THE PLEO-ANTIGENICITY OF A VARIANT OF PROTEUS X19

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The purpose of this paper is to report the finding of an unusual spontaneously occurring variant in a stock strain of Proteus X19. Because of the unusual biological reactions and because the organism presented a potential source of trouble in using the Weil-Felix reaction for laboratory diagnosis, we believe a detailed report is desirable.

The Weil-Felix reaction has been in use since Weil and Felix (1916) demonstrated that a culture of a proteus-like organism isolated from the urine of a patient with typhus fever would agglutinate to a high titer not only in the patient’s serum but also in the sera of other patients suffering from typhus. Fletcher and Lessler (1926) later recorded a series of typhus cases in the Federated Malay States in which the patients’ sera failed to agglutinate the classical X19 strain, but did agglutinate a variant strain. The variant (Kingsbury strain) differed from the typical Proteus X strains in failing to ferment maltose or sucrose, in not producing indol, and in being serologically distinct as tested by direct agglutination. Besides these cases, however, there were others occurring with equal frequency which gave the Weil-Felix reaction with the X19 strain.

Weil and Felix (1920) demonstrated that the agglutinogen of typhus fever virus was identical with the specific main receptor (O receptor) of Proteus X19 and hence that the agglutinins in
typhus patients for Proteus X19 were due to a specific immunologic response. These results were confirmed and extended by Castaneda and Zia (1933) who concluded from their investigation there is a common antigenic factor in the Mexican Rickettsiae and Proteus X19 which explains the Weil-Felix reaction.

Studies by Weil (1920) demonstrated that variants of a Proteus X19 strain were serologically new organisms. In the same year Loewenhardt (1920) demonstrated that the sera of typhus fever patients frequently showed the presence of agglutinins for various kinds of bacteria particularly those of the typhoid-dysentery group. He believed that co-agglutination with typhoid was due to a previously formed antibody. This was not true in the case of dysentery. Recently an editorial in the Journal of the American Medical Association (1934) discussed the pleo-antigenicity of bacteria emphasizing the importance of this phenomenon to clinicians. The editor commented on the work of Führth (1923) who demonstrated that some variants of the paratyphoid group gained antigenic components not present in the original parent culture.

The original culture used in this investigation was obtained ten years ago from The American Type Culture Collection. It was carried on standard "pneumo-agar" until September, 1932, at which time "Savital-agar"—a yeast-extract, meat-free medium—was used. No change was noted in the original culture until one year later at which time it was noted that several Weil-Felix reactions were positive although there was nothing clinically which would indicate typhus fever. Control tests of supposedly normal sera were used and a high titer agglutination was found. A re-check of the supposedly pure stock strain revealed the presence of another organism and it was the attempt to explain this apparent dissociate which led to the following work.

In this investigation the authors have studied a variant of Proteus X19 with antigenic components that could not be demonstrated by similar direct agglutinations in the original parent culture. The variant agglutinated markedly with a variety of

PLEO-ANTIGENICITY OF VARIANT OF PROTEUS X19

sera and on animal inoculation had the ability to produce agglutininins for the homologous organisms of these sera.

To determine the relationship of the variant found to the Proteus X19 strain, studies were made of the morphological, physiological and serological characteristics of the parent strain and of its spontaneous variant. By growth in a Savita-serum broth using a pure culture of Proteus X19 as a parent strain and the homologous serum in varying dilutions, another variant was induced which had some characteristics in common with the variant originally found.

Throughout this article the pure strain of Proteus X19 found in the original culture will be referred to as the parent strain, the variant found in the original culture as Variant A and the laboratory-induced variant will be referred to as Variant B.

MORPHOLOGY

Microscopic. The parent strain and both its variants were similar microscopically. The organisms were fairly uniform, non-motile, Gram-negative rods. Occasional filamentous forms were found in all three cultures although the variants appeared to be uniformly longer than the parent strain. Some of the filamentous forms showed Gram-positive granules.

Macroscopic. In general, the parent strain and its variants grew more readily on agar which had been standing long enough to lose its water of condensation and to present a very dry surface.

The parent strain showed a glistening, white growth on agar slants. This growth was slightly spreading with erose edges. In broth a fairly even turbidity was produced in twenty-four hours although a flocculent precipitate appeared in the bottom of each tube. On eosin-methylene blue agar the colonies, measuring 2 to 5 mm. in diameter, were raised and had rough edges and surfaces. On this medium the colonies had black centers indicating dye precipitation, and white, undulate peripheries.

* Six months after completing the morphological studies, Variant B showed some motile forms. Both the Proteus X19 (parent strain) and Variant A strain were definitely non-motile in three, eight and twenty-four hour broth cultures.
On Krumwiede’s triple sugar agar an acid and gas butt and an acid slant were produced.

On agar slants, Variant A produced a dull, raised, filiform growth. An even turbidity without sediment was produced in broth. On eosin-methylene blue agar the colonies, measuring 1 to 3 mm. in diameter, were raised, smooth, glistening and entire after forty-eight hours. No precipitation of the dye was observed. On Krumwiede’s triple sugar agar an acid butt and an alkaline slant were produced.

Variant B grew very meagerly on agar at first but appeared to adapt itself to this medium with serial transplantation. On agar slants the growth was smooth, raised, glistening and filiform. On eosin-methylene blue agar the colonies, measuring 2 to 3 mm. in diameter, were smooth, raised, glistening and entire with discrete blue centers. In broth a slight turbidity, with a moderate amount of flocculent precipitate occurred after twenty-four hours. No change was produced in Krumwiede’s triple sugar agar.

**BIOCHEMICAL REACTIONS**

The parent strain gave the typical biochemical reactions of the Proteus type. The reactions of both variants, characterized chiefly by failure to produce gas in carbohydrate media, were sufficiently distinct to differentiate them with ease from the parent strain. A summary of the reactions of each strain follows:

**Parent strain.** Acid and gas were produced in glucose, sucrose, salicin, maltose, raffinose, cellibiose, xylose and glycerol. No change was noted in lactose, mannitol, rhamnose, arabinose, inositol, dulcitol, sorbitol, galactose, adonitol and dextrin. The methyl red and nitrate tests were positive. The indol test was negative. The reaction in litmus milk varied from neutral to slightly alkaline. In lead acetate media $\text{H}_2\text{S}$ was produced. Gelatin was liquefied.

**Variant A.** Acid was produced in glucose, lactose, maltose, mannitol, rhamnose, arabinose, raffinose, cellibiose, galactose, and glycerol. No change was noted in sucrose, salicin, inositol,
dulcitol, xylose, sorbitol, adonitol and dextrin. The methyl red and nitrate tests were positive. Indol was not produced. Litmus milk was rendered slightly acid. In lead acetate media H₂S was produced. Gelatin was not liquefied.

**Variant B.** This organism grew poorly and even after eighteen days in carbohydrate media acid without gas was produced in only one sugar, glucose. The methyl red, nitrate, and indol tests were negative. H₂S was not produced in a lead acetate medium. Litmus milk showed no change. Gelatin was liquefied in twenty-four to forty-eight hours.

**SEROLOGICAL STUDIES**

After several transplantations and reisolations, Variant A was tested against diagnostic sera representative of the Salmonella, Eberthella and Shigella groups of organisms, all of which had been prepared during the past year. At the same time the parent strain and Variant B were tested against similar sera. All antigens used were prepared to correspond to a turbidity of 7.6 to 7.8 with the Gates’ (1920) apparatus. The macroscopic tube test (1.0 ml. total volume) was used in all instances.

The parent strain agglutinated in an homologous serum but failed to agglutinate with representative sera of the Salmonella, Eberthella or Shigella groups of organisms. On the other hand Variant A not only showed a marked agglutination with the Proteus serum but also with the sera of *S. Schottmuelleri*, *S. paratyphi*, *S. enteritidis*, *S. Hirschfeldii*, *E. typhi*, *E. typhi O*, *Shigella dysenteriae*, *Shigella paradysenteriae* Sonne, Flexner and Army. This also was true to a lesser degree with Variant B. The marked antigenicity of these variants might suggest the possibility of spontaneous agglutination but in some of the sera used no agglutination occurred. Control tests showed no tendency toward spontaneous agglutination. In addition, work on agglutinin absorption reported below definitely showed that the agglutination was not of the spontaneous variety.

Since both Variant A and Variant B showed marked agglutination with several heterologous sera, the question arises as to whether these agglutinogens were acquired in the course of dis-
association, or existed in the parent strain in such a state that they did not identify themselves by direct agglutination in heterologous sera. The parent strain showed no agglutination with similar sera. Without further study it would appear that the dissociates had acquired new antigenic components similar to those reported by Fürth (1923) for the paratyphoid group. Since the parent strain showed no agglutination in heterologous sera and since variants of this strain had this property, it appeared advisable to determine whether on injection into animals this parent strain could produce agglutinins corresponding to the agglutinogens shown to be present in the variants. Accordingly three rabbits were injected respectively with the original Proteus X19 culture (this culture contained both the parent and Variant A strains), the parent strain in pure culture and the Variant A strain in pure culture. These rabbits were injected every day with increasing doses of live organisms.

After ten injections the Proteus X19 culture (parent strain plus Variant A) as well as the parent strain in pure culture produced titers of about 1:2000. Ten injections of the Variant A in pure culture, however, failed to develop agglutinins for its homologous organism but agglutinins were present, at this time, for the parent strain. It was only after 18 injections that a low-titered Variant A serum was produced for its homologous organism. Even after 18 injections, however, the titer of the Variant A serum with the parent strain was greater than the titer of this serum for its homologous organism.

Because the original culture had present in it both the parent and Variant A strains, it seemed advisable to test only a short series of organisms in this serum, since no definite information could be obtained as to the source of any agglutinins found. Accordingly this serum was tested against its homologous culture, the parent strain (in pure culture), Variant A, *S. Schottmuelleri*, *E. typhi O* and *Shigella paradysenteriae* Sonne. Definite agglutination was obtained with the heterologous organisms in dilutions of 1:320 to 1:640.

The sera prepared for the parent strain (in pure culture) and for Variant A were tested against several heterologous organisms.
These organisms corresponded to the heterologous sera mentioned above. A summary of the reciprocal serological relationships of the parent and Variant A strains to heterologous organisms is given in table 1.

**TABLE 1**
Summary of results obtained with Proteus Variant A organism vs. heterologous sera and with Proteus X19 serum vs. corresponding heterologous organisms

<table>
<thead>
<tr>
<th>HETEROLOGOUS SERA</th>
<th>AGGLUTINATION RESULTS</th>
<th>HETEROLOGOUS ORGANISMS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proteus Variant A</td>
<td>Proteus X19 (parent strain) serum</td>
</tr>
<tr>
<td>S. Schottmuelleri (Ufford)</td>
<td>+        0 0 0</td>
<td>S. Schottmuelleri (Ufford)</td>
</tr>
<tr>
<td>S. paratyphi (Michigan)</td>
<td>+        + + +</td>
<td>S. paratyphi (Michigan)</td>
</tr>
<tr>
<td>S. Hirschfeldii</td>
<td>+        + + +</td>
<td>S. Hirschfeldii</td>
</tr>
<tr>
<td>S. enteritidis</td>
<td>+        + + +</td>
<td>S. enteritidis</td>
</tr>
<tr>
<td>E. typhi (Michigan)</td>
<td>+        + + +</td>
<td>E. typhi (Michigan)</td>
</tr>
<tr>
<td>E. typhi &quot;O&quot; (New York)</td>
<td>+        + + +</td>
<td>E. typhi &quot;O&quot; (New York)</td>
</tr>
<tr>
<td>Shigella dysenteriae Shiga</td>
<td>+        + + +</td>
<td>Shigella dysenteriae Shiga</td>
</tr>
<tr>
<td>Shigella paradysenteriae Sonne</td>
<td>+        + + +</td>
<td>Shigella paradysenteriae Sonne</td>
</tr>
<tr>
<td>Shigella paradysenteriae Flexner</td>
<td>+        + + +</td>
<td>Shigella paradysenteriae Flexner</td>
</tr>
<tr>
<td>Shigella paradysenteriae Army</td>
<td>+        + + +</td>
<td>Shigella paradysenteriae Army</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>0        0 0 0</td>
<td>Pseudomonas</td>
</tr>
<tr>
<td>Br. abortus (Mulford)</td>
<td>0        0 0 0</td>
<td>Br. abortus (Mulford)</td>
</tr>
</tbody>
</table>

Note: The titers of heterologous sera with Variant A varied between dilutions of 1:80 and 1:640; the titers of heterologous organisms with Variant A and Proteus X19 (parent strain) sera varied between dilutions of 1:80 and 1:320.

It will be noted (table 1) that both the parent and Variant A strains, on injection into animals, are capable of producing agglutinins for the members of the Salmonella, Eberthella and Shigella groups of organisms that were used with the single exception of *S. Schottmuelleri*. As previously noted, *S. Schottmuelleri* was agglutinated in the serum produced by injection of the parent strain plus Variant A. This was not the case with antisera pro-
duced by injection of pure cultures of either of these strains separately. However, since *S. Schottmuelleri* serum does agglutinate Variant A and Variant B but not the parent strain and since the Variant A serum has definitely a low titer, it would appear to us that this does not indicate the loss of an antigenic component of the Variant A strain but is probably due to the concentration of antibodies in the serum produced by this strain. Further studies on agglutinin absorption showed definitely antigenic components for *S. Schottmuelleri* in both the parent and Variant A strains.

Briefly summarizing the results obtained, we find that the parent strain alone does not agglutinate in heterologous sera. The variants of this parent strain both show a remarkable ability to agglutinate in a variety of heterologous sera. On injection of the parent strain or its variant into rabbits, agglutinins are produced which in general correspond to the agglutinogens shown for the variants. Apparently agglutinogens for a variety of sera are present in the parent strain but do not manifest themselves by direct agglutination of this strain in heterologous sera. These agglutinogens may be demonstrated either in the variants directly or in the parent strain by the production of antiserum. The ability of an organism to produce an agglutinin for another strain when it cannot be shown to agglutinate in the sera of such a strain has been demonstrated previously in the dysentery group of organisms by Welch and Mickle (1932).

Of the twelve heterologous sera used to agglutinate Variant A, all but the Pseudomonas and *Brucella abortus* sera showed ability to agglutinate this organism. Correspondingly, when Pseudomonas and *Brucella abortus* strains were tested against sera of the parent or the Variant A strains, negative results were obtained. Although *S. Schottmuelleri* serum was shown to agglutinate Variant A, neither Variant A nor the parent strain sera agglutinated *S. Schottmuelleri* (table 1). In view of these results we cannot consider that Variant A did acquire new antigenic components as was first thought to be the case early in this study except possibly in the case of *S. Schottmuelleri*. With this latter organism further study is necessary for definite proof.
AGGLUTININ ABSORPTION STUDIES

In order to establish more definitely the relationship of the parent Proteus X19 strain to Variant A and, further to explain the results obtained with the *S. Schottmuelleri* strain, a series of agglutinin absorption tests were made. Representative sera and organisms of the Salmonella, Shigella and Eberthella groups were used in this series.

Agglutinin absorption method. The agglutinating serum was diluted to a degree depending on its original titre. The dilutions varied from 1:10 to 1:50. Six milliliters of the diluted serum were added to a twenty-four-hour agar slant of the organism under study. With a platinum loop, the suspension was carefully emulsified in the serum. A small pledget of sterile absorbent cotton was dropped into the test tube and the suspension drawn up into a pipette through the cotton which acted as a filter for gross particles. The suspension was pipetted directly to a sterile centrifuge tube, incubated in a water bath at 37°C. for two hours, and then centrifuged at high speed for one hour or until the supernatant fluid was clear. (The cotton plugs were fixed firmly in the centrifuge tubes with elastic bands.) The supernatant fluid was then pipetted under sterile conditions onto a second twenty-four-hour slant culture and the procedure repeated. The procedure was then repeated on a third slant. (This third suspension may be placed in the icebox over night with equally good results.) Absorption with homologous organisms using this technique is invariably complete.

Results. The results obtained by agglutinin absorption are given in table 2. The parent strain serum was absorbed with Variant A, *E. typhi* "O" (New York) and *S. Schottmuelleri*, respectively. The Variant A serum was absorbed with the parent strain, *E. typhi* "O" (New York) and *S. Schottmuelleri*. The *S. Schottmuelleri* serum was absorbed with Variant A and with the parent strain. The sera of *S. enteritidis*, *E. typhi* (Michigan) and *Shigella paradysenteriae*, both Sonne and Army varieties, were absorbed with the parent strain. Duplicate unabsorbed control sera were titrated at the same time these absorptions were made.

It will be noted in table 2 that both unabsorbed parent strain and Variant A sera agglutinated all organisms used except *S.
### TABLE 2
Agglutinin absorption tests

<table>
<thead>
<tr>
<th>UNABSORBED PROTEUS X19 (PARENT STRAIN) SERUM</th>
<th>ORGANISMS</th>
<th>ABSORBED PROTEUS X19 (PARENT STRAIN) SERUM WITH:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Variant A</td>
</tr>
<tr>
<td>1280</td>
<td>Protein X19 (parent strain)</td>
<td>1280 1280 1280</td>
</tr>
<tr>
<td>640-1280</td>
<td>Variant A, <em>S. enteritidis, E. typhi, Shigella paradysenteriae Sonne</em></td>
<td>0 0 0</td>
</tr>
<tr>
<td>160-320</td>
<td><em>S. paratyphi, S. Hirschfeldii, E. typhi “O,” Shigella dysenteriae, Shigella paradysenteriae, Sonne, Army, Flexner</em></td>
<td>0 0 0</td>
</tr>
<tr>
<td>0</td>
<td><em>S. Schottmuelleri</em></td>
<td>0 0 0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>UNABSORBED VARIANT A SERUM</th>
<th>ORGANISMS</th>
<th>ABSORBED VARIANT A SERUM WITH:</th>
</tr>
</thead>
<tbody>
<tr>
<td>640</td>
<td>Protein X19 (parent strain)</td>
<td>0 640 1280</td>
</tr>
<tr>
<td>320</td>
<td><em>S. paratyphi, S. enteritidis, S. Hirschfeldii, E. typhi, E. typhi “O,” Shigella dysenteriae, Shigella paradysenteriae, Sonne, Army, Flexner</em></td>
<td>0 0 0</td>
</tr>
<tr>
<td>0</td>
<td><em>S. Schottmuelleri</em></td>
<td>0 0 0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>UNABSORBED SERA</th>
<th>TITRES</th>
<th>ABSORBED WITH:</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Homologous organism</td>
<td>Protein X19 (parent strain)</td>
</tr>
<tr>
<td><em>S. Schottmuelleri</em></td>
<td>2560 0 320</td>
<td>Protein X19 (parent strain)</td>
</tr>
<tr>
<td><em>S. enteritidis</em></td>
<td>1280 0 640</td>
<td>Protein X19 (parent strain)</td>
</tr>
<tr>
<td><em>E. typhi</em></td>
<td>1280 0 640</td>
<td>Protein X19 (parent strain)</td>
</tr>
<tr>
<td><em>Shigella paradysenteriae Sonne</em></td>
<td>1280 0 320</td>
<td>Protein X19 (parent strain)</td>
</tr>
<tr>
<td><em>Shigella paradysenteriae Army</em></td>
<td>2560 0 320</td>
<td>Protein X19 (parent strain)</td>
</tr>
</tbody>
</table>

*Note:* Table shows final significant titers of the unabsorbed and absorbed sera used.

532
Schottmuelleri even though the serum produced by the injection of these organisms (parent strain and Variant A) together contains agglutinins for this strain (S. Schottmuelleri). Since, also, the S. Schottmuelleri serum itself agglutinates Variant A but not the parent strain it would appear, as noted previously, that this lack of ability of the parent strain and Variant A sera to agglutinate the S. Schottmuelleri strain is due to a weakness of these sera rather than a lack of antigenicity inherent in the parent or Variant A strains. This is borne out when S. Schottmuelleri serum is absorbed with either the parent or Variant A strains. These absorptions show definitely that both the parent and Variant A strains have an antigenic component in common with S. Schottmuelleri since the absorption of S. Schottmuelleri serum with the parent strain removes agglutinins for Variant A. In this connection it has already been shown that Variant A agglutinates in S. Schottmuelleri serum (table 1).

The absorption of the parent strain serum with Variant A organism removed all but the homologous agglutinins of this serum. The absorption of the parent strain serum with E. typhi “O” or with S. Schottmuelleri gave identical results; i.e., the agglutinins present in this serum for Variant A, S. paratyphi (Michigan), S. Hirschfeldii, S. enteritidis, E. typhi (Michigan), E. typhi “O” (New York), Shigella dysenteriae and for Shigella paradysenteriae Sonne, Army and Flexner varieties were completely removed.

The absorption of Variant A serum with the parent strain removed all agglutinins including the homologous agglutinins from this serum. The absorption of Variant A serum with E. typhi “O” or S. Schottmuelleri removed all agglutinins except those present in this serum for the parent strain. These results correspond to those obtained when the parent strain serum was similarly absorbed. It would appear then that the heterologous agglutinins found in the Proteus X19 (parent strain) and Variant A sera are group agglutinins.

Since a variety of heterologous sera were shown to agglutinate the Variant A strain but not the parent strain, it seemed advisable to absorb certain of these sera with the parent strain in
order to determine whether this organism, although unable to agglutinate in these sera, could remove from them the agglutinins present for its variant. The results of these absorptions with controls are given in table 2. All the sera were absorbed with the parent strain. The serum of S. Schottmuelleri was also absorbed with Variant A. (This latter absorption, run as a control, removed completely the Variant A agglutinins.) In each case where a serum, known to agglutinate Variant A, was absorbed with the parent strain, the agglutinins present in such sera for Variant A were removed in spite of the fact that the absorbing organism did not have the ability per se to agglutinate in these sera.

HETEROPHILE ANTIGEN STUDIES

In view of the fact that a number of heterologous sera were shown to be capable of agglutinating variants of a Proteus X19 strain in contrast to a corresponding lack of ability to produce a similar reaction with the parent strain, it seemed possible that these results might be explained on the basis of the presence of a heterophile antigen in the variant that was absent in the parent Proteus X19 strain. In favor of this possibility is the investigation of Abe (1924) who demonstrated that Proteus vulgaris X19 lacked the heterophile or Forssman antigen.

Method. Sera were tested for heterophile antibody (hemolysin) by diluting with sterile isotonic salt solution 1:4 through 1:1024. Guinea pig complement used in the regular Wassermann test and titrated daily was added in the proper dilution in 0.4 ml. amounts to 0.5 ml. of the serum dilutions. (The complement dilution varied from 1:10 to 1:12.) Washed (5 per cent) sheep cells (0.1 ml.) were then added giving a final concentration of 0.5 per cent sheep cells and making a total volume of 1.0 ml. The tubes were shaken and incubated in the 37°C. water bath for one hour and read. Appropriate controls were incubated at the same time.

The organisms under study were tested for heterophile antigen by direct agglutination in a known heterophile antiserum. The heterophile serum was prepared by the injection of guinea pig kidney emul-

3 We are indebted to Dr. C. A. Stuart, Brown University, Providence, R. I., who furnished this serum.
tion into rabbits and had a titer of approximately 1:40,000. The bacterial antigens had a turbidity corresponding to 7.6 to 7.8 on the Gates (1920) apparatus. Dilutions were made from 1:100 to 1:12,800 in a regular sequence. The total volume in each tube was 1.0 ml.

Results. On testing the Proteus X19 (parent strain), Proteus Variant A, E. typhi, E. typhi "O," S. Schottmuelleri, S. enteritidis, Shigella paradysenteriae Sonne and Army sera for presence of heterophile antibody (hemolysin) it was found that all sera (including parent-strain serum) tested except Variant A serum showed the presence of a heterophile antibody, the titers varying from 1:32 to 1:512 (0.5 per cent sheep cell suspensions final concentrations were used). The presence of heterophile antibody in the parent strain serum obviates the possibility of explaining the contrasting results obtained in agglutinating the parent strain and Variant A in heterologous sera. However, the apparent absence of heterophile antibody in Variant A serum does not necessarily demonstrate the lack of heterophile antigen in the organism used to produce this serum. Because of the difficulty encountered in producing the Variant A serum it seemed advisable to test the homologous organisms of the sera mentioned above for heterophile antigen. (Later work, using more dilute sheep cell suspensions, demonstrated the presence of small amounts of heterophile hemolysin but not heterophile agglutinin in Variant A serum.)

On testing Proteus X19 (parent strain), Proteus Variant A, E. typhi, E. typhi "O," S. Schottmuelleri, S. enteritidis, Shigella paradysenteriae varieties Sonne and Army for the presence of heterophile antigen using agglutination in a known heterophile serum as an index (homologous titer 1:40,000) titers of 1:400 to 1:800 were obtained in all instances with the single exception of Proteus X 19 (parent strain). No heterophile antigen was demonstrated in the parent strain even though the serum of this organism contained heterophile hemolysin. The reaction of the parent strain in heterophile serum is similar to its reaction in heterologous sera, i.e., it fails to agglutinate in such sera but produces agglutinins on animal injection.

If we correlate the above results with those obtained pre-
viously (where it was shown that Variant A would agglutinate in a series of heterologous sera), it would appear that these agglutinations of Variant A might be due to the presence of heterophile antibody in heterologous sera. The presence of heterophile antibody in the serum of the parent strain might also explain the agglutination of heterologous organisms with this serum.

In order to determine whether the agglutination of Variant A by heterologous sera and the agglutination of heterologous organisms with the parent strain serum were due to heterophile relationships, or to definite bacterial antigenic components of the parent and Variant A strains, the heterologous sera used throughout this study were absorbed with sheep cells to remove the heterophile antibody and titrated, after absorption, against Variant A. Assuming that the agglutination of Variant A by heterologous sera was due to the presence of heterophile antibody, the absorption of these sera with sheep cells would remove the Variant A antibodies.

In view of the fact that the agglutination of the Variant A strain might possibly be due not to heterophile agglutinin but to isophile agglutinin, it appeared advisable also to absorb the heterologous sera with boiled sheep cells, since it is known that boiling sheep cells for thirty minutes destroys the isophile antigen.

Method. The method used for the absorption of sera with sheep cells was similar to the method used for the absorption of sera with bacteria reported elsewhere in this paper. Each serum was absorbed three times with washed sheep cells using 0.5 ml. of packed cells in each instance. (The packed sheep cells were prepared from a 5 per cent suspension in isotonic salt solution.) The sera were decanted onto the packed sheep cells in sterile centrifuged tubes and the cells resuspended. The centrifuge tubes were then placed in a 37°C. water bath for two hours. The sheep cells were then packed again in the centrifuge (fifteen minutes at 1500 r.p.m.) and the sera decanted into a fresh centrifuge tube containing 0.5 ml. of packed sheep cells. This process was repeated twice similarly to the absorption with bacteria. The absorption of heterophile antibody using the above method was complete in each instance. The same method was used in the absorption of sera with sheep cells from which the isophile antigen had been removed by boiling.
All absorbed sera were tested for both heterophile agglutinin and heterophile hemolysin since we found the latter test more sensitive. Unabsorbed control sera and sera absorbed with boiled sheep cells were titrated at the same time. It was found that with each serum after absorption with sheep cells, the heterophile antibody was completely removed but that these absorbed sera continued to agglutinate Variant A undiminished in some degree. Whether the sera studied were absorbed with fresh or with boiled sheep cells the same results were obtained indicating that we were dealing with heterophile and not isophile antibody in these sera. It would appear from these results that the agglutination of Variant A by heterologous sera and the agglutination of heterologous organisms in the parent strain serum is not due to heterophile relationships but to the pleo-antigenicity of these Proteus strains.

DISCUSSION

The importance to diagnostic laboratories in the public health field of the finding of pleo-antigenic variants of Proteus X19 in stock cultures, when such cultures are used daily in the diagnosis of typhus fever, is obvious. The particular culture studied in this investigation, containing a variant of a Proteus X19 strain, was discovered when it was found that the original culture used in the Weil-Felix reaction was giving positive results on patients with no symptoms of typhus fever. On further study, the culture used for diagnosis was found to be made up of two strains—a true Proteus X19 and a variant strain. It was further shown that the variant strain was responsible for the false positive results obtained and that the Proteus X19 strain, when used alone, agglutinated only in its homologous serum and did not give false positive results in the Weil-Felix reaction. The Proteus X19 strain apparently dissociated spontaneously after being carried over a long period of time on artificial media. Since it

\[4\] Instead of using 0.5 per cent sheep cells for the hemolysin test a 0.02 per cent suspension was used. Small amounts of heterophile hemolysin were demonstrated in this case for unabsorbed Variant A serum in contrast to the results obtained previously when using the 0.5 per cent suspensions.
was found possible to induce in the laboratory another variant of the parent Proteus X19 strain in some respects similar to the variant that appeared spontaneously in this stock culture, it is reasonable to expect that other diagnostic laboratories may have to deal with a similar situation.

The marked pleo-antigenicity of the variants of the Proteus X19 strain studied appeared at first to be an example of the acquisition of new antigenic components since the variants would agglutinate with a variety of heterologous sera (dysentery, typhoid, paratyphoid and enteritidis) whereas the parent strain agglutinated only in its homologous serum. However, further study of the sera of the parent and Variant A strains indicated that this was not the case. The parent strain produced agglutinins for several heterologous organisms. It would seem that this is not a demonstration of acquired antigenic components but that the parent strain does have in its make-up antigenic components for the heterologous sera used. It would appear to us of fundamental significance that the dissociation of an organism brings about a "freeing" or rearrangement of agglutinogens of the induced variant so that they can be demonstrated in an agglutination test where previously such results were not demonstrable. For example, when the Proteus X19 organism is dissociated, the antigenic structure of the induced variant strain is such that direct agglutination in heterologous sera becomes possible. A similar freeing of agglutinogens is obtained on animal injection of the parent strain resulting probably from hydrolytic cleavage of the bacterial antigen, in the sense of Manwaring (1928), since heterologous agglutinins are found in the serum produced.

A definite antigenic relationship between the parent Proteus X19 strain and its variant was demonstrated by agglutinin absorption. On absorbing heterologous sera, which were capable of agglutinating the variant strain but not the parent strain, with Proteus X19, the agglutinins for the variant strain were removed from these sera.

Heterophile antigen was demonstrated in both Proteus strains—in the parent strain by demonstration of heterophile hemolysin
in the serum produced by this organism—in Variant A by a similar observation, as well as by demonstration of a heterophile agglutinogen in the antigenic structure of this organism by agglutination in a heterophile serum. The marked pleo-antigenicity of these Proteus strains was demonstrated to be due to bacterial group agglutinogens and not solely, if at all, to the presence in these strains of heterophile antigen.

Further studies of Proteus X19 and Proteus Variant strains obtained from various sections of this country and Canada are now in progress.

SUMMARY AND CONCLUSIONS

1. A pleo-antigenic variant of a Proteus X19 strain was shown to be the cause of false positive reactions in the Weil-Felix test.
2. The Proteus X19 strain, although inagglutinable in certain heterologous sera, was shown, by agglutinin absorption, to have agglutinogens in common with the organisms used to produce these sera.
3. The heterologous agglutinogens, shown in the variant strain by direct agglutination, were not acquired in the process of dissociation but were present in the parent strain although not demonstrable by the same method.
4. The heterologous relationships of the Proteus X19 strain and its variant were shown to be due to bacterial group agglutinogens and not soley, if at all, to the presence of heterophile antigen in these strains.

REFERENCES


