FOOD-POISONING DUE TO *BACILLUS SUIPESTIFER* (SUB-GROUP II).

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Introduction.

SAVAGE and BRUCE WHITE (1925)[1] remark upon the rarity of food-poisoning due to *B. suipestifer*. They point out that their only experience of it was in an outbreak due to imported cheese (Harrogate, 1922) in which no living bacilli could be found, though the authors proved the presence in the cheese of antigens characteristic of the suipestifer type. They suggest (1) that *B. suipestifer* is probably the commonest Salmonella to be ingested by human beings since it is a frequent inhabitant of the pig, (2) that it is probably of low virulence to man, and (3) that it can set up an outbreak of food-poisoning only under exceptionally favourable conditions such as very massive infection of foodstuff.

Recently, however, there have been four outbreaks due to infection with bacteria which cannot be distinguished from the "G" strain of *B. suipestifer*[2]. This is not absolute proof, in my opinion, that they are to be identified as belonging to the *B. suipestifer* type, as I shall explain, but I feel that the outbreaks should be recorded and the strains responsible described. If the infecting bacteria are to be classed as *B. suipestifer*, Savage and Bruce White agree[3] that this type should no longer be regarded as specially innocent, though its pathogenicity for man is probably lower than that of *B. aertrycke* or the *B. enteritidis* of Gaertner.

I shall first give a short account of the epidemiological and clinical features of each of the four outbreaks and shall then describe the serological and other characters of the bacteria responsible.

The Pendleton outbreak.

This occurred on June 1st, 1924. Forty persons became ill, all of whom had consumed beef paste prepared by a local confectioner. This paste consisted of "the best beef" stewed, cooled, passed through a mincing machine and mixed with seasoning and butter. It was sold by weight wrapped in paper. The yard in which cooling took place was small and contained the W.C. and refuse bins. The mincing machine and receptacles were not sterilised after use.

[1] *Med. Res. Council, Special Report Series*, No. 91, pp. 108 and 143.

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

[3] Personal communication.

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There had been trouble with mice on the premises. No other suspicious circumstance could be found.

The incubation period was 36 hours. The symptoms were headache, thirst, and abdominal pain with repeated vomiting and diarrhoea especially among the children; almost all the cases were mild and all made a rapid recovery.

None of the paste remained for examination, but Dr H. Osborne, the Medical Officer of Health (from whose reports the above information has been obtained) sent specimens of excreta to the Public Health Laboratory in Manchester. There Professor Topley isolated from the faeces of three of the patients a Salmonella which he was kind enough to send to me. The blood serum of four of the convalescents in the outbreak was found by Professor Topley to agglutinate this Salmonella in a dilution of 1 in 200.

The Todmorden outbreak.

This occurred on May 21st, 1925, and was confined to one family, so far as can be ascertained; three of the cases were young women and one a boy of 16, while the father escaped without symptoms. All had partaken of a polony, but the boy, disliking the taste, threw most of his portion away. The polony, one of a batch of 16 lbs., was made from pork killed by a local butcher, but the actual pig from which the pork was taken could not be identified; so far as is known its meat did not cause other cases of illness. The premises in which the polony was manufactured were not satisfactory as regards cleanliness and suitability for the preparation of food. The incubation period was about 20 hours in the case of the three young women but was prolonged to 3 days in the boy.

The symptoms were of the usual character, vomiting and diarrhoea with some rise of temperature, and recovery was rapid.

For these facts, I am indebted to the Medical Officer of Health, Dr C. L. Williams, who reported the outbreak.

The remains of the polony yielded no suspicious colonies on direct plating on MacConkey's medium but after culture in brilliant-green peptone water a fair number of colonies appeared of a Salmonella. Faeces from the four sufferers were obtained on May 26th, *i.e.*, 5 days from onset of symptoms. From one specimen the same Salmonella grew but only after enrichment in brilliant-green peptone water: the others were negative.

The serum of three of the patients (not the boy) was obtained on June 3rd, 13 days from onset. In each case it agglutinated the Salmonellas isolated from the polony and from the faeces in dilution of 1 in 300.

The Scunthorpe outbreak.

This occurred about July 5th, 1925, and affected at least 54 persons. Of these 45 had potted meat, 7 sausages and 1 roast pork, all purchased from a local pork butcher; one case was probably merely coincidental as no con-

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nection with this shop could be established. It is remarkable that only three of the 56 persons who were known to have eaten the suspected food failed to develop gastro-enteritis. Twenty-six persons who belonged to infected households, but did not eat any of the food which fell under suspicion, remained well.

The potted meat, the chief vehicle of infection, was prepared from South American canned beef by mincing and mixing with liquid gelatine. Three successive batches were implicated, purchased on July 2nd, 3rd and 4th. Only three cases were ascribed to the first of these, the great majority being associated with the batch of July 4th. The way in which the infection was introduced into the shop was not discovered, but there were numerous possibilities. Sausage-making from freshly killed pork was performed in close proximity—a few feet away—to the mincing for potted meat. Mice were numerous and a nibbled portion of potted meat was seen in the shop at the inspection following upon the outbreak. The mincer was not habitually cleaned immediately after use and flies had access to it and to the prepared food. The gelatine solution was also under suspicion, as there was reason to believe that a quantity remaining over from one day might be kept and used the next. It is evident, at any rate, that multiplication of the infecting bacteria was taking place at some point in the shop with a consequent great increase of the infecting dose on the third day; the contamination had extended to at least two other articles of food, sausage and roast pork.

The incubation period was almost constant, 16 hours elapsing between the time of eating and the appearance of symptoms in nearly all the patients: in one case, a man who ate potted meat which had been kept in his house overnight, symptoms appeared in about 6 hours.

The symptoms were characteristic and in more than half the cases were severe with sudden onset, abdominal pain, vomiting, diarrhoea, fever ranging from 101° to 103° F. and great prostration. All the sufferers, however, recovered.

The circumstances surrounding the outbreak were investigated on the spot by my colleague of the Foods Branch, Dr Hancock, who has given me the benefit of his careful and laborious enquiries.

The specimens available for bacteriological investigation were (A) a portion of the suspected potted meat (found in a refuse bin) and a potted meat sandwich, (B) faeces from nine of the patients all obtained on the third day of illness and all containing much mucus.

(A) MacConkey plates inoculated with the potted meat and with the sandwich both showed many colonies which failed to ferment lactose but in each case only a few of these were Salmonellas. Culture of these infected materials in brilliant-green peptone water failed to increase the proportion of the Salmonellas; in fact, it appeared as if the other non-lactose-fermenting bacteria were at least equally and perhaps more favoured by the differential medium, for no Salmonella colonies were recovered from it.

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(B) Seven of the faecal specimens were negative as regards Salmonellas, both by direct plating and by differential culture though again in the latter other non-lactose-fermenting bacteria appeared. But with one specimen a considerable number of Salmonella colonies appeared on the direct plate (as well as in that from the differential culture), and with another, though no non-lactose-fermenting colonies were detected in the direct plate, a few appeared on the plate from the differential medium and were typical Salmonellas. In all cases the colonies which turned out to be typical strains were unusually small and flat.

Serum was obtained from four of the convalescents 17 days after the outbreak. One specimen, from one of the more serious potted meat cases, agglutinated the potted meat strain and the stock strains of *B. suipestifer* "G", *B. para* C (Hirschfeld) and *B. aertrycke* (Mutton) to a titre of 1 in 1600, but was negative to the "specific phase" of *B. aertrycke* (Mutton) even at 1 in 50 dilution. Two others agglutinated the *B. suipestifer* "G" and the potted meat strain to a titre of 1 in 400, the *B. para* C (Hirschfeld) and *B. aertrycke* (Mutton) to a titre of 1 in 200 and again failed entirely to agglutinate the latter in its specific phase; the fourth, from the patient apparently infected by roast pork and seriously ill, agglutinated all the strains mentioned to a titre of 1 in 100 only and again contained no agglutinin for the specific phase of *B. aertrycke* (Mutton).

The Fulham outbreak.

This occurred on October 30th, 1925, and was confined to one family. Both parents and three out of seven children became ill and the youngest child, aged 15 months, died. All the sufferers had eaten some brawn which had been in the house for two days; the four children who escaped had had none. The brawn had been cooked in the home of a relative and other portions of it were eaten by other people without symptoms. The portion sent to the affected house was stored in a cupboard close to the kitchen fire in company with miscellaneous rubbish salvaged from town refuse by the father who was a labourer at a Refuse Destructor. There was possible access by mice though actual access was not proved. In any case it appears that infection with, and certainly multiplication of, the causal organisms took place in the house, since the father ate some a day before the others without being ill, though he fell ill along with the others after his second partaking.

The incubation period in all cases was about 20 hours.

The symptoms were those of "food poisoning," colic, vomiting and diarrhoea of a severe type; except in the case of the infant who died, recovery was rapid.

For these facts, I am indebted to Dr A. M. Hewat, the Medical Officer of Health for Fulham, and to Dr J. Sullivan his successor.

Liver, spleen and rectum from the infant and faeces from the two other children were sent for examination. None of the suspected brawn was obtainable. The faeces specimens, obtained two days after the illness began, did not

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yield any suspicious colonies, but in the spleen of the fatal case, though not in the liver, a Salmonella was present in pure culture and may be presumed to represent the fatal infection. From the rectum similar colonies were obtained, but these, though they fermented the same sugars, were non-motile, did not split any of the organic salts and failed to agglutinate to a significant degree with any serum of the paratyphoid-aertrycke-suipestifer-gaertner group; their presence may perhaps be safely regarded as accidental. No agglutination tests were made with the blood serum of the survivors.

Identification. (*A*) *Cultural.*

The strains investigated are eight in number; one from the Pendleton outbreak to which I have given the same name; two from that at Todmorden, one called Polony from its source and the other Savage from the name of the patient; four from the Scunthorpe outbreak, the one from the potted meat called Scunthorpe, that from the sandwich called Sandwich and those from the two patients called Gunthorpe and Ellison respectively; finally one from the Fulham outbreak called Gore, the name of the child it killed. All these strains have been deposited in the National Collection of Type Cultures.

The morphological and cultural characters of the strains isolated from the four outbreaks are almost exactly alike. All grew rather less vigorously on nutrient agar than the usual strains of *B. aertrycke*; they formed smaller colonies and gave a scantier yield of culture. All blackened lead acetate paper suspended over a growing broth culture. The fermentation reactions are those typical of *B. suipestifer* (Table I).

Table I.

Fermentation of Sugars.

Strain	Glucose	Lactose Sucrose Dulcite	Mannite
Pendleton	AG	0	AG
Polony	Ag	0	A
Savage	Ag	0	A
Scunthorpe	A	0	A
Sandwich	Ag	0	Ag
Gunthorpe	A	0	A
Ellison	Ag	0	A
Gore	Ag	0	A
B. suipestifer			
Indiana 49	A	0	A
"G"	A	0	A

Incubation for 10 days at 37° C. in peptone water containing 0·5 per cent. of the different sugars. A signifies acidity to litmus, G indicates gas production to half the volume of the Durham's tube or more, while g means a small bubble of gas only.

Not only do they all fail to ferment dulcite but they also resemble the stock suipestifer strains in having a very limited capacity for gas production from the fermented sugar. The last feature has scarcely a diagnostic value, since occasionally strains of otherwise typical *B. paratyphosus* B and *B. aertrycke* may be found failing to produce gas. But the absence of dulcite fermentation

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points strongly to *B. suipestifer*. The other Salmonellas rarely, if ever, fail to attack this sugar; I have several times found typical strains of *B. aertrycke* give a negative result with dulcite even after three to five days' incubation, but invariably thereafter a sudden violent fermentation occurred as if a dulcite-fermenting race had at last emerged.

Their behaviour towards salts of organic acids[1] is equally interesting. I have omitted fumarate as the results appeared to me to be irregular.

Table II.

Decomposition of Organic Salts.

Strains	Citrate	Tartrates Dextro-	Laevo-	Meso-	Mucate
Pendleton, Polony, Scunthorpe, Gore	+	+	0	0	0
B. paratyphosus C, *B. suipestifer*	+	+	0	0	0
B. paratyphosus B	+	0	+	0	+
B. aertrycke (Mutton), Thompson	+	+	+	+	+

They all decompose citrate and dextro-tartrate, but appear unable to attack either the meso- or the laevo-tartrate: unlike *B. paratyphosus* B, *B. aertrycke* and the other food-poisoning types, they all fail to decompose mucate. They behave, in fact, exactly like the stock *B. suipestifer* strains.

Identification. (B) Serological.

Preliminary serological identification was easy. With each of the eight strains characteristic loose flocculi appeared in all the stock sera of the paratyphoid-aertrycke-suipestifer group. Comparative titration showed that they agglutinated to the full titre with the serum of *B. suipestifer* "G",[2] almost to full titre with Reading serum and to about two-thirds titre with a *B. aertrycke* (Mutton) serum containing chiefly group agglutinin. Absorption experiments showed that they could not achieve complete absorption of agglutinin from any of the stock sera except that of *B. suipestifer* "G". But this "G" serum can be robbed of all its agglutinin by several different types so that, as a means of identification, its value is slight; it contains merely group agglutinin. To identify a type a "specific" antigen must be detected as distinguished from the group antigen common to several types. The task of identification should, therefore, be begun by selection of the "specific phase" of the strain under investigation. In most cases this can readily be accomplished by plating out and testing colonies for agglutination with a serum containing chiefly group agglutinin. Those colonies which fail to agglutinate, or agglutinate only feebly, are in the "specific phase." This was the discovery

[1] Brown, Duncan and Henry (1924). *Journ. of Hgy.* xxiii. 1.

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

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of Andrewes (1922)[1] which has done so much to put the classification of Salmonellas on a firm foundation. I have discussed it in my preceding article (p. 398), so need say no more here.

But with these eight food-poisoning Salmonellas this procedure failed. No specific phase could be detected. Repeated platings of all the eight strains yielded only colonies of "group" character, *i.e.*, agglutinating well with all the sera of different types which contained abundant group agglutinin. Subcultures from these colonies would agglutinate in almost the maximum dilutions of stock sera of different types and from these sera would absorb the agglutinin responsible for cross-agglutination with non-homologous strains. But even when applied in great quantities, these cultures could not lower appreciably the titre of any of these sera for the homologous strain in its specific phase.

All the strains appeared to be permanently in the "group" condition. Such a state has already been recognised both by Andrewes and by Bruce White (1925)[2] in connection with certain strains of *B. suipestifer*. The strains originally described by Andrewes and Neave (1921)[3] as the Group II subdivision of this type are now regarded by both Andrewes and Bruce White as *B. suipestifer* permanently in the "group phase"; the stock example is the "G" strain already referred to.

But "permanent group phase" strains are not to be accepted simply on the basis that their colonies appear invariably to contain predominantly group antigen. Two further tests can be applied: (1) the sera prepared with such strains must not contain even a trace of specific agglutinin, *i.e.*, they must be robbed completely of agglutinating power for the homologous bacillus by contact with the group phase of one or more of the other Salmonellas, and (2) growth in broth containing group agglutinating serum must not induce the appearance of colonies of specific phase even after several passages. The necessity for such tests is well illustrated by the behaviour of the "Thompson" strain, the subject of my previous article (p. 398), and its immediate relative "Maidstone." Both these strains were "group" in phase on isolation and after many subcultures, but (1) they produced a serum in which a small residue of homologous agglutinin (about 1 in 100 out of a titre of 1 in 30,000) persisted even after very heavy absorption with heterologous strains in the group phase, and (2) they produced colonies in the specific phase on passage through agglutinating serum-broth.

I have applied both these tests to strains from the first three of the four outbreaks under consideration.

(1) The sera (Pendleton, Polony and Scunthorpe) behaved very much alike: each agglutinated the other strains to approximately full titre and agglutinated almost equally well *B. suipestifer* "G" and *B. paratyphosus* C (Hirschfeld) in the group phase. Absorption tests showed that each strain and also the strain Gore easily removed all the agglutinin from all three sera;

[1] *Journ. of Path. and Bact.* xxv. 505.

[2] *Medical Research Council, Special Report,* No. 91, p. 36.

[3] *Brit. Journ. Exp. Path.* II. 157.

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further, all or all but the merest trace of agglutinin could be removed by sufficient amounts of *B. suipestifer* (Indiana 49) and the Thompson strain in their group phases as well as by *B. suipestifer* "G". *B. aertrycke* (Mutton), on the other hand, even in its group phase, absorbed only insignificant amounts of agglutinin for the homologous strain while the specific phases of Thompson and *B. suipestifer* (Indiana 49) had apparently no absorbing activity whatever.

The sera were prepared by injecting chloroformed broth cultures intravenously into rabbits, a total of 1 c.c. divided into four doses being administered within 10 days. One week after the last dose the serum had a titre of 25,000 in each case.

Absorption experiments were conducted by emulsifying the moist growth from agar plates in serum diluted 1 in 50. The relative quantities varied from a minimum of 30 mg. of bacterial growth in 1·5 c.c. of the diluted serum to a maximum of 150 mg. in the same quantity. The maximum quantity was necessary for Thompson and *B. suipestifer* (Indiana 49); there were minor differences in the ease with which these two strains absorbed the homologous agglutinin from the different sera, indicating that the antigenic complex of the three strains probably differed slightly in the quantitative distribution of its different components: for example, Pendleton was more easily cleared by *B. suipestifer* (Indiana 49) and Polony by Thompson.

The result of the first test, the agglutinin response, is that no evidence of specific agglutinin and therefore of specific antigen has appeared.

(2) Pendleton, Polony, Scunthorpe and Gore, as well as *B. suipestifer* "G" itself, were cultivated in nutrient broth containing in 15 parts 1 part of serum prepared with *B. suipestifer* "G". After five passages in this, and at each passage, plate cultures showed only "group" colonies, *i.e.*, colonies agglutinating with "G" serum, "group" Mutton serum and paratyphoid B serum.

Colonies from the fifth passage of each were inoculated into paratyphoid B serum (1 in 15); at the same time the persistent group strain Thompson already mentioned was similarly inoculated. After the second passage the latter produced specific colonies only while the five strains under test produced still only "group" colonies. A third passage similarly failed. Growth in undiluted "G" serum was also tried with *B. paratyphosus* C (Hirschfeld) in mixed phase as a comparison. Plates made after 4 and 8 days' incubation in the undiluted serum gave only group colonies with all the strains, but when these cultures in neat serum were diluted with broth (to make about 1 of serum in 10) and incubated overnight, plate culture gave nothing but specific colonies in the control strain, *B. paratyphosus* C, and still nothing but group colonies in the strains Pendleton, Polony, Scunthorpe, Gore and *B. suipestifer* "G". The most careful examination of strains from different colonies failed to show differences which could suggest change in the serum broth culture. With the Polony strain, in one or two plates, colonies were found producing clumps finer than normal in the group sera used for testing. But titration showed that this fine clumping continued to the full titre of the test serum (both "G" serum and group Mutton serum) and absorption tests with growth from

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such colonies showed that they cleared the "G" serum of agglutinin equally as well as colonies giving normal clumps.

The result of the second test, the attempt to force change of phase from group to specific, is thus that no such change was achieved. The four strains from the four outbreaks of food-poisoning behaved exactly like the *B. suipestifer* "G"; they persisted in reacting only with agglutinin common to different Salmonella types, *i.e.*, with group agglutinin, and showed no differentiation into more specialised strains.

There is here, then, a group of strains isolated from rather mild outbreaks of food-poisoning and identical with the *B. suipestifer* "G". Like "G" they are simplified as compared with the two-phase *B. suipestifer* strains; they contain none of the specific antigen which makes *B. suipestifer* a serological type and, indeed, if the passage experiments recorded have any heuristic value, they have lost even the power to grow it; even the "Anlage," to use an embryological analogy, seems to have vanished.

It is difficult to give a name to such a group of strains. "Type" is obviously unsuitable and perhaps the best solution of the difficulty for the present is to return to the name given to them by Andrewes and Neave (*loc. cit.*), before their full serological significance was appreciated, and call them the "Group II subdivision of the *B. suipestifer* type," or "sub-group II *B. suipestifer*"; some justification for the name *B. suipestifer* is given by the identity of their antigenic complex with the usual group antigen of the *B. suipestifer* type, but it must be remembered that, so far as serology can tell, they might be degraded descendants from other types such as the Thompson type described on pp. 398 *et seq.*

On the other hand, the fermentation reactions both for sugars and organic salts suggest that they are really examples of *B. suipestifer*. Assuming this to be so, it is evident from the four outbreaks described that the ability of *B. suipestifer* to produce gastro-enteritis is by no means small and infection may even be fatal to a young child as in the Fulham outbreak. Nor does a large dose appear to be necessary, *i.e.*, it is not merely accumulated bacterial products which act as irritants; in all the outbreaks infection with living bacteria is evident as the cause and, in fact, except for the low mortality, the outbreaks are indistinguishable from those caused by *B. aertrycke* and *B. enteritidis* (Gaertner), the classical food-poisoning types.

Summary.

Four outbreaks of food-poisoning are described in which over 100 cases in all of gastro-enteritis occurred, one being fatal. In each outbreak the infecting bacteria were indistinguishable culturally and in serological behaviour from the "G" strain of *B. suipestifer*.

The reasons are given for following Andrewes and Neave and classifying these as *B. suipestifer* (sub-group II).

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