The differentiation of Enterobacteriaceae infecting the urinary tract

A study in male paraplegics

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SYNOPSIS Methods adopted in a routine bacteriology laboratory for the rapid identification of Enterobacteriaceae isolated from urine are described. The incidence of various bacteria causing infection in paraplegics after catheterization of the bladder is recorded, *Klebsiella* accounting for the majority of infections caused by lactose fermenters and *Providencia* for the majority of 'para-colon' infections. The importance of these bacteria in cross-infection is discussed.

The investigation of urinary tract infections may involve the clinical bacteriologist in problems of identification and classification of Gram-negative bacilli of the family Enterobacteriaceae. Methods involving the prolonged incubation of a long series of biochemical tests are impracticable for routine purposes and it is necessary to have a few tests, with a high degree of specificity, which will clearly differentiate several groups or species after a short period of incubation.

During an investigation into the incidence of urinary cross-infections in patients with paraplegia and paralysed bladders at the National Spinal Injuries Centre, Stoke Mandeville Hospital, we adopted certain rapid tests for the routine identification of the different members of the family Enterobacteriaceae which have proved their value over a two-year period. The purpose of this report is to describe and justify the use of these methods and to give a brief account of the bacterial flora which can infect the urinary tract under conditions of repeated catheterization.

CRITERIA OF INFECTION

Because a standard loopful of uncentrifuged fresh urine was used routinely it was possible to assess the significance of the growth by the number of bacterial colonies on the blood agar plates, the purity of the growth being also taken into consideration. In specimens collected by intermittent catheterization 50-100 colonies was considered significant representing 10^4

bacteria per millilitre of urine (Merritt and Sanford, 1958). Doubtful growths were repeated or disregarded. That the bacteria discussed in this paper are truly infecting organisms, *i.e.*, multiplying within the urinary tract, is further supported by the presence of pyuria in most cases, and by repeated isolation in consecutive specimens from the same patient. In the case of per-urethral specimens in follow-up studies only heavy growths were considered significant. Kass (1960) found that urines with less than 10^5 bacteria per millilitre were generally free of Gram-negative rods.

MATERIAL AND TECHNIQUES

SAMPLING OF URINE Initial bladder management entailed intermittent catheterization and specimens of urine were obtained through the catheter. In those cases where a Foley type catheter was subsequently inserted specimens were obtained, at the time of changing the catheter, through a clean catheter. From those patients capable of voiding urine, per-urethral specimens were collected after cleansing the surrounding area.

All specimens were sent to the laboratory within 30 minutes of collection and cultured without delay.

CULTURE OF URINE The uncentrifuged urine was cultured using a standard wire loop which held 0.005-0.01 ml. of water by weight depending on how it was loaded. The loaded loop was streaked on the surface of blood-agar and MacConkey agar plates and on a segment of a Lemco agar containing 0.03% cetrimide for the isolation of *Pseudomonas pyocyanea* (Lowbury and Collins, 1955). An agar plate containing 7% laked horse blood was evenly spread with a loopful of urine and paper discs con-

taining various antibiotics and sulphonamides were applied to it. The plates were incubated at 37°C. over night.

EXAMINATION OF CULTURE PLATES Staphylococci were identified by the colonial appearance, by Gram's stain, and by coagulase tests. *Streptococcus faecalis* was identified by colonial appearance and fermentation of aesculin. *Ps. pyocyanea* was identified by colonial appearance, pigment formation, growth on Lowbury's medium, and the oxidase test of Kovacs (1956). All other Gram-negative bacilli were divided arbitrarily into lactose-fermenters and non-lactose fermenters according to the appearance of their colonies on MacConkey agar after overnight incubation, as this was found to be a useful dividing point, the lactose-fermenting strains forming a smaller group and requiring a shorter set of tests to identify them. Both types were subcultured to peptone water for further investigation and cultures were maintained on nutrient agar slants in bijou bottles.

ROUTINE BIOCHEMICAL TESTS All tests were performed at 37°C. The media were inoculated from young peptone water cultures which were also plated out for purity. With the exception of certain tests mentioned below, media were contained in screw-capped bottles.

H₂S and indole production Dried lead acetate papers were suspended over freshly inoculated peptone water cultures. Results were read after overnight incubation and the culture then tested with Kovacs reagent.

Motility Semi-solid agar stab medium (test no. 16, Report 1958).

Urease test Christensen's (1946) weakly buffered urea agar was used, adjusted to pH 7.0.

Carbohydrate fermentation Andrade's indicator was used in media consisting of 0.5% carbohydrate in 1% peptone water.

Citrate test Simmons' citrate agar (Oxoid) was used and inoculated with a straight wire from a peptone water culture.

Gluconate test The Dysentery Reference Laboratory method was used with Shaw and Clarke's medium (1955) modified by using Clinitest reagent tablets instead of Benedict's qualitative reagent (Carpenter, 1961). Screw-capped bottles were found to be unsatisfactory for this test. Size 4 × ½ in. glass tubes are convenient as they admit the Clinitest tablet.

Phenylalanine and malonate tests The combined medium of Shaw and Clarke (1955) was used dispensed in bijou bottles in 2 ml. quantities. The cap of the bottle must be loosened during incubation.

Gelatin liquefaction Nutrient gelatin stabs were incubated at room temperature. For the identification of organisms which produce gelatinase quickly, the method of Smith and Goodner (1958) was adopted. This is an agar plate medium incorporating gelatin and the following modification gave very satisfactory results:—

Proteose peptone (Difco No. 3)	..	0.4%
Yeast extract	..	0.1%
Agar (Difco)	..	1.5%
Gelatin (Difco)	..	3.0%

Add gelatin before agar and dissolve by heating to 56°C. Adjust the pH to 7.2 and autoclave at 20 lb. for

20 minutes and pour plates. The surface should be well dried.

Gelatinase-producing colonies are surrounded by a wide halo of opacity after overnight incubation at 37°C.

OTHER TESTS Certain other tests were used but not as a routine.

Christensen's (1949) citrate agar test (Report, 1958).

KCN test Møller (1954), modified by using bijou bottles with caps tightly screwed.

Nitrate reduction Using nitrate peptone water (Report, 1958).

Methyl red and Voges-Proskauer tests On Oxoid Voges-Proskauer broth cultures tested at two days using Barritt's (1936) method for acetylmethylcarbinol production.

Decarboxylase tests Strains were tested for lysine and ornithine decarboxylase and arginine dihydrolase using the simplified tests of Møller (1955).

IDENTIFICATION OF ENTEROBACTERIACEAE

LACTOSE-FERMENTING COLONIES ON MACCONKEY The routine short set of tests is given in Table I. The first five tests listed form a combination which we have found to be reliable in dividing lactose-fermenting colonies into three species, *E. coli*, *Klebsiella*, and *Citrobacter*.

TABLE I

ROUTINE TESTS FOR THE DIFFERENTIATION OF STRAINS PRODUCING LACTOSE-FERMENTING COLONIES ON MACCONKEY AGAR

	<i>E. coli</i>	<i>Klebsiella</i>	<i>Citrobacter</i>
Gluconate	—	+	—
Malonate	—	++	d
Simmons' citrate	—	—	+
Indole	++	—	d
H ₂ S	+	—	+
Adonitol	—	+	d
Inositol	—	++	—
Cellobiose	—	—	++
Motility	+	—	+

± = positive within 24 hours

d = some strains positive, some negative

— = negative after 48 hours

Not infrequently, strains of *E. coli* gave mucoid colonies indistinguishable in appearance from typical *Klebsiella* colonies, and occasionally low convex non-mucoid colonies gave the biochemical reactions of typical *Klebsiellae*.

The gluconate test, which we have found simple to use, is of great value. In a recent review of *Klebsiella* strains, Cowan, Steel, Shaw, and Duguid (1960) found 100% of '*K. aerogenes*' types to give oxidation of gluconate while 94% produced alkali in malonate. *Escherichia* and *Citrobacter* strains, on the other hand, do not oxidize gluconate, which makes it the most reliable single test for the differentiation of

typical *Klebsiella* from other lactose-fermenting strains.

Koser's (1924) citrate test was abandoned for technical reasons well explained by Talbot, Cunliffe, and Gower (1957). Simmons' medium was substituted and proved very reliable as an overnight test for citrate utilization. Christensen's (1949) citrate medium was tried but found to have no advantages over Simmons' in rapid identification. It had the disadvantage that if properly inoculated it took 48 hours to give a result, and also that a small proportion of otherwise typical *E. coli* strains, not growing in Koser's or on Simmons' were positive on it. It was, however, useful as an additional test for atypical strains within the *Escherichia* group.

Indole-positive *Klebsiella* were isolated occasionally from female patients but they were not isolated from male patients.

The inclusion of lead acetate papers over peptone water cultures was found to be helpful in identifying lactose-fermenting colonies belonging to the *Citrobacter* group, but more reliance was placed on citrate utilization with a negative gluconate test. Although malonate fermentation is not regarded as a feature of the group as a whole (Report, 1958), we found some strains which were positive. Likewise, indole production was a variable feature, but cellobiose fermentation was a remarkably constant feature of *Citrobacter* strains and for this reason alone it is worth a place in the short set.

The KCN test of Møller (1954) was tried in the short set of tests but did not increase their reliability. As an additional test, in distinguishing intermediate strains from *Escherichia* it is valuable. Voges-Proskauer and methyl red tests are time consuming and were not used routinely.

MacConkey (quoted by Malcolm, 1938) used the fermentation of inositol and adonitol to distinguish '*B. coli*' from '*B. lactis aerogenes*' (*Klebsiella*). Kauffmann (1954) stated that 'adonitol and inositol are fermented by all typical *Klebsiella* strains'. Hormaeche and Munilla (1957) reported that all their strains and the 72 capsular type strains fermented cellobiose. In our experience *Klebsiella* strains which do not ferment all three sugars overnight are rare, but those *Klebsiella* isolated from urine show a more uniform and easily recognized behaviour than strains isolated from the respiratory tract (Ørskov, 1955). It is difficult to decide the frequency with which otherwise typical *E. coli* strains ferment these sugars if the intermediate strains, which we regarded as *Citrobacter* (Report, 1956), are excluded. Talbot *et al.* (1957) suggested the use of adonitol and inositol, in place of Koser's citrate, to delineate two groups, citrate-positive and citrate-negative, as they found that most organisms which

fermented only one of these sugars was citrate negative. The use of gluconate and Simmons' citrate obviates the difficulties they encountered and is better than relying on carbohydrate tests alone. The differentiation of *Citrobacter* from *E. coli* may sometimes be difficult and here the addition of cellobiose is most useful. In our experience *E. coli* strains which ferment cellobiose are rare and the reactions are delayed.

Lactose-fermenting *Citrobacter* strains were uncommon and their presence in pure culture on primary plates was extremely rare in our material. They could be divided into several biochemical types but in the day-to-day follow up of infected patients this division had no practical value.

The question of motile strains which behave as *Klebsiella* in the tests listed in Table I has to be considered. Hormaeche and Edwards (1960) suggest that these strains should be called *Enterobacter aerogenes* as they are more closely related to *Enterobacter cloacae* than to *Klebsiella*. They have a colonial appearance very like that of a *Klebsiella* but are motile as well as capsulated. On further study they do not decompose urea and have lysine and ornithine decarboxylases which distinguishes them clearly from *Klebsiella*. We did not detect any of these strains in paraplegic patients but it may be that the motility stab has a place in routine investigations.

NON-LACTOSE-FERMENTING COLONIES These were subcultured in the first instance on Christensen's urea slopes and incubated at 37°C. Strains which decomposed urea within about four hours were regarded as *Proteus* species and the first six tests listed in Table II were set up routinely. Strains which did not decompose urea quickly were regarded as 'paracolons' and subcultured to a different set of tests as described below.

PROTEUS The differentiation of biochemical types or species within this group has been fully discussed by Kippax (1957). Differentiation into four species was obtained with a short set of tests (Table II) but by the addition of sucrose, salicin, and xylose further biochemical types could be recognized. The indole-negative strain of *P. vulgaris* was noted by Dutton and Ralston (1957) in their study of urinary cross-infection and is easily recognized by the fermentation of maltose. A strain of *P. mirabilis* which fermented sucrose overnight was also distinguished. Decarboxylase tests confirmed the correctness of placing these biochemical types in their respective species. Indole-positive *P. mirabilis* strains ('atypical 2', Kippax, 1957) and his 'atypical 3', which is a xylose fermenting strain of *P. morganii*, were not en-

TABLE II
BIOCHEMICAL REACTIONS OF 130 CONSECUTIVELY ISOLATED PROTEUS STRAINS¹

	P. mirabilis		P. vulgaris		P. morganii	P. rettgeri	
H ₂ S	++	+	++	+	+	—	—
Indole	—	—	+	—	+	—	—
Glucose	Ag	Ag	Ag	Ag	Ag	A	A
Mannite	—	—	—	—	—	A	A
Maltose	—	—	Ag	Ag	—	—	—
Gelatinase	++	+	+	+	—	—	—
Sucrose	—	Ag	Ag	Ag	—	—	—
Salicin	—	—	—	—	—	—	A
Xylose	Ag	Ag	Ag	Ag	—	—	—
Simmons' citrate	(—)	(++)	—	—	—	—	—
Decarboxylase							
Arginine	—	—	—	—	—	—	—
Ornithine	+	+	—	—	—	—	—
Lysine	—	—	—	—	—	—	—
Number of strains	79	3	2	9	6	23	4
	82		11		6	31	

¹All strains produced urease rapidly at 37°C.

++ = positive within 24 hours

(+) = positive in two to three days

— = negative after 10 days' incubation

Ag = acid and gas produced within 24 hours

A = acid only

TABLE III
DIFFERENTIATION OF 'PARACOLON' STRAINS ISOLATED FROM URINE

	Providence	Cloacae	Serratia	B. anitratum	Escherichia	Citrobacter
<i>Routine tests</i>						
H ₂ S	—	—	—	—	—	—
Indole	—	—	—	—	—	—
Simmons' citrate	—	—	—	—	—	—
Gluconate	—	—	—	—	—	—
Malonate	—	—	—	d	—	—
Phenylalanine	—	—	—	—	—	—
Glucose	A	Ag	A	A	Ag/A	Ag/A
Mannite	— ¹	Ag	A	—	Ag/A	Ag/A
<i>Confirmatory tests</i>						
Cellobiose	—	Ag	—	—	—	Ag/A
Salicin	—	— ¹	A	—	d	d
Xylose	—	Ag	— ¹	A	Ag/A	Ag/A
Oxidase test (Kovacs)	—	—	—	—	—	—
Methyl red	—	—	—	—	—	—
Voges-Proskauer	—	—	—	—	—	—
Gelatin liquefaction	—	(—)	+	—	—	—
Nitrate reduction	—	—	—	—	—	—
<i>Decarboxylases</i>						
Arginine	—	+	—	—	—	+
Lysine	—	—	—	—	—	—
Ornithine	—	+	—	—	+	—

A = acid only

Ag = acid and gas produced

++ = positive (routine tests within 24 hours)

(+) = gelatine liquefied in 2 to 3 weeks

d = some strains positive, some negative

—¹ = some strains produced acid on prolonged incubation

countered but some of our *P. rettgeri* strains corresponded to his 'atypical 4' and fermented xylose.

'PARACOLON BACTERIA' Under this heading are included all strains producing non-lactose-fermenting colonies which did not decompose urea promptly. Strains belonging to the *Shigella*, *Salmonella*, *Arizona*, and *Hafnia* groups of Enterobacteriaceae were not encountered and are not considered here.

The differentiation of these 'paracolon' strains

into various 'species' obviates further use of the term 'paracolon' which taxonomically is not justifiable. It is used here for simplicity because the term has been applied to certain non-lactose and late lactose-fermenting organisms, other than *Salmonella*, *Shigella*, or *Proteus* species, and is generally understood by hospital bacteriologists.

The tests used to differentiate the 'paracolon' strains which we encountered are listed in Table III. Confirmatory tests, not used routinely but of value

when a strain was first isolated from a patient, are also given.

Providencia The term *Providencia* was first used by Kauffmann (1951) to designate strains which Stuart, Wheeler, Rustigian, and Zimmerman (1943) called 'anaerogenic paracolon 29911'. Henriksen (1950), Shaw and Clarke (1955), and Singer and Volcani (1955) showed that *Providencia* strains share with the *Proteus* group the ability to form an l-amino acid oxidase. This acts on a wide range of amino-acids converting them to the corresponding α -keto acid, some of which give distinctive colours with ferric chloride. All other Enterobacteriaceae are negative in this test. Shaw and Clarke's medium was found to be practical for routine use enabling *Providencia* to be identified with certainty after overnight incubation as a non-lactose-fermenting colony which, although urease negative, was phenylalanine positive. Further confirmatory tests were not necessary but adonitol and inositol were included routinely in our short set in order to detect different biotypes.

Ewing, Tanner, and Dennard (1954) studied 611 strains of *Providencia* and found that they fell into two distinctive biochemical groups. Group I strains fermented adonitol and produced some gas in glucose, while group II strains were anaerogenic and fermented inositol but not adonitol. Most of Ewing's strains were isolated from faeces and 86.5% belonged to biochemical group I. He further divided his groups into 31 biotypes. *Providencia* strains were common in our material and without exception they all belonged to biochemical group II. This finding is another point in favour of the theory that infections of the urinary tract with this organism are nosocomial rather than autogenous infections from the patient's own bowel flora. Middleton (1958), reporting the antibiotic sensitivity of *Providencia* strains, noted that all those isolated from urine belonged to biotype 27. Of 50 cultures we studied in detail, the majority belonged to this biotype (Table IV).

Enterobacter cloacae Strains belonging to this

group were all isolated as non-lactose-fermenting colonies on MacConkey plates. They gave positive malonate and gluconate tests which clearly differentiated them from other non-lactose fermenters. They were all motile and did not ferment inositol or adonitol. Tests for decarboxylases confirmed the identification made by overnight tests and gelatin was liquefied after two to three weeks at room temperature.

Serratia Members of this group of bacteria are generally regarded as producers of red pigment (Breed, 1957) and are often called *B. prodigiosus*. Davis, Ewing, and Reavis (1957) studied 50 strains from a great variety of sources including wounds, sputa, and urine, and found that they formed a fairly homogeneous group within the family Enterobacteriaceae. Only 16, or 32%, of their strains produced a red or pink pigment and they pointed out that non-pigmented strains are often mistakenly identified as *Cloaca*, *Hafnia*, or intermediate coliform bacteria. Fulton, Forney, and Leifson (1959) review the criteria for pathogenicity of this group of bacteria and also stress the frequency with which non-pigmented strains are mistaken for other coliforms.

All the strains encountered from the urine of our paraplegics were non-pigmented on first isolation and they failed to produce pigment on subculture on the medium of Williams, Green, and Rappoport (1956) which was modified from Bunting (1940). On MacConkey plates colonies of *Serratia* are recognizable, after overnight incubation, as densely opaque colonies, 1 to 2 mm. in diameter, consisting of very short Gram-negative rods. The medium around the colonies shows an alkaline change. On blood agar they cause darkening of the medium and to the sensitive nose there is a characteristic smell reminiscent of damp hay. Biochemically their outstanding characteristic is very rapid liquefaction of gelatin and, in contrast to *Cloaca* strains, they are malonate and cellobiose negative, and ferment salicin rapidly. Most strains produce a small bubble of gas in glucose but the majority of our strains were anaerogenic. The medium devised by Smith and Goodner (1958) for the detection of gelatinase was most useful for the identification of *Serratia*. Many strains can be 'spotted' on one plate, gelatinase producers becoming surrounded by opaque halos after overnight incubation. By this method, *Serratia* is a strong gelatinase producer, *Ps. pyocyanea* and some staphylococci are slightly weaker but *Cloaca* strains produce gelatinase too slowly to give the reaction.

Bacterium anitratum Although Stuart, Formal, and McGann (1949) regarded this organism, referred to as B₃W, as a 'paracolon' it is not usually considered a member of the group Enterobacteriaceae. However, we included it in our study because there were several

TABLE IV
BIOCHEMICAL REACTIONS OF 50 PROVIDENCE CULTURES

	Biotype	
	21	27
Urea	Negative	Negative
Phenylalanine	+	+
Indole	+	+
Glucose	A	A
Mannite	Negative	A ³⁻⁷
Adonitol	Negative	Negative
Inositol	A	A
Sucrose	A ²⁻³	A ²⁻³
No. of cultures	12	38

50

All tests incubated for 15 days

unequivocal infections with it. In their original description of this organism Schaub and Hauber (1948) studied 15 human strains, of which 10 came from the urinary tract and, of these 10 patients, seven had clinical evidence of urinary tract infection. Brooke (1951) studied 84 strains, 54 of which had been isolated from urine. On MacConkey plates, after overnight incubation, the salmon-pink colonies are composed of what appear in Gram-stained smears to be perfectly regular cocci indistinguishable from *Neisseria*. The organism is, however, oxidase negative and in hanging drop preparations can be seen to be bacillary. They are not motile. Capsules can be demonstrated in Indian ink preparations on first isolation when the organism is in the 'M' phase. In biochemical tests nitrates are not reduced and few carbohydrates are broken down. Acid but not gas is usually produced in glucose, arabinose, and xylose; the malonate and Simmons' citrate tests are positive. This organism tends to become rough in stored cultures with loss of capsules giving a smaller, flatter, and drier colony.

OTHER 'PARACOLON' STRAINS Occasionally non-lactose-fermenting colonies proved to be *Escherichia* or *Citrobacter* in biochemical tests and were readily recognized (Table III). *Alkalascens-Dispar* strains were occasionally encountered. They can be confirmed by their ability to change the indicator on Christensen's citrate but not on Simmons' medium (Edwards and Ewing, 1955). One strain with which we became familiar presented as a non-lactose-fermenting colony of 2-3 mm. diameter. In biochemical tests it produced H_2S and indole, was citrate positive, gluconate negative, and positive in cellobiose and malonate but negative in KCN. It was a late lactose fermenter and, in spite of the negative KCN result, we regarded it as belonging to the *Citrobacter* group (Table III). *Citrobacter* organisms of the Bethesda-Ballerup type were not encountered but would be easily detected by the short set of tests and could be confirmed by a positive KCN test.

FURTHER DIVISION WITHIN SPECIES OR GROUPS

The incidence of infection with various bacteria is given in Table V. This was a fairly representative group of 50 male patients who were admitted to hospital within two or three days of the onset of traumatic paraplegia and whose urine on admission was sterile. It will be seen that *Klebsiella* infected the largest number of patients and that *Ps. pyocyanea*, *P. mirabilis*, and *Providencia* also had a high incidence.

An attempt was made to type the *Klebsiella* strains using the 'capsular swelling' or 'Quellung' reaction. Sera were prepared in rabbits against unknown strains isolated from patients on different wards and,

TABLE V
INCIDENCE OF VARIOUS BACTERIA CAUSING URINARY TRACT INFECTIONS IN 50 MALE PATIENTS WITH TRAUMATIC PARAPLEGIA¹

Organism	Percentage of Patients Infected
<i>Staph. aureus</i>	16
<i>Strep. faecalis</i>	46
<i>Ps. pyocyanea</i>	72
<i>Klebsiella</i>	80
<i>Esch. coli</i>	4
<i>Citrobacter</i>	0
<i>P. vulgaris</i> indole positive	4
<i>P. vulgaris</i> indole negative	10
<i>P. mirabilis</i>	48
<i>P. morgani</i>	6
<i>P. rettgeri</i>	8
<i>Providencia</i>	38
<i>Cloacae</i>	2
<i>Serratia</i>	6
<i>B. anitratum</i>	6

¹All infections acquired in hospital. All these infections were either acute, i.e., accompanied by gross pyuria and symptoms, or established, i.e., same organism isolated on several consecutive occasions.

by a process of elimination, five specific sera were finally available. The antigenic strains for these sera were examined by Dr. Ida Ørskov at the Statens Serum Institut, Copenhagen, and their capsular type determined. With these five sera we examined strains from 92 patients of which strains from 77 (84%) could be typed, the remainder being acapsular or not reacting. On the main admitting ward, over a period of two years, 93% of the *Klebsiella* infections were due to capsular types 20 and 25. Other types found on the male wards were 16, 31, and 54, each ward having a predominant type. Ørskov (1952, 1954) found types 7, 8, 9, 10, 24, and 38 predominating in different male surgical clinics in Denmark. In many instances she isolated the same type from 100% of the specimens from one ward. The technique is not difficult and the information it provides is of considerable value in controlling cross-infection.

An attempt to differentiate *P. mirabilis* strains by Dienes' phenomenon (Dienes, 1946) was not successful in our hands, and the procedure was abandoned. The typing of *Ps. pyocyanea* by bacteriophage and serology has been shown by McLeod (1958) to give reliable information in the study of urinary cross-infection, but we did not attempt to type our *Ps. pyocyanea* strains. *B. anitratum* can be typed by 'capsular swelling' reaction and 10 serological types are recognized (Ferguson and Roberts, 1950).

DISCUSSION

The identification of the bacteria commonly found in urine from patients with acute cystitis or pyelitis presents few problems. *Escherichia coli*, *Streptococcus faecalis*, and the common species of *Proteus* account for the great majority of such infections (Garrod, Shooter, and Curwen, 1954). In contrast, infections

acquired in hospital following instrumentation, operation, or the insertion of an indwelling catheter are associated with a high incidence of *Ps. pyocyanea* (Pyrroh, Goldie, Parsons, and Raper, 1955; McLeod, 1958); atypical strains of *Proteus* (Kippax, 1957; Edebo and Laurell, 1958; Omland, 1960); *Providencia* (Dutton and Ralston, 1957) and other 'paracolony' bacilli. The incidence of typical *E. coli* is low (Dutton and Ralston, 1957) while the incidence of *Klebsiella* is high (Warner, 1948; Coleman and Taylor, 1949; Wilhelm, Schloss, Orkin, Seligmann, and Wassermann, 1949). These 'coliforms' and 'paracolony' bacilli are often resistant to several antibiotics. That cross-infection is mainly responsible for these infections acquired in hospital has been shown by the studies of Ørskov (1952, 1954), Kippax (1957), McLeod (1958), Miller, Gillespie, Linton, Slade, and Mitchell (1958, 1960), and Gillespie, Linton, Miller, and Slade (1960).

In hospital urinary infections, and where cross-infection is occurring, the same strain may be isolated repeatedly from several patients and their environment. It is in such circumstances that identification tests involving only a few tubes are of value. It is a distressing feature of these paraplegic patients that, no sooner is one infection apparently controlled by antibiotic therapy, than another organism appears in the urine, resistant to the current antibiotic. In so far as this is due to cross-infection, it is more easily demonstrated if consistent results can be achieved in identification tests. Accurate identification and some practical typing method can be of assistance in the management of patients with paraplegia and bladder dysfunction who have to be repeatedly catheterized while in hospital.

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