Effects of Carbohydrates in Growth Medium on Agglutination of Several Species of *Salmonella* with Polyvalent H Antiserum

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The effect of various carbohydrates in the growth medium on agglutination of salmonellae with polyvalent H antiserum was studied. There appeared to be a relationship between fermentation of the carbohydrate by the organism and resultant agglutination with the antiserum. It is recommended that the tube test for flagellar antigens be allowed to remain in a water bath for 2 hr before the final observation is made. Sorbitol, dulcitol, mannose, maltose, rhamnose, or trehalose, when included in the growth medium for *Salmonella*, yielded high percentages of positive agglutinations with all conditions of the experiment.

A procedure for testing *Salmonella* for the "H" antigen was described by Hajna and Damon (4). They devised a special broth, later named "H broth," to help develop the H antigens of the *Salmonella* during growth. The reasons for including any particular ingredients in the broth were not explained. In a study of the fluorescent-antibody system to detect *Salmonella* (3), mannitol was substituted for glucose in the H broth since a brighter fluorescence was produced with this modification. Sperber and Deibel (6) tested mixed cultures for *Salmonella* H antigens and substituted mannose for glucose in an attempt to prevent false-positive agglutinations.

Apparently carbohydrates in the medium are thought to affect the production of H antigens by *Salmonella* and, hence, also affect agglutination with H antiserum.

This study was undertaken to determine what effect, if any, various carbohydrates in the growth medium might have on the agglutination of *Salmonella* H antigens with *Salmonella* polyvalent H antiserum.

**MATERIALS AND METHODS**

Test organisms were *S. saintpaul*, *S. heidelberg*, *S. derby*, *S. reading*, *S. typhimurium*, *S. typhimurium* var. *copenhagen*, *S. montevideo*, *S. oranien-

burg*, *S. tennessee*, *S. infantis*, *S. choleraesuis*, *S. newport*, *S. muenchen*, *S. enteritidis*, *S. anatum*, *S. meleagridis*, *S. newington*, *S. senftenberg*, *S. wortthington*, and *S. gaminara*. These organisms were selected to represent different somatic O groups and to produce various fermentations in carbohydrates.

Carbohydrates tested were dulcitol, mannitol, glucose, sucrose, xylose, salicin, maltose, lactose, sorbitol, arabinose, inositol, raffinose, trehalose, rhamnose, and D-mannose.

The basic medium was purple broth base. Test broths were made by adding carbohydrates at the rate of 5 g per liter (w/v) to purple broth base, tubing in 9-ml amounts, and sterilizing at 121 C for 15 min.

To provide growth to inoculate the test broths, the organisms were inoculated into purple broth base and incubated at 37 C for 24 hr. The growth was diluted in 0.1% peptone (7), and 1 ml of the 10⁻⁷ dilution was transferred to each tube of test broth. To determine the number of cells inoculated, appropriate dilutions were plated on plate count agar and incubated at 37 C for 24 hr before counting.

The inoculated tubes of test broth were incubated at 37 C, observed for fermentation, and sampled for the H agglutination tests at 24 and 48 hr. Tube agglutination tests were conducted essentially as outlined by Difco (1) with *Salmonella* H polyvalent antiserum (Difco). Tubes were removed from the water bath at 50 C and observed for agglutination at 1 and 2 hr. The agglutination test was given a value of 0 for no observable reaction and 1 for an observable agglutination.

Each test was replicated three times. Analysis of variance and chi square values were determined (5), and differences among carbohydrate means were tested by the method of Duncan (2).
RESULTS AND DISCUSSION

Carbohydrates were selected so that some were fermented and others were not fermented by *Salmonella*. All *Salmonella* species fermented mannitol, glucose, maltose, sorbitol, rhamnose, and D-mannose. None of the species fermented sucrose, salicin, lactose, or raffinose. Reactions of the *Salmonella* species in other carbohydrates are shown in Table 1. Three failed to ferment dulcitol. Although there was apparent fermentation in xylose, three did not produce enough acid to completely change the color of the indicator from purple to yellow. Only *S. choleraesuis* failed to ferment either arabinose or trehalose. Fermentation of inositol was mixed; 10 species fermented it and 10 did not.

The number of agglutinations observed for each *Salmonella* species in each replication is listed in Table 2. There were 64 tests for each *Salmonella* in each of the three replications. The range of the number of positives was a low of 27 to a high of 64. Statistical analysis revealed that there was no significant difference among the three replications.

Of 192 agglutination tests for each of the salmonellae, the number of positive tests ranged from 133 for *S. infantis* to 191 for *S. typhimurium*. For statistical analysis, each incubation time (24 or 48 hr) with each serological test time (1 or 2 hr) was analyzed individually. For the 24-hr incubation, no significant differences were attributed to *Salmonella* species. This was also true for the 48-hr incubation period with a 1-hr serological test. However, when the salmonellae were incubated for 48 hr with a 2-hr agglutination test, there was a significant difference (1% level) due to species. Table 3 shows the effect of the addition of carbohydrates to the pure broth base on the agglutination tests of *Salmonella* after incubation. With some exceptions, addition of carbohydrates to the growth medium increased the number of agglutinations. When raffinose, inositol, or arabinose was added to the growth medium, there were fewer total agglutinations than when no carbohydrate was added to the purple broth base. None of the salmonellae fermented raffinose, which gave the least number of agglutinations. All salmonellae fermented glucose, which gave fewer agglutinations than lactose which none of the salmonellae fermented. If carbohydrates are listed in order of descending number of agglutinations, those carbohydrates at the top are generally those that are fermented by the salmonellae.

### Table 1. Reactions of *Salmonella* in five carbohydrates showing mixed fermentation results

| *Salmonella*         | Dulcitol | Xylose | Arabanose | Inositol | Trehalose |
|----------------------|----------|--------|-----------|----------|-----------|
| saintpaul            | A*       | A      | A         | A        | A         |
| heidelberg           | A        | A      | A         | -        | A         |
| derby                | A        | A      | A         | A        | A         |
| reading              | A        | A      | A         | A        | A         |
| *typhimurium*        | A        | A      | A         | -        | A         |
| *typhimurium* var.   | -        | A      | A         | A        | A         |
| copenhagen           | A        | A      | A         | -        | A         |
| oranienburg          | A        | A      | A         | A        | A         |
| tenessee             | A        | A      | A         | A        | A         |
| *choleraesuis*       | -        | A      | A         | -        | -         |
| newport              | A        | A      | A         | -        | A         |
| *enteritidis*        | A        | A      | A         | -        | -         |
| *anatum*             | A        | (A)^c  | A         | A        | -         |
| *meleagridis*        | A        | A      | A         | A        | A         |
| newington            | A        | (A)    | A         | A        | -         |
| senftenberg          | A        | A      | A         | A        | -         |
| *worthington*        | A        | A      | A         | A        | A         |
| *gaminaria*          | A        | A      | A         | A        | A         |

* Carbohydrate fermented, acid formed.
* No reaction observed.
* Not enough acid formed to completely change the color of medium.

### Table 2. Number of agglutinations observed in each of the replications with each *Salmonella* species

| *Salmonella* species | Replicate 1 | Replicate 2 | Replicate 3 | Total |
|----------------------|-------------|-------------|-------------|-------|
| *S. saintpaul*       | 51          | 61          | 49          | 161   |
| *S. heidelberg*      | 61          | 63          | 52          | 176   |
| *S. derby*           | 50          | 52          | 64          | 166   |
| *S. reading*         | 54          | 59          | 61          | 174   |
| *S. typhimurium*     | 64          | 64          | 63          | 191   |
| *S. typhimurium* var.| copenhagen  | 63          | 63          | 53    | 179   |
| *S. montevideo*      | 51          | 42          | 58          | 151   |
| *S. oranienburg*     | 50          | 60          | 64          | 174   |
| *S. tennesse*        | 50          | 46          | 59          | 155   |
| *S. infantis*        | 27          | 52          | 54          | 133   |
| *S. choleserusis*    | 63          | 40          | 64          | 167   |
| *S. newport*         | 62          | 40          | 58          | 160   |
| *S. muenchens*       | 58          | 55          | 61          | 174   |
| *S. enteritidis*     | 64          | 48          | 61          | 173   |
| *S. anatum*          | 61          | 38          | 58          | 157   |
| *S. meleagridis*     | 61          | 64          | 53          | 178   |
| *S. newington*       | 61          | 49          | 56          | 166   |
| *S. senftenberg*     | 64          | 64          | 51          | 179   |
| *S. worthington*      | 59          | 54          | 59          | 172   |
| *S. gaminaria*       | 64          | 56          | 59          | 179   |
| Total                | 1,138       | 1,070       | 1,157       | 3,365 |

Vol. 23, 1972  AGGLUTINATION OF SALMONELLA  63
TABLE 3. Number of agglutinations observed with various carbohydrates in the growth medium, at each incubation time and serological test time

| Carbohydrate added | At 24 hr of incubation | At 48 hr of incubation | Total agglutinations |
|--------------------|------------------------|------------------------|----------------------|
|                    | 1-hr STT               | 2-hr STT               | Total STT            | 1-hr STT               | 2-hr STT               | Total STT            |
| None               | 36 (d,e)*              | 54 (b,c)               | 90                   | 50 (b,c,d)             | 59 (a)                 | 109                   | 199                 |
| Dulcitol           | 50 (a,b)               | 59 (a)                 | 109                  | 57 (a)                 | 60 (a)                 | 117                   | 226                 |
| Mannitol           | 51 (a,b)               | 58 (a,b)               | 109                  | 49 (c,d,e)             | 58 (a,b)               | 107                   | 218                 |
| Glucose            | 47 (b,c)               | 58 (a,b)               | 105                  | 46 (d,e)               | 55 (b)                 | 101                   | 206                 |
| Sucrose            | 38 (d,e)               | 58 (a,b)               | 96                   | 45 (e)                 | 59 (a)                 | 104                   | 200                 |
| Xylose             | 44 (b,c,d)             | 57 (a,b,c)             | 101                  | 49 (c,d,e)             | 57 (a,b)               | 106                   | 207                 |
| Salicin            | 39 (c,d,e)             | 57 (a,b,c)             | 96                   | 46 (d,e)               | 58 (a,b)               | 104                   | 200                 |
| Maltose            | 51 (a,b)               | 60 (a)                 | 111                  | 54 (a,b)               | 59 (a)                 | 113                   | 224                 |
| Lactose            | 44 (b,c,d)             | 58 (a,b)               | 102                  | 49 (c,d,e)             | 60 (a)                 | 109                   | 211                 |
| Sorbitol           | 53 (a)                 | 60 (a)                 | 113                  | 55 (a)                 | 60 (a)                 | 115                   | 228                 |
| Arabinose          | 39 (c,d,e)             | 53 (c)                 | 92                   | 47 (d,e)               | 56 (a,b)               | 103                   | 195                 |
| Inositol           | 39 (c,d,e)             | 54 (b,c)               | 93                   | 46 (d,e)               | 58 (a,b)               | 104                   | 197                 |
| Raffinose          | 34 (e)                 | 53 (c)                 | 87                   | 47 (d,e)               | 56 (a,b)               | 103                   | 190                 |
| Trehalose          | 53 (a)                 | 58 (a,b)               | 111                  | 53 (a,b,c)             | 59 (a)                 | 112                   | 223                 |
| Rhamnose           | 51 (a,b)               | 58 (a,b)               | 109                  | 53 (a,b,c)             | 58 (a,b)               | 111                   | 220                 |
| Mannose            | 53 (a)                 | 58 (a,b)               | 111                  | 54 (a,b)               | 58 (a,b)               | 112                   | 223                 |
| Total              | 722                    | 913                    | 1,635                | 800                    | 930                    | 1,730                 | 3,365                |

* Letters in parentheses (a, b, c, d, e) indicate the following: values of those carbohydrates with the same postscripts are not significantly different at the 1% level (2).

None of the media tested yielded an agglutination in all of the 240 tests. Totals varied from the low of 190 for raffinose to a high of 228 for sorbitol. The result for sorbitol meant that 95% of the tests showed a positive agglutination reaction, whereas less than 80% of the samples with raffinose showed a positive agglutination.

Analysis of variance at each incubation time and serological testing time showed significant differences (1% level) in the number of positive agglutinations resulting from carbohydrate in the growth media, except at 48 hr of incubation with a 2-hr serological test time (Table 3). There is no significant difference between carbohydrates with the same postscript following the values.

To test for differences in the results of the incubation times or serological test times, the chi square test was used. Difference was not significant between incubation times (24 or 48 hr) of the organism but was significant (1% level) between the 1-hr and 2-hr serological test times. With a 1-hr serological test period, there was a significant difference (5% level) between growth at 24 and 48 hr. The data in these experiments suggest that the serological test should be allowed to progress for 2 hr in a water bath at 50 C before final determination of agglutination is made.

These experiments show that the type of carbohydrate in the growth medium does make a difference in the effectiveness of the test with Salmonella H polyvalent antiserum. Rather than using glucose, as in the H broth of Hajna and Damon (4), some other carbohydrate, such as dulcitol, sorbitol, maltose, mannose, trehalose, or rhamnose, should be considered. The serological tube test for Salmonella H antigens should be allowed to run for 2 hr before the final reading. With a 2-hr serological test time, incubation time of the organisms made no difference in the results. Although not recommended, if a 1-hr serological test is used, organisms should be allowed to grow for 48 hr before serological testing with the polyvalent H antiserum.

In these experiments, a combination of 16 growth media and 20 salmonellae was replicated three times, for a total of 960 trials which were incubated for 24 or 48 hr and serologically tested for 1 or 2 hr. In these 960 trials, we failed to detect the Salmonella only five times with either the 24- or 48-hr incubation or the 1- or 2-hr serological test. S. infantis was not detected three times, once with no carbohydrate and once each with inositol or raffinose present. S. cholerasuis was not detected in two instances, once with raffinose added and once with trehalose added. Even in
these cases, a very slight reaction was observed, but it was not sufficient to be considered as a positive agglutination reaction for the purposes of these experiments. It was evident that the type of carbohydrate present in the growth medium did affect the production of H antigens by the salmonellae as evidenced by the agglutination reaction of the organisms with polyvalent H antiserum.

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