ANTIGENIC STUDIES OF A GROUP OF PARACOLON BACTERIA (BETHESDA GROUP)

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In spite of continuous improvements in methods for the isolation of recognized pathogenic groups of Enterobacteriaceae, many outbreaks of gastrointestinal disease still occur in which only organisms of unknown significance are found. Often cases of diarrhea that are obviously of common origin yield only paracolon bacteria with identical or closely related biochemical characters. There is an increasing tendency to attribute etiological significance to these forms. The literature dealing with the role of paracolons in diarrhea has been reviewed by Neter and Clark (1944), Stuart and Van Stratum (1945), and Barnes and Cherry (1946).

One of the greatest difficulties in assessing the importance of paracolon bacteria in the causation of diarrheal disease is the lack of an exact method of identifying the cultures involved. In only a few instances has it been demonstrated that cultures from the same outbreak were serologically identical or even closely related. Rhodes (1942) found that several cultures isolated from one food-poisoning outbreak were identical. Stuart and Van Stratum (1945) found a high percentage of serologically identical coliform and paracolon strains in each of two institutional outbreaks of diarrhea in children. Barnes and Cherry (1946) noted that 28 strains isolated from an outbreak of gastroenteritis were serologically related, but the antigenic properties of the cultures were not studied intensively.

Although the Arizona group of paracolon bacteria has been classified and antigenic types have been established (Edwards, West, and Bruner, 1947), it composes only a very small fraction of the cultures usually classified as paracolon bacteria. No systematic work has been done on the antigenic analysis of other groups of these bacteria, although the work of Stuart, Wheeler, Rustigian, and Zimmerman (1943) demonstrated that a high degree of serologic relationship exists between biochemically similar strains. It therefore seemed worth while to study intensively the antigenic relations of a group of epidemiologically related strains in the hope of arriving at some conclusions regarding their significance. Also, such a study might serve as a basis for the classification of a further group of paracolons. Cultures previously described by Barnes and Cherry and similar cultures isolated from other small outbreaks were generously supplied by Comdr. L. A. Barnes. Of the 32 cultures studied, 22 were

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isolated from 12 patients in one outbreak, 6 from 4 patients and 2 food handlers in a second outbreak, and 2 from patients in a third outbreak. The remaining 2 cultures were isolated from milk and from a person who developed diarrhea after drinking it. The cultures all belonged to the group classified by Stuart, Wheeler, Rustigian, and Zimmerman (1943) as paracolon intermediates and most closely resembled their type 14011. According to the scheme of Borman, Stuart, and Wheeler (1944), they would be classed as *Paracolobacterium intermedium*. Since the majority of the cultures were isolated in Bethesda, Maryland, it is proposed to refer to this group of serologically related paracolons as the "Bethesda group."

**BIOCHEMICAL PROPERTIES OF CULTURES**

All the cultures were methyl-red-positive, Voges-Proskauer-negative types that failed to form indole but produced abundant H₂S. Barnes and Cherry (1946) found that they failed to grow in Koser's citrate medium, but all grew slowly on Simmons' citrate agar so that the medium became alkaline within 48 hours. All produced acid and gas from glucose, xylose, arabinose, rhamnose, maltose, trehalose, sorbitol, mannitol, and dulcitol within 24 hours. Lactose was acidified in 3 to 6 days, and slight gas production was eventually noted in all but 2 cultures. Eleven cultures fermented sucrose within 24 hours, but the remainder did not attack the sugar after 3 serial transfers at intervals of 30 days. Sucrose fermentation was confined to one serologic type, although not all cultures of that type fermented sucrose. Sucrose-positive and sucrose-negative cultures that were serologically identical were isolated from the same individual. Salicin was fermented by half of the cultures after 10 to 25 days' incubation. There was no correlation between salicin fermentation and serologic type, and salicin-positive and salicin-negative strains were isolated from the same individual. All but 3 of the cultures fermented cellobiose after 3 to 10 days. Inositol was not fermented. Gelatin was not liquefied after 90 days' incubation. Litmus milk was slowly acidified but was not coagulated during 60 days' incubation.

**SEROLOGIC STUDIES**

Any study of the antigens of paracolon bacteria must be approached with some caution since it has been shown by Kauffmann (1943, 1944) and by Knipschildt (1945) that coliform bacteria contain heat-labile and heat-stable somatic antigens that inhibit O agglutination. Therefore, it is necessary to ascertain whether such antigens are present in cultures being studied. O serums were prepared from broth cultures that had been heated at 121 C for 2.5 hours. The serums were then used in agglutination tests in which living broth cultures and broth cultures heated to 100 C served as antigens. Tests were incubated at 50 C for 1 hour and read after standing overnight. In the strains under consideration it was found that living cultures and heated cultures were agglutinated equally well and in high dilution by the O serums, a fact which indicated that they did not contain L or A (K) antigens. Though tube agglutina-
tion was used throughout the work, slide agglutination tests with properly diluted serums gave comparable results.

The H antiserums were prepared from formalinized broth cultures of strains that had been passed repeatedly through semisolid medium to insure optimum development of H antigens. Similar cultures were used as antigens in the H agglutination tests, which were read after incubation for 1 hour at 50 °C.

By agglutination and absorption the organisms were divisible into four O groups, the reactions of which are given in Table 1. Fifteen cultures fell into group 1, 7 into group 2, 10 into group 3, and 1 into group 4. The O antigens of all group 1 cultures were identical, as were also those of group 2. In group 3 minor differences were found among the cultures and their relationships were complicated. Reciprocal absorptions revealed minor differences that were not clear cut. For the present it seems best to place these strains in one O group.

The O antigens of the cultures were tested with serums for Kauffmann’s coli O groups 1 to 11 inclusive, 18, and 21 to 25 inclusive. These serums were obtained through the kindness of Dr. Kauffmann. The paracolons of O groups 1 and 3 were agglutinated to 5 to 10 per cent of the titer of coli O group 9 serum. No other reactions were noted. Coli 9 was not agglutinated by the paracolon serums. The cultures included in this study were not agglutinated by any Salmonella O serums. Other strains of the Bethesda group have been studied but that are not included here have somatic relationships to Salmonella onderstepoort.

Five groups of H antigens were established by agglutination and absorption tests. These are set forth in Table 2. The 15 cultures included in H group 1 were identical, as were the 3 cultures of H group 2, and the 5 strains of H group 4. The 6 strains included in H group 3 were very closely related but minor differences were found in absorption tests. The 2 cultures of H group 5 cross-agglutinated in high dilution, but even in the agglutination tests it was apparent that differences existed between them. These differences were clearly revealed by absorption tests, but until more is known about the antigens of the group it seems best not to separate them.

\[ \text{TABLE 1} \]

\[ \text{Somatic agglutination and O factors} \]

<table>
<thead>
<tr>
<th>ANTIGENS</th>
<th>Na 1A (1)</th>
<th>Na 4 (2)</th>
<th>Na 11 (3)</th>
<th>Na 19 (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unabsorbed</td>
<td>Absorbed</td>
<td>Unabsorbed</td>
<td>Absorbed</td>
<td>Unabsorbed</td>
</tr>
<tr>
<td>Na 1A (1)</td>
<td>12,800</td>
<td>6,400</td>
<td>3,200</td>
<td>0</td>
</tr>
<tr>
<td>Na 4 (2)</td>
<td>3,200</td>
<td>0</td>
<td>12,800</td>
<td>12,800</td>
</tr>
<tr>
<td>Na 11 (3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Na 19 (4)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

0 indicates no agglutination 1:100.
Figures in parentheses indicate 0 factors.
When the O and H groupings of the 32 cultures were combined, 8 types were established. These types were designated simply by the numbers applied to the O and H groups to which they belonged. Thus a member of O group 2 that had group 3 H antigens was represented by the formula Be.2:3. The letters preceding the formula indicate that the culture belonged to the Bethesda group. The distribution of the types is given in Table 3. Fifteen cultures of Be.1:1 were recovered from 9 of 12 patients in outbreak 1. One of these patients also yielded a Be.2:3 culture. Four cultures of Be.3:2-3 were recovered from one patient. These cultures are discussed later. Types Be.3:3 and Be.3:5 were each recovered from one patient. In the second outbreak 3 of 4 persons affected yielded a...
type Be.2:4, but a nonmotile strain of O group 2 was recovered from the fourth. Two food handlers who were not ill yielded types Be.3:3 and Be.4:3, respectively. The third outbreak was composed of only 2 cases of diarrhea thought to be of common origin. One patient yielded type Be.3:3, the other type Be.3:5. The fourth outbreak was represented by only 2 cultures, one from milk that obviously was unfit for consumption and the second from a person who developed diarrhea after drinking the milk. The cultures were identical and belonged to type Be.2:4.

VARIABILITY IN CULTURES

The reactions of the O groups given in table 1 suggest that form variation similar to that described in the Salmonella group by Kauffmann (1940) exists in these strains. This possibility has not been investigated. Certain observations were made on variation in the H antigens, in which the O antigens were unaffected. One patient in outbreak 1 yielded 4 cultures of Be.3:2-3. Actually, three of the cultures were isolated as Be.3:2 and one as Be.3:3. Since the O antigens of the cultures were identical, this strongly suggested that H antigens 2 and 3 had a common origin, although, as shown in table 2, there was very little relationship between them. By growing the three Be.3:2 cultures in semisolid agar that contained appropriately absorbed H group 2 serum, it was possible to change them to Be.3:3. Likewise, it was possible to change the Be.3:3 culture recovered from this patient to Be.3:2 by cultivation of the culture in semisolid medium containing absorbed serum of H group 3. That the H antigens of the cultures were actually reversed was shown by reciprocal absorption tests. Thus, these cultures must be considered as one type that has two H phases, and the formula must be written 3:2-3. This observation is very similar to that of Kristensen and Bojlen (1936), who isolated two cultures with the formula VI, VII:1.5 and one culture with formula VI, VII: c from the stools of a patient infected with Salmonella cholerae-suis var. kunzendorf.

Within type Be.3:5 are two cultures, Na 11 and Na 22. Both the O and H antigens of these cultures are closely related, but neither the O nor H components of the two are identical. It was found that Na 11 was related to Md. 2, a culture received from Mr. A. A. Hajna. When a single colony culture of Na 11 was plated, two distinct forms were isolated, the reactions of which are given in table 4. These forms, which are called phase 1 and phase 2, were both agglutinated in high dilution by serum derived from the whole culture. Phase 1 (H antigen 6) was agglutinated in high dilution by Md. 2 serum but was unaffected by Na 22 serum. With phase 2 these reactions were reversed. Colonies of mixed phase were found that were agglutinated by both serums. Upon plating these mixed colonies, mixed colonies and colonies of phase 2 appeared. When phase 2 colonies were placed in semisolid medium that contained suitably absorbed Na 22 serum, they spread through the medium and phase 1 was isolated from the spreading growth. Phase 1 has not been similarly reversed, nor has either phase been found to revert naturally to the other.

As originally received, Na 22 was agglutinated actively by Na 11 serum, and the two cultures were included in the same H group. When this culture was
placed in semisolid medium that contained suitably absorbed Na 11 serum, it spread through the medium, and from the spreading growth a form was isolated that was no longer agglutinated by Na 11 serum and reacted only slightly with serum derived from the parent strain. The changed culture was agglutinated to the titer of serum derived from Md. 10, another strain received from Mr. Hajna. This component was called phase 1 and designated as H antigen 7.

**TABLE 4**

*Variation in H antigens*

<table>
<thead>
<tr>
<th>ANTIGENS</th>
<th>H FACTORS</th>
<th>Na 11</th>
<th>Na 22</th>
<th>Md 2</th>
<th>Md 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na 11, whole culture</td>
<td>5, 6</td>
<td>12,800</td>
<td>6,400</td>
<td>3,200</td>
<td>0</td>
</tr>
<tr>
<td>Na 11, phase 1</td>
<td>6</td>
<td>12,800</td>
<td>0</td>
<td>6,400</td>
<td>0</td>
</tr>
<tr>
<td>Na 11, phase 2</td>
<td>5</td>
<td>12,800</td>
<td>12,800</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Na 22, original</td>
<td>5</td>
<td>3,200</td>
<td>12,800</td>
<td>0</td>
<td>200</td>
</tr>
<tr>
<td>Na 22, induced</td>
<td>7</td>
<td>0</td>
<td>200</td>
<td>0</td>
<td>6,400</td>
</tr>
<tr>
<td>Md 2</td>
<td>6</td>
<td>800</td>
<td>0</td>
<td>12,800</td>
<td>0</td>
</tr>
<tr>
<td>Md 10</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6,400</td>
</tr>
</tbody>
</table>

0 indicates no agglutination at 1:100.

**TABLE 5**

*Antigenic table*

<table>
<thead>
<tr>
<th>ANTIGENIC SYMBOLS</th>
<th>O antigens</th>
<th>H antigens</th>
<th>TYPE STRAINS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phase 1</td>
<td>Phase 2</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>Na 1a</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>3</td>
<td>Na 23</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>4</td>
<td>Na 4</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>3</td>
<td>Na 2C</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>6</td>
<td>Na 11, Na 20, Na 30</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>7</td>
<td>Na 22</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>3</td>
<td>Na 19</td>
</tr>
</tbody>
</table>

The only variation noted in Na 22 was that which occurred under the influence of serum.

**ANTIGENIC TYPES**

The antigenic types established are given in table 5. The 4 O groups were divided into 8 serologic types. Type 3:3 was not merged with type 3:2-3 nor designated as a subtype of the latter since the cultures were not identical with phase 2 of 3:2-3 nor with each other. It was stated above that O group 3
and H group 3 each contained closely related but distinct antigens. Thus cultures Na 12, Na 20, and Na 30 possess slightly different O and H antigens. At present these types are placed under the same antigenic formula because the specific factors that distinguish them are rather weak. Later it may be found advisable to distinguish between them. As yet these strains have not been found diphasic. It is possible that they later may be distinguished by the isolation of other phases.

DISCUSSION

The question of the pathogenicity of the Bethesda group is not definitely answered by this study, but certain suggestive results were obtained. Although the paracolons isolated from outbreak 1 were divisible into 5 types, one type (Be.1:1) was predominant and was present in at least 9 of 12 patients from which cultures were available. The presence of this type in 75 per cent of the individuals suggests that it was responsible for the epidemic.

In the second outbreak 3 of the 4 persons affected yielded identical cultures, but from the fourth patient was isolated a nonmotile culture of the same O group. Asymptomatic food handlers connected with this outbreak yielded distinctly different types. It seems highly probable that type Be.2:4 was responsible for this infection.

The third outbreak consisted of only 2 cases of diarrhea thought to be of common origin. These yielded types of the same O group that had distinct H antigens. One of these cultures was Na 11, mentioned above. Since so little is known of variation in these organisms, it is impossible to say whether the 2 cultures originated from the same type. The fourth outbreak was represented only by 2 identical cultures, one from obviously spoiled milk and the other from the stools of a patient who became ill after drinking it.

Although the results suggest that these paracolon bacteria may incite outbreaks of disease, it was found that many cultures of the Bethesda group occurred in individuals with no history of diarrhea. This group is widely distributed, and the writers are in possession of a large number of cultures from both normal persons and individuals affected with diarrhea. If the organisms are pathogenic, it must be recognized that the carrier-case ratio is rather high, apparently much higher than in the Salmonella and in the Shigella types of recognized pathogenicity.

In the present state of knowledge it is difficult to establish the limits of the Bethesda group. As in other enteric bacteria it undoubtedly will be found to merge with other groups. Nevertheless, until more is known of the serology of the Enterobacteriaceae it seems worth while to set it apart for the moment so that it may be studied and correlated with other groups. Such a procedure has yielded excellent results with the true Escherichia coli types and with the Arizona paracolons. Therefore, the Bethesda group is tentatively described as follows:

Slow lactose-fermenting cultures that are methyl-red-positive, Voges-Proskauer-negative, indole-negative, H2S-positive, d-tartrate-positive and Simmons’ citrate-positive. Urea utilization is negative when tested by the method of Rustigian and
Stuart (1941), but most cultures give positive tests by the method of Christensen (1946). Dulcitol usually is fermented promptly, although dulcitol-negative strains occur. Sucrose generally is not fermented, although occasional cultures may ferment sucrose.

Undoubtedly, cultures with other biochemical characters will be found to be serologically related to this group. However, it is within this biochemical group that serologic relationships most frequently are found. It is known that the antigens characterized here are only a few of many that occur within the group and that the establishment of the few serologic types delineated here constitutes only a very meager beginning in its classification. However, if these cultures that prove so troublesome to the worker in enteric bacteriology are to be understood, a start toward their classification must be made.

The few instances of serologic variation noted above indicate that the classification of the group will not be easily accomplished. For lack of landmarks the work proceeded slowly, and it was necessary to support each step by reciprocal absorption tests. As the group is better understood its classification should proceed much more rapidly.

**SUMMARY**

Thirty-two paracolon cultures isolated from 4 distinct outbreaks of diarrhea were studied. The organisms formed a rather uniform biochemical group. They were divided into 8 serologic types through the examination of their O and H antigens. In general, the types established by serologic examination agreed fairly well with the epidemiologic data. The H antigens of some cultures were variable. The cultures studied belonged to a group of paracolon bacteria that were designated as the Bethesda group.

**REFERENCES**


Christensen, W. B. 1946 Urea decomposition as a means of differentiating Proteus and paracolon cultures from each other and from Salmonella and Shigella types. J. Bact., 52, 461–466.


