THE VALUE OF THE SODIUM DEXTRO-TARTRATE FERMENTATION TEST IN THE DIFFERENTIATION OF *SALMONELLA* ORGANISMS

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The identification of *Salmonella* cultures from cases of intestinal infection forms an important part of the routine duties of many bacteriological laboratories, and in this connexion the most important problem is to differentiate between *B. paratyphosus* B and the closely related food-poisoning bacilli. To the public health officer this differentiation is often a matter of great importance, for it is essential for him to know whether the case is one of paratyphoid fever or of infection by one of the food-poisoning organisms. The routine carbohydrate fermentation tests do not help to distinguish between these organisms but, as a general rule, agglutination tests are of service. Consequently, preliminary agglutination tests with "O" sera are carried out, and serve to place the organism in one of several subgroups. In this paper we are mainly concerned with organisms falling into that "O" subgroup containing *B. paratyphosus* B, *B. aertrycke*, and the "Stanley", "Heidelberg", "Chester", "Derby", "Reading", *Abortus equi* and certain other strains of *Salmonella* (see Kauffmann, 1937). Later, tests with specific "H" sera can be performed and the cultures often accurately identified, but always the point of practical importance in such investigations is to distinguish between *B. paratyphosus* B and the food-poisoning group. While it is often not of any practical importance to know the precise name of a food-poisoning bacillus, it is important to exclude the possibility of its being a strain of *B. paratyphosus* B.

While such serological tests with specific sera are frequently sufficient for identification, a very real problem arises if the organism proves to be in the group phase, as shown by agglutinability by an "H" antiserum to the Kunzendorf bacillus. To convert such a culture into the specific phase, when it may be more readily identified, often involves several days, if not weeks, of subculturing, and in the meantime the report is being withheld.

It is to enable a differentiation to be made between cultures of *B. paratyphosus* B and related food-poisoning organisms (such as *B. aertrycke*), in the group phase, that we wish to recommend a reaction involving the fermentation of sodium dextro-tartrate. This test was originally introduced by Brown, Duncan & Henry (1924–5), who investigated the fermentation by various bacteria of a number of salts of organic acids. Although we have actually carried out tests with six of the sodium salts recommended by Brown *et al.*

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(viz. citrate, dextro-tartrate, laevo-tartrate, meso-tartrate, fumarate and mucate), we have found that, to distinguish between *B. paratyphosus* B on the one hand and food-poisoning bacilli on the other, only dextro-tartrate need be used. Brown *et al.* showed that *B. paratyphosus* B fails to ferment this salt, while *B. aertrycke*, and the "Stanley", "Reading" and *Abortus equi* strains do cause fermentation. By the application of this single fermentation test, we have found it possible to distinguish sharply between *B. paratyphosus* B and certain food-poisoning organisms.

Methods

Fermentation tests

The medium used for the tests contains a pure peptone (1 % bactopeptone, which itself gives no precipitate with saturated lead acetate solution) and a 1 % concentration of sodium *d*-tartrate. The reaction is adjusted to *p*H 7·4 and the medium is then tubed in 5 c.c. quantities. Sterilization is effected by steaming for 20 min. on each of three consecutive days.

At the outset of this investigation, as recommended by Brown *et al.*, a broth culture of the particular intestinal bacillus was used to inoculate 5 c.c. of the organic salt medium, one loopful being employed. For the majority of the tests, however, we found it sufficient to inoculate the organic salt medium directly from an agar slope culture of the bacillus (itself inoculated directly from a McConkey plate), without the necessity of first subculturing this into broth. This modification resulted in a saving of 24 hr.

The medium was then incubated for 24–48 hr., and, in order to ascertain whether or not decomposition of the salt had occurred, saturated lead acetate solution was added (0·6 c.c. per 5 c.c. culture). Lead acetate solution was also added to a control uninoculated tube of the *d*-tartrate medium. In the control tube a bulky white flocculent precipitate formed. If fermentation had occurred in the test, a small amount only of a granular precipitate formed, whereas if no fermentation had taken place the bulky white precipitate formed.

Brown *et al.* recommended that the medium should be incubated for 48 hr. We compared the fermentation results of a number of strains after 24 and 48 hr. and found that in one case only was any discrepancy observed, and here the strain (46032) failed to ferment sodium *d*-tartrate in 24 hr. at 37° C., but did so after 48 hr. If, therefore, results are required as soon as possible, observations may be made after 24 hr. growth. If the test is negative at this time, a 48 hr. growth should be examined, so as not to miss a slowly developing positive reaction.

Agglutination tests

The sera used in these tests were *B. paratyphosus* B specific "H" serum (titre 1 : 250), *B. aertrycke* specific "H" serum (titre 1 : 250), *B. paratyphosus* B "O" serum (titre 1 : 250), as supplied by the Oxford Standards Laboratory. An "H" antiserum to the Kunzendorf strain of *Salmonella* (titre 1 : 1600) was

also employed, to detect organisms in the group phase. On a few occasions a less specific "H" serum for *B. paratyphosus* B (titre 1 : 3200) was used; this serum gave agglutination also with *B. aertrycke*. "H" tests were incubated at 37° C. and "O" tests at 55° C. The tests were all straightforward agglutination tests, no agglutinin-absorption experiments were carried out.

RESULTS

One hundred strains of *Salmonella* organisms were investigated by the fermentation of sodium dextro-tartrate as well as by serological tests. The results, given in the accompanying tables, show that the organisms fall into various groups. Table I gives the reactions of ten strains that serologically proved to be *B. aertrycke* in the specific phase. All these strains fermented the organic salt.

Table I. *Strains of* B. aertrycke (*specific phase*)

		Agglutination tests			
No. of strain	Sodium *d*-tartrate	*Paratyphosus* B "H" titre 1 : 250	*Aertrycke* "H" titre 1 : 250	Kunzendorf "H" titre 1 : 1600	*Paratyphosus* B "O" titre 1 : 250
239	+	—	1 : 240	—	1 : 240
244	+	—	1 : 120	—	1 : 120
249	+	—	1 : 120	—	1 : 240
250	+	—	1 : 120	1 : 50	1 : 240
254	+	—	1 : 120	—	1 : 240
259	+	—	1 : 120	—	1 : 120
260	+	—	1 : 120	—	1 : 240
261	+	—	1 : 60	—	1 : 120
262	+	—	1 : 120	—	1 : 240
268	+	—	1 : 120	—	1 : 240

Note. In this and succeeding tables a + sign in the column headed "Sodium *d*-tartrate" means that decomposition (i.e. fermentation) of the salt has taken place.

Table II. *Strains of* Salmonella (B. aertrycke "*O*" *subgroup*) *in the group phase*

		Agglutination tests			
No. of strain	Sodium *d*-tartrate	*Paratyphosus* B "H" titre 1 : 250	*Aertrycke* "H" titre 1 : 250	Kunzendorf "H" titre 1 : 1600	*Paratyphosus* B "O" titre 1 : 250
246	+	—	—	1 : 30	1 : 120
258	+	—	—	1 : 1600	1 : 120
263	+	1 : 1600*	—	1 : 960	1 : 120
264	+	—	—	1 : 800	1 : 240
43792	+	1 : 60	1 : 480	1 : 1600	1 : 480
45451	+	—	—	1 : 960	1 : 240
45491	+	—	—	1 : 1600	1 : 120

* The serum used had a titre of 1 : 3200.

Table II refers to seven strains of *Salmonella* in the group phase. On serological evidence alone it was difficult to classify them except into their "O" subgroup but, on the evidence of fermentation of sodium *d*-tartrate, no hesitation was felt in accepting these strains as *B. aertrycke*, or one of the other

closely related *Salmonella* organisms, the important point being that they were definitely not strains of *B. paratyphosus* B.

In Table III four strains of *Salmonella* organisms are shown, which by "O" agglutination did not belong to the same subgroup as *B. paratyphosus* B and *B. aertrycke*. Strains 42438 and 44256 were shown to belong to Kauffmann's (1937) subgroup C ("O" antigens VI and VII), and strain 46032 to group D ("O" antigen IX). The precise types to which they belong have not as yet been determined, three of the four being in the group phase.

Table III. *Strains of food-poisoning bacilli (not in the* B. aertrycke *"O" subgroup)*

		Agglutination tests			
No. of strain	Sodium *d*-tartrate	*Paratyphosus* B "H" titre 1 : 250	*Aertrycke* "H" titre 1 : 250	Kunzendorf "H" titre 1 : 1600	*Paratyphosus* B "O" titre 1 : 250
42438	+	—	—	1 : 1600	—
44144	+	—	—	1 : 1600	—
44256	+	—	—	1 : 1600	—
46032	+	—	—	1 : 30	—

Table IV. *Strains of* B. paratyphosus *B in the specific phase*

		Agglutination tests			
No. of strain	Sodium *d*-tartrate	*Paratyphosus* B "H" titre 1 : 250	*Aertrycke* "H" titre 1 : 250	Kunzendorf "H" titre 1 : 1600	*Paratyphosus* B "O" titre 1 : 250
217	—	1 : 240	—	—	1 : 240
230	—	1 : 240	—	—	1 : 240
236	—	1 : 240	—	—	1 : 240
237	—	1 : 240	—	—	1 : 120
238	—	1 : 240	—	—	1 : 240
242	—	1 : 240	—	—	1 : 240
243	—	1 : 240	—	—	1 : 240
247	—	1 : 240	—	—	1 : 240
252	—	1 : 240	—	—	1 : 60
265	—	1 : 800*	—	—	1 : 240
267	—	1 : 120	—	—	1 : 240
44231	—	1 : 120	—	—	1 : 60
44263	—	1 : 120	—	1 : 30	1 : 120
44264	—	1 : 120	—	—	1 : 60
44372	—	1 : 120	—	1 : 120	1 : 120
44455	—	1 : 120	—	—	1 : 240
44514	—	1 : 240	—	—	1 : 60
44515	—	1 : 120	—	—	1 : 120
44574	—	1 : 120	—	—	1 : 120
44615	—	1 : 240	—	—	1 : 120
44616	—	1 : 480	—	—	1 : 120
44627	—	1 : 240	—	—	1 : 120
44694	—	1 : 120	—	—	1 : 120
44696	—	1 : 120	—	—	1 : 120
44697	—	1 : 120	—	—	1 : 120
44698	—	1 : 120	—	—	1 : 60
44700	—	1 : 120	—	—	1 : 120
44701	—	1 : 120	—	—	1 : 120
44703	—	1 : 120	—	—	1 : 120
44716	—	1 : 240	—	—	1 : 60
45134	—	1 : 120	—	—	1 : 120

* Serum used had a titre of 1 : 3200.

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In Table IV are shown the reactions of thirty-one strains of *B. paratyphosus* B in the specific phase. Although on the basis of serological tests alone it was possible to be quite certain of their nature, failure to ferment the organic salt was regarded as useful confirmatory evidence.

Table V shows the results of tests with thirteen strains which by serological tests proved to be in the group phase. In the majority of these, difficulty would have been experienced in deciding whether or not they were *B. paratyphosus* B strains but, as they failed to ferment the organic salt, they were considered to be true strains of this organism.

Table V. *Strains of* B. paratyphosus *B (group phase)*

		Agglutination tests			
No. of strain	Sodium *d*-tartrate	*Paratyphosus* B "H" titre 1 : 250	*Aertrycke* "H" titre 1 : 250	Kunzendorf "H" titre 1 : 1600	*Paratyphosus* B "O" titre 1 : 250
219	—	—	—	1 : 400	1 : 60
220*	—	—	—	1 : 400	1 : 240
231	—	—	—	1 : 400	1 : 120
235*	—	1 : 60	—	1 : 400	1 : 120
269	—	—	—	1 : 30	1 : 120
43952	—	1 : 200†	1 : 200	1 : 400	1 : 240
44164	—	—	—	1 : 480	1 : 120
44194*	—	1 : 60	—	1 : 400	1 : 60
44370*	—	—	—	1 : 100	1 : 60
44631*	—	1 : 60	—	1 : 240	1 : 120
44699*	—	1 : 60	—	1 : 240	1 : 60
44704*	—	1 : 60	—	1 : 120	1 : 240
45671	—	—	—	1 : 120	1 : 240

* These strains were later shown to become more specific (see Table VI).
† Serum used had a titre of 1 : 3200.

Table VI. *Results of re-examination, after subculturing, of certain strains in Table V*

	Agglutination tests			
No. of strain	*Paratyphosus* B "H" titre 1 : 250	*Aertrycke* "H" titre 1 : 250	Kunzendorf "H" titre 1 : 1600	*Paratyphosus* B "O" titre 1 : 250
220	1 : 120	—	1 : 120	1 : 120
235	1 : 120	—	1 : 60	1 : 120
44194	1 : 120	—	1 : 120	1 : 240
44370	1 : 120	—	1 : 240	1 : 240
44631	1 : 120	—	1 : 480	1 : 240
44699	1 : 240	—	1 : 120	1 : 240
44704	1 : 120	—	1 : 240	1 : 240

A number of the strains referred to in Table V were subcultured and their serological reactions retested. It was found that many of these, although still showing some group reactions, now reacted to a high titre with specific "H" *B. paratyphosus* B serum (see Table VI).

It is evident, therefore, that *Salmonella* organisms in that "O" subgroup containing *B. paratyphosus* B and *B. aertrycke* which fail to ferment sodium *d*-tartrate and which prove serologically to be in the group phase may, on the basis of these fermentation results, be regarded as genuine strains of *B. paratyphosus* B. It is in such cases that this test is clearly of great value, for a report

can be submitted without waiting until subculturing has converted the strain into the specific phase.

Table VII describes thirty-five strains on which, for various reasons, only incomplete serological tests were performed. The failure of all these strains to ferment the salt, together with the serological results, was regarded as sufficient evidence of their being *B. paratyphosus* B.

Table VII. *Other strains of* B. paratyphosus *B* (*incomplete serological tests only performed*)

		Agglutination tests			
No. of strain	Sodium *d*-tartrate	*Paratyphosus* B "H" titre 1 : 250	*Aertrycke* "H" titre 1 : 250	Kunzendorf "H" titre 1 : 1600	*Paratyphosus* B "O" titre 1 : 250
265	—	1 : 800*	—	—	1 : 240
42400	—	1 : 120	—	—	1 : 60
42605	—	1 : 240	—	1 : 30	1 : 240
44263	—	1 : 120	—	1 : 30	1 : 120
44264	—	1 : 120	—	—	1 : 60
44630	—	1 : 60	.†	.	1 : 60
44684	—	1 : 120	.	.	1 : 120
44692	—	1 : 60	.	.	.
44693	—	1 : 120	.	.	.
44695	—	1 : 60	.	.	.
44702	—	1 : 240	.	.	1 : 120
44799	—	1 : 120	.	.	.
44805	—	1 : 120	.	.	1 : 120
44812	—	1 : 120	.	.	1 : 120
44813	—	1 : 120	.	.	1 : 120
44814	—	1 : 120	.	.	1 : 120
44815	—	1 : 120	.	.	1 : 120
44816	—	1 : 120	.	.	1 : 120
44817	—	1 : 60	.	.	.
44819	—	1 : 120	.	.	1 : 240
44820	—	1 : 120	.	.	1 : 240
44821	—	1 : 120	.	.	1 : 120
44822	—	1 : 240	.	.	1 : 120
44824	—	1 : 60	.	1 : 20	1 : 120
44854	—	1 : 240	.	.	.
44945	—	1 : 120	.	.	1 : 60
44948	—	1 : 240	.	.	1 : 120
44951	—	1 : 120	.	.	1 : 120
44952	—	1 : 240	.	.	1 : 120
44957	—	1 : 240	.	1 : 30	1 : 120
44961	—	1 : 240	.	.	1 : 120
44975	—	1 : 120	.	.	.
45138	—	1 : 240	.	.	.
45157	—	1 : 60	.	.	.
45671	—	—	—	1 : 120	1 : 240

* Serum used had a titre of 1 : 3200.
† A point (.) means no test carried out.

Discussion

It has been shown that a number of strains of *B. aertrycke*, in the specific phase, ferment sodium dextro-tartrate. By contrast, strains of *B. paratyphosus* B, in the specific phase, uniformly fail to ferment this substance. The test therefore affords valuable additional evidence on the nature of such organisms.

A number of strains were isolated in the group phase, and, on the basis of the primary serological tests, difficulty was experienced in deciding on their precise nature. Application of the *d*-tartrate fermentation test, however, divided these strains sharply into two groups. Those strains which caused fermentation were naturally regarded as *B. aertrycke*, or one of the rarer types in the same "O" subgroup. Those which failed to cause fermentation were regarded as strains of *B. paratyphosus* B. That this was a justifiable assumption was proved, for, after subculturing, a number of strains became more specific and reacted to high titre with specific "H" antiserum to *B. paratyphosus* B. We believe that the main value of this fermentation test lies in the differentiation of strains in the group phase, and it is for this purpose that we desire to recommend the introduction of this reaction as a routine into bacteriological laboratories.

SUMMARY

1. The authors have confirmed the observations of Brown *et al.* (1924–5) that *B. paratyphosus* B fails to ferment, while *B. aertrycke* and food-poisoning organisms do ferment, sodium dextro-tartrate.

2. By a slightly modified procedure provisional results are obtained 48 hr. sooner than when the exact technique of Brown *et al.* is followed.

3. The application of this test is of definite practical value in differentiating between *B. paratyphosus* B and other *Salmonella* strains in the group phase.

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