URINARY TRACT INFECTION IN PATIENTS
WITH SPINAL CORD INJURY

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I am also indebted to Mrs. Barbara Bertels, Mr. Michael Fitzgerald, Mrs. Sandra Stirling and Miss Rhonda Watson for technical assistance.
A road sign in the grounds of the Austin Hospital, Heidelberg, Melbourne, Victoria.
ABBREVIATIONS

The abbreviations which are used without definitions are based on the instructions to contributors to the Journal of Medical Microbiology, Great Britain and Ireland (J. Med. Microbiol. 1974, 7, 1-4).
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ABSTRACT

This study was made to ascertain the aetiology, origin and pathways of infection as well as the reason for recurrence of urinary tract infection in male patients with permanent indwelling catheter, at the Spinal Injuries Unit, Austin Hospital, Australia. There has been no detailed bacteriological report published since the Unit opened in 1956.

Since urinary infections are frequent during the life of spinal paralytics, it is important in the management of such patients to determine whether it is relapse or re-infection which plays the major role of infection. The main body of this thesis presents the findings of a detailed search for wall-defective bacteria (L-forms) in an attempt to confirm or deny the hypothesis that persistent L-forms play a part in the repeated isolation of the same organism from a patient over a number of years.

The aetiology of urinary infection occurring in new admissions was a reflection of the ward flora present at different times of survey, which in turn depended on the disinfection measures taken. Gram-negative, urea splitting organisms such as Klebsiella, Proteus, and Providence as well as the nosocomial bacteria Acinetobacter, Pseudomonas and Serratia were found to be more common in causing urinary infection than the traditional Escherichia coli and Streptococcus faecalis.

The chlorhexidine solution used for bladder irrigation was shown to be the source of Acinetobacter infections acquired during 1971-2
in new admissions. From weekly examinations during 1971-2 of new admissions there was little evidence that the urinary infections came from the patients' own faecal or nasal flora even when Klebsiella, Proteus or Pseudomonas were regularly isolated from such material.

The type of urine drainage bag used and its care, influenced the length of time before the appearance of the initial bladder infection.

That the persistence of specific strains in individual patients was due to the continuing presence of the same bacteria rather than due to re-infection, was shown by weekly examination of urine. However it was in chronic spinal paralytics that this persistence of early infection was most convincingly demonstrated. Species and strains which were not detected in more recent patients were regularly isolated from this group.

Re-infection with new species did occur over a background of the permanent organism. Clinical urinary disease due to re-infection would be treated, and when the new organism had been eliminated, the permanent organism would often remain. It is postulated as a result of the current study, that a patient with an indwelling catheter usually maintains a low grade infection with one particular organism for many years and that such strains are always resistant to the antibacterial in use.

This study shows that L-forms did not account for the persistence of a particular bacterial species in a patient. The method of L-form isolation was validated by a parallel study with a group of non-spinal patients receiving chemotherapy for renal disease, and L-forms and mycoplasmas were recovered from this group. The reasons for the absence
of L—forms in the spinal patients are given as: the infrequent use of antibiotics; a fluid output resulting in low urine osmolality (90-600 mOsm/kg); the presence of formaldehyde in urine; and the low hydrogen ion concentration of urine.

Reasons for persistence, other than L—form production, were investigated:

(a) Successful antibiotic therapy did not eradicate the organism in most cases.
(b) Several organisms which acquired antibiotic resistance were found to possess transferable resistance factors (R factors).
(c) Urinary acidification and disinfection were not effective since the concentration of formaldehyde did not reach 10 μg/ml in many of the patients examined. E. coli was the only species with a M.I.C. of formaldehyde < 10 μg/ml.

The traditional, antibiotic-sensitive species E. coli and Strep. faecalis were not common probably because of their relative sensitivity to:

(a) the chlorhexidine disinfectant used in bladder irrigation, urethral irrigation and catheter lubrication and sprayed at the junction of the catheter and drainage tubing;
(b) lysol used to swab the anal area;
(c) the formaldehyde released as a result of methenamine mandelate or hippurate medication. The species commonly found in the patients were much more resistant to the
antibiotics and disinfectants used. An animal experiment designed to test the lowered infectivity of *E. coli* compared with *Pr. mirabilis* and *Kl. aerogenes* in the face of acid urine and the presence of formaldehyde, adds further evidence to the selection pressures acting against *E. coli*.

A small group of patients with neurogenic bladders caused by spina bifida were examined to test the aetiological findings in the spinal patients. In this group *E. coli* and *Strep. faecalis* were rare, but the nosocomial species were absent. Persistence of the same species was present for many years.

The study concludes with a recommendation for increased vigilance in the care of the drainage bag as well as improved methods for disinfection of equipment in hospital and at home. A simple and accurate method has been developed for use in the ward and at home, to monitor the level of total formaldehyde in the urine after acid hydrolysis. This should assist in the choice of a therapeutic dose of the urinary disinfectant.
SECTION 1

INTRODUCTION

1.1 HISTORY OF SPINAL INJURIES
From the Edwin Smith Surgical Papyrus, 17th Century B.C.

Translated by Breasted (1930) who considered the papyrus to be a transcription of a physician of 3,000 B.C.

Case 31

Instructions concerning a dislocation in a vertebra of (his) neck.

Examination

If thou examinest a man having a dislocation in a vertebra of his neck, shouldst thou find him unconscious of his two arms (and) his two legs on account of it, while his phallus is erected on account of it, (and) urine drops from his member without his knowing it; his flesh has received wind; his two eyes are bloodshot; it is a dislocation of a vertebra of his neck extending to his backbone which causes him to be unconscious of his two arms (and) his two legs. If, however, the middle vertebra of his neck is dislocated, it is an emissio seminis which befalls his phallus.

Diagnosis

Thou shouldst say concerning him: "One having a dislocation in a vertebra of his neck while he is unconscious of his two legs and his two arms, and his urine dribbles. An ailment not to be treated".
"An ailment not to be treated" was bluntly stated by the physician in 3,000 B.C. (Breasted, 1930); and this typifies the pessimistic attitude of the medical profession until the end of the Second World War of 1939-45. For 5,000 years the literature contains references to methods of treatment to alleviate symptoms following spinal cord injury (See Table 1). The mortality within three months of injury was described as 80% as late as 1937 (Thompson Walker), and those who survived the injury were expected to be helpless cripples. This attitude was perpetuated firstly by the defeatist view of physicians and secondly by society's concept that a totally disabled person could not work and indeed might lose pension and compensation if remunerative work were attempted. When persons of prominence such as Admiral Nelson at Trafalgar and James A. Garfield (20th President of the United States of America) sustained spinal injuries, the hopeless future predicted for these victims is shown by comments recorded at the time (Warner, 1966; Guttman, 1973).

The causes of death in the spinal patient had been well described by 1900; these were renal failure due to ascending urinary infection, exhaustion due to bedsores, and amyloidosis due to a combination of the first two (Curling, 1836; Dickinson, 1877; Thorburn, 1889 and Fagge, 1891). Tribe and Silver (1969) have reviewed the literature on mortality of allied soldiers in the First World War of 1914-18 and found that approximately 80% of spinal injured patients died in the first few weeks in consequence of infection from bedsores and catheterisation.
TABLE 1

HISTORY OF TREATMENT OF SPINAL INJURIES

3000 B.C. Egyptian physician Imhotep described five types of spinal injuries.

1700 Edwin Smith Papyrus: transcription of Imhotep (Breasted, 1930).

460 Hippocrates described complications of spinal injury: constipation, dysuria and bedsores. Advocated large fluid intake, special diet and reduction of dislocations by hyperextension (Adams, 1849).

300 A.D. The Hindu Sushruta-Samhita advocated immobilization of the spinal column. Differentiated between fractures and dislocations (Bender and Thom, 1961).

600 Paul of Aegina originated the laminectomy (Adams, 1844-7).

1000 Avicenna of Persia reduced cervical vertebrae by compression, abstersion and immobilization (Castiglioni, 1958).

1200 Roland of Parma in his "Chirurgia" described manual extension for dislocation.

1564 Ambroise Paré (France) revived laminectomy, introduced the modern flexion-rotation method of reduction. Described catheters, diuretics and invented a satin condom for incontinent urine.

1600 Fabricius of Hilden introduced the modern technique of open reduction of a fracture dislocation of the lumbar spine.

1793 Sömmerring in Germany wrote a monograph on injuries of the spine (Castiglioni, 1958).

1806 Bozzini of Frankfurt-on-Main described the first candle-illuminated endoscope to view the bladder cavity (Murphy, 1972).

1814 Cline in London performed a laminectomy (Guttmann, 1973).

1841 Reybard in France designed a self-retaining catheter, the prototype of the Foley catheter (Murphy, 1972).

1851 Simon in London performed the first urinary diversion operation from ureter to rectum (Murphy, 1972).

1895 Roentgen developed X-rays.

1910 Ehrlich described the first modern chemotherapeutic, salvarsan; and screening techniques to detect natural and synthetic antibacterials (Fleming, 1946).
In 1943 Riddoch with the British Medical Research Council advocated giving spinal cord sufferers a comprehensive treatment commencing within hours of injury, and combining clinical procedures with social and occupational rehabilitation. With this concept in mind, Guttmann opened a Spinal Injuries Centre at Stoke Mandeville, England in 1944 and was able for the first time in history, to discharge patients able to resume a social and economic life of personal and community usefulness. Legislation was passed the same year in England to provide training for disabled persons and compelling employers to give work to a quota of persons handicapped by disablement (Disabled Persons Employment Act, 1944 — Halsbury, 1962).

The goal of comprehensive management of patients from the start and throughout all stages is slowly being achieved. The United States of America in 1971 set up the first civilian comprehensive centre, although the war veterans had been under the care of Bors and Comarr at Long Beach since the end of the Second World War. Guttmann has visited 38 countries to give advice to Spinal Centres. In Australia, centres and associated residential hostels were established at Perth in 1954 and at Melbourne in 1956, while Adelaide, Sydney and Brisbane opened centres later. All the Australian centres treat civilians (irrespective of income) and all ex-servicemen; and admit patients within hours of injury, in contrast to many other countries.

Guttmann (1967) initiated competitive sports for paraplegics with the Stoke Mandeville Games of 1948. Basketball had been introduced by Bors and Comarr at Long Beach the year before. During the Melbourne
Olympic Games (1956) the Fearnley Cup for achievement in the service of the Olympic idea was awarded to the Stoke Mandeville Games. Bedbrook, the Director of the Perth Spinal Injuries Centre instituted the first British Commonwealth Paraplegic Games in Perth in 1962.

Guttmann (1973) has described the Perth and Melbourne centres as outstanding among institutions for comprehensive care; nevertheless Australia has been less progressive in other fields. For example, legislation has not been passed directing employers to offer work to disabled persons. Legislation or Ministerial directives have been promulgated in some Australian States, but not all, that public building designs comply with the Australian Standard CA 52 (Design for Access by Handicapped Persons). There is no universal insurance scheme, at present, to compensate all persons accidently injured; although the National Compensation Bill (1975) is presently before the Federal Parliament.

Burke (1971) stated that the average incidence in peacetime of new traumatic spinal lesions in a western community is approximately 20 cases per million population per year. The Spinal Centres in Perth, Melbourne and Adelaide have, to date, enough beds to receive all spinal cord injuries immediately after injury; this situation is not seen in many other countries.

1.2 SPINAL INJURIES UNIT, AUSTIN HOSPITAL

A continuous examination of the patients and the Spinal Injuries Unit at the Austin Hospital, Melbourne, conducted during
the past four years, forms the basis of this study. The Unit receives all civilian and Service spinal injuries from the States of Victoria and Tasmania as well as from the Riverina district of New South Wales, and thus provides a cover for four million people within a radius of 300-400 miles. The patients are transported by road or air ambulance to Melbourne, the majority arriving within eight hours of injury.

The Austin Unit consists of 65 beds in three wards, housed in two separate buildings — in themselves far from modern. The Davies Block was built during 1889-91; Figure 2 shows patients in the main ward (now admission Wards 7 and 8) in December 1891. The Bowen Block was completed in 1898 and the rehabilitation Ward 17 now occupies the ground floor of this block. Cheshire (1968) has described the physical arrangements of the wards. Table 2 gives a summary of the comprehensive care at this Unit and indicates the part each ward plays.

1.3 SPINAL CORD INJURY

A brief explanation will be given of the terms used to describe spinal cord lesions. The anatomical and physiological data have been drawn from Bors (1957), Boyar (1967), Burke and Murray (1973), Guttmann (1973) and Pearman and England (1973).

The spinal column consists of 33 vertebrae, viz. seven cervical, twelve thoracic, five lumbar, five sacral and four coccygeal. In the adult the spinal cord ends opposite the first lumbar vertebra, therefore there is a progressive discrepancy between the spinal cord segments and vertebral body levels; the spinal cord is made up of
FIGURE 2

The main ward of the Davies Block, December, 1891. This ward is now the admission Wards 7 and 8.

(Courtesy Professor E.W. Gault, Historian, Austin Hospital.)
TABLE 2

COMPREHENSIVE CARE OF SPINAL INJURIES

ADMISSION
WARD 7 & 8
1 week

10-12 weeks

REHABILITATION
WARD 17

3-6 months

Discharge

Post hospital follow up

WARD 8 & 17 < complications

Acute intensive care

Acute to Sub—acute

MANAGEMENT OF ASSOCIATED COMPLICATIONS & INJURIES

POSITIONS & TURNS
BLADDER CARE
BOWEL CARE
PSYCHOLOGICAL SUPPORT
PHYSIOTHERAPY
OCcupational therapy

CARE OF ANAESTHETIC SKIN
BLADDER TRAINING
BOWEL TRAINING
PSYCHOLOGICAL ADJUSTMENT
PHYSICAL RE—EDUCATION
LEARNING INDEPENDENCE :
ACTIVITIES OF DAILY LIFE
PREVENTION OF COMPLICATIONS
USE OF APPLIANCES
SOCIAL ADJUSTMENT
VOCATIONAL GUIDANCE

PATIENT HAS TOTAL RESPONSIBILITY FOR OWN CARE

GENERAL ASSESSMENT
UROLOGICAL REVIEW
PRESSURE SORES
URINARY INFECTION
FAECAL IMPACTION
SPASM & CONTRACTURES
FRACTURES
COMPLICATIONS :
MEDICAL & SURGICAL
30 segments, viz. eight cervical, twelve thoracic, five lumbar and five sacral.

Spinal cord injury is described in terms of the level and degree of the neurological lesion. It is usual to describe the functional spinal cord level rather than the bony anatomical level, and to use the terms complete for transection of the spinal cord, and incomplete for partial injury. Paraplegia is the loss of lower limb function resulting from damage to the thoracic, lumbar and sacral cord segments. Tetraplegia (quadriplegia) is the loss of function in all four limbs resulting from damage to cervical segments. In both paraplegia and tetraplegia with complete lesions, there is impairment of autonomic function, including the bladder, whereas patients with incomplete lesions may preserve some neurological function.

Table 3 shows the correlation of skeletal muscles and tendon reflexes with the major spinal cord segments.

The majority of patients at the Austin Hospital Spinal Injuries Unit are traumatic spinal paralytics; Appendix 1 indicates the aetiology of the non-traumatic paralytics; the small group of spina bifida patients from the Queen Victoria Memorial Hospital, Melbourne (Appendix 3) are also non-traumatic paralytics.

1.4 THE NEUROGENIC BLADDER

A brief description of the normal bladder is appropriate to the understanding of the neurogenic bladder.
### TABLE 3

**CORRELATION OF MAJOR SPINAL CORD SEGMENTS WITH SKELETAL MUSCLES AND TENDON REFLEXES**

| Major Spinal Cord Segmental Supply | Skeletal Muscles
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C 1 - 3</td>
<td>Neck muscles</td>
</tr>
<tr>
<td>C 4</td>
<td>Diaphragm, trapezius</td>
</tr>
<tr>
<td>C 5</td>
<td>Deltoid, biceps</td>
</tr>
<tr>
<td>C 6</td>
<td>Extensor carpi radialis</td>
</tr>
<tr>
<td>C 7</td>
<td>Triceps, extensor digitorum</td>
</tr>
<tr>
<td>C 8</td>
<td>Flexor digitorum</td>
</tr>
<tr>
<td>T 1</td>
<td>Hand intrinsics</td>
</tr>
<tr>
<td>T 2 - 12</td>
<td>Intercostals</td>
</tr>
<tr>
<td>T 7 - L 1</td>
<td>Abdominals</td>
</tr>
<tr>
<td>L 2</td>
<td>Ileopsoas, adductors</td>
</tr>
<tr>
<td>L 3</td>
<td>Quadriceps</td>
</tr>
<tr>
<td>L 4</td>
<td>Medial hamstrings, tibialis anterior</td>
</tr>
<tr>
<td>L 5</td>
<td>Lateral hamstrings, tibialis posterior, peroneals</td>
</tr>
<tr>
<td>S 1</td>
<td>Extensor digitorum, extensor hallucis, gastrocnemius and soleus</td>
</tr>
<tr>
<td>S 2</td>
<td>Flexor digitorum, flexor hallucis</td>
</tr>
<tr>
<td>S 2 - 4</td>
<td>Bladder, lower bowel</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tendon Reflexes</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C 5</td>
<td>Biceps</td>
</tr>
<tr>
<td>C 6</td>
<td>Supinator</td>
</tr>
<tr>
<td>C 7</td>
<td>Triceps</td>
</tr>
<tr>
<td>L 3</td>
<td>Quadriceps</td>
</tr>
<tr>
<td>S 1</td>
<td>Gastrocnemius and soleus</td>
</tr>
<tr>
<td>S 2 - 4</td>
<td>Anal and bulbocavernosus reflexes (important in bladder and bowel function).</td>
</tr>
</tbody>
</table>
The bladder and urethra are coated, without interruption, with smooth muscle (detrusor urinae) and so when the detrusor contracts the bladder neck is simultaneously pulled open and the ureters are pulled inferiorly thus assisting in the prevention of vesicoureteric reflux. Normal micturition is entirely secondary to voluntary active contraction of the bladder. Continence is maintained passively by the intrinsic tone of the smooth muscle and fibroelastic tissue of the urethra. In the male there is an external urethral sphincter composed of the striated muscles of the urogenital diaphragm. When a catheter is in place the urine is retained by the elastic tissue of the sphincter.

The spinal reflex centre for micturition resides in the sacral 2-3-4 spinal cord segments. The somatic nerve fibres also leave these segments and play a role in the voluntary interruption of the urinary stream when voiding. Although the sympathetic nerve fibres of the bladder arise from T 11 to L 2 cord segments, their function is not exactly known (Pearman and England, 1973). Sensory nerves from the bladder run with the parasympathetic and sympathetic nerves. The physiological function of micturition in the adult is thus a complex mechanism of interacting cortical and spinal reflex actions.

A neurogenic bladder is one whose function has been modified by interference with its nerve supply. Bors (1957) based his classification of the neurogenic bladder on the nerve pathways involved (sensory, motor or both) and the level and degree of cord damage. Taking into account the time interval after injury, Burke and Murray (1973) suggest four main types of neurogenic bladder.
1. During **spinal shock**: areflexic, flaccid paralysis below the level of spinal cord lesion includes bladder function. There are no reflex contractions of the detrusor, and acute retention with overflow incontinence results.

2. **Upper motor neurone bladder**: When spinal shock passes, reflex activity returns. In a lesion above the conus medullaris (containing the sacral segments of the spinal cord), the spinal micturitional reflex arc is intact, and an automatic (reflex) bladder results. The bladder capacity may be small and with no sensation of fullness, however it will empty involuntarily as it fills and there is good voiding pressure at capacity (300 ml).

3. **Lower motor neurone bladder**: Injuries at or below the conus medullaris produce interruption in the spinal micturitional reflex and an autonomous (flaccid) bladder results. In this situation, voluntary control of micturition is lost as with upper motor neurone, but reflex function is also lost. As bladder volume increases there is a linear increase in pressure until capacity is reached; urine may then flow past the sphincter by overflow incontinence.

4. **Mixed upper and motor neurone bladder**: It is possible with a conus-cauda equina lesion to have a lower motor neurone detrusor of the bladder and an upper motor neurone sphincter. The reverse may also occur. Other patterns of mixed neurogenic bladder may occur reflecting the varying degrees of partial voluntary control overriding reflex functions.
1.5 **UROLOGICAL MANAGEMENT**

The aim of urological management of spinal paralytics is to preserve renal function by preventing chronic pyelonephritis. All measures taken are aimed at producing a balanced bladder where the residual urine is approximately 10% of the voided volume; at preventing the entry of micro-organisms into the urinary tract; and at eradicating any infection if it occurs.

In the initial period of spinal shock, overdistension of the bladder must be avoided because of the risk of permanent damage to smooth muscle and nerve fibres (Bradley *et al.*, 1963, 1967 and Lloyd-Davies, 1970). The methods used to empty the bladder are:

1. Suprapubic cystostomy, which has been discontinued at most centres, although still used at Craig Rehabilitation Hospital, Denver, U.S.A. (Jones, 1974).

2. Manual compression was advocated by Golding (1968) but most workers prefer to avoid the dangers of overdistension inherent in the technique (Pearman and England, 1973).

3. It is with the techniques of intermittent and continuous urethral catheterisation that the most recent reports are concerned. Pearman and England (1973) from the Royal Perth Hospital Spinal Unit have prepared a detailed monograph describing their program and success with intermittent
catheterisation. Burke and Murray (1973) have also produced a handbook setting out the present method of continuous catheterisation used at the Austin Hospital Spinal Injuries Unit.

In an attempt to prevent the entry of bacteria into the urinary tract most urologists working with paralytics favour the disinfection of the urethra and lubrication of the catheter prior to catheterisation. Disinfectants recommended include savlon and chlorhexidine (Guttmann and Frankel 1966); benzalkonium chloride and polymyxin-B (Linden, 1969) and chlorhexidine (Pearman and England, 1973; Burke and Murray, 1973).

Most workers advocate the instillation of disinfectants for a short period to clear the catheter and to irrigate the bladder, particularly in cases of indwelling urethral catheters. Guttmann (1973) recommends 0.5% acetic acid, 4% boric acid, benzalkonium chloride 1:1,000, potassium permanganate 1:2,000 or 2.4% phenoxetol. Chlorhexidine 1:5,000 has been used twice daily at the Austin Hospital Spinal Unit until recently. Permanent instillation into the bladder at each intermittent catheterisation, of 25 ml of sterile water containing 150 mg kanamycin and 30 mg colistin has been described by Pearman and England (1973) in 36 patients. They report 25 episodes of significant bacteriuria with a variety of micro-organisms, 23 of the isolations were resistant to kanamycin and colistin whereas the other two were resistant to colistin alone.

Antibacterial agents taken orally to acidify the urine and liberate formaldehyde in acid urine, were first described by
Nicolaier (1894). The current use of a combination of methionine and hexamine-mandelate (Guttmann, 1973), ammonium chloride and methenamine mandelate (Pearman and England, 1973) and ascorbic acid and methenamine mandelate (Burke and Murray, 1973) are examples of this daily therapy still in vogue. The chemotherapeutic management of paralytics varies. Units such as the Austin Spinal Unit avoid the routine or prophylactic use of antibiotics in a patient on catheter drainage, unless there are systemic signs of ascending urinary infection. However infections in patients without catheters, are treated to produce bacteria-free urine; the choice of antibiotic being made after sensitivity tests. Guttmann (1973) strongly advocates streptomycin even with unfavourable sensitivity tests on isolated organisms. He states that the therapeutic effect of this antibiotic on the patient's clinical condition is unquestionable. The rationale of treatment by antibiotics is set out by Pearman and England (1973) for patients at the Perth Spinal Unit. It involves a knowledge of the site of infection and quantitative antibiotic sensitivity of the infecting organism, followed by treatment and subsequent prophylactic administration if deemed necessary.

A number of diagnostic tests on the spinal paralytic after the period of bladder shock, allow a bladder profile to be made. These tests provide the basis for treatment and are regularly performed at the Austin Hospital Spinal Injuries Unit.
The tests are:

Response to the neurologic tests of the perineal somatic nervous supply and autonomic supply of the detrusor can usually classify the neurogenic bladder.

Cystometrogram indicates type and capacity of bladder, but is useful as a guide to therapy.

Renal function is monitored regularly by intravenous pyelogram and blood urea and creatinine estimations.

Voiding cystourethrogram and retrograde urethrogram indicate abnormal structures, vesicoureteric reflux and bladder capacity.

Cystoscopic examination is used to inspect the bladder mucosa, outlet area, stone identification and removal.

Routine culture of urine to detect bacteria, examination of urine for cells, casts and crystals.

Measurement of residual urine.

Twenty per cent of Austin patients (Burke, private communication) retain an indwelling catheter in the rehabilitation stage. It was the males with indwelling catheters who were selected for prolonged study during the course of these experiments.
SECTION 2

MATERIALS AND METHODS
2.1 PATIENTS

Studies were performed on two groups of patients other than the spinal patients. The three groups are defined below:

2.1.1 SPINAL INJURED PATIENTS

The patients from the Spinal Injuries Unit, Austin Hospital, studied during 1970-74 were males with indwelling catheters. Appendix 1 identifies these patients by the Hospital serial number and gives the date of onset of illness which is also the commencement of catheterisation. Only those patients with indwelling catheters of at least four months' duration were selected for L-form isolation studies (see Figure 3). They consisted of 16 outpatients, who returned to the rehabilitation Ward 17 for catheter changes or were re-admitted at anytime because of complications; in addition 23 patients living in Ward 17, who had progressed from the admission Ward 7 were examined for L-forms.

Fifteen patients, admitted to Ward 7 usually within eight hours of injury, had "virgin bladders" with reference to urinary infection. Urine, faeces and nasal swabs were examined weekly from this group to determine whether their initial urinary infection was of endogenous origin. During the period of study, all patients admitted to Ward 7 who retained catheters for at least two months were examined to determine the possible influence on acquisition of infection by different commercial drainage bags, daily bladder irrigation and the use of a urinary disinfectant.

2.1.2 PATIENTS FROM GENERAL PRACTITIONERS AND UROLOGISTS

The validity of the L-form isolation methods used, was tested by examining during 1970-72 the urines of a group of 39 patients who
FIGURE 3

A patient in the admission Ward 7. The indwelling catheter is connected to the closed drainage system.
were being treated with antibiotics for urinary tract infection. It could be reasonably predicted that L-forms would be present in this group. Twenty-six of these patients were managed at home by a General Practitioner and 13 were managed in hospital by Urologists. Appendix 2 lists these patients who are identified by their initials.

2.1.3 SPINA BIFIDA PATIENTS

A group of seven spina bifida patients who had ileal conduits, and were outpatients at the Queen Victoria Memorial Hospital, Melbourne was examined in 1974-75 to test the conclusion drawn from the spinal patients, that a low grade infection with the same organism usually persists in the neurogenic bladder, without clinical signs of urinary infection. Appendix 3 identifies these patients by the Queen Victoria Memorial Hospital serial number.

2.2 COLLECTION OF SPECIMENS

2.2.1 URINE

Catheter specimens from the patients in the Spinal Injuries Unit were collected in the following manner: After a fresh catheter was passed and connected to the drainage tubing, a clamp was applied to the upper end of the drainage tubing for 10-15 minutes. The area of the catheter below the junction of the inflator tube and the catheter was then disinfected with "Cetal spray" (1% chlorhexidine in alcohol, Grapharm Products, Aust.), and from this site 10 ml of urine was withdrawn by needle and syringe. Half of the urine sample was added to 5 ml of 20% sucrose solution and held at room temperature for no longer than one hour, prior to examination for L-forms. The remaining 5 ml of urine was added to a sterile container, refrigerated and after examination within one hour for casts and cells, was cultured quantitatively.
Separate catheter specimens of urine were obtained in the same manner for estimation of osmolality, acidity and formaldehyde concentration. Urine was also collected from the drainage bags. This was refrigerated and examined within one hour for classical bacteria.

Midstream urine was collected from ambulant patients in group 2.1.2 above who presented to the General Practitioner. The Urologists sent midstream and catheter specimens. Disinfectants were not used to wash the meatus prior to collection. Each sample was divided into two portions as above, sent to the laboratory without delay, and examined in the same way. Ureteric urine was collected from three patients. In this instance an osmotic stabilizer was not added, but the urine was processed within 10 minutes of collection. From one of these patients, portion of a kidney received 10 minutes after resection, was homogenized in an equal volume of 20% sucrose solution and examined for L-forms and classical bacteria.

The urine from the spina bifida patients in group 2.1.3 was collected after a catheter was passed through the ileal conduit. Distilled water was used to swab the stoma before collection.

2.2.2 Faeces

Faecal samples from the spinal patients for endogenous flora identification, collected on a plain cotton wool swab inserted into the rectum, were inoculated onto horse blood agar and MacConkey agar within one hour of collection.
2.2.3 NASAL FLORA

Nasal flora was sampled by inserting a plain cotton wool swab into each nostril from the vestibule to a point just below the middle turbinate, and rotating it in situ. The specimens were sown onto horse blood agar and MacConkey agar within one hour of collection.

2.2.4 WARD FLORA AND DISINFECTANTS

Plain swabs, moistened with sterile saline, were used to sample the bacterial flora of floors, walls, beds, tables, bathrooms, wheelchairs, physiotherapy equipment, urine disposal rooms, preparation rooms, nurses' hands and gowns, urinary appliances, and measuring jugs just prior to use. The specimens were inoculated onto horse blood agar and MacConkey agar immediately after sampling.

Samples of chlorhexidine (Hibitane, I.C.I., diluted 1 in 5,000 with distilled water) prepared in Ward 17 for bladder irrigation were filtered to test for sterility; the residual filtrate was used to assess bactericidal activity against organisms isolated from the urine of patients in Ward 17 including as controls Staphylococcus aureus N.C.T.C. 4163 and Pseudomonas aeruginosa N.C.T.C. 6749. Samples of "Saf-Sol" (mixed halogen) and "Wescodyne" (polyethoxyethanol-iodine), were removed from the plastic containers held in Ward 17, for disinfecting urinary appliances and measuring jugs. These solutions were similarly assessed for bactericidal activity.
2.3 MEDIA

The chemicals used in media preparation were of high analytical grade and unless indicated, were manufactured by British Drug Houses, Ltd., Poole, England. The following abbreviations are used for manufacturers of dehydrated media:

- Albimi: Albimi Laboratories, Inc., Brooklyn, U.S.A.
- BBL: Baltimore Biological Laboratory, Balt., U.S.A.
- Difco: Difco Laboratories, Michigan, U.S.A.
- Oxoid: Oxoid Ltd., England

Blood and sera were supplied by the Commonwealth Serum Laboratories, Parkville, Australia (C.S.L.).

2.3.1 ISOLATION AND IDENTIFICATION MEDIA FOR BACTERIA

Classical bacteria as opposed to L-forms, were isolated on horse blood agar, containing defibrinated horse blood in nutrient broth (Oxoid). MacConkey agar No. 2 (Oxoid) which permits the growth of enterococci was also used. Proteus did not spread on this medium.

Media for identification were prepared according to the formulae of the authorities quoted in paragraph 2.4 "Identification of Organisms", page 34.

2.3.2 GLYCEROL BROTH

Colonies of classical bacterial isolates were stored in this broth and held at \(-20^\circ\text{C}\).

Glycerol 20% in distilled water

Tryptose (Difco) 1.5% (w/v)
2.3.3 PRIMARY ISOLATION L-FORM MEDIUM

Freake (1970, personal communication) devised a broth to provide a biochemical environment similar to normal plasma; his broth had a higher osmolality (340 mOsm/kg) than plasma and a lower glycine level. Klieneberger-Nobel (1965) states glycine is inhibitory to cell wall synthesis.

The isolation L-form medium used in this study is basically that of Freake, except the osmolality is raised further to 650 mOsm/kg.

Yeast extract (Oxoid) 2g
Peptone (BBL) 8g
Lab. lanco (Oxoid) 0.5g
NaCl 5g
MgCl₂ 0.16g
NaHCO₃ 3g
Distilled water to 1 litre

Prior to use, 15% foetal calf serum, glucose 50 mg% and urea 20 mg% were added.

This medium was prepared as broth, biphasic medium and solid medium; agar (Difco) if required, being added at a concentration of 1.2%.

2.3.4 SECONDARY ISOLATION L-FORM MEDIA

Sucrose L-form medium

The sucrose broth of Lederberg and St. Clair (1958) was
modified; solid, biphasic and liquid media being made from the following formula:

Nutrient broth No. 2 (Oxoid) 25g
Glucose 1g
Sucrose 100g
Distilled water 1 litre

After autoclaving, 10ml of 20% MgSO_4\cdot7H_2O solution was added.

Osmolality was 690 mOsm/kg.

Agar (Difco) if required, was added at a concentration of 1.2%.

Casamino acids L-form medium

Conner et al. (1968) described a medium, which was used without modification.

Sucrose 100g
K_2HPO_4 16g
KH_2PO_4 2g
Sodium citrate 1g
MgSO_4\cdot7H_2O 0.2g
Nicotinic acid 35mg
Vitamin-free casamino acids (Difco) 10g
Distilled water to 1 litre

Prior to use 10% (v/v) horse serum was added, plus agar (Difco) 1.5% if required.
**Brucella medium**

**Brucella broth (Albimi)**
56g

Distilled water to
1 litre

After autoclaving, 20% sucrose (v/v) was added, and for solid medium 1.75% Noble agar (Difco).

**Brain heart L-form medium No. 1**

Brain heart infusion (Difco)
37g

Sucrose
100g

Yeast extract (Oxoid)
5g

Agar No. 3 (Oxoid)
12g

Distilled water to
1 litre

Prior to use 10% (v/v) inactivated sterile horse serum was added.

**Brain heart L-form medium No. 2**

Brain heart infusion (Difco)
37g

Sucrose
270g

MgCl₂
2g

Agar (Difco)
12g

Distilled water to
1 litre

Prior to use 10% (v/v) sterile horse serum was added.

**Proteus synthetic medium**

Medill and O'Kane (1954) demonstrated a synthetic medium which
supports the growth of L-form colonies of *Proteus*. This medium was used when patients with persistent *Proteus* infection were examined. The medium consists of three parts.

(a) Basal synthetic medium:

- **Glucose** 1.0%
- **Sodium lactate** 2.0%
- **K₂HPO₄** 0.9%
- **KH₂PO₄** 0.1%
- **Nicotinamide** 1 μg/ml
- **Salt mixture** 0.2%

The salt mixture contained per litre:

- **MgSO₄·7H₂O** 40.0g
- **NaCl** 2.0g
- **FeSO₄·7H₂O** 2.0g
- **MnSO₄·4H₂O** 8.0g

Glucose was autoclaved separately in 10% solution.

(b) The following amino acid mixture which was added to the basal synthetic medium, is given in mg per 10ml:

- **Glycine** 0.2
- **L-Tyrosine** 3.0
- **L-Histidine** 1.0
- **L-Alanine** 1.0
- **DL-Valine** 4.0
- **DL-Phenylalanine** 2.0
- **DL-Lysine** 4.0
- **DL-Valine** 2.0
(c) An autoclaved solution of 2.2% agar (Difco) in distilled water was added to an equal volume of the basal plus amino acid mixture, giving a final concentration of 1.1% agar.

2.3.5 PENNASSAY BROTH

This broth was used to grow bacteria prior to antibiotic treatment for production of spheroplasts.

Antibiotic medium No. 3 (Oxoid) 1.75%

2.3.6 CLASSICAL MYCOPLASMA MEDIUM

This medium formulated by Cottew (1971, private communication) was used to screen urine samples for classical mycoplasmas.

Base:

PPLO (Difco) 3.5%
Albimi yeast autolysate (Difco) 0.6%
Adjusted before autoclaving to pH 6.5.

An alternative base used was:

Albimi brucella medium (Albimi)
Adjusted before autoclaving to pH 6.5.

Additives to autoclaved base:

Yeast extract (Herderschee, 1963) 0.5%
Penicillin G (C.S.L.) 100 units/ml
Thallous acetate 0.25%
Inactivated horse serum 20%
D.N.A. 20 μg/ml
Actidione (Upjohn Ltd.) 0.02%
### 2.3.7 Mycoplasma T-Strain Medium

A medium described by Shepard (1969) for the primary isolation of T-strains from clinical material was used.

**Base:**
- Trypticase soy broth (BBL) \(30g\)
- Distilled de-ionized water \(1\) litre
- Adjusted to pH 5.5
- Ionagar No. 2 (Oxoid) \(11.33g\)

**Additives:**
- Horse serum \(20\%
- Penicillin G (C.S.L.) 200 units/ml
- Adjusted to pH 6.0

### 2.3.8 Antibacterial Activity Medium

This spore medium was used routinely in 1973-5 to check antibacterial activity in urine.

**Diagnostic sensitivity test agar (Oxoid) 4%**

**Bacillus subtilis** A.T.C.C. 6633 spore suspension 0.1%

The spore suspension was prepared by harvesting a 4-6 day culture grown at 26°C on Diagnostic sensitivity test agar in distilled water, heating at 80°C for 10 min and washing three times in distilled water. The suspension was stored at 4°C.
2.3.9 SENSITIVITY TEST MEDIA

Isolations were screened by the multi-disc method, using Diagnostic sensitivity test agar (Oxoid) containing 5% lysed horse blood.

Multodisk U4 (Oxoid) was used and contained in μg per ml:

- Gentamicin: 10
- Cephaloridine: 25
- Colistin sulphate: 10
- Sulphafurazole: 500
- Ampicillin: 25
- Carbenicillin: 100
- Sulfamethoxazole/Trimethoprim: 25
- Tetracycline: 50

The Minimal Inhibitory Concentration values were estimated on all isolations from the Spinal Unit patients, using solid media.

The following antibacterials (concentrations in μg per ml) were incorporated into MacConkey agar No. 2 (Oxoid):

- Ampicillin: 20, 50, 100, 200, 300
- Carbenicillin: 25, 50, 100
- Cephalothin: 50, 100, 250
- Chloramphenicol: 10, 25, 50, 100, 200
- Gentamicin: 4, 8, 16
- Kanamycin: 12.5, 25, 50, 100, 200, 300
- Nalidixic acid: 12.5, 25, 50, 100, 200, 300
Neomycin 12.5, 25, 50, 100, 200, 300
Nitrofurantoin 50, 100, 200
Rifamycin 25, 50, 100
Streptomycin 10, 25, 50, 100, 200, 300
Tetracycline 25, 50, 75, 100, 200, 300

The following antibacterials (concentrations in µg/ml)
were incorporated into a minimal medium of Peptone (Difco) 0.5% in saline with agar (Difco) 1.5%:

Formaldehyde 10, 50, 100, 125
Sulphathiazole 50, 100, 150, 200
Trimethoprim 50, 100, 150, 200

The antibacterials used were obtained from the following sources, and except where indicated, the solutions were made up aseptically in distilled water and stored at -15°C.

Ampicillin: Crystalline sodium ampicillin from C.S.L.
Carbenicillin: Pyopen (disodium salt) from Beecham (Australia) Pty. Ltd.
Cephalothin: Keflin (sodium cephalothin) from Eli Lilly (Aust. & Co.).
Chloramphenicol: "Chloromycetin Recrystallized" from Parke-Davis, dissolved in 70% ethanol.
Formaldehyde: Formalin: 37.5% formaldehyde solution.
Gentamicin: Garamycin (gentamicin sulphate) from Schering Corporation, U.S.A.
Kanamycin: Kanasig (kanamycin sulphate) from Sigma Co. Ltd.
Nalidixic Acid: Pure salt from Sterling-Winthrop Laboratories, was dissolved in hot 0.125M NaOH solution.
Neomycin: Mycifradin (neomycin sulphate) from Upjohn Ltd.
Nitrofurantoin: Furadantin (sodium nitrofurantoin) from Smith Kline and French Lab. (Aust.) Ltd.
Rifamycin: from Lepetit Labs.
Streptomycin: Streptomycin sulphate from Glaxo-Allenburys.
Sulphathiazole: Pure salt from Roche Products Pty. Ltd. was dissolved in 0.05M NaOH at 60°C.
Tetracycline: Tetracycline hydrochloride powder from E.R. Squibb and Sons.
Trimethoprim: Pure salt from Roche Products Pty. Ltd.

2.4 IDENTIFICATION OF ORGANISMS

Bacteria were identified as fully as the study required. The systematic tables recommended by Cowan and Steel (1970) were used for staphylococci, streptococci and Gram-negative bacilli. Reference was made to Pickett and Pedersen (1970) and to Gilardi (1971) for characterization of the non-fermentative bacilli. Candida specimens were identified with reference to Ajello et al. (1966) and mycoplasmas with reference to Shepard (1969). All other species were identified according to criteria set out in Bergey's Manual (Breed, Murray and Smith, 1957).
All isolates of *Proteus mirabilis* and *Proteus vulgaris* were classified according to the Dienes phenomenon (Story, 1954), whereas all isolates of *Pseudomonas aeruginosa* were pyocine typed by the method of Gillies and Govan (1966) together with the modifications of Tagg and Mushin (1971), Jones *et al.* (1973) and Lovrekovich *et al.* (1972). Serological typing of strains of *Escherichia coli* (Turck *et al.*, 1969) was carried out, and confirmed by the Reference Laboratory, Institute of Medical and Veterinary Science, Adelaide, South Australia.

### 2.5 EQUIPMENT

#### 2.5.1 MICROSCOPY

Plates were examined for colonies of L—forms or mycoplasmas with an Olympus Stereoscopic Zoom Microscope Model (SZ-111, Olympus Optical Co., Ltd., Tokyo, Japan). By using objective 1X and eyepieces 10X and 20X, magnifications up to 80X were obtained and the working distance was 88 mm. Two side illuminators facilitated the examination of the surface of opaque media.

Urine and liquid media were examined under phase contrast illumination for L—forms. For this purpose Leitz phase contrast equipment using the annular stop turret No. 2 on the condenser and objective Phaco 40/0.65 gave a free working distance of 0.71 mm. This arrangement did not allow the use of a haemocytometer for quantitative estimations, so wet mounts were examined under phase contrast microscopy and quantitative estimations of casts and cells were made with a Neubauer haemocytometer, using bright light.
2.5.2 **OPTICAL DENSITY**

A Bausch and Lomb Spectrophotometer, model Spectronic 20, was used to determine the optical density of,

(a) bacterial suspensions, using wavelength 660 nm and
(b) urine containing formaldehyde, using wavelength 415 nm.

The optical density was recorded as absorbance.

2.5.3 **OSMOLALITY**

The osmolality of the patients' urine and of the L-media expressed in mOsm/kg, was measured by the freezing point depression method, using the Osmometer model Halbrimko made by Knauer and Co., Berlin.

2.5.4 **pH MEASUREMENT**

The pH of media was measured with a pH meter Model E 520 by Metrohm (Switz.).

The pH of urine was measured using the same pH meter and in addition the indicator papers:

Neutralit pH 5-10 and Spezialindikator pH 4-7 made by E. Merck, Darmstadt, Germany.

Multistix (Ames Co., Miles Lab., Inc., Australia) which is based on methyl red and bromphenol blue indicators. The nursing staff tested the pH of patients' urine in the Spinal Unit using Multistix indicator paper.

The pH of rat urine was measured with the Spezialindikator paper.
2.5.5 ULTRA-VIOLET LIGHT IRRADIATION

Ultra-violet irradiation was used to sterilize pyocine typing plates of *Pseudomonas aeruginosa* (Lovrekovich *et al.*, 1972) and for the differentiation of pseudocolonies from L-colonies on solid media.

The UV lamp used was a Blak-Ray Model UVL-22 (Ultra-violet Products Inc.) at wave length 2537 Å and at an intensity of 8 watts per sq. ft. if held at a perpendicular distance of six in. from the plate.

2.5.6 INCUBATION OF CULTURES

Tosca (Thomas Optical and Scientific Co. Pty. Ltd., Australia) incubators were used for incubation. Plates were inserted into plastic bags when prolonged incubation was required. Increased aeration, by means of a Baird and Tatlock Ltd., (England) shaker attachment in a 37°C water bath enabled the comparison of the optical density of various organisms to be completed within six hours. A flask shaker (Griffin and George Ltd.) situated in an incubator room was also used to increase aeration.

2.6 ISOLATION OF L-FORMS FROM URINE

Table 4 shows the method of processing urine for L-form isolation and the simultaneous estimation of casts, cells and bacteria. This flow sheet combines the method of direct isolation of L-forms on to solid media with the indirect method of producing reverting parental forms on horseblood agar and MacConkey agar.
TABLE 4
L-FORM ISOLATION FROM URINE

DAY 1

10 ml urine

Add 5 ml to
5 ml 20% sucrose

5 ml in dry container
Microscopy
Culture: count and identification

filter (0.45μ membrane)

filtrate

Membrane
replicate upper surface to HBA and MacC for identification

duplicate

<table>
<thead>
<tr>
<th>1 ml</th>
<th>0.5 ml</th>
<th>0.1 ml</th>
<th>1 drop</th>
<th>1 drop</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-broth</td>
<td>L-biphasic</td>
<td>L-solid</td>
<td>HBA</td>
<td>MacC</td>
</tr>
</tbody>
</table>

DAILY PROCEDURES FROM DAY 2 to DAY 7:

Examine cultures for growth, macroscopically and with stereoscopic microscope. If broth is turbid, examine wet preparation under phase contrast and examine Gram stain.

Subculture all broths whether turbid or not to:

HBA  MacC  L-solid  L-broth

Set up drug sensitivity tests when growth is detected.
The equal quantity of 20% sucrose solution used provided osmotic stability for the urine specimens. All cultures were incubated aerobically at 37°C except the biphasic and solid L-media which were incubated under CO₂. The L-cultures were incubated for one month before being discarded. The 0.45μ filter membrane enabled classical bacteria to be retained on the upper surface of the membrane, but allowed L-forms to pass through with the filtrate. The inoculation of the final filtrate on horse blood agar and MacConkey agar provided a check for possible rupture of the membrane since rupture would invalidate the process. Sensitivity tests were set up at each sub-culture so that a comparison could be made of any changing pattern during reversion of the L-forms to the parent bacteria.

Irradiation of half the L-solid media with ultra-violet light (2537 Å) for 5 min served as a control for pseudocolony formation, because UV light allows pseudocolony formation but prevents the growth of L-colonies, mycoplasmas and classical bacteria.

2.7 INVESTIGATION OF URINE FOR BACTERIA, CASTS AND CELLS

A semi-quantitative technique was used to count bacteria by transferring standard loopfuls (0.02 ml) of uncentrifuged urine on to horse blood agar and MacConkey agar. After aerobic incubation at 37°C for 24-48 hours, counts were made and expressed as organisms per millilitre. Organisms were identified to species level, and then typed where possible. After concentrating the urine ten times by centrifugation, quantitative estimations were made of casts and cells, using a Neubauer haemocytometer.
2.8 ESTIMATION OF DRUG SENSITIVITY

2.8.1 DISC DIFFUSION TECHNIQUE

The Disc Diffusion Technique was used to screen isolates for drug resistance by preparing lawn cultures in the logarithmic phase of growth (3 hours incubation in nutrient broth) on 5% lysed horse blood medium. The plates were well dried before multidiscs were placed on the surface and sensitivity or resistance was recorded after incubation at 37°C for 18 hours.

2.8.2 MINIMAL INHIBITORY CONCENTRATION

MIC values for the drugs tested were determined by the agar plate dilution method, which involves spotting a light suspension (100,000 organisms/ml) diluted in phosphate buffer after three hours' incubation, as well as a heavier suspension (approximately 1 x 10^6 organisms/ml). Using an aluminium handle with 38 prongs, 19 strains could be replicated at one time. Plates were incubated overnight and all negative tests were reincubated for a further 24 hours. Resistance was taken as the level at which single colonies of the lower dilution showed good growth. The method set out by Tolhurst et al. (1972) was followed.

2.9 DETERMINATION OF FORMALDEHYDE IN URINE

2.9.1 LABORATORY METHOD

Each spinal patient receives a urinary acidifier and disinfectant, commencing several weeks after admission and the present policy is to continue this indefinitely, after discharge. Methenamine mandelate (William R. Warner and Co.) and methenamine hippurate (Riker Laboratories), the disinfectants used at the Austin Unit, are both hydrolysed in acid urine to the broad spectrum formaldehyde. The
maximum amount of formaldehyde (HCHO) released in urine was shown by Jackson and Stamey (1971) to be dependent on the quantity of methenamine; the volume, composition and pH of urine; and a period of time between 3 to 6 hours. The reaction was then slowed by the release of ammonia.

\[ N_4(CH_2)_6 + 6H_2O \rightarrow 4NH_3 + 6HCHO \]

Under the usual urinary acidity, only 2 to 10% of methenamine in urine was converted to HCHO. Methods to determine HCHO are sensitive to less than 1 µg/ml but are not applicable in the presence of methenamine when the methods themselves can convert up to 30% of the residual methenamine to HCHO. Theoretically, 1,400 µg/ml of methenamine could yield 1,800 µg/ml of HCHO. To achieve a bactericidal level of 50 µg/ml of HCHO in the urine, a concentration of at least 1,200 µg/ml of HCHO after acid hydrolysis is required.

Formaldehyde was estimated by the Nash reaction. It is simple, fast and accurate (Cochin and Axelrod, 1959; La Du et al., 1971). The Nash reagent consists of:

- Ammonium acetate 30 g
- Acetyl acetone 0.4 g
- Distilled water to 100 ml

A standard HCHO curve was prepared using 0.5, 1, 2 and 4 µg/ml concentrations. 5ml quantities of each formaldehyde concentration were mixed with 2 ml quantities of the Nash reagent and heated in a water bath at 60°C for 30 minutes. The absorbance was determined in a colorimeter at 415 nm.
The free HCHO in the patients' urine was determined immediately after sampling. First, the residual methenamine was precipitated out with mercuric chloride (2 g of HgCl₂ added to 10 ml of urine, diluted 1 in 10), and after centrifugation the estimation was carried out as above.

The total HCHO, which is the free and bound HCHO, was later determined in the urine of patients and of rats after hydrolysis of the residual methenamine with normal H₂SO₄.

2.9.2 METHOD OF MONITORING TOTAL FORMALDEHYDE IN URINE

Since patients at home and nurses in hospital wards do not have access to a colorimeter, a simple method was devised to measure the total HCHO in urine after acid hydrolysis. This measurement relates the total HCHO to the methenamine in urine and this in turn relates to the amount of free HCHO which exerts the bacteriostatic or bactericidal activity. A few drops of normal H₂SO₄ are added to the urine before commencement of the test. Its complexity can be equated with the urine sugar test used daily by diabetics (Clinitest by the Ames Company). Figure 20 illustrates this method which is based on the Nash reaction, and where the colour developed is matched on a colour chart.
2.10 ANTIBACTERIAL ACTIVITY IN URINE

A high quality 6mm filter paper disc (Schleicher and Schuell, U.S.A.) was dipped into well-mixed, uncentrifuged urine and placed on the surface of the antibacterial activity medium. Using 14cm diameter petri dishes (Camelec), 20 urines could be screened on each petri dish. After an overnight incubation a zone of inhibition developed if antibacterial activity was present due to antibiotic therapy, barbiturates, salicylates, high bilirubin or disinfectant-contaminated urine. The zone of inhibition was not measured, but the presence of antibacterial activity was noted for assessment of the quantitative count from urine, (Figure 21).

2.11 ANTIBACTERIAL ACTIVITY OF DISINFECTANTS

2.11.1 BACTERICIDAL

Disinfectants used in ward procedures were evaluated by a surface disinfection method. The organisms used were the standard Staphylococcus aureus NCTC 4163 and Pseudomonas aeruginosa NCTC 6749, as well as species isolated from the urine of patients viz. Acinetobacter sp., Escherichia coli, Klebsiella aerogenes, Proteus mirabilis, Pseudomonas aeruginosa and Serratia sp.

Glass slides 1.3 by 3.5cm carried approximately 5 x 10^6 organisms mixed with horse blood, spread on a 5mm square area. Methods were standardised for preparing the inoculum on carrier slides, and for washing in tap water and an inactivator. Controls were included to check for loss of organisms from slides during washing, for viability on carriers and for carry-over of disinfectant. Lack of growth in liquid medium was verified by subculture to solid media. The inactivators recommended by Rubbo and Gardner (1967) were used.
2.11.2 BACTERIOSTATIC

The bacteriostatic effect of the urinary disinfectant methenamine mandelate and acidifier ascorbic acid on bacterial isolates from the spinal patients' urine was investigated. Standard inocula were added to varying concentrations of methenamine mandelate, ascorbic acid and combinations of the two, using as diluent buffered nutrient broth, as well as filtered, pooled, normal urine. Incubation in a water bath with shaker attachment enabled growth (absorbance) to be measured at hourly intervals until completion of six hours' incubation.

2.12 INDUCTION OF L-FORMS

L-forms were induced in liquid culture by the method of Lederberg and St. Clair (1958) from species of bacteria isolated from the urine of the spinal patients. One drop of an overnight culture in Pennassay broth was inoculated into 10 ml of sucrose L-form broth containing 1000 μg/ml of penicillin.

To test the hypothesis that urine containing methenamine mandelate and with a high hydrogen-ion concentration and low osmolality, will adversely affect the induction of L-forms, comparative observations were made in sucrose L-form broth and in sucrose L-form broth with additives, to simulate the spinal patient urine. Penicillin induction was attempted from Escherichia coli, Klebsiella aerogenes, Proteus mirabilis and Pseudomonas aeruginosa, using broths at pH 5, 6 and 6.5. Ascorbic acid (0.5 mg/ml) and methenamine mandelate (0.1 mg/ml) were added to the three broths of varying pH. In all the test broths the osmolality had been reduced to 450 mOsm/kg. The cultures were incubated for 48 hours and examined at frequent intervals under phase contrast microscopy.
2.13 ESTABLISHMENT OF URINARY INFECTION IN RATS

An in vivo experiment was conducted to test the hypothesis that acidification of urine with ascorbic acid and the use of a urinary disinfectant, methenamine hippurate, producing free and bound formaldehyde in urine at concentration between 500-900 µg/ml, prevented the establishment of urinary tract infection.

2.13.1 RATS

All rats were randomly bred in the laboratory from a Sprague-Dawley strain. Male rats of the same age (10 weeks) and approximately the same weight (180-200g) were used. The feeding, positioning of water bottles and handling of test and control rats were identical and they were housed in plastic cages. The pH of urine from these rats ranged from 7.5 to 8.

2.13.2 INGESTION

Each rat consumed 4 to 5 pellets of Barastoc Mouse Breeder Ration (KMM Pty. Ltd., Melbourne) daily. There was free access to water bottles of 150 ml capacity and these were changed every second day. Each rat in the control group drank approximately 18 ml of water per day throughout the whole experiment. For three weeks after the bacterial challenge and during the next seven days before death, each rat in the test group also drank approximately 18 ml of water per day, with additives of ascorbic acid 1.5 mg/ml and methenamine hippurate 4 mg/ml. This concentration of additives was chosen as it produced urine similar to that of a group of spinal injured patients, viz. pH 6 to 6.5 and free and bound formaldehyde level of 500-900 µg/ml.
2.13.3 EXCRETION

Urine was collected after natural voiding. Each rat was placed in a plastic cup three inches in diameter by four inches in depth, with plastic wire netting held one inch above the base to separate the animal from voided urine. Urine was also collected at post mortem by clamping the penis before abdominal wall incision; after exposing the bladder, urine was aspirated by needle and syringe for culture and estimation of pH and formaldehyde.

2.13.4 URINARY TRACT INFECTION

Preliminary experiments established the optimal infective dose, route of infection and criteria of establishing urinary tract infection in rats, using bacterial species isolated from the patients. Control and test rats were challenged by intravenous injection of $10^6$ organisms contained in 0.5 ml of Ringer's solution (1.5 x $10^6$ in 0.75 ml for Proteus) prepared from overnight incubation in nutrient broth. The inoculum size was always confirmed by surface plate counts (Miles and Misra, 1938). Urine obtained after natural voiding was cultured daily for recovery of the organisms. A healthy rat excreting the inoculated organisms seven days after challenge was deemed to be infected, and the control and test rats were sacrificed at this time.

Each rat was killed in a chloroform jar and the abdominal wall opened aseptically. Urine was sampled and the kidneys placed in 20 ml of saline for homogenization in a Waring Blender, held at 4°C. To avoid overheating, homogenization of one min. followed by a cooling period of two min. was repeated until a total of four minutes blending was achieved. Bacterial counts were performed as before and the urine
examined for bacteria, pH and formaldehyde. The method consistently avoided contaminating bacteria and those bacteria grown were always identified as the particular species of the challenge.

2.14 TRANSFERABLE ANTIBIOTIC RESISTANCE FACTORS IN BACTERIA FROM PATIENTS

Selected antibiotic-resistant bacterial strains from the spinal patients were investigated for the presence of transferable drug resistance factors (R-factors). The method and media used in this study have been described by Davey and Pittard (1971). The recipient strains used were female mutants of *E. coli* K12 kindly supplied by Davey and Pittard, and carried multiple auxotrophic markers as well as resistance to nalidixic acid. The minimal media described by these workers was made available for characterisation of the repliconates.
SECTION 3

AETIOLOGY, ORIGIN AND PATHWAYS OF URINARY TRACT INFECTION

IN SPINAL PATIENTS
3.1 INTRODUCTION

Infections of the urinary tract include many clinical entities described variously as asymptomatic bacteriuria, cystitis, pyelitis and acute and chronic pyelonephritis; Kunin (1970) points out that bacteriuria is the most common denominator of all these types of urinary tract infections. It is nearly 20 years since Kass (1956) introduced the concept of "significant bacteriuria" and stimulated surveys in various population groups (Asscher et al., 1969; Bailey, 1970; Freeman and Sindhu, 1974 and Robertson, 1968). It is generally believed that persistent infection, however insignificant clinically, is a precursor of renal deterioration. Kincaid-Smith et al. (1971) state that unless some anatomical, functional or pathologic abnormality is present, there is little evidence that urinary tract infection leads to progressive renal damage.

Any assessment of the literature relating to the bacterial aetiology of urinary tract infection should consider the following variants in patients:

Age and sex

Anatomical level of infection

Abnormalities of the urinary tract

Hospitalisation and instrumentation

Acute or chronic episode

Relapse or recrudescence is considered to be the recurrence of infection with the same organism. Re-infection however implies recurrence with a different organism. Turck et al. (1968) have shown that patients with renal infection have a high proportion of relapse with the same
bacterial strain, whereas patients with lower urinary tract infection (cystitis) have a high proportion of re-infections with different bacteria. Garrod and O'Grady (1971) indicate that an acute infection is almost always caused by a single bacterial species, but chronic infections, usually associated with abnormalities of the urinary tract, are frequently caused by more than one species. Dawborn et al. (1973) by extending the incubation time, found a double aetiology in 18% of acute infections in general practice, a finding not unexpected if faecal organisms are the origin of urinary infection in patients without abnormalities.

The majority of acute urinary infections in all ages and groups of patients seen in general practice is due to Escherichia coli, whereas Proteus mirabilis, Klebsiella aerogenes, Streptococcus faecalis, and Staphylococcus epidermidis are isolated at a much lower frequency (Stamey, 1974). Gould (1968) has shown that in chronic infections E. coli is less common and the Proteus, Klebsiella and Pseudomonas species predominate. Hospitalized patients with instrumentation are reported to show a high percentage of infections due to bacteria found in the ward and a lower percentage from the endogenous source. This is reflected in the Klebsiella, Proteus and nosocomial species of Pseudomonas and Acinetobacter being more frequently found than the faecal E. coli (Gould, 1968; Gillespie et al., 1962).

The normal bladder has self sterilizing mechanisms such as complete emptying (Tribe and Silver, 1969) so that infection is not necessarily the consequence of introducing pathogenic bacteria into the bladder. In the spinally injured patient with a neurogenic bladder, incomplete emptying usually results in infection when pathogenic bacteria are introduced. Bladders of patients without neurological disorders cope
successfully with infection after removal of a catheter (Cox and Hinman, 1965; O'Grady and Cattell, 1966).

The literature contains reports from spinal centres giving the overall range of bacteria isolated from patients with neurogenic bladders. The order of frequency differs in the reports but the same organisms form the majority. From Stoke Mandeville, Milner (1963), Guttman and Frankel (1966), and Silver (1974) list \textit{Kl. aerogenes, Strep. faecalis, Ps. aeruginosa, Proteus, Providence, E. coli, Serratia} and \textit{Acinetobacter (B. anitratum)} as the most frequent isolates. In a study of 50 males, \textit{Kl. aerogenes} was isolated in 72% and \textit{Ps. aeruginosa} in 80% of patients. In the United States, Morales and Tsou (1962) at New York State Rehabilitation Hospital found streptococci to be the most common organisms, whereas Bors and Comarr (1971) reporting from Long Beach, California found in 33 patients only 2.4% of isolates were Gram-positive cocci. This difference may well be only a reflection of the increase of Gram-negative organisms causing hospital infections seen throughout the world.

McLeod \textit{et al.} (1965) noted that few investigators have explored the varieties of bacteria responsible for infection in the urine of the spinally injured. This section of my thesis reports a detailed identification of the isolates from the Austin Spinal Unit. Section 5 suggests that the characteristics of the isolates, coupled with some features in the management of the patients may directly influence the range of organisms found.

The speed at which patients, admitted with sterile urine acquire their first infection has been studied in detail by Walsh (1968), Stickler \textit{et al.} (1971), Pearman and England (1973) and Silver (1974)
particularly in relation to the method of intermittent catheterisation. Stickler et al. found (in four out of nine cases) the organisms eventually responsible for the infection could be isolated from the urethra or its external meatus. More frequent screening for urinary infection has accompanied the technique of intermittent catheterisation; Pearman and England in Perth, Australia have cultured every specimen of urine collected by this method. This close surveillance showed that infection had been present for at least 48 hours before urine became turbid or pyrexia occurred. The acquisition of the first infection in a previously healthy bladder can be delayed by scrupulous care taken with each technique relating to the indwelling or intermittent catheterisation.

Gillespie et al. (1960, 1967), Lindan (1969), Guttmann (1973) and Pearman and England (1973) have shown that it is possible to reduce the overall rate of infection by surveillance of all techniques concerned with bladder care, as well as the use of skilled technicians for catheter changing.

There are few reports in the literature which relate statistically significant numbers of patients admitted without delay, of the same sex, similar neurological bladder and antibiotic therapy to the source of the infecting organisms. It is important to note that the current survey presents patients selected with uniformity in the following:

- Admitted within 24 hours of injury
- Male sex
- Permanent indwelling catheter during the study
- Bladder irrigation regime
- Type of drainage bag
- Urinary acidification and disinfection
- High fluid intake (approximately 3 litres per day)
- No prophylactic antibiotics
- Skilled catheter technicians to change catheters
3.2 RESULTS

3.2.1 AETIOLOGY OF INITIAL URINARY TRACT INFECTION

Table 5 lists the organisms, in order of frequency, isolated from the first urinary tract infection acquired by patients who had been admitted with sterile bladders to the admission Ward 7. The survey was conducted from 1971-74 and is the result of weekly examinations of 74 male patients with indwelling catheter.

TABLE 5

INITIAL INFECTION IN 74 MALE SPINAL PATIENTS

<table>
<thead>
<tr>
<th>Organism</th>
<th>Incidence in 74 patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of patients</td>
</tr>
<tr>
<td>Pr. mirabilis</td>
<td>17</td>
</tr>
<tr>
<td>Acinetobacter anitratus</td>
<td>12</td>
</tr>
<tr>
<td>Ps. aeruginosa</td>
<td>8</td>
</tr>
<tr>
<td>Kl. aerogenes</td>
<td>6</td>
</tr>
<tr>
<td>E. coli</td>
<td>5</td>
</tr>
<tr>
<td>Serratia</td>
<td>3</td>
</tr>
<tr>
<td>Strep. faecalis</td>
<td>2</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>2</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>2</td>
</tr>
<tr>
<td>E. coli</td>
<td></td>
</tr>
<tr>
<td>Kl. aerogenes</td>
<td>2</td>
</tr>
<tr>
<td>E. coli</td>
<td></td>
</tr>
<tr>
<td>Serratia</td>
<td>1</td>
</tr>
<tr>
<td>Acinetobacter anitratus</td>
<td>1</td>
</tr>
<tr>
<td>Staph. epidermidis</td>
<td>1</td>
</tr>
<tr>
<td>Serratia</td>
<td></td>
</tr>
<tr>
<td>Ps. aeruginosa</td>
<td>1</td>
</tr>
<tr>
<td>Pr. mirabilis</td>
<td></td>
</tr>
<tr>
<td>Kl. aerogenes</td>
<td>1</td>
</tr>
<tr>
<td>No infection</td>
<td>11</td>
</tr>
</tbody>
</table>

Criteria for reported isolation:
- Polyuria greater than 50,000 per ml
- Organisms greater than 100,000 per ml
- Clinical signs (e.g. pyrexia) of infection.
3.2.2 OVERALL AETIOLOGY OF URINARY INFECTION

Table 6 lists the organisms, in order of frequency, isolated from urinary tract infections in 60 male patients resident in the rehabilitation Ward 17. These patients had progressed from the admission ward, some with urinary infections, or had been readmitted for complications or checkup. The survey was conducted during 1971-72.

The patients were uniform with respect to:

- Sex (male)
- Indwelling catheter
- Rehabilitation Ward 17
- Manual chlorhexidine bladder washouts twice daily
- Oral ammonium chloride 2-4g per day
- Oral methenamine mandelate 4g per day
- High oral fluids, 2500-3500ml per day
- Same catheter technicians

The criteria for reported isolations were:

- Polyuria greater than 50,000 per ml,
- Organism greater than 100,000 per ml,
- More than one isolation of organism,
- Clinical signs (eg. pyrexia) of infection.
<table>
<thead>
<tr>
<th>Organism</th>
<th>Incidence in 60 patients</th>
<th>No. of patients</th>
<th>% of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ps. aeruginosa</td>
<td></td>
<td>28</td>
<td>47</td>
</tr>
<tr>
<td>Serratia</td>
<td></td>
<td>24</td>
<td>40</td>
</tr>
<tr>
<td>Pr. mirabilis</td>
<td></td>
<td>22</td>
<td>37</td>
</tr>
<tr>
<td>Kl. aerogenes</td>
<td></td>
<td>20</td>
<td>33</td>
</tr>
<tr>
<td>Acinetobacter anitratus</td>
<td></td>
<td>19</td>
<td>32</td>
</tr>
<tr>
<td>E. coli</td>
<td></td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>Providence B</td>
<td></td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>Pr. rettgeri</td>
<td></td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>Alcaligenes faecalis</td>
<td></td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Candida albicans</td>
<td></td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Ps. fluorescens</td>
<td></td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Strep. faecalis</td>
<td></td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td></td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Pr. vulgaris</td>
<td></td>
<td>1</td>
<td>1.6</td>
</tr>
</tbody>
</table>
3.2.3 ISOLATIONS FROM OUTPATIENTS

Monthly examinations were made from spinal patients attending the Outpatients' Clinic. Three patients are reported here because the complete records are available of regular examinations performed at the Austin Hospital since their first admission more than 10 years ago.

Patient 266 (disseminated sclerosis) admitted 1955.

Patient 266 was reported to have Pr. vulgaris causing a urinary tract infection in 1956. Regular isolations of this species are reported in the Austin Hospital records each year since 1956, either alone or in association with "Paracolon" or E. coli. There have been two episodes of Ps. aeruginosa infection. During 1971-73 my monthly examinations showed 19 out of 21 consecutive isolations were Pr. vulgaris. The patient did not show clinical signs nor was he treated for urinary infection; oral ammonium chloride and methenamine mandelate were taken daily. The isolate Pr. vulgaris was conspicuous in that it was my only isolation of the species at the Spinal Unit during this time. The antibiotic profile was constant, showing resistance to ampicillin (300 µg/ml) and sulpathiazole (200 µg/ml). The same organism was isolated from the drainage bags at each examination during this study.

It could be reasonably assumed that Pr. vulgaris was acquired at a date prior to 1971, and was present in the bladder for at least two years without causing clinical symptoms; it is tempting to postulate that it has been present since 1956, but the early isolations were not preserved so comparison is impossible.

Patient 4753 (T4/5 fr., paraplegic) admitted 1958.

Patient 4753 was reported to have a Proteus infection in 1958, Klebsiella was first seen in 1960. Treatment had been given for urinary infections caused by Staph. aureus, E. coli and Ps. aeruginosa as well as
Pr. mirabilis and Kl. aerogenes, until 1969. The examinations of urine during 1970-74 have revealed these last two organisms alone or together. After 34 consecutive monthly examinations of isolating Pr. mirabilis and/or Kl. aerogenes, there was an episode of Strep. faecalis. This was treated and when antibacterial activity was no longer detected in the urine, Kl. aerogenes was isolated again. The Pr. mirabilis was characterised by Dienes typing and was not compatible with any other isolate of this species from the Spinal Unit. The antibiotic profile was constant, resistance was shown to tetracycline (100 µg/ml) and nitrofurantoin (50 µg/ml). The same organism was isolated from the drainage bags during the study. The Kl. aerogenes isolates from the catheter specimens of urine and from the drainage bags were characterised by the M.I.C. of the 15 antibacterials routinely tested and were identical, showing resistance to kanamycin (500 µg/ml), nitrofurantoin (200 µg/ml) and tetracycline (75 µg/ml).

It could be reasonably assumed that for four years Pr. mirabilis and Kl. aerogenes have been present at a sub-clinical level and again it is tempting to postulate that these organisms have been present for at least 15 years having never been eradicated by any antibacterial therapy.

Patient 25007 (disc prolapse, paraplegic) admitted 1963.

Patient 25007 has had 31 isolations of Proteus since 1963-73. Pr. rettgeri was identified in 1964 and since then isolation of this organism associated with E. coli, "Paracolon" and Strep. faecalis has been a regular observation. Strep. faecalis has featured in 14 associations with Proteus. My monthly examinations during 1970-73 produced 22 isolations of Pr. rettgeri from the catheter urine and at the same time from the drainage bags. The isolates had similar M.I.C. of the antibacterials routinely tested and showed resistance to ampicillin (200 µg/ml), carbenicillin (25 µg/ml), cephalothin (50 µg/ml) and chloramphenicol (25 µg/ml). Again it could be reasonably assumed that this patient acquired Pr. rettgeri prior to 1970 and it had been maintained without showing clinical signs of infection.
3.2.4 AETIOLOGY OF URINARY INFECTION IN WARD 7 DURING TWO PERIODS

Table 7 shows organisms causing the initial infection in patients in the admission Ward 7 during two periods, viz. October 1970 to May 1971 and the following October 1971 to May 1972. Usually there was only one species causing the infection; Table 5 shows only 8% of patients acquired their initial infection with double aetiology. It would appear from Table 7 that there was a change in the incidence of species suggesting an exogenous source of infection and reflecting the prevalent ward flora at the different times of study.

The patients were uniform with respect to:

- Sex (male)
- Admitted with sterile urine
- Indwelling catheter for period of study
- Admission Ward 7
- Manual chlorhexidine bladder washouts twice daily
- Oral ammonium chloride 2-4g per day
- Oral methenamine mandelate 4g per day
- High oral fluids, 2500-3500 ml per day
- Same catheter technicians

The criteria for reported isolations were:

- Polyuria greater than 50,000 per ml
- Organisms greater than 100,000 per ml
- More than one isolation of organism
- Clinical signs of infection
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kl. aerogenes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pr. mirabilis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Providence B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ps. aeruginosa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serratia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>10</td>
<td>15</td>
</tr>
</tbody>
</table>
3.2.5 INFECTION WITH ACINETOBACTER

The weekly examination of urine from 12 male patients with indwelling catheter, admitted from 26.10.71 to 5.5.72 into Ward 7 showed an unusually high isolation rate of *Acinetobacter anitratus*. Five of these patients developed clinical symptoms of urinary infection and were treated with antibiotics. Table 8 identifies the patients and sets out the date of admission, the date of the first isolation of *Acinetobacter anitratus*, the number of consecutive weekly isolations of this organism and the date of the clinical signs of urinary disease such as malaise, pyrexia and turbid urine.

All 12 patients had sterile urine on admission and received manual bladder irrigations, twice daily, with chlorhexidine diluted 1 in 5,000 with sterile water. The chlorhexidine was diluted in the preparation room of the ward and the irrigations carried out by trainee nurses. *Acinetobacter anitratus* was isolated from samples of the diluted chlorhexidine ready for use. Since June 1972 the Austin Hospital Pharmacy has prepared the chlorhexidine solutions, diluted with filtered water and autoclaved. Samples examined since that time have not grown bacteria. At the end of February 1973, routine chlorhexidine irrigation was discontinued; now the procedure is performed when catheters are blocked by debris resulting from infection (Jones, private communication).

The isolates of *Acinetobacter anitratus* gave similar M.I.C. of the antibiotics tested. Resistance was shown to the following:

- ampicillin >300 µg/ml neomycin 200
- carbenicillin >100 nitrofurantoin 200
- cephalothin 250 streptomycin >300
- gentamicin 32 sulphathiazole 100
- kanamycin 300 tetracycline >200

There has been a marked reduction in the incidence of *Acinetobacter anitratus* infection in the patients from Ward 7 since June 1972 and as this coincides with sterile chlorhexidine available for irrigation the source of this infection is clear.
### Table 8

**Isolation of Acinetobacter from Patients in Admission Ward 7**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Admission date</th>
<th>Days prior to first isolation of Acinetobacter</th>
<th>No. of weekly isolations</th>
<th>Date of clinical infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>97059</td>
<td>26.10.71</td>
<td>26</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>97281</td>
<td>2.11.71</td>
<td>41</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>97310</td>
<td>2.11.71</td>
<td>41</td>
<td>15</td>
<td>4.5.72</td>
</tr>
<tr>
<td>97406</td>
<td>7.11.71</td>
<td>87</td>
<td>5</td>
<td>8.2.72</td>
</tr>
<tr>
<td>99819</td>
<td>25.1.72</td>
<td>81</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>99985</td>
<td>1.2.72</td>
<td>7</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>100155</td>
<td>6.2.72</td>
<td>36</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>100390</td>
<td>11.2.72</td>
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<td>4</td>
<td>3.3.72</td>
</tr>
<tr>
<td>101743</td>
<td>26.3.72</td>
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<td>3</td>
<td>4.4.72</td>
</tr>
<tr>
<td>102056</td>
<td>4.4.72</td>
<td>7</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>102819</td>
<td>28.4.72</td>
<td>5</td>
<td>9</td>
<td></td>
</tr>
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<td>103186</td>
<td>5.5.72</td>
<td>10</td>
<td>13</td>
<td>26.6.72</td>
</tr>
</tbody>
</table>

**Isolation:** Polyuria 50,000/ml

*Acinetobacter anitratus* 100,000/ml

**Clinical infection:** Pyrexia, malaise and turbid urine
3.2.6 INFECTION WITH ESCHERICHIA COLI

The E. coli isolated from the spinal patients with clinical infection, were serologically typed and the M.I.C. of 15 antibacterials were estimated. The isolations which occurred between 1971-72 are set out in Table 9.

Infection with E. coli was not common. Patient 96544 (admitted 8.10.71) is typical of this group. He had the first urinary infection on 8.11.71 and E. coli type 06:H1 was isolated. Weekly examinations isolated this strain until January 1972; the patient was treated with ampicillin. In March 1972, P8. aeruginosa, with multiple antibacterial resistance, caused infection, and the sensitive E. coli did not reappear after treatment with gentamicin.

When E. coli infection did persist, as in patients 263 and 91941, multiple resistance was shown by this species.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Serological type of E. coli</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>263</td>
<td>0 18ac; H 1</td>
<td>nalidixic acid, sulphamethoxazole, tetracycline</td>
</tr>
<tr>
<td>63810</td>
<td>0123 ; H 6</td>
<td>ampicillin, carbenicillin, streptomycin, sulphathiazole, tetracycline, trimethoprim</td>
</tr>
<tr>
<td>91941</td>
<td>0 6 ; H31</td>
<td></td>
</tr>
<tr>
<td>93026</td>
<td>0 2 ; H 4</td>
<td></td>
</tr>
<tr>
<td>96544</td>
<td>0 6 ; H 1</td>
<td></td>
</tr>
<tr>
<td>97406</td>
<td>0 smooth non-typable; H 4</td>
<td></td>
</tr>
</tbody>
</table>
3.2.7 INFECTION WITH PROTEUS MIRABILIS

The 70 separate isolations of *Pr. mirabilis* from 15 patients, as well as isolations from equipment in the wards, were typed by the method of Dienes and designated A to M.

*Pr. mirabilis* Dienes type A was isolated regularly during 1970-74 from the outpatient 4753. No other isolation of this type was made. The antibiotic sensitivity pattern did not change during this time.

Dienes type C was regularly isolated from the urine of nine patients:

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Isolation Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>9219</td>
<td>100390</td>
</tr>
<tr>
<td>13790</td>
<td>100868</td>
</tr>
<tr>
<td>90086</td>
<td>101743</td>
</tr>
<tr>
<td>90637</td>
<td>104150</td>
</tr>
<tr>
<td>96544</td>
<td></td>
</tr>
</tbody>
</table>

The sensitivity of this type was uniform in all isolations, viz.

- **Resistances:**
  - Carbenicillin: 100 μg/ml
  - Chloramphenicol: 50
  - Kanamycin: >300
  - Neomycin: >300
  - Sulphathiazole: >200
  - Tetracycline: 200
  - Trimethoprim: >200

Dienes type C was acquired as nasal flora in patient 102056 after 10 weeks in Ward 7 but this patient did not acquire a urinary infection from *Pr. mirabilis*. Patient 103186 was also shown to have Dienes type C, this time in faeces, but he did not develop a urinary infection.

Other Dienes types were identified from the faecal isolates of *Pr. mirabilis*; all the types had sensitive antibiotic patterns as compared with the table given by Garrod and O'Grady (1971).

*Pr. mirabilis* could be isolated from bed rails and floors before disinfection, but was never isolated on urinary equipment. The Dienes type found on bed rails and under the bed could be related to the type isolated from the patient occupying the bed. This organism appeared to have a poor survival on fomites in the wards.
3.2.8 INITIAL PROVIDENCE B INFECTION IN PATIENTS

Providence B was frequently isolated during 1970-71 compared with 1972-74. Table 10 lists 14 patients and the date at which Providence B was first isolated from their urine and when clinical infection was deemed to be present. All the patients were males with indwelling catheters and resident in the rehabilitation Ward 17. Other patients in the ward had Providence B infections at the same time, but the patients listed in Table 10 are well documented and had not grown this species before.

At the time of this survey, 1970-72, disinfectant was not placed in the urine drainage bag, and the urine taken from the bag was also cultured. Providence B was isolated from the bags of all patients who had the infection. As well, this species was isolated from the bags of two patients who did not have an infection from this organism. One of these patients (90637) was later shown to have Providence B in urine. One pathway of infection is indicated here, viz. contamination of a re-used disinfected (not sterile) drainage bag.

All the isolates of Providence B showed the same antibiotic resistance pattern as listed in Table 10.
### TABLE 10

**INITIAL PROVIDENCE B INFECTION IN PATIENTS FROM REHABILITATION WARD 17**

<table>
<thead>
<tr>
<th>Patient Identification</th>
<th>Date of Infection with Providence B</th>
</tr>
</thead>
<tbody>
<tr>
<td>79348</td>
<td>1.10.70</td>
</tr>
<tr>
<td>9842</td>
<td>10.10.70</td>
</tr>
<tr>
<td>84335</td>
<td>5.12.70</td>
</tr>
<tr>
<td>83245</td>
<td>17.1.71</td>
</tr>
<tr>
<td>86420</td>
<td>6.2.71</td>
</tr>
<tr>
<td>86324</td>
<td>2.3.71</td>
</tr>
<tr>
<td>89339</td>
<td>4.3.71</td>
</tr>
<tr>
<td>86349</td>
<td>12.4.71</td>
</tr>
<tr>
<td>88028</td>
<td>12.4.71</td>
</tr>
<tr>
<td>86728</td>
<td>6.5.71</td>
</tr>
<tr>
<td>88579</td>
<td>7.5.71</td>
</tr>
<tr>
<td>86529</td>
<td>11.5.71</td>
</tr>
<tr>
<td>91941</td>
<td>30.7.71</td>
</tr>
<tr>
<td>90006</td>
<td>3.8.71</td>
</tr>
</tbody>
</table>

The evidence that the isolates are the same strain is shown by their similar antibiotic resistance pattern.

- Resistant to ampicillin: >300 μg/ml
- Gentamicin: >32
- Kanamycin: >300
- Neomycin: >300
- Streptomycin: 300
- Tetracycline: 200
3.2.9 ISOLATION OF PSEUDOMONAS AERUGINOSA

Infections with \textit{Ps. aeruginosa} were usually characterised by the clinical signs of turbid urine, pyrexia and malaise. Ward 7 and 17 were swabbed regularly to determine the sites of survival and the pathways of infection with this organism. All isolates from the patients and the wards were classified by pyocine typing and antibiotic sensitivity patterns.

\textbf{Table 11} sets out the isolation of \textit{Ps. aeruginosa} from the patients and the wards; the patients from Ward 17 who had progressed from Ward 7 did not show evidence of infection with \textit{Pseudomonas} when they first arrived at Ward 17. Clusters of isolations with respect to time and place are shown in Table 11.

The sensitivity of this organism was variable but the resistance was correlated with the pyocine type. For example:

Pyocine type 5 : resistant to carbenicillin $>$100 $\mu$g/ml

Pyocine type 22 : resistant to gentamicin 32
  kanamycin $>$300
  neomycin $>$300
  streptomycin $>$300
  tetracycline 300

Further reports will show that \textit{Ps. aeruginosa} survived some disinfection procedures used to prepare rubber leg bags and stainless steel urinals for re-use.
<table>
<thead>
<tr>
<th>Ps. aeruginosa Pyocine type</th>
<th>Ward</th>
<th>Source of Isolation</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>Patient 26609</td>
<td>11.8.71</td>
</tr>
<tr>
<td>1</td>
<td>17</td>
<td>90086</td>
<td>31.8.71</td>
</tr>
<tr>
<td>1</td>
<td>17</td>
<td>13790</td>
<td>1.9.71</td>
</tr>
<tr>
<td>1</td>
<td>17</td>
<td>3137</td>
<td>10.2.72</td>
</tr>
<tr>
<td>1</td>
<td>17</td>
<td>Sink in lavatory</td>
<td>1.9.71</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>Patient 97316</td>
<td>27.11.71</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>97281</td>
<td>20.12.71</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>105845</td>
<td>15.8.72</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>Sink in ward</td>
<td>20.12.71</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>Urine measure</td>
<td>20.12.71</td>
</tr>
<tr>
<td>5</td>
<td>17</td>
<td>Patient 91941</td>
<td>14.7.72</td>
</tr>
<tr>
<td>5</td>
<td>17</td>
<td>103186</td>
<td>9.8.72</td>
</tr>
<tr>
<td>5</td>
<td>17</td>
<td>103923</td>
<td>11.8.72</td>
</tr>
<tr>
<td>5</td>
<td>17</td>
<td>Ledge under rack for drainage bags</td>
<td>24.7.72</td>
</tr>
<tr>
<td>22</td>
<td>17</td>
<td>Patient 90142</td>
<td>5.10.71</td>
</tr>
<tr>
<td>22</td>
<td>17</td>
<td>89481</td>
<td>5.10.71</td>
</tr>
<tr>
<td>22</td>
<td>17</td>
<td>68516</td>
<td>3.11.71</td>
</tr>
<tr>
<td>22</td>
<td>17</td>
<td>93826</td>
<td>9.12.71</td>
</tr>
<tr>
<td>22</td>
<td>17</td>
<td>63810</td>
<td>9.12.71</td>
</tr>
<tr>
<td>22</td>
<td>17</td>
<td>Disinfected urine bottle</td>
<td>2.12.71</td>
</tr>
</tbody>
</table>
3.2.10 INVESTIGATION OF ENDOGENOUS SOURCE OF INFECTION

The identity of organisms isolated from 15 patients is set out in Table 12. Each patient arrived at Ward 7 within 24 hours of injury and had sterile urine. Weekly examinations of the nasal, faecal and urine flora were carried out during the 12 week period that these patients retained indwelling catheters. Table 12 lists the organisms in order of isolation for each patient; single species were usually isolated at each examination of urine.

It can be seen that two patients acquired E. coli infections and as the same serological type was present in their faeces, it could be reasonably assumed that the endogenous source was implicated.

Serratia and Acinetobacter were not isolated from nasal or faecal flora from any of the patients. As Acinetobacter was found in the chlorhexidine irrigation solution, this species is probably not acquired from the patients' own bacterial flora.

Kl. aerogenes was frequently isolated from all three sites. A comparison of the strains has been made on the antibiotic profile. The strain isolated from four of the patients with urinary tract infection and from the nose of a fifth patient showed:

<table>
<thead>
<tr>
<th>Resistance to:</th>
<th>400 µg/ml</th>
<th>100 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>ampicillin</td>
<td>carbenicilllin &gt;100</td>
<td>sulphathiazole &gt;200</td>
</tr>
<tr>
<td>streptomycin</td>
<td>kanamycin 300</td>
<td>tetracycline 100</td>
</tr>
<tr>
<td>neomycin</td>
<td>200</td>
<td>trimethoprim &gt;200</td>
</tr>
<tr>
<td>nitrofurantoin</td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>

The Kl. aerogenes strains isolated from the faeces of these patients usually only showed resistance to carbenicillin, sulphathiazole and trimethoprim. The Kl. aerogenes infection could not be shown to be endogenous in origin.

Ps. aeruginosa type 3 was isolated from the nose and faeces of patient 105845 after 14 days in Ward 7. Although this type was isolated from the patient's nose and faeces for the following five weeks he did not acquire a urinary infection caused by Ps. aeruginosa.

Pr. mirabilis Dienes type C could not be isolated from the nose or faeces of the three patients who acquired urinary infections from this strain.
<table>
<thead>
<tr>
<th>Patient</th>
<th>Isolates During First 12 Weeks after Injury</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nose</td>
</tr>
<tr>
<td>96544</td>
<td>E. coli (06;H1)</td>
</tr>
<tr>
<td></td>
<td>Strep. faecalis</td>
</tr>
<tr>
<td>97059</td>
<td>Staph. aureus</td>
</tr>
<tr>
<td>97281</td>
<td>E. coli</td>
</tr>
<tr>
<td>97310</td>
<td>E. coli</td>
</tr>
<tr>
<td>97406</td>
<td>Pr. mirabilis (H)</td>
</tr>
<tr>
<td>99985</td>
<td>E. coli</td>
</tr>
<tr>
<td>100155</td>
<td>Staph. aureus</td>
</tr>
<tr>
<td>100390</td>
<td>E. coli</td>
</tr>
<tr>
<td>100868</td>
<td>E. coli</td>
</tr>
<tr>
<td>101743</td>
<td>E. coli</td>
</tr>
<tr>
<td>102056</td>
<td>Pr. mirabilis (C)</td>
</tr>
<tr>
<td>102819</td>
<td>E. coli</td>
</tr>
<tr>
<td>102958</td>
<td>E. coli</td>
</tr>
<tr>
<td>103186</td>
<td>Kl. aerogenes</td>
</tr>
<tr>
<td>105845</td>
<td>Ps. aeruginosa (3)</td>
</tr>
</tbody>
</table>

|         | Faeces                                      |
| 96544   | E. coli (06;H1)                           |
| 97059   | Strep. faecalis                           |
| 97281   | Strep. faecalis                           |
| 97310   | Strep. faecalis                           |
| 97406   | Strep. faecalis                           |
| 99985   | Strep. faecalis                           |
| 100155  | Strep. faecalis                           |
| 100390  | Strep. faecalis                           |
| 100868  | Strep. faecalis                           |
| 101743  | Strep. faecalis                           |
| 102056  | Strep. faecalis                           |
| 102819  | Strep. faecalis                           |
| 102958  | Strep. faecalis                           |
| 103186  | Strep. faecalis                           |
| 105845  | Ps. aeruginosa (3)                        |

|         | Urine                                   |
| 96544   | E. coli (06;H1)                         |
| 97059   | Pr. mirabilis (C)                       |
| 97281   | Acinetobacter                           |
| 97310   | Staph. aureus                           |
| 97406   | E. coli (0 sm;H4)                       |
| 99985   | Acinetobacter                           |
| 100155  | Acinetobacter                           |
| 100390  | Acinetobacter                           |
| 100868  | Acinetobacter                           |
| 101743  | Acinetobacter                           |
| 102056  | Acinetobacter                           |
| 102819  | Acinetobacter                           |
| 102958  | Ps. aeruginosa (1)                      |
| 103186  | Ps. aeruginosa (5)                      |
| 105845  | Ps. fluorescens                        |

Abbrev.: Ps. aeruginosa (pyocine type), E. coli (serological type), Pr. mirabilis (Dienes type), Kl. aerogenes (resistance)
3.2.11 WARD FLORA

Regular sampling of flora in the admission Ward 7, where patients do not move about in wheelchairs, yielded organisms similar to those isolated from the faeces and urine of patients resident at the time of sampling.

*K. aerogenes* was isolated from the bevelled skirting boards, a visitor's chair, the nurses' wash basin in the ward and the Charge Sister's desk. *Ps. aeruginosa* and *Acinetobacter* were grown from the wash basin in the ward and from the urine disposal room - on the floor, in the hopper and inside a stainless steel urinal. *E. coli* and *Proteus* were only isolated in the immediate environment of patients. *Serratia* was not isolated.

A typical result of sampling is shown:

<table>
<thead>
<tr>
<th>Date: 6.11.71</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Bed rails</td>
</tr>
<tr>
<td>Wooden pack</td>
</tr>
<tr>
<td>Sand bag cover</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Floor under bed before emptying drainage bag</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Floor under bed after emptying drainage bag</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Floor polisher</td>
</tr>
<tr>
<td>Occupational therapy</td>
</tr>
<tr>
<td>typewriter keys</td>
</tr>
</tbody>
</table>
The following pages (Figures 4 and 5) show areas where the urinary pathogens were isolated in the rehabilitation Ward 17 and illustrate the difficulty in disinfecting wooden floors, chipped paint on wheelchairs, and perpetually damp bathroom equipment such as ropes and wooden floor mats.

The wards are cleaned by the Austin Hospital cleaning staff and the policy of "cleaning without disinfectants is better than disinfectants without cleaning" is practiced.

Accidents with urine drainage bags occur, urine is spilt on 
wooden floors, gymnasium equipment and on wheelchairs. Urine was mopped up with the cleaning equipment stored in the wards for the use of the cleaning staff. Observations were that on occasions cotton mops reserved for the kitchen were used for drainage bag spills. In 1973 the wooden floor of the balcony recreation room was replaced by vinyl sheeting. However one area still remains as shown in Figure 4.

In December 1974 when this photograph was taken, a member of the nursing staff was sitting on a commode chair and was using the telephone. The combination of storage area for commode chairs, patients' telephone and wooden floor is unsatisfactory.

Organisms causing urinary tract infection survived on damp equipment in the bathroom (Figure 5). Wooden foot stools should be replaced with plastic material as is used for the bench under the shower. It is recommended that ropes be pasteurised or autoclaved and dried each night.
FIGURE 4

PATIENTS' TELEPHONE AND STORAGE AREA FOR COMMODE CHAIR

REHABILITATION WARD 17

<table>
<thead>
<tr>
<th>Site</th>
<th>Organisms Isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wooden floor</td>
<td>Acinetobacter</td>
</tr>
<tr>
<td></td>
<td>Bacillus subtilis</td>
</tr>
<tr>
<td>Commode chair</td>
<td>Alcaligenes</td>
</tr>
<tr>
<td></td>
<td>Staph. epidermidis</td>
</tr>
<tr>
<td>Telephone</td>
<td>Staph. epidermidis</td>
</tr>
<tr>
<td></td>
<td>Yeasts</td>
</tr>
</tbody>
</table>
FIGURE 5

BATHROOM IN WARD 17

Bacteria isolated from the floor, rope and wooden foot stool:

Acinetobacter
Kl. aerogenes
Ps. aeruginosa
Staph. epidermidis

From the interior of the clothes washing machine in the same bathroom, bacteria isolated:

Acinetobacter
Staph. epidermidis
3.2.12 DISINFECTION OF URINARY EQUIPMENT

Until 1972, "Wescodyne" (polyethoxyethanol-iodine) was used in eight gallon plastic bins to disinfect urinals, measuring jugs and urinary appliances such as rubber and plastic drainage bags. The solution was changed every 3 or 4 days depending on a colour change which was related to free iodine content. The staff and patients were instructed to leave articles in the bin for 10 minutes before removing them for use. This was not practicable as the past history of an article was not known when a utensil was removed. It can be seen from Table 13 that when the solution was more than one day old, *P.* aeruginosa survived 10 minutes, and "Wescodyne" at the concentration used was ineffective against *Staph. aureus* for 10 minutes.

**TABLE 13**

BACTERICIDAL ACTION OF "WESCODYNE" SAMPLED FROM URINAL DISINFECTANT BIN

<table>
<thead>
<tr>
<th>Date of Sampling</th>
<th>Fresh Solution</th>
<th>Contact Time (min) Pseudomonas aeruginosa NCTC 6749</th>
<th>Contact Time (min) Staphylococcus aureus NCTC 4163</th>
</tr>
</thead>
<tbody>
<tr>
<td>28.10.71</td>
<td>*</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>29.10.71</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1.11.71</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2.11.71</td>
<td>*</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3.11.71</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.11.71</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5.11.71</td>
<td>*</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8.11.71</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ growth
"Saf-Sol", a mixed halogen, was compared with "Wescodyne" and as a result of a number of tests showing the same effect as set out in Table 14, "Saf-Sol" was substituted as the utensil disinfectant.

Three organisms isolated from patients were used in the test as well as two standard species.

The concentrations compared were:

- "Wescodyne": 1 oz to 1 gallon water
  100 p.p.m. of available iodine
- "Saf-Sol": 1 oz to 1 gallon water
  150 p.p.m. of available halogen

<table>
<thead>
<tr>
<th>Test Organism</th>
<th>&quot;Wescodyne&quot;</th>
<th>&quot;Saf-Sol&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Contact time (min)</td>
<td>Contact time (min)</td>
</tr>
<tr>
<td></td>
<td>1 2 4 6 10</td>
<td>1 2 4 6 10</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>+ + + + +</td>
<td>1 2 4 6 10</td>
</tr>
<tr>
<td>Kl. aerogenes</td>
<td>+ + + + +</td>
<td>+</td>
</tr>
<tr>
<td>Pr. mirabilis</td>
<td>+ + + +</td>
<td></td>
</tr>
<tr>
<td>Ps. aeruginosa</td>
<td>+ + +</td>
<td></td>
</tr>
<tr>
<td>Ps. aeruginosa NCTC 6749</td>
<td>+ +</td>
<td></td>
</tr>
<tr>
<td>Staph. aureus NCTC 4163</td>
<td>+ +</td>
<td>+ + + +</td>
</tr>
</tbody>
</table>

+ growth

When the "Saf-Sol" solution was 24 hours old tests showed that the Gram-negative species did not survive 6 minutes contact. As a result of this advice to the Spinal Unit, fresh solutions of "Saf-Sol" are now prepared each morning.
Figure 6 shows the method of disinfection of urinals and urine measuring jugs. The plastic articles were often observed to be floating on the "Saf-Sol" solution, and even the heavier stainless steel utensils had trapped air and were not immersed. Bacteria were recovered from inside the utensils and from the shelf above the baths they were drained.

The re-used drainage bags from the patients with catheters were disinfected in individual plastic containers (Figure 7). This was an attempt to prevent cross infection, but as can be seen the lids were placed together for storage. The bags were hung on a drainage rack so designed that cross infection was inevitable (Figure 8).

In 1973 it was recommended that a pasteurising tank be installed for disinfection of all urinary equipment.
Utensils are emptied into a hopper, rinsed with water and placed in either bath of "Saf-Sol" for at least 15 minutes before being inverted on the shelf above.

Note that the utensils are not immersed in the disinfectant. Three different types of *Ps. aeruginosa* have been isolated from stainless steel and plastic urinals during 1971-74.
An attempt has been made to disinfect separately the drainage bags of those patients who are at present re-using plastic or rubber bags. The bag is emptied, rinsed with water, filled and immersed in the jar of 'Saf-Sol' for 15 minutes. It can be seen that the lids rest on each other when the jars are not in use.
From 1970-75 this method of draining leg bags has operated. To ensure that the bag dries out, the stopper from the tail end is inserted at the top of the bag where the valve is situated. It is considered that the valve end will then remain disinfected until the autoclaved tubing is connected.

The bags were not disinfected by this method. Organisms such as *Ps. aeruginosa* and *Kl. aerogenes* could be recovered from bags belonging to patients with infections caused by these species. Organisms could be recovered from the outside of bags belonging to patients without infections and from the bench below the drainage rack.
3.2.13 EFFECTIVENESS OF DIFFERENT TYPES OF DRAINAGE BAGS IN PREVENTING INFECTION

Until 1973 the acute patients in the admission Ward 7 used a closed circuit, one-use only, plastic drainage bag which had to be disconnected at the catheter to be discarded each day. It was also disconnected twice daily for manual bladder irrigation. Since 1973, patients have had 7-day drainage bags which can be emptied at the side or at the base of the bag. This type of bag is discarded when the catheter is removed each week. In Ward 7 the bag has also been disconnected twice daily for bladder irrigation, so the concept of a 7-day drainage bag is here misplaced, (Figures 9 - 11). 

The ambulant patients in Ward 7 and 17 have used one-use only disposable bags at night and in the daytime they have used leg bags made either of plastic or of rubber. The leg bags can be emptied from the bottom. Since 1974 most leg bags have been made of plastic to which 20 ml of Savlon Concentrate (I.C.I.) is added each time the bag is emptied.

During 1973, the Shenton Park Urodrain (Pearman and Cox, 1972) was available and used for the ambulant patients. This bag is claimed to be superior to all others because of the improved non-return flutter valve, the tail for emptying urine and refilling with disinfectant, and above all because of the shape and tubing. This bag could be described as a truly 7-day drainage bag, as it can be strapped to the leg for daytime or attached to the bed at night, so it need not be disconnected at all. It was used in this fashion and discarded when the catheter was removed each week, (Figure 12).
One-use disposable plastic bag, which must be disconnected from the catheter to be discarded.
The flutter valve in the sterile one-use drainage bag used without disinfectant.

It was shown that the ascent of bacteria from the bag to the tubing was not barred by the valve.
FIGURE 11

The 7-day drainage bag used from 1973–1975 for the acute patients. It is used by active ambulant patients as a daytime leg bag and disinfected at night.
FIGURE 12
The Shenton Park Urodrain. This bag has a tail for emptying urine and refilling with disinfectant, built in tubing for use in bed at night, straps for use as a leg bag and an excellent flutter valve.
Table 15 illustrates the improvement in preventing the initial infection in patients admitted with sterile urine, by the use of a closed system, 7-day drainage bag such as the Shenton Park Urodrain and without breach of the circuit for manual bladder irrigation. Pearman (private communication, 1975) has been unable to make an assessment of this bag in use, because of the small number of patients with permanent catheters at the Perth Spinal Centre. Table 15 shows that at the Austin Spinal Unit during 1973, when the Shenton Park Urodrain was used, the acquisition of the initial infection was delayed for at least 80 days in 9 of the 14 patients. In 1972, with the same nursing supervisor, but with one-use only plastic drainage bags, the acquisition of infection in 13 of the 14 patients was less than 40 days.

The complexity of the re-usable rubber leg bags is shown in Figure 13. The valves are difficult to disinfect. It was found that outpatients usually had two rubber leg bags in use and these were replaced once a year. Figure 14 shows a leg bag which was being used by an outpatient. It had been repaired with adhesive plaster and from the interior of this bag *Ps. aeruginosa* was isolated. Heat disinfection by the method of pasteurisation is preferable to chemical disinfection, particularly when metal and rubber joints are present.

My experience in 1975, with the disinfection of rubber teats from babies' nurseries has led me to recommend that in spinal centres rubber leg bags should be cleaned by immersion in an ultrasonic bath for five minutes followed by disinfection by pasteurisation.
**TABLE 15**

**EFFECTIVENESS OF DIFFERENT TYPES OF BAGS IN PREVENTING INITIAL URINARY TRACT INFECTION**

<table>
<thead>
<tr>
<th></th>
<th>Bag A</th>
<th>Bag B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>101493</td>
<td>114432</td>
</tr>
<tr>
<td>2</td>
<td>101743</td>
<td>115284</td>
</tr>
<tr>
<td>3</td>
<td>102056</td>
<td>115504</td>
</tr>
<tr>
<td>4</td>
<td>102819</td>
<td>115522</td>
</tr>
<tr>
<td>5</td>
<td>102958</td>
<td>115562</td>
</tr>
<tr>
<td>6</td>
<td>103186</td>
<td>116294</td>
</tr>
<tr>
<td>7</td>
<td>104063</td>
<td>116844</td>
</tr>
<tr>
<td>8</td>
<td>104190</td>
<td>118534</td>
</tr>
<tr>
<td>9</td>
<td>105845</td>
<td>119034</td>
</tr>
<tr>
<td>10</td>
<td>106022</td>
<td>119092</td>
</tr>
<tr>
<td>11</td>
<td>106551</td>
<td>119129</td>
</tr>
<tr>
<td>12</td>
<td>107350</td>
<td>119726</td>
</tr>
<tr>
<td>13</td>
<td>65602</td>
<td>119881</td>
</tr>
<tr>
<td>14</td>
<td>107936</td>
<td>120775</td>
</tr>
</tbody>
</table>

**Legend:**

*Sterile Urine.*
TO EMPTY LEG BAG
Turn outlet cap only two turns. Urine then will flow from hole in center.

TO WASH OUT BAG—Unscrew and remove outlet cap.

IF FLUTTER VALVE STICKS TOGETHER...
Unscrew adapter, push blunt instrument in opening and separate flutter valve.

FIGURE 13

Rubber leg bags used by the outpatients with indwelling catheters, until 1974. Since then, plastic leg bags as illustrated in Figure 11 have been used by some of the patients.
FIGURE 14

A rubber leg bag used by an outpatient. The rubber leg bags have been re-used for up to 12 months and are sometimes repaired by patients with adhesive plaster as illustrated here. *P. aeruginosa* was recovered from the interior of this bag.
3.3 DISCUSSION

3.3.1 AETIOLOGY

The range of micro-organisms causing clinical urinary tract infection in patients at the Austin Spinal Unit confirms the observation of Gardner et al. (1970) that infections acquired in the last 15 years are predominantly due to the Gram-negative bacilli. The overall incidence in 60 male spinal patients who were admitted with sterile bladders and retained indwelling catheters for at least four months was found (using the data from Table 6) to be:

**Enterobacteriaceae**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteus - Providence</td>
<td>24%</td>
</tr>
<tr>
<td>Serratia</td>
<td>15%</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>12.5%</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>7%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>58.5%</strong></td>
</tr>
</tbody>
</table>

**Non-fermentative bacilli**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ps. aeruginosa</em></td>
<td>18%</td>
</tr>
<tr>
<td>Nosocomial species</td>
<td>17.5%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>35.5%</strong></td>
</tr>
</tbody>
</table>

**Gram-positive cocci**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida</em></td>
<td>3%</td>
</tr>
</tbody>
</table>

There have been no reports from other centres listing the incidence of micro-organisms causing urinary infection in a similar group of patients, partly because at other centres admissions were often transfers from hospitals and partly because intermittent catheterisation was used. Gardner and his co-workers found that the non-fermentative Gram-negative bacilli (other than *Ps. aeruginosa*) accounted for 4.4% of isolates from all conditions in a general hospital and they point out that the reservoirs and methods of spread of these species remain largely unknown.
Milner (1963) and Guttmann (1973) from Stoke Mandeville, Hunt and Hader (1966) from Canada and Bors and Comarr (1971) from the United States of America have reported that less than 2% of urinary infection in spinal patients (unspecified) was due to the nosocomial species. The higher 17.5% found in the Austin spinal patients relates to a group characterised by complete lesions and permanent indwelling catheter.

The same range of organisms was responsible for the initial infection in the acute Austin spinal patients (Table 5) as in the chronic patients still with indwelling catheter, but the incidence was different. The latter group had a higher incidence of infection with multiple drug resistant organisms such as Pr. rettgeri, Providence B, Ps. aeruginosa and Serratia.

Identification of isolates to species level and typing of strains where possible, provided evidence that one strain of an organism persisted in a patient for many months. In outpatients polyuria and bacteriuria due to identified strains were found repeatedly during the four years of study. Clinical symptoms of pyrexia and malaise were not observed in some patients during this time. It is postulated that a low grade infection is maintained by one or more organisms and that the infection is insufficient to cause turbid urine, pyrexia and malaise in the patient. It is unlikely that re-infection from an exogenous source would account for the continued isolations of Pr. rettgeri, Pr. vulgaris and Pr. mirabilis Dienes type A, because the particular strains of these species were never isolated from other patients or the environment.

When a relapse of clinical urinary tract infection did occur, an indication of this event was seen in retrospect by the slow increase
in the number of bacteria grown in the successive weekly examinations. Despite the semi-quantitative method of counting, there was good correlation between high polyuria, bacteriuria and turbid urine, malaise and pyrexia. This supports the observation of Morales and Tsou (1962) that the urine sampled after passing a fresh catheter is a reasonable representative of bladder urine uncontaminated by bacterial growth from within the lumen of the old catheter. A cumulative bacteriological report sheet for urine examination was introduced to the Spinal Unit in 1971 by the Austin Hospital Pathology Department and this has assisted in the assessment of the relevance of polyuria and bacteriuria. Regular examination is superior to an isolated examination of urine taken when clinical symptoms are present and identification and sensitivity tests are requested.

A change in the incidence of organisms was noticed over a period of time. This was seen with Providence in particular. Silver (1974) reported the same change in incidence and attributed the virtual disappearance of Providence to more frequent culture of urine and earlier treatment with antibiotics. This implies that the organism has no reservoir outside infected patients.

3.3.2 ORIGIN AND SPREAD OF INFECTION

The mode of acquisition of urinary infection is controversial. Silver as recently as 1974(a) stated that the patients' own bowel flora are the primary source of infection. These bacteria are assumed to spread to the perineum, colonize the anterior urethra and with catheterisation are pushed into the bladder. Pearman and Cox (1972) also suggested that organisms enter the bladder from the external urethral meatus by way of the thin space between the catheter and the
urethral mucosa. Linton and Gillespie (1962), Roberts et al. (1965) and Gillespie et al. (1967) have shown that organisms enter the bladder on unsterile catheters, pass up the catheter when the connection to the drainage system is broken or are carried up the drainage tubing and catheter from the drainage bag itself by air bubbles and eddy currents.

In the study of flora from 15 acute spinal patients, without bladder infection at the time of admission (summarised in Table 12), the deduction can be made that two of the patients acquired endogenous E. coli because of the similar serological and drug sensitivity pattern. The other 13 patients did not acquire E. coli infections, nor did any of the 15 patients show infection with Strep. faecalis. Most of the patients received ampicillin and/or penicillin for several weeks after admission. The fact that these two species might not survive in the face of the chemotherapy given is acceptable, but the patient with repeated isolations of Ps. aeruginosa from nose and faeces, and yet not developing a urinary infection is difficult to account for. Three of the patients acquired infection with a relatively drug-resistant Kl. aerogenes, but this was not isolated from the faeces of the 15 patients, although it did appear in the nasal flora of one of them. A conclusion can be drawn from this work that endogenous infection has not been shown except for two patients with initial infections with E. coli. Indeed the work indicates an exogenous source for 31 of the 33 separate urinary infections found in the 15 patients.

In the case of Acinetobacter anitratus infection, the study of the Austin Spinal Unit showed that one pathway of infection was the direct introduction of this organism into the bladder with chlorhexidine irrigant.
This species is reported to be a commensal of skin (Taplin et al., 1963), however it was found to have remarkable ability to survive and multiply in other situations. It was readily isolated in this Unit from the sinks, damp ropes and foot mats, the patients' washing machine and urinals. The ability of this species to establish clinical infection was shown in Table 8, as 5 out of 12 patients with repeated isolations of this species developed turbid urine and pyrexia. With improved sterilization methods of the irrigant and the discontinuation of irrigation as a daily routine, infection with this species has decreased.

A second pathway was evident when *Providence* B was isolated from the drainage bag of one patient and the subsequent first isolation of this species from his urine. Frequent breach of the continuous closed drainage circuit occurs when irrigation, replacement of a new bag or change to a leg bag, provides opportunities for the entry of bacteria carried passively by the staff or equipment. The urine in a drainage bag is an excellent medium for the growth of bacteria, particularly when a disinfectant cannot be added to the one-use bag illustrated in Figure 9. Tests on all the bags used at the Austin Unit revealed that the valves are not a barrier to bacteria ascending from the bag to the tubing above the valve. An exception was the Shenton Park Urodrain which did prevent the ascent of bacteria. The assumption in this case of *Providence* B infection is, that the organism entered the drainage bag during a disconnection of the catheter, grew in the urine medium in the bag, ascended the tubing and catheter and established infection. At the time of this infection *Providence* B was seen isolated frequently in Ward 17 as shown in Table 10.

A third pathway could be seen with *Ps. aeruginosa* infection.
Pyocine typing of the strains enabled a study to be made of the survival of known strains in the personal leg bags of patients infected with \textit{Ps. aeruginosa}. The species survived disinfection measures taken with urinals, sinks, bathroom equipment and re-usable rubber and plastic leg bags. This species was recovered from the inside of leg bags of patients with infection, and the same pyocine type was isolated from the outside of bags hanging to drain underneath an infected bag, as well as from the bench below the drainage rack. On inspection of the rack at frequent intervals a bag could be found which did not have the valve end of the bag plugged with a stopper, hence entry of the \textit{Ps. aeruginosa} to the bag was possible.

Many researchers in this field have published warnings on the danger of infection following catheterisation. Thornton and Andriole (1970) and Lindan (1972) advocate the use of special catheter care technicians for any change of catheter or breach of the closed drainage system. All the literature on this subject stresses the need for eternal vigilance when managing indwelling catheters.

Since the commencement of this study methods to break the pathways of infection in the Austin Spinal Unit include:

(a) An education programme instituted to involve all staff, including the orderlies who position the patients every two hours. One senior nurse visited six centres in other countries to study methods of nursing the spinally injured patient.
(b) Chlorhexidine irrigant prepared and sterilised by the Hospital Pharmacy Department.

(c) Discontinuation of the routine chlorhexidine bladder irrigation.

(d) Substitution of polyethoxyethanol-iodine ("Wescodyne") for the more effective mixed halogen "Saf-Sol" for the disinfection of urinary equipment.

(e) Application to the Board of Management for a pasteurising bath to disinfect urinary equipment.

(f) Introduction of a 7-day closed drainage bag. This allows disinfectant to be added to the bag and for bed patients at least, the circuit need not be broken during the life of the 7-day catheter.

Section 4 is a study designed to answer the question often posed in relation to the spinal patients with persistent infection (Pearman and England, 1973) i.e. "why do bacteria persist in the spinal patient?".
SECTION 4

L-FORMS IN URINE OF PATIENTS FROM SPINAL UNIT
4.1 INTRODUCTION

Bacterial variation that displays itself in morphological change has been studied for a long time. The "smooth" to "rough" variation and the loss of capsule have both been correlated with reduction in virulence, whereas spore formation results in resistance to heat and other physical agents. Another variant was documented when Klieneberger-Nobel (1935) described L-forms of bacteria. She honoured the Lister Institute by using its initial letter to designate the aberrant stage of Streptobacillus moniliformis. Clinically, this variant might be considered a survival form in adverse conditions.

The International Conference entitled "Microbial Protoplasts, Spheroplasts and L-forms" held at Kalamazoo, Michigan, U.S. in 1966 opened with a session on nomenclature. Prolonged discussion provided no agreement on the meaning of commonly used terms and the result is that at this conference (Guze, 1968) and in some subsequent literature investigators have tried to avoid further confusion by defining their terms relating to L-forms. Examples of this situation can be seen with Kagan (1968) who commences "In the work to be described here the terms L-forms, protoplasts, spheroplasts and L-form variants have been considered to have essentially one and the same meaning" and with Charache (1968) who defines "the phrase atypical bacterial forms ... includes spheroplasts, protoplasts, L-forms and non-spherical forms", whereas Godzeski et al. (1967) state "the term L-phase bacteria is not used as the equivalent of the L-form bacteria".

McGee et al. (1971) make a strong plea for the acceptance and use of defined terms to assist the critical evaluation of published
work and to improve the design of experiments for studying wall-defective micro-organisms. Unless the recognizable differences of these micro-organisms are appreciated, speculations concerning the relationship of L-forms to the infection process may be ill founded.

Some clinical microbiologists, eg. Brem (1969), Conner et al. (1968) and Domingue and Schlegel (1970) when reporting isolations of L-forms from clinical material do not distinguish between stable and unstable L-forms. They rely on growth of typical L-colonies on agar and the subsequent production of classical bacteria to establish the presence of L-forms in the specimens from patients.

The geneticists, Wyrick et al. (1973) place emphasis on the difference between stable and unstable L-phase variants when they describe the genetic transfer of a character from a donor stable L-phase variant to a classical bacterial type resulting in the stable L-phase of the recipient.

The literature relating to microbial variants is marred by the indiscriminate use of the terms. Godzeski et al. (1957) point out that it is difficult to attract investigators into this area of research, and perhaps what is even worse, the literature has created a sense of uncertainty and even scepticism amongst readers of this discipline.

A discussion of the terminology follows to indicate the nuances in terms used by major workers.
4.2 TERMINOLOGY

4.2.1 L

Prefixed to any word this letter can relate to the morphology or cultural characteristics of bacteria which exhibit plasticity of shape due to defective cell walls.

L-body, L-colony, L-culture, L-cycle, L-element, L-form, L-phase, L-strain, L-transformation and L-variant are examples of this use. Only two of these terms, L-form and L-phase, have specialized meanings acceptable to microbiologists. In two consecutive sentences Klieneberger-Nobel (1960) uses three terms of which only the third has a precise meaning. "Thus, aging had favored the development of L-like colonies in S. moniliformis. By a process of repeatedly cutting out these L-type colonies and transferring them to new plates L-form cultures were obtained".

4.2.2 L-TYPE BACTERIA

This is a general term used by most microbiologists to denote organisms showing cell wall defects. L-types are pleomorphic wall-defective bacteria which occur spontaneously in bacterial culture (probably induced with toxic end-metabolites) or are induced with some known bacteriotoxic agent. Because they may or may not be capable of growth in this wall-defective state, this term includes non-propagating protoplasts and spheroplasts. The L-type phenomenon seems to be typical of most bacterial species.

4.2.3 L-FORM

This term initially coined by Klieneberger-Nobel in 1935, is used to describe any wall-defective variant which is capable of
dividing and giving rise to typical L-colonies on media supplemented with serum and osmotic stabilizers.

Many authors restrict the term L-form to organisms which can no longer revert to the classical bacterial form; so that L-form is synonymous with "stable L-form"; whereas the term L-variant refers to the "unstable L-form" (Feingold, 1969 and Godzeski et al., 1967).

Dienes (1968) who has been a giant in this field, does not use the term L-phase at all. He describes wall-defective bacteria in terms of L-forms of two different types, called A and B, which differ in morphology, in ultra structure and in the ability to resume bacterial form. He points out that the terms stable and unstable have no definite meaning because almost all L-forms are unstable after isolation from bacteria and then lose the ability to reproduce bacteria after long cultivation.

The term L-form will be used in this thesis to denote propagating L-types; the adjectives stable and unstable will be prefixed if reversion is known.

4.2.4 L-PHASE

The majority of workers use the term L-phase to describe the cell wall-defective, osmotically sensitive, propagating, bizarre forms of bacteria which are capable of reversion to the classical parental bacteria upon removal of the inducing agent. The L-phase has been called "transitional form", "soft-walled", "pleomorphic" and "aberrant".
Dienes does not use the term at all, but other workers such as Smith (1971) use the term L-phase as the non-reverting L-form of bacteria, and McGee et al. (1971) extend the use of the term L-phase to include stable and unstable L-phase variants.

4.2.5 PROTOPLAST

A protoplast is defined as the structure derived from a vegetative cell after removal of the entire outer cell wall by artificial means. It cannot reproduce or revert to its classical bacterial form (Klieneberger-Nobel, 1960). A group of 13 experts (Brenner et al., 1958) recommended that the term protoplast be restricted to this precise definition, and my thesis will follow the concept.

Weibull (1953, 1965) showed that lysozyme could induce protoplasts and that the phenomenon is restricted to Bacillus megaterium, a few other Gram-positive bacteria and yeasts. Protoplasts are spherical and discrete; they require osmotic protection or they lyse. The loss of characteristics one would expect to accompany the absence of the cell wall has been confirmed, viz., no positive staining reaction, absence of cell-wall antigens (Tomcsik, 1956) and failure to adsorb bacteriophage (McQuillen, 1960).

Feingold (1969) includes in his definition of protoplasts the need to test for any residual cell wall: "atypical bacterial forms which have no remaining cell wall as shown morphologically, chemically and by absence of phage receptor sites".

The word protoplast is borrowed from the botanical vocabulary where it serves to distinguish the living content of a plant cell from the
lifeless cellulose wall. Bacterial walls are not so defined either chemically or morphologically. Whereas most bacteriologists now agree to the Klieneberger-Nobel definition of a protoplast, the literature unfortunately contains a large number of reports of clinical conditions where so-called protoplasts have been induced by antibiotics and show variable ability to revert to the parent bacteria (Braude et al., 1961; Gutman et al., 1967). Any conclusions drawn from reports of this kind should allow for the possible mis-use of terms.

4.2.6 SPHEROPLAST

This term is used to describe those globular forms of bacteria in which the cell wall structure has been modified rather than totally removed. A term is sometimes prefixed so as to indicate the method of induction, eg. the "protoplasts" of Lederberg and St. Clair (1958) would now be called penicillin-spheroplasts. The definition does not specify multiplication (McQuillen, 1960). Dienes (1968) however considers the term spheroplast an unnecessary complication of nomenclature as spheroplasts do not differ from "large bodies" which have been described for 40 years. Authors do not use the latter noncommittal name at present, despite the opinion of Dienes that they are probably the connecting link both in the transformation of bacteria to L-forms and in the retransformation of L-forms to bacteria. Instead, the use of the term spheroplast has increased in the last decade and this thesis will make use of the term where applicable.

4.2.7 GYMNOPLAST

A term used by Stähelin (1954) and Colobert and Lenoir (1957) to denote the structure remaining when the cell wall is removed from
a bacterial cell. This term was suggested for use when proof of absolute loss of the cell wall (protoplast) was not evident. Fortunately the terminology of L-forms has not been further complicated by acceptance of this term.

4.3 GROWTH OF L-FORMS

4.3.1 L-MEDIA

The medium requirements for L-forms differ considerably from those of the parent forms.

(a) Inactivated serum at a concentration of 10-20% is usually required for L-forms from most of the bacterial species although those from Gram-negative rods require less serum. The precise role of serum is yet to be determined, but it is unlikely to be required as a source of nutrients because of the high concentration used. Hijmans et al. (1969) having failed to isolate a growth-promoting factor from serum, postulate that serum merely protects L-forms from toxic substances in the medium. After many subcultures most L-forms can grow without serum.

(b) An agar concentration between 0.7 and 1.2% in solid media and between 0.1 and 0.2% in liquid media or diphasic media, is required by L-forms. Agar is considered to give mechanical support to L-forms and no other substance has been found to substitute for it.

(c) Osmotic stabilizers such as salt and sucrose are essential for L-form growth in order to avoid cell lysis.
4.3.2 L-COLONY

The characteristic "fried egg" colony of L-forms is a small (0.3 mm diam.) round, umbonate colony with a dense centre, embedded in the medium, and having a clear, flat peripheral zone. It is independent of the parent bacterial colony, and will grow only on solid media supplemented with serum and osmotic stabilizers. Agar block preparation of colonies stain blue with Dienes' stain and can be examined by the oil immersion objective (Dienes, 1967). The colony is composed of three basic morphological elements. This colony form is not unique to L-forms since it is similar to many species of Mycoplasma. Relative differences include the larger size of L-colonies, slight differences in smoothness of colony outline and in granularity (Smith, 1971).

4.3.3 G-COLONY (GONIDIAL)

These are small colonies that can grow on media suitable for the parent species and consist of granules of various sizes, coccal forms, large globules and swollen or deformed bacteria. After several subcultures these colonies and elements revert to the characteristic bacterial species.

Hadley et al. (1931) described G-colonies of Shigella, and Quie (1969) has reviewed the G-variation in Staph. aureus. Klieneberger-Nobel (1951) postulates that the G-colony is the intermediate form between a bacillary colony and an L-colony. The G-colonies grow on standard media, therefore they do not fulfil one of the criteria of L-colonies.

4.3.4 PSEUDOCOLONIES

These are non-viable artifacts which mimic colonies of both mycoplasmas and L-forms. They can have a dense granular central mass surrounded by a swirl of fibrillar chains, showing a strong resemblance
to the so-called "fried egg" colonies. Smith (1971) considers the pseudocolonies are accumulations of sterols, together with insoluble calcium and magnesium salts of fatty acids.

As early as 1925, Laidlaw wrote describing the appearance of pseudocolonies on sterile agar, and there are now several reviews on the subject, eg. Hijmans et al. (1969) and Clasener (1972). The effect is noticed with:

(a) a high serum or ascites content in the media,
(b) the presence of cholesterol (eg. in one of the commercial mycoplasma media, Difco Bacto - PPL0),
(c) hypertonic media,
(d) pH of the medium above 7.4,
(e) incubation for some days at 37°C,
(f) media drying after prolonged incubation,
(g) use of inocula such as urine, blood and epithelial cells,
(h) mechanical disturbance on the surface of the media, eg. the "push block" method of subculture, or the addition of liquid.

Pseudocolonies are a hindrance in the isolation of L-forms from clinical material. Uninoculated incubated media that grow no pseudocolonies do not provide adequate controls for inoculated isolation media because it is often the inoculum that provides the nucleus for pseudocolony formation. Nor can transferability of the colonies always distinguish true from pseudocolonies, because mechanical disturbance is involved.
The prevention of pseudocolony formation is difficult. The use of agamma horse serum was suggested by Hayflick and Stanbridge (1967) and again by Hubbard and Kite (1971). Lowering the pH below 7.4 is advocated by Shepard and Lunceford (1965).

Despite all these measures, pseudocolonies do occur and must be differentiated from true colonies. Organick (1966) suggests that morphological differences seen with phase contrast microscopy and the failure of pseudocolonies to stain with either Dienes' stain or DNA stain can assist in the differentiation. Furthermore, some inhibitory measures for true colonies fail to inhibit pseudocolonies, eg. higher incubation temperature, incorporation of phenol, merthiolate or formalin in the media.

4.4 ELEMENTS

4.4.1 L-ELEMENTS

Three basic types of elements found in cultures from solid and liquid media are described from light and electron microscope studies (Freundt, 1969). Each of the types shows variation in size, and together they form a continuous range of diameters from the smallest (0.05μ diameter) to the largest element of 50μ diameter.

Large bodies are spherical structures of great plasticity, ranging in size from 1μ to 50μ in diameter. They contain a homogeneous cytoplasm or may be vacuolated and even transformed to empty blebs. The cytoplasm and vacuoles may contain granules.

Granules are round or elongated elements of size from 0.1μ to 1.0μ, and occur singly or in masses within or outside the large bodies.
Small elements (corpuscles) are spherical elements less than 0.3\(\mu\) in diameter; they are densely filled with ribosomes and surrounded by a cytoplasmic membrane. Recent studies have been directed towards estimating the DNA of these elements. Since Weibull and Beckman (1961) found no appreciable content of DNA, this strengthens the theory that the small elements cannot reproduce.

Dienes (1967) leads the school which considers that small elements develop and multiply inside large bodies, but Panos (1967) and others believe binary fission of elements greater than 0.6\(\mu\) in diameter is the mechanism of reproduction.

4.4.2 PSEUDELEMENTS

Artifacts resembling morphological elements of L-forms and mycoplasmas can develop in fluid media containing serum and tissue, and to a lesser extent on solid media. Electron-microscope studies can show a structural difference between pseudoelements and L-elements, but it is a difficult task if one is using phase contrast or oil immersion techniques.

Nelson (1958) and others who studied the formation of these artifacts have concluded that they consist of over 80% protein, plus DNA and lipid; and that their formation is an enzymatic process. Lederberg and St. Clair (1958) showed that nutrient gelatin agar is also a source of pseudoelements. Kang and Casida (1967) found that structures similar to large bodies in size, morphology and fluorescence-staining characteristics occur in various normal tissues and in egg yolk; since they could reconstruct their artifacts, they considered them to be accumulations of lipoidal cellular by-products, interacting with medium components which had assumed definite outer boundaries.
4.5 FILTRATION

The ability of L-types to pass through cellulose filters of pore size 0.45μ or less enables them to be separated from their parent bacteria. The filterability of L-elements appears to be due to their plasticity rather than to their size. van Boven et al. (1968) have made direct measurements with phase contrast microscopy. Two aspects of filtration have been studied:

(a) The comparison has been made of the numbers of filterable elements before and after filtration through membranes of varying porosity. This classical method based on the concept of a rigid particle (Elford, 1938) has not proved successful in estimating element size, due both to plasticity and to lack of viability of the smallest elements.

(b) L-forms have the ability to grow through cellulose membranes. Various pore-sized inoculated filters can be placed uppermost on solid media and the growth underneath examined by microscopy. The entire spore space is usually filled by L-form growth; larger elements can pass through small pores because filtration is slow and the elements are plastic.

4.6 CELL WALL

The bacterial cell wall is no longer considered to be an inert structure and architectural adornment, but rather a structure with physiological and biochemical function. The wall follows a general scheme with a major distinction between Gram-positive and Gram-negative bacteria; minor differences accounting for some characteristics of individual species.
Salton (1964), Martin (1966) and Hijmans et al. (1969) have discussed wall structure in terms of L-form induction and stability.

The bacterial wall has three basic layers.

(a) **The cytoplasmic membrane**

This unit of approximately 50 Å acts as the only cell-supporting membrane in protoplasts. It provides an osmotic barrier and to prevent lysis of the cell, media which effect osmotic counter-pressure are used, e.g. media incorporating NaCl, phosphate ions, succinate ions and sucrose. Stabilization of the membrane itself by magnesium ions (Lederberg and St. Clair, 1958) or spermine (Tabor, 1962) can reduce the osmotic effect.

(b) **The rigid layer**

There is a thin (30-60 Å), dense layer close to the cytoplasmic membrane which is responsible for rigidity. In Gram-positive bacteria the rigid layer is composed of several sheets, linked by short polypeptide chains. In Gram-negative bacteria the rigid sheet is a thinner two-dimensional structure. The biopolymer which confers rigidity is termed murein (Weidel and Pelzer, 1964) and consists of repeating "mucopeptide" or "polytidoglycan" units. These repeating units are composed of N-acetylglucosamine linked to muramic acid, to which is attached a tetrapeptide side chain. In Gram-negative bacteria the tetrapeptide contains alanine, glutamic acid and diaminopinic acid (DAP). Gram-positive bacteria contain lysine instead of DAP and cross linking is due to the presence
of a pentapeptide. The dissolution of murein by enzymes such as lysozyme or interference with its synthesis by antibiotics is the first stage in the production of wall-defective organisms.

(c) The plastic outer layer

In Gram-negative bacteria the plastic layer is composed of lipopolysaccharides and lipoproteins, whereas in Gram-positive bacteria teichoic acids and polysaccharides are present. This layer may be covered by capsular material, flagella or other material.

4.7 REPRODUCTION OF L-FORMS

Two conflicting concepts of reproduction occur in the literature.

(a) Binary fission

The division process in L-forms is essentially the same as in bacteria, i.e., binary fission where the cell mass is distributed over the daughter cells and, after separation, free new cells are produced. When the cell walls are defective, physical factors in the environment may allow unequal divisions producing elements with a wide range of sizes. As well, the divergent morphology observed under dissimilar experimental conditions can be explained by the different physical factors.

The observed incapacity of small elements less than 0.7 µ (van Boven et al., 1968) to reproduce would fit this theory if these elements with little or no content of DNA, are functionally "debris" (Hijmans et al., 1969).
(b) **Cyclical mechanism**

In the alternative cyclical concept a fundamental change from the binary fission of bacteria is envisaged. Small elements develop and multiply inside large bodies, which then disintegrate. The small elements later coalesce or multiply into large bodies, and so on.

Dienes (1967) and others have observed this phenomenon in solid media, i.e. with agar gel or inside porous filters but not in fluid media. The time-lapse cinematographic studies made by Gumpert and Taubeneck (1966) on L-form cultures suggest the physical pressure of agar fibrils and lack of pressure in adjacent spaces in the agar gel may account for "swellings" of bodies which are mistaken for growth.

The current weight of evidence favours reproduction by binary fission and the view that division proceeds irregularly, with environmental influences dictating the size of the elements produced.

4.8 **INDUCING AGENTS**

Inducing agents transform bacteria into L-types. This implies transformation into totally wall-deficient protoplasts, modified wall-deficient spheroplasts, stable L-forms and unstable L-forms (L-phase variants).

Induction has three requirements.
The first is the disappearance of the rigid cell wall. This can be achieved by the dissolution of the existing murein layer or by prevention of the formation of murein. Lysozyme has a broad spectrum, so is most widely used to dissolve the murein layer of bacteria. It catalyzes the hydrolysis of the bond between acetylmuramic acid and acetylglucosamine (Strominger and Ghuysen, 1967); even some Gram-negative species can be made sensitive to the action of lysozyme by the addition of EDTA (Repaske, 1958). The phage lysate of Group C streptococci (Maxted, 1957) can also dissolve murein. However, Hijmans et al. (1969) consider reports of the inductive effect of dissolution with antisera and complement may be dependent on lysozyme in the systems used by various workers.

Prevention of murein synthesis can be achieved by antibiotics which interfere with the cross-linkage between peptide chains of the murein. Penicillin is a structural analogue of part of the peptide chain of muramic acid and D-cycloserine is a structural analogue of D-alanine (Richmond, 1966). Bacitracin (Siewert and Strominger, 1967), vancomycin and ristocetin (Roberts, 1967) inhibit the utilization of lipid intermediates. Amino-acids such as glycine (Dienes, Weinberger and Madoff, 1950), autolytic enzymes (Falkow, 1957) and filtrates of antagonistic strains (Dienes, 1946) have all been implicated as inducing agents.
(b) The second requirement is prevention of osmotic lysis. The use of sera, NaCl and sodium succinate is described in the literature. However, there is a sharp difference between the osmotic value of the cell contents of the Gram-negative and Gram-positive bacteria. This parallels the ability to produce L-forms from most Gram-negative species when the NaCl concentration is about 0.5% whereas Gram-positive species require at least 2% (Hijmans et al. 1969).

Stabilization of the cytoplasmic membrane by the use of magnesium ions can minimize the osmotic effect.

(c) The third requirement is multiplication of the wall-defective, osmotically protected cells. It is expressed in yield as shown on solid and to a lesser extent in liquid media. Hijmans et al. (1969) describe the yield as the percentage of bacteria which enter the L-phase. Even when bacteria have been converted to 100% spheroplasts by penicillin, Scheibel and Assandri (1959) show only 0.0005% Clostridium tetani give rise to L-colonies; Hamburger and Carleton (1966) estimate a low 0.002-0.7% from staphylococci. The yield can reflect many variables such as concentration of the inducing agent, osmotic value of the bacterium, agar concentration, water content of the medium, anaerobiosis, type of serum used and growth phase of the bacterium.

4.9 REVERSION

Reversion to the parent bacterial form occurs with unstable L-forms (L-phase) as soon as the inducing agent is removed or
inactivated. Ryter and Landman (1964) and Ryter (1968) correlated the presence of mesosomes with the capacity to form cell walls. Since the elements of L-forms do not contain mesosomes these workers postulate membrane invagination as the primary factor in reversion, followed by wall and septation priming. On electron micrograph evidence, Landman and Halle (1963) and Landman (1968) have suggested that the physical properties of the media, e.g., agar fibrils, aid invagination of L-elements and this may be the crucial step in reversion.

With the stable L-forms, reversion has been found to occur after more than 200 subcultures (Schönfeld, 1961). Many attempts have been made to find a precursor of cell wall synthesis, without success.

4.10 MYCOPLASMA

Mycoplasma are the smallest (125-500μm) of the free living organisms and occur as parasites or commensals in most mammalian species, or as saprophytes in nature. The organisms are pleomorphic, lack a rigid cell wall, possess DNA and are inhibited by specific antibody. Growth on solid media is usually a characteristic "fried-egg" colony. This genus is defined because of the similarity to L-forms in morphology and colonial characteristics.

The first mycoplasma was isolated by Nocard and Roux in 1898, and although the term Mycoplasma was introduced in 1929, the organisms have been known by a variety of names including PPLD (pleuropneumonia-like organisms) and Eaton Agent. Kleneberger-Nobel (1935) postulated that the L-forms of Streptobacillus moniliformis were mycoplasmas but later conceded that whereas L-forms are always derived from bacteria, mycoplasmas are never derived from bacteria.
Edward and Freundt (1956) presented a classification scheme for the mycoplasmas, and this is the basis of various systems used. Stanbridge (1971) has reviewed the position of over 30 distinct species of mycoplasmas with the generic name *Mycoplasma*. They have been classified into two separate families.

(a) the sterol-requiring *Mycoplasmataceae*
(b) the sterol-nonrequiring *Acholeplasmataceae*

The T-strain mycoplasmas, so called because of the tiny colonies, are unclassified. In addition to their sterol requirement, they have a unique requirement for urea.

Eaton (1965) has summed up the possible relationship of mycoplasmas to other micro-organisms thus:

"The present knowledge of the group of large filterable organisms suggests an evolutionary continuum from bacteria through spheroplasts and protoplasts ..... to L-forms, PPLO (mycoplasma) ..... to rickettsia and psittacosis-like organisms".

4.11 **PERSISTENCE**

This is the ability of micro-organisms to survive in the presence of adverse environmental factors.

McDermott (1958) relates the survival of bacteria in vivo during antibiotic treatment to inaccessibility to drugs, to genotypic antibiotic resistance or to bacterial plasticity (induced L-forms).
The consensus of opinion is that L-forms induced in vitro are not virulent unless they revert to the parent bacteria which are themselves virulent. In fact, the cell wall-defective L-forms are more vulnerable to the environment than their parents because the associated wall-defective characters of mechanical and osmotic fragility make their survival difficult.

The persistence of bacterial antigens found in chronic urinary tract infection when no bacteria can be recovered has focused attention on the hypertonic renal medulla as one of the few possible survival sites of L-forms in vivo (Clasener, 1972). So far, there are few convincing reports relating persistence of bacterial infection to L-forms, and the present thesis further discounts this view.

4.12 L-FORMS IN VIVO

4.12.1 INDUCTION

The main agents inducing wall-defective bacteria in vitro are some of the antibiotics, enzymes and antisera. They are the subject of many reviews; however the analogy with the in vivo situation can only be speculative as the experimental evidence is not convincing.

As the great variety of antibiotics are capable of producing L-forms in most bacterial species, it is reasonable to postulate that they do the same in vivo. Schmitt-Slonska and Lucel-Varnier (1969) report in vivo induction of streptococcal L-forms with penicillin, but other laboratories have failed to reproduce this work as well as other reported inductions.

Enzymes such as lysozyme, leucozyme, acid phosphatase and phospholipase acting alone, or synergistically with amino acids or with
antibiotics, can produce wall-defective bacteria in vitro from Gram-positive (McQuillen, 1960), Gram-negative (Weibull, 1958) and acid-fast bacilli (Willett and Thacore, 1966). It is a temptation to postulate that these bacteria when phagocytized in vivo might not be killed but induced to L-types by the enzymes. Work with staphyloccocal enzymes and bacteriophage has been promising; Schaffner et al. (1967) appear to have shown induction in vivo with these agents.

Specific antibodies with complement can induce L-forms in vitro (Dienes et al., 1950); and Carey et al. (1960) report in vivo induction. The observations of Prockop and Davidson (1964), and Myrvik and Weiser (1951) should not be forgotten, viz. that during some infections and in response to certain antigens the lysozyme concentration in tissue, blood and urine may rise to levels high enough to induce L-forms, at least, in vitro. The in vivo level is not yet established.

Conversion to L-forms is also determined by several characteristics of the bacteria. Mortimer (1965) has shown that streptococci of low virulence are more readily induced in vivo than those of high virulence, and that reverted bacteria are more readily induced than the original parent and do so in response to a wider range of agents.

4.12.2 PERSISTENCE

The survival of L-forms in vivo has been studied in normal and immunized mice (Wittler, 1952) and the indication is that L-form survival is enhanced in the immune host despite in vitro evidence of inhibition of L-form growth by specific antibody and nonspecific serum factors. Organisms with cell wall-deficiencies may survive in the presence of specific opsonins to cell wall or capsule, and withstand ingestion and
destruction by phagocytes (Jenkin, 1963). Mims (1964) has shown that smaller particles are cleared more slowly than larger particles in the reticulo-endothelial system, suggesting L-forms may have a survival advantage over their parent bacteria in vivo.

The more recent work of Haller and Lynn (1968) has shown that L-forms of Strep. pyogenes do not survive in diffusion chambers implanted in rabbits, nor do enterococci in the mouse kidney (Watt, 1970). Klieneberger-Nobel's observation (1962) that stable L-forms are a laboratory phenomenon has not yet been convincingly disproved.

4.12.3 PATHOGENICITY

As the biochemistry of L-forms is essentially identical to the parent bacteria, the factors which determine pathogenicity except for cell wall determinants are probably similar. Mechanisms which have been studied are the production of cellular and extracellular toxins from Clostridium tetani (Scheibel and Assandri, 1959), Vibrio sp. (Minck, 1951) and the production of M protein by streptococci (Mortimer, 1965). L-forms can produce experimental hypersensitivity (Kagan et al., 1964) and this has led to the search for L-forms in naturally occurring hypersensitivity diseases. Winterbauer et al., (1967) have shown that unstable L-forms injected into animals produce pyelonephritis but when the reversion to the parent bacteria is prevented there is no infection produced.

The traditional notion of pathogenicity when applied to L-forms per se is in doubt as L-forms are not convincingly demonstrated in diseased tissue nor demonstrated to initiate disease. McGee and Wittler (1967) suggest widening the concept of "pathogenicity" to include organisms such as L-forms of pathogenic bacteria because they can
maintain residence in a host under conditions (eg. antibiotic therapy) normally adverse to pathogenic bacteria. When the inducing agent (antibiotic) is removed, these L-forms revert to the pathogenic bacteria and the disease process is re-established.

4.13 CRITERIA FOR ISOLATION AND IDENTIFICATION

The following criteria have been used in this thesis to evaluate the literature pertaining to L-forms in urinary tract infection and in designing isolation methods for L-forms from the urine of patients with spinal injuries.

(a) A knowledge of the characteristics of the different L-types is important, eg. protoplasts cannot replicate as such, and are sensitive to changes in osmolality whereas L-forms can replicate and those from Gram-negative bacteria are more resistant to changes in osmolality.

(b) False positive isolations.

The appearance of pleomorphic bacterial forms in clinical specimens or even immunofluorescence demonstration of these forms is not proof of the existence of L-forms; they could be fragments of degenerating bacteria.

Growth of L-forms in vitro does not constitute proof of existence of these forms in vivo. Bacteria can undergo conversion on media if the specimen contains host lysozyme or antibiotics.
Bacteria may exist in the classical phase in a diseased site and undergo conversion to L-forms when shed into blood, urine and other body fluids, thus L-forms isolated from a source distant from the site of the lesion is not proof of the pathogenic role of the L-forms.

The media and techniques employed in culturing L-forms favour accidental contamination with fastidious, pleomorphic species, e.g., *Mycoplasma* sp.

The production of artifacts in liquid and on solid media, and even the "transferability" of colonies from solid to fresh media is a common occurrence.

(c) False negative isolations.

Recently induced L-form colonies often require many transfers to give regular and dependable growth, hence frequent subcultures must be made before a negative isolation is reported.

(d) Ideally, identification of the isolated L-form requires:

Visualization of L-types in specimens of the diseased tissue.

Separation by filtration of the L-type from contaminating bacteria.

Evidence of its viability by growth.

Isolation of the same species on more than one occasion from the same patient.

Identification of the parent species after controlled reversion, or by immunologic or molecular-genetic techniques.
Immunologic evidence in the patient of infection with the particular species.
Exclusion of the isolation being a contaminant by knowledge of the aetiological agents in the disease process.
Control media used and handled identically with each technical step in the isolation method.

4.14 L-FORMS IN URINARY TRACT INFECTION

The possible role of L-forms in urinary tract infection has attracted the attention of many workers because of the following facts:

(a) Relapse or episodic bacteriuria with recurrence of the same organism is frequent.
(b) In some chronic pyelonephritis no bacteria are isolated.
(c) Concentration of lysozyme in the kidney and urine reach a level high enough to induce L-forms in vitro.
(d) The renal medulla provides the hypertonicity needed to stabilize L-forms.
(e) Antibiotics capable of inducing L-forms are used in the treatment of urinary tract infections and are concentrated in the kidney.

Investigations have been directed towards assessing the stability of the urinary tract pathogens to osmolality, urea concentration, pH, glucose concentration and spermine concentration in urine. Other workers have attempted to isolate L-forms from urine and another group has experimented with mice and rats in an attempt to establish infection with L-forms. These studies have been reviewed by Wittler (1968), McGee and Wittler (1969), Sharp (1970) and Clasener (1972).
The first approach is illustrated by Gnarpe and Edebo (1967) who showed that penicillin-induced spheroplasts of _E. coli_ and _Pr. vulgaris_ remain viable in hypertonic urine, and acidification to pH 5 provides almost the same protection for these organisms as a 0.5M sodium chloride concentration. Braude _et al._ (1968) demonstrated that without sodium chloride, urea was ineffective in preventing lysis of spheroplasts of _E. coli_ and _Pr. mirabilis_. Watt (1970) found that osmotic protection by the addition of sodium chloride to give 750mOsm/kg was required to support the growth of penicillin-induced _L_-forms of _Strep. faecalis_ on serum agar medium. Spermine or acidification of the medium did not reduce this requirement.

The second approach described from many laboratories has been attempts to isolate _L_-forms from the infected human urinary tract and has applied the conclusions drawn from the work reviewed before. One way the problem has been tackled is to culture unfiltered urine in parallel with 0.45μ filtrates of the same urine protected osmotically. Comparisons are then made between isolates of classical bacteria and _L_-forms as well as revertants appearing after successive subcultures made up to one month. Conner _et al._ (1968) did not increase the osmotic value of the urine specimens, relying on early morning urine which has a high osmotic value; did not filter urine if it was relatively free of bacteria; used only 0.1ml of urine as inoculum, did not inactivate horse serum despite its implication in pseudocolony formation. They could not differentiate between blood cells and spheroplasts. This report lacks any quantitative colony data, photographs of _L_-colonies, and investigation of antibacterial activity of the urine. Of the 100 cases studied, 19 had _L_-forms grown from urine but 12 of these had "antibiotic therapy just before cultures were made". This report does not satisfy all the criteria one would desire.
Gutman et al. (1965) discount growth in L-broth or on solid L-media unless supported by microscopic colony formation and reduction of tetrazolium-red indicator in the medium. Unfortunately there are no photographs of stained surface colonies from the 20% of pyelonephritis patients from whom isolates are claimed. These workers consider isolations obtained from the site of infection important; they isolated classical \textit{E. coli} from a kidney biopsy and L-forms from the urine. However, from another patient, also having ampicillin therapy, L-forms were recovered from the kidney. Reversion to \textit{P. mirabilis} was claimed after incorporation of serial serum concentrations in media and also after passage in rats. Domingue and Schlegel (1970) reject animal passage as a medium prior to reversion because of the possibility of contamination. They produce convincing photographs of L-colonies, and claim a 20% isolation of L-forms from the urine of pyelonephritis patients compared with no isolations from normal individuals and other urinary tract problem patients, as distinct from pyelonephritis. This work lacks details, eg. media, time of antibiotic therapy and although the authors state "infected urine may contain antibiotics, antibodies, lysozyme, glycine and bacteriophage", there is no attempt to establish the presence of any of these. All the workers quoted conclude that L-forms are usually recovered in a higher proportion from urine where spheroplasts are observed, than from other urine.

Another aspect which has bearing on my own experience is a report from Kalmanson and Guze (1968) who processed renal biopsy material and showed that bacterial colonies could be isolated on enriched and osmotically protective medium but not on standard medium. Their conclusions that these were revertants from L-forms is not persuasive because the identified species were unusual urinary tract pathogens, and control media with penicillin to prevent revertants was not used.
Swierczewski and Reyes (1970) do not produce convincing evidence to distinguish their L-form isolates from artifacts or *Mycoplasma*. Csonka *et al.* (1967) have shown that *Mycoplasma* can be isolated from the urine in 10% of healthy subjects, so the distinction is pertinent when revertants are not obtained.

Young and Armstrong (1969) and Mortimer (1965) report that though L-forms in broth culture are able to pass through 0.45µ filters, it is an inefficient process leading to a loss of over 99% of colony forming units. This quantitative aspect of colony formation before and after filtration has not been used as evidence of L-forms in biopsy material or urine. Unfiltered urine could be so diluted to quantitate L-colonies even in the presence of bacterial contamination.

The third approach to the problem of pathogenicity has been to induce L-forms in animals or to inoculate animals with *in vitro* induced L-forms. Young and Dalquist (1967) injected rabbits intravenously with a lethal dose of *Staph. aureus* and after penicillin therapy found the kidney and urine the only site of bacterial or L-form survival. The renal milieu of the rabbit appears to provide osmotic stability for L-forms. In rats and mice the picture is not so clear. Guze and Kalmanson (1964) using penicillin to treat rats suffering from enterococcal pyelonephritis, recovered a greater number of bacteria on osmotically protective media than on standard media, but failed to grow L-colonies. Their inference is that the revertants were also growing on the special media. Watt (1970) failed to produce experimental enterococcal pyelonephritis in mice with a high inoculum
of stable L-forms. He concludes that the lysis of the L-forms could be attributed to the lack of any system in mouse tissues capable of giving osmotic stability to L-forms. After investigating phagocytosis and specific immunity he concludes that L-forms of enterococci are not virulent for mice.
4.15 RESULTS

Consecutive monthly examinations of the urine of 39 spinally injured patients failed to isolate any L—forms. Pleomorphic forms resembling L—forms were not seen in urine when examined under phase contrast microscopy.

4.15.1 METHOD OF ISOLATION

The reasons for the failure to isolate L—forms were investigated. Each step in the isolation method (Table 4) was tested using in vitro penicillin—induced spheroplasts of bacterial isolates from the patients. Since the same organism or organisms were regularly isolated from each patient, it was possible to induce spheroplasts and use their survival and propagation as criteria to validate the method of transporting urine to the laboratory, the filtration of urine in the presence of large numbers of white blood cells and bacteria, and as well, the method of culture.

(a) Spheroplasts were added to urine with and without the sucrose osmotic stabiliser. In some cases, the spheroplasts disappeared when placed in urine without sucrose, but in all cases the spheroplasts survived in the osmotically protected urine. The rise in osmolality appeared to be the factor responsible for survival of spheroplasts at this stage.

(b) The second step in the isolation method, that of filtration, was investigated by filtering the induced spheroplasts and culturing the filtrate on sucrose L—form solid medium with and without added penicillin and on classical bacterial medium (MacConkey). Growth of revertants was seen on the sucrose L—form medium without penicillin. L—colonies grew on this medium with penicillin. There was no growth detected on MacConkey agar. It was considered that the use of the
0.45μ membrane was satisfactory as it allowed L-forms to pass through but held back the bacteria.

(c) The third factor investigated was the media and conditions of culture. Kalmanson et al. (1968) had reported "serum bactericidal activity against protoplasts" by a range of mammalian sera and had found that increased osmolality or inactivation of sera reduced the bactericidal effect. Human, horse, bovine and foetal calf sera, with and without heat inactivation were incorporated into the primary isolation solid medium. Patient-derived spheroplasts were filtered and cultured on media with and without penicillin. Foetal calf serum without inactivation was chosen as the serum ingredient as it proved satisfactory for these in vitro L-forms. However as the study of isolation of L-forms from the urine of patients progressed without producing L-forms and as in vivo variants may require other conditions, the secondary isolation media were used in addition to the primary media. The media have been described in Section 2. The atmospheric conditions of incubation were tested and incubation under 10% carbon dioxide was chosen as slightly better than aerobic or anaerobic conditions.

The method of isolation was considered to be suitable for any L-forms which might have been in the urine of the patients examined.

4.15.2 PARALLEL EXAMINATION OF NON-SPINAL PATIENTS

A parallel study was made of non-spinal patients who were receiving chemotherapy for urinary tract infection, and from whom it might reasonably be expected to isolate L-forms. Appendix 2 identifies these patients.
A group of 26 female patients presenting with urinary symptoms to a medical practitioner were treated with nitrofurantoin 50 mg four times daily. The aetiology and treatment of their urinary tract infection has been reported by Dawborn et al. (1973). Three weeks after commencement of treatment 16 of the 26 patients who were examined for classical bacteria and L-forms were considered to be free of infection. Of the remaining 10 patients, the filtrate from the urine of 2 patients, inoculated into L-broth, showed growth after 3 successive subcultures. Phase contrast microscopy showed structures resembling L-elements. However it was not until the sixth serial subculture that revertants could be grown on solid L-media. L-form colonies were not grown. In the case of J.C., Strep. faecalis was isolated and the revertant from M.R. was identified as E. coli. Not all the criteria for classification of these two isolates as L-forms has been met viz. growth of L-colonies on solid medium and controlled reversion to the parental type with an associated change in the antibiotic sensitivity.

A second group of 13 patients who had recurrent urinary tract infection and were managed by urologists, had urine examined at irregular intervals. All these patients had been treated with antibacterials including ampicillin, nitrofurantoin, nalidixic acid and a sulphonamide-trimethoprim mixture. It was with this group that many of the difficulties of L-form isolation were encountered.

Early in the study it was difficult to distinguish pseudocolonies from L-colonies. A technique was developed to facilitate this differentiation. It consisted of irradiating portion of an inoculated
plate with ultraviolet light and comparing the irradiated with the unirradiated surface. Mycoplasmas and L-colonies did not grow after U.V. irradiation, whereas pseudocolony formation was unaffected.

Examination of urine under phase contrast microscopy was not definitive in differentiating L-forms from leucocytes. However the daily L-form broth subcultures were also examined under phase contrast and from 5 of these patients, irregular structures were seen. The identity of these structures was confirmed after growth on the appropriate solid media. The results are listed below:

Patient B.M. T-strain mycoplasma. Figure 15 shows a colony stained with carbol fuchsin.

Patient D.H. Classical mycoplasma. Figure 16 shows colonies photographed on the solid medium.

Patient E.K. Haemophilus vaginalis from urine and from homogenised kidney.

Patient D.M. Ps. aeruginosa L-form. The spheroplasts could be seen in the urine, and both L-colonies and parental type grew on solid medium without penicillin after subculture from the first L-broth (depicted on the flow sheet, Table 4).

Patient S.B. E. coli L-form. Figure 17 shows the L-colonies amongst the parental revertants after subculture from the first L-broth.

The isolation of H. vaginalis could be accounted for because of the small size (0.7 x 0.3 μ) and therefore there was the possibility of this organism passing through the membrane. It required 3 days for visible
FIGURE 15

(Magnification 1600x)

T-strain mycoplasma colony showing the vacuolated large bodies. This is a cover-slip impression of a colony, fixed with methanol and stained with carbol fuchsin. (Patient B.M., Appendix 2)
Classical mycoplasma colonies, growing on sucrose L-form medium, after 48 hours incubation under 10% CO₂. The so-called "fried egg" appearance is similar to L-form colonies. (Patient D.H., Appendix 2)
FIGURE 17
(Magnification 400x)

L-colonies (arrowed) of *E. coli* growing amongst confluent parental *E. coli* growth, on sucrose L-form medium after 48 hours, aerobic incubation. (Patient S.B., Appendix 2)
growth on first isolation. The luxurious ingredients in the L-media may have promoted the growth of this organism which could have been missed on the routine media used (blood agar and MacConkey agar). The two patients for whom L-form isolations are claimed, did show pleomorphic forms in urine and antibiotics were received at the time of examination. Although L-forms were recovered from the urine of these patients with renal infections no inference can be drawn that the L-forms were from the renal medulla. The urine sampled from the bladders of these patients had conditions which would allow induction of L-forms viz. pH 6.5-6.7, osmolality of > 850 mOsM/kg and antibacterial activity due to ampicillin. In fact it was demonstrated that when a broth culture of the E. coli from patient S.B. was introduced into a mouse by the intraperitoneal route, followed by penicillin 500 units/ml, the peritoneal fluid contained spheroplasts within 4 hours.

The purpose of this investigation of L-forms in selected patients with urinary infection who were receiving antibacterials, was to test the processing method. As a result of the isolations reported here, the method was considered satisfactory. However it required time and patience and could not be regarded as suitable for routine work in a diagnostic laboratory.
4.15.3 FACTORS IN URINE OF SPINAL PATIENTS

As the isolation method for L-forms was successful in the survey of non-spinal patients, factors which might inhibit L-form induction and survival were sought in the urine of spinal patients.

There were three characteristics of the urine:

- Low osmolality
- Low pH
- Presence of formaldehyde

The osmolality of urine in normal healthy adults has been estimated by Isaacson (1960) to range from 900-1400 mOsm/kg. Reeves and Brumfitt (1968) have shown impaired urine concentration in patients with bacteriuria. The osmolality of the urine from 20 spinal patients ranged from 90-600 mOsm/kg when urine sampled at 8 a.m. was tested on 14 consecutive days. The fluid intake ranged from 150-200 ml per hour except for 2-hour intervals at night, and the output was 2500-3500 ml per 24 hours. The result of the regular intake and constant urine drainage was reflected in small variations in a particular patient's urine osmolality.

Acidification with ammonium chloride was found to reduce the pH of the patients' urine to 5.5-5.7 and with ascorbic acid to 5.7-6.2. The patients were treated with methenamine mandelate and from the measured total formaldehyde concentration of 500-900 µg/ml, they achieved a methenamine value of only 390-500 µg/ml. This is far short of the 1000 µg/ml estimated by Pearman and England (1973) to be necessary for bactericidal action.
An experiment was carried out to test the hypothesis that spheroplasts may not be induced in conditions combining low pH and formaldehyde. The results of induction of spheroplasts from 4 species isolated from spinal patients is shown in Table 16. It can be seen that L-forms are induced at a much less rate as the pH is lowered. With methenamine mandelate therapy, the lower the pH, the greater the concentration of formaldehyde, so that with success in acidification the induction of L-forms would fall.

Figures 18 and 19 are preparations of induced spheroplasts seen under phase contrast microscopy. They show the reduction in number and the incomplete formation of the L-forms when the pH is reduced and when formaldehyde is present in the induction medium.
### TABLE 16

<table>
<thead>
<tr>
<th>INDUCTION BROTH</th>
<th>E. coli</th>
<th>K1. aerogenes</th>
<th>Pr. mirabilis</th>
<th>Ps. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 5</td>
<td>50</td>
<td>10</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>pH 5 with HCHO</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>pH 6</td>
<td>70</td>
<td>10</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>pH 6 with HCHO</td>
<td>40</td>
<td>—</td>
<td>5</td>
<td>—</td>
</tr>
<tr>
<td>pH 6.5</td>
<td>90</td>
<td>30</td>
<td>80</td>
<td>40</td>
</tr>
<tr>
<td>pH 6.5 with HCHO</td>
<td>50</td>
<td>20</td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>

**Induction broth:** sucrose L-form broth with penicillin 500 units/ml

**HCHO:** methenamine mandelate 500 μg/ml

**pH:** adjusted with ascorbic acid

**Spheroplasts:** counted after 6 hours under phase contrast microscopy
Comparison of penicillin-induced spheroplasts of *E. coli* using induction broth at pH 6 with and without formaldehyde, shown under phase contrast microscopy.
Comparison of penicillin-induced spheroplasts of *Pr. mirabilis* using induction broth at pH 6 with and without formaldehyde, shown under phase contrast microscopy.
4.15.4 REASONS FOR FAILURE TO ISOLATE L-FORMS FROM URINE

The reasons for failure to isolate L-forms from the urine of the patients from the Austin Spinal Unit are now proposed:

Patients were not receiving antibacterials (except methenamine mandelate) prophylactically.

Bizarre forms were not observed in the urine of the patients at each monthly examination.

The presence of formaldehyde associated with an acid urine prevented the induction of osmotically fragile L-forms.

The osmolality was maintained at a level low enough to lyse any L-forms which might form.

The conclusion drawn from this section is that L-forms do not account for persistence of bacterial infection in the patients treated at the Austin Spinal Unit.
SECTION 5

SELECTION OF URINARY PATHOGENS
5.1 INTRODUCTION

Human urine is considered to lack the humoral and cellular defensive mechanisms which other body fluids possess (Asscher et al. 1968). Lysozyme and immunoglobulin have not been reported in urine. Moreover, the hyperosmolar urine renders phagocytes ineffective and the presence of ammonium ions interferes with the action of complement. Human urine readily supports bacterial growth. Over 75% of urinary infection seen in general practice is due to \textit{E. coli}.

Section 3 has shown that \textit{E. coli} was isolated from the spinal patients less frequently than other species and that those strains of \textit{E. coli} which did survive in the urine possessed resistance to a number of antibacterials.

Section 5 is a study to investigate the hypothesis that the initial infecting organisms are selected from a range of potential urinary tract pathogens, by a combination of medication and ward procedures which directly affect the urine of the patient. The persistence of a particular strain is partly accounted for by inadequate therapy coupled with routine bacteriological techniques not sensitive enough to confirm eradication of the strain; and above all the deliberate policy of using chemotherapy as little as possible.

During the period of this study 1970-74, the majority of the patients received all the measures listed below:

(a) Antibiotic therapy (usually penicillin or ampicillin) for associated injuries.
(b) Chlorhexidine, prior to insertion of a Gibbon catheter, to wash the external meatus and irrigate the urethra. One-use only chlorhexidine lubricant was used on the catheter.

(c) Connection of catheter to a drainage bag which did not contain disinfectant (prior to 1973).

(d) Emptying of the drainage bag which involved breach of the closed drainage system (prior to 1973).

(e) Twice daily chlorhexidine bladder irrigation which involved breach of the closed drainage system and spraying the catheter junction with alcoholic chlorhexidine aerosol.

(f) Monitoring of high intake and output of fluids to achieve an output of 3000 to 3500 ml of urine per 24 hours.

(g) Lysol (cresols) to disinfect the anal area.

(h) Administration of ammonium chloride or ascorbic acid (1974) to acidify the urine and methenamine mandelate or methenamine hippurate to provide a disinfecting concentration of formaldehyde in the urine.

5.2 RESULTS AND DISCUSSION

5.2.1 ANTIBIOTIC THERAPY

As mentioned above, penicillin and ampicillin were the drugs of choice when a patient was admitted. 80% of all urinary isolates during the years of study were found to be resistant to ampicillin.

An investigation of urinary isolates for the presence of transferable resistance factors (R factors) was undertaken in two of the species - *E. coli* and *Kl. aerogenes*. Reference was made to the
work of Datta (1969), Davey and Pittard (1971) and Anderson et al. (1972).
The female mutant of E. coli K12 strain JP990 kindly supplied by Davey
and Pittard was the recipient for ampicillin, kanamycin and neomycin
resistance factors found in the isolates from 6 patients.

Table 17 sets out the organisms isolated from one of these
patients (97310) and indicates the time when the resistant organisms
were first found.

**TABLE 17**

**ISOLATION OF ANTIBIOTIC RESISTANT SPECIES**

<table>
<thead>
<tr>
<th>Weeks after admission</th>
<th>Species Isolated</th>
<th>Source of Species</th>
<th>M.I.C.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ampicillin</td>
</tr>
<tr>
<td>1 E. coli</td>
<td>Faeces</td>
<td>&lt;20</td>
<td>&lt;12.5</td>
</tr>
<tr>
<td>2 E. coli</td>
<td>Faeces</td>
<td>&lt;20</td>
<td>&lt;12.5</td>
</tr>
<tr>
<td>3 E. coli</td>
<td>Faeces</td>
<td>&lt;20</td>
<td>&lt;12.5</td>
</tr>
<tr>
<td>4 E. coli</td>
<td>Faeces</td>
<td>&lt;20</td>
<td>&lt;12.5</td>
</tr>
<tr>
<td>Serratia</td>
<td>Urine</td>
<td>200</td>
<td>12.5</td>
</tr>
<tr>
<td>Ps. aeruginosa</td>
<td>Urine</td>
<td>300</td>
<td>50</td>
</tr>
<tr>
<td>5 E. coli</td>
<td>Faeces</td>
<td>&lt;20</td>
<td>&lt;12.5</td>
</tr>
<tr>
<td>6 E. coli</td>
<td>Faeces</td>
<td>&gt;300(R)</td>
<td>300(R)</td>
</tr>
<tr>
<td>K1. aerogenes</td>
<td>Faeces</td>
<td>&gt;300(R)</td>
<td>&gt;300(R)</td>
</tr>
<tr>
<td>7 E. coli</td>
<td>Nose</td>
<td>&gt;300(R)</td>
<td>300(R)</td>
</tr>
</tbody>
</table>

R = transferable resistance factor
A similar finding was observed in patient 102056 whose organisms acquired resistance to ampicillin, kanamycin and neomycin after 5 weeks in the admission ward. During the third week, Acinetobacter was present in the urine and was found to be resistant to ampicillin, carbenicillin, cephalothin, gentamicin, kanamycin, neomycin, nitrofurantoin and streptomycin. Unsuccessful crosses were carried out with this strain of Acinetobacter using Ps. aeruginosa PA02 (from Holloway, personal communication) as the recipient. It is tempting to speculate that the ubiquitous Acinetobacter is the source of many R factors.

The conclusion drawn from this sub-section is that the majority of urinary tract pathogens isolated from the Spinal Unit were resistant to ampicillin, a finding not unexpected because this antibiotic is used for associated injuries. R factors were found in isolates but as shown in Section 3, specific multiple resistant strains could not be found in endogenous sources before their isolation in the urine. However the fact that R factors are present in the endogenous flora suggests that characterisation of a strain by resistance is not sufficient evidence of dissimilarity.

5.2.2 BACTERICIDAL ACTIVITY OF CHLORHEXIDINE AND LYSOL

The bactericidal activity of chlorhexidine and lysol at the concentrations used in the procedures listed above, was investigated using a surface test with bacteria suspended in whole blood and dried on glass carriers (Rubbo and Gardner, 1965). The test organisms were isolates from the urine of patients and two standard strains were included. The Minimal Inhibitory Concentrations of chlorhexidine were
also ascertained. Table 18 summarises the findings.

**Table 18**

**Bactericidal Activity of Lysol and Chlorhexidine**

<table>
<thead>
<tr>
<th>Species</th>
<th>Time (minutes) for complete kill</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.2% acq. lysol</td>
</tr>
<tr>
<td>E. coli</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Strep. faecalis</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Staph. aureus NCTC 4163</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Acinetobacter anitratus</td>
<td>15</td>
</tr>
<tr>
<td>Kl. aerogenes</td>
<td>10</td>
</tr>
<tr>
<td>Pr. mirabilis</td>
<td>10</td>
</tr>
<tr>
<td>Ps. aeruginosa</td>
<td>15</td>
</tr>
<tr>
<td>Ps. aeruginosa NCTC 6749</td>
<td>15</td>
</tr>
</tbody>
</table>

It can be seen that *E. coli*, *Strep. faecalis* and *Staph. aureus* are sensitive to the concentrations of these two disinfectants used on the skin and mucous membranes of the patients. *Kl. aerogenes* is relatively sensitive to chlorhexidine. *Pr. mirabilis*, *Ps. aeruginosa* and *Acinetobacter anitratus* are resistant to chlorhexidine for such periods which would allow survival after disinfection procedures. The survival of *Acinetobacter anitratus* in the bladder irrigant chlorhexidine solution is explained by the high M.I.C.
Lysol, a mixture of o-, m- and p- cresols (Sykes, 1965) is probably effective in reducing *E. coli* and *Strep. faecalis* in the anal area but the other resistant species were frequently isolated on bedding and therefore may be selected and survive on the skin and bedding of the patients.

5.2.3 URINARY DISINFECTANT

The patients' urine was examined to determine the concentration of formaldehyde achieved after ascorbic acid and methenamine mandelate medication. The concentration was lower than the optimal amount recommended by Pearman and England (1973). Table 19 sets out the findings from the examination of 5 patients. An inter-relationship appears to exist between the amount of fluids excreted and the acidification achieved, and this in turn determines the amount of formaldehyde produced in urine. The estimation of osmolality was performed as an added guide to urine dilution.

The pH was estimated using a pH meter, and various pH papers as described in Section 2. The recommendation of Tolhurst et al. (1972) was confirmed, viz. that litmus paper is not to be recommended; in fact Multistix was not accurate enough for an important estimation. When formaldehyde concentration is dependent on dosage and acidity, pH estimation is important. A pH meter estimation of 6.5 was recorded by the nursing staff as 6.0. A high quality paper manufactured by Merck gave a similar level as with the pH meter.
**TABLE 19**

**INTER-RELATIONSHIP OF FLUID OUTPUT AND FORMALDEHYDE LEVEL**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Urine Output over 24 hours (ml)</th>
<th>Analysis of Urine Sampled at 0600 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Osmolality mOsm/kg</td>
</tr>
<tr>
<td>136813</td>
<td>2220</td>
<td>420</td>
</tr>
<tr>
<td>137060</td>
<td>4210</td>
<td>137</td>
</tr>
<tr>
<td>137734</td>
<td>3280</td>
<td>240</td>
</tr>
<tr>
<td>137813</td>
<td>2450</td>
<td>445</td>
</tr>
<tr>
<td>138872</td>
<td>2850</td>
<td>271</td>
</tr>
</tbody>
</table>

Patients received 2 mg ascorbic acid and 4 mg methenamine mandelate daily.

Figure 20 illustrates the method used to detect antibacterial activity in the urine of the spinal patients. The free formaldehyde in urine did not produce a zone of inhibition. It was considered that the amount of formaldehyde being achieved in the urine was such that the species of *E. coli* was usually prevented from establishing infection, whereas other species were unaffected. The need for a method which could be used to measure free formaldehyde concentration in routine bacteriology laboratories, hospital wards or by patients at home, was evident. A method to detect total formaldehyde after acid hydrolysis was investigated.

Figure 21 depicts the method developed. The complexity can be equated with the glucose estimation in urine performed by diabetics.
Measurement of Antibacterial Activity in Urine

This method depends on the inhibition of growth of Bacillus subtilis seeded in the media, by the antibacterial activity of urine contained in small discs placed on the surface of the medium.
FORMALDEHYDE IN URINE

DIRECTIONS

1. Put hot water into large test tube, up to the red mark.

2. Insert capillary tube into its bulb, dip tube into urine and allow urine to fill to the ½ way mark on the capillary tube.

3. Place finger on hole in bulb and transfer urine to large tube. Squeeze bulb and urine will flow into hot water.

4. Using the dropper in the formaldehyde reagent, add drops to large tube until the blue mark is reached.

5. Put clean finger on top of tube and shake 2 or 3 times. Allow tube to stand for 30 minutes.

6. Compare the colour in the tube with the colour chart and record the result.

COLOUR CHART

NEGATIVE 250ug/ml 500ug/ml 1000ug/ml

FIGURE 21

A method for use by patients at home or staff in a hospital ward.
The accuracy of the colour chart match was assessed by comparison with readings on the colorimeter and was found to be excellent for the concentrations selected.

Using the levels obtained in the majority of patients viz., pH 6.0-6.5 and total formaldehyde 500-900 µg/ml, an experiment was designed to test the infectivity of different species in the urinary tract. This was carried out in rats and is described in the next sub-section.

5.2.4 ESTABLISHMENT OF URINARY INFECTION IN RATS

Rats with urine acidified to pH 6 to 6.5 after ingestion of ascorbic acid and with formaldehyde concentration between 500-900 µg/ml after ingestion of methenamine hippurate, were challenged with three species isolated from the patients. Control rats which had no oral additives were infected in the same way.

Bacteria used:

Pr. mirabilis from urine of patient 96544
   Resistant to kanamycin, neomycin and tetracycline
   M.I.C. of formaldehyde 50 µg/ml
   Dienes type C (Spinal Centre notation)

Kl. aerogenes from urine of patient 97059
   Resistant to ampicillin, chloramphenicol, kanamycin, neomycin, streptomycin, sulphathiazole and tetracycline
   M.I.C. of formaldehyde 50 µg/ml

E. coli from faeces of patient 97059
   Resistant to ampicillin, cephalothin and nitrofurantoin
   M.I.C. of formaldehyde 10 µg/ml

Serological type 021 : H non typable.
Table 20 sets out the number of bacteria recovered from the homogenized kidneys and the pH and formaldehyde concentration of urine.

The results indicate that establishment of a urinary tract infection in rats with the three species was possible. When formaldehyde was present and the urine pH lower than normal, infection with *E. coli* was retarded.

Infection with *Kl. aerogenes* produced enlarged spleens in test and control rats but three of the test rats had abscesses on the kidneys and this may account for slightly larger numbers of bacteria. This is an indication that the slightly lower pH in the test animals might favour the multiplication of this organism.

*Pr. mirabilis* raised the pH of the urine in the test rats and infection in both groups seemed identical.

The conclusion drawn from this experiment is that in rats, infection with *E. coli* is reduced by the conditions of lower acidity and formaldehyde. Infection with *Pr. mirabilis* and *Kl. aerogenes* is not prevented. This is not proof that a similar situation exists in man but evidence that the use of the urinary disinfectant coupled with disinfection measures using chlorhexidine and lysol select against *E. coli* and allow infection with other species.
## TABLE 20

**ESTABLISHMENT OF URINARY INFECTION IN RATS**

<table>
<thead>
<tr>
<th>CHALLENGE SPECIES</th>
<th>CONTROL RATS</th>
<th>TEST RATS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Kidneys</strong></td>
<td><strong>Urine</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Bacteria</strong></td>
<td><strong>pH</strong></td>
</tr>
<tr>
<td><strong>Pr. mirabilis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$1 \times 10^5$</td>
<td>$7.5$</td>
<td>$0$</td>
</tr>
<tr>
<td>$3 \times 10^4$</td>
<td>$7.5$</td>
<td>$0$</td>
</tr>
<tr>
<td>$1 \times 10^4$</td>
<td>$7.5$</td>
<td>$0$</td>
</tr>
<tr>
<td>$1 \times 10^2$</td>
<td>$8$</td>
<td>$0$</td>
</tr>
<tr>
<td>$1 \times 10^3$</td>
<td>$7.5$</td>
<td>$0$</td>
</tr>
<tr>
<td>$1 \times 10^4$</td>
<td>$7.5$</td>
<td>$0$</td>
</tr>
<tr>
<td>$6 \times 10^3$</td>
<td>$8$</td>
<td>$0$</td>
</tr>
<tr>
<td>$0$</td>
<td>$8$</td>
<td>$0$</td>
</tr>
<tr>
<td><strong>Kl. aerogenes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$3 \times 10^4$</td>
<td>$7$</td>
<td>$0$</td>
</tr>
<tr>
<td>$1 \times 10^5$</td>
<td>$6.5$</td>
<td>$0$</td>
</tr>
<tr>
<td>$1 \times 10^4$</td>
<td>$7.5$</td>
<td>$0$</td>
</tr>
<tr>
<td>$2 \times 10^4$</td>
<td>$7.5$</td>
<td>$0$</td>
</tr>
<tr>
<td>$3 \times 10^2$</td>
<td>$7$</td>
<td>$0$</td>
</tr>
<tr>
<td>$1 \times 10^4$</td>
<td>$6.5$</td>
<td>$0$</td>
</tr>
<tr>
<td>$1 \times 10^2$</td>
<td>$7$</td>
<td>$0$</td>
</tr>
<tr>
<td>$1 \times 10^5$</td>
<td>$7.5$</td>
<td>$0$</td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$2 \times 10^5$</td>
<td>$7$</td>
<td>$0$</td>
</tr>
<tr>
<td>$5 \times 10^4$</td>
<td>$7$</td>
<td>$0$</td>
</tr>
<tr>
<td>$2 \times 10^5$</td>
<td>$7.5$</td>
<td>$0$</td>
</tr>
<tr>
<td>$1 \times 10^5$</td>
<td>$7.5$</td>
<td>$0$</td>
</tr>
<tr>
<td>died</td>
<td>$-$</td>
<td>$0$</td>
</tr>
<tr>
<td>died</td>
<td>$-$</td>
<td>$0$</td>
</tr>
<tr>
<td>died</td>
<td>$-$</td>
<td>$0$</td>
</tr>
</tbody>
</table>

* Abscesses on kidneys
5.2.5 INFLUENCE OF pH ON GROWTH OF ISOLATES FROM URINE

Species isolated from the patients were cultured with increased aeration for 6 hours, in pooled sterile urine from laboratory volunteers. The pH was adjusted to levels ranging from 4 to 8, and buffered with acetate/NaOH (Sørensen, 1909). The growth achieved is shown in Table 21. It can be seen that *P. aeruginosa* attained greatest opacity at pH 5 and 6, whereas *E. coli* grew better when the pH was at 6 or above.

5.2.6 SPINA BIFIDA PATIENTS FROM THE QUEEN VICTORIA MEMORIAL HOSPITAL

This group were included in the study because the author had the opportunity to examine the urine of this group of 7 patients, who had been born at the Queen Victoria Hospital, had been readmitted for surgery and now attend a monthly Outpatients' Clinic. These patients are the only children with urinary diversions who have been totally managed at this hospital.

Appendix 3 sets out the isolations before and after the ileal conduit was formed. Most of the patients have had continuous prophylactic sulphonamide/trimethoprim or the urinary disinfectant nitrofurantoin. Methenamine mandelate has not been used.

The species listed represent a great number of continuous isolations of those species. Therapy instituted did not entirely eradicate the organism concerned, although the clinical symptoms disappeared.
TABLE 21
GROWTH OF ISOLATES IN URINE OF VARYING pH

![Graph showing growth of isolates in urine of varying pH](image-url)
The *P. aeruginosa* from patients 66268 and 85278 have been pyocine typed, and for 4 months the same types occur in the 2 patients indicating relapse rather than re-infection.

The three features which bear relation to the main study of this thesis are:

(a) The low incidence of *E. coli* and *Strep. faecalis* comparable with the spinal patients at the Austin Unit.

(b) The persistence of the same species after the urinary diversion, indicating a low grade infection which may be of long duration, again comparable with the findings at the Austin Spinal Unit.

(c) The dearth of nosocomial species even when these patients have had up to ten readmissions to the Queen Victoria Hospital. It should be noted that none of these patients had indwelling catheters, the diversions were performed because of incontinence or infection.

5.2.7 RECOMMENDATIONS FOR AUSTIN SPINAL INJURIES UNIT

1. Improved sterilisation and disinfection measures.
   (a) All bladder and urethral irrigants to be prepared and sterilised in the Austin Hospital Pharmacy Department.
   (b) Catheters and silastic tubing to be cleaned in an ultrasonic bath prior to autoclaving. The Austin Hospital Central Sterile Supply should clean and sterilise these articles.
(c) Urinary utensils and re-usable plastic and rubber bags should be cleaned in an ultrasonic bath and then disinfected by pasteurisation.

(d) Wet ropes in bathrooms should be pasteurised preferably at night. Cotton mop heads should be boiled or autoclaved.

(e) The floor polisher brush should have regular disinfection preferably by pasteurisation.

2. Removal of infected porous material. The wooden floor area in Ward 17 should be covered by vinyl sheeting. The wooden foot rests in the bathrooms should be replaced by plastic.

3. All patients to use the Shenton Park Urodrain. This should not be disconnected from the catheter. 20 ml of a mixture of centrimide 15% and chlorhexidine 10% should be added to the bottom of the bag each time it is emptied. If disconnection is required the catheter orderlies should be the only staff to perform this procedure.

4. pH estimations should be performed daily on urine, using Merck paper. The formaldehyde concentration should be estimated to indicate the dosage of the urinary disinfectant prescribed. The patient should be educated to plan a diet which aids acidification of the urine and at the same time adds indigestible fibre to the faeces to facilitate assimilation of nutrients (including the urinary disinfectant tablets).
5. Education of the staff. The reintroduction of the post
graduate course and the introduction of practical demonstrations
to the nurses, nursing aides, orderlies, turning team and
catheter orderlies.
The demonstration of techniques designed to prevent cross
infection in the wards should be made available to all levels
of staff. The Sister in Charge of the Austin Hospital Central
Sterile Supply should be consulted on all aspects of
sterilisation and disinfection and the methods standardised
and demonstrated.

5.3 CONCLUSION

The original hypothesis in this section was that the initial
infecting organisms are selected from a range of potential urinary
tract pathogens by a combination of medication and ward procedures.
The above results provide evidence to suggest the hypothesis is correct.
The traditional urinary tract pathogens, *E. coli* and *Strep. faecalis* were
sensitive to the antibacterials used, as well as to the disinfectants
chlorhexidine, lysol and formaldehyde. The infectivity of *E. coli* in rats
was reduced when the urinary disinfectant was used. In addition the
growth rate of this species was reduced when cultured in urine at pH
less than 6.

In contrast, the other pathogens belonging to the Entero-
bacteriaceae and to the nosocomial group of organisms did survive the
influence of antibacterials, disinfectants and reduced hydrogen ion
concentration. There was no reduction in infectivity of the two
species tested in the animal system.
## APPENDIX 1

### PATIENTS FROM THE AUSTIN SPINAL INJURIES UNIT

#### L-FORM EXAMINATION  PATIENTS 227 - 94192

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>CLASSIFICATION</th>
<th>ADMISSION</th>
</tr>
</thead>
<tbody>
<tr>
<td>227</td>
<td>P</td>
<td>31.7.54</td>
</tr>
<tr>
<td>263</td>
<td>P</td>
<td>14.9.54</td>
</tr>
<tr>
<td>266</td>
<td>P, Dissem. scler.</td>
<td>23.2.55</td>
</tr>
<tr>
<td>4753</td>
<td>P</td>
<td>4.4.58</td>
</tr>
<tr>
<td>9219</td>
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### URINARY DISINFECTANT STUDY

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## APPENDIX 2

### THE NON-SPINAL PATIENTS PROVIDED BY MEDICAL PRACTITIONERS AND UROLOGISTS

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**THE NON-SPINAL PATIENTS PROVIDED BY MEDICAL PRACTITIONERS AND UROLOGISTS**

**UROLOGISTS**

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<td>D.H.</td>
<td>Bactrim</td>
<td>Urine</td>
<td>Pr. mirabilis, Kl. aerogenes</td>
<td>Mycoplasma</td>
</tr>
<tr>
<td>J.H.</td>
<td>Nitrofurantoin</td>
<td>Urine, Bladder</td>
<td>Staph. epidermidis</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. ureter</td>
<td>Staph. epidermidis</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R. ureter</td>
<td>Staph. epidermidis</td>
<td>-</td>
</tr>
<tr>
<td>J.J.</td>
<td>Nitrofurantoin, Ampicillin</td>
<td>Urine</td>
<td>Kl. aerogenes</td>
<td>-</td>
</tr>
<tr>
<td>E.K.</td>
<td>Ampicillin</td>
<td>Urine, Bladder</td>
<td>H. vaginalis</td>
<td>H. vaginalis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. ureter</td>
<td>H. vaginalis</td>
<td>H. vaginalis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. kidney</td>
<td>H. vaginalis</td>
<td>H. vaginalis</td>
</tr>
<tr>
<td>B.M.</td>
<td>-</td>
<td>Urine</td>
<td>-</td>
<td>T-strain Mycoplasma</td>
</tr>
<tr>
<td>A.R.</td>
<td>Ampicillin, Nitrofurantoin</td>
<td>Urine</td>
<td>Candida albicans</td>
<td>-</td>
</tr>
<tr>
<td>M.S.</td>
<td>Nitrofurantoin</td>
<td>Urine</td>
<td>Kl. aerogenes</td>
<td>-</td>
</tr>
</tbody>
</table>

**Abbrev: H. (Haemophilus)**

Bactrim, Septrin (trimethoprim with sulphamethoxazole)
## APPENDIX 3

**SPINA BIFIDA PATIENTS FROM QUEEN VICTORIA MEMORIAL HOSPITAL**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Birth</th>
<th>Species causing infection before urinary diversion</th>
<th>Urinary diversion</th>
<th>Species causing infection after diversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>31711</td>
<td>11. 7.63</td>
<td>Strep faecalis Pr. mirabilis</td>
<td>8. 8.72</td>
<td>Pr. mirabilis</td>
</tr>
<tr>
<td>57773</td>
<td>15. 9.66</td>
<td>Pr. mirabilis E. coli Strep. faecalis Kl. aerogenes</td>
<td>26.11.71</td>
<td>Pr. mirabilis Ps. aeruginosa</td>
</tr>
<tr>
<td>66268</td>
<td>19. 8.67</td>
<td>Pr. mirabilis E. coli</td>
<td>9. 3.72</td>
<td>Pr. mirabilis Ps. aeruginosa</td>
</tr>
<tr>
<td>66709</td>
<td>9. 10.67</td>
<td>Pr. mirabilis Strep. faecalis Kl. aerogenes</td>
<td>20. 4.72</td>
<td>Pr. mirabilis</td>
</tr>
<tr>
<td>85278</td>
<td>27. 8.69</td>
<td>Kl. aerogenes E. coli Pr. mirabilis Ps. aeruginosa Strep. faecalis</td>
<td>8.10.73</td>
<td>Pr. mirabilis E. coli Strep. faecalis Ps. aeruginosa</td>
</tr>
<tr>
<td>85883</td>
<td>15. 9.69</td>
<td>E. coli Kl. aerogenes Pr. mirabilis</td>
<td>27.11.74</td>
<td>Pr. mirabilis Ps. aeruginosa</td>
</tr>
<tr>
<td>94023</td>
<td>3. 10.70</td>
<td>Kl. aerogenes Pr. mirabilis</td>
<td>29.10.74</td>
<td>Staph. aureus</td>
</tr>
</tbody>
</table>
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ROLAND OF PARMA. 1210. Chirurgia.


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