SEROLOGICAL VARIANTS OF *SALMONELLA TYPHI-MURIUM* WITH SPECIAL REFERENCE TO *S. TYPHI-MURIUM* VAR. *BINNS*[1]

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In his work in the paratyphoid B. group, Schütze (1920) called attention to two strains (Binns and Timson) which he found serologically identical and designated as the Binns type. The close relationship of these cultures to *Salmonella typhi-murium* (Mutton type) was noted and commented upon by Schütze. White (1925) concluded that the Binns type was in all likelihood a permanently non-specific variant of *S. typhi-murium* to which Schütze's (1921) term "substrain" could be applied correctly. White also noted that the strain, Greifswald, U 3 of Hecht-Johansen (1923) was serologically identical with the cultures Binns and Timson. White (1926), after further study of these cultures, concluded that they occurred only in the non-specific phase, since all his efforts to isolate a specific phase from the strains consistently failed. Another peculiarity of these cultures was their inability to exhaust completely the somatic agglutinins from *S. typhi-murium* antiserum. The conclusion of this worker was that "...; 'Binns', 'Greifswald' and 'Timson' permanently coincide with the non-specific phase of typical Aertrycke save that they seem to contain quantitatively a little less of the specific factor and qualitatively a little less of the Aertrycke 'O' complex." Due largely to the work of White, the Binns cultures have been accepted as a variety of *S. typhi-murium* and have been assigned the antigenic formula: IV, V : — : 1, 2, 3.

In an attempt to prepare a strictly specific *S. typhi-murium* antiserum an ordinary specific-non-specific serum was absorbed with the Binns strain. While Binns is known to lower the titre of *S. typhi-murium* serum to a marked degree, according to previous publications, a considerable residue of specific agglutinins should remain. The surprising result obtained in this instance was the complete removal of agglutinins for several strains of *S. typhi-murium* tested. Upon further examination of the Binns culture it was found to produce a rather large proportion of specific colonies. Since this finding was contrary to the generally accepted description of this variety a number of cultures of the Binns type were collected and studied.

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

MATERIAL AND METHODS

The cultures included in the work were as follows:

Binns:

73. Original Binns from the National Collection of Type Cultures, Lister Institute. Received 1935.

73 K. A culture of the same strain from Dr F. Kauffmann. Received 1935.

B. A culture of the original Binns strain received from Dr H. Schütze in 1924, which has been in the writer's possession since that time.

Timson:

314. Received from Miss Georgia M. Cooper, 1935.

Tim S. From the collection of Dr W. M. Scott. Received from Dr H. Schütze, 1935.

Greifswald:

U 3 of Hecht-Johansen. From the collection of Dr W. M. Scott. Received from Dr H. Schütze, 1935.

The cultures were plated and the phase of the resulting colonies determined. Preliminary examination was made by slide agglutination. A colony was emulsified in carbolized saline and the suspension divided into two parts. To one portion of the suspension was added an equal amount of a 1 to 100 dilution of specific *S. typhi-murium* antiserum. The other portion was similarly treated with a non-specific *S. typhi-murium* antiserum. Very definite results were obtained by this method. Agglutination with one of the serums was apparent within a few seconds. Occasionally a colony composed of non-motile organisms was encountered. These gave no reaction with either serum. In a few instances colonies of mixed phase occurred which agglutinated with both serums. The remaining portions of the colonies which were agglutinated by specific serum were transferred to beef infusion broth. After incubation for 4 or 5 hours at 37° C. an equal amount of normal saline containing 0·5 per cent. formalin was added to the broth cultures. The formalinized cultures were used as antigens in expanded agglutination tests with specific and non-specific serums. The results of these tests invariably confirmed the results of slide agglutination.

RESULTS

The proportion of specific colonies found in the different cultures varied widely. The results obtained are summarized briefly below.

Binns

73. Four hundred colonies of this strain were tested, only one of which was specific. In an effort to isolate additional specific colonies the organisms were cultivated in broth containing group serum. The only effect noted was a rapid roughening of the bacilli after a few daily transfers. Plates inoculated from these tubes yielded only group colonies. The organisms were exposed to the combined action of non-specific serum and complement.

A marked bactericidal action was noted but no specific races could be isolated from plates inoculated from tubes showing this bactericidal action.

73 K. Six per cent. of the colonies tested were specific.

B. Thirty per cent. of the colonies examined were specific.

Timson

Tim. S. Twenty-two per cent. of the colonies tested were specific, in two per cent. both type and group antigens were present.

314. This culture was quite rough. Suspensions of the colonies in carbolized saline were agglutinated by both specific and non-specific serums so that the determination of phase by slide agglutination was impossible. Broth tubes were inoculated with twenty-five colonies which appeared less rough than the majority of the colonies produced by this strain. These were incubated for 5 hours at 37° C. Fifteen of these colonies produced a granular deposit in broth tubes. The broth cultures of the remaining ten colonies appeared suitable for use as antigens. They were diluted with an equal amount of formalinized distilled water, giving a final salt concentration of 0·25 per cent. These cultures furnished satisfactory antigens for the determination of phase. Seven of the antigens flocculated rapidly to the titre of a specific serum and reacted only in low dilutions of a non-specific serum after 2 hours incubation at 52° C. The other three antigens gave strong non-specific reactions and reacted only slightly with specific serum. After standing overnight all tubes exhibited a considerable degree of granular settling but the original reactions were still perceptible. Due to the extreme roughness of this culture no further work was done with it. If one may judge by the small number of colonies examined, the culture was largely specific. Certainly specific components were present.

Greifswald

U 3. The one culture of the Greifswald strain available for study produced 10 per cent. specific colonies. There was a relatively poor development of the flagellar antigens in this culture. This paucity of flagellar development was reflected both in agglutination and absorption tests. While type and group strains could be distinguished with ease, the organisms were not all agglutinated, even in low dilutions of the serums. Incubation at lower temperatures did not increase the development of flocculating antigen. When observed microscopically the organisms were only very sluggishly motile. Plating in semisolid agar indicated that the large majority of the colonies were made up of motile organisms but the colonies were much smaller than those formed by actively motile strains of the *Salmonella* group.

The absorptive capacities of specific strains isolated from the various cultures were studied. In performing absorption tests the serum was exposed to large doses of the absorbing antigen, sixty plate cultures per c.c. of undiluted serum being the amount used in most instances. In general it may be said that the specific components isolated from cultures of the Binns type

behaved like specific races of *S. typhi-murium*. In most instances they were able to absorb completely the agglutinins from specific serums. The only exception was the specific variant of U 3. As noted above, this culture contained a relatively small amount of flagellar antigen. This fact probably accounts for its failure to exhaust a specific serum of agglutinins.

Agglutinating serums were prepared from the specific phase of Binns and Timson. These serums flocculated the specific phase of *S. typhi-murium* to the titre limits, agglutinated non-specific antigens to only 10 per cent. of the titres, and caused flocculation of the non-specific phases of *S. newport* and *S. suipestifer* only in very low dilutions. Through the courtesy of Dr W. M. Scott the writer received a sample of serum prepared in 1924 from the Binns strain. This serum offered unmistakable evidence of the presence of specific components in the culture from which it was prepared. The serum received from Dr Scott flocculated the specific phase of *S. typhi-murium* rapidly and in high dilution. While the group titre of the serum exceeded the specific titre, the serum possessed specific agglutinins far in excess of those contained in a serum of like titre prepared from the non-specific phase of *S. typhi-murium*. In a personal communication to the writer, Dr Scott stated that he worked with the Binns strain several years ago and found it to be diphasic.

Somatic antigens

Since White (1926) noticed certain peculiarities in the somatic complex of cultures of the Binns type, these antigens have been carefully examined. The O antigens used in this work were prepared by the method of Gardner (1929). The Binns strains were unable to exhaust the agglutinins responsible for granular agglutination from *S. typhi-murium* serum. They were able, however, to remove all O agglutinins acting upon *S. abortus equi* and a variant of *S. typhi-murium* isolated from pigeons which contained only the somatic factor IV. Likewise *S. abortus equi*, *S. reading* and the variant from pigeons were able to remove all O agglutinins acting on the Binns strains from *S. typhi-murium* antiserum. Similarly *S. abortus-equi*, *S. reading* and the variant from pigeons caused a complete exhaustion of somatic agglutinins from Binns serum. It is obvious, therefore, that the Binns type contains only the somatic factor IV and lacks antigen V. The somatic antigens of the specific phases were identical with those of the cultures from which they were derived.

DISCUSSION

It is quite possible that the authenticity of the cultures under discussion may be questioned. That these cultures are actually descendants of the cultures studied by Schütze and White, and not merely strains of *S. typhi-murium* which have been mislabelled, is evidenced by the character of the somatic antigens. Strains of *S. typhi-murium* lacking the somatic factor V are found infrequently (Kauffmann, 1934; Zahn, 1935). It is hardly conceivable that so many cultures maintained in different laboratories would display this pecu-

liarity unless it had been present in the original stock. It is much more likely that the absence of factor V was responsible for the qualitative deficiency noted by White in the somatic antigens of the Binns type. Landsteiner and Levine (1932) and Kauffmann (1934) found that Binns lacked factor V. In this connection it is interesting that another culture (7998 of the Institute Robert Koch) received from Dr Kauffmann contained both antigens IV and V. This culture had been classified as a member of the Binns type. In the hands of the writer it was diphasic, but less than 1 per cent. of the colonies tested were specific. The culture probably should be regarded merely as a stubbornly non-specific *S. typhi-murium.*

The reason for the extreme non-specificity of certain of the cultures is perplexing. Of especial interest is the widely varying proportion of specific colonies occurring in different cultures supposedly descended from the same parent strain. Strains B, 73 K and 73 are probably all derived from the Binns strain of Schütze, yet they are quite different in the proportions of specific colonies produced. Strain B resembles an ordinary diphasic culture in its content of specific and non-specific components. On the contrary, it was apparently only through good fortune that a specific phase was isolated from strain 73, since no other specific colonies could be found. Culture 73 K lies midway between these two extremes. The answer to this problem seemed to lie in a study of the behaviour of specific and non-specific components of the cultures when they were maintained under ordinary laboratory conditions and transferred at regular intervals. No reason for the predominance of non-specific elements was apparent from the study. One would expect to find a greater stability in the non-specific components than in the specific. This was not true. The two phases differed little in stability through four to eight transfers on agar at monthly intervals. Both phases were equally as stable as those of typical cultures of *S. typhi-murium* used for comparison. In this connection it may be stated that certain stock cultures of *S. typhi-murium* were found to remain almost entirely in the group phase for many months, while others were just as persistently specific. This part of the work is being continued in the hope of arriving at some conclusion regarding the dominance of the non-specific elements in certain cultures of the Binns type.

From the foregoing observations it is obvious that the cultures originally described as the Binns type, and more recently called *S. typhi-murium* var. *binns*, should be characterized by the antigenic formula IV : i : 1, 2, 3. This is identical with the formula of the organism called *S. typhi-murium* var. *copenhagen* by Kauffmann (1934). Also the writer (Edwards, 1935), not being aware of the publication of Kauffmann, described variants having the same antigenic formula as *S. aertrycke* var. *storrs*. It seems that these three varieties should be designated by the same varietal term. Since the term *binns* possesses priority it is probably advisable to designate all these organisms *S. typhi-murium* var. *binns* and emend the serological formula from IV, V : — : 1, 2, 3 to IV : i : 1, 2, 3. While it is true that certain of the cultures called *S. aertrycke*

var. *storrs* did not ferment maltose, this should not exclude them from the Binns group. The inability to ferment maltose has no epidemiological significance, since maltose positive and maltose negative strains were isolated from the same outbreak of disease. Non-maltose fermenting cultures were incubated in 5 per cent. maltose broth and plated after varying intervals on Endo's agar containing maltose. Two cultures yielded secondary colonies which, when transferred to maltose broth, produced acid and gas within 24 hours. The failure of certain strains to attack maltose is probably a characteristic too variable upon which to base a variety. The writer is inclined to agree with Boecker (1936) that varieties should not be based on the action of an organism on one carbohydrate unless it can be demonstrated that this difference in carbohydrate metabolism is epidemiologically significant.

On the contrary, the lack of antigen V in strains of *S. typhi-murium* may be of epidemiologic significance. Thirteen cultures isolated from pigeons in eight widely separated flocks possessed only the somatic factor IV. Apparently this type is prevalent in infections of pigeons in the eastern United States. Thirty-seven cultures of *S. typhi-murium* isolated from mice, guinea-pigs, canaries, chickens, ducks and horses all possessed both factors IV and V. While the number of cultures examined was small as compared with the more than 300 strains tested by Kauffmann (1934), the findings may be significant. Further work is necessary to determine whether the variant is enzootic among pigeons.

While it is possible that totally and permanently non-specific forms of *S. typhi-murium* exist, there is no reason for including them in the classification of the genus *Salmonella* until they are thoroughly studied and described. The present work, like that of Scott (1926) on *S. thompson*, well illustrates the necessity of careful examination of cultures over a long period of time before concluding that they are monophasic. At the time the Binns cultures were examined by White, Andrewes, and others the specific phase was apparently entirely absent. To-day the same cultures, without having undergone any special procedures to induce variation, contain relatively large amounts of the specific component.

SUMMARY

Studies of several cultures of *Salmonella typhi-murium* var. *binns* revealed the fact that all contained specific components characteristic of *S. typhi-murium*. These cultures lacked the somatic factor V. It is proposed, therefore, to emend the antigenic formula of *S. typhi-murium* var. *binns* from IV, V : — : 1, 2, 3, to IV : i : 1, 2, 3. Under the emended variety would also be included the organisms described by Kauffmann as *S. typhi-murium* var. *copenhagen* and by Edwards as *S. aertrycke* var. *storrs*.

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