A STUDY OF TWO HUNDRED CULTURES OF GRAM-NEGATIVE BACILLI ISOLATED FROM CASES OF GENITO-URINARY INFECTION

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Received for publication, October 17, 1928

We have hoped that a further study of the bacteria found in genito-urinary infections might be of value, both in increasing our knowledge of these organisms, and as a basis for later work. We present here the characteristics of two hundred cultures of Gram-negative bacilli, isolated from two hundred cases. As far as possible, these have been taken in their order of admission, but, in the four years' duration of this work, some cultures have been lost and a few have been discarded because of insufficient clinical data. *Pseudomonas* cultures, included in the preliminary report¹ have finally been omitted, in order to limit this series to organisms of the colon group, or related forms. Studies of the anaerobes and of the Gram-positive cocci found in genito-urinary infection are separate problems, about which almost nothing is yet known.

Our interest in studying bacillary infections has been first, to determine the bacteriological characteristics of the organisms, and, second, to consider any possible clinical correlation. The cultural characteristics of the organisms have been studied on a variety of media and the findings compared with those of other observers on bacteria from similar sources, or on related organisms. In a limited number of cases, it has been possible to study organisms present in the blood simultaneously with a genito-urinary infection, and a summary of these findings has been

¹ Paper read before the Society of American Bacteriologists, Philadelphia, December, 1926.

made. In regard to any bacteriological-clinical correlation, comparisons have been made between the type of the culture and the nature of the infection. There is reason to believe that a consideration of the wide differences of the strains might readily explain some of the marked variations in response to treatment of different "*B. coli*" infections, a point which is seldom made clinically.

PREVIOUS STUDIES OF GENITO-URINARY INFECTION

Although the literature on this subject is extensive, many of the articles are now of only historical interest, while in others, the emphasis is clinical rather than bacteriological, so that there are relatively few studies available for comparison. We have prepared a bibliography of the more important general articles in which bacillary infections are discussed, and a few of these reports may be cited briefly.

Pasteur (1862) early stated his belief that the ammoniacal decomposition of urine was due to an organized ferment. Later (1874) he is quoted as follows:

M. Pasteur, à l'occasion de l'intéressante note de MM. Gosselin et A. Robin, fait observer qu'il y aurait une grand utilité à rechercher si, dans tous les cas, ou dans des cas particuliers, la qualité ammoniacale de l'urine par la présence du carbonate d'ammoniaque n'est pas liée à l'existence d'un ferment organisé, notamment du ferment ammoniacal de l'urine si bien étudié par M. van Tieghem, ou de bactériidies, ferments dont les germes seraient apportés de l'extérieur par le canal de l'urèthre, or par le sang qui aurait pu lui-même prendre ce germe dans quelque partie du corps, par exemple, par une blessure quelconque, ou communication avec le canal intestinal; enfin ce germe, organisé vivant, peut être apporté souvent par une soude ou par un instrument chirurgical.

One may read many articles on this subject, which say less than has been expressed by Pasteur in these few words.

Early interest continued to center on *Proteus* infections. Roberts (1881) divided the bacillary infections into those with, and without, ammoniacal decomposition. Attention was called to the polymorphic nature of the bacilli which did not liquefy

gelatin, to the pyogenic nature of infections with these organisms, and to the possibility of blood stream infection by Albarron and Hallé (1888). Doyen (1888) made an early study of renal infection. Guyon (1889) emphasized the rôle played by retention. The studies of Clado (1887a) (1887b) and of Krogius (1890) should be noted. Morelle (1891) included an examination of direct smears of specimens in his study of cystitis. The large number of infections due to non-gelatin-liquefying bacilli was emphasized by Krogius (1892), by Schmidt and Aschoff (1893), by Escherich (1894), by Savor (1894) and by Bastianelli (1895). The books of Melchior (1895) and of Rovsing (1897a) were important. Autointoxication was discussed by Goldburg (1895) and Hutinel (1896) also emphasized the probable intestinal origin of these infections.

Young (1898) made an interesting study of cystitis, cultures being obtained by suprapubic punctures, in order to avoid urethral contamination. Albarron, Hallé and Legrain (1898) summarized the literature. At the same time Rovsing (1897b) (1898) continued his studies, as did Melchior (1897) (1898).

Rostoski (1898) differentiated Colon group organisms and reported cystitides due to *Bact. acidilactici*, *Bact. lactis-aerogenes* and to *Bact. coli*. Maxwell and Clarke (1899) emphasized the secondary nature of *Bact. coli* infections, Suter (1901) studying them as secondary to tuberculosis. Parascandolo (1900) made both a clinical and experimental study. T. R. Brown (1902) provided bacteriological details in his careful report. Baisch (1903) studied post-operative cystitides in women and stated his belief that such catheter infections occurred only in pathological bladders. Von Hofmann (1904) made a thorough review of the literature. Raskai (1905) and Lenhartz (1907) differentiated their cultures bacteriologically.

Suter (1907) reported 211 cases of urinary infections, differentiating between those of endogenous and instrumental origin. Albeck (1907) studied the bacteriuria of pregnancy. Goldburg (1907) made a careful study of cystitis in infants, Jeffreys (1911) reporting 121 cases of urinary infection in children. Kodama and Krasnogorski (1913) found *Bact. coli* but seldom in extra-

renal infections and emphasized the necessity of considering kidney involvement when this organism was present. Tanaka (1909) studied 50 cases of cystitis, correlating the examination of smears with cultures, the latter being bacteriologically incomplete. Alsberg (1910) advised using the more general term "Colon Group" and noted morphological variations.

More recently, several valuable reports, such as those of Wolff (1912), of David (1914), of Brunnich (1918) and of Herrold and Culver (1919) are not available for purposes of comparison because no Voges-Proskauer tests were made. These authors, however, describe forms closely resembling those found by us. The high percentage of Colon group bacilli in renal infections has been emphasized by Campbell and Rhea (1918), by Mackenzie (1921) and by Mackenzie and Cochrane (1924a) (1924b). The work of Dudgeon (1908) and of Dudgeon, Wordley and Bawtree (1922) on *Bact. coli* infections of the urinary tract are of great importance, as are the studies of Kowitz (1915) and of Bitter and Grundel (1924). Murray, Williams and Wallace (1910), Beckman and van der Ries (1925) and others have demonstrated the immunological reaction of patients infected with *Bact. coli*.

METHODS OF STUDY AND FINDINGS

The routine laboratory methods used in the Brady Urological Institute have been described elsewhere by Young and Davis, (1926). They were closely followed in this series. We wish to acknowledge most gratefully the constant encouragement of Dr. Hugh H. Young and the clinical coöperation of Dr. Young and his associates in securing specimens with great care to prevent contamination. The method of collecting specimens consisted of cleansing the urinary meatus with alcohol and the anterior urethra by an injection of Meroxyl, 1:500. The first urine, in voided specimens, was allowed to escape and a specimen was then obtained in a sterile glass tube. The tubes used had been plugged with cotton and their tops wrapped in brown paper before sterilization. Many of the specimens were obtained by catheterization of bladder or kidneys. In the laboratory, rigid precautions were taken to avoid contamination. Specimens were

transferred for centrifugalization by means of sterile pipettes and were always kept in sterile tubes. Whenever the amount of the specimen allowed, it was centrifugalized at 2200 revolutions a minute for at least three minutes, often longer. This was sometimes possible even with prostatic secretions by using a finely pointed centrifuge tube.

We are convinced that there must be a careful correlation of thorough examinations of direct smears with cultural results, if accurate bacteriological findings are to be obtained from such specimens. Rarely, direct smears may fail to show organisms, which may be recovered regularly by culture, usually from cases undergoing intensive treatment. But, in general, an organism which develops but scantily on culture, usually only after forty-eight hours of incubation, and which has not been seen in the direct smear, can be discarded as a urethral contamination. Such contaminations, in the male, however, are almost invariably cocci. No culture has been included in which Gram-negative bacilli were not seen in the direct smear. One cannot, without special precautions, place any value on the estimation of the number of bacilli in such smears, because of the rapidity with which these organisms grow in urine. The recent report of Ver Mooten (1928) in which he reports that in over one third of his cases the examinations of direct smears and cultures do not correspond, is not in accord with our experience.

A Gram stain was done on all direct smears, a modification of Nicolle's (1895) one minute stain being used. This consisted of staining twenty seconds with gentian violet, (one part of saturated alcoholic gentian violet, freshly diluted with nine parts of 2.5 per cent aqueous solution of phenol), and then twenty seconds with Gram's iodine solution, decolorizing carefully with a mixture of one part of acetone in three parts of 95 per cent ethyl alcohol, washing in water and counterstaining twenty seconds with one part of carbol-fuchsin in twenty parts of distilled water.

MORPHOLOGICAL VARIATION

Although it is true that, in general, organisms of the genus *Aerobacter* differ from those of the genus *Escherichia* morpho-

logically, in being shorter and thicker and sometimes appearing in the form of streptobacilli or of diplococci, no presumptive diagnosis should be made from examinations of direct smears alone. Schmidt (1892) emphasized the diplococcoid forms which may be assumed by colon bacilli, a point also made by Adami, Abbott and Nicholson (1899) and by Konrich (1910). On the other hand, colon bacilli may assume equally confusing filamentous forms. The experimental work on morphological variation made by Péju and Rajat (1906), by Scales (1921) and by Henrici (1926) seems to correlate many of these variations with environmental changes. There is every reason to believe that such changes occur in different specimens of urine. Fishch (1926) has recently called attention to morphologic changes of *Bact. coli* in this medium. Clark and Ruehl (1919) have noted the difference in morphological characteristics between young and old cultures, which is of interest in view of the fact that many cases of genito-urinary infection have retention, the bladder serving as an excellent incubator for the *in vivo* growth of organisms.

Before sub-culturing, the organisms were plated by streaking lactose agar plates, and single colonies were fished to form the initial stock cultures. The fermentation of carbohydrates was studied on agar slants containing 1 per cent of the carbohydrate and the double indicators brom-cresol purple and cresol red (Manual of Methods for Pure Culture Study, 1926, A 22, B-25 and B-10). The agar was made sugar-free before the addition of the carbohydrate and care was taken not to overheat in sterilization. Inoculations were made both on the surface of the slant and deep into the butt. Observations were made for at least one week before negative cultures were discarded and tests were repeated whenever indicated.

For convenience our 200 cultures have been divided into 4 groups, as follows:

- Group I. 100 *Escherichia* cultures, fermenting lactose with acid and gas, not producing acetyl-methyl-carbinol, but methyl red positive.

Group II. 79 *Aerobacter* cultures, fermenting lactose with acid and gas, producing acetyl-methyl-carbinol, but methyl red negative.

Group III. 5 *Proteus* cultures.

Group IV. 16 miscellaneous cultures.

The following carbohydrates were used:

I. Monosaccharides:

a. pentoses,

1. arabinose, utilized by all forms in Groups I and II, by none in Group III.
2. xylose, utilized by all forms in Groups I and II, and III.
3. rhamnose, utilized by 96 per cent of Group I, by all of Group II and none of Group III.

b. hexoses,

1. glucose, utilized by all of Groups I, II and III.
2. galactose, utilized by all of Groups I, II and III.
3. levulose, utilized by all of Groups I, II and III.

II. Disaccharides:

- a.* lactose, utilized by all of Groups I and II, by none of Group III.
- b.* maltose, utilized by all of Groups I and II, and III.
- c.* sucrose, utilized by 55 per cent of Group I, and by all of Groups II and III.

III. Trisaccharides:

- a.* raffinose, utilized by 51 per cent of Group I, by 96.2 per cent of Group II, and, slowly, by 40 per cent of Group III.

IV. Polysaccharides:

- a.* dextrin, utilized by 32 per cent of Group I, by 74.6 per cent of Group II and 80 per cent of Group III.
- b.* inulin, utilized by 6 per cent of Group I, by 15.1 per cent of Group II and none of Group III.
- c.* starch, utilized by 29 per cent of Group I, by 68.3 per cent of Group II and 60 per cent of Group III.

V. Glucosides:

- a.* salicin, utilized by 31 per cent of Group I, by 97.4 per cent of Group II and 40 per cent of Group III.

VI. Alcohols:

- a. trihydric alcohols.
 1. glycerol, utilized by all of Groups I, II and III.
- b. hexahydric alcohols,
 1. dulcitol, utilized by 69 per cent of Group I, by 63.2 per cent of Group II and 40 per cent of Group III.
 2. mannitol, utilized by all of Groups I and II and none of Group III.
 3. inositol, utilized by 8 per cent of Group I, by 87.3 per cent of Group II and by none of Group III.
- c. sorbitol, utilized by 80 per cent of Group I, by 93.6 per cent of Group II and by 10 per cent of Group III.
- d. adonitol, utilized by 13 per cent of Group I, by 83.5 per cent of Group II and by 10 per cent of Group III.

The value and correlations of these carbohydrate reactions will be discussed later in comparison with our findings.

THE PRODUCTION OF ACETYL-METHYL-CARBINOL

This was tested by adding 3 cc. of 10 per cent potassium hydroxide solution to 5 cc. of a five-day old broth culture. The medium used was a 1 per cent glucose, 1 per cent peptone and 0.5 per cent sodium chloride broth. After the addition of the hydroxide, the tubes were thoroughly shaken, the plugs removed and the cultures kept under observation for at least twenty-four hours. By making readings in comparison with negative controls, very satisfactory results were obtained. We preferred this method to allowing the color to appear only as a superficial ring, because the entire culture was colored and often remained so for several days, insuring a more satisfactory reading than could be obtained from the more transitory surface reaction.

Because it is an inconvenience to wait five days for this test, daily tests for five days were done with all of our *Aerobacter* cultures, inoculating the same lot of media with eighteen hour broth cultures of the organisms, using for inoculation a standard size of platinum loop, but with no other effort to equalize the inocula. The results of these tests are plotted in figure 1. By

this it will be seen that within twenty-four hours, forty-one cultures, or 51.8 per cent of this group showed definite production of acetyl-methyl-carbinol. There was little difference between the second and third day readings, sixty-eight cultures, or 86 per cent being positive the second day and sixty-nine cultures, or 87.3 per cent on the third. By the fourth day, seventy-one

FIG. 1. PRODUCTION OF ACETYL-METHYL-CARBINOL BY SEVENTY-NINE CULTURES

cultures, or 89.8 per cent were positive and all were positive on the fifth day. Since 86 per cent were positive by the third day, we believe that it would be advisable to test part of a culture on the third day. This early production of acetyl-methyl-carbinol has been shown by Levine, Weldin and Johnson (1917).

The recent article by Paine (1927), in which the transitory nature of the Voges-Proskauer reaction is studied is of interest

here, since two of the three cultures which he found were positive from fourteen to thirty-six hours and then negative from three to ten days, were of renal origin. In our seventy-nine cultures, we have not observed this transitory action, but Paine's observation should certainly be noted in the study of atypical cultures.

In our series the Voges-Proskauer test has been satisfactory and has served as a valuable aid in identification. Correlated with citrate utilization, it might be of value in the classification of some of the encapsulated forms about which there remains so much confusion. There is evidence that at least some of the viscid, heavily encapsulated cultures do not utilize citrate and do not form acetyl-methyl-carbinol, while other cultures, similar in other reactions, utilize citrate rapidly and are Voges-Proskauer positive.

METHYL RED TEST

The medium used for this test was the glucose, potassium phosphate, peptone broth described in the A.P.H.A. Standard Methods of Water Analysis (1923). The addition of methyl red was carried out as there described, that is, by adding five drops of indicator (0.1 gram of methyl red in 300 cc. of alcohol, diluted to 500 cc. with distilled water) to 5 cc. of a five-day broth culture. Correlations of methyl red and of Voges-Proskauer tests in our *Escherichia* and *Aerobacter* groups were entirely satisfactory.

CITRATE UTILIZATION

This has proven to be one of the most valuable of all of the available tests. Three media were tried, Koser's (1924b) two citrate broths and Simmons' (1926) citrate agar. This last has now been adopted for routine use in preference to the broths and is invaluable in giving rapidly a presumptive generic classification, as citrate is utilized promptly by *Aerobacter* cultures and either not at all, or scantily and belatedly, by *Escherichia* forms. There are, of course, a few exceptions to this, which are found almost invariably in the types which are also intermediate in some of their other characteristics. One of our most typical *Aerobacter* strains, however, utilized citrate only slightly, al-

though repeated tests have been made with both freshly isolated and old stock cultures. The confusion which reigns at present in regard to the encapsulated forms makes it possible that further subdivision of these organisms will, in time, eliminate such inconsistencies. In general, the citrate test is so accurate, that one suspects the atypical nature of the culture rather than the test when results are contradictory. Paran (1925) has found the citrate test of value in tropical water analysis.

We have been interested in observing the different types of growth in citrate agar. Simmons (1926) found with the strains he studied that *Bact. coli* either failed to grow on citrate, or grew only as pin point colonies, the reaction of the medium being either unchanged or superficially acidified. Fifty-four of our one hundred *Escherichia* cultures failed to produce any visible growth on citrate agar in four days, with incubation at 37.5°C. Eighteen of the cultures produced only slight growth within four days, without visibly changing the reaction of the medium. Seventeen cultures definitely acidified the medium within four days, but only four of these grew well within two days. These four cultures belong to the *Escherichia* sub-group C, which seems to be nearest to the *Aerobacter* cultures. Eleven of the *Escherichia* cultures produced a deep blue coloration of the medium, this alkalinity developing in 4 cases only after forty-eight hours or longer. In the others,—all cultures of the intermediate sub-group,—it occurred promptly.

Although Simmons found that the *Bact. aerogenes* strains he tested uniformly produced an alkaline reaction on citrate agar, our seventy-nine *Aerobacter* cultures varied markedly in this respect. Thirty of our cultures or 38 per cent of the group, did produce prompt and permanent alkalinity. Fifteen cultures, or 19 per cent produced equally prompt and permanent acidity. Thirteen cultures, or 16 per cent, after an initial alkalinity, reverted to neutrality within seven days. Eight cultures, or 10 per cent, although growing promptly, were at first neutral and became alkaline only after forty-eight hours. Similarly, four cultures, or 5 per cent, growing promptly, remained neutral for forty-eight hours and then gradually became acid. Three cul-

tures, or 3.8 per cent, although growing well, remained neutral during seven days of observation. Two cultures, or 2.5 per cent, after an initial alkalinity, became acid within a week. Two other cultures, after an initial acidity, reverted to neutrality. One culture, after an initial acidity, became permanently alkaline and one culture utilized citrate only after forty-eight hours, the

TABLE 1
Citrate utilization by 100 cultures of the genus Escherichia and 79 cultures of the genus Aerobacter

	ESCHERICHIA		AEROBACTER	
	Number of cultures	Per cent	Number of cultures	Per cent
No growth.....	54	54	0	0
Prompt growth with acidification.....	4	4	15	19
Prompt growth with alkalization.....	7	7	30	38
Prompt growth, medium remaining neutral.....	0	0	3	3.8
Prompt growth with alkalization, reverting to neutrality.....	0	0	13	16
Prompt growth with delayed alkalization.....	0	0	8	10
Prompt growth with delayed acidification.....	0	0	4	5
Prompt growth with alkalization, reverting to acidity.....	0	0	2	2.5
Prompt growth with acidification, reverting to neutrality.....	0	0	2	2.5
Prompt growth with acidification, reverting to alkalinity.....	0	0	1	1.1
Growth only after 2 days incubation:				
Neutral.....	18	18	0	0
With acidification.....	13	13	0	0
With alkalization.....	4	4	1	1.1

growth even then being macroscopically invisible, but the medium becoming alkaline. No correlation of these variations could be made.

In our series, therefore, the value of the citrate medium has been more in the lack of, or in the rate of, growth than in the reaction changes of the medium. The difference between our *Escherichia* and *Aerobacter* strains was uniformly striking in

that the *Escherichia* strains either did not utilize citrate or did so only after forty-eight hours, while the *Aerobacter* strains in general utilized this medium promptly and heavily. On the other hand, both alkali and acid formers were found in both genera. These findings are summarized in table 1.

The stability of this citrate reaction, as shown by Koser (1924a) has been confirmed as most of the cultures have been tested both when originally isolated and after months of artificial cultivation.

LIQUEFACTION OF GELATIN

The medium was prepared as described in the Standard Methods of Water Analysis, A.P.H.A., (1923) page 95. After heavy inoculations, the cultures were placed in the incubator at 37.5°C. They were removed from the incubator at three or four day intervals, cooled on ice, and read in comparison with uninoculated controls. Incubation was continued for at least two weeks before cultures were considered as not liquefying gelatin.

None of the *Escherichia* cultures liquefied gelatin, but twenty-one of the *Aerobacter* cultures and all of the *Proteus* forms did liquefy. Two of the latter lost this power after prolonged cultivation on artificial media. The gelatin liquefiers of the miscellaneous group will be discussed later.

PRODUCTION OF INDOL

Dunham's peptone broth, with Bacto-Difco peptone was used. The cultures were tested after two days of incubation, the Ehrlich method being used. (Manual of Methods, B. 31.) As excellent results were obtained in this way, neither tryptophane broth nor tryptic digestion of the peptone was tried. Cultures showing the characteristic pink color were shaken up with a little chloroform and not considered as indol positive unless the color was chloroform soluble. The fact that only eleven of our *Escherichia* cultures failed to form indol, while seventy-three, or 92.4 per cent of the *Aerobacter* series were indol negative is in accordance with the previous findings of others working with similar organisms.

MILK

After trying both litmus milk and milk containing brom-cresol purple, the former was selected as preferable. This was prepared by adding the indicator to cream-free milk, which was then tubed and autoclaved. Cultures in milk were observed for ten days. All of the *Aerobacter* cultures both acidified and coagulated milk. Ninety-six of the one hundred *Escherichia* cultures caused acidification and coagulation, three of them acidified without coagulation and one culture, No. 14, was alkaline. All of the *Proteus* cultures peptonized this medium. The action of the miscellaneous cultures on milk will be discussed later.

MOTILITY

This was studied by the examination of hanging drops of broth cultures. Tests were made with various ages of cultures, from eight to eighteen hours, and negative tests were always repeated. In the *Escherichia* series, twenty-nine of the one hundred cultures were motile. Of the *Aerobacter* cultures, twenty-one or 26.6 per cent were motile. Of these twenty-one cultures, however, sixteen belonged in the gelatin liquefying group and three of the remaining five showed only very slight motility. All five of the *Proteus* cultures were actively motile. De Stoecklin (1894), studying 300 undifferentiated strains of Colon group bacilli from faeces, found 116 motile forms and 184 non-motile.

UREA DECOMPOSITION

This was determined by the use of plain broth containing 2 per cent urea, phenolphthalein being used as an indicator and the medium adjusted to acidity. With the decomposition of urea and the production of alkalinity, there developed a pink color and a strong ammoniacal odor. Only one *Escherichia* culture decomposed urea, and this organism was in an intermediate group. Two cultures in the *Aerobacter* series, both of them gelatinliquefiers, decomposed urea. One of them has been isolated repeatedly from the same patient and always decomposes urea when freshly isolated, losing this power upon prolonged artificial cultivation. Unfortunately, we have been unable to follow the other culture

with repeated isolations. It has been carried for four years, however, and still decomposes urea promptly. All of the *Proteus* strains decompose urea energetically.

Pasteur's previously cited studies are of interest in this connection. Guiard's (1883) thesis was incomplete bacteriologically. Renault (1893) concluded that bacteria which do not liquefy gelatin do not decompose urea, a belief which cannot always be confirmed.

CAPSULE FORMATION

At the suggestion of Dr. J. H. Brown, the milk agar, India ink method was finally adopted for the demonstration of capsules. This was found to be much simpler than other methods and, if carefully prepared and controlled, entirely satisfactory. The medium consisted of one part of milk in nine of plain agar, only fresh, moist slants being used. By means of a platinum needle, a small amount of an eighteen-hour milk agar culture was transferred to a carefully cleaned glass slide, on which had been previously mixed one drop of sterile physiological salt solution and one drop of Higgins' American India Ink. The organisms were stirred gently but thoroughly into this large drop and a cover slip inverted over the mixture. Capsules could then be demonstrated beautifully by examining the preparation under the high dry magnification, manipulating the light and the fine adjustment screw simultaneously. The capsules stood out clearly as colorless halos around the bacteria and against the darker background of the ink. Negative results were checked by repeated examinations. The only difficulty with this method is that many of the freshly opened bottles of ink contain large encapsulated bacilli, such as were observed by Hamilton (1898) in similar inks. It is therefore necessary to run careful ink controls with every series of tests.

In view of the tendency of certain bacteriologists to place the encapsulated forms in a separate group, as in Castellani and Chalmers' (1919) "Tribe 10, Encapsulateae," of the Bacilliaceae, and Bergey's (1925) "Tribe Klebsielleae Trevisan, Genus Klebsiella," we have been interested in analyzing our cultures from this point of view. Our findings are summarized in table 2.

In our Group I, *Escherichia* cultures, fifty-five of the one hundred forms were not encapsulated, thirty-nine showed thin, but definite capsules, and six had thick capsules. These six cultures, however, all belonged to the intermediate sub-group D, which utilized citrate promptly. Four of these six cultures did not form indol and four of them were non-motile. The latter might be placed in Bergey's Genus *Klebsiella*.

In the Group II, *Aerobacter*, cultures, there was a marked increase in the number of encapsulated forms. Here only eight cultures or 10 per cent of the group were not encapsulated, while fifty-eight cultures, or 73 per cent showed small but definite capsules, and thirteen cultures, or 17 per cent were thickly encapsulated.

TABLE 2
Summary of capsule formation

	NO ENCAPSULATION		THIN CAPSULES		THICK CAPSULES	
	Number	Per cent	Number	Per cent	Number	Per cent
Group I, <i>Escherichia</i>	55	55	39	39	6	6
Group II, <i>Aerobacter</i>	8	10	58	73	13	17
Group III, <i>Proteus</i>	1	20	4	80	0	0
Group IV, Miscellaneous.....	1	6	6	37	9	56

Considering together these heavily encapsulated forms from both the *Aerobacter* and the *Escherichia* series, as would be necessary were they to be united in a genus of encapsulated forms, it is found that thick encapsulation is the only characteristic these organisms have in common, with the unimportant exception of inability to break down urea. Therefore, if such a genus or tribe is to be of value, it should be widened to include the forms described, of which one liquefied gelatin and four of which were motile. Taken as a whole, classification of the bacteria by their other characteristics, by which they fall easily into genera containing similar forms, would seem more satisfactory. Otherwise, we have widely differing organisms placed together in one group on the basis of a single characteristic. Moreover, if we include in a tribe of encapsulated organisms all of the forms which show any definite encapsulation, 45 per cent of the *Escherichia* cul-

tures and 90 per cent of the *Aerobacter* cultures would have to be included. If there is to be any quantitative division on a basis of thick or thin encapsulation, by what standards is such a division to be made?

The relation of capsule formation to infection, studied by Preisz (1909) is further emphasized by Zinsser (1923). Reference should also be made to Furst's (1910) study and to the recent work of Avery, Heidelberger and Goebel (1925) on the isolation of a specific polysaccharide from a strain of Friedländer bacillus.

HAEMOLYSIS

Blood agar plates were prepared by adding two-thirds of a cubic centimeter of rabbit red blood cells, washed once with physiological salt solution to twelve cubic centimeters of agar. The plates were streaked with diluted twelve- or eighteen-hour cultures of the organisms and readings were made after incubation at 37.5°C. for twenty-four and forty-eight hours. Doubtful tests were repeated. We were surprised to find that sixty of the one hundred *Escherichia* cultures were haemolytic and also fifty-nine, or 74 per cent of the *Aerobacter* cultures. Only one of the *Proteus* cultures was haemolytic, although Kline (1925) has reported a haemolytic *Proteus* which was highly pathogenic for rabbits.

Table 3 shows the incidence of haemolytic organisms in different clinical conditions. It indicates, as was to be expected from the number of haemolytic cultures, that, in general, over 50 per cent of the organisms in any type of infection considered here are haemolytic.

Kayser (1903) in an interesting study of haemolytic colon group bacilli described a thermostabile colilysin, for which some normal sera contained an anticolilysin. Burk (1908) noted haemolytic colon group bacilli in both urine and faeces of suspected typhoid cases. Schmidt (1909) concluded that haemolysis was not correlated with fermentation reactions or virulence. Meyer and Lowenburg, (1924) expressed the opinion that the haemolytic colon bacilli could not be considered as one serologic group. Lowenberg (1924) found that haemolytic colon bacilli

played a rôle in pyelitis and were increased in the faeces in gastro-intestinal disturbances. Jinozzi (1925), on the other hand, agreed with Schmidt (1909) in considering the haemolytic properties of colon group bacilli in the gastric tract unrelated to their virulence. Bawtree (1923) found thirteen haemolytic strains among thirty *Escherichia* cultures from urinary infections and emphasized their relation to the intestinal flora. Klingenstein

TABLE 3
Incidence of haemolytic organisms in various clinical conditions

CLINICAL CONDITION	TOTAL NUMBER OF CULTURES	HAEMOLYTIC CULTURES		NON-HAEMOLYTIC CULTURES	
		Number	Per cent	Number	Per cent
Renal infection.....	57	38	67	19	33
Abscess formation.....	4	3	75	1	25
Blood stream invasion.....	12	7	58	5	42
Epididymitis.....	23	13	56	10	43
Seminal vesiculitis.....	11	7	64	4	36
Orchitis.....	1	1	(100)	0	0
Prostatitis.....	60	41	68	19	32
Prostatic hypertrophy.....	62	40	64	22	35
Cancer of prostate.....	7	5	71	2	28
Stricture, etc.....	13	9	69	4	31
Cystitis, male.....	115	82	71	33	29
Cystitis, female.....	9	8	89	1	11
Urethritis.....	10	6	60	4	40
Wound infections.....	11	9	82	2	18
Bacilluria, male.....	6	3	50	3	50
Pneumaturia.....	1	0	0	1	(100)
Arthritis.....	7	5	71	2	28

(1925), studying forty-two cultures from the intestines, found that twenty-two were haemolytic. Of these, nine were from the jejunum, fourteen from the ileum and nineteen from the colon. Bitter and Grundel (1924) believed that haemolytic strains are found in acute cases, non-haemolytic in chronic cases. Dudgeon (1924) has described acute genito-urinary infections due to haemolytic bacilli which fermented lactose slowly.

FINDINGS

Our four main groups of organisms will be considered separately before the general discussion.

As soon as any attempt was made to arrive at a specific identification of these organisms, difficulties were encountered. These will be discussed more fully under classification. We believe that it is more important to describe the characteristics of our cultures than to try to fit them into species which are either incompletely described or differently defined by several authors. (We have limited ourselves, therefore, to giving the closest specific identification.) It is impossible to publish the charts necessary to give full descriptions of all of our two hundred cultures, but the record of these is available for anyone who wishes to pursue the subject in greater detail than it is presented here.

Group 1. Escherichia

This group, consisting of one hundred cultures, or fifty per cent. of our series, was composed of cultures which fermented lactose with the production of both acid and gas, which failed to produce acetyl-methyl-carbinol, but which were methyl red positive. These cultures utilized citrate either scantily or not at all. Gelatin was not liquefied. Milk was usually both acidified and coagulated, but, in three instances, it was acidified without coagulation, and, in one case, it remained alkaline. The cultures varied in motility, encapsulation and haemolytic power. They regularly formed both acid and gas from arabinose, xylose, glucose, galactose, levulose, lactose, maltose, glycerol and mannitol. They usually fermented rhamnose and sorbitol. They varied in the fermentation of sucrose, raffinose, dextrin, starch, salicin and dulcitol. They seldom fermented inulin, adonitol or inositol.

These characteristics make these organisms conform in general to Winslow, Kligler and Rothberg's (1919) Group V., and to Bergey's (1925) or Weldin's (1927) Genus *Escherichia*. There were a few heavily encapsulated organisms which might have been placed in Castellani and Chalmers' (1919) Tribe Encapsulateae, or Bergey's (1925) Tribe Klebsielleae, perhaps wisely

omitted by Winslow, Kligler and Rothberg (1919) and, more recently, by Weldin (1927).

Summary of Group 1 cultures. These 100 cultures fall into the following sub-groups.

- A. Typical sucrose negative cultures, 43, or 43 per cent of the group.
- B. Typical sucrose positive cultures, 40, or 40 per cent of the group.
- C. Atypical sucrose positive, citrate utilizers, 11, or 11 per cent of the group
- D. Atypical, heavily encapsulated, viscid cultures, 6, or 6 per cent of the group.

Niwa (1918-19) has reported a case of cystitis in which the infection was due to a bacillus which was sucrose negative, but which fermented raffinose, dulcitol and adonitol. This organism is an interesting exception to the general correlation of sucrose and raffinose fermentation, to which we have only one exception. Niwa's organism is even more unusual in its fermentation of adonitol. We had only one adonitol fermenter in our sucrose negative *Escherichia* cultures, our strain differing from Niwa's in being dulcitol and raffinose negative. Riesman and Bergey (1908) have reported a case of pyelonephritis due to *Escherichia grnthalii*.

GROUP II. AEROBACTER

These cultures correspond to those described by Winslow, Kligler and Rothberg (1919) in their Group VI and by Bergey (1925) and Weldin (1927) in the genus *Aerobacter*. They all formed acetyl-methyl-carbinol and were methyl red negative. They fermented carbohydrates more readily than the *Escherichia* cultures. All of our seventy-nine *Aerobacter* cultures fermented sucrose and rhamnose as well as the carbohydrates utilized by all of Group I, namely, arabinose, xylose, glycerol, glycose, galactose, levulose, lactose, maltose and mannitol. Many more of Group II than of Group I fermented sorbitol, raffinose, dextrin, salicin, starch, adonitol, inositol, and inulin, the difference being especially marked with sucrose, salicin, adonitol and inositol. On the other hand, more of Group I than Group II fermented

FIG. 2

dulcitol, this being the only carbohydrate showing this difference. The Group II forms were more generally indol negative than were the Group I cultures, an expected difference. More of the Group II cultures were haemolytic and many more were encapsulated and utilized citrate. Twenty-one Group II cultures liquified gelatin. These differences between the reactions of Group I and Group II are expressed in figure 2.

The Group II cultures have been divided into three sub-groups, as follows:

Sub-Group A, 21 gelatin-liquefying cultures.

Sub-Group B, 36 cultures, not liquefying gelatin, all fermenting all of the carbohydrates tested except dextrin, starch and inulin, on which the reactions varied.

Sub-Group C, 22 cultures, not liquefying gelatin, all fermenting all the carbohydrates tested, except adonitol, sorbitol, inositol, dextrin, starch and inulin, on which the reactions varied.

Sub-groups B and C might be considered together, having been separated as a matter of convenience, on account of the large number of cultures included.

Classification of Group II cultures. Although Grimbert and Lgros (1900) and others have believed that the Friedlnder group and the *Bact. aerogenes* group were identical, Coulter (1917) and others have emphasized the inability of true Friedlnder group organisms to ferment lactose. Fitzgerald (1914) described the organisms of the Mucosus Capsulatus Group as non-motile, but some of them liquefied gelatin and 8 of his 43 cultures produced acetyl-methyl-carbinol. He could get no classification from a study of fermentation reactions. Small and Julianelle (1923) also found carbohydrate tests of little value in the classification of respiratory and granuloma strains of *B. mucosus-capsulatus*. Eight of their cultures, however, produced acetyl-methyl-carbinol, four of them being also methyl red positive. This interesting finding, we have not encountered in studying bacilli, but one of us (Stadnichenko) has found cocci which both produced acetyl-methyl-carbinol and were methyl red positive. We see no way, however, of differentiating non-motile, typical

Aerobacter cultures from the Voges-Proskauer positive forms of the *B. mucosus-capsulatus* group described by these authors.

The work of Levine (1916b) (1916c) (1917) (1921) and his colleagues is by far the most helpful contribution which has been made. The study by Levine and Linton (1924) of 123 strains of *Bact. aerogenes*, 76 from soil, and 47 from human dejecta, is very valuable. They confirmed the previously shown correlation between motility, gelatin liquefaction and glycerol fermentation. Their cultures were arranged in three groups. None of our gelatin liquefiers could be placed in their Group I, "Cloacae," because ours fermented glycerol. But our 21 gelatin liquefiers from urine fitted closely into Levine and Linton's (1924) intermediate group of cultures, which both liquefied gelatin and fermented glycerol. The only exceptions were that our cultures did not always ferment adonitol or inositol. We found adonitol of value in subdividing our gelatin liquefiers, as 12 of them utilized this alcohol and 9 did not. Our 58 Group II cultures which did not liquefy gelatin correspond very closely to the characteristics of Levine and Linton's (1924) Group II, or *Aerogenes*. Only four of these 58 cultures failed to ferment adonitol and would therefore be considered as "probably not of human origin." Two of these were thickly encapsulated and perhaps should be placed in another genus, should one be sufficiently defined. We also had two inositol negative cultures, but otherwise these organisms fit well into the classification of Levine and Linton (1924). The close correlation of our gelatin liquefiers with the intermediate group of Levine and Linton (1924) is confirmation of the value of their description of these organisms.

In addition to the articles cited in the general review of genito-urinary infections, a few may be noted which are of interest because the organisms described belong in these groups. Denys (1892) reported *Bact. aerogenes* in urine and tried to correlate its presence with similar findings in the faeces of children. Kidney infection, due to an encapsulated bacillus, was noted by Nicholaier (1894). Heyse (1894) reported pneumaturia due to *Bact. aerogenes*, only one case in our series being of this type. Trumpp (1896) described 29 cases of cystitis in children due to *Bact.*

aerogenes. Montt-Saavedro (1896) reported cystitides due to the Friedländer bacillus. Other reports have been made by Barlow (1893), Warburg (1899), Wildbolz (1901), Wolf (1908) and Lenhartz (1906). Klieneberger (1908) discussed encapsulated bacilli as the cause of chronic cystitis. Luetscher (1911) gave an excellent review of the literature, but his belief that the percentage of *Bact. aerogenes* infections had been overestimated now seems based on incomplete bacteriological differentiation. The work of Meyer and Hinman (1920) and of Lacy and Murdoch (1922) on lactose negative, encapsulated bacilli is related more closely to the encapsulated forms we have described in our Group IV.

GROUP III. PROTEUS

There were only five cultures in this group. They all fermented xylose, glucose, sucrose, maltose and glycerol. None fermented arabinose, rhamnose, lactose, inulin, mannitol or inositol. They varied in the fermentation of dulcitol, salicin, sorbitol, adonitol, raffinose, dextrin and starch. Although van Loghem and van Loghem-Pouw (1912), Bertholet (1913) and Wenner and Rettger (1919) have found that their *Proteus* strains generally formed indol, and Steensma (1906) has observed a pseudo-indol reaction, none of our strains formed indol by the method used. This agrees with the findings of Groot (1918), of Besson and Ehringer (1923) and of van Loghem (1918). None of our strains formed acetyl-methyl-carbinol, although Archibald (1913) has described a *Proteus* form which did. All of our cultures were methyl red negative.

After an initial acidification, these cultures showed an alkaline reaction in milk, with partial or complete peptonization. Gelatin was always liquefied by freshly isolated cultures. Urea was always decomposed and much more actively than in any other group. The utilization of citrate agar varied, all cultures showing but scant growth on this medium, four giving an alkaline reaction and one remaining neutral. All cultures were actively motile. Only one was haemolytic.

According to both Bergey (1925) and Weldin (1927), all of

these cultures were *Proteus vulgaris*, differentiated on the basis of their action on maltose and mannitol. Our forms differed, however, from the specific definition of Weldin (1927) in that they were indol negative and sometimes fermented dextrin.

The carbohydrate reactions of this group were in some cases much less clear-cut than in Groups I and II and gas production by these *Proteus* strains was somewhat irregular and in no case marked. For example, in sucrose, although acid was produced by all, in some cases gas was not evident until from the third to the fifth day, and in one case no gas was noted. In both maltose and glycerol, although acidification was produced by all cultures, gas production was variable.

The loss of ability to liquefy gelatin is in accordance with the findings of Theobald Smith (1894), of Herter and Ten Broeck (1911) and others. Three of our *Proteus* cultures have retained their power to liquefy gelatin, while two have lost it entirely.

No pigment production was exhibited by any of our strains. They were cultured for fourteen days on plain broth by the method of Bengtson (1919) and on potato for five days as suggested by Jordan (1903), but failed to show pigment by either method.

Only one of the *Proteus* cultures was haemolytic. This differed from the haemolytic strain described by Kline (1925) in that ours did not ferment mannitol or arabinose, and did ferment xylose. There were also some differences in gas production.

Ability to grow under anaerobic conditions was tested on chopped meat medium, incubated at 37.5°C. for fourteen days. Growth was obtained in all cases. The meat became slate-colored and there was a slight reduction in the size of the meat particles. No gas was produced and in no case was there evidence of proteolytic action.

Many authors have reported urological infection due to *Proteus*. Lenhartz (1923) found it causing septicaemia of renal origin. Wolff (1912) found that 8 per cent of his one hundred cultures were *Proteus*. He divided these eight cultures into 5 types, only one of which fermented mannitol. The recent work of Hagar and Magrath (1925) (1926) (1928) is of special interest.

The organism which they find as the etiological factor in encrusted cystitides they now call *Proteus ammoniae*, as it seems more closely related to this genus than to *Salmonella*, although sucrose negative. We have not found this organism in our series, but the increase in the incidence of *Proteus* cultures in lithiasis, as shown in the clinical summary, and the promptness with which they all decompose urea makes them of importance in alkaline infections. The problem is complicated by the fact that such infections are almost always mixed, although Hagar and Magrath have obtained pure cultures from some of their cases.

Group IV. Sixteen miscellaneous cultures

These may be placed in the following genera:

<i>Alcaligenes</i>	1 culture
<i>Eberthella</i>	5 cultures
<i>Shigella</i>	9 cultures
<i>Salmonella</i>	1 culture

Alcaligenes. This culture, motile and gelatin liquefying, may be considered as *Alcaligenes bookeri*, Bergey (1925, page 258), or Weldin (1927, page 187). It was associated with a coccus in a case of pyonephrosis and epididymitis. Beckmann and van der Reis (1925) have described cystitis due to this organism. Straub and Kraus (1914) and others have found it in urinary infections, Mackenzie and Cochrane (1924b) reporting it in 63 of their 241 ureteral cultures, an unusually high incidence.

Eberthella. Using the classification of Bergey (1925), both motile and non-motile organisms which ferment glucose without gas belong in this genus, while Weldin (1927) places the non-motile forms in *Shigella*. Fourteen of our Group IV cultures may be placed in these genera, seven cultures not fermenting lactose and seven fermenting lactose without gas.

Four of our cultures were practically identical. They were motile, liquefied gelatin, and in other ways resembled Ford's (1901) *Eberthella chylogena* except for their action in milk, and resembled his *Eberthella dubia*, except for their action in milk and their failure to form indol. These organisms differed from *Eberthella lewisii* in the liquefaction of gelatin, Weldin (1927, page

172). They seem, therefore, more closely related to the organism described by Ford (1901).

One motile culture was considered as *Eberthella talavensis*. One non-motile culture could not be classified. It resembled *Shigella dysenteriae* closely and, although differing in its fermentation of mannitol from *Shigella paradysenteriae* Flexner, it showed an immunological relationship to this organism, agglutinating in its serum in a dilution of 1:40. Another culture, non-motile and lactose negative, was apparently *Eberthella alkalescens*, or *Shigella alkalescens*.

The remaining seven cultures in Group IV, which all fermented both glucose and lactose without gas, as well as mannitol and xylose, were non-motile. The four dulcitol negative forms were considered as *Shigella madampensis*, Weldin (1927, page 181). The dulcitol positive forms seemed to be *Shigella ceylonensis*, Weldin (1927, page 182). They satisfied the definitions of this species much more closely than that of *Eberthella viscosa*, the closest resemblance to be found in any of the forms described by Bergey (1925, page 254).

One culture has been considered *Salmonella paratyphä*, in spite of its fermentation of sucrose, because it was agglutinated by *Salmonella paratyphi* immune serum in a dilution of 1:320.

Dudgeon (1906) has described an organism from a case of prostatitis which closely resembled some of our Group IV cultures, although we had no salicin positive, sucrose negative forms and his organism was slightly motile. Lacy and Murdoch (1922) have reported encapsulated non-gas-forming bacilli from the urinary tract. One of our cultures resembles the organism described in its fermentative reactions. Meyer and Hinman (1920) have described a similar encapsulated organism from a case of hydronephrosis.

BACTERIOLOGICAL SUMMARY AND DISCUSSION

The 200 hundred cultures fall into 65 types, if grouped according to their 14 most clearly differentiating characteristics, that is, their behavior in regard to the Voges-Proskauer test, methyl red, citrate, gelatin, milk, indol, encapsulation, motility

and the fermentation of glucose, lactose, sucrose, dulcitol, salicin and adonitol. Our 100 *Escherichia* cultures form 30 of these 65 types, while the 79 *Aerobacter* cultures form 20 types. The largest number of cultures in any *Escherichia* type was 15, while the largest number in any *Aerobacter* type was 29. That is, the *Aerobacter* cultures tended to be less differentiated. The 5 *Proteus* cultures formed 3 types, differing only in their fermentations of dulcitol, salicin and adonitol. The 12 types of the miscellaneous cultures are not significant.

While these 65 types represent only a few of the number theoretically possible on the basis of 14 characteristics, there seems no reason why many others should not be found in addition to those previously reported from other sources. The use of some outline which includes the possible variations of whatever characteristics may be considered essential, and which gives each variation a place, would seem simpler than the available systems of classification. Levine (1918), considering the possibilities presented by Bergey and Deehan (1908) and by others too flexible, on account of the large number of possible variations, has emphasized the necessity of studying correlations of characteristics. Such a statistical plan would offer some way of quickly making comparisons and of placing organisms not previously listed, while the demands of nomenclature could be satisfied by naming these types as found.

In regard to classification, a review of the enormous literature on the subject has yielded comparatively few studies that are helpful in the light, or rather darkness, of present knowledge. Moreover, these few disagree in so many ways or have such omissions, that the unfortunate bacteriologist who tries to identify cultures by them, is at once thrown into confusion. There are, however, some valuable guides, which will be discussed briefly.

Winslow, Kligler and Rothberg (1919), basing their study on 160 cultures of the colon-typhoid group, offer a grouping which is valuable in the very breadth of its classification, which allows it to be used many times when the more detailed definitions of others exclude cultures on minor characteristics. However, they frankly offer no assistance in regard to encapsulation. They

have no place for anaerogenic lactose fermenters. Their Group V, in which most of our Group I, *Escherichia*, cultures belong, has no place for motile species which ferment both salicin and sucrose. This omission excludes nine of our cultures. In their Group VI, in which most of our Group II, *Aerobacter*, cultures belong, the species *B. cloacae* is defined as non-motile, so excluding sixteen of our twenty-one gelatin liquefying cultures. By the addition of such groups to their classification, the fundamental groupings of Winslow, Kligler and Rothberg (1919) could be made to include these and other forms which may be found.

Castellani and Chalmers (1919), emphasizing gelatin liquefaction and encapsulation in tribal differentiation, would force us to remove the 45 encapsulated cultures from our Group I, *Escherichia*. While the advisability of taking away from this group the 6 heavily encapsulated, viscid cultures may be seriously considered, it seems illogical to remove the 39 thinly encapsulated cultures from forms which they so closely resemble in every other way. In their Tribe Encapsulateae, Castellani and Chalmers (1919) describe only two species which ferment both glucose and lactose with acid and gas. Of these, *Encapsulatus acidi-lactici* is Voges-Proskauer negative, and does not ferment inositol. Seven of our encapsulated Group I cultures did ferment this alcohol, although three of these were thickly encapsulated and most fitted to be placed in this tribe. The only other glucose and lactose fermenting species of the Tribe Encapsulateae, or *Encapsulatus lactis-aerogenes*, is described as Voges-Proskauer positive, and would, therefore, include all of the encapsulated forms in our Group II which did not liquefy gelatin. Further division of this tribe would be useful. The gelatin-liquefiers, according to Castellani and Chalmers (1919) would be placed in the Tribe Proteae, Genus *Cloaca*, species *cloacae*. This is a difficult transition, in view of the fact that these organisms seem more closely related to the other Voges-Proskauer positive, lactose fermenting, milk coagulating cultures, than to the Voges-Proskauer negative, lactose negative, milk peptonizing *Proteus* cultures. Castellani and Chalmers (1919) are more helpful than others in the identification of atypical or unusual forms.

Bergey's (1925) Manual is of value in its genera of the tribe Bacterieae, but confuses by such mistakes as dividing the genus *Escherichia* on the fermentation of lactose and glucose, (page 217, AA. 1 and AA.1a bb c), when the key describes the genus as forming both acid and gas in these carbohydrates. The problem of encapsulation offers much of the confusion found in Castellani and Chalmers (1919). The non-motile, encapsulated organisms are placed in the genus *Klebsiella*, but no way is given of clearly differentiating the glucose and lactose fermenters in this genus from *Aerobacter* cultures, as there is no statement as to the production of acetyl-methyl-carbinol by the *Klebsiellae*. There is no place for motile encapsulated organisms in this tribe, or any way to distinguish Voges-Proskauer negative, encapsulated, lactose fermenters of this tribe from certain *Escherichia* forms. It is seldom possible to make more than a generic classification by Bergey's (1925) system, but his genera are of value.

Weldin (1927) is more helpful than Bergey (1925) in many ways, but again throws one into confusion by putting the encapsulated forms under *Proteus*. This is inconsistent if any encapsulated lactose fermenters are to be included. Weldin (1927) offers no species of the Genus *Escherichia* which differs in the fermentation of dulcitol and salicin. Our 17 cultures which did differ in the fermentation of these carbohydrates form such a large and consistent group that we believe such a subdivision should be made. Weldin (1927) omits the use of salicin in his definition of *Escherichia communior*, defined as not fermenting this carbohydrate by Winslow, Kligler and Rothberg (1919) and as salicin positive by Bergey (1925). This is a typical example of the perplexities which confront anyone who endeavors to use the present methods of classification. Weldin (1927), however, remains the most useful at present for specific identification.

COMPARISON WITH RELATED FORMS FROM INTESTINAL CONTENTS

In view of the many studies which have been made of Colon group bacilli from other sources, certain comparisons may be made. The findings of others in studying faecal strains of the Colon group show a relatively high percentage of *Bact. coli*, or

Escherichia cultures, in proportion to the percentage of *Bact. aerogenes* or *Bact. cloacae* (*Aerobacter*) strains. These findings have been summarized in table 4.

From this it will be seen that, in the fourteen reports tabulated, representing nearly 7000 cultures, some authors found no *Aerobacter* strains, others from 0.06 to 16 per cent. Rogers, Clark and Lubs (1918), using special enrichment methods, obtained 26 per cent *Aerobacter* cultures in faeces. These results are in striking contrast to the presence of *Aerobacter* strains in 39.5 per cent

TABLE 4
Colon bacilli in faeces

AUTHOR	TOTAL NUMBER OF CULTURES	BACT. COLI		BACT. AEROGENES AND BACT. CLOACAE	
		Number	Per cent	Number	Per cent
Chen and Rettger (1920).....	173	173	100	0	0
Rogers, Clark and Evans (1914).....	150	149	99.9	1	0.06
Levine (1916a).....	117			0	0
Koser (1924).....	118	109	92.4	9	7.6
Schöbl and Ramirez (1925).....	20	20	100	0	0
Rogers, Clark and Lubs, (1918).....	177	131	74	46	26
MacConkey (1909).....	316	306	96.6	11	3.4
Darling (1919).....	113	113	100	0	0
Stokes, (1919).....	156	131	83.9	25	16
de Magalhaes (1924).....	1,290		96.5		3.1
Robinson (1920).....	2,100		93.4		6.1
Clemesha (1912).....	2,236			184	8.0
Hulton (1916).....	13	13	100	0	0

of the urological infections, or in 44.1 per cent of the 179 true *Colon* group infections in our series.

If the urological infections are of intestinal origin, as has been believed by many, including Heitz-Boyer (1919), Bawtree (1923), and Chynoweth (1926), it is possible that the *Aerobacter* strains respond to some selective action in the genito-urinary tract. More probably, these forms, being more resistant than *Escherichia* strains, survive when introduced into the genito-urinary tract, while the *Escherichia* organisms, although far outnumbering the *Aerobacter* forms in faeces, are more easily killed when trans-

planted. This same point may be made in regard to Colon group invasions of the blood stream, as will be shown later. The problem of whether or not there is any increase in the *Aerobacter* content of the intestines before or during genito-urinary infection, is one about which we have no information.

CLINICAL SUMMARY

By comparing the incidence of our four main groups of cultures to their proportion in the whole series of 200 cultures, some estimate may be made of variation in different clinical conditions. We may state briefly the few correlations which seem important.

1. *Renal infections, 57 cases, 49, or 86 per cent pure cultures.* The only variation to be noted here is the increase in incidence of gelatin liquefying forms of the genus *Aerobacter*. Of the 21 *Aerobacter* strains isolated from renal infections, 9, or 42.8 per cent liquefied gelatin, although only 26.5 per cent of all of the 79 *Aerobacter* cultures did so. The fact that in 8 of the 9 cases of renal infection due to gelatin-liquefying bacilli, the organisms were obtained in pure culture, seems to offer evidence that these strains, although commonly considered harmless, are not lacking in pathogenicity when established in the kidney.

2. *Urolithiasis, 25 cases, 17, or 68 per cent pure cultures.* Here there was a sharp drop in the incidence of the *Escherichia* cultures to 28 per cent, less than half of these being pure cultures. There was a corresponding increase in the incidence of cultures from Group II, *Aerobacter*, and Group III, *Proteus*. The *Aerobacter* cultures had an incidence of 48 per cent in lithiasis, 83.3 per cent of them being pure. The *Proteus* cultures, forming only 2.5 per cent of the total of 200 cultures, occurred in 12 per cent of the cases with stone formation.

3. *Abscess formation, 4 cases, 3, or 75 per cent pure cultures.* One prostatic abscess, due to a pure *Escherichia* strain was encountered. The other three were cases of peri-urethral abscesses. Two of these three cases had blood stream invasions, a pure culture of a *Shigella* strain being obtained from both blood and abscess in one instance and a pure culture of a gelatin-liquefying *Aerobacter* strain being obtained from the blood in the second

case, although associated with a Gram positive coccus in the abscess. A pure, non-gelatin-liquefying *Aerobacter* strain was obtained from the third case of peri-urethral abscess.

4. *Epididymitis*, 23 cases, 19, or 82.8 per cent pure cultures. All of the 11 *Escherichia* cultures obtained from cases of epididymitis were pure. There was a relative increase of sucrose negative *Escherichia* strains. No gelatinliquefying bacilli were found among the 9 *Aerobacter* strains encountered. There was 1 pure *Proteus* culture. One *Alcaligenes*, mixed with a coccus, was found, and 1 pure culture of a *Shigella* strain.

5. *Seminal vesiculitis*, 11 cases, 10, or 90.9 per cent pure cultures. The incidence of *Escherichia* cultures here was above normal, being 72.7 per cent. The percentage of heavily encapsulated Group I, *Escherichia*, cultures was increased, being 37.5 per cent as compared with only 6 per cent in the total Group I series. There was a drop in the incidence of Group II, *Aerobacter*, cultures, to 27 per cent, all pure cultures. No gelatin liquefiers were found, but two of the *Aerobacter* cultures were heavily encapsulated, bringing the number of heavily encapsulated cultures found in seminal vesiculitis to 5 or 45.4 per cent.

6. *Prostatitis*, 60 cases, 48, or 80 per cent pure cultures. There was no specific incidence in these infections, nearly every type of organism being found. The studies of Young, Geraghty and Stevens (1906), of Dudgeon (1906), (1907), of Culver (1916), of Rosen (1919), and of Baker (1925) may be cited here, although bacteriologically incomplete.

7. *Prostatic hypertrophy, or carcinoma of prostate with retention*, 69 cases, 45, or 65.2 per cent pure cultures. There was no specific incidence. The relatively small number of pure cultures was to be expected.

8. *Stricture, contracture of the vesical orifice, with retention*, 13 cases, 12 or 92.3 per cent pure cultures. There was a slight increase in the number of *Aerobacter* cultures, to form 53.9 per cent of the group.

9. *Cystitis, male*, 115 cases, 85, or 73 per cent pure cultures. Some difficulty was encountered in deciding what to include in this group. Using clinical diagnoses, and cystoscopic reports,

but omitting doubtful cases, some of which perhaps should have been included, these 115 cases may be considered as representative. There was no significant variation in incidence of culture groups. While 83.8 per cent of the 62 *Escherichia* cultures were pure, only 38.2 per cent of the 43 *Aerobacter* cultures were pure, a proportion which by no means held in other clinical types. There were 2 *Proteus* infections, 1 pure and 1 mixed. There were 4 *Eberthella* infections, only 1 pure, and 4 *Shigella*, all pure.

10. *Cystitis, female, 9 cases, 8, or 88.8 per cent pure cultures.* There was a relative increase of *Aerobacter* cultures, to form 55.5 per cent of the infections.

11. *Urethritis, 10 cases, all pure cultures.* Ninety per cent of the cultures were *Escherichia*.

12. *Bacilluria, male, 6 cases, 5, or 83.3 per cent pure cultures.* *Escherichia* cultures formed half of these infections.

13. *Wound infections, 11 cases, no pure cultures.* No *Escherichia* cultures were found in wound infections, 81.8 per cent being *Aerobacter*. There was 1 *Proteus* infection and 1 *Shigella*.

14. *Arthritis, 7 cases, 6, or 85.7 per cent pure cultures.* Four of these were *Escherichia*, all pure, and 3 *Aerobacter*, 2 pure. Kauntze (1924) has recently discussed the rôle of *Bact. coli* in arthritis.

15. *Blood stream invasions, 13 cases, 12 of bacillary infection, all pure cultures.* These thirteen cases had blood stream invasions simultaneously with genito-urinary infection. In one of these, *Staphylococcus aureus* was recovered from the blood, but not from the urine. The other twelve cases showed the same bacillus in the blood which was also found in the genito-urinary tract. Of these twelve cultures, only one, or 8.3 per cent was from Group I, *Escherichia*. That is, there was a great drop in the incidence of this group, which was normally 50 per cent. On the other hand, nine of the twelve bacillary blood invasions, or 75 per cent were by organisms of Group II, *Aerobacter*. There was no blood stream invasion by *Proteus* in our series and only two, or 16.6 per cent from Group IV organisms. One of these was an *Eberthella* and the other a *Shigella*. It is evident, therefore, that, just as in general there is a marked increase of the in-

cidence of *Aerobacter* cultures in genito-urinary infections in proportion to the known incidence of these organisms in the body, there is a similar strikingly high percentage of these *Aerobacter* species in blood stream invasions. This is a small series of cases, but further evidence is gradually being accumulated which supports this finding. Its significance cannot be explained unless on the grounds previously discussed.

A review of the many articles on Colon group invasions of the blood stream fails to reveal any previous attempt to identify such cultures generically, except in a few separate cases. The importance of the genito-urinary tract as the focus for such invasions, however, has long been emphasized, especially by Felty and Keefer (1924), by Jacob (1909) and by Maciag and Olbrycht (1926). Liebermeister (1906) cites Barlow's early case of *Bact. coli* sepsis and death in a case of urethral stricture. Krencker (1907), Helmoltz (1926), Rooke (1925) and Roux and Lemaire (1925), among others, have reported *Bact. coli* septicaemias of urinary origin, without further bacteriological differentiation. Friedländer bacillus septicaemias have been reported by Brouardel (1926), by Lereboullet and Pierrot (1927), by Caussade, Joltrain and Surmont (1924) and others. Berg and Libman (1902), Longcope (1902), and others, have reported paracolon septicaemias.

FINAL SUMMARY

1. Two hundred cultures of the Colon group, or related organisms, isolated from two hundred cases of genito-urinary infection, have been studied.

2. These cultures may be divided as follows:

Group I. One hundred cultures, or 50 per cent of the series, were methyl red positive, Voges-Proskauer negative, and may be considered as *Bact. coli*, or closely related forms. They belong in Winslow, Kligler and Rothberg's Group V, or in Bergey or Weldon's Genus *Escherichia*. They may be subdivided into 4 subgroups, namely: (A) 43 typical sucrose negative cultures; (B) 40 typical sucrose positive cultures; (C) 11 typical sucrose positive cultures which utilized citrate promptly; (D) 6 atypical,

heavily encapsulated cultures, which might have been placed in another genus.

Group II. Seventy-nine cultures, or 39.5 per cent of the series, were methyl red negative, Voges-Proskauer positive, and may be considered as *Bact. cloacae*, *Bact. aerogenes*, or closely related forms. They correspond to Winslow, Kligler and Rothberg's Group VI, and to Bergey's or Weldin's Genus *Aerobacter*. They may be subdivided on the bases of gelatin liquefaction into two groups, as follows: (A) 21 gelatin liquefying cultures, corresponding closely to Levine and Linton's intermediate group; (B) 58 cultures, which did not liquefy gelatin, and which resemble closely the organisms described by Levine and Linton in their Group II.

Group III. 5 cultures, or 2.5 per cent of the series were *Proteus*.

Group IV. 16 cultures, or 8 per cent of the series composed our miscellaneous group, which may be subdivided as follows:

<i>Alcaligenes</i> ,.....	1 culture
<i>Eberthella</i>	5 cultures
<i>Shigella</i>	9 cultures
<i>Salmonella</i>	1 culture

3. *Encapsulation.* 135 cultures, or 67.5 per cent of the series showed definite encapsulation. Of these, 28 had thick capsules. A comparison of the encapsulated forms, however, showed that, if they were placed in a separate genus, it would have to include species varying in every other characteristic but encapsulation. We do not believe, therefore, that until more is known about encapsulation, it is a satisfactory basis for generic differentiation.

4. *Citrate utilization.* This has been found to be a very valuable means of differentiation, Simmons' citrate agar being more useful than Koser's citrate broths. Citrate was used scantily or not at all by our Group I (*Escherichia*) cultures, with the exception of 11 strains which were also intermediate in their other characteristics. Citrate was used rapidly by all but 1 of our Group II (*Aerobacter*) cultures, and by all of our *Proteus* cultures. It was not utilized by our one *Alcaligenes* culture, or

our one *Salmonella paratyphi* culture. The correlation between citrate utilization and the Voges-Proskauer test was excellent. The citrate test is of great value in obtaining within twenty-four hours a presumptive generic differentiation between *Escherichia* and *Aerobacter*.

5. *Voges-Proskauer test*. It was found by testing our 79 *Aerobacter* cultures daily for five days, that 51.8 per cent were positive within twenty-four hours, 86 per cent were positive in two days, 87.3 per cent in three days, 89.9 per cent in four days and 100 per cent in five days. It is therefore evident that in about 86 per cent of such cultures, testing for the production of acetyl-methyl-carbinol on the third day would give a positive result, saving 2 days time in making generic identification. Cultures negative on the third day could be held until the fifth day, as is customary.

Haemolysis. The number of haemolytic cultures was higher than had been anticipated, 131 cultures, or 65.5 per cent of the series being haemolytic. In group I (*Escherichia*,) 60 per cent of the cultures were haemolytic, in Group II (*Aerobacter*) 74 per cent. In Group III (*Proteus*) only 1 of the 5 cultures was haemolytic. In Group IV, the haemolytic cultures included the one *Alcaligenes* found, 2 of the 5 *Eberthella* cultures, 7 of the nine *Shigella* cultures and the one *Salmonella* culture.

A comparison of haemolytic action with clinical types of infection shows a high percentage of haemolytic cultures in many types of infection, but with no significant selective pathogenicity on the part of these strains.

Urea decomposition. This was found most markedly in the *Proteus* cultures, 2 of the Group II (*Aerobacter*) strains also possessing this power although their action on urea was much weaker than that of the *Proteus* cultures. Although the *Proteus* strains did not lose their ability to decompose urea after artificial cultivation, one of the *Aerogenes* strains could decompose urea only when freshly isolated from urine.

Comparison with intestinal Colon group cultures. A review of the literature on colon group strains from intestinal contents, revealed the fact that in urological infections the incidence of

Aerobacter cultures is much higher than in the intestines. The reason for this is unknown.

Clinical summary. The most significant facts found in comparing our culture groups with different clinical infections were as follows:

1. Seventy-five per cent of the blood stream invasions were due to organisms of Group II (Aerobacter), in the 12 cases in which the same organism was recovered from blood and urine, the incidence of Group I (Escherichia) cultures being only 1 case, or 8.3 per cent. This high proportion of Aerobacter cultures in such blood stream invasions parallels the high incidence of this group of cultures in genito-urinary infections, as compared with organisms present in the intestinal flora. The incidence of Aerobacter cultures in the blood, however, is even higher than in the urine. So far as we have been able to determine, this differentiation of the Colon group in blood stream invasions has not been made previously and further data should be obtained.

2. There was also a drop in the incidence of Group I (Escherichia) cultures in the 25 cases of lithiasis, with a corresponding increase of Group II (Aerobacter) cultures to 48 per cent and of Proteus to 12 per cent.

3. Two of the three cases of abscess formation developed blood-stream invasions.

10. We hope that by this analysis of 200 cultures, some knowledge of the nature of the bacillary infections has been obtained which will be of value as a basis of further comparison and a point of departure for further study.

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