COLIFORM BACTERIA

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The oldest members of the coliform group of bacteria, as the reviewer conceives it, are Klebsiella pneumoniae, or Friedländer's bacillus, described in 1882, and K. rhinoscleromatis, which v.Fritsch recorded the same year. Next come Escherichia coli and Aerobacter aerogenes, both of which were ushered into the bacteriological world in 1885 by Escherich. Somewhat younger is A. cloacae, described in 1890 by Jordan. Proteus morgani, the problem child of the group, dates from 1908 and the juveniles are E. freundii, recorded in Braak's Delft thesis of 1928, and K. paralytica, the etiological agent of "moose disease," described in 1932 by Cahn, Wallace, and Thomas.

These are the principal members of the coliform group as listed in the fifth edition of Bergey's Manual of Determinative Bacteriology (12), a great simplification of the genera Escherichia, Aerobacter and Klebsiella given in the fourth edition (1934)
which contained 35 species. In the latest edition these three genera comprise but ten species.\(^1\)

With this simplification we are in heartiest accord, but the problem has several aspects. Among them, the principle of simplification, or "lumping," as Skinner and Brudnoy (149) term it, must be defended. The inclusion of the genus *Klebsiella*\(^2\) with the coliform bacteria has to be explained. To claim the Morgan bacillus as a coliform organism will require justification. The broadening of the concept of *Escherichia coli* to include such forms as *E. coli-mutabile* and the paracolon bacilli which ferment lactose slowly or not at all is a new development and there are many who will want to know how the "coli-aerogenes intermediates" (180, 165, 25, 123) are classified and why.

**THE TERM "COLIFORM"

The term "coliform" has long been in use by British bacteriologists (4, 23, 59, 92, 100, 132, 133, 160). In America, Breed and Norton (16) suggested the term to describe the lactose-fermenting bacteria used as a measure of the pollution of water. In 1937 H. E. Jordan advised that as Editor of the Journal of the American Water Works Association his policy would be to substitute "coliform" bacteria for "*B. coli*" or "colon group" in papers submitted to him (70). As Jordan also stated, the term "coli-aerogenes" continues as official in water analysis since the eighth edition of Standard Methods of Water Analysis (157) uses it. And "*Escherichia-Aerobacter*" is also official since that is the terminology employed in the sixth edition of Standard Methods of Milk Analysis (156).

**SEPARATION OF FRIEDLÄNDER GROUP FROM OTHER COLIFORM BACTERIA**

As noted above, the first members of the coliform group to be described were *Klebsiella pneumoniae*, from acute fibrinous

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\(^1\) The other three species are *Klebsiella ozaenae*, described by Abel in 1893; *K. granulomatis* of Aragäs and Vianna (1912); and *K. capsulata* recorded in 1889 by Pfeiffer.

\(^2\) These organisms have also often been called the "*Bacillus mucosus capsulatus* group."
pneumonia, and *K. rhinoscleromatis* from rhinoscleroma. The typhoid bacillus had been described less than two years earlier and the emphasis in early bacteriology on its medical aspects favored the finding of pathogens. But even at this time the inclusion of the encapsulated forms found in the upper respiratory tract in a group with coliform bacteria from the intestine and from milk was urged (31). From the first all studies of the gram-negative, aerobic, encapsulated bacilli, of which the Friedländer bacillus is the type known to pathologists, have also included *Aerobacter aerogenes* and all studies of coliform bacteria have likewise included *A. aerogenes*.

The close relationship of *Klebsiella* and *Aerobacter* has been asserted or demonstrated down to the present time (72, 74, 75, 76, 77, 41, 59, 170, 93). Perkins maintained that the prototype of the encapsulated group was *A. aerogenes* and that the other members were variants which had lost the power, in whole or in part through modification in environment (125), to ferment certain sugars. Edwards stated that *A. aerogenes* is so closely related to the other encapsulated forms that they should be classified in the same genus (41).

Notwithstanding, the Friedländer group and the rest of the coliform bacteria have been kept apart in the minds of most bacteriologists. This unfortunate point of view has separated two groups which must be considered together and it has minimized our understanding of the potentialities for pathogenicity possessed by *Escherichia* and *Aerobacter*. As we shall see, these forms, particularly *Escherichia*, are important in pathology, especially in infections of the urinary tract, in all age groups, and of the gastro-intestinal tract in the very young.

As a result we shall have to trace the steps in the history of the classification of coliform bacteria bearing in mind that *Klebsiella* is probably not under consideration and that the emphasis is mainly on the sanitary aspects of the organisms in question. Later we shall bring the Friedländer group back into the dis-

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3 In our own discussion in this review the terminology of the Bergey Manual will be employed but we believe that the use of one genus for all coliform bacteria is biologically more sound than the use of three.
cussion for it has a definite place in the concept of the coliform group.

DEVELOPMENT OF THE CLASSIFICATION AND CHARACTERIZATION OF COLIFORM BACTERIA

Escherich (42, 43) characterized *Bacterium coli-commune* as a bacillus of feeble motility which coagulated milk but did not liquefy gelatin; which fermented milk-sugar and grape-sugar with the disengagement of gas; which produced a moist growth on potato of a color varying from corn yellow to pea yellow; and which produced in animals a rapidly fatal disease characterized by diarrhea, somnolence and coma. *Aerobacter aerogenes* was first described as *Bacterium lactis-aerogenes* by Escherich who noted that it was shorter and plumper than *B. coli*, coagulated milk more actively and was non-motile. He stated that it fermented milk-sugar, cane-sugar and grape-sugar, both aerobically and anaerobically, and he pointed out the prominence of lactic acid among the products of fermentation.

A decade later Theobald Smith (150) suggested a biological division of *E. coli* when he redefined *B. coli-communis* stating it could be divided into alpha and beta varieties based on sucrose fermentation. Smith is usually credited with pioneer work in determining the gas ratios produced in the fermentations. The results were corrected by later workers using more delicate methods; but it is curious that this promising field has not been exploited more thoroughly. Durham (39) named the sucrose-fermenting variety of the colon bacillus *Bacillus coli-communior*. Among the other species of coliform bacteria early described and frequently encountered in the literature may be mentioned the "Milchsäurebacterium" of Hueppe (1884) described in 1885 by Zopf as *Bacterium acidi-lactici*, and Flügge's *B. neapolitanum* (1885). These two species and *B. communior* appear in Bergey (12) as varieties of *Escherichia coli*.

MacConkey (96, 97) first placed classification of coliform organisms on a comprehensive, biochemical basis. He established

4 In the review of a reference its terminology is used even if outdated or incorrect as some are.
four primary groups of the lactose fermenters based on the fermentation of sucrose and dulcitol. These groups were further subdivided on the basis of tests for motility, indole production, gelatin liquefaction, the Voges-Proskauer reaction and the dissimilation of adonitol, inositol and inulin. MacConkey’s scheme called for 128 types of which he actually isolated 36. By 1928 other workers had described 35 additional types, making a total of 71 to be recognized by that date (133). Climaxing taxonomic work of the MacConkey type, Mackie (100) studied the coliform bacteria of feces and urine, forming four principal groups on the basis of gas production, indole production and inositol fermentation. Subdivisions of these groups allowed for many types of which 73 were found. This contribution is of value today because of its emphasis on the use of inositol and as a further illustration of the difficulties encountered by taxonomists whose schemes of classification proceed on the 2-4-8-16-32 ... or “2ⁿ principle.”

As pointed out by Topley and Wilson (170), one important correlation between biochemical activity and natural habitat was early recognized. The A. aerogenes type was found to be a relatively infrequent inhabitant of the intestine, but was frequently isolated from certain grasses and from the soil, while B. coli-communior and B. coli-communis were noted to be typically intestinal parasites (185). This correlation was of practical as well as of theoretical importance. The presence or absence of “B. coli” in water supplies, and the relative number of this organism if present, soon came to be recognized as a very valuable indication of the presence and degree of fecal pollution, and it became desirable to differentiate between those types which were of intestinal origin, and those which might occur in unpolluted waters.

Thus, editions of Standard Methods of Water Analysis published in the second decade of this century included a classification in which “the following group reactions indicate the source of the culture with a high degree of probability:” and there followed a classification of “fecal” B. coli, “fecal” B. aerogenes, B. aerogenes probably not fecal, and B. cloacae which might or might not be
fecal, based on the methyl-red and Voges-Proskauer reactions, the liquefaction of gelatin, the production of indole and the fermentation of sucrose and adonitol. This material has not appeared in recent editions because no confirmation could be found for the early reports that *A. aerogenes* of intestinal origin fermented adonitol whereas *A. aerogenes* from grains and soil did not. Indeed, as we shall see, the prolonged search for tests which differentiate between fecal and non-fecal coliform bacteria has so far been in vain.

As early as 1912 principles of simplification of coliform classification were beginning to appear. Thus Howe (64) showed that there was no correlation between certain features, such as motility, fermentation of dulcitol and mannitol, indole production and nitrate reduction; and he recognized only two species, *B. communis* and *B. communior*. Kligler (79) subdivided the lactose fermenters according to their sucrose and salicin reactions rather than by sucrose and dulcitol reactions, as proposed by MacConkey, since sucrose-salicin sub-groups correlated more highly with the indole, Voges-Proskauer and gelatin reactions than did the sucrose-dulcitol subgroups. The chief emphasis in coliform taxonomy on simplification through the application of statistical methods of correlation of related characters has been expressed by Levine (89, 90). His classification also was one of the first to make use of the Voges-Proskauer test as the primary feature of division of the strains studied.

From quite another point of view Clemesha (28) grouped coliform bacteria according to the resistance shown toward storage. This work represents the most important early contribution to the ecology of the coliform bacteria, possibly of any group, and treats in detail such problems as viability, competition and succession.

Although the first edition of Bergey’s Manual of Determinative Bacteriology (1923) allocated 22 species to the genus *Escherichia*, six to *Aerobacter*, and six to *Encapsulatus (Klebsiella)* classified mainly on the basis of reactions with fermentable substances, ground had been broken for a somewhat different and more basic approach to taxonomy. The Committee of the Society of Amer-
ican Bacteriologists on the Characterization and Classification of Bacterial Types, appointed at the 1915 annual meeting at Urbana, projected, among other tasks, a study of the colontyphoid group of bacteria, "a group which, together with certain sharply defined species, includes many puzzling intermediate forms, difficult of classification and yet of fundamental medical and sanitary importance" (186). Out of the interest of this committee, two sets of data emerged dealing with coliform bacteria.

These have been summarized by Yale as follows: "During the period 1914 to 1921, Rogers and associates published a series of outstanding papers on the characteristics and distribution of the coliform group. An especially important contribution was the separation of the group into a low ratio section in which the CO₂:H₂ ratio was approximately 1:1 and a high ratio group in which it was approximately 2:1. In a report summarizing these studies in 1921, Rogers made this statement, 'So far as data are available, the low ratio or B. coli group appears to be a very definite and circumscribed entity and there is no apparent reason for separating it into species.' In addition he recognized B. aerogenes and B. cloacae as separate species in the high ratio group."

"Winslow, Kligler and Rothberg (186) made extensive classification studies and decided that in the low ratio group, only four species were justified (Bacterium coli, B. communior, B. acidilactici and B. neapolitanus). In the high ratio group, two species were accepted (Bacterium aerogenes and B. cloacae)."

The methyl-red test (27) was a logical outcome of work in Rogers' laboratory on the fundamental nature of the fermentation of glucose by coliform bacteria. These Washington workers found that the low gas-ratio E. coli section produces under suitable conditions a lower pH in glucose broth than do the high gas-ratio A. aerogenes organisms. As a result, the former give a red color to methyl red (+) and the latter a yellow color (−)

in the glucose broth. The formation of acetyl-methyl-carbinol, demonstrable by the Voges-Proskauer test, occurs when \textit{A. aerogenes} is grown in glucose broth but does not occur with \textit{E. coli}. This inverse correlation (M.R. + V.P. – and M.R. – V.P. +) was emphasized by Levine (88). For some years it was held to be well-nigh perfect, but it is now known that coliform organisms occur which are positive to both tests and also those which are negative to both. Thus Stuart and co-workers (161) found that almost ten per cent of their coliform cultures did not correlate.

Another important development has been the recognition of "intermediates," organisms resembling both \textit{E. coli} and \textit{A. aerogenes} but identical with neither. In a study of the utilization of simple nitrogenous compounds, Koser (82) observed that \textit{A. aerogenes} was able to multiply and grow luxuriantly in a medium containing uric acid as the only source of nitrogen. In this medium \textit{E. coli} failed to develop. Later work by Koser (83) developed another, and better, utilization test, i.e., growth in a medium in which the only available carbon source is the citrate radical. In this medium \textit{A. aerogenes} develops very well, whereas \textit{E. coli} fails to grow noticeably (140). It was soon found that some methyl-red-positive organisms, presumably \textit{E. coli}, grew as well in the citrate medium as \textit{A. aerogenes} (85). For a time such organisms were spoken of as "soil coli" and there was again revived the hope that sanitarians had a test which would set off fecal from non-fecal organisms. Such organisms are now known as "intermediates" and are found in feces as well as in man's environment.

Werkman and Gillen proposed the genus \textit{Citrobacter} for coliform bacteria producing trimethylene glycol, and described seven species (180). Since such bacteria utilize citrate and are methyl-red-positive, it was thought that "\textit{coli-aerogenes} intermediates" might be allocated to the genus \textit{Citrobacter}, but the genus has not

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\* Pesch (1921) evidently somewhat anticipated the work of Koser on citrate utilization but apparently did not follow it up with further investigations, and he dealt more primarily with other members of the colon-typhoid group than the coliform bacteria.
been recognized by the leading taxonomists. Tittsler and Sandholzer (165), and Carpenter and Fulton (25), favor classifying "intermediates" with *Escherichia* and *Aerobacter*; Minkewitsch (106) calls them *B. coli-citrovorum* Koser; and Parr (123) suggests that they be made a species in the genus *Bacterium*, which would include also *B. coli*, *B. aerogenes* and *B. cloacae*. The fifth edition of Bergey's Manual designates them as *Escherichia freundii*.

In an analysis of data presented in recent papers (1924–1937) dealing with coliform bacteria, Parr (123) found that the reactions earlier used for classification, such as the fermentation of sucrose, dulcitol, salicin, and raffinose, have largely been replaced by other tests. If this analysis be brought down to date it will be found that in 22 of the most recent projects the following tests have been used to establish coliform classification:

<table>
<thead>
<tr>
<th>Test</th>
<th>Times</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indole production</td>
<td>15</td>
</tr>
<tr>
<td>Methyl-red reaction</td>
<td>20</td>
</tr>
<tr>
<td>Voges-Proskauer reaction</td>
<td>22</td>
</tr>
<tr>
<td>Citrate utilization</td>
<td>20</td>
</tr>
<tr>
<td>Uric acid utilization</td>
<td>6</td>
</tr>
<tr>
<td>Cellobiose fermentation</td>
<td>4</td>
</tr>
<tr>
<td>Gelatin liquefaction</td>
<td>3</td>
</tr>
<tr>
<td>Eijkman test</td>
<td>2</td>
</tr>
<tr>
<td>H₂S production</td>
<td>2</td>
</tr>
<tr>
<td>Sucrose fermentation</td>
<td>1</td>
</tr>
<tr>
<td>Inositol fermentation</td>
<td>1</td>
</tr>
<tr>
<td>Alpha-methyl-d-glucoside fermentation</td>
<td>1</td>
</tr>
</tbody>
</table>

There is, therefore, justification for the creation of the "Imvic" quartet of tests (119). Imvic is a mnemonic which fixes in order the four tests now in greatest use in classifying coliform bacteria: (I) indole production; (M) methyl-red reaction; (V) Voges-Proskauer test; and (C) the utilization of citrate as a sole carbon source. Four characters give 16 possible combinations. Three of these (+ + − −, − + − −, + − − −) Parr called the *E. coli* section, three (− − + +, − − + −, − − − +) the *A. aerogenes* section, and ten (+ + + −, + + − +, − + − +, + + − −, − − − −, + − − +, − + + −, + + + +, + + + −, + + − +, + − + +, + + − −, − − + −, − + − +, − − + −, − − − +, − + − +, + − + +, + − + −) the *A. aerogenes* section, and ten (+ + + −, + + − +, − + − +, + + − −, − − − −, + − − +, − + + −, + + + +, + + + −, + + − +, + − + +, + + − −, − − + −, − + − +, − − + −, − − − +, − + − +, + − + +, + − + −) the *A. aerogenes* section.
+ - + +, - + + +) constitute the "intermediate" section, and he stated that 14 of the 16 types have been reported.

Recently, Stuart and co-workers (161) used the Imvic group of tests plus the fermentation of cellobiose in the study of a large series of coliform bacteria. Cellobiose may be fermented with acid production or with the production of both acid and gas, or it may not be fermented at all. Hence, recording three possibilities for cellobiose brings the possibilities of the Stuart scheme to 48 types of which the Brown University group found 21 among the 3247 cultures studied. These could be assigned to nine of the 16 Imvic types. Of the 48 Stuart types Parr has found 16, Skinner and Brudnoy (149) 14, and Oeser (115) 13.

Malcolm (103) classified 1636 coliform strains isolated from milk and bovine feces into eight groups on the basis of the Voges-Proskauer test, citrate utilization, inositol fermentation and indole production. He called one group B. coli, three groups "intermediates," one B. friedländeri, one B. cloacae, one B. oxytocus, and one B. aerogenes, and he insisted that all should belong to one genus. In addition he encountered 39 anomalous strains which did not fit into his scheme of classification. This work illustrates again the difficulty of classification in this field. The group is so complex and intergrading that each form recovered cannot be assigned an exact name without making the number of such names well-nigh legion. It is refreshing, therefore, to find that Yale in the fifth edition of Bergey's Manual has kept the number of species small, listing some forms as varieties and disregarding scores of names that have confused workers down to the present day.

That the field of biochemical classification has been by no means exhausted is shown by Mitchell and Levine (109), who studied coliform bacteria to determine if nitrogen utilization was as distinctive for differentiation as carbohydrate dissimilation is thought to be. Nucleic acid and its degradation products were employed as nitrogen sources in synthetic media with glucose as the carbohydrate source and indicator. Over 350 strains were tested with yeast nucleic acid, uric acid, allantoin, hydantoin, uracil, urea, adenine and xanthine. Organisms giving positive
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reactions with yeast nucleic acid, uric acid, allantoin and hydantoic acid correlated with the positive Voges-Proskauer test and formed the section *Aerobacter*. *Escherichia* and the "intermediates" were negative to these tests but among them *Escherichia* utilized uracil and failed to utilize urea, whereas the "intermediates" metabolized urea but could not break down uracil. Since both *Escherichia* and *Aerogenes* can utilize uracil and the "intermediates" cannot, Mitchell and Levine feel that they have additional evidence that the "intermediates" should constitute a separate genus. Other evidence is that many "intermediates" produce hydrogen sulphide and those tested yield trimethylene glycol in a suitable medium.

Returning to the Friedländer group it is to be noted that these encapsulated coliform bacteria have not been satisfactorily classified on any such basis as has been used for the other members of the group. Perkins (125) stated that organisms of this group which show no fermentative power are probably degenerate rather than definite entities and can in many cases be reactivated to their original type. Fitzgerald (46) studied 44 cultures of *Bacillus mucosus-capsulatus* and found satisfactory biochemical classification difficult. He believed that mutations, based on the necessity of maintaining a parasitic existence, have caused gram-negative bacilli, found normally in the body elsewhere than in the intestinal tract, to develop capsules for protection, and a new group has arisen designated as *B. capsulatus-mucosus*, connected by the varieties *B. aerogenes* and *B. acidi-lactici* with the non-encapsulated gram-negative bacilli belonging to the colon group.

Winslow, Kligler and Rothberg (186) in commenting on the encapsulated pathogenic forms, said: "It seems evident, either that we are dealing with an extraordinarily variable group, or that forms which are not really related have been identified as of this type merely because of possession of capsule." It should be recalled that *Escherichia coli* not infrequently occurs heavily encapsulated (151, 118).

*Parr* (123) maintains that some "intermediates" are Voges-Proskauer positive.
THE TRIBE ESCHERICHEAE AND RELATED FORMS ACCORDING TO BERGEY

In the fifth edition of Bergey's Manual of Determinative Bacteriology (12)* Family X. Enterobacteriaceae Rahn is divided into five tribes, viz.,

Tribe I. Escherichae: 3 genera, 10 species.
Tribe II. Erwineae: 1 genus, 13 species.
Tribe III. Serrateae: 1 genus, 6 species.
Tribe IV. Proteae: 1 genus, 8 species.
Tribe V. Salmonelleae: 3 genera, 65 species.

The three genera of the tribe Salmonelleae are Salmonella with 37 species and 12 additional varieties, Eberthella with 14 species, and Shigella with 14 species. With the exception of Proteus morgani, the coliform group is found in and includes all of the tribe Escherichae which we shall next consider.

Escherichae trib. nov.—ferment dextrose and lactose with the formation of acid and visible gas. In only one genus, Aerobacter, is gelatin liquefied and that but slowly.

"Genus I. Escherichia.—Methyl red test positive. Voges-Proskauer test negative. Citric acid may or may not be used as sole source of carbon.

I. Citric acid not utilized as sole source of carbon.
   A. Hydrogen sulphide not produced.
      1. Escherichia coli.
   II. Citric acid utilized as sole source of carbon.
      A. Hydrogen sulphide produced.
         2. Escherichia freundii.

Genus II. Aerobacter.—Methyl red test negative. Voges-Proskauer test positive. Citric acid used as sole source of carbon.

I. Glycerol fermented with acid and gas.
   A. Gelatin not liquefied.
      1. Aerobacter aerogenes.
   II. Glycerol not fermented with acid and gas.
      A. Gelatin liquefied.
         2. Aerobacter cloacae.

Genus III. Klebsiella.—Methyl red test usually positive. Voges-Proskauer test usually negative. Citric acid usually (?) used as sole

* For both galley and page proofs of the new Bergey Manual dealing with the family Enterobacteriaceae I am indebted to Dr. Robert S. Breed.
source of carbon.  Capsulated forms from respiratory and other mucous membrane regions.

I. Litmus milk acid, but not coagulated.
   A. No acid and gas from maltose or mannitol.
      1. *Klebsiella pneumoniae*.
   B. Acid and gas from maltose and mannitol.
      2. *Klebsiella ozaenae*.

II. Litmus milk acid and coagulated.
   3. *Klebsiella granulomatis*.
   4. *Klebsiella capsulata*.
   5. *Klebsiella paralytica*.

III. Litmus milk unchanged.
   6. *Klebsiella rhinoscleromatis*.

**OCCURRENCE AND SIGNIFICANCE OF COLIFORM BACTERIA, INCLUDING PATHOLOGY**

In the discussion which follows an effort will be made to present and preserve the ecological point of view which should help to explain why encapsulated coliform bacteria occur in one environment and non-encapsulated in another. A shift of environment may provide opportunity for the development in large numbers of species which survive in small numbers and with difficulty in another milieu. Or, if we are dealing with pure cultures, changes in environment may favor the survival of the descendants of one variant or mutant over those of another. Natural selection has probably not caused organic evolution but it is one of the most important factors in its direction. What happens to an association of bacteria or to a pure culture of a single organism with capacity for variation or mutation will in large part depend on the environment.

The entire family *Enterobacteriaceae* Rahn, with the exception of the plant parasites, and the red pigment-producing chromogens, are thought of as intestinal bacteria, having, however, in the case of the more saprophytic species a considerable distribution outside the body of man and animal in nature.

*Animal pathology.* Very little attention was paid to the coliform group in veterinary medicine until comparatively recently.

Citations include *Proteus morgani* infections of zoo animals, gaseous emphysema, abortion in sheep, a disease of white rats associated with parasitic infestation, "moose disease," diarrhea in foals and in young pigeons, calf "scours," infectious enteritis of young lambs, a fatal disease of carp, metritis of mares, pneumonia in beavers, mastitis in cows, infectious diarrhea of chicks and abortion in ewes.

TenBroeck recently stated (1938) that it is extremely difficult to evaluate the importance of the colon group in animal pathology but that they take the place of the streptococci in man, i.e., they are often secondary invaders that complicate infection. Plastridge (1938) involved coliform organisms as responsible for navel ill in chicks, navel ill in calves, and a limited number of acute cases of mastitis in cows, and Hitchner (1938) stated that slow lactose-fermenting coliform bacteria have caused several epidemics of intestinal disease among chicks in Maine.

It will be noted that coliform bacteria produce "scours" in calves, diarrhea in foals and pigeons, infectious enteritis in young lambs, and infectious diarrhea in chicks. This pathogenicity for the young of animals is significant. Calf "scours" has been the subject of a classical investigation by the Rockefeller group at Princeton. The coliform bacteria concerned are usually in the mucoid phase (151, 95) but they may not be (Dollahite, 1938). Certain points are significant. The disease is produced by a soluble, diffusible substance of exotoxic nature (153, 131). This toxin is a capillary toxin and is far less effective in the usual laboratory animal than in the calf. Colostrum or maternal serum will protect the calf. The disease is one of intoxication and diarrhea with resultant dehydration. "If we put all the facts together the inference seems admissible that scours is associated with special races of *Bacillus coli* and that such races are developed and maintained in large herds. This will account for the different races of *Bacillus coli* which have been charged by other observers as the cause of scours. Each large herd through the presence of calves below par at birth may thus develop and maintain its own type of scours organism which, however, is not virulent enough to make any headway in naturally
strong calves properly cared for as regards food and housing" (Smith and Orcutt, 151). Somewhat the same view is held by Lovell (131) who concluded that special races of Bact. coli pathogenic for young calves exist, but that more than one race may be present in a herd and sometimes more than one type may be isolated from an individual calf.

The importance of this disease may be inferred from the statement of Dollahite (1938), released by Schoening (1938), that during the first five months of 1938 in a large government-owned dairy 68 calves were born, of which 33 (49 per cent) died with acute dysentery before they were five days old. Many of them died within 48 hours after birth.

In this outbreak Escherichia communior, E. acidi-lactici, and rarely E. coli, were encountered with E. communior occurring in about 60 per cent of the cases. From June 15 to November 15, 1938, cow serum prepared with a pooled E. communior-E. acidi-lactici antigen was given intravenously twice on successive days to 35 new-born calves with the result that none of the calves died, whereas of 32 others born during the same period and left untreated, ten died of acute dysentery before they were seven days old.

The coliform strains most concerned in animal disease are E. coli, often in the mucoid phase and often atypical as to lactose fermentation, and members of the genus Klebsiella.

Human pathology. Proteus morgani has been reported as the etiological agent in summer diarrhea of infants, infectious diarrhea of the new-born, diarrhea and dysentery in adults, infections of the urinary tract, meningitis, chronic discharging wounds, ulcerative colitis, war wounds, fatal septicemia and a paratyphoid-like infection. Rauss (130) placed Morgan's bacillus with Proteus, the British System of Bacteriology (1929) put it in the dysentery group, and Winslow, Kligler and Rothberg (186) considered it a paratyphoid (Salmonella) which was the classification given it in the third edition of Bergey (1930), an allocation approved by Levine, Ajwani and Wedin (91), Havens and Mayfield (57), and sanctioned by the French Dictionary (56). Thjøtta (164), d'Aunoy (3), Waaler (177), and Jordan, Crawford
and McBroom (69) think of it as a coliform type because it is
gas-producing, actively motile, strongly positive for indole, ex-
tremely heterogeneous serologically and distributed in a number
of environments, including soil, water and normal stools (80).
Since the fermentation of lactose is delayed or even absent, it
seems logical to consider the Morgan bacillus as standing with
the paracolon bacilli on the border of the coliform group next to
the paratyphoid group, Salmonella.

Organisms of the genus Klebsiella are reported from diseases of
the respiratory tract, rhinoscleroma, war wounds, suppuration,
meningitis, gaseous emphysema, septicemia, fetid nasal catarrh,
infections of the urinary tract, infectious diarrhea of the new-
born, and bronchial asthma. The Friedländer type of coliform
organism is not prominent in diarrhea, dysentery, cystitis, pyel-
itis, cholecystitis and cholangitis, nor is it as relatively prevalent
in post mortem invasion of the body as many have supposed.
Mackennon, Turner and Khayat (98) reported a study on bron-
chial asthma in which 28 strains of "mucoid encapsulated or-
ganisms" were studied. They confirmed the generally accepted
view that such strains show very variable cultural reactions and
that when present in bronchial asthma there was an associated
hypersensitivity of the patients towards the intradermal test
with vaccine prepared from the bacilli. The presence of gas in
the tissues discovered on autopsy is usually attributed to Clos-
tridium welchii, but coliform bacteria can cause this condition.

Coliform organisms not specifically labelled as Klebsiella or
the Morgan bacillus have been reported from such conditions as
pyelitis, cystitis, cholecystitis, cholangitis, suppuration, septi-
cemia, war wounds, Winckel's disease or hemorrhagic septicemia
of the new-born, sepsis neonatorum, infectious diarrhea of the
new-born, gastro-enteritis, food poisoning, peritonitis, diarrhea,
meningitis, arthritis, intestinal intoxication, gaseous emphysema,
and rare cases of infectious dermatitis.

The importance of coliform bacteria in cystitis and pyelitis is
attested to by a very large literature on the subject. The two
points of chief concern to the bacteriologist working in urology
are: (1) the mechanism of the invasion of the urinary tract by
bacteria; and (2) the types of coliform bacteria concerned. For two decades Dudgeon and his co-workers represented the most active group in this field (35, 36, 37). Their early work emphasized the property of hemolytic power as characterizing most of the coliform types involved, particularly in the male, and somewhat later they called attention to the slow lactose-fermenting coliform bacteria in urine. Although practically all of the atypical coliform bacteria were first isolated from feces, they were early found in urine. Thus Mair (101) described the paracolon bacillus from urine, W. J. Wilson (183) reported “anaerogenous” coliform bacteria from that source, and Kennedy, Cummings and Morrow had in their series of slow lactose-fermenters four strains from urine (78).

Hill, Seidman, Stadnichenko and Ellis (62) made an exhaustive report on the coliform bacteria isolated from cases of genito-urinary infection. They classified 200 cultures into Escherichia, 50 per cent; Aerobacter, 39.5 per cent; Proteus, 2.5 per cent; and miscellaneous, 8 per cent. Their data permit a breakdown of the 179 coliform strains (89.5 per cent) into Escherichia, 27 per cent; “coli-B bacteria,” 23 per cent; and Aerobacter, 39.5 per cent. More than half the strains were hemolytic.

Burke-Gaffney (19) studied 1000 strains of coliform organisms isolated from 126 specimens of urine. Classified by MacConkey groups 18 per cent were B. acidi-lactici; 7 per cent, B. coli-communis; 27 per cent, B. coli-communior; and 48 per cent, A. aerogenes. On the basis of indole production, methyl-red reaction and citrate utilization, 33 per cent were E. coli, 52 per cent A. aerogenes, 10 per cent “intermediates,” and 5 per cent “atypical.” Sandholzer (142) reported on 530 cultures of coliform bacteria isolated from 283 patients with urinary infection. Of the 530 strains 83 per cent were Escherichia belonging to 27 species or types, and 13 per cent were Aerobacter belonging to 14 species or types. The relative abundance of Aerobacter strains in urinary infections is striking. Hill et al. (62) reviewed the literature on the prevalence of Aerobacter in feces and found that among 14 reports totalling nearly 7000 cultures there were five reporting no Aerobacter; and in the other nine the percentage
occurrence of this genus ranged from 0.06 to 16.0 per cent. Their own data showed 39.5 per cent of Aerobacter in urological infections, and they stated that if the source of such infection is intestinal it is possible that the fecal organisms finding their way into the urine respond to some selective action in the genitourinary tract which operates to favor the genus Aerobacter over Escherichia since in the bowel Aerobacter is far outnumbered by Escherichia. This point is well supported by the data of Burke-Gaffney and to a lesser degree by those of Sandholzer. If the analysis be made on the basis of citrate utilization the difference is even more marked. Ruchhoft, Kallas, Chinn and Coulter (140) summarized the findings of six workers on 2534 coliform cultures from feces. Only 9.2 per cent of these were citrate-positive. In the Hill series from urine there were 62.5 per cent of citrate utilizers. Here is indeed a nice example of the operation of ecological factors.

Food poisoning is not thought of as of coliform etiology but Buchanan and Megrail (17) in Ohio, and Gilbert, Coleman and Laviano (51) in New York have reported outbreaks apparently due to organisms of the genus Aerobacter. If coliform organisms can produce a toxin, as seems amply demonstrated, it is a little odd that more intoxications with this toxin have not occurred. It may well be that the human adult is relatively resistant to it.

In recent years considerable interest has been aroused by the occurrence of outbreaks of epidemic diarrhea and gastro-enteritis, apparently water-borne, but in which no definite pathogen as etiological agent can be demonstrated (176, 181, 50, 188). This problem has assumed such proportions that a special symposium on gastro-enteritis was held by the American Water Works Association in connection with its annual meeting at Buffalo in 1937 (29).

Infectious diarrhea of the new-born is a disease in which emphasis should be placed on the coliform group as probable etiological agents. This disease is highly fatal and infectious in nature and when it invades a hospital nursery it is sometimes brought under control only by closing the maternity service of the institution. The nature of the disease indicates a potent intoxica-
tion which induces a diarrhea and results in extreme dehydration. Its analogy to the clinical course of Asiatic cholera has occurred to some of the students of the problem and comparative pathology calls to mind the calf scours situation. Among recorded outbreaks Dick, Dick and Williams (33) reported *Proteus morgani*, Jampolis, Howell, Calvin and Leventhal (68), a form of *Klebsiella*; Dulaney and Michelson (38) found *B. coli-mutabile*; McKinlay (99) recovered an organism thought to be a paratyphoid but which was probably a coliform organism of paracolon type; and Randall (1938) encountered coliform organisms in his study of two cases.

Theobald Smith's philosophy concerning *E. coli* is pertinent at this point. He stated (152) that in the gradual evolution of pathogenic or invasive types of bacteria, the beginnings of parasitism may have been made possible by a soluble diffusible toxin, but that in later stages this primary offensive, more or less accidental, mechanism is either partly or wholly suppressed and some different mechanism developed with which the bacteria protect themselves against the body-foreign forces of the host. The process may be regarded as shifting from the destructive, predatory to the parasitic, from the offensive to the defensive type. According to this hypothesis, *E. coli* represents the early predatory toxic stage with, however, a certain specialization towards protection from anti-foreign activities in the digestive tract. It represents in many respects the cholera vibrio in its activities.

In a recent discussion on staphylococci (Levine, B. S., 1938) it was stated that probably all of the *Staphylococcus aureus* type possess the capacity to produce exotoxin. Only certain strains produce enough to induce food poisoning in man when ingested in cream puffs or other food. There is evidence that somewhat the same situation may hold among coliform bacteria.

In all considerations of gastro-intestinal disease of infectious nature one should not lose sight of the possibility that symptoms are being manifested in the bowel whereas the inciting cause is elsewhere in the body. Felsen (45) stated that the indirect hematogenous excretory mechanism of the intestine is important in explaining many poorly understood, non-specific intestinal
infections or so-called infectious diarrheas. The primary cause often exists outside the intestine, and search for specific noxious agents in the bowel is then futile. Focal intestinal symptoms often cease abruptly after the primary extra-enteric focus of infection is eliminated, but they may persist for a longer period if necrosis and ulceration have been produced. The possible rôle of upper respiratory infection on the bacteriology of the intestinal tract has recently been discussed by Lieb and Chapman (94). In cases where the intestinal manifestations are incited extraneously the bacteria of the bowel may well respond to the new conditions with an altered flora which might serve as an "indicator" (121). Sufficiently studied, such bacterial types might be resolved into instruments of diagnosis almost as surely as if they were of primary etiological significance. We have then to study infectious diarrhea of the new-born either as a locally incited disease produced in a susceptible host by toxin-producing coliform bacteria, or to consider it as of other etiology, probably viral, with the avenue of infection by way of the respiratory tract.

Plant pathology. In this connection it is desired merely to emphasize that the recognized coliform bacteria and the 13 species of Erwinia, listed in Bergey, are very closely related. F. D. Chester (1938) stated that the genera Erwinia and Phytomonas were established on a purely utilitarian basis and have no genetic standing. Stanley (158) was of the opinion that the soft rot bacteria undoubtedly belong to the colon-typhoid-dysentery group of bacteria. Stuart, Griffin and Baker (161) studied 200 "coliform" cultures obtained from decayed portions of a number of fruits and vegetables. Serological investigations, in progress, seem to show an antigenic relationship between the plant, atypical and typical coliform organisms.

Occurrence in the intestine. Coliform organisms (Escherichia and Aerobacter) were first isolated from the intestinal tract of man. They were shortly recognized, though not without considerable research, as occurring in the intestinal tract of all higher animals. In examinations of meconium, commonly considered as sterile, it has been shown (22, 55, 155) that coliform
organisms may be present in a certain percentage of specimens. Throughout life, man is rarely without demonstrable coliform bacteria in his gastro-intestinal tract.

Much research has been expended in the effort to discover tests which will select fecal from non-fecal coliform bacteria (149, 18, 140, 6, 25, 122, 123, 103, 161). Certain facts emerge from this mass of data. All types of coliform bacteria may occur in feces but *Escherichia coli* (Imvic + + − −) is the most typical, numerous and constant type, with *Aerobacter aerogenes* (− − + +) next, and coliform intermediates (− + − + most common type) third. *A. cloacae*, paracoli, slow-fermenters, and *Klebsiella* may also be recovered. There is not much point in making comparisons of data unless identical methods of isolation have been used. In a certain number of cases *E. coli* may be absent, and there are even fecal specimens which yield no coliform bacteria at all (25, 123). Furthermore the coliform flora of an adult in good health and on a constant diet may show considerable change from day to day (122). When the usual fecal specimen is stored in saline suspension in the ice box considerable change occurs in the coliform flora, *E. coli* decreasing, and "intermediates" and *A. aerogenes* and *A. cloacae* increasing with, after many months, a complete change in flora often ending up with slow lactose-fermenting varieties of citrate utilizers. In about 14 per cent, however, there is no such change, the original *E. coli* persisting for months in competition with the other fecal bacteria and still presenting the characteristics of *E. coli* from fresh feces. These data are interpreted by Parr (119) to mean that in the latter case the specimens were originally pure cultures of *E. coli* so far as coliform bacteria are concerned.

The significance of these findings for sanitary science is that all of the coliform bacteria must be thought of as possibly fecal in origin. Where pollution derives from several sources one may expect to find *Escherichia coli* if the pollution be fresh; where pollution is from a single source there is no certainty that *E. coli* will be present; and the finding of typical *E. coli* may not indicate fresh pollution, particularly if that pollution be derived from a single source. Despite these qualifications, the presence of
significant numbers of *E. coli* in water remains our best test for fecal pollution.

**Occurrence in milk.** The recent literature on coliform bacteria in milk is even more voluminous than that for these organisms in feces (81, 44, 102, 182, 6, 7, 187, 10, 115, 162). Stark\(^\text{10}\) has stated that, due to what we may call the “living conditions of cows,” most raw milk contains coliform bacteria. These organisms in milk are assumed to come from barnyard manure, and since cows do not have typhoid fever, the presence of coliform bacteria in raw milk is known not to be of the same public health significance as is their presence in water. They are uniformly regarded as undesirable bacteria to have in milk and dairy products because they produce gas and undesirable flavors and odors. Their significance is largely proportional to the numbers present. It is important to remember that, unless some inhibiting condition is present, these bacteria grow well in milk. Although bacteria belonging to this group are occasionally found able to resist the heat treatment of the pasteurizing process, their presence in pasteurized milk is usually interpreted to indicate recontamination. The seriousness of permitting pasteurized milk to become recontaminated with any kind of bacteria is readily recognized. The pasteurizing processes applied to cream for buttermaking and ice cream mixes are generally accepted as adequate to destroy coliform bacteria. Their presence in these products is also believed to indicate recontamination of a pasteurized product. The types of coliform bacteria present in milk will vary with the flora of the feces, soil, or grain dust contaminating it.

**Occurrence in soil.** Coliform organisms are common in soil. Minkewitsch, Rabinowitsch and Joffe (108) believe that these bacteria are not found in the soil where there is no animal life. As pointed out by Thom (1938), it has been assumed that the presence of the colon group in soil is due to fecal contamination, and for that reason coliform bacteria have not particularly engaged the attention of soil microbiologists. In soil *A. aerogenes* is more abundant than *E. coli*, and “intermediates” and atypical

\(^{10}\) Coliform Round Table, 1937.
forms are present. This picture will vary with the character and use of the soil from the *E. coli*-sparse, virgin, protected soil to the *E. coli*-rich pasture grazed over by animals. From some quarters there is evidence that some, at least, of the citrate utilizers and atypical forms in soil are derived from fecal *E. coli* and typical intestinal forms. Minkewitsch, Rabinowitsch and Joffe (108) report the change of *E. coli* seeded in soil into citrate-utilizing "intermediates." This is far from the production of *A. aerogenes* from *E. coli* which, so far as we are aware, has never been reported, but it does indicate a step in coliform evolution. Parr (124), working with one of Koser's original soil strains, V5, in laboratory cultivation for more than a decade, has derived *E. coli* (+ + − −) from the strain called originally an "intermediate" (+ + − +). Despite the fact that citrate utilizers predominate there is evidence that *E. coli* can survive for a considerable time in soil (121).

*Occurrence in urine.* The striking thing in urine, as in soil, is the shift in the coliform picture from what it is in fresh feces to a predominance of citrate-positive coliform bacteria (62, 19, 142). The same shift occurs, as we have shown, when the usual fecal specimen is stored. The mechanism of these shifts may be a matter of variation, but is more likely succession, conditioned by ecological factors.

*Occurrence in water.* The literature of water bacteriology is much too complex to be reviewed here, as it touches on mediums, tests, interpretations and standards (139, 140, 18, 52, 169, 24, 48, 8, 60, 128, 14). Personal communications (1938) from Kulp, Levine, Norton, Butterfield, Mickle, McCrady, Norcum and G. F. Edwards have called attention to many sanitary problems connected with the coliform bacteria. Kulp feels that an attempt should be made to differentiate between *E. coli* and *A. aerogenes* especially when dealing with private water supplies, whereas Butterfield states that it is his policy to attach equal sanitary importance to the presence of each member of the coliform group since all are found in feces and since they are about equally susceptible to the forces of natural and artificial purification processes. Norcum is concerned over the increasing prevalence
of gastro-enteritis, apparently water-borne, but with questioned etiology. Norton mentions the significance of the work of Heathman, Pierce and Kabler (60) from which it appears that \textit{E. coli} may be no more (or even less) resistant to chlorine than the typhoid bacillus, and he feels the chlorine resistance of coliform bacteria should be restudied. McCrady is also anxious to have the significance of atypical coliform bacteria cleared up, and the New England workers are particularly concerned over the necessity for generous interpretations of standards to avoid condemning too many water sources epidemiologically satisfactory. Coliform bacteria do not occur ordinarily in water except from contamination with soil washings and fecal material from man and animal. When the pollution is from feces these bacteria survive for some time but generally with a shift from citrate-negative predominance to citrate-positive predominance. There are, however, both theoretical and actual conditions under which \textit{E. coli} may persist with typical reactions for long periods of time. Usually, though, the numbers of coliform bacteria decrease and in the absence of recontamination the group is usually lost sight of after a few weeks.

"\textit{Pump infection}," and \textit{paper and wood pulp}. It is well established that coliform bacteria grow well on leathers and other organic pump-parts, on swimming-pool ropes, and in pipe slime (24, 87, 1, 144). The growth of these bacteria in water distribution systems, of course, affects the analysis of the water. L. S. Stuart (1938) has reviewed the bacteriology of the tanning process from which it is apparent that modern leather is not itself the source of these coliform growths. The forms which are likely to occur naturally are "intermediates" and \textit{A. aerogenes}, but other organisms will grow on jute and leather (\textit{Serratia, Escherichia}, and even the typhoid bacillus).

The part played in the paper and wood pulp industries by coliform bacteria is but seldom mentioned. Tonney and Noble (168) have noted the persistence of \textit{E. coli} and \textit{A. aerogenes} on wood. The quality of water in contact with wood may be impaired by a high coliform count under conditions somewhat analogous to pump "infection." In 1931 Beckwith (11) re-
ported on the bacteriology of pulp slime and pointed out the importance of *A. aerogenes*. In 1938 he stated that it was his opinion that pulp slime has as one of its important causes the growth of capsular bacteria, nearly all of which are coliform. He showed, with Morgan, that the mucoid type appears frequently if the incubation temperature is low, and, of course, in the presence of a certain amount of carbohydrate. In "white water" the temperature is low, and it frequently contains appreciable amounts of carbohydrate which possibly are produced by inversion of the cellulose. Sanborn (1938) indicated the need for a detailed study of the coliform organisms found in pulp and paper mill systems and stated that he had seen pulp wood logs coated with gelatinous slime due to the development of organisms related to the genus *Aerobacter*. Chlorination and the high temperature of drying eliminate the bacteria so there is but little danger of the spread of bacteria by paper containers; but pulp containing slime organisms works up into defective finished products so that the problem is one of economic importance.

**Olives.** Alvarez (2) studied the blister-covered olives commonly called "floaters" and found that the condition was caused by atypical organisms "closely allied to, but not identical with the colon group." One strain, "H," resisted 80°C. for 45 minutes. Ten per cent salt solution was required to kill it in 24 hours. Again, Tracy (171) emphasized the spoilage of olives by colon bacilli. The reviewer has been given to understand that the coliform group constitute the most important olive spoilage organisms and that recoveries are mostly "intermediates" and *Aerobacter*, but occasionally *Escherichia*.

**Shellfish.** The presence or absence of fecal pollution in oysters and mussels is determined by examination for coliform bacteria (13, 126). In 1938, Perry stated that the examination of shellfish and their growing waters cannot be considered in the same category with drinking water which can be filtered, chlorinated or protected. Perry holds that many coliform bacteria, particularly of the *E. cloacae* type, are present in shucked market oysters or shell oysters when the temperature exceeds 60°F., that they are without significance as indicating pollution, and
that *E. coli* is the logical indicator of fecal pollution in shellfish and shellfish growing waters.

**Foodstuffs; miscellaneous.** It would appear from the nature of the processing procedures involved that canned foods do not contain coliform bacteria, a surmise confirmed by Williams (1938). Crossley (30) found 88 per cent of 14,365 samples of meat and fish pastes sterile, with coliform bacteria having small significance among the positives. In other types of foods their importance is greater as shown by the report of Griffiths and Fuller (53) on the detection and significance of *E. coli* in commercial fish and fillets, and that of Hunter (65) who found coliform bacteria important in salmon spoilage. One of the “believe it or nots” of bacteriology is the record of Simonds (148) that in a World War depot in Belgium three barrels of soft soap exploded due to growth of bacteria of the genus *Klebsiella* in the soap. The work of Burkey (20) on the fermentation of corn stalks and their constituents by bacteria of the genus *Aerobacter* has further extended our appreciation of the ubiquity of coliform bacteria. Lastly, Minkewitsch (107) has pointed out the part that insects play in the spread of coliform bacteria in the soil and on plants.

**ATYPICAL COLIFORM BACTERIA**

The significance of atypical coliform bacteria was early recognized, for in 1899 a committee composed of Veranus A. Moore, J. G. Adami, Elmer G. Horton and J. Monjares, was appointed by the section of bacteriology and chemistry of the American Public Health Association to study variations of the colon bacillus in relation to public health.

For convenience we may divide the atypical coliform bacteria into two classes. There are, first, those forms which give most of the reactions peculiar to a particular species but differ from it in some slight degree not sufficient to be named as another species. Such, for instance, are chromogenic *E. coli* (116, 120, 167, 161); encapsulated *E. coli* (153, 118); the sugar-tolerant coliform organism described by James (67); the organism giving common
serological reactions at high titre with the *Salmonella* (54); *A. transcapsulatus* (163), in which the organism lies at right angles to the greatest diameter of the capsule; *E. coli* with polar flagella (66); a heat resistant form (2); the organisms which are methyl-red positive and also Voges-Proskauer positive, or "double negative"; gelatin-liquefying *E. coli*; cellobiose fermenting *E. coli*; and hydrogen-sulfide positive *E. coli*. The property of hemolysis is hardly an atypical feature for it is common to many strains of *E. coli* both from the urine and the bowel. Such atypical forms are confusing to the taxonomist but probably not as much so to sanitarians as the second class of atypical coliform bacteria.

In the second category we place the instances of fermentation irregularities encountered in these bacteria. It will suffice to consider only irregularities encountered with lactose. If one seeds a tube of lactose broth with a typical coliform organism, within 24 hours full acid and gas production will appear. In water analysis, a positive tube must show acid and gas production within 48 hours. What about the tube which has acid and only a bubble of gas in 24 hours but never any more, or one which has full gas production but requires 72 hours to produce it? These are the organisms concerning which McCrady (1938) circularized workers interested in coliform bacteriology.

Moreover, coliform organisms are frequently encountered which fail to ferment lactose for a considerable number of days. Such strains are often confused with paratyphoid bacteria. They are the true slow fermenters and in many cases can be trained to rapid fermentation. Many of them are *Bacterium coli-mutabile* or mutabile types of *Aerobacter* and as such appear to be unstable variants as described by Deskowitz (32), earlier called "mutants."

Other atypical forms are those strains which ferment lactose producing acid but failing to produce gas. For these the term "anaerogenous" is used. Again there are strains which give all of the reactions for *Escherichia* except the fermentation of lactose and which fail to give serological reactions with *Salmonella*. 
These are called "paracoli" and may not ferment lactose no matter how long cultured. One further variant is the strain that ferments lactose at room temperature but not at 37°C.

The prevalence of such strains is indicated by Malcolm's work (103) with 1636 cultures of which 3 per cent were atypical. Kline (81) found 126 "anaerogenous" E. coli among 325 cultures isolated from raw and pasteurized milk. He expressed the opinion that these organisms are really members of the colon group which may have become modified through the influence of an unfavorable environment. We believe the evidence warrants the view that the slow fermenters, the "anaerogenous" strains, Morgan's bacillus and "paracoli" strains are all coliform bacteria which may be placed with whichever species they have the most characters in common.

It is much more difficult to assess the significance of the slow fermenters and other atypical coliform bacteria. It has been suggested that there is some relationship between the power to ferment lactose and virulence, as shown by the fact that the pathogens of the colon-typhoid group do not ferment lactose, and also by Dudgeon's (37) account of 49 cases of very severe acute infection of the genito-urinary tract in which all the strains of B. coli showed delayed fermentation of lactose. It has been found that atypical forms are likely to occur in stool specimens from subjects showing evidences of gastro-intestinal ill health (47). If these points of view be true, it would seem that the atypical strains encountered in water analysis should have more significance as indicators of dangerous pollution from feces or urine than more typical strains. Difficulty arises from the fact that atypical strains are also found in many environments in which coliform bacteria without pathological significance survive. This is known to the water bacteriologist who is inclined to look upon slow lactose-fermenting coliform organisms as "attenuated" or "devitalized" forms.

VARIATION IN THE COLIFORM GROUP

The "unstable variant" is by far the most interesting of bacterial variants. Bacterium coli-mutabile (113, 104) is a good
example. When this organism is cultured on lactose indicator-agar it appears not to ferment lactose. After some days, however, papillae appear growing on or out of the original colonies. Sub-cultures from these secondary colonies give typical lactose fermentation but sub-culture from the primary colony, avoiding contact with the papillae, gives delayed fermentation and will, when again plated, reproduce the original picture of colonies, negative to lactose, but on which lactose-fermenting secondaries eventually appear. One may take such a strain and plate it serially hundreds of times. It will still produce non-fermenting colonies on which fermenting papillae later appear. Such strains Deskowitz called "unstable variants." The early workers (113) thought of them as de Vriesian mutations, Stewart (159) attempted to explain them on Mendelian principles, and Mellon (105) has considered *Bacterium coli-mutabile* as a transitional developmental stage between the normal strain of *E. coli* and wild, non-lactose-fermenting *E. coli*. Such "unstable variants" are not uncommon and their peculiar type of variation is manifested in changes in colony type as well as in biochemical reactivity. Thus Deskowitz was working with the R-S colony type variation as manifested by certain strains of *Salmonella aertrycke*; and what appear to be "unstable variant" phenomena are recorded by Koser and Vaughan (86) in their paper on the utilization of d-arabinose by bacteria. It is possible, also, that the citrate "mutant" described by Parr may be another instance of "unstable" variation. It should be stated that except in the case of capsulated forms the discussion deals, so far as the records show, with smooth phase cultures.

Recent work with variations in the ability to ferment sucrose have interested workers in the coliform field and challenged taxonomists. Sherman and Wing (146) found that certain recently isolated strains of *E. coli* and *A. aerogenes*, seeded in pure culture in salicin and sucrose broths, gave rise to progeny which varied from the parent strains used. For example, from a culture of *E. coli*, which fermented salicin but not sucrose, progeny of four fermentative types were obtained which would by some terminologists be named as four different species. Treg-
oning and Poe (172) confirmed the production of sucrose variants, whereas Fulton (49) was unable to do so. Minkewitsch, Rabinowitsch and Joffe (108) have also reported the production of sucrose-fermenting strains from sucrose-negative antecedents. There seems to be marked difference in the facility with which strains of these bacteria vary and the frequency of appearance of strains capable of variation. Thus in our work we encountered up to the summer of 1938 only 29 instances of coliform strains giving the citrate "mutation." But, June 2, a fecal specimen was examined in which 54 of 60 colonies, picked, purified and studied, were E. coli which gave off in each instance small numbers of variants that one would have to classify as atypical E. freundii since they were citrate-positive and hydrogen-sulfide negative.

Nyberg, Bonsdorff and Kauppi (114) reported that two of their strains changed from Escherichia to Aerobacter. This statement was made on the basis of a change from M.R.+V.P.− to M.R.−V.P.+ after isolation. Citrate was not used in this work. Such changes were observed by Koser (84) who reported eight soil coliform strains which reversed their methyl-red and Voges-Proskauer reactions. Koser's cultures were, however, all positive utilizers of citrate so that the change observed was not from Escherichia to Aerobacter but a shift of type within the "intermediate" group. Minkewitsch, Rabinowitsch and Joffe (1936) reported changes in vitro and in the soil of E. coli to "intermediates." On the basis of their findings they suggested that it might be argued that all coliform bacteria arise from fecal E. coli. Most workers, however, seem to feel that the direction of evolution in the coliform group has been from the highly reactive, ubiquitous A. aerogenes to the less reactive, more specialized parasitic types.

Passing over numerous interesting references to the alteration of cultural finding in the coliform group through the use of chemicals, immune serum, and the like, we next discuss "shifts." Nyberg, Bonsdorff and Kauppi (114) in 1935 studied 200 cultures isolated at Helsingfors in 1933. They found that 68 strains were not viable and that only 25 of the 132 viable cultures had the
original colony type and cultural reaction. Fifty-nine cultures had changed in both colony type and reaction and 11 more, although retaining the original colony type, gave different reactions. Stuart, Griffin and Baker (161) studied "shifts" in the reactions of 191 cultures and found that in 47 instances changes occurred. They suggest that it might be better to use the term "stabilization" rather than "purification" for the treatment to be accorded cultures, and hold that "purification" implies contamination by a foreign species whereas "stabilization" implies a reasonable constancy of reaction without excluding the possibility of variation under suitable conditions.

SEROLOGY

Van Loghem (173) early emphasized the serological heterogeneity of the coliform group, stating: "Das individuelle Benehmen der Coli-Bazillen bei serologischen Untersuchungen ist bekannt. Stellt man ein Immunserum her mit einem bestimmten Coli-Stamme, dann findet man selten andere Coli-Stamme, welche von diesen Serum agglutiniert werden." Even fecal strains isolated from the same plate will not generally be influenced by the antisera prepared from any of the others. There are two possibilities. Either the number of kinds of E. coli is very considerable or the serological variability is very great. Such extreme variability could only be conceived of on some such theory of antigenic flux or instability as van Loghem had in mind: "der Rezeptorenapparat des B. coli sich in einem Zustande stätiger immer Verschiebung befindet, so dass sie Characterzüge, welche bei anderen Bakterien die spezifischen Serumreaktionen ermöglicht haben, bei Coli-Bazillen bald wieder ausgewischt werden."

Mackie (100) stated that, while an immune serum to a particular strain of typhoid bacillus will agglutinate most strains of B. typhosus with but little variation in degree, immune serum to certain B. coli types, on the other hand, have been found to exert little or no action on other strains identical as regards cultural reactions with that used for immunization. Smith (154) said: "The relation between a strain of B. coli and its mutant
with reference to the production of agglutinins and protective antibodies may be expressed by the statement that the original strain when injected into cows develops antibodies both toward itself and the mutant, whereas the mutant produces them only towards itself.” The citrate “mutant” reported by Parr (124) reacted to the same titre as the parent strain with a serum prepared against the parent. On the other hand, Sievers (147) reported that a coliform strain gave two variants, a gas former and a strain which did not form gas. Sera were prepared from both variants and these sera were not identical. Havens and Irwin (58) also observed an antigenic change coincidental with the acquisition of sucrose fermentation in the Morgan bacillus, “no cross-agglutination” occurring between the sucrose-fermenting and the non-sucrose-fermenting “strains from the same culture.”

Lovell (95), employing the precipitin test, found that 79 of 110 strains of coliform bacteria from diseased calves fell into eight groups. Hitchener (1938) prepared sera against eight slow lactose-fermenting strains and tested 19 cultures of these organisms against the eight sera. Four strains were encountered which fell into one group but the others were individualistic. It is true that Dudgeon, Wordley and Bawtree (36) found that most of their hemolytic strains of coliform bacteria isolated from acute urinary infections were agglutinated by a serum prepared from one of them. However, the non-hemolytic cultures from the same source showed no such relationship.

Serological work with coliform bacteria of the genus Klebsiella has, however, led to more satisfying results. In 1926 Julianelle (71, 72, 73) established the fact that the specificity of these organisms resides in their capsular materials. He studied a series of Friedländer bacilli and classified them into three specific types A, B, and C, and a heterogeneous group X. Edwards (40) tested 50 strains of encapsulated bacilli and was able to place 43 in two serological groups, seven remaining untyped. In 1929 he found that five cultures of Bact. aerogenes were serologically identical (as then tested) with type B Friedländer bacilli and two were identical with a strain of the granuloma bacillus.
In 1934 Morris and Julianelle studied rhinoscleroma strains and found them serologically identical with type C Friedländer bacilli. Barnes and Wight (9) studied a hemolytic, encapsulated strain of E. coli which appeared to have antigenic identity with pneumococcus type I. In 1937 Julianelle (75) examined strains of Bacterium aerogenes and showed there were three type-specific immunological entities among them, one common to pneumococcus type II, one common to both pneumococcus type II and Friedländer's bacillus type B, and a third which was individualistic. He also showed that strains of Bacterium aerogenes differ serologically when encapsulated, but become antigenically the same when de-capsulated. This leads us to the most important recent advance in the serology of the coliform group. Studies by Julianelle (76) on the immunological reactions of the unencapsulated cell supplied the hypothesis that the different organisms once deprived of the ability to elaborate capsular polysaccharide might be more readily amenable to systematization. Accordingly, unencapsulated, or "R" strains, were derived from the encapsulated "S" strains by continued cultivation of the "S" form in homologous anti-S serum.

With such unencapsulated strains and such sera, Julianelle studied some of the encapsulated coliform bacteria (No type B Friedländer bacilli were included) and found that they fell into two main groups: one including Friedländer bacilli types A and C; and the other rhinoscleroma, ozaena, A. aerogenes, and granuloma strains. More recently (1938) Julianelle has tested three strains of E. coli and two of A. aerogenes in a preliminary study. The former fell into two groups and the latter into one. Such serological work is laborious and time-consuming, but it is possible that some such approach as this will prove very fruitful.

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Malcolm (103) has stated that the coliform group of bacteria consists of a gradation of types so closely linked together as to render it undesirable to divide the group into two genera. The

11 The designations of the culture phases are those of Julianelle (76).
reviewer hopes to convey a concept of coliform bacteria as a group of closely related, closely intergrading bacteria in which, by the dropping of one character or the acquisition of another, an organism appears as a new strain. It is only reasonable to suppose that at intervals along the gamut of numerous varieties one can pick out a strain that will differ in a number of respects from another selected from another locus in the series.

The error of past classifications has been to dignify each recognizable variety encountered with a name. We now know that some of our most cherished measuring rods, such as the methylred and Voges-Proskauer reactions, sugar fermentations, indole production, and the utilization of citrate as a sole carbon source, are not to be depended upon to give with the same organism at all times the same reaction. Coliform bacteria are particularly restless when compared with most other groups of bacteria. It is among them that the most interesting and numerous instances of variation, and "shifts" occur.

The most fundamental objection, from our point of view, to the establishment of more than one genus for the coliform bacteria is that to do so will obscure for all save a few who are unusually conversant with the group its essentially intergrading nature. The correct orientation and stimulus which this point of view provides should result in further needful research in the field.

It seems, furthermore, that the concept of the coliform "intermediates" is such that if we are to have more than one genus we must also recognize one for the "intermediates." To classify these forms with Escherichia is to obscure the significant points that characterize "intermediates" as such, and to lose sight of their essential intermediate nature.

For some time we have regarded Klebsiella as coliform strains derived from the more definitely recognized types of the group, such as Aerobacter, and differentiated from them by ecological factors to manifest a lessened and variable biochemical activity, a more distinct encapsulation and, in some instances at least, enhanced virulence. Serology indicates their close relationship to Aerobacter. Aerobacter is less a toxin producer than is Escherichia, and it is more easily degraded by environmental
influences. Such a form as Friedländer's bacillus is, according to Smith, a more advanced or developed pathogen than the toxin-producing *E. coli* which Smith speaks of as "a primitive aggressive form."

If the Friedländer bacillus has evolved beyond the primitive toxin stage, as conceived by Theobald Smith, it would require some sort of specialization in order to maintain its position successfully as an invader of respiratory membranes. This the capsule supplies. It would seem that there is little to be gained in setting up a number of species in this group based on very little else than host source.

Personally, we should like to follow Jordan (General Bacteriology, 11th edition, 1935) in retaining the genus *Bacterium* for the entire group of coliform bacteria. It seems, however, that there are taxonomic difficulties preventing this. For a genus name to be valid its type species must be recognizable, and *Bacterium triloculare* Ehrenberg, 1828, is of course unrecognizable. *Bacterium* was retained for some time as a temporary genus but it is now felt that the time has passed for a continuation of such a status (15). Were the taxonomists to propose one genus for all coliform bacteria, it is our suggestion that it should comprise the two species, now called *E. coli* and *E. freundii*; the two now designated as *A. aerogenes* and *A. cloacae*; and one of the six now listed under *Klebsiella*, presumably *K. pneumoniae*.

CONCLUSION

The coliform group of bacteria has presented distinct problems in classification in the past. As early as 1893 Denys and Martin (31) indicated the two main reasons for the encumberment of bacteriology with false species as, first, a paucity of comparative studies, and second, the variations to which a single species is subject. Sufficient data have now accumulated to warrant revision of former classifications. An understanding of the complexity of the group as revealed by the application of a wide variety of biochemical tests and an appreciation of its variability and changeability has convinced workers of the futility of attempting to give species rank to more than a few of the many
types that have been described. This point of view has been strengthened by the apparent failure thus far to demonstrate that certain strains are peculiar to a given environment or activity. It appears rather that coliform bacteria must be thought of as a well-nigh ubiquitous group of organisms. Of these, that

![Diagram of coliform classification]

FIG. 1. A CONCEPT OF COLIFORM CLASSIFICATION

type will predominate in a given environment which is best adapted to the conditions of living found there.

The recent revision of Bergey's Manual of Determinative Bacteriology (12) has wisely taken cognizance of these principles and radically revised coliform classification in the direction of simplicity. The opportunity which this review offers for comment on that classification has been taken advantage of and suggestions have been made for further progress which our ac-
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quaintanceship with the organisms and their literature would seem to warrant.

The coliform group is a large one made up of closely related, highly intergrading, and somewhat unstable bacteria which form a fairly wide gamut or continuum extending from the lactose-negative paracolon forms at one extreme to the highly reactive A. aerogenes at the other. Standing with the paracolon forms next to Salmonella, one finds the Morgan bacillus. In about the same position, and leading to Eberthella and Shigella, are located the anaerogenous E. coli. Next to these varieties come the slow lactose-fermenting E. coli so likely to be manifested as "unstable variants." Completing one side of the picture, one finds the typical E. coli which bridge over to the A. aerogenes side by way of the "intermediates." Below A. aerogenes we find A. cloacae which appears to point toward the genus Proteus, and above all forms, but particularly above A. aerogenes, are located the Friedländer organisms. We have tried to represent this concept graphically in figure 1.

It is felt that our present understanding of the coliform group requires for its best expression the allocation of all these bacteria to five species within one genus.

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