

LABORATORY STUDIES ON BACTERIURIA

by

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INTRODUCTION

INTRODUCTION

Capot and Grabarec (1916) in a review of the aetiology and pathology of non-tuberculous renal infections stated that "the literature of the subject is stupifying both in quantity and in complexity, and anyone who has attempted to master it will, we think, be convinced of the fact that it is more likely to confound than to enlighten the reader. There is no subject in which there is so little uniformity of opinion and so much confusion." This statement is even more relevant when applied to the present day literature on this subject.

INTRODUCTION

Records show that 4000 B.C. Babylonian physicians made observations on the colour and consistency of urine. Examination of urine as a valuable aid to the diagnosis of human disease was also recognised early by the Greeks. Hippocrates (460 to 377 B.C.) wrote one of the first organised accounts of diseases of the urinary tract in his Prognostica and Aphorisms. The physicians of these times, however, confined their examination of the urine to observations of changes in colour, transparency, quantity, sediments, odour and even taste of urine. In England a book, written by Dr. J. Fletcher in 1541, on the Differences, Causes, and Judgments of Urine, states that "amongst all signs of sickness and health whereby the skillful physician is

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led into the knowledge of the state of the body two are of most general and certain signification which are taken from the pulse and urine, without which all the knowledge of physicke besides is obscure, doubtful, and uncertain" (Dukes, 1939).

Although the spontaneous ammoniacal fermentation of freshly voided urine left exposed to the air was recognised for centuries, and microscopic creatures were seen in it by the lenses of Leeuwenhoek from 1675 onwards, these creatures were thought to be yet another example of the so-called "spontaneous generation of life". In other words, they came into the world spontaneously, as the result of the combined action of heat, water, air and putrefaction. Leeuwenhoek himself, however, was an opponent of the spontaneous generation theory but believed that the source of these "animalcules" was the air, where they existed in the form of seeds.

Theodor Schwann (1810 to 1882) should be regarded as the founder of the germ theory of putrefaction and fermentation. In a crucial experiment he showed that it was not the air itself which brought about putrefaction but something in the air. However, the name of Louis Pasteur (1822 to 1895) will remain as the father of the science of modern bacteriology, particularly on the theory of fermentation. Pasteur also recognised the usefulness of urine as a culture medium and grew anthrax bacilli in it (Bullock, 1938).

A very accurate and up-to-date description of the significance of bacteria in the urine, the difference between contamination and true multiplication of bacteria within the urinary tract is given in an article by Roberts in 1881 which appeared in the British Medical Journal. I can do no better than to quote relevant parts from this article entitled "On the occurrence of micro-organisms in fresh urine." "The fresh and healthy urine is perfectly free from bacteria or other minute organisms. But there are conditions in which the urine, at the moment of emission, contains bacteria, that is to say, conditions in which the urine breeds, or becomes contaminated with, bacteria during its sojourn in the urinary passages." Roberts also recognised the entity of true bacteriuria which was asymptomatic, and as being especially common among women. He stated that this condition may persist for years without symptoms, but its presence renders these subjects liable to a graver form of urinary infection, with abundant discharge of pus and sometimes blood during micturition. The present concept of the ascending route in the pathogenesis of urinary infection was also embodied in the following statement: "We must assume that the offending organisms gain access to the bladder from without by the urethra. In the female, the short and comparatively wide urethra offers obvious facilities to wandering bacteria to penetrate into the viscus from the external

genitals. In the male, the long and narrow urethra forbids this mode of entrance in the healthy state. A dirty catheter is a most efficient infective agent." Yet it was 75 years later that Kass (1956) succeeded in focusing attention on the importance of significant asymptomatic bacteriuria.

REVIEW OF LITERATURE

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etiology of Urinary Infections

The relative frequency of the occurrence of any single bacterial species as a pathogen in the urinary tract is largely dependent on the clinical material studied. In most series of uncomplicated urinary infections including acute and chronic pyelonephritis reported, Escherichia coli or closely related organisms are the commonest pathogens isolated. In uncomplicated urinary infections this organism alone accounts for about 70 to 80% of the infections. In chronic or complicated infections the frequency of occurrence of other bacterial species, Staphylococcus aureus, Pseudomonas, staphylococci and enterococci, is increased. These complicated infections of the urinary tract occur in association with congenital anatomical abnormalities of the urinary tract, instrumentation and catheterisation, and conditions involving incomplete evacuation of the bladder, as in paraplegia. A single species in pure culture is found in 80 to 100% of cases of acute uncomplicated infections, whereas in complicated infections, this occurs in only about 20% of the infections (Kass, 1955; Coleman and Taylor, 1949; Erlanson and Jansson, 1953). Coleman and Taylor (1949) examined the causative organisms in 50 uncomplicated urinary infections and found that Escherichia coli accounted for 82% of these

REVIEW OF LITERATUREAetiology of Urinary Infections

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infections whereas in 40 complicated infections only 18% were caused by this organism.

In symptomatic or asymptomatic infections of the urinary tract during pregnancy the commonest causative organism is Escherichia coli, which again accounts for 70 to 90% of these infections (Kincaid-Smith et al., 1964; Turner, 1961). Recently it has been suggested by a number of workers that a comparatively few serological groups within the 145 known 'O' groups of Escherichia coli are more invasive within the urinary tract and that these cause clinically evident pyelonephritis more frequently than other groups. The term "nephropathogenic" strains has been suggested to these serogroups (Rantz, 1962; Ujvary, 1958; McGeachie, 1965). However, Turck and Petersdorf (1962) maintained that these serogroups occurred more frequently in urinary infections only because they were also more prevalent in the environment and argued against special nephropathogenicity of Escherichia coli strains.

Pathology of Pyelonephritis

Since the classical works of Weiss and Parker (1939) and Longcope and Winkenwerder (1933), most pathologists have come to recognise a morphological complex in renal biopsy or in post-mortem material as "chronic pyelonephritis", with the implication that such a disease was

the result of bacterial infection. Much of the present confusion in this field of pathology exists because in a proportion of these cases at autopsy no bacteria are seen in the kidney tissue or urine, although the histological picture is otherwise similar. Most of these cases are also undiagnosed during life. This group should perhaps be called "inactive" chronic pyelonephritis (Kass, 1965). There is a further possibility that in a proportion of cases of "active or infective" chronic pyelonephritis, diagnosed on the basis of a co-existent positive urine culture at autopsy, this bacterial infection has only been a terminal event before death, superimposed on a kidney already scarred by some other process. Perhaps some of the considerable variation which is seen in the percentage incidence of chronic pyelonephritis in various reported consecutive hospital autopsy series can be attributed, in part, to confusion among pathologists as to what they call "chronic pyelonephritis". To quote a few of these reported incidences, Kimmelsteil et al. in 1961 found a 2.8% incidence among 3,393 routine hospital autopsies, Jackson et al. in 1955 reported a figure of 9% out of 4,425 autopsies and Rhoads et al. in 1952 reported a figure of 20%. The true figure probably lies somewhere between 3 and 9%.

The derivation of the word "pyelonephritis" from Greek is ΠΥΕΛΟΣ = pelvis, ΝΕΦΡΟΣ = kidney, ΤΙΣ =

inflammation, and hence is defined as a pathological condition resulting from the inflammation of the kidney and pelvis, primarily due to bacterial invasion of the renal substance. Where such an aetiology cannot be definitely implicated, the term "inactive" as suggested by Kass (1965) is a useful classification. However, different authors mean one or more of the following by the term "pyelonephritis", e.g. a kidney lesion resulting from phenacetin abuse, a clinical syndrome or a radiologic appearance.

At the Second International Symposium on Pyelonephritis in 1964 held in Boston, Kass (1965) suggested that it would be useful to start by accepting the criteria laid down by Weiss and Parker in 1939.

Weiss and Parker based their conclusions on the study of 100 selected cases of pyelonephritis with necropsies. In 86 of these there was an adequate clinical history and post-mortem examination failed to reveal any other complicating renal pathology. On the basis of the histological picture, pyelonephritis was classified into four groups, all of which represent various stages of the same disease:

1. Acute pyelonephritis.
2. Chronic pyelonephritis.
3. Healed pyelonephritis.
4. Healed and recurrent acute pyelonephritis.

This last diagnosis was not feasible unless a

detailed clinical history was available. The authors concentrated mainly on the study of the chronic stage of the disease with particular emphasis on the vascular lesions encountered and their relationship to the development of arterial hypertension.

Acute pyelonephritis

The clinical picture was often not sufficiently specific to indicate an acute inflammation of the kidney; at times bacteraemia and a tendency to anaemia were the main features. At post-mortem examination the kidneys were usually enlarged, with small abscesses under the renal capsule. Microscopically there was pus within the tubules and an infiltration of leucocytes in the interstitial tissues. It was primarily an inflammatory disease of the interstitial tissue. Bacteria might be present in the tubules or in the interstitial tissue. In almost all cases diagnosed during life as "pyelitis" there was an inflammation of the renal pelvis and parenchyma.

Chronic pyelonephritis

The clinical history could be one of persistent bacteriuria and pyuria for many years following an initial acute attack of pyelonephritis. In one group the only clinical manifestations could be a loss of appetite, pallor, lassitude and obscure fever occurring at intervals and lasting a few days. In this group the diagnosis was made only after culture of the urine,

which was often positive. In this condition, "arterial hypertension frequently develops" and death was usually caused by uraemia. At post-mortem examination the kidneys could be normal, enlarged or reduced in size. The larger kidney was seen particularly in association with hydronephrosis. The renal surface was irregular with scars and nodules which represented areas of compensatory dilatation of the tubules. The scars were 'U'-shaped in contrast to 'V'-shaped scars of vascular origin. The renal capsule was adherent and stripped with difficulty, tearing the tissue with it. Microscopically the scarred areas contained tubules filled with "colloid casts" and lined with flattened epithelium, the so-called "thyroidization" of the kidney. The glomeruli showed concentric pericapsular fibrosis. The interstitial tissue was increased in amount and contained lymphocytes and plasma cells and, if the process was still active, also polymorphonuclear leucocytes. The non-scarred areas could be essentially normal. The authors attach special significance to the vascular changes which were characterised by proliferative, hyperplastic, arteriosclerotic, arteriolar changes rather than the degenerative hyaline arteriolar changes associated with benign nephrosclerosis. The authors estimate that in about 20% of cases of malignant hypertension pyelonephritis was aetiologically responsible. However, this question is still widely debated.

Hence it is not possible, on morphological grounds alone, to determine whether any particular case has a bacterial aetiology, but the presence of neutrophils and plasma cells is taken as suggestive evidence to define these cases as "chronic active pyelonephritis". Since this involves histological examination of both kidneys, to prove this diagnosis in a living patient is impossible. It is alarming to consider that only about 20% of the cases diagnosed as chronic pyelonephritis at autopsy are detected before death (Jackson et al., 1955; Rhoads et al., 1952). This situation can be remedied to some extent by epidemiological follow-up studies on a group of patients with an added risk of developing chronic pyelonephritis, namely, patients with chronic asymptomatic bacteriuria without clinical evidence of renal involvement. Young pregnant women make an ideal catching ground for such a "high risk" study population. This would also help us in understanding the pathogenesis of the chronic "inactive" pyelonephritis, to which at present it is not possible to assign a definite aetiology.

Healed pyelonephritis

The clinical diagnosis of healed pyelonephritis was difficult. In the majority of cases this represented an end stage of acute focal pyelonephritis. At post-mortem examination the kidneys were usually reduced in size and showed multiple scars in the cortex with

nodular areas between them. The variation in the size of the two kidneys and the irregular size of the scars differentiated this kidney disease from nephrosclerosis and glomerulonephritis. On microscopic examination the presence of colloid casts, which were indicative of non-functioning nephrons, marked infiltration of the inter-tubular tissue with lymphocytes and plasma cells, the presence of pericapsular fibrosis and inflammation of the renal pelvis were seen.

Healed and recurrent pyelonephritis

The clinical and histological changes here represented a combined picture of healed pyelonephritis and acute or chronic "active" pyelonephritis. Clinical separation of this group from chronic pyelonephritis was often not possible.

It is worth reviewing the observations made by Brun et al. (1965) on renal biopsy material from 79 patients with a clinical diagnosis of chronic pyelonephritis. Fifty-seven per cent. of these patients had bacteriuria at the time of the biopsy. On culture of the material from renal biopsy, bacteria could be grown in only 24 of them (30.4%). But in a control group of 105 renal biopsies on various non-bacterial nephropathies only 5 showed growth on culture, presumed to be due to bacterial contamination during the procedure. The authors stated that definite histological changes were present in nearly

all patients with clinical pyelonephritis and impairment of a 24 hour creatinine clearance test to less than 70 ml. per minute. All 48 patients with a kidney function below 20% by creatinine clearance test, showed pathological changes in the renal biopsy specimen. The criteria used for the diagnosis of chronic pyelonephritis on the histology of the biopsy material were primarily the infiltration of the interstitial tissue with plasma cells and lymphocytes, and "less seldom" polymorphonuclear leucocytes. Casts containing polymorphonuclear leucocytes, although regarded as a more specific sign when present, occurred in only a third of the patients with renal function below 20%. Although periglomerular fibrosis was considered pathognomonic of chronic pyelonephritis, this change was absent in a fourth of the specimens from advanced cases. Tubular dilatation with a "thyroid-like" appearance was found more frequently in those with severe reduction in kidney function. They concluded that these changes were to a large extent non-specific and no single change was present in all specimens. They felt that presence of cell casts and infiltration of interstitial tissue with polymorphs were the most valuable diagnostic criteria. The authors claimed that their technique was superior to the Vim-Silverman technique since it picked up a larger amount of kidney tissue. However, the value of a biopsy of the kidney in a disease process which may be focal and

patchy in distribution, or even unilateral, is open to question!

Pathogenesis of Infections of the Urinary Tract as evidenced by Laboratory Animal Experimental Methods

Our knowledge of the factors involved in the pathogenesis of upper or lower urinary tract infections in man are grossly inadequate. We can, however, draw some conclusions from common clinical factors found in association with upper and lower urinary infections in man. Some clues may also be gained from the animal experimental models used, which are unfortunately beset with many drawbacks.

Animal experimental models

In order to obtain a satisfactory animal experimental model on which to study the disease as it occurs in man, we ideally need the following: (a) a satisfactory, small, laboratory animal in which spontaneous pyelonephritis occurs with some frequency; (b) the causative organism used in the animal model should be similar to the causative organism in man; (c) these organisms should be easily isolated from the animal and be amenable to antibiotic therapy; and (d) there should be evidence, in some cases, of physiologic or pathologic factors which precipitate the disease in the animal.

However, so far, there is no well documented example of

the occurrence of spontaneous urinary infection in animals. Even in the few cases claimed to be so infected, the causative organism is far removed from the human pathogen, e.g. Corynebacterium renale. Animals in fact seem to be singularly resistant to experimentally induced urinary infection. To establish a renal disease in them usually requires quite drastic interference with the flow of urine, along with the administration of large numbers of bacteria, usually by the intravenous route. Bearing in mind the above limitations to the application of these results to the pathogenesis of the human disease, some of the methods used and results obtained may be reviewed.

Lepper in 1921 carried out the classical experiment, in rabbits, and showed that a kidney from which the flow of urine was obstructed, due to ligation of one ureter, developed pyelonephritis if E. coli in large numbers was injected intravenously. The non-obstructed, normal kidney remained uninfected. This experiment has been repeatedly confirmed by subsequent workers, who used various minor modifications in the actual method of producing an obstruction to urine flow. Intravenous injection of Escherichia coli alone in the normal animal, unlike injection of Staphylococcus aureus, does not produce renal infection.

Guze and Beeson (1958), in rats and rabbits, produced a partial ureteric obstruction by irradiation of

the abdomen. This was verified by subsequent intravenous pyelography done in these animals. These animals were then challenged intravenously with an Escherichia coli in one group and a coagulase negative staphylococcus in another group. Renal infection occurred in only about 20% of the animals with partial ureteric occlusion of one ureter, whereas in animals with complete ureteric obstruction almost all developed pyelonephritis. Similar results were obtained in rats and rabbits irrespective of the organism used.

The factors operating in the obstructed kidney that predispose to infection are not clear. Asher and Sokol (1941) postulated that stasis of the renal circulation in an obstructed kidney was the factor responsible for the localisation of circulating bacteria and infection in this kidney. This claim has been challenged by Guze and Beeson (1956) and Brumfitt and Heptinstall (1958). Guze and Beeson challenged rats intravenously using Escherichia coli 24 hours after ligation of one ureter and did whole organ counts on both kidneys at various intervals after challenge. There was no difference in the numbers of organisms trapped in the obstructed and unobstructed kidney until 4 to 6 hours after intravenous challenge, when the number of organisms in the obstructed kidney rapidly increased. Brumfitt and Heptinstall obtained similar results also using rats. Hence these findings lend no support to the concept that there is an

initial increased deposition of organisms in the obstructed kidney.

De Navasquez (1956), in his model using rabbits, produced an intrarenal, as opposed to the usual ureteric, obstruction. An intravenous injection of Staphylococcus aureus, unlike Escherichia coli, can produce pyelonephritis in the normal unobstructed rabbit kidney. These animals recover, with scarring of the kidneys. It was found that subsequent intravenous injection of Escherichia coli produced pyelonephritis only in those animals with pre-existing renal scars due to previous staphylococcal infection. Histologically, in the affected kidneys the purulent exudate was confined to the distended tubules of the scarred areas. It was suggested that the tubular obstruction in relation to the pre-existing scar tissue caused an "intrarenal hydro-nephrosis" and subsequent infection.

Further experimental evidence seems to indicate that obstructive lesions on the renal medulla are more prone to infection in comparison with similar lesions situated in the renal cortex. Beeson et al. (1957), using rabbits, showed that a small obstructive lesion produced in the renal medulla by means of an electric cautery rendered an entire segment of the kidney, associated with the tubules passing through that portion, susceptible to subsequent coliform infection. These coliform organisms could be injected intravenously or

introduced into the urinary bladder. The challenge by the former route produced an infection in 85% and by the latter route in 50% of the animals used. A similar injury in the renal cortex failed to render these kidneys susceptible to coliform infection. Freedman and Beeson (1958) also made observations on the relative susceptibility of the renal cortex and medulla to infection. They inoculated coliform bacilli directly into each of these zones in rabbits. Introduction of fewer than 10 bacilli into the medulla resulted in an infection, whereas 10^5 or more were usually required to establish a similar infection in the renal cortex. The area of infection following a medullary inoculation was wedge-shaped, including both medulla and cortex, apparently involving all nephrons which drained through the site of medullary injury. The lesion that followed a cortical inoculation was an abscess, confined to the site of inoculation.

Rocha et al. (1960) studied the susceptibility, in rats, to haematogenous coliform pyelonephritis following a previous chemical injury to the kidneys. The chemicals used were mercuric chloride in one group of 20 rats and sodium-N-acetyl sulphathiazole in another group of 34 rats. These animals were challenged by the intravenous administration of about 10^8 organisms of Escherichia coli about 2 to 6 weeks after the above chemical renal damage. A control group of animals was

also sacrificed about 2 and 6 weeks after chemical renal damage alone. The group of rats with sulphathiazole-induced renal damage showed an increased susceptibility to subsequent development of coliform pyelonephritis whereas the group with mercuric chloride-induced renal damage did not develop coliform pyelonephritis. The authors believed that one, if not the main, reason for this difference was the site of renal damage induced by these two drugs. Mercuric chloride produced damage confined to the convoluted tubules of the renal cortex with no detectable alteration of the medulla. Sodium-N-acetyl sulphathiazole, on the other hand, was deposited in the collecting tubules in the medulla with resulting intra-renal obstruction and an increased susceptibility to coliform infection.

The common factor in most of these procedures used to produce experimental pyelonephritis is tubular obstruction. The way in which such obstruction causes an increased susceptibility to infection is not evident. Rocha et al. (1958) held the view that tubular obstruction did not act by preventing the excretion of organisms from that kidney segment since the kidney, normal or obstructed, was a poor trap for circulating bacteria since only one organism in 10,000 lodged in the kidney. There was no evidence that these trapped bacteria were excreted in the urine via the tubules unless, like staphylococci, they were capable of setting

up an infection in a normal kidney. They suggested that obstruction might lead to increased susceptibility to infection through biochemical changes in the cells and in the fluid around the tubules, but they made no comment as to the nature of these changes.

Only in the kidney do Escherichia coli produce chronic and often intractable infection. Beeson and Rowley (1959) investigated the peculiar properties of kidney tissue in comparison with other tissues and made a very interesting observation. Kidney tissue was unique in its capacity to interfere with the killing of Escherichia coli by normal serum. This mechanism of interference was mediated through the inactivation of the fourth component of complement, probably due to ammonia formed in the kidney tissue. Ammonia in the kidney is largely derived from glutamine, this process being activated by the enzyme glutaminase I and also in the presence of acidosis. The authors suggested that in circumstances of greatly increased renal ammonia production, as in acidosis, complement inactivation might become sufficient in some parts of the kidney to permit bacterial growth and hence a true infection. They also commented on pyelonephritis being more common and severe in diabetics, in whom there was likely to be an increased production of ammonia due to acidosis.

Kass (1959) succeeded in producing progressive, bilateral pyelonephritis in normal rats by a retrograde

ascending route of challenge. He used a strain of Proteus vulgaris which when introduced into the bladder of a normal rat produced bilateral kidney infection within 24 hours in 34% of the animals challenged. If a glass bead was also introduced into the bladder the infection rate rose to 70% of the animals challenged. In addition, if one of the ureters was ligated and divided, infection developed only in the opposite kidney, indicating that the organism probably reached the kidney through the lumen of the ureter.

Jackson et al. (1965) also made attempts to initiate renal infection, through the retrograde route of challenge, in the kidney of a normal animal. They used a strain of Klebsiella and produced persistent bacteriuria and pyelonephritis in about 60% of the animals. A retrograde infusion of the culture of the organism into the urinary tract was made by placing the hub of a 20-gauge hypodermic needle, from which the shaft had been removed, firmly against the urethral orifice of female rats, and injecting with minimal pressure. This procedure resulted in a reflux of urine from the bladder into the ureters and renal pelvis in more than 50% of the animals. It was claimed that the above procedure did not produce anatomical damage or significant bacteraemia. Initially it was believed that the capsule and fimbriae of the Klebsiella were important factors in establishing an adhesion and proliferation of the organisms on the

bladder wall or renal pelvic epithelium. Unfortunately, they did not investigate this further but remarked that, later, similar renal lesions were produced with a non-encapsulated, 'K'-negative strain of Escherichia coli.

Pathogenesis of Human Renal Infection

Much argument and discussion has been devoted to the exact route by which bacteria lodge in the human kidney. Beeson (1956) has reviewed some of the relevant literature on this subject. Bacteria can reach the kidney by the ascending route through the urethra, bladder and ureters or by the descending route, through the blood stream. Theories are also put forward that bacteria travel directly from the lumen of the large intestine to the kidney through lymphatic channels and also that they may travel from the lower to the upper urinary tract through lymphatic vessels rather than through the lumen of the urinary passages. These latter claims lack convincing experimental proof (Franke, 1910; Sweet and Stewart, 1914; Eisendrath and Kahn, 1916; Winsbury-White, 1933).

The descending or haematogenous route of infection could be considered likely in only a small proportion of patients with renal infection. Although bacteraemia occurs in a significant proportion of the patients undergoing instrumentation or an operation of the urinary

tract (Barrington and Wright, 1930), this route is unlikely in the majority of patients where no abnormality of the urinary tract is demonstrable. It is also worth recalling that in animals a severe degree of obstruction to the flow of urine is usually necessary before a kidney infection can be established with Escherichia coli injected by the intravenous route.

An ascending route is at present thought to be the most likely mode of human renal infection in the majority of patients. Several findings lend support to this claim. The greater incidence of urinary infection among females, in childhood and adult life, has been amply confirmed in many population surveys (Kass, 1956; Kunin, 1960, 1964). One of the main factors which would account for this female preponderance and favour an ascending route of infection is the fact that the urethra in the female is short and relatively straight, being only about 4 cm. in length, in comparison to a length of 20 cm. in the average male. It is also well established that the lower few centimetres of the urethra is far from sterile. Helmholtz (1950) reported recovery of bacteria at a depth of 5 cm. in 49% of 72 normal male urethras. Shackman and Messerant (1954) state that the normal urethra may contain E. coli, bacilli of the Proteus group, Streptococcus faecalis, and staphylococci. Beeson (1956) is in no doubt that organisms which cause pyelonephritis in man originate in

the intestinal tract since similar organisms are isolated from both sites. Jensen (1952) could reduce the incidence of urinary infection following urological operations if he first reduced the intestinal bacterial flora by prior oral antibiotic therapy. Although exact figures are not available, it is also well recognised that there is a high incidence of acute pyelonephritis in children between 6 months and 3 years of age: the female to male ratio is about 3:1 (Dunn et al., 1964). In this age group an ascending infection favoured by a soiled diaper is thought to be causative. More study of urinary infection in this age group is needed.

Heptinstall (1950), in experimental studies in the rat, failed to confirm this claim. The role of fimbriae and

Factors involved in the Initiation and Perpetuation of Urinary Infection in Man by an Ascending Route

Although pathogenic organisms are introduced into the normal human bladder from time to time from the lower urethra, only about 1 to 4% of a normal population subsequently develop a urinary infection. A number of factors seem to operate in the normal healthy person which helps to eradicate bacteria that have gained entry into the bladder. Some of these factors may be related to the virulence and pathogenicity of the infecting organism, the factors of complete or incomplete evacuation of the bladder contents during micturition, local defence mechanisms operating on the bladder wall such as

the presence of specific antibodies or the presence of opsonic substances in the urine which would accelerate phagocytosis of invading organisms. We do not have answers to many of these questions at the present time.

Virulence of the infecting organism

The finding that a relatively small group of about 10 Escherichia coli serotypes are found more commonly in urinary tract infections needs further study. Do these strains have a special ability to initiate urinary infection? Kauffman (1947) stated that Escherichia coli strains containing the 'K' or envelope antigen were more pathogenic in the human urinary tract. Brumfitt and Heptinstall (1960), in experimental studies in the rat, failed to confirm this claim. The role of fimbriae and its relationship to pathogenicity of Escherichia coli strains has not been fully investigated. The fimbriate variants could perhaps adhere to the bladder mucosa and resist evacuation during micturition.

Local defence mechanisms in the bladder

The work of Cox and Hinman (1965) has made a substantial contribution to our understanding of the factors involved in the resistance of the normal bladder to infection. The salient features of their experiments are:

(a) The rate of growth of bacteria in urine in vitro was similar to the rate of growth of the same organism in nutrient broth. This fact has been known and made

use of by Pasteur who used urine as a culture medium. They concluded from this experiment that the urine, under normal conditions, contained no antibacterial substances.

(b) Using an experimental flask model of the human bladder, and a subject voiding directly into it, the growth rate of an inoculum of Escherichia coli introduced in the flask was determined during the next 48 hours.

It was found that although emptying the flask contents at each micturition reduced the bacterial counts, this was insufficient to completely remove bacteria from the simulated flask bladder. This they attributed to the small amount of residual urine containing bacteria, which remained in the flask, enough to wet the inside walls and perpetuate infection.

(c) About 8 million organisms of Escherichia coli were introduced into the bladders of each of 4 human volunteers by means of a urethral catheter and the rate of growth of these bacteria was compared with the multiplication in vitro in a simulated flask bladder of a similar inoculum; the contents of the flask were emptied at each micturition. The bacteria in the human bladder, unlike the simulated bladder flask, were inhibited in growth and completely eliminated by 72 hours. This they concluded was due to two factors, namely, voiding and a specific vesical factor inhibiting bacterial growth. This vesical antibacterial factor was not present in the urine and seemed to be more effective the longer the

invading bacteria were in contact with the bladder wall.

The authors further investigated the mode of infection of the bladder following minor degrees of bladder dysfunction, which were not obvious as anatomical changes in the urinary tract. Normal voiding consists of a co-ordinated action between three muscular components, namely, perineal relaxation, reduction in muscular resistance in the urethra and contraction of the bladder detrusor muscle. The authors put forward radiological evidence for an inco-ordination in these factors resulting in a two stage or "hesitant voiding" in which, due to intermittent perineal relaxation combined with active urethral resistance, the urethra was partially filled with urine or radiographic contrast medium which was then emptied back into the bladder, thus washing bacteria from the distal urethra into the bladder and initiating infection.

In the normal female the bladder emptied all but about 1 ml. of urine, which wet about 25 sq. cm. of bladder surface. If, however, there was about 10 ml. of residual urine initially containing only 100 bacteria, the calculated number of bacteria in the bladder after 24 hours, allowing for about 400 ml. voidings at four hour intervals, was 7×10^7 organisms! Hence the presence of residual urine which may be due to incomplete bladder contraction, or as a result of vesico-ureteral reflux fostered and perpetuated urinary infection.

MacGregor and Wynne Williams (1966) investigated the incidence of residual bladder urine after voiding in a group of children with recurrent urinary infection, unassociated with any obstruction. In a series of 63 children on whom micturating cystograms were performed 17 or 27% showed the presence of residual contrast medium in the bladder in an X-ray film taken 24 hours after cystography. They also described a simpler modification of the phenol-red test which could be used as a screening-test in these patients to reveal those who should have a micturating cystogram besides an excretory pyelogram. The authors felt that in this group of children with chronic residual urine and recurrent infections a variety of minor causes may have led to a habit of incomplete emptying of the bladder. Urethritis, or a meatal ulcer, incomplete micturition in a hurrying child, repeated bladder overdistension in class could be some of these causative factors. The female child, with a shorter urethra and susceptibility to urethritis, would be specially at risk, as confirmed by clinical experience. The device of "triple micturition" described by Stephens (1963) was particularly recommended as a means of reducing residual bladder urine and controlling infection.

Reflux could be caused by congenital ureteral anomalies, in which case it could predispose to urinary infection, or it could be a

Vesico-ureteral Reflux

It is now generally accepted that a reflux of urine from the bladder into one or both ureters during micturition does not normally occur in the healthy individual. This is due to a valve mechanism at the junction of the entry of the ureters into the bladder which prevents regurgitation of bladder urine into one or both ureters occurring during the contraction and evacuation of the bladder contents (Edwards, 1965; Hutch et al., 1965). Yet even this explanation is not unanimously accepted. Forsythe and Whelan (1958) suggest that vesico-ureteric reflux occurs in normal individuals, and Johnston (1962) claims that volunteers for micturating cystography have so far been either young children or convicted criminals! It further appears that the only satisfactory method of demonstrating the presence of reflux is by micturating cysto-urethrography, the bladder being filled to capacity via a urethral catheter, with contrast medium, and cine or still films being taken of the resting bladder and the whole act of micturition (Caine and Edwards, 1958).

Reflux may occur due to various causes but only two need be considered here as contributing to the development of urinary infection. Reflux could be caused by congenital ureteral anomalies, in which case it could predispose to urinary infection, or it could be a

secondary phenomenon due to an inflammatory process of the bladder wall with resulting oedema between the bladder mucosa and the muscle wall, thus rendering a marginally competent valve temporarily incompetent. Whether it is the cause or effect of infection, there seems little doubt that the presence of reflux contributes to kidney infection as well as to residual urine in the bladder at the end of micturition, which again acts as an infecting inoculum of bacteria. It is interesting here to review briefly the incidence of demonstrable reflux in various reported series of urinary infection. Edwards (1961) examined 55 patients with radiological signs of chronic pyelonephritis and demonstrated vesico-ureteral reflux in 42 or 76% of them. Edwards (1965) also examined 165 children with urinary infection and found reflux in 56 or 34% of them. In 22 or 40% of these children with demonstrable reflux a radiologic evidence of chronic pyelonephritis was also present. Hutch et al. (1965) also reported reflux in 92 out of 190 (48%) children with urinary infection. These workers, however, felt that their patients were selected since many of them were known to have reflux before investigation. It was concluded that reflux was present in about half the number of children with recurrent urinary infections. Hanley (1962) studied 50 patients with an acute or subacute cysto-urethritis of a non-specific aetiology

and observed a transitory occurrence of reflux in 10 of them. He felt that this could be a fleeting occurrence and would be missed unless the cystogram was performed under X-ray intensifier control. This was thought to be caused by oedema, with some resulting rigidity of the vesico-ureteral orifice, and was a temporary phenomenon.

Vesico-ureteral reflux could cause pyelonephritis of pregnancy. Hutch et al. (1961) obtained micturating cystograms in 12 pregnant women during an attack of acute pyelonephritis that occurred during the latter half of pregnancy and puerperium and demonstrated reflux in 5 of them. Bunge (1953), on the other hand, obtained cystograms in 24 normal pregnant women during the last trimester of pregnancy and was unable to demonstrate reflux in any of them.

Evidence in favour of a causal rather than a coincidental relationship between the presence of reflux and development of chronic pyelonephritis is offered by Williams (1962). He examined 24 patients with complete duplication of the ureter and duplex kidney. In 2 patients this congenital anomaly was bilateral, hence 26 such kidneys were studied. Twenty-five kidneys showed pyelonephritis of the lower pole and where micturating cystograms were performed, a vesico-ureteric reflux into this lower pelvis was demonstrated. The author concluded that since the ureter draining the upper kidney pelvis usually entered the bladder nearer the bladder

neck, it had a longer intramural course through the bladder wall and hence a better valvular protection against the occurrence of reflux.

Congenital Faulty Development of the Nephron

The work of Darmady and Stranack (1961) suggests that the faulty development of the nephron as seen by a technique of microdissection of the kidney at post-mortem examination may be associated with pyelonephritis in children. He examined 4 children from two families between the ages of 4 and 6. All these children died in early life with a history of recurrent pyelonephritis. On microdissection of these kidneys there appeared to be an undue number of premature proximal convoluted tubules, some of which showed a "cystic" change. Similarly microscopic dysplasia of tubules has been suggested as a primary cause of focal scars on the surface of adult kidneys (Marshall, 1956). Hence it is conceivable that these congenitally faulty nephrons could be a source of an intramedullary tubular obstruction and an increased susceptibility to bacterial infection, leading to healing and scar tissue which further continues the cycle of events. This could account for some cases of clinically undetected instances of pyelonephritis occurring in infancy.

Excretion of Urine that is a better Medium forBacterial Growth

Jackson and Griebble (1957) compared the growth of Escherichia coli and Enterococcus in a pool of urine from normal individuals and another pool of urine from 22 patients with urinary infection. The resulting growth was expressed as a percentage of the maximum growth obtained in an "enriched bacteriologic medium". After 3 hours of incubation at 37° C. it was noted that growth was more rapid in urine from patients with urinary infection. However, the initial inoculum and subsequent growth was not quantitatively estimated and the authors suggested that the composition of urine could make a person more vulnerable to infection by encouraging a faster growth of bacteria.

Roberts and Beard (1965) investigated the efficiency of urine as a culture medium at different stages of pregnancy and puerperium in comparison with urine from non-pregnant women. All these women were healthy. At the start of each experiment about 1,000 organisms from a 24 hour culture of Escherichia coli in peptone water were inoculated into 25 ml. of midstream Seitz-filtered urine from each patient. The pH was measured using a universal indicator and a pour plate technique was used to estimate the viable count at the beginning and after 6 hours' incubation at 37° C. The results of analysis

of about 80 patients indicated that the mean bacterial count of Escherichia coli after 6 hours of incubation in the urine from pregnant women was double that which occurred in the urine from non-pregnant women. This increase was greatest shortly after delivery and declined to the non-pregnant rate 6 weeks after delivery. It was interesting to note that the mean residual bladder urine at the end of micturition in antenatal women was similar to the non-pregnant, being about 3 ml. However, 2 days after a spontaneous delivery this mean residual volume was 33 ml. This increased residual bladder urine after delivery could account for the incidence of symptomatic infection in asymptomatic bacteriuric patients seen at this time.

Presence of an Auto-immune Mechanism in the Kidney

A morphological similarity in the histology of transplanted kidneys and chronic pyelonephritis has suggested an aetiological similarity (Simonsen et al., 1953). So far the search for the presence of auto-antibodies in the sera of bacteriuric and chronic pyelonephritic patients has been unrewarding (Kramer et al., 1961).

DiagnosisThe Concept of "Significant Bacteriuria"

Kass (1956) developed the concept of true or significant bacteriuria by counting the numbers of viable organisms present in 1 ml. of urine. Initially this sample of urine from female patients was collected by catheterisation, but similar and reproducible results were later obtained by examining carefully taken mid-stream voided specimens. By means of quantification of the numbers of bacteria in the urine it was possible to distinguish contamination of the urine by bacteria, during its passage through the urethra and perineum, from a state of actual multiplication of bacteria within the urinary tract. A count of 10^5 organisms per ml. or above was used as an arbitrary dividing line to distinguish between these two categories. Thus any normal population examined in this way falls into a bimodal distribution curve with the majority of them having counts below 10^4 organisms per ml. of urine. A few fall in the doubtful range between 10^4 and 10^5 per ml. and need further examination. The number of patients found to have significant bacteriuria (10^5 or more organisms per ml. of freshly voided urine) is a function of the clinical material studied. Kass (1956) found that 95% of 75 patients in whom a clinical diagnosis of pyelonephritis had been made had urine bacterial counts

of over 10^5 organisms per ml. An additional 3% had counts between 10^4 and 10^5 per ml. Using these criteria it is now possible to determine the incidence of true or significant bacteriuria in any population group and it enables us to detect patients who have no symptoms of urinary infection, the so-called "asymptomatic bacteriurics", in a population. Symptoms in infections of the urinary tract are a very poor guide to the presence of significant bacteriuria. Hence quantitative methods have now been widely accepted as a routine method of examining urine for the presence of infection (see later).

Pyuria

The significance of the presence of pus cells in voided urine specimens as an indication of inflammation of the urinary tract is widely accepted. However, the meaning of pyuria differs from laboratory to laboratory. The arbitrary finding of five or more pus cells per high power field of the microscope, using a centrifuged specimen of urine, is notoriously prone to variables. The speed and time of centrifugation is one of the factors which is liable to vary from one laboratory to another and one person to another. Kass (1956) estimated that pyuria thus defined occurred in only 33% of women with significant bacteriuria.

White cell excretion rate

The best method at present of detecting small

significant increases in the excretion of white cells in the urine is by determining the white cell excretion rate, expressed as the number of white cells excreted per ml. of urine per hour. Addis (1925) originally described the technique whereby it was necessary for women to be catheterised and also hold their urine for 12 hours! During this holding period a number of white cells were lysed and the errors of sampling were greatly multiplied (Houghton and Pears, 1957).

Hamburger et al. (1950) described a more simplified technique. They estimated an excretion rate of 30,000 cells per hour as the normal maximum. No mention was made as to the number of normal individuals examined.

Houghton and Pears (1957) examined urine specimens from 41 normal males and 28 normal females aged between 20 and 64 years and estimated that the normal white cell excretion rate varied between 18,000 and 196,000 cells per hour. There was no correlation between the rate of urine flow and the cell excretion rate. Hutt et al.

(1961) examined 60 normal male and female subjects and only 2 of them had excretion rates over 200,000 per hour.

However, Houghton and Pears and Hutt et al. catheterised their female patients. The risk of infection following a single catheterisation is estimated to be about 4 to 6% (Marple, 1941; Brumfitt et al., 1961). Little

(1962) estimated the white cell excretion rate in normal men and women. He, however, used a "clean" midstream

sample of urine instead of a catheterised specimen from women. Fifty men and 17 women were examined. The white cell excretion rate in men ranged from 0 to 220,000 cells per hour, with a mean excretion rate of 46,000 cells per hour. In normal females the range was 0 to 574,000, only 2 of whom had an excretion rate above 300,000 cells per hour. The mean here was 74,000 cells per hour in women. The upper limit of normal urinary white cell excretion is hence taken as 400,000 per hour (Bradley and Little, 1963).

Bradley and Little (1963) examined 28 patients with acute pyelonephritis, as diagnosed by the presence of loin pain, loin tenderness and fever and a positive urine culture. In all of them the cell excretion rate was over 400,000 per hour. However, out of 32 patients with chronic pyelonephritis, as evidenced by a renal biopsy or intravenous pyelography, only 18 had a raised cell excretion rate. Similarly, out of 29 patients with significant but asymptomatic bacteriuria only 14 had a raised cell excretion rate.

Little (1964) compared a simplified technique, namely, the estimation of "urinary white cell concentration", with the above standard white cell excretion rate in 958 specimens of urine. The urinary white cell concentration was estimated by counting the number of white cells per cu. mm. in a counting chamber using an uncentrifuged, midstream specimen of urine. There was a

good correlation between the above cell concentration and the cell excretion rate in these urines. If more than 10 cells per cu. mm. was the concentration the excretion rate was always greater than 400,000. Also if the concentration was below 3 per cu. mm. the excretion rate was always below 400,000. But in the range of 3 to 10 cells per cu. mm. the correlation was variable. This simplifies the procedure of the test considering that even a cell excretion rate at best gives only a 50% indication of the presence of renal infection. The incidence of an increased cell excretion rate in 17% of urines from control normal adults, that is, a false positive cell excretion rate, is also alarming (Kennedy, Ormonde and Murdoch, 1964).

Value of provocation tests

Pears and Houghton (1959) were pioneers in this field when they noticed that 17 patients with chronic pyelonephritis, unlike normal individuals, had a markedly increased urinary white cell excretion rate following the intravenous injection of a purified lipopolysaccharide pyrogen. This substance, "Pyrexal", which was derived from a strain of Salmonella abortus equi, appeared to induce an inflammatory reaction which could not be correlated to factors such as reactivation of a latent focus of renal infection, increase in the urine flow, changes in the renal blood flow or fever. However, in 2 of these 17 cases bacteria were seen in

the urine following the injection where none was found before. The authors could give no evidence as to which part of the renal tract these white cells originated from except to say that, since the women were catheterised, it came from a level above the urethra. They felt that it was probably a manifestation of the hypersensitivity of an infected tissue to an unrelated bacterial product, similar to the Shwartzman reaction.

Little and De Wardener (1962) modified the above test by using prednisolone phosphate intravenously instead of pyrogen. A positive provocation test was here defined as a rise over the pre-injection cell excretion rate of 100% or above, to a final excretion rate over 400,000 per hour within 3 hours after injection. This test was positive in only 8 out of 27 patients with chronic pyelonephritis as evidenced by a positive intravenous pyelogram and/or renal biopsy evidence. The pyrogen provocation test was less successful in 18 of these patients who were tested by both methods. The side effects of headache, backache, malaise and occasionally vomiting and rigors were an added drawback following the pyrogen injection.

In acute pyelonephritis the above provocation test is of no value until the very high excretion of white cells and bacteria has subsided. Little and De Wardener (1962) felt that this test was a good indicator of the response to treatment in these cases. The provocation

test was not positive following successful therapy, presumably because the inflammation had subsided. They suggested that the test be done 1 or 2 days after cessation of treatment.

In patients with significant bacteriuria of pregnancy the incidence of increased white cells in the urine varies somewhat with the methods used. Kincaid-Smith and Bullen (1965) estimated the white cell excretion rate in 120 pregnant women with bacteriuria and found that 80% of them had excretion rates above 200,000 per hour. However, 8% of 50 pregnant women without bacteriuria also excreted over 200,000 cells per hour. They found that they could predict a clinical attack of pyelonephritis in several patients by a rise in the cell excretion rate as well as the urine bacterial count. Brumfitt and Percival (1964) examined 163 women with bacteriuria of pregnancy by estimating the white cell concentration and found that 25% had counts below 3 cells per cu. mm., indicating a normal cell excretion rate.

Hence, as a screening programme these tests are time-consuming and laborious and detect only about 70% of women with significant bacteriuria. Moreover, a raised cell excretion rate gives no definite indication as to the anatomical site of inflammation within the urinary tract. As with other diagnostic methods in this disease, a negative cell excretion test or provocation

test does not exclude active renal disease. When other tests are negative a positive provocation test may give an added clue in a problematic case. Kennedy et al. (1964) felt that, done as a routine procedure, these tests were of limited value.

Gram stain

Although the presence of bacteria in the Gram stain of an uncentrifuged specimen of urine usually indicates the presence of significant bacteriuria, it is not a very reliable guide. Kass (1956) found only 80% of urines with significant bacteriuria to give a positive Gram stain. The Gram stain was also falsely positive in 20% of cases with viable counts below 10^5 organisms per ml. The value of a Gram stain is particularly dependent on the skill with which the slide is stained and interpreted. The results, when the technique is left to inexperienced technicians, are of doubtful value.

Collecting Methods used to obtain Specimens of Urine from Antenatal Women

Kass initially determined the incidence of significant bacteriuria in pregnant women by examining catheterised specimens of urine. He found that after a single catheterisation about 2% of these female patients developed frank dysuria and true bacilluria (Kass, 1956). Later, Kass discarded the use of catheters for collection

of specimens and a "clean voided" urine specimen was used instead. Patients were asked to clean the perineum with soap and water before voiding into a sterile beaker (Kass, 1960).

Monzen et al. (1958) compared the rates of contamination using three methods of urine collection in a group of 34 patients. In each patient a suprapubic needle aspiration of the distended bladder was followed by a midstream voided specimen, followed again by a catheterised specimen of urine. The following results were obtained:

Method of urine collection	Count of organism per ml. of urine		
	0 to 5	5 to 10^5	Over 10^5
Aspiration	27	2	5
Catheter	23	6	5
Clean-voided	6	23	5

The above results indicated that colony counts below 10^5 per ml. were usually due to contamination in a voided specimen. It was also significant that when counts over 10^5 per ml. were considered all three methods of collection were equally good.

Although it is now widely accepted that a clean voided specimen is sufficient to estimate the presence of significant bacteriuria in pregnant women, the degree of preparation of the perineum before voiding has given rise

to much argument. It is worth recalling some of the so-called simple preparative techniques used, if only to illustrate how impractical these methods would be at a busy antenatal clinic where about 90 women are seen in one afternoon. Boshell et al. (1958), for example, described these steps - the patient at first sits in a sitz bath filled with a green soap solution and washes the anogenital region, followed by cleaning of the urethral orifice with sponges soaked in "zephiran", patient then moves to a commode, separates the labia and collects the middle of a stream of urine.

The findings of Turner (1961), that there was no significant difference in the incidence of contaminated urines from a group of 200 "prepared" women and 200 women with no preliminary cleansing procedure before collection of a midstream specimen of urine, has been a further practical simplification in antenatal collection procedures.

Sleigh et al. (1964) confirmed these findings. Kincaid-Smith et al. (1964, 1965) collected midstream specimens of urine from 3,000 antenatal women without any prior cleansing and found that when the presence of significant bacteriuria was confirmed in two consecutive urine specimens this state usually persisted throughout pregnancy, if untreated.

Screening Methods

The use of quantitative culture methods to screen large numbers of routine antenatal women, for the purpose of detecting patients with significant bacteriuria is impractical. Hence methods of simplified, semi-quantitative culture or chemical screening tests are now widely used.

The advantage of chemical screening tests is that they need only about 4 hours of incubation and that they are usually interpreted adequately by technical assistants and do not necessarily need the services of trained bacteriologists. The various semi-quantitative bacteriological screening procedures, on the other hand, necessitate the use of culture media and the facilities of a bacteriological laboratory and hence are not applicable in the surgeries of general practitioners or even schools or large industrial establishments. Their results also need to be interpreted by a trained bacteriologist particularly in recognising heavily contaminated urines with a high bacteriological count.

However, irrespective of the particular semi-quantitative method used, this method does not replace quantitative culture methods. In other words, the presence of significant bacteriuria in a positive screening test should always be followed by confirmatory quantitative culture techniques. As a first step these

simplified screening procedures make the examination of about 500 individuals in a day quite within possibility. Semi-quantitative bacteriological screening methods used for detecting significant bacteriuria

It is worth noting that these methods are still routinely employed in many laboratories in this country as the only bacteriological culture method used in the examination of infected urines. Here a loopful of uncentrifuged urine is plated out on MacConkey and blood agar plates and the resulting growth is reported as "scanty growth", "moderate growth", "growth" or "heavy growth". Bradley and Little (1963) compared the above routine technique with a quantitative pour plate technique of culture on 205 urine samples. Fifty-six of these having more than 10^5 organisms per ml. by the quantitative technique were reported as having "growth" in 50, "scanty growth" in 4 and "moderate growth" in 2. Out of 134 specimens with a count of less than 10^4 organisms per ml., 53 were also reported as having "growth". It was evident that here a "growth" did not distinguish between contamination and a true infection. The situation was not improved by identifying the causative organism since 41 out of the 53 urines with less than 10^4 organisms per ml. contained a urinary pathogen. Standard loop culture

Here a fused wire loop of usually 2 mm. internal diameter is immersed into a well mixed, undiluted

specimen of urine and discharged as fully as possible on to the surface of well dried solid culture medium. The number and type of colonies are counted after overnight incubation. Cattell and Leeford (1963) related the results of culture by the above method to the results of a quantitative pour plate culture technique. A urine containing 10^5 bacteria per ml. by the pour plate technique gave about 176 colonies using the standard loop technique. Only 2 out of 38 urine specimens with counts over 10^5 per ml. gave less than 176 colonies by the loop method. Guttman and Stokes (1963) used a loop with an external diameter of 5 mm. and also found close agreement between the quantitative and semi-quantitative culture technique used.

McGeachie and Kennedy (1963) used a slightly modified technique whereby a standard loopful of undiluted urine was plated out across one edge of the plate to a width of about 1 cm. Using a second sterile loop another 1 cm. wide area was plated out at right angles to the first. This procedure was carried out twice more to give a final box-like inoculum. One hundred and sixty-five specimens of urine were cultured by the above method and also the quantitative pour plate technique. There was good correlation between the two techniques in urines with high and low bacterial concentrations. 85.8% of urine specimens containing more than 10^5 organisms per ml. produced growth on three or

four sides of the box in the above "stroke-plate" method.

Brumfitt and Percival (1964) claimed that better results were obtained with the technique of Ryan, Hoody and Luby (1962) of using strips of sterilized absorbent filter paper, $\frac{1}{4}$ inch wide with a fold $\frac{1}{2}$ inch from one end, folded at an angle to form a "foot". This foot is dipped into the urine, excess drained off by touching the side of the container, and an impression of it made on well dried MacConkey agar. A growth of 30 or more colonies from such an inoculum corresponded to 10^5 or more organisms per ml., and no colonies to less than 10^4 organisms per ml. The above test puts any growth below 30 colonies into the doubtful range of 10^4 to 10^5 organisms per ml. although the mixed character of colonies in a heavily contaminated urine could be easily distinguished. Using this method Leigh and Williams (1964) were able to detect 96% of 60 urines with significant Gram negative bacteriuria and 77% of 9 urines with significant Gram positive bacteria, an overall positive detection rate of 94%. However, the low counts obtained if only part of the "foot" of the paper was placed on the medium and also a falsely high count given if a few seconds were not allowed after sampling to allow the urine to be completely absorbed into the paper brings in added variables into this technique, which at best can only be regarded as semi-quantitative.

Chemical Screening Tests in the urineThe nitrite test

Cruickshank and Moyes (1914) first noticed that nitrites were not present in normal urine, but were associated with the presence of coliform bacteriuria. This nitrite, when present, is a result of bacterial action on the substrate nitrate which is present in varying quantities in normal urine. Hence the presence of nitrite in a specimen of voided urine usually indicates significant bacteriuria of over 10^5 organisms per ml. The presence of nitrite is detected by the Ilosva modification of the Greiss test, which consists of the addition of a solution of alpha-naphthylamine and sulphanic acid in 30% acetic acid to 1 ml. of freshly voided urine. A concentration of nitrite as low as 1 microgramme per ml. of urine causes the formation of a pink to red colouration in a few seconds. The colour is formed by a diazotization reaction in which a red azo dye is formed (Fregl, 1960). When this test is applied to a series of voided urine specimens containing more than 10^5 organisms per ml. only 40 to 80% of them give a positive test. This figure has varied from 35% (Kincaid-Smith et al., 1964), 40% (Sleigh et al., 1964), 50% (Smith et al., 1961), to 80% (Turner, 1961). Some of the reasons for the occurrence of the false negative nitrite tests are:



- (a) that the substrate nitrate is absent in the urine due perhaps to an inadequate dietary intake;
- (b) that nitrate is present in such small amounts that during the period of incubation in the bladder it is reduced to nitrite which is further decomposed by bacterial action resulting in a negative test;
- (c) voiding frequently, as occurs in clinical urinary infection, so that bacteria lack the necessary 4 to 6 hours to reduce nitrate to nitrite when their concentration in the urine is about 10^5 organisms per ml.

Some of these shortcomings in a direct nitrite test, performed on voided urine, have been overcome by the modification introduced by Sleigh (1965). Here excess substrate in the form of a solution of sodium nitrate is added to the specimen of voided urine, which is then incubated in a water bath at 37° C. for 4 hours, before testing for the presence of nitrite in the usual way. By this method 69 out of 71 (97%) of the urines with significant bacteriuria gave a positive screening test, whereas out of 912 urines with counts of less than 10^5 organisms per ml., only 1.6% gave a false positive test.

The ability to reduce nitrates to nitrites is dependent on the action of an adaptive enzyme, nitrate reductase, possessed by Gram negative bacilli belonging to genera Escherichia, Klebsiella, Citrobacter, Proteus, Salmonella, Shigella, Pseudomonas aeruginosa, Staphylococcus aureus and albus. This property is not

possessed by enterococci and hence in chronic infections caused by this organism or by a slow nitrate reducer like Pseudomonas the above test may be unsuccessful.

The Triphenyl Tetrazolium Chloride (T.T.C.) test

This chemical test depends on the ability of respiring bacteria to reduce a colourless alkaline solution of triphenyl tetrazolium chloride to a red precipitate form. This test was originally claimed to detect about 90% of infected urines (Williams and Simmons, 1963). Leigh and Williams (1964) reported that in a later series this test detected only 81% of significant Gram negative bacillurias and only 74% of all urines with significant bacteriuria including Gram positive cocci. Other reports on the use of this test include detection of 86% out of 184 infected urines by Kincaid-Smith et al. (1964), 75% of 71 infected urines by Sleigh (1965), using 10^5 or more organisms per ml. as the criterion of infection. The preparation of the T.T.C. reagent was laborious and when the results were interpreted even a slight red or pink colouration of the precipitate was regarded as positive. This could be missed by an inexperienced eye, specially in a darkly coloured urine.

Other chemical screening tests include the disc floatation test for the detection of catalase of infected urines described by Brande and Berkowitz (1961), which

was far less successful than the above tests (Kincaid-Smith et al., 1964).
 Mallinckrodt Pharmaceuticals in the United States have marketed a "stat-nitrite test" using a stabilised nitrite test reagent. Here the patient dips an ampule containing the reagent into a specimen of voided urine and observes any immediate pink colouration of the cotton wool plug. Hence it is feasible that a normal population could take an oral tablet of sodium nitrate and test the first morning specimen of urine, thus conducting their own survey, and report to the general practitioner's surgery for confirmation of any positive test.

The Importance of Bacteriuria in Clinical Medicine

Kass et al. (1965) estimated that about 10 to 20% of the total female population would experience a urinary infection at some time during their life. This makes significant bacteriuria the commonest human bacterial infection, when the communicable diseases are excluded.

Significant bacteriuria and active pyelonephritis appear to be causally related. Animal experiments and studies on human autopsy material and follow-up studies on pregnant women with significant asymptomatic bacteriuria lend support to this claim (Macdonald et al., 1957; Kass, 1960). Acute pyelonephritis of pregnancy

is a preventable disease, if treatment is instituted early in pregnancy (Kass, 1960; Little, 1965; Kincaid-Smith and Bullen, 1965). There are about 850,000 deliveries in Great Britain every year. It is estimated that about 21,000 women suffer from acute pyelonephritis during pregnancy every year (Little, 1965). These and many other related considerations emphasise the importance of urinary infection and pyelonephritis in clinical medicine. The best way to study and tackle this vast problem is first to detect the population at risk by determining the incidence of significant bacteriuria before these have developed into symptomatic disease.

Incidence of bacteriuria of pregnancy

Dodds, as early as 1931, examined 406 antenatal women and estimated that 7.6% of them had persistent coliform bacteriuria. It was further noted that this condition preceded a clinical attack of urinary infection in many instances. The interest in this field was revived and put on a more scientific basis by Kass in 1956 when he defined significant bacteriuria on a quantitative basis as the presence of 10^5 or more viable organisms per ml. of urine. Kass estimated that about 7% of women attending the routine antenatal clinic at the Boston City Hospital had significant bacteriuria, which was silent or asymptomatic. He further established that

the majority of this infection was acquired by the second month of pregnancy. Turner (1961) in Aberdeen reported a 7% incidence, Sleigh et al. (1964) in Edinburgh reported a 6.6%, Little (1965) 5.5% in the London area, and Kincaid-Smith and Bullen (1965) in Australia also reported a 6% incidence among pregnant women. The need to confirm the presence of significant bacteriuria in two consecutive specimens is stressed by most workers in this field since about 20 to 30% of those found to have significant bacteriuria at the first visit are found to be negative when a subsequent urine specimen is examined (Little, 1965; Kincaid-Smith and Bullen, 1965). It is particularly important to identify the asymptomatic patients with significant bacteriuria since the others with urinary symptoms may be detected and treated by the clinicians.

Acute pyelonephritis and asymptomatic bacteriuria of pregnancy

Kass (1960) established, by means of a controlled trial, that pyelonephritis of pregnancy was a preventable disease. Forty per cent. of an untreated group of pregnant women with significant bacteriuria developed acute pyelonephritis in the last trimester or the immediate postpartum period of pregnancy. None of the patients in a similar concurrent group of women treated with sulphonamides throughout pregnancy developed this

disease. This finding has since been widely confirmed. Little (1965) noted an incidence of acute pyelonephritis in 36.6% of the untreated and in only 5% of the treated group. Kincaid-Smith and Bullen (1965) also reported similar comparative figures from Australia. There is little doubt about the urgency in treating this group of asymptomatic women if only to reduce the incidence of acute pyelonephritis which in itself is a painful and debilitating condition and usually requires admission into hospital for care and treatment. It probably also carries with it risks to the mother and foetus, the nature and magnitude of which are still widely debated.

Renal tract abnormalities and bacteriuria of pregnancy

Renal function tests

Winberg (1959) first drew attention to an early loss of urine concentrating ability in children with clinically mild urinary infections. He reported that 17 out of the 22 children tested showed a subnormal renal concentration capacity, which was restored to normal following successful therapy. This defect was attributed to damage to the parts of the nephron situated in the renal medulla, mainly to the distal tubules and collecting ducts. The finding that a creatinine clearance test done on these children was within normal limits in all but 2 of them suggested that the glomerular filtration rate, in these mild infections, was relatively

normal. This test for renal urine concentration capacity consisted of measuring the osmolal concentration of a specimen of urine obtained after a 12 to 17 hour period of water deprivation, combined with an injection of pitressin tannate in oil. Since the concentrating capacity of the kidneys is limited by the total number of dissolved particles (osmolal concentration) and not by the weight of urinary solutes a measurement of the osmolality, rather than of the specific gravity, is a more correct index of the renal concentrating power. The probable damage to the distal medullary parts of the nephron in these instances raises the possibility of subsequent scar formation causing micro-obstructive lesions in the kidney with a resulting susceptibility to further infections. It also shows that even in clinically mild urinary infections the renal parenchyma may be involved.

Kaitz (1961) made the observation that 45% of pregnant women with asymptomatic bacteriuria showed an impaired urine concentrating ability. Here an inability to concentrate urine above 700 milli-osmols per kilogram of water was chosen as the point of demarcation between normal and impaired concentrating ability. Norden and Tuttle (1965) and Elder and Kass (1965) have confirmed these findings in pregnant women with significant bacteriuria. Norden and Tuttle also observed that the above defect was corrected following successful chemo-

therapy. Bacteriuria was not associated with alterations in either creatinine clearance, osmotic clearance, or tubular reabsorption of water. These studies suggest that an intrarenal infection was probably causative in producing a renal concentration impairment in these women with bacteriuria of pregnancy.

Kincaid-Smith and Bullen (1965) tested the renal concentration capacity in a group of bacteriuric and another control group of non-bacteriuric women in the post-partum period but could detect no significant difference in these two groups. Vasopressin was administered and the authors felt that there probably was some degree of resistance to vasopressin at this time of pregnancy. Blood urea levels were, however, significantly raised in the bacteriuric group when tested during pregnancy. The creatinine clearance results in this series were discarded since, in a large number of cases, the specimens were not complete 24 hour collections.

Radiological evidence of renal involvement in bacteriuria of pregnancy

Kincaid-Smith and Bullen (1965) reported the incidence of radiological renal tract abnormalities in 51% of 148 women with bacteriuria of pregnancy when they were examined 6 weeks after delivery. Even if only the major renal abnormalities, such as calculi, chronic

pyelonephritis, duplex kidneys and renal papillary necrosis, were considered 30% of the radiographs from the bacteriuric group fell into one of these categories. A difference in renal length of 2 cm. or more was regarded as abnormal in the above series since Hodson (1960) did not see a difference of this magnitude among 500 patients with normal renal radiographs.

Pinkerton et al. (1961) re-examined 43 women who had had pyelonephritis of pregnancy 5 years previously and found some abnormality in the intravenous pyelograms in 19 of them. However, the significance of a number of these abnormalities such as "fundal indentation of the bladder" could not be determined.

Other Complications thought to be related to

Bacteriuria of Pregnancy

1. Toxaemia and hypertension

Peters, Lavietes and Zimmerman (1936) reported that 11 or 44% of 25 patients with known toxaemia of pregnancy revealed the presence of pyelonephritis at autopsy.

They also reviewed the hospital records of 320 patients with toxaemia and found only 41 or 13% with a clinical evidence of pyelonephritis. The failure to recognise the role of pyelonephritis in toxaemias of pregnancy was thought to be due to the fact that in a great proportion of them clinical signs of such an infection were

insignificant or even entirely lacking. The authors saw little justification for the casual attitude with which pyelitis of pregnancy was regarded by obstetricians and suggested that something more than the usual prenatal urine and blood pressure examination be done if pyelonephritis was to be recognised.

Finnerty (1956) again drew attention to the finding that pyelonephritis could "masquerade as toxæmia" during pregnancy. He examined 1,130 antenatal women with toxæmia at weekly intervals till delivery. This examination, at a special clinic, included a complete urine analysis and culture every week. Seventy-three women, with no urinary symptoms, had pyuria and a positive urine culture. With chemotherapy, in addition to the improvement in the urine findings, toxæmia and persistent albuminuria in this group also cleared up. Finnerty felt that this was a preventable toxæmia and only a diligent urine examination could reveal this otherwise asymptomatic group.

Stuart et al. (1965) investigated the possible relationship between symptomatic or asymptomatic bacteriuria and the hypertensive disorders of pregnancy in 88 bacteriuric and 729 non-bacteriuric control women in Jamaica. Hypertensive disorders in this study included patients who developed a diastolic blood pressure above 100 mm. of mercury for the first time during pregnancy and patients with hypertension occurring

in association with albuminuria, oedema, and/or convulsions. 18.2% of the bacteriuric and 4.9% of the non-bacteriuric patients developed hypertensive disorders of pregnancy. Kass et al. (1961) earlier reported higher blood pressure levels in Jamaican women with bacteriuria in comparison to non-bacteriuric controls in the general population. They suggested that bacteriuria could be a cause of hypertension in toxaeemias of pregnancy.

Kincaid-Smith and Bullen (1965) reported a significantly higher incidence of pre-eclamptic toxæmia among pregnant bacteriuric women in comparison with non-bacteriuric controls. However, this increased incidence of toxæmia was not significantly altered in a proportion of bacteriuric pregnant women under successful, prolonged chemotherapy. This could be due to an inability to start treatment early in pregnancy since in a small proportion of bacteriuric women treated before the 16th week of gestation this incidence was reduced (Kincaid-Smith, 1965). Norden and Kilpatrick (1965) also reported a significant difference in the incidence of pre-eclampsia among bacteriuric and non-bacteriuric pregnant women, their figures being 8% of 110 bacteriuric and 2% of 109 matched control non-bacteriuric pregnant women. However, Le Blanc and McGanity (1965) found no such relationship between bacteriuria and toxæmia of pregnancy. They examined 110 bacteriuric and 1,150 non-bacteriuric pregnant women.

2. Prematurity and foetal loss in bacteriuria of pregnancy

Kass (1961) reported an unusually high infant mortality rate among babies born to untreated, asymptomatic bacteriuric women. This perinatal mortality was eliminated in a concurrent, similar group who were successfully treated during pregnancy. The cause of death in these babies included hyaline membrane disease, atelectasis and other findings associated with prematurity. In a subsequent publication (Kass et al., 1965) of a controlled treatment trial, the authors claimed proof that "bacteriuria was a cause of prematurity and not merely an associated event." Kass stated that about 8 to 10% of prematurity was preventable by early elimination of bacteriuria during pregnancy. Some of these figures quoted in 1961 were:

Patient group	% Neonatal death	% Premature infants
Bacteriuric untreated (48)	17%	24%
Bacteriuric treated (43)	0%	10%
Non-bacteriuric control (1,000)	3%	9%

Kass suggested that the hormonal changes occurring in late pregnancy sensitized the uterus to the effects of bacterial endotoxin and that it reacted by sustained severe contractions. Some animal experimental proof is available for this theory. Zahl and Bjerknes (1943)

found that pregnant mice reacted to an intraperitoneal injection of Gram negative endotoxin by abortion. However, the ensuing controversy in the subsequent literature on this subject can best be described as a "well planned tennis match", in the words of Petersdorf (1965).

There is confusion in the reported literature as to the definition of prematurity. Although it can be taken to describe babies born prematurely by gestation, i.e. before 36 weeks of gestation, the internationally accepted criterion is a birth weight of below 5 lb. 8 oz. regardless of the period of gestation. Some authors (Turner, 1961) fail to define prematurity, and others (Henderson and Reinke, 1965; Sleigh et al., 1964) have analysed their data using both criteria separately. It is certainly less confusing to speak of "small baby" in this context.

It is interesting to recall some of the earlier literature on this subject although the criteria used for the diagnosis of urinary infection were less quantitative. Dodds (1932) reported an incidence of prematurity in the offspring of 38.8% of women with pyelitis of pregnancy. Prematurity here probably referred to the period of gestation since these patients "went into premature labour spontaneously." Dugald Baird (1935, 1936) in an extensive study recorded that in pyelitis of pregnancy there was double the normal

incidence of small babies. Twenty-six per cent. of women with right-sided pyelitis and 42.3% of women with bilateral pyelitis of pregnancy had small babies.

Henderson et al. (1962) found an increased incidence of small babies among white and negro women with asymptomatic bacteriuria of pregnancy in comparison with non-bacteriuric women in each group. 17.9% of 39 white and 23.3% of 73 negro bacteriuric women had small babies. The comparative figures for non-bacteriuric controls were 6.6% of 604 white and 14.8% of 921 negro pregnant women.

Stuart et al. (1965) examined 2,713 consecutive pregnant women attending the hospital at Jamaica. Of these, 22.8% of 88 women with bacteriuria had small babies whereas only 11.4% of 729 control non-bacteriuric women had small babies. These authors could not determine whether this increased incidence was a result of the urinary infection only or was perhaps due to the higher incidence of hypertensive disease also found in this group.

Le Blanc and McGanity (1965) also reported an increased incidence of prematurity in the bacteriuric group; this incidence was significantly reduced in another group of bacteriuric women on continuous therapy during pregnancy. In 1,141 non-bacteriuric patients the incidence of prematurity was 11.6%, compared with 22.1% of 27 bacteriuric patients who were untreated, and

6.9% of 101 bacteriuric but treated patients. In this study the untreated bacteriuric group is particularly small. It is surprising that drug therapy in the bacteriuric group not only reduced the incidence of prematurity but that this was reduced to half the "normal" incidence in the non-bacteriuric control women.

Kincaid-Smith and Bullen (1965) in their series found that 13.3% of 240 bacteriuric women had small babies whereas only 5% of 500 random controls with no bacteriuria had small babies. However, in a double blind controlled treatment trial there was no significant reduction in the incidence of prematurity or perinatal mortality in a group who were successfully treated. In this study the term "foetal loss" was used due to difficulties in terminology relating to still-birth and abortion and also the difficulty experienced in obtaining accurate figures for abortion in the first trimester.

These results are shown in the table below:

Patient group	Foetal loss 13 to 27 wks.		Foetal loss 28 to 40 wks.		Total foetal loss	
	No.	%	No.	%	No.	%
Non-bacteriuric controls (500)	8	1.6%	8	1.6%	16	3.2%
Bacteriuric (200)	10	5%	10	5%	20	10%

On the other hand, there are an almost equal number of workers in this field who found no correlation between

bacteriuria and the incidence of prematurity or foetal loss. Turner (1961) stated that of 79 women with significant bacteriuria of pregnancy 4 had premature infants, a rate which was not considered abnormally high. However, it is worth recalling that in her series there is no mention of the gestation of pregnancy at which these patients had their bacteriuria detected and hence probably gives equal weight to bacteriuric women who have acquired bacteriuria late in pregnancy when it is too late to have any effect on premature delivery. Also no definition of the term "prematurity" is given but "premature birth of baby" probably indicates the duration of gestation. In this analysis not all bacteriuric women had 2 consecutive positive urine cultures.

Kaitz and Hodder (1961) also failed to find an increased incidence of small babies in untreated bacteriuric women. His series comprised only 17 bacteriuric and 500 non-bacteriuric women and no significant conclusion can be drawn from this sample although these findings are repeatedly quoted.

Turck, Goffe and Petersdorf (1962) also found no correlation between prematurity and bacteriuria. All the specimens of urine in this series were catheterised specimens obtained on the delivery table and are hence open to the following criticisms. Bacteriuric patients who delivered before the 28th week of pregnancy may not come to the delivery table and also this series probably

includes a number of women who may have acquired bacteriuria ^{too} late in pregnancy for any effect on prematurity. It also fails to consider women who were probably treated for symptomatic urinary infection before delivery and hence are non-bacteriuric but have a small baby, at the time of delivery. Turck et al. noted a higher incidence (9.7%) of prematurity among the women of a low socio-economic class when compared with the incidence (3.7%) in a high socio-economic group.

Norden and Kilpatrick (1965) felt that the bacteriuric pregnant population was not homogeneous but that the women who developed clinical symptoms were more likely to have small babies. He found a 15% incidence of small babies in 114 bacteriuric cases and a 13% incidence in 109 non-bacteriuric matched controls. However, in a small group of 25 women with bacteriuria, who developed symptomatic urinary infection and were treated, the small baby incidence was 23%.

Whalley (1965) was also of the similar opinion that the higher incidence of small babies occurred in the subgroup of bacteriuric pregnant women who became symptomatic prior to delivery and required treatment.

It is evident from the above literature that not all studies were analysed using the same diagnostic criteria. Other causes of prematurity, such as twins, pre-eclamptic toxæmia, placenta prævia, abruptio placenta, induced labour, and erythroblastosis foetalis, should be

considered before implicating bacteriuria as the cause of small baby or foetal loss. Controlled treatment trials conducted on a large series of patients would be a better solution to this controversy.

3. Anaemia and bacteriuria

Giles and Brown (1962) investigated the incidence of urinary tract infection in anaemic pregnant women and in those with a normal haemoglobin level. They found that 7.2% of 447 normal patients without anaemia had urinary infection whereas 15.8% of 436 women with anaemia had a urinary infection. Anaemia in this group tended to respond when the urinary infection was successfully treated. The diagnosis of urinary infection in this study was, however, based on the presence of pus cells and organisms in the urine and not on a quantitative basis.

Layton (1964) suggested that a urinary tract infection probably interfered with the absorption of iron from the intestine. In his study anaemia, defined as a haemoglobin level below 70%, was found in 31.3% of 67 women with untreated bacteriuria of pregnancy and in 19.5% of 118 control non-bacteriuric pregnant women. The control group was here selected blindly by taking the two patients with registration numbers following the bacteriuric patient.

A controlled treatment trial would again throw more light on this problem.

Chemotherapy of Bacteriuria of Pregnancy

There appears to be general agreement by all workers in this field that adequate treatment of asymptomatic bacteriuria early in pregnancy prevents the development of acute pyelonephritis later in pregnancy, which would otherwise occur in about 36 to 40% of these women (Kass, 1960; Little, 1965; Kincaid-Smith and Bullen, 1965). Turner (1961) in her study found that 60% of 79 women with untreated asymptomatic bacteriuria of pregnancy developed clinical symptoms of a urinary tract infection. There is some evidence that successful treatment of bacteriuria early in pregnancy may also reduce the incidence of toxæmia, prematurity, abortion and perinatal mortality to a significant extent in these women. However, there is no uniformity of opinion regarding this latter claim. It is nevertheless necessary to treat women with asymptomatic bacteriuria in early pregnancy if only to save them from the painful and debilitating disease of acute pyelonephritis which usually requires the admission of the patient into hospital (Kincaid-Smith and Bullen, 1965). There is, however, no agreement regarding the drug of choice and the duration of treatment.

Kass (1960) initially treated all women with asymptomatic bacteriuria in early pregnancy with a short, one or two week course of sulphamethoxyypyridiazine in a

dosage of 0.5 g. daily. Kass stated that in these early observations where the treatment was discontinued before term, the bacteriuria returned within a month or two and hence it was decided to continue therapy till delivery. No actual figures as to the relapse rate after a short term of chemotherapy is given; perhaps the numbers involved were too few.

Little (1965) also followed a similar treatment regime in a group of antenatal bacteriuric women who were under monthly supervision from about the 12th week of pregnancy until 6 weeks after delivery. In a group of 57 women with significant bacteriuria given 0.5 g. sulphamethoxypyridiazine daily throughout pregnancy and the puerperium, only 3 or 5% developed acute pyelonephritis. Two of these 3 women were probably not taking their tablets since the monthly specimens of their urine did not contain sulphonamide. In a concurrent group of 52 women with untreated bacteriuria, 19 or 36% developed acute pyelonephritis. Little did not mention the rate of failure or relapse on this regime of therapy except to say that most infections which failed to respond to sulphonamides were successfully treated with ampicillin 250 mg. six-hourly or preferably with nitrofurantoin 100 mg. at night. No serious side effects on the mother or infant were reported in this long-term, long-acting sulphonamide therapy given during pregnancy.

Kincaid-Smith and Bullen (1965) also used a long-acting sulphonamide, namely sulphamethoxydiazine, 0.5 g. daily till the 30th week and a short-acting sulphadimidine for the last 10 weeks of pregnancy in order to avoid possible hazard to the foetus from a long-acting sulphonamide. All women with significant bacteriuria, who attended the antenatal clinic before 26 weeks of gestation, received either treatment or a placebo tablet. In a double blind controlled trial it was found that acute pyelonephritis which occurred in 20 or 36.6% of 55 untreated women occurred in only 2 or 3.3% of 61 treated women, again confirming that acute pyelonephritis of pregnancy is preventable. The rate of failure on this regime of treatment is again not mentioned.

Williams, Brumfitt, Leigh and Percival (1965) reported the results of a short course of sulphonamide therapy given to 127 pregnant bacteriuric women. Although all received treatment for 8 days, four different sulphonamides were used, dividing this group further. No mention of the method of choice of drug in each instance was made.

Number Treated	Drug	Dosage
73	Sulphadimidine	2 g. daily for 8 days
7	Sulphafurazole	Do.
17	Sulphamethoxydiazine	0.5 g. daily for 8 days
30	A mixture of Sulphadimidine and sulphamethoxypyrimidine	1 g. daily for 8 days
<u>127</u>	Total	

In the last two groups a higher loading dose of drug was given on the first day of treatment. The follow-up scheme after treatment consisted of the following:

Urine was examined bacteriologically

1st - 1 week after cessation of therapy.

2nd - 6 weeks later.

3rd - at about 36 weeks of gestation.

4th - 1 week after delivery.

Unfortunately this follow-up could not be instituted in all cases reported and

all 127 women were examined 1 week after therapy;

only 80 women were examined 6 weeks after therapy;

only 58 women were examined 1 week after delivery.

These results certainly emphasise the difficulty experienced in a complete follow-up of this group of patients. The results of the immediate post-treatment examination indicate an immediate "cure" rate of about 77%. These results for short-acting and long-acting sulphonamides were 75% and 79% respectively. When the urine was examined 6 weeks after treatment 4 or 5% of those who showed an immediate cure relapsed with infection. The authors stated that when the urine was clear at 1 and 6 weeks after treatment, there were no further relapses during pregnancy and after delivery. But from the few women examined at these times it is difficult to conclude that the women who were not followed up did not have a relapse.

Of the 34 women in this study who failed to respond to sulphonamides, 15 failed due to resistance of the infecting organism to sulphonamides. A further 9 women showed renal abnormalities by radiology and a further 2 women were unable to take the drug due to vomiting, thus accounting for 22 out of 34 failures. It is interesting to note that 43% of 28 women with a significantly raised serum antibody response failed treatment, whereas 20% of 54 women with a normal or insignificant antibody response failed treatment. The authors felt that since a short course of treatment was successful in about 70% of women it was preferable to long-term suppressive therapy which might harm mother and foetus.

From these results it is evident that sulphonamides are effective in the majority of women with asymptomatic bacteriuria of pregnancy. Although short-term therapy carries less hazards, it is clear that a strict follow-up scheme to detect women who relapse or re-infect themselves during the remainder of pregnancy is essential. It is probably easier to persuade women to attend a regular follow-up scheme if they are on continuous long-term suppressive therapy. It is more essential to follow up those women who have relapsed or become re-infected since it is this group that is likely to have renal involvement. There appears to be no easy way out of this problem, and the proper care and investigation of women who have failed to respond to initial treatment

is the responsibility of obstetricians and family doctors.

Some indication of the efficacy of any one form of therapy can be obtained by determining whether those patients who become re-infected after a period of eradication of bacteriuria following therapy, do so due to a recrudescence of an inadequately eradicated infection or due to infection by a new organism. When this infection is due to a different species of organism there is no doubt about a re-infection. However, in those instances where the re-infecting organism is of the original species methods such as serological typing of Escherichia coli may be helpful. Only a limited analysis of this sort is at present possible since all the 145 typing sera cannot be made by each laboratory. McGeachie (1965a) investigated 108 recurrent urinary infections due to Escherichia coli which occurred in 49 patients, using serological and colicine typing methods. In most of these patients there was no demonstrable abnormality of the urinary tract. There was only one man in this group. It was found that following short-term chemotherapy of about 1 week's duration, 27.8% of these recurrent infections occurred within one month, a further 42.6% within one to three months and 19.4% within three to six months. Hence 90% of these 108 recurrences had occurred in a period of 6 months following the initial infection. Of these 108 recurrences only 17 or 15.8%

were due to a relapse or recrudescence of the original strain and 84.2% had a strain of Escherichia coli which was different from the strain isolated at the previous infection. It may be argued that if the majority of recurrences are due to a different organism, there is no justification for a prolonged course of therapy. The author felt that during the period following an attack of pyelonephritis, in spite of clinical and bacteriological cure, the kidney was more liable to re-infection for a period which was not likely to be less than six months. McGeachie was in favour of supplying antimicrobial "cover" during this period. A similar study of instances of re-infection following initial successful therapy of asymptomatic bacteriuria of pregnancy is required.

Some of the reported hazards following chemotherapy during pregnancy may be briefly discussed. Black (1962) reviewed some of the literature on this subject. The critical periods, when the foetus could be adversely affected, were the first 12 weeks and the last few weeks of gestation. Odell (1959) stated that sulphonamides could induce jaundice and kernicterus in the newborn, especially in the premature. The underlying mechanism appeared to be due to the displacement of protein-bound bilirubin in plasma, making free bilirubin available for diffusion into the tissues and the brain. Short-acting sulphonamides, namely sulphafurazole and sulphadiazine,

and the long-acting sulphamethoxine and sulphamethoxy-pyrimidine, are mentioned. The long-acting sulphonamides, when given to the mother before delivery, persisted in effective levels in the blood of the newborn for about 6 days. Tetracyclines, during pregnancy, had the danger of causing discolouration and maldevelopment of the teeth of the child (Shwachman et al., 1958). Streptomycin given during pregnancy could cause deafness in the foetus due to toxic labyrinthine injury (Conway and Birt, 1965).

The Serum Antibody Response in Infections of the Urinary Tract

A urinary tract infection caused by coliform organisms in man may elicit a measurable antibody response. This was shown by Pfaundler as early as 1898, using a bacterial agglutination technique. Further studies by Siede and Luz (1941) using a similar technique indicated a certain difference in the level of this antibody response between cases of pyelitis and those with cystitis. Kaarsalo et al. (1962) have been critical of the usefulness of antibody estimations in cases of human urinary tract infections. They examined the sera of 63 female patients with pyelonephritis. Only 17 of these had an "active pyelonephritis", another 17 had "recurrent pyelonephritis" and 29 had a "chronic pyelonephritis".

Using a bacterial agglutination technique and the infecting urinary organism as antigen, they found no serum antibodies in over half of these patients. This is hardly surprising in the light of later findings (Percival et al., 1964; Neter et al., 1965) that these antibody results yield information only on relatively recent infection since the antibody titre decreases from its peak within about 12 months. Moreover, the criteria on which the diagnosis of significant bacteriuria and the presence of renal involvement were based in the above study are inadequate.

Percival, Brumfitt and De Louvois (1964) were the first to do a systematic study using this particular technique. They estimated the serum antibody response in 293 patients with urinary infection and in 20 control normal subjects. In 19 out of 20 control normal subjects the serum antibody levels, tested against 11 standard Escherichia coli 'O' types used as antigen, did not rise above 1 in 160. These particular 'O' types were previously reported as being most commonly associated with urinary infections, namely 'O' types 1, 2, 4, 5, 6, 7, 9, 11, 18, 39 and 75 (see later). In another group of 41 patients with significant bacteriuria and clinical features of an acute pyelonephritis, the titre was 1 in 320 or above in 38 or 93% of them. On the basis of these findings a titre of 1 in 160 or below was regarded as normal. Twenty-five of

these patients with acute pyelonephritis were subsequently examined by intravenous pyelography and 17 showed renal evidence of chronic pyelonephritis, although many of them had no previous history of urinary infections. Hence in subsequent estimations of the serum antibody response in various groups with a urinary infection, the presence of a serum antibody titre of 1 in 320 or above was taken as evidence of probable renal damage. In 86 patients in general practice with asymptomatic bacteriuria or with symptoms confined to the lower urinary tract 71 (82%) showed serum antibody titres of 1 in 160 and below, being similar to the control negative group. In a group of 126 pregnant women with mainly asymptomatic significant bacteriuria, 40 (32%) showed significantly raised antibody titres. A rising titre during pregnancy was found in only 10 of them. In a small group among these of 13 women, with chronic bacteriuria throughout pregnancy in spite of treatment, 9 showed significantly raised antibody levels and in 5 there was a significant rise in this level during the study period. Also 8 out of these 9 women when examined about 3 months after delivery showed radiological evidence of chronic pyelonephritis. It was also interesting that there was a greater incidence of treatment failures in the group of 40 women with high antibody titres. Hence it appeared that a significantly high serum antibody response, instead of being protective to

the host, seemed to demarcate a group of patients with more severe infection probably involving the renal parenchyma which was also less amenable to treatment. In patients with asymptomatic significant bacteriuria of pregnancy this test is a possible means of differentiating patients with clinically inapparent renal involvement. Coliform infections of other parts of the body could also stimulate a significant antibody response and should be considered. These authors also stressed that a significant antibody response was dependent on a recent infection and would be of no value as an indication of past infection and chronic pyelonephritis.

Seneca, Peer and Hampar (1963) investigated the possibility of an immunological diagnosis of upper urinary tract infections using the agar gel diffusion technique without success. They used antigen extracts of the infecting urinary organism as well as a number of common urinary pathogens. Only 74 out of 127 pathogens isolated from 100 patients with an upper urinary tract infection gave a correct band with the homologous patients' serum. There were also numerous additional bands, the nature of which could not be determined. The authors felt that this technique was of no value until it was possible to eliminate these collateral reactions. The criteria for the diagnosis of infection were not mentioned in this group.

A more sensitive method of estimating this antibody

response appears to be the enterobacterial, indirect, passive, haemagglutination test first investigated by Neter et al. in 1952. However, Australian workers Keogh, North and Warburton (1948) were the first to report that a variety of bacterial polysaccharide antigens were readily adsorbed by red blood cells and that these cells were then specifically agglutinated by homologous bacterial antibodies. Neter et al. (1952a) demonstrated that both the sediment and supernate of a boiled suspension of Escherichia coli were capable of sensitising red cells of man, dog, rabbit, guinea pig, sheep, rat and chicken so that they then became specifically agglutinable by homologous Escherichia coli antisera. Neter et al. (1952) further observed that boiling of the sterile Escherichia coli broth culture filtrate also rendered the filtrate active in the above test. It is well known that in the broth culture itself, the heat labile, capsular 'L' or 'B' or 'A' antigen are the cause of inagglutinability of these organisms in specific 'O' antisera, in the bacterial agglutination test. The authors suggested that 'L' or 'B' and 'O' antigens may be present in the intact bacterial cell as well as in the culture filtrates as a complex and that the 'L' or 'B' antigens block the reactive group or groups of the 'O' antigen unless heated. Neter, Gorzynski, Gino, Westphal and Lüderitz (1956) repeated these observations using purified Escherichia

coli polysaccharides as well as the corresponding crude antigen extracts. Minor cross-reactions observed with crude antigens of 'O' groups O₁₁₁, O₅₅ and O₂₆ and rabbit antiserum to O₁₁₁ were not encountered with purified lypopolysaccharides in the above test.

Kunin, Beard and Halmagyi (1962) have since then tested 137 of the 145 standard 'O' group strains of Escherichia coli and their corresponding rabbit antisera by means of the haemagglutination test, using the crude 'O' antigen extracts, and observed only a small number of cross-reactions. These cross-reactions were according to the general pattern already described with the standard bacterial agglutination technique used in the serological typing of these organisms.

In studies employing human serum, the haemagglutination test appeared to be more sensitive than the simpler bacterial agglutination test in estimating the antibody response to Escherichia coli. This was in marked contrast to the results obtained with hyperimmunised rabbit antisera, where there was no added advantage in using the haemagglutination test (Neter et al., 1953; Kunin, 1962).

Kunin et al. (1962) also described the presence of a common cross-reacting hapten associated with endotoxin fractions of Escherichia coli and other members of the family Enterobacteriaceae. This cross-reacting "Kunin antigen" was first noticed when they tested the 137

standard, crude 'O' antigen extracts and their corresponding rabbit antisera in the above haemagglutination test. It was found that the rabbit antiserum to Escherichia coli O₁₄ agglutinated erythrocytes coated individually with almost all of the other 137 crude 'O' antigen extracts and even similar extracts from Salmonella, Shigella and Proteus. Red cells coated with O₁₄ antigen, however, reacted only with O₁₄ antiserum. This cross-reacting antibody present in O₁₄ antiserum was completely inhibited by pre-incubation with any one of the other 137 unrelated 'O' antigen extracts except extracts from O₆₂ and O₉₂. The possibility that the cross-reacting antibody in O₁₄ antiserum could be against a common factor in the bacterial growth medium used, such as polypeptides and agar, was also eliminated by using extracts of organisms grown on a defined synthetic medium. Even purified lipopolysaccharides, extracted by the aqueous ether or phenol-water method, showed similar cross-reactions with O₁₄ antiserum (Whang and Neter, 1962). Whang and Neter (1963) studied the properties of this cross-reacting antigen and the presence of the non-specific heterogeneous antibody in human serum samples was also investigated. The available data suggests that the lipopolysaccharide fractions of Escherichia coli and other enterobacteriae may possess 2 types of antigenic determinants. One is the classical 'O' antigen, the other is a hapten

common to almost all the other 145 'O' groups and others of this family. The lipopolysaccharide from Escherichia coli O₁₄ appears to differ from the others in that this hapten is situated or structured in such a manner that it can behave as a complete antigen and induce antibody formation in the rabbit in this case. The exact nature of this antigen has not yet been established, but it appears unrelated to the Frossmann antigen, human blood group substances, Rantz factor described with various Gram positive organisms, the 'C' antigen of Brodhage, or to the haemagglutinating fimbrial antigens. Moreover, only the haemagglutination test was involved in the O₁₄ phenomenon since no response of bacterial agglutination was observed. These authors felt that a hapten rather than a complete antigen was involved.

Since purified "Kunin" antigen was not available Whang and Neter (1963) used a haemagglutination inhibition test to detect the presence of heterogenetic or cross-reacting antibodies in any particular serum sample. The heterogenetic antibody titre could be reduced by more than 95% by pre-incubation of the serum with an equal amount of undiluted crude 'O' antigen extracts from various unrelated enteric bacteria. The difference in end titre between two series of titrations of a serum, one pre-incubated with an unrelated 'O' antigen and the other with buffer only, represented the amount of heterogenetic antibody present. The authors emphasised that

heterogenetic antibodies could be detected by this procedure only if their titre was higher than the titre of 'O' antibodies against the 'O' antigen coated on the erythrocytes, used as an indicator. Neter et al. (1965) analysed the sera of 461 children for antibody titres against Shigella, Salmonella and enteropathogenic Escherichia coli and found that in practice the presence of the heterogenetic antibody did not interfere with the test unless present in high titre, which happened rarely. Needell et al. (1955) used the haemagglutination test to estimate the antibody response in 20 patients with urinary infection. No attempt was made to correlate the level of antibody titre to the severity or anatomical site of infection. Details of the methods used to diagnose infection were also not given. However, antibody titres over 1 in 160, considered significant, were demonstrated against 50% of 40 infecting organisms isolated from 20 patients. Slifkin et al. (1959) and Ehrenkranz and Carter (1962, 1964) also used this technique to analyse serum from patients with urinary infection. Winberg et al. (1963), however, were the first to do a systematic study in a small group of 20 children with urinary infection and 25 controls with no infection using the above haemagglutination test. Diagnosis of urinary infection was based on a quantitative viable count of the bacteria in the urine. Evidence of renal involvement was also

determined by an estimation of the renal concentration capacity according to Winberg (1959a). Serum from control negative children was titrated using Escherichia coli antigens derived from homologous faecal strains. None of the control children had titres over 1 in 64. In 10 children with acute pyelonephritis and reduced renal concentration capacity there was a rapid and marked antibody response. However, in 4 children with cystitis and normal renal concentration capacity, the level of antibody was similar to the control group. This work also suggested that the level of antibody response, in children over 2 months of age, might permit differentiation between urinary infections with and without involvement of the kidney.

Kunin and Beard (1963) found that antibody to Escherichia coli in human serum appeared predominantly in the γ -globulin fraction. In dogs, pigs, cows and horses it was primarily in the β fraction. The authors stated that, like typhoid 'O' antibodies, antibodies to Escherichia coli may be exclusively in the 19S fraction in man since these antibodies were poorly transmitted to the foetus across the placenta, which appeared to be quite permeable to 7S but not to 19S globulin fractions. The antibody in the foetus was estimated by the haemagglutination test done on the blood from the umbilical cord.

Serological Typing of Urinary Strains of
Escherichia coli

Early attempts at a serological classification of the Escherichia coli group of organisms failed largely because sufficient attention was not paid to the fact that newly isolated strains are mostly capsulate and hence inagglutinable in homologous antiserum. Kauffman and later Knipschildt and Vahlne, his collaborators, were the pioneers in this field although the earlier work of Theobald Smith et al. (1927) is also recognised.

Kauffman (1943, 1944) demonstrated that the inagglutinability of Escherichia coli strains in homologous antiserum was in most instances due to the presence of a capsular antigen named 'L'. In addition to inhibiting the somatic 'O' agglutination, this antigen was fortunately heat labile so that these strains could be rendered agglutinable in homologous antiserum if the suspension was first boiled. Vahlne (1945) made a comprehensive review of the earlier literature and Edwards and Ewing (1962) have summarised the present status of serological typing of Escherichia coli in a very useful and practical way.

In order to completely identify a given strain of Escherichia coli serologically it is necessary to analyse three classes of antigens, namely

'O' or Somatic Antigen

'K' antigen or Capsular Antigen

This antigen 'H' or Flagellar Antigen

The 'O' antigen

This somatic antigen is thermostable and is not inactivated by heat at 100° C. or 121° C. It is not influenced by treatment with 50% ethyl alcohol or with 1 N hydrochloric acid. Chemically it is a lipopolysaccharide and contains the endotoxin moiety.

The 'K' antigens

Kauffmann and Vahlne (1944) suggested the term 'K' antigen to include a group of 3 capsular antigens. These antigens occur as envelopes or sheaths and inhibit agglutination of the living bacteria in homologous 'O' antiserum. The 'K' antigens are further subdivided into 3 varieties based on their physical behaviour. These can be considered individually as 'L', 'B' and 'A' antigens.

'L' antigen

This antigen was first described by Kauffmann in 1943. 'L' antigen is completely inactivated by heat at 100° C. for 1 hr. After this inactivation organisms containing it do not agglutinate in 'L' antiserum, but agglutinate in homologous 'O' antiserum. The inactivated 'L' antigen is no longer antigenic and fails to bind 'L' antibody. Antigenic in this context refers to the ability to induce a specific antibody response in rabbits.

'B' antigen

This antigen is only partially inactivated by heat. It is similar to 'L' antigen in that organisms containing it are rendered 'O' agglutinable after heating the suspension at 100° C. for 1 hr. Although 'B' antigen is no longer antigenic after such heat treatment, its ability to combine with 'B' antiserum is not inactivated by heat at 100° C. for $2\frac{1}{2}$ hrs. or 121° C. for 2 hrs. Hence one cannot prepare a specific 'B' antiserum from an 'OB' antiserum by absorbing it with a heated suspension of the homologous strain since both 'O' and 'B' antibody are thus absorbed out leaving the resulting serum inactive.

'A' antigen

Antigens of this group require a temperature of 120° C. for about $2\frac{1}{2}$ hours for even partial inactivation. Organisms containing them are agglutinable in homologous 'O' antiserum after such heat treatment. However, like the 'B' antigens, the antibody-binding ability of 'A' antigen is not inactivated by this heat.

In brief, the 'K' antigenic differences after heating suspensions at 100° C. for 1 hour can be summarised as:

L	B	A
Not antigenic	Not antigenic	Antigenic
O-agglutinable	O-agglutinable	O-inagglutinable
No antibody binding	Antibody binding	Antibody binding

As far as 'O' antigenic analysis is concerned strains containing 'L' and 'B' capsular antigens are rendered agglutinable after heat at 100° C. for 1 hour; the strains containing 'A' antigen require autoclaving at 120° C. for 2½ hours.

The 'H' antigen

This antigen is contained in the flagella of motile strains of Escherichia coli. 'H' antigen is inactivated by heat at 100° C. but is not inactivated by formalin.

In 1947 Kauffmann published a scheme for the antigenic analysis of Escherichia coli consisting of 25 'O' antigen groups, 55 'K' antigens and 20 'H' antigens.

It should be understood any 'O' group is divided into types depending on the 'H' and 'K' antigen combination.

This scheme has since been greatly enlarged and now consists of

145 - 'O' antigen groups

86 - 'K' antigens

49 - 'H' antigens

Hence the complete typing of an unknown Escherichia coli serotype consists of the determination of its 'O', 'K' and 'H' antigens. Fortunately, during the identification of Escherichia coli strains isolated from patients with urinary infection, it was found that relatively few 'O' antigen groups accounted for about 50% of such isolates.

Vahlne (1945) using the first 25 'O' antisera

investigated the occurrence of these 'O' groups in Escherichia coli isolated from 54 patients with urinary infection. Sixty per cent. of these urinary isolates were typed using the 25 'O' antisera, in comparison to 42% typed from Escherichia coli isolated from the faeces. A certain preponderance of 'O' group 4 was also noticed among the typed urinary strains. It was further noted that whereas 95% of the colonies from any one infected urine fell into the same 'O' group, 73% of normal faecal Escherichia coli isolates contained 2 or more 'O' serotypes, thus representing a more heterogeneous population.

Kauffmann (1947) also noted that there was a frequent change of Escherichia coli serotypes in the normal gut over a period of time. He examined faecal specimens from two normal individuals, every two weeks, for 6 months. Ten and 22 different serotypes were isolated from each of these individuals respectively during this time. Kauffmann also designated 'O' groups 2, 4 and 6 as particularly pathogenic to man and animals.

Vosti et al. (1962) also made similar observations. They examined 100 urine samples from patients with urinary infection and 100 normal faecal specimens. Fifteen to 25 colonies from each faeces and 5 colonies from each urine sample were serotyped. Over 90% of the urine samples contained a single 'O' group per specimen. Fifty per cent. of faecal specimens contained a single 'O' group of Escherichia coli, 30% had 2 different 'O'

groups and 20% had between 3 and 5 different 'O' groups at one time.

The Rantz (1962) was able to serologically type 75% of Escherichia coli strains isolated from 156 patients with significant bacteriuria. Forty-five per cent. of these infections were caused by strains belonging to the first 10 'O' groups. It was further noted that certain 'O' groups predominated in urinary isolates in contrast to findings in normal faeces. 'O' groups 2, 4, 6 and 75 accounted for 49.3% of urinary infections but occurred in only 20.1% of faecal isolates. The authors felt that 'O' groups 4 and 6 were particularly nephropathogenic since these groups occurred in 72.5% of incidences of clinical acute pyelonephritis. Ujvary (1958) also noted that 48% of urinary infecting Escherichia coli belonged to the first 10 serological groups.

Turck et al. (1962) in a very interesting study on the epidemiology of Escherichia coli infections made the following observations. He used 137 standard 'O' antisera. 76.8% of 522 Escherichia coli strains were typed. These strains were isolated from urinary, pulmonary and other non-enteric infections. They found no significant differences between the serological patterns among urinary and extra-urinary coliforms. 'O' groups 1, 4, 6 and 75 occurred most frequently in all infections and accounted for 57% of groupable urinary strains, 72.3% of groupable urethral contaminants, 60.9% of groupable

extra-urinary strains. These 4 serogroups also accounted for 51.5% of typable normal faecal strains. The authors suggested that the frequent occurrence of 'O' groups 1, 4, 6 and 75 in human infections was because they were more prevalent in the environment. A group of 35 patients with significant Escherichia coli bacteriuria was subjected to special investigation and had stool cultures performed on the same day as urine culture. Twenty-five of these patients had the same 'O' group of Escherichia coli in the urine and faeces at the same time. In 17 of these cases the common 'O' group was the predominant isolate in the faeces which also contained several other 'O' groups. These studies also suggest that the route of infection is from the faeces via the urethra into the bladder.

McGeachie (1965) made the following 12 antisera belonging to 'O' groups 1, 2, 4, 5, 6, 7, 8, 9, 11, 18, 39 and 75. Using the above sera 56.6% of 534 urinary Escherichia coli strains were typed. 6.5% of these strains were not typable due to roughness. 47.4% of the typed strains belonged to 'O' groups 1, 2, 4, 6, 18 and 75. It was further suggested that since the same serological groups were isolated from infected and contaminated urines, the origin of urinary infection is through an ascending pathway, from the contaminating urethral flora.

MATERIALS AND METHODS

Patients Examined during this Study

9,741 consecutive antenatal women who attended the out-patient clinic of the Simpson Memorial Maternity Pavilion were examined for the presence of significant bacteriuria. About 100 new antenatal patients attended the "booking clinic" each week. All women had a mid-stream specimen of urine examined at their first visit.

595 of the above women were found to have significant bacteriuria. The present study involves a detailed study of the first 475 of these women with significant bacteriuria.

MATERIALS AND METHODS

Method of collection of urine specimens

At their first visit to the hospital, all new antenatal women were asked to collect the middle of a stream of urine into a sterile, wide mouthed, metal screw capped bottle. These bottles, called "one pound honey pats", of 300 ml. capacity were easy to use and sterilise.

Each specimen had a gummed label indicating the name of the patient and her antenatal number. A list of the names of these women, their gestation of pregnancy in weeks and the particular antenatal unit that they were booked under, accompanied these urine specimens to the laboratory. All specimens were transported to the laboratory on a wheeled trolley as soon as they were

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collected. These specimens were refrigerated and examined the next morning.

Chemical screening tests used to detect urine specimens with significant bacteriuria

In an initial experiment two different screening methods were tried on each of 1,000 urine specimens. Each specimen also had a quantitative urine culture done by the method of Miles and Misra (1938) (see later).

The chemical screening tests evaluated were:

1. The triphenyl tetrazolium chloride test (T.T.C.) by the method of Simmons and Williams (1962).
2. The modified nitrite test by the method of Sleigh (1965).

The modified nitrite test was found to be more successful than the T.T.C. test, detecting 97% of 71 urine specimens with significant bacteriuria as evidenced by quantitative viable count technique (see results). Hence all subsequent screening was done by the modified nitrite test.

Procedure for modified nitrite screening test

Each specimen of urine from one antenatal booking clinic was given a serial laboratory number. Each urine specimen was well mixed by shaking and a 1 ml. aliquot transferred into a chemically clean small Wasserman test-tube kept in a numbered metal rack. An automatic pipette with a sterile Pasteur pipette attached to it was

used for this transfer. Two 0.02 ml. drops of a 5% solution of potassium nitrate in sterile distilled water, were also added to each test-tube of urine. These urine specimens in a rack were shaken and placed in a water-bath at 37° C. for incubation for 4 hours. Each specimen, following incubation, was tested for the presence of nitrite by the Ilosvay modification of the Greiss test. The reagents for this test consisted of:

Solution A - 1.25 g. of sulphanilic acid in 500 ml. of 30% acetic acid.

Solution B - 2.5 g. of alpha-naphthylamine in 500 ml. of 30% acetic acid.

Solution B was kept in a dark bottle away from sunlight and discarded if any tinge of pink appeared in it. Equal volumes of solutions A and B were mixed immediately before each test and 1 ml. of this mixture was added to each tube of incubated urine. The immediate development of a red or pink colour following the above addition was regarded as positive. Any light pink colouration was considered of only doubtful significance but was nevertheless confirmed by viable count technique.

Confirmation of all +ve screening tests

All specimens of urine positive by the above screening test had a quantitative culture done on the same day, the urine specimen having been refrigerated during this 4 hour period.

It was important to use culture medium which was

Method of quantitative urine culture

The Miles and Misra (1938) technique was used to enumerate the numbers of viable bacteria present in each ml. of urine. In addition to all positive screening tests, all urine specimens received from women with asymptomatic, significant bacteriuria in their subsequent follow-up during pregnancy were also cultured by this method. About 2,000 urine specimens have been examined by this technique.

The following dilutions of each urine specimen were made in 99 ml. amounts of diluting fluid using an automatic pipette to transfer 1 ml. volumes: neat urine, a 10^{-2} dilution, a 10^{-4} dilution and a 10^{-6} dilution of the neat urine. Starting with the most dilute suspension, known volumes of each dilution were inoculated, as discrete drops, on one half of a blood agar plate, using two plates for each specimen of urine. A sterile, calibrated dropping pipette was used to deliver the inoculum in 0.02 ml. volume in a drop. Five drops of each dilution were thus inoculated on each half of a plate. These inoculated plates were incubated overnight at 37° C. and examined the next day. The dilution showing a growth of discrete, single colonies was enumerated and the pure or mixed nature of the resulting culture was also noted, in the interpretation of the results.

It was important to use culture medium which was

well dried and warmed in the incubator for about 1 hour before inoculation. Under these conditions the delivered drops were immediately absorbed, preventing any surface multiplication within the drops during incubation. A specially levelled working bench surface was also used so that the drops were discrete and did not coalesce before incubation.

Diluting fluid used for quantitative culture of urine

Sodium chloride 8.5 g.
Distilled water 1 litre
40 ml. of saline were removed and replaced with 40 ml. of nutrient broth (Oxoid No. 2). This fluid was dispensed in 100 ml. amounts in screw-capped bottles and sterilised by autoclaving.

Capillary pipettes delivering measured 0.02 ml. drops

Made and sterilised according to Cruickshank (1965, p. 792). These pipettes were mostly used only once, unless the tip remained unbroken during resterilisation.

Blood agar medium

Made according to Cruickshank (1965, p. 745). A 10% concentration of horse blood was used. This medium rather than MacConkey agar was used to avoid any inhibitory effect on the growth of non-intestinal bacteria in the urine.

Preservation of organisms isolated from positive urine cultures

A single, discrete colony from each urine with

significant bacteriuria was used. If it was not possible to obtain a pure culture, as in the case of Proteus, a subculture on appropriate medium was made initially. All positive cultures were inoculated on to the surface of one nutrient agar slope medium and one egg saline slope medium contained in bijou bottles. Each isolate was numbered on the caps and sides of the bottle. Strains thus stored were subcultured once a year to avoid selection of rough variants. It was vital to ensure that all metal caps were lined with new rubber liners and that the caps were screwed tightly before storage at room temperature in a dark cabinet with wooden partitions.

Egg saline medium for preservation of cultures

This modification of Dorset's egg medium, in which the broth was replaced by saline and no malachite green was added, was made according to Cruickshank (1965, p. 778).

Preservation of cultures by freeze-drying

About 100 positive culture strains were freeze-dried by the Greave's centrifugal method, in accordance with Cruickshank (1965, p. 806). An overnight culture of the organism in sucrose serum broth, containing 7.5% of sucrose was used. About 0.1 ml. of this culture was placed in each sterile ampule. Each isolate was put into 3 ampules and these ampules were freeze-dried in batches. One of each batch was later tested by

subsequent culture to ensure purity and survival of these organisms. It was hoped to minimise "rough" variation among isolates of Escherichia coli by this method.

Chemotherapy trial for the treatment of asymptomatic bacteriuria of pregnancy

Definition of asymptomatic bacteriuria: for the purposes of this study all antenatal women with significant bacteriuria who were not regarded by those responsible for their care as having active infections of the urinary tract were termed "asymptomatic".

Criteria used to include asymptomatic bacteriuric women in the trial

1. Only women attending the hospital early in pregnancy, to allow a two-week treatment with either drug before the 20th week of gestation, were included. In the latter half of this study this was extended to include women who could be treated before the 28th week of gestation, since it was found that a great proportion of bacteriuric women was rejected as unsuitable by the above criteria. The results were analysed separately in these groups.

2. The drugs used were cycloserine and sulphadimidine.

3. The allocation of drugs to consecutive patients included in the trial was on an alternative random basis.

Dosage given

Each drug was given for a duration of 14 days.

Cycloserine: capsules of 250 mgm. each. One capsule was given twice daily, 500 mgm. per day, for 14 days.

Sulphadimidine: tablets of 0.5 g. each. One tablet was given every 6 hours, 2 g. per day, for 14 days.

The follow-up scheme, in short, consisted of clinical and urine examinations at the following periods during pregnancy:

1. Specimen 1.
2. Specimen 2 at about 12th week.
3. Followed by 2 weeks of treatment.
4. Post-treatment urine specimen 1 week after cessation of therapy.
5. Follow-up specimen of urine at 24 or 28 weeks depending on the gestation when treated.
6. Specimen of urine at 32 weeks.
7. Specimen of urine at 38 weeks.

In order to put the above scheme into operation the following steps were also necessary, since these patients were distributed in three different units for antenatal care:

1. All patients in the trial had their antenatal cards stamped "U.T.I. Survey", so that it was easy to identify them.
2. All "booked" patients, booked for a hospital delivery, were asked to return to the antenatal clinic when 12 weeks pregnant, to avoid therapy during the early period of pregnancy.

3. If not booked, a letter was sent to the patient offering hospital booking, and asking to report back when about 12 weeks pregnant.

A booking form was also sent to the General Practitioner (G.P.) concerned.

4. If under the G.P. for antenatal care, a letter was sent to the G.P. explaining the trial and another to the patient.

5. All patients in the trial also had a letter to their respective G.P.s regarding the drugs given.

6. A cyclostyled "trial form" was completed at each visit of a patient in the trial so that clinical details and complications were accurately recorded.

7. If any patient in the trial developed overt symptoms of urinary infection during the survey, this infection was confirmed by a urine culture and the patient "re-treated" at the discretion of the clinician in charge. This patient was classed as "failed treatment" for the purposes of the trial, and follow-up continued as before.

Collection and storage of serum samples

8. During the latter half of this study it was possible to organise a special "bacteriuria follow-up clinic" where these women were seen and a sample of blood taken at each visit, along with the specimen of urine, for serum antibody studies.

These sera were numbered and stored in 0.5 ml.

aliquots in small plastic tubes with cork stoppers.

These sera were stored at -40° C. and all samples from one patient were titrated simultaneously at the end of pregnancy.

Samples of serum were also obtained, for control purposes, from antenatal women without bacteriuria.

This was an aliquot from the usual amount taken for routine antenatal tests such as Wasserman test.

Laboratory recording of data

Each patient in the trial had a card measuring 8 inches by 5 inches on which a standard form was stamped by a small hand duplicator and stencil. At each visit of the patient the following data were recorded, namely:

1. Date.
2. Gestation of pregnancy in weeks.
3. Viable count of organisms per ml. of urine.
4. Organism isolate number if culture is positive.
5. Biochemical identity of the organism.
6. Drug sensitivity by the tube dilution method.
7. Number of serum sample.
8. Reciprocal titre of antibody detected.
9. Sero-type of organism isolated.

Each card also carried the name of the patient and the drug given and was filed according to the antenatal number.

Other Clinical Groups Examined

1,443 women attending the infertility clinic had urine specimens examined by the modified nitrite test followed by culture of positive screening tests, using the same urine specimen. It was possible to re-examine some of these women during their subsequent visits to this clinic.

Biochemical identification of organisms isolated from urines with significant asymptomatic bacteriuria

All isolates from the patients in the chemotherapy trial were identified by this method. The great majority of these strains belonged to the family Enterobacteriaceae and were identified in accordance with the scheme proposed by the International Enterobacteriaceae Subcommittee (1958). About 50 organisms were tested in one batch, including known controls belonging to each of the common members of the family found in urine. It was thus possible to detect variations in the composition of batches of culture media.

Each organism was plated out on blood agar medium from the bijou slope on which it was initially stored. This ensured purity of the culture and also enabled the recognition of staphylococci and streptococci by their colonial morphology and pattern of haemolysis on blood agar. All Gram negative bacilli were further identified by inoculating a single colony into a tube of sterile

normal saline, followed by the inoculation of a tube of about 7 ml. of nutrient broth (Oxoid No. 2).

These broths were incubated overnight and 2 drops of the respective broth culture were used to inoculate each of the media as described below. The saline suspension was used to inoculate only citrate agar slopes, thus minimising carry-over of nutrient material in this inoculum. Bromothymol blue was used as an indicator of change of pH in medium where it was necessary.

Sugar fermentation tests

The sugars routinely used were lactose and inositol. The medium in each case was made up according to Cruickshank (1965, p. 813) using a 0.5% concentration of the sugar and bromothymol blue as indicator. These media, after inoculation, were incubated at 37° C. and observed for acid and gas production on the 1st, 2nd, 3rd and 7th days of incubation. The day on which these changes occurred was also noted.

Indole test

This test was done according to Cruickshank (1965, p. 826). The medium, after inoculation, was incubated for 48 hours and then tested for the presence of indole using Kovac's reagent.

Methyl-red test

This test was done according to Cruickshank (1965, p. 818). The inoculated medium was incubated for 5 days before adding the methyl-red reagent. The resulting

colour of the culture, after mixing, was read as follows:

A bright red = +ve
 A red-orange = \pm (weak positive)
 Yellow = -ve

Voges-Proskauer test

Test done according to Cruickshank (1965, p. 818). The medium, after inoculation, was incubated for 48 hours and then tested by the Barritt's modification.

Ammonium-citrate utilisation test

Simmonds' citrate medium with agar and bromothymol blue as indicator was used according to Cruickshank (1965, p. 829). The inoculated citrate agar slope was incubated and examined daily for 4 days. A positive test was indicated by a change from green to a dark blue and a streak of growth on the medium. All positive tests were confirmed by subculture on to a second citrate agar slope.

Urease test

Christensen's urea agar medium was used according to Cruickshank (1965, p. 826). All inoculated tubes were examined after 4 hours of incubation and thereafter daily for 4 days before being ascribed negative.

Phenylalanine deaminase test

This test was done according to Cruickshank (1965, p. 825). The inoculated media were tested after 24 hours' incubation and interpreted as described in the above reference.

Sulphonamide: Since there was no satisfactory method

Malonate utilisation test

Done according to Cruickshank (1965, p. 831). The change in the colour of the bromothymol blue indicator from green to blue due to a rise in pH was regarded as positive.

In some instances where atypical results were obtained in any of the above tests, the manual on Identification of Enterobacteriaceae, edited by Edwards and Ewing (1962), was found to be of practical value.

Identification of Gram positive cocci

Staphylococci: The colonial morphology on blood agar plate was noted. All strains had tests for coagulase production done by the slide method. The tube coagulase test was also done in instances where the results of the slide test were doubtful (Cruickshank, 1965, p. 138).

Streptococci: The colonial morphology and the nature of haemolysis on fresh blood agar medium were noted. These strains were also cultured on MacConkey agar. Strains producing minute, magenta-coloured colonies on MacConkey agar were called Streptococcus faecalis or enterococcus. Further classification of these organisms was not done, due to the small numbers of them isolated during this study.

Methods for testing sensitivity of organisms to antimicrobial agents used

Sulphonamide: Since there was no satisfactory method

for assessing the sensitivity of urinary pathogens to sulphonamides, the following standardised technique was developed (see results).

Tube dilution sensitivity test using sulphadimidine:

Injection sodium sulphadimidine (I.C.I.) in ampules containing 1 g. of the compound was used. A stock solution was made by dissolving 1 g. in 100 ml. of sterile sensitivity test broth (Oxoid). A series of doubling dilutions of the above stock solution was made in 20 ml. amounts of the same broth; a fresh sterile pipette was used for each transfer. The following concentrations of the drug were used in the test: 625 $\mu\text{g.}$, 312 $\mu\text{g.}$, 208 $\mu\text{g.}$, 156 $\mu\text{g.}$, 104 $\mu\text{g.}$, 52 $\mu\text{g.}$, 39 $\mu\text{g.}$, 19 $\mu\text{g.}$, of sulphadimidine per ml. of the solutions, these figures being corrected to the nearest whole number. Aliquots (0.5 ml.) containing these concentrations were transferred by automatic pipette into sets of small sterile stoppered tubes.

Inoculum size: All test organisms were initially plated out on (Oxoid) sensitivity agar to ensure purity and single colonies were inoculated into 10 ml. of sensitivity test broth. An 18 hour liquid culture of this broth was serially diluted in 99 and 9 ml. amounts of 0.85% sterile saline using an automatic 1 ml. pipette. Inocula contained about 2×10^3 organisms in 0.02 ml. volumes delivered by a standard calibrated dropping pipette.

The tubes containing the required concentrations of sulphadimidine were each inoculated with the above inoculum of the test organism. All tubes were examined after 18 hours' incubation at 37° C. to determine the minimum inhibitory (bacteriostatic) concentration (MIC) of sulphadimidine used. This was the lowest concentration that inhibited growth as judged by a lack of turbidity in comparison with the turbidity produced by growth under similar conditions in a control tube of the medium which contained no sulphonamide. End points were confirmed by the addition of 1 drop of 0.2% bromothymol blue to all tubes; acid production associated with significant bacterial growth was indicated by a colour change from blue to yellow. An MIC level of 52 µg. per ml. or below was considered sensitive (see results).

Disc diffusion sensitivity test

Discs impregnated with 250 µg. of sulphadimidine were prepared as described by Cruickshank (1965, p. 894). All discs were used in the wet state as recommended by Gould and Bowie (1952). A blue dye, Cr. Chlorazol sky blue F.F. 200 (I.C.I.), was incorporated in these discs to distinguish them from other discs used in the laboratory. Plastic petri dishes of 3½ inch diameter containing Oxoid sensitivity test agar were inoculated with a 10⁻³ dilution of an overnight sensitivity broth culture of the test organism in 0.85% sterile saline. A uniform growth of discrete colonies was obtained by surface

seeding well dried plates with 3 ml. of the above dilution (about 10^5 organisms per ml.) and discarding any excess fluid using a Pasteur pipette. The discs were placed after allowing a few minutes and the resulting zone of inhibition of growth, after overnight incubation, was measured as mm. diameter, including the diameter of the disc. When clear plastic plates were used, these zones were easily measured by dividers when viewed from the back of the plate against a good source of light.

Cycloserine tube dilution sensitivity tests

Ampules containing 100 mg. of cycloserine in powder form, for laboratory sensitivity tests, were kindly supplied by Eli Lilly and Co. A stock solution of 100 mg. of cycloserine in 100 ml. of sensitivity test broth was made. A series of doubling dilutions of the above stock solution in 20 ml. amounts of sensitivity test broth were made. Aliquots (0.5 ml.) containing the following concentrations were used for each test organism, namely, 1,000 $\mu\text{g.}$, 500 $\mu\text{g.}$, 250 $\mu\text{g.}$, 125 $\mu\text{g.}$, 62.5 $\mu\text{g.}$, 31 $\mu\text{g.}$, 15.5 $\mu\text{g.}$, and 8 $\mu\text{g.}$ per ml. These tubes were inoculated with about 2×10^4 organisms of the test strain, in 0.02 ml. volume as delivered by a standard calibrated dropping pipette. All tubes were examined after 18 hours' incubation to determine the minimum inhibitory (bacteriostatic) concentration of cycloserine used, as described in the above test for sulphadimidine. An MIC level of 125 $\mu\text{g.}$ per ml. or less

was considered sensitive (Welch et al., 1955).

Cycloserine disc sensitivity tests

Discs containing 75 ^{μ} g. per disc of cycloserine were supplied by Mast Laboratories for trial purposes. The antibiotic was unstable on these discs and the zone sizes were not reproducible under identical conditions using the same organism. Hence its use was abandoned in favour of tube dilution tests.

Serological typing of strains of *Escherichia coli*

Standard type cultures

Type cultures belonging to all 145 'O' antigen groups of *Escherichia coli* were kindly supplied, as freeze-dried samples, by the National Collection of Type Cultures, Colindale, London.

Preparation of rabbit antisera to selected 'O' antigen types from the above collection

The schedule of immunisation of rabbits was according to the scheme used at the Central Public Health Laboratory, Colindale. This scheme is similar to the one recommended by Edwards and Ewing (1962).

Cultures employed in antiserum production were plated out on 0.05% glucose infusion agar plates and smooth colony forms were selected. In order to test that suspensions were homogeneous and not autoagglutinable, an overnight infusion broth culture of each strain was steamed at 100° C. for 2½ hours. Only strains that

remained as homogeneous suspensions in broth after heating were used to inoculate rabbits.

Rabbits were given 5 intravenous injections in all, the first three of formalinised broth cultures and the last two consisting of live organisms. All injections were given into the marginal ear vein. The interval between injections was 5 days.

1st injection: 0.5 ml. of a formalinised 5-hour infusion broth culture containing about 10^6 organisms per ml.

Commercial formalin was added to a 6-hour broth culture to a final concentration 0.5%. These cultures were mixed and refrigerated overnight, the rabbits being inoculated the next day.

2nd injection: 1 ml. of a formalinised, 5-hour broth culture.

3rd injection: 1 ml. of a formalinised, 5-hour broth culture.

4th injection: 1 ml. of a live, 5-hour broth culture.

5th injection: 2 ml. of a live, 5-hour broth culture.

After 2 test bleeds at 5 day intervals following the last injection, the animal was exsanguinated by cardiac puncture, about 10 days after the last injection.

All blood was collected into sterile test-tubes lined with a thin film of saline agar, in order to promote good clot retraction and separation of serum. The serum was separated after overnight refrigeration.

Preservation of typing sera

Each of the sera was preserved at a working dilution of 1 in 50. The diluent used was physiological saline containing 1:10,000 "Merthiolate" made by Eli Lilly and Co. Only about 2 ml. of each serum were diluted thus. The rest of the serum was stored in glass ampules containing about 2 ml. each, the tips of which were sealed in a flame. These sera, after labelling, were stored in a deep freeze at -40° C. till required.

Preparation of 'O' antigen suspensions for testing'O' antisera

Each of the 145 standard 'O' strains were plated out on 0.05% glucose infusion agar. Five smooth colony forms from each plate were selected and inoculated into 20 ml. amounts of sterile infusion broth contained in screw-capped bottles. These bottles were incubated for 18 hours at 37° C. and then heated in free steam at 100° C. for 1 hour in order to inactivate the 'L' or 'B' surface antigen. After steaming these suspensions were preserved, at room temperature, by adding 0.06 ml. of a 40% solution of formaldehyde to each 20 ml. of culture.

Titration of each prepared antiserum against the homologous 'O' antigen suspension to determine its titre

All titres here refer to the reciprocal of the final serum dilution, after the addition of the antigen.

Glass test-tubes with rims, 3" x $\frac{1}{4}$ ", with rounded bottoms made by "Sameo" were used for these agglutination tests.

Procedure

A series of doubling dilutions of each antiserum, in 0.3 ml. amounts, was made in 0.85% saline. The initial serum dilution in this test was 1 in 50. An automatic pipette was used to transfer constant volumes. An equal volume of saline only was also included as a control tube. The homologous 'O' antigen suspension was added to all tubes including the control, using an equal 0.3 ml. volume in each. These tubes were incubated in a waterbath at 50° C. overnight, then kept at 4° C. for about 1 hour before examining for agglutination. These titrations were repeated four times for confirmation.

A simple viewing box, 22" x 14" x 10" of wood, was constructed and a 10" filament strip lamp was fixed in it. The inside of this box was painted black. This ensured reproducible, uniform lighting conditions for reading agglutination tests with the naked eye.

Criteria used for a positive agglutination test

All tubes were dipped in xylol to clean the outer surface, before viewing. Agglutination was considered to have occurred only if there was a sediment of granules of clumped organisms with a completely clear supernatant. All doubtful reactions were neglected. It was also essential that the control tube containing saline only with the antigen suspension showed a uniform turbidity with no granules.

Dilution of above antisera to give a final titre of 1 in 800 against the homologous strains

Each antiserum was diluted with merthiolated saline in a proportion as calculated from its initial titre. Hence, if an antiserum had an initial titre of 1 in 6400, the 1 in 50 dilution of the antiserum was further diluted 1 + 7 parts of diluent. The end titre of 800 in each instance was verified by further titrations of this second dilution of each serum, using the homologous 'O' antigen suspensions. Hence all sera, diluted to give a final titre of 800, were used in order to determine cross reactions with each of the 144 other 'O' antigens within the genus.

Titration of antisera against each of 145 standard 'O' antigen suspensions in order to detect cross-reacting antigens within the genus

Test for homogeneity of 'O' suspensions

Each 'O' antigen suspension was tested to ensure that it did not autoagglutinate. This test was carried out by adding 0.2 ml. of normal saline to 0.2 ml. of each 'O' antigen suspension in an agglutination tube. These tubes were incubated overnight in a waterbath at 50° C. and viewed the next day for agglutination. If agglutination was present the strain was considered as probably "rough" and attempts were made to isolate a smooth colony from the original culture.

Titration of antisera against each of 145 standard 'O' antigens

Using the serum diluted to give a titre of 800 with the homologous strain, a one tube screening titration was done with one volume of above serum (0.2 ml.) and an equal volume of a homogeneous 'O' antigen suspension. Hence each antiserum was titrated against all 145 'O' antigens. All positive cross-reactions in these screening tests were titrated out. It was thus possible to determine the titre of cross-reactions in comparison with the titre against the homologous strains. It was then also possible to determine sera that had to be absorbed with cross-reacting strains in order to produce a serum which was monospecific for the homologous strain. The absorption was not, however, undertaken at this stage.

Identification of the somatic 'O' antigen of Escherichia coli strains isolated from urine

Preparation of 'O' antigen suspension

The technique was the same as that used to prepare the standard 'O' suspensions from type cultures.

Preliminary screening

A screening test was done using one volume of each antiserum, at a titre of 800, and one volume of the unknown antigen suspension. A control tube containing one volume of saline and one volume of antigen was

included to ensure that the unknown strain was not rough or autoagglutinable. Since there were 10 rabbit antisera it was possible to screen in one step against all sera rather than use "pooled" sera. These agglutination tubes were incubated overnight at 50° C. and viewed for agglutination as above.

Autoagglutinable or rough: If the control tube with saline and antigen only showed agglutination, granules and clearing of the supernatant, this organism was called rough or autoagglutinable. In this case all other tubes containing antisera also showed agglutination.

Provisional type O 'X': If the control 'O' suspension was homogeneous and one of the tubes containing antisera showed agglutination, the unknown strain was called provisional type O 'X', 'X' here being the number of that particular 'O' antiserum. If no agglutination occurred in any tube the strain was "non-typable".

After a batch of strains were thus screened using one tube of each serum, all strains provisionally typed as, say, O₂ were confirmed by titrating each antigen in the O₂ antiserum to its full titre. If the standard O₂ suspension showed agglutination up to the 4th tube (titre 800) the unknown strain should also agglutinate to this titre, or one tube less only, to be called type O₂. A one tube difference was allowed as acceptable experimental error.

well mixed by shaking. The contents, in a sterile,

Use of absorbed mono-specific antisera

This was only done in the case of those antisera which showed cross-reactions with other 'O' antigens to the full titre as given against the homologous strains, or to one tube less. Thus, if a cross-reacting strain only agglutinated to a titre of 200 and the titre against the homologous strain was 800, then this cross-reaction would not interfere with the final typing of an unknown strain.

Preparation of mono-specific antisera by absorptions with cross-reacting strains

The method used was as recommended by the Central Public Health Laboratories, Colindale.

Overnight infusion broth culture of the cross-reacting strain was flooded on to the surface of well dried infusion agar plates and the excess inoculum discarded using a Pasteur pipette. These plates were incubated overnight and the growth from about $1\frac{1}{2}$ plates of $4\frac{1}{2}$ inch diameter was used to absorb out each anti-serum titre of 1000. The neat rabbit antiserum was used. The growth from the required number of plates was suspended in mercuric iodide solution. The volume of the solution used here depended on the titre of the neat serum, so as to dilute it to give a rough titre of 1000. The neat serum was added to the suspension of organism in mercuric iodide solution and the contents well mixed by shaking. The contents, in a sterile,

screw-capped bottle, were incubated at 50° C. for 2 hours and then centrifuged at 2000 g. for 20 minutes and the clear supernatant used. Each absorption was tested by titration against the homologous and the cross-reacting, now absorbed, strain. If absorption was incomplete, the antiserum was re-absorbed with the growth from additional infusion agar plates. When there were two or more strongly cross-reacting strains, these absorptions were done one after the other.

Preparation of mercuric iodide solution

Stock solution A: Mercuric iodide 1 g.
Potassium iodide 4 g.
Distilled water 100 ml.

Stock solution B: 2% commercial formalin in physiological saline brought to pH 7.6 with disodium hydrogen phosphate.

Solution for use in absorption of antisera

Stock solution A 10 ml.
Stock solution B 2.5 ml.
Physiological saline 90 ml.

Acridine test to detect incipient or complete roughness of *Escherichia coli* strains

Acridine solution was made by dissolving 1 g. of acridine in 500 ml. of physiological saline. This solution was stored in the dark and discarded if any deposit formed.

The test was done by using a straight wire and emulsifying the growth from a single colony in a drop of the above acriflavine solution on a glass slide. The suspension remained homogeneous if the organism was serologically smooth. Granularity or clumping indicated incipient or complete roughness.

Haemagglutination technique used for detecting antibodies to urinary strains of *Escherichia coli* in the serum of patients with significant bacteriuria

A modification of Neter's haemagglutination technique (Neter et al., 1952) was used (see results).

'O' antigen extract

The urinary strain of *Escherichia coli*, used as antigen, was plated out on a horse digest agar plate. A single smooth colony was inoculated into 10 ml. of horse digest broth. This broth was incubated overnight and 2 ml. of it flooded on to the surface of each of 3 horse digest agar plates. It was essential to ensure that these plates were not contaminated by previously incubating them overnight and examining the surface for contaminants before use. When well dried, warm digest agar plates were used, the inoculum was mostly absorbed. Excess fluid was discarded using a sterile pipette. The plates were incubated overnight and the resulting surface growth removed and suspended in 10 ml. of sterile normal saline in a screw-capped bottle. This suspension was

heated at 100° C. for 1 hour, and then centrifuged at 2000 g. for 20 minutes. The clear supernatant, or the crude 'O' antigen extract, was used to coat erythrocytes. These antigen extracts were kept at 4° C. and used within a week, to avoid contamination. If the extracts became turbid during storage within this time, they were discarded, but this only occurred on prolonged storage.

Sensitisation of sheep cells

Fresh sheep blood was collected from the slaughterhouse in Alsever's solution. The blood was centrifuged and the supernatant discarded. The packed cells were washed three times in a screw-capped glass container, twice with 0.85% sodium chloride and once with phosphate buffered saline at pH 7.2. These cells were finally centrifuged at 750 g. for 15 minutes, to pack the cells. The supernatant buffer was discarded.

Sensitisation of washed, packed sheep cells

The above washed and packed cells were suspended in the 'O' antigen extract. 0.1 ml. of packed sheep cells was pipetted into a one ounce universal container and 1 ml. of the sensitising antigen extract was added to it. The contents were gently mixed and incubated in a water-bath at 37° C. for 15 minutes. These cells were then washed 3 times in about 20 ml. of phosphate buffered saline to remove excess uncoated antigen and finally re-suspended in 10 ml. of phosphate buffered saline thus making a 1% suspension of coated or sensitised sheep

cells in phosphate buffer.

About 1 ml. of packed, washed, uncoated cells was kept aside from the same batch of cells to absorb out any antibody against sheep cells in human sera tested.

Titration of human serum samples

All specimens of sera collected from one patient over the period of gestation, about 5 samples during pregnancy, were titrated at the same time against the same batch of antigen-coated sheep cells so that any rise in titre, more than fourfold, was considered significant.

Sera were removed from the deep freeze and allowed to thaw at room temperature. All initial dilutions were made using a specially drawn, wide bore, Pasteur pipette. The diluent used was 0.5% bovine serum albumen in phosphate buffered saline at pH 7.2. An initial 1 in 20 dilution of each serum was made by adding 2 drops of the neat serum to 38 drops of the diluent using the same pipette. The pipette was rinsed seven times in buffer and dried on clean blotting paper before another serum was diluted. The antisera, diluted 1 in 20, in clean, small, glass test-tubes, were incubated at 56° C. for 30 minutes in a water-bath, to destroy complement. After inactivation 1 drop of packed, uncoated sheep cells was added to each serum with the same pipette. The contents were mixed repeatedly and left at room temperature for about 30 minutes and then centrifuged and the

clear supernatant was titrated.

Titration

W.H.O. plastic haemagglutination plates with 80 wells were used. The first row of each plate was used to make 9 doubling dilutions of the above serum in buffer containing 0.5% bovine serum albumen as above. About 0.5 volumes of each dilution were made using an automatic pipette and the tenth well contained only the diluent as control. Aliquots of each dilution, beginning with the control and then the most dilute serum, were transferred into each of 3 rows, transferring only one drop into each well using the same wide bore pipette. Aliquots of these dilutions were also transferred into sets of agglutination tubes at the same time to do a comparative titration by bacterial agglutination technique.

After rinsing the pipette, one volume of the antigen extract, used to coat the cells, was added to each well in the middle of the 3 rows including the control well. One volume of the diluent was added to the first and third rows to make up to the same dilution. The contents were mixed by shaking the plate and 1 volume 1% suspension of control, normal, uncoated sheep cells in buffer only was added to the third row. The first and second rows had 1 volume of a 1% sensitised sheep cell suspension. The plates were shaken for about 2 minutes to ensure a uniform suspension of sheep cells. These plates were now left covered and undisturbed at 4° C.

overnight and the agglutination pattern read the next day. Hence each antiserum titrated against one infecting urinary antigen used 4 rows of 10 wells each. The first row was used for making the doubling dilutions in bulk, the second row gave the haemagglutination titre against the infecting antigen, the third row showed a specific inhibition of haemagglutination in all wells by pre-incubation of this antiserum with antigen, and the last row detected any unabsorbed antibody to normal sheep cells. The controls were sensitised cells in diluent alone, sensitised cells in antigen and diluent alone, and unsensitised cells in diluent only and in all dilutions of the serum.

Cleaning of W.H.O. plates

After a test all plates were rinsed under running tap water and completely immersed in a plastic bucket containing a solution of "Pyronex". After being soaked overnight each plate was rinsed in tap water by filling all wells and shaking and emptying all wells. It was important to rinse thoroughly to avoid lysis of red cells used in the test. The plates were then shaken dry and kept inverted on clean sheets of blotting paper.

Immediately before use each well was cleaned with cotton wool soaked in phosphate buffer at pH 7.2.

Each antiserum was titrated twice independently to confirm the end titre.

Titration of human sera against infecting urinary organisms using the bacterial agglutination technique

The bacterial agglutination technique was similar to the technique used in the serological typing of these organisms. The 'O' antibody titre in each serum was determined by using 'O' antigen suspensions of the urinary strain of Escherichia coli isolated initially from that patient. Since aliquots of the same series of doubling dilutions were used for the haemagglutination and bacterial agglutination tests carried out at the same time, the end points were comparable and indicated the sensitivity of each test. In each series a control tube containing saline and the respective 'O' antigen suspension served to indicate roughness or autoagglutination of the strain used.

Titration of control normal human sera for antibody against common urinary strains of Escherichia coli

Samples of serum were obtained from women at their first attendance at the antenatal clinic, along with a specimen of urine. Hence these are sera from pregnant women who did not have significant bacteriuria.

The following standard 'O' antigens were tested against each serum: O₁, O₂, O₄, O₆, O₇, O₈, O₁₈, O₂₅, O₇₅, O₇₇, using the haemagglutination and bacterial agglutination techniques.

Screening of control sera by the haemagglutination method

Ten sensitised 1% sheep cell suspensions were prepared as described above. Hence these cells were sensitised individually with standard 'O' antigens of groups 1, 2, 4, 6, 7, 8, 18, 25, 75 and 77. A control 1% suspension of unsensitised sheep cells was also prepared.

Each control serum was initially screened at a dilution of 1 in 80 using each of the above sensitised cells. All positive haemagglutinations were titrated out to their full titres.

All sera were inactivated for complement, and absorbed with normal, washed, packed cells before screening for antibody against the above 'O' antigens.

Screening of control sera by the bacterial agglutination technique

An aliquot of the same 1 in 80 dilution of each control serum initially made for the haemagglutination test was used for this test. 0.2 ml. volumes of each serum were put into each of 10 agglutination tubes. An equal volume of the respective standard 'O' antigen suspension was added to each tube. A control tube of each antigen suspension in saline only was also included. Tubes were incubated and read as previously described. All positive reactions were titrated out to their full titres.

Method of Analysis of Clinical Data

1. Clinical data from 127 asymptomatic bacteriuric women in the treatment trial

The clinical and laboratory data from this group of patients were coded and transferred to punch cards made by The Copeland Chatterson Co. Ltd. These cards were then analysed manually.

2. Clinical data from 1,974 non-bacteriuric women were also put into code form and transferred on to Hollerith cards by the firm of International Computers and Tabulators, Glasgow. These cards were analysed by The Data Processing Unit of the Department of Social Medicine, Edinburgh.

Statistical methods

The statistical methods used were:

1. Mean and standard deviation for the age distribution of the patients in this study. A comparison of the mean age of the patients in the groups, i.e. treated and untreated bacteriuric and control non-bacteriuric patients using the "students' t test" (Fisher, 1958; Hill, 1966). A similar comparison of the mean age of the patients **treated** with sulphonamide or cycloserine was also undertaken.
2. The comparison of the overall incidence of the various complications during pregnancy and the results of treatment were possible by using the "chi-square test

(χ^2) " as suggested by Fisher (1958) and Hill (1966).

The partitioning of chi-square into the separate degrees of freedom was done by the method of Brandt and Snedecor as quoted by Maxwell (1961) and Mather (1964). The tables consulted for levels of significance were those of Fisher and Yates (1963).

An example of the method of partitioning chi-square into its several degrees of freedom in the analysis of the incidence of anaemia during pregnancy in the various groups is given below:

The incidence of anaemia

Groups	Anaemia present	Anaemia absent	Total	Proportion present
	x_j	$n_j - x_j$	n_j	p_j
Control	169	1805	1974	0.0856
Success	6	59	65	0.0923
Relapse	7	24	31	0.2258
Failed	8	23	31	0.2581
Untreated	32	192	224	0.1429
Total	$T_x = 222$	$T - T_x = 2103$	$T = 2325$	

$$\hat{p} = T_x / T = 0.0955$$

$$\hat{q} = (1 - \hat{p}) = 0.9045$$

$$\hat{p} \times \hat{q} = 0.0864$$

Hence there is a difference between these two groups.

$$\text{Overall } \chi^2_{(4)} = \frac{\sum x_{jp} - \hat{p} T_x}{\hat{p} \hat{q}}$$

$$= \frac{(169)(0.0856) + \dots + (32)(0.1429) - (222)(0.0955)}{0.0864}$$

$$= \frac{2.0374}{0.0864}$$

$$= 23.5810$$

$$P < 0.001$$

	Anaemia present	Anaemia absent	Total	Proportion present
Relapse	7	24	31	0.2258
Failed	8	23	31	0.2581
Untreated	32	192	224	0.1429
Total	47	239	286	

Partitioning χ^2

Method of Brandt and Snedecor for 2XN contingency

table:

1. Comparing the incidence of anaemia in the successfully treated group and the control non-bacteriuric group:

Groups	Anaemia present	Anaemia absent	Total	Proportion present
Control	169	1805	1974	0.0856
Success	6	59	65	0.0923
Total	175	1864	2039	

the relapse, failed, untreated groups together:

$$\hat{p}_1 = \frac{175}{2039} = 0.0858$$

$$\chi^2_{(1)} = \frac{(169)(0.0856) + (6)(0.0923) - (175)(0.0858)}{0.0864}$$

$$= 0.0601$$

$$P > 0.80$$

Hence there is no significant difference between these two groups.

2. Comparing the incidence of anaemia among patients who relapsed or failed to respond to treatment with the untreated bacteriuric group:

Groups	Anaemia present	Anaemia absent	Total	Proportion present
Relapse	7	24	31	0.2258
Failed	8	23	31	0.2581
Untreated	32	192	224	0.1429
Total	47	239	286	

$$\hat{p}_2 = \frac{47}{286} = 0.1643$$

$$\chi^2_{(2)} = \frac{(7)(0.2258) + \dots + (32)(0.1429) - (47)(0.1643)}{0.0864}$$

$$= 5.7419 \quad P > 0.05$$

Hence there is no difference in the incidence of anaemia between these three groups.

3. Comparing the incidence of anaemia in the control plus successfully treated group with the incidence in the relapse, failed and untreated groups together:

There is a significant difference in the incidence of anaemia between the following two groups:

1. Control non-bacteriuric + successfully treated bacteriuric group.

Groups	Anaemia present	Anaemia absent	Total	Proportion present
Control + success	175	1864	2039	0.0858
Relapse + failed + untreated	47	239	286	0.1643
Total	222	2103	2325	

$$\hat{p} = \frac{222}{2325} = 0.0955$$

$$\chi^2_{(1)} = \frac{(175)(0.0858) + (47)(0.1643) - (222)(0.0955)}{0.0854} = 17.7789 \quad P < 0.001$$

Components of χ^2 due to (groups)	χ^2	Degrees of freedom	Significance level
1. Comparing control with successfully treated group	0.0601	1	Not significant
2. Comparing relapse, failed treatment and untreated	5.7419	2	Not significant
3. Comparing control + successfully treated with relapse + failed treatment + untreated group	17.7789	1	$P < 0.001$
Overall χ^2	23.5809	4	$P < 0.001$

There is a significant difference in the incidence of anaemia between the following two groups:

- Control non-bacteriuric + successfully treated bacteriuric group.

2. Relapse + failed treatment + untreated bacteriuric groups.

There is no significant difference in the incidence of anaemia within either of the above two groups.

Definitions of Terms used in this Thesis

1. Anaemia

Haemoglobin level tested three times during pregnancy and under 70% on two occasions or an unequivocal clinical diagnosis of anaemia confirmed by further investigation. A 100% haemoglobin level in this case was 14.8 g. per 100 ml.

2. Abortion

If the menstrual history is clear, any delivery before 28 weeks of gestation. If the menstrual history is in doubt, a foetal birth weight of under 2½ lbs.

3. Toxaemia and hypertension

This group includes women with "essential hypertension" and "hypertension with superimposed toxaemia". A blood pressure persisting at 140/90 or over in the first 20 weeks of pregnancy is classified as "essential hypertension". An elevation of the blood pressure level at the first visit of the patient to hospital is not considered significant. "Hypertension with superimposed toxaemia" is applied to patients with essential hypertension before 20 weeks who subsequently develop proteinuria.

4. Acute urinary infection with symptoms in the antenatal or immediate post-natal period

Includes all cases of bacteriologically proven urinary infection with symptoms occurring in the antenatal period or during the 7 to 10 days following delivery, while the patient was in hospital.

5. Stillbirth

The birth of any child after the 28th week of pregnancy which did not show any signs of life after being expelled from its mother.

6. Neonatal death

Death of a baby during the first 28 days of life.

7. Prematurity by weight

A birth weight below 5 lbs. 8 ozs.

8. Prematurity by gestation

Delivery before the 36th week of gestation.

9. Infertility

Women who have failed to become pregnant having had the desire and the opportunity.

RESULTS

The present work is a continuation and expansion of a study which was started by Dr. J.D. Sleight in association with the clinical and nursing staff of the Simpson Memorial Maternity Pavilion, Edinburgh.

The general scope of this study was to determine the incidence of bacteriuria among pregnant and infertile women, using the extended nitrite test for the initial screening of urine specimens. A chemotherapeutic trial was undertaken in order to assess the value of a short course of therapy in the treatment of asymptomatic bacteriuria of pregnancy. It was hoped to study the pattern of recurrence of

RESULTS

aria after treatment. This was done by means of serological typing of Escherichia coli strains isolated before and after treatment. It was also hoped to estimate the serum antibody response to the infecting urinary organisms in these women.

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2. Bacteriuria of doubtful significance, consisting of all urines containing coliform organisms, in pure culture, between 10^4 and 10^5 organisms per ml.

3. Significant bacteriuria, which included all urines containing a pathogen at a concentration of 10^5 or more organisms per ml.

Some heavily contaminated urine specimens gave a count of mixed organisms at 10^5 organisms per ml. and were considered in the insignificant group.

CHAPTER I

Evaluation of Two Different Chemical Screening Tests
in the Detection of Significant Bacteriuria in
Specimens of Urine

One thousand "mid-stream" urine specimens were obtained from antenatal and infertile women attending these respective clinics at the Simpson Memorial Maternity Pavilion. All urine specimens were tested within two hours of collection or after overnight refrigeration. A quantitative viable bacterial count was done on each urine specimen. The modified nitrite test (Sleigh, 1965) and the T.T.C. test (Simmons and Williams, 1962) were also carried out on each urine. On the results of the quantitative urine culture, these urine specimens were divided into the following three groups:

1. Insignificant bacteriuria, which included all urines with a growth of mixed contaminants at or below the level of 10^4 organisms per ml.
2. Bacteriuria of doubtful significance, consisting of all urines containing coliform organisms, in pure culture, between 10^4 and 10^5 organisms per ml.
3. Significant bacteriuria, which included all urines containing a pathogen at a concentration of 10^5 or more organisms per ml.

Some heavily contaminated urine specimens gave a count of mixed organisms at 10^5 organisms per ml. and were considered in the insignificant group.

Table 1

The results of quantitative culture of 1,000 specimens
and of urine

Result of quantitative culture	Number of urine specimens
Insignificant bacteriuria	912
Bacteriuria of doubtful significance	17
Significant bacteriuria	71
Total	1000

Biochemical identity of the organism in 71 urine
specimens with significant bacteriuria

<u>Organisms</u>	<u>Number of urine specimens</u>
Escherichia coli	50
Escherichia coli (atypical)	16
Proteus mirabilis	2
Klebsiella	2
Enterococcus	1
Total	71

All subsequent prenatal and infertility clinic urine specimens were initially screened by the modified nitrite test. Each urine specimen giving a positive screening test was quantitatively cultured the same day. All specimens containing significant bacteriuria, as defined above, were reported as positive.

Table 2

Comparison of the efficacy of the modified nitrite
and T.T.C. test

Result of quantitative urine culture	No. of specimens	Modified nitrite test		T.T.C. test	
		Positive	Negative	Positive	Negative
Significant bacteriuria	71	69 (97%)	2	54 (76%)	17
Bacteriuria of doubtful significance	17	13	4	6	11
Insignificant bacteriuria	912	15	897	17	895

The infecting organisms in two instances where the modified nitrite test was negative were Escherichia coli and Enterococcus, both at a concentration of 10^5 organisms per ml. The number of false positive reactions were 1.6% with the modified nitrite test and 1.9% with the T.T.C. test.

In addition to being more successful in detecting significant bacteriuria, the modified nitrite test was simple to carry out and easy to read.

All subsequent antenatal and infertility clinic urine specimens were initially screened by the modified nitrite test. Each urine specimen giving a positive screening test was quantitatively cultured the same day. All specimens containing significant bacteriuria, as defined above, were reported as positive.

The Incidence of Significant Bacteriuria in Pregnant
and in Infertile Women

Incidence of bacteriuria in pregnant women

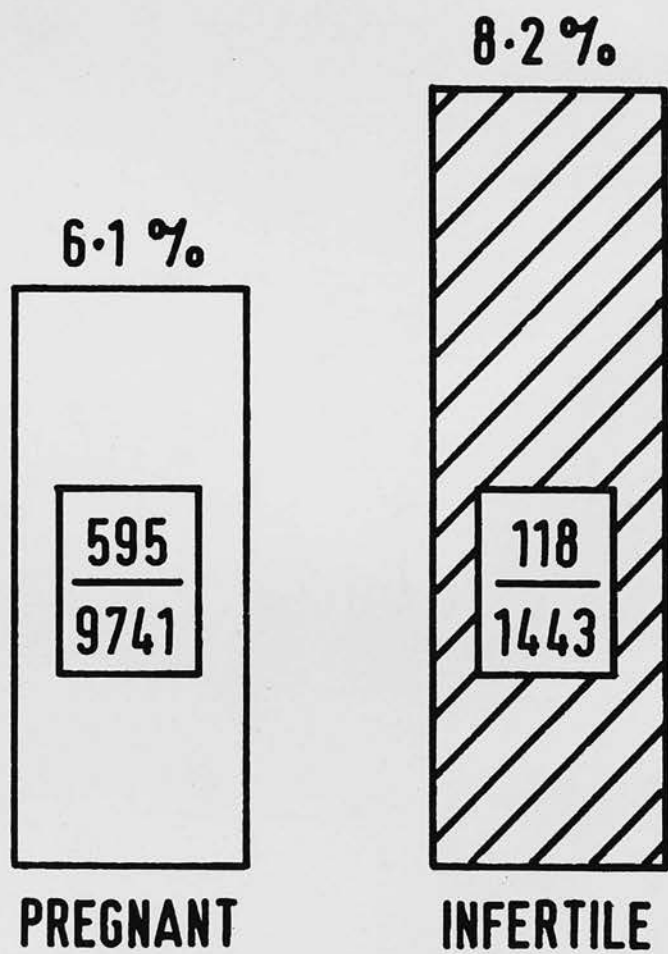
Total number of women examined	9,741
Number found to have significant bacteriuria	595
% Incidence in Simpson Maternity Pavilion	6.1%

It should be emphasised that this incidence is based on the examination of one specimen of urine from each antenatal woman and would be less if the usual definition of confirmed bacteriuria in two separate urine specimens is taken.

Incidence of bacteriuria in infertile women

Specimens of urine from 1,443 married women attending the Infertility Clinic were examined. One hundred and eighteen of them or 8.2% were found to have significant bacteriuria.

The incidence of significant bacteriuria in normal pregnant and infertile women in this study are shown in Figure 1.



Incidence of bacteriuria in normal pregnant and infertile women.

Figure 1

CHAPTER II

The Results of the Chemotherapeutic Trial for
Asymptomatic Bacteriuria of Pregnancy

The first 475 of the above 595 women with bacteriuria at their first antenatal visit were allotted, on a random alternative basis, to a two week therapy with either cycloserine or sulphadimidine.

Details of 475 bacteriuric pregnant women

Women in the trial	217
Withdrawn from trial	258
Total	475

Reasons for not including 258 bacteriuric women in the trial

	<u>No. of patients</u>
(1) Attending the hospital too late to allow treatment before the 28th week of gestation	135
(2) Had symptoms of urinary infection at the first visit (hence not asymptomatic)	32
(3) Under the General Practitioner for antenatal care - mainly due to distance from the hospital - unable to attend the "follow-up"	35
(4) Other reasons	56
Total	258

The "other reasons" for not including 56 bacteriuric women in the trial include women who were not pregnant or who had a previous history of hypersensitivity to these drugs, women who were already on an antibiotic for other medical reasons and women who aborted before the treatment was started.

The group of 258 untreated bacteriuric women were, however, followed up clinically and served as an "untreated control bacteriuric group" for comparison with the treated groups as regards the incidence of various urinary and other antenatal complications. Thirty-four of these 258 women were delivered outside Edinburgh and hence 224 untreated bacteriuric women were available for study.

Details of 217 women with asymptomatic bacteriuria of pregnancy who were in the chemotherapy trial

	<u>Number</u>
1. Women who have completed pregnancy after treatment with either drug	137
2. Women whose second urine specimen, taken immediately before starting treatment, was negative on quantitative culture (the 2nd specimen negative group), who were nevertheless treated with either drug	64
3. Women who left Edinburgh and were delivered elsewhere	4
4. Women who have not yet completed their pregnancy	12
Total	<u>217</u>

Non-bacteriuric control group of antenatal women

A group of 1,974 women without bacteriuria, as evidenced by a negative nitrite screening test, was also studied for comparison with the groups of treated and untreated bacteriuric pregnant women. This control group consisted of the first 1,974 consecutive antenatal women examined, after excluding the women with significant bacteriuria.

The results of the treatment trial

The results of treatment have been assessed using the following criteria based on the examination of urine specimens by a quantitative culture technique, after treatment with either drug. All patients here had bacteriuria confirmed on two separate occasions before treatment was started.

Table 3

Criteria used to evaluate the result of treatment

Result of treatment	Result of urine culture					
	Pre-treatment specimens of urine		Post-treatment follow-up specimens of urine			
	1st	2nd	1 week after treatment	At 24 to 28 weeks	At 32 weeks	At 38 weeks
1. Success	+	+	-	-	-	-
2. Failure	+	+	+	any finding here		
3. Initial success but relapse or reinfection	+	+	-	+	+	-
4. Initial success but inadequate follow-up	+	+	-	?	?	-

In this analysis only 5 patients on each drug did not have a complete follow-up, and these were not included in this analysis.

Number treated with sulphadimidine 62

Number treated with cycloserine 65

Total 127

The identity of organisms following cultural and biochemical tests isolated from the urine of 127 women with asymptomatic bacteriuria in the treatment trial is shown in Table 4.

Table 4

Biochemical identity of organisms isolated before
treatment in asymptomatic bacteriuric
pregnant women

Organism isolated	Sulphonamide treated (No. of women)	Cycloserine treated (No. of women)	Total
Escherichia coli	51	54	105
Escherichia coli (atypical)	7	6	13
Proteus mirabilis	3	2	5
Staphylococcus albus	0	1	1
Staphylococcus aureus	0	1	1
Klebsiella aerogenes	1	1	2
Total	62	65	127

The two groups of treated women have been compared as regards age distribution, parity distribution, and the number in each group who gave a past history of urinary tract infection and a past history of abortion.

Table 5
Age distribution

Age group (years)	Sulphonamide treated	Cycloserine treated	Total
19 years or less	9	5	14
20 - 24 years	27	28	55
25 - 29	15	21	36
30 - 34	8	7	15
35 and over	3	4	7
Total	62	65	127

$$\chi^2 = 1.251, P > 0.80$$

There is no significant difference in the age distribution of these two groups which are very similar. It can be further noted that 91 out of 127 or 71.7% of these women were between the ages of 20 and 30 years.

Table 6
Parity distribution

Parity	Sulphonamide treated	Cycloserine treated	Total
1st Pregnancy	41	39	80
2nd or more	21	26	47
Total	62	65	127

$$\chi^2 = 0.212, P > 0.50$$

As shown in Table 6, the two treated groups are again very similar in their parity distribution, there being no significant differences between them. Eighty out of 127 or 62.9% of these women were primiparous. Only two women, both treated on cycloserine, were "grand multiparous" or were in their fifth or more pregnancy.

Table 7

Past history of urinary tract infection

Past history of urinary infection	Sulphonamide treated	Cycloserine treated	Total
Yes	11	15	26
No	51	50	101
Total	62	65	127

$$\chi^2 = 0.278, P > 0.50$$

There was no significant difference between these groups as regards the incidence of urinary infection in the past. It is difficult to evaluate clinically a past history of urinary infection and only those who had infections which required treatment in the past are considered; vague incidences of "chill in the bladder" have not been included. In this asymptomatic bacteriuric group 26 out of 127 or 20.5% gave a past history of urinary infection.

Table 8

Past history of abortion

Past history of abortion	Sulphonamide treated	Cycloserine treated	Total
Yes	6	7	13
No	56	58	114
Total	62	65	127

$$\chi^2 = 0.014, P > 0.90$$

There is no significant difference between these groups. Thirteen out of 127 or 10.2% gave a past history of abortion.

Comparative results of treatment with sulphadimidine and treatment with cycloserine

The results have been considered as overall results, and then grouped together as short-term results evaluated one week after cessation of therapy, and long-term results as evidenced by culture of three further "follow-up" urine specimens.

Table 9

Overall results of treatment trial

Result of treatment	Sulphonamide treated	Cycloserine treated	Total
1. Success	28	37	65
2. Failure	18	13	31
3. Initial success with relapse or re-infection	16	15	31
Total	62	65	127

$$\chi^2 = 1.394, P = 0.50$$

There is no significant difference between the group treated with sulphonamide and the group treated with cycloserine as regards treatment success, treatment failure or the incidence of relapse/re-infection.

Table 10

Immediate or short-term results of treatment

Result of treatment	Sulphonamide treated	Cycloserine treated	Total
Success	44	52	96
Failure	18	13	31
Total	62	65	127

$$\chi^2 = 0.982, P > 0.30$$

These results (Table 10) are evaluated on the result of a quantitative culture of the urine one week after cessation of therapy. There is no significant difference in the results obtained with the two drugs. The immediate success rate is 96 out of 127 or 75.6%. If no further follow-up specimens were taken this regime of treatment would be falsely considered adequate.

Table 11

Long-term results of treatment

Result of treatment	Sulphonamide treated	Cycloserine treated	Total
Success	28	37	65
Failure or late relapse/re-infection	34	28	62
Total	62	65	127

$$\chi^2 = 1.290, P > 0.20$$

These results indicate that the effectiveness of the two drugs used in this trial was statistically similar in the treatment of asymptomatic bacteriuria of pregnancy. However, only 65 out of 127 or 51.2% of those treated remain uninfected during the remainder of pregnancy. The failure and relapse/re-infection rate is 62 out of 127 or 48.8%. These results are surprising and will be discussed more fully in the next chapter.

The Incidence of Complications in the Treated
Bacteriuric, Untreated Bacteriuric and
Non-bacteriuric Control Pregnant Women

The incidence of the following complications in the present pregnancy have been compared in these groups:

1. Bacteriologically proven acute urinary infection with symptoms in the antenatal or immediate post-partum period.
2. Anaemia.
3. Toxaemia or hypertension.
4. Prematurity by weight.
5. Prematurity by gestation.
6. Abortion.
7. Total foetal loss, including abortions.

The age and parity distributions in the above groups have also been analysed.

Table 12

Age distribution

Age group (years)	No. of patients with bacteriuria who were treated	No. of patients with untreated bacteriuria	No. of non- bacteriuric control patients
≤ 19	14	28	176
20 - 24	55	73	537
25 - 29	36	57	601
30 - 34	15	37	334
35 and over	7	29	326
Total	127	224	1974
Mean age (years)	25.5	27.1	28.2
\pm standard deviation (years)	\pm 5.4	\pm 6.7	\pm 6.7

There was a difference in the average age of the patients in these three groups. The treated group was younger, due to a higher proportion of women between the ages of 20 and 24 years in this group, in comparison with the untreated or non-bacteriuric groups.

Table 13

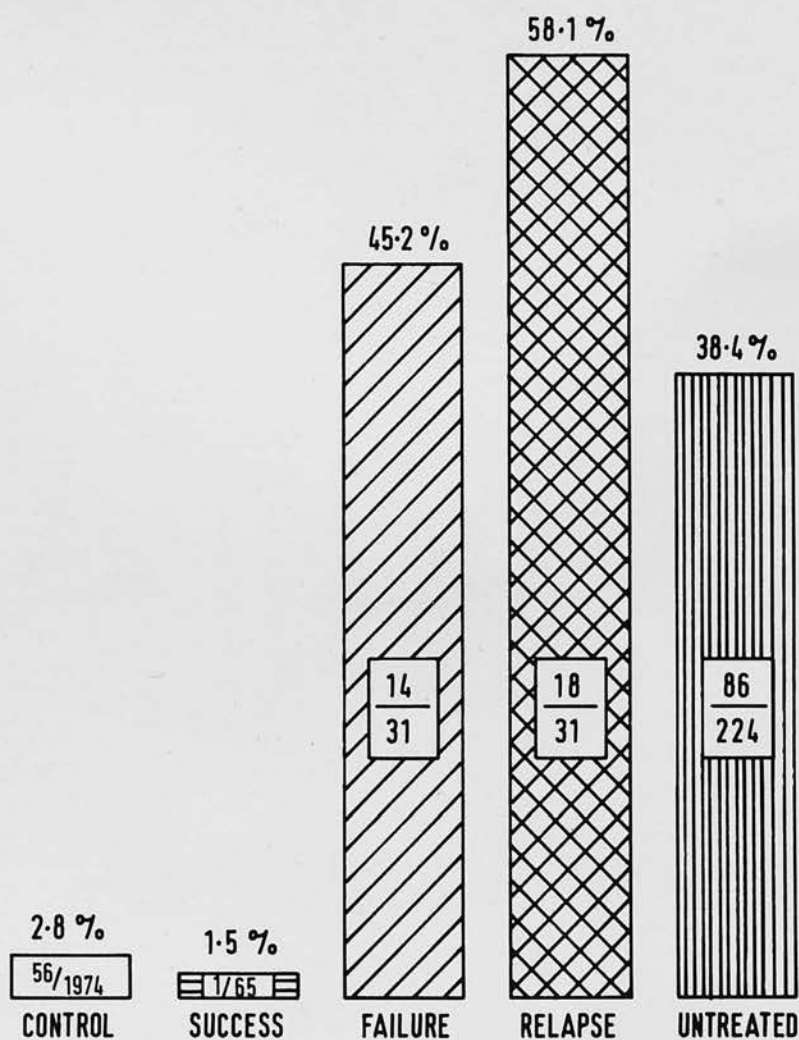
Parity distribution

Parity distribution	No. with treated bacteriuria	No. with untreated bacteriuria	Non-bacteriuric control women	Total
Primiparous	80	90	817	987
Multiparous	47	134	1157	1338
Total	127	224	1974	2325

There was no significant difference in the parity distribution between the untreated bacteriuric and non-bacteriuric control women. There was, however, a significantly higher incidence of primiparous women in the treated group ($\chi^2 = 23.081$, $P < 0.001$).

Symptomatic urinary infection

Table 14 and Figure 2 show the frequency of symptomatic, acute, urinary infection in the different groups:



Incidence of symptomatic urinary tract infection in treated and untreated bacteriuric patients and control subjects.

Figure 2

Table 14

The incidence of urinary infection with symptoms

Groups	Total No.	No. with symptomatic urinary infection
Non-bacteriuric pregnant women	1974	56 (2.8%)
Untreated bacteriuria of pregnancy	224	86 (38.4%)
Successfully treated bacteriuria of pregnancy	65	1 (1.5%)
Failed treatment for bacteriuria of pregnancy	31	14 (45.2%)
Relapsed after treatment for bacteriuria of pregnancy	31	18 (58.1%)

There was a highly significant difference in the incidence of bacteriologically proven, acute urinary tract infection with symptoms in these groups (overall $\chi^2 = 549.0933$, $P < 0.001$). These groups were further compared by partitioning the overall chi-square into its four degrees of freedom as seen in Table 15. The method of Brandt and Snedecor as quoted by Maxwell (1961) was used as described in the Materials and Methods Section. By this method it was possible to determine the sub-groups in this study which contributed to the raised incidence of acute urinary infection. It was also possible to compare the various groups with each other.

Table 15

Incidence of urinary infection with symptoms

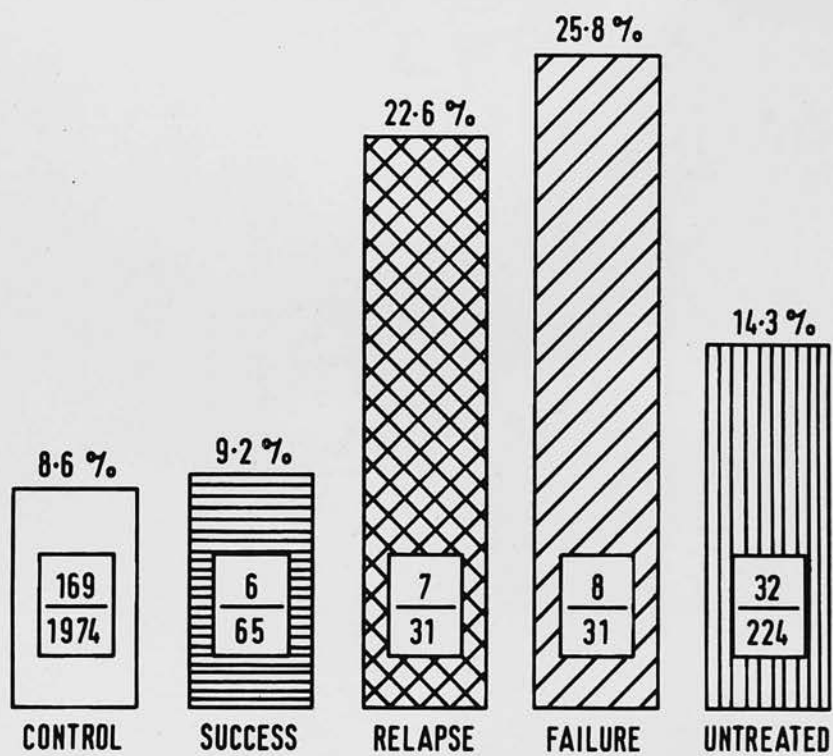
Components of χ^2 due to (groups)	χ^2	Degrees of freedom	Significance level
1. Comparing control group with successfully treated group	0.1408	1	Not significant
2. Comparing relapse after treatment with treatment failure group	3.7069	1	Not significant
3. Comparing relapse and failed treatment with the untreated group	12.1236	1	$P < 0.001$
4. Comparing success and control with relapse, failed and untreated groups	533.1221	1	$P < 0.001$
Overall χ^2	549.0934	4	$P < 0.001$

Hence successful treatment of asymptomatic bacteriuria was highly effective in preventing symptomatic urinary infection during pregnancy. There was no significant difference in the incidence of urinary infection in the successfully treated and the control non-bacteriuric groups. The incidence was significantly higher in the groups of patients who failed or relapsed after treatment for asymptomatic bacteriuria when compared with the untreated bacteriuric patients ($\chi^2 = 12.1236$, $P < 0.001$). When the groups who failed or relapsed after treatment and the untreated were

compared with the successfully treated and control non-bacteriuric groups there was a highly significant difference in the incidence of urinary infection, being high in the former groups ($\chi^2 = 533.1221$, $P < 0.001$). The duration of pregnancy, when these urinary tract symptoms were first noticed in the groups who failed or relapsed after treatment for asymptomatic bacteriuria, have been analysed. Out of 127 women who were treated, 33 developed symptomatic infections. Of these 33, 14 developed symptoms in the third trimester of pregnancy only, 7 in the second trimester only, 1 in the second and third trimesters, 5 during pregnancy and also after delivery and 6 after delivery only. The only patient who developed urinary symptoms in the successfully treated group did so on the day that her cycloserine therapy was commenced.

Anaemia

Table 16 and Figure 3 show the incidence of anaemia during pregnancy in the various groups:



Incidence of anaemia in treated and untreated bacteriuric patients and control subjects.

Figure 3

Table 16

The incidence of anaemia during pregnancy

Groups	Total No.	No. with anaemia during pregnancy
Non-bacteriuric pregnant women	1974	169 (8.6%)
Women with untreated bacteriuria of pregnancy	224	32 (14.3%)
Successfully treated bacteriuria of pregnancy	65	6 (9.2%)
Failed treatment for bacteriuria of pregnancy	31	8 (25.8%)
Relapsed after treatment for bacteriuria of pregnancy	31	7 (22.6%)

There was a highly significant difference in the incidence of anaemia in these groups (overall $\chi^2 = 23.5810$, $P < 0.001$). The incidence within these groups was further compared as shown in Table 17.

Table 17

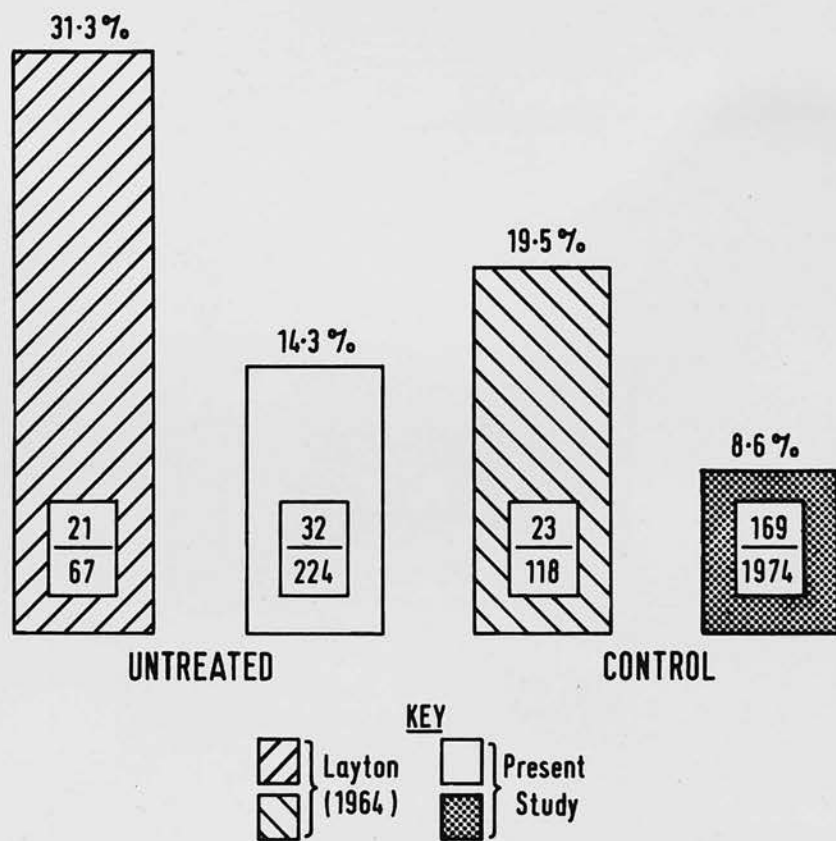
The incidence of anaemia during pregnancy

Components of χ^2 due to (groups)	χ^2	Degrees of freedom	Significance level
1. Comparing control with successfully treated group	0.0601	1	Not significant
2. Comparing relapse and failed treatment and the untreated group	5.7419	2	Not significant
3. Comparing the control and successfully treated groups with relapse, failed and untreated groups	17.7789	1	$P < 0.001$
Overall χ^2	23.5809	4	$P < 0.001$

The incidence of anaemia was significantly higher in pregnant women with bacteriuria which was untreated or unsuccessfully treated when compared with non-bacteriuric or successfully treated women ($\chi^2 = 17.7789$, $P < 0.001$). Hence successful treatment of asymptomatic bacteriuria appeared to have significantly reduced the incidence of anaemia during pregnancy. In other words, the presence or persistence after treatment of bacteriuria of pregnancy was associated with a higher incidence of anaemia.

The results obtained in the present study have been compared in Figure 4 with those reported by Layton (1964). It is seen that in both studies the incidence of anaemia is higher among the bacteriuric pregnant women than among the non-bacteriuric controls. The incidence of anaemia in Layton's study is higher than that found in the present study. This could be due to the small numbers examined by Layton or because of the difference in the two populations examined.

The results of the present study further suggest that the incidence of anaemia is particularly high among those women who have persistent bacteriuria after treatment. It is possible that this group of women have a chronic kidney infection which could cause anaemia. The results of radiological examination of the kidneys, which is at present being undertaken, would elucidate this further.



Incidence of anaemia in untreated bacteriuric and non-bacteriuric subjects. The results of the present study compared with those of Layton (1964).

Figure 4

Toxaemia or hypertension

The incidence of toxaemia or hypertension in these groups is seen in Table 18:

Table 18

The incidence of toxaemia or hypertension
during pregnancy

Groups	Total No.	No. with toxaemia or hypertension
Non-bacteriuric pregnant women	1974	99 (5.0%)
Untreated bacteriuria of pregnancy	224	13 (5.8%)
Successfully treated bacteriuria of pregnancy	65	7 (10.8%)
Failed treatment for bacteriuria of pregnancy	31	7 (22.6%)
Relapsed after treatment for bacteriuria of pregnancy	31	5 (16.1%)

There was again a highly significant difference in the incidence of toxaemia or hypertension during pregnancy among these groups (overall $\chi^2 = 28.0508$, $P < 0.001$). Its incidence in the successfully treated women was compared with that in the groups who either failed or relapsed after treatment; there was no significant difference between these groups. However, when all the treated bacteriuric women were compared with the untreated bacteriuric women, the incidence in the treated group was significantly higher ($\chi^2 = 12.7683$, $P < 0.001$).

This finding will be discussed later. The treated and untreated bacteriuric groups were next compared with the control non-bacteriuric group and the incidence of toxæmia or hypertension was significantly higher in the bacteriuric women ($\chi^2 = 9.6591$, $P < 0.010$).

These results, as obtained by the partitioning of the overall χ^2 , are seen in Table 19.

Table 19

The incidence of toxæmia or hypertension
during pregnancy

Components of χ^2 due to (groups)	χ^2	Degrees of freedom	Significance level
1. Comparing successful treatment with relapse and with failed treatment for bacteriuria of pregnancy	5.6233	2	Not significant
2. Comparing treated with untreated groups in bacteriuria of pregnancy	12.7683	1	$P < 0.001$
3. Comparing treated and untreated bacteriuric with non-bacteriuric pregnant women	9.6591	1	$P < 0.010$
Overall χ^2	28.0507	4	$P < 0.001$

In this study the group of patients who were treated for bacteriuria of pregnancy had a higher incidence of toxæmia or hypertension compared with the untreated or control non-bacteriuric groups. It was further noted,

in a separate analysis, that the incidence of toxæmia or hypertension was not significantly different in the untreated bacteriuric and the non-bacteriuric groups.

Prematurity by weight

There was no significant difference in the incidence of premature babies born to women in these groups ($\chi^2 = 1.112, P > 0.50$).

Table 20

The incidence of prematurity by weight

Groups	Total No.	No. with premature infants
Non-bacteriuric control women	1900	128 (6.7%)
Women with untreated bacteriuria of pregnancy	205	14 (6.8%)
Women with bacteriuria who were treated	127	5 (3.9%)

In the above analysis women who had abortions were excluded.

Prematurity by gestation

The above groups have been compared in Table 21:

Table 21

The incidence of prematurity by gestation

Groups	Total No.	No. with premature infants
Non-bacteriuric control women	1900 [*]	58 (3.1%)
Women with untreated bacteriuria of pregnancy	205 [*]	11 (5.4%)
Women with bacteriuria who were treated	127	2 (1.6%)

^{*} Excluding abortions

There was no significant difference in the incidence of prematurity in the babies born to women in these groups ($\chi^2 = 4.2922$, $P > 0.10$).

Abortion

The frequency of abortions occurring to the women in these groups can be seen in Table 22:

Table 22

The incidence of abortions

Groups	Total No.	No. of women who had abortions
Non-bacteriuric pregnant women	1974	74 (3.8%)
Women with untreated bacteriuria of pregnancy	224	19 (8.5%)
Women with bacteriuria of pregnancy who were treated	127	Nil
Total	2325	93

There was a very significant difference in the incidence of abortions occurring in these groups ($\chi^2 = 12.9116$, $P < 0.001$). The incidence of foetal loss due to abortions was significantly higher in those women with untreated bacteriuria of pregnancy in comparison with the treated bacteriuric women and the non-bacteriuric control groups of pregnant women.

Incidence of total foetal loss

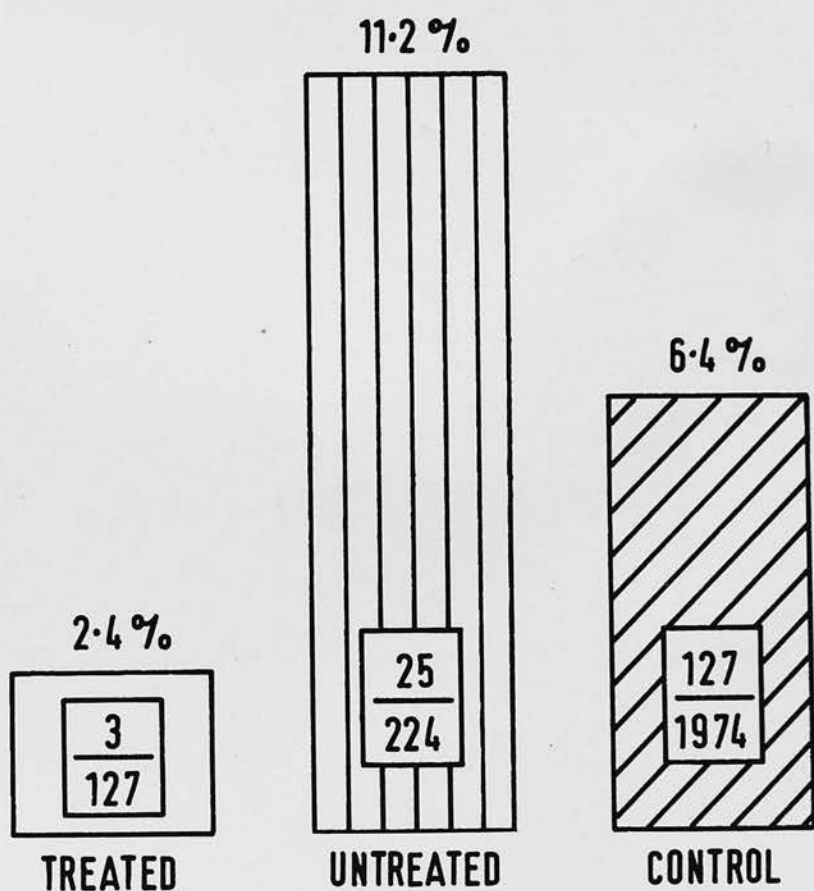
In this analysis foetal loss due to abortions, stillbirths and neonatal death have been considered together in Table 23 and Figure 5.

Table 23

Incidence of total foetal loss

Groups	Total No.	Total foetal loss in relation to mothers in each group
Non-bacteriuric control group of pregnant women	1974	127 (6.43%)
Women with untreated bacteriuria of pregnancy	224	25 (11.16%)
Women with bacteriuria of pregnancy who were treated	127	3 (2.36%)
Total	2325	155

The above incidence of total foetal loss is in relation to the mothers in each group and has been adjusted for multiple pregnancies. There was a



Incidence of total foetal loss in treated and untreated bacteriuric patients and control subjects.

Figure 5

significant difference in the incidence of foetal loss in these groups (overall $\chi^2 = 11.0674$, $P < 0.01$). These groups were further compared by partitioning the overall chi-square into its degrees of freedom. There was no significant difference in the incidence of foetal loss in the control non-bacteriuric group and the group of women who were treated for bacteriuria of pregnancy. However, there was a significantly higher incidence of foetal loss in the untreated bacteriuric pregnant women in comparison with the other groups ($\chi^2 = 8.0144$, $P < 0.01$).

Table 24

The incidence of total foetal loss,
including abortions

Components of χ^2 due to (groups)	χ^2	Degrees of freedom	Significance level
1. Comparing treated bacteriuric with control non-bacteriuric group	3.0530	1	Not significant
2. Comparing control + treated with untreated bacteriuric group	8.0144	1	$P < 0.01$
Overall χ^2	11.0674	2	$P < 0.01$

CHAPTER III

Standardisation of a Method for Sulphonamide Sensitivity Tests and its Evaluation in Patients with Asymptomatic Bacteriuria of Pregnancy

This work was undertaken to compare the disc diffusion and tube dilution methods for assessing the sensitivity of urinary pathogens to sulphonamides and to correlate these results with the clinical responses to sulphadimidine therapy in women with asymptomatic bacteriuria of pregnancy.

Differences in the in vitro antimicrobial activity apparent with the various sulphonamide compounds are quantitative rather than qualitative. The relative potencies of the different compounds are compared by determining the minimum concentration required to inhibit the growth of a standard inoculum of a test organism in a suitable test medium. Sulphanilamide, the parent substance, is sufficiently soluble in water or in liquid growth medium to allow direct testing. The heterocyclic derivatives, e.g. sulphadimidine, sulphadiazine, are almost insoluble in water at neutral pH. The powder form of the latter compounds can be more readily dissolved at an alkaline pH.

Materials and methods

The liquid media tested in the standardisation of the

tube dilution sensitivity test using sulphaniilamide were:

1. Synthetic medium of Sahyun et al. (1936).
2. Sensitivity test broth (Oxoid).
3. Nutrient broth (Oxoid).
4. 1% glucose broth (Cruickshank, 1965).
5. 1% peptone water (Cruickshank, 1965).

As the synthetic medium containing glucose, ammonium sulphate and asparagin did not support an adequate growth of all urinary pathogens tested, it was not included after preliminary tests.

Inoculum size in tube dilution sensitivity tests

All test organisms were initially preserved in pure culture on bijou agar slopes. These were plated out again on sensitivity test agar (Oxoid) to ensure purity and single colonies were inoculated into the various liquid media tested. An 18-hour liquid culture was serially diluted in 99 and 9 ml. amounts of 0.85% sterile saline using an automatic 1 ml. pipette. Confirmatory viable counts were made on these dilutions by the method of Miles and Misra (1938). Inocula tested contained about 2×10^3 and 1×10^5 organisms in 0.02 ml. volumes delivered by a standard calibrated dropping pipette.

Sulphonamide solutions

The following compounds were tested:

1. Sulphanilamide Powder (May & Baker Ltd.).
2. Sulphadimidine Powder (May & Baker Ltd.).
3. Injection Sodium Sulphadimidine (I.C.I.).

4. Injection Sodium Sulphadiazine (May & Baker Ltd.).

Stock solutions of the above compounds were made by dissolving 1 g. of the compound in the liquid growth medium. The solutions of weighed quantities of the powder forms were Seitz filtered to ensure sterility, but this step was omitted when the sterile injection form was used. As all compounds except sulphanilamide crystallised in stock solution, about 1.5 ml. of 40% solution of potassium hydroxide in distilled water was added drop by drop to alter the pH to about 9. A similar quantity of potassium hydroxide was also added to a control amount of liquid medium without sulphonamide to ensure that this addition alone produced no bacteriostatic effect. The stock solution of injection sodium sulphadimidine was stable and did not require added alkali provided that it was used immediately after preparation.

All patients involved in these studies were the asymptomatic bacteriuric pregnant women in the treatment trial, treated with sulphadimidine for two weeks between the 12th and 28th weeks of gestation.

Effect of inoculum size and sulphonamide inhibitor content of the liquid test medium

The parent substance sulphanilamide was used in this experiment; varying concentrations of it were separately prepared in tubes of the five liquid test

media and each tube was inoculated with 0.02 ml. of a saline suspension of a known sensitive strain of Escherichia coli. In two identical series of tests performed in parallel two different inocula of 2×10^3 and 1×10^5 viable organisms in 0.02 ml. volumes were tested. A control tube of each liquid medium without sulphaniamide was also inoculated and included in each series. The inoculated tubes were then incubated at 37°C . for 18 hours and thereafter examined for inhibition of growth.

Table 25

The effect of inoculum size and inhibitor content on sulphaniamide tube dilution sensitivity tests

Medium	Inoculum (organisms)	MIC in $\mu\text{g/ml}$.
Sensitivity test broth	2×10^3	312
"	1×10^5	over 2500
Nutrient broth (Oxoid)	2×10^3	over 2500
"	1×10^5	"
1% glucose broth	2×10^3	over 2500
"	1×10^5	"
1% peptone water	2×10^3	over 2500
"	1×10^5	"
Synthetic medium	2×10^3	No visible growth in control or other tubes
"	1×10^5	

These results (Table 25), which were confirmed in a repeated experiment, indicate that the tube dilution sensitivity tests performed with sulphanilamide diluted in peptone water, 1% glucose broth, or nutrient broth, yielded misleading results at the two levels of inoculum used. This is presumably attributable to the presence of sulphonamide inhibitor in these media (MacLeod, 1940; Jawell and Pearmain, 1954). The synthetic medium did not support growth of the test organism. Results obtained with Oxoid sensitivity test broth were valid only when the smaller inoculum of the test organism (2000 organisms) was used. This is consistent with the findings of Garrod (1959); the test is clearly influenced by the size of inoculum. In all subsequent tests, sensitivity test broth was routinely employed with a standard inoculum of about 2×10^3 organisms.

The MIC levels of different sulphonamide compounds, for the same test organism, as determined by the tube dilution test

In order to compare the relative potencies of various sulphonamide compounds, stock solutions were prepared and diluted as described. Six different strains of Escherichia coli isolated from patients with urinary infection were tested. The inoculum size in each case was verified by means of viable counts.

Table 26

The relative potencies of various sulphonamides as determined
by the tube dilution technique
 (MIC levels in μg per ml.)

Strains of Escherichia coli	Sulphanilamide (Powder)	Sulphadimidine (Powder)	Sulphadimidine (Injection)	Sulphadiazine (Injection)	Broth and KOH (Control I)	Broth only (Control II)
DT - 123	625	39	19	< 9	No bacteriostasis	Good growth
DT - 16	158	19	< 9	< 9	"	"
DT - 139	625	39	19	< 9	"	"
DT - 159	625	158	78	< 9	"	"
DT - 167	625	39	19	< 9	"	"
DT - 167A	625	39	39	< 9	"	"

These results (Table 26) were confirmed in two further experiments; they showed that of the three compounds tested, sulphanilamide is the least and sulphadiazine the most potent in these in vitro tests. The powder and injection forms of sulphadimidine are both of intermediate potency and show only minor differences in MIC level when tested in this way. The potassium hydroxide used to increase the solubility of some preparations showed no bacteriostasis in the concentrations used.

The growth inhibition zone sizes obtained with different sulphonamide compounds in the disc diffusion test

Filter paper discs containing 250 μ g. of the various sulphonamide compounds were prepared as described by Cruickshank (1965). The powder forms, which were not soluble in distilled water at neutral pH, required the addition of potassium hydroxide. In the case of the injection forms, discs impregnated with sulphonamide solutions with and without potassium hydroxide were tested. Large pyrex petri dishes of 5½ in. diameter were used so that eight discs containing different preparations could be tested simultaneously against the same inoculum of a test organism. The six strains of Escherichia coli that were used in the tube dilution tests were again used as test strains.

Table 27

Relative potencies of various sulphonamides as determined by the disc diffusion technique
(Zones of inhibition in mm. diameter)

Strains of Escherichia coli	Sulphanilamide (Powder)	Sulphadimidine (Powder)	Sulphadimidine (Inj.)		Sulphadiazine (Inj.)		Disc with dye	
			+	-	+	-	+	-
DT - 123	Nil	24	21	22	29	29	Nil	Nil
DT - 16	PI	27	27	28	32	33	Nil	Nil
DT - 139	PI	20	22	22	28	28	Nil	Nil
DT - 159	Nil	22	22	22	22	22	Nil	Nil
DT - 167	Nil	27	30	28	30	32	Nil	Nil
DT - 167A	Nil	26	26	27	33	32	Nil	Nil

PI - Zone of partial inhibition

These results (Table 27 and Figure 6) confirm that there is the same variation in potency of the three sulphonamide compounds as was shown in the tube tests, but these differences are much less evident with the disc test. Sulphanilamide shows virtually no bacteriostatic effect when tested in this way. This is perhaps a result of poor diffusion of the drug through the medium. Potassium hydroxide and the blue identifying dye (see Methods) produced no bacteriostatic effects when tested alone.

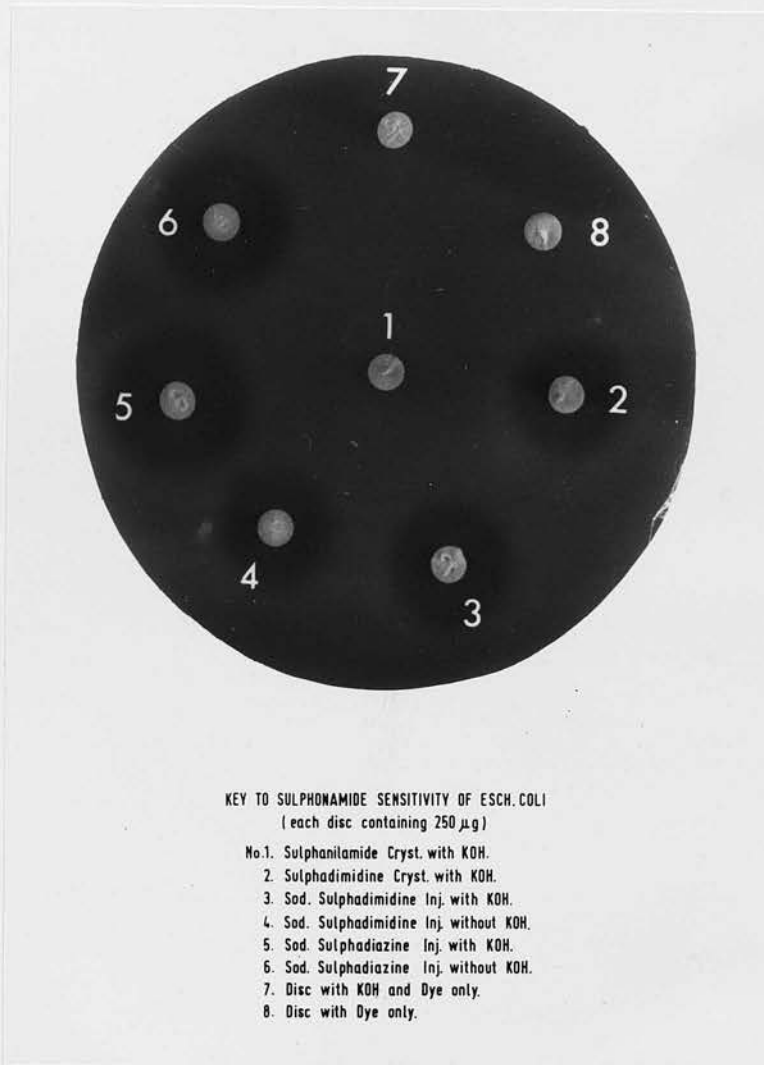


Figure 6

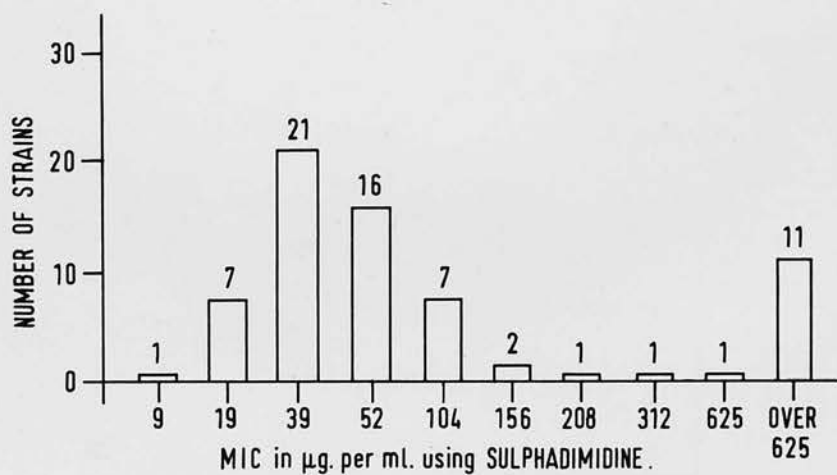
A comparative evaluation of the tube and disc diffusion tests as measures of the sensitivity of representative strains of urinary pathogens to sulphadimidine

Injection sodium sulphadimidine was used in the following in vitro tests. This drug, in tablet form, was also used in the clinical treatment trial. The MIC levels of this compound, as judged by tube dilution sensitivity tests, are readily attained in vivo in patients on ordinary dosage schedules.

Discs impregnated with 250 μg . and 500 μg . of sulphadimidine and discs containing 250 μg . of sulphadiazine were tested simultaneously against each of 68 strains. The zone sizes produced with the more potent discs were larger in each instance, but all disc tests showed poor correlation with MIC determinations in tube dilution tests with the same strains. Hence representative data obtained in experiments using discs containing sulphadimidine 250 μg . are given as illustration.

Sixty-eight representative strains isolated at various times from 24 women with asymptomatic bacteriuria of pregnancy were tested. These strains were biochemically identified as follows:

46	-	<u>Escherichia coli</u>
16	-	<u>Escherichia coli</u> (atypical)
2	-	<u>Proteus mirabilis</u>
2	-	<u>Klebsiella aerogenes</u>
2	-	<u>Enterococcus</u>
68		Total
==		



The distribution of sensitivity of 68 urinary pathogens to sulphadimidine. (expressed in — minimum inhibitory concentration determined by tube dilution tests.)

Figure 7

The results of tube dilution sensitivity tests are presented in Figure 7.

The lack of correlation between the MIC levels and the corresponding zone diameters as determined by the disc test is evident in Table 28 and Figure 8.

Table 28

Correlation of results of single-disc and tube dilution sensitivities of 68 strains of urinary pathogens with sulphadimidine

MIC in $\mu\text{g}/\text{ml}.$	Number of strains	Zones of inhibition to single discs of 250 $\mu\text{g}.$ sulphadimidine (mm. diameters)
Less than 9	Nil	
9	1	30
19	7	25, 26, 27, 27, 30, 30, 30
39	21	20, 21, 22, 23, 24, 25, 26, 27, 29 20, 22, 24, 25, 26, 27, 29 20, 22, 24, 26, 27
52	16	19, 20, 22, 23, 24, 25, 26, 27, 28 20, 22, 24, 27 20, 24 24
104	7	18, 19, 21, 24, 25, 31 19
156	2	24, 27 (both Proteus)
208	1	18
312	1	10
625	1	14
Over 625	11	Nil, Nil, Nil, Nil Nil, Nil, Nil, Nil Nil, Nil, Nil

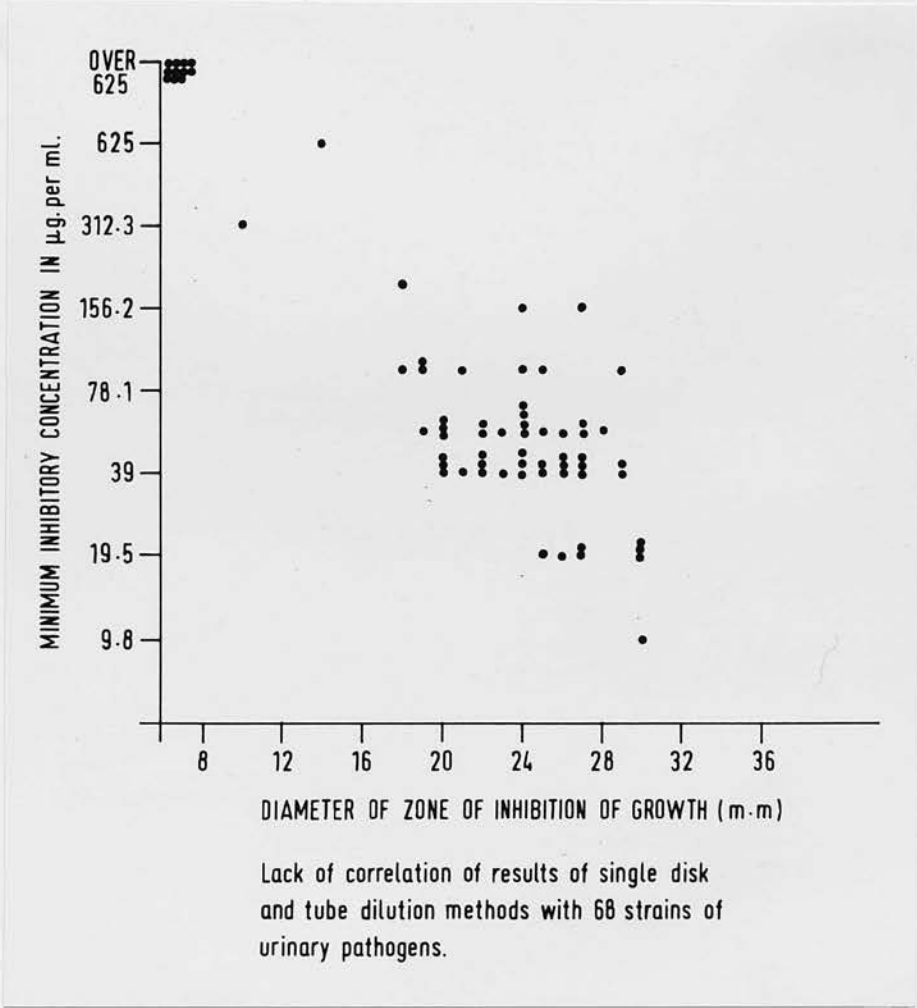


Figure 8

Although there is good correlation between the two tests when the test organism is either very sensitive or very resistant, there is no correlation in tests with strains of intermediate sensitivity. An organism that is relatively resistant in the tube test gives zones well within the sensitive range in the disc test. It was further noticed that with some of the relatively resistant strains (MIC levels above $104 \mu\text{g./ml.}$ and below $625 \mu\text{g./ml.}$) there was a growth of very small colonies within the zone of inhibition in the disc test. Addition of lysed horse blood to the solid medium did not prevent this. When the small colony forms were sub-cultured and tested again by the tube dilution and disc test, they behaved exactly like the parent strain and showed no increased resistance. Thus, the small colonies did not seem to represent resistant mutants. This phenomenon may be caused by sulphonamide inhibitors produced by the inoculum itself when the zone of inhibition is relatively small.

Correlation of results of *in vitro* sensitivity tests with clinical responses to treatment in patients

The clinical and laboratory data obtained from the first 35 patients in the treatment trial for asymptomatic bacteriuria of pregnancy, who were each treated with sulphadimidine for two weeks at some time during the 12th to 28th week of their pregnancies have been analysed in

detail. The immediate result of treatment and the result of subsequent follow-up examinations of urine were analysed as specified in the Materials and Methods Section.

The "immediate" results (Table 29) show that treatment was successful in 28 or 80% and unsuccessful in 7 or 20% of these cases. The sensitivity results obtained in tube tests with the organisms isolated from these patients correlate well with the result of treatment. Injection sodium sulphadimidine was used in the in vitro tests. We have considered that MIC levels up to 52 $\mu\text{g./ml.}$ indicate sensitivity; MIC values around 104 $\mu\text{g./ml.}$ indicate that the organism is of relative resistance; higher levels indicate definite resistance (see Discussion). The zones of inhibition in disc tests with these organisms do not correlate well with the clinical response to treatment. On the basis of response to treatment, these patients can be grouped in the following categories:

1. All 25 patients, who were infected with sensitive organisms, were successfully treated.
2. Two patients, both infected with Proteus mirabilis (MIC: 156 $\mu\text{g./ml.}$), were also successfully treated.
3. In three patients infected with resistant strains, treatment failed.
4. In two patients infected with initially sensitive strains which became relatively resistant during treatment, treatment also failed.

Table 29

Patient number	Pre-treatment isolate - 1		Pre-treatment isolate - 2		Result of treatment		
	Organism	Zone size (mm. diam.)	MIC µg/ml.	Organism		Zone size (mm. diam.)	MIC µg/ml.
1	<i>Esch. coli</i>	26	19	<i>Esch. coli</i>	27	19	Success
2	<i>Esch. coli</i>	20	52	<i>Esch. coli</i>	Died before test		Success
3	<i>Esch. coli</i>	20	104	<i>Esch. coli</i>	20	104	Success
4	<i>Esch. coli</i>	28	39	<i>Esch. coli</i>	26	39	Success
5	<i>Esch. coli</i>	26	39	<i>Esch. coli</i>	27	39	Success
6	<i>Esch. coli</i>	24	52	<i>Esch. coli</i>	24	52	Success
7	<i>Esch. coli</i>	22	52	<i>Esch. coli</i>	20	52	Success
8	<i>Esch. coli</i>	26	19	<i>Esch. coli</i>	27	39	Success
9	<i>Esch. coli</i>	23	39	<i>Esch. coli</i>	26	39	Success
10	<i>Esch. coli</i>	20	52	<i>Esch. coli</i>	20	52	Success
11	<i>Esch. coli</i>	20	39	<i>Esch. coli</i>	22	52	Success
12	<i>Esch. coli</i>	20	39	<i>Esch. coli</i>	24	52	Success
13	<i>Esch. coli</i>	27	52	<i>Esch. coli</i>	26	52	Success
14	<i>Esch. coli</i>	22	39	<i>Esch. coli</i>	23	52	Success
15	<i>Esch. coli</i>	27	39	<i>Esch. coli</i>	24	39	Success
16	<i>Klebsiella</i>	23	52	<i>Klebsiella</i>	24	52	Success

Table 29 (contd.)

Patient number	Pre-treatment isolate - 1		Pre-treatment isolate - 2		Result of treatment		
	Organism	Zone size (mm. diam.)	MIC μ g/ml.	Organism		Zone size (mm. diam.)	MIC μ g/ml.
17	Esch. coli	23	52	Esch. coli	24	52	Success (late relapse)
18	Esch. coli	20	39	Esch. coli	20	39	Success
19	Esch. coli	22	52	Esch. coli	19	52	Success (late relapse)
20	Esch. coli	23	39	Esch. coli	24	52	Success (late relapse)
21	Esch. coli	22	39	Esch. coli	25	19	Success (late relapse)
22	Esch. atypical	19	104	Esch. atypical	20	52	Success
23	Esch. coli	29	39	Esch. coli	29	39	Success (late relapse)
24	Esch. coli	21	39	Esch. coli	25	39	Success
25	Esch. coli	22	52	Esch. coli	21	104	Success
26	Proteus	26	156	Proteus	25	104	Success
27	Proteus	27	156	Proteus	24	156	Success
28	Esch. coli	18	104	Esch. coli	19	104	Failed (Esch. coli MIC 208 μ g.)
29	Esch. coli	Nil	over 625	Esch. coli	Nil	over 625	Failed (Esch. coli MIC over 625 μ g.)
30	Esch. coli	26	104	Esch. coli	25	156	Failed (Esch. coli MIC 312 μ g./ml.)

Table 29 (contd.)

Patient number	Pre-treatment isolate - 1		Pre-treatment isolate - 2		Result of treatment		
	Organism	Zone size (mm. diam.)	MIC $\mu\text{g}/\text{ml.}$	Organism		Zone size (mm. diam.)	MIC $\mu\text{g}/\text{ml.}$
31	Esch. coli	35	19	Esch. coli	37	19	Failed (Esch. coli MIC 104 $\mu\text{g.}/\text{ml.}$)
32	Esch. atypical	30	19	Esch. atypical	30	19	Failed (Esch. atypical MIC 104 $\mu\text{g.}/\text{ml.}$)
33	Esch. coli	25	39		Died on storage		Failed (Esch. coli MIC 52 $\mu\text{g.}/\text{ml.}$)
34	Esch. coli	20	52	Esch. coli	21	52	Failed (Esch. coli MIC 52 $\mu\text{g.}/\text{ml.}$)
35	Esch. atypical	Nil	over 625	Esch. atypical	Nil	over 625	Success (positive one month later with Esch. atypical MIC over 625 $\mu\text{g.}/\text{ml.}$)

5. In two patients treatment failed although they were shown to be infected with strains that remained sensitive after treatment. Perhaps this is due to structural abnormalities within the renal tract.
6. In one patient who was infected with a resistant strain, the immediate post-treatment specimen of urine was clear, but a specimen taken one month later showed a recurrence of infection with a serologically identical, type 'O'₁, resistant strain.

Hence there was complete correlation between the results of tube dilution sensitivity tests and the clinical response in 30 out of 35 patients treated. In the remaining five cases the sensitivity test results were apparently misleading, but it should be noted that these included two patients who were infected with Proteus mirabilis (MIC 156 $\mu\text{g.}/\text{ml.}$) and both were successfully treated. In another patient infected with Escherichia coli serotype 'O'₁, with an MIC over 625 $\mu\text{g.}/\text{ml.}$, the treatment was only apparently successful. Thus the result in only two cases out of 35 is unexplained. Further investigations, including intravenous pyelograms, in these two patients may be helpful.

Correlation of results of *in vitro* tube dilution sensitivity tests with clinical responses to treatment with sulphadimidine in 62 patients with asymptomatic bacteriuria of pregnancy

Table 30

The "immediate" result of treatment	Organism sensitive	Organism relatively resistant	Organism resistant	Total
Success	35	7	2	44
Failure	8	1	9	18
Total	43	8	11	62

$$\chi^2 = 7.4058, P < 0.01$$

There is a significant difference ($P < 0.01$) between the incidence of sensitive as compared with the incidence of resistant and relatively resistant organisms in the two groups of patients. When the organism was sensitive the treatment was successful and when the organism was resistant or relatively resistant the treatment was unsuccessful. On the basis of the response to treatment these 62 patients can be grouped in the following categories:

1. In 35 patients infected with sensitive organisms, the treatment was successful.
2. In 7 patients, infected with relatively resistant organisms, treatment was also successful.

3. In one patient, infected with a relatively resistant organism, the treatment was a failure. The organisms isolated immediately before and after treatment were serologically typed as Escherichia coli O₇₇.
4. In five patients the infecting organism was resistant and treatment was a failure. In three of these instances the same serological type of Escherichia coli was isolated from the pre- and post-treatment specimens of urine.
5. In four patients the organism isolated before treatment was sensitive but these became resistant during treatment. There was a 4 - 8 fold rise in the MIC levels, e.g. from 19 µg. per ml. to 156 µg. per ml., in these cases. In one instance the strain isolated before and after treatment was typed as Escherichia coli O₆.

There was a very good correlation in 52 out of 62 patients treated. In one other patient the infecting organism was a resistant strain of Escherichia coli type O₁; the treatment in her case was only apparently successful since a urine examination one month later showed a relapse due to the same resistant strain.

Correlation of the results of in vitro sensitivity tests and the clinical responses to treatment with cycloserine in 65 women with asymptomatic bacteriuria of pregnancy

The correlation between the sensitivity of the infecting urinary organism and the result of treatment with cycloserine is shown in Table 31.

Table 31

Result of treatment with cycloserine	Infecting organism sensitive (No. of women)	Infecting organism resistant (No. of women)	Total
Success	51	1	52
Failure	11	2	13
Total	62	3	65

There was no significant relationship between the sensitivity of the infecting organism and the result of treatment ($\chi^2 = 1.769$, $P > 0.10$). The three strains resistant to cycloserine in this group were biochemically identified as two strains of Proteus mirabilis and one strain of Escherichia coli. The MIC in these three instances of resistance to cycloserine was 250 $\mu\text{g.}$ per ml.

CHAPTER IV

Serological Typing of Urinary Strains of
Escherichia coli

Typing antisera

Rabbit antisera to the following standard Escherichia coli 'O' types were made, i.e. O₁, O₂, O₄, O₆, O₇, O₈, O₁₈, O₂₅, O₇₅, and O₇₇. These strains were chosen from the reported literature as being the most frequent strains encountered in urinary infections.

Titres of rabbit antisera against homologous 'O'
antigen suspensions

The initial dilution of serum used was 1 in 50. End titres were here confirmed in four independent titrations. A one tube difference in end titre was taken as reasonable experimental error. All titres here refer to the reciprocal of the final serum dilution, after the addition of 1 volume of antigen.

Table 32

Titres of rabbit antisera against
homologous 'O' antigens

Rabbit antiserum to 'O' type	Homologous 'O' antigen	End titre
1	1	6400
2	2	12800
4	4	6400
6	6	6400
7	7	12800
8	8	3200
18	18	6400
25	25	3200
75	75	6400
77	77	800

These results indicate that the end titres in all antisera except O₇₇ lay between 3200 and 12800. The rabbit immunised with strain O₇₇ was almost moribund before the immunisation schedule was completed. The other rabbits did not react to the intravenous injections except by some loss of weight. Two rabbits died after the second injection and post-mortem failed to reveal any obvious pathology. Since only one rabbit was used for each antiserum, these immunisations were repeated using two other animals.

Each of the above antisera was diluted further to give a final titre of 1 in 800 against the homologous strains, as described in the Materials and Methods Section.

Cross-reactions detected within the other 144 standard 'O' types in the genus Escherichia

All 145 standard 'O' antigen suspensions of Escherichia coli were first tested for autoagglutination in saline.

Each antiserum, diluted to a screening titre of 1 in 800, was tested against one volume of each of the other 144 'O' antigens. Any positive reactions in this test were titrated to its full titre, along with a titration using the homologous 'O' antigen. The results are seen in Tables 33 and 34.

Table 33

Cross-reacting antigens detected

Rabbit antiserum 'O' type	Cross-reacting 'O' antigens
1	50
2	74
4	Nil
6	100
7	5 and 71
8	15 and 40
18	Nil
25	Nil
75	Nil
77	17

Table 34

Titration of rabbit antisera against the standard homologous and cross-reacting 'O' antigen suspensions

(The diluted serum of final titre 1 in 800 was used in these tests)

Rabbit antiserum 'O' type	Titrated against 'O' antigens	Titre
1	1	800
1	50	400
2	2	800
2	74	400
6	6	800
6	100	100
7	7	800
7	5	100
7	71	100
8	8	800
8	15	800
8	40	800
77	77	800
77	17	800

From the above results it was decided that anti-serum to strains O_1 , O_2 , O_8 , and O_{77} would need absorptions to be done to prepare monospecific antisera in the final typing of urinary strains.

Result of serological typing of *Escherichia coli* strains isolated from 113 patients in the treatment trial

332 strains of *Escherichia coli* were isolated during the follow-up of 113 patients with asymptomatic bacteriuria of pregnancy. Of these 81 strains were found to be rough or autoagglutinable, hence only 251 strains were suitable for typing. Using 10 typing 'O' antisera it was possible to type 139 or 55.4% of these strains. These typed strains were obtained during the follow-up of 51 patients with asymptomatic bacteriuria of pregnancy.

Table 35

Number of strains	'O' type
12	1
22	2
9	4
29	6
21	7
3	8
7	18
5	25
22	75
9	77
139 strains	

In this study (Table 35) 'O' types 2, 6, 7 and 75 account for 94 of these 139 typed strains, i.e. 67.6%.

The distribution of *Escherichia coli* 'O' type strains in 47 patients with asymptomatic bacteriuria of pregnancy, before treatment

Although 139 strains of *Escherichia coli* isolated during the follow-up of 51 of these patients were typed, in four of them only the strains isolated after treatment were serotyped. Hence these are excluded from this analysis. The distribution of the 'O' types as seen in these 47 patients is illustrated in Table 36.

Table 36

The distribution of *Escherichia coli* 'O' types in 47 patients with asymptomatic bacteriuria of pregnancy

Number of patients	'O' type
3	1
8	2
4	4
11	6
4	7
1	8
1	18
2	25
9	75
4	77
47 patients	

In 44 out of these 47 patients the same 'O' type was isolated in both urine specimens examined before treatment. From this analysis it appears that serotypes 'O' 2, 6 and 75 accounted for the infections in 28 out of these 47 patients.

Analysis of strains of *Escherichia coli* typed before and after treatment

In 21 patients it was possible to determine by serological tests whether patients who failed or relapsed after treatment did so due to a recurrence of the original infecting strain, or due to re-infection with a different strain.

Table 37

"Immediate" result of treatment	Relapse with 'O' type	Re-infection 'O' type	
		Before therapy	After therapy
Success (11 patients)	1	4	7
	7	Non typable	1
	75	75	Non typable
	75	6	Non typable
	25	6	18
	8		
Failure (10 patients)	1		
	2		
	6		
	6		
	6		
	6		
	7		
	7		
18			
77			
Total: 21 patients	16 patients	5 patients	

All 10 patients who failed to respond to treatment showed a relapse of the original infection. Out of 11 patients who responded with success, one week after treatment, 6 patients had an apparent relapse and 5 patients had a re-infection during the follow-up period.

Standardisation of the Indirect Bacterial
Haemagglutination Test to detect Antibody
Response to *Escherichia coli*

In initial experiments the standard *Escherichia coli* type strains and their homologous rabbit antisera were used. The method of the test and the controls used were as described in the Materials and Methods Section. All titres refer to a reciprocal of the final serum dilution.

Effect of the container, concentration of sensitised cell suspension and duration of incubation on the haemagglutination test

The containers tested were W.H.O. plastic plates, agglutination tubes and larger 5" x 9/16" test-tubes. The concentrations of the sensitised sheep cells which were tested were 1% and 2.5%. The mixtures of sensitised cell suspensions and serially diluted antiserum were incubated at 37° C. in an incubator and examined after $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2 and 18 hours. The results are shown in Table 38.

These results were confirmed in a second experiment. The test carried out in W.H.O. plates using a 1% concentration of sensitised sheep cells gave the highest titre. The tests carried out in tubes were extremely difficult to read even with the help of a concave mirror. Incubation for approximately $1\frac{1}{2}$ hours was considered adequate.

Table 38

Effect of the container, the concentration of sensitised sheep cells and the duration of incubation in the haemagglutination test

Container	Sensitised sheep cell concentration	Titre after 30 min.	Titre at 1 hour	Titre after 1 hour 30 min.	Titre after 2 hours	Titre after 18 hours	Controls
W.H.O. plates	1%	< 100	< 100	3200	3200	3200	Negative
	2.5%	< 100	800	1600	1600	1600	"
Agglutination tubes 3" x 1/4"	1%	200	200	200	200	200	"
	2.5%	< 100	< 100	< 100	< 100	< 100	"
Test tubes 5" x 9/16"	1%	200	400	400	400	200	"
	2.5%	< 100	< 100	< 100	< 100	< 100	"

Effect of variation in temperature in the
haemagglutination test

Tests were carried out in W.H.O. plates, using a 1% concentration of sensitised cells. These experiments carried out at 4° C. and at room temperature gave no variation in the results. The test conducted at 4° C. had the advantage of preventing evaporation of fluid in the wells, when small volumes were used. The end points were clearly defined at both temperatures.

Effect of heat on the activity of the sensitising
antigen used

The supernatant and sediment of an unheated and a heated overnight broth culture of Escherichia coli type O₂ were used to sensitise red cells for haemagglutination in homologous rabbit antiserum. Heat treatment applied was exposure to steam for one hour in a Koch steamer. Human group 'O' and sheep red cells were tested. The results are seen in Table 39.

Table 39

Effect of heat on the activity of the sensitising antigen used to sensitise human and sheep red cells

Source of washed red cells	Sensitising antigen used	Reciprocal titre of rabbit O ₂ antiserum	Controls
Human (group O)	Unheated supernatant O ₂	Nil	-
Sheep	"	"	-
Human	Unheated sediment O ₂	Nil	-
Sheep	"	"	-
Human	Heated supernatant O ₂	800	-
Sheep	"	800	-
Human	Heated sediment O ₂	400	-
Sheep	"	400	-

These findings confirm those of Neter et al. (1952). Heat treatment activates the supernatant and sediment fractions of this culture. The unheated antigens are inactive in sensitising red cells. This is perhaps due to a blocking of the reactive group or groups of the somatic 'O' antigen by capsular 'L' or 'B' antigens in the unheated culture. The one tube difference in end titre between the heated sediment and heated supernatant antigens can be considered significant in this instance since both antiserum dilutions were aliquots of the same doubling dilution series. The use of human red cells gave no advantage over sheep cells.

Effect of varying the concentration of antigen used to sensitise sheep cells

A concentrated antigen extract was made by suspending the surface growth of ten digest agar plates in 100 ml. of sterile 0.85% saline and the antigen extracted as described. This extract was serially diluted and each dilution used to sensitise sheep red cells. The results are shown in Table 40.

Table 40

Effect of varying the concentration of antigen used to sensitise sheep cells for haemagglutination in homologous rabbit antiserum

Concentration of antigen used to sensitise red cells	Haemagglutinin titre of homologous rabbit antiserum	Controls
Neat antigen extract	12800	Negative
Antigen diluted 1 in 8	6400	"
" " 1 in 16	6400	"
" " 1 in 32	6400	"
" " 1 in 64	1600	"
" " 1 in 128	800	"

These results indicate that the undiluted antigen in this instance gave the highest antibody titre, but a dilution of 1 in 64 or more of this antigen was insufficient to sensitise the red cell suspension. There is hence a minimum antigen concentration below which the

red cells are not adequately sensitised.

In a repeated experiment a more concentrated solution of antigen was used, namely, growth from 20 plates suspended in 10 ml. The test was repeated using serial dilutions of this antigen to sensitise sheep cells.

The results are seen in Figure 9.

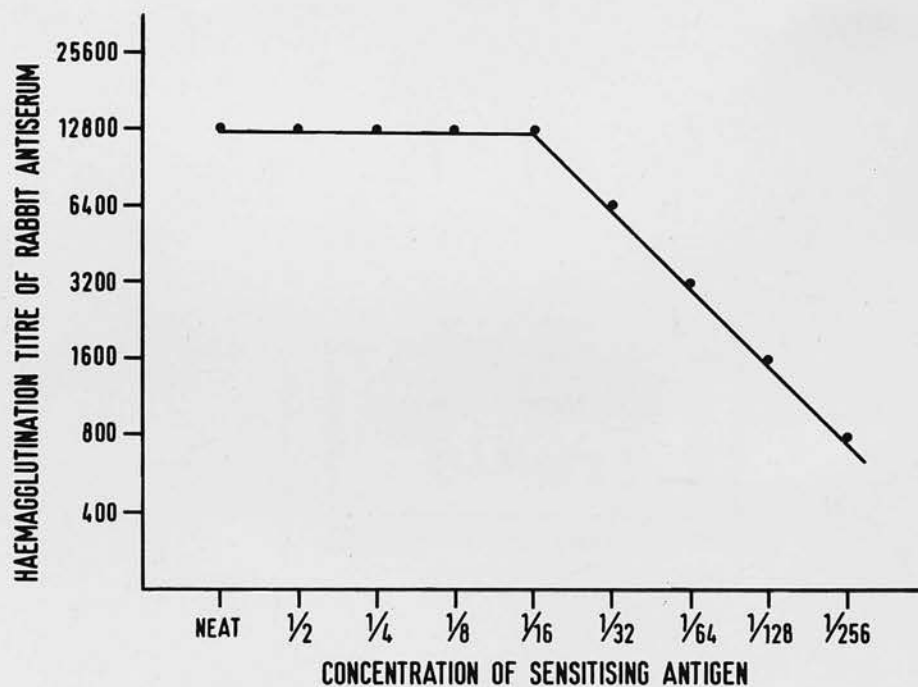
In this experiment no fall in end titre was recorded till a 1 in 32 dilution of the above antigen was used. There was also no adverse effect in using a very concentrated antigen solution since the excess in this case was probably removed in the washing procedure. When sub-optimal concentrations of sensitising antigen were used the fall in antibody titre bore a direct relationship to the antigen concentration used.

Effect of different growth media

Antigens were extracted from Escherichia coli, type O₂ and type O₄, grown on each of the following growth media:

Nutrient agar (Oxoid), 10% blood agar, chocolate agar, lysed blood agar, salt milk agar, and MacConkey's agar.

The results using all these antigens showed no variation in end titres provided that the optimal minimum antigen concentration was exceeded except when the growth on MacConkey's agar was used. The antigens extracted from organisms grown on MacConkey's agar were, however, completely inactive in tests using both strains of



THE PASSIVE HAEMAGGLUTINATION TITRES OF RABBIT ANTISERUM OBTAINED WITH SHEEP CELLS SENSITISED BY VARYING CONCENTRATIONS OF THE ESCHERICHIA COLI CRUDE 'O' ANTIGEN.

Figure 9

Escherichia coli. This is perhaps due to an inhibitory effect of the indicator, neutral red, used in this medium.

Selection of an optimal antigen concentration for routine use

Two antigen concentrations were prepared, one using the growth from ten digest agar plates suspended in 20 ml. of sterile 0.85% saline, and another using the growth from three plates in 10 ml. These antigens were serially diluted and tested.

Table 41

Experiment to select an optimal antigen concentration for sensitising red cells in further haemagglutination tests

Concentration of antigen	Haemagglutinin titre of homologous rabbit antiserum	Controls
Undiluted extract of growth from 10 plates	12800	Negative
1 in 4 dilution	12800	"
1 in 8 dilution	12800	"
1 in 16 dilution	12800	"
Undiluted extract of growth from 3 plates	12800	"
1 in 4 dilution	12800	"
1 in 8 dilution	12800	"
1 in 16 dilution	12800	"

These results (Table 41) indicate that the growth from three plates is adequate even when diluted to 1/16th of its original concentration. Hence in further tests the extract from three plates in 10 ml. was used.

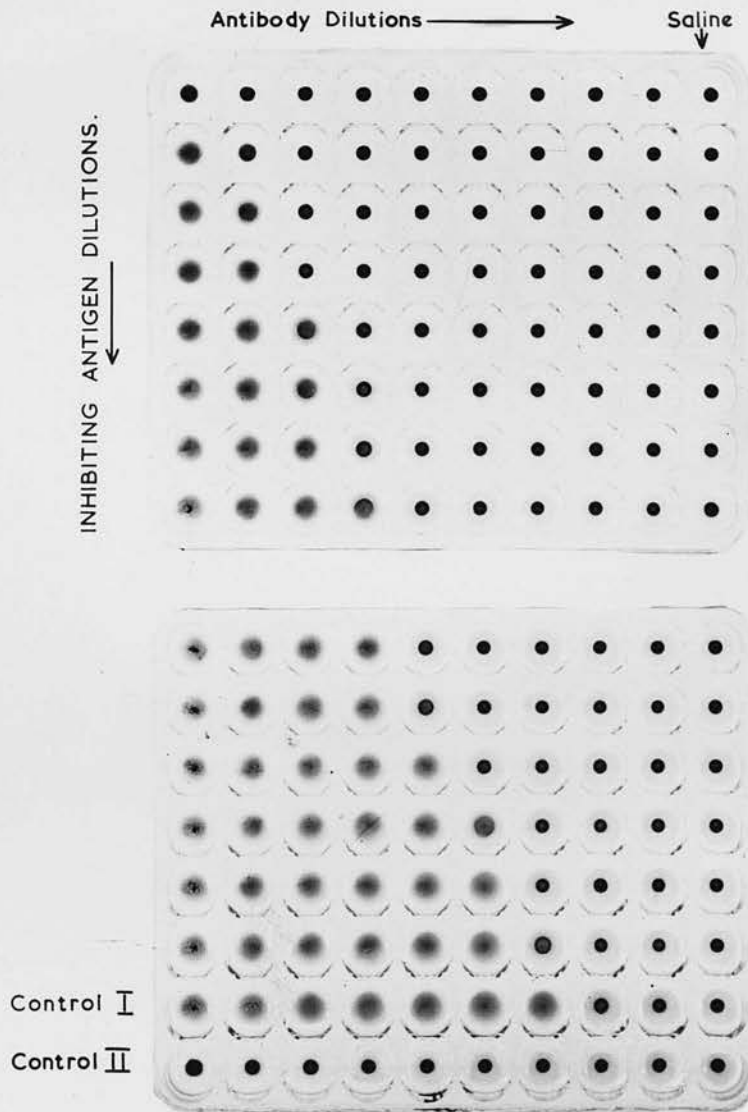
Haemagglutination inhibition test

This test was used to demonstrate the specificity of the haemagglutination test. The procedure was the same as above except that an equal volume of the sensitising antigen was added to each dilution of the serum before the addition of sensitised sheep cells. Since the inhibition was almost instantaneous the sheep cells were added soon after addition of the inhibiting antigen.

Effect of variation of the concentration of the inhibiting antigen in the haemagglutination inhibition test

These results (Figure 10) show a direct relationship between the concentration of the inhibiting antigen used and the drop in haemagglutinin titre. Control I in this instance consisted of the test done without addition of inhibiting antigen, one volume of buffer being used to make up to the same dilution. Control II consisted of the addition of normal, unsensitised sheep cells.

PASSIVE HAEMAGGLUTINATION INHIBITION TEST.



The effect of varying the concentration of the inhibiting antigen in the haemagglutination inhibition test.

Key. Control I = Titre of serum without added inhibiting antigen.

Control II = Unsensitised sheep cells in serum.

Figure 10

The effect of pre-incubation of rabbit antiserum to *Escherichia coli* type O₄ with unrelated antigens in the above haemagglutination test

Inhibiting antigen extracts used

A strain of *Shigella sonnei* (reference No. 30761) and a strain of *Salmonella typhimurium* (reference No. 29964) freshly isolated from patients in the routine diagnostic laboratory were used to extract antigens by the same method as was used for *Escherichia coli*. In each instance a 1% sensitised sheep cell suspension was prepared using the shigella and the salmonella antigens. The activity of these sensitised cells and their specificity were tested in a series of doubling dilutions of their respective rabbit antisera.

Salmonella antiserum. This was a specific antiserum to salmonella 'O' antigen 4 that was supplied for routine diagnostic work by Colindale Laboratories.

Shigella sonnei antiserum. This antiserum to types 1 and 2 was a sample of the serum made by Burroughs Wellcome Ltd.

These results (Table 42) show the activity and specific inhibition of the salmonella antigen extract used. Similar results were obtained in an experiment using the antigen extract from *Shigella sonnei*.

Table 42

Specificity of an extract of Salmonella typhimurium in sensitising red cells for haemagglutination in rabbit antiserum to Salmonella

(Initial dilution of antiserum in the first well was 1 in 20)

Antigen extract used to sensitise sheep cells	Inhibiting antigen used	Haemagglutinin titre of antiserum	Controls
Salmonella typhimurium	1 volume of buffer only to all wells	1280	Negative
Salmonella typhimurium	1 volume of Salmonella antigen extract to all wells	Less than 20	"

Table 43

Effect of pre-incubation of rabbit antiserum to Escherichia coli type O₄ with "unrelated" antigens

(Serum dilution in the first well was 1 in 150)

Antigen used to sensitise sheep cells	Inhibiting antigen	Haemagglutinin titre of rabbit antiserum	Controls
Escherichia coli type O ₄	1 volume of buffer only	9600	Negative
"	1 volume of Esch. coli type O ₄ antigen	Less than 150	"
"	1 volume of Salmonella antigen	9600	"
"	1 volume of Shigella antigen	9600	"

Effect of pre-incubation of rabbit antiserum to
Escherichia coli type O₄ with Salmonella and Shigella
antigen extracts

The results shown in Table 43 indicate that the titre of the rabbit antiserum to Escherichia coli O₄ was specifically reduced by pre-incubation with the homologous antigen. Pre-incubation with Salmonella and Shigella antigens in this instance did not reduce the titre to the homologous organism.

Titration of a sample of serum from an unselected
patient with asymptomatic bacteriuria of pregnancy

The urinary organism isolated from this patient was an Escherichia coli serologically typed as O₂. The presence of antibody to the urinary strain of Escherichia coli and also to Shigella sonnei was investigated. The patient's serum was pre-incubated with antigen from her infecting organisms as well as shigella antigen.

The initial dilution of serum in the first well here was 1 in 60. The results (Table 44) indicate that this patient had a haemagglutinin titre against her infecting urinary Escherichia coli of 960. This reaction was completely inhibited in all wells by pre-incubating her serum with an extract of this organism. This patient also showed an antibody response to Shigella sonnei of a titre of 1 in 240. Pre-incubation with shigella antigen did not reduce the haemagglutinin titre to Escherichia

Table 44

Titration of a sample of serum from patient (No. 34) with asymptomatic bacteriuria of pregnancy, caused by *Escherichia coli* type O₂, by the haemagglutination test

Antigen used to sensitise sheep cells	Inhibiting antigen	Haemagglutinin titre	Controls
<i>Escherichia coli</i> type O ₂	1 volume of buffer only	960	Negative
"	1 volume of <i>Esch. coli</i> O ₂	Less than 60	"
"	1 volume of <i>Shigella</i> antigen	960	"
<i>Shigella sonnei</i>	1 volume of buffer only	240	"
"	1 volume of <i>Shigella</i> antigen	Less than 60	"

coli, but did specifically reduce the titre against Shigella sonnei. Hence in routine practice a test of specificity was included in all titrations of human serum samples, consisting of a specific inhibition of haemagglutination by pre-incubation of the serum with 1 volume of the infecting antigen extract (see Materials and Methods).

Results of serum antibody estimations by the haemagglutination and bacterial agglutination techniques in pregnant women with and without bacteriuria

The serum antibody response to the infecting urinary organisms was studied in each of the following groups of pregnant women:

1. In 81 pregnant women without bacteriuria. The ten common 'O' types of Escherichia coli seen in urinary tract infections were used as antigens in these cases.
2. In 54 women with bacteriuria of pregnancy who were successfully treated with either cycloserine or sulphadimidine.
3. In 21 women with bacteriuria of pregnancy who had a relapse or re-infection after successful treatment.
4. In 9 women with bacteriuria of pregnancy who failed to respond to treatment.

In all women with bacteriuria of pregnancy five samples of serum were tested during pregnancy and only the highest reciprocal titre found in each patient is given.

Haemagglutination and bacterial agglutination titres
in 81 control pregnant women with no bacteriuria

The results in 81 control non-bacteriuric pregnant women are shown in Tables 45 and 46.

Table 45

The highest haemagglutination titres
in 81 non-bacteriuric pregnant women

Highest haemagglutination titre	No. of pregnant women
< 240	28
240	9
480	24
960	15
1920	5
Total	81

Table 46

The highest bacterial agglutination titres
in 81 non-bacteriuric pregnant women

Highest bacterial agglutination titre	No. of pregnant women
< 160	76
160	5
Total	81

It was further found that there was no correlation between the end titres as detected by these two methods. All five women who had titres of 1920 by the haemagglutination test had titres below 160 by the bacterial agglutination test. In five patients who had bacterial agglutination titres of 160, the corresponding haemagglutination titres ranged from 480 to 960.

Haemagglutination and bacterial agglutination titres in 54 women with asymptomatic bacteriuria of pregnancy who were successfully treated

The highest titres as obtained in 54 successfully treated bacteriuric women are seen in Tables 47 and 48.

Table 47

The highest haemagglutination titres in 54 successfully treated bacteriuric women

Highest haemagglutination titre	No. of pregnant women
< 60	15
120	5
240	10
480	13
960	9
1920	2
Total	54

Table 48

The highest bacterial agglutination titres in
54 successfully treated bacteriuric women

Highest bacterial agglutination titre	No. of pregnant women
< 40	30
80	10
160	4
320	1
Autoagglutinable antigens	9
Total	54

It was found that in nine instances where the antigen was autoagglutinable by the bacterial agglutination test, the corresponding haemagglutination titres using the same organisms ranged between 120 and 1920 in six of them; in the other three instances the haemagglutination titre was less than 60. It is evident from these results that the antibody response in this group is similar to the antibody response seen in control non-bacteriuric women.

The haemagglutination and bacterial agglutination titres
in 21 women with bacteriuria who had a relapse or re-
infection after successful treatment

These results are as seen in Tables 49 and 50.

Table 49

The highest haemagglutination titres in 21 women with bacteriuria who had a relapse or re-infection after successful treatment

Highest haemagglutination titre	No. of pregnant women
< 60	3
60	2
120	3
240	6
480	4
960	3
Total	21

Table 50

The highest bacterial agglutination titres as seen in 21 women with bacteriuria who had a relapse or re-infection after successful treatment

Highest bacterial agglutination titre	No. of pregnant women
40	12
80	4
160	None
320	2
Autoagglutinable antigens	3
Total	21

It would appear from these results that the antibody response in this group also is similar to the successfully treated bacteriuric women and the non-bacteriuric women. The two instances out of 21 where the bacterial agglutination titres were 320 and the corresponding haemagglutination titres were 960 need further investigation as to renal involvement. In one instance, patient No. 82, there was an eight-fold rise in antibody titre, by both techniques, during pregnancy. In three instances where the antigens were autoagglutinable by the bacterial agglutination test, the corresponding haemagglutination titres were between 120 and 960.

The haemagglutination and bacterial agglutination titres in 9 women with bacteriuria of pregnancy who failed to respond to treatment

These results in 9 women who failed to respond to treatment are as seen in Tables 51 and 52.

Table 51

The highest haemagglutination titres in 9 bacteriuric women who failed to respond to treatment

Highest haemagglutination titre	No. of pregnant women
120	1
240	2
480	None
960	1
1920	2
3840	1
7680	2
Total	9

Table 52

The highest bacterial agglutination titres in 9 bacteriuric women who failed to respond to treatment

Highest bacterial agglutination titre	No. of pregnant women
< 40	2
80	2
160	2
320	1
640	1
1280	1
Total	9

A detailed analysis of 5 patients with a raised antibody response in the group who failed to respond to treatment

1. Patient No. 70

Date	Result of urine culture	H.A. titre	B.A. titre	Symptoms and treatment
22. 9.65	+	240	< 40	Nil
1.10.65	+	240	< 40	Given sulphadimidine
22.10.65	+	7680	160	Acute pyelonephritis, given tetracycline
17.12.65	+	3840	40	On tetracycline
11. 2.66	-	3840	40	Retreated with ampicillin
8. 4.66	-	1920	40	Nil

H.A. = Haemagglutination, B.A. = Bacterial agglutination

2. Patient No. 39

Date	Result of urine culture	H.A. titre	B.A. titre	Symptoms and treatment
11. 8.65	+	240	< 40	Nil
9. 9.65	+	480	40	Given sulphadimidine
30. 9.65	+	480	40	Nil
25.11.65	+	960	80	Nil
13. 1.66	+	1920	640	Acute pyelonephritis, admitted
17. 2.66	+	1920	640	Ampicillin and furadantin given

3. Patient No. 84

Date	Result of urine culture	H.A. titre	B.A. titre	Symptoms and treatment
6.10.65	+	240	< 40	Nil
19.10.65	+	240	< 40	Given cycloserine
16.11.65	+	240	< 40	Nil
11. 1.66	-	960	320	Given ampicillin on 10.12.65 for symptoms
15. 3.66	-	960	320	On long-term cycloserine from 17.2.66 till delivery
19. 4.66	-	960	320	

4. Patient No. 93

Date	Result of urine culture	H.A. titre	B.A. titre	Symptoms and treatment
13.10.65	+	960	40	Nil
11.11.65	+	960	40	Given sulphadimidine
2.12.65	+	960	40	Nil
3. 3.66	+	7680	1280	Had right loin pain only, 8 weeks ago
13. 4.66	+	3840	1280	Nil

5. Patient No. 99

Date	Result of urine culture	H.A. titre	B.A. titre	Symptoms and treatment
20.10.65	+	No serum		Nil
25.11.65	+	960	40	Given cycloserine
16.12.65	+	480	< 40	Nil
10. 2.66	+	960	< 40	Treated by general practitioner for acute pyelonephritis
5. 5.66	+	3840	80	Nil

A detailed analysis of the antibody response to the infecting urinary organism in five patients shows the rise in antibody titre during pregnancy which coincided in all instances with the onset of clinical symptoms. In three of these patients a definite clinical diagnosis of acute pyelonephritis was made. It appears from these results that a high titre of antibody response is often found in women with persistent bacteriuria even after treatment, and also in women with clinical symptoms of acute pyelonephritis.

Since the number of women with a high antibody response in this study was small, it was not possible to test the significance of these results by statistical methods. However, these women are at present being radiologically examined for evidence of kidney damage, and it may be possible to evaluate the significance of these antibody titres with regard to the extent or site of infection when this examination is complete.

DISCUSSION

DISCUSSION

Despite recognition of the existence of asymptomatic bacteriuria among women from the year 1881 (Roberts, 1881), it was only 75 years later that Kass (1956) succeeded in focusing attention on its importance in clinical medicine. Even today its pathological significance is questioned and few surveys are done. It is estimated that about 21,000 women suffer from acute pyelonephritis during pregnancy every year in Britain (Little, 1965). Acute pyelonephritis is considered a preventable disease if treatment is instituted early in pregnancy for asymptomatic bacteriuria (Kass, 1960; Little, 1965; Kincaid-Smith and Bullen, 1965). It was hoped that the results of the present work would evaluate the significance of bacteriuria of pregnancy.

In the present study the term "acute urinary infection" has been applied to these symptomatic episodes during pregnancy in initially asymptomatic bacteriuric women. Kass (1960) used the term "pyelonephritis" for similar clinical episodes during pregnancy without defining the criteria on which he made such a diagnosis in 42% of untreated bacteriuric pregnant women. Turner (1961) stated that over 60% of 79 asymptomatic bacteriuric pregnant women developed a subsequent clinical urinary infection. She did not use the term "pyelonephritis" although the clinical symptoms encountered in

this study included loin pain, fever and dysuria. Little (1965) stated that "acute pyelonephritis" developed during pregnancy in 36% of 52 untreated bacteriuric women. This diagnosis was made when the women had loin pain, tenderness, a temperature above 100° F. and an infected urine. Kincaid-Smith and Bullen (1965) called these clinical episodes "symptomatic pyelonephritis". This diagnosis was made in the presence of the following symptoms: "loin pain and tenderness, with or without pyrexia, and rigors, with or without symptoms of dysuria and frequency." There is hence a lack of uniformity in the criteria used to make a clinical diagnosis of acute pyelonephritis. From the results of the present study it appears unlikely that all these symptomatic urinary infections are an indication of an acute bacterial infection of the kidney. It would be safer to call them "acute urinary infection" during pregnancy and to make the diagnosis of pyelonephritis on the evidence of radiological investigation or the results of renal function tests.

Incidence and possible factors in the pathogenesis of significant bacteriuria

In the Edinburgh area 595 out of 9,741 pregnant women or 6.1% were found to have significant bacteriuria at their first visit to the hospital. This finding was based on the results of an initial screening done using

the modified nitrite test. It is possible that this incidence is somewhat lower than the true one since bacteriuria due to organisms such as enterococci will not be detected and those due to Pseudomonas are unlikely to be detected by this test. Sleigh et al. (1964) examined urine from 1,684 pregnant women from the same hospital by a quantitative culture technique and found that 111 or 6.6% of them had significant bacteriuria. Hence it appears that the nitrite test has been able to detect almost all these bacteriuric women. Bacteriuria due to faecal streptococci occurred in four out of 100 women and none had a bacteriuria due to the Pseudomonas species in Sleigh's study. The use of the extended nitrite screening test has made the examination of about 120 women every week a practicable procedure.

The incidence in this series was based on the examination of a single specimen of urine from pregnant women. It compared well with the reported incidences of significant bacteriuria among pregnant women, e.g. an incidence of 7% by Turner (1961), 5.5% by Little (1965) and 6% by Kincaid-Smith and Bullen (1965). It has also been reported that about 20 to 30% of those with significant bacteriuria at their first visit are found to be negative when a subsequent urine specimen is examined (Kass, 1962; Little, 1965; Kincaid-Smith and Bullen, 1965). Kass (1962) stated that the demonstration of significant bacteriuria in two consecutive urine

specimens had a 96% confidence level in indicating the presence of true bacteriuria. In 64 out of 217 or 29.5% of bacteriuric women in this study, a subsequent examination failed to detect significant bacteriuria. But all these women had received a two-week course of treatment before the results of culture of this second specimen were known, in order to complete therapy before the 28th week of pregnancy. Hence it is difficult to evaluate the significance of this finding. It was further noted that seven of these pregnant women had a recurrence of bacteriuria during the subsequent follow-up period after treatment. This finding indicates that some of those with negative second specimens probably had a true bacteriuria. An analysis of the bacterial counts obtained by the examination of the second specimen of urine from these 64 pregnant women gave the following results:

Number of Pregnant Women	Count of organisms per ml. of urine (mixed contaminants)
11	10^5
26	$10^3 - 10^5$
27	below 10^3
Total 64	

In a proportion of these, contamination of the urine by perineal organisms during the collection procedure

may have been responsible. Turner (1961) examined urine specimens from 200 pregnant women who had a preliminary washing of the vulva with soap and water and from 200 women without such preparation and found no significant difference in the incidence of contamination found in these two groups of urine specimens. It was also noted that the degree of contamination in sterile urine specimens obtained by either of the above collection methods was similar when they were examined after a period of six hours at room temperature. Murdoch et al. (1966) confirmed these findings in Edinburgh. Hence it appears that an adequate specimen of urine for laboratory examination can be obtained from antenatal women without a preliminary cleansing of the vulva.

A factor regarding which there is at present insufficient knowledge is the intermittency of bacterial excretion in cases of established urinary infection. Could the above finding be an example of such an intermittency? A proper evaluation of this factor necessitates the frequent examination of urine specimens from a group of bacteriuric women without the administration of antimicrobial agents. Such a study is more feasible in a group of patients in hospital. The effect of frequent bladder evacuation and consumption of fluid on the bacterial concentration in the urine should also be considered. Since all these specimens were obtained during the afternoon it is possible that insufficient

time was allowed for the bacterial concentration to reach the critical figure of 10^5 per ml. In spite of these drawbacks it is preferable to treat these women for two weeks since a proportion of them may have true bacteriuria. Kincaid-Smith and Bullen (1965) in a controlled trial found a high incidence of symptomatic infection and radiological renal abnormalities including calculi in women in whom the second urine specimen did not confirm significant bacteriuria. In the present study a radiological investigation of the urinary tract in these women will be undertaken in the near future. This investigation is at present being undertaken in women who failed to respond to chemotherapy in the treatment trial.

Incidence of significant bacteriuria among
infertile women

The incidence among married but infertile women in this study was 118 out of 1,443 women examined, or 8.2%. Sleigh (1964) examined 397 infertile women and found that 8% had significant bacteriuria. In mature but unmarried women, e.g. in 100 pupil midwives, the incidence was 1%. A 1% incidence has also been reported in 9,878 school-children (Kunin and Halmagyi, 1962). Hence there is an increased incidence of bacteriuria in women after marriage. Sexual intercourse could be considered as a causative factor. Although "honeymoon pyelitis" is a

well recognised clinical entity, its incidence among newly married women is unknown. Bacteria could be introduced from the perineum and lower end of the urethra into the bladder during sexual intercourse. It is also possible that the associated minor trauma could initiate a bacteriuria at this time, with or without symptoms. In this situation a haematogeneous route of infection of the urinary tract could be considered possible. Barrington and Wright (1930) found that a bacteraemia occurred in a significant proportion of patients undergoing instrumentation or an operation of the urinary tract. The syndrome of "catheter fever" is also well recognised in clinical medicine. A culture of a specimen of blood along with the culture of urine in young married women with acute urinary infection would be helpful in investigating this problem. Kass (1960) claims that almost all women with bacteriuria of pregnancy acquire it in the first two months of pregnancy. Although it is true that pregnant women have established bacteriuria during the first trimester, it is more likely that they had this bacteriuria before the onset of pregnancy. It is also possible that symptoms of urinary infection in this mainly asymptomatic group occur during the latter half of pregnancy when the anatomical changes could be contributory. Baird (1935) examined 150 normal women once during pregnancy and 94 women at monthly intervals from the second month of pregnancy till

delivery. An investigation of the anatomical and functional changes occurring within the urinary tract during pregnancy was done by chromocystoscopy, intravenous pyelography, an estimation of the urea concentration in the urine from each kidney and also an estimation of the tone of the ureteral musculature by a special apparatus. Analysis of the results of the indigo carmine excretion test showed that some degree of stasis of urine in the upper urinary tract was present in 72.8% of primigravidae and in only 44.9% of multiparae. In primigravid women there was a delay in excretion by both kidneys beginning at the fourth month and reaching its maximum at the fifth or sixth month and then diminishing as pregnancy progressed further. In some cases there was evidence of delay in excretion and irregular action of the ureters as early as the second month of pregnancy. In nearly every case this delay in the excretion of the dye was due to an obstruction at or above the level of the brim of the bony pelvis. Baird stated that the two factors which caused this stasis within the urinary tract were an atony of the ureter and compression of the atonic ureter by the pregnant uterus.

In this study only 32 out of 475 bacteriuric women had symptoms of a urinary infection at the time when the first specimen of urine was taken.

Results of a two-week course of treatment for asymptomatic bacteriuria in 127 pregnant women

These women were treated with either cycloserine or sulphadimidine on an alternative, random sampling basis. The 62 women on sulphadimidine and 65 on cycloserine therapy were similar as regards age and parity distribution. About 20% in each group had a past history of urinary infection and about 10% had a past history of abortion.

The immediate response to treatment was evaluated by the culture of a specimen of urine taken one week after treatment was stopped. Both drugs were equally effective in controlling bacteriuria in about 76% of those treated. There were no obvious adverse effects on either the mother or the baby due to the drugs used.

The long-term results were evaluated on the results of culture of three further specimens of urine during pregnancy. It was found that only 51% of those treated for two weeks remained free of recurrences during the remainder of pregnancy. Hence women who had a recurrence of bacteriuria would remain undetected if a regular follow-up was not done on all bacteriuric women till the completion of pregnancy.

These results may be compared with those of other workers in this field. Kass (1960) stated that in a pilot study on the treatment of asymptomatic bacteriuria of pregnancy, bacteriuria commonly recurred after a

short course of treatment. He gave no figures as to this rate of recurrence after a short course but used suppressive sulphonamide therapy throughout pregnancy in his reported series. Little (1965) followed a similar regime and treated 57 women with bacteriuria on sulphamethoxy-pyridazine given daily throughout pregnancy and the puerperium. Kincaid-Smith and Bullen (1965) also used long-term sulphonamide therapy. In all these three reported studies no figures as to the rate of treatment failure or recurrence were mentioned. In only one reported study was a short-term chemotherapy for bacteriuria of pregnancy evaluated. Williams et al. (1965) reported the results of an eight-day course of sulphonamide therapy given to 127 women with bacteriuria of pregnancy. Four different sulphonamide compounds were used, e.g. sulphadimidine in 73 women, sulphafurazole in 7, sulphamethoxydiazene in 17 and a mixture of sulphadimidine and sulphamethoxypyrimidine in 30 women. No mention of the method of choice of the particular compound in each group was made. The results of the examination of a specimen of urine one week after therapy indicated "success" in about 77% of those treated. These authors found that when the urine was examined six weeks later only 5% showed a recurrence. They claimed that when the urine was clear at one and six weeks after treatment there were no further recurrences of bacteriuria during pregnancy or after delivery. It was,

however, noted that although all 127 women were examined one week after treatment only 80 were examined at six weeks and only 58 were examined one week after delivery. Hence it is difficult to conclude that the women who were not seen at these times had no recurrences. In the present study a short course of chemotherapy was effective in about 51% of women with asymptomatic bacteriuria and complete follow-up of all treated women was essential in order to detect all recurrences of bacteriuria.

In the present series, out of 31 women, who had a recurrence of bacteriuria after an initially successful therapy, it was possible to type serologically the Escherichia coli strains isolated before and after therapy in 11 cases. In six women the recurrence of bacteriuria was due to a relapse with the original serotype and in five women a re-infection with a different serotype was found. In addition, the recurrence of bacteriuria was due to a different species of infecting organism in five women. It could be argued that a relapse due to the same serotype of Escherichia coli indicates inefficient treatment and that a longer course of therapy would be more successful. Yet there is no reason to suppose that an entirely new infection did not occur by an organism of the same serotype. The superiority or otherwise of a longer course of therapy would have to be established in a comparative trial

where alternate bacteriuric women are given either a long or short course of treatment. From the results of the present study it appears that in about 50% of these women a short course of treatment with either sulphadimidine or cycloserine is sufficient to eradicate bacteriuria during pregnancy. Hence in about half of pregnant women with bacteriuria prolonged antimicrobial therapy throughout pregnancy would not be justified.

Correlation of the results of sensitivity tests and the response to treatment with sulphadimidine

In a recent report of an antibiotic sensitivity test trial organised by the Bacteriology Committee of the Association of Clinical Pathologists (J. clin. Path., 1965, 18, 1), it was found that more errors were made in the in vitro assessment of bacterial sensitivity to sulphonamides than were made in assessing sensitivity to any other antimicrobial agent in common use. This was because the methods varied in important details and there was no standardisation. The results of the present work confirm that sulphonamide sensitivity tests by the tube technique are markedly influenced by the test inoculum size and by the inhibitor content of the liquid medium used. Moreover, the particular sulphonamide compound used in the test further influences the results obtained by either the disc or tube dilution procedure. It is necessary in practice to standardise these variables

using one of the more active sulphonamide compounds. The results may then be applicable to other sulphonamide compounds in clinical use if important considerations concerning in vivo activity are borne in mind. In the present study it was found that of the three sulphonamide compounds tested, sulphanilamide was least and sulphadiazine was the most potent in in vitro tests. The powder and injection forms of sulphadimidine were both of intermediate potency and showed only minor differences in MIC level when tested in vitro. It was decided to use injection sodium sulphadimidine in the in vitro sensitivity tests done in the present study since this compound was more soluble than the powder form. This compound, in tablet form, was also used in the chemotherapeutic trial and the MIC levels as judged by tube dilution sensitivity tests are readily attained in vivo in patients on ordinary dosage schedules. Known sensitive and resistant strains should be included as controls in each batch of tests so that errors arising from variations in inoculum size, in the constituents of the liquid medium or in the potency of the sulphonamide stock solution do not pass undetected.

The term "resistant" may be applied to an organism under two different circumstances: (1) when the concentration of the antimicrobial agent that an organism is able to withstand is appreciably higher than the concentration obtained in vivo; (2) when an organism tolerates

an antimicrobial agent in concentrations appreciably higher than those that inhibit the growth of the majority of strains in the same species. If the first of these is taken as the criterion of resistance, the sulphonamide level attainable in vivo is the deciding factor. In patients with a urinary tract infection this level is open to question because the levels in the urine are appreciably higher than are the blood levels. In so-called lower urinary tract infections, an effectively bacteriostatic level in the urine would be sufficient to control infection. The urine level of sulphathiazole attained in man on a normal fluid intake after a dose of only 1 g. by mouth is 500 $\mu\text{g./ml.}$ (Hawking and Lawrence, 1950). Kass (1955) maintained that organisms with sulphonamide MIC levels of over 500 $\mu\text{g./ml.}$ were likely to be resistant to treatment in infections of the urinary tract. On the other hand, for the effective treatment of parenchymal infections of the kidney, it seems that a bacteriostatic level must be achieved in the tissues. As we still have inadequate information regarding tissue levels of drugs, an adequate blood or plasma level may be regarded as a reasonable index. Levels easily attainable in blood on normal dosage with sulphadiazine^a (2 g. per day) are about 50 $\mu\text{g./ml.}$ After vigorous treatment (4 g. per day) it is possible to produce blood concentrations of about 150 $\mu\text{g./ml.}$ (Kass, 1955).

Unfortunately, we have at present no simple way of

differentiating an upper from a purely lower urinary tract infection in any patient. Investigations of such indices as the serum antibody response may help to differentiate these patients (see later). Until we have a reliable guide, it is safer to adopt the somewhat extreme view that every urinary infection should be considered as pyelonephritis unless otherwise proved; at least this may be advisable as far as treatment is concerned.

The commonest causative organism in cases of uncomplicated urinary tract infection is Escherichia coli. In 118 out of 127 isolates in the present treatment trial for asymptomatic bacteriuria of pregnancy, the causative organism was typical or atypical Escherichia coli. Since sulphonamides will sterilise the urine in about 76% of such infections, it can be regarded as the most suitable drug with which to start treatment. The reported MIC values for sulphonamides against Escherichia coli cover such a wide range that the clinician has no useful guide. Indeed, the bewildering number of conflicting reports have caused some to abandon sulphonamides as unreliable and useless. For sulphadimidine against strains of Escherichia coli, reported MIC values range from 1 $\mu\text{g./ml.}$ (Garrod, 1959), 50 $\mu\text{g./ml.}$ (Brumfitt et al., 1965), to 2500 $\mu\text{g./ml.}$ (Schweinburg and Rutenburg, 1949). It is therefore essential to have individual strains tested for sulphonamide sensitivity,

but the lack of standardisation renders many laboratory reports misleading.

The lack of correlation between the results of the disc test and the clinical response is evident from the results of the present investigation and findings of other workers who used this method (Gould and Edmond, 1964). Further standardisation of the inoculum size may give better results in the disc test, but this is not justified because a disc test should at least be quicker and easier to perform. The tube test, described here, can be developed so that a competent technician is able to carry it out routinely, but it is more time consuming and expensive than the usual disc test.

In the clinical trial reported above, Escherichia coli infections caused by strains with an MIC level of $52 \mu\text{g./ml.}$ or below were successfully treated. Treatment of infections caused by organisms having an MIC of $104 \mu\text{g.}$ of sulphadimidine per ml. was unsuccessful in one out of eight such instances. The results of the tube dilution sensitivity tests using sulphadimidine thus appear to be consistent with the clinical response in 52 out of 62 patients treated for asymptomatic bacteriuria of pregnancy in the present study.

Correlation of results of *in vitro* sensitivity tests and the response to treatment with cycloserine in 65 women with asymptomatic bacteriuria of pregnancy

Cycloserine, an antibiotic produced by Streptomyces orchidaceous, was isolated in 1954. Welch et al. (1955) investigated the antibacterial activity and blood and urine concentrations of this antibiotic in man. In the above study a calorimetric method was used to determine the quantity of cycloserine in the blood and urine. Forty normal subjects were examined after the oral administration of various single doses of cycloserine. Blood samples were collected at 4, 8, 12 and 24 hours and the total output of urine over 48 hours after medication was also collected. The average blood concentration 4 hours after the administration of a dose of 500 mg. was about 6 $\mu\text{g./ml.}$ The peak urine concentration on this dosage, reached after about 8 hours, was around 100 $\mu\text{g./ml.}$ These authors also investigated the *in vitro* sensitivity of ten strains of Escherichia coli to cycloserine by the tube dilution method. The MIC level in nine of these strains was 100 $\mu\text{g./ml.}$ and in one strain it was 125 $\mu\text{g./ml.}$ Garrod (1959) reported the MIC level of this antibiotic for Escherichia coli as 64 $\mu\text{g./ml.}$ and for Proteus strains as 256 $\mu\text{g./ml.}$ Garrod further stated that the average urine concentration of this antibiotic in man was about 250 $\mu\text{g./ml.}$, presumably on a dose of 250 mg. four times a day.

The MIC levels obtained during the present study were similar to the above reported values for Escherichia coli. These results are seen in Table 53.

Table 53

The results of in vitro sensitivity tests using cycloserine in organisms isolated from 65 women with asymptomatic bacteriuria of pregnancy

No. of strains	MIC levels ($\mu\text{g./ml.}$)
16	31 or less
41	62
5	125
3	250
Total 65 strains	

In this study two out of three strains with MIC levels of 250 $\mu\text{g./ml.}$ were identified as Proteus mirabilis and one strain as Escherichia coli.

The clinical response to cycloserine treatment in these 65 women did not correlate with the results of in vitro sensitivity tests. In only two out of 13 treatment failures in this study was an in vitro resistance to the infecting urinary organism demonstrated. It appears that the results of in vitro tests with this antibiotic are not a reliable guide to the clinical response. It is evident that its antimicrobial activity

in vitro and in vivo differ markedly. However, from the results of this trial it appears that this antibiotic is therapeutically effective in infections caused by Escherichia coli in the urine. These results with cycloserine were no better than those obtained with sulphadimidine.

Murdoch et al. (1966) reported the result of treatment of urinary infections with cycloserine in 404 female patients, the majority of whom were under 40 years of age. Most of these infections were caused by Escherichia coli. The antibiotic was given for two weeks in a dose of 250 mg. twice daily to 108 of these women. All women were followed up for varying periods of time, between one month and two and a half years. A relapse or re-infection of bacteriuria occurred in 47% of women thus treated. These results are in agreement with the result of therapy in asymptomatic bacteriuric pregnant women in the present study. Murdoch et al. found that in a further group of 296 women with urinary infection, who were treated with a long-term suppressive therapy with cycloserine, the relapse rate was 20%. The results of tube dilution sensitivity tests in this study showed an in vitro resistance to cycloserine in only 4% of 555 strains of Escherichia coli tested.

Serological typing of urinary strains of Escherichia coli

A complete serologic identification of strains of

Escherichia coli requires the determination of the 'O', 'K' and 'H' antigens. For the purposes of the present study, a determination of the 'O' antigen alone was considered sufficient. Serologic identification was carried out on a single colony isolated from each urine specimen since Vosti et al. (1962) found that over 90% of urine samples contained a single 'O' type per specimen regardless of the number of colonies examined. In the present study it was possible to type 139 or 55.4% of 251 strains of Escherichia coli by using only ten typing antisera. McGeachie (1965) used 12 antisera and was able to type 56.6% of 534 urinary strains. Hence the results in the present series are in agreement with the findings of McGeachie. It was further noted that 'O' types 2, 6, 7 and 75 accounted for 94 or 67.6% of the 139 typed strains in the present study. Since more than five strains of the same 'O' type were isolated during the follow-up of each of six women, the incidence of each 'O' type was related to the number of women examined. Only the Escherichia coli strains isolated before treatment were considered in order to determine the relative frequency of occurrence of each 'O' type in 47 women with asymptomatic bacteriuria of pregnancy. It was found that 'O' type 6 occurred most frequently, occurring in 11 out of 47 women. 'O' type 75 was isolated from nine women and 'O' type 2 from eight women. Rantz (1962) found that 'O' types 2, 4, 6 and 75

accounted for 49% of the isolates from 156 patients with significant bacteriuria. Turck et al. (1962) found that 'O' types 1, 4, 6 and 75 occurred most frequently in 522 strains of Escherichia coli isolated from cases of urinary, pulmonary and other non-enteric infections. These four 'O' types also accounted for 57% of the typed urinary strains. Hence it was suggested that the prevalence of these serotypes reflected their predominance in nature rather than the pathogenicity of these strains. Jackson et al. (1965) found that urinary infections due to Escherichia coli 'O' type 6 caused urinary symptoms more often than other 'O' types. 'O' type 75 was found more frequently in cases of urinary infection during pregnancy.

The predominance of 'O' types 2, 6 and 75 was also found in the present study of 47 women with asymptomatic bacteriuria of pregnancy. From this finding or from the available literature it is not possible to make conclusions regarding the invasiveness or pathogenicity of these 'O' types of Escherichia coli at the present time.

In 21 antenatal women it was possible to type serologically the Escherichia coli strains isolated before and after treatment. In ten of these patients, who failed to respond to treatment, a relapse with the original serotype was detected in the urine obtained after therapy. The same serotype also persisted during pregnancy in subsequent urine samples examined in eight

of them. In 11 patients, in whom bacteriuria recurred after an initially successful treatment, a relapse due to the original serotype was seen in six and a re-infection due to a different 'O' type in five. Hence it appears that in women with persistent bacteriuria the same 'O' serotype is likely to persist throughout pregnancy. After an initially successful course of treatment, recurrences of bacteriuria may or may not be due to the same serotype. It would be interesting to assess different forms of treatment by means of such serological identification. To make such a study worthwhile it is necessary to have access to all 145 typing antisera.

The incidence of complications during pregnancy in treated and untreated bacteriuric and non-bacteriuric women

The present trial was designed to compare two types of drug therapy in the treatment of asymptomatic bacteriuria of pregnancy. Since it was not a "controlled" trial, in the sense of having a similar untreated group, the untreated bacteriuric group consisted of all women who were not included in the treatment trial for the reasons stated earlier. It would have been preferable if alternate bacteriuric women in the trial were given a placebo and observed during pregnancy but for a variety of reasons this was not thought to be feasible. Untreated bacteriuric women in this study had

significant bacteriuria detected in only one urine specimen and perhaps includes those who failed to show bacteriuria in a subsequent specimen. Hence it can be argued that the size of this untreated bacteriuric group is larger than the numbers with true bacteriuria and that only a repeated urine examination could establish the degree to which contamination or intermittency of bacterial excretion led to these findings. The incidence of complications in the untreated group should be viewed in the light of these discrepancies.

The incidence of bacteriologically proven acute urinary infection during pregnancy

Successful treatment for asymptomatic bacteriuria was highly efficacious in preventing the occurrence of symptomatic infection later in pregnancy. The incidence of symptomatic infection was highest in those women who failed to respond or had a recurrence of bacteriuria following treatment, and occurred in 32 out of 62 of these women. The term "acute pyelonephritis" was not used to describe these incidences since it was difficult to arrive at this diagnosis from the clinical details available in most instances. All 32 women were re-treated for their symptoms by the obstetrician in charge of them. Eight of the women required two such courses of re-treatment during pregnancy. In untreated bacteriuric women the incidence was 86 out of 224 or

38.4%. Only one out of 65 successfully treated women developed urinary symptoms. Hence it can be stated that symptomatic urinary infections occurring during pregnancy can be prevented by successful treatment early in pregnancy for asymptomatic bacteriuria. These results confirm the findings of Kass (1960), Little (1965) and Kincaid-Smith and Bullen (1965) that acute pyelonephritis or acute urinary infection of pregnancy is a preventable disease.

Anaemia

Giles and Brown (1962) reported that urinary infections occurred more frequently in anaemic pregnant women and that anaemia tended to respond when the urinary infection was successfully treated. Layton (1964) reported an incidence of anaemia in 31.3% of 67 untreated bacteriuric and in 19.5% of 118 control non-bacteriuric pregnant women. He suggested that a urinary infection probably interfered with the absorption of iron from the intestine. In the present study a significantly higher incidence of anaemia was found in untreated and unsuccessfully treated bacteriuric pregnant women than in successfully treated or control non-bacteriuric women. The cause of the high incidence of anaemia in bacteriuric pregnant women merits further investigation.

Toxaemia and hypertension

Kincaid-Smith and Bullen (1965) and Norden and

Kilpatrick (1965) reported a significantly higher incidence of pre-eclamptic toxæmia among pregnant bacteriuric women in comparison with the incidence in non-bacteriuric controls. In the former study this increased incidence was not altered in a proportion of bacteriuric women under successful prolonged chemotherapy during pregnancy. Finnerty (1956) found that successful treatment of bacteriuria in his study also improved the signs of toxæmia and persistent albuminuria in this group. The results of the present study are more in agreement with those of Kincaid-Smith and Bullen (1965). The incidence of toxæmia or hypertension was significantly higher in the group of asymptomatic bacteriuric women in the trial in comparison with the non-bacteriuric control group. Successful treatment in a proportion of these women did not alter the incidence of this complication. It was further noted that the incidence in untreated bacteriuric women was similar to the incidence in non-bacteriuric controls. One factor which could contribute to this finding is the fact that the untreated bacteriuric women in this study did not have significant bacteriuria confirmed in two separate urine specimens. Hence this group probably includes a number without true bacteriuria.

Prematurity by weight or by gestation

The present study failed to find any correlation

between the incidence of prematurity by either of these definitions and the presence of bacteriuria during pregnancy. A controlled chemotherapeutic trial would be useful in investigating this further.

Abortions and total foetal loss

There was a significantly higher incidence of abortion and total foetal loss in the untreated bacteriuric group in comparison with the incidence in treated bacteriuric and non-bacteriuric pregnant women. These findings confirm those reported by Kass (1961) and Kincaid-Smith and Bullen (1965). Kincaid-Smith and Bullen, unlike Kass, did not find a significant improvement in the foetal loss in a group of successfully treated bacteriuric women. Kass suggested that the increased foetal loss in bacteriuric women could be due to the effects of bacterial endotoxin on the pregnant uterus. Hence, although the exact mechanism by which foetal loss occurs is in doubt, it appears that untreated bacteriuric pregnant women have a higher risk of losing their babies.

Radiological abnormalities of the urinary tract

The investigation of intravenous pyelography in this study has been possible only in the last six months. All 62 women who failed to respond to a short course of chemotherapy for asymptomatic bacteriuria are being investigated. So far 17 women have completed this

examination. Only four of these women had radiological abnormalities of the urinary tract. One woman had a nephrectomy done for unilateral chronic pyelonephritis, another is being treated for chronic bilateral pyelonephritis, a third patient had a cyst in the right kidney and the fourth showed a diverticulum associated with the lower end of the right ureter.

The serum antibody response to bacteriuria
caused by Escherichia coli

The haemagglutination technique of Neter et al. (1952) for detection of antibodies to Escherichia coli has been successfully modified in this study. It was found that this test carried out in W.H.O. plastic plates using a 1% suspension of sensitised sheep cells gave better results than the tube method described by these authors. It was further found that the concentration of the sensitising antigen used in this test had a considerable effect on the end results. After testing various concentrations an optimal concentration for routine use was determined and a simple procedure for the extraction of crude 'O' antigen from strains of Escherichia coli is described.

In comparison with the bacterial agglutination technique, the haemagglutination technique was more sensitive in detecting the antibody response to Escherichia coli in pregnant women. This was seen

particularly in the level of antibody detected by each of these methods in 81 control non-bacteriuric pregnant women. By the bacterial agglutination technique only five of these 81 women had antibody levels of 160 to standard 'O' type strains of Escherichia coli. The haemagglutination technique showed antibody levels in 53 of these women ranging from 240 to 1,920 against the same strains. The specificity of this haemagglutination titre was demonstrated in each instance by a haemagglutination inhibition test. It was also found that there was no correlation between the end titres as detected by each of these two techniques. All five women with titres of 1,920 by the haemagglutination test had titres below 160 by bacterial agglutination. It is hence possible that these two tests measure different fractions of the antibody response to Escherichia coli. It was possible, by examining the serum from this control non-bacteriuric group, to state that an antibody response by Escherichia coli up to a titre of 960 by the haemagglutination technique was normal. A titre of 1920 was considered of doubtful significance.

The above criteria were used to analyse the serum antibody response in 54 women who were successfully treated and in 21 women who had a recurrence after an initially successful treatment for asymptomatic bacteriuria of pregnancy. In these women the infecting urinary organism isolated before treatment was used as

antigen. The antibody levels obtained in both these groups were similar to those found in control non-bacteriuric women. It is possible that in women who were successfully treated for the whole or a part of pregnancy, the transient bacteriuria was an insufficient antigenic stimulus for a significant antibody response.

In 12 women the infecting urinary strains were unsuitable for use in the bacterial agglutination technique since they were autoagglutinable. These antigens were successfully used to sensitise red cells in the haemagglutination technique.

In five out of nine bacteriuric women who failed to respond to treatment the haemagglutination titre against the urinary organism was between 1920 and 7680. A significant, or over four-fold, rise in antibody titre during pregnancy was found in these women. In three women the rise in titre coincided with the onset of clinical symptoms of acute pyelonephritis. In three of these five instances the titre by bacterial agglutination technique was also raised, being 320 or above. It is possible that the use of a purified polysaccharide extract of Escherichia coli strains as antigen in the haemagglutination test may give better results but this is not practical as a routine procedure in the investigation of patients with a urinary infection. Neter et al. (1956) used purified Escherichia coli polysaccharides as well as the corresponding crude antigen

extracts of 'O' groups O₁₁₁, O₅₅ and O₂₆. The haemologous antisera were examined with these antigen preparations. Minor cross-reactions observed with the crude antigens were not encountered with purified antigens used in the haemagglutination test.

Varying levels of antibody response to Escherichia coli in patients with urinary infection and controls have been reported by other workers using the haemagglutination technique. Needell et al. (1955) considered titres over 160 by this technique as significant in a group of 20 patients with a urinary infection. No attempt was made to correlate the level of antibody response with the severity or anatomical site of infection in the urinary tract. Winberg et al. (1963) estimated the antibody response by this technique in 20 children with a urinary infection and in 20 controls with no such infection. The level in the control group was a titre of 64 or below. In ten children with acute pyelonephritis a rapid and marked antibody response was seen. It is possible that children show a lower antibody response to Escherichia coli in comparison with the response in adults. It is also possible that these lower levels were obtained due to the method used, namely, that of Neter et al. (1952).

Percival, Brumfitt and De Louvois (1964) used the bacterial agglutination technique and found a correlation between an antibody titre of 320 or above and the

presence of an active renal infection. These workers examined the serum antibody response in a group of 41 patients with significant bacteriuria and the clinical features of an acute pyelonephritis. The highest antibody titre was 1 in 320 or above in 38 or 93% of them. Twenty-five of these patients were subsequently examined by intravenous pyelography and 17 of them showed a renal evidence of chronic pyelonephritis. Hence in subsequent estimations of the serum antibody response in various groups of patients with a urinary infection, the presence of a serum antibody titre of 1 in 320 or above was taken as evidence of probable renal damage. In 19 out of 20 control normal subjects the serum antibody levels, tested against 11 standard Escherichia coli 'O' type strains used as antigen, did not rise above 1 in 160. These authors found a raised serum antibody titre in 32 out of 126 bacteriuric pregnant women examined. A rising titre during pregnancy was found in only ten of them.

The results of the present study confirm the findings of Percival, Brumfitt and De Louvois (1964) that a high serum antibody response to the urinary strain of Escherichia coli probably indicates renal involvement. The results of a radiological investigation in these patients, which is at present being undertaken, will confirm this further. The standardised haemagglutination test appears to be more sensitive than the bacterial

agglutination technique in the detection of this response. The fact that Escherichia coli infections in other parts of the body can also stimulate an antibody response should be recognised. The number of women with a significant antibody response in the present study is extremely small to make any further conclusions. These techniques should be further evaluated in a group of patients with active renal disease. The presence of renal involvement in patients should also be proved by such tests as radiological examination, estimation of renal concentration capacity and renal biopsy.

SUMMARY

SUMMARY

1. The literature regarding the etiology and pathology of human urinary tract infections has been reviewed.

Particular reference was paid to factors involved in the initiation and perpetuation of urinary infection in man by an ascending route.

2. Animal experimental models used to study the human disease were considered in detail. Some of the limitations in the application of these results were considered.

3. The literature regarding methods used to diagnose the presence and the severity of human urinary infection was considered, namely: quantitative and semi-quantitative culture of urine, Gram stain of uncentrifuged urine, pyuria, white cell excretion rate, estimation of white cell concentration, "pyrexal" and prednisolone tests, radiology of the urinary tract including the demonstration of vesico-ureteral reflux, serological typing of urinary strains of Escherichia coli and the serum antibody response to a urinary infection.

4. The concept of "significant bacteriuria", developed by counting the numbers of viable organisms present in 1 ml. of voided urine, was considered in detail with reference to the findings of Kass (1956). The importance of asymptomatic bacteriuria in pregnancy and the possible complications associated with the presence

of this condition were considered.

5. Chemical and bacteriological screening tests used to detect women with significant bacteriuria were considered.

6. The modified nitrite test and the triphenyl tetrazolium chloride (T.T.C.) test to detect significant bacteriuria were evaluated in 1,000 specimens of urine from antenatal and infertile women. The former test detected 69 out of 71 or 97% of urine specimens with significant bacteriuria as determined by a quantitative culture technique. The T.T.C. test detected only 54 or 76% of these infected urines.

7. A total of 9,741 consecutive pregnant women attending the Simpson Memorial Maternity Pavilion had urine specimens examined by the extended nitrite test. All positive by this test had a confirmatory quantitative culture of this urine specimen done. Of these, 595 or 6.1% were found to have significant bacteriuria.

8. 1,443 married but infertile women attending the Infertility Clinic also had urine specimens examined. Of these, 118 or 8.2% were found to have significant bacteriuria. This finding was compared to the reported incidence in mature unmarried women and school-children. It was postulated that marriage and sexual intercourse was a factor in initiating the occurrence of significant bacteriuria in pregnant women. Kass's claim (1960) that all women with bacteriuria of pregnancy acquire it in

the first two months of pregnancy was considered unlikely.

9. A group of 127 women with asymptomatic bacteriuria were included in a treatment trial. Sulphadimidine or cycloserine was given for two weeks to alternate women in the trial and the results evaluated during the remainder of pregnancy by examination of specimens of urine by quantitative culture technique.

10. The immediate result of treatment in each case was evaluated by examination of a specimen of urine one week after cessation of therapy. In 96 out of 127 or 75.6% the treatment was successful.

11. The long-term results were more disappointing since only 65 or 51.2% of these 127 women remained uninfected during the rest of pregnancy. In 31 women there was a recurrence of bacteriuria during the follow-up period.

12. There was no significant difference in the results obtained by these two drugs.

13. The occurrence of the following complications during pregnancy, viz., bacteriologically proven acute urinary infection with symptoms, anaemia, toxæmia or hypertension, prematurity by weight, prematurity by gestation, abortion, and total foetal loss was determined in treated and untreated women with bacteriuria and a non-bacteriuric control group of pregnant women.

14. There was a highly significant difference in the incidence of symptomatic infections in the treated and

untreated women with bacteriuria and the non-bacteriuric control group. Successful treatment of asymptomatic bacteriuria was highly effective in preventing symptomatic infection during pregnancy.

15. The incidence of anaemia was significantly higher in pregnant women with bacteriuria which was untreated or unsuccessfully treated when compared with non-bacteriuric or successfully treated women.

16. The incidence of toxæmia or hypertension was not significantly reduced by successful treatment of asymptomatic bacteriuria of pregnancy. The incidence of this complication was significantly higher in bacteriuric women when compared to non-bacteriuric controls.

17. No correlation between the incidence of prematurity and bacteriuria of pregnancy was found.

18. The incidence of abortion and total foetal loss was significantly higher in the untreated bacteriuric pregnant women in comparison with the other groups.

19. A standardised method for estimating the in vitro sensitivity of urinary pathogens to sulphadimidine has been developed.

20. There was good correlation between the results of in vitro sensitivity tests and the clinical response to treatment in 52 out of 62 patients in the trial.

21. There was no significant relationship between the results of in vitro sensitivity tests and the clinical

response to treatment in 65 women treated with cyclo-serine. These findings are discussed.

22. Rabbit antisera to the following standard

Escherichia coli 'O' types were prepared, i.e. O₁, O₂, O₄, O₆, O₇, O₈, O₁₈, O₂₅, O₇₅, O₇₇.

23. It was possible to serologically type 139 or 55.4% of 251 urinary strains of Escherichia coli with the above antisera. These typed strains were obtained during the study of 51 patients with asymptomatic bacteriuria of pregnancy.

24. The relative frequency of occurrence of each of these 'O' types in 47 women with asymptomatic bacteriuria were determined. 'O' type 6 occurred most frequently, in 11 of these women, 'O' type 75 in nine women and 'O' type 2 in eight women. These results are discussed with reference to results obtained by other workers in this field.

25. The haemagglutination technique of Neter et al. (1952) for the detection of antibodies to Escherichia coli has been modified in this study.

26. The bacterial agglutination technique and haemagglutination technique for the estimation of antibodies to urinary strains of Escherichia coli have been evaluated.

27. The haemagglutination technique was found to be more sensitive in estimating the antibody response in 81 non-bacteriuric pregnant women. Ten standard 'O' type strains of Escherichia coli were used as antigens in

each of these patients.

28. The antibody response ^{was} also estimated in 54 women who were successfully treated for asymptomatic bacteriuria of pregnancy, 21 women who had a recurrence of bacteriuria following treatment and in nine bacteriuric women who failed to respond to treatment.

29. In five women who failed to respond to treatment there was a significant antibody response in three of whom the rise in antibody titre coincided with the clinical onset of symptoms of acute pyelonephritis.

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