A CULTURE OF SALMONELLA INFANTIS OF COMPLEX ANTIGENIC CONSTITUTION

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ABSTRACT

Edwards, P. R. (Communicable Disease Center, U.S. Public Health Service, Atlanta, Ga.), A. C. McWhorter, and G. W. Douglas. A culture of Salmonella infantis of complex antigenic constitution. J. Bacteriol. 84:95-98. 1962—An antigenically complex Salmonella serotype (\(\beta,7:z_0,\gamma:z_0,1,\beta\)), in which the antigen \(z_0\) is a major component of both phases, is described. Through loss variation, this form gives rise to cultures identical with S. infantis (\(\beta,7:z_0,1,\beta\)). Attention is drawn to the similarity of its behavior to that of S. montgomery and S. salinatis, and the possible origin of such complex forms is discussed briefly. The organism is not assigned a name, but is considered simply as a complex form of S. infantis.

Salmonella cultures, which possessed complex flagellar (H) antigens and which gave rise to simpler forms through loss variation, occasionally have been encountered. Edwards and Bruner (1942) described S. salinatis (4,12:de,h : de,n,\(z_{13}\)), which was changed irreversibly to Salmonella sandiego (4,12:e,h : e,n,\(z_{13}\)) during migration through semisolid medium containing Salmonella typhosa H (d) antiserum. In the original culture, the d factor was the major component of both phases, and it was only through examination of the minor antigenic components that the diphasic nature of the culture was established. Similarly, Edwards (1950) described a salmonellalike culture which fermented sucrose and possessed the antigenic formula 4,12 : d,e,h : -. Like S. salinatis, this culture gave rise to 4,12 : e,h : - forms on passage through d serum, and spontaneous loss variants of the latter form were found rarely by simple colony selection. Edwards, Kaufmann, and Huy (1957) described S. montgomery (11 : d,a : d,e,n,\(z_{13}\)), which gave rise to a form indistinguishable from S. luciana (11 : a : e,n,\(z_{13}\)) on passage through d serum. Again, antigen d was the major component of both phases. The purpose of the present paper is to describe cultures of S. infantis, which displayed complex H phases and behaved in a manner similar to the cultures mentioned above.

MATERIALS AND METHODS

The strains studied consisted of two cultures (2783-61 and 2784-61), isolated in the Texas State Department of Health, from two different fecal specimens from a child affected with gastroenteritis displaying typical symptoms of acute salmonellosis. The materials used for comparison were the standard cultures distributed by the International Salmonella Center, Copenhagen, Denmark, and antisera derived from them. The methods used were those described by Edwards and Ewing (1955).

RESULTS

Since the results obtained in the study of the two cultures were identical, only one of them (2783-61) is described. Upon examination, the culture was found to be a member of Salmonella O group C \(_1\) which, in absorption tests, removed all agglutinins from O antiserum derived from S. thompson (\(\beta,7\)). In the examination of the H antigens, agglutination in diagnostic dilutions was observed only in S. thompson, phase 2 (1,6) serum and in sera for the related 1,2; 1,6; and 1,7 phases. When tested with single factor sera for H factors 2, 5, 6, and 7, agglutination was observed only in factor 5 serum, indicating that the organism contained H antigens 1,5 in phase 2. The organism was then passed repeatedly through semisolid medium containing 1,5 serum in an effort to obtain phase 1. The resultant growth was not agglutinated in diagnostic dilutions of antisera for the known H antigens of the Salmonella group, although the cultures were very actively motile. Antisera prepared from these forms agglutinated S. thompson phase 2 and related forms due to the known 1,5 components.
of the cultures. The sera, when absorbed with *S. thompson* phase 2, agglutinated the homologous cultures in undiminished dilutions, but failed to agglutinate any of the known antigens of *Salmonella*. It was apparent that a hitherto unrecognized H antigen was involved and to this antigen the symbol *z* was assigned. (We are indebted to F. Kauffmann for assigning the symbol to this antigen as well as for examination of the culture.)

The phase of culture 2783-61, obtained by passage through 1,5 serum, was placed in semi-solid medium which contained homologous serum, and the organisms promptly migrated through the medium. Surprisingly, antigens prepared from the spreading growth were agglutinated not by 1,5 serum, but by serum derived from phase 1 of *S. rubislaw* (r). Since the results indicated unusual complexity of H antigens, the original culture received for diagnosis was plated and colonies tested on slides with suitable dilutions of r; 1,5; and absorbed *z* sera. A total of 250 colonies was tested, and all were agglutinated actively by *z* serum, which had been absorbed thoroughly with phase 2 of *S. thompson*. In addition, a large number of the colonies were agglutinated by *S. thompson* phase 2 (1,5) serum. Equal numbers of colonies, which were agglutinated only by *z* serum and of those which were agglutinated both by *z* and 1,5 sera, were selected and H antigens prepared from them. These antigens were used in expanded tube-agglutination tests. The results obtained with a colony of each sort are given in Table 1.

It was quite apparent that two distinct H phases were present in the culture. One, designated as phase 1, was agglutinated in low dilution by *S. rubislaw*, phase 1 serum (r) and in high dilution by *z* serum. The other, designated phase 2, was agglutinated in high dilution both by *z* and 1,5 sera. Ten colonies of each phase were passed through semisolid medium which contained *z* serum. The colonies of phase 1 gave rise to antigens which were agglutinated to the titer of *S. rubislaw* serum, but were unaffected by *z* and 1,5 sera. With one exception, the phase 2 colonies, after passage through *z* serum, yielded typical 1,5 phases, which were not agglutinated by *r* or *z* sera. One phase 2 colony reverted to a typical *r* phase on passage through *z* serum. The reactions of the phase 1 and phase 2 colonies, after passage through *z* serum, are also included in Table 1.

Study of the *r* and 1,5 phases, thus obtained, revealed that the *r* phase absorbed all H agglutinins from *S. rubislaw* phase 1 serum, and the 1,5 phase removed all agglutinins for *S. infantis* phase 2 (1,5) from *S. thompson* phase 2 serum, but left a slight residue of agglutinins for the homologous strain. Further, the *r* and 1,5 phases were easily reversible when cultivated in appropriate sera and, in fact, reverted spontaneously, one to the other. On the contrary, when ten colonies of each of the *r* and 1,5 phases, obtained by passage of the original culture through *z* serum, were cultivated in semisolid medium containing both *r* and 1,5 sera, the organisms were immobilized. Serial passage through ten tubes of *r + 1,5* sera over a 3-month period failed to indicate antigenic change. Thus, it became apparent that, from a complex form represented by the antigenic formula 6,7 : *z* : 1,5, a culture indistinguishable from *S. infantis* (6,7 : *r* : 1,5) had been derived. It was not possible again to obtain the *z* antigen from the 6,7 : *r* : 1,5 forms.

**DISCUSSION**

The behavior of the cultures described here is completely comparable to that of *S. montgomery* and *S. salinaris*. It is remarkable that, in all of these, the components of phase 1 emerging as major antigens after passage through serum are present only in small amounts in phase 1 of the original cultures. On the contrary, the comparable components of phase 2 are much more easily demonstrable. This fact is illustrated by the behavior of the *z* serum in *r* and *z* sera, as

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**Table 1. Agglutination reactions of H antigens of *Salmonella infantis* 2783-61**

<table>
<thead>
<tr>
<th>Antigens</th>
<th><em>S. rubislaw</em>, 2783-61, phase 1 (r)</th>
<th><em>S. infantis</em>, 2783-61, phase 1</th>
<th><em>S. thompson</em>, 2783-61, phase 1 (1,5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2783-61, phase 1</td>
<td>200</td>
<td>6,400</td>
<td>&lt;100</td>
</tr>
<tr>
<td>2783-61, phase 2</td>
<td>&lt;100</td>
<td>3,200</td>
<td>12,800</td>
</tr>
<tr>
<td>2783-61, <em>r</em> phase</td>
<td>3,200</td>
<td>&lt;100</td>
<td>&lt;100</td>
</tr>
<tr>
<td>2783-61, 1,5 phase</td>
<td>&lt;100</td>
<td>&lt;100</td>
<td>12,800</td>
</tr>
</tbody>
</table>

* The *r* and 1,5 phases of 2783-61 were obtained by passage of phase 1 and phase 2, respectively, through *z* serum. The *z* serum was absorbed with *S. thompson*, phase 2.
contrasted with the reactions of the \( z_{20}f,5 \) phase in \( z_{20} \) and \( 1,5 \) sera. Occasional colonies of phase 1 were not agglutinated by \( r \) serum in a dilution of 1 to 100, yet were fully agglutinable in \( z_{20} \) serum. Such colonies invariably yielded \( r \) phases when passed through \( z_{20} \) serum.

When phase 1 colonies (\( z_{20}r \)) were passed through \( r \) serum they remained unchanged. Apparently, there was not sufficient effective \( r \) antigen present to arrest the migration of the organisms. However, when \( z_{20}f,5 \) phases were passed through \( 1,5 \) serum, \( z_{20}f \) phases were isolated from the spreading growth. This behavior also was entirely comparable to that of \( S. montgomery \) and \( S. salinatis \).

The question arises as to whether a form like that described here should be designated as a new serotype and a name applied to it, as has been the custom with other serotypes. No doubt it deserves designation to the same extent as do \( S. montgomery \) and \( S. salinatis \). However, experience gained since those forms were described, and unpublished data on a number of salmonellae with complex phases, indicate that it is advisable simply to designate the organism as a complex form of \( S. infantis \) and to be prepared to recognize the \( z_{20}f \) antigen when it again appears in \( S. infantis \) or in other \( Salmonella \) serotypes.

The question also arises as to the origin of such antigenically complex forms. When \( S. salinatis \) and \( S. montgomery \) were found, they were thought to be primitive forms in the sense of White (1926), who thought that specialized, host-adapted salmonellae were derived from more complex, nonadapted forms through loss variation. \( S. typhi \), \( S. pullorum \), \( S. gallinarum \), \( S. abortus-equ 

Although no normal colonies of \( S. infantis \) were found among the 250 colonies examined, it seems most probable that such forms are split off spontaneously in relatively small numbers, and that cultivation in a medium containing \( z_{20}f \) serum simply serves as a very sensitive method of selection. This seems much more likely than that the serum actually exerts any specific effect to induce variation. It should be remembered that spontaneous loss variation from \( d,e,h \) to \( e,h \) phases has been observed.

The possibility that forms similar to the form described here may arise from recombination or transduction must be considered. It now is well known, through the work of Baron, Carey, and Spilman (1959), Baron, Spilman, and Carey (1959), Zinder (1960a, b) and Ørskov, Ørskov, and Kaufmann (1961), that salmonellae are capable of recombination with other groups of \( Enterobacteriaceae \), and it is possible that antigenically complex forms result from recombination. The methods generally used in transduction of antigens (Zinder and Lederberg, 1952) were designed to demonstrate substitutional rather than additive changes in \( H \) antigens, although the results of Spicer and Datta (1959) on reversion in transductants indicate that the capacity of salmonellae to produce multiple \( H \) antigens may result from transduction. Further, it is known from the work of Baron, Formal, and Washington (1957) and Harada (1956a, b) that additive changes in \( O \) antigens may result from the action of phages. Until antigenic recombinations are studied more thoroughly, the origin of antigenically complex forms must remain in doubt.

**LITERATURE CITED**


