# THE "THOMPSON" TYPE OF SALMONELLA.

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An apology is, perhaps, necessary for adding still another to the list of Salmonella types pathogenic for man. But it is plainly desirable that the full serological range of this group should be ascertained and, after all, since Schütze (1920)[1] analysed the paratyphoid B group with so much success, few additions to it have been made, in spite of the increased attention paid both in this country and in America[2]. Further, a description of the difficulties met with in establishing the novelty of this type "Thompson," as I propose to call it, will, I hope, be of use to other workers.

## *The source of the type strain.*

There are two outbreaks of gastro-enteritis from which strains of this type have been isolated and the circumstances under which each occurred may briefly be described.

The first outbreak occurred in July, 1924, in the household of a farmer named Thompson living near Guisborough in Yorkshire. Dr C. R. Gibson, the Medical Officer of Health, reported the outbreak and to his account I owe the following facts.

The Sunday supper of the family included a pie made with beef and rabbit; the three small rabbits put into it had been shot on the farm two days before, cooked the same day as shot and made into the pie the following day, the cooking and storage of the food being under conditions of unimpeachable domestic cleanliness. Fourteen persons who partook of that pie suffered from gastro-enteritis while three others who were present at the meal but did not taste the pie escaped. The incubation period varied from 2 to 24 hours, the severer cases having the shorter periods. In two cases the symptoms were quite grave, with high fever, delirium, strabismus and arrhythmic breathing lasting for 48 hours, besides the vomiting and diarrhoea common to all the patients.

One specimen of faeces represents the only material available for bacteriological examination; it was passed 48 hours after the onset of symptoms and contained a Salmonella in almost pure culture. The specimen came from one of the two severe cases. The serum of this patient obtained a month later

[1] *Lancet*, I. 93.

[2] Among the additions are the "L" type of Bruce White, the Dar-es-Salaam type of Schütze and the group phase para-A strains of Aoki, etc.

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agglutinated her own Salmonella in a dilution of 1 in 40; it also agglutinated the stock *Bacillus aertrycke* in a dilution of 1 in 100.

The source of infection was undoubtedly the pie. Unfortunately, none of it nor any of the dishes with which it had been in contact was available. There were no suspicious surroundings in the kitchen or larder to suggest infection there. There were suspicious circumstances about the beef so that it could not be completely exonerated, though no other cases were caused by the same carcase so far as could be found. The remaining possibility is that the source of the Salmonella was a naturally infected rabbit. Other rabbits from the same warren were asked for but not obtained, so the existence of such a natural infection could not be proved.

The second outbreak occurred on August 31st, 1925, in an asylum in Maidstone. Fifteen patients in one ward became ill within 24 hours with abdominal pain, vomiting, diarrhoea and rise of temperature; there was great prostration which lasted long after the acute symptoms subsided but all recovered. Faeces from 10 of the patients were sent to the Kent County Council Public Health Laboratory, and Dr C. Ponder isolated from one a Salmonella which he was kind enough to send to me. I am indebted to him also for making further enquiries as to the outbreak. Unfortunately, a long time elapsed before I appreciated the interest of the strain and asked for details, so that little information about the food consumed is available. There is no evidence that rabbit formed part of a meal on the day preceding the outbreak though, in answer to a leading question, it was stated that rabbit had been a frequent article of diet about that time. The medium of infection remains, therefore, unknown. I have called the single strain isolated "Maidstone." Both it and "Thompson" are deposited in the National Collection of type cultures at the Lister Institute.

### *Identification.*

These two strains, Thompson and Maidstone, behave in all respects alike. They have all the cultural characters of *B. aertrycke* and, in particular, ferment glucose, mannite and dulcite with gas production. They behave like *B. aertrycke* towards the salts of organic acids, fermenting citrate, mucate, dextro-, laevo- and meso-tartrate, as *B. aertrycke* (Mutton) does (Brown, Duncan and Henry, 1924)[1].

Before I discuss the agglutination reactions which determine the type of these two Salmonellas I must give a short account of the discoveries of Andrewes (1922)[2] as to antigenic "phases" in the Salmonella group. These discoveries have enormously increased the facility and precision with which serological type can be determined among these bacteria. Andrewes has shown that they, the Salmonellas, can form culture masses of two very different sorts according as the growth is started from a colony in the "specific" or in the "group" phase. From the former a mass of culture develops, whether in

[1] *Journ. of Hyg.* XXIII. 1.

[2] *Journ. Path. and Bact.* XXV. 505.

liquid or on solid media, in which the antigen is highly specialised, is characteristic of the type and almost completely free from admixture with those antigenic components which are not confined to the type but occur among other Salmonellas belonging to the same group. From a "group phase" colony, on the other hand, a culture mass appears containing mainly antigenic components of the latter kind and only small amounts of the special antigen confined to the type. With certain unimportant exceptions the distinction between the special antigen characteristic of the type and the group antigen common to several types is absolute throughout the Salmonella group.

Subcultures from such culture masses do not retain this purity of phase, so that the usual stock cultures are of "mixed phase" and produce a mass of bacteria in which the two kinds of antigen may be present in equal amount or one or other may predominate as chance determines.

When such "mixed phase" cultures are plated out, the colonies appear again in pure specific or pure group phase, readily distinguishable by their different behaviour in serum containing only group agglutinin. Agglutinating serum prepared by the injection of rabbits with culture masses in pure specific phase contains only insignificant amounts of group agglutinin and that prepared with pure group phase culture relatively small amounts of specific agglutinin. Serum prepared with culture masses from mixed phase subcultures, *i.e.*, the usual stock cultures, may contain chiefly group agglutinin or chiefly specific agglutinin according as the corresponding antigens predominated in the injected mass of culture. But the usual stock rabbit sera prepared with mixed phase culture generally contain plenty of both kinds of agglutinin since, (*a*) the response of the animal to a mixture of antigens is not strictly in proportion to their relative quantity, and (*b*) it is customary to give much larger doses of antigen than are actually necessary so that there is sufficient of the less abundant antigen present in the dose to excite a full response to it as well as to the more abundant components.

The use in serological work of culture masses in the mixed phase has undoubtedly caused serious difficulties in the past in the differentiation of *B. paratyphosus* B, *B. aertrycke* and *B. suipestifer*. Many workers have found that subcultures from the same strain agglutinate at one time to the full titre of a serum prepared with *B. paratyphosus* B, as well as with a serum prepared with *B. aertrycke*, while at another time they give barely perceptible agglutination with the former but retain full agglutinability with the latter. And all possible permutations can be found with strains isolated from paratyphoid fever, food-poisoning and swine fever so long as the existence of pure specific, pure group and mixed phases is ignored.

For this reason German and English-speaking bacteriologists have held divergent views as to the taxonomy of these strains for nearly 20 years—with equal reason or lack of reason in each case. As it turns out, the English bacteriologists were right and there will be, no doubt, general agreement when the principles discovered by Andrewes are universally applied.

Proceeding now to the classification of the two unknown strains, Thompson and Maidstone, the best plan appears to be to describe the successive tests and the deductions from them as they were performed.

(1) From the primary plate, inoculated with the faeces of Thompson, 12 colonies were subcultured in broth and tested with the stock agglutinating sera. All colonies agglutinated alike in 1 in 10,000 dilution or higher with the stock sera of *B. paratyphosus* B, and of the food-poisoning types Binns, Newport, Reading and Stanley (*vide* Schütze, *loc. cit.*); they agglutinated in 1 in 2000 dilution with the stock serum of *B. aertrycke* (Mutton), a serum which agglutinates "Mutton" strains, in whatever phase, to 1 in 12,000.

(2) Absorption tests with each of these stock sera showed that Thompson in excess could lower the titre for the homologous strain by a slight amount in the case of Newport and Reading sera (from 1 in 25,000 to 1 in 12,000) and not at all in the sera of Binns, Mutton and Stanley.

(3) Subculture in broth and on agar followed by replating gave again only colonies behaving as in (1) and (2).

The conclusions were that Thompson was in the group phase, that it was almost certainly not of the Mutton type and that it most probably differed also from the other known types.

(4) Rabbit serum prepared by injection of broth culture (killed by chloroform) from one of these colonies—two injections of 0·5 c.c. and 1·0 c.c. intravenously followed by bleeding 14 days after the first injection—agglutinated its homologous culture to a titre of 32,000, Binns, Mutton and Stanley to 4000, Newport to 8000 and Reading to 32,000.

(5) Absorption tests on this Thompson serum showed that none of the stock cultures mentioned affected the titre appreciably except Reading which happened to be mainly in its group phase and lowered the titre from 32,000 to 400.

These findings confirmed the impression that I was dealing with a strain permanently in the group phase, possessing very little, if any, specific antigen and possessing group antigen similar to that of Reading.

(6) Many platings and the examination of some 200 colonies in all failed to find a colony differing at all in its antigenic phase. All agglutinated to high titres with "mixed phase" sera and with pure "group" sera.

(7) Further absorption tests were made. A pure "group" Mutton serum agglutinated Thompson to rather over half its titre and when absorbed by Thompson culture in excess lost only a little of its agglutinin for "group" Mutton, the titre falling from 6000 to 3000. On the other hand, Thompson serum absorbed by excess of "group" Mutton culture lost all its agglutinin for *B. paratyphosus* B, Newport and Stanley as well as for Mutton, but retained its titre unimpaired for *B. paratyphosus* C (Hirschfeld) and for Thompson itself.

Again, the serum of *B. suipestifer* "G"[1], which is an example of a pure

[1] National Collection of Type Cultures, No. 91.

"group" serum, "G" having no specific phase and apparently no specific antigen, agglutinated Thompson to full titre and was absorbed by it completely. But when "G" culture was applied in excess to Thompson serum complete absorption could not be achieved though it reduced the titre for the homologous Thompson strain from 32,000 to 100 (cf. Table I).

At this stage all that serological analysis had shown was (*a*) that the group antigen in Thompson was made up chiefly of the group elements in Reading, *B. paratyphosus* C and *B. suipestifer*, and (*b*) that there was present in very small amount an antigen not represented in any of the other types and therefore probably specific.

(8) But here the strain "Maidstone" came under observation. It, too, behaved as a pure "group" strain, agglutinating with all the "group" agglutinins in the same way as Thompson and obstinately refusing to produce "specific" colonies. But, when applied to Thompson serum, it achieved what no other strain had so far done; it easily absorbed all the agglutinin for the homologous "Thompson" strain and, of course, for itself and all the other strains responding to the group agglutinin.

It was fairly obvious at this stage that these two strains were serologically of a new type but, in default of sufficient quantities of the specific antigen on which differentiation depended, the existence of the type was a matter of inference and not of demonstration.

(9) It occurred to me that the development of excess of group antigen (characteristic of the group phase in which these two strains persisted in appearing) might be suppressed if the strains were made to grow in the presence of a powerful group serum. So, with a minimal inoculation, cultures were made of both these strains in nutrient broth containing in fifteen parts one part of stock *B. paratyphosus* B serum, "G" serum, Mutton serum and the original Thompson serum. After 24 hours these cultures all showed clear supernatant fluid (with or without a pellicle) over a thick deposit of growth. They were centrifuged and from the surface of the fluid a plate culture was made from each and a small loop transferred to similar fresh tubes of "group serum" broth. After 6 hours' incubation the procedure was repeated and again next day.

There were thus three successive passages, (*a*) of 24 hours, (*b*) of 6 hours, and (*c*) of 18 hours, with plate cultures from each.

In the first passage no alteration was found; 10 colonies from each serum broth all behaved as "group phase" strains. But from the second passage in *B. paratyphosus* B, "G" and Thompson sera the plates showed colonies which no longer agglutinated in heterologous group serum, even at 1 in 50 dilution, but still agglutinated in Thompson serum at 1 in 100 though not higher. From the third passage in these three group sera five out of six of the colonies on plates were of this new variety. By the fifth passage pure cultures of the new phase were obtained; the serum broth no longer gave growth in the form of a deposit and plate cultures from it showed none but colonies of the new phase.

The "Mutton" serum was less active in producing the alteration but occasional colonies of the new phase appeared in it also after the third passage.

(10) Subcultures of these new strains in ordinary broth did not undergo reversion to the original phase, for, in plates made from such broth cultures, every colony (within practicable limits of examination) behaved in the new way. Agar subcultures gave growth which differed similarly from the original "Thompson" and "Maidstone" strains. Even applied in large amount the new strain failed to absorb the homologous agglutinin from Thompson serum.

(11) Rabbit sera prepared by injection of cultures of Thompson and Maidstone in their new phase agglutinated their own strains to a dilution of 1 in 12,000 and agglutinated each other to exactly the same titre. The titre for the original strains of Thompson and Maidstone was 200 with one serum and 500 with the other. There was no agglutinin perceptible for the "G" strain of *B. suipestifer* and only a trace for the specific phase of *B. suipestifer* (Indiana 49). None of the "food-poisoning" types, whether in their specific or group phase, showed any agglutination with these new Thompson and Maidstone sera.

(12) Absorption tests with these new sera showed that their homologues were identical with each other, cross-absorption being complete, whereas the original Thompson and Maidstone cultures had no effect on the titre except when applied in enormous quantities. No other strain has been found to absorb the agglutinin from these two new sera even when gross amounts were applied; this includes in particular both group and specific phases of *B. paratyphosus* C (Hirschfeld) and *B. suipestifer* (Indiana 49) which strains I had suspected of some affinity with Thompson and Maidstone.

The conclusions to be drawn from the experiments in (9) to (12) are (*a*) that these new cultures from Thompson and Maidstone are the specific phase of the strains which were isolated originally in quasi-permanent group form, and (*b*) that they are specifically identical and represent a new type of the same status as Mutton, Stanley, *B. paratyphosus* C, etc.

### *Definition of the "Thompson" type.*

This type is defined as one possessing (*a*) in its specific phase an antigen identical with that of the specific phase of Thompson deposited in the National Collection, and (*b*) in its group phase a complex identical with *B. suipestifer* and, like it, containing elements present in the group phase of *B. aertrycke* (a small proportion only), of Type Reading (a considerable amount) and of *B. paratyphosus* C. Table I serves as a chart delimiting this group complex in terms of standard strains.

I have not found a way of charting the specific antigen of Thompson; like most other specific antigens it appears exclusively in the type and is not discoverable in the antigenic complex, group or specific, of strains belonging to other types. All I can say is that, in its specific phase, Thompson (or Maidstone) is not agglutinated at 1 in 50 dilution or higher with the sera, mixed or

Table I.

*Absorption of Agglutinin from Thompson (group) Serum.*

| Culture agglutinated | Un-absorbed | Absorbed by 1 *B. para-typhosus* B | 2 Mutton | 3 Newport | 4 Stanley | 5 Reading | 6 *B. para-typhosus* C (Hirschfeld) *B. sui-pestifer* (Indiana 49 and G) | 7 Pendleton Polony Scunthorpe Gore |
|---|---|---|---|---|---|---|---|---|
| Thompson (Group) | 90 | 90 | 75 | 80 | 90 | 5 | 1 | 1 |
| *B. paratyphosus* C (Hirschfeld) | 85 | 70 | 40 | 40 | 70 | 1 | 0 | 0 |
| *B. suipestifer* "G" | 85 | 85 | 60 | 60 | 85 | 1 | 0 | 0 |
| Reading | 65 | 65 | 40 | 45 | 65 | 0 | 0 | 0 |
| Newport | 5 | 2 | 0 | 0 | 4 | 0 | 0 | 0 |
| Mutton | 6 | 2 | 0 | 0 | 4 | 0 | 0 | 0 |
| Stanley | 1·5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *B. paratyphosus* B | 1·0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

The serum employed was the original Thompson serum which contains almost exclusively group agglutinin and thus reflects the composition of the group complex in Thompson. It had been kept for 2 years at room temperature; the titre which was originally 32,000 had dropped to 9000.

The absorbing culture in each case was in the group phase and contained abundance of its appropriate group complex. In 8 c.c. of a 1 in 50 dilution of the serum (Thompson "Group") the growth from three agar plates was emulsified directly (moist growth about 600 mg.): this quantity represents excess of culture, *i.e.*, increase will not produce more absorption.

The emulsions employed to test for the persistence of agglutinin were chloroformed broth cultures in the group phase in each case. The strains referred to as Pendleton, Polony, Scunthorpe and Gore were examples of *B. suipestifer* in the group phase and are more fully described in the succeeding article, p. 406. They behaved exactly alike as absorbing cultures so that the four absorptions can be put in one column. Similarly, *B. paratyphosus* C, *B. suipestifer* (Indiana 49) and *B. suipestifer* "G" behaved alike and are put together. The figures multiplied by 100 in each case represent the final dilution of serum still giving clumps visible to the naked eye after 2 hours' incubation at 50° C.

specific, of 17 different Salmonellas in my possession. The specific serum of Thompson (or Maidstone) similarly fails to agglutinate any of these. Finally, the fermentation reactions of the Thompson type must not be neglected in the definition. Although its group phase is serologically almost indistinguishable from *B. suipestifer* "G" and the group phase of *B. paratyphosus* C, its power of fermenting dulcite, absent in the former, and its power of splitting laevo- and meso-tartrate and mucate, absent from both, are useful distinguishing characters.

*Transformation of phase by culture in agglutinating serum.*

The effect of culture in group serum broth in producing the specific phase appears to apply generally among the two-phase Salmonellas. I have done the experiment, described for "group phase" Thompson, on *B. paratyphosus* B, *B. aertrycke* (Mutton), *B. paratyphosus* C and Reading with success, though the effect is less striking since passage in ordinary broth without serum usually

results in a certain amount of spontaneous reversion from group phase to specific with those strains.

On the other hand, as will be related in the succeeding article (p. 406), there are permanent group strains like the "G" strain of *B. suipestifer* which remain unaffected by growth in group serum. They appear to have lost entirely the specific antigen from their hereditable complex; it has been torn out by the root, as it were, so that they can no longer react to the influence of group agglutinin by developing the concealed antigen and appearing in the specific phase.

The converse experiment is also possible, transformation of the specific phase into group by culture in broth containing pure specific serum. In the case of Thompson and Maidstone it is particularly striking because the specific phase of these two strains is, curiously enough, at least as permanent as the original group phase, for subculture in ordinary broth has so far not led to the appearance of "group phase" characters. But reversion can be induced by growing in broth containing in ten parts about one part of the specific serum. The culture, at first in the form of a large deposit in clear supernatant fluid, gradually becomes turbid and after about 4 days' incubation at 37° C. will sometimes, though not always, yield colonies of the group phase. I have done the experiment also with a particularly stable specific *B. suipestifer* which in ordinary culture invariably produced only specific colonies. It has seemed to me, however, that the process is less easy than the converse transformation of group into specific. I have been struck also by the tendency for specific strains grown in specific serum to become "rough" though this change does not always occur. Speculations as to the teleological value of the phase phenomenon to Salmonellas in infection are tempting but would certainly be premature.

SUMMARY.

(1) The source, cultural characters and serological properties of a new food-poisoning type of Salmonella are described.

(2) The value is emphasised of Andrewes' discovery of the group and specific phases in Salmonellas.

(3) Alteration of phase from group to specific can be produced by growth in group serum and *vice versa*. This device is useful in determining the serological type of strains which persistently appear in the group phase only.

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