A "NEW" SALMONELLA FROM A CASE OF ENTERIC FEVER.

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(With 4 figures.)

The list of bacteria recorded as causative agents in enteric fever and "food-poisoning" increases steadily, and the proved pathogens already number eleven types, of which three have been found in both continued fever and food-poisoning, three in continued fever alone, and five in food-poisoning alone. This list will undoubtedly be added to when cultural methods for the diagnosis of these conditions are more widely employed, especially in the tropics.

Unfortunately bacteriologists are not agreed on the delimitation of the salmonella group which includes nine of the eleven types (*B. typhosus* and *B. paratyphosus* A excluded); some workers restrict the genus (?) *Salmonella* to bacilli having certain cultural characters in common, but more or less distinctive serological reactions, others include, on serological grounds, organisms such as *B. gallinarum*, *B. glässer*, and *B. voldagsen* which do not present the cultural characters of the "true" salmonellas, and American writers have included *Morgan's bacillus* which has neither cultural nor serological connection with the group and could scarcely be admitted even on the ground of pathogenicity.

For the purposes of this paper the salmonellas may be regarded as presenting the following common characters: Gram-negative non-sporing bacilli, usually actively motile, which do not ferment lactose, saccharose or salicin, do not liquefy gelatin, and never give the indol reaction. In litmus-milk they cause a transient acidity followed after about 48 hours by alkalinity. They ferment glucose, mannitol, and maltose with production of acid and gas. These general reactions cover salmonellas in the restricted sense (Topley, Weir and Wilson, 1921).

In the case to be described a bacillus presenting definite affinities with the salmonella group was isolated from the circulating blood of a patient suffering from typical enteric fever. The apparent absence of the various bacilli commonly causing this disease, and the fact that the patient's serum did not react with any of them, but only with the homologous bacillus, seem to justify the assumption that this bacillus played the role of pathogen.

The case. On October 14th, 1922, an Indian seaman, aged 28, was admitted to the Royal Albert Dock Hospital under the care of Dr G. C. Low. The patient presented all the common clinical features of enteric fever, and during his first

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week in hospital suffered two attacks of intestinal haemorrhage. After this he made an uninterrupted recovery; the illness running the usual course of a mild typhoid. Unfortunately no history prior to his admission to hospital is obtainable, but as he was admitted directly from his ship in the early stage of the disease, it seems probable, taking into account the duration of the voyage, that the infection was contracted in Bombay, if the man were not already a carrier.

Widal tests made during the first and third weeks in hospital were negative with different strains of *B. typhosus*, *B. paratyphosus* A, *B. paratyphosus* B, *B. paratyphosus* C, *B. aertrycke* (Mutton and Newport types) and *B. enteritidis* Gärtner, but *B. suipestifer* (Hog Cholera Ten Broeck, No. 16) was agglutinated in titres up to 1/80.

The bacillus. A blood-culture was successfully made on the day of admission and a bacillus (hereinafter referred to as the *Alphonso* strain) having the following characters was obtained in pure culture. Morphologically like *B. typhosus* and the salmonellas. Actively motile, Gram-negative, gelatin not liquefied, and the indol reaction not given. Lactose, salicin and dulcitol were not fermented. Litmus-milk was rendered acid, changing to an alkaline reaction after 48 hours. Glucose, mannitol and maltose were fermented with acidity and gas evolution. It therefore presents the general characters of the salmonella group, and in its failure to ferment dulcitol it resembles *B. suipestifer*.

Agglutination tests. The patient's serum taken five weeks after admission to hospital agglutinated the bacillus up to a titre of 1/500, but agglutination at all titres was incomplete, a portion of the suspension remaining unagglutinated. Agglutination also occurred with certain stock sera for the group: with *B. enteritidis* Gärtner serum to 1 per cent. of full titre, *B. paratyphosus* B serum 16 per cent., *B. paratyphosus* C serum 10 per cent., *B. aertrycke mutton* serum 10 per cent., *B. glässer* serum 10 per cent. It was not agglutinated by sera for *B. typhosus*, *B. paratyphosus* A, or the *Newport bacillus*, nor did it absorb the specific agglutinin from any of the stock sera mentioned.

Pathogenicity to albino rats. Tests were made when the strain had been cultivated under artificial conditions for three months. Oral feeding was apparently without effect, but 0·25 c.c. of a very young broth culture introduced into the peritoneum caused death in four days. Post-mortem the only macroscopic changes noticed in the animals were petechiae in the lungs and some enlargement of the spleen; the peritoneum appeared normal. The bacillus was recovered in pure culture from the spleen, liver, and heart and was not found to have been modified in any respect by its passage through the animal.

Tests with high-titre sera. An *Alphonso* agglutinating serum, (rabbit), having an end-titre for the homologous bacillus of 1/20,000, was prepared, and tested with the following 11 types obtained from the National Collection of Type Cultures. The numbers refer to the catalogue of the National Collection.

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B. paratyphosus A (Schottmüller). No. 13.
B. paratyphosus B (Tidy). No. 14.
B. paratyphosus C (East Africa) Salmonella type Hirschfeld, No. 777.
Hog Cholera bacillus (Ten Broeck No. 19) Salmonella type Hirschfeld. No. 357.
B. enteritidis Gärtner (Danyz). No. 205.
B. aertrycke, Salmonella type Mutton. No. 115.
Salmonella type Newport. No. 129.
Salmonella type Binns. No. 73.
Salmonella type Stanley. No. 92.
Salmonella type Reading. No. 72.
Salmonella type "G." No. 91.

These strains were all agglutinated in varying degree by the *Alphonso* serum; see Fig. 1.

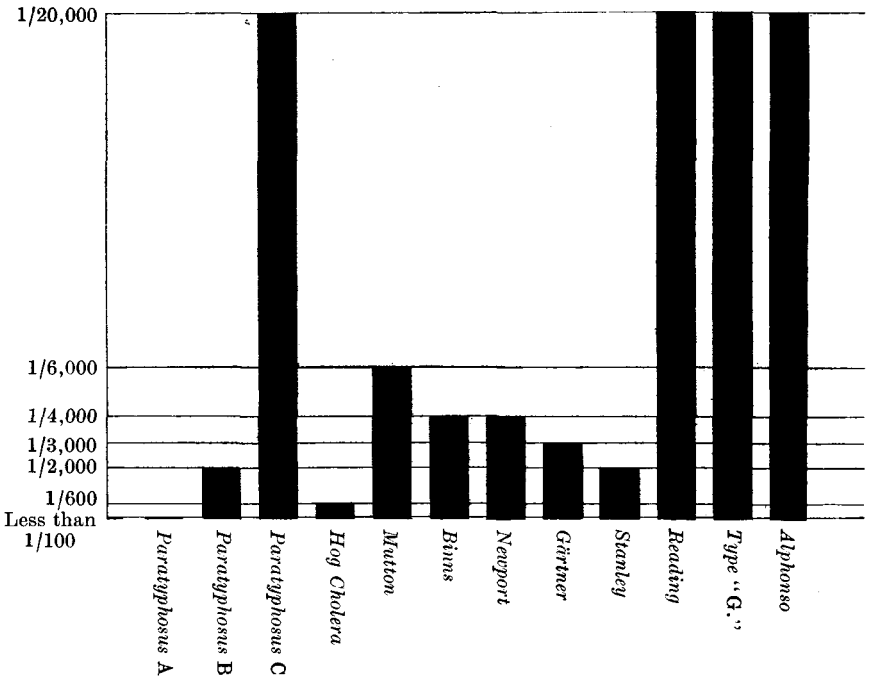

Fig. 1. Agglutination of twelve salmonellas by *Alphonso* serum. End-titres of agglutination: *B. paratyphosus* A less than 1/100. *B. paratyphosus* B 1/2000. *B. paratyphosus* C 1/20,000. *Hog Cholera bacillus* 1/600. *Mutton* type 1/6000. *Binns* type 1/4000. *Newport* type 1/4000. *B. enteritidis* Gärtner 1/3000. *Stanley* type 1/2000. *Reading* type 1/20,000. *Type* "G." 1/20,000. *Alphonso* 1/20,000.
The individual agglutinability of all these strains, except that of the Hog Cholera bacillus, was good. Agglutination in every case was incomplete, especially in the higher titres.

Absorption of *Alphonso* serum by the various types separately affected no reduction of end-point for the homologous bacillus except in the cases of *Type* "G.," *Reading*, and *Newport* (Fig. 2). *Type* "G." removed *all* agglutinin at 1/50, *Reading* reduced the end-titre for *Alphonso* to 1/3000, but did not

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completely remove all group-agglutinin for any of the types except itself and *gärtner*.

Although absorption by *Paratyphosus* B, *Paratyphosus* C, *Stanley*, *Binns*, and *Mutton* caused no apparent reduction in end-titre for *Alphonso*, the quality and the velocity of agglutination were affected; as compared with a control (unabsorbed) serum agglutination was much slower and more incomplete, the flocculi being very minute and fewer in the higher titres especially after absorption by *Paratyphosus* C. This phenomenon is well known and was noticed by Castellani in 1902, in the case of a *B. typhosus* serum of titre 1/10,000 which

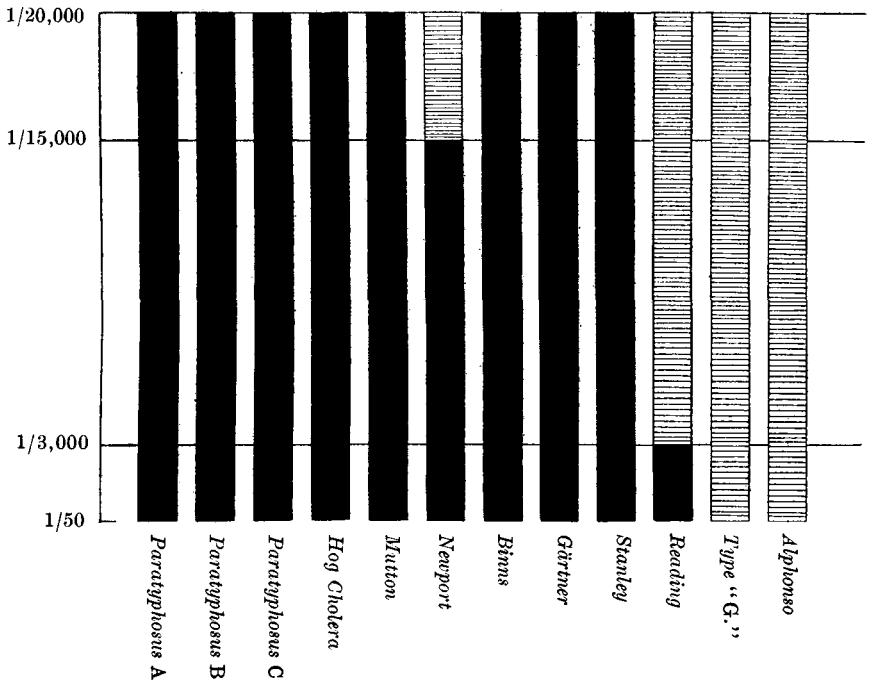

Fig. 2. Effect of absorption of *Alphonso* serum by twelve salmonellas separately, on end-titre for homologous bacillus. Absorption was carried out in a titre of 1/50 and was complete in the cases of *Type* "G." and *Alphonso*.

also agglutinated *B. coli* up to 1/800. Absorption by *B. coli* removed all of the *coli* agglutinin without lowering the end-titre for *B. typhosus*, but the clumps of *B. typhosus* were small as compared with the clumps formed in the unabsorbed serum.

The five types mentioned therefore appear capable of removing some of the agglutinins effective for *Alphonso* without lowering the end-point. These agglutinins evidently represent the elements by which the organisms are themselves agglutinated and seem to correspond to the more remote type of group-agglutinin or *co-agglutinin*. That this co-agglutinin is not a single element but a mixture of agglutinins corresponding to the apparently complex antigenic

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mosaic of the *Alphonso* bacillus is suggested by the fact that each of the five types can remove completely only its own co-agglutinin, but not completely those for other types. This is illustrated in Fig. 3 which shows the effect of absorption of *Alphonso* serum by *Paratyphosus* C on agglutination for the group.

It will be seen that the results of the absorption tests arrange themselves into three groups: (1) complete absorption of all agglutinins by *Alphonso* and *Type* "G."; (2) reduction of end-titre for the homologus bacillus by *Reading*

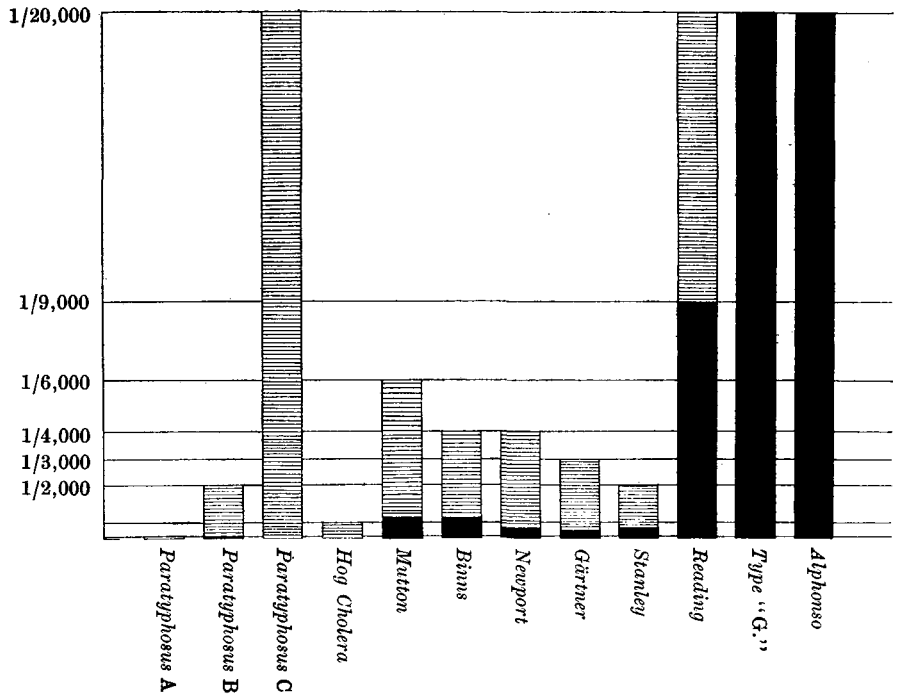

Fig. 3. *Alphonso* serum absorbed by *B. paratyphosus* C at 1/50. Effect on end-titres of agglutination for the twelve salmonellas. The light shading indicates the reduction in titre, the dark shading the titre of agglutination.

Alphonso and *Type* "G." no reduction in end-titre. *Reading* reduced from 1/20,000 to 1/9000. *Stanley* from 1/2000 to 1/400. *Gärtner* from 1/3000 to 1/300. *Newport* from 1/4000 to 1/400. *Binns* from 1/4000 to 1/800. *Mutton* from 1/6000 to 1/800. *Hirschfeld* type (*Paratyphosus* C and *Hog Cholera*) complete absorption. *B. paratyphosus* B from 1/2000 to 1/100.

and *Newport*; (3) alteration in the quality of agglutination without reduction of end-titre by each of the others.

The *Alphonso* serum therefore may be said to contain a "*specific agglutinin*" common to *Alphonso* and *Type* "G." an "*intimate group-agglutinin*" removable by *Reading* and to a slight extent by *Newport*, and a more remote type of group-agglutinin or *co-agglutinin* affecting the other types. It has already been suggested that the co-agglutinin is made up of several different elements

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corresponding to the different antigenic types, and these elements appear to run parallel so that absorption by a single type, removing chiefly its own co-agglutinin, does not lower the end-titre, but if two types be employed together the effect is increased; that is the degree of agglutination in the higher titres is still further lessened, and agglutination at full-titre is very feeble or doubtful. When combined absorption by four of these types (*Paratyphosus* B, *Paratyphosus* C, *Mutton* and *Binns*) was attempted there was a definite lowering of the end-point; agglutination at 1/15,000 being a mere trace and even at 1/10,000, the flocculation was not more than half as marked as at 1/20,000 of the control (unabsorbed) serum. This lowering of the end-point by combined absorption cannot be attributed to the greater mass of bacteria employed, as absorption by a similar mass of any of the types separately was without such effect.

Combined absorption at 1/10 by *Paratyphosus* B, *Paratyphosus* C, *Gärtner*, *Mutton*, *Newport*, *Stanley*, *Binns*, and *Reading* removed all agglutinin except that for *Alphonso* and *Type* "G." and the end-titre for these two was reduced to 1/400. This probably represents the full titre of *specific agglutinin*. *Reading* and *Newport* together did not lower the titre below the *Reading* figure 1/3000, and the further reduction to 1/400 must have been brought about by the other types.

A *Type* "G." serum having an end-titre of 1/30,000 was prepared. This serum was as catholic in its affinities as the *Alphonso* serum and gave, even with the homologous bacillus, a similar incomplete type of agglutination. Fig. 4 shows the agglutination of the group by this serum, and it will be seen that, in the types most affected, its action is very similar to that of the *Alphonso* serum.

At a titre of 1/100 *Type* "G." serum was completely absorbed by *Alphonso*.

Salmonella *Type* "G." was isolated at the Lister Institute (1917) from a mesenteric gland of a monkey which died in the course of a dietetic experiment.

Some time before the examination of the *Alphonso* strain was completed, a culture and some of the serum were sent to Sir Frederick Andrewes who tested them with *mono-specific* and *ultra-specific* sera and *specific* (sensu stricto) types of the common salmonellas (but not *Type* "G."). Sir Frederick Andrewes has very kindly given me permission to state that he has not been able to identify *Alphonso* strain with any of these. Unfortunately, repeated attempts to obtain the *specific* type of the bacillus were unsuccessful.

The results of the serum tests described may be taken as definitely establishing the identity of the *Alphonso* bacillus as a strain of *Type* "G." It should however be mentioned that when cross-absorptions were attempted at a serum dilution of 1/10, it was found in the case of the *Alphonso* serum that although absorption by *Type* "G." removed all agglutinin for this strain, a trace active for *Alphonso* remained even after repeated re-absorption by *Type* "G."; the homologous bacillus itself sometimes failed to remove this trace. Similarly absorption of *Type* "G." serum at this titre by *Alphonso* left a trace of agglutinin

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which however was active for both strains. This should not be regarded as indicating any racial or sub-strain difference between the organisms but rather as the natural consequence of attempts to absorb from too great a concentration of a high-titre serum: Eisenberg (1903) showed that absorption of a high-titre serum by a single dose, no matter how great, of the homologous bacillus would not remove *all* agglutinin, and that repeated absorption may sometimes leave a trace of *residual* agglutinin.

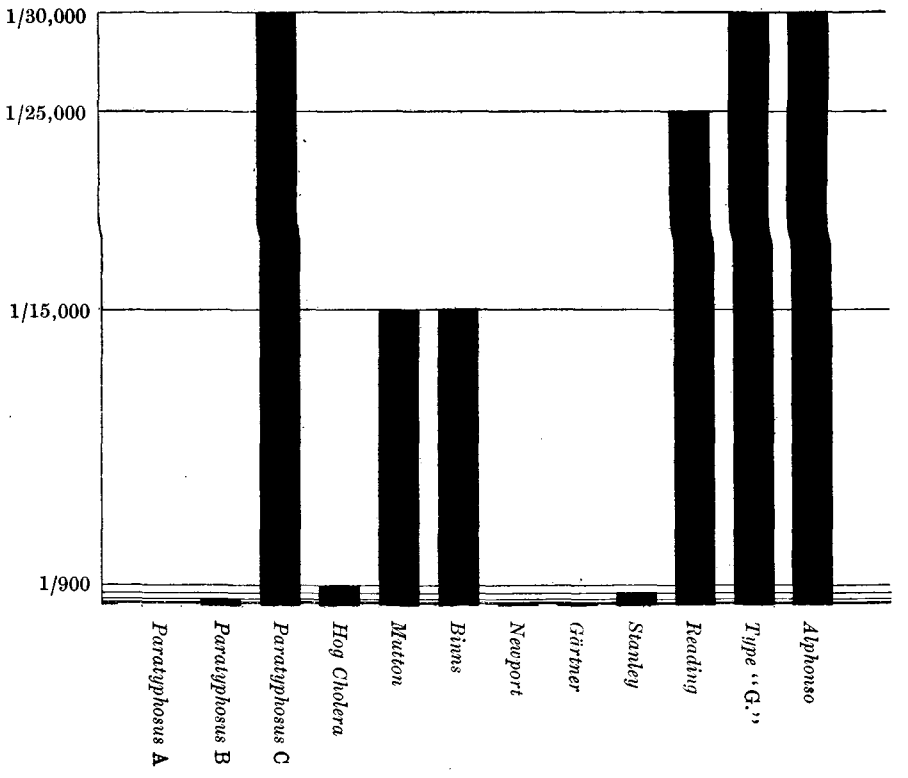

Fig. 4. Agglutination of twelve salmonellas by *Type* "G." serum. End-titres of agglutination: *Type* "G." 1/30,000. *Alphonso* 1/30,000. Type *Reading* 1/25,000. Type *Stanley* 1/600. *B. enteritidis* Gärtner 1/100. Type *Newport* 1/150. Type *Binns* 1/15,000. Type *Mutton* 1/15,000. *Hog Cholera bacillus* 1/900. *B. paratyphosus* C 1/30,000. *B. paratyphosus* B 1/300. *B. paratyphosus* A negative. Agglutination, as in the case of the *Alphonso* serum, was *incomplete* with all strains.

Since its isolation the *Alphonso* strain has always been very *unspecific* and it is desirable that the results of the serum tests should receive confirmation from the examination of a *specific* type. Up to the present all attempts to isolate the specific type have failed, and as the strain in my hands has recently undergone further antigenic degradation, evidenced by a marked reduction in agglutinability and absorbing power, the probability of obtaining such a type seems more remote.

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Examined by new biochemical tests devised by Major H. C. Brown, C.I.E. and myself for the differentiation of the salmonellas, *Alphonso* shows some relationship with *Paratyphosus* C and *Reading*, and it is indistinguishable from *Type* "G." and the Hog Cholera bacillus; these three also give similar reactions with the common fermentation tests. In this connection it is worth repeating that the Hog Cholera bacillus was the only heterologous organism agglutinated by the patient's serum, and although the tests with the high-titre serum did not establish any connection between it and *Alphonso*, it should be mentioned that the only Hog Cholera strain available has undergone great retrogressive change and for serological work is of little value. It is not possible therefore to say what relationship, if any, may exist between *Alphonso-Type* "G." and the Hog Cholera bacillus.

In evaluating the tests a few points in the technique must be stated. In the preparation of the high-titre sera formolised or carbolised suspensions, *sterilised in the cold*, were used. For absorption 6 in. agar plates were sown from agar cultures. The preliminary absorbing dose used was a 48 hours' growth from three or four of these plates to 3·0 c.c. of a 1/50 dilution of serum; the growth being either washed off with the already diluted serum or scraped off and added to it. The absorbing mixture was kept in the cold chamber for two days before centrifuging. In titres below 1/50 re-absorption was done. For all agglutination tests stock suspensions of 20 hour peptone water cultures fixed with 0·1 per cent. formol and diluted to a density about 100 per cent. greater than that of the "Standard" suspensions of the paratyphoids were used, and the tests were incubated at 55° C. for three hours. The denser suspensions were made necessary by the low specificity of the *Alphonso* and *Type* "G." strains.

I wish to express my indebtedness to Sir Frederick Andrewes for kindly allowing me to mention the results of his *specific* tests, to Dr G. C. Low for permission to refer to the clinical case, and to Dr P. H. Manson-Bahr from whom I received the culture.

CONCLUSIONS.

(1) The *Alphonso* bacillus is a Salmonella and apparently a strain of *Type* "G."

(2) It was the cause of a typical enteric fever.

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