THE ISOLATION OF B. TYPHOSUS FROM INFECTED WATER, WITH NOTES ON A NEW PROCESS.

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IT has been estimated that 75% of typhoid epidemics are water-borne, and it is not surprising, therefore, that many attempts have been made to prove the presence of the specific bacillus in the suspected waters, although the bacteriological examination for this purpose has always been recognised as most difficult and uncertain.

The required investigation consists of two distinct processes; first, the isolation of the bacillus, and second, its identification. As regards the process of identification, the application, during the last few years, of the specific reactions of agglutination (Widal-Gruber) and bacteriolysis (Pfeiffer) has rendered the diagnosis reasonably certain, and these two tests together with the morphological and cultural characters now constitute what may be termed a working standard.

For the isolation of the organism, however, there is at present no established method, despite the large amount of attention that has been given to the subject by Continental workers. In the present paper I propose, firstly, to consider very briefly some of the more recent methods that have been advocated for the isolation of the typhoid bacillus; secondly, to enumerate some instances in which the bacillus has been successfully recovered from infected water supplies; thirdly, to describe a precipitation method upon which I have been working, with notes of experiments.

I.

Methods for isolating B. typhosus.

In an infected water the typhoid bacilli are likely to be present in relatively small number, and hence a considerable volume of the water must be examined. It is true that in two or three instances (to be mentioned later) the organism was isolated by direct plating of the water without any preliminary treatment; but the circumstances were exceptional, and as a general rule concentration of the bacterial content of the water must be attempted. The following methods have been used or suggested.

(1) *Filtration.* By passing the water through a bacterial filter, under pressure, all the organisms contained in one or more litres can, in theory, be gathered together in a few c.c. In practice, however, it is certain that a considerable proportion of the organisms is not recovered from the filter. For this and other reasons filtration is being abandoned in the routine bacteriological examination of water for *B. coli*, and therefore cannot be recommended in the more difficult search for *B. typhosus.* (See below, Experiment 9.)

(2) *Chemical precipitation.* The basis of this method is the formation in the water to be examined of an inert precipitate which entangles the bacteria and carries them down.

The following process was devised by a French army surgeon, Vallet[1], and further developed by Schüder[2].

(a) *Vallet-Schüder method.* To two litres of the water to be examined are added 20 c.c. of a 7·75 % solution of sodium hyposulphite and 20 c.c. of a 10 % solution of lead nitrate. A precipitate forms, and this is allowed to settle, or is centrifugalized. The clear fluid above is then drawn off and the precipitate dissolved in a saturated solution of the hyposulphite. From this solution plates of suitable media are inoculated.

Schüder states that a water containing 1,366,000 organisms per c.c., after treatment by this method had only 646 per c.c., left.

(b) *Ficker's method*[3]. Here the precipitating agent is ferrous sulphate. Two litres of water are rendered faintly alkaline with soda solution and 7 c.c. of a 10 % solution of ferrous sulphate added. The precipitate after

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[1] *Archives de Médecine expérim.*, 1901, p. 55.

[2] *Zeitschr. f. Hyg.*, Bd. XLII. 2, pp. 317—326.

[3] *Hygienische Rundschau*, Bd. XIV. No. 1, 1904, pp. 7–9.

settling or being centrifugalized, is dissolved in 25 % solution of neutral potassium tartrate. Ficker claims that 97 to 98 % of the organisms contained in the water are carried down by the precipitate, and that it has no germicidal action on *B. typhosus.*

(c) *Alum method.* When alum (double sulphate of aluminium and potassium) is added to tap water, a gelatinous precipitate of aluminium potassium hydrate forms and slowly sinks to the bottom of the vessel. The remarkable power that this precipitate possesses of entangling and carrying down bacteria has been long known. In 1903 I carried out some experiments to test its action on *B. typhosus*[1]. I found that it had practically no germicidal effect on this organism, and it occurred to me that it might be used, therefore, in examining water for the presence of *B. typhosus.* A description of the process, with experimental results, forms the third part of the present paper.

(3) *Serum agglutination.* The use of anti-typhoid serum for the isolation of the bacillus was first suggested by Windelbandt, in a Russian journal. He added 1 c.c. of the infected water to a number of broth tubes, which were then incubated at 37° C. for 3 or 4 days. To the tubes showing a diffused turbidity some drops of an active anti-typhoid serum were added. If clumps formed they were separated out by centrifugalizing, and the clear fluid above drawn off. The deposit was then emulsified in water, and plates prepared from the emulsion. He claims to have thus recovered the bacillus from a broth culture diluted 10 to 30,000,000 times.

Schepilewsky carried out experiments[2] to test Windelbandt's process. The principle remained the same, but he added 10 to 20 c.c. of the infected water to flasks containing 50 c.c. of broth, and used a better medium for plating out. He claims to have recovered the bacillus from tap water inoculated in the following proportions; (i) 1 loop (diam. = 2 mm.) of a typhoid broth culture in 50 litres; (ii) 1 loop (diam. = 1 mm.) of an agar culture in 10,000 litres; (iii) the same in 100,000 litres. (I quote these figures, as I shall comment on them later.)

Altschüller[3] first converted the water under examination into a nutrient medium by adding peptone and salt, then incubated at 37° C., for 24 hours, and drew off quantities of 10 c.c., to which he added the serum. He claims to have isolated the bacillus from 1 litre of water

[1] *Journ. State Medicine*, Sept. 1903.
[2] *Centralbl. f. Bakt.* Orig. xxxiii. No. 5, 1903.
[3] *Centralbl. f. Bakt.* Orig. xxxiii. No. 9.

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containing 150 typhoid bacilli and two loopfuls of an agar culture of *B. coli.*

Next for consideration are those methods whose aim is to allow the *B. typhosus* to multiply before attempting isolation, or, as they have been termed,

(4) *Methods of enrichment.* The principle here is the utilisation of a fluid medium which will allow *B. typhosus* to multiply and at the same time prevent, or at least retard, the growth of other organisms, above all of *B. coli.* Various fluids have been devised which more or less inhibit the growth of many water and sewage organisms, but all have proved more favourable to *B. coli* than to *B. typhosus,* and hence in the presence of the former organism have been of little value for the "enrichment" of the latter.

Such was the position when, in 1903, Roth announced[1] that by the addition of caffeine to broth it was possible to check the growth of *B. coli* without interfering much with that of *B. typhosus.* His discovery was fully investigated by Hoffmann and Ficker[2] and applied by them in the examination of faeces and of water for the presence of *B. typhosus.* For the latter purpose they convert the water itself into a nutrient medium by adding 1 % of nutrose, 0·5 % of caffeine, and 0·001 % of crystal violet. The mixture is incubated at 37° C. for not more than 12 or 13 hours. At the end of this time typhoid bacilli will be sufficiently numerous to be isolated on plates without difficulty, whereas colon bacilli will be almost or wholly restrained in their growth. In one experiment they took $1\frac{1}{2}$ litres of water containing 63,000 bacteria per c.c. To this they added 1,500,000 *B. coli* and 2000 *B. typhosi.* The water was treated as just described, and they succeeded in isolating *B. typhosus.* In a second case they recovered *B. typhosus* from water in which it was present in the ratio of 1 to 30,000 of other bacteria.

As to the value of the method of Hoffmann and Ficker there can be no doubt, since it has twice given a successful result in actual practice (see Cases 5 and 6 below); but there is experimental evidence to show that the action of caffeine is by no means uniform.

Kloumann[3] selected three strains of *coli* and typhoid, respectively of different age and origin, and tested the action of caffeine upon them in amounts varying from 0·1 to 1 % of the medium employed. The results showed that on the whole the *coli* strains were more restrained

[1] *Hygienische Rundschau,* xiii. pp. 489—91.

[2] *Hygienische Rundschau,* xiv. pp. 1—7, 1904.

[3] *Centralbl. f. Bakt.* Orig. xxxvi. p. 312, 1904.

in growth than the typhoid, but he concludes by stating that there is no strength of caffeine which will at the same time effectively prevent the growth of *B. coli* and allow the increase of *B. typhosus*; the action of caffeine is relative only, not specific; but although not ideal, the caffeine method is a distinct step forward.

Rietsch[1] tried the effect of caffeine on 22 strains of typhoid and 4 of *coli*. He found that the maximum strength of caffeine which permitted the growth of each strain varied exceedingly, and in the final table of order which he drew up the strains of *coli* did not occupy a detached position.

Courmont and Lacomme[2] find that while caffeine will inhibit *coli*, most strains of typhoid will grow. But typhoid bacilli which have been long isolated and also those which have been recently isolated from blood are very sensitive to its action. They mention a case in which typhoid bacilli isolated from the urine of a patient grew well in caffeine media, but when isolated from the blood of the same patient they would not grow.

I have myself tried the action of caffeine on two strains of typhoid, two of *coli*, and one of *enteritidis* Gärtner. Using broth containing $0·5 °/_0$ caffeine, I found that Gärtner's bacillus grew very well, the *coli* strains rather feebly, and the typhoid not at all. None of the organisms were of recent origin.

One must conclude, then, that caffeine cannot be entirely relied upon for the elimination of *B. coli* and its allied forms.

One more process must be mentioned, since it is stated to have been frequently used in Paris in the campaign against typhoid fever.

(5) *The process of Cambier*[3]. This author maintains that under given conditions actively motile bacteria will pass more rapidly through a bacterial filter than feebly motile or non-motile bacteria. He first devised a special peptone solution, containing caustic soda, which he found to be favourable to *B. typhosus*, both as regards growth and motility, but unfavourable to *B. coli* and other organisms in both these respects. In a glass vessel containing this peptone solution is placed a small Chamberland filter candle. The interior of the candle is half-filled with the same solution, to this is added 1 c.c. of the fluid under examination, and the whole apparatus incubated at 37° C. Cambier states that under these conditions, if typhoid bacilli are

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[1] See *Bull. de l'Institut Pasteur*, Vol. II. p. 597, 1904.
[2] *Journ. de Physiol. et de Pathol. génér.* t. VI. 1904.
[3] *Revue d'Hygiène*, 1902, p. 64.

present, they will, owing to their motility, pass through the filter before colon bacilli or other organisms, and will appear in pure culture in the outer peptone solution. To meet obvious objections, he maintains that while a Chamberland filter does not allow bacteria to pass through at ordinary temperatures, it will do so at 37° C. and under the special conditions mentioned. Cambier's process has been tried by several workers, always with results more or less unfavourable. To give one instance, L. Jacqué[1] found that *coli* grew better than typhoid in the peptone solution, and nearly always appeared first in the outer fluid (1—4 days). From the stools of typhoid patients he generally obtained *B. coli* but never *B. typhosus*, and twice he found in the outer fluid a pure culture of a non-motile coccus.

The truth seems to be that the appearance of bacteria in the external solution is not due to direct transit but to growth through the filter at a favourable temperature, and it is not surprising therefore to find that *B. typhosus* is usually shut out by more hardy forms.

(6) *Solid media.* Whatever process may be used for the preliminary treatment of the water under examination, for the final isolation of the bacillus solid media must be employed. Of these media no description has been given so far, as it seemed more convenient to consider them in one group. Mention must be made of Elsner's and carbolic gelatin, since *B. typhosus* has two or three times been isolated from water on these media. (See Cases 2, 3, 4, below.) Despite these successes, however, for obvious reasons gelatin does not commend itself in the search for typhoid, and during recent years special agar media have been devised, the best known of which are (1) bile-salt agar (MacConkey), (2) alkaline glucose agar (Horrocks), (3) neutral lactose agar, and (4) Drigalski-Conradi agar. All these media aim at the same result, viz., the differentiation of typhoid and *coli* by the naked-eye appearance of their colonies. In this they are all four more or less successful, but by general consent the Drigalski-Conradi medium is the best, and is now nearly always employed in the search for *B. typhosus*. As this is the medium adopted by me in the experiments recorded below I will here state my experience of its use and diagnostic value.

The medium of Drigalski and Conradi may be described as "nutrose-lactose-litmus agar with a trace (0·001 %) of crystal violet." Although it has not fulfilled early expectations in the diagnosis of typhoid fever, it is none the less valuable for water work.

[1] *Centralbl. f. Bakteriol.* Orig. xxxvi. p. 300.

After preparation, it is melted and plates are poured in the ordinary way and allowed to set. Before use they should be dried for a few hours in the warm incubator. They are then inoculated by spreading the suspected fluid over the surface and should be incubated at 42° C.

The growth of many saprophytic organisms is prevented or restrained, but *coli*, typhoid, and allied forms grow readily and produce characteristic colonies. After 24 hours coli colonies are well developed, glistening white by *reflected* light, bright red by *transmitted* light, whereas typhoid colonies are smaller, more delicate in appearance, and bluish-white in colour, never producing any change in the medium. *B. enteritidis* (Gärtner) closely resembles typhoid in its colonies; and certain varieties of *B. coli* which have little or no action on lactose are, in my experience, very difficult to distinguish from typhoid until they have grown for at least 48 hours at 42° C. Then a slight redness in the centre of the colony may be seen, but the growing margin remains blue, and the medium is practically unchanged. Other organisms which I have met with on this medium are *B. fluorescens non-liquefaciens, B. pyocyaneus*, streptococci, vibrios, and members of the subtilis and mesentericus groups. (i) The fluorescens and pyocyaneus colonies resemble typhoid, but the extraordinary motility of these bacilli in a hanging-drop preparation generally gives one a clue, and a few subcultures soon settle the diagnosis. (ii) Streptococci are readily distinguished by their very thin, delicate colonies, usually faintly red in colour. (iii) Some vibrio and spirillum forms appear as bluish colonies, but they are not likely to be confused with typhoid. (iv) The subtilis and mesentericus groups present no difficulty. They grow slowly, often not appearing until after 30 hours' incubation, at 42° C., and if the surface of the medium is dry, they do not spread—a point of real value in plate work.

To sum up, this medium is superior to others for separating *coli* and typhoid but is in no sense specific for *B. typhosus*, since several organisms produce upon it colonies of a bluish-white colour which are not easily distinguishable.

Recognizing the limitations of the Drigalski-Conradi medium, further attempts have been made to produce one which shall differentiate *B. typhosus* not only from *B. coli*, but at the same time from closely-allied forms. Among these, the medium devised by Endo, of Tokio[1], has attracted much attention.

Medium of Endo. This is an alkaline lactose agar containing fuchsin, but rendered colourless by the addition of sodium sulphite. Upon it

[1] *Centralbl. f. Bakteriol.* xxxv. 1903, No. 1.

typhoid colonies remain colourless, but *coli* colonies after 20 hours' growth are sufficiently acid to produce a bright red colour.

Petkowitsch[1] instituted a comparison between this medium and that of Drigalski and Conradi. He selected 16 organisms, 6 varieties of so-called paratyphoid bacilli, and 10 varieties of Gärtner's bacillus. He made separate cultures of all these on each medium, and compared the colonies. On the Drigalski-Conradi medium they all produced blue colonies, indistinguishable from each other and from typhoid. On Endo's medium the Gärtner group could be distinguished from the "paratyphoids"; and of these latter three showed slight red coloration, the others, however, resembling typhoid. He considers this medium to be a step forward in the differential diagnosis of *B. typhosus*.

Marschall[2] considers that Endo's medium is in some respects superior to that of Drigalski and Conradi, but Ruata[3] was not favourably impressed, and thinks that it does not rank above other coloured media.

Having now briefly described the principal ways and means for attempting the isolation of *B. typhosus*, I shall next mention the instances I have been able to collect in which the organism has been successfully recovered from infected water-supplies.

II.

According to Lösener, up to 1895 there were 65 cases in which it was claimed that *B. typhosus* had been isolated from water. It is possible that in some of these the bacillus was really that of typhoid, but the evidence of identity is now known to be insufficient and none of these cases can be accepted as conclusive. In the following list only those cases are given in which the diagnosis was confirmed both by agglutination tests and by Pfeiffer's reaction.

(1) Lösener, 1895, from the Berlin water-works. The isolated organism exhibited all the then known characters of *B. typhosus* and was afterwards confirmed as such by Pfeiffer's reaction.

(2) Kübler and Neufeld[4], 1898, from a well on a farm. The bacillus was isolated by direct plating of the water in Elsner's medium, and must have been present in great number. *B. coli* was not found and the well was thought to have been infected by the urine of a typhoid patient.

[1] *Centralbl. f. Bakteriol.* xxxvi. 1904, No. 2.
[2] *Centralbl. f. Bakteriol.* Orig. xxxviii. 1905, No. 3.
[3] *Centralbl. f. Bakteriol.* Orig. xxxvi. 1904, No. 4.
[4] *Zeitschr. f. Hyg.* xxxi.

(3)   Fischer and Flatau[1], 1901, from a well in Schleswig-Holstein.   The bacillus was isolated on carbol-gelatin plates.

(4)   D. Konradi[2], 1902, from a well in Hungary.   The bacillus was isolated by direct plating in carbol-gelatin.   *B. coli* was not found.   Konradi considers that the well was infected by urine.

(5)   Jaksch and Rau[3], 1904, at Prague.   This case is the most interesting and important of the series.   Typhoid fever had been endemic at Prague for years with occasional epidemic outbursts, one of which occurred in the spring of 1904.   Jaksch and Rau determined to examine the water for *B. typhosus*.   They took a sample of the town supply from a tap in the laboratory, treated it by the caffeine process of Hoffmann and Ficker, and plated out on the Drigalski-Conradi medium.   A few red and many blue colonies appeared on the plates.   Two of the latter were selected and eventually identified as *B. typhosus*.   The bacilli answered to every test, including agglutination and Pfeiffer's reaction, and were highly virulent to guinea-pigs and rabbits.

They next examined the water of the river Moldau, which flows through Prague. In the course of eight days they took five samples of river water and from two they isolated bacilli in every respect identical with those from the tap water, and therefore true typhoid organisms.

(6)   Ströszner[4], 1904, from a shallow well near Budapest.   He used the process of Hoffmann and Ficker, and for plating out the mediums of Drigalski-Conradi, and of Endo.   From both he obtained bacilli which answered to all the standard tests for typhoid.   The well had probably been contaminated by urine.

In the following five cases, the isolated organisms reacted positively to agglutination tests, but Pfeiffer's reaction was apparently not applied, so that proof of identity cannot be regarded as complete.

(*a*)   Wilson and Wesbrook[5], 1897, from a public water-supply in Minnesota.

(*b*)   Hankin[6].   From wells, etc. in India.   He used a modification of Parietti's method.

(*c*)   Genersich[7], 1899, from public supply at Pécs in Hungary.   He made carbol-gelatin plates direct from the water.

(*d*)   Tavel[8], 1902, at Olten.

(*e*)   Bonhoff[9], 1902, from a well near Marburg.

[1] *Centralbl. f. Bakteriol.* xxix. No. 8.
[2] *Centralbl. f. Bakteriol.* Orig. xxxv. No. 5.
[3] *Centralbl. f. Bakteriol.* Orig. xxxvi. No. 4.
[4] *Centralbl. f. Bakteriol.* Orig. xxxviii. No. 1.
[5] See *Brit. Med. Journ.* Dec. 1897.
[6] *Centralbl. f. Bakteriol.* xxvi. 1899.
[7] *Centralbl. f. Bakteriol.* xxvii. 1899.
[8] *Centralbl. f. Bakteriol.* Orig. xxxiii. p. 166.
[9] *Ibid.* p. 461.

<div align="center">III.</div>

<div align="center">*Alum Precipitation Process.*</div>

I now proceed to describe the experiments that I have carried out for the isolation of *B. typhosus* from infected water by precipitation with alum. Before coming to details, however, a few words must be said upon the number of typhoid bacilli added to water for experimental purposes. If investigations of this kind are to be of any value some attempt must be made to reproduce numerical conditions that are likely to actually occur in practice. The bacilli must be added to the water in very small amount and the number should be determined.

In describing Schepilewsky's serum process I called attention to the fact that he recovered the typhoid bacillus from water infected in the proportion of 1 loopful (internal diam. = 1 mm.) of an agar typhoid culture to 100,000 litres of water. This statement conveys no real idea of the quantitative value of the process. Using a loop 1 mm. in external diameter, I found that one loopful of an agar typhoid culture contained about 10,000,000 bacilli. Assuming that Schepilewsky in his experiments added the same amount the bacilli were present in the ratio of 1 bacillus to 10 c.c. of water. If it were so, his result was by no means unfavourable, but probably few, even among bacteriologists, would realise that so small a loopful of culture contained so many organisms, as the following instance will show. Schüder's precipitation method was tested experimentally by a bacteriologist in this country, who reported that he had succeeded in recovering *B. typhosus* from 5 litres of water to which one loopful of an agar culture had been added. Assuming that the loop was very small and contained only 5,000,000 bacilli, this would give 1000 bacilli to each c.c. of water, and by plating a few drops on any simple medium the organism could have been at once isolated. The experiment was in fact useless as a test of the value of Schüder's process.

<div align="center">*General details of method employed.*</div>

A stock solution of alum was first prepared in *distilled* water (10 grm. to 100 c.c.). A known quantity of the infected water was placed in a glass vessel and alum added in the proportion of 0·5 grm. to the litre. As soon as the precipitate had completely formed, the vessel was well shaken to evenly distribute its contents and measured quantities were withdrawn and centrifugalized for 15 minutes, at about 2000

revolutions per minute. The clear water of each centrifugalized tube was then syphoned or poured off without risk of disturbing the small mass of precipitate firmly wedged in the conical extremity, about 0·5 to 1 c.c. of fluid being left behind. In this the precipitate was well stirred, taken up in a sterile pipette, and spread over the surface of one or more Drigalski-Conradi plates. These were incubated at 42° C. for 24 to 48 hours.

Occasionally, after adding the alum the water was allowed to stand in a tall cylindrical vessel until the precipitate had completely settled The clear top water was then syphoned off, leaving about 80 c.c. behind. The precipitate was stirred up in this, centrifugalized and plated out as before.

Notes of Experiments.

(D.-C. = Drigalski-Conradi medium. t. b. = Typhoid bacilli.)

No. 1. (Preliminary) 1·5 litres of tap water were placed in a glass flask. A platinum needle was dipped into a 24 hours' broth culture of typhoid to a depth of $1\frac{1}{2}$ inches, and stirred into the flask. Alum was added and precipitate allowed to form. After shaking, 8 c.c. were withdrawn and centrifugalized, and the sediment spread on D.-C. plates and incubated at 42° C. In 24 hours, plates were covered with typhoid colonies in pure culture. From their number, it was estimated that 300,000 bacilli were introduced into flask by needle. After 2 days, flask was well shaken and 0·1 c.c. plated direct on D.-C. medium. 220 typhoid colonies formed, indicating that flask now contained 3,000,000 t.b. After 9 days, t.b. were not found in 8 c.c. of contents.

Remark. Despite the presence of alum, t.b. multiplied for a day or two.

No. 2. A thin needle was dipped for 1 inch into an old t.b. broth culture and stirred into 10 c.c. of water. Of this, 0·1 c.c. was added to 1 litre of tap water, which was well shaken, and 1 c.c. plated direct on D.-C. No typhoid colonies grew. After precipitation and centrifugalization, t.b. were recovered from the water in the proportion of 1 bacillus to 4 c.c.

No. 3. On same lines as Nos. 1 and 2, but 1/50 of a medium-sized loop of t.b. broth was put into the flask. It was found that 1/50 loop contained 7000 bacilli, which were recovered in abundance by precipitation etc.

No. 4. One loopful of t.b. broth (14 days old) was put into 10 c.c. of sterile water. From this dilution 3 loopfuls were put into 1 litre of tap water, and 3 also into a tube of glucose peptone water. This tube took 72 hours to become acid, so that 3 loopfuls of the dilution evidently contained very few bacilli. Flask of tap water, after inoculation, was treated as usual, and eventually 3 typhoid colonies were obtained from 100 c.c. of water.

No. 5. Into 1 litre of tap water typhoid and colon bacilli were introduced, 1 c.c. was immediately plated on D.-C., and was found to contain 15 typhoid and 4 colon bacilli. After precipitation and centrifugalization the two organisms were recovered

in about the same ratio. Thus, a little of the centrifugalized sediment spread on D.-C. gave 36 typhoid and 9 colon colonies. After 24 hours, flask well shaken and again examined. The organisms were recovered in ratio of 17 typhoid to 18 coli. After 3 days, 1 c.c. from flask contained 3 coli but no typhoid. The latter were evidently rapidly dying out.

No. 6. 1/200 loopful of t.b. broth (14 days old) was added to 1 litre of tap water. T.b. were found to be absent from 1 c.c.

A comparison was instituted between the precipitation method and filtration through a Chamberland filter, 500 c.c. being used for each purpose. The final result was that filtration indicated 1 t.b. in 20 c.c., whereas precipitation indicated 3 t.b. in 20 c.c.

No. 7. To determine percentage of t.b. carried down by the precipitate. 1 litre of sterile tap water was inoculated with typhoid, well shaken, and agar-plates prepared, each containing 0·5 c.c. of the infected water. Alum was then added, and some of the water withdrawn and centrifugalized. From the clear water above the sediment agar-plates were prepared as before, each with 0·5 c.c. Both sets of plates were incubated at 37° C. for 40 hours, and the colonies then counted.

Before precipitation, 1 c.c. of water = 125 bacilli.
After   „   „   „   = 16   „   .

Therefore, 87 % of bacilli were carried down by precipitation.

The satisfactory results obtained thus far led me to test the method on a larger scale, and in the remaining experiments here recorded a galvanized iron tank was used.

No. 8. 130 litres of tap water were run into tank, to which was added 1 litre of sterile water containing 120,000 t.b., and the whole well stirred up. After 24 hours, 250 c.c. were removed from the tank, precipitated, centrifugalized and plated out as usual. Typhoid colonies appeared on the plates in due course, but the number suggested that the bacilli had undergone rapid diminution in the 24 hours following their introduction into the tank. This was confirmed later (see No. 10). After 10 days, tank water was again examined, but no t.b. found in 500 c.c.

No. 9. Tank with 130 litres of tap water to which were added 70,000 t.b. well distributed. After 3 hours, (*a*) 500 c.c. were withdrawn and filtered through a hollow Chamberland filter-candle, (*b*) 250 c.c. were withdrawn and treated by alum process. As in No. 6, numerical comparison was instituted between the two methods by counting number of colonies on the two sets of plates. It was found, (*a*) by filtration, 80 c.c. of water averaged 9 to 10 colonies, (*b*) by alum process, 80 c.c. of water averaged 28 colonies—ratio of about 1 to 3, closely agreeing with result in No. 6.

Two days later, tank water was again examined by alum method. T.b. not found in 100 c.c.

No. 10. Tank with 90 litres of tap water and 180,000 t.b. A few hours after introducing t.b. a sample of water was withdrawn and treated as usual. 40 c.c. of sample yielded 45 typhoid colonies. 24 hours later a second sample of water was withdrawn and treated precisely as before. 400 c.c. now yielded 15 typhoid colonies.

Two days later 40 c.c. failed to yield one t. colony. Six days after introduction of bacilli none were recovered from as much as 400 c.c. of water.

Remark. The rapid dying out of t. b. was probably due to two causes: (i) the presence of numerous other organisms, (ii) chemical or electrical action of the zinc-iron coating of the tank[1]. Twice, after the water had been standing in the tank for two or three weeks, a greyish-white film was observed all over the surface. This film was found on examination to consist of zinc hydrate and carbonate.

No. 11. Tank with 100 litres of tap water to which were added 61,000 coli and 35,000 typhoid. 160 c.c. were withdrawn and examined by usual method. In this instance lactose litmus agar had to be used for plating. This medium proved unsatisfactory, much inferior, indeed, to that of D.-C. Most of the plates were spoilt by the rapid growth of "spreaders." One plate, however, on which a very small quantity of precipitate had been spread, yielded four well isolated colonies, two being coli, and two typhoid.

Remark. Despite some unfavourable circumstances t. b. were recovered from water containing twice as many colon bacilli.

Throughout these experiments there was as a rule no difficulty in identifying typhoid colonies on the D.-C. plates. From time to time, however, a colony was selected at random and tested by the agglutination reaction and on various culture media. In any doubtful case these tests were systematically used. In No. 11, for example, the two colonies isolated on lactose agar were fully tested, their appearance, although typical, not being considered sufficient evidence of identity under the conditions of the experiment.

The strain of typhoid used throughout was isolated post-mortem from the spleen a few months before the experiments commenced. The bacillus was actively motile and agglutinated readily with typhoid serum.

The tap water came from an exposed cistern on the roof of the laboratory and usually contained many bacteria per c.c. These gave no trouble on the D.-C. plates, at 42° C. A few sporing forms, streptococci, and occasionally *B. coli*, were most frequently met with.

After the first experiment a large centrifugal machine was used,

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[1] Since the above was written P. W. Bassett-Smith (*Journ. Prevent. Med.* July, 1905) has recorded some experiments on the germicidal action of various metals. He finds that "zinc or iron coated with zinc...after 24 to 48 hours appears to free the water from typhoid organisms," and he considers therefore that a galvanized iron tank is most valuable for storage purposes.

containing 12 tubes, and capable of dealing with 500 c.c. of water at a time.

Conclusions.

Recent methods for isolating the typhoid bacillus have been duly considered, successful results in actual practice have been recorded, and a new precipitation method described. An important question remains to be answered. Out of all these methods and processes which should be chosen to-day for the examination of a water suspected of typhoid pollution? In the routine examination of water for the colon bacillus where a considerable volume has to be searched it is now recognised that the conversion of the water itself into a nutrient medium is the best method to adopt, and there can be no doubt that this is also the best method for the typhoid organism. As already shown, Hoffmann and Ficker have devised a process, dependent on the use of caffeine, by which the typhoid bacillus can be "enriched" at the expense of most other organisms; and further, their process has twice given a successful result in practice. The first choice, then, should undoubtedly fall on the process of Hoffmann and Ficker.

But since caffeine varies in its action and does not favour all races of typhoid bacilli alike, it seems advisable to supplement this process by some further proceeding, to use a popular phrase, "to have two strings to one's bow." Of other methods, precipitation is probably the best, and is certainly the most practicable. I have shown that the alum process is capable of giving good results, and in its chemical details it is rather simpler than Schüder's or Ficker's process.

To sum up. In the examination of a suspected water for the presence of the typhoid bacillus there should be employed side by side the method of Hoffmann and Ficker, and some method of chemical pre-cipitation. This combination would seem to offer the best chance of successfully isolating the organism, thus demonstrating the water to be the actual carrier of infection.

Tabular Summary.

Exam. of water for T. B.

Isolation
- (1) Filtration.
- (2) Chemical precip.
  - Schüder's process.
  - Ficker's "
  - Alum "
- (3) Serum agglutination.
- (4) Enrichment—Hoffmann and Ficker's process.
- (5) Cambier's process.
- (6) Solid media
  - Gelatin (Elsner's, etc.).
  - Bile-salt agar.
  - Glucose and lactose agars.
  - Drigalski-Conradi medium.
  - Endo's medium.

Identification
- Morphological and cultural characters, etc.
- Specific reactions
  - Agglutination.
  - Pfeiffer's, etc.