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FOOD-POISONING DUE TO BACILLI OF THE TYPE *B. MORBIFICANS BOVIS* (BASENAU).

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INTRODUCTION.

B. MORBIFICANS BOVIS was isolated in 1893 in Amsterdam by Basenau (1893)[1] from the flesh and the internal organs of a cow which had been slaughtered while suffering from puerperal metritis. The meat was condemned and destroyed, so that no cases of food-poisoning occurred, but Basenau was struck by the similarity of the bacillus to that found in another animal, the meat of which had caused an extensive outbreak of gastro-enteritis. He compared these strains with others which had caused food-poisoning, including the then recently described *B. enteritidis* Gaertner, and decided that the differences in culture and pathogenicity were sufficient to distinguish the first-mentioned bacillus which he named *B. morbificans bovis*.

The strain has since been preserved in various hands and has been referred to by several bacteriologists, including Savage (1908[2] and 1912[3]), in the course of comparative studies (chiefly cultural) of similar food-poisoning strains. Recently Bruce White (1926)[4] has investigated the antigenic composition of the strain obtained originally from Kràl and long maintained in subculture by Savage. He has shown that it is serologically a distinct type of Salmonella, diphasic and resembling closely in its group phase *B. suipestifer*, *B. reading* and *B. thompson*, its specific phase being quite distinct from all these.

The type had not emerged again or, at least, had not been identified, so far as we can find, since its original isolation in 1893 until the outbreak to be described.

THE FOOD-POISONING OUTBREAK IN SWANSEA.

Between June 26th and June 28th, 1926, 39 cases of gastro-enteritis occurred in 11 households in Swansea in all of which eating "pressed beef" from a certain shop was ascribed as the cause; this unanimity was probably due to the fact that, as several consumers remarked, the meat was "not very nice." Only three people were known to have eaten this beef without becoming ill. Those members of the households attacked who had not partaken of the meat escaped.

[1] *Archiv f. Hygiene*, **20**, 242.
[2] *Local Govt. Board, Medical Officer's Report* (1907–8), p. 425.
[3] *J. Hygiene*, **12**, 1.
[4] *Med. Res. Council, Special Report Series*, No. 103, p. 124.

The suspected article was prepared by a firm at Burton-on-Trent, the meat being chilled beef obtained from London, but pickled and pressed at Burton. The process of manufacture is said to include boiling of the meat for ten hours. About twenty times the amount of the Swansea consignment in question was prepared along with it and was distributed to various parts of the country without any resulting complaint.

The 22 lb. lot received at the Swansea shop on June 24th was packed in grease-proof paper with wooden discs at top and bottom. On arrival it was noted that the lower portion of the meat was "soft and jelly-like" and, in fact, a complaint to that effect was lodged with the vendors. The meat was, nevertheless exposed for sale at the reduced price of 1*s.* 8*d.* a lb. It was eaten without further cooking. On June 29th the outbreak came to the knowledge of the Medical Officer of Health but, by this time, none of the meat remained either at the shop or with the purchasers.

The shortest interval recorded between the suspected meal and the symptoms was five hours (in one case); the general incubation period was from 12 to 15 hours; in a few cases it was as long as 24 hours. It may be concluded that preformed toxin was, for the most part, not responsible for the gastro-intestinal disturbance.

The symptoms were, in all cases, headache, abdominal pain and diarrhoea of varying degree of severity and persistence with vomiting usually late in the course of the illness. In nearly all cases there was considerable prostration. Two patients, M. and S. were admitted to hospital; their illness lasted three weeks with slight fever (under 100° F.) and some collapse during the first week, but the general course of the disease hardly simulated an attack of typhoid or paratyphoid fever. In the faeces of these two, Salmonellas were present on admission but were not found on a second search at the end of a fortnight. Most of the other sufferers were well within a week. There were no fatal cases.

We are indebted to Dr Thomas Evans, the Medical Officer of Health, for most of the above information.

The specimens obtained were

(*a*) Faeces from five patients (three examined in Swansea and two in London). In four of these, B., F., M. and S., a Salmonella was isolated which is the subject of this communication.

(*b*) The two wooden discs between which the meat had been packed. These were examined in London; from one of them great numbers of Salmonella-like colonies were obtained, but though some 50 of these were examined, no true Salmonella was detected. The incrimination of the pressed beef rests, therefore, upon circumstantial evidence only.

(*c*) Blood-serum from the two hospital patients was examined on the 9th and 16th days of their illness with positive agglutination results, the details of which will be given below.

Identification.

Cultural.

All the strains isolated were closely similar in all respects. As usual with Salmonellas, they fermented glucose and mannite with gas production and failed to ferment lactose and saccharose. They all fermented dulcite and arabinose, producing acid and gas, and all blackened lead acetate. In these respects the strains behaved like the stock strain of *B. morbificans bovis*. As regards inosite fermentation, the latter displayed distinctly greater activity, producing acid and gas in 24 hours, whereas the Swansea strains had produced no change in 48 hours; three of the four, however, formed acid with a little gas in 72 hours and the remaining one in 96 hours.

The six organic salts used for the differentiation of Salmonellas by Brown, Duncan and Henry (1924)[1] differentiate *B. morbificans bovis* more definitely from the Swansea strains; whereas the former ferments all six (citrate, dextro-, meso-, and laevo-tartrates, fumarate and mucate), the latter four strains all failed to ferment meso-tartrate and were much more feeble in their attack on fumarate.

Serological.

(1) *The double phase.* All four strains from Swansea appeared, on plating out, in colonies showing the two different phases described by Andrewes for Salmonellas. The colonies obtained on direct plating of two of the faecal specimens which were examined as to this point also showed the two phases. In one case (S.) the great majority of these direct colonies were of group phase (about 90 per cent.); in the other case (F.) only 20 per cent. were of this phase. With these group-phase colonies both the bacterial suspension made from the colony itself and the first broth subculture from it agglutinated with the group Salmonella sera (Paratyphoid, Aertrycke (mutton), Newport, Reading, Suipestifer, etc.). The specific colonies, similar in appearance and in culture, failed to agglutinate with these sera and with sera containing abundant specific agglutinin for the various Salmonella types (including all the rarer types at our disposal) with the sole exception of the serum prepared with the stock strain of *B. morbificans bovis*.

(2) *Agglutination.* With this Morbificans Bovis serum, broth cultures from both specific and group colonies of the Swansea strains agglutinated to high titre, coarse flocculi visible to the unaided eye appearing in 2 hours at 50° C. in dilutions which varied with the different cultures from 10,000 to 16,000. The titre of the serum for *B. morbificans bovis* itself at the time of comparison varied from 10,000 to 25,000, the former being the titre for the specific phase and the latter for the group phase. A similar range of difference in the titre appeared with the Swansea strains; S., for example, agglutinated in its specific phase in 10,000 dilution and in its group phase in 16,000. It may be said, thus, that the Swansea strains agglutinated to the full titre of

[1] *J. Hygiene*, **23**, 1.

B. morbificans bovis serum except that in their group phase they agglutinated a little less strongly than did the homologous strain in its group phase (*vide* Table I).

(3) *Agglutinin production.* Agglutinating serum was prepared by intravenous injection into a rabbit of mixed phase broth culture of the S. Swansea strain. This serum, as was to be expected, agglutinated the other three Swansea strains and also *B. morbificans bovis* to approximately full titre. It contained, as it happened, rather more specific than group agglutinin, the titre of group phase cultures being slightly lower than those in the specific phase. The group-phase of *B. morbificans bovis* agglutinated to a definitely lower titre than the group phase of the Swansea strains (*vide* Table I).

Table I. *Agglutination titres.*

Unheated broth cultures		Morbificans Bovis serum	S. serum
Morbificans bovis	Specific	10,000	40,000
	Group	25,000	20,000
Swansea B.	Specific	10,000	50,000
	Group	12,000	40,000
Swansea F.	Specific	10,000	50,000
	Group	15,000	40,000
Swansea M.	Specific	10,000	50,000
	Group	15,000	35,000
Swansea S.	Specific	10,000	40,000
	Group	16,000	35,000
Aertrycke (mutton)	Specific	400	400
	Group	8,000	8,000
Newport	Specific	400 g.	1,600 g.
	Group	6,000	6,000
Reading	Specific	400	400
	Group	20,000	16,000
Para-C. (Hirschfeld)	Specific	<100	<100
	Group	8,000	12,000
Thompson	Specific	<100	<100
	Group	12,000	15,000
Para-B.	Specific	<100	<100
	Group	1,000	1,000

g. = granular agglutination only.

(4) *Absorption of agglutinin—Identity of Swansea strains and* B. morbificans bovis. Cross-absorption experiments confirmed the antigenic identity of the Swansea strains with *B. morbificans bovis*. In 2 c.c. of 1 in 50 dilution of the S. serum described above, about 200 mg. of the agar growth of *B. morbificans bovis* (mixed phase) was emulsified and the mixture centrifuged after standing at room temperature for half an hour; the supernatant fluid mixed with an equal volume of S. broth culture gave no agglutination at 1 in 100; untreated S. serum agglutinated the S. culture to a titre of 40,000. A similar experiment, with the Morbificans Bovis serum absorbed with S. culture, again resulted in the complete absorption of all agglutinin, the supernatant serum producing no agglutination with a broth culture of *B. morbificans bovis* at 1 in 100 dilution, the control titre being 1 in 25,000. Similar absorption experiments with

Morbificans Bovis serum and the other three Swansea strains were also successful, complete removal of the homologous agglutinin being obtained.

(5) *Analysis of the group antigen.* The group antigen of *B. morbificans bovis* has been analysed by Bruce White (1926)[1]. He shows that it corresponds closely with the group complexes of the Suipestifer, Reading and Thompson types. We have confirmed this and have shown that the Swansea strains are similar in their group antigenic composition, *i.e.* in the composition of their non-specific labile antigen.

Table II. *S. serum—absorption of agglutinin.*

Cultures agglutinated		Unabsorbed	Absorbed Aertrycke (mutton)	Absorbed Reading	Absorbed Suipestifer	Absorbed Thompson
Morbificans Bovis	Specific	40,000	40,000	40,000	40,000	40,000
	Group	20,000	20,000	10,000	8,000	4,000
Swansea S.	Specific	40,000	40,000	40,000	40,000	40,000
	Group	35,000	30,000	20,000	10,000	6,000
B. paratyphosus B.	Group	1,000	<100	<100	<100	<100
B. aertrycke (mutton)	Group	8,000	<100	100	200	100
B. newport	Group	6,000	<100	100	100	100
B. reading	Group	30,000	6,000	<100	<100	<100
B. suipestifer	Group	12,000	8,000	800	<100	<100
B. thompson	Group	25,000	8,000	800	1,600	<100

Table II shows that *B. suipestifer*, *B. thompson* and *B. reading* remove group agglutinin almost entirely from the Swansea S. serum for all the other Salmonellas; a good deal of agglutinin is left, it is true, for the group phases of *B. morbificans bovis* and "S." itself, but this is to be explained, most probably, by the presence of moderate amounts of specific antigen in the group phase of these bacteria, the agglutination thus depending not on persisting group agglutinin but on the specific agglutinin which heterologous bacteria are incapable of removing. Absorption of the Morbificans Bovis serum in a similar manner gave a table closely resembling Table II except that absorption with *B. reading* removed group agglutinin still more completely, all the agglutinin for *B. suipestifer* and *B. thompson* disappearing, whereas in S. serum Reading left appreciable, though small, amounts of agglutinin for these two strains.

(6) *Heat-stable O-antigen.* The agglutination so far discussed has been solely the flocculating variety, characterised by the appearance of voluminous fluffy clumps in the mixture of bacterial culture and agglutinating serum; this is dependent, as is known, on the interaction of the H-antigen, the heat-labile flagellar material, with the corresponding antibody. But the heat-stable O-antigen can be obtained free from H-antigen by prolonged steaming of cultures or emulsions of Salmonellas, by extraction of bacterial masses with alcohol or by cultivating the bacteria on phenol-agar (1 in 800). The suspensions so obtained agglutinate in the form of small firm granules resembling those produced by dysentery bacilli in their homologous anti-serum; the agglutination is most probably the result of interaction between the

[1] *Med. Res. Council Spec. Rep.* No. 103, p. 124.

protein of the bacillary bodies, the "somatic" antigen, with corresponding agglutinin. This O-agglutinin is rarely present in large amount in the ordinary Salmonella agglutinating sera; a serum which produces the H-form of agglutination in a dilution of 1 in 50,000 may fail to give the O-form in dilutions over 1 in 1000.

O-agglutination is not specific among the Salmonellas in the sense that individual types may be founded upon it; for example, *B. paratyphosus* B, *B. aertrycke* (mutton) and *B. stanley* have practically the same O-antigen but are sharply differentiated by their specific H-antigen. Though the O-antigen is group in character, its distribution does not coincide with the group H-antigen; for example, as Bruce White has shown, *B. newport* differs sharply in its O-antigen from *B. aertrycke* (mutton), although their group H-antigen is nearly identical. To establish the antigenic identity of two strains, it is therefore necessary to show that the heat-stable O-antigen is the same in both.

In the case of the Swansea strains and *B. morbificans bovis* we have succeeded in doing this. Emulsions grown on phenol agar and heated to 100° C. agglutinated with Newport and Morbificans Bovis sera to titres varying from 800 to 1500 but gave, at most, only traces of agglutination with the sera of other types. Bruce White has already shown (*loc. cit.*) that the O-antigen of *B. morbificans bovis* is identical with that of Newport. We have found that this is so; the O-agglutinin in the serum of Newport can be completely removed by saturation with *B. morbificans bovis* and *vice versa*. Newport serum can also be stripped of its O-agglutinin by the Swansea S. strain. S. serum contains agglutinin for the O-forms of the other Swansea strains, of *B. morbificans bovis* and of *B. newport*, giving dense granular clumps up to 1 in 3000 dilution after incubation for 4 hours at 50° C. With the O-form of *B. suipestifer* and Thompson it agglutinates to about 1 in 200 and contains only traces of agglutinin for *B. aertrycke* (mutton) and *B. reading*. This O-agglutinin in the S. serum can be completely removed by saturation with the O-form of Newport and Morbificans Bovis and the O-agglutinin in Morbificans Bovis serum by saturation with the S. strain.

(7) *Agglutinin production as the consequence of infection of human beings.* Samples of serum were obtained from the two patients M. and S. on both the 9th and the 16th day after the onset of their illness. Neither patient had previously had a typhoid infection or inoculation. The sera were examined in Swansea and gave the results shown in Table III.

The high titre for *B. paratyphosus* B shown with both Oxford and Tidy strains is rather surprising, in view of the small degree to which paratyphoid B group antigen is represented in the infecting strains. Swansea M strain was found to remove practically all *B. paratyphosus* B agglutinin from the 9th day serum of patient M., an Oxford emulsion being used for testing. The negative result with the Bainbridge strain is probably due to its being in the specific phase. Can it be that the human immunity mechanism has a natural

tendency to select and respond more freely to the human type of Salmonella antigen?

In other respects Table III gives satisfactory confirmation of the fact that *B. morbificans bovis* was the infecting strain.

Table III. *Agglutinating power of patient's serum.*

Culture and Source	End-point titre with M serum 9th day	M serum 16th day	S serum 9th day	S serum 16th day
Paratyphoid B (Oxford)	1000	800	400	1600
Gaertner (Oxford)	200	100	25	0
Aertrycke (mutton) (Oxford)	12	0	<10	0
Aertrycke (Newport) (Oxford)	0	0	0	0
Paratyphoid A (Oxford)	0	—	—	—
Paratyphoid B (Bainbridge) (National collection)	<40	<40	—	<200
Paratyphoid B (Tidy) (National collection)	—	—	160	800
Aertrycke (mutton) (Swansea)	<40	<80	15	25
Morbificans bovis (specific phase)	200	200	400	1200
B (Swansea)	80	60	100	200
M (Swansea)	125	60	80	160

The exact identification of the infecting bacillus in this outbreak had more than a purely scientific interest; within five days of the outbreak another group of food-poisoning cases occurred in Swansea which, on epidemiological evidence, appeared, at first, as if it could be linked up with the pressed-beef group. In the second outbreak, however, the infecting bacillus was a typical *B. aertrycke* (mutton), so that the separate nature of the epidemic was at once declared. It was later confirmed by epidemiological inquiry.

SUMMARY.

An outbreak of food-poisoning is described in which the *B. morbificans bovis* (Basenau) was the causal agent. The position of this type in the Salmonella group as classified by Bruce White has been confirmed by analysis of the antigenic properties and agglutination reactions (including absorption of agglutinin) of the strains isolated in the outbreak.

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