previous infection.

A history of one or more episodes of urinary tract infection was found in the past history of about 50% of all female patients under the age of seventy years. The table below sets out the numbers of patients in the age groups of 20 to 49 years, and those above 50 years, who gave a history of previous urinary tract infection.

	Below 50 yrs.	Above 50 yrs.	
Number of patients included in study	148	121	
Number with history of infection	80	53	
expressed as a percentage	54.0	43.7	P = 0.09
Number of patients +ve in Stage 1	27	52	
Number with history of infection	17	21	
expressed as a percentage	62.9	40.4	P = 0.03
Number of patients +ve in Stages 1 and 2	15	30	
Number with history of infection	11	12	
expressed as a percentage	73.3	40.0	P = 0.01

Female Patients

It can be seen that, in the case of female patients included in the study, the difference in the numbers in each age group who

give a past history of urinary tract infection is not significant. In the case of those female patients who were positive in Stage 1 and those who were positive in Stage 2, a significantly higher percentage of the younger age group gave this previous history. The numbers of elderly patients who admitted to a previous urinary infection were small, and the numbers who showed positive nitrite tests were large as compared with younger patients, and, as has been stated above, the number of patients who admitted to a previous renal history may be inaccurate due to forgetfulness about previous infections.

In the group of patients under the age of fifty years, there is no significant difference between the numbers who gave a previous history of infection in those who showed a positive test in Stage 1 compared with the female study population as a whole ($P = 0.36$), and similarly, between those who showed positive tests in both Stage 1 and Stage 2, and the study population ($P = 0.11$). Again, there is no significant difference between the numbers with a positive test in Stage 1 and a history of previous infection, and those with a positive test in Stage 1 and Stage 2 and a history of infection ($P = 0.50$). In the group of older patients there is no significant difference between any of the three percentages quoted in the table on Page 90. From these figures it may be said that a history of previous infection was not found more frequently in those with positive nitrite tests.

The value of the Study.

This study of certain aspects of asymptomatic bacteriuria has been of great interest and value to the author. It has provided information about the pattern of bacteriuria in a group of adult patients of all ages who presented for consultation at the Surgery. Although some similarity exists between the distribution in age of the patients seen and those in the Practice as a whole, those included in the study cannot be regarded as representitive of the Practice population as a whole. In this

respect, the actual figures and percentages must be taken as referring to the particular group of self-selected patients included in the study, but the overall relationships of bacteriuria to age, sex, and parity compare favourably with the results published in other studies.

If bacteriuria is to be diagnosed by one isolated finding of a positive urine, as determined by both the modified nitrite test and a positive growth on culture, then in this group of patients, the incidence is 7.5% among adult males of all ages and 29.3% among females. These figures are high, and compare only with the figures obtained by Kass (1957) from certain groups of hospital in-patients, and by Mestitz (1965) in certain selected patients from General Practice. If the conditions for diagnosing bacteriuria are made more strict and two consecutive positive urines are required, the incidence in male patients becomes 3.7% and in females, 17.7%. If the female patients are divided into those of child-bearing age and those above this age, the percentages become 10.1 in the younger group and 27.6 in the older group, and these figures are more comparable with other published results.

Throughout this study the number of patients whose nitrite results varied between positive and negative was impressive, particularly as in most cases, a pure growth of the same organism was obtained regardless of the result of the chemical test. It seems likely that patients who have a focus of infection within their urinary tract and who are excreting bacteria in their urine, may vary the numbers of organisms that are excreted from time to time. The numbers of bacteria in the voided urine could then alternate between a figure below 100,000 organisms per millilitre and a figure above this level, and the significance of a negative nitrite result would be in doubt. A negative nitrite result could then mean one of three things - that the urine was sterile,

that the urine contained organisms of a number below 100,000 per millilitre which were contaminants in the urine, or that the urine contained organisms of a number below 100,000 per millilitre which were pathogens released from an infective source within the urinary tract, and whose numbers might be found to be more than 100,000 per millilitre if the urine were to be tested a few days or a few weeks later.

As all chemical tests for bacteria in the urine are designed to become positive at the accepted 'significant' level of organism concentration, doubts must be expressed as to the reliability of their use in screening programmes. A positive chemical test is of value, but a negative result, if of variable interpretation, would require a bacterial count to indicate whether the bacterial level in the urine was approaching the significant level, or whether it was so low as to indicate that contamination was more likely. If bacterial counts have to be made to test the validity of a chemically negative result, there is little point in utilising the chemical method.

If the concept of a varying bacterial excretion rate is accepted, then a bacterial count should be done on all urines in a screening programme, and the importance of the count assessed in relation to other factors, such as the sex, age, and parity of the patient. In doubtful cases, repeat urine testing would be necessary, or the repeat test could be evaluated by a leucocyte count after the injection of a febrile substance such as Pyrexal or Pyrasid (Padelt, H. et al. 1965). Alternatively, if laboratory facilities were limited, screening programmes could be limited to those patients considered to have an increased risk of harbouring an asymptomatic bacteriuria, such as those with a repeated history of acute infections, elderly patients, young patients of high parity, diabetics, and those who were pregnant.

The question of contamination of the urine specimen can

be eliminated by the use of the suprapubic bladder puncture (Beard, R.W. et al., 1965) , but this method, although relatively simple in operation, would only be suitable for the screening of a small number of patients at a hospital department and has no place in domiciliary practice. This study has shown that patients can be relied on to carry out simple instructions prior to the collection of a mid-stream sample, and contamination did not prove to be an important cause of false positive results.

As a result of this study, a policy has been developed in the author's Practice for the identification and treatment of asymptomatic bacteriuria. A full screening programme, using quantitative bacteriological methods will be started when hospital laboratory facilities in the area can cope with the large number of urine specimens that would be obtained. At present, screening is limited to patients attending ante-natal and post-natal clinics, to diabetics, and to those with a past history of repeated episodes of acute urinary tract infection, and quantitative bacteriological methods are used rather than chemical tests. If bacteriuria is discovered, or an acute infection proved by bacterial counts, a two week course of an appropriate antibiotic is given and further urine analysis is done two weeks after the end of the course of treatment, and again four weeks later. If both the follow-up tests are negative the patient is screened six months later, and a further course of treatment given if infection is again discovered. All patients under the age of sixty years who again relapse, and all those in this age group who have persistently positive urines, are investigated more fully, in particular with regard to intravenous pyelography, haemoglobin, blood film, and blood urea estimations. In the absence of any surgically remediable pathology, treatment, preferably with cycloserine, is then continued on a long-term basis, until three consecutive monthly urine specimens are found to be negative. Elderly patients are not routinely screened as they are more likely to have an established infection which is

more resistant to treatment, and it is more necessary to detect and treat the younger patient who might otherwise progress to an established pyelonephritis. Although this programme will fail to discover some who have a symptomless bacteriuria, it is hoped that some reduction will be made in the numbers of patients who would eventually present with one or more of the complications of chronic pyelonephritis.

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APPENDIX

STATISTICAL METHODS

In experiments in clinical medicine, results are complicated by the fact that variable factors such as sex, age, height, weight, and others cannot be controlled, and attention must be paid to several factors in assessing the results. No two patients are alike, and no two react in exactly similar fashion to disease or treatments, and it is necessary to apply statistical methods to the interpretation of figures which may be influenced by a variety of variable factors.

In this study, as many variables have been controlled as possible by subdividing patients into age decades, into the two sexes, or into parity groups, and then discussing a particular result in groups which are, as far as possible, comparable. What cannot be controlled is that variations in results might have occurred by chance, and, while the statistical methods used cannot prove or disprove a result, they can indicate how likely the result was due to chance and therefore how much significance can be placed on it.

Where a single percentage result has been given, as in the percentage number of patients in each age group showing bacteriuria, the Standard Error (S.E.) has been calculated to show how far the sample value is liable to differ from the true value in the population sampled (Bradford Hill, A.,1966, Principles of Medical Statistics, 8, Chap. 10.). The S.E. is estimated from the equation $S.E. = \sqrt{\frac{p \times q}{n}}$ where p is the percentage in one category, q is equal to 100- p , and n is the number in the sample.

When a difference in percentages has been found from two comparable samples, the S.E. of the difference has been estimated from the equation $S.E. = \sqrt{\frac{p \times q}{n_1} + \frac{p \times q}{n_2}}$ where

as before, p and q are the percentages in each category and n_1 and n_2 are the numbers in the two samples. It is generally accepted that the observed difference is significant if it exceeds twice the standard error but, as this is only an approximate guide, the 'P' or Probability factor has been estimated in addition. The 'P' factor is estimated from the figure obtained by dividing the observed difference in percentages by the standard error of this difference. From the normal distribution tables, a value for this figure is obtained, which, when subtracted from unity gives the Probability factor. The generally accepted significant value for 'P' is 0.05, which means that the variation would have been obtained as a result of the play of chance, once in twenty samples, and although any critical value for 'P' can be adopted, this is the value that has been used in this study. The degree with which the estimated 'P' factor differs from 0.05 gives a more accurate estimation of the significance of the result (Bradford Hill, A., 1966, Principles of Medical Statistics, 8, Chap. 12; Documenta Geigy, 1962, pps. 28-29.).

When a series of figures have to be interpreted, as in the incidence of bacteriuria with age or with parity, the chi-squared test is applied. Two steps are required, firstly to test whether the numbers in each group vary from each other in a way that would have been unlikely to have arisen by chance, and secondly to test the significance of an observed progression of the figures. Having found that the figures do differ from each other in a significant manner, the order or trend of the figures is then evaluated and the 'P' factor estimated. Again, this test only gives an assessment of how likely the progression would have arisen by chance (Bradford Hill, A., 1966, Principles of Medical Statistics, 8, Chap. 14.).

In the estimation of the standard error, the numbers in the sample are important. With percentages in the

25 to 75 range, the S.E. estimation is accurate for samples of 30 or more, but if the percentages are outwith this range larger samples are required to maintain accuracy. With small numbers, as in the estimation of the results of treatment in the two small groups numbering seven and seventeen patients, the exact treatment of the four-fold table has been applied using Yate's correction, and this affords an accurate method of estimating the 'P' factor (Bradford Hill, A., 1966, Principles of Medical Statistics, 8, Chap. 14 ; Fisher, R.A., 1944, Statistical Methods for Research Workers, 9, Chap. 4).

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