STUDIES ON GROUP G OF THE GENUS SALMONELLA

P. R. EDWARDS, ALMA C. McWHORTER, AND MARY A. FIFE

Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Atlanta, Georgia

Received for publication September 21, 1953

I. O antigens of group G. The first member of Salmonella group G to be described was Salmonella poona (Bridges and Scott, 1935). The O antigens of this type were not related to those of other Salmonella types then known, and the O antigen of S. poona was designated by the single symbol 13. Later, Salmonella worthington was studied (Edwards and Bruner, 1938) and found to possess O antigens related to, but not identical with, those of S. poona. Therefore, S. poona was assigned O antigens 13,22, and S. worthington was given symbols 1,13,23 since each possessed a specific O antigen and, in addition, S. worthington contained antigen 1 which occurs in several O groups. Edwards et al. (1943) described Salmonella mississippi and assigned to it O antigens 1,13,23 but noted that the O antigens of this type were not identical with those of S. worthington. Likewise, Hormaeche et al. (1944) designated the O antigens of Salmonella grumpensis as 13,23 but stated that certain peculiarities of the O antigens were not expressed by these symbols.

In view of this confused situation, Kauffmann (1944) studied the O antigens of group G more closely, characterized two antigens, 36 and 37, which caused the unusual reactions of S. mississippi and S. grumpensis, and assigned formulas to the O antigens as follows: S. poona—13,22,36; S. worthington—1,13,23,37; S. grumpensis—13,23,36; S. mississippi—1,13,23,36,37.

The antigenic formulas delineated above satisfactorily explained the behavior of the O antigens of the types mentioned and proved to be adequate for the characterization of the O antigens of more recently recognized serotypes of the group. However, experience in serologic typing indicated that the expected antigenic combinations did not always occur in given serotypes, particularly in Salmonella atlanta (13,23,36: b), a monophasic type described by Saphra and Seligmann (1948). For this reason it was decided to study closely a number of cultures of the group. In this work, single factor serums of satisfactory titer for each of the O antigens were used in slide agglutination tests. In those instances in which the results departed from the anticipated pattern, conventional agglutination tests and agglutinin absorptions were done. In one instance, an agglutinating serum was prepared in order that reciprocal absorption tests might be done. Only smooth cultures were used so that changes in O antigens due to S-R variation were avoided.

A total of 103 cultures collected over a period of four years was examined. Of these, 97 cultures contained O antigens which would be expected to occur in combination with the H antigens observed. These types, their antigenic formulas, and the number of cultures studied were as follows:

<table>
<thead>
<tr>
<th>Type</th>
<th>Antigenic Formula</th>
<th>No. of cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. poona</td>
<td>13,22,36: z-1,6</td>
<td>17</td>
</tr>
<tr>
<td>S. worthington</td>
<td>1,13,23,37: 1,w-z</td>
<td>43</td>
</tr>
<tr>
<td>S. mississippi</td>
<td>1,13,23,36,37: b-1,5</td>
<td>2</td>
</tr>
<tr>
<td>S. grumpensis</td>
<td>13,23,36: d-1,7</td>
<td>8</td>
</tr>
<tr>
<td>S. atlanta</td>
<td>13,23,36: b</td>
<td>6</td>
</tr>
<tr>
<td>S. cubana</td>
<td>1,13,23,37: z59</td>
<td>19</td>
</tr>
<tr>
<td>S. wichita</td>
<td>1,13,23,37: d</td>
<td>2</td>
</tr>
</tbody>
</table>

On the contrary, there were six cultures which possessed unexpected antigenic combinations and did not correspond to recognized types. These were as follows:

<table>
<thead>
<tr>
<th>Formula</th>
<th>No. of cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 13,23,36: b-1,5</td>
<td>2</td>
</tr>
<tr>
<td>2. 1,13,23,37: b-1,5</td>
<td>2</td>
</tr>
<tr>
<td>3. 1,13,23,36,37: 1,5 monophasic</td>
<td>1</td>
</tr>
<tr>
<td>4. 1,13,22,36,37: z-1,6</td>
<td>1</td>
</tr>
</tbody>
</table>

As stated above, the indicated formulas were confirmed by numerous absorption tests which it seems unnecessary to delineate here. In every
instance the results of absorption tests confirmed the results obtained by slide and tube agglutination tests with single factor serums. This also was true of results obtained with an O serum prepared with the 1,13,22,36,37: z-1,6 form.

The first aberrant form listed might be considered either as a diphasic variety of *S. atlanta* or as *S. mississippi* which lacked antigens 1 and 37. The second form was a culture which resembled *S. mississippi* but which lacked the antigen 36 possessed by typical strains. It had the O antigens of *S. worthington* et al. but contained the H antigens of *S. mississippi*. The third form probably was simply a culture of *S. mississippi* which lacked phase 1 of the H antigen. The fourth type possessed a combination of O antigens not hitherto recognized. The H antigens were those of *S. poona*, and the writers considered the organism as an aberrant culture of that type.

From the results cited above, it may be concluded that, while in general the O antigens of types within *Salmonella* group G follow expected patterns, certain irregularities occur. These irregularities chiefly affect the organisms resembling *S. atlanta* (13,23,36: b) and *S. mississippi* (1,13,23,36,37: b-1,5). It is evident that forms intermediate between these two types occur, and for that reason it does not seem logical to separate them. Therefore, the writers favor placing these organisms in one serotype designated as *S. mississippi* since that term has priority.

II. Diphasic cultures of *Salmonella wichita* with some observations on the motility of flagellar phases. A *Salmonella* culture (6093-52) isolated from the stools of a three year old child affected with diarrhea was received from the Arizona State Department of Health. The organism possessed O antigens 1,13,23,37. When first received it failed to flocculate with antisera for the recognized *Salmonella* H antigens. After colony isolation and passage through three tubes of semisolid medium, each containing a column of medium approximately 10 cm in depth, the culture flocculated rapidly with *Salmonella typhi* H (d) serum. It then was diagnosed provisionally as *S. wichita* (Schiff and Strauss, 1939).

Such results might have been attributable simply to poor motility and lack of development of H antigens in the culture as originally received. However, the culture spread through the first tube of semisolid medium with a speed which indicated that failure to flocculate was not due to lack of motility. Therefore, the culture was placed in semisolid medium which contained d serum to determine whether other phases were present. The organism promptly spread throughout the medium but not with the speed observed in the medium without serum. After three serial passages through medium containing d serum, a highly motile form which failed to flocculate with antisera for known *Salmonella* H antigens was obtained. The O antigens of this culture were identical with those of the original culture. An antiserum prepared from this form flocculated the homologous phase at 1:20,000 but failed to agglutinate the recognized *Salmonella* H antigens at 1:100. Further, this serum flocculated broth cultures made from the original tube received from Arizona to the titer of the serum. The hitherto undescribed H antigen present in the culture was assigned the symbol zS.

In order to assure that zS was a normal antigen and not an "artificial" antigen as are the j phases produced by the growth of *S. typhi* in d serum (Kauffmann, 1936), it was necessary to demonstrate that natural phase variation occurred in the culture. Fortunately, this was accomplished quite easily due to a difference in the motility of the two phases. Eight colonies of the zS phase were passed serially six times through semisolid medium. Broth cultures of the sixth passage from all of them reacted strongly in d serum but failed to react in zS serum. Simultaneous serial passage of the eight colonies in broth brought about no change in the H antigens.

Upon another occasion a phase 2 (zS) colony was passed serially through semisolid medium and the H antigens examined after each transfer. After the first passage through semisolid medium, the culture flocculated equally well with d and zS serums. After the second passage, it reacted rapidly with d serum but failed to react with zS. On a third occasion, a phase 2 colony (zS) changed completely to phase 1 (d) after a single passage through semisolid medium.

From the results, it was evident that culture 6093-52 was diphasic and that change in phase occurred without the influence of agglutinating serum. Since the biochemical reactions, the O antigens, and the H antigens of phase 1 were identical with those of *S. wichita*, the organism must be considered a diphasic form of that serotype having the antigenic formula 1,13,23,37:
Only three additional cultures of *S. wichita* had been recognized since the laboratory was established. Examination of these cultures revealed that one of them, received in 1949, was diphasic and was identical with 6093-52. The remaining two cultures, as well as the original culture of Schiff and Strauss, were monophasic forms.

The peculiar behavior of the H phases of culture 6093-52 clearly indicated that phase 1 was more actively motile and migrated more rapidly in semisolid medium than did phase 2 since the change in phase was not due simply to back mutation of phase 2 as shown by serial transfer in broth. This behavior resembles that of a culture of *Salmonella typhimurium* characterized by Seligmann, Saphra, and Wassermann (1945), which was studied by one of the writers at the time of its description. Recently a transfer of this strain was received through the courtesy of Dr. Bruce Stocker, and the behavior of the culture again was investigated.

Seligmann et al. (1945) stated that this particular culture of *S. typhimurium* contained three H phases instead of the one or two contained by conventional *Salmonella* cultures since, upon plating the original culture, phase 2 colonies and colonies which agglutinated neither with phase 1 nor phase 2 serum were found. The latter colonies were designated as the "X" phase. Cultivation of the X phase in Gard plates which contained phase 2 serum resulted in the recovery of a typical phase 1 (i). Since no special agglutinin for their X phase could be demonstrated, and since the culture was immobilized by growth in semisolid medium which contained both phase 1 (i) and phase 2 (1,2) serums, the authors concluded that the X phase was composed of a mixture of phase 1 and phase 2.

Examination of the *S. typhimurium* culture of Seligmann et al. (1945) upon two occasions in 1945 and 1953 yielded the same results, which may be summarized as follows:

1. Upon plating, the culture yielded colonies which agglutinated with 1,2 serum but not with i serum, and colonies which agglutinated with neither i nor 1,2 serum (X colonies of Seligmann et al., 1945).

2. When the two types of colonies were transferred to broth and agar slants and incubated at 30 C, the 1,2 cultures were found to be actively motile and to agglutinate in a normal manner with 1,2 serum. On the contrary, the X cultures were made up almost entirely of cells which appeared to be nonmotile or only very sluggishly motile. A very few motile elements were seen. Formalized broth cultures of the X form cultivated at 30 C gave a very faint agglutination with i serum but did not react with 1,2 serum. In slide agglutination tests, suspensions from the agar slant cultures of the X form cultivated at 30 C gave distinct reactions with i serum but not with 1,2 serum. Serial transfer of X forms in broth gave rise to cultures which, after six passages, agglutinated in 1,2 serum. Upon plating, the cultures yielded approximately equal numbers of poorly motile, almost inagglutinable phase 1 colonies and normal, actively motile phase 2 colonies.

3. X forms passed through one tube containing a 10 cm column of semisolid medium gave rise to typical phase 2 (1,2) cultures. This observation was repeated with many X colonies, and they invariably changed to phase 2. This indicated that the majority of the few actively motile elements present in X form cultures are phase 2 cells which have appeared as a result of phase variation.

4. Motile, agglutinable phase 1 forms were obtained from the spreading of the X form in semisolid medium containing 1,2 serum. These maintained themselves when transferred serially in broth. This indicated that motile, agglutinable phase 1 elements occurred in small numbers in the culture or that the motility of the poorly motile phase 1 cells was enhanced when phase 2 was immobilized. When once isolated, the motile phase 1 forms behaved in a normal manner. It should be emphasized that actively motile phase 1 cultures were obtained only by cultivation in phase 2 serum. Inasmuch as neither Seligmann et al. (1945) nor the writers found such forms by direct plating, they must have comprised only a very small proportion of the culture if they were present as such. When transferred serially in semisolid medium, the majority of such cultures gradually changed to phase 2, indicating that the phase 1 forms obtained by the use of 1,2 serum still were somewhat less actively motile than the normal phase 2 form. Upon plating, these changed cultures were found to contain a few actively motile phase 1 elements and a preponderance of phase 2 components.

From the foregoing it was obvious that *S. wichita*, 6093-52, and the *S. typhimurium* culture of Seligmann et al. (1945) behaved in a com-
parable manner although the difference in motility of the two phases was much greater in the S. typhimurium culture than in S. wichita. These observations indicated that it is not always sufficient to speak of the motility of a diphasic culture but that it sometimes is necessary to consider separately the motility of the two phases. Many cultures which exhibited a difference in the motility and agglutinability of the two phases have been observed by the writers. This phenomenon should not be confused with the nonmotility of fully flagellated, agglutinable cultures described by Kauffmann (1941), by Edwards et al. (1946), and by others.

SUMMARY

Unexpected combinations of O and H antigens occurred in group G of the genus Salmonella. Forms intermediate between Salmonella atlanta and Salmonella mississippi were found which made it impractical to separate these serotypes. It was recommended that they be combined and called S. mississippi since that serotype has priority.

Two diphasic cultures of Salmonella wichita (1,13,23,37: :d-za) were described.

Differences in the motility of the two phases of diphasic cultures were found to be responsible for the anomalous behavior of certain cultures.

REFERENCES


Kauffmann, F. 1941 Die bakteriologie der Salmonella gruppe. Einar Munksgaard, Copenhagen.


