THE ANTIGENS OF SALMONELLA ANATUM

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Rettger and Scoville (1920) isolated a number of cultures of paratyphoid bacilli from a fatal disease of young ducklings. These organisms were described and characterized as Bacterium anatum, N.S. Further study of these cultures by Edwards and Rettger (1927) revealed that they constituted two distinct types, one identical with Salmonella aertrycke, the other an apparently independent type of paratyphoid bacillus unlike any of the cultures with which it was compared. The name, Salmonella anatum, was retained for the latter type. Edwards (1929) isolated S. anatum from an outbreak of paratyphoid infection in young chicks. Again the organism was found to be associated with S. aertrycke. Gaiger and Davies (1930) isolated an organism which they referred to as S. anatum, from young chicks. According to Lovell (1932) the organism isolated by Gaiger and Davies was not S. anatum, but Salmonella enteritidis.

While S. anatum had gained recognition as a distinct type of Salmonella, no detailed report of its antigenic relationships was published until Lovell (1932) tentatively assigned it an antigenic formula based on the scheme of White (1926). Lovell stated that S. anatum possessed the stable antigen of the S. enteritidis group or of the London type of Salmonella. This conflict of evidence probably arose through certain English workers mistaking S. enteritidis for S. anatum. Lovell represented the specific phase of S. anatum as similar to the specific phases of the

1 The investigation reported in this paper was conducted in connection with a project of the Kentucky Agricultural Experiment Station, and is published by permission of the Director.
Newport and Reading types, with the possibility that *S. anatum* possessed additional specific factors. The non-specific phase of the organism was given as that of *Salmonella suipestifer*, with the possibility of the presence of additional factors.

Since some doubt existed as to the exact relations of *S. anatum*, it was thought worth while to determine more exactly the antigenic structure of the species. During the course of the work a paper by Kaufmann and Silberstein (1934) appeared in which were given the results of their work with *S. anatum*. These investigators included in this species three cultures isolated from cases of gastro-enteritis and food poisoning in man. This is the first record of the isolation of this type of *Salmonella* from man. Further reference to the work of Kaufmann and Silberstein will be made later in the paper.

**MATERIALS AND METHODS**

The cultures used in the investigation were as follows:

*S. anatum* C1, C2, C5—isolated from ducklings in 1918 by Dr. L. F. Rettger. See Rettger and Scoville (1920).

*S. anatum* MC2—isolated from baby chicks, 1928.


*Salmonella*, Reading type 316–316 Reading. From Dr. Georgia M. Cooper.

*S. abortus-ovis* H—*B. abortus-ovis* from Dr. H. Miessner, Hannover.

In the characterization of the organisms and in the definition of antigenic factors the recommendations of the Salmonella Subcommittee of the Nomenclature Committee of the International Society for Microbiology (1934) have been followed.
The methods used in the preparation of antigens for agglutination and absorption tests were largely those recommended by White (1926) and Lovell (1932). In testing for the presence of flocculating agglutinins, formalinized broth cultures were used, since it was found that they were much more sensitive to the action of flocculating agglutinins than most suspensions of agar-grown organisms. Considerable difficulty was encountered in preparing agar-grown suspensions which would flocculate quickly in the presence of appropriate antisera. This was true even when motile colonies were picked from semi-solid agar and sown on moist, freshly prepared agar. Broth cultures flocculated in a few minutes at 50°C. in the presence of antiserum. The reaction was practically completed in two hours, whereas many agar-grown suspensions had only just started to flocculate in that length of time. A further advantage in the use of formalinized broth cultures is the fact that they do not react to somatic agglutinins to any appreciable extent. There is no difficulty in distinguishing floccular from granular agglutination, as there may be when agar-grown antigens are employed.

In the preparation of antigens for the detection of somatic agglutinins the alcohol-treated antigens of White (1926) were used. White's methods of isolating specific and non-specific races, preparation of suspensions for agglutinin absorption, preparation of antiserums, and conducting agglutination tests were followed closely.

RESULTS

Somatic antigens. The results obtained in the study of the somatic antigens are given in table 1. The cultures C5, MC2 and 7089 possess the same somatic factors as the London type. These cultures all possess the factor III of the Senftenberg type as well as the distinctive factor X. Strains C1 and C2 possess the factor III of the Senftenberg type but in them the factor X of the London type is lacking. In its place is a well-developed factor which, as far as could be determined, is not possessed by any of the known salmonellas. This factor is designated as XII.

Specific Antigens. In preliminary tests it was found that, as
Lovell (1932) stated, the specific phase of *S. anatum* closely resembled the specific phases of the Reading and Newport types. Tests to determine definitely the specific factors of strains C1 and MC2 are given in Table 2. Since the same results were obtained with cultures C2, C5 and 7089 they are not included in the table.
It will be noted that *S. anatum* completely removed the specific agglutinins from the Newport serum. Likewise Newport completely removed the specific agglutinins from *S. anatum* MC2 serum. In absorption of *S. anatum* C1 serum by Newport and Reading and in the absorption of *S. anatum* MC2 serum with Reading, small residues of agglutinins for *S. anatum* remain. These are thought to be due to the presence of small amounts of group components present in the reagents employed. The se-

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Non-specific antigens of <em>S. anatum</em></th>
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<tbody>
<tr>
<td><strong>ANTIGENS</strong></td>
<td><strong>BERA</strong></td>
</tr>
<tr>
<td></td>
<td><strong>S. anatum C1 non-specific</strong></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. anatum</em> C1 non-specific</td>
<td>8000</td>
</tr>
<tr>
<td>London 1946 non-specific</td>
<td>8000</td>
</tr>
<tr>
<td>Kunzendorf/1713</td>
<td>8000</td>
</tr>
<tr>
<td><em>S. anatum</em> var. muenster 7089 non-specific</td>
<td>8000</td>
</tr>
<tr>
<td><em>S. abortus-ovis</em> H</td>
<td>8000</td>
</tr>
<tr>
<td>Newport 1947 non-specific</td>
<td>8000</td>
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</table>

rums were shown to possess a slight amount of group agglutinin after absorption and the antigens were not absolutely specific.

**Non-specific Antigens.** Preliminary tests revealed that the group factors of *S. anatum* were closely related to those of the London and Kuzendorf types. The results obtained in their study are given in table 3. It may be seen that strain C1 possesses non-specific factors identical with the London type and with *S. abortus-ovis*, as evidenced by mutual absorption. Cultures C2, C5 and MC2 also possess these factors. The formula for the group factors of these cultures is 1, 4, 6.
Culture 7089 is lacking in a factor common to C1, C5, MC2, and London. This factor is also absent in Kunzendorf and therefore must be factor 6. Strain 7089 possesses a factor in common with Kunzendorf which is lacking in C1, C5, MC2, London and Newport. This must be the factor 5. The formula of the non-specific phase of 7089, then, is 1, 4, 5.

DISCUSSION

The facts pertaining to the antigenic analysis of the Salmonellas have been so thoroughly investigated by the English and Continental workers that there is little need to discuss the present work. However, one point calls for comment. Earlier in the paper it was stated that Kaufmann and Silberstein (1934) published an antigenic analysis of *S. anatum* while the present work was in progress. The results obtained here are in agreement with the conclusions of Kaufmann and Silberstein regarding the labile or flocculating antigens. However, we cannot agree with their conclusions regarding the stable antigens. Kaufmann and Silberstein found that all of their cultures except strain C1 had the stable complex possessed by the London type. The presence of these antigens in cultures C5, 7089 and MC2 has been confirmed. The last-named culture was not studied by the European workers. They found that culture C1 possessed the factor III in common with the London and Senftenberg types, and in addition exhibited a "poorly-developed, apparently rough antigen." In the present work it has been demonstrated that there is a well developed independent antigen in strain C1. Culture C2, which was not studied by Kaufmann and Silberstein, possesses the same independent antigen as strain C1.

In the opinion of the writer the independent factor in cultures C1 and C2 is not due to roughness. The form of growth in broth, colony formation, and saline stability of the *S. anatum* cultures were examined. In testing saline stability the methods of White (1926) and Wilson (1930) were used. Cultures C1 and C2 exhibited less tendency to roughness than the other *S. anatum* strains. Only one culture, MC2, was decidedly rough. This culture produced flat, irregular colonies, formed a pellicle and pro-
duced copious sediment in broth, and was much less stable in high concentrations of salt than the other strains. Yet the rough culture, MC2, apparently possessed the same stable antigens as London, C5 and 7089. In addition, the cultures C1 and C2 do not exhibit the "serological cosmopolitanism" attributed by Schütze (1921) to rough variants. It seems obvious, therefore, that it is erroneous to attribute the independent antigens of strains C1 and C2 to roughness. Since the factor XI has been used by Smith (1934) to denote the stable complex of the Aberdeen type, the factor XII has been applied to the independent antigen of S. anatum, C1 and C2. The antigenic formulae of the organisms studied is as follows:

<table>
<thead>
<tr>
<th>Somatic Antigens</th>
<th>Floculating Antigens</th>
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<tbody>
<tr>
<td></td>
<td>Specific</td>
</tr>
<tr>
<td>S. anatum C1, C2</td>
<td>III, XII</td>
</tr>
<tr>
<td>S. anatum C5, MC2</td>
<td>III, X</td>
</tr>
<tr>
<td>S. anatum var. muenster</td>
<td>III, X</td>
</tr>
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</table>

**SUMMARY**

Salmonella anatum possesses the specific antigens of the Reading and Newport types of Salmonella. With the exception of one culture studied, the non-specific factors of S. anatum were identical with those of the London type and of S. abortus-ovis. The non-specific antigens of one culture more closely resembled those of the Kunzendorf type. In three of the cultures the somatic antigens were identical with those of the London type. Two cultures possessed a well-developed somatic antigen unlike those of any other Salmonella studied.

**REFERENCES**

SMITH, J. 1934 Jour. Hyg., 34, 351.