ON THE VARIATION OF THE *Bacillus Typhosus*.

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In a previous paper¹ I showed that under the influence of the bacteria-free toxins present in a filtered mixture of typhoid urine and water, the *Bacillus typhosus* might change into a bacillus indistinguishable from the *B. fecalis alkaligenes*, and that when this organism was passed through the peritoneal cavity of a guinea-pig, evidences of further change were obtained. I also pointed out that the factors concerned in the passage experiments could not be completely controlled, so that, while a re-conversion of *B. fecalis alkaligenes* into *B. typhosus* would have a definite signification, a conversion of the alkaligenes type into *B. coli* or streptococci must be open to doubt, as these variants are normal inhabitants of the guinea-pig’s alimentary canal, and it is conceivable that the injections might cause changes in the walls of the intestines leading to the passage of intestinal microbes into the peritoneal cavity. Variation resulting from the action of bacteria-free toxins is not open to the same objection, as definite controls can be provided for these experiments. Consequently, in the work which I am about to describe, the experiments were mainly limited to the study of the effects of bacteria-free toxins on the *B. typhosus*. The experiments may be divided into three groups:—

*Group I.*—To study the action on the *B. typhosus* of bacteria-free toxins present in a mixture of typhoid urine and water.

¹ *Journal of the Royal Army Medical Corps, March, 1911.*
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Group II.—To study the combined effects on the B. typhosus of the toxins and living bacteria present in a mixture of Kent well water and typhoid urine.

Group III.—To determine whether it is possible by animal experiment to re-convert the B. fecalis alkaligenes into the B. typhosus.

Experiments in Group I.

To study the action on the B. typhosus of the toxins developed in a mixture of typhoid urine and water.

Forty-five specimens of urine were obtained from twenty-seven recent cases of enteric fever and from one “carrier.” One part of each specimen was at once mixed with nine parts of tap-water, then put in a flask plugged with cotton wool and exposed to light at the laboratory temperature. At periods varying from seventeen to seventy-four days the contents of each flask were filtered through a sterilised Doulton white candle, no pressure being employed. At the end of thirty-six hours the filtrate collected was treated as follows:

(a) One cubic centimetre was added to broth and incubated at 37° C.; if no growth occurred at the end of five days, the filtrate was considered to be sterile and the broth tube was then removed from the incubator and kept in a cupboard, exposed to light, during the whole course of the experiment.

(b) Ten cubic centimetres were placed in a sterile test-tube and inoculated with a small particle of a twenty-four hours’ agar growth of B. typhosus (R), the stock culture which has been used for some years in the manufacture of vaccine in the Royal Army Medical College. The inoculated tube was also placed in a cupboard exposed to light.

(c) The portion of the filtrate remaining in the flask, after removal of the 11 c.c. for tubes (a) and (b), was also kept in a cupboard exposed to light, and control tests were made with it as will be explained later.

Five of the inoculated filtrates showed a growth in the control tube (a) and were at once discarded. The remaining forty filtrates which had been inoculated with B. typhosus (R) were examined weekly; a standard loopful (0.002 grn.) from each tube being spread over the surface of a glucose neutral red bile salt agar plate, which had been previously incubated at 37° C. for forty-eight hours in order to dry the surface of the medium and make certain of its sterility.
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The typhoid bacilli present in thirty-two of the filtrates never showed any deviation from the standard type; but in six of the filtrates changes occurred which will now be described in detail.

Filtrate No. 1.—The urine in the case of this filtrate was obtained from a boy suffering from typhoid fever; he was considered to be in the ninth day of disease when the specimen was collected. Typhoid bacilli could not be isolated from the urine. The mixture of one part of urine and nine parts of tap-water was kept for nineteen days at the laboratory temperature exposed to light and then filtered. The sterile filtrate was inoculated with a very small particle of a twenty-four hours' agar growth of _B. typhosus_ (R). Fourteen days later all the typhoid bacilli appeared to have died out, as no growth was obtained on the plate inoculated with the standard loop, and 1 c.c. of the filtrate added to broth and incubated at 37° C. did not show the slightest turbidity. The filtrate was therefore re-inoculated with a slightly larger quantity of a twenty-four hours' growth of _B. typhosus_ (R). A week later, one standard loopful of the filtrate spread over a glucose neutral red bile salt agar plate produced a pure culture of typhoid bacilli. Fourteen days after the re-inoculation of the filtrate a glucose neutral red bile salt agar plate prepared in the same manner showed red and white colonies. The red colonies when investigated gave all the reactions of typhoid bacilli. The white colonies, however, consisted of highly motile, Gram-negative bacilli which did not ferment glucose, lactose, mannite, cane sugar, salicin, dulcite, or sorbite, after incubation at 37° C. for fourteen days. After twenty-nine days' incubation a slight reddening of the glucose tube was noticed, but the other sugars were quite unaffected. The bacilli did not liquefy gelatine or change neutral red; but in litmus milk they produced a characteristic reaction, the medium being first rendered acid and then strongly alkaline. The bacilli grew well in broth, which acquired a somewhat sickening odour, but indol was not produced. When tested with a potent anti-typhoid serum the bacilli showed no real agglutination; occasionally some aggregation of the bacilli was noticed with a low dilution of the serum, but the clumps never had the appearance of a true agglutination. Moreover, the bacilli had no power of absorbing the typhoid agglutinins from the serum, and when injected into a rabbit failed to produce any typhoid agglutinins in the animal's serum.

There seems to be no doubt that some of the typhoid bacilli added to the filtrate had changed their biological characters and assumed those of the _B. fecalis alkaligenes_. It was always
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noted that the subcultures of the changed bacilli were remarkably vigorous, so the failure to ferment the sugars cannot be attributed to feebleness of growth. By subculturing from agar to agar once a month I have preserved a culture of these bacilli for more than a year, and when last tested they gave the same cultural reactions as I have just described. I do not think there can be any question of the purity of the typhoid culture, which was the parent of the B. faecalis alkaligenes. The culture of B. typhosus (R) has been used for the preparation of anti-typhoid vaccine for some years and has been repeatedly tested by bacteriologists of acknowledged repute. The culture could not possibly have contained any bacilli of the alkaligenes type when I commenced my experiments. It should also be noticed that when the inoculated filtrate was first tested only pure typhoid bacilli were isolated.

Now as to controls: The broth containing 1 c.c. of the original filtrate was kept during the whole time of the experiment and the medium never showed the slightest trace of growth. Moreover, at the moment the change in the typhoid bacilli occurred 0.5 c.c. of the original uninoculated filtrate was plated on glucose neutral red bile salt agar; the plate, however, remained sterile. As the standard loop employed to inoculate the plate containing the alkaligenes type of bacilli only held 0.5 c.c., I think it must be admitted that the amounts used in the control tests were sufficient to prove the sterility of the original filtrate. As the plates also were incubated for forty-eight hours before use there does not seem any loophole through which a contamination could have gained access to the plates or media employed.

Filtrate No. 2.—The urine in the case of this filtrate was passed by a woman who was supposed to be convalescing from enteric fever. The specimen when examined in the usual manner was found to contain the B. coli and a microbe which gave the sugar reactions of B. typhosus, but produced alkali in milk and indol in broth. It was, however, non-motile and quite unaffected by a powerful anti-typhoid serum even in low dilutions. It failed to absorb the specific agglutinins from an anti-typhoid serum, and when injected into a rabbit failed to produce any typhoid agglutinins. A second specimen of urine obtained from the same patient a week later contained, however, B. coli and a B. typhosus which answered to all the usual cultural and serological tests. In view of this discovery I thought it would be interesting to try the effect of intraperitoneal passage on the aberrant microbe. Accordingly twenty-three passages through guinea-pigs were made, but the microbe remained quite unchanged.
The first specimen of urine when received at the laboratory was diluted 1 in 10 with tap water and kept at the room temperature for seventeen days. It was then filtered and the filtrate tested for sterility in the usual manner. The test being satisfactory the filtrate was then inoculated with a small particle of a twenty-four hours' agar growth of *B. typhosus* (R). Fourteen days later all the inoculated bacilli had died out; the filtrate was then re-inoculated with a larger quantity of a twenty-four hours' growth of *B. typhosus* (R). A loopful (0.002 grm.) of the inoculated filtrate when plated a week later on glucose bile salt neutral red agar produced a pure culture of typhoid bacilli answering to all the usual tests. A similar result was obtained at the next testings. But a month after the filtrate had been re-inoculated a loopful (0.002 grm.) plated on glucose neutral red bile salt agar showed the presence of red and white colonies. The red colonies were found to be composed of typical typhoid bacilli, but the white colonies gave exactly the same reactions as the white colonies found in the plate made from filtrate No. 1. The control tests were made as described on p. 504, but the broth tube and plates showed no signs of growth. Evidently some of the typhoid bacilli present in filtrate No. 2 had changed to the alkaligenes type.

**Filtrate No. 9.**—The urine was obtained from a recent case of enteric fever, but no typhoid bacilli could be isolated from the specimen, which appeared to contain *B. coli* in large numbers. The urine was diluted 1 in 10 with tap water and left exposed to light at the laboratory temperature for forty days. It was then filtered and the filtrate tested for sterility. The control broth showing no signs of growth, the filtrate was inoculated with a small particle of a twenty-four hours' agar growth of *B. typhosus* (R). The inoculated filtrate was examined in the usual manner, and at the end of three weeks all the typhoid bacilli had disappeared and had been replaced by bacilli giving all the reactions of the *B. faecalis alkaligenes* isolated in Experiments Nos. 1 and 2, except that the glucose was never acidified after prolonged incubation at 37° C. The original control broth tube, however, showed no growth and 1 c.c. of the uninoculated filtrate planted in broth remained sterile after fourteen days' incubation at 37° C.

**Filtrate No. 11.**—The urine was obtained from a recent case of enteric fever and contained large numbers of typhoid bacilli, a few Gram-staining cocci, but no *B. coli*. It was diluted 1 in 10 with tap water, left exposed to light at the laboratory temperature for forty days and then filtered. The filtrate, having been proved
to be sterile, was inoculated with a small particle of a twenty-four hours' agar growth of *B. typhosus* (R). Through an over-sight the inoculated filtrate was not examined for three weeks, when all the bacilli appeared to have died out. The filtrate was then re-inoculated with a larger quantity of a twenty-four hours' growth of *B. typhosus* (R). A week later one standard loopful of the filtrate was plated on glucose bile salt neutral red agar, and a pure culture of *B. fecalis alkaligenes*, which did not acidify glucose, was obtained. No signs of typhoid bacilli could be discovered on the plate. At this date the original broth control tube showed no growth and 1 c.c. of the uninoculated filtrate planted in broth also proved to be sterile.

The inoculated filtrate was examined from time to time and bacilli of the alkaligenes types were found in gradually decreasing numbers at each examination. No other microbe was ever found even when 1 c.c. of the inoculated filtrate was planted in broth and then plated on glucose bile salt neutral red agar.

**Filtrate No. 21.**—The urine was obtained from a recent case of enteric fever and found to contain many typhoid bacilli, a few *B. coli* and a few Gram-staining cocci. The urine was diluted 1 in 10 with tap water, left at the laboratory temperature exposed to light for fifty days and then filtered. The sterile filtrate was inoculated with a small particle of a twenty-four hours' agar growth of *B. typhosus* (R). Seven days later one standard loopful of the inoculated filtrate was plated on a large glucose bile salt agar plate when only white colonies appeared. These colonies, when fished to agar and planted in the various differential media, were found to be made up of the *B. fecalis alkaligenes*, which did not produce acid in glucose; no signs of typhoid bacilli could be discovered. Meanwhile the control broth tube had shown no change, and 1 c.c. of the uninoculated filtrate planted in broth caused no growth in that medium after fourteen days' incubation at 37° C.

The inoculated filtrate was kept under observation for ten months and plated at frequent intervals; on every occasion the *B. fecalis alkaligenes* was recovered in pure culture, no signs of typhoid bacilli were ever detected.

**Filtrate No. 29.**—The specimen of urine was obtained from a recent case of enteric fever and contained typhoid bacilli, Gram-staining cocci and coliform bacilli. The mixture of urine and water (1 in 10) was kept at the laboratory temperature for fifty-six days and then filtered. The sterile filtrate was inoculated with a small
particle of a twenty-four hours' agar growth of *B. typhosus* (R). Controls were made in the usual manner. The inoculated filtrate was examined at frequent intervals, but only *B. typhosus* in gradually decreasing numbers was isolated. At the end of a month 1 c.c. of the inoculated filtrate had to be planted in broth before a growth of typhoid bacilli could be obtained. At the next examination 1 c.c. of the inoculated filtrate again produced a growth in broth, but the microbe present proved to be a pure culture of *B. fecalis alkaligenes*, which produced no change in glucose. The controls were then examined; the original broth tube containing 1 c.c. of filtrate showed no growth, and 1 c.c. of the uninoculated filtrate when planted in broth produced no growth after three weeks' incubation at 37° C.

It will be noticed that the strains of *B. fecalis alkaligenes* isolated in these experiments fall into two groups:

(a) The strain, isolated in Experiments Nos. 1 and 2, which produced a small amount of acid in glucose. The strain No. 2 was only subcultured for three months after it was isolated, but strain No. 1 has been kept for more than a year, and the growth on agar is now somewhat granular and difficult to emulsify and has acquired a faint yellow colour. In other respects it is unchanged; it is still a highly motile bacillus, slightly smaller than the *B. typhosus*, causes a markedly alkaline reaction in milk after a few days' incubation at 37° C., and produces a small amount of acid in glucose.

(b) The strains isolated in Experiments Nos. 9, 11, 21 and 29. These strains never produced a trace of acid in glucose after incubation for one month at 37° C. As regards morphology and motility they were identical with Nos. 1 and 2. Nos. 11 and 21 have been kept for a year and regularly subcultured once a month. They are quite unchanged, and the growth on agar still resembles that of *B. typhosus*.

Serological tests were then made to ascertain if there were any differences in the reactions of the two groups with specific sera.

As regards anti-typhoid serum both groups gave the same reaction, viz., an aggregation of bacilli, but no real agglutination, with the serum in low dilutions. Both groups also failed to absorb the typhoid agglutinins. The strain isolated in Experiment No. 1 was then injected into two rabbits. Both animals produced a serum which, when diluted 1 in 1,000, readily agglutinated strain No. 1, but failed to agglutinate Nos. 11 and 21, even when the serum was only diluted 1 in 20. The serum, however, when diluted 1 in 100 agglutinated the typhoid bacillus, but higher dilutions
caused no trace of a reaction. The serum was then absorbed with strains Nos. 1, 11 and 21, and with the *B. typhosus*. The results are shown in the following tables:

**Rabbit Serum produced by Strain No. 1. Absorbed with Strain No. 1.**

<table>
<thead>
<tr>
<th>Dilution of serum</th>
<th>1-20</th>
<th>1-100</th>
<th>1-500</th>
<th>1-1,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tested with—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strain No. 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>B. typhosus</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**The same Serum absorbed with *B. typhosus*.**

<table>
<thead>
<tr>
<th>Dilution of serum</th>
<th>1-20</th>
<th>1-100</th>
<th>1-500</th>
<th>1-1,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tested with—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strain No. 1</td>
<td>+</td>
<td>±</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>B. typhosus</em></td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**The same Serum absorbed with Strain No. 11.**

<table>
<thead>
<tr>
<th>Dilution of serum</th>
<th>1-20</th>
<th>1-100</th>
<th>1-500</th>
<th>1-1,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tested with—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strain No. 1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>B. typhosus</em></td>
<td>+</td>
<td>±</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Rabbits were also injected with strains Nos. 11 and 21. It was found difficult to produce agglutinins for these bacilli. At the present time the sera in higher dilutions than 1 in 100 do not agglutinate their own bacilli, but in this low dilution they appear to react equally with the two strains, viz., serum 11 agglutinates strain No. 21 and serum 21 agglutinates strain No. 11.

**Group II.**

To study the effects on the *B. typhosus* of the toxins and bacteria present in a mixture of typhoid urine and unsterilized Kent well water.

Twenty-six specimens of water from the Kent wells were obtained through the kindness of Dr. Houston. To each specimen one-tenth of its volume of typhoid urine, obtained from Carrier I,
was added and the mixture exposed in sterile test-tubes to sunlight at the laboratory temperature. Controls of the water and specimens of urine were carefully tested for the presence of *B. faecalis alkaligenes* and *B. coli*. Neither of these microbes could be detected, although ten times the volume of water and urine placed in the test tubes was subjected to examination. Some of the specimens of urine contained enormous numbers of typhoid bacilli, so that the number of these bacilli added to the samples of Kent well water varied from 5,000 to 5,000,000 per cubic centimetre of the mixture.

The inoculated specimens of well water were examined at frequent intervals for several months.

Sample No. 8 is of peculiar interest as in it after the disappearance of the *B. typhosus* in the fifth week after inoculation with the typhoid urine, a bacillus appeared which corresponded to the *B. faecalis alkaligenes*, isolated in the experiments described in Group I (Nos. 9, 11, 21 and 29), except that the production of alkali was not quite so marked. As regards the appearance of colonies, sugar tests, morphology and motility the bacilli appeared identical.

In the remaining twenty-five specimens of inoculated well water the *B. faecalis alkaligenes* was not found. The *B. typhosus* gradually died out, Gram-staining cocci and bacilli of the *B. fluorescens liquefaciens* group being detected. Both these organisms were present in the typhoid urine. The persistence of the typhoid bacilli in some of the specimens is worthy of note. In the first sample, to which 5,000 bacilli per cubic centimetre were added, 500 typhoid bacilli per cubic centimetre were isolated five and a half months later. In the third specimen containing 6,000,000 bacilli per cubic centimetre 1,000 typhoid bacilli per cubic centimetre were isolated at the end of four months.

**GROUP III.**

To determine whether it is possible by animal experiment to re-convert the *B. faecalis alkaligenes* type (Group I), into *B. typhosus*.

(a) *Intra-peritoneal Passage in Guinea-pigs and Rabbits.*

It has already been pointed out that the conversion of *B. faecalis alkaligenes* into *B. coli* or Gram-staining cocci which are normal inhabitants of the alimentary canal of guinea-pigs and rabbits must be open to doubt. A conversion to *B. typhosus* would, however, be significant, and it appeared worth while to test
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one of the specimens of *B. faecalis alkaligenes* by intraperitoneal passage.

The bacillus isolated in Experiment No. 9, Group I, was selected and subjected to thirteen passages, ten in guinea-pigs and three in rabbits. No change, however, occurred except that the production of alkali in milk appeared to be delayed. The *B. faecalis alkaligenes*, when first isolated in Experiment No. 9, produced a strong alkaline reaction in milk after seventy-two hours' incubation at 37° C., but after intra-peritoneal passage the production of alkali was not marked until after fourteen days' incubation at 37° C.

(b) Intravenous Injection into Rabbits.

Four rabbits were injected intravenously with one-quarter of an agar slope of the *B. faecalis alkaligenes* isolated from filtrate No. 1. The rabbits showed no symptoms; two of them were killed four days and two nine days after the injection. Cultures were made from the bile, scrapings of the gall-bladder, liver, spleen and blood; no growth occurred in any of the tubes. Two other rabbits were injected intravenously with one-quarter of an agar slope of *B. faecalis alkaligenes* isolated from filtrate No. 11. Eight days later the rabbits were killed and subcultures made from the blood, spleen, liver, bile and scrapings of the gall-bladder. No growth occurred in any of the tubes.

(c) Feeding Experiments.

(1) A small black monkey was fed with milk containing the growths on two plates of *B. faecalis alkaligenes* isolated in the first and eleventh experiments described under Group I. No rise of temperature or sign of illness resulted. The stools were perfectly normal and no signs of *B. faecalis alkaligenes* or *B. typhosus* were found in the faeces. Thirteen days after feeding blood was removed from a vein in the leg and planted in a large quantity of broth. The *B. faecalis alkaligenes* was isolated in pure culture after the broth had been incubated for four days at 37° C.

The monkey was kept under observation for several weeks and the blood and faeces were examined at frequent intervals. No signs of *B. typhosus* were detected in the blood or in the excreta.

(2) A small baboon from the West Coast of Africa was fed on three successive days with milk containing a forty-eight hours' growth of *B. faecalis alkaligenes* isolated in Experiment No. 1 of Group
I. An examination of the stools was commenced a week later and blood was also removed from a vein of the left leg and planted in a large quantity of broth. No growth occurred in the broth, and the stools showed only organisms of the \textit{B. coli} type. A week later after a dose of calomel the \textit{B. facalis alkaligenes} was isolated from the faeces. The faeces were examined for a period of four weeks, but no signs of the \textit{B. typhosus} appeared, nor was the \textit{B. facalis alkaligenes} again isolated, although calomel was given on several occasions.

A month later the baboon was fed on milk containing the growth of strain No. 11 on two large agar plates. The stools were examined twice a week for a month, but no signs of strain No. 11 or of the \textit{B. typhosus} were ever detected.

**Conclusions.**

The results of the experiments described in Group I show clearly that under the conditions therein described the \textit{B. typhosus} may be converted into \textit{B. facalis alkaligenes}.

I am unable to afford any evidence as to the epidemiological significance of this variation. The \textit{B. facalis alkaligenes} appears to have no pathogenic effect on ordinary laboratory animals, and it has not been possible to re-convert this variant into the \textit{B. typhosus}. 