A COMPARATIVE BACTERIOLOGICAL STUDY OF A GROUP OF NON-LACTOSE-FERMENTING BACTERIA ISOLATED FROM STOOLS OF HEALTHY FOOD-HANDLERS

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The bacterial strains reported in this paper were isolated during a routine carrier examination of the stools of a group of normal food-handlers. Though culturally a heterogeneous group, these bacteria are interesting chiefly because of their confusing resemblance to the pathogenic intestinal bacteria when first isolated on differential media. A detailed report of their reactions and possible taxonomic position is considered valuable.

Though unclassified gram-negative bacteria, with slight or no ability to ferment lactose, are frequently encountered in stool examinations, they are, in the majority of cases, isolated from hospitalized groups, or from individuals concerned in outbreaks of intestinal disorders of various kinds. For example, Meinicke and Neuhaus (1909) and Burri and Duggeli (1919), found strains in human feces which were like B. coli, but did not ferment lactose, though they could be trained to do so. Many "atypical" cultures were isolated by the British during the last war from soldiers who had attacks of intestinal disease. Douglas (1917), Dobell, Gettings, Jepps and Stephens (1918), and Dean, Adamson, Giles and Williamson (1917) all examined dysentery convalescents who were free of symptoms, and found a total of 130 "unnamed" strains, none of which fermented lactose, and the majority of which produced indol. None agglutinated in paratyphoid sera. Fildes (1917) and Glyn and Robinson (1918) report a total of fifty-one "unknown" paratyphoid-like strains iso-
lated from healthy soldiers. None fermented lactose, almost all produced indol, motility was variable, all fermented glucose, and some fermented sucrose. None agglutinated in paratyphoid A or B sera. Fildes reports eighteen additional strains which were able to ferment lactose after three or four weeks cultivation on this sugar. In 1924, Trawinski, studying a group of typhoid and dysentery convalescents and healthy control subjects, isolated ninety-one strains of intestinal organisms which produced indol, but did not ferment lactose. These cultures did not agglutinate in paratyphoid B serum, and para B did not agglutinate in serum made from a few of the strains. He concludes that these organisms are saprophytes which can become pathogenic for man under the most favorable conditions. Dudgeon in 1926 found 2 per cent of slow lactose-fermenting bacilli in stools of a group of subjects which included typhoid patients, people suffering from dietetic errors, and infections of the respiratory tract, and healthy subjects. He says that such strains "have been mistaken for paratyphoid bacilli, though the indol test distinguishes them." But he concludes that such organisms may give rise to intestinal disorders. Fothergill in 1929 cultured the stools of 104 infants during July to October, most of whom had diarrhea. The organisms he isolated from these cases did not ferment lactose, were non-motile, did not liquefy gelatin, and fermented a considerable number of sugars with acid and gas. Some of his strains finally fermented lactose after seven to ten days incubation. The group was heterogeneous both culturally and antigenically, and evidence for pathogenicity was definite in only two cases. In 1932 Kennedy, Cummings and Morrow described twenty-two strains of slow lactose fermenters which they were unable to classify. Since their group of organisms resembles so closely the strains to be described here, they will be discussed later.

**Routine Procedure**

The stools of 127 food-handlers were examined in groups of about 25 each week over a period of five weeks during October and November, 1932. A portion of the morning stool was collected
by the subject on a swab, and placed in a test tube containing about 2 cc. of distilled water. The tubes were collected about nine o'clock in the morning, and were brought directly to the laboratory, where the specimens were streaked on differential plates. The stools, in so far as the small specimen would permit description, appeared normal in color and consistency. None indicated a diarrheal condition.

Eosin-methylene-blue agar plates were used as the first differential medium. After twenty-four hours incubation, all colorless colonies were fished to Russell double-sugar agar slants. The growth on the Russell slants was used for Gram stains, agglutination reactions, and for inoculation of the following media: Hiss semi-solid agar for motility determinations, 1 per cent peptone water for indol test, gelatin stabs for liquefaction test, and 1 per cent carbohydrate-peptone water for fermentation reactions. All media were sterilized in the autoclave, sugar-containing media at 10 pounds pressure for twenty minutes, and the other media at 15 pounds pressure for fifteen minutes. The Russell slant cultures were restreaked on eosin-methylene-blue plates to check the purity of the culture, and also to afford an adequate description of the colonies. The cultures were preserved on infusion slants, and were transferred regularly every two weeks. All media were controlled with stock strains of B. coli, B. typhosus, and B. paratyphosus B.

GENERAL CULTURAL REACTIONS

Of the 127 stools examined, 102 were entirely negative, yielding only the common lactose-fermenting bacteria. Twenty-five of the stools, or roughly 20 per cent, produced non-lactose-fermenting colonies resembling pathogenic bacteria. These colonies were few in number. In some cases only 1 or 2 appeared, and never more than 10 or 12 were present on any one plate. B. coli was always present in great numbers. In a twenty-four-hour growth these colonies were small, gray in color, and were fairly transparent. When fished to Russell double-sugar slants, the reaction was typical of the paratyphoid group: acid and gas in the butt, and a colorless slant. Two specimens,
strains 38 and 100, produced no gas, resembling the typhoid or dysentery groups. All the cultures were small, gram-negative bacteria, so short as to appear coccoid in form. None of the strains liquefied gelatin in fourteen days. A forty-eight-hour growth on semi-solid agar showed 17 of the 25 cultures to be non-motile, and twenty-one of the strains produced indol. Cultures in infusion broth, and on blood plates, showed the strains to be essentially smooth in character.

Fourteen carbohydrates were used in studying the fermentation reactions. The following results were obtained. None of the strains fermented lactose in twenty-four hours, though some of them exhibited a latent fermentation in two to seven days incubation in 1 per cent lactose. A further discussion of the affinity for lactose will be given later. All the strains fermented a considerable number of carbohydrates with acid and gas production (strains 38 and 100 produced no gas). Glucose, mannitol, maltose and arabinose were universally fermented. Dextrine and xylose were fermented by all but a few strains. Inositol and inulin were not fermented at all, and raffinose was fermented by only two strains. A wide variation was exhibited in the reactions to the other sugars. Table 1 gives the reactions of all the 25 strains after forty-eight hours incubation in the various sugar solutions.

An inspection of the reactions listed in table 1 shows at once that these organisms constitute a very heterogeneous collection. They fall nearer to the colon-paratyphoid series than to any other type of intestinal bacteria, but can be identified completely with neither group. There are seven general groups. A small number of sucrose fermenters are further divided on the basis of dulcitol and salicin fermentation, following the basis of classification of the colon group. The majority of the strains do not ferment sucrose, and these are likewise grouped on the basis of dulcitol and salicin reactions. It will be noted that divisions II, IV and VI each contain two strains which are identical, and groups III and V contain three strains each, which are identical.

Division I contains only one strain, which ferments sucrose and salicin, but not dulcitol. This culture corresponds in this
NON-LACTOSE-FERMENTING BACTERIA

respect to *B. neapolitanum* of the Colon group, and to *B. guimai* of the intermediate groups of Topley and Wilson (1932), but

**TABLE 1**

Reactions of strains isolated from healthy food handlers

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here the likeness ceases. Division II, containing three cultures, ferments sucrose and dulcitol, but not salicin. These strains resemble *B. coscoroba* (which differs from *B. coli-communior* only
in being non-motile), but not completely. This grouping corresponds to no member of the Salmonella group. Division III, with six members, ferments dulcitol and salicin, but not sucrose, which fact suggests *B. immobile* (which differs from *B. coli-communis* only in being non-motile). Further comparison shows the identity to fail from this point on, and the resemblance to the Salmonellas is even less. Division IV has four strains which ferment neither sucrose, salicin nor dulcitol, and which resemble *B. acidi-lactici* in this respect, but in very little else. Division V, with six cultures, does not ferment sucrose or dulcitol, and does ferment salicin. *B. guimai* fits this grouping, but only this far. Division VI has three strains which ferment dulcitol, but do not ferment sucrose or salicin. Several members of the Salmonella group, including *B. paratyphosus* A, *B. paratyphosus* B, *B. abortus-equus*, *B. enteriditis*, *B. suispestifer*, and *B. aertrycke* may be classified in this way, but again complete resemblance is lacking. Strains 38 and 100 are grouped together because of their lack of gas production, but they do not belong to the typhoid or dysentery groups.

In the hope that definite reactions which would identify these organisms more clearly would be elicited by the use of other differential media, the following were employed: lead acetate agar stabs, Jordan's tartaric acid stabs, Endo's agar plates, and Krumwiede's brilliant green agar plates. No consistent reactions were obtained.

Agglutination tests with specific sera showed that none of the twenty-five strains agglutinated in paratyphoid A or B sera in dilutions beginning at 1:100 and continuing up to titre, though seven cultures had given a partial agglutination reaction when mixed on a slide with undiluted serum. Strains 38 and 100, which had produced no gas, were tested with typhoid, Flexner, Hiss and Sonne dysentery sera, and were entirely negative. The conclusion is that none of these strains show antigenic identity with the pathogenic intestinal bacteria which they resemble.

In mid-winter, three to four months after the original isolation of the organisms described, twenty-one of the subjects were recultured, but colorless colonies were recovered from only three
of the stools. For purposes of comparison, the original numbers were used, with the addition of an A. A contrast of tables 1 and 2 will show that strains 26A and 32A differ markedly from their corresponding strains 26 and 32. Stool 100A yielded two types of colony, 100A2, which was identical with the original strain 100, and strain 100A which differed only in producing gas from the sugars fermented. These four new strains were non-motile, produced indol, were gram-negative, coccoid bacteria, which did not ferment lactose. Strains 26A and 32A did not agglutinate in paratyphoid A or B, typhoid or Flexner sera. Strain 100A agglutinated in such low dilution in typhoid serum, and 100A2 so slightly in Flexner serum, that they were considered negative.

A brief survey of the reactions of these “atypical” organisms so far shows that they are not only a heterogeneous group, but that they fall into no known grouping of intestinal bacteria. On eosin-methylene blue and Russell double-sugar media, they resemble the pathogenic paratyphoids in appearance, but their fermentation reactions, general indol production, and lack of motility place them nearer to the colon group.

LACTOSE FERMENTATION

Several experiments were tried with media containing lactose to see whether or not these strains would finally ferment this sugar.

Soon after the twenty-five strains were isolated, they were
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placed on Russell’s double-sugar slants, and were transferred daily for eight days on this medium. Nineteen of the strains did not change in their reactions, giving acid and gas in the butt of the slant only. However, six strains produced acid and gas throughout the slant in from two to five transfers, and in each case the culture then fermented 1 per cent lactose with acid and gas in twenty-four hours. These strains were strains 34, 50, 54, 83, 106 and 111, and it will be noted that they differ in their reactions to the other sugars. All the strains were then put on 1 per cent lactose and were incubated for seven days. Eight strains (34, 42, 50, 54, 62, 83, 90 and 111) fermented lactose with acid and gas in from two to four days, and two others (42 and 100) fermented it with acid only. The fifteen strains which had not fermented lactose were then transferred daily in this medium for fourteen days. Four more strains fermented this sugar with acid and gas in from four to eleven days. These were strains 7, 10, 106 and 125. This makes a total of fourteen out of twenty-five strains which had shown a latent power to ferment lactose.

Five months after the original isolation of these strains, they were all plated on eosin-methylene-blue plates to check the purity and the stability of the cultures. A typical colony was fished from each plate to a Russell slant. These cultures gave the same reactions as they had originally, and were continued on infusion slants as stock cultures which were used in further experimentation with lactose fermentation.

Bronfenbrenner and Davis (1918), in the course of a bacteriological examination of foods, reported the use of higher percentages of lactose to convert to lactose-fermenters some organisms (later identified as B. coli) which they had isolated from Endo’s plates because they did not ferment lactose. Though their cultures did not react in six days growth in 1 per cent lactose-peptone water, they did ferment 2 and 3 per cent lactose in ten transfers. In accordance with the suggestion obtained from this work, lactose solutions were made up in 1, 2, 3, 5 and 10 per cent concentrations, and tubes of each were inoculated with twelve strains chosen from each of the sugar groups. Each series was controlled with two stock strains of paratyphoid B, one rough and
the other smooth. Cultures were incubated for one week, and reactions were recorded each day.

It was immediately apparent that 5 per cent lactose was more effective in producing a change than the other concentrations. Lower percentages generally took a longer time, and 10 per cent lactose produced irregular results, often with no gas production. In no case did the paratyphoid cultures ferment any lactose solution. Seven of the twelve test cultures fermented 5 per cent lactose with acid and gas in from two to five days. Once any given culture fermented the lactose, it also fermented 1 per cent lactose regularly. When inoculated into Russell double sugar, all the fermenting cultures produced a typical coli reaction with acid throughout the tube, and generous gas production. When plated on E.M.B., four of these cultures produced two types of colonies, one, the original pinkish-gray colony, and the other a typical coli colony, deep blue, with a metallic sheen.

Since all these cultures had been freshly isolated from individual colonies on homogeneous plates, the conclusion must be that dissociation of the culture had taken place in lactose solution, toward a coliform bacterium. This conclusion was further corroborated when the four dissociated cultures were plated again on eosin-methylene-blue following a seven days continuous incubation after fermentation had taken place. At this time the cultures were 99 per cent typical coli, with about 1 per cent retaining the original appearance.

All the other strains, seventeen in number, were then incubated continuously in 5 per cent lactose. The total result was that twelve more strains were converted to lactose fermenters in from one to fourteen days, with the average falling at about four days. These strains all produced coli reactions on Russell double sugar, but exhibited a variety of reactions on eosin-methylene-blue. One culture produced a large per cent of typical coli colonies. The other showed some colonies with deep blue centers and some with the original appearance on each plate. Evidence for dissociation was still apparent, though it was evident that some cultures were more stable than others.

There remained ten strains which fermented lactose very
slightly. These strains were not more amenable to daily transfer than they were to continuous incubation. In one to two days, they all showed a faint acid reaction throughout the tubes, with slight gas production. However, this result contrasts with the action of the paratyphoid B controls which produced no gas, and not the faintest acid reaction at any time. These ten slightly fermenting cultures all produced their original reactions on Russell double sugar and on E.M.B. plates.

This experimentation with lactose fermentation had yielded as its most significant result, the fact that these strains may be definitely excluded from the pathogenic groups, on the basis of their tendency to dissociate toward the coli group. It is suggested that when such organisms are isolated during routine stool examinations, they be placed in 5 per cent lactose solution as the simplest means of determining their characters.

At all events, this group of organisms isolated from healthy food-handlers remains very heterogeneous, even in the ability to ferment lactose with rapidity.

DIARRHEAL STRAINS

During the study of the organisms isolated from the stools of food handlers, eleven already isolated cultures were received from various unrelated sources for further study. These organisms had all been isolated from cases of diarrhea and vomiting of unknown origin. Their reactions are given in table 3. Strains 11F, 12F, 13F, 19F, 22F and 24F came from an epidemic of diarrhea in a tuberculosis sanitarium in November, 1932. Cultures 66F and 68F came from a family epidemic in November, 1932. Strain BF was isolated from an employee, ill with diarrhea and vomiting, in a hospital where two patients had a para-typhoid like fever. Strains CB and CU were from the blood and urine, respectively, of a baby who died of a para-typhoid-like fever of three weeks duration in March, 1933. The feces were reported to be an almost pure culture of this organism, and no agglutination took place in para B serum.

When replated on eosin-methylene-blue agar in this laboratory, these strains all produced small, smooth, grayish colonies, some
with faint bluish cast. On Russell double-sugar slants, they all gave a typical paratyphoid reaction: acid and gas in the butt, and a negative slant. All were gram-negative, coccoid bacteria, giving a smooth growth on blood plates, and an even turbidity in broth. All but one culture produced indol, and all but four were non-motile. Gelatin was not liquefied in fourteen days. Inspection of table 3 shows that none of the strains fermented lactose, inositol or inulin. All fermented glucose, mannitol, maltose and arabinose. A considerable variety of reactions are

### TABLE 3

<table>
<thead>
<tr>
<th>Strain</th>
<th>Lactose</th>
<th>Sucrose</th>
<th>Dulcitol</th>
<th>Salicin</th>
<th>Glucose</th>
<th>Mannitol</th>
<th>Maltose</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Inositol</th>
<th>Inulin</th>
<th>Motility</th>
<th>Indol</th>
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<tr>
<td>11 F...</td>
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</tbody>
</table>

exhibited toward the other sugars. It will be noted that the first group in table 3 corresponds to group II of table 1, and that strains 22F and 24F are identical with strains 90 and 17. It will also be noted that strains 11F and 19F are identical as are 22F and 24F, and 66F and 68F. Strains CB and CU fall into group III of table 1, though they are not identical with any strains in this group. Strain 13F falls into group IV of table 1, but is not identical with any strains in that group. Strain 12F is identical in sugar reactions with 46 and 111 of group VI, but differs in motility and indol. Generally, it can be said that these diarrheal strains resemble more closely the strains obtained from a group of
healthy people than they do any of the accepted groups of intestinal bacteria.

When these strains were tested with specific sera, 12F gave a fine partial agglutination on the slide in para A, para B, and typhoid serum, and when set up in diluted serum, agglutinated partially after a night in the ice box, in para A and typhoid serum in dilution 1:100 to 1:800. Strains 22F and 24F gave a partial agglutination in Flexner serum on the slide, but were negative in diluted serum. All the others were negative in all sera.

In this connection it must be mentioned that stools from twelve individuals suffering from diarrhea of varying severity were plated directly in this laboratory, and none of them produced any suspicious colonies on eosin-methylene-blue agar.

When grown in 5 per cent lactose, these diarrheal strains all fermented it with acid and gas production in from two to seven days. Strains 13F and BF did not give a strong reaction, but fermentation was definite. Fermentation of 1 per cent lactose was also present, in from three to nine days, but four strains did not produce gas in this concentration. When inoculated in Russell double sugar, these strains produced a coli reaction after they had fermented lactose and a definite dissociation into coli was apparent on the eosin-methylene-blue plates. Briefly, the diarrheal strains are very similar in their behaviour to the strains isolated from healthy people.

DISCUSSION

Since failure to ferment lactose is the first criterion for differentiating pathogenic intestinal bacteria from the common inhabitants of the bowel, any colonies which do not ferment lactose require further attention. The literature on intestinal bacteria, as well as several personal communications, reveals that the isolation from human feces of bacteria which can be neither identified nor eliminated by the customary procedure, is not a rare occurrence. The chief importance of these strains in routine bacteriological work lies in their confusing resemblance to the pathogenic forms. For example, Kennedy, Cummings and Morrow (1932) describe 22 strains of slow lactose-fermenters
NON-LACTOSE-FERMENTING BACTERIA encountered during routine procedure in a diagnostic laboratory. Their cultures were isolated as colorless colonies on Endo's plates, streaked mostly for routine, carrier, and typhoid stool examination. On the solid differential media, i.e. Endo's medium, Russell double sugar, triple sugar and eosin-methylene-blue agar, these strains resembled the paratyphoid group, but when grown in lactose broth, they all fermented that sugar in from two to fourteen days. Daily transfer in lactose broth speeded up the fermentation reaction. Furthermore, all but one of their strains produced indol. They conclude that these strains must be placed in the genus Escherichia since they ferment lactose, but that they can hardly be classified as colon bacilli though "they must be closely allied to that group probably as variants forming one of the connecting links between the colon-aerogenes group, and the paratyphoid group."

The strains described by these authors resemble so closely the strains reported in this paper, that it seems certain that they belong to the same group of bacteria. Variability within the groups in indol production, motility, and carbohydrate fermentation runs parallel. Eight of their strains were classified as B. coli-mutabile because of the production of true-breeding red daughter colonies on Endo's plates. When the remaining fourteen strains were compared in cultural reactions with the strains described here, it was apparent that the similarity was very close, though complete identity was lacking. Eleven of their strains fell into several of the sugar groups reported here.

Though the classification of this group of organisms is obviously uncertain, such bacteria must now be recognized as a group characterized by irregular lactose fermentation, and closely related to the colon bacillus.

The presence of these organisms in the feces of a group of normal people is more confusing than their presence in a diseased condition, where they might logically be assumed to be aberrant forms of previous etiological agents in an infection. The problem of what conditions in the intestine favor the loss of the power to ferment lactose, and whether a postulated loss of this power accompanies an increase in pathogenicity, must remain hypo-
thetical for the present. The fact that these organisms were
recovered on later examination in only three of the twenty-five
original subjects, suggests that they were transient, of no patho-
genic significance, and perhaps the result of purely physiological
conditions. However, the occurrence of similar strains in cases
of diarrhea, indicates that the question is far from settled. It is
suggested that the apparent etiological rôle of similar strains in
intestinal disturbances of infants may be due to a higher sus-
ceptibility in the infant than in the adult. If this be so, we must
accordingly admit that such organisms may have a pathogenic
effect even in the adult, given the proper intestinal conditions of
susceptibility.

It seems highly improbable, in view of the close similarity of
these bacteria to other groups reported in the literature, that
such organisms constitute a separate, and hitherto unrecognized
group of intestinal bacteria. It is more reasonable to assume
that we are concerned with a dissociative process in which well
defined classes may have produced such variants. In 1928,
Dulaney reported the dissociation of *B. coli-communis*, after long
incubation in broth, into a rough variant which was colorless on
Endo's medium and Russell double sugar, and a smooth variant
which produced the customary red reactions on both media.
Otherwise the two forms were identical in sugar fermentation.
However, a contradictory report was published by Nungester
in 1931. He describes the dissociation of a *B. coli*-like organism
isolated from a gall bladder empyema, which, after 6 days of
continuous incubation in broth, produced two forms, a rough
variant which fermented lactose, and a smooth variant which
did not attack this sugar. Both forms produced indol. It was
possible to change the non-lactose-fermenting smooth variant
into a lactose-fermenting organism, but the reverse change with
the rough form was not effected. Though it is obvious that the
question of dissociation of these forms is still in the realm of
conjecture, it is suggested that further work with this group of
organisms from this point of view will serve to clarify the subject.
NON-LACTOSE-FERMENTING BACTERIA

SUMMARY

1. Twenty-nine strains of gram-negative, non-lactose-fermenting intestinal bacteria were isolated from a group of healthy food-handlers during a routine carrier examination.

2. These strains produced non-lactose-fermenting colonies on eosin-methylene-blue plates, and on Russell double sugar, resembling the paratyphoid group.

3. None of the strains fermented 1 per cent lactose solution in forty-eight hours, though they all fermented a considerable number of other carbohydrates.

4. The organisms could be divided into seven groups by sugar fermentation reactions. In some groups there were identical strains.

5. Twenty-five strains produced indol, and twenty-one strains were non-motile.

6. Agglutination reactions with sera of established pathogenic types were negative.

7. Continued growth in 5 per cent lactose solution resulted in varying degrees of dissociation into lactose-fermenting variants, some of which produced typical colon colonies when plated on eosin-methylene-blue agar.

8. The chief importance of these bacteria lies in their confusing resemblance to the pathogenic intestinal organisms on the first differential media.

9. The conclusion is drawn that these strains are closely related to the colon group, possibly as variants, since they tend to disassociate into lactose-fermenting coli-like organisms.

REFERENCES

BURRI AND DUGGELI 1919 Kolle u. Wassermann, Handbuch der Path. Org., III.