STUDY OF BACTERIA FOUND IN INFECTIONS OF THE URINARY TRACT.

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This is a study of the different types of bacteria found in 102 infections of the urinary tract. The purpose is to identify, as completely as possible, the various types of organisms, hence more emphasis will be placed on the bacteriological, than on the clinical aspect. Material was obtained from the Multnomah County Hospital, The Doernbecher Memorial Hospital for Children, The Portland Free Dispensary, and from the private practices of Dr. J. Guy Strohm, Dr. A. M. Boyd, Dr. E. J. Nitschke, Dr. C. U. Moore and Dr. R. E. Watkins. Specimens were obtained by cystoscopic examination in a majority of the cases; the remainder were from bladder catheterization. The study is limited as nearly as possible to cases in which the bladder and kidney pelvis were involved. Cases of known tuberculous or gonorrheal origin were not included, though no routine method was used to rule out these types of infection. The clinical diagnoses accompanied the specimens.

Previous Studies on Urinary Infections.

In 1874, Pasteur, in commenting on a paper by Gosselin and Robin on the subject of ammoniacal decomposition of urine, stated his belief that this decomposition was probably due to the action of bacteria. He further states that the origin of these bacteria may be from the exterior by way of the urethra, from the blood stream, from communication with the intestinal tract, and from passage of surgical instruments. Thus we see that with the exception of the theory of lymphatic extension, Pasteur anticipated all the theories concerning the origin of urinary infections that are discussed today. Since this time many reports on the bacteriology of urinary infections have been made, and practically

every pathogenic organism, as well as many non pathogens, has been reported at some time or another as the cause of such infections.

Roberts, (1881) in a study of the relation of bacteria to ammoniacal decomposition of the urine, was the first to describe cocci occurring in long chains in urinary infections. Ogston (1881) had described these chain cocci in abscesses, but it was left to Fehleisen (1883) and Rosenbach (1884) to identify these chain cocci as Streptococci and study them in pure culture. Rovsing (1890), Melchoir, (1898), and Brown (1902) reported studies on the bacteriology of urinary infections and their results indicated that B. coli communis was by far the most frequent offender. However, we must remember that the classification of Gram negative bacilli was very incomplete at this time, and also that methods of identification were not as well worked out as they are today. For example, the following is quoted from the paper of Brown: "The methods of making the cultures and identifying the organisms are those usually in vogue, and will not be dwelt upon here. Two or three loops of the shaken-up urine are plated on agar or smeared over slant agar and from the colonies therefrom cultures are made on the usual media, gelatine, potato, litmus milk, glucose agar, bouillon and peptone". Faltin (1902) called attention to the polymicrobic nature of urinary infections and the tendency to change in flora.

V. C. David (1914) made a study of 50 cases of non-tuberculous infections of the urinary tract and described 59 aerobes and 14 anaerobes. Among the aerobes B. coli and Staphylococcus albus were the most frequently encountered, the former occurring 23 times, the latter 15. Cabot and Crabtree (1916), in a study of suppurative nephritis reported 118 cases and the preponderating organisms found by them belong to the Gram positive coccus group. Brunnich (1918), in a study of 79 cases with special reference to pyelitis, found a large preponderance of Gram negative bacilli; in the 68 urines Staphylococci were found in only 2 instances in pure culture. Herrold, Culver, and Phifer (1918), in 116 cases of urinary

infection found B. coli in 85% of the cases, 74% being in pure culture. Staphylococci were present in 19%, only 9% being in pure culture. The following year Herrold and Culver (1919 endeavored to classify the Gram negative bacilli in more detail and of the 86 strains, 50% were true colon bacilli and the remaining strains were classified as para colon bacilli. Dudgeon, Wordley, and Bawtree, (1921), reported 69 cases with special relation to hemolytic types of B. coli. In this series a total of 38 strains were hemolytic, 20 occurring in males and 11 in females. These authors also examined feces for hemolytic types and found them present in 11% of normal persons. The same authors in a second communication (1921b) on hemolytic B. coli infections, report 91 additional cases. In this series 37 hemolytic strains were isolated. Again there was a marked predominance of hemolytic strains in males. They also call attention to the fact that these hemolytic types are associated with acute infections in a majority of instances. They were unable to find organisms in the stool that corresponded to the organisms isolated from the urine. Maths and Belt (1922) reported a case of bilateral pyelitis due to Pseudomonas aeruginosa and gave an excellent review of the literature on infections due to this organism. In their review of 835 cases, B. coli was found 631 times, Staphylococcus aureus 89 times and Streptococcus 34 times. Mitchell (1922) reported 400 cases of urinary infection, occurring in children the organisms were studied in 183 cases. Colon bacilli were found 106 times, Staph. aureus 24, Pneumococcus 18, B. pyocyaneus, 15, and Streptococci 12. Duhig (1922), in 128 cases, found B. coli 97 times, 80 times in pure culture and 17 times in mixed culture. Dudgeon (1924) collected 49 additional hemolytic slow lactose fermenting types of B. coli and calls attention to the fact that these organisms are associated with very severe types of infection. He states that although the severe symptoms are apt to persist longer than with the ordinary types, ultimate freedom from infection is more likely to occur. Bitter and Grundel, (1924), are of the opinion that the hemolytic types of B. coli are associated with acute infections while the non-hemolytic are

associated with the more chronic infections. MacKenzie and Cochrane, in a preliminary report on the bacteriology of ureteral cultures, examined the large number of 1728 specimens from 897 cases. Their findings were, organisms of the Coliform group 468; Gram positive bacilli 32; organisms of the coccal group 211; Sarcinae and yeasts (contaminations) 9; mixed cultures 18; sterile 990. The last group included specimens from non-urological conditions, as, from the uninvolved side in unilateral lesions, from cases of lower urinary involvement only, prostatitis, urethral stricture, etc., and from cases of renal tuberculosis, congenital anomalies, etc. In the Coliform group the following types were found: B. coli communis 241; B. coli communior 83; B. fecalis alcaligenes 63; Coli vulgaris 33; B. lactis aerogenes 15. In the coccal group they found Staphylococci 200 times, Streptococci 11 times and Enterococci once. Scholl (1926) reports 2 cases of "cohabitation cystitis" due to B. coli and reviews the literature on the etiology of this type of cystitis; he states that practically all cases are due to members of the Colon group. Keilty (1926), in a study of 163 cases, reports 50 of them in detail. He studied specimens from cystoscopic examinations and reported the organisms found in the bladder and each ureter. He found a high incidence of Gram positive cocci; in the bladder specimens a total of 13 strains of cocci were found, and 19 Gram negative bacilli; in the right ureteral specimens the 2 types were found in equal numbers; in the left ureteral specimens 6 strains of Gram negative bacilli were found and 5 strains of Gram positive cocci. In 1927 Dudgeon and Pulvertoft made a further study of the slow lactose fermenting, hemolytic types of B. coli. In this study they endeavored to show the experimental effects of injection of the toxin of these organisms. Beef broth cultures were cultivated for month at 37 degrees and filtered through a Berkfeld filter. When this filtrate was injected intravenously into rabbits no ill effects were seen. But when injected into a closed cavity, abscess formation rapidly resulted. In the opinion of these workers the acute febrile illness in man, produced by these organisms, is due to

an inflammation of the urinary passages which causes a partial or complete obstruction to the outflow of urine.

Caldwell (1928) reported the Gram negative bacilli occurring in 112 cases of urinary infection. She found B. coli 47 times, B. lactis aerogenes 12 times, B. pseudotetanicus 12 times, slow lactose fermenters 9 times, B. fecalis alkaligenes 5 times, fluorescent bacilli 12 times and B. proteus 7 times. This is the first series in which B. pseudotetanicus was reported as an etiological agent in urinary infections, although Dick and Dick (1915) had mentioned it occurring in the urine on one occasion.

Hill, Seidman, Stadnichenko and Ellis, (1929), made a complete bacteriological study of 200 cultures of Gram negative bacilli isolated from cases of urinary infections. The organisms were classified into 4 groups. Group 1 included the Escherichia cultures fermenting lactose with acid and gas, not producing acetyl-methyl carbinol, and methyl red positive. This group contained 100 cultures. Group 2 included the Aerobacter cultures, fermenting lactose with acid and gas, producing acetyl-methyl-carbinol, but methyl red negative; 79 cultures belonged to this group. Group 3 contained the Proteus cultures, 9 in number. The last group contained the miscellaneous cultures, 16 in all. Group 1 was further divided into (1) 45 typical sucrose negative cultures, (2) 40 typical sucrose positive cultures, (3) 11 typical sucrose positive cultures which utilized citrate promptly and (4) 6 typical, heavily encapsulated cultures. The 79 cultures in Group 2 were subdivided on the basis of gelatine liquefaction into 21 gelatine liquefying and 58 non-liquefying. The 16 cultures in Group 4 consisted of 5 Eberthella cultures, 9 Shigella cultures, 1 Salmonella culture and 1 Alkaligenes fecalis culture. They also report some interesting data on hemolysis. In group 1 60% of the cultures were hemolytic; in Group 2 74% were hemolytic; in the third or Proteus group only one of the cultures was hemolytic; and in the last group, the single Alkaligenes culture, 2 of the Eber-

thella cultures, 7 of the Shigella cultures and the single Salmonella produced colonies which had the ability to hemolyse rabbit blood. They state that there is no selective pathogenicity on the part of these strains. Only 11 of the Escherichia cultures failed to produce indol, and 94% of the Aerobacter cultures were indol negative. This study shows an unusually high incidence of Aerobacter cultures compared with previous papers. In this connection they noted in 12 cases of blood stream invasion simultaneously with genito-urinary infection that 9 of the cultures belonged to the Aerobacter group while only 1 belonged to the Escherichia group. Their paper represents studies made over a period of 4 years and shows the importance of complete bacteriological classification. Their paper also contains an exhaustive review of the literature on urinary infections, as well as pertinent laboratory methods.

METHODS.

Specimens were collected in sterile containers, under aseptic technique, and examined as soon as possible there-after. The urine was transferred to sterile tubes and centrifuged at moderate speed for 20 minutes. If the specimen was grossly turbid this procedure was not considered necessary. Gram stains were then made of the sediment and examined for micro-organisms; these stains were kept as a check on the later cultural reactions. In case only an occasional Gram positive coccus was seen in the original smear, and a few colonies of Staphylococci later developed on plates, the organisms were considered contaminations and not reported. In practically every case there was a close relationship between the organisms seen in the direct smear and the colonies that later developed on plate media.

The sediment was streaked on horse blood agar plates and incubated at 37 degrees Centigrade for 24 hours. The plates were then examined and isolated colonies were transferred to plain extract agar or blood agar slopes and used as stock cultures. In cases which showed Gram negative bacilli in the direct smear streaks were made on Endo's media in addition to the blood agar. If a specimen

showed no organisms in the direct smear, and no colonies were produced on blood plates, it was reported sterile.

Utilization of Carbohydrates.

All the Gram negative bacillus cultures were inoculated into glucose, lactose, sucrose, maltose and mannite broth tubes. The media contained 1% of the respective carbohydrate, in extract broth adjusted to pH 7.2, and was carefully sterilized to assure the sugar was not broken down by excessive exposure to high temperatures. The media was put in Durham fermentation tubes. In addition to the carbohydrate each tube contained Andrade's indicator in concentration of 1%. All tubes were incubated for 1 week, unless positive reactions were obtained. This procedure is of special value in detecting the slow lactose fermenting types. In certain instances additional fermentative mediums were used; these were dulcite, xylose, rhamnose, inulin and arabinose.

Hemolysis.

Organisms were streaked on plates containing one and one-half per cent extract agar and 1.0 cc. of defibrinated horse blood to each 10.0 cc. agar, plates were incubated for 24 hours at 37 degrees. Areas of laked cells around the bacterial colonies were used as a basis for hemolytic power. Streptococci were classified according to the diameter of the laked area.

Motility.

Cultures were inoculated into sugar free extract broth and incubated for 24 hours. Hanging drop preparations were then made and examined under a 4 mm. objective.

Indol Production.

Tubes containing 5 cc. of Bacto-Tryptophane broth in 1% concentration were inoculated and allowed to incubate for 3 days. The presence of indol was determined by the method of Salkowski and the Vanillin test.

Gelatine Liquefaction.

Tubes containing 10 cc. of 13% gelatine in extract broth adjusted to pH 7.2 were used. Stabs were made and left at room temperature for at least 14 days before being discarded.

Methemoglobin Production.

Colonies which produced areas of greenish discoloration on blood plates were placed in inulin serum water tubes and incubated for 1 week or until a positive reaction was obtained. Andrade's indicator was used to denote the presence of acid. In addition, 24 hour extract broth tubes were tested for bile solubility.

Agglutination Reactions.

The macroscopic method was used. Tubes were incubated for 1 hour in a 37 degree water bath, and then placed in the ice box over night.

General Summary of Bacteriological Data.

One hundred eight different strains of bacteria, representing 16 distinct species, were isolated in this series. In 86 instances, only 1 species was present in a single specimen; in 10 cases, more than 1 species was found, and in 1 case 3 separate species were present. More than 1 specimen from a single case was obtained in only 3 instances; in 2 of these a second examination was made, and in 1 case 3 separate examinations were made. In 5 of the specimens no organisms were seen in the direct smear, and no growth was obtained on blood agar plates.

In the following lists are indicated the incidence of the various species of bacteria encountered in this study. The first table lists the Gram positive cocci, and the Gram negative bacilli in order of incidence in pure culture. The second table gives similar data on the mixed cultures.

Table 1.

Gram positive Cocci.		Gram negative Bacilli.	
1.	Staphylococcus aureus - - - - - - - - - 22	1.	Escherichia coli - - - - - - - - 19
2.	Staphylococcus albus - - - - - - - - - 13	2.	Escherichia communior- - - - - - 8
3.	Streptococcus viridans - - - - - - - - 3	3.	Pseudomonas aeruginosa - - - - - 6
4.	Streptococcus hemolyticus (Beta) - - 1	4.	Aerobacter aerogenes - - - - - - 4
5.	Streptococcus anhemolyticus - - - - - 1	5.	Alcaligenes fecalis - - - - - - 3
6.	Streptococcus fecalis - - - - - - - - 1	6.	Proteus vulgaris - - - - - - - - 2
		7.	Eberthella typhi - - - - - - - - 1
		8.	Aerobacter cloacae - - - - - - - 1
		9.	B. pseudotetanicus - - - - - - - 1
	Total- - - - 41		Total - - 45.

Table 2.

Mixed Cultures.			
1.	Staphylococcus aureus - - - - - - - - - 8	1.	Escherichia coli - - - - - - - - 3
2.	Streptococcus anhemolyticus - - - - - 4	2.	Escherichia communior- - - - - 1
3.	Staphylococcus albus - - - - - - - - - 3		
4.	Streptococcus hemolyticus (Beta)- - - 3		
	Total - - - - 18		Total - - - 4.

Note: One species, Neisseria catarrhalis, was obtained in mixed culture.

Detailed Bacteriological and Clinical Findings.

1. Staphylococcus aureus.

Organisms of this type were isolated 22 times. All the cultures were Gram positive and showed the typical group morphology and pigment production on extract agar slopes. All the strains liquefied gelatine. Eight strains showed definite zones of hemolysis around the colonies on horse blood agar. Of the 8

hemolytic strains, 3 were from cases of chronic pyelitis, 2 from acute pyelitis, 1 from acute exacerbation of a chronic cystitis and in 1 case no diagnosis was obtained. The remaining 14 strains of Staphylococcus aureus did not produce hemolysis on blood agar. The diagnoses in these cases were, chronic pyelitis 8; acute cystitis 2; acute pyelitis 1; acute pyelocystitis 1; chronic pyelocystitis 1; chronic infection not diangosed 1.

In 6 cases of pyelitis the organisms were present in both ureteral specimens; in two the right specimen only contained the organism. One of the acute cases was diagnosed "cohabitation cystitis". In the case of chronic infection in which no diagnosis was given a previous examination had revealed a different type of organism.

2. Staphylococcus albus.

This organism was found 13 times. All cultures gave the typical Gram positive staining reaction, group morphology, and absence of pigment on extract agar slopes. All strains liquefied gelatine. Only 1 of the strains developed colonies which produced hemolytic areas on blood agar plates. This strain was obtained from a case of chronic cystitis. Of the 12 remaining strains, 5 were associated with acute pyelitis, 5 with chronic pyelitis, 1 with chronic cystitis, and in 1 case no diagnosis was obtained. In 3 instances specimens from both ureters were obtained; in 2 of these the right specimens only were positive, and in 1,organisms were present only in the left specimen.

3. Streptococcus viridans.

This type of Streptococcus was found 8 times. The cultures showed Gram positive cocci occurring usually in short chains, and their colonies produced an area of greenish discoloration on blood agar plates. None of the strains fermented inulin, and the broth cultures were not bile soluble. All of these strains were found in cases in which a diagnosis of chronic pyelitis had

been made. One of the strains was obtained by bladder catheterization, the remaining 2 drom ureteral catheterization. In these cases the organisms were obtained from the right specimens; the left were negative in both instances.

4. Streptococcus anhemolyticus.

Micrococci occurring in chains, whose colonies produce neither hemolysis or green color on blood agar plates was found in only 1 instance in pure culture. This strain was associated with a case of pyonephrosis, and subsequent examination of the same case failed to reveal the organism.

5. Streptococcus hemolyticus.

This type of Streptococcus was found once in pure culture. The organisms were Gram positive cocci which appeared in long chains, and their colonies produced a definite area of hemolysis on blood agar plates. The area corresponded to the Beta type. The strain was isolated from the case of pyonephrosis cited above and was present in the second examination. A third examination of this case revealed Staph. aureus in addition to this hemolytic streptococcus.

The Gram Positive Cocci.

Staphylococcus aureus.

Identification. Typical morphology, pigment production on plain agar and liquefaction of gelatine.

Group 1. Hemolytic type in pure culture - - - - - - - - 8

Associated with chronic pyelitis (3), acute pyelitis (2), acute exacerbation of a chronic cystitis (1), no diagnosis (1).

Group 2. Hemolytic type in mixed culture- - - - - - - -

Group 3. Non hemolytic type in pure culture - - - - - - 14.

Associated with chronic pyelitis (8), acute pyelocystitis (1) acute cystitis (2), acute pyelitis (1), chronic pyelocystitis (1), chronic infection undiagnosed (1).

In 6 cases of pyelitis the organisms were present in both ureteral specimens. In 2 cases the right specimen only contained the organisms. One of the acute bladder infections was diagnosed "cohabitation cystitis"; the staphylococci were present in pure culture and in large numbers; this is rather unusual because practically all the cases of this type of cystitis are said to be due to members of the Colon group. (Scholl). Another interesting point was brought out by the case of undiagnosed chronic infection in that a previous examination had shown a pure culture of B. pseudotetanicus.

Group 4. Non hemolytic type in mixed culture - - - - - - - -8

a. Non hemolytic streptococcus. (chronic cystopyelitis) 1.

b. Streptococcus hemolyticus. (pyonephrosis)
(acute cystitis)
(acute pyelocystitis)

c. Staphylococcus albus. (chronic cystopyelitis).

d. Non hemolytic streptococcus and Esch. coli. (chronic cystopyelitis).

e. Neisseria catarrhalis. (acute pyelocystitis).

f. Escherichia communior. (chronic cystitis).

It may be seen that the non hemolytic type of Staph. aureus occurred in mixed culture in one-third of the cases. The hemolytic type was not found in mixed culture.

The Gram negative Bacilli.

1. Escherichia coli. (B. coli communis.)

Escherichia coli was found 19 times. All of the strains were small motile rods, and produced acid and gas on lactose, glucose, maltose and mannite but did not attack sucrose. Brom-cresol-purple milk showed an acid reaction and curd formation. Indol was produced in tryptophane broth by all the strains. Gelatine was unaffected by any of the cultures. Two atypical strains were isolated. One produced hemolytic colonies on blood agar plates, but fermented lactose within

24 hours. This culture was isolated from a case of chronic p elitis with an acute exacerbation. The other atypical strain was likewise a slow lactose fermenter but did not develope hemolytic colonies on blood agar. This was isolated from a case of acute pyelitis. Of the remaining 17 strains, 9 were from cases of chronic cystitis, 6 from cases of chronic pyelocystitis, 1 from a case of acute cystitis, and in 1 case no diagnosis was given. In only 1 case were specimens from both ureters obtained and this showed the organisms in each.

2. Escherichia communior. (B. coli communior).

Organisms belonging to this species were found 8 times. All the strains were short motile rods and fermented glucose, lactose, sucrose, maltose and mannite with the production of acid and gas. All of the different cultures produced indol from tryptophane broth, and none liquefied gelatine. There were 3 atypical types isolated; 1 strain developed colonies which produced hemolysis on blood agar, but fermented lactose in 24 hours. This was isolated from a case of acute pyelitis. Two of the strains, in addition to hemolytic properites, did not ferment lactose for 72 hours. Both of the strains were isolated from cases of acute pyelitis. The remaining 5 strains were non hemolytic and fermented lactose within 24 hours. Two of these were from cases of chronic cystitis, 2 from chronic pyelitis, and 1 from acute pyelitis.

3. Pseudomonas aeruginosa. (B. pyocyaneus).

Six specimens revealed this species. All of the strains were actively motile bacilli, liquefied gelatine rapidly and produced typical pigment on extract agar slopes. Five of the strains showed no fermentation in glucose, lactose, sucrose, maltose, or mannite media; one however, produced acid in glucose, lactose and maltose broth, the Andrades indicator becoming definitely changed in 24 hours. This is the only lactose fermenting strain ever encountered in this laboratory. Five of the strains were isolated from cases of chronic pyelitis, 1 was from a case of

typhoid in which the urinary carrier condition was suspected. In 4 of the cases of chronic pyelitis the organisms were present in both ureteral specimens; in 1 the involvement was confined to the right kidney; the left having been removed some months previously.

4. Aerobacter aerogenes. (B. lactis aerogenes).

Four strains were isolated. All showed the ability to ferment glucose, lactose, sucrose, maltose and mannite, with the production of acid and large amounts of gas in 24 hours. The colonies on agar plates were raised, moist, and showed a tendency to be stringy. Indol was not produced from tryptophane broth by any of the strains, and none liquefied gelatine. Two of the cultures were associated with chronic pyelitis, 1 with chronic cystitis, and 1 with acute pyelitis. In both cases of chronic pyelitis the organisms were present in both ureteral specimens. No atypical strains, such as slow lactose fermenting or hemolytic types were encountered.

5. Alcaligenes fecalis. (B. fecalis alcaligenes).

Three strains were isolated. All showed motility in hanging drop preparations, and although there was abundant growth in glucose, lactose, sucrose, maltose and mannite broth there was no acid formed. None of the cultures liquefied gelatine. Two of the strains were associated with chronic pyelitis, and 1 with chronic cystitis. In 1 of the cases of chronic pyelitis the organisms were present in both ureteral specimens, in the other they were found only in the right specimen.

6. Porteus vulgaris. (B. proteus).

Two strains were isolated; both showed active motility, diffuse growth on the surface of extract agar plates, and rapid liquefaction of gelatine. Acid and gas was formed in glucose, maltose, and sucrose, lactose and mannite were not attacked. One of the strains was associated with a case of chronic pyelitis and the organisms were found only in the left ureteral specimen. The other strain was obtained from a case which was undiagnosed.

7. Eberthella typhi. (B. typhosus).

Only 1 strain was encountered; this showed actively motile rods, which produced acid but no gas in glucose, maltose and mannite. It gave positive agglutination with immune serum. Gelatine was not liquefied. This organism was isolated from a case of chronic cystitis.

8. Aerobacter cloacae. (B. cloacae).

Only 1 strain was isolated; this organism was motile and produced acid and gas in lactose, glucose, sucrose, maltose and mannite broth. It produced a slow craterform liquefaction of gelatine. The culture was obtained from a case of chronic pyelocystitis.

9. Bacillus pseudotetanicus.

Only 1 strain of Gram negative spore forming bacilli was isolated; these were long motile rods and the spores were terminal and subterminal. There was good growth in glucose, lactose, sucrose, maltose and mannite but there was no fermentation. There was no liquefaction of gelatine. The organisms were present in large numbers. The culture was obtained from a case of chronic infection in which no focal diagnosis was given. Subsequent examination of the same case failed to reveal this organism.

Group 2.

Specimens from which more than 1 species were isolated at a single examination.

Mixed infections were encountered 11 times; in 10 of these 2 different species were represented; in 1 instance 3 different species were found. Staphylococcus aureus, nonhemolytic type, was found 8 times; Staphylococcus albus, nonhemolytic type, 3 times; Streptococcus hemolyticus, Beta type, 3 times; Streptococcus anhemolyticus, 4 times; Escherichia coli, 3 times; Escherichia communior, once, and Neisseria catarrhalis, once. The following table gives the mixtures and the conditions with which they were associated.

1. Staphylococcus aureus and Neisseria catarrhalis, acute pyelocystitis.
2. " " " Streptococcus hemolyticus, acute cystitis.
3. " " " " " , acute pyelocystitis.
4. " " " " " , pyonephrosis.
5. " " " Streptococcus anhemolyticus, chronic cystopyelitis.
6. " " " Escherichia communior, chronic cystitis.
7. " " " Staphylococcus albus, chronic cystopyelitis.
8. Escherichia coli and Streptococcus anhemolyticus, acute pyelitis.
9. Staphylococcus albus and Streptococcus anhemolyticus, chronic pyelitis;
10. Escherichia coli and Staphylococcus albus, no diagnosis.
11. Escherichia coli, Streptococcus anhemolyticus and Staphylococcus aureus, chronic pyelitis.

Number 5 represents a third examination of a single case, 2 previous examinations having revealed pure cultures.

Discussion and Summary.

Staphylococcus aureus was the most frequently encountered species of bacteria, occurring as it did 22 times in pure culture, and 8 times in mixed culture. Therefore, it occurred in association with other organisms in one-third of the cases. The hemolytic type never occurred in association with other organisms, while in pure culture it was found in 8 instances. The organisms when found in pure culture were associated practically as frequently with chronic conditions, as they were with the acute. This is contradictory to the statement so often seen that the acute infections are more likely to be coccal in origin, and the chronic to be bacillary. One of the acute cases was diagnosed "cohabitation cystitis"; this is rather out of the ordinary, in that this type of cystitis is said to be due to members of the Colon group in the majority of cases (Scholl). The presence

of Staphylococcus aureus in second examinations is interesting; in 1 case a previous examination had shown a pure culture of B. pseudotetanicus. In the next examination, 1 month later, these organisms were not found, having been replaced by Staphylococcus aureus. In another case Staph. aureus was found on the first examination, and the next specimen, taken a few weeks later, was sterile. In still another case Staph. aureus was not encountered until the third examination and then it was in association with another organism, 2 previous specimens having revealed pure cultures of Strep. anhemolyticus and Strep. hemolyticus respectively. This case will be discussed more fully later.

Staphylococcus albus was third in frequency, and what has been said about Staph. aureus is practically true for the white Staphylococcus. However, in this group only one of the strains was hemolytic. Furthermore the incidence of this organism in mixed culture was not as high as in the Staph. aureus group. The ratio of pure culture to mixed culture was 13:3.

The Streptococcus viridans group occurred only in pure culture, and in each instance was associated with a chronic condition. Blood culture and further clinical data would have been valuable in this group.

Streptococcus hemolyticus occurred only once in pure culture, and only 3 times in mixed culture. With such a high incidence of Staphylococci one would have expected to find the chain cocci in larger numbers. The single pure culture and one of the mixtures were obtained from a single case, from specimens taken at different times.

The anhemolytic type of Streptococcus was likewise found only once in pure culture. The subsequent history of the case from which this organism was isolated is interesting enough to warrant a detailed description. The case was one of infected hydronephrosis, occurring in a young male. The first specimen was

obtained by bladder catheterization, and revealed a pure culture of non hemolytic Streptococcus. Four days later a second bladder catheterization was made; this specimen showed a pure culture of an hemolytic type of Streptococcus; careful examination of the blood plates failed to reveal the nonhemolytic type found on the first occasion. Either the anhemolytic culture had become hemolytic during the interim, or had been replaced by the hemolytic type. The latter explanation seems the most plausible, in the light of our present knowledge of bacterial species. One week later a third specimen was obtained. This time the ureters had been catheterized. Material from the left ureter and the bladder contained the hemolytic Streptococcus described above, however, in addition Staph. aureus colonies in approximately equal numbers were found. The material from the right ureter contained no demonstrable organisms, either by direct smear or blood plates. Therefore in this case 3 different examinations over a period of 11 days had revealed 3 different species of bacteria. This is a rather striking example of the change of flora in urinary infections, a point brought out years ago by Faltin, but which has not been stressed in late years.

Escherichia coli was present 19 times, being next in frequency to Staph. aureus. In only 3 instances was this type associated with other organisms, differing quite markedly in this respect from the Staph. aureus cultures. It is also interesting to note that only 2 atypical types of non-sucrose fermenting coli were encountered, and each was associated with an acute infection. In the remaining 17 cases all were associated with chronic infections; this point is also in contrast to the Staph. aureus group. No attempt was made to associate the types of Esch. coli found in the urine with those which might have been present in the stools. No data was obtained on blood cultures or stool cultures in this group.

Escherichia communior occurred only once in mixed cultures and 8 times in pure culture, or one half as often as Escherichia coli. The incidence of su[illegible]ose

fermenting to non sucrose fermenting is not as high, therefore,as in the report of Hill and his workers. But although Esch. communior was found in pure culture in only 8 instances there was a very high incidence of atypical cultures. Two of the atypical strains were slow lactose fermenters and produced hemolysis on blood plates, while 1 culture produced hemolysis but lactose was fermented within 24 hours. All the above strains were associated with acute infections. The number of hemolytic types of Escherichia was not as large in this series as in the reports of Dudgeon, or that of Hill and his associates. Dudgeon used human blood for his determination of hemolysis, and Hill used rabbit blood; in this series horse blood agar was used and this factor may have been responsible for the smaller number of hemolytic types found. One of the cases of acute pyelitis in which a slow lactose fermenting hemolytic type of Escherichia was found was associated with nephroptosis resulting in an acute kinking of the corresponding ureter. This case would come under the classification of acute urinary fever according to Dudgeon. The case was an unusually severe one and seems to bear out the experimental work of Dudgeon cited previously, namely, the effects of hemolytic slow lactose fermenting types of coli when placed in a more or less closed cavity.

Pseudomonas aeruginosa cultures were third in frequency in the Gram negative bacillus group, and fifth in frequency in the total series. This is an unusually high incidence if we consider the report of Mathe and Belt as representative. All of the organisms in this group conformed to the usual description with one exception. This organism was isolated from a case which had had typhoid fever a few weeks previously and was having a routine examination of the urine for typhoid bacilli before being dismissed from the hospital. The specimen was negative for Eberthella typhi and Pseudomonas aeruginosa was present in pure culture. The organism produced typical pigment on extract agar slopes; rapid liquefaction of gelatine, and in addition fermented lactose, glucose and mannite. The colonies on Endo's agar were very similar to those of the colon group and without further

cultural data, undoubtedly would have been reported as B. coli. There seems to be little doubt that these organisms have been mistaken many times for members of the colon group especially when the diagnosis rested upon such insecure grounds as a Gram stain. I believe that this organism would have occurred more frequently in the literature as a urinary infectant had the trouble been taken to study each specimen completely. Mathe and Belt bring out the seriousness of pyelitis due to this organism and how important it is to make an early diagnosis of its presence in the urinary tract.

Cultures belonging to the Genus Aerobacter were found in 5 cases and were the only organisms present in each case. All except one culture belonged to the species Aerobacter aerogenes; the exception liquefied gelatine and was classified as Aerobacter cloacae. The relation of Aerobacter types to Escherichia types is not as high in this series as it is in the report of Hill and co-workers, in which the ratio was 79 to 100. However, it does correspond to the ratio given by Caldwell which is 12:47, or roughly one Aerobacter culture to four Escherichia cultures. In this series there was roughly one Aerobacter culture to six Escherichia cultures. None of the Aerobacter cultures isolated showed any atypical reactions such as hemolysis or slow lactose fermentation.

The remaining Gram negative bacilli are relatively unimportant from a bacteriological standpoint, with one exception, namely the B. pseudotetanicus culture. Dick and Dick (1915) reported a Gram negative spore forming aerobe found in urine but did not discuss it further. Caldwell (1928) found B. pseudotetanicus 12 times in a series of 112 Gram negative bacilli. She gives a complete description of the organism and states that it has probably been overlooked frequently in routine smears and cultures of catheterized specimens and probably also in reported bacteriological studies of urine. The culture isolated in this laboratory was obtained from a chronic infection of the urinary tract and was present in the first examination. The organisms were present in pure culture and in large numbers.

A second examination of the same case made a few weeks later showed Staphylococcus aureus. This is another example of the change in bacterial flora from time to ti e that occurs in the urinary tract. A single e amination of any given case does not appear to be sufficient to warrant the statement that the organism found is the inciting agent.

Conclusions.

1. In the 86 cases in which only 1 type of organism was found Gram negative bacilli occurred 45 times, Gram positive cocci 41 times. However, in the total number of cultures (108) Gram positive cocci were found 69 times, while the Gram negative bacilli occurred 59 times.
2. Staphylococcus aureus was the type most frequently encountered, occurring 30 times. Escherichia coli was next in frequency, occurring 22 times.
3. Five atypical strains of Escherichia were isolated, and in each case, was associated with an acute infection.
4. In 3 cases where repeated examinations of a single case were made, there was a tendency to change of the bacterial flora.
5. Among the 108 cultures studied, 16 different species were represented, showing the poly-microbic nature of infections of the urinary tract.

Bibliography.

Bergey, D. H., Manual, Williams and Wilkins. 1923.

Bitter, L., and Gundel, M., (1924) Klin. Woch. 3 pt 2 p, 2141.

Brown, T. R., (1902) Johns Hopkins Hosp. Rep., 10, 12.

Brumnich, K., (1918) Med. Jr. Australia., 2, 23.

Cabot, H., and Crabtree, E. G., (1916) Surg. Gyn. and Obs., 22, 495.

Caldwell, J. A., (1928) Jr. Inf. Dis., 43, 353.

David, V. C., (1914) Surg. Gyn. and Obs., 18, 432.

Dick and Dick (1915) Jr. Am. M.A., 65, 6.

Dudgeon, L. S., Wordley, E., and Bawtree, F., (1921) Jr. Hyg., 20, 137.

Dudgeon, L. S., Wordley, E., and Bawtree, F., (1922) Jr. Hyg., 21, 168.

Dudgeon, L. S. (1924) Jr. Hyg., 22, 119.

Dudgeon, L. S., and Fulvertoft, R. J. V., (1927) Jr. Hyg., 26, 285.

Duhig, J. V., (1922) Med. Jr. Australia, 2, 437.

Faltin, R., (1902) Ann. des Mal. des Org. Gen. Urin. p. 176.

Herrold, R. D., Culver, H., and Phifer, F. M., (1918) Jr. Am. M. A., 70, 1444.

Herrold, R. D., and Culver H., (1919) Jr. Inf. Dis., 24, 114.

Hill, J. H., Seidman, L. R., Stadnichenko, A. N. S., and Ellis, M. G., (1929) Jr. Bact., 17, 205.

Keilty, R. A., (1926) Jr. Lab. and Clin. Med., 11, 601.

MacKenzie, D. W., and Cochrane, W. J., (1924) Jr. Urol., 12, 113.

Mathe, C. P., and Belt, A. E. (1922) Jr. Urol., 8, 281.

Melchoir, M. (1898) Monatsb. f. die Krankheit der Harn u. Sem. Appar. Bd. 3, 584.

Mitchel, E., (1922) Southern Med. Jr., 15, 836.

Ogston, A., (1881) Brit. Med. Jr., 1, 369.

Pasteur, L., (1874) Comp. Rend. des sea. De Lacad. des Sci., 78, 46.

Rovsing, (1898) Ann. d. Mal. Genito Urin., 16, 179 and 278.

Roberts, W., (1881) Brit. Med. Jr., 2, 623.

Schell, A. J., (1926) Jr. Am. M.A., 87, 1794.

Sherwood, N. D., Johnson, T. L., and Radotinsky, I., (1926) Univ. Kansas Sci. Bull. 16, 91.