ANTIGENIC RELATIONSHIPS OF 765 PARACOLOBACTRUM INTERMEDIUM CULTURES

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In a study of the antigenic relationships of Paracolobactrum,1 Stuart, Wheeler, et al. (1943) found that 48 per cent of 140 Paracolobactrum aerogenoides strains were identical or closely related to one or another of 8 of the strains used to produce antiserums. Fifty-three per cent of 40 Paracolobactrum intermedium cultures were identical with one or another of 6 strains used to prepare antiserums; and 67 per cent of 223 Paracolobactrum coliforme strains were identical or closely related to one or another of 13 strains used to produce antiserums. These results indicated that the investigation should be continued.

In 3 years more than one thousand additional cultures comprising all three groups were investigated. At this time certain conclusions could be drawn. As the number of P. coliforme strains increased, the percentage of cultures identified by the original 13 antiserums decreased rapidly. Sixty-five per cent of the first 250 strains isolated were identified in these serums. But only 46 per cent of the next 250 strains, and 19 per cent of the last 250 strains could be identified. A similar situation was encountered in isolating and identifying various strains of P. aerogenoides. Biochemical type 32011 (Stuart and Rustigian, 1943), an exception to this observation, was encountered throughout the investigation.

In addition, it was observed that over a certain time interval a given serological type was isolated consistently from any one institution or group of institutions. At a later period, however, all types encountered, although antigenically identical or closely related to one another, differed markedly from those isolated over the previous time interval. The limits of each period varied with the type and with its source. There was also a certain degree of overlapping among the types involved. For example, all 100 strains of P. coliforme (31611) were isolated in Providence, Rhode Island, or the immediate vicinity, between 1939 and 1944. Since that time this type seems to have disappeared entirely. Twenty-three strains of type 111 came from a single institution over a period of 3 years, but...

1 Borman, Stuart, and Wheeler (1944) proposed the term "Paracolobactrum" for that large group of organisms possessing physiological and serological characteristics common to coliform and Salmonella bacteria. It was further suggested that the Paracolobactrum be divided into three sections or groups, P. aerogenoides, P. intermedium, and P. coliforme. For a more detailed description of these terms see the sixth edition of Bergey's Manual of Determinative Bacteriology (Breed, Murray, and Hitchens, 1948). In order to correlate the terms used in the present work with those of the past (Stuart, Wheeler, et al., 1943; and other publications), Paracolobactrum would be interpreted as paracolon; P. aerogenoides as paracolon Aerobacter; P. intermedium as paracolon intermediate; and P. coliforme as paracolon Escherichia.
this type was not encountered again after that period. On the other hand, a single biochemical type occasionally persisted in one institution over periods of several years. In such instances, however, a succession of serological types was usually encountered. This "temporal and topographical antigenic continuity" was found also in Escherichia coli (Stuart and Carpenter, 1948).

The number of new P. intermedium isolates was small, but the percentage identified in 5 of the 6 original antiserums remained high. It was decided, therefore, to concentrate on this group to the exclusion of the other aerogenic Paracolobactrum groups.

All Paracolobactrum cultures isolated in the Florida State Public Health Laboratories and the Paracolobactrum cultures received at Brown University from state and municipal laboratories as far west as California totaled 1,957 in the year 1946–1947. These cultures were isolated in the various laboratories on Salmonella-Shigella, Wilson-Blair, brilliant green, and eosin-methylene-blue agar. They were replated on eosin-methylene-blue agar, the least inhibitory of these four mediums, and a well-isolated colony was transplanted to an agar slant. Strains selected for serological study produced acid and gas rapidly in glucose, maltose, and mannitol, almost always attached lactose slowly, seldom fermented sucrose, and frequently fermented salicin slowly. They were motile, grew on citrate agar, and produced hydrogen sulfide. They failed to produce indole or acetyl-methylcarbinol and were urea-negative in the medium of Rustigian and Stuart (1941). A few strains producing acid but not gas in lactose in 24 hours were included in this investigation.

Of the 1,957 cultures obtained, 765 or 39 per cent were selected for further study and were classified as P. intermedium. About 40 per cent of all Paracolobactrum cultures isolated in Florida were P. intermedium, but only 5 to 10 per cent of Paracolobactrum from other areas were P. intermedium. These percentages have remained fairly consistent in the areas involved over periods of from 2 to 3 years and in the Rhode Island area for 10 years.

In general the biochemical reactions of these P. intermedium cultures were quite consistent. An occasional strain failed to ferment lactose; many strains were anaerogenic in lactose and some required 60 days to attack this carbohydrate. Salicin, when positive, was attacked more slowly than lactose. The great majority, however, produced acid or acid and a bubble of gas in lactose in from 3 to 9 days and in salicin in 1 to 3 weeks. Large gas volumes, 20 to 40 per cent, were seldom encountered in lactose and salicin. Sucrose was fermented slowly by some strains. Several strains produced variants that utilized citrate slowly or not at all. A few strains yielded variants that failed to produce \( \text{H}_2\text{S} \) or produced it slowly. Two strains produced indole-positive variants, and another, an acetyl-methylcarbinol-positive variant. There was good antigenic agreement among strains producing acid rapidly in lactose, among strains failing to ferment lactose, and among strains fermenting sucrose. However, there were sufficient serological exceptions to render biochemical classification impractical. Moreover, some lactose-negative strains were trained to ferment lactose. Occasionally sucrose-negative variants were obtained from sucrose-positive parents.
A large number of strains positive in salicin when freshly isolated were negative on subsequent tests. For the most part, plating such cultures on EMB agar containing salicin instead of lactose failed to establish salicin-positive variants. A similar situation was found for Shigella alkalescens with respect to sucrose (Stuart and Rustigian, 1944). When definite serological types were established, additional biochemical reactions were determined on representative numbers of the different types. Table 1 shows the biochemical reactions of the 12 most common types.

**TABLE 1**

*Biochemical reactions of the 12 most common serological types of Paracolobactrum intermedium encountered in the present work*

<table>
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<tr>
<th>TYPE</th>
<th>NUMBER</th>
<th>STRAINS</th>
<th>GLUCOSE</th>
<th>GALACTOSE</th>
<th>MALTOSE</th>
<th>MANNITOL</th>
<th>BROMINOL</th>
<th>ARABINOSIDE</th>
<th>XYLOSE</th>
<th>SORBITOL</th>
<th>INOSITOL</th>
<th>ADONITOL</th>
<th>LACTOSE</th>
<th>SUCROSE</th>
<th>MALTON</th>
<th>DOPHOL</th>
<th>RUTENOSIDE</th>
<th>CITRATE</th>
<th>TRIS</th>
<th>H2S</th>
<th>MOTILITY</th>
<th>INDOL</th>
<th>V-P</th>
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+ = rapid production of acid and gas.
+* = slow production of acid or acid and gas.
± = some strains positive and others negative.
W = some strains weakly positive and others negative.

As fast as new strains were isolated they were tested in 5 P. intermedium anti-serums prepared in the previous work. Serial dilutions of the antiseraums ranging from 40 to 40,960 were prepared. Living saline suspensions of 16-24-hour agar transplants served as antigens. Results were recorded after 2 hours in a 37 C water bath and again after holding overnight in a 55 C water bath. Certain advantages of this incubation system over the conventional 2 hours at 50 C followed by an overnight period at 2 C have been discussed in another report (Stuart and Carpenter, 1948). Of the first 150 strains tested, many gave strong reactions in antiserum 14011 and a few in antiserum 12611. An occasional strain reacted in 2 other sera but none reacted in the fifth serum. Upon adsorption no strain was found to be antigenically identical with any of the 5 used to produce antiseraums. From the results of these tests 15 cultures were selected for the preparation of additional antiseraums. After considerable exploratory
work 9 antiserums giving the highest titers with the greatest number of strains were selected as "basic types." Only one of the 5 original antiserums (14011) was included in the nine. Each of the 765 cultures was tested in the 9 basic antiserums. In these tests 0.1 ml of a saline suspension of living antigen was added to 0.5-ml portions of 80, 640, and 5,120 dilutions of each antiserum. Tubes were incubated as previously described.

Many definite antigenic patterns were established by the 9 basic antiserums. Table 2 shows some of the more common patterns. All strains having an antigenic pattern comparable to 5883, 9466, 13304, and 20158, etc., were used to adsorb the antiserums of these respective strains. Two hundred forty-one strains were identified with the 9 basic antiserums. Other antigenic patterns were studied, and, when a sufficient number of strains showed a comparable pattern, an antiserum was prepared from one of the strains. In this way a total of 34 antiserums was used to study this group of _P. intermedium_ organisms.

Of the 765 cultures, 453 or 59 per cent were antigenically identical with one or another of the 34 cultures used to produce antiserums. Strains were called antigenically identical when they agglutinated to titer in an antiserum and removed all agglutinins upon adsorption. They were considered closely related when they agglutinated to titer and markedly reduced the titer upon adsorption. The most commonly encountered type was 20158 (table 1) with 99 strains. This type was found in Rhode Island, Florida, California, and elsewhere. Sixty-seven strains of type 1805 (table 1) having the same somatic antigens as 20158 were also widespread. Sixty-one additional strains, comprising 11 different serotypes, possessed somatic antigens related to or identical with those of 20158. Therefore 227 or 52 per cent of the identified strains or 29 per cent of all _P. intermedium_ strains had the same or related somatic antigens. On the other hand, 312 strains or 41 per cent of the total could not be identified by any of the 34 antiserums, although many of the 312 strains were closely related to one or another of the 9 basic strains used to produce antiserums. Moreover, despite the fact that 140 strains possessed the antigenic pattern of S-519 (table 2), only 16 were identical with S-519. Five more antiserums were prepared from cultures in this group, but only 50 or 36 per cent of the 140 strains were identified in these 6 antiserums.

Thirty-two strains failed to agglutinate in any of the 9 basic antiserums even when tests were repeated in a dilution of 20. Twenty-five of these constituted the only group that rapidly produced acid in lactose. An antiserum prepared from one of the 25 identified 13 strains; an antiserum prepared from another strain identified 2; and an antiserum from a third identified only its homologous strain.

**DISCUSSION**

The general antigenic relationships of 765 strains of _P. intermedium_ were determined in the present investigation. No attempt was made to set up a diagnostic typing scheme. Typing systems are a great aid in diagnostic bacteriology but if carried too far may impede progress in the field as a whole. Like the Cohn-Koch dogma of monomorphism they tend to create a false impression of security and
<table>
<thead>
<tr>
<th>ANTIGEN</th>
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<td></td>
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<td>37</td>
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Antiserum dilutions

4, 3, 2, 1, - = degrees of agglutination from complete to negative.

? = unidentified strain.
finality. Studies involving the unrestricted use of single factor sera probably err as much by missing important relationships as the unrestricted use of whole antiseraums confuses the issue by uncovering too many unimportant relationships. Gard (1937), Gard and Ericson (1939), Schiff, Bornstein, and Saphra (1941), Wheeler, Stuart, et al. (1943), and many others found labeled H and O Salmonella antigens in coliform bacteria. Stuart, Wheeler, et al. (1943) found a number of coliform cultures agglutinating to high titer, occasionally to the homologous titer, in whole Salmonella antiseraums. These cross reactions were due to unlabeled antigens that in a study of taxonomic relationships could be as important or more important than labeled antigens.

The 34 strains used to produce antiseraums, tested in Salmonella single factor sera with the slide test, showed no significant cross reactions.

Previous to the present work, Edwards, West, and Bruner (1948) made an intensive study of 32 P. intermedium cultures isolated in four outbreaks of gastroenteritis. Agglutination and adsorption tests established 4 groups of O and 5 groups of H antigens. The 32 cultures were divisible into 8 types. For convenience these investigators called their strains the "Bethesda" group. Dr. Edwards kindly furnished us with unadsorbed Bethesda antiseraums and their homologous cultures. Agglutination and adsorption tests with the Bethesda cultures and antiseraums and the 9 basic cultures and antiseraums of the present work revealed that 3 of the 9 basic cultures, including strain 20158, were identical with one or another of the Bethesda cultures. All of the 6 remaining basic cultures were related antigenically to the Bethesda strains. Obviously a very high percentage of the 765 cultures studied in the present work belonged to the Bethesda group.

The hope that P. intermedium cultures would show marked antigenic continuity irrespective of the time and place of isolation was not realized completely. Only one of the 5 antiseraums (14011) prepared in 1939 was of real value in the present work. Although a number of strains were related antigenically to 14011, only one strain was identical with it. Among the first 100 Paracolobactrum cultures isolated in Florida were 21 P. intermedium (type 9466) strains (table 2). Sixteen of the 21 strains came from normal children in one institution and the remainder from different sources. This type was not encountered again in Florida nor was it found in any other part of the country. Therefore there is some temporal and topographical antigenic continuity in P. intermedium, but it seems much less marked than in the other Paracolobactrum groups or the coliform groups.

P. aerogenoides and P. coliforme cultures for the most part can be distinguished easily from Salmonella by positive Voges-Proskauer and indole reactions, respectively, in 24 hours or less. Most P. intermedium cultures cannot be differentiated from some Salmonella species by biochemical reactions for several days or more. The nuisance value of the P. intermedium group far exceeds that of the other Paracolobactrum groups. Holding lactose, sucrose, and salicin tubes until one or another is positive may cause prolonged delay in "reporting out" the culture, whereas running any considerable number of these cultures in Salmonella typing seraums results in a waste of time and expensive typing seraums.
Although *P. intermedium* organisms, including the Bethesda group, doubtless can cause acute gastroenteritis of varying duration, on the whole their pathogenicity does not seem very great. For every type isolated from gastroenteritis patients a greater number of the same type, where significant numbers were involved, were isolated from normal individuals in food handler and industrial surveys. The antigens of certain Bethesda types because of their wide distribution and frequent occurrence, if for no other reason, might well be included in some diagnostic typing scheme. How extensive and inclusive such a scheme should be must depend to some degree on further investigations.

**Acknowledgment**

We are indebted to Dr. K. M. Wheeler, deceased, who tested in single factor *Salmonella* typing serums the 34 *P. intermedium* strains used to produce antisera in the present work.

**Conclusions**

Of 765 strains of *Paracolobacterium intermedium*, 453 or 50 per cent were antigenically identical with one or another of 34 strains used to produce antisera. Two hundred twenty-seven strains, 52 per cent of the 453 identified or 29 per cent of the total of 765 strains, possessed the same or closely related somatic antigens. Antigenic continuity appears to be much greater in the *Paracolobacterium intermedium* group than in the *Paracolobacterium coliforme* and *Paracolobacterium aerogenoides* groups. Although strains of *P. intermedium* doubtless can cause acute gastroenteritis, the group as a whole does not present a serious public health problem.

**References**


Stuart, C. A., and Carpenter, P. L. 1948 Flagellar antigens of *Escherichia coli*. In manuscript.


