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# THE DISTRIBUTION OF BACTERIUM COLI COMMUNE

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THIS work was undertaken at the suggestion of Professor Boyce, in order to study Bacterium coli commune, with special reference to its distribution. The significance and origin of the bacillus, when present, especially in sources of water supply, were points also which required clearing up, viz. :—How far its presence might be considered as an index of pollution by sewage, in which fluid the bacillus is known to exist in large numbers. The experiments described later in this paper were undertaken with these two objects :—(1) To examine carefully, material well-known to be polluted, and, at the same time, material that might be considered unpolluted, in order to see if the respective presence or absence of the B. coli could be established. It was especially important to definitely ascertain whether, for instance, the brooks and rivers of the water supply of a large city like Liverpool normally contained the B. coli commune, and, if so, in what numbers. (2) To see if there was evidence of multiplication of this bacillus in water, soil, dust, etc., if any of these became contaminated; or whether, on the other hand, the presence of the bacillus might be taken as indicative of recent pollution.

A method had to be adopted by means of which the presence of B. coli commune could be demonstrated and a quantitative estimation made. A solid medium was found to be much more convenient than a liquid one; and a carbolized nutrient agar, of which a full description is given later, incubated at a high temperature, has been found most useful for isolating and enumerating the bacillus.

It has frequently been stated that, since B. coli commune is widely distributed in nature and is often to be found in air, soil, and water from all sources, its presence cannot be taken as direct evidence of pollution. Lehmann* describes an experiment in which he found, in yeast, a bacillus closely resembling the typical B. coli commune, and brings this forward as a reason why the presence of such a bacillus is not a sure sign of contamination. Other authors, Kruse,† Stoddart,‡ Lunt,§ think that these examples of B. coli commune, found in unpolluted material, are probably only 'coli-like' bacilli, and that the presence of the typical, intestinal, B. coli is rarer than has been supposed, although various organisms, resembling it more or less closely, are

* *Centralbl. f. Bakt.* xv, p. 350.

† *Zeitschrift für Hygiene*, xvii, p. 1.

‡ *Journ. of Path.* 1897, p. 422.

§ *Proc. Jenn. Inst.*, 2nd series, 1899, p. 249.

undoubtedly to be found very widespread in nature. These 'coli-like' organisms have been described at length by various observers, most recently by LUNT, in the paper already referred to, and by GORDON.* THEOBALD SMITH† is of the opinion that many statements of the widespread distribution of B. coli are without experimental foundation. He considers that, when found, its presence may be regarded as due to direct contamination. But actual experiments on this subject are rarely met with, and it therefore seemed worth while to make systematic experiments with polluted and unpolluted material, to see what significance might be attributed to the presence of B. coli commune. I have taken care to identify the bacillus whenever met with, and I have not found that 'coli-like' bacilli are at all general; among a very great number of examples of B. coli isolated, there were only three that were not typical. In the Tables the presence of B. coli commune always refers to the typical bacillus; and, I think, these investigations also tend to show that its presence is a good index of pollution.

Samples of rain, snow, hail, air, dust, grains and many foods were found to be free from B. coli commune—virgin moorland soil, similarly. Streams and rivulets, not obviously polluted, showed an absence of the bacillus in most cases in the quantities analyzed; while, in the cases in which it was present, it was there in very small quantity. Water from pipes draining the land was also generally free from it. Sewage and sewage effluents, on the other hand, contain B. coli commune in great numbers, and its presence is also easily demonstrated in rivers polluted by sewage.

These experiments have also shown the efficiency of sand and earth as filtering material for removing the bacillus, and have added a few more facts to what is already known‡ as to the vitality of B. coli commune under unfavourable circumstances. These confirm the conclusions of other observers, that, owing to its low vitality, the B. coli undergoes little multiplication in nature outside its normal habitat, viz., in water, dust, etc. This being so, the presence of the bacillus may be taken as evidence not only of pollution but also of recent pollution.

I wish to take this opportunity of expressing my indebtedness to Professor BOYCE for his invaluable help and kind advice during the course of this investigation.

## I. METHOD

The first method tried, which proved to be unsatisfactory, was the well-known one which consists in incubating sewage, etc., properly diluted, with a peptone-salt broth, to which enough phenol has been added to make its concentration in the mixture equal to 1 in 1,000. In the case both of sewage and of sewage effluents the growth obtained consisted largely of liquefying organisms, which outgrew the B. coli commune, although it must undoubtedly have been present. Often, after passage

* *Journ. of Path.*, 1897, p. 438.

† 13*th Ann. Report of State Board of Health for New York*, 1893, p. 712.

‡ Frankland and Marshall Ward, *Proc. Roy. Soc.*, 1893, p. 315. Walliezek, *Centralbl. f. Bakt.* xv, p. 949. Von Freudeureich *Centralbl. f. Bakt.* xx, p. 522.

through a second culture in broth carbolized in the same proportion, a pure culture was obtained by stroking on agar; in some cases, however, the culture so obtained was obviously mixed. Table I gives the details of the former cases—10 samples of crude sewage from West Derby, Chorley, and Dewsbury, yielded pure cultures, all of which were liquefying organisms which also coagulated milk, and 4 of which were spore-bearing. Out of 29 cases of sewage effluents examined, 9 only gave a pure culture of B. coli commune, while 9 gave a liquefying organism, and in 11 cases no growth at all was obtained.

TABLE I

| Description of sample | No. of samples examined | No. surviving 1st incubation, 24 hrs. at 42° C. 1 c.c. in 50 c.c. containing 39 c.c. sterile water and 10 c.c. broth carbolized in the proportion of 5 in 1,000 | No. surviving 2nd incubation, 24 hrs. at 42° C. 1 drop of latter added to 10 c.c. broth carbolized in the proportion of 1 in 1,000 | No. giving liquefying organism, which also coagulated milk, but gave no indol or gas in glucose gelatine | No. giving a spore-bearing liquefying organism | No. giving an organism which agreed with B. coli commune, showing coagulation of milk, indol, gas in glucose gelatine, and no liquefaction, motile bacillus |
|---|---|---|---|---|---|---|
| West Derby Sewage... | 5 | 5 | 5 | 5 | 2 | |
| Dewsbury Sewage ... | 3 | 3 | 3 | 3 | 2 | |
| Chorley Sewage ... | 2 | 2 | 2 | 2 | | |
| Total Sewages... | 10 | 10 | 10 | 10 | 4 | |
| Leeds Dibdin (No. 4) Bed Effluent ... | 1 | 1 | 1 | | | 1 |
| Leeds Lime Bed Effluent ... | 2 | 2 | 2 | 2 | | |
| Leeds Cameron Bed Effluent ... | 2 | 2 | 2 | 1 | | 1 |
| West Derby Farm Land Effluent... | 17 | 7 | 6 | 4 | | 2 |
| Dewsbury Effluent ... | 2 | 2 | 2 | | | 2 |
| Chorley Tank Effluent ... ... | 1 | 1 | 1 | 1 | | |
| Chorley Filter Effluent ... ... | 2 | 2 | 2 | | | 2 |
| Ashton Hall (L'pool) (Experimental) 1st Effluent ... | 1 | 1 | 1 | | | 1 |
| (Experimental) 2nd Effluent ... | 1 | 1 | 1 | 1 | | |
| Total Sewage Effluents ... | 29 | 19 | 18 | 9 | | 9 |
| Total ... | 39 | 29 | 28 | 19 | 4 | 9 |

In the method which I finally adopted, nutrient agar, carbolized in the proportion of 1 in 1,000, was used as a medium for the isolation of the B. coli. I first undertook a series of experiments to determine how great a proportion of phenol might be used without inhibiting too much the growth of the B. coli itself. Control plates of ordinary uncarbolized agar were employed for comparison, and the inoculations were made from pure cultures of B. coli commune. I found that the presence of 2 parts of phenol in 1,000 parts of the medium rendered it quite sterile and 1.75 parts rendered it nearly so; 1.5 parts of phenol exercised great inhibition (Plate I, Fig. 4), and 1.33 parts caused the colonies to be distinctly smaller and fewer in number (Fig. 3). It appeared that 1 part of phenol in 1,000 parts of the medium was the limiting quantity which could safely be employed (Fig. 2).

The medium is prepared as follows:—Nutrient agar is prepared in the usual way, and filtered. While still melted one gram of pure phenol is added to each 900 c.c. of the liquid, which is at once distributed, 9 c.c. being introduced by means of a Lode's distributer into each test tube. These should have been previously plugged and sterilized. The tubes containing the carbolized agar are sterilized for twenty minutes, in a Koch's sterilizer, at 100°, and this has always been found to render tubes and medium quite sterile.

That there is no loss of carbolic acid during this sterilization has been proved in the following way:—Tubes of agar, containing phenol in the proportion of 5 in 1,000, were placed in a Koch's sterilizer; the end of the tube was closed by an india-rubber stopper and delivery tube, the other end of which dipped beneath the surface of water in another test tube. After 20 minutes the water in the receiving test tube, and washings from the delivery tube, were tested with ferric chloride and found to give no trace of colouration, this test having previously been proved delicate enough to demonstrate the presence of phenol in water in a solution containing only .5 parts per 1,000. A 4 per cent. solution of phenol in water, similarly treated, gave the same result; and, even when boiled, gave no trace of phenol in the distillate. Hence one may assume that no phenol is lost during the sterilization, nor during subsequent melting of the tubes before plating.

When a sample is to be analyzed tubes of carbolized agar, prepared as above stated, are melted by being plunged into boiling water, and are then placed in a large water bath, the temperature of which remains constant at 45° to 47° C. One cubic centimetre of the sample, or of the diluted sample, is added to the melted tube, which is then poured. By this addition the concentration of the phenol in the tube is lowered from 1 in 900 to 1 in 1,000. The plates are incubated at 42° C. for 48 hours, and then are examined, experience having shown that practically no more colonies develop after that time.

It was at first feared that the numbers of B. coli on this medium might be diminished, and experiments were made to see if this were so. Pure cultures of

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C. S. SHERRINGTON
RUBERT BOYCE

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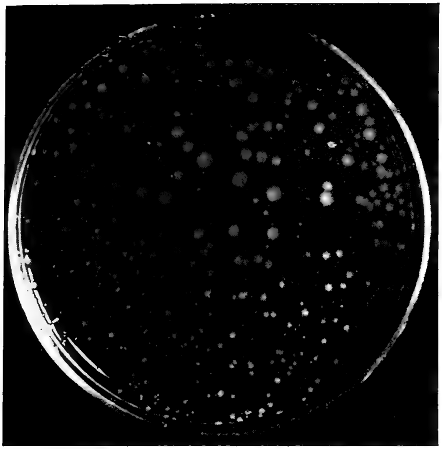

FIG. 1

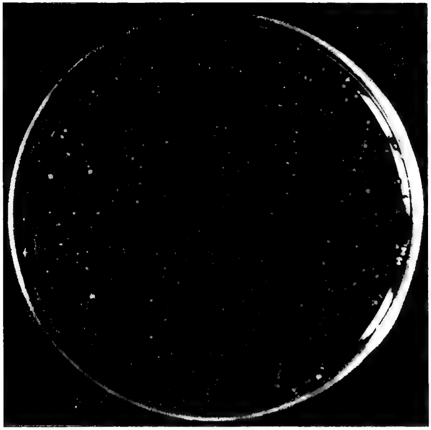

FIG. 2

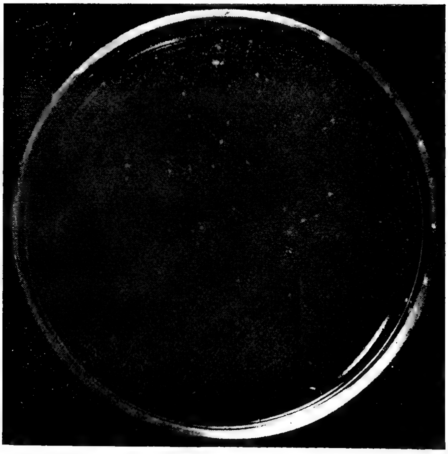

FIG. 3

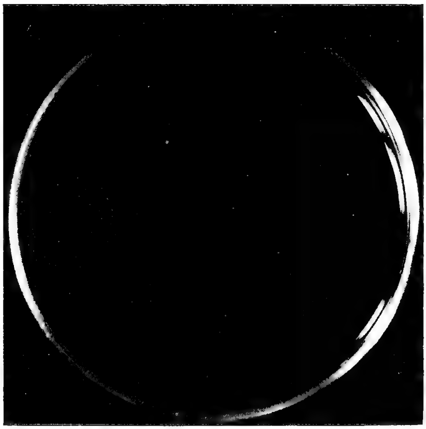

FIG. 4

Photographs of plates made from a pure cultivation of *B. coli commune*, after 48 hours' incubation at 42° C., approximately the same number of organisms being introduced in each case.

Fig. 1—Ordinary nutrient agar.
Fig. 2 „ „ „ carbolized in proportion of 1 in 1000.
Fig. 3 „ „ „ „ „ „ 1.3 in 1000.
Fig. 4 „ „ „ „ „ „ 1.5 in 1000.

B. coli, in broth or peptone-salt solution, were diluted with sterile tap-water (1 in 1,000,000 or 1 in 10,000,000) and plates made of 1 c.c. Comparison was made with ordinary agar, and it was found that, when freshly made dilutions of fresh cultures were used, there was no diminution in numbers on the carbolized medium (Experiments 1, 5, 6, Table II). In Experiment 4 an old culture was used, and in Experiments 2 and 3 old dilutions were used. In this latter case the numbers of B. coli, counted on ordinary agar, decreased in the dilution from 3,505 per c.c., on the day the dilution was made, to 500 two days later, and to 81 at the end of three days. At the same time there was great diminution in the numbers on carbolized agar as compared with those on ordinary agar. It would seem as though—the vitality of the bacillus being lowered by remaining so long in water—it was unable to withstand the carbolized medium. This method, then, gives the number of robust individuals rather than the total number; this, I think, is a point in its favour. It may also be seen from

TABLE II

| Experiment No. | Date | Description of culture and dilution | Temperature | † No. of colonies on ordinary agar plates | † No. of colonies on carbolized agar plates |
|---|---|---|---|---|---|
| 1 | March 24, 1900 | 48 hrs. culture of B. coli commune, diluted 1 in 1,000,000, sterile water and 1 c.c. taken | *37° C. | 3505½ | 5307 |
| | | | *42° C. | 3187 | 2790 |
| 2 | March 26, 1900 | Dilution of Experiment 1, diluted yet 1 in 100, and 1 c.c. taken | 37° C. | 5 | 2 |
| | | | 42° C. | 2 | 0 |
| 3 | March 27, 1900 | Dilution of Experiment 1 used | 37° C. | 81 | 30 |
| | | | 42° C. | 61 | 3 |
| 4 | March 28, 1900 | 8 days peptone-salt culture, diluted 1 in 10,000,000, and 1 c.c. taken | 37° | 11 | 5 |
| | | | 42° | 16 | 4 |
| 5 | April 6, 1900 | 24 hrs. broth culture, diluted 1 in 10,000,000, and 1 c.c. taken | 37° | 469 | 585 |
| | | | 42° | 438 | 422 |
| | | | 20° | | |
| | | | (1) 3 days incub | 455 | 0 |
| | | | (2) 4 days incub. | 505 | 403 |
| 6 | April 7, 1900 | 24 hrs. broth culture, diluted 1 in 100,000,000, and 1 c.c. taken | 37° | 89 | 104 |
| | | | 42° | 114 | 119 |
| | | | 20° | | |
| | | | (1) 3 days | 147 | 0 |
| | | | (2) 23 days | 157 | 84 |

* All plates incubated at 37° or 42° were counted after 48 hrs. incubation.

† Numbers refer to the mean of three enumerations in each case.

Table II that, when incubated at a low temperature, the carbolic acid in the medium has a very marked inhibitive effect (see Experiments 5 and 6), even when no such effect can be observed at a higher temperature.

If B. coli commune is present in fair quantity, and a high dilution is used, as in sewage, the plates yield an almost pure culture of the bacillus and the numbers are easily counted. The plates also give the very characteristic smell which a culture of B. coli upon agar always has. The appearance of the colonies, both large and small, on the surface and in the depth of the medium is always very characteristic, and can usually be at once identified, especially under the low power of the microscope; the typical colony from these plates has, with two exceptions, always proved to be a typical B. coli commune. In material, such as river water, soil, &c., B. coli commune is present, if at all, in very small quantity, and a very low dilution, or none at all, is used. Here the plates by no means show pure cultures of B. coli; they have to be very carefully examined and separate colonies cultivated, in order to be sure whether they are B. coli or not. The commonest organisms occurring along with B. coli, or in its place, are certain micrococci, one of which agrees with M. candicans, and some spore-bearing organisms of the 'subtilis' type. These, if small and in the depth, may look a little like colonies of B. coli, but can be at once detected as different when grown on agar. Others, however, even in such cases, have an appearance at once distinguishable by hairy mycelium-like outgrowths. Constant use has been made of subcultures on the various media to test the B. coli, when found, to see if it is typical. With these precautions the medium has been found useful to demonstrate the presence of B. coli and its quantity with fair accuracy, even in these latter cases.

The B. coli isolated was in almost every case typical. For example, in experiments from January 12th to February 24th, 1900, made for the most part on river waters, &c., which were not highly polluted, 87 bacilli, resembling B. coli, were isolated and their characteristics observed; 84 were typical B. coli commune; one did not coagulate milk, and two gave no gas in glucose gelatine, although they showed typical coagulation of milk. These three bacilli were, in many other respects, similar to the typical colon bacillus. As to the importance of the various tests in deciding whether a bacillus is B. coli commune or not, a few words are necessary. The coagulation of milk has been considered a necessary property of typical B. coli, and many authors have considered that a form, which only differed in not possessing it, was a separate variety. Pioskowski,* however, considers it to be by no means a specific property, but one which can be modified by previous environment. For example, B. coli obtained from his urine media failed to coagulate milk; but if grown many times on ordinary agar, and then in milk, would give the usual coagulation. I have found that all the examples of B. coli isolated gave coagulation of milk, with the exception of the

* *Centralbl. f. Bakt.* xix, p. 686.

one instance already noted. A good deal, however, depends on the character of milk used; it is very necessary that it should not have reached too high a temperature during sterilization. Hence it is best to sterilize three times in KOCH's sterilizer at 100° C., rather than in the autoclave. In the latter case, I found frequently that no coagulation took place, in cases in which it was very marked in milk, which had not been heated above 100°. All the examples of B. coli isolated gave indol to a greater or less degree; whether this test, also, is as useful as has been supposed has been questioned.*

The occurrence of fermentation, formation of gas and acid, in media containing certain sugars seems to be the first and most important of these characteristics; this is what one would expect, as the reaction appears to be the simplest. For example, in a glucose-gelatine shake culture, when the gas is once produced, its

TABLE II*a*

| Description | Date | Dilution | No. on Plate | No. per c.c. |
|---|---|---|---|---|
| West Derby Sand Bed Effluent ... ... ... | Dec. 11, 1899 | ·01 | 1 258<br>2 272<br>3 276 | 25,800<br>27,200<br>27,600 |
| West Derby Sand Bed Effluent (another sample) ... ... | Dec. 11, 1899 | ·01 | 1 158<br>2 225<br>3 193 | 15,800<br>22,500<br>19,200 |
| Effluent from West Derby Dibdin Bed, filtered through experimental Filters. | | | | |
| No. 1 Filter ... | Dec. 12, 1899 | undiluted | 1 18<br>2 18<br>3 31 | |
| No. 2 Filter ... | Dec. 12, 1899 | undiluted | 1 11<br>2 13 | |
| No. 3 Filter ... | Dec. 12, 1899 | undiluted | 1 8<br>2 10<br>3 5 | |
| No. 4 Filter ... | Dec. 12, 1899 | undiluted | 1 14<br>2 15<br>3 16 | |
| Manchester Crude Sewage ... | Dec. 12, 1899 | ·01 | 1 44<br>2 53<br>3 37 | †4,400<br>5,300<br>3,370 |
| Open Septic Tank Liquor, Manchester ... ... | Dec. 12, 1899 | ·01 | 1 26<br>2 27<br>3 34 | 2,600<br>2,700<br>3,400 |
| West Derby Dibdin Bed Effluent ... ... ... | Nov. 8, 1899 | ·01 | 1 114<br>2 105<br>3 111 | 11,400<br>10,500<br>11,100 |
| West Derby Dibdin Bed Effluent ... ... ... | Nov. 9, 1899 | ·01 | 1 34<br>2 37<br>3 35<br>4 33 | 3,400<br>3,700<br>3,500<br>3,300 |

* A. W. Peckham, *Abstr. Centralbl. f. Bakt*, xxiii, p. 986.

† A very low figure for crude sewage.

evidences will remain free from any visible alteration for any length of time. The formation of indol and the coagulation of milk seem to result from much more complicated reactions, and hence are much more likely to be influenced by external conditions. A more exclusive property of B. coli commune than the fermentation of glucose is the fermentation of lactose ; this separates it, for example, from B. enteritidis (Gärtner), which bacillus it closely resembles.* I should add that of the 84 examples of typical B. coli referred to on page 6, 75 were inoculated into lactose-broth, and all that were thus tested showed fermentation of lactose as well as of glucose. In the former case the test was made by inoculating with the organisms fermentation tubes containing broth, from which all other sugars had been excluded, and to which 1 per cent. lactose had been added. Of the three 'coli-like' organisms, mentioned on page 6, the first produced neither acid nor gas in lactose broth in 48 hours, and thus resembled B. enteritidis (Gärtner), while the two others produced acid but no gas in the same medium, even after 12 days.

Except for the three cases already noted, the B. coli isolated have always proved to be typical. The other organisms appearing upon the carbolized agar plates could easily be distinguished from B. coli commune. Out of 183 such organisms which were isolated, 163 were sharply marked off by such unmistakable characteristics, as spore-bearing or a coccus form, while the remaining 20 were unlike, though in a less noticeable manner. Thus, with a few exceptions, such as those noted above, I have not come across examples of the 'coli-like' organisms often described and said to have been found widespread in nature. It may be that this medium inhibits their growth.

Three plates were always poured, and the mean of the three countings was taken for the result ; in most cases fairly concordant numbers were obtained (see Table II*a*, which contains the results of a few enumerations taken at random).

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* H. E. Durham. *Trans. Path. Soc.*, London, 1899, p. 262.

## II. DISTRIBUTION OF B. COLI COMMUNE

### A. HIGHLY POLLUTED WATERS

The highly polluted waters examined included several examples of crude sewage and of septic tank liquors, as well as of effluents from various sewage disposal works. In all these cases the bacillus was present in very large numbers, though filtration, if properly applied, seems to lessen the number very much.

The sewage at West Derby contained B. coli to the extent of 52,000 per c.c. on one occasion, and of 27,470 on another (see Table III). The sewage of Manchester showed a distinctly lower figure, 5,000 per c.c. being the mean of 5 determinations, while that of Leeds gave 19,700 as the mean of 6 determinations.

At Manchester the number of B. coli in the sewage was compared with the number in the septic tank liquors, both of the open septic tanks and of the Cameron. It was found that no increase took place, rather that there was a decrease. It must be noted, however, that the sewage had a distinct tarry odour, and probably contained many substances with antiseptic properties. The same thing was noticed in the case of the sewage and septic tank liquor at Leeds. Whether such a diminution would also take place in cases where the sewage is purely domestic is an interesting point. So far it would seem that the influence of the septic tank in sewage disposal was beneficial in reducing the numbers of B. coli.

The B. coli in sewage or in septic tank liquors can largely be removed by filtration. In the Dibdin system it is by no means eliminated (see Table III); the effluent from the experimental Dibdin beds at West Derby showed nearly 10,000 B. coli per c.c. A second filtration, which was continuous, improved the character of this effluent very much; the numbers decreased to 239 per c.c. when a small experimental sand filter was used, and to 89 per c.c. when earth was used as the filtering material. This latter figure was the average of 18 determinations, and often B. coli was absent altogether, the diminution depending largely on the rate of flow. This result is confirmed by the fact that a very small number of B. coli per c.c. was found in the effluents from the land drains of the West Derby Sewage Farm. Here the average from 7 different drains on January 22nd was only 50 B. coli per c.c.

Under the head of polluted waters one can consider rivers which receive much untreated sewage. The River Severn, at Shrewsbury, showed an average of 55 B. coli per c.c. on one occasion when the river was in flood; on a second occasion the average number per c.c. was 180. The River Alt, which is contaminated by sewage,

## TABLE III

Numbers of B. Coli Commune present in Sewage and other Highly Polluted Waters

| Description | Date drawn | Analyzed | Dilution used | B. coli commune per c.c. | Remarks |
|---|---|---|---|---|---|
| West Derby Crude Sewage ... | 19-10-99 | 21-10-99 | ·01 | 52,000 | ... |
| „ „ | 26-10-99 | 27-10-99 | ·005 | 27,470 | ... |
| Manchester Roscoe Beds, Effluent ... ... ... | 5-12-99 | 6-12-99 | ·01 | 3,900 | Mean from 3 different beds |
| „ Final Contact Bed Effluent ... | Mean of 3 analyses | in Dec., 99, and Ap., 00 | ·01 | 590 | |
| „ Crude Sewage ... | „ 5 „ | in Dec., 99 | ·01 | 5,000 | These samples were plated the same day as they were drawn. Weather very cold, sewage, etc., smelling strongly of tar. |
| „ Cameron Tank Liquor ... | „ 4 „ | „ Dec., 99 | ·01 | 2,100 | |
| „ Open Septic Tank Liquor ... | „ 5 „ | „ Dec., 99 | ·01 | 2,130 | |
| Leeds Crude Sewage ... | Mean of 6 analyses | in Dec., 99, and Jan., 00 | ·01 | 19,700 | Samples came by post and were plated a day after they were drawn. |
| „ Cameron Tank Liquor | „ 7 „ | in Dec., 99, and Jan., 00 | ·01 | 6,500 | |
| „ Septic Tank Liquor (No. 1 Tank) ... | „ 8 „ | in Dec., 99, and Jan., 00 | ·01 | 4,060 | |
| „ Septic Tank Liquor (No. 2 Tank) ... | „ 8 „ | in Dec., 99, and Jan., 00 | ·01 | 4,300 | |
| West Derby Sand Bed Effluent ... | Mean of 5 analyses | ... | ·01 | 9,300 | ... |
| „ Dibdin Bed Effluent ... | „ 19 „ | ... | ·01 | 10,000 | ... |
| Above Dibdin Effluent, after subsequent filtration through small experimental filters. | | | | | |
| No. 1 Sand Filter ... | „ 17 „ | ... | Undiluted | 240 | ... |
| No. 2 Earth Filter ... | „ 18 „ | ... | Undiluted | 90 | ... |
| Land Effluents from West Derby Sewage Farm ... | From 7 different 22-1-00 | drains | Undiluted | 50 | ... |

## HIGHLY POLLUTED RIVERS, Etc.

| Description | Date drawn | Analyzed | Dilution used | B. coli commune per c.c. | Remarks |
|---|---|---|---|---|---|
| Manchester Ship Canal— | | | | | |
| Above sewer outfall ... | 5-12-99 | 6-12-99 | ·01 | 6,100 | ... |
| At sewer outfall ... | „ | „ | ·01 | 3,650 | ... |
| Below sewer outfall ... | „ | „ | ·01 | 3,800 | ... |
| River Alt ... ... ... | 22-1-00 | 22-1-00 | ·01 | 277 | ... |
| River Severn at Shrewsbury... | 11-1-00 | 12-1-00 | ·1 | 55 | River was in flood. Mean of 5 dets. at different places in the town. |
| „ „ „ ... | 14-3-00 | 15-3-00 | ·1 | 190 | Mean of 8 dets. at different places in the town. |
| „ „ „ ... | 30-3-00 | 31-3-00 | ·1 | 180 | Mean of 6 dets. at different places in the town. |

showed B. coli to the number of 277 per c.c. The Manchester Ship Canal is not materially fouled by the addition of the Manchester sewage, containing, as it does, 6,100 B. coli per c.c. before the sewage enters it, and 3,800 afterwards.

## B. NON-POLLUTED SUBSTANCES

The same method of plating with carbolic agar has also been found convenient for the examination of substances that are not obviously polluted. In such cases a very low dilution, or none at all, has been employed, and the plates contain for the most part colonies that are not B. coli; any colony that resembles B. coli, when looked at under the microscope, is investigated further by means of subcultures. As a rule, the distinction can be seen by merely observing the colony, and subcultures are made as a means of confirmation. The colony of B. coli is extremely typical on this medium, especially by its blue opalescence seen by transmitted light, and the oil-drop-like, colourless, appearance when viewed by reflected light. No colonies resemble those of B. coli exactly, though they may be a little like; in these cases a subcultivation on agar and coverslip preparation will often settle the point. As will be seen in cases where there has been no obvious pollution no B. coli is found, and in these cases the plates are often found to be quite sterile.

The substances examined were the following :—

(*a*) Air.
(*b*) Snow.
(*c*) Rain and hail.
(*d*) Dust.
(*e*) Virgin soil.
(*f*) Streams and rivulets.
(*g*) Sand of the sand filters of the Liverpool Waterworks.
(*h*) Filtered and other drinking waters.
(*i*) Grain and other food stuffs.

(*a*.) *Air*.—In the first experiments plates were poured and exposed to the air for 5, 15, 30 minutes. No B. coli were found, either in the air of the laboratory or in the air outside, by these means.

To make a more complete search for the presence of the bacillus the following plan was adopted :—

The air to be examined was drawn through a measured quantity of water in an Erlenmeyer flask, by means of a water pump, and measured by a gas meter. In the tube leading the air from the Erlenmeyer flask to the meter was a wool plug, and after the experiment was completed this plug was added to the water and well washed. Then 1 c.c. of this water was taken and plated. The air of the laboratory was analyzed in Experiments I and II.

*Experiment I.*—250 litres were drawn through in about 25 hours; three plates were made of 1 c.c. of the 100 c.c. in the flask, and all found to be sterile, so that B. coli may be said to be absent in 7.5 litres. Also, 40 c.c. of the water was taken, 10 c.c. of (5 in 1,000) carbolized broth was added, and the mixture incubated for 24 hours at 42°, the proportion of carbolic acid in the mixture being equal to 1 in 1,000. After 24 hours there was slight turbidity in the broth, which when stroked on agar gave a growth which was unlike B. coli, and proved to be a micrococcus. By the phenol-broth method one can say that B. coli was absent in 100 litres ($\frac{40}{100}$ of 250 litres).

*Experiment II.*—A second experiment, in which 200 litres were drawn through at a rate of 10 litres per hour, gave a similar result. By plating on carbolic agar B. coli was found to be absent in 8 litres, and by incubation with carbolized broth in 80 litres.

*Experiment III.*—249 litres of the air outside were drawn through at a rate of about 11 litres per hour, and treated similarly. By carbolized agar plates, B. coli was found to be absent in 15 litres, and, by the phenol-broth method, absent in 199 litres.

In the next experiment the air was drawn through at a much greater rate, so that a great deal more was analyzed. It was found, in the previous experiments, that, of the cotton wool plug between the wash flask and the meter, the end nearest the wash flask became very dirty, showing that the water had but little effect in scrubbing the air, and hence in retaining the bacteria. The wash flask was therefore discarded and the air was passed through a plug of glass wool, which had been tightly packed in a glass tube and then sterilized. Glass wool was used instead of cotton wool, because the former can be so much more thoroughly washed. At the conclusion of the experiment the plug was pushed out, and the tube was washed down with sterile water into a sterile flask ; 10 c.c. of water was used, and the whole thoroughly well shaken and then analyzed. On this occasion the air outside was examined, and in all 2,893 litres were drawn through in about 48 hours, from February 17th to February 19th, 1900. The water was incubated with carbolic broth, and also plated on carbolized agar ; in neither case was B. coli found. By the first method it was found to be absent in 1,157 litres, 4 c.c. being used for analysis; and by the second absent in 867 litres. Adding the two results together, one may say that B. coli was absent in 2,025 litres. The weather at the time was very cold, and snow was on the ground. I am hoping to repeat the experiment in dry, dusty weather.

(*b*) *Snow.*—On February 10th, 1900, snow was collected off the ground in sterile petri dishes, brought into the laboratory and allowed to melt, ·5 c.c. and 1 c.c. being taken for analysis. Four plates were poured, and they were all found to be sterile.

(*c*) *Rain.*—On three occasions rain water was analyzed in order to see if the B. coli was present, but each time no B. coli was found. On January 17th, 1900, the first analyses of rain water were made; rain was collected in sterile petri dishes and 1 c.c. plated on carbol-agar. The plates, after incubation, were found to contain no B. coli. On the same day plates were poured and exposed to the rain with the same result.

On January 24th five samples of rain water were collected from gutters at different places on the roof of the laboratory. B. coli was found to be absent in .5 c.c. in each case.

The last analysis was made on April 3rd, 1900, three samples of rain were collected, as it was falling, in sterile petri dishes. In each case no B. coli was found in 1 c.c.

*Hail* was also analyzed with the same result. Unfortunately, the storm (January 27th, 1900) lasted a very short time, so that only a small quantity could be collected. The B. coli was, however, found to be absent in 1 c.c.

(*d*) *Dust.*—In all five samples of dust were examined; they were obtained on two occasions (February 27th and April 3rd) from different places in the laboratory, from the tops of cupboards, &c., where it was lying thick and undisturbed. The samples were taken by means of a sterile swab, 10 c.c. of sterile water was then poured into the test tube containing the swab and the whole thoroughly well shaken, and 1 c.c. of the mixture taken for analysis. This was the method adopted in the case of four samples, and no B. coli was found. In the case of the fifth sample, the swab was itself shaken successively in three tubes of melted carbolized agar, and these tubes then poured; these plates were also found to be free from B. coli, though by no means sterile. The plates had to be very carefully examined, and many subcultures made of colonies of which it could not definitely be said that they were not B. coli, although they had not the typical appearance. In every case these cultures proved to be an organism other than B. coli. However, for analyzing such substances as dust, earth, sand of filter beds, &c., it must be confessed that the method of plating with carbolized agar is by no means ideal, and requires a great deal of tedious work in examination of separate colonies before definite conclusions can be drawn as to the presence or absence of the B. coli.

On one occasion also the dust underneath the laboratory floor was collected and analyzed, one gram being weighed and shaken up with 20 c.c. sterile water. From this, typical B. coli were isolated, and they were present approximately to the extent of 10 per gram. The fact that the bacillus was found here and not in the dust lying upon the cupboards, etc., may probably be explained by the possibility of a culture of B. coli having at some time been dropped and broken on the floor, and may be traced to a direct infection.

(*e*) *Virgin Soil* (*moorland*).—Samples of moorland soil were obtained from different places on the high ground of the Lake Vyrnwy and Rivington watersheds. In all 15 samples were examined, and the details are given in Table IV. The analysis was made as follows: 1 gram of the soil was weighed into a sterile flask with the help of a sterile platinum spatula; 10 c.c. of sterile water were added in some cases, and 50 c.c. in others. The whole was then thoroughly well shaken and 1 c.c. taken for analysis, and, as usual, three plates of each were poured. The B. coli was found to be absent in all 15 samples, in some absent in approximately .1 gram, in others in .02 gram.

(*f*) *Streams and Rivulets.*—The following experiments have reference to the water from sources which form the main Liverpool water supply. These are two in number ; Lake Vyrnwy acts as one reservoir, while the rest of the water of the city is taken from the Rivington Reservoirs. The water is filtered through sand filter beds at Oswestry and Rivington respectively, and the water supplied to the city consists of a mixture of the two supplies. A very complete analysis was made of the streams and rivulets of the watershed supplying Lake Vyrnwy. In the foregoing sets of analyses the material examined was obviously unpolluted, and B. coli was found absent in every case in the quantities analyzed ; of the previous history of these streams and rivulets one cannot be so sure. The water of thirty-eight streams was examined, 1 c.c. being taken for analysis in each case and three plates poured. The B. coli was found to be absent in thirty-one cases and its presence was demonstrated in seven. In these, however, it was present in very small quantities ; one organism per c.c. in one case, and less than this amount in the other six cases. The details of the sources, etc., of the water examined are given in Table V, and it is very interesting to notice that in nearly all the cases in which B. coli was found, there is very good evidence of the possibility of sewage pollution, and in many instances this had previously been suspected and the analyses were made with a view to clearing up this point.

It was found that, although on the whole the rivulets of the Vyrnwy watershed were free from the B. coli, yet that the sand of certain of the sand filters at Oswestry (see Table VI), used for the subsequent filtration of the Vyrnwy water, contained the bacillus to a considerable extent, so it was thought desirable to examine larger quantities of the water. About 2½ litres were concentrated by filtration through a Chamberland and Berkefeld Filter, the filter brushed with a measured quantity of water and 1 c.c. plated. These experiments have no great numerical accuracy, the object being rather to demonstrate the mere presence or absence of the colon bacillus. The surface water of Lake Vyrnwy, at Masonry Dam, was analyzed in this way two days after having been drawn. The water contained approximately 158 B. coli per litre. The water of the Cedig River, analyzed four days after having been drawn, showed 133 per litre. Probably these figures are rather lower than they should be, as the B. coli tends to decrease in numbers when kept in fairly pure water in a closed bottle. This has been pointed out previously in this paper and is further confirmed by the following experiment. Water from the surface of the lake near the Tower was examined 25 days after having been taken ; B. coli was found to be absent in at least 514 c.c. Unfortunately the same water was not analyzed at the time it was drawn, or shortly afterwards; but it is probable it would then have contained the B. coli, though probably not in 1 c.c.

The rivulets of the watershed of the Lower Rivington Reservoir were similarly examined. The plates of the analyses of January 17th were full of colonies, contrasting with those made from the Vyrnwy waters, which were, in many cases completely sterile. On this occasion the B. coli, when present, was found in greater numbers, about 10 per c.c. in some cases, though definite enumerations were not made. On the other occasions the numbers of B. coli when found were as low as was the case in the Vyrnwy waters. Fifty-three samples were analyzed; B. coli was found to be absent in thirty-eight cases, and present in eleven cases; in four cases its presence was doubtful (see Table V*a*). Here, as was the case with the rivulets of the Vyrnwy watershed, many of the streams shown to contain B. coli have long been suspected of pollution, and in the other cases there is distinct evidence of the possibility. The water of the Rivington Reservoir was also examined in quantity, about $2\frac{1}{2}$ litres being used for analysis. Water from the surface of the reservoir showed the B. coli to be absent in 119 c.c., while water from the depth contained the bacillus to the extent of 27 organisms per litre (approx.). These analyses were made one and three days respectively after the samples had been taken.

The streams and drains flowing into the River Severn near Shrewsbury were also analyzed (see Table V*b*). Seventeen analyses were made, and in twelve cases B. coli was absent in 1 c.c.; in the others it was present to a greater or less degree. Number 11, which contains B. coli commune to the extent of 19 per c.c., enters the River just below Wroxeter, and, from the map, it seems probable that it would be contaminated with domestic sewage. Number 15 is a stream which receives the sewage from Atcham Union Workhouse. There are considerable difficulties in the way of finding out exactly the previous history of such streams and drains as these; probably all those containing B. coli have received some definite pollution. The fact that the majority of the land drains are free from B. coli is, I believe, contrary to a statement that is often made, that this bacillus is to be found in quantity in all cultivated lands, and hence in their drainage. Of course, such water has been subjected to a very fair filtration before it enters the drains.

(*g*) *Sand of Filters used for filtering Drinking Water.*—The water of the Vyrnwy Lake is filtered through sand beds before being used for drinking purposes. It contains the Bacterium coli commune, though to a very small extent. All attempts to detect the presence of the bacillus in the filtered water have failed, even when large quantities have been used for analysis (see below). Hence it seemed probable that the sand beds used for filtering might retain the bacillus, and this has proved to be the case, confirming other experiments on the efficiency of sand as a medium for filtration. The sand of the filter beds was analyzed in the same way as the soils previously described; in these cases a dilution of 1 gram in 10 c.c. of sterile water was invariably used. The details are given in Table VI. Out of 15 samples analyzed, B. coli was absent in .1 gram in 8 cases and present in 7. In one of these cases the approximate number of B. coli per gram of sand was as high as 2,055, while in another place on the same bed it was 20.

Similar analyses were made of the sand of the filters at Rivington, which filter the water of the Rivington Reservoir. The sand of three of the beds was examined; in all 14 analyses were made, and the B. coli was found to be absent in each case (see Table VI*a*.)

(*h*) *Drinking Water.*—Eighteen samples of filtered water from the filters a' Rivington were examined, and in every case B. coli was found to be absent in 1 c.c. (see Table VII). The tap water of the laboratory was also found to contain no B. coli in 1 c.c. This water was also analyzed in quantity on two occasions (March 9th and March 13th, 1900), about $2\frac{1}{2}$ litres being concentrated by means of a Chamberland and Berkefeld Filter respectively, and a portion of the concentrated water used for analysis. In the one case B. coli was found to be absent in 900 c.c., and in the other in 823 c.c. Of course, the Liverpool water supply is unusually excellent.

On February 26th the dead ends of the pipes supplying certain streets in Liverpool were cleaned out by flushing with water, and samples were taken at certain intervals of the dirty-looking water which came out (see Table VII*a*). Sixteen samples were taken and analyzed, and B. coli was found to be absent in every case.

The drinking water of Shrewsbury was analyzed (November 18th, 1899) and showed the presence of B. coli. The water supplied to the town by conduits from a distant source contained 2 B. coli per c.c. The river water, filtered, is also largely used in the town, and contained 17 B. coli per c.c.; this water is of an undeniably polluted origin. The water of the river, at the point where the town supply is drawn off, has on several occasions been analyzed and has always contained the B. coli.

(*i*) *Grain and other Foodstuffs.*—These analyses were made in order to institute a comparison between the respective distribution of B. coli commune and B. enteritidis sporogenes. The duplicate analyses were made by Dr. Balfour Stewart, who also investigated the significance and comparative frequency of the latter organism (see this volume, pp. 31 and 34). In all 19 samples of grain were examined, including English and foreign wheat, both before and after malting, rice, oats, &c. The analyses were made thus:—Different quantities of the grain were placed in a sterile flask, and well washed with 10 c.c. sterile water, 1 c.c. of this being used for plating. B. coli commune was found to be absent in every case (see Table IX). Some samples of crushed grain were also examined; no particular quantities were taken, a little was well shaken up with 10 c.c. sterile water, and 1 c.c. plated as before. B. coli was absent in crushed malt, oat husks, bran, and also in a sample of flour, which was the coarsest quality sold.

The comparative infrequency of the occurrence of B. coli commune in foods may also be seen from Table IX, which contains a summary of food analyses made in the Thompson Yates Laboratories for the Liverpool Corporation, extending over a period of about six months. It will be seen that out of a total of 440 foods analyzed B. coli commune was present only in 19 cases; 17 of these referred to milk, where its presence could easily be explained by contamination occurring in the cowsheds. The remaining two cases were those of mussels, very possibly procured from an estuary, where pollution by sewage is a common occurrence.

## TABLE IV

SAMPLES OF SOIL FROM MOORLAND

| Sample Number | Date | Source | B. coli |
|---|---|---|---|
| 1 | Jan. 27th | Bryn Gwyn Hill ... ... | Absent in .02 gram. |
| 2 | " " | " " " ... ... | " " |
| 3 | " " | " " " ... ... | " " |
| 4 | " " | Fawnog, at back of Tyn-y-Garry, N.E. Side of Lake | " " |
| 5 | " " | | " " |
| 6 | " " | | " " |
| 7 | Feb. 6th | S. side of Hirddu River ... | Absent in .1 gram. |
| 8 | " " | W. side of Eunant River ... | " " |
| 9 | " " | S. side of Iddew River ... ... | " " |
| 10 | " " | N.E. side of Nadroedd River ... | " " |
| 11 | " " | N.E. side of Garneddwen Stream... | Absent in .1 gram. |
| 12 | " " | W. side of Cedig River... ... | " " |
| | March 17th | From that portion of the Rivington watershed which drains into the Yarrow, or 2nd Rivington Reservoir | |
| 13 | " " | 1. A small watercourse running through flat boggy ground ... | Absent in .1 gram. |
| 14 | " " | 2. From steep bank of stream which falls rapidly over a rocky course near the boundary of the watershed ... ... | Absent in .1 gram. |
| 15 | " " | 3. From the top slope of Rivington Pike, a steep bank of exposed soil which drains into River Douglas ... ... | Absent in .1 gram. |

## TABLE V

### Streams of the Lake Vyrnwy Watershed

| No. of sample bottle | Date | Source | B. coli commune | Remarks |
|---|---|---|---|---|
| 49 | Jan. 11, 1900 | Llettyreos Stream ... | Less than 1 per c.c. | |
| 9 | " " | Nant-y-Maes Stream ... | " " | |
| 51 | " " | Pistill-y-Ceunant Stream... | Absent | |
| 59 | " " | Llwynrhiw Stream ... | Less than 1 per c.c. | Very possible contamination from a farm-house on the margin of the stream ; a suspected case |
| 135 | " " | Hirddu River... ... | Absent | |
| 45 | " " | Stream S.E. of Eunant Quarry ... ... | Bottle broken | |
| 34 | " " | Stream N.W. of Eunant Quarry ... ... | Absent | |
| 32 | " " | Eunant River... ... | Absent | |
| 13 | " " | Nant Llwyn ... ... | 1 per c.c. | |
| 143 | " " | Nadweddor Rhiwargor River ... ... | Less than 1 per c.c. | There are dwelling houses near, but their drainage has been carefully attended to ; no apparent cause for suspicion |
| 140 | " " | Iddew or Blaen-y-Coed River ... ... | " " | " " |
| 35 | " " | Alltforgan Stream (taken near house) ... | Absent | |
| 44 | " " | Ceunant Moel-y-Nant Stream ... ... | " | |
| 55 | " " | Garneddwen Stream ... | " | |
| 56 | " " | Ceunant-y-Ffinnant ... | " | |
| 5 | " " | Llechwedd ... ... | " | |
| 11 | " " | Cedig River ... ... | " | |
| 17 | " " | Surface of Lake at Tower | " | |
| 72 | " " | Stream W. of Hendre ... | " | |
| 33 | " " | Stream N. of Hendre ... | " | |
| 67 | " " | Compensation water drawn from near bottom of Lake ... ... | " | |
| 96 | Jan. 22, 1900 | Stream Glyn-du ... | " | There was a gang of men working close to this stream at this date |
| 48 | " " | Stream N.W. of old Nursery ... ... | Present<br>Less than 1 per c.c. | |

## TABLE V—*continued*

| No. of sample bottle | Date | Source | B. coli commune | Remarks |
|---|---|---|---|---|
| 25 | Jan. 22, 1900 | Stream N.W. of Shanty .. | Absent | |
| 64 | " " | Stream N.W. of new Nursery ... ... | " | |
| 50 | " " | Stream between Nant-y-Maes and Pistill-y-Ceunant ... ... | " | |
| 28 | " " | Stream passing through Llwynrhiw House (above house) ... | " | |
| 26 | " " | Stream N.W. of Craig-y-Gribin ... ... | " | |
| 53 | " " | Stream 180 yards S.E. of Eunant Quarry ... | " | |
| 65 | " " | Stream 100 yards S.E. of Eunant Quarry ... | " | |
| 71 | " " | Stream N.W. of Fyn-y-Coed ... ... | " | |
| 132 | " " | Stream 190 yards N.W. of Eunant Quarry ... | " | |
| 57 | " " | Stream S.E. of Garneddwen | " | |
| 1 | " " | Stream by Gwyddaran and Ty-mawr or Ffynnon Dogfan ... ... | " | |
| 8 | " " | Stream by Gwyddaran or 4 miles 4 chains ... | " | |
| 22 | " " | Stream N.W. of Llechwedd | " | |
| 42 | " " | Stream S.E. of Llechwedd | " | |
| 46 | " " | Stream between Cedig Bridge and Glanvyrnwy | " | |
| 18 | " " | Stream S.E. of Vrongoch | " | |

## TABLE V*a*

STREAMS OF THE WATERSHED OF THE LOWER RIVINGTON RESERVOIR

| Date | Source | B. coli commune in 1 c.c. | Remarks |
|---|---|---|---|
| Jan. 17, 1900 | River Douglas, opposite flood stop planks ... ... | Absent | *The carbolized agar plates of this date were so full of colonies that in some cases, where B. coli is stated to be absent it is doubtful whether there may not have been present one or two very small colonies. In cases where it was present, it was frequently found to the extent of 10 per c.c. |
| " " | River Douglas, just below stream through Jephson's Farmyard | Absent | |
| " " | Stream into Douglas diversion, just above ornamental water | Absent | |
| " " | Stream into head of Ettock's Creek... ... ... | Absent | |
| " " | Stream into Ryder's Creek from Ward Hill Farm... ... | Doubtful | There is a house close to the stream |
| " " | Stream between Top-of-Hill Farm and Tanpits ... ... | Present | Pollution from farmyard drainage has been suspected |
| " " | Stream into Taylor's Creek below Great House Farm ... | Absent | |
| " " | Stream below School Brow Farm | Present | A suspected case of pollution |
| " " | School Brook ... ... | Present | Vicarage and cottages close to the stream ; a suspected case of pollution |
| " " | Ditch below 'Black-a-Moor's' Cesspool, inside Reservoir... | Doubtful | A suspected case of pollution |
| " " | Iron outlet pipe inside Reservoir walls ... ... ... | Absent | |
| " " | Outlet of stone drain a few yards to the S. of last ... ... | Absent | |
| " " | Stream below Anderton Hall Stables ... ... ... | Absent | |
| " " | Drain below Anderton Hall Stables ... ... | Present | A suspected case of pollution |
| " " | Land drain in bend of Reservoir | Absent | |
| " " | Similar outlet in next bend to south | Doubtful | No suspicion |
| " " | Outlet of iron drain below Anderton Hall Cesspool ... | Present | |
| " " | Stream, N. boundary of ornamental grounds, Anderton Hall ... ... ... | Absent | |
| " " | Outfall drain at Reservoir gate, Anderton Hall ... ... | Absent | |
| " " | Outfall of land drains below Tanpits Farm ... ... | Absent | |
| " " | Drain which receives overflow from pond in yard of Tanpits Farm ... ... | Present | Has been suspected of receiving Farmyard drainage |

TABLE V*a*—*continued*

| Sample bottle No. | Date | Source | B. coli commune in 1 c.c. | Remarks |
|---|---|---|---|---|
| 110 | * Jan. 29, 1900 | River Yarrow at Corporation boundary ... ... ... | Absent | *The carbolized agar plates were not at all overgrown on this occasion, and contained on an average less than 10 colonies on a plate, and certainly no B. coli in cases in which it is stated to be absent |
| 120 | " " | Trough on Farm Road to Parson Bullvagh's Farm ... ... | Absent | |
| 62 | " " | Outlet end of tunnel from Dean Wood to Yarrow Reservoir ... | Absent | |
| 135 | " " | Water supply to Anderton's Farm, Yarrow Reservoir ... ... | Absent | |
| 128 | " " | Trough in yard of 'Black-a-Moor's Head' Hotel ... ... | Absent | |
| 118 | " " | Public drinking trough, Rivington Village ... ... ... | Absent | |
| | " " | Drinking trough at Haddock's Fold, Anglezark ... ... | Absent | |
| 132 | " " | Stream from Yarrow Reservoir ... | Absent | |
| 7 | " " | Drinking trough at Brook House, Anglezark ... ... | Absent | |
| 89 | " " | Stream at or from Chorley Reservoir | Absent | |
| 126 | Jan. 31, 1900 | Farm trough on bank of Dean's Reservoir (west side) ... | Absent | |
| 73 | " " | Stream flowing into Dean's Reservoir from Peter's Farm ... | Absent | |
| 82 | " " | Small Stream which joins last before flowing into Dean's Reservoir... | Absent | |
| 69 | " " | Well, behind Royal Farm, on Darwen watershed ... ... | Absent | |
| 28 | " " | Stream flowing into Upper Roddlesworth Reservoir, at outlet end of culvert under road, Hollingshead Mill ... ... | Absent | |
| 86 | " " | Stream below Hollingshead Terrace, flows into Upper Roddlesworth | Absent | |
| 22 | " " | Drinking water supply Halliwell Fold Farm ... ... | Present, 1 per c.c. | Suspected pollution from farmstead |
| 93 | " " | Stream flowing down Farm Road from Halliwell Fold into River Roddlesworth ... ... | Present, 1 per c.c. | " " " |
| 129 | " " | River Roddlesworth, Bridge from Halliwell Fold ... ... | Present, 1 per c.c. | " " " |
| 0 | Feb. 1, 1900 | Drain emptying into Lower Rivington Reservoir ... ... | Absent | |
| 61 | " " | Outlet drain emptying into Lower Rivington Reservoir, being overflow from water supply to Tanpits Farm ... ... | Absent | |

## TABLE V*a*—*continued*

| Sample bottle No. | Date | Source | B. coli commune in 1 c.c. | Total Nos. of bacteria per c.c. | Remarks |
|---|---|---|---|---|---|
| 18 | * Feb. 23, 1900 | Stream by roadside, Lower Roddlesworth Farm ... ... | Absent | — | *All the samples of this date were suspected cases with the exception of No. 34 |
| 34 | " " | Small Spring on slope of field in front of Aushaw's Farm ... | Absent | 184 | |
| 132 | " " | Stream below Well in same field as last... ... ... | Absent | 312 | |
| 22 | " " | Overflow from Well in middle of field between Deal Fold Farm and Rake Brook... ... | Present, 1 per c.c. | 1005 | Presence of B. coli agrees with a large increase in total numbers |
| 43 | " " | Overflow from Well below Snape's Farm ... ... ... | Absent | 56 | |
| 122 | " " | Small Stream down hedgeside from Botany Bay Farm to Rake Brook ... ... | Present, 1 per c.c. | Plate liquefied. | Presence of B. coli corresponds with increase in total numbers |
| 48 | " " | From Rake Brook, below inflow of last Stream ... ... | Absent | 392 | |
| 37 | " " | Water lying in grip on field in front of Botany Bay ... | Doubtful (prob. absent) | 1184 | |
| 127 | " " | Stream by roadside issuing from rock, Norcross Farm ... | Absent | | |
| 143 | " " | Standpipe at No. 2 Bridge ... | Absent | | |
| 118 | " " | From Goit below No. 2 Bridge... | Absent | | |

## TABLE V*b*

RIVULETS, DRAINS, ETC., DRAINING INTO THE RIVER SEVERN IN THE NEIGHBOURHOOD OF SHREWSBURY

| No. | Date | Source | B. coli commune per c.c. |
|---|---|---|---|
| 1 | Mar. 14, 1900 | Land Drain, L. bank above Penley Rough ... ... ... | Absent |
| 2 | " " | Land Drain, R. bank opposite Penley Rough ... ... | Absent |
| 3 | " " | Land Drain, above Uffington, L. bank ... ... ... | Absent |
| 4 | " " | Mill Brook, Uffington, L. bank ... | 16 |
| 5 | " " | Land Drain, R. bank, Ford ... | Absent |
| 6 | " " | Land Drain ... ... ... | Absent |
| 7 | " " | Land Drain, Railway Bridge ... | Absent |
| 8 | " " | Land Drain, at Municipal Boundary, below Preston ... | 25 |
| 9 | " " | Land Drain, below R. ... ... | Absent |
| 10 | " " | Land Drain, Bell Brook ... | Absent |
| 11 | " " | Land Drain, below Wroxeter ... | 19 |
| 12 | " " | Cound Brook ... ... | 1 |
| 13 | Mar. 30, 1900 | Land Drain, L. bank ... ... | Absent |
| 14 | " " | Land Drain, Burton's ... ... | Absent |
| 15 | " " | Drain, from Atcham Union Workhouse ... ... ... | 19,000 |
| 16 | " " | Land Drain, Coton's ... ... | Absent |
| 17 | " " | Land Drain, R. Bank, Lower Cound Farm ... ... | Absent |

## TABLE VI

### Sand Filters at Oswestry

| Number of Sample Bottle | Date | Source | B. coli commune Approx. per grm. |
|---|---|---|---|
| 33 | Jan. 26, 1900 | Surface of Test Filters (No. 6) ... | 10 |
| 43 | " " | 6″ Pipe Filters (C) ... ... | Absent. |
| | | Taken from three different places | |
| 44 } | " " | No. 1 Large Filter... ... ... | 38 |
| 72 } | " " | " " | 57 |
| 143 } | " " | " " | 73 |
| 43 | Feb. 10, 1900 | No. 2 Filter ... ... ... | Absent in .1 grm. |
| 33 | " " | No. 2 Filter (a different place) ... | 3 |
| 127 | " " | No. 4 Filter ... ... ... | Absent in .1 grm. |
| 120 | " " | No. 4 Filter (a different place) ... | Absent in .1 grm. |
| 114 | " " | No. 5 Filter ... ... ... | 2055 |
| 40 | " " | No. 5 Filter (a different place) ... | .20 |
| 143 | " " | No. 7 Filter .. ... ... | Absent in .1 grm. |
| 122 | " " | No. 7 Filter (a different place) ... | Absent in .1 grm. |
| 118 | " " | No. 8 Filter ... ... ... | Absent in .1 grm. |
| 18 | " " | No. 8 Filter (a different place) ... | Absent |

## TABLE VI*a*

### Filters at Rivington

| Number of Sample Bottle | Date | Source | B. coli commune |
|---|---|---|---|
| 5 | Jan. 29, 1900 | From surface of No. 6 Filter. Taken with full head of water on bed. This bed has been worked up for 60 days since last cleaning ... ... | Absent in .02 grm. |
| 141 | " " | Same as last ... ... ... | Absent in .1 grm. |
| 68 | " " | From surface of No. 7 Filter. Taken after head of water had been reduced to 1 foot. This bed has been in work for 38 days since last cleaning ... | Absent in .02 grm. |
| 7 | " " | Same as last ... ... ... | Absent in .1 grm. |
| 57 | Feb. 24, 1900 | Surface of Filter No. 7, bed in use since Dec. 22nd, 1899 ... | " " |
| 134 | " " | " " " ... | " " |
| 57 | " " | " " " ... | " " |
| 134 | " " | " " " ... | " " |
| 65 | Mar. 10, 1900 | Six different Places in No. 5 Filter Bed, along centre line equally distant from each other | " " |
| 64 | " " | | " " |
| 115 | " " | | " " |
| 17 | " " | | " " |
| 1 | " " | | " " |
| 137 | " " | | " " |

## TABLE VII

### Filtered Waters, Rivington

*Analyzed March 5th,* 1900

| Bottle No. | Description of Sample | B. coli commune in 1 c.c. |
|---|---|---|
| 118 | No. 8 Filter, working since January 1st ... ... | Absent |
| 122 | Well Hole ... ... ... ... .. ... | " |
| 31 | Clear Water Tank No. 2 ... ... ... ... | " |
| 43 | Feeder No. 2... ... ... ... ... ... | " |
| 22 | No. 1 Filter, working since December 23rd, 1899 ... | " |
| 33 | Well Hole ... ... ... ... ... ... | " |
| 121 | Clear Water Tank No. 1 ... ... ... ... | " |
| 80 | No. 1, Filter working since January 3rd ... ... | " |
| 19 | Water Hole ... ... ... ... ... ... | " |
| 140 | No. 3, Filter working since January 6th ... ... | " |
| 135 | Well Hole ... ... ... ... ... ... | " |
| 49 | No 4, Filter working since December 11th, 1900 ... | " |
| 132 | Well Hole ... ... ... ... ... ... | " |
| 48 | No. 5, Filter working since December 9th, 1899 ... | " |
| 47 | Well Hole ... ... ... ... ... ... | " |
| 127 | No. 6, Filter working since December 1st, 1899 ... | " |
| 114 | Well Hole ... ... ... ... ... ... | " |
| 34 | Feeder No. 1 ... ... ... ... ... | " |

## TABLE VII*a*

### Dead Ends

| Date | Place | Time | B. coli commune in 1 c.c. |
|---|---|---|---|
| Feb. 26, 1900 | Pembroke Place ... ... Ashton Street, Liverpool | 1st minute ... | Absent |
| | | 2nd „ | „ |
| | | 3rd „ | „ |
| | | 5th „ | „ |
| | | 6th „ | „ |
| „ „ | Prinfield Street, and of Chatham Place, Liverpool | 1st minute ... | Absent |
| | | 2nd „ ... | „ |
| | | 3rd „ ... | „ |
| | | 4th „ ... | „ |
| | | 5th „ ... | „ |
| | | 6th „ ... | „ |
| „ „ | Smithdown Road, Liverpool | 1st minute ... | Absent |
| | | 2nd „ | „ |
| | | 3rd „ | „ |
| | | 4th „ | „ |
| | | 5th „ | „ |

## TABLE VIII

### Grains

| Description | Quantities used for Analysis | | B. coli commune |
|---|---|---|---|
| | Sterile water | No. of grains | |
| Wheat (English)... ... ... | 10 c.c. | 3 | Absent |
| Wheat (Foreign) ... ... ... | 10 c.c. | 3 | Absent |
| Barley ... ... ... | 10 c.c. | 3 | Absent |
| Barley (Malted) ... ... ... | 10 c.c. | 3 | Absent |
| Barley (Malted and Crushed) ... | 10 c.c. | — | Absent |
| Rice (Rangoon) ... ... ... | 10 c.c. | 5 | Absent |
| Rice (Java) ... ... ... | 10 c.c. | 24 | Absent |
| Peas (English) ... ... ... | 10 c.c. | 1 | Absent |
| Peas (Wisconsin) ... ... | 10 c.c. | 4 | Absent |
| Peas ... ... ... ... | 10 c.c. | 4 | Absent |
| Haricots (Foreign) ... ... | 10 c.c. | 4 | Absent |
| Green Lentils (Whole) ... ... | 10 c.c. | 12 | Absent |
| Rye ... ... ... ... | 10 c.c. | 24 | Absent |
| Oats ... ... ... ... | 10 c.c | 24 | Absent |
| Oat Husks ... ... ... | 10 c.c. | — | Absent |
| Bran ... ... ... ... | 10 c.c. | — | Absent |
| Flour (very Coarse) ... ... | 10 c.c. | — | Absent |
| Hemp ... ... ... ... | 10 c.c. | 12 | Absent |
| Linseed ... ... ... | 10 c.c. | 24 | Absent |

# TABLE IX

LABORATORY ANALYSES OF FOODS, NOV. 11TH, 1899, TO APRIL 30TH, 1900

*(See also this volume, p. 37)*

| Description of Food | No. of samples examined | No. of samples in which B.coli commune was present |
|---|---|---|
| Drinking Water ... ... ... | 53 | 0 |
| Milk (New) ... ... ... | 239 | 17 |
| Milk (Skim) ... ... ... | 3 | 0 |
| Milk (Condensed) ... ... ... | 10 | 0 |
| Butter ... ... ... ... | 12 | 0 |
| Cheese ... ... ... ... | 4 | 0 |
| Margarine... ... ... ... | 4 | 0 |
| Oatmeal and Flour ... ... ... | 11 | 0 |
| Tinned Meat, Fish, &c. ... ... | 22 | 0 |
| Shell-fish (Fresh) ... ... ... | 66 | 2 |
| Sausages ... ... ... ... | 1 | 0 |
| Fluid Beef ... ... ... | 1 | 0 |
| Suet ... ... ... ... | 1 | 0 |
| Worcester Sauce, &c. ... ... | 2 | 0 |
| Preserved Fruits and Jams ... ... | 11 | 0 |
| TOTAL ... ... ... | 440 | 19 |

# ON THE DISTRIBUTION OF BACILLUS ENTERITIDIS SPOROGENES

By C. BALFOUR STEWART, M.A., M.B. Camb., Assistant Bacteriologist to the Liverpool Corporation

Plate II

In connection with some fish poisoning cases which occurred in Liverpool last September, where death was due to enteritis associated with the presence of Bacillus enteritidis sporogenes of Klein, I had occasion to examine samples of fish procured from the same stores that supplied the fish which was incriminated in these cases, and from that I was led to examine other substances with a view to discover, if possible, the source from which the germ is distributed in nature.

That the germ occurs very widely spread has been observed by its discoverer, Klein, and others; but so far it has been looked for and found chiefly in what may be called pathogenic substances, as diarrhoeal stools, horse dung, sewage, etc., and in milk, where its presence was taken as denoting contamination.

The object of this paper is to draw attention to the fact that Klein's Bacillus enteritidis sporogenes occurs very widely distributed in quite innocent substances. Any remarks I may have to make on the cultural characteristics of the bacillus will be reserved for a future occasion; but I may here mention that I have found it by no means so easy to obtain a pure culture of the bacillus on solid media as Klein states in his paper in the Local Government Board Report for 1897-8. The reason may be that, from the nature of my samples, there were many other spore-bearing micro-organisms present which ran through the milk cultures and appeared on the glucose agar tubes inoculated from the milk; and even, in some cases, appeared in solid media cultures inoculated from the exudation of an animal killed by an injection of the milk culture. Often no growth at all on solid media was obtained from the exudation of a dead animal or from a milk culture, although a milk tube inoculated with the same grew and showed the typical reaction. Milk is a particularly good medium for the growth of the Bacillus enteritidis; whereas, as is well known, anaerobes in general do not grow well on solid media. It was found, by experiment, that if a milk tube was inoculated with B. enteritidis and some other micro-organism, as B. mesentericus, B. cadaveris (Klein), B. coli, the tube would show the typical reaction of B. enteritidis sporogenes after incubation for 18 hours.

In all these experiments litmus milk tubes were inoculated with a small portion of the sample; in the case of the milk which was done in the ordinary course of analysis for the Liverpool Corporation about 10 c.c. was placed in a sterile test tube.

The litmus milk, or plain milk tubes, were then placed in an apparatus described by me in another part of these Reports, where they were kept automatically at a temperature between 75° C. and 80° C. for 20 minutes. They were afterwards placed in wide mouth stoppered bottles, into which the requisite amount of pyrogallic acid and caustic potash was poured to insure anaerobiosis ; the bottles were then placed in the incubator at 37° C.

Usually all the tubes that 'went' typically had 'gone' after 18 hours' incubation. By 'typical' I mean the reaction described by KLEIN : the milk is clotted, and the clot turned into clear whey with an acid reaction, a broken or scattered layer of cream remaining on the top broken up by the gas which may be seen evolved in bubbles from the whey. If litmus milk was used, the whey and cream which was bleached in the anaerobic bottle will be seen gradually to assume a pink colour on being exposed to the air, instead of its original violet colour ; this is due to the altered reaction. The tube generally smells more or less of butyric acid.

With many of my samples the butyric acid smell was masked by a putrid smell ; sometimes both could be distinguished ; this occurred chiefly in the samples of grains, and was probably due to the presence of some other spore-bearing micro-organism causing putrefactive changes. On making one or two subcultures in milk, or a milk culture from the exudation of an animal killed by injection of a culture with a putrid smell, the smell would often be found to have disappeared and the milk culture to be in every respect typical. A certain proportion of tubes 'went' in the ways KLEIN describes as atypical and intermediate. An atypical tube is one devoid of gas formation, and, consequently, the layer of cream remains unbroken ; in the intermediate type the clot is not fully peptonised into whey, the tube contains one to two-thirds excavated clot and the rest is whey, there is also less gas formation.

With the following samples whenever a tube 'went' typically, atypically, or in the intermediate way, a portion of the whey was inoculated into Guinea-pigs beneath the skin over the abdomen ; 2 c.c. of the whey were inoculated in most cases, and, as the Guinea-pigs were all fully grown, this dose would correspond to KLEIN's dose for standard virulence, namely, 1 c.c. per 200-300 grams of Guinea-pig. According to KLEIN, the pathogenicity to Guinea-pigs is the means of distinguishing his Bacillus enteritidis sporogenes from BOTKIN's butyric acid bacillus, which gives the same reaction in milk cultures, but is non-pathogenic. As a result of subcutaneous inoculation, if the culture is virulent, the Guinea-pig will be found dead in 18 to 36 hours. On examination it will be found that the skin is separated from the subjacent tissues over a considerable area by dissolution of the connective tissue ; the skin may have sloughed through to the outside, or the muscles of the abdominal wall may have sloughed into the abdominal cavity. The space between the skin and the abdominal and thoracic walls contains more or less badly-smelling sanguineous fluid, but the fluid may be very scanty or almost absent in the most rapidly fatal cases.

If the culture is less virulent the post-mortem appearances will be modified, the separation of skin will be over a less extensive area, and if the culture is very little pathogenic there may only be a slight separation of skin with only a small quantity of fluid in the space. In these cases the fluid is gradually absorbed and the separated skin dries, and after one to two weeks sloughs off, leaving an ulcer. In a few cases the whey is quite non-pathogenic ; it is absorbed, and the animal shows no signs of being ill.

1. *Dried Salted Codfish.*—A litmus milk tube was inoculated with a small shred ; after 48 hours' incubation it had 'gone' typically. 2 c.c. whey were inoculated into a Guinea-pig. Result—Died in 18 hours.

2. *Dried Salted Haddock.*—A litmus milk tube was inoculated with a small shred ; after 18 hours it had 'gone' typically. 2 c.c. whey were inoculated into a Guinea-pig. Result—It was found dead in 16 hours, the skin over the abdomen and thorax was completely separated from the subjacent muscular tissue and there was a considerable quantity of badly-smelling sanguineous fluid in the space.

3. *Boiled Cod and Potatoes.*—Litmus milk tubes were inoculated with small portions of the fish and of the potato with negative results for B. enteritidis sporogenes.

4. *Salted Ling.*—A litmus milk tube was inoculated with negative results for B. enteritidis sporogenes.

5. *Salted Cod.*—A litmus milk tube was inoculated with a small shred ; after 18 hours' incubation it had 'gone,' but the whey was slightly yellow and had a putrid smell, a subculture of this in litmus milk 'went' and smelt of butyric acid. A Guinea-pig was inoculated with 1 c.c. of the whey from the first tube. Result—Non-pathogenic.

6. *Salted Haddock.*—A litmus milk tube was inoculated with a small shred with negative result for B. enteritidis sporogenes.

7. *Salted Cod.*—A litmus milk tube was inoculated with a small shred with negative result for B. enteritidis sporogenes.

8. *Salted Cod.*—A litmus milk tube was inoculated with a small shred from a clean part of the fish with negative results for B. enteritidis sporogenes. Another tube was inoculated with a shred from a spot where there was a mark as if from a dirty finger ; after 18 hours' incubation this tube had 'gone' typically. A Guinea-pig was inoculated with 1 c.c. of the whey. Result—Died in 30 hours and had separation of the skin and red fluid in the space.

9. *Salted Cod.*—A litmus milk tube was inoculated with a small shred. There was little change after 18 hours' incubation, but after some days it had 'gone.' 2½ c.c. whey were inoculated into a Guinea-pig. Result—Died in about 40 hours with complete separation of skin over thorax and abdomen.

10. *Salted Cod.*—A litmus milk tube was inoculated with a small shred ; after 18 hours incubation it had 'gone' atypically and had a putrid smell. A Guinea-pig was inoculated with 2 c.c. whey. Result—Died in 48 hours with considerable separation of skin and sloughing of abdominal muscle.

11. *Salted Cod.*—A litmus milk tube was inoculated with a small shred with negative results for B. enteritidis sporogenes.

12. *Salted Haddock.*—A litmus milk tube was inoculated with a small shred ; after 18 hours' incubation it had 'gone' typically and well, the cream being scattered. A Guinea-pig was inoculated with 2 c.c. Result—Pathogenic but not fatal.

13. *Salted Cod.*—A litmus milk tube was inoculated with a small shred with negative results for B. enteritidis sporogenes.

14. *Salted Haddock.*—A litmus milk tube was inoculated with a small shred; after several days incubation it had 'gone.' A Guinea-pig was inoculated with 2½ c.c. whey. Result—Non-pathogenic.

15. *Salted Ling.*—A litmus milk tube was inoculated with a small shred with negative results for B. enteritidis sporogenes.

16. *Salted Cod.*—A litmus milk tube was inoculated with a small shred; after several days' incubation it had 'gone.' 3 c.c. whey were inoculated into a Guinea-pig. Result—Non-pathogenic.

Thus, in 16 samples of fish 6 gave cultures of B. enteritidis of which 5 were fatal when inoculated into Guinea-pigs.

## Grains taken from Sample Boxes in a Saleroom of a Wholesale Firm

(See Plate II)

*Inoculated into milk incubated anaerobically* 18 *hours at* 37° *C.*

1. *English Wheat.*—Milk 'went' in intermediate way; putrid smell. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours; extensive separation of skin; very little exudation.

2. *Foreign Wheat.*—Milk 'went' typically; cream scattered; slight putrid smell. Guinea-pig inoculated with 2 c.c. whey. Pathogenic but not fatal.

3. *Bran.*—Milk 'went' well; cream raised to the cotton plug. Guinea-pig inoculated with 2 c.c. whey. Died in 18 hours.

4. *Oats.*—Milk 'went' slowly and not typically. Guinea-pig inoculated with 2 c.c. whey. Pathogenic but not fatal.

5. *Ground Oats.*—Milk 'went' typically. Guinea-pig inoculated with 2 c.c. whey; died 36 hours; much separation of skin; little or no fluid.

6. *Oat Husks.*—Milk after re-incubation had clotted; there was very little whey and no gas. Guinea-pig inoculated with 1 c.c. whey; non-pathogenic. Not B. enteritidis.

7. *Barley.*—Milk 'went' well; cream raised to the cotton plug. Guinea-pig inoculated with 2 c.c. whey; sloughing, ulcer. Pathogenic but not fatal.

8. *Malt*, *i.e.*, *Kilned Barley.*—Milk 'went' only after re-incubation, and then not well. Guinea-pig inoculated with 2 c.c. whey; ulcer. Pathogenic but not fatal.

9. *Crushed Malt.*—Milk did not 'go'; a little whey formed, rest was clot; putrid smell. Guinea-pig inoculated with 1 c.c. whey; non-pathogenic. Not B. enteritidis.

10. *Rye.*—Milk did not 'go' till after re-incubation, when a yellowish whey formed with a putrid smell. Guinea-pig inoculated with 2 c.c. whey; died in 36 hours with much separation of tissues.

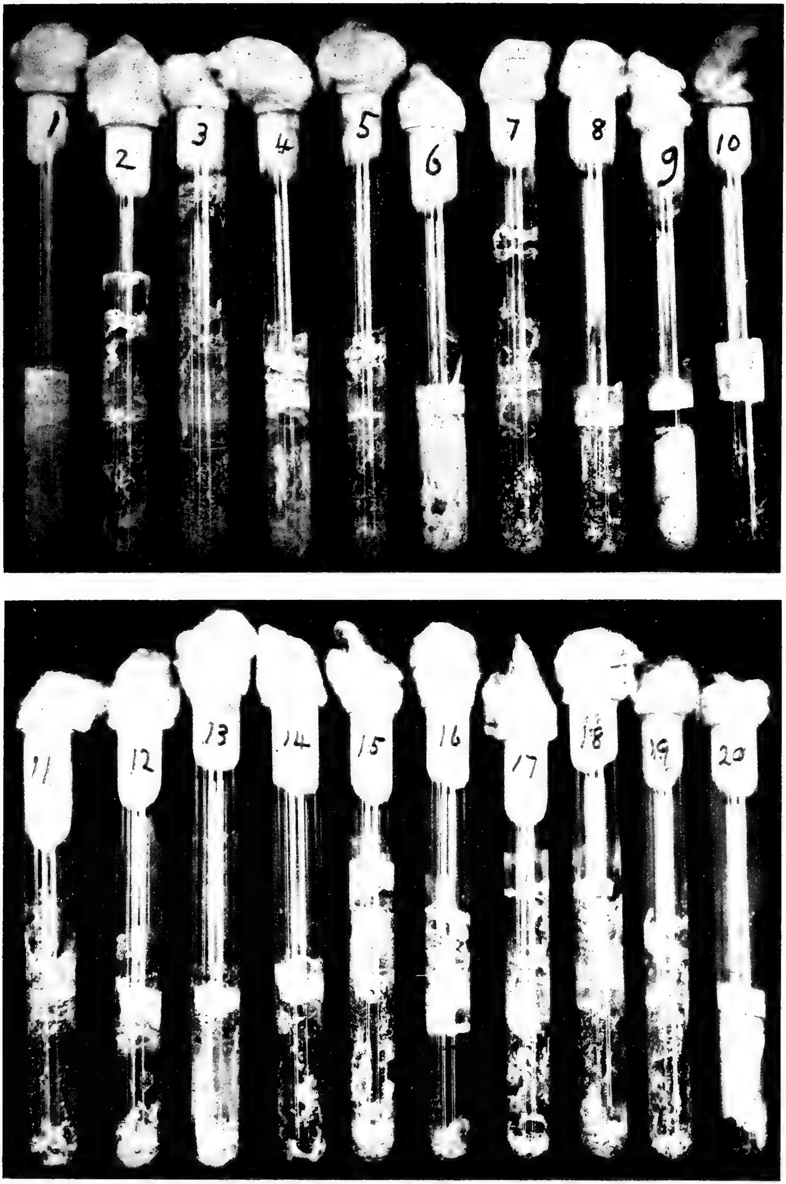
11. *Maize.*—Milk 'went' typically. Guinea-pig inoculated with 2 c.c. whey; died in 36 hours with separation of skin and sanguineous fluid in the space.

12. *Rice (Java).*—Milk 'went'; the whey had slightly putrid smell. Guinea-pig inoculated with 2 c.c. whey; died in 36 hours with separation of skin and red fluid in the space.

13. *Rice (Rangoon).*—Milk 'went'; whey had butyric acid smell. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours with very extensive separation of skin. There was little or no fluid in the space.

14. *Lentils.*—Milk 'went'; there was a little yellowish whey with a butyric acid and putrid smell. The cream layer was not raised. Guinea-pig inoculated with 2 c.c. whey; ulcer slightly pathogenic.

15. *Haricot Beans (Foreign).*—Milk 'went' typically; the cream layer was much raised. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours; very extensive separation of skin; little or no fluid.




Litmus milk tubes inoculated with grains incubated for 18 hours at 37° C., anærobically.

16. *Dried Peas (English).*—Milk 'went' typically; no butyric smell. Guinea-pig inoculated with 2 c.c. whey; died in 36 hours with separation of skin; very little fluid in the space.

17. *Dried Peas.*—Milk 'went' typically; no butyric smell. Guinea-pig inoculated with 2 c.c. whey; died in 36 hours with separation of skin.

18. *Dried Peas (Wisconsin).*—Milk 'went' typically; slight butyric acid smell. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours with extensive separation of skin; abdominal muscles sloughed through.

19. *Hemp.*—Milk 'went' typically; slight aromatic smell. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours with extensive separation of skin; little or no fluid.

20. *Linseed.*—Milk not changed after re-incubation. There was only a firm clot. Not inoculated.

## Meals and Grains taken from Freshly Opened Sacks in a Wholesale Warehouse

*Flour in five grades of quality, No.* 21 *finest,* 25 *the lowest.*

21. *Flour.*—Milk not changed.

22. *Flour.*—Milk not changed.

23. *Flour.*—Milk 'went' atypically after re-incubation. Not inoculated.

24. *Flour.*—Milk 'went'; butyric acid smell. Guinea-pig inoculated with 2 c.c. whey; died in 16 hours; not much separation of skin.

25. *Oats (English).*—Milk 'went'; no smell. Guinea-pig inoculated with 2 c.c. whey; ulcer. Pathogenic but not fatal.

26. *Groats (whole oat grain without husk).*—Milk not changed.

27. *Oatmeal (coarse).*—Milk not changed.

28. *Oatmeal (medium).*—Milk not changed.

29. *Oatmeal (finest Irish).*—Milk 'went' violently; cream raised to cotton plug; butyric acid smell. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours; not much separation of skin.

30. *Wheat (White English).*—Milk 'went' violently; cream raised to cotton plug; butyric acid smell. Guinea pig inoculated with 2 c.c. whey; died in 18 hours. Not much separation of skin.

31. *Wheat (Red English).*—Milk 'went' well; butyric acid smell. Guinea-pig inoculated with 2 c.c. whey; ulcer. Pathogenic, not fatal.

32. *Flour (lowest grade).*—Milk 'went' well; butyric acid smell. Guinea-pig inoculated with 2 c.c.; ulcer. Pathogenic, not fatal.

33. *Flour* (*finest quality*).—Milk 'went' violently; cream raised to cotton plug; butyric acid smell. Guinea-pig inoculated with 2 c.c. whey; died in 2 days with much separation of skin and considerable quantity of fluid in the space.

34. *Flour* (*Hungarian, finest quality*).—Milk tube not changed.

35. *Flour* (*American*).—Milk not changed.

36. *Barley* (*English*).—Milk 'went' well; cream raised to cotton plug; butyric acid smell. Guinea-pig inoculated with 2 c.c. whey; died in 2 days with much separation of skin and considerable amount of fluid in the space.

37. *Darri* (*an Indian Grain*).—Milk 'went' well. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours with considerable separation of skin.

38. *Maize.*—Milk not changed.

39. *Maize* (*Russian*).—Milk 'went' violently; butyric acid smell. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours with considerable separation of skin and bad-smelling fluid in the space.

40. *Beans* (*Egyptian.*)—Milk 'went' well; butyric acid smell. Guinea-pig inoculated with 2 c.c. whey; killed; had considerable separation of skin, which had sloughed through to outside.

41. *Beans* (*English*).—Milk not changed.

42. *Rice* (*Rangoon*).—Milk 'went'; slight butyric acid smell. Guinea-pig inoculated with 2 c.c. whey; ulcer, pathogenic.

43. *Bran.*—Supposed to be the cause of illness in some cattle; brought by farmer for examination; milk 'went' well; butyric acid smell. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours with considerable separation of tissues.

### Specimens of Flower Seeds from a Seed Shop

44. *Peas.*—Milk not changed typically; putrid smell; not inoculated

45. *Peas.*—Milk not changed typically; putrid smell; not inoculated.

46. *Beans.*—Milk not changed.

47. *Timothy Grass.*—Milk not changed; clot, no gas.

48. *Cow Grass.*—Milk not changed.

49. *Rye Grass.*—Milk 'went'; putrid smell. Guinea-pig inoculated with 2 c.c. whey; died in 2 days.

50. *Rye Grass* (*Perennial*).—Milk not changed.

51. *Rib Grass.*—Milk not 'gone' typically; putrid smell. Guinea-pig inoculated with 2 c.c. whey; died in 2 days.

52. *Quaking Grass.*—Milk 'went'; putrid smell. Guinea-pig inoculated with 1 c.c. whey; ulcer. Pathogenic, not fatal.

53. *Statice* (*a fluffy grass*).—Milk 'went'; putrid smell. Guinea-pig inoculated with 2 c.c. whey; ulcer. Pathogenic, not fatal.

54. *Corn Flower.*—Milk 'went'; putrid smell. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours, skin separated.

55. *Clover* (*White*).—Milk 'went'; putrid smell. Guinea-pig inoculated with 1 c.c. whey; ulcer. Pathogenic, not fatal.

56. *Clover* (*Red*).—Milk 'went'; putrid smell. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours with very considerable separation of skin and fluid in the space.

57. *Fennel.*—Milk 'went'; putrid smell. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours with considerable separation of skin and fluid in the space.

58. *Nasturtiums.*—Milk 'went'; much gas; putrid smell. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours with considerable separation of skin and fluid in the space.

59. *Beet.*—Milk 'went'; much gas; putrid smell. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours with considerable separation of skin.

60. *Samples of dust from the top of a cupboard in the Laboratory.*—Milk inoculated 'went' typically. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours with much separation of skin.

From the above 60 samples 41 gave an enteritidis-like growth in the milk; of these 30 were fatal to Guinea-pigs when inoculated, and 11 were pathogenic but not fatal.

### Milk Samples (Liverpool Corporation)

Each of these samples were tested for B. enteritidis and B. coli, and all those giving an enteritidis-like growth in milk were inoculated into Guinea-pigs. All the tubes which caused a fatal result, or were pathogenic but not fatal, were accepted as

B. enteritidis. Those that were non-pathogenic were not accepted as B. enteritidis, they may have been B. enteritidis in a non-pathogenic form of BOTKIN's butyric acid bacillus.

| | B. ent.? alone. | B. ent.? + B. coli. | B. coli alone. | B. ent. fatal. | B. ent. pathogenic. | B. ent. non-pathogenic. |
|---|---|---|---|---|---|---|
| Nov., 12 Samples ... | 6 | 0 | 2 | 1 | 5 | 0 |
| Dec., 50 Samples ... | 18 | 2 | 0 | 12 | 5 | 3 |
| Jan., 62 Samples ... | 7 | 0 | 8 | 2 | 0 | 5 |
| Feb., 55 Samples ... | 7 | 1 | 1 | 0 | 5 | 3 |
| March, 34 Samples ... | 5 | 3 | 0 | 3 | 5 | 0 |

Samples sent by the Liverpool Corporation during the first four months of the year. They were all tested for B. enteritidis sporogenes and B. coli in the manner described above.

| | Number of samples. | B. enteritidis alone. | B. enteritidis + coli. | B. coli alone. |
|---|---|---|---|---|
| Shell-fish—Cockles ... | 17 | 7 | 0 | 0 |
| „ Mussels ... | 21 | 4 | 2 | 0 |
| „ Periwinkles ... | 4 | 4 | 0 | 0 |
| „ Oysters ... | 21 | 0 | 0 | 0 |
| Tinned Meats and Fish ... | 24 | 0 | 0 | 0 |
| Potted Meats and Sausage ... | 3 | 1 | 0 | 0 |
| Butter ... ... ... | 11 | 0 | 0 | 0 |
| Margarine ... ... | 3 | 1 | 0 | 0 |
| Cheese ... ... | 3 | 0 | 0 | 0 |
| Sauces ... ... | 2 | 0 | 0 | 0 |
| Condensed Milk ... ... | 3 | 0 | 0 | 0 |
| Jams and Preserves ... | 6 | 0 | 0 | 0 |
| Tinned Fruits ... ... | 4 | 1 | 0 | 0 |
| Flour ... ... | 6 | 3 | 0 | 0 |
| Oatmeal ... ... | 5 | 2 | 0 | 0 |

The first 20 samples of grains and the sample of dust were kindly examined by Miss CHICK for B. coli. The milk samples were examined in the usual course; the method adopted to separate the B coli was to inoculate phenol-agar plates (1-1000), which were incubated at 42° C.

If B. enteritidis had got into the samples from water or dust recently polluted with sewage matter one would have expected to find B. coli associated with it; in the case of the grains it is possible that B. coli was there and had died out, leaving the more resistant spore-bearing B. enteritidis, so the fact that no B. coli were found in the samples of grains and flour examined does not necessarily prove it never was present.

With respect to the milk and shell-fish, however, it is different; if milk is polluted with sewage matter it could only have been recently, so here one would expect to find some relation between the presence of B. enteritidis and B. coli.

In the above samples there does not appear to be any uniform relation between the two, for in the larger proportion of samples B. enteritidis occurred alone.

It is noteworthy that in the samples of grains a higher proportion of extremely virulent cultures were obtained than were got from the samples of milk and from other samples examined in the laboratory; indeed, to obtain a culture of virulent B. enteritidis it is only necessary to drop a few grains of barley or a little bran into a milk tube, and, after heating it for 20 minutes at 80° C., to incubate it at 37° C. anaerobically. The milk tube will probably be found to have undergone the typical change and to be virulent if inoculated.

Considering the frequency of its presence in the spore state on grains and seeds and its virulence when injected into Guinea-pigs, it seems certain that the B. enteritidis got there as spores and from a habitation where it preserved its virulence to a high degree.

Dust from the soil seems to be the most probable infecting agent for the grains and for most of the samples of milk; and here it may be mentioned that Klein found the microbe in soil and in horse manure, but not in cow manure, which may possibly be accounted for by the fact that horses are generally fed on grains, while cows seldom are.

Seeing that B. enteritidis sporogenes is even more widely distributed than has hitherto been shown and on innocent substances, two questions arise —

1. Should its presence be taken as indicating anything more than contamination by dust? and

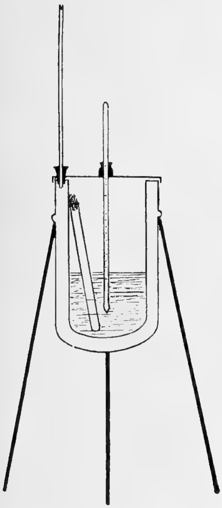
2. Is it, after all, the causal agent of some forms of diarrhœa?

The presence of the germ in the intestinal canal may easily be accounted for both in unhealthy and healthy conditions. I am kindly allowed by Dr. Glynn, who is working in this laboratory, to mention that he has recently found the germ in normal fæces as well as in cases of diarrhœa, and cultivations from the former were at least equally virulent.

The fact of its being virulent when injected beneath the skin of Guinea-pigs does not show that it would be pathogenic if taken by the mouth. I have fed Guinea-pigs with milk cultures, and with bran known to contain the germ capable of giving milk cultures virulent when injected, without any result.

# APPARATUS FOR HEATING CULTURES TO SEPARATE SPORE-BEARING MICRO-ORGANISMS*

By C. BALFOUR STEWART, M.A., M.B. Camb., Assistant Bacteriologist to the Liverpool Corporation



Those who have occasion to work with spore-bearing micro-organisms must often have felt the want of an apparatus which would keep at a constant temperature of between 70° C. and 80° C.

An application of Meyer's hot air bath, such as is used in chemical laboratories, suggested itself as being most applicable to the purpose; several of these were made and are now kept in every-day use in this laboratory. They maintain a constant temperature of 80° C. without any attention, and are always ready for placing tube cultures in, and, what is more important, the cultures may be left without any fear of their becoming heated above 80° C.

The apparatus is similar in construction to Meyer's hot air bath, except that it is larger than those generally used and has no outlet from the bottom of the inner chamber.

The inner chamber is 18 c.m. deep and 9 c.m. in diameter, and will take 7 or 8 tubes; it is advisable to have a condensation tube of 1 m. in height.

In use.—A small quantity of pure bensole BP 80° C. is poured into the outer jacket through the hole for the condensation tube, and the tube is re-inserted. A small flame below will keep the bensole boiling, and as the vapour condenses in the condensation tube and runs back very little is lost.

The inner chamber is filled to about one-third of its depth with water at 80° C.; the water retains its heat when the lid is removed and acts as a good conductor of heat to the culture tubes when they are placed in it. If water is not put in the inner chamber the heated air escapes when the lid is removed and it takes a long

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time for the culture tubes to become heated; whereas, if water is in the inner chamber, and the culture tubes put in when the thermometer registers 80° C., it is found that there is only a fall of 10 to 15° C., and the thermometer rises to 70° C., in a few minutes.

The tubes are left in for 15 to 20 minutes after the thermometer has risen again to 70° C., so during that time they will have been kept at between 70° C. and 80° C., and for most of the time over 75° C.

The apparatus should be made in beaten copper and spun afterwards; any local coppersmith should be able to make one at the cost of about £1 5s.

## DESCRIPTION OF PHOTOGRAPHS OF CULTURES OF B. PESTIS IN BROTH SHOWING 'STALACTITE' FORMATION

By Balfour Stewart, M.A., M.B. Camb.

Plate IV. Figs. 7 and 8

Fig. 7 is a photograph of a culture of B. pestis in a flask of broth; several 'stalactites' are seen growing near the side where they come into focus.

Fig. 8. 'Stalactite' growth of B. pestis in a flat flask (Soyka's flask). The light was behind, and was thrown in parallel rays by means of a lens. Between the lens and the flask was an alum bath to stop the heat rays from the incandescent lamp; without this it was found that diffusion currents were set up, and the 'stalactites' moved during the time of exposure. In front of the flask was a black screen to cut off the reflection from the edges of the flask, an opening was cut corresponding to the lower half of the flask, the upper edge of the cut out portion just allowed the surface of the broth to show.

At the left are seen several long thread-like growths ('stalactite' growth of Haffkine) hanging from small particles of butter floating on the surface.

At the right is seen a denser growth, which does not show individual 'stalactites.'

The flask was inoculated by means of a glass rod infected with plague culture. The rod was pushed down into the broth. The rod is seen to be covered with growth, which has crept up the rod and spread out over the surface of the broth, the substance of the broth remaining clear, except where some isolated colonies have grown on the sides of the flask.

EXPERIMENTS ON

# THE DIFFERENTIATION AND ISOLATION FROM MIXTURES OF THE BACILLUS COLI COMMUNIS AND BACILLUS TYPHOSUS BY THE USE OF SUGARS AND THE SALTS OF BILE

By ALFRED MacCONKEY

Physiologists tell us that in the bile of animals the salts occur in proportions different to those in the human subject, and bacteriologists find that animals are not susceptible to enteric fever. It has also been noticed that intestinal fermentation is greatly lessened by the administration of 'sodium taurocholate' in keratin coated pills.

These considerations suggested a research into the possibility of using the salts of bile as a means of—

1. Differentiating the *B. c. c. from the B. t. a.
2. Isolating the B. t. a. from mixtures of organisms.
3. Finding an index of pollution of drinking waters.

For many months experiments were made to ascertain whether 'sodium taurocholate' had a different effect to 'sodium glycocholate.' The results were always the same, and it was concluded that these two salts behaved alike, until, quite accidentally, it was discovered that the chemist from whom the salts were obtained considered that the names were synonymous and had supplied mixed salts. Consequently these experiments must be considered as having been made with a mixture of the salts. The pure salts might give different results.

Elsner's medium suggested the use of potato juice as a basis in the first series of experiments, but as potato juice has not always the same composition, later an attempt was made to secure uniformity by using pure salts.

The effect of bile salts upon B. c. c. and B. t. a. may be considered under three headings:—

I. Motility.
II. Morphology.
III. Duration of life.

The medium consisted of slightly alkaline potato juice, with varying percentages of bile salts, and the incubation temperature was 37° C.

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B. c. c. = Bacillus coli communis. B. t. a. = Bacillus typhi abdominalis.

### Bacillus Typhi Abdominalis

*I. Motility*

1 p.c. Only the shorter forms are motile, and the movement is chiefly rotatory.

2 p.c. The movements are most active, and the motility is not confined to the short forms.

3 p.c. A greater number of the bacilli are actively motile.

4 p.c., 5 p.c., 6 p.c. The bacilli are actively motile in each percentage.

*II. Morphology*

*A.* Hanging drop.—The short forms preponderate. There are a few long rods and filaments. Most of the bacilli have a beaded appearance, and resemble a row of cocci. The long forms are segmented, and the segments appear to be composed of cocci. As the percentage of bile salts increases the long forms disappear.

*B.* Stained preparations.—The appearance of individual bacilli is much the same in each percentage. The longer forms are segmented into chains of very short bacilli, and some of the latter appear square-shaped, owing to the extreme ends not taking up the stain.

### Bacillus Coli Communis

*I. Motility*

1 p.c. Actively motile.

2 p.c. Motility about the same.

3 p.c. Slight decrease in motility.

4 p.c., 5 p.c., 6 p.c. Motility decreases with the increased percentage of bile salts until it is doubtful whether any of the bacilli are motile. They shew a tendency to 'clump.'

*II. Morphology*

*A.* Hanging drop.—1 p.c. The bacilli are not to be distinguished from the B. t. a. except in that the short forms have a more oval appearance.

2 p.c. to 6 p.c. With the increase in amount of bile salts the length of the bacilli decreases until they appear to be cocci and very short rods.

*B.* Stained preparations.—The staining is very irregular. Much more so than in the case of B. t. a. Frequently the ends of a bacillus do not stain and are somewhat swelled, while in the centre is a very short stained portion which often looks like a coccus.

A bile salt potato juice gelatine was used for plate, stab, and slant cultures, but there was nothing characteristic, either in the mode of growth or in stained preparations, except in the case of one strain of B. c. c., which always, after 24 hours' growth, showed many huge fantastic-shaped involution forms which stained deeply. Many other strains were grown, but the same result was never obtained. Lactose, glucose, and sucrose were added to the gelatine and used in slant cultures, but no characteristic differences were visible.

## Duration of Life

As bile salts had an inhibiting effect upon the motility of an actively motile B. c. c., while that of B. t. a. was unaffected, it was thought that possibly B. t. a. might live longer than B. c. c. in the bile salt medium.* In the first experiment the two strains of B. c. c. used died in 9 days, while the B. t. a. lived for some time longer. In this and subsequent experiments the incubation temperature was 42° C., so that if a successful result was obtained the temperature would assist in inhibiting the growth of other organisms. The various media used and the duration of life of B. c. c. and B. t. a. are given in the appended Table:—

| Organism and Source | Bile salt 4% Pot. Juice | Bile salt 4% Pot. Juice | Bile salt 4% Glucose 2.5% Pot. Juice | Bile salt 5% Glucose 2.5% Pot. Juice | Bile salt 5% Glucose 2.5% Pot. Juice | Bile salt 5% Na $NO_3$ 1.5% Water | Bile salt 5% $KNO_3$ 1.5% Water |
|---|---|---|---|---|---|---|---|
| B. t. a. (Laboratory) ... ... | L 16 | L 12 | D 4 | D 5 | D 5 | D 4 | D 5 |
| „ Human fæces ... ... | L 16 | L 12 | D 2 | D 5 | D 5 | D 4 | D 5 |
| B. c. c. isolated by Eyre from $H_2O$... | D 6 | L 12 | D 7 | D 8 | D 6 | D 7 | D 6 |
| „ given by Pakes ... ... | D 6 | D 4 | D 4 | D 8 | D 6 | D 5 | D 11 |
| „ isolated from $H_2O$ by Pakes | L 16 | L 12 | L 8 | D 8 | D 4 | D 7 | D 5 |
| „ „ „ human fæces... | L 16 | D 8 | L 8 | D 8 | D 4 | D 7 | contaminated |
| „ „ „ „ „ ... | L 16 | L 12 | D 4 | D 8 | D 7 | D 8 | D 10 |
| „ „ „ horse „ ... | L 16 | D 8 | L 8 | D 8 | D 4 | D 7 | D 5 |
| „ „ „ cow „ ... | L 16 | L 12 | D 8 | D 8 | D 4 | D 7 | D 5 |
| „ „ „ sheep „ ... | D 6 | D 6 | L 8 | D 8 | D 5 | D 7 | D 5 |
| „ „ „ rabbit „ ... | L 16 | L 12 | D 7 | D 8 | D 6 | L 11 | D 5 |
| „ „ „ dog „ ... | — | — | D 6 | — | D 6 | — | D 5 |
| „ „ „ „ „ ... | — | — | D 6 | — | D 4 | — | D 5 |
| „ „ „ mouse „ ... | — | L 12 | D 4 | D 8 | D 8 | L 11 | D 13 |
| „ „ „ Ice Cream by Pakes ... | D 6 | L 12 | L 8 | D 8 | D 11 | D 5 | D 5 |
| „ „ „ blood of case of Infective Endocarditis ... | — | — | D 7 | — | D 4 | — | D 7 |
| Typhoid stools ... ... ... | — | — | D 5 | — | — | — | L 15 |
| Gærtner, given by Durham... ... | L 16 | L 12 | L 8 | D 8 | D 12 | L 11 | L 15 |

L = living. D = dead. The numbers indicate the days after inoculation.

In order to ascertain whether a culture was living the bile salt tube was well shaken, and a loopful transferred to ordinary broth, and this broth tube incubated at

* A percentage of 4 was used because the effect upon the motility of B. c. c. was distinct.

37° C. If the broth cultures did not show any growth the transplantations from the bile salt medium were still continued for several days, so as to make quite certain that there were no viable organisms present.

The results are negative as regards both differentiating B. t. a. from B. c. c., and isolating B. t. a. from mixtures. But they show that 2.5 p.c. of glucose has a distinctly inhibiting effect upon the organisms used.

This action of glucose is confirmed by the following experiment. Fæces from

1. A case of Bright's Disease,
2. A case of Mitral Stenosis,
3. A case of Cirrhosis with ascites,

were inoculated into tubes containing

*A*. Bile salt 5 p.c. $KNO_3$ 1.5 p.c. Water,
*B*. Bile salt 5 p.c. $NaNO_3$ 1.5 p.c. Water,
*C*. Bile salt 5 p.c. Glucose 2.5 p.c. Potato Juice,

and incubated at 42° C.

1 *A*. Was living on 14th day.
1 *B*. Was living on 14th day.
1 *C*. Was dead on 5th day.
2 *A*. Was living on 14th day.
2 *B*. Was living on 14th day.
2 *C*. Was dead on 5th day.
3 *A*. Was living on 14th day.
3 *B*. Was living on 14th day.
3 *C*. Was dead on 6th day.

The latest culture which shewed any growth was plated in ordinary nutrient gelatine.

The organisms isolated were—From

1 *A*. An organism which looked like a streptococcus, and which gave
*Broth*—General turbidity,
*Litmus Milk*—Acid, but no clotting,
*Potato*—Raised whitish growth,
*Gel. S. tab*—Very slight growth on surface, but good growth all down the needle track.

1 *B*. B. c. c.

1 *C*. (*a*) B. c. c.
(*b*) A bacillus which produced slight fluorescence in gelatine, caused no change in milk, and gave a moist yellowish growth, with discolouration of potato.

2 *A*. Organism similar to 1 *A*.

2 *B*. B. c. c.

2 *C*. (*a*) B. c. c.
(*b*) Organism similar to 1 *C*.

3 *A*. Liquefying motile bacillus, which peptonizes milk, turning it brownish yellow, gives slight indol, but no gas; produces general turbidity in broth, and a moist brownish-yellow growth, with discolouration of potato.

3 *B*. Same as 3 *A*.

3 *C*. B. c. c.

From these three cases, then, there were isolated four organisms which would grow in bile salt 5 p.c. media at 42° C., and the most frequent was the B. c. c.

It was thought that an increased percentage of bile salt might inhibit all but B. c. c. [That B. c. c. would grow in 7 p.c. B. s. was proved by the experiment given in the following Table :—

Medium—Bile salt 7 p.c., $KNO_3$, $NaNO_3$, AmCl., each 0.5 p.c. Made up with water. Temperature—42° C.

| | |
|---|---|
| B. t. a. fæces . . . | Dead 5th day. |
| B. c. c. . . . . | Living 10th day. |
| „ Infec. Endocarditis . . | „ „ |
| „ Ice Cream . . . | „ „ |
| „ Cow . . . | „ „ |
| „ Horse . . . | „ „ |
| „ Sheep . . . | „ „ |
| „ Dog . . . | „ „ |
| Gærtner . . . . | „ „ ] |

So fæces from (1) a case of phosphorous poisoning, (2) a case of (?) influenza, were inoculated into tubes containing the above medium, and incubated at 42° C. for 5 days, and then plates were made from these tubes.

From case 1 were obtained (*a*) a liquefying motile bacillus, similar to the one which had been isolated from previous case; (*b*) a non-typical B. c. c. From case 2, only B. c. c. was isolated.

Fæces from the Rabbit, Sheep, and Cow were inoculated into the same medium, and incubated at 42° C., for 48 hours, and then plated in ordinary gelatine.

The organisms isolated were:—From Rabbit, only B. c. c. group; Sheep, B. c. c., and the same liquefying motile bacillus as before; Cow, B. c. c. group only.

The experiment was repeated with two specimens of Rabbit fæces, and the organisms isolated were:—(*a*) B. c. c.; (*b*) a motile liquefying bacillus having the same characteristics as before.

A repetition with fæces of the Cow and Sheep resulted in the isolation of B. c. c. only.

As glucose seemed to have an unfavourable effect upon the duration of life of B. c. c. and B. t. a. a liquid medium was made, consisting of potato juice containing varying percentages of glucose, and B. c. c. and B. t. a. were grown in this medium at 37° C. The amounts of the sugar used were 1, 2, 3, and 4 per cent.

## Bacillus Typhi Abdominalis

*Motility*

The lowest percentage causes a decrease in the motility as compared with ordinary broth, and as the percentage increases the amount of movement decreases.

*Morphology*

In 1 p.c. the bacilli have much the same appearance as in ordinary broth, but the size decreases with the increase in the amount of glucose.

### Bacillus Coli Communis

*Motility*

A fairly motile B. c. c. was used, and it was found that motility decreased with an increased percentage, until in 4 p.c. it was doubtful whether the bacilli were motile.

*Morphology*

The bacilli become shorter as the percentage rises.

### B. Enteritidis of Gærtner

The effect of glucose upon this organism is the same as upon B. t. a. and B. c. c.

[All the experiments up to this point were carried out in the Bacteriological Laboratory at Guy's Hospital during the winter of 1897 and the first half of 1898. Then an interruption occurred and they were not resumed until 1900, in the Thompson Yates Laboratories, University College, Liverpool.]

The conclusion arrived at from the first series of experiments was, that by using a liquid bile salt medium, an incubation temperature of 42° C., and subsequently plating in ordinary nutrient gelatine, one could inhibit the growth of most of the organisms usually found in fæces. The thought naturally arose that possibly some of the organisms isolated were merely existing in the bile salt medium without multiplication, and that it was the subsequent plating in a more favourable medium that enabled them to grow. Therefore an agar medium was tried ; at first without peptone, but later, when it was seen that peptone did not increase the number of species, a slightly alkaline agar, composed as follows, was used :—

| | |
|---|---|
| Bile salt . . . . | 1 gramme. |
| Peptone . . . . | 0.2 ,, |
| $KNO_3$ . . . | 0.5 ,, |
| $Na_2HPO_4$ . . . | 0.5 ,, |
| AmCl . . . . | 0.5 ,, |
| Agar . . . . | 2 ,, |
| Tap water . . . | 100 c.c. |

When used for sewage effluents this medium gave an almost pure culture of the B. c. c. group ; but when used for pure cultures of B. c. c., and compared with nutrient agar, the bile salt medium frequently inhibited too much.

The following are examples of the results obtained :—

I. About two grammes of garden earth were shaken up with about 100 c.c. of tap water in a flask, which was then allowed to remain at rest until the larger particles had settled to the bottom. The supernatant liquid was plated, 1 c.c. being put into each plate. The plates were incubated at 42° C., and examined after 48 hours.

| | |
|---|---|
| *Nutrient Agar* . | The plate was covered with colonies of various kinds. |
| *Bile Salt Agar* . | There was no growth in this plate. |

II. Pure cultures of B. c. c. (*A*, *B*, *C*, and *D*) were diluted with sterile water, and 1 c.c. of the dilution was put into each plate.

| | | | |
|---|---|---|---|
| A. *Nutrient Agar* | | | |
| 3 months old. | 1 . . | 135 colonies | Average 138 |
| | 2 . . | 104 " | |
| | 3 . . | 175 " | |
| *Bile Salt Agar* | | | |
| | 1 . . | 65 colonies | Average 103 |
| | 2 . . | 151 " | |
| | 3 . . | 94 " | |
| B. *Nutrient Agar* | | | |
| 1 month old. | 1 . . | 356 colonies | Average 562 |
| | 2 . . | 912 " | |
| | 3 . . | 420 " | |
| *Bile Salt Agar* | | | |
| | 1 . . | 570 colonies | Average 536 |
| | 2 . . | 500 " | |
| C. *Nutrient Agar* | | | |
| 12 days old. | 1 . . | 35 colonies | Average 27 |
| | 2 . . | 29 " | |
| | 3 . . | 19 " | |
| *Bile Salt Agar* | | | |
| | 1 . . | 16 colonies | Average 23 |
| | 2 . . | 28 " | |
| | 3 . . | 25 " | |
| D. *Nutrient Agar* | | | |
| 24 hours old. | 1 . . | 116 colonies | Average 118 |
| | 2 . . | 136 " | |
| | 3 . . | 102 " | |
| *Bile Salt Agar* | | | |
| | 1 . . | 57 colonies | Average 77 |
| | 2 . . | 121 " | |
| | 3 . . | 53 " | |

When endeavouring to estimate the value of this medium, in the case of mixtures containing B. c. c., a difficulty arose from the fact that nutrient agar cannot be used as a standard. So for sewage, river water, &c., carbolic agar, 1 ‰, was taken as the standard, because it is said that carbolic acid in this percentage practically gives the same results as nutrient agar in the case of pure cultures of B. c. c.

Several samples (about 12) of Severn water, from the neighbourhood of Shrewsbury, were plated in this bile salt agar, and in carbolic agar 1 °/₀₀. The results showed that the bile salt agar inhibited some 70 p.c. of the B. c. c. The problem, then, was to find out the composition which would give results equal to carbolic agar.

Numerous alterations in, and additions to, the medium were made, and in the course of these experiments it was found that—

Bile Salts—1 p.c. and over—have an inhibitory effect upon the great majority of organisms, but favour the growth of moulds.

$KNO_3$, AmCl, NaCl—0.5 p.c.—exercise an unfavourable influence upon B. c. c. while encouraging the growth of other organisms.

$Na_2HPO_4$—0.5 p.c.—seems specially favourable to earth organisms.

Glucose—0.5 p.c.—tends to inhibit the ordinary earth and water bacteria, and, if in a larger percentage, B. c. c. also; but assists the growth of moulds and yeasts. A small percentage of glucose benefits B. c. c., especially the colonies in the depth.

One example will show the effect of glucose and of salts upon B. c. c. A pure culture of B. c. c. was taken and diluted with sterile water, plated, and incubated at 42° C.

| | Nutrient Agar | Bile salt 1% Peptone 0.2% | Bile salt 1% Peptone 0.2% AmCl. 0.5% $KNO_3$ 0.5% | Bile salt 1% Peptone 0.2% Glucose 0.5% | Bile salt 1% Peptone 0.2% Glucose 1% | Bile salt 1% Peptone 0.2% Glucose 1.5% |
|---|---|---|---|---|---|---|
| | 870 | 440 | 260 | 960 | 496 | 270 |
| | 700 | 230 | 200 | 800 | 600 | 240 |
| | 960 | 300 | 168 | 650 | 484 | 228 |
| Average No. of Colonies per plate ... ... ... | 844 | 323 | 209 | 803 | 527 | 246 |

If B. c. c. be plated in a medium containing bile salts, peptone, and glucose only, the colonies at the end of 24 hours appear much larger than usual, and have a hazy outline, owing to the formation of a precipitate round each colony. If there are many colonies the plate looks 'milky,' and has a yellowish colour. This precipitate is caused by an acid produced by the organism. If a drop of $NH_3$ solution be placed upon the colony the haze soon disappears, and the colony is seen to be of the usual size. The addition of salts to the medium tends to prevent the formation of this precipitate, and thus to remove a means of distinguishing one kind of colony from another. Further experiments showed that a percentage of glucose not greater than 0.5 was most favourable, and that an increase of the peptone to 1 p.c. did not cause an increase in the number of organisms other than B. c. c. So a medium composed of bile salts 1 p.c., peptone 1 p.c., glucose 0.1 p.c., agar 2 p.c., and tap water, was compared with carbolic agar 1 p.c.; 16 samples of Severn water being plated. The results were, however, disappointing as there was no definite correspondence between the numbers in the two media. In a few of the samples the numbers were practically the same, but in the others the bile salt agar results were much too low.

An increase in the amount of bile salt caused a decrease in numbers, and the addition of 0.5 p.c. of carbolic acid decidedly inhibited. Thus a pure culture of B. c. c. gave—

| | Nutrient Agar | Bile salt 1% Peptone 1% Glucose 0.3% Carb. Acid 0.5% | Bile salt 1% Peptone 1% Glucose 0.3% | Bile salt 1.5% Peptone 1% Glucose 0.3% | Bile salt 2% Peptone 1% Glucose 0.3% |
|---|---|---|---|---|---|
| | 109 | 20 | 67 | 30 | 33 |
| | 102 | 15 | 101 | 23 | 36 |
| | 78 | 12 | 47 | 21 | 31 |
| Average No. of Colonies per plate ... ... ... | 96 | 15 | 71 | 24 | 33 |

On some occasions the addition of $KNO_3$ 0.1 p.c. seemed to increase the numbers of B. c. c., and on other occasions the numbers were decreased. So a series of trials was made with percentages of $KNO_3$, varying from 0.1 to 0.5, and the conclusion come to was that this salt had an unfavourable effect upon the growth of B. c. c. Just prior to coming to this conclusion 20 samples of Severn water were plated in bile salt 0.5 p.c., peptone 2 p.c., glucose 0.5 p.c., $KNO_3$ 0.1 p.c., agar 1.5–2 p.c., tap water q. s. The results corresponded more closely than before with those given by carbol agar; but the inhibition was still too great.

At this stage Dr. Herbert E. Durham suggested that I should once more try the effect of different kinds of sugars. The hypothesis he enunciated (as far as I understood it) was that if an organism ferments a sugar it presumably gives rise to products which are inimical to its own growth, and that, consequently, if of two organisms *A* and *B*, *A* ferments both glucose and lactose, while *B* only ferments glucose, then a medium containing lactose should be more favourable to *B* than to *A*. Glucose, lactose, and mannite were the sugars tried, and it seemed that when employed in the same percentage—0.5—glucose was most favourable to B. c. c.; but that 1 p.c. of lactose gave results equal to 0.5 p.c. glucose. It was doubtful whether mannite had an equal inhibitory effect upon other organisms.

As nitrates, chlorides, and phosphates seemed unfavourable, it was thought that possibly Liebig's Extract might be of use in giving the extra amount of nourishment apparently required. So garden earth and road sweepings were thoroughly shaken up with tap water, and the mixture plated in

I. B. s. 0.5 p.c., peptone 2 p.c., lactose 0.5 p.c., agar 1.5 p.c.

II. B. s. 0.5 p.c., peptone 2 p.c., lactose 0·5 p.c., agar 1.5 p.c., Liebig 0.1 p.c. Nutrient agar being used as a control.

The control plate was covered with all kinds of colonies.

I. The plates appear to contain only one kind of organism. On one of them there were 12 surface colonies. Eleven of these were subcultured in broth, and afterwards in milk, glucose jelly, and potato.

| | Broth | Milk | Glucose Jelly | Potato | Indol |
|---|---|---|---|---|---|
| 1. . . . . . . | General turbidity | Acid and clotting | Profuse gas No liquefaction | Moist, grey growth. P.D. | |
| 2. Actively motile. Short bac. | ,, | ,, | ,, | Yellowish P.D. | + |
| 3. ,, ,, ,, | ,, | ,, | ,, | ,, ,, | + |
| 4. Motile ,, | ,, | ,, | ,, | ,, ,, | + |
| 5. Actively motile ,, | ,, | ,, | ,, | ,, ,, | + |
| 6. ,, ,, ,, | ,, | ,, | ,, | ,, ,, | + |
| 7. Motile ,, | ,, | ,, | ,, | ,, ,, | + |
| 8. Actively motile ,, | ,, | ,, | ,, | Almost invisible | + |
| 9. ,, ,, ,, | ,, | ,, | ,, | Moist, grey. P.D. | + |
| 10. ,, ,, ,, | ,, | ,, | ,, | Yellowish ,, | + |
| 11. ,, ,, ,, | ,, | ,, | ,, | ,, ,, | + |

P.D. = discolouration of potato.

II. The plates appear to be a pure culture. The numbers are practically the same as in I. There were 11 surface colonies on one plate. These were all subcultured in broth, and later in milk, glucose jelly, and potato.

| | Broth | Milk | Glucose Jelly | Potato | Indol |
|---|---|---|---|---|---|
| 1. Motile. Short bac.. | General turbidity | Acid and clotting | Gas No liquefaction | Moist, grey yellow. P.D. | + |
| 2. ,, ,, | ,, | ,, | ,, | ,, ,, | + |
| 3. Slightly motile. Short bac. | ,, | ,, | ,, | Almost invisible | + |
| 4. Actively ,, ,, | ,, | ,, | ,, | Moist, yellow. P.D. | + |
| 5. ,, ,, ,, | ,, | ,, | ,, | ,, ,, | + |
| 6. ? Motile ,, | ,, | ,, | ,, | ,, ,, | + |
| 7. Actively motile ,, | ,, | ,, | ,, | ,, ,, | + |
| 8. Slightly ,, ,, | ,, | ,, | ,, | Moist, grey yellow. P.D. | + |
| 9. Motile ,, | ,, | ,, | ,, | Moist, grey. P.D. | + |
| 10. Actively motile ,, | ,, | ,, | ,, | Almost invisible | + |
| 11. Motile ,, | ,, | ,, | ,, | Moist, yellowish. P.D. | + |

P.D. = discolouration of potato.

Again, 5 c.c. of the Ashton Hall Septic Tank liquor were mixed with 95 c.c. of the earth, road sweepings, and tap water mixture used in the previous experiment, and which had been standing on the laboratory bench. 1 c.c. of the mixture was put into each plate, and the medium used was B. s. 0.5 p.c., peptone 2 p.c., lactose 1 p.c., agar 1.5 p.c., tap water. The average of three plates was 1194 colonies per plate. The whole of the plate was 'milky,' except for a small clear space round some of the colonies. The colonies with a clear space round them appeared to be of two kinds. One was finely and uniformly granular, with a regular, well-defined margin; the other had an irregular edge, and was covered with fine wavy lines. Cultures in broth were made from eight colonies.

| | | Broth | Milk | Glucose Jelly | Potato | Indol |
|---|---|---|---|---|---|---|
| 1. Granular colony with clear space. | Short B., motile | General turbidity | Acid and clotting | Gas No liquefaction | Moist, grey P.D. | + |
| 2. ,, ,, | ,, ? motile | ,, | ,, | ,, | ,, | + |
| 3. ,, ,, | ,, ? motile | ,, | ,, | ,, | ,, | + |
| 4. ,, ,, | ,, motile | ,, | ,, | ,, | ,, | + |
| 5. Hazy colony. | ,, ,, | ,, | ,, | ,, | ,, | + |
| 6. ,, ,, | ,, actively motile | ,, | ,, | ,, | ,, | + |
| 7. Lined ,, | ,, ,, | ,, | ,, | ,, | ,, | + |
| 8. ,, ,, | ,, motile | ,, | ,, | ,, | ,, | + |

The flask containing the mixture of earth, road sweepings, and tap water was allowed to stand on the laboratory bench for several days. Then 100 c.c. of the supernatant liquid were poured into another flask, and shaken up with about 2 grammes of fresh garden mould. This mixture was inoculated with 1 loopful of a culture of B. t. a., and the flask, unplugged, placed in a dark cupboard for two days at ordinary temperature. Plates were then made, about 0.25 c.c. being put into each plate, in

I. B. s. 0.5 p.c., peptone 2 p.c., glucose 0.5 p.c., agar 1.5 p.c.
II. B. s. 0.5 p.c., peptone 2 p.c., lactose, 0.5 p.c., agar 1.5 p.c.
III. B. s. 0.5 p.c., peptone 2 p.c., lactose 1 p.c., agar 1.5 p.c.

I. 45 colonies on the plate. On the surface were 9, which were all transferred to broth, and subcultures made.

| | Broth | Milk | Glucose Jelly | Potato | Indol |
|---|---|---|---|---|---|
| 1. Short bac. Actively motile | General turbidity | Acid, clotting | Gas<br>No liquefaction | Moist, yellowish P.D. | ? |
| 2. " " " | " | No change | No gas<br>No liquefaction | 'Invisible' | None |
| 3. " " " | " | " | " | " | " |
| 4. " " " | " | " | " | " | " |
| 5. " " " | " | " | " | " | " |
| 6. " " " | " | " | " | " | " |
| 7. " " " | " | " | " | " | " |
| 8. " " " | " | Acid, clotting | Gas<br>No liquefaction | Moist, grey. P.D. | ? |
| 9. " " " | " | No change | No gas<br>No liquefaction | 'Invisible' | None |

II. 51 colonies, of which 7 were on the surface.

| | Broth | Milk | Glucose Jelly | Potato | Indol |
|---|---|---|---|---|---|
| 1. Short bac. Actively motile | General turbidity | No change | No liquefaction<br>No gas | 'Invisible' | ? |
| 2. " " " | " | " | " | " | None |
| 3. " " " | " | Acid, clotting | No liquefaction<br>Gas | Grey yellow, moist. P.D. | " |
| 4. " " " | " | No change | No liquefaction<br>No gas | 'Invisible' | " |
| 5. " " " | " | " | " | " | " |
| 6. " " " | " | " | " | " | " |
| 7. " " " | " | " | " | " | ? |

This plate was exposed to light for several days, when one of the surface colonies became yellow.

III. There were 122 colonies on this plate. It was exposed to light for several days. Three of the surface colonies turned yellow; the rest remained greyish and translucent. The 3 yellow colonies and 3 others were transferred to broth.

| Colony | Organism | Broth | Milk | Glucose Jelly | Potato | Indol |
|---|---|---|---|---|---|---|
| 1. Yellow . . | Short bac., actively motile | General turbidity | Clotting and acid | No liquefaction Gas | Moist, yellow. P.D. | + |
| 2. ,, | ,, ,, | ,, | ,, | ,, | ,, | + |
| 3. ,, | ,, ,, | ,, | ,, | ,, | ,, | + |
| 4. Grey . . . | ,, motile | ,, | Slight acid No clotting | No liquefaction No gas | Just visible | + |
| 5. ,, | ,, ,, | ,, | ,, | ,, | ,, | + |
| 6. ,, | ,, ,, | ,, | ,, | ,, | ,, | + |

The mixture of earth, &c., remained in the cupboard for five more days, when it was again plated on nutrient agar and on lactose bile salt agar. On the nutrient agar plate there were about 70 colonies of various kinds, of which five or six might have been B. c. c. or B. t. a.

On the bile salt agar plate there were four colonies. Cultures were made from two of them. Both gave cultural characteristics corresponding to the B. t. a. One was tested with serum and gave a positive reaction; it also gave the indol reaction.

Five days later this mixture was again plated in bile salt lactose agar, 1 c.c. being put into the plate. Only one colony, besides a few moulds, appeared, and this was B. c. c.

One c.c. of urine, from a case* of enteric fever, was put into a plate of bile salt lactose agar. The result was an almost pure culture of B. c. c. A few of the colonies seemed as if they might be B. t. a., and these were transferred to broth and subcultured.

| | Broth | Milk | Glucose Jelly | Agar | | Indol |
|---|---|---|---|---|---|---|
| 1. Slender bac. Extremely motile | General turbidity Greenish yellow fluorescence | Acid clotting Peptonization Greenish yellow liquid | No Gas liquefaction Greenish yellow colour | Bac. Pyocyaneus | | + |
| 2. ,, ,, ,, | ,, | ,, | ,, | ,, | ,, | + |
| 3. ,, ,, ,, | ,, | ,, | ,, | ,, | ,, | + |
| 4. Short bac. Slightly motile . | General turbidity | Acid and clotting | No liquefaction Gas | Dry, filmy, greyish translucent growth | No spores. Not by Gram. | + |
| 5. ,, ,, ,, | ,, | ,, | ,, | ,, | ,, | + |

* This case was a typical case subsiding by lysis.

The specimen was taken on the 24th day. The temperature was normal. The serum reaction had been tried 3 times, with 2 negative results and 1 doubtful.

Urine from another enteric case, which was convalescing from a relapse, was taken, and 1 c.c. put into a plate of bile salt lactose agar. Five cultures were made from this plate.

| | Broth | Milk | Glucose Jelly | Potato | Indol |
|---|---|---|---|---|---|
| 1. Short bac. Actively motile. | General turbidity | Acid | Gas No liquefaction | Just visible | + |
| 2. „ ? motile . . | „ | | „ | Moist, yellow growth with discolouration | + |
| 3. „ „ . . | „ | Acid, clotting | „ | „ | + |
| 4. „ „ . . | „ | | „ | „ | + |
| 5. „ „ . . | „ | | „ | „ | + |

None of these formed spores; nor did they stain by Gram's method.

Again, fæces from a case of enteric, which was in the 3rd week of a relapse and 10th week of the disease, were diluted 30,000 times with sterile water and plated. 1 c.c. of this dilution was put into each plate. The plates were overcrowded with what looked like a pure culture of the B. c. c. group. Subcultures were made from 19 surface colonies.

| | Motility | Broth | Glucose jelly | Milk | Potato | Indol |
|---|---|---|---|---|---|---|
| 1. Short bacillus | ? | General turbidity | Gas No liquefaction | Acid clotting | Moist, transparent | + |
| 2. „ | + | „ | „ | „ | Moist, yellow. P.D. | + |
| 3. „ | ? | „ | „ | „ | „ „ „ | + |
| 4. „ | + | „ | „ | „ | „ grey, yellow. P.D. | + |
| 5. „ | ? | „ | „ | „ | „ transparent | + |
| 6. „ | – | „ | „ | „ | „ „ | + |
| 7. „ | – | „ | „ | „ | „ grey, yellow. P.D. | + |
| 8. „ | – | „ | „ | „ | „ „ „ „ | + |
| 9. „ | + | „ | „ | „ | „ „ „ „ | + |
| 10. „ | + | „ | „ | „ | „ „ „ „ | + |
| 11. „ | + | „ | „ | „ | „ „ „ „ | + |
| 12. „ | ? | „ | „ | „ | „ „ „ „ | + |
| 13. „ | ? | „ | „ | „ | 'Invisible' | + |
| 14. „ | ? | „ | „ | „ | Moist,grey,yellow. P.D. | + |
| 15. „ | ? | „ | „ | „ | „ „ „ „ | + |
| 16. „ | ? | „ | „ | „ | „ transparent | + |
| 17. „ | + | „ | „ | „ | „ „ | + |
| 18. „ | + | „ | „ | „ | „ grey, yellow. P.D. | + |
| 19. „ | + | „ | „ | „ | „ „ „ „ | + |

P.D. = discolouration of potato.

The following samples were plated in Bile salt 0.5 p.c., peptone 2 p.c., lactose 1 p.c., agar 1.5 p.c. :—

I. Road puddle diluted with sterile water. Carbol agar (Miss CHICK's analysis).

1. 200 B. c. c.
2. 212 „
3. 174 „

} = 195 . . Average 211

II. Road dust, shaken up with sterile water.

1. 13 B. c. c.
2. 11 „
3. 9 „

} = 11 . . Average 3

III. Earth from a potato field, shaken up with sterile water.

1. 2. 3. } 0 B. c. c. . . . . Average 0

IV. Earth from another potato field, shaken up with sterile water.

1. 2. 3. } Sterile, except for a few moulds . Average 0

V. Garden earth, in which turnips were growing, shaken up with sterile water.

1. 1 B. c. c.
2. 1 „
3. 1 „

} = 1 . . . Average 3

Samples I, II, and III contained, besides B. c. c., another kind of colony which was very small, round, grey, slightly raised, and looked finely granular under the microscope. Eleven cultivations were made from these colonies, and all turned out to be yeasts.

Plates were also made with water from the aqueducts at Vyrnwy and Rivington. Three plates were made from each sample, 1 c.c. of water being put into each plate.

There was no growth on any of these plates.

A sample of water from a pond, in which there were 20,000 young trout, was plated in the same medium, 1 c.c. being put into each of 3 plates. There was no growth on any of the plates.

Countings were made of 13 samples of Severn water, and it was found that when there were not many colonies on a plate the number agreed with those on carbol agar; but when the colonies were more than about 100 per plate other organisms interfered with the proper development of the B. c. c.

These results are a fair reason for concluding that by means of a lactose bile salt agar, and an incubation temperature of 42° C., one can inhibit almost all the intestinal organisms except B. c. c., and that B. c. c. will grow as well on this medium as on carbol agar.

But the correctness of this conclusion is rendered doubtful by the following experiments :—

A pure culture of the Escherich strain of B. c. c. (given me by Dr. HERBERT DURHAM) was plated in nutrient agar, carbol agar, and lactose bile salt agar.

In the first experiment the solution was not dilute enough, and the nutrient agar and carbol agar plates were overcrowded, there being more than 1,600 colonies on a plate. But even with this large number present in the solution not more than 20 had grown on a lactose agar plate.

In a second experiment the numbers were :—

| Nutrient agar | Carbol agar 1 °/₀₀ | Bile salt lactose agar |
|---|---|---|
| 324 | 52 | 5 plates |
| 338 | 51 | No growth |
| 332 | 40 | |
| 331 | 48 | |

In a third similar experiment the numbers were :—

| Nutrient agar | Carbol agar 1 °/₀₀ | Bile salt lactose agar |
|---|---|---|
| 161 | 9 | 3 plates |
| 172 | 9 | No growth |
| 89 | 0 | |
| 140 | 9 | |

Similar experiments made with a pure culture of B. t. a. gave results :—

I

| Nutrient agar | Carbol agar 1 °/₀₀ | Bile salt lactose agar |
|---|---|---|
| Overcrowded, more than 3,000 per plate. | 2 per plate. | Overcrowded, more than 3,000 per plate. |

II

| Nutrient agar | Carbol agar 1 °/₀₀ | Bile salt lactose agar |
|---|---|---|
| 139 | 0 | 129 |
| 166 | 0 | 143 |
| 133 | 0 | 142 |
| 146 | 0 | 138 |

FIG. 1

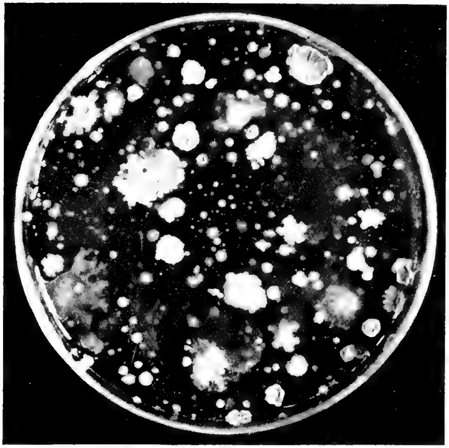

FIG. 2

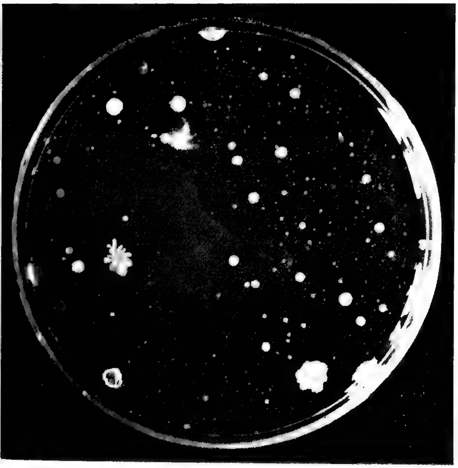

FIG. 3

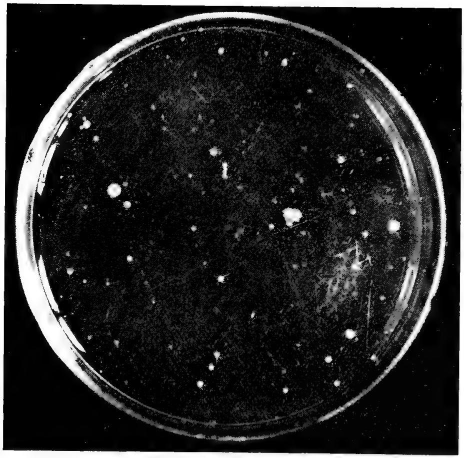

FIG. 4

Fig. 1—Garden mould + tap water + B. coli com. Nutrient agar at 42° C.
Fig. 2 „ „ „ Nutrient agar at 37° C.
Fig. 3 „ „ „ Carbol agar 1 in 1000 at 37° C.
Fig. 4 „ „ „ Bile-salt agar at 37° C.

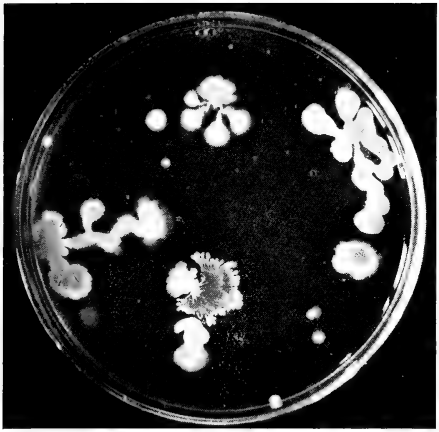

Fig. 5

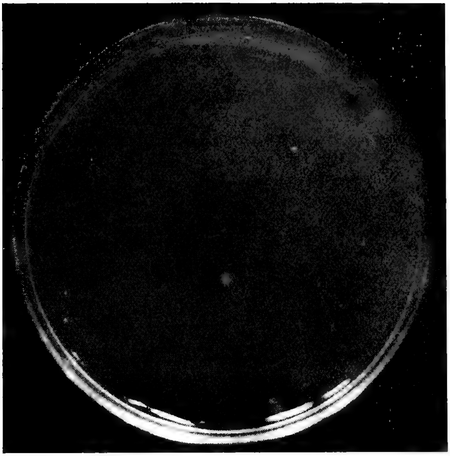

Fig. 6

Fig. 7

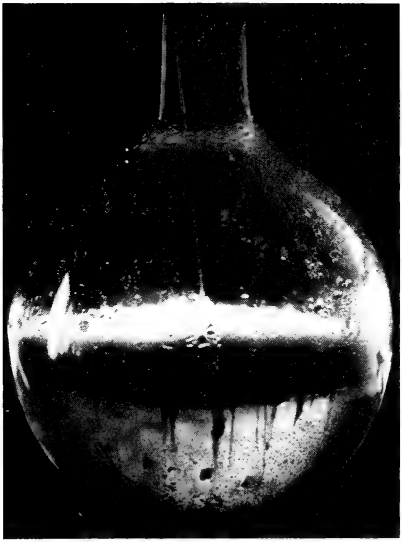

Fig. 8

Fig. 5—Sewage contaminated mud. Carbol agar 1 in 1000.
Fig. 6—Sewage contaminated mud. Bile-salt agar.
Fig. 7—Stalactite growth of B. pestis, natural size.
Fig. 8—Stalactite growth of B. pestis.

These experiments are so few that a definite conclusion cannot be framed, but they suggest that working on these lines it may be ultimately possible to devise a simple method of isolating the Bacillus typhi abdominalis.

In making up the medium great care must be taken not to overheat it after adding the lactose, as, in that case, a change takes place, and B. t. a. produces a haze like B. c. c. The composition which has given the best results so far is :—Bile salt 0.5 p.c., peptone 2.0 p.c., lactose 1.0 p.c., agar 1.5 p.c., tap-water q. s.

NOTE UPON THE

# ACTION OF THE DIBDIN CONTACT BEDS CONSTRUCTED BY THE CORPORATION OF LIVERPOOL AT WEST DERBY

The following account of the Sewage Farms at West Derby and Walton, and of the subsequent construction of Storm Water Filter Beds, and of Dibdin Beds, has been kindly furnished by the City Engineer. I desire to express my thanks both to the City Engineer, the Assistant Engineer (Mr. Cooper), and the Farm Bailiff (Mr. Smith), and more recently to Mr. Edge, for their cordial co-operation in enabling me to conduct the bacteriological investigations.

The interest attaching to the experiments lies in the comparison of the sand effluents with the Dibdin effluents, and with the action of the rough storm water filter beds, which have been in use since April, 1896. The bacteriological analyses have been made by Drs. Hill, MacConkey, and Miss Chick.

Rubert Boyce.

## COPORATION SEWAGE FARMS AT WEST DERBY & WALTON

The system adopted on these Farms is broad irrigation, and filtration without chemical treatment. On the West Derby Farm special contact beds have been constructed.

The West Derby Farm has an area of 207 acres, which, at the present time, takes the sewage of about 35,000 inhabitants, spread over an area of about 2,000 acres. The sewage is conducted on to the Farm by means of two sewers, one a high-level brick sewer, 5ft. 3in. by 3ft. 6in. in size, outlets at the highest point of the land, whence, if desired, the sewage could be carried to any part of the Farm. It is a gravitation sewer throughout, with a ruling gradient of 5 feet per mile, or 1 in 1,056. The second sewer, which is also of brick, but 4 ft. 6 in. by 3 ft. internal dimensions, with a gradient of 1 in 1,120, serves the low-lying portion of the district, and discharges into a tank some 13 feet below surface level at the lowest part of the land. It has here to be raised by pumping to a height of about 10 feet above the ground level, to permit of its distribution over the land by gravitation. This distribution is effected both for the high and low level sewers by means of open carriers (or conduits), which are generally constructed of concrete, but where the transverse depressions in the land are, of galvanized iron, supported on tressels. Syphons are provided under any cartways which are encountered in the course of the carriers. Special provision is made for storm water, which mostly enters the sewers (as few surface water drains exist), and in storm time reaches the Farm more rapidly than it can be filtered through the land.

The provision for storm water consists of a plot of land some 20 acres in extent, which is banked round to the height of 3 feet. The storm water accumulates on this plot, and is gradually run off through a Coke Screening Chamber with double screens, filled with coke of small size, which suffices to arrest most of the suspended matter in the diluted sewage; and also filter beds composed of burned clay ballast of about 2 feet in depth. The area of these beds is about 1.75 acres, and they give a satisfactory effluent compared with the brook when filled with storm water. The Farm is underdrained with agricultural drain pipes, which are laid at depths varying between 4 and 6 feet.

In the case of the Walton Farm, this takes at the present time the sewage of about 35,000 inhabitants spread over an area of 1,650 acres. It has an acreage of 183 acres. There is only one outfall sewer to this Farm, the internal dimensions of which are 3 ft. 6 in. by 2 ft. 4 in. It has a gradient of 1 in 1,300. This sewer discharges through a 38-inch and also an 18-inch pipe into a tank elevated some 6 or 8 feet above the highest point of the Farm. From this tank underground pipe carriers are laid to the different parts of the Farm, where they terminate in chambers provided with penstocks, and the sewage is distributed from these chambers into the various parts of the Farm which require the irrigation.

No special provision is made on this Farm for dealing with storm water, which must all be accommodated upon the surface of the land. This Farm, like the West Derby Farm, is extensively underdrained, and these drains also are collected in the main effluent drains, which discharge into a natural watercourse.

Both the West Derby and the Walton Farms are used for the purpose of raising crops, principally ryegrass, cabbages, potatoes, mangel-wurzels, and turnips.

## WEST DERBY FARM

Dry weather flow per 24 hours :—

| | Gallons | Total |
|---|---|---|
| High-level sewer... ... | 725,000 | 976,000 |
| Low-level sewer ... ... | 251,000 | |
| Walton Farm ... ... | 660,000 | |

## STORM WATER PLOT & BEDS—WEST DERBY SEWAGE FARM

In April, 1896, the then City Engineer obtained authority from the Works Committee to form banks about 3 feet high round plot L, which is about 20 acres in area and reasonably level. The object of this was to impound storm water from the western outfall sewer, and to gradually run it off into the 'Alt' through a special coke screening chamber, which was constructed on the intake to the 24-inch pipes which provided the outlet to the 'Alt.' This arrangement was much improved subsequently by providing a 15-inch pipe at a higher level than the 24-inch outlet pipes from the screening chamber; and this 15-inch pipe discharged on to the surface of a burned ballast filter bed about $\frac{1}{4}$ acre in extent, and thence into the 'Alt.' A much clearer effluent was thus obtained than through the coke screens only.

A considerable amount of sludge was deposited all over plot L, although there was only 1 part of sewage in each 36 of storm water, and, as no crops could be raised on any part of this plot, authority was obtained in January, 1897, to form three sludge beds, each rather over 1 acre in area, which should discharge alternately into a common channel and through the coke screening chamber. The effect of these beds was to concentrate the sludge, and to enable a paying crop to be raised on half of the remaining area of plot L. The other half remainder (about 8 acres) was to be devoted to storage of storm water. These two plots of about 8 acres have been changed about each year, being one year under cultivation and next year under water.

The cost of the sludge beds was about £250

## BACTERIOLOGICAL FILTER BEDS

In the year 1898 the Corporation of Liverpool decided to put down filter beds, with the object of assisting the irrigation area of the West Derby Sewage Farm. The original proposal was to construct four coarse and four fine beds of a cheap construction, the excavations being out of the solid clay, and the floors and sides being faced with concrete. Owing, however, to difficulties in connection with levels, and other local conditions, the number of beds was reduced to six in three series. The first contact beds in each series had also to be built above the natural surface of the ground, the secondary beds only being in the solid ground. The beds in use, and which comprise three systems, are numbered 1, 1*a*, 2, 2*a*, 3, 3*a*.

### BEDS Nos. 1 and 1*a*.

In this pair, the first contact bed (No. 1) was made up of burnt clay to a depth of about 2 ft. 8 in., and the second contact bed (No. 1*a*) has burnt clay in the bottom to a depth of about 14 inches, and on the top of this coke broken to about the size of the ballast, making a total depth of 2 ft. 8 in.

Bed No. 1 has an area of 230 square yards, and No. 1*a* an area of 254 square yards, with a capacity of 205 and 226 cubic yards respectively. The area of the two beds combined equals one-tenth acre.

These beds commenced working on the 28th June, 1899, and were charged with crude sewage three times per 24 hours, each charge being allowed to remain in contact with the material in the bed for at least two hours. This rate of working was maintained up to the 25th October, 1899, without any period of absolute rest, with the exception of an occasional charge or two which were missed in times of heavy rainfall. On the 25th October, owing to the surface of Bed No. 1 becoming very foul, the charges were reduced to one per day, with the result that an improvement in the surface began at once to be noticeable. At the present time the charges on these beds are two per day, the increase taking place from the 13th December, 1899.

On the 23rd January, 1900, a section of the bed was exposed and little if any sludge was found in the bottom; but the whole of the material was uniformly and somewhat heavily coated with dark-coloured sewage matter, and worms were found in considerable numbers. The beds opened up sweet, and apparently without any bad smell whatever. Owing to the action of the weather upon the clay ballast, it was necessary on the 16th March, 1900, to remove about 3 inches from the surface and to replace it with coke.

During the whole period the second contact bed No. 1*a* had worked well in conjunction with No. 1, as regards quantities, and has kept very clean on the surface.

The following statement shows the capacity of the bed at the various times at which it has been tested by measurement :—

| Date | Cubic feet | Gallons |
|---|---|---|
| *June 28th, 1899 ... ... | 2,900 | 18,120 |
| July 6th, " ... ... | 2,500 | 15,620 |
| October 6th, " ... ... | 2,050 | 12,810 |
| November 29th, " ... ... | 1,900 | 11,870 |
| December 13th, " ... ... | 1,850 | 11,560 |
| January 23rd, 1900 ... ... | 1,760 | 10,940 |
| February 23rd, " ... ... | 1,700 | 10,620 |
| March 19th, " ... ... | 1,600 | 10,000 |
| April 23rd, " ... ... | 1,500 | 9,370 |

* On this date, which was the first time on which the bed was charged, the material was dry.

## BEDS Nos. 2 AND 2*a*

The first contact bed (No. 2) is made up of red sandstone obtained from Wavertree, and has a depth of about 3 feet. Bed No. 2*a* is constructed of coke broken to about the size of coke breeze.

The area of bed No. 2 is 230 square yards and of No. 2*a* 254 square yards, with a capacity of 230 cubic yards and 254 cubic yards respectively.

These beds commenced working on the 17th August, 1899, and were charged, as in the case of No. 1, with three charges per day, the only relief being during times of heavy rainfall, when the sewers were delivering practically nothing but storm water. This rate of working was reduced to two charges per day on the 24th February, 1900, owing to the surface of the bed showing signs of foulness, and this reduced rate at present continues.

On the 23rd January, 1900, the bed was opened up, and about 1½ inches of sludge was found at the bottom, but in other respects it was in a similar condition to No. 1 bed.

A noticeable feature in the case of this bed was the length of time the sandstone remained clean and bright upon the surface.

The following statement shows the capacity of the bed at the various times on which it has been tested by measurement :—

| Date | Cubic feet | Gallons |
|---|---|---|
| * August 17th, 1899 ... ... | 2,450 | 15,310 |
| ,, 18th, ,, ... ... | 2,300 | 14,370 |
| October 6th, ,, ... ... | 2,150 | 13,440 |
| November 29th, ,, ... ... | 2,100 | 13,120 |
| December 19th, ,, ... ... | 2,050 | 12,810 |
| January 24th, 1900 ... ... | 1,950 | 12,190 |
| February 23rd, ,, ... ... | 1,900 | 11,870 |
| March 19th, ,, ... ... | 1,800 | 11,250 |
| April 23rd, ,, ... ... | 1,700 | 10,620 |

## BEDS Nos. 3 AND 3*a*.

In this instance the first contact bed is composed of ordinary gas coke placed in position as carted from the works, and has a depth of about 3 feet.

The second contact bed (No. 3*a*) is filled with sand and gravel, the greater part consisting of sand containing a certain amount of grit. This is, however, imported into Liverpool as Wyre Gravel.

* First time of charging, material dry.

As in the case of the other beds, the combined area of the bed is one-tenth of an acre ; No. 3 bed being 230 square yards in extent, and No. 3*a* 254 yards, with a capacity of 230 cubic yards and 254 yards respectively. Owing to the nature of the material in 3*a*, it has been found that the area of the sand bed should be twice that of the coke bed.

These beds commenced working on November 14th, 1899, at the rate of one charge per day through No. 3, this being equal to filling 3*a* twice. On November 20th the rate was increased to two fillings of No. 3, or four for No. 3*a*, which was continued until the 16th January, 1900, when the rate was reduced to one charge for No. 3, owing to the difficulty in getting the water through the sand. As the rate of working No. 3*a* did not improve, about 2 inches in depth of the surface was removed, and on restarting a great improvement was noticed, which, however, only continued for 12 or 14 days, the surface becoming gradually clogged up again. The operation of removing the surface of the bed has had to be repeated on two occasions, and digging over the surface has also been tried, the result in each case being a temporary improvement.

Both beds were opened up for examination at the same time as Nos. 1 and 2. No sludge was found in the bottom of No. 3, and no worms were to be seen, but a slight covering of sewage matter was on the material of the bed, and large patches of a reddish-brown colour, resembling iron-rust, were noticed, especially near the surface. No. 3*a* was opened carefully to the bottom, and showed signs of clogging for about 3 inches from the surface, below which the material appeared as fresh as when put in.

The following statement shows the capacity of No. 3 bed at the various times on which it has been tested by measurement :—

| Date | Cubic feet | Gallons |
|---|---|---|
| *November 14th, 1899 ... ... | 3,500 | 21,870 |
| „ 16th, „ ... ... | 3,200 | 20,000 |
| „ 29th, „ ... ... | 3,150 | 19,690 |
| December 20th, „ ... ... | 2,800 | 17,500 |
| January 25th, 1900 ... .. | 2,700 | 16,870 |
| February 23rd, „ ... ... | 2,700 | 16,870 |
| March 19th, „ ... ... | 2,700 | 16,870 |
| April 23rd, „ ... ... | 2,700 | 16,870 |

The effect produced by the working of the bacteriological beds was most marked, not only on the Sewage Farm but on the purity of the River Alt, into which

*First time of charging, material dry.

all effluent drains from the Farm discharge. It was the subject of general remark that the River Alt had not been so clean for many years.

NOTE.—On May 3rd, 1900, the beds 1, 2, and 3 were opened up. There was a considerable amount of sludge on the surface of the bed 1, and the burnt ballast was throughout uniformly covered with one-eighth inch sludge. On bed 2 there was a considerable amount of sludge on the surface, and the stones were coated to the bottom with half-an-inch of sludge. In bed 3, which was only doing half of the work of the other beds, the material had only a very slight coating of sludge.

## BACTERIOLOGICAL INVESTIGATIONS

### NUMBER OF BACTERIA PER C.C. PRESENT IN THE CRUDE SEWAGE DISCHARGED AT THE WEST DERBY AND WALTON SEWAGE FARMS

### West Derby Sewage Farm. Eastern Outfall, Low Level

| Date | Dilution | No. of Plates | Average No. of Bacteria per c.c. |
|---|---|---|---|
| November 7th, 1898 | $\frac{1}{10,000}$ | 6 | 587,000 |
| | $\frac{1}{100,000}$ | 5 | 820,000 |
| " 11th, " | $\frac{1}{10,000}$ | 6 | 3,307,000 |
| | $\frac{1}{100,000}$ | 6 | 5,250,000 |
| " 15th, " | $\frac{1}{100,000}$ | 6 | 17,200,000 |
| " 18th, " | $\frac{1}{100,000}$ | 3 | 2,000,000 |
| " 21st, " | $\frac{1}{100,000}$ | 6 | 19,640,000 |
| " 28th, " | $\frac{1}{10,000}$ | 4 | 265,000 |
| | $\frac{1}{100,000}$ | 5 | 420,000 |
| June 27th, 1899 ... | $\frac{1}{100,000}$ | | 5,640,000 |
| July 23rd, " ... | $\frac{1}{100,000}$ | | 650,000 |
| " 25th, " ... | $\frac{1}{100,000}$ | | 3,650,000 |

### Western Outfall, High Level

| Date | Dilution | No. of Plates | Average No. of Bacteria per c.c. |
|---|---|---|---|
| November 7th, 1898... | 1/10,000 | 6 | 3,160,000 |
| | 1/100,000 | 6 | 5,760,000 |
| " 11th, " | 1/10,000 | 6 | 32,480,000 |
| | 1/100,000 | 5 | 35,960,000 |
| " 15th, " | 1/100,000 | 6 | 64,600,000 |
| " 21st, " | 1/100,000 | 6 | 4,217,000 |
| June 27th, 1899 ... | 1/100,000 | | 3,140,000 |
| July 10th, " | 1/100,000 | | 6,540,000 |
| " 13th, " | 1/100,000 | | 22,834,000 |
| " 28th, " | 1/100,000 | | 6,000,000 |
| August 23rd, " | 1/100,000 | | 3,700,000 |
| September 2nd, " | 1/100,000 | | 1,340,000 |

## WALTON SEWAGE FARM

### Crude Sewage

| Date | Dilution | No. of Plates | Average No. of Bacteria per c.c. |
|---|---|---|---|
| November 11th, 1898 | 1/10,000 | 6 | 9,554,000 |
| | 1/100,000 | 6 | 32,200,000 |
| " 15th, " | 1/100,000 | 6 | 20,800,000 |
| " 21st, " | 1/100,000 | 6 | 4,170,000 |
| " 28th, " | 1/100,000 | 4 | 425,000 |

From the above tables it will be seen that the average number of bacteria per c.c. varies very greatly. The average of 29 analyses is 1,090,726.

## The Contact Beds

The effluent from the final contact beds, 1*a* and 2*a*, has always contained a considerable amount of matter in suspension, and in large quantity is opaque. The effluent also undergoes slight decomposition on standing. The effluent from bed 3*a* (sand bed) has on the other hand been clear, and has not undergone decomposition on standing, or after incubation. In the case of all three effluents, the following figures show that the total number of organisms, as well as the numbers of the B. coli, are very high.

Table showing the number of bacteria per c.c., the number of B. coli per c.c., and the presence or absence of the B. enteritidis sporogenes in the effluent from bed 2*a* :—

| Date | No. of Organisms per c.c. | B. coli per c.c. | B. enteritidis sporogenes in 0.01 c.c. |
|---|---|---|---|
| November 8th, 1899 | 270,000 | 11,030 | Present |
| „ 9th, „ | 325,000 | 3,475 | „ |
| „ 10-11th, „ | 220,000 | 21,370 | „ |
| „ 13th, „ | 4,327,000 | 12,380 | „ |
| „ 14th, „ | 937,000 | 24,070 | „ |
| „ 15th, „ | 2,060,000 | 12,770 | „ |
| „ 16th, „ | 2,187,000 | 7,433 | „ |
| „ 17th, „ | 1,440,000 | 14,570 | „ |
| „ 18th, „ | 1,284,000 | 13,000 | (in 0.1 c.c.) |
| „ 27th, „ | 20,000 | 100 | Present |
| „ 28th, „ | 667,000 | 22,500 | „ |
| „ 29th, „ | 1,196,700 | | „ |
| „ 30th, „ | 1,690,000 | 14,367 | „ |
| December 1st, „ | 840,000 | 20,100 | „ |
| „ 5th, „ | 2,460,000 | 11,000 | „ |
| „ 7th, „ | 720,000 | 1,600 | „ |
| „ 8th, „ | 23,000 | | |
| „ 9th, „ | 13,000 | Doubtful if any 200 (?) | „ |
| „ 12th, „ | 48,000 | 2,700 | „ |
| „ 13th, „ | 13,700 | 407 | „ |
| „ 14th, „ | 23,700 | | |
| „ 15th, „ | 7,700 | Absent in 1 over 100 c.c. | Absent |
| „ 16th, „ | 12,200 | | „ |
| „ 18th, „ | 3,870 | | Present |
| „ 19th, „ | 157,200 | 6,633 | „ |
| January 8th, 1900 | 81,400 | 2,000 | Absent |
| „ 10th, „ | 360,000 | 13,400 | „ |
| „ 15th, „ | 64,470 | | |
| „ 19th, „ | { 114,000 / 41,700 } | | |
| „ 20th, „ | 14,700 | 1,333 | |
| „ 23rd, „ | 162,000 | | |
| „ 24th, „ | 89,400 | 14,000 | Present |
| „ 26th, „ | 91,700 | 600 | |
| „ 29th, „ | 119,470 | | „ |
| „ 30th, „ | 124,800 | | |

From the above table it will be seen that the average number of bacteria per c.c., as the result of 36 analyses, is 614,158.

The average number of B. coli in 23 analyses is 10,036.

The number of times in which the B. enteritidis sporogenes is present in .01 c.c., in 28 analyses, is 23.

## Bacteriological Analyses of the Land Drain Effluent of the West Derby Farm

Of 8 determinations the average number of bacteria per c.c. was 17,887, and the average number of the B. coli 50 per c.c. The B. enteritidis sporogenes was present in the 1 c.c. in 2 out of 5 determinations. It will therefore be seen that the land filtration gives better results than filtration through the artificial beds.

## Observations upon the Sand Filter Bed (Bed 3*a*)

As previously stated the effluent from this bed has been very much clearer than those derived from the other 2 contact beds. It has also not undergone decomposition upon incubation. On the other hand bacteriological analyses show that the numbers of B. coli still remains high.

### West Derby Sand Bed Effluent

| Date | Bacteria | B. coli | B. enteritidis sporogenes |
|---|---|---|---|
| November 28th, 1899 ... | | 11,400 | |
| December 11th, 1899 ... | | 26,867 | |
| " " " ... | | 20,850 | |

### Sand Filter Bed

January 22nd, 1900.—The bed had become clogged by deposit on the surface. An analysis of the effluent gave the following results :—

| January 22nd, 1900 | Bacteria per c.c. | B. coli per c.c. | B. enteritidis sporogenes |
|---|---|---|---|
| Effluent from Rough Coke Bed running on to Sand... | 1,085,000 | 777 | Absent in 0.01 c.c. |
| Sand Effluent ... ... | 23,800 | 533 | Present in 1 c.c. |
| " after 1 hour... | 28,600 | 663 | Absent in 0.01 c.c. |

January 23rd.—An analysis of the sand of the bed was made.

| January 23rd, 1900 | Bacteria per gram. | B. coli | B. enteritidis sporogenes |
|---|---|---|---|
| Top of Bed ... ... | 34,000,000 | 33,600 | Present in 0.01 gram. |
| Middle ... ... ... | 2,000,000 | 300 | Absent in 0.01 gram. |
| Bottom ... ... ... | *46,000,000 | 700 | Absent in 0.01 gram. |

January 24th, 1900.—The surface of the filter was skimmed off. An analysis of the first filtration gave the following results :—

| January 24th, 1900 | Bacteria per c.c. | B. coli | B. enteritidis sporogenes |
|---|---|---|---|
| First 10 minutes ... ... | 75,100 | 6,100 | Absent in 0.8 c.c. (clot) |
| After 1 hour ... ... | 63,500 | 5,900 | Absent in 0.9 c.c. (clot) |

January 31st, 1900.—Samples of the effluent were taken after the bed had been working for a week.

| January 31st, 1900 | Bacteria per c.c. | B. coli | B. enteritidis sporogenes in 1 c.c. |
|---|---|---|---|
| Average of Samples taken every $\frac{1}{4}$ hour for 3 hours... | 40,800 | 6,733 | Present |
| Last of effluent coming from bed ... ... | 30,300 | 4,800 | Present |

Bacteriological Investigations of the Storm Water Filter Beds

On January 22nd, 1900, during a period of heavy rainfall, bacteriological analyses were made of the effluents of the Storm Water Filter Beds.

| Source | Bacteria per c.c. | B. coli per c.c. | B. enteritidis sporogenes |
|---|---|---|---|
| Crude Sewage and Storm Water, W. outfall ... ... ... | 5,360,000 | 31,400 | Present in 1 c.c. |
| Crude Sewage and Storm Water, E. outfall ... ... ... | 946,700 | 9,300 | |
| Effluent from Burnt Ballast Filter ... ... ... | 149,600 | 4,133 | Absent in .01 c.c. |
| Effluent from Cinder Bed Filter | 290,600 | 733 | Absent in .01 c.c. |

* One organism chiefly.

From these figures it will be seen that the storm water filters produce a very considerable bacterial purification. The satisfactory action of these beds is probably explained by the very long periods of rest which they obtain.

## Clogging of Filter Beds

A year's constant working having very considerably reduced the capacity of the beds, it has been decided to give them a rest from June 9th, 1900. Before doing so, however, analyses were made on June 9th to show exactly the bacterial condition of the effluents after the one year's working. The following table shows the number of bacteria per c.c., the number of B. coli, and the presence or absence of the B. enteritidis sporogenes. It will be seen that the numbers still remain very high.

| Source | Bacteria per c.c. | B. coli per c.c. | B. enteritidis sporogenes .01 c.c. |
|---|---|---|---|
| Bed 1*a* ... ... | 1,270,000 | 7,000 | Present |
| Bed 2*a* ... ... | 837,000 | 21,100 | Absent |
| Bed 3*a* ... ... | 740,000 | 12,000 | Present |

NOTE UPON THE

# TWO SPECIES OF 'FUNGUS' COMMONLY FOUND IN SEWAGE CONTAMINATED WATER

By RUBERT BOYCE

The 'Sewage Fungus' is a gelatinous, cotton wool-like and wavy, white or reddish growth, which is found in shallow running water, covering stones, lining drain pipes, or attached to water plants and débris. Its presence indicates sewage contamination, and it is therefore usually to be found in the drains and streams of sewage farms and sewage works, and in the sewage contaminated brooks of villages. The appearance of the fungus is characteristic, and it cannot be readily mistaken for other growths, such as those produced by Streptothrix and Beggiatoa. It may occur in enormous quantities and produce blocking of drain pipes and small streams; or, as it becomes readily detached, accumulations sometimes occur, which are prone to undergo decomposition and give rise to black putrescent masses.

Great interest attaches to the fungus because it indicates sewage pollution. The growth, however, is not found in crude sewage; it is found in the effluents of sewage farms, resulting from the filtration of the crude sewage through the earth, and in brooks which receive small house drains. It indicates, therefore, a certain degree of pollution. In Germany, owing chiefly to the researches of Mez and Schorler, attention has been drawn to the significance of the 'fungus.' It became apparent that at least two distinct growths were included in the term 'sewage fungus,' one *Leptomitus lacteus*, the other *Sphærotilus natans*, the former present in very slightly contaminated water, the latter in highly polluted water.

## Leptomitus lacteus (Agardh)*

This fungus belongs to the family of the Saprolegniaceæ. It forms long wavy tufts, springing from the sides of drains or attached to stones. It is very soft, almost gelatinous, and may form white, rusty, or black masses. The white appearance is the natural colour of the growing filaments, but very soon this colour is replaced by a deposit on the hyphœ of oxide of iron. The rusty colour of the fungus is very characteristic, and is an indication that the stream in which it is found is well oxygenated. If the oxygen is absorbed, as in the interior of masses of the growth, or

* Hildebrand, *Jahrb. für wissensch. Bot.* Bd. vi; Pringsheim, *Jahrb. für wissensch. Bot.* Bd. ii; Cohn, *Jahresber. d. Schles. Gesellsch. für Vaterländ*, Cultur, 1852.

in stagnant water, sulphide of iron is formed, and the fungus assumes a black colour and undergoes putrefactive decomposition.

Microscopic examination shows that it consists of long branching filaments, which are constricted at regular intervals; the branches bud off below the constrictions, and each segment has a very characteristic refractile nucleus. Zoospores are formed in the terminal segments.

### Sphærotilus natans

This organism may be readily confounded with the preceding. It forms quite as long wavy masses in the drains and streams as does Leptomitus. It is, however, usually white and cotton wool-like when seen in the water. It is much more gelatinous than Leptomitus. On stones in shallow streams, or coating the sides of drain pipes, it forms low velvety or feather-like growths. Like Leptomitus, it requires oxygen, and grows best in shallow water-courses and where there is plenty of movement. Its presence indicates much greater pollution than does the former organism, and therefore it is of importance to be able to distinguish the two forms. In two cases where bacterial analyses were made of the water in which both forms of 'fungus' were found, that in which Leptomitus occurred contained at least less than 100 Bacillus coli per c.c., whilst that in which Sphærotilus was found contained over 19,000 Bacillus coli per c.c. With the introduction of the bacterial bed method of treatment, this organism has also made its appearance. In one case, where in the method of treatment the bacterial filter is warmed, I found that the Sphærotilus had made its appearance in large quantity, the food materials—circulation, aeration, and warmth—being the conditions most favourable to its development. It is, therefore, an organism which may cause blocking of aerobic contact beds.

Sphærotilus has been long confounded with Beggiatoa. It is allied to the Leptothrix forms. As the microscopic preparations show, it occurs in chains of short bacilli, or as long undivided filaments. It is, therefore, very much more minute than Leptomitus; this is readily seen from the photomicrographs, which are equally magnified. The filaments and rods are surrounded with a gelatinous capsule.

In crude, undiluted sewage, a skin-like growth may form at the sides of the conduit in contact with the air, or at those points where crude sewage passes over a 'lip.' The growth, which has a coarse velvety appearance, consists of club-shaped zooglea masses of bacilli, and it seems probable that this bacterium, if not identical with Sphærotilus, is closely allied to it.

Sphærotilus may also be confounded with Carchesium. I have found *Carchesium Lachmanni* coating the surface of the stones in a polluted stream and producing the white cotton wool-like appearance of the fungus; in fact, on the farm in question, it was always regarded as the 'sewage fungus'; microscopic examination, Plate VI., Fig. 6, showed, however, a striking difference.

FIG. 1. LEPTOMITUS LACTEUS, nat. size.

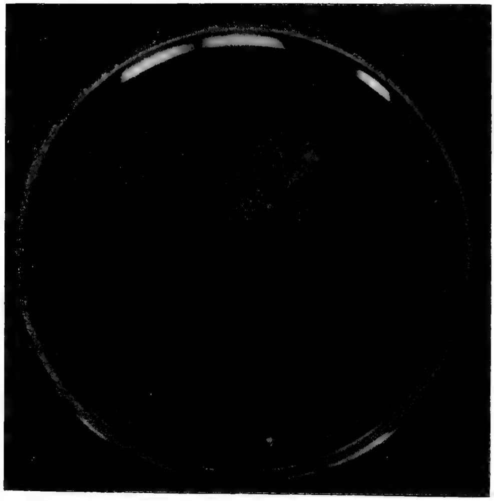

FIG. 2. SPHÆROTILUS NATANS, nat. size.

FIG. 3. LEPTOMITUS LACTEUS, showing branching.

FIG. 4. LEPTOMITUS LACTEUS, showing constriction and nucleus.

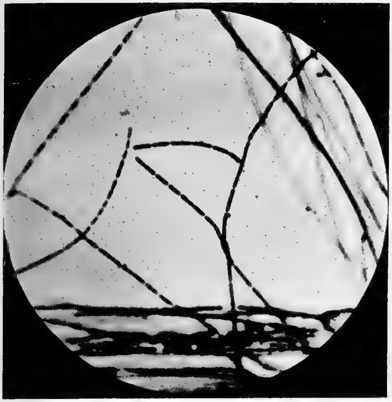

FIG 5. SPHÆROTILUS NATANS.

FIG. 6. CARCHESIUM LACHMANNI.

Leptomitus, Sphærotilus, the zooglea forming bacteria, and Carchesium Lachmanni, all indicate a water which contains special food stuffs derived from sewage; they also indicate aeration. Leptomitus indicates the least amount of contamination and the greatest amount of aeration; the zooglea masses are present in concentrated sewage, and Sphærotilus and Carchesium hold intermediate places.

These organisms perform a very important function—they follow up and complete the work of the bacteria in breaking up organic matter; they are succeeded by higher forms of ~~animal~~ vegetable life—the Diatomaceæ and the Green Algæ, whilst these in their turn give place to the fishes and green plants. The 'sewage fungus,' therefore, plays a very considerable share in the self-purification of water.

# PRESERVATIVES AND COLOURING MATTERS IN FOODS

By E. W. HOPE, M.D., D.Sc.

The importance of avoiding waste in regard to food stuffs of any kind does not need to be emphasized, but it is of greater importance to insure that the means taken to prevent waste are not calculated to injure health.

The preservation of meat and other perishable foods by means of cold is, as is well known, largely resorted to in this country. The successful application of cold as a preservative receives its best illustration in the case of the Copenhagen milk supply, into the details of which it is not now necessary to enter.

In this country the use of chemical preservatives is exceedingly common, and in the case of some very perishable articles, such as milk and cream, it is to be feared, takes the place of care and cleanliness.

It is established beyond dispute that chemical preservatives, whilst checking putrefactive changes in the food, may also check the fermentative processes of digestion.

Boracic acid or borates, as a preservative, is found in margarine, butter, ham, bacon, pork, fish, cream, and milk. In margarine and butter the use of it has been common for many years; it is usually more or less uniformly mixed with these articles, and about 30 grains to the pound have been found. It is also met with in sausages, pork pies, and pastry. An article sold as 'Arcticanus Special Cream Compound' is a mixture of boracic acid and borax.

Salicylic acid and salicylates are met with in jam, but the manufacturers appear to be bonâ fide anxious to minimise the quantity. In the case of British wines, however, as well as in the case of some unfermented drinks, sometimes both boracic acid and salicylic acid have been found, and there are not evidences of the same care on the part of the manufacturers to limit the amount, the quantity varying from 7 grains to 150 grains of salicylic acid to the gallon, and 4 grains to 100 grains of boracic acid to the gallon.

It is perfectly plain that if it is absolutely necessary to add either of these drugs, and if 7 grains to the gallon is enough in one case, there is no necessity to throw in 150 grains to the gallon in another case.

In prosecutions which have been undertaken in regard to British wines, the defendants have actually had the effrontery to put forward the defence that the chemicals employed were useful as drugs, and they had actually induced medical men to go into the witness-box to prove to the court the value of these drugs when used medicinally, the evidence proving nothing more than that a dose of physic can be administered without injury in certain diseased conditions of the human frame; but the promiscuous administration of doses of physic of this character at meal times is known to have a very prejudicial effect, especially upon young infants.

The addition of formalin, or boracic acid, to milk, appears to be exceedingly uncommon in Liverpool, a growing tendency last year to the use of these materials in milk having been checked by a prosecution under the Food and Drugs Act.

It is abundantly plain that there can be no real necessity for the use of these drugs in milk; if it were a real necessity the sale of milk could not go on as it does, without their use. Of all articles in which the use of chemical preservatives is likely to be attended with mischief, milk is the most likely, and it is in this case that the use of preservatives is most indefensible. The experiments conducted in the Bacteriological Department of the Thompson Yates Laboratories are sufficient to establish the dangers of the practice, even if they stood alone. There are numerous cases of injury resulting from the use of milk so preserved. One may be quoted:—Mrs. E. T. voluntarily offered her milk dealer twice the price he asked to send to her pure milk to be used for her infant. The double price, however, did not deter the dealer on one occasion from sending milk containing boracic acid preservative, the presence of which was immediately detected by its effect upon the child. The dealer made no secret of his action when taxed with it. In cases such as this the use of the preservative is distinctly toxic, hence, if the use be allowed by law, the fact should be made known to the purchaser, since of all casual dangers to health, those arising from the use of poisons in food are the most difficult to defend one's self from.

### Colouring Matters

Colouring matters seem to have two distinct uses in foods—in the one case, merely to give an attractive appearance to an article, but without imitating any other article; and in the other case, it is added to increase the resemblance of the article to that which it is intended to simulate.

The commonest colouring matter, which is usually used for sausages, especially German sausages, consists of a mixture of borax, red coal tar dye of the class known as sulphonated diazol, with a little salt or saltpetre, and sometimes ground rice or bread crumbs mixed with it. Armenian bole consists of oxide of iron with a siliceous matter.

The use of these colouring matters by no means necessarily implies fraudulent intent—purchasers might be supposed to know that no natural food stuff could have the remarkable colour of some German sausages; but there is another colouring matter which might, without injustice, be suspected of bordering upon the fraudulent; that is 'Smokene.' This is a mixture of borax, salt, creosote, and red coal tar dye, which is used for brushing over hams, bacon, tongue, fish, &c., and which gives the article the appearance of having been perfectly and carefully smoked, the operation, like the name, being very ingenious. Amongst other common fraudulent colourings may be mentioned the use of burnt sugar with dilute acetic acid to resemble vinegar, and the use of glucose with a very common black treacle to imitate the brightness of golden syrup.

The dirt, or staleness of goods may be concealed by colouring matters, as, for example, dirty rice, used to make egg powders, coloured with a yellow coal tar dye which takes away the dirty appearance; similarly, stale milk coloured with a slightly yellow dye, gets a richer look. With regard to egg powders, which in reality are merely baking powders, these are coloured yellow and labelled 'each packet equivalent to one egg.' Cases have actually come under notice in which the purchaser has believed that the packet did actually contain the equivalent in food of a desiccated egg, possibly the yellow colour completed the delusion.

It does not appear that the Food and Drugs Act, which is essentially framed on commercial lines, discountenances the use of colouring matters and preservatives, unless it can be proved that such ingredients are injurious to health.

Every person adding any colouring matter, or preservative whatever, to articles of food, should state on a plain, simple and conspicuous label—

1. The material used.
2. The quantity used.
3. The date at which the material was added.

A form of label for supplying this information might be suggested.

Heat and cold as preservatives are exceedingly common, and many articles are now sold as sterile. An article sold as sterile ought certainly to be sterile; if it is not, it possesses the disadvantage of giving a false sense of security to the purchaser. But the sterility should not be secured by using chemicals.

In procuring samples with a view to obtaining information as to the extent to which any given preservative, colouring matter, or adulterant is used, it is of the utmost importance that persons trained in the procedure of obtaining samples should be employed; they, of course, can make use of the services of agents when necessary, but a case may be instanced in which a gentleman not conversant with the methods obtained a considerable number of samples, and on causing them to be analysed found all of them to contain the same preservative. Inquiry, however, showed that all these samples had been supplied from one and the same source.

## Preservatives and Cold

Last year chemical preservatives were substituted for cold to a very great extent in the case of imported pork. This pork is packed in small boxes of 50 lbs. each, which are placed in a refrigerator on board ship with other 'chilled' meat, and kept at a temperature of 32 or 35 to 38 degrees. Previous to packing it is treated in some way with boracic acid, which finds its way into the flesh. The reason for putting it in is to check accidental decomposition, which would result from any defect in the cooling process. If pork is frozen it spoils the appearance and deteriorates the value; hence it can only be 'chilled.'

As illustrating the sudden increase of this trade last year, although the number of pigs slaughtered in the City was well maintained—in fact, was considerably in excess of the previous year—the imports amounted to no less than 29,000 more than in the preceding year, the total 36,227 being nearly five times as large as it was in the preceding year, when the trade appears to have commenced. Prior to that the carcases of dead pigs imported did not exceed a few dozen annually, the trade being practically nil.

Another important aspect of the question of chemical preservatives arises in connection with the increasing demand for, and supply of, sterilized milk, more especially for infants, all over the country.

Many of the firms supplying sterilized milk are not milk-producing firms, the milk being supplied by contract from wholesale milk dealers.

It is obvious that if the milk dealers have added chemical preservatives to the milk sent to the sterilizing companies, the greatest danger to the health of infants will be incurred. It is not reasonable to expect that the sterilizing companies will systematically, day by day, cause analyses to be made to ascertain the presence of these preservatives; but their use should be altogether prohibited, and cleanliness and cold relied upon to ensure preservation of the milk.

The following special samples of home and imported goods were submitted during 1899 for special examination, in order to determine the character and amount of the preservative and colouring matter used:—

SUMMARY

| | | | |
|---|---|---|---|
| Bacon ... ... ... | 5 | Meat Juice... ... ... | 1 |
| Brawn ... ... ... | 2 | Pork Pie ... ... ... | 2 |
| Cheese ... ... ... | 6 | Port Wine ... ... ... | 3 |
| Chicken Broth ... ... | 1 | Arcticanus ... ... ... | 1 |
| Claret ... ... ... | 1 | Armenian Bole ... ... | 1 |
| Cocoa and Milk ... ... | 1 | Bi-Sulphite of Lime ... ... | 1 |
| Condensed Milk ... ... | 2 | Indian Red ... ... | 1 |
| Fruit Jelly ... ... ... | 1 | Meat Preservative ... ... | 1 |
| Ham ... ... ... | 5 | Poloney Dye ... ... | 1 |
| Jams ... ... ... | 7 | Rose Pink ... ... ... | 1 |
| Kidney Soup ... ... | 1 | Sausage Colouring ... ... | 1 |
| Meat Jelly ... ... ... | 1 | Smokene ... ... ... | 1 |

# REPORT TO THE MEDICAL OFFICER OF HEALTH OF THE INVESTIGATIONS AND ANALYSES MADE BY THE CORPORATION BACTERIOLOGIST

The work of the Bacteriologist comprises :—

(*a*) Examination of food stuffs of various kinds.

(*b*) Regular examination of water supplied to the City.

(*c*) Examinations into suspected cases of rabies, anthrax, glanders, &c.

(*d*) Examination for diagnostic purposes in suspected cases of diphtheria, typhoid fever, tubercular sputum, &c.

(*e*) Special investigations.

In the examination of food stuffs the following general methods are employed :—

Samples of milk and samples of water are collected in sterilized bottles, and if they cannot be examined immediately they are kept in a refrigerating chamber.

(1) *Preservatives in Food.* In view of the increasing use of preservatives of various kinds in food stuffs, and the difficulty which has been experienced in proving in Courts of Law the limit beyond which it was dangerous to extend their use, the Medical Officer requested the Bacteriologist to undertake some exact experiments into the physiological action of boracic acid and formalin, as these, especially the latter, were found in foods likely to be used for infants and young children.

A series of experiments were conducted by Dr. Annett, which consisted in feeding kittens of three weeks old upon milk containing minute but definite proportions of the preservatives in question, and the observations extended in each case over a period of from six to seven weeks. With regard to the boracic acid, (*a*) Five kittens were fed upon milk containing 10 grains to the pint ; (*b*) Five kittens were fed upon milk containing 5 grains to the pint ; (*c*) Five kittens were fed upon perfectly pure milk, the kind of food being the only difference in the treatment of the animals. In every case a supply of milk was always available for the kittens to lap.

*Results.* The group (*a*) fed with 10 grains of boracic acid to the pint showed rapid emaciation, diarrhœa, and death resulted in from three to four weeks ; the kittens treated with 5 grains showed results almost similar. The remaining five kittens fed with pure milk increased in weight and remained healthy.

With regard to the second series of milk, viz., that treated with formalin, the proportions of formalin, which is a very powerful antiseptic, were used as follows :— Five kittens were fed with milk containing 1 part in 50,000 of milk, five with 1 in 25,000 of milk, and five with 1 in 12,500. As in the preceding cases, there was a continuous supply for the kittens to lap. Four kittens were fed at the same time with wholesome milk.

The results showed (*a*) the five kittens fed with the weakest solution (1 in 50,000) showed an average increase of weight per week equal only to 70 per cent. of the healthily fed kittens; those fed with the next strongest solution (*b*) (1 in 25,000) increased in weight only to 55 per cent. of that of the healthily fed kittens; (*c*) those animals fed with the proportion containing the largest amount of formalin, namely, 1 in 12,500, increased in weight only from 20 to 25 per cent. of the amount which the healthily fed kittens increased. Two of the number died after six weeks, suffering from emaciation, diarrhœa, and other evidence of disturbed digestion and starvation. The whole of the animals were kept under careful observation, and it appeared that the dosed animals showed general evidences of ill-health, inactivity, and so on, and a disinclination to feed on the preserved milks.

There is not the slightest reason to doubt that the use for infants of milk or cream containing these preservatives will have precisely the same effects upon the infants as they have upon kittens, and the inquiry once more emphasizes the absolute necessity for feeding infants as nature intended they should be fed, or if that is impracticable, to employ pure and natural substitutes.

Formalin, fortunately, is not largely used by milk dealers as a preservative adulterant. About 1,500 samples of milk were chemically examined for the specific purpose of ascertaining if formalin was present, but it was only found in .8 per cent. of these samples.

(2) *Anthrax in Tanneries.* Several cases of Anthrax having occurred during 1899 amongst the workers of the tan-yards, four of which proved fatal, a thorough investigation of the vats, hides, and dust of the tan-yards was made by the Bacteriologist and Dr. Grünbaum. The bacillus anthracis was found in large quantities in the gloves which the workmen used whilst cleaning the hides. The bacillus was also found in the vats used for macerating the hides, and in the dust of the yards.

It was evident that the Anthrax was introduced from the hides taken from infected animals, and a special investigation was then made of the ships which brought over the hides from Eastern ports. This resulted in finding the anthrax bacillus in the dust of a hold from a ship which had contained bales of hides. Subsequently an investigation was made to ascertain the kind of disinfectant which could be relied upon to destroy the anthrax bacilli (which is one most tenacious of life), but without injuring the hides.

(3) *The Tuberculin Reaction.* Some cows which reacted to tuberculin were slaughtered, and the subsequent inoculations proved the presence of the tubercle bacillus, although the lesions were very small, and confined to minute nodules in the lungs and bronchial glands.

(4) *Sewage Investigations.* The importance of bacteria in the treatment and disposal of sewage matter has occupied much attention recently. A large proportion of the work of the Royal Commission on Sewage Disposal is done at the THOMPSON YATES LABORATORIES, and the Health Committee authorized a very large number of experiments to be carried out at the Sewage Farms at West Derby. These investigations have been very useful. The effluents from the Sewage Farm drains have been carefully examined from time to time, and have shown a very good average purification. Experimental filter beds were constructed out of various materials, and have now been at work many months. The effluents yielded by some of the beds have been very good, showing a considerable amount of purification.

(5) *Plague Investigations.* During the summer, rats taken from ships in which plague had occurred were carefully investigated for the presence of the plague bacillus. They proved to be free. Two cases of Bubo, also from suspected ships, were examined for the presence of the plague bacillus, but careful bacteriological examination of the glands clearly demonstrated that the swelling was not due to this cause. The bacteriological diagnosis in these cases proved of the greatest importance in demonstrating the nature of the diseases suspected to be plague. The Assistant Corporation Bacteriologist (Dr. BALFOUR STEWART) has published during the year several papers dealing with his researches upon plague.

(6) *Fish Poisoning.* A bacteriological investigation into the cases of fish poisoning resulted in the discovery of organisms kindred to those found in the putrefying contents of infants' feeding bottles. It is very difficult to say whether the organisms alone are capable of producing the irritant symptoms, or whether these are due to some product resulting from the growth of the bacilli. Be it as it may, in either case the importance of absolute cleanliness is emphasized by the investigation.

## RABIES

During the year there have been no cases of Rabies, but eight cases of suspected Rabies, mostly reported by the police, have been carefully investigated; in none of the cases could the presence of that disease be ascertained.

The Medical Officer has arranged with the Board of Agriculture that in cases of suspected Rabies the inoculation test shall be made by the Corporation Bacteriologists, which obviates the necessity for sending parts of the bodies of dogs suspected of having that disease to London.

## THE BACTERIOLOGICAL DIAGNOSIS OF CASES OF TYPHOID AND DIPHTHERIA

The total number of diagnostic examinations made for the City hospitals was 201. It is of the greatest importance to be able to state whether the organism which causes Diphtheria is present in the throat or not. With regard to the bacteriological diagnosis of Typhoid Fever, evidence is accumulating of the value of the test.

### Pathological Diagnosis Society

This society is formed by a number of medical men, who desire diagnostic investigations to be carried out in respect to patients under their care. It is an extremely useful society, and numbers about 200 medical men.

The work of this society supplements that done for the Corporation. For an annual subscription of 10s. 6d. any practitioner can place any number of cases of Diphtheria, Typhoid, Tubercle, and other infective processes, urine and tumours for bacteriological examination. The number of investigations in this connection has nearly reached 1,000 during the year.

## SPECIAL INVESTIGATIONS

### Summer Diarrhœa

The Medical Officer requested the Bacteriologist to make investigations into the condition of the artificial foods supplied to infants during the season of exceptional mortality from summer diarrhœa.

The homes of the poor where sickness was known to exist were visited, and the feeding bottles found in use, together with their contents, were removed to the laboratories, new bottles being given in their place at the cost of the Health Committee. Furthermore, the same practice was adopted at the dispensaries, the feeding bottles actually in use, and their contents, being taken away for examination, and other bottles given to replace them.

The bacteriological examination confirmed the evidence of the senses, the putrefying and offensive contents of the bottles containing innumerable putrefactive organisms, amongst them being the bacillus enteritidis sporogenes, which, judging from the effects of the injection of bacilli of this description into Guinea-pigs, is virulently destructive of life.

The tubes connected with the bottles were also found to contain putrefactive matter. The examination added confirmation to the neglect, ignorance, and carelessness of the parents.

### Cases of Food Poisoning

On Thursday, September 28th, 1899, the Deputy Coroner held inquests on five persons who died from acute food poisoning.

The facts shortly are as follows :—The deceased persons, all women between 44 and 70 years of age, had partaken of either cooked salt fish or pig's cheek at the stall of Mrs. B——, in St. Martin's Market, on the 14th, 15th, or 16th of September. In a few hours after eating the food each person became seriously ill with symptoms of acute food poisoning, and eventually died, four of them the day after taking the food, and the other the next day but one.

After consultation with the Medical Officer, a post-mortem and bacteriological examination was ordered by the Coroner to be made in each case by the Corporation Bacteriologist.

The result of the examination showed that in all the cases the naked eye appearances were identical, and indicated a very rapid and intense infection. In three cases the Bacteriologist was immediately able to discover the presence of the bacillus enteritidis sporogenes, and in the other two, although this bacillus was not definitely proved to exist at the date of inquest, there appeared strong evidence that it was or had been present.

The bacillus enteritidis sporogenes is found in sewage, foul air and water, foul vessels and filthy clothes, and in decomposing horse manure, &c. It is very resistant to heat, and its spores will survive the action of boiling water for some time.

The Bacteriologist has also recently found it in dirty feeding bottles of infants suffering from acute diarrhœa.

In the case of the persons who died after eating food in St. Martin's Market, there was no evidence to prove how the food became contaminated by the bacillus. It may have been in the food before cooking, though in the case of the salt fish that is unlikely, or it may have infected the food afterwards. Inquiry showed that the food was kept under insanitary conditions by the vendor, at her house, and by alternate wetting and drying, cooking, and carrying through the streets, it would be in a condition suitable for the growth and development of the bacillus.

The Bacteriologist carried out experiments with portions of similar cooked and uncooked food, and with scrapings of stalls, &c., on which it lay. No examination of the actual offending food in these cases was possible, as the deceased persons ate their portions in the market, and by the time their deaths were known the stall holder had no further sample of the same lot left. She, in fact, stated that she never carried over any cooked food from day to day.

Frequent observations by the Fish Inspectors have failed to show any naked eye appearances of diseased food on the stall.

Besides the five fatal cases, three other cases of illness came under the notice of the Medical Officer. These persons ate some of the same food in company with one or other of the deceased persons, and afterwards suffered with serious symptoms of food poisoning.

A table giving particulars of all the cases is appended. It is probable that a considerable number of persons ate food from Mrs. B——'s stall on the dates in question without suffering any ill effects.

| Name | Age | Date of Illness | Result of Illness | Nature of Food | Bacteriologist's Report |
|---|---|---|---|---|---|
| Catherine K... ... | 48 | Sept. 14th... | Death, Sept. 15th... | Pig's Cheek... | No Bacillus Enteritidis Sporogenes |
| Teresa D. ... ... | 44 | „ 15th... | „ „ 16th... | Fish ... ... | No Bacillus Enteritidis Sporogenes |
| Ellen M. ... ... | 54 | „ 15th... | „ „ 17th... | „ ... ... | Bacillus Enteritidis Sporogenes found |
| Margaret McG. ... | 66-70 | „ 16th... | „ „ 17th... | „ ... ... | Bacillus Enteritidis Sporogenes found |
| Ann G. ... ... | 62 | „ 16th... | „ „ 17th... | Fish and Pea Soup ... | Bacillus Enteritidis Sporogenes found |
| Charles D. ... ... | 15 | „ 15th... | Recovery ... ... | Fish ... ... | |
| Catherine C.... ... | — | „ 15th... | „ ... ... | „ ... ... | |
| Esther K. ... ... | — | „ 16th... | „ ... ... | Fish and Pea Soup ... | |

## Samples taken for Bacteriological Analysis or Examination

| | | | |
|---|---|---|---|
| Anchovy Paste ... ... | 1 | Olive Oil ... ... | 1 |
| Bloater „ ... ... | 2 | Oysters ... ... | 4 |
| Bovril ... ... ... | 1 | Periwinkles ... ... | 1 |
| Camp Coffee ... ... | 1 | Potted Beef ... ... | 2 |
| Cheese ... ... | 6 | „ Game ... ... | 1 |
| Chicken, Ham, and Tongue ... | 3 | „ Ham ... ... | 3 |
| Cockles ... ... | 4 | „ Shrimps ... .. | 3 |
| Crab ... ... ... | 1 | „ Tongue ... ... | 2 |
| Cream ... ... ... | 2 | „ Venison ... ... | 1 |
| Desiccated Egg Food ... | 1 | „ Lobster ... ... | 2 |
| „ Soup ... ... | 1 | Preserved Peas ... ... | 1 |
| Dry Antiseptic ... ... | 1 | „ Peaches ... ... | 1 |
| Fruit Juice Lemonade ... | 1 | „ Pears ... ... | 1 |
| Ham and Tongue (Tinned) ... | 1 | „ Pineapple... ... | 1 |
| Ham and Chicken ... ... | 1 | „ Tomatoes ... | 1 |
| Ice ... ... ... | 1 | Salmon (Tinned) ... ... | 7 |
| Jams ... ... ... | 17 | Salted Haddock ... ... | 6 |
| Jellies ... ... ... | 4 | „ Cod ... ... | 8 |
| Ketchup ... ... | 1 | „ Ling ... ... | 2 |
| Lemon Cheese ... ... | 1 | Sardines (Tinned) ... ... | 9 |
| Lobster (Tinned) ... ... | 1 | „ (Smoked, Tinned) ... | 1 |
| Malted Milk ... ... | 1 | Sausages (Smoked) ... ... | 1 |
| Margarine ... ... | 14 | Sauces ... ... ... | 4 |
| Marmalade ... ... | 5 | Sterilized Milk ... ... | 5 |
| Milk ... ... ... | 353 | Turkey and Tongue (Tinned) ... | 2 |
| „ Condensed ... ... | 4 | Veal and Ham ... ... | 2 |
| Mussels ... ... | 5 | Total ... ... | 506 |

*Milk.*—The total number of samples analyzed has been 352. Of these 159 have been taken at the railway stations on arrival from the country, and 162 from the town.

Of the 159 railway samples, in 27 cases the inoculated animals died before the observation was completed, and of the remaining 132, 15 were proved to be tubercular, giving the percentage of tubercle in railway-borne milk as 11.3 per cent.

Of the town milks, 23 inoculated animals died before the observation was completed; of the remainder 1 was tubercular, thus giving a percentage of .7, or less than 1 per cent.

There were 31 examinations of special samples of milk. None of these were tubercular.

In the following table the + sign denoted the presence of tubercle, the — sign the absence of tubercle, and the word negative indicates that the animal died before the tubercular reaction had time to develop, if it were present in the milk which was inoculated.

MILKS

| No. | Source | Date | Tubercle present or absent |
|---|---|---|---|
| 450 | Town | January 4th | — |
| 451 | Town | January 4th | Negative |
| 455 | Rail | January 6th | — |
| 456 | Rail | January 6th | Negative |
| 459 | Town | January 12th | — |
| 460 | Town | January 12th | — |
| 461 | Town | January 12th | — |
| 462 | Town | January 12th | — |
| 467 | Rail | January 17th | — |
| 468 | Rail | January 17th | — |
| 469 | Rail | January 17th | + |
| 470 | Rail | January 17th | + |
| 471 | Town | January 17th | — |
| 475 | Rail | January 25th | — |
| 476 | Rail | January 25th | — |
| 477 | Rail | January 25th | — |
| 478 | Rail | January 25th | — |
| 479 | Town | January 26th | — |
| 480 | Town | January 26th | — |
| 484 | Town | January 30th | — |
| 485 | Town | January 31st | — |
| 486 | Town | January 31st | — |
| 487 | Town | January 31st | — |
| 488 | Town | January 31st | — |
| 492 | Rail | February 8th | + |
| 493 | Rail | February 8th | + |
| 494 | Rail | February 8th | — |
| 495 | Rail | February 8th | — |
| 496 | Rail | February 10th | — |
| 503 | Town | February 16th | — |

MILKS—*continued*

| No. | Source | Date | Tubercle present or absent |
|---|---|---|---|
| 504 | Town | February 16th | — |
| 505 | Town | February 16th | — |
| 507 | Town | February 16th | — |
| 508 | Rail | February 23rd | — |
| 509 | Rail | February 23rd | — |
| 510 | Rail | February 23rd | — |
| 511 | Rail | February 23rd | — |
| 512 | Rail | February 23rd | — |
| 519 | Rail | March 2nd | — |
| 520 | Rail | March 2nd | — |
| 521 | Rail | March 2nd | Negative |
| 522 | Rail | March 2nd | — |
| 523 | Rail | March 2nd | — |
| 524 | Rail | March 9th | — |
| 525 | Rail | March 9th | — |
| 526 | Rail | March 9th | — |
| 527 | Rail | March 9th | — |
| 528 | Rail | March 9th | — |
| 532 | Town | March 6th | — |
| 533 | Town | March 6th | — |
| 534 | Town | March 6th | — |
| 535 | Town | March 6th | — |
| 536 | Town | March 6th | — |
| 548 | Town | March 29th | — |
| 549 | Town | March 29th | — |
| 550 | Town | March 29th | — |
| 551 | Town | March 29th | Negative |
| 552 | Town | March 29th | — |
| 540 | Rail | March 23rd | — |
| 541 | Rail | March 23rd | — |
| 542 | Rail | March 23rd | — |
| 543 | Rail | March 23rd | — |
| 544 | Rail | March 23rd | — |
| 561 | Rail | April 7th | + |
| 562 | Rail | April 7th | + |
| 563 | Rail | April 7th | — |
| 564 | Rail | April 7th | — |
| 565 | Rail | April 7th | — |
| 569 | Town | April 13th | — |
| 570 | Town | April 13th | — |
| 571 | Town | April 13th | — |
| 572 | Town | April 13th | — |
| 573 | Town | April 13th | — |
| 580 | Rail | April 21st | — |
| 581 | Rail | April 21st | + |
| 582 | Rail | April 21st | + |
| 583 | Rail | April 21st | + |
| 584 | Rail | April 21st | — |
| 588 | Town | April 27th | Negative |
| 589 | Town | April 27th | — |
| 590 | Town | April 27th | — |

MILKS—*continued*

| No. | Source | Date | Tubercle present or absent |
|---|---|---|---|
| 591 | Town | April 27th | — |
| 592 | Town | April 27th | — |
| 593 | Rail | May 4th | — |
| 594 | Rail | May 4th | — |
| 595 | Rail | May 4th | Negative |
| 596 | Rail | May 4th | Negative |
| 597 | Rail | May 4th | Negative |
| 601 | Town | May 11th | — |
| 602 | Town | May 11th | Negative |
| 603 | Town | May 11th | — |
| 604 | Town | May 11th | Negative |
| 605 | Town | May 11th | — |
| 610 | Park Style Farm | May 16th | — |
| 611 | P.S.F. | May 16th | — |
| 612 | P.S.F. | May 16th | — |
| 613 | P.S.F. | May 16th | — |
| 614 | P.S.F. | May 16th | — |
| 615 | P.S.F. | May 16th | — |
| 616 | P.S.F. | May 16th | — |
| 617 | P.S.F. | May 16th | — |
| 618 | P.S.F. | May 16th | — |
| 619 | P.S.F. | May 20th | Negative |
| 620 | P.S.F. | May 20th | Negative |
| 621 | P.S.F. | May 20th | — |
| 622 | P.S.F. | May 20th | — |
| 623 | P.S.F. | May 20th | — |
| 624 | P.S.F. | May 20th | — |
| 625 | P.S.F. | May 20th | — |
| 626 | P.S.F. | May 20th | — |
| 627 | P.S.F. | May 20th | — |
| 630 | Rail | May 26th | — |
| 631 | Rail | May 26th | Negative |
| 632 | Rail | May 26th | — |
| 633 | Rail | May 26th | — |
| 634 | Rail | May 26th | — |
| 635 | Town | June 2nd | — |
| 636 | Town | June 2nd | — |
| 637 | Town | June 2nd | — |
| 638 | Town | June 2nd | — |
| 639 | Town | June 2nd | — |
| 643 | Rail | June 6th | — |
| 644 | Rail | June 6th | — |
| 645 | Rail | June 6th | — |
| 646 | Rail | June 6th | Negative |
| 647 | Rail | June 6th | — |
| 651 | Girls' Indus. Sch. | June 14th | — |
| 652 | Seamen's Orphan. | June 15th | — |
| 653 | Town | June 15th | — |
| 654 | Town | June 15th | Negative |
| 659 | Rail | June 22nd | — |
| 660 | Rail | June 22nd | — |

MILKS—*continued*

| No. | Source | Date | Tubercle present or absent |
|---|---|---|---|
| 661 | Rail | June 22nd | — |
| 662 | Rail | June 22nd | — |
| 663 | Rail | June 22nd | — |
| 669 | Town | June 29th | — |
| 670 | Town | June 29th | — |
| 671 | Town | June 29th | — |
| 672 | Town | June 29th | — |
| 673 | Town | June 29th | — |
| 676 | Rail | July 6th | — |
| 677 | Rail | July 6th | — |
| 678 | Rail | July 6th | — |
| 679 | Rail | July 6th | Negative |
| 680 | Rail | July 7th | — |
| 683 | Town | July 14th | Negative |
| 684 | Town | July 14th | — |
| 685 | Town | July 14th | — |
| 686 | Town | July 14th | — |
| 687 | Town | July 14th | — |
| 691 | Rail | July 20th | — |
| 692 | Rail | July 20th | — |
| 693 | Rail | July 20th | — |
| 694 | Rail | July 20th | Negative |
| 695 | Rail | July 20th | — |
| 698 | Special | July 21st | — |
| 701 | Town | July 27th | Negative |
| 702 | Town | July 27th | — |
| 703 | Town | July 27th | — |
| 704 | Town | July 27th | — |
| 705 | Town | July 27th | — |
| 707 | Country | July 31st | Negative |
| 708 | Country | July 31st | — |
| 709 | Country | July 31st | — |
| 710 | Country | July 31st | — |
| 711 | Country | July 31st | Negative |
| 712 | Town | August 2nd | + |
| 713 | Town | August 1st | — |
| 714 | Town | August 1st | — |
| 715 | Town | August 2nd | — |
| 716 | Town | August 2nd | — |
| 717 | Town | August 3rd | — |
| 718 | Town | August 3rd | — |
| 719 | Country | August 3rd | Negative |
| 720 | Country | August 3rd | — |
| 721 | Rail | August 10th | — |
| 722 | Rail | August 10th | — |
| 723 | Rail | August 10th | — |
| 724 | Rail | August 10th | — |
| 725 | Rail | August 10th | — |
| 726 | Town | August 17th | — |
| 727 | Town | August 17th | — |
| 728 | Town | August 17th | — |

MILKS—*continued*

| No. | Source | Date | Tubercle present or absent |
|---|---|---|---|
| 729 | Town | August 17th | — |
| 730 | Town | August 17th | — |
| 735 | Rail | August 26th | — |
| 736 | Rail | August 21st | — |
| 737 | Rail | August 26th | — |
| 738 | Rail | August 26th | — |
| 739 | Rail | August 26th | — |
| 740 | Town | August 30th | — |
| 741 | Town | August 31st | — |
| 742 | Town | August 30th | — |
| 743 | Town | August 30th | Negative |
| 744 | Town | August 30th | — |
| 748 | Rail | September 7th | — |
| 749 | Rail | September 7th | — |
| 750 | Rail | September 7th | Negative |
| 751 | Rail | September 7th | — |
| 752 | Rail | September 7th | — |
| 759 | Town | September 15th | — |
| 760 | Town | September 15th | Negative |
| 761 | Town | September 15th | — |
| 762 | Town | September 15th | — |
| 763 | Town | September 15th | — |
| 764 | Rail | September 21st | — |
| 765 | Rail | September 21st | — |
| 766 | Rail | September 21st | — |
| 767 | Rail | September 21st | — |
| 768 | Rail | September 21st | — |
| 772 | Town | September 28th | — |
| 773 | Town | September 28th | — |
| 774 | Town | September 28th | — |
| 775 | Town | September 24th | — |
| 776 | Town | September 28th | — |
| 780 | Netherfield Road Hospital | October 2nd | — |
| 781 | Mill Lane Hospital | October 3rd | — |
| 782 | Priory Rd. Hospital | October 4th | — |
| 783 | Town | October 4th | — |
| 787 | Town | October 5th | — |
| 788 | Rail | October 6th | — |
| 789 | Rail | October 6th | + |
| 790 | Rail | October 6th | — |
| 791 | Rail | October 6th | + |
| 792 | Rail | October 6th | — |
| 793 | Town | October 12th | — |
| 794 | Town | October 12th | — |
| 795 | Town | October 12th | — |
| 796 | Town | October 12th | — |
| 797 | Town | October 12th | — |
| 798 | Town | October 12th | — |
| 799 | Town | October 12th | — |
| 800 | Town | October 12th | — |

MILKS—*continued.*

| No. | Source | Date | Tubercle present or absent |
|---|---|---|---|
| 801 | Town | October 12th | Negative |
| 802 | Town | October 19th | — |
| 809 | Rail | October 19th | Negative |
| 810 | Rail | October 19th | — |
| 811 | Rail | October 19th | — |
| 812 | Rail | October 19th | — |
| 813 | Rail | October 19th | Negative |
| 814 | Rail | October 19th | — |
| 815 | Rail | October 19th | — |
| 816 | Rail | October 19th | — |
| 817 | Rail | October 19th | — |
| 818 | Rail | October 19th | — |
| 819 | Town | October 27th | — |
| 820 | Town | October 27th | — |
| 821 | Town | October 27th | — |
| 822 | Town | October 27th | — |
| 823 | Town | October 27th | — |
| 824 | Town | October 27th | — |
| 825 | Town | October 27th | — |
| 826 | Town | October 27th | — |
| 827 | Town | October 27th | — |
| 828 | Town | October 27th | — |
| 833 | Rail | November 3rd | — |
| 834 | Rail | November 3rd | — |
| 835 | Rail | November 3rd | — |
| 836 | Rail | November 3rd | — |
| 837 | Rail | November 3rd | Negative |
| 838 | Rail | November 3rd | — |
| 839 | Rail | November 3rd | Negative |
| 840 | Rail | November 3rd | — |
| 841 | Rail | November 3rd | Negative |
| 842 | Rail | November 3rd | Negative |
| 843 | Town | November 9th | — |
| 844 | Town | November 9th | — |
| 845 | Town | November 9th | Negative |
| 846 | Town | November 9th | — |
| 847 | Town | November 9th | Negative |
| 848 | Town | November 9th | — |
| 849 | Town | November 9th | — |
| 850 | Town | November 9th | — |
| 851 | Town | November 9th | — |
| 852 | Town | November 9th | — |
| 855 | Rail | November 14th | Negative |
| 856 | Rail | November 14th | — |
| 857 | Rail | November 14th | — |
| 858 | Rail | November 14th | — |
| 859 | Rail | November 14th | Negative |
| 860 | Rail | November 14th | — |
| 861 | Rail | November 14th | — |
| 863 | Town | November 14th | Negative |
| 864 | Rail | November 16th | Negative |

MILKS—*continued*

| No. | | Source | | Date | | Tubercle present or absent |
|---|---|---|---|---|---|---|
| 865 | .. | Rail | ... | November 16th | ... | — |
| 871 | ... | Town | ... | November 24th | ... | — |
| 872 | ... | Town | ... | November 24th | ... | — |
| 873 | ... | Town | ... | November 24th | ... | — |
| 874 | ... | Town | ... | November 24th | ... | — |
| 875 | ... | Town | ... | November 24th | ... | — |
| 876 | ... | Town | ... | November 24th | ... | Negative |
| 877 | ... | Town | ... | November 24th | ... | — |
| 878 | ... | Town | ... | November 24th | ... | — |
| 880 | ... | Town | ... | November 24th | ... | Negative |
| 881 | ... | Town | ... | November 24th | ... | — |
| 882 | ... | Town | ... | November 28th | ... | — |
| 883 | ... | Town | ... | November 29th | ... | Negative |
| 884 | ... | Town | ... | November 29th | ... | — |
| 885 | ... | Rail | ... | November 30th | ... | — |
| 886 | ... | Rail | ... | November 30th | ... | — |
| 887 | ... | Rail | ... | November 30th | ... | — |
| 888 | ... | Rail | ... | November 30th | ... | — |
| 889 | ... | Rail | ... | November 30th | ... | — |
| 890 | ... | Rail | ... | November 30th | ... | Negative |
| 891 | ... | Rail | ... | November 30th | ... | — |
| 892 | ... | Rail | ... | November 30th | ... | — |
| 893 | ... | Rail | ... | November 30th | ... | — |
| 894 | ... | Rail | ... | December 1st | ... | Negative |
| 895 | ... | Town | ... | December 1st | ... | — |
| 896 | ... | Town | ... | December 6th | ... | — |
| 897 | ... | Town | ... | December 6th | ... | — |
| 898 | ... | Town | ... | December 6th | ... | Negative |
| 899 | ... | Town | ... | December 6th | ... | — |
| 900 | ... | Town | ... | December 6th | ... | Negative |
| 901 | ... | Town | ... | December 6th | ... | — |
| 902 | ... | Town | ... | December 6th | ... | — |
| 903 | ... | Town | ... | December 6th | ... | — |
| 904 | ... | Town | .. | December 6th | ... | Negative |
| 905 | ... | Town | ... | December 6th | ... | — |
| 906 | ... | Rail | ... | December 14th | ... | Negative |
| 907 | ... | Rail | ... | December 14th | ... | + |
| 908 | ... | Rail | ... | December 14th | ... | — |
| 909 | ... | Rail | ... | December 14th | ... | Negative |
| 910 | ... | Rail | ... | December 14th | ... | — |
| 911 | ... | Rail | ... | December 14th | ... | Negative |
| 912 | ... | Rail | ... | December 14th | ... | — |
| 913 | ... | Rail | ... | December 14th | ... | — |
| 914 | ... | Rail | ... | December 14th | ... | Negative |
| 915 | ... | Rail | ... | December 14th | ... | — |
| 918 | ... | Town | ... | December 19th | ... | — |
| 920 | ... | Town | ... | December 20th | ... | — |
| 921 | ... | Town | ... | December 20th | ... | — |
| 922 | ... | Town | ... | December 20th | ... | Negative |
| 925 | ... | Town | ... | December 21st | ... | — |
| 926 | ... | Town | ... | December 21st | ... | — |

MILKS—*continued*

| No. | | Source | | Date | | Tubercle present or absent |
|---|---|---|---|---|---|---|
| 927 | ... | Town | ... | December 21st | ... | — |
| 928 | ... | Town | ... | December 21st | ... | Negative |
| 930 | ... | Rail | ... | December 29th | ... | — |
| 931 | ... | Rail | ... | December 29th | ... | — |
| 932 | ... | Rail | ... | December 29th | ... | — |
| 933 | ... | Rail | ... | December 29th | ... | Negative |
| 934 | ... | Rail | ... | December 29th | ... | + |
| 935 | ... | Rail | ... | December 29th | ... | + |
| 936 | ... | Rail | ... | December 29th | ... | Negative |
| 937 | ... | Rail | ... | December 29th | ... | — |
| 938 | ... | Rail | ... | December 29th | ... | + |

*General Conclusions.*—It will be seen from the analysis of milk that the occurrence of tubercle in the milk obtained from the shippons in the city is diminishing. The presence of the bacillus tuberculosis in the railway-borne milk is, however, still too frequent, although there is no doubt that many of the milk dealers realize the importance, in their own interests, of banishing tubercle.

## Preserved Milks, Cheese, Butter, and Margarine

*Condensed Milks.*—As in the previous year, the condensed tinned milks are not always found to be sterile, or free from organisms. This form of milk ought always to be sterile; if it is not it has no advantage over raw milk, from a bacteriological point of view, and it may be the means of distributing pathogenic germs.

*Sterilized Milks.*—The analyses have revealed the presence of bacteria. This is most unsatisfactory, and shows that sufficient care is not taken to sterilize the milk, and that the manufacturers do not employ proper means to ensure the efficacy of their sterilizing processes, nor do they adopt tests to prove that their methods are effectual. Unless milk sold as sterilized is actually so it has no advantage over non-sterilized milk; on the contrary, the consumers are entirely misled, and an article is supplied which is not of the nature, substance, and quality demanded.

TINNED MILKS

| No. | | Date | | | Result |
|---|---|---|---|---|---|
| 517 | ... | February 28th | ... | ... | Sterile |
| 628 | ... | May 24th | ... | ... | Not Sterile |
| 831 | ... | November 2nd | ... | ... | No B. Coli Communis<br>No B. Enteritidis Sporogenes |
| 832 | ... | November 2nd | .. | ... | No B. Enteritidis Sporogenes<br>No B. Coli Communis |

MALTED MILKS

| No. | | Date | | | Result |
|---|---|---|---|---|---|
| 518 | ... | February 28th | ... | ... | Sterile |

## CREAM

| No. | | Date | Result |
|---|---|---|---|
| 699 | ... | July 23rd ... ... ... | Non-tubercular |
| 771 | ... | July 21st ... ... ... | Non-tubercular |

## STERILIZED MILK

| No. | | Date | | Name | | Result |
|---|---|---|---|---|---|---|
| 700 | ... | July 26th | ... | Sterilized Milk ... | ... | Not Sterile |
| 731 | ... | August 24th | ... | Sterilized Humanized Milk | ... | Not Sterile |
| 732 | ... | August 24th | ... | Sterilized Humanized Milk | ... | Not Sterile |
| 733 | ... | August 24th | ... | Sterilized Milk ... | ... | Not Sterile |
| 734 | ... | August 24th | ... | Sterilized Milk ... | ... | Not Sterile |

## MARGARINE

| No. | | Date | | Result |
|---|---|---|---|---|
| 500 | ... | February 14th | ... | No Tubercle |
| 501 | ... | February 14th | ... | No Tubercle |
| 502 | ... | February 14th | ... | No Tubercle |
| 528 | ... | March 10th | ... | No Tubercle |
| 535 | ... | March 29th | ... | No Tubercle |
| 556 | ... | March 29th | ... | No Tubercle |
| 559 | ... | April 5th | ... | No Tubercle |
| 560 | ... | April 5th | ... | No Tubercle |
| 574 | ... | April 14th | ... | Non-tubercular |
| 578 | ... | April 20th | ... | No Tubercle |
| 579 | ... | April 20th | ... | Suspicion of Tubercle, Doubtful |
| 608 | ... | May 12th | ... | No Tubercle |
| 830 | ... | October 27th | ... | No Tubercle |

## CHEESE

| No. | | Date | | Result |
|---|---|---|---|---|
| 553 | ... | March 29th | ... | Non-tubercular |
| 554 | — | March 29th | ... | Non-tubercular |
| 575 | ... | April 14th | ... | Non-tubercular |
| 606 | ... | May 12th | ... | Non-tubercular |
| 607 | ... | May 12th | .. | Non-tubercular |
| 809 | ... | October 27th | ... | Non-tubercular<br>No Enteritidis Sporogenes |

*Jams, etc.*—A large number of samples have been examined and no pathogenic bacteria found. A very considerable number of the jams are sterile, a condition which is very satisfactory, the result of careful preparation. But the use of preservatives for this purpose, by lessening digestibility, lessens or destroys the nutritious properties of the food.

## JAMS, ETC.

| No. | | Date | | Name | | Result |
|---|---|---|---|---|---|---|
| 489 | ... | February 3rd | ... | Strawberry ... | ... | Spores Mould Fungi |
| 490 | ... | February 3rd | ... | Black Currant | ... | Sterile |
| 491 | ... | February 3rd | ... | Raspberry ... | ... | Sterile |
| 513 | ... | February 23rd | ... | Marmalade ... | ... | Sterile |
| 585 | ... | April 26th | ... | Gooseberry and Apple ... | | Sterile |
| 586 | ... | April 26th | ... | Damson and Apple | ... | Sterile |

JAMS, ETC.—*continued*

| No. | Date | Name | Result |
|---|---|---|---|
| 587 | April 26th | Plum and Apple | Sterile |
| 598 | May 5th | Damson and Apple | Sterile |
| 599 | May 5th | Marmalade ... | Sterile |
| 600 | May 5th | Peach and Lemon | Sterile |
| 648 | June 9th | Plum and Apple | Mould |
| 649 | June 9th | Damson and Apple | Mould |
| 650 | June 9th | Apple Jelly ... | Sterile |
| 465 | June 14th | Preserved Pears | Sterile |
| 658 | June 16th | Fruit Juice ... | Not Sterile |
| 664 | June 22nd | Marmalade ... | Sterile |
| 665 | June 26th | Fig and Lemon | Sterile |
| 666 | June 22nd | Gooseberry and Apple | Sterile |
| 688 | July 15th | Table Jelly ... | Not Sterile |
| 689 | July 15th | Table Jelly ... | Numerous Organisms |
| 690 | July 15th | Table Jelly ... | Numerous Organisms |
| 745 | September 1st | Marmalade ... | Sterile |
| 746 | September 1st | Damson and Apple | Sterile |
| 747 | September 1st | Pineapple ... | Not Sterile |
| 869 | November 24th | Rhubarb ... | Sterile |
| 870 | November 24th | Plum and Apple | Sterile |

*Potted Meats.*—A considerable proportion of these were found to be sterile; those which were not, with a single exception, contained no objectionable organism.

POTTED MEATS

| No. | Date | Name | Result |
|---|---|---|---|
| 454 | January 5th | Shrimps ... | No B. Coli Communis |
| 474 | January 17th | Beef ... | Not Sterile |
| 516 | February 28th | Bloater Paste | Sterile |
| 556 | April 11th | Shrimp Paste | Sterile |
| 567 | April 11th | Anchovy Paste | Not Sterile |
| 568 | April 11th | Bovril ... | Sterile |
| 577 | April 20th | Potted Ham ... | Sterile |
| 640 | June 2nd | Ham ... | Sterile |
| 641 | June 2nd | Tongue ... | Sterile |
| 642 | June 2nd | Game ... | Sterile |
| 656 | June 16th | Ham, Chicken, and Tongue Sausage | Sterile |
| 657 | June 16th | Smoked Sausage | Sterile |
| 706 | July 27th | Venison ... | Not Sterile |
| 756 | September 12th | Beef ... | Sterile |
| 757 | September 12th | Ham ... | Sterile |
| 758 | September 12th | Tongue ... | Sterile |
| 769 | September 21st | Lobster ... | Not Sterile |
| 770 | September 21st | Shrimps ... | Not Sterile |
| 917 | December 14th | Lobster ... | Not Sterile |

*Tinned Meats.*—These ought to be sterile in every case, otherwise they may become a serious source of danger owing to slow fermentation taking place in the contents of the tin and leading to the production of poisonous products.

## TINNED MEATS

| No. | Date | Name | Result |
|---|---|---|---|
| 457 | January 6th | Salmon | Numerous Organisms<br>No B. Coli Communis |
| 458 | January 6th | Sardines | Sterile |
| 464 | January 14th | Sardines | Not Sterile |
| 466 | January 14th | Lobster | Not Sterile |
| 472 | January 17th | Ham, Chicken, and Tongue | Not Sterile |
| 473 | January 17th | Ham and Tongue | Not Sterile |
| 497 | February 10th | Salmon | Not Sterile |
| 498 | February 10th | Salmon | Sterile (?) |
| 545 | February 24th | Sardines | Not Sterile |
| 546 | February 24th | Turkey and Tongue | Sterile (?) |
| 547 | February 24th | Veal and Ham | Not Sterile |
| 557 | April 5th | Ham, Chicken, and Tongue | Sterile |
| 558 | April 5th | Turkey and Tongue | Sterile |
| 576 | April 20th | Veal and Ham | Sterile |
| 681 | July 7th | Sardines | Sterile |
| 682 | July 7th | Sardines | Sterile |
| 696 | July 21st | Sardines | Sterile |
| 697 | July 21st | Salmon | Sterile (?) |
| 753 | September 8th | Bloater Paste | Sterile |
| 754 | September 8th | Ham and Chicken | Sterile |
| 755 | September 8th | Sardines | Sterile |
| 778 | September 28th | Sardines | Sterile |
| 853 | November 11th | Sardines | Sterile |
| 854 | November 11th | Salmon | Sterile |
| 867 | November 18th | Salmon | Not Sterile |
| 868 | November 18th | Salmon | Not Sterile |
| 916 | December 14th | Sardines | Sterile |

## TINNED FRUITS AND VEGETABLES

| No. | Date | Name | Result |
|---|---|---|---|
| 465 | January 14th | Peas (Preserved) | Sterile |
| 513 | February 23rd | Tomatoes | Sterile |
| 674 | July 1st | Pears (Preserved) | Sterile |
| 675 | July 7th | Peaches (Preserved) | Sterile |
| 866 | November 15th | Pine Apple (Preserved) | Not Sterile |

*Shell-fish.*—Unlike preserved foods which have been submitted to processes intended to destroy bacteria, shell-fish, owing to the fact that they are eaten raw, may be a very serious source of infection. None of the samples, however, examined showed evidence of disease-producing organisms. It is to the interest of the oyster merchant to see that the oyster beds are absolutely free from sources of possible pollution. The same remark applies to dealers in other kinds of shell-fish.

### SHELL-FISH

| No. | Date | Name | Result |
|---|---|---|---|
| 452 | January 5th | Oysters | No B. Coli Communis |
| 453 | January 5th | Oysters | No B. Coli Communis |
| 463 | January 12th | Mussels | No B. Coli Communis |
| 481 | January 27th | Cockles | No B. Coli Communis |
| 482 | January 27th | Mussels | No B. Coli Communis |
| 483 | January 27th | Oysters | No B. Coli Communis |
| 537 | March 17th | Mussels | No B. Coli Communis |
| 538 | March 17th | Cockles | No B. Coli Communis |
| 805 | October 18th | Oysters | No B. Enteritidis Sporogenes |
| 806 | October 18th | Mussels | No B. Enteritidis Sporogenes |
| 807 | October 18th | Cockles | No B. Enteritidis Sporogenes |
| 808 | October 18th | Periwinkles | No B. Enteritidis Sporogenes |
| 923 | December 21st | Cockles | No B. Enteritidis Sporogenes<br>No B. Coli Communis |
| 924 | December 21st | Mussels | No B. Enteritidis Sporogenes<br>No B. Coli Communis |

### SAUCES

| No. | Date | Name | Result |
|---|---|---|---|
| 617 | June 28th | Mint Sauce | Not Sterile |
| 668 | June 28th | Chutney | Not Sterile |
| 784 | October 6th | Chutney | Not Sterile |
| 803 | October 13th | Worcester Sauce | Sterile |

### MISCELLANEOUS

| No. | Date | Name | Result |
|---|---|---|---|
| 479 | February 10th | Desiccated Soup | Not Sterile |
| 514 | February 23rd | Desiccated Egg Food | Numerous Organisms |
| 539 | March 17th | Crab | Not Sterile |
| 629 | May 24th | Ice | Numerous Organisms |
| 777 | September 29th | Camp Coffee | Not Sterile |
| 785 | October 5th | Lemon Cheese | Not Sterile |
| 786 | October 5th | Olive Oil | Sterile |

## WATER ANALYSES

During the year 1899 systematic examinations of the drinking water have been made. In collecting the samples the usual precautions to prevent extraneous contamination have been taken. The analyses show that the water is uniformly very good. No pathogenic forms have been isolated, and the average number of bacteria per cubic centimetre has been well below 100 (Koch's Standard). A very large number of analyses have also been made for the purposes of the Water Committee, and these appear in the separate report of the Water Committee.

The following are the sources which have been examined :—

FORTNIGHTLY EXAMINATIONS

Ashton Hall Tap

MONTHLY EXAMINATIONS

PRESCOT— { Lake Vyrnwy Water, Rivington Water, The Mixed Water

WELLS— { Windsor Well, Dudlow Lane Well, Green Lane Well

ASHTON HALL—FORTNIGHTLY SAMPLES

| Source | Date | Time of collecting | Time of investment | Bacteria present Gelatine | Agar |
|---|---|---|---|---|---|
| Ashton Hall | January 7th | 10 am. | 10 5 am. | 25 | 3 |
| ,, | January 20th | 2 25 pm. | 2 30 pm. | 120 | 50 |
| ,, | February 6th | 2 pm. | 2 7 pm. | 104 | 11 |
| ,, | February 15th | 10 15 am. | 10 25 am. | 21 | 12 |
| ,, | March 2nd | 4 pm. | 4 15 pm. | 89 | 21 |
| ,, | March 20th | 12 noon | 12 7 pm. | 68 | 15 |
| ,, | April 1st | 10 10 am. | 10 15 pm. | 87 | 26 |
| ,, | April 17th | 2 pm. | 2 7 pm. | 1550 | 240 |
| ,, | April 21st | 10 5 am. | 10 9 am. | 143 | 67 |
| ,, | May 12th | 5 pm. | 5 7 pm. | 9 | 2 |
| ,, | May 26th | 4 pm. | 4 7 pm. | 63 | 10 |
| ,, | June 10th | 10 am. | 10 7 pm. | 31 | 5 |
| ,, | June 21st | 4 pm. | 4 5 pm. | 75 | 16 |
| ,, | July 5th | 2 pm. | 2 7 pm. | 77 | 22 |
| ,, | July 19th | 2 pm. | 2 8 pm. | 81 | — |
| ,, | August 19th | — | — | 100 | — |
| ,, | August 29th | 2 35 pm. | 2 45 pm. | 88 | 96 |
| ,, | September 15th | 9 45 am. | 9 54 am. | 50 | — |
| ,, | September 27th | 2 30 pm. | 2 45 pm. | 19 | 17 |
| ,, | October 10th | 11 30 am. | 11 45 am. | 30 | — |
| ,, | October 30th | 9 am. | 9 5 am. | 40 | 10 |
| ,, | November 10th | 9 30 am. | 10 am. | 15 | 12 |
| ,, | November 25th | 1 pm. | 5 30 pm. | Liquefied | — |
| ,, | December 7th | 10 am. | 10 10 am. | 24 | 12 |
| ,, | December 21st | 2 30 pm. | 2 40 pm. | 23 | — |

PRESCOT—VYRNWY WATER—MONTHLY SAMPLES

| Source | Date | Time of collecting | Time of investment | Bacteria present Gelatine | Agar |
|---|---|---|---|---|---|
| Vyrnwy | January 17th | 12 30 pm. | 1 pm. | 52 | 16 |
| ,, | February | — | — | — | — |
| ,, | March 29th | 11 am. | 12 30 pm. | 112 | 128 |
| ,, | April 26th | 2 pm. | 3 30 pm. | 83 | 4 |
| ,, | May 15th | — | 3 35 pm. | 13 | 8 |
| ,, | June 21st | 2 30 pm. | 4 10 pm. | 58 | 13 |
| ,, | July 17th | 2 15 pm. | 4 pm. | 43 | — |
| ,, | August 28th | 2 pm. | 4 pm. | 24 | 48 |
| ,, | September 29th | 2 2 pm. | 3 30 pm. | 8 | 13 |
| ,, | October 27th | 12 30 pm. | 4 pm. | 30 | — |
| ,, | November 24th | 12 45 pm. | 3 30 pm. | 10 | 7 |
| ,, | December 20th | 2 5 pm. | 3 45 pm. | 15 | — |

PRESCOT—RIVINGTON WATER—Monthly Samples

| Source | Date | Time of collecting | Time of investment | Bacteria present Gelatine | Agar |
|---|---|---|---|---|---|
| Rivington | March 1st | 11 am. | 12 noon | 15 | 170 |
| ,, | March 29th | 11 am. | 12 30 pm. | 104 | 368 |
| ,, | April 26th | 2 pm. | 3 30 pm. | 22 | 15 |
| ,, | May 15th | 2 15 pm. | 3 35 pm. | 21 | 11 |
| ,, | June 21st | 2 30 pm. | 4 10 pm | 70 | 20 |
| ,, | July 17th | 2 17 pm. | 4 pm. | 25 | — |
| ,, | August 28th | 2 5 pm. | 4 pm. | 2 | 24 |
| ,, | September 29th | 2 7 pm. | 3 30 pm. | 30 | 3 |
| ,, | October 27th | 12 35 pm. | 4 pm. | 30 | 8 |
| ,, | November 24th | 12 50 pm. | 3 pm. | 9 | — |
| ,, | December 20th | 2 8 pm. | 3 45 pm. | 20 | — |

PRESCOT—MIXING WELL—Monthly Samples

| Source | Date | Time of collecting | Time of investment | Bacteria present Gelatine | Agar |
|---|---|---|---|---|---|
| Mixing Well | January 17th | 12 30 pm. | 1 pm. | 50 | 23 |
| ,, | February | — | — | — | — |
| ,, | March 29th | 11 am. | 12 30 pm. | 40 | 43 |
| ,, | April 26th | 2 pm. | 3 30 pm. | 51 | 73 |
| ,, | May 15th | 2 15 pm. | 3 35 pm. | 53 | 200 |
| ,, | June 21st | 2 30 pm. | 4 10 pm. | 120 | 47 |
| ,, | July 17th | 2 20 pm. | — | 107 | — |
| ,, | August 28th | 2 10 pm. | 4 pm. | 72 | 80 |
| ,, | September 29th | 2 10 pm. | 3 30 pm. | 88 | — |
| ,, | October 27th | 12 40 pm. | 4 pm. | 50 | 45 |
| ,, | November 24th | 12 55 pm. | 3 pm. | Liquefied | 3 |
| ,, | December 20th | 2 10 pm. | 4 pm. | 10 | — |

WINDSOR WELL—Monthly Samples

| Source | Date | Time of collecting | Time of investment | Bacteria present Gelatine | Agar |
|---|---|---|---|---|---|
| Windsor Well | January 20th | 2 15 pm. | 2 30 pm. | 105 | 6 |
| ,, | March 2nd | 8 40 am. | 9 5 am. | 93 | 31 |
| ,, | March 30th | 8 35 am. | 9 20 pm. | 39 | 37 |
| ,, | April 29th | 8 30 am. | 9 am. | 114 | 38 |
| ,, | May 26th | 9 am. | 9 20 am. | 400 | 57 |
| ,, | June 20th | 2 pm. | 2 20 pm. | 12 | 8 |
| ,, | July 19th | 8 15 am. | 9 am. | 28 | — |
| ,, | August 29th | 10 42 am. | 11 20 am. | 10 | 11 |
| ,, | September 27th | 9 35 am. | 2 pm. | 22 | 5 |
| ,, | October 26th | 2 30 pm. | 11 am. Oct. 27 | 70 | — |
| ,, | November 25th | 11 am. | 5 30 pm. | 44 | 2 |
| ,, | December 19th | 3 30 pm. | 4 pm. | 80 | 4 |

DUDLOW LANE—Monthly Samples

| Source | Date | Time of collecting | Time of investment | Bacteria present Gelatine | Agar |
|---|---|---|---|---|---|
| Dudlow Lane | January | — | — | — | — |
| ,, | February | — | — | — | — |
| ,, | March 1st | 2 48 pm. | 4 10 pm. | 16 | 8 |
| ,, | March 29th | 2 45 pm. | 4 15 pm. | 12 | 7 |
| ,, | April 28th | 10 am. | 11 45 am. | 31 | 22 |

DUDLOW LANE—Monthly Samples—*continued*

| Source | Date | Time of collecting | Time of investment | Bacteria present Gelatine | Agar |
|---|---|---|---|---|---|
| Dudlow Lane | May 26th | 3 pm. | 4 20 pm. | 350 | 41 |
| ,, | June 21st | 10 4 am. | 12 50 pm. | 6 | 3 |
| ,, | July 18th | 2 30 pm. | 4 pm. | 20 | — |
| ,, | August 28th | 11 15 am. | 2 pm. | 72 | 56 |
| ,, | September 27th | 10 20 am. | 2 pm. | 10 | 10 |
| ,, | October 26th | 12 15 pm. | 11 am. Oct. 27 | 30 | — |
| ,, | November 25th | 11 30 am. | 5 30 pm. | 152 | — |
| ,, | December 19th | 3 10 pm. | 4 pm. | 80 | — |
| Woolton—Tap in Engine Room | January 18th | 3 pm. | 3 30 pm. | 28 | 4 |
| Reservoir | January 18th | 3 pm. | 3 30 pm. | 320 | 13 |
| ,, | February | — | — | — | — |
| ,, | March 1st | 2 40 pm. | 4 10 pm. | 20 | 13 |
| ,, | March 29th | 3 10 pm. | 4 15 pm. | 14 | 12 |

GREEN LANE WELLS—Monthly Samples

| Source | Date | Time of collecting | Time of investment | Bacteria present Gelatine | Agar |
|---|---|---|---|---|---|
| G. Holt | January | — | — | — | — |
| ,, | February | — | — | — | — |
| ,, | March 1st | 3 30 pm. | 4 10 pm. | 35 | 3 |
| ,, | March 29th | 3 3 pm. | 4 15 pm. | 26 | 24 |
| ,, | April 28th | 11 am. | 11 45 am. | Colonies too numerous to be counted | |
| ,, | May 26th | 2 pm. | 4 15 pm. | 104 | 70 |
| ,, | June 21st | 11 30 am. | 12 50 am. | 200 | 500 |
| ,, | July 18th | 3 30 pm. | 4 pm. | 640 | — |
| ,, | August 28th | 10 45 am. | 2 pm. | 296 | 512 |
| ,, | September 27th | 11 17 am. | 2 pm. | 17 | 12 |
| ,, | October 26th | 1 5 pm. | 11 am. Oct. 27 | 240 | 40 |
| ,, | November | — | — | — | — |
| ,, | December 19th | 1 45 pm. | 4 pm. | 85 | — |
| J. Holmes | January | — | — | — | — |
| ,, | February | — | — | — | — |
| ,, | March 1st | 3 33 pm. | 4 10 pm. | 20 | 340 |
| ,, | March 29th | 3 35 pm. | 4 15 pm | 73 | 64 |
| ,, | April 28th | 11 am. | 11 45 am. | Colonies too numerous to be counted | |
| ,, | May 26th | 2 10 pm. | 4 15 pm. | 112 | 160 |
| ,, | June | — | — | — | — |
| ,, | July 18th | 3 30 pm. | 4 pm. | 364 | — |
| ,, | August 28th | 10 45 am. | 2 pm. | Liquefied | — |
| ,, | September 27th | 11 21 am. | 2 pm. | 37 | 340 |
| ,, | October 26th | 1 pm | 11 am. Oct. 27 | 50 | 3200 |
| ,, | November 25th | 12 noon | 5 30 pm. | 240 | — |
| ,, | December 19th | 1 45 pm. | 4 pm. | 94 | — |

The following gentlemen have assisted in the bacteriological work :—Dr. Balfour Stewart, Assistant Bacteriologist; Dr. Grünbaum, Dr. Warrington, Dr. Annett, and Dr. Hill.

# THE DISTRIBUTION OF TUBERCULOSIS IN LIVERPOOL

J. H. ELLIOTT, M.B. (Tor.)

Considering the great importance of the study of the distribution of Tuberculosis in our large cities, I have taken advantage of the opportunity afforded me by a short residence in Liverpool to make a few notes regarding the occurrence of the disease throughout the city.

The incorporated area of the city is 13,236 acres, over 700 of which are occupied by docks and quays. The estimated population in 1898 was 668,645; the figures given by the last census were 629,443. This population of 50.5 to the acre is only exceeded in Great Britain by London, West Ham, and Glasgow, while Brighton, Bolton, and Edinburgh have about the same number per acre.

The city is divided into 11 Wards, the population of these varying from 9.8 per acre in Wavertree to 160.4 per acre in Everton. Four of the Wards—Walton, West Derby (rural), Wavertree, and Toxteth (rural) were added to the city in 1895. They are practically suburban; Walton, with 29.8 per acre, being the most densely populated. The remaining Wards have a population of over 100 per acre, with the exception of Abercromby and Exchange; but considering the areas in these Wards, occupied by large retail shops, warehouses, municipal and other public buildings, the actual population per acre, in the part occupied by householders, will be much above the stated figures.

The following table shows the population per acre for the various Wards, as estimated by the Medical Officer of Health for the years 1895-1898 inclusive :—

TABLE I

| Population per acre | 1895 | 1896 | 1897 | 1898 |
|---|---|---|---|---|
| Scotland ... ... ... | 122.3 | 119.0 | 115.5 | 128.0 |
| Exchange ... ... | 85.8 | 82.5 | 99.5 | 89.2 |
| Abercromby ... ... | 76.0 | 74.4 | 73.3 | 75.1 |
| Everton ... ... ... | 159.7 | 159.7 | 159.7 | 160.4 |
| Kirkdale ... ... ... | 96.0 | 96.4 | 96.7 | 102.8 |
| West Derby ... ... | 117.1 | 117.5 | 117.8 | 125.3 |
| Toxteth ... ... | 117.8<br>134.5 | 123.5 | 125.4<br>173.4 | 124.6 |
| Walton ... ... ... | 27.4 | 29.1 | 30.7 | 29.8 |
| West Derby (rural) ... ... | 12.9 | 13.1 | 13.2 | 14.0 |
| Wavertree ... ... | 8.2 | 8.4 | 8.6 | 9.8 |
| Toxteth (rural) ... ... | 21.9 | 23.5 | 25.1 | 25.2 |

The districts of Walton, West Derby (rural), Wavertree, and Toxteth (rural), having been added in 1895, I have in these notes only made observations on the statistics published since then, so as to be enabled to make comparisons of the various districts. The reports available previous to 1895 cover only the 7 remaining districts, an area of 5,210 acres instead of the 13,236 as at present, and having an average population of 113 per acre.

Also, previous to 1894, the deaths occurring in public institutions were not referred to the districts from which the patient was admitted, as is now done. The statistics for 1899 were not available for incorporation with those of the previous four years.

The following table shows the death rate per 1,000, from all causes, for the four years 1895-1898 inclusive :—

TABLE II

| District | Death Rate per 1,000 population | | | | Average of these rates |
|---|---|---|---|---|---|
| | 1895 | 1896 | 1897 | 1898 | |
| Scotland ... ... | 38.4 | 34.5 | 37.7 | 36.4 | 36.7 |
| Exchange ... ... | 41.2 | 37.2 | 39.8 | 36.9 | 38.8 |
| Abercromby ... ... | 27.0 | 22.4 | 24.6 | 24.5 | 24.6 |
| Everton ... ... | 27.6 | 24.3 | 25.9 | 24.5 | 25.6 |
| Kirkdale ... ... | 24.4 | 19.5 | 22.1 | 18.8 | 21.2 |
| West Derby ... ... | 24.3 | 21.4 | 22.9 | 21.1 | 22.4 |
| Toxteth... ... ... | | 20.8 | 22.9 | 21.3 | 22.2 |
| Walton ... ... ... | 15.7 | 12.7 | 12.9 | 12.9 | 13.5 |
| West Derby (rural) ... | 17.7 | 17.5 | 17.0 | 16.3 | 17.1 |
| Wavertree ... ... | 16.2 | 14.6 | 16.4 | 16.3 | 16.0 |
| Toxteth (rural) ... ... | 10.4 | 9.8 | 10.2 | 9.9 | 10.1 |

We find the death rate to be greatest in Scotland and Exchange Wards, where, in the parts which are occupied by dwelling-houses, the people are very much crowded, and even the simpler rules of hygiene are much neglected. The death rate here, too, is increased by the fact of there being many lodging-houses in the two districts which are the temporary abodes of the floating population incident to a large city, and increased in Liverpool by its immense shipping. The death rate is markedly less in the less congested districts.

An idea of the class of people in parts of the city is conveyed by the fact that of 1,002 deaths from Tuberculosis in Exchange Ward, from 1895 to 1899, 563 occurred in the Workhouse and Infirmary, and only 439 in the patients' houses.

The death rate per 1,000 population from Tuberculosis in the various districts is as follows :—

## TABLE III

| District | Death Rate per 1,000 from Tuberculosis | | | | Average of these rates |
|---|---|---|---|---|---|
| | 1895 | 1896 | 1897 | 1898 | |
| Scotland ... ... | 3.68 | 3.91 | 3.90 | 3.51 | 3.75 |
| Exchange ... ... | 5.36 | 5.21 | 4.48 | 4.39 | 4.86 |
| Abercromby ... ... | 3.00 | 2.84 | 2.81 | 2.64 | 2.82 |
| Everton... ... ... | 2.55 | 2.69 | 2.43 | 2.86 | 2.63 |
| Kirkdale ... ... | 2.77 | 1.94 | 2.00 | 1.91 | 2.15 |
| West Derby .. ... | 3.13 | 2.78 | 2.88 | 1.81 | 2.65 |
| Toxteth ... ... | 2.11 | 2.16 | 2.22 | 1.94 | 2.11 |
| Walton... ... ... | 1.76 | 1.45 | 1.42 | 1.33 | 1.49 |
| West Derby (rural) ... | 1.76 | 1.74 | 1.62 | 1.54 | 1.64 |
| Wavertree ... ... | 2.51 | 1.42 | 1.52 | 1.54 | 1.75 |
| Toxteth (rural) ... ... | 1.12 | 1.25 | 1.46 | 1.22 | 1.26 |

The death rate from Tuberculosis in Scotland is seen to be 3 times as great as in Toxteth (rural), while in Exchange the rate is nearly 4 times as great. In Abercromby, Everton, and West Derby, which are also crowded districts, it is more than twice as great. This bears out our generally accepted belief that Tuberculosis is a disease which is fostered by and varies with the density of the population and the general hygiene observed by the inhabitants of the district. But, on studying Table II, it is seen that the general death rate per thousand is more than 3 times as great in Scotland as in Toxteth (rural), and in Exchange almost 4 times as great ; while in Abercromby, Everton, and West Derby it is more than twice as great. So that the same influences which increase Tuberculosis in the crowded districts are also active agents in the increase of other diseases, and in about the same proportion. This appeared to me to be unusual. Although we expect the general death rate to be greater in the poorer parts, and with it the Tuberculosis death rate also increased, we would be inclined to look for more than a proportionate increase in the deaths from Tuberculosis.

To judge more fairly the occurrence of the disease in the various Wards I prepared the following table, showing what percentage of deaths is due to Tuberculosis;

TABLE IV

| District | Population per acre, 1898 | Deaths from Tuberculosis—Percentage of all Deaths | | | | Average of these |
|---|---|---|---|---|---|---|
| | | 1895 | 1896 | 1897 | 1898 | |
| Scotland ... ... | 128.0 | 9.5 | 11.3 | 10.3 | 9.1 | 10.0 |
| Exchange ... ... | 89.0 | 12.9 | 14.0 | 11.2 | 11.2 | 12.3 |
| Abercromby ... ... | 75.1 | 11.1 | 12.6 | 11.4 | 10.7 | 11.4 |
| Everton ... ... | 160.4 | 9.2 | 11.0 | 9.4 | 11.2 | 10.2 |
| Kirkdale ... ... | 102.8 | 11.3 | 10.0 | 9.0 | 10.2 | 10.1 |
| West Derby ... ... | 125.3 | 12.8 | 13.0 | 12.5 | 9.1 | 11.9 |
| Toxteth ... ... | 124.6 | 8.8 | 10.4 | 9.7 | 9.1 | 9.5 |
| Walton ... ... ... | 29.8 | 11.8 | 11.3 | 10.9 | 10.3 | 11.1 |
| West Derby (rural) ... | 14.0 | 10.0 | 10.8 | 9.4 | 9.5 | 9.9 |
| Wavertree ... ... | 9.8 | 15.5 | 10.2 | 9.3 | 9.4 | 11.1 |
| Toxteth (rural) ... ... | 25.2 | 10.9 | 12.7 | 8.9 | 12.3 | 11.2 |

Percentage for the whole city for the four years 10.93.

With an average throughout the city for the four years of 10.93 deaths in each 100, it is seen that of the 5 Wards with the greatest population, each having over 100 to the acre, 4 fall below the average; while of 4 Wards with the least number to the acre, a population of from 9.8 to 29.8 per acre, 3 are above the average.

This is, perhaps, only a coincidence, and should not carry much weight in localizing Tuberculosis in one district rather than another, but it is certainly interesting.

However, it is remarkable that throughout the city, with Wards varying in density of population, and in the general attention paid to personal and general household hygiene, the number of deaths in every 100 due to Tuberculosis should be so uniform—all lying between 9.5 and 12.3. Although in individual years it has varied more than this, as in 1895, from 8.5 in Toxteth to 15.5 in Wavertree. In 1896 the rate was generally high, the lowest rate being 10.0 and the highest 14.0; while in 1898 the rate was generally low, with the exception of 1 Ward, and only 3 Wards had a mortality greater than 10.7.

The great number of deaths each year reported as due to Bronchitis makes it doubtful if these figures are to be entirely relied upon. Were these cases of Bronchitis more carefully diagnosed it is very probable many of them would be cases of Pulmonary Tuberculosis. In 1898 there were 402 more deaths from Bronchitis than from Phthisis and Tuberculosis; there being 1,808 from the former, and 1,406 from the latter. Of these cases, there occurred in the various institutions 534 cases of Phthisis and Tuberculosis, and 439 of Bronchitis; while in the houses throughout the city there were 872 deaths from Phthisis and Tuberculosis, with 1,349 from Bronchitis, showing a marked preponderance of the latter.

The following table shows the mortality reported under Phthisis and Tuberculosis, as compared with Bronchitis. These are the deaths occurring in the houses throughout the city, and do not include deaths in public institutions:—

TABLE V

| | Scotland | Exchange | Abercromby | Everton | Kirkdale | West Derby | Toxteth | Walton | West Derby (rural) | Wavertree | Toxteth (rural) | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1898 | | | | | | | | | | | | |
| Tuberculosis & Phthisis | 83 | 52 | 62 | 175 | 80 | 148 | 137 | 43 | 44 | 20 | 28 | 872 |
| Bronchitis ... ... | 162 | 138 | 111 | 251 | 111 | 147 | 258 | 61 | 70 | 23 | 17 | 1344 |
| 1897 | | | | | | | | | | | | |
| Tuberculosis & Phthisis | 79 | 53 | 65 | 152 | 84 | 151 | 133 | 51 | 42 | 19 | 31 | 860 |
| Bronchitis ... ... | 225 | 175 | 117 | 334 | 146 | 147 | 242 | 50 | 68 | 18 | 22 | 1549 |
| 1896 | | | | | | | | | | | | |
| Tuberculosis & Phthisis | 62 | 53 | 67 | 142 | 88 | 128 | 125 | 49 | 32 | 16 | 20 | 752 |
| Bronchitis ... ... | 208 | 137 | 90 | 289 | 142 | 163 | 222 | 44 | 58 | 16 | 23 | 1392 |

With the exception of West Derby, Wavertree, and Toxteth (rural), every district shows a marked preponderance of Bronchitis, in fact, so much that it makes one feel very doubtful as to the accuracy of the stated cause of death.

On an ordnance survey map of Exchange District I have marked the houses in which deaths have occurred from Tuberculosis, including deaths in the various Hospitals and Infirmaries of patients admitted from this district, referring the same to the houses from which they were received. Under Tuberculosis I have included all deaths reported under the terms Phthisis, Consumption, Tuberculosis, and Tabes mesenterica. The five years, 1895-1899 inclusive, have been taken. In some few cases, principally deaths in institutions, an incorrect or incomplete address has been given, and it has been impossible to locate these cases. In a few, when the number of the court is given but no house number, a red dot has been placed in the court, simply approximating the locality.

As noted previously, a considerable part of Exchange District is occupied by public buildings, warehouses, and large shops, and in these areas the population is very sparse. Consequently only a portion of the Ward is here reproduced, giving a fair idea of the distribution of the cases throughout other similarly inhabited areas.

What is very striking is the grouping of cases—either two or more in one house, or contiguous houses, with cases occurring in each.

Some of the houses where many cases have occurred are large lodging-houses, *e.g.*, 30 Byrom Street, 131 and 132 Richmond Row, 107 St. Anne Street. In 131 Richmond Row are about 100 lodgers, while 107 St. Anne Street has about 200 ; many of these are transient, only remaining a few days.

So many cases occurring in the crowded parts, and it being doubtful what precautions are taken in the care of the sputum—and it is known that in a great number of instances practically no care is taken—it is simply incalculable what number of cases receive in such rooms their primary infection.

Although the infective properties of the dust of rooms in which consumptives have slept have been demonstrated over and over again, I asked the Medical Officer of Health in January of this year for a list of houses in which deaths from consumption had recently occurred. Of ten houses in which deaths had occurred 7 to 14 days previously, I was able to secure dust in four. In the others, from being vacant or from objections by the occupants, I was unable to secure any.

The following are my notes at time of taking the dust:—

*House A.*—Patient slept on lounge 2 to 3 weeks before death. Rooms very clean ; difficult to secure dust. Three specimens—No. 1, from lounge between seat and back ; No. 2, from cracks in floor at base of wall ; No. 3, washings of wall paper above doorway.

*House B.*—Patient slept in room, rising about 8 a.m., was up each day until day preceding death. House very clean. Sputa all burned. Room thoroughly cleaned after patient's death. Could only secure dust in floor cracks near base of wall, and from washings of wall paper. One specimen. No. 4.

*House C.*—Patient in room for 3 weeks previous to death. Very careless regarding sputa. House very dirty. One sample collected from mantel, wall paper, and floor at base of wall near where bed stood. No. 5.

*House D.*—Patient in room for a week only. Sputa slight. Room carefully cleansed. Secured dust from under base-board, and from washings. No. 6.

These six specimens of dust were washed with sterilized water, and the washings injected into Guinea-pigs.

No. 2 died on the day after injection, with an extensive subcutaneous extravasation of limpid serum about the seat of injection. The abdominal muscles also were extensively involved.

No. 4, a big brown male, died four weeks after. The cause of death could not be found ; there were no evidences of Tuberculosis.

No. 5 died March 16th, two months after injection, with caseating inguinal glands, in which tubercle bacilli were demonstrated. There were also extensive tubercular involvement of liver and spleen.

Nos. 1, 2, and 6 were apparently quite healthy on March 20th, and showed no enlarged glands.

It would not be right to draw conclusions from these experiments in themselves, but as they bear out the results of many other experiments we may safely do so.

The one house, the dust of which proved infective, was the only one in which there had been carelessness in the disposal of the sputa, and in which ordinary cleanliness had not been observed during and after the patient's death.

It shows what a menace the consumptive's room may be to those who spend any time in it, either during the patient's illness or for some time after. When a case has occurred in a lodging-house or hotel many may become infected by occupying, for a short time only, the room in which the patient lay.

It is satisfactory to know that the death rate throughout England from Tuberculosis has gradually decreased during the past 40 or 50 years with improved sanitation. With proper care in the disposal of the sputa it could be made remarkably less.

It is first essential that the patient understands thoroughly his disease is consumption. A patient who is expectorating, if told by his physician that his lungs are 'weak' or 'slightly affected,' will not see the necessity for destroying his expectoration. The physician should tell him frankly what his disease is, and, at the same time, give him to understand that it is not necessarily fatal, or at any rate not immediately so; that he has good chances for recovery, if in an early stage, and if the physician's advice is carefully followed.

The statement generally accepted is that Tuberculosis, directly or indirectly, causes the death of one in every seven throughout England, and one in every four or five of the working classes. In Liverpool, as we have seen, about one death in every nine is directly ascribed to Tuberculosis. These figures impress us with the necessity of doing everything in our power to control its spread.

In order to reach as many cases as possible, it should be a rule to examine the chest of every case presenting for treatment at the out-patient department of the hospitals and dispensaries, whether medical or surgical. This was begun some time ago in some of the American dispensaries, and so many unsuspected cases were discovered that it is now done as a matter of routine, and is bearing excellent results.

It would be advisable, also, to have a small pamphlet for distribution amongst such cases, instructing them about the disposal of their expectoration. It should be carefully prepared, so as to avoid alarming them or their friends, for if the danger be made too prominent it may result in making them practically outcasts, to be avoided. I have seen this in other places, where people with a slight knowledge of the danger of consumptives in a community have so exaggerated matters as to forbid them in certain hotels and boarding-houses. The pamphlet should advise them as to diet, clothing, and hygiene, as well as to care with the expectoration; and I am confident this would materially add to the influences at work in the decrease of the mortality from this disease.

Also, if it were possible, for the Health Department of all large cities to undertake the thorough disinfection of all rooms in which consumptives have died, a great source of danger would be averted. In the absence of any legislation on the matter, a great deal could be done by the Health Department undertaking to do this work when requested by the householder, making a reasonable charge where possible, and in other cases doing the work free of cost. Many people would gladly avail themselves of this were it within their reach, especially as the apparatus for the work is beyond their reach. Should the Health Departments announce to the citizens that they were ready to do this work we might look for a steadily increasing demand for the use of the apparatus, and with this a lessening in the mortality.

The whole question of decrease in the death rate from Tuberculosis will depend upon proper treatment of those affected, and on the education of both the masses and the classes in the importance of prophylaxis.

I am pleased to know that Liverpool is making preparations for the reception of part of its consumptive poor in special sanatoria. This is an excellent start, and one which deserves all support. Not only will the patients there treated receive marked benefit, and many of them be able to return to their work, but what appears to me the most important function of a sanatorium will then bear fruit, that is, its work as a centre from which information regarding prophylaxis will radiate. Every patient who returns to his home will be the means of spreading amongst his own circle the knowledge of the necessity for care of a patient and his expectoration.

With the erection of these sanatoria throughout the kingdom we may hope to see in a few years most beneficial results, and Liverpool should be congratulated on taking the first step in this work.

In conclusion, I must express my thanks to Dr. Hope, the Medical Officer of Health for Liverpool, and to the officials of his department, through whom I have had access to the weekly returns of deaths covering the past five years and to other vital statistics of the city.

NOTE ON

# EXPERIMENTS ON SEWAGE DISPOSAL IN GERMANY

By A. S. GRÜNBAUM, M.A., M.D., D.P.H.

During the past few years the subject of sewage disposal has received much attention in Germany. The two annual supplements to the quarterly journal of Forensic Medicine and Hygiene* contain no less than nineteen papers on this subject. The experiments have been carried out in various directions by sanitary officials and engineers, partly independently but for the greater part conjointly with Government experts, local authorities, and practising engineers. To this system of co-operative experiment by Government and municipality much of the success and reliability of the investigations is due. Nobody has any object in falsifying the results, the municipality sees that the Government has an interest in its affairs, the inventor is encouraged by the subsidy which he receives, and any improvement suggested by the official experts becomes public property, even in the case of a patented process. It seems to me an error in this country that the local authority is required to place a definite scheme before the Local Government Board, instead of the latter offering expert advice for the particular circumstances.

Most of the sewage disposal schemes which have been tried in Germany had their origin in Great Britain, but are often re-christened with a German name. Thus the closed sewage tank (so-called 'septic tank') system is known as SCHWEDER's system, and CAMERON's name is entirely omitted. The variations and changes are rather in mechanical details than of principle ; into these I do not propose to enter, but to limit my descriptions to the experiments on the three newer systems of sewage disposal, omitting entirely any reference to the merely mechanical or chemical precipitation methods, since practically all of them have been found wanting.

These three systems are—

(1) Contact beds—Hamburg and Charlottenburg ;

(2) Closed sewage tank (SCHWEDER's system)—Gr. Lichterfelde ;

(3) Lignite paste (ROTHE-DEGENER system)—Potsdam and Tegel ;

and concerning them the following notes of the results obtained may be of interest. In Hamburg, Potsdam, and Tegel I have seen the installations, but the data of all the experiments are taken from the published results.

* *Vierteljahrschrift f. gerichtl. Medicin u. oeffentliches Sanitätswesen, Jahrgänge* 1898 and 1900. Supplement.

## I. CONTACT BEDS

These are only of experimental size, and, so far as I know, the system has not been tried on a large scale like in several English towns. The results are in agreement, for the most part, with those obtained in Great Britain ; but the amount of purification attained seems to have been less, and the technique of the investigation less complete. Experiments have been made with various filtering materials. The two places, Hamburg and Charlottenburg, also form a valuable contrast and comparison, since the former supplies only domestic, and the latter both domestic and trade sewage to the beds.

### Hamburg

The experimental installation of contact beds (under cover) for the treating of the sewage of the General Hospital (2,000 persons) has been very thoroughly investigated by Dunbar and Zirn*, chiefly from the chemical aspect, since no bacteriological analyses are given in their published results. The sewage was purely domestic and was screened. It comes only a short distance, consequently the solids do not get much broken up. Yet with double filtration the best purification was not more than 86 p.c. diminution of oxygen absorbed, and the effluents which I saw myself were far from clear. (At Leeds, with a stronger sewage, purification up to 93 p.c. has been obtained). The authors, however, consider their results favourable to the system and draw the same conclusions as English observers, viz.: (1) clogging of the beds will occur sooner or later ; (2) the diminution in capacity, due to clogging by inorganic sludge, will not be much improved by rest ; (3) the diminution due to clogging with organic sludge will be relatively more improved by rest, but such sludge should not be allowed on to the beds in properly arranged works. The experiments with various materials all pointed to coke of appropriate size being the best. Both from a theoretical as well as from a chemical point of view, these critical and well written papers deserve careful study from those interested in the question, and it is much to be regretted that more complete chemical and some bacteriological analyses were not made.

### Charlottenburg

The experimental beds had a gross capacity of 6 cbm.† and a net capacity of 5 cbm. ; they took about two hours to fill and to empty. Experiments were made with (1) coke and sand ; (2) granite, gravel, and sand ; (3) sand and brick breeze. The coarser solids were screened off.

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* *Vierteljahrschr. f. gerichtl. Medic.*, 1900 : Suppl. pp. 178 et seq.

† 1 cbm. = 1,000 litres = 220 gallons.

The conclusions which the Government experts who made the investigations arrived at may be stated as follows :—*

(1) Gravel, or gravel with brick breeze, is not an efficient medium ; coke of a definite size seems to be the best.

(2) Two hours is the best period for contact.

(3) Settling for 24 hours for 'septic' action is of no import.

(4) Cold (– 10° C.) does not interfere with the action of the beds.

(5) Aeration is necessary.

(6) The reduction in the number of bacteria is of no appreciable importance. Consequently the effluents, *if they are to enter streams, require similar precautions and treatment to merely settled sewage.*

Whilst the conclusions seem quite correct, it is evident from the protocols of the experiments that these filters were not so efficient as they can be made. Taking, for example, an experiment from the sand and coke bed after it had been working three months (Experiment III), the following numbers were obtained on analysis :—

| | (Nitrogen in mgr. p. litre) | | | | O absorbed |
|---|---|---|---|---|---|
| | Ammoniacal | Organic | Nitrate | Nitrite | Mgr. p. litre |
| Crude Sewage ... ... | 73 | 13 | 0 | 0 | 145 |
| Effluent (after two hours) ... | 14 | 0 | 2 | present | 23 |

The absence of organic nitrogen was quite exceptional, and, although in this case the effluent was clear, it was by no means always so ; and occasionally it was putrescible.

| | No. of bacteria | |
|---|---|---|
| | Ordinary gelatine | KI-gelatine |
| Crude Sewage... ... ... | 2,700,000 | 210,000 |
| Effluent (after two hours) ... | 225,000 | 10,000 |

In many cases the plates of ordinary gelatine had liquefied (through insufficient dilution) ; the KI-gelatine is used to indicate the numbers of B. coli.

* Op. cit. pp. 262, et seq.

## 2. CLOSED SEWAGE TANK

### Gr. Lichterfelde (near Berlin).* *(Extracted from Official Report).*

This experimental installation was worked for over a year and then dismantled. It was merely a modification of Cameron's system, and the effluent was run on to a contact bed. The investigation was conducted by Government officials, and the inventor was subsidised. The bacteriological and chemical analyses extended over nearly the whole of the working period. The results do not differ essentially from those obtained elsewhere. The alteration in quantity of solids in suspension in the effluent is not recorded ; the total solids increased rather than diminished.

On dismantling the closed tank it was found that sludge had accumulated to the extent of 13.32 cbm., the total capacity of the tank being 90 cbm. The sludge did not differ chemically from that obtained from fresh sewage. Consequently *no 'mineralisation' had occurred.* The dilute nature of the sewage accounted for the sludge not having made itself very evident during the working period. In another installation the sludge had to be removed twice within six months from the tank.

The experiment showed the possibility of dealing with the sludge of a dilute sewage for a certain time without nuisance ; and the system may be useful for hospitals, barracks, &c.

These results are hardly so good as those obtained in England. But they all point in the same direction, viz., that, except to avoid smell, the closed presents no advantage over the open sewage tank, whilst it is considerably more expensive, and it does not in many cases dispose of the sludge in the effectual way that was expected and affirmed.

## 3. THE LIGNITE PROCESS (Rothe-Degener Patent)

### Potsdam and Tegel

I do not know of any similar process in use in this country. The sewage is first mixed with crushed lignite and then precipitated with ferric sulphate. The sludge settles and the effluent is drawn up into a Rothe-Roeckner tower. The former is drawn off from below, pressed, and finally used as fuel in the boiler furnace ; the latter is mixed with a small quantity of bleaching powder, and, thus sterilised, is run into the river. The free chlorine can, if desired, be easily removed by filtering through a shallow bed of coke or gravel.

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* Op. cit. pp. 288 et seq. ; also 1898, Suppl., pp. 99 et seq.

The quantities used are : 1 kg. lignite to 1 cbm. sewage and then 170 grammes ferric sulphate, and finally bleaching powder to the amount of 0.15 ‰.

As examples of the results the following, taken at random, may be quoted :—

| | Ammon. N. | Organic N. | Oxygen absorbed |
|---|---|---|---|
| Crude Sewage ... ... | 92.4 | 33.6 | 409.55 |
| Settled „ ... ... | 50.4 | 8.4 | 101.86 |

| | Ordinary gelatine | KI-gelatine |
|---|---|---|
| Crude Sewage... ... ... | Liquefied | 320,000 |
| Settled „ ... ... ... | „ | 140,000 |
| Bleaching Powder „ ... ... | Sterile | Sterile |

The effluent was not putrescible. Allowing the effluent to go through a filter bed usually caused a re-appearance of B. coli ; and since running the chlorine-containing effluent straight into the river (Havel) caused no harm to the flora and fauna, nor even appreciably affected the amount of chlorides, the filtration seems to be superfluous.

This process is of considerable importance because it demonstrates the practicability of obtaining a bacteriologically perfect effluent, and of disposing of the sludge. The cost is rather considerable (1s. 3d. per head), but it seemed to me that the mechanical arrangements might be simplified and made more economical. In Potsdam domestic sewage from over 50,000 inhabitants is treated in this way.*

The problem of 'sewage disposal' does not present so many difficulties in Germany as in this country. The rivers are mostly of greater size, and there is more land available for sewage farms. According to Mr. Lindley, who has probably the greatest experience in the construction of sewage schemes, it would require three of the largest English rivers in their non-tidal portion to make a volume equal to that of the Main at Frankfurt. It is, consequently, more easy to obtain the minimum dilution of 1 in 16 necessary to obviate putrefaction. In many instances the dilution reaches 1 in 1,000, or more, so that putrefaction hardly enters into consideration, and the deposit of solid matter on the banks only occasionally. Nevertheless, the undesirability of allowing untreated sewage to enter even a large river is being recognised, and the city of Cologne has been compelled to erect works for treating its sewage.

* Whittaker and Ducat Filters have not, so far as I am aware, been tried anywhere in Germany.

Yet the legal enactments regarding sewage disposal are hardly so definite as in this country. There is no special Act relating to Rivers Pollution, and local by-laws cannot exceed the common law. Moreover, there is an unfortunate decision of the Supreme Court of June 2nd, 1886, which says that a pollution not exceeding what is customary ('gemeinueblich') must be permitted, even if it damage the utility of the water. Consequently there is no standard, and each case is judged upon its merits. There are, however, indications of impending changes and improvement.* A committee of experts has been formed, with official recognition, for the complete (biological, chemical, &c.) investigation of rivers, and there is a possibility of legislative regulations being introduced in the near future.

It has been possible to lay down certain rules without statutory authority. Thus, in all cases where the sewage is treated by chemical means, the effluent is required to be free from B. coli. But this condition has not been applied to other methods of treatment, and no regulations have been laid down for sewage farms. Consequently the sewage fungus Leptomitus occurs in the effluents from the Berlin sewage farm, and it grew so luxuriantly that a bathing place in the River Panke, which received the effluent, became unusable. Yet the owner could obtain no redress, although he tried to do so, in face of the decision of 1886.

On the other hand certain trades, and more particularly the sugar refineries, have been compelled to purify their waste; and this has been done without any obvious detriment to the trade. A friendly co-operation between manufacturers and the Government exists, and advice and direction are given in each individual case by a committee composed of a commissary and an expert of the State and of the Directorate of the Union of German Sugar Industry. In spite of all these experiments it is, of course, recognised that, where possible, the proper method of sewage disposal is on a farm, and that only when this is unattainable, should other methods be adopted.

Although it would appear that, both in technique and in results, the investigations undertaken in Germany fall behind those of this country, it is the harmonious association, to which allusion has been several times made, between the Administration and the municipality or the trade, the readiness of the Government to give advice instead of only criticism, which forms the most pleasant and striking feature of the present experimental stage of sewage disposal in Germany. To quote the words† of Dr. Schmidtmann, the principal medical officer of the Cultus Ministerium:—

'This appropriate union of State and private objects and interests, the definite co-operation of science, technique and practice, promises most successful results,

* Op. cit. 1900, Suppl., p. 308.

† Op. cit. 1900, Suppl., p. 319.

and puts before us a picture of how in the future the great economic questions regarding water supply and sewage disposal, as yet unanswered, may be solved. The direct participation, by the experts of the State departments concerned, in the foregoing problems, has to a large extent put an end to existing uncertainty and given stimulus and support to purposeful honest exertion and work, and, with comparatively small sacrifice on the part of the State, the latter has been able to make use of the large means at the disposal of the municipalities and trades concerned for the common weal.'


# Kanthack Medal in Pathology

A Bronze Medal, designed by Mr. Allen and Mr. McNair, has been presented to the College by a Liverpool Shipowner, to commemorate the late Professor Kanthack, of Cambridge, formerly Student of University College. The Medal is awarded to the best Student in Pathology and Bacteriology in the year.

THE

# THOMPSON YATES LABORATORIES REPORT


EDITED BY

RUBERT BOYCE AND C. S. SHERRINGTON

WITH THE CO-OPERATION OF

A. S. GRÜNBAUM AND J. MACDONALD
CHEMICAL PHYSIOLOGY AND PHYSIOLOGY

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C. A. HILL, A. T. MACCONKEY, A. S. GRIFFITH, AND E. E. GLYNN
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HYGIENE


*WITH ILLUSTRATIONS AND PLATES*


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# CONTENTS

# THE DISTRIBUTION OF B. COLI COMMUNE

By HARRIETTE CHICK, B.Sc., 1851 Exhibition Scholar

## PART II

THE following experiments are a continuation of those described in a previous paper (*T. Y. Reports*, *Vol. III.*, *p.* 1), and will, I hope, serve to render them more complete. They were undertaken in order, if possible, to answer with more certainty the question whether substances containing B. coli might be considered to be polluted or not, and the only answer to such a question seemed to be a further study of the distribution of the bacillus in question.

The following material has been carefully studied in addition to that already described :—

1 Air.

2 Cultivated manured land.

3 Road dust and sweepings, as well as road puddles.

The experiments substantiate the view I have already put forward, and my present opinion is that the occurrence of this organism, as isolated by the method employed, may be looked upon as useful evidence of recent faecal contamination. At the same time, they show very strikingly a characteristic of B. coli, to which I have already drawn attention, viz. :—the very low resistance which it can offer to unfavourable conditions.

I am aware that this opinion is not general among bacteriologists ; the statement of Kruse (*Zeitschrift f. Hyg. u. Infect. XVII, S.* 53) is constantly quoted, viz. :—that bacilli, closely resembling B. coli, are to be found everywhere, in air, soil, and water from every source. Weissenfeld (*Zeitsch. f. Hyg. u. Infect., XXXIII, S.* 78), has lately shown that in 100 samples of waters he found a 'coli-like' organism in 'good' waters as well as 'bad,' if one litre were taken for analysis. Using one cubic centimetre as a standard, I have found B. coli to be absent in that quantity in 'good waters,' and absent in one litre in specially good, filtered, drinking water (*T. Y. Reports, vol. III, pp.* 14 *and* 16). A good sand filtration seems to be effectual in removing the bacillus, retaining it in the top layer. (*See Table VI, T. Y. Reports, vol. III, p.* 15).

*Air.*—I have endeavoured to find any satisfactory experimental foundation for the statement that B. coli is to be found in the air, and the only description of such experiment I have found is in a paper by Concornotti. (*Centralbl. f. Bakt. XXVI, S.* 492). He exposed in various places agar plates that had already been poured, and incubated them ; he then made an emulsion with sterile water of the mixed cultures he obtained, and injected it intravenously into rabbits. In five cases out of forty-six he obtained cultures of B. coli from the organs and heart blood of the dead animal ; he concluded that this bacillus was present in the emulsion injected, and, hence, in the original air examined. The conclusion, however, appears to be quite unwarranted, for the researches of Lesage and Macaigne (*Arch. d. Med. Exp.* 1892, *p.* 250), Achard and Phulpin (*Arch. d. Med. Exp.* 1895, *p.* 25), and notably those of Beco (*Ann. d. l'Inst. Past.* 1895, *p.* 199), have conclusively proved that B. coli, normally found only in the intestines, is in the habit, both just before and also after death, of invading the organs and even the peripheral circulation. It is significant that most of the experiments of the last-named author were also made upon rabbits. Thus the presence of B. coli, as found by Concornotti, by no means proved that this bacillus had been the cause of death, or that it had been artificially introduced into the circulation.

I have made a careful examination of the air in different places, but only on one occasion have I isolated B. coli, and that was from the air of an ill-ventilated stable.

*Soil, Road Dust.*—I have never found the bacillus in virgin soil, and in cultivated manured lands, where it had been added artificially, I have also found it to be absent, a fact which I take to be due to the low power of resistance which B. coli possesses. This is also shown by the almost invariable absence of B. coli in dry samples of road dust, while it occurs in quantity in road puddles or in wet samples of dirt taken during rainy weather. This led me to make some special experiments to illustrate this point, and I found, for example (Table IV, b) that a mixture of a B. coli culture with soil, which contained more than 1,000,000 organisms per grm., after seven and a half hours' drying in the sun had all its organisms killed, while a similar sample, which was kept damp, had its numbers lowered only to 380,000 per grm. These experiments are fully described at the end of the paper, and indicate a conclusion that the presence of B. coli not only is significant of pollution, but shows that the pollution must have been recent.

The method used for these experiments has been already described in detail, viz. :—plating the sample with carbolized agar, incubating for forty-eight hours at 42° C., counting the colonies which resemble B. coli, if present, and isolating and identifying them. For identification I have found the coagulation of milk with the production of acid and the fermentation of lactose the two most distinguishing reactions of B. coli.* A medium containing the latter is conveniently made by simply adding to tap water 2% of peptone and 1% pure lactose ; this is distributed in Durham's

* Harvey Cushing, *Johns Hopkins Hospital Bulletin*, July, 1900.

fermentation tubes, and usually contains no other sugar but lactose. It should, however, always be tested with a culture of B. enteritidis (Gärtner), for example, to see if traces of glucose are present. An organism which, while generally resembling B. coli, coagulates milk and ferments lactose may, I think, be considered to belong to the coli group.

The fermentation of saccharose does not appear to be a constant quality in the group ; while Escherich's original B. coli commune, of which I tested a culture, gave no fermentation in a saccharose broth, many isolated from different sources showed production both of acid and gas, while otherwise indistinguishable from the original Escherich culture. For example, out of thirty-seven examples that were thus examined, seventeen fermented saccharose with production of acid and gas, two produced acid but no gas, while sixteen showed no change at all in the medium. The duration of the experiments was from eight to eleven days.

## I AIR

The experiments with air, already described,* being somewhat inadequate, the following were undertaken with a view to making the investigation more complete. The original apparatus was discarded in favour of one which was both more convenient and more accurate. As before, a measured quantity of air was drawn through a wool plug, the plug well washed with water, and a portion of the latter plated, and so analysed. As a rule the plug consisted of two parts ; the air first went through a glass wool plug and then through one of cotton wool, the former being much easier to wash thoroughly, while the latter is a more efficient filter. The air was drawn through by means of a pump, which was worked by a hot air engine, and each revolution of the engine wheel was recorded by a cyclometer. The record of the cyclometer was calibrated by aspirating a known volume of air, and it was found to give concordant results ; the apparatus was usually left running for about twenty-four hours. I am much indebted for the use of the machine to Dr. E. E. Glynn, under whose direction the whole apparatus was put together.

*Experiment I*—The air outside the laboratory was analysed on the night of April 30-31, 1900, after wet weather. B. coli was found absent in 240 litres ; a bacillus resembling B. fœcalis alcaligenes was, however, isolated on this occasion. The details of this and of all the other experiments are given in Table I.

*Experiment II*—The air of the College grounds was analysed for five consecutive days, in dry, dusty weather, from May 14 to May 19, 1900. B. coli was found invariably to be absent.

*Experiment III*—A dusty street corner was chosen as the place for analysis of the air, which was examined for seven consecutive days ; the air was drawn

* *T. Y. Reports*, vol. III, p. 11.

through continuously, the plug being removed each day for analysis. B. coli was absent in every analysis.

*Experiment IV*—In this experiment the air analysed was that of two of the wards of the Royal Infirmary, and B. coli was also found to be absent.

*Experiment V*—The air was that of an ill-ventilated stable, and was examined on three occasions ; on the first B. coli was isolated and found present in 238 litres. The air was drawn through during the night in each instance, and the plug analysed early on the following morning.

## TABLE I

### Experiment I—Air of College Grounds

| Date | Quantity of air aspirated | Quantity analysed | Remarks |
|---|---|---|---|
| 1900<br>April 30-31 | 596 litres | B. coli absent in 240 litres | Rain on 29<br>Bacillus closely resembling B. fœcalis alcaligenes isolated |

### Experiment II—Air of College Grounds

| Date | Quantity of air aspirated | Quantity analysed | Remarks |
|---|---|---|---|
| May 14-15 | 3,570 litres | B. coli absent in 890 litres | No rain since May 12 |
| May 15-16 | 2,580 litres | B. coli absent in 646 litres | No rain since May 12 |
| May 16-17 | 3,960 litres | B. coli absent in 1,950 litres | No rain since May 12, days from May 16-18 were dry and dusty, and much dust was blowing about |
| May 17-18 | 1,790 litres | B. coli absent in 880 litres | |
| May 18-19 | 3,130 litres | B. coli absent in 620 litres | |

### Experiment III—Air at the Corner of Brownlow Hill and Ashton Street Liverpool

| Date | Quantity of air aspirated | Quantity analysed | Remarks |
|---|---|---|---|
| May 24-25 | 3,856 litres | B. coli absent in 1,930 litres | Machine calibrated May 24 ; rain on May 23 ; May 25, a dry, dusty day |
| May 25-26 | 3,670 litres | B. coli absent in 1,840 litres | May 26, windy, dusty day |
| May 26-28 | 12,400 litres | B. coli absent in 4,135 litres | |
| May 28-30 | 7,650 litres | B. coli absent in 3,826 litres | |
| May 30-31 | 4,520 litres* | B. coli absent in 2,260 litres | |

* At the conclusion of Experiment III, it was found that the washer of the piston of the pump was slightly worn out, so that at the end of the experiment the registered quantity of air may be a little too large, the friction of the machine being less. A calibration after a new washer had been fitted gave the same number as on May 24.

EXPERIMENT IV—AIR OF THE ROYAL INFIRMARY, LIVERPOOL

| Date | Quantity of air aspirated | Quantity analysed | Remarks |
|---|---|---|---|
| June 8th | 856 litres | B. coli absent in 430 litres | Ward I (Medical), well ventilated |
| June 11th | 2,000 litres | B. coli absent in 1,000 litres | Ward XII (Medical), well ventilated |

EXPERIMENT V—AIR OF A STABLE

| | | | |
|---|---|---|---|
| July 11-12 | 2,380 litres | B. coli present in 240 litres | Stable badly ventilated. |
| July 12-13 | 2,480 litres | B. coli absent in 743 litres | Stable badly ventilated. |
| July 17-18 | 1,600 litres | B. coli absent in 800 litres | Stable badly ventilated. |

## II CULTIVATED SOILS (MANURED)

Samples of ploughed land were taken in the neighbourhood of Shrewsbury on June 23 and June 24, 1900; the weather was rainy, and most of the samples were damp, both when taken and when analysed. As will be seen from Table II, B. coli, which must have been added in quantity to the land when manured, was absent in almost every case in the quantity analysed. In No. 3, where B. coli was present, the sample was taken from a garden close to the shore of the river Severn, where the ground would probably be always kept moist. The samples were taken about an inch below the surface, as a rule, and were all in neighbourhoods which drain into the river Severn; this is interesting in connection with the analyses of a number of land drains running into that river (*T. Y. Reports, vol. III, p.* 25), in which B. coli was also found to be absent. The crops were only just showing, so that the time of manuring was probably not very far distant, but the B. coli had apparently been unable to withstand the drying, &c., it had undergone in the meantime.

## TABLE II

### Cultivated Soil

| No. | Description of the Field, &c. | B. Coli Commune, per grm. | Remarks |
|---|---|---|---|
| 1 | Potato field, Buildwas | Absent in ·02 grm. | Samples taken June 23, and plated June 25, 1900 (Wet weather) |
| 2 | Same field, another place | Absent in ·01 grm. | Samples taken June 23, and plated June 25, 1900 (Wet weather) |
| 3 | Garden, growing turnips, Buildwas, on the shore of the river Severn | 205 per grm. (approx.) | Samples taken June 23, and plated June 25, 1900 (Wet weather) |
| 4 | Ploughed field, crops just growing | Absent in ·02 grm. | Samples 4-13 taken on June 24, and plated on June 26 (Wet weather) |
| 5 | Ploughed field, turnips | Absent in ·03 grm. | Sample dry when analysed |
| 6 | Ploughed field, crops just growing | Absent in ·02 grm. | Sample dry when analysed |
| 7 | Ploughed field | Absent in ·02 grm. | Sample wet when analysed |
| 8 | Ploughed field | Absent in ·02 grm. | Sample dry when analysed |
| 9 | Ploughed field, crops just growing | Absent in ·006 grm. | Sample dry when analysed |
| 10 | Ploughed field, Pitchford | Absent in ·025 grm. | Sample dry when analysed |
| 11 | Ploughed field, cabbages, crops very small | Absent in ·04 grm. | Sample wet when analysed |
| 12 | Ploughed field, crops just growing | Absent in ·02 grm. | Sample dry when analysed |
| 13 | Ploughed field, crops just growing | Absent in ·02 grm. | Sample wet when analysed |
| 14 | Garden, Grange - over - Sands, July 8, 1900 | Absent in ·04 grm. | Sample was taken from the base of a rose-bush, where there were obvious remains of manure ; a damp sample |

## III ROAD DUST, &c.

In all thirty-three samples of road dust and puddles were examined, the latter, and those of the former taken during wet weather, usually contained B. coli in quantity, for example, those examined on May 7 (*see Table III*, where the numbers must be considered as approximate only). The dirt was collected in sterile test tubes, weighed out into sterile flasks, the requisite amount of sterile water added for the required dilution, the whole well shaken, and 1 c.c. plated on carbolized agar. This method has been the one ordinarily followed, and the preceding samples of soil were analysed exactly in the same way. Dry samples, as a rule, contained no B. coli in the quantities analysed, and it is significant that when among a number of dry samples, wet ones were examined on the same day, the latter invariably contained B. coli (*e.g.*, *No.* 18,

*Table III*). The dampness may have been caused by artificial watering of the street in question, or by the constant trickling of water from a fish shop serving to keep a gutter moist, as in No. 14.

Whether a moist substratum would serve to resuscitate B. coli, which had apparently died out, or whether in such dry samples the bacillus was quite dead, was answered in the following manner. The dilutions of May 31 (*see Nos.* 26-31, *Table III*) were kept for three days and then analysed again. The results showed that in the cases in which B. coli was absent on May 31, it was also absent after the sample had been thus kept wet and favourable for the growth of the bacillus; in those cases in which it was present on May 31, its numbers were much reduced on June 2. One must conclude that B. coli, constantly being thrown upon the roads, is able to survive in dry weather for a short time only (*No.* 33); if, however, the weather is wet or the road kept damp artificially, it can live much longer.

These facts suggested the enquiry whether sunshine or drying was the important factor in the short life of B. coli in nature under these conditions. The effect of drying has been studied by Walliezek (*Centralbl. f. Bakt. XV*, *S.* 949), in whose experiments 17 hours' exposure to drying over sulphuric acid killed almost all the bacteria on a piece of filter paper that had been dipped in a culture of B. coli and then dried; a similar effect was obtained after 45 minutes' drying in a vacuum. Billings and Peckham (*Science*, *Feb.*, 1895, *Abstr. Centralbl. f. Bakt. XIX*, *S.* 244), found that threads moistened with a broth culture of B. coli and then dried, showed living bacteria for a period of 152-229 days, while sunlight alone proved fatal in 3-6 hours; they concluded that death by sunlight is on no account to be attributed to drying. Buchner (*Centralbl. f. Bakt. XI*, *S.* 781), also showed the importance of sunlight in killing B. coli; in one experiment 100,000 B. coli suspended in water and exposed to sunlight were all found dead after one hour.

The experiments of Wittlin (*Wiener Klin. Wochenschr.*, 1896, *No.* 52, *S.* 1,229), however, indicate that drying must be a factor in the death of B. coli in nature, as important as sunshine, if not more so; and the experiments I am about to describe confirm his observations.

An emulsion was made with a twenty-four hours' culture of B. coli on agar and a little water, and this was used to moisten some sterilised earth, which was then distributed into six Petri dishes. The soil in all of these was analysed for B. coli in the way already described; in the subsequent analyses, where the earth was really wet, it was dried before weighing on sterile filter paper. This method, I think, makes the numbers of the wet samples rather lower than they should be, and renders the differences a little less striking than they really are. After analysis, water was added to three of the six samples, and these were kept thoroughly wet throughout the course of the experiment, the other three were allowed to dry. One of each was placed in the sunshine, two also in the shade, and two were kept in the dark. The details are given in Table IV; after exposure to six hours' sunshine, both wet and dry samples contained no B. coli in the quantities analysed; of the shade samples, the wet one contained 247,000 per grm., while in the dry sample there was no B. coli. No 5 was scarcely dry by the evening of the first day; on the next day, however, it also contained no B. coli. The two samples 5 and 6 were then left for nearly two months, at the end of that time, while No. 5 was sterile as regards B. coli, No. 6, which had been kept moist all the time, contained 566,000 per grm. Though at the commencement of the experiments care was taken to exclude all other organisms by sterilising, no attempt was made to keep the contents of the dishes sterile afterwards, thus attempting to approximate to the conditions which obtain in nature.

*Experiment II*—(*Table IV*, *b*), consisting of two samples only, a wet and a dry one, both exposed to sunshine, was made to correct a discrepancy in Experiment I. After the analysis on July 27, the two Petri dishes with their contents were placed in a dark cupboard, and after nearly two months were again analysed. The result was the same as in Experiment I; while in the dry sample B. coli was absent, in the wet one it was present to the extent of 47,000 per grm.

*Experiment III* was made when there was not so much sunshine, and that rather intermittent; it, however, also illustrates the marked difference in the length of life of B. coli under dry and wet conditions.

Some experiments were also made with a pure culture of B. coli in order to find its length of life when dried upon filter paper. Scraps of sterile filter paper, about an inch square, were dipped into a 'peptone-salt' culture, twenty-four hours old, of B. coli, and these were drained and then laid upon a sterile Petri dish. The pieces of paper were just the same size, and hence retained approximately the same number of organisms. They were placed under a tightly-fitting bell-jar, and a current of dry air was drawn over them by means of a pump, so that they were quickly dried. From time to time, one of these pieces of paper was removed and incubated with 10 c.c. nutrient broth; after twenty-four hours plates of ordinary nutrient agar were made of 1c.c. of the broth and these examined, after forty-eight hours' incubation, to see if they were sterile. It was found that there had remained no B. coli in the piece of paper which had been dried for twenty-eight hours, while B. coli was almost entirely killed after twenty-three and a quarter hours exposure to the current of dry air. A culture of B. coli commune from the original strain of Escherich was used for these experiments, kindly given me by Dr. H. E. Durham. *Experiment II* gave a similar result; after twenty-four hours' exposure of the pieces of paper to a current of dry air, all the organisms were found to have been killed. An interesting series of plates can be obtained by taking the dried pieces of filter paper at different times, moistening them with sterile water and pressing them on the surface of an already poured agar plate for a few minutes; then the paper should be removed and the plate incubated. At first the shape of the paper is shown after incubation as a solid mass of growth, after six hours drying (*No.* 4, *Table V*, *Exp. II*) there were only a few scattered colonies, and after ten hours drying (*No.* 5, *Exp. II*) the patch remained quite sterile. This test, of course, is not so searching as incubating the paper with broth for twenty-four hours and then plating the broth culture.

This makes it easy to understand how it is that one frequently does not find any trace of the B. coli in places where it must undoubtedly have been present in quantity not so very long before, *e.g.*, in roads, manured land, &c. The effect of drying, shown in the above experiments, would account for its absence. A question of practical interest arising out of this is whether the streets should be watered or not in the dry weather. Mazuschita (*Archiv. f. Hygiene*, 1899, 35, *S.* 252), found in Freiburg that the life of certain bacteria in watered dust was three times as long as in dry dust, and also that on an average there were twice as many organisms in the dust of a watered street as in that of one which was unwatered. He considers that although the watering of streets lengthens the life of the bacteria in the dust, that it is still useful in laying it, and thus keeping it away from the respiratory organs ; as an improvement he would suggest thoroughly flushing the streets at frequent intervals.

In conclusion, I wish again to give my best thanks to Professor Boyce, at whose suggestion the work was originally undertaken, and to whom I am indebted for much kind help and advice during the course of the investigation.

## TABLE III

| No. | Date | Description and Source of Sample | Dilution used | Presence or Absence B. coli in quantity analysed | Remarks |
|---|---|---|---|---|---|
| | 1900 | | | | |
| 1 | May 1 | Dust of roads | | Absent in .2 grm. | Dry weather |
| 2 | „ 1 | | | „ .16 „ | Dry weather |
| 3 | „ 1 | | | „ .15 „ | Dry weather |
| 4 | „ 1 | | | „ .15 „ | Dry weather |
| 5 | „ 7 | Dirt, side of road, Bedford St., L'pool. | | *65,000 per grm. | Damp weather |
| 6 | „ 7 | „ „ Walnut Street, „ | | * 1,200 „ „ | Damp weather |
| 7 | „ 7 | Puddle water, Ashton Street, „ | $\frac{1}{50}$ | 500 „ c.c. | Damp weather |
| 8 | „ 7 | „ „ Huskisson Street, „ | $\frac{1}{50}$ | * 4,200 „ c.c. | Damp weather |
| 9 | „ 7 | „ „ Bedford Street, „ | $\frac{1}{50}$ | 750 „ c.c. | Damp weather |
| 10 | „ 7 | „ „ „ „ „ | Neat | 25 „ c.c. | Damp weather |
| 11 | „ 15 | Dirt, middle of road, Edge Lane, „ | .2 grm. in 50 c.c. | Abs. in .012 grm. | Dry sample |
| 12 | „ 15 | „ side of road, „ „ „ | 1.18 „ „ 50 c.c. | Abs. in .07 „ | Dry sample |
| 13 | „ 15 | „ „ Marmaduke Street, „ | .98 „ „ 50 c.c. | Abs. in .06 „ | Dry sample |

TABLE III—*continued*

| No. | Date | Description and Source of Sample | Dilution used | Presence or Absence B. coli in quantity analysed | Remarks |
|---|---|---|---|---|---|
| 14 | 1900 May 15 | Dirt, side of Paddington, L'pool. | 1.05grm. in 100c.c. | * 4,400 per grm. | Damp sample, taken at side of road, opposite a fish shop, by no means clean, where the gutter is always wet |
| 15 | „ 15 | „ „ Brownlow Hill, „ | 1.2 „ „ 50c.c. | Probably absent in .07 grm. | Dry sample |
| 16 | „ 17 | „ Crosshall Street, „ | 1.04 „ „ 100c.c. Water | Abs. in .03 „ | No rain since May 12. May 17 a dry, dusty day; dry sample |
| 17 | „ 17 | „ Victoria Street, „ | .65 „ „ 100c.c. | Abs. in .02 „ | Dry sample |
| 18 | „ 17 | „ St. John's Lane, „ | 1.91 „ „ 100c.c. Water | 1,200 (at least) per grm. | Damp sample |
| 19 | „ 17 | „ South Gate, St. George's Hall, „ | 1.05 „ „ 100c.c. Water | Abs. in .06 „ | Dry sample |
| 20 | „ 17 | „ London Road, Seymour St., „ | .82 „ „ 50c.c. | Abs. in .05 „ | Dry sample |
| 21 | „ 17 | „ Seymour Street, „ | .16 „ „ 20c.c. | Abs. in .02 „ | Dry sample |
| 22 | „ 17 | „ Russell Street, „ | .53 „ „ 100c.c. | Abs. in .016 „ | Dry sample |
| 23 | „ 17 | „ Brownlow Street, „ | .85 „ „ 100c.c. | Abs. in .025 „ | Dry sample |
| 24 | „ 31 | „ Ling Street „ | .49 „ „ 100c.c. | Abs. in .03 „ | Dry sample |
| 25 | „ 31 | „ Brae Street „ | 1.51 „ „ 100c.c. | Abs. in .045 „ | Dry sample |
| 26 | „ 31 | „ Kensington Park, „ | .94 „ „ 50c.c. | (*a*) Abs. in .06 „ | Dry sample |
| „ (*b*) | June 2 | „ „ „ „ | .94 „ „ 50c.c. | (*b*) Abs. in .06 „ | Dry sample |
| 27 | May 31 | „ Jubilee Drive, „ | .49 „ „ 50c.c. | *(*a*) 29,900 per grm | Wet sample |
| „ (*b*) | June 2 | „ „ „ „ | .49 „ „ 50c.c. | †(*b*) 2,350 „ „ | Fewer total organisms and many fewer B. coli than on May 31 |
| 28 | May 31 | „ Edge Lane, „ | 1.07 „ „ 50c.c. | *(*a*) 2,100 „ „ | Wet sample |
| „ (*b*) | June 2 | „ „ „ „ | 1.07 „ „ 50c.c. | †(*b*) 327 „ „ | More total organisms, but not so many B. coli |
| 29 | May 31 | „ Marmaduke Street, „ | .87 „ „ 100c.c. | *(*a*) 340 „ „ | Wet sample |
| „ (*b*) | June 2 | „ „ „ „ | .87 „ „ 100c.c. | (*b*) abs. in .001 grm | |
| 30 | May 31 | „ Brownlow Hill, „ | .55 „ „ 50c.c. | (*a*) abs. in .03 „ | Dry sample |
| „ (*b*) | June 2 | „ „ „ „ | .55 „ „ 50c.c | (*b*) abs. in .03 „ | |
| 31 | May 31 | „ College Grounds, University College, L'pool. | .71 „ „ 50c.c. | (*a*) abs. in .04 „ | Dry sample |
| „ (*b*) | June 2 | „ „ „ „ „ | .71 „ „ 50c.c. | (*b*) abs. in .04 „ | |
| 32 | July 7 | Dust, middle of road, Grasmere | 1 in 100 | Abs. in .04 „ | Dry sample (Weather July 6, very wet) |
| 33 | „ 8 | „ „ „ Grange-over-Sands | 1 in 100 | 3,900 per grm. | Dry sample |

* Numbers are only approximate. † These numbers are a maximum. The dilutions of May 31 kept and plated again.

## TABLE IV (A)

### EXPERIMENT I

| | July 24, 1900, 11-45 a.m., beginning of Expt. B. coli per grm. | July 24, 5-45 p.m., after 6 hours' exposure to sun in case of 1 and 2. B. coli per grm. | July 25, 6 p.m., after 6½ hours' exposure to bright sun in case of 1 & 2. B. coli per grm. | July 27, 12 p.m. B. coli per grm. | Sept. 21, Nos. 5 and 6 kept in dark since July 27. B. coli per grm. |
|---|---|---|---|---|---|
| Contents of Petri Dish— | | | | | |
| No. 1 (exposed to sun and allowed to dry) | 1,210,000 | Absent in .24 grm. | Absent in .5 grm. | | |
| No. 2 (exposed to sun and kept wet) | 592,400 | Absent in .008 grm. | ‡1,170 | | |
| No. 3 (in shade and allowed to dry) | 850,000 | Absent in .04 grm. | Absent in .6 grm. | | |
| No. 4 (in shade and kept wet) | 336,000 | 247,000 | 3,360,000 | | |
| No. 5 (in dark and allowed to dry) | 390,000 | 1,020,000* | Absent in .02 grm. | Absent in .5 grm. | †Absent in .24 grm. |
| No. 6 (in dark and kept wet) | 508,500 | 56,800 | 1,870,000 | 1,180,000 | 566,000 |

* Was not then properly dry. † It was impossible to take more for analysis on account of the extraneous organism which had found their way in. ‡ There appears to be a discrepancy here, to correct which Experiment II was undertaken.

## TABLE IV (B)

### EXPERIMENT II

| | July 26, 1900, 10-15 a.m. B. coli per grm. | July 26, 5-45 p.m., after 7½ hours' sunshine. B. coli per grm. | July 27, 12 noon, after 5½ hours' sunshine. B. coli per grm. | Sept. 21, in dark since July 27. B. coli per grm. |
|---|---|---|---|---|
| Petri-dish— | | | | |
| No. 7 (exposed to sun and allowed to dry) | 1,050,000 | Absent in .13 grm. | Absent in .5 grm. | †Absent in .28 grm. |
| No. 8 (exposed to sun and kept wet) | 1,220,000 | 381,000 | 1,584,000 | 47,000 |

† It was impossible to take more for analysis, owing to the extraneous organisms which had found their way in.

## TABLE IV (c)

### Experiment III

| | Sept. 25, 1900, 10-15 a.m. B. coli per grm. | Sept. 25, 1-50 p.m., after 3½ hours' intermittent sunshine. B. coli per grm. | Sept. 25, 5 p.m., after 4¾ hours' intermittent sunshine. B. coli per grm. | Sept. 26, 5 p.m., no sun all day. B. coli per grm. | Sept. 28, after 6¾ hours' sunshine. B. coli per grm. | Oct. 6, C. and D. after about 38 hours' light. B. coli per grm. | Oct. 22, D had exposure to 55 hours light. B. coli per grm. |
|---|---|---|---|---|---|---|---|
| Petri-dish— | | | | | | | |
| (A) Exposed to sunlight, and allowed to dry | 3,800,000 | 42 | 9 | Absent in .3 grm. | | | |
| (B) Exposed to sunlight, and kept wet | 4,250,000 | 1,015,000 | 547,000 | 354,000 | 1,090,000 | plate broken | |
| (C) In the shade, and allowed to dry | 2,250,000 | | Absent in .03 grm. | 5 | Absent in ·3 grm. | Absent in ·3 grm. | |
| (D) In the shade, and kept wet | 3,640,000 | | 1,030,000 | 1,450,300 | 1,982,000 | 800,000 | 82,500 |
| (E) In the dark, and allowed to dry | 4,300,000 | | 420 | 7 | Absent in .3 grm. | Absent in .6 grm. | |
| (F) In the dark, and kept wet | 3,420,000 | | 405,000 | 1,650,000 | 1,912,000 | 1,600,000 | 166,000 |

## TABLE V (a)

### Experiment I

| No. | Time | No. of hours' exposure to current of dry air | Appearance on plate (piece of paper incubated for 24 hours with 10 c.c. broth and 1 c.c. plated). |
|---|---|---|---|
| 1 | Oct. 1, 1900, 10-55 a.m. | 0 | ∞ Colonies |
| 2 | " " 12-45 noon | 2 | ∞ " |
| 3 | " " 2-55 p.m. | 4 | ∞ " |
| 4 | " " 5-10 p.m. | $6\frac{1}{4}$ | ∞ " |
| 5 | " 2, " 10 a.m. | $23\frac{1}{4}$ | 9 " |
| 6 | " " 2-45 p.m. | 28 | Sterile |
| 7 | " 3, " 9-40 a.m. | $4\frac{3}{4}$ | Sterile |

## TABLE V (B)

### Experiment II

| No. | Time | No. of hours' exposure to current of dry air | Appearance on plate (piece of paper incubated for 24 hours with 10 c.c. broth and 1 c.c. plated). |
|---|---|---|---|
| 1 | October 4, 10-40 a.m. | 0 | ∞ Colonies |
| 2 | ” ” 12 noon | $1\frac{1}{3}$ | ∞ ” |
| 3 | ” ” 2-30 p.m. | 4 | ∞ ” |
| 4 | ” ” 4-30 p.m. | 6 | ∞ ” |
| 5 | ” ” 8-45 p.m. | 10 | ∞ ” |
| 6 | ” 5, 10-35 a.m. | 24 | Sterile |

## APPENDIX—TABLE VI

This table shows the efficiency of sand filtration for removing B. coli commune from water *(see this vol., pp.* 15 *and* 16). The samples were taken from a sand filter bed while it was being cleaned. The bed was used to filter the Rivington supply of the Liverpool drinking water.

| Date | No. | Description of Sample | B. coli per gram | Remarks |
|---|---|---|---|---|
| Sample taken July 2, 1900, analysed July 3, 1900 | 1 | Sand from unscraped surface of filter bed | 20 | Sand appeared very dirty |
| Sample taken July 2, 1900, analysed July 3, 1900 | 2 | Sand from same bed, after the surface (about ½ inch deep) had been scraped off | Absent in ·3 grm. | |
| Sample taken July 2, 1900, analysed July 3, 1900 | 3 | Sand, similar to No. 1, after washing | ” ” | |

# THE RELATION BETWEEN BACILLUS ENTERITIDIS SPOROGENES OF KLEIN AND DIARRHOEA*

By E. E. GLYNN, M.A., M.B.

The bacillus enteritidis sporogenes was discovered by Dr. KLEIN during certain investigations concerning the cause of an epidemic of diarrhoea. The following is an abstract of the epidemic taken from the report by Dr. ANDREWES, Health Officer to St. Bartholomew's Hospital.† 'On the night of October 27, 1895, a sudden outbreak of diarrhoea of a severe type occurred amongst the in-patients of St. Bartholomew's Hospital. Out of twenty-eight wards fifteen were affected. The total number of cases during the night and following morning was fifty-nine. The outbreak ceased as suddenly as it began, and by mid-day on October 28 it was practically over.' Clinically, the earliest symptom in nearly all cases was abdominal pain. 'This was followed in the course of half an hour or more by diarrhoea. Vomiting was conspicuously absent in the great majority of the cases. The disorder was intestinal, not gastro-intestinal. In some instances the bowels were opened six or eight times in the course of twelve hours, but in the majority of cases only two or three times. The stools were in most cases liquid and watery, and a considerable amount of mucous was present. In the more severe cases a considerable degree of prostration occurred. But in no case was the attack fatal, even when the patient had been previously suffering from severe disease.'

On microscopical examination of the evacuations and also of the mucous discharge, a very large number of oval glistening spores were detected. These, Dr. KLEIN demonstrated by anaerobic cultivation, were derived from a hitherto unknown, and 'uncommon,'‡ organism, the bacillus enteritidis sporogenes. Moreover, milk cultures of this bacillus inoculated subcutaneously into guinea-pigs led to severe haemorrhagic oedema with gangrene, and death in about twenty-four hours.

The main features of the outbreak pointed to 'the toxic character of some article of diet supplied to the patients alone,' for the nurses, with one exception, escaped. By process of exclusion the milk was suspected. Since none of this incriminated milk was available for bacteriological examination a sample 'taken from the same source as that supplied on October 27 was furnished by the company

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* Part of this article was presented to the University of Cambridge as a thesis for the degree of Bachelor of Medicine.

† *Medical Officer's Report*, Local Government Board, for 1895-96, p. 197-198.

‡ *Medical Officer's Report*, Local Government Board, for 1895-96, p. 204.

supplying the hospital, on October 30.' This sample contained the spores of bacillus enteritidis, and the cultures were virulent to guinea-pigs. 'Inquiry into the source of the milk of the company which supplied it, elicited the facts that on Sunday, October 27, seventeen gallons out of a total of 190 gallons came from a certain farm at Melton Mowbray, and the sample furnished on October 30 was from the same farm.' Dr. ANDREWES also remarks, 'that on the supposition that the milk was the cause of the diarrhoea outbreak, the irregular and capricious distribution of the disease in the hospital need excite no surprise, for the manner in which the milk was sent out to the different wards would well explain it. It was poured through a strainer into a wooden tub, from which the ward vessels were filled, and this tub was never allowed to get empty, but a fresh churn was poured into it when it got low, thus leading to an irregular mixture of the milk from one churn with that of the others.'*

It was concluded from these observations that the outbreak in question 'appeared referable'† to the bacillus enteritidis sporogenes, 'a diarrhoeal organism.'‡

I have attempted to state as clearly as possible what seem to be the main reasons for this conclusion—

(1) The spores of bacillus enteritidis were demonstrated in the evacuations of the persons attacked.

(2) The outbreak was attributed to milk from a certain farm, and it was assumed, inasmuch as the spores of the bacillus enteritidis were detected in a sample of milk obtained from this farm three days after the outbreak, that they were also present in the original toxic milk.

(3) The bacillus enteritidis was regarded as an 'uncommon' organism, and had not been detected in the normal evacuations of healthy individuals.

(4) The milk cultures obtained from the evacuations and from the hospital milk were pathogenic to guinea-pigs.

It is curious that milk obtained from the Melton Mowbray farm 'was supplied both before and since the outbreak' at St. Bartholomew's 'to other customers without producing any harmful effect, so far as the company's knowledge went.'§

At present B. enteritidis can only be detected in a given material by cultivating its spores, consequently if the bacillus is present in a non-sporing condition, the result of cultivation will be negative. However, as B. enteritidis very readily forms spores, their absence must be taken, in practice, to indicate the absence of the bacilli also.

In order to isolate the spores from faeces, a small particle is inoculated into a tube of *recently* sterilised litmus milk, which is heated for fifteen minutes from 70°C.

* *Medical Officer's Report*, Local Government Board, for 1895-96, p. 198.
† *Medical Officer's Report*, Local Government Board, for 1895-96, p. 197.
‡ *Medical Officer's Report*, Local Government Board, for 1895-96, p. 204.
§ *Medical Officer's Report*, Local Government Board, for 1895-96, p. 198.

up to 80°C. to kill non-sporing organisms. The tube is then cooled and placed with a certain quantity of a fresh solution of pyrogallic acid and caustic potash in a Buchner's cylinder, tightly corked, and incubated at 37°C. In from twenty-four to forty-eight hours, if the bacillus is present, a most striking and characteristic change occurs. The milk is 'curdled with copious evolution of gas, the spongy curd floating in a fairly clear transparent whey;' the reaction is acid, with a smell of butyric acid. Microscopical examination reveals multitudes of bacilli in the form of 'rods and cylinders,' which stain by the method of Gram.

The virulence of the organism is classed as normal by Dr. Klein, if the subcutaneous inoculation of one cubic centimetre of whey from a recently changed milk culture into a guinea-pig weighing from 200-300 grammes is fatal in about thirty hours or less, the animal dying with 'severe haemorrhagic oedema and gangrene of the subcutaneous tissues, spreading widely from the point of inoculation.' The virulence of the organism is diminished if the guinea-pig dies slowly, or recovers with extensive sloughing of the skin in the neighbourhood of inoculation. Lastly, if the only result of inoculation is a transient swelling without subsequent necrosis, the milk culture contains the bacillus of Botkin; an organism which is said to resemble the bacillus enteritidis sporogenes in almost every respect save one, viz., that its cultures are non-pathogenic when inoculated subcutaneously into guinea-pigs or rabbits.

The only other anaerobic sporing organism with which bacillus enteritidis might be confounded is the bacillus of malignant oedema. This organism, as Klein points out, does not stain by Gram's method, and does not produce in anaerobic milk cultures, or when inoculated into guinea-pigs, the changes which are characteristic of bacillus enteritidis sporogenes.* According to Klein, the presence of the bacillus enteritidis is proved if an anaerobic cultivation of its spores in milk produce certain reactions, and if the resulting whey exerts certain definite pathogenic effects when inoculated subcutaneously into the groin of a guinea-pig. Microscopical examination of the organism and subcultivation is not necessary.

I have, however, during observations on the spores of bacillus enteritidis obtained from various sources, examined microscopically as a matter of routine a large number of typical milk cultures, and in every instance detected, usually in pure culture, the characteristic bacilli which stained by the method of Gram.

Subcultivation of bacillus enteritidis, like other anaerobic organisms, on solid media, is unsatisfactory. Although I have obtained in blood serum, glucose agar, and glucose gelatin, pure colonies of the bacillus, the usual result of transplantation into milk is not a 'typical' culture, but one deficient in whey and gas formation, and containing many spores. 'This ability to cause atypical change in milk occurs,' according to Klein, 'sooner or later to bacillus enteritidis from any given source.'†

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* *Medical Officer's Report*, Local Government Board, 1897-98, pp. 227-229.
† *Medical Officer's Report*, Local Government Board, 1897-98, p. 224.

On the night of Sunday, March 6, 1898, a second epidemic of diarrhoea similar to the first broke out among the in-patients of St. Bartholomew's Hospital, there being 144 severe cases. The milk was proved by Dr. ANDREWES to be the 'vehicle of infection.' On bacteriological examination spores of bacillus enteritidis of a virulent character were demonstrated in the evacuations of the patients affected, and also in a sample of Sunday morning's milk.*

On Friday night, August 5, 1898, the hospital was again attacked, eighty-six persons were affected, the majority of whom had eaten certain rice pudding. Bacillus enteritidis was isolated in a sporing condition from the diarrhoeal evacuations, and in a non-sporing condition from a sample of the suspected rice pudding ;† while Dr. KLEIN remarked, 'the conclusion becomes irresistible that this microbe was the direct cause of the disease.'‡ Clinically, the second and third epidemics resembled the first, and every patient recovered.

Obviously it is important to discover whether a particular organism—the bacillus enteritidis sporogenes—is probably or certainly a cause of diarrhoea.

Now, in the first place, if the existence of enteritidis spores in the evacuations of the patients at St. Bartholomew's can be used as an argument that the bacillus was, or probably was, the cause of the diarrhoea, it is clear that the spores should be almost or entirely absent in the evacuations of healthy persons, and of those suffering from diarrhoea which is casual and commonplace, or secondary to organic disease, as cirrhosis of the liver or typhoid.

Again, as the virulence of B. enteritidis to guinea-pigs is subject to considerable variation, one would expect, even if the spores were constantly present in all evacuations, that their virulence would be considerably less than the virulence of those obtained from the intestines of individuals suffering from diarrhoea directly due to the activity of the microbe.

Therefore it is essential to discover, firstly, whether the spores of enteritidis exist in normal and diarrhoeal evacuations generally ; and secondly, if present, whether they are equally virulent.

In the summer of 1897 Dr. KLEIN made some observations regarding the presence and virulence of bacillus enteritidis in infantile diarrhoea.§ He examined the stools of eleven cases ; in nine the diarrhoea was acute, generally with symptoms of gastro-enteritis ; in the remaining two it was more chronic ; ten of the infants died. It was demonstrated that the spores occurred in a virulent form in four out of the ten fatal cases, while in the remaining six and in the one that recovered, cylindrical rods were discovered on microscopical examination of the bowel contents, which might

* *Medical Officer's Report*, Local Government Board, 1897-98, p. 235.
† *Lancet*, January 7, 1899, p. 8.
‡ *Medical Officer's Report*, Local Government Board, 1898-99, p. 336.
§ *Medical Officer's Report*, Local Government Board, 1897-98, p. 230-233.

have been bacillus enteritidis in a non-sporing state. Further, spores of normal virulence were also isolated from six out of six cases of cholera nostras in adults, three being fatal.*

Now, if bacillus enteritidis produced the infantile diarrhoea, why were the spores apparently absent in the evacuations of seven out of eleven patients? But assuming six cases of cholera nostras and four of infantile diarrhoea were due to this bacillus, why did seven of them die, whereas all the 291 persons attacked at St. Bartholomew's recovered, although at the time several were seriously ill from intercurrent diseases? Why, also, were the cultures of enteritidis obtained from the mild cases which recovered equally virulent to those obtained from the severe cases which died? Indeed, considering the presence or absence of enteritidis spores in the evacuations, some evidence in support of the proposition that the bacillus was the agent producing the St. Bartholomew's epidemics and also, in certain instances, infantile diarrhoea and cholera nostras,† was the fact that Dr. Klein believed they were absent in the normal evacuations; a statement which he has since modified.‡

Dr. Andrewes, in 1896, investigated the anaerobic organisms existing in twenty consecutive cases of diarrhoea.§ From one to three loopfuls of faecal matter were inoculated into grape sugar gelatine, heated between 78° and 80° for ten minutes, and incubated anaerobically at 20° C. The only microbe constantly present was bacillus enteritidis sporogenes, or a 'variety'; it was isolated from twelve individuals. The 'variety,' as Dr. Klein pointed out, was most probably the true enteritidis growing atypically, as frequently obtains under certain conditions. I have attempted to summarise Dr. Andrewes' results. The spores of enteritidis were present in—

(*a*) Four out of ten cases of acute diarrhoea with symptoms, often severe, of intestinal or gastro-intestinal irritation.

(*b*) Four out of six cases of diarrhoea without enteritis.

(*c*) In diarrhoea associated with catarrhal jaundice, also with gastric ulcer.

(*d*) In a case of fatty diarrhoea, and in the watery offensive stool of a child.

The results of inoculation are not stated in six instances; in four the cultures were of diminished virulence, in one non-pathogenic, while an anaerobic milk culture from the evacuations of a man who died with choleraic symptoms was exceedingly virulent.

The explanation of the diminished virulence is the fact that the gelatin cultures, liquefied by the bacillus, were injected, and as the gelatine sometimes liquefied slowly, 'it is probable that some such cultures had lost their virulence.'¶

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* *Medical Officer's Report*, Local Government Board, 1897-98, p. 234.
† *Medical Officer's Report*, Local Government Board, 1897-98, p. 211.
‡ *Medical Officer's Report*, Local Government Board, 1898-99, p. 330.
§ *Medical Officer's Report*, Local Government Board, 1896-97, p. 255-62.
¶ *Medical Officer's Report*, Local Government Board, 1896-97, p. 260.

Dr. Andrewes was led to this conclusion, among others, 'Whether the bacillus in question is actually a cause of acute diarrhoea is not so far positively established, but there seems to be a distinct probability that such is the case.'†

There is, however, in my opinion, one striking feature in these experiments, *i.e.*, the spores were apparently absent in five cases of acute sporadic diarrhoea, three being choleraic ; and present in two instances at least, in which the diarrhoea was merely looseness of the bowels without any acute symptoms.

Professor Boyce suggested to me, during some observations on bacillus coli commune in diarrhoeal evacuations, that I should also ascertain whether the spores of bacillus enteritidis were present. Now, it was necessary for the isolation of bacillus coli, by means of agar plates, to use very dilute solutions of stool ; and it was these dilute solutions which were first tested for the spores of enteritidis, and consequently the quantity of stool examined was very minute. The dilution was the following :—One platinum loopful of faecal matter was well mixed with one cubic centimetre of sterile water, and as a rule from one to five loops of this solution were inoculated into litmus milk, subsequently heated for fifteen minutes from 70° C. up to 80° C., and inoculated anaerobically. The size of the platinum loop used on different occasions was approximately the same, for it was made by using a piece of wire as a gauge. I have found by careful measurement that one cubic centimetre of water contains about thirteen hundred such loopfuls, and consequently a rough comparison of the dilution used may be obtained by adopting the following standards :—One loopful of a solution of one loopful of stool in one cubic centimetre of water represents a dilution of 1,300 ; while if five loopfuls are taken the dilution is 260 ; if half a cubic centimetre the dilution is two, &c.

The samples of stools obtained from hospital patients were kindly provided in sterilised vessels by Dr. Hay and Dr. Fletcher. Unfortunately, I did not in many instances ask for a brief account of the disease with which the diarrhoea was associated ; but in no case was it the result of typhoid or of the action of drugs, and only one patient had distinct symptoms of enteritis. Often the diarrhoea was slight, the stools being semi-solid.

The results of the examination of seventeen samples of evacuations from different individuals are classified below for the sake of brevity. In cases 1 to 11 the dilution represents the minimum dilution in which the spores were absent ; in the remainder the maximum dilution in which the spores were present. The whey of typical milk cultures only was inoculated subcutaneously into guinea-pigs in the proportion of 1 c.c. to every 200 grms. of guinea-pig. If the animal died in about thirty hours or less, the virulence of the culture is described as 'normal ;' if death occurred in two to four days it is diminished. If the animal recovered with sloughing of the skin at

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† *Medical Officer's Report*, Local Government Board, 1896-97, p. 260.

the site of inoculation, the term pathogenic is used to distinguish from the transient swelling resulting from inoculation with the non-pathogenic bacillus of Botkin.

In all, except possibly two or three of the earlier experiments, the milk inoculated had been recently sterilised by not more, usually much less, than five days previously ; a necessary precaution, because stale milk becomes again charged with air (oxygen) by absorption, and is consequently a bad anaerobic medium.

| Cases | Enteritidis Spores | Dilution | Nature of Stool | Result of Inoculation |
|---|---|---|---|---|
| 1 to 9 | Absent | 260 | ... | ... |
| 10 | Absent | 4 | ... | ... |
| 11 | Absent | 2 | ... | ... |
| 12 | Present | 1300 | Fluid | Diminished virulence |
| 13 | Present | 1300 | Solid | Pathogenic |
| 14 | Botkin ? | 1300 | Semi-solid | Non-pathogenic |
| 15 | Present | 260 | Semi-solid | Diminished virulence |
| 16 | Present | 1300 | Fluid | Normal virulence |
| 17 | Present | 65 | Fluid | Pathogenic |

Cases 1 to 11 were suffering from the following diseases amongst others—rheumatism, morbus cordis, phthisis, acute bronchitis, and pneumonia.

Cases 14 and 15 had cirrhosis of liver, and 17 enteritis.

In cases 1 to 11 enteritidis spores were only proved to be absent in very small portions of stool, while they were occasionally present in still smaller portions ; when the number per gramme must have been enormous. If comparatively large quantities of stool, say from one to three loopfuls, had been tested, it is highly probable the spores would have been isolated in almost every instance.

Having therefore, contrary to expectation, found enteritidis spores in the diarrhoeal evacuations of patients, only one of whom had symptoms of intestinal irritation, several normal evacuations were next examined, but the amount tested was larger than the first series. Samples 1 to 5 were obtained from hospital patients whose bowels were moved about once a day ; number 6 was a healthy individual.

| Cases | Enteritidis Spores | Dilution | Nature of Stool | Result of Inoculation |
|---|---|---|---|---|
| 1 | Present | 150 | Relaxed | Diminished virulence |
| 2 | Botkin ? | 2 | Relaxed | Non-pathogenic |
| 3 | Present | 150 | Normal | Normal virulence |
| 4 | Present | Small particle | Normal | Diminished virulence |
| 5 | Present | Small particle | Normal | Normal virulence |
| 6 | Present | 2 | Normal | Pathogenic |

Thus the spores were present in five out of six samples of relaxed or normal stools obtained from different individuals, while they were absent in a large percentage of the diarrhoeal cases, because, I believe, a relatively smaller quantity was tested.

But assuming that the same number of enteritidis spores were present in an equal quantity of faecal material from two persons, one only suffering from diarrhoea, it is incorrect to infer that, therefore, enteritidis is sporing with equal rapidity, and that the spores are equally numerous in the intestines of each ; because the total quantity of diarrhoeal stool passed in a given time will, in most instances, greatly exceed the total quantity of normal stools.

The former is diluted with fluid, and consequently its bulk is increased, so the organisms present are also diluted. In other words, one loopful of a diarrhoeal evacuation represents a much smaller portion of the whole quantity passed in twenty-four hours than one loopful of the normal evacuation. In order, therefore, to compare accurately the number of enteritidis spores present in the intestines of two persons passing normal and diarrhoeal motions, respectively, it would be necessary to collect and measure the total quantity passed in twenty-four hours, minus urine ; then to ascertain the smallest measured portion in which the spores were present, and from that to calculate the total number of spores passed per diem : a tedious proceeding not devoid of practical difficulties.

Now the total quantity of diarrhoeal stool passed in twenty-four hours exceeds that of the normal, partly because it is diluted with water which would tend to lessen the specific gravity. I therefore thought that if diarrhoeal and normal evacuations were made into an emulsion with sterile water and diluted to a standard specific gravity, the bulk of the former would be slightly increased and the bulk of the latter greatly increased ; and then if the same quantity of both emulsions were examined for the spores of enteritidis, a rough comparison of the total number in both kinds of stool might be obtained without collecting and measuring the whole quantity passed in a day. But I have found the method unsatisfactory, because the specific gravity of a diarrhoeal evacuation does not always vary with its consistency.

Below are appended the results of the examination of fourteen consecutive samples of faecal material, the first series were from patients suffering from diarrhoea, the remainder from perfectly healthy individuals who were not in hospital. Each sample was made into an emulsion with sterile water and reduced to a specific gravity of 1005. Next, one cubic centimetre of the emulsion having been previously well stirred, was inoculated into a freshly sterilised tube of litmus milk. Some of the emulsion was also diluted 100, 10,000, and 1,000,000 times, and one cubic centimetre of each dilution was also inoculated into milk. The four milk tubes from each sample were then heated, incubated anaerobically, and at the end of thirty-six to forty-eight hours the whey of a typical milk was inoculated into a guinea-pig in the proportion of one cubic centimetre to every 200 grammes.

## DIARRHOEA

| Cases | Spores in Dilution of | | | | Result of Inoculation |
|---|---|---|---|---|---|
| | 1 | 100 | 10,000 | 1,000,000 | |
| 1 | Present | Present | ... | ... | Pathogenic |
| 2 | Present | Present | ... | ... | Normal virulence |
| 3 | Atypical | ... | ... | ... | Not inoculated |
| 4 | Botkin (?) | Botkin (?) | ... | ... | Non-pathogenic |
| 5 | Present | Present | ... | ... | Normal virulence |
| 6 | Present | Present | Present | ... | Normal virulence |
| 7 | Present | ... | ... | ... | Normal virulence |

Case 1 had ovarian cyst, 2 pneumonia, 6 tubercular enteritis, and 7 parenchymatous nephritis.

## NORMAL

| Cases | Spores in Dilution of | | | | Result of Inoculation |
|---|---|---|---|---|---|
| | 1 | 100 | 10,000 | 1,000,000 | |
| 1 | Present | ... | ... | ... | Normal virulence |
| 2 | Present | Present | ... | ... | Pathogenic |
| 3 | Present | ... | ... | ... | Normal virulence |
| 4 | Present | Present | Present | ... | Pathogenic |
| 5 | Present | Present | ... | ... | Pathogenic |
| 6 | Botkin (?) | Botkin (?) | ... | ... | Non-pathogenic |
| 7 | Present | Present | ... | ... | Pathogenic |

In order to ascertain whether enteritidis spores were constantly present in normal faeces, five consecutive examinations were made at intervals of about a fortnight from the stools of another healthy individual. The quantity tested was a platinum loopful or less.

In all five examinations the milk tube presented the changes characteristic of enteritidis; three cultures were inoculated.

| Examination | Enteritidis Spores | Results of Inoculation |
|---|---|---|
| 1 | Present | Death in about twelve hours |
| 2 | Present | Death in three days |
| 3 | Present | Not inoculated |
| 4 | Present | Death in about thirty hours |
| 5 | Present | Not inoculated |

To ascertain how long enteritidis spores in faecal material survived, four samples of stool from the same individual were enclosed in sterile Petri dishes and kept for a period varying from one week to three months. Milk tubes inoculated with one loopful of the faecal matter from the centre of each sample and treated in the usual way, presented the changes typical of enteritidis. Two cubic centimetres of whey obtained from the oldest sample were inoculated into a guinea-pig of four hundred grammes. The animal died in twelve hours with characteristic oedema.

It will be noted in these experiments that the virulence of the cultures, although made from recently sterilised milk, was often sub-normal. However, this diminution may be more apparent than real. I have frequently observed, when a few hours after inoculation the large subcutaneous tumour forms, an escape of the enteritidis-containing fluid from the site of the puncture. If the leak is considerable the guinea-pig usually recovers with local sloughing of the skin, and the virulence of the culture, which may have been normal, is apparently diminished. Leaking is especially liable to occur when, as obtained in the majority of my experiments, the skin of the animal was shaved over an area of the size of a two shilling piece, and, consequently, the protection afforded by the hair removed. That accidental diminution of the virulence may take place is proved by the following experiment. On one occasion two guinea-pigs were inoculated at the same time with milk cultures obtained from the same material. One cubic centimetre of an emulsion of diarrhoeal stool, reduced to a specific gravity of 1005, was diluted 100 and 10,000 times, and inoculated into tubes of recently sterilised milk, *a* and *b* respectively. At the end of two days both tubes presented the characteristic changes, and on cover slip examination, using Gram's stain, were found to contain apparently pure cultures of enteritidis; spores were absent. Two cubic centimetres of whey from tube *a* were inoculated into a guinea-pig of 415 grammes, and 2·25 cubic centimetres from tube *b* into a guinea-pig of 465 grammes; both animals were previously shaved. Next day the second guinea-pig was dead with the usual oedema; while the enteritidis-containing fluid was freely escaping through the punctured skin of the first, which recovered with slight necrosis. Thus the apparent difference in the virulence of two similar cultures from the same material was almost certainly due to the accidental enlargement of the inoculation puncture in the case of one guinea-pig: an accident more prone to occur when the animal has been shaved.

It has been shewn that enteritidis spores were isolated in ten out of twenty-four samples of diarrhoea evacuations from different patients, one only having symptoms of intestinal irritation. In five the virulence was normal, and in two diminished, while three were pathogenic, but not fatal. The spores were also demonstrated in the normal evacuations of eleven out of thirteen persons; they were of normal virulence in four cases, diminished in two, and pathogenic in five.

Apparently the bacillus of Botkin was present in two samples of diarrhoeal and two of normal stools.

Lastly, the normal evacuations of the same healthy individual were examined on ten occasions at intervals of weeks or months, and enteritidis-like cultures were invariably obtained. Four such cultures were tested by inoculation, in three the virulence was normal, and in one diminished.

The spores were isolated in a larger percentage of normal than of diarrhoeal stools, because in the former the quantity tested was relatively larger than in the latter. The virulence was often subnormal, partly because the site of the inoculation was shaved.

Occasionally the milk cultures were 'atypical.' Such were not inoculated, but they are, according to Klein, of diminished virulence or non-pathogenic.

Recently Klein isolated the spores of enteritidis of normal virulence in one loopful of stool from one case of sudden fatal diarrhoea, and from a sample of diarrhoea associated with pneumonia; also in from one to three loopfuls of six samples of the 'more or less fluid contents of the colon transversum obtained, post-mortem, from six persons' who died from various causes as epilepsy, peritonitis, cerebral abscess, etc. Lastly, in good-sized lumps of faecal matter obtained, post-mortem, from three out of five individuals in whom 'neither the history or the actual state of the bowel and contents of the colon suggested diarrhoea.'*

Dr. Klein also tested the evacuations of forty-three persons suffering from typhoid at different periods of the disease and in convalescence, estimating the minimum number of spores present in the diarrhoeal and non-diarrhoeal stages. He found that 'whereas in the phases of enteric fever associated with typical fluid typhoid stools, these spores of bacillus enteritidis are as a rule *numerously present*, in a

* *Medical Officer's Report*, Local Government Board, 1898-99, p. 329.

considerable percentage of instances (about thirty-nine per cent.) of the typhoid cases wherein these stools had become, owing to constipation or convalescence, normally formed and solid, the spores were altogether absent in relatively considerable amounts of faecal matter. And even in those instances of formed stools in which these spores were found to be present they could only be demonstrated by using large amounts of the faecal matters.' *

Do these results indicate some cause or connection between the presence of enteritidis spores and the formation of the typical fluid typhoid stools? If so, it is curious that the virulence of enteritidis cultures obtained from the cases of 'diarrhoea' with 'typical' stools was normal in twenty,†[1] and diminished in three[2], instances; while on the other hand, it was normal in fourteen[3] and diminished in two[4] cultures from 'solid' stools, diarrhoea being absent.

In my opinion the simplest explanation of the numerical increase of enteritidis spores in typhoid diarrhoea is the fact that as a rule all micro-organisms normally inhabiting the intestines tend to multiply exceedingly when the character of the bowel contents is modified by disease. So it appears highly probably that the increase of enteritidis spores in typical stools is a result, not a cause, of the abnormal conditions of the evacuations.

In conclusion, it is proved that the spores of bacillus enteritidis are constantly present, not only in the abnormal evacuations of those suffering from diarrhoea, whether it is acute or chronic, mild or severe, arising *de novo*, or secondary to organic disease, but also in the formed evacuations of healthy individuals; consequently, bacillus enteritidis, frequently of 'normal virulence,' must be regarded as a common inhabitant of the human intestine.

Since writing the above my attention has been drawn to some observations by Dr. Hewlett at the Jenner Institute of Preventive Medicine. Adopting Klein's method of isolating the spores by heat incubating them anaerobically in recently sterilised milk and testing the cultures by inoculation of guinea-pigs, Dr. Hewlett sums up his investigations as follows:—'I have isolated the bacillus enteritidis sporogenes from the dejecta in twelve cases of ulcerative colitis, in one case of ordinary diarrhoea, one case of chronic dysentery, and in eleven out of thirteen specimens of normal dejecta from ten healthy individuals, and the conclusion I have formed is that *this* organism is probably an inhabitant of the normal digestive tract, and frequently to be found in the dejecta.'

Dr. Legge also examined a number of samples of dejecta from healthy individuals 'by means of anaerobic milk cultures and microscopical examinations therefrom,' and bacillus enteritidis sporogenes 'seem to be constantly present.'‡

* *Medical Officer's Report*, Local Government Board, 1898-99, p. 312-328.
† *Medical Officer's Report*, Local Government Board, 1898-99, p. 312-328:
1 Cases 1, 3, 6, 7, 9, 11, 14, 19, 20, 24, 25, 28, 31, 32, 35, 40.
2 Cases 7, 22, 33.
3 Cases 4, 5, 6, 8, 14, 15, 16, 21. 37, 43, 44.
4 Cases 3, 11.
‡ *Transactions of the Jenner Institute of Preventive Medicine*, second series, p. 73.

The second main reason for thinking the epidemic of 1895 was probably due to bacillus enteritidis, was the discovery of the virulent spores of this supposed 'uncommon'* organism in a particular sample of milk. Obviously, it is essential to ascertain the distribution of the bacillus, whether it is common or uncommon, and whether its spores are frequently or rarely present in food.

In the year 1898, KLEIN points out that among the sources of bacillus enteritidis sporogenes are the evacuations of cases of diarrhoea, sewage water, soil and dust polluted sewage, as also horses' dung. But normal evacuations must also be regarded as a source. Again, Dr. BALFOUR STEWART in Liverpool and Dr. HEWLETT in London have isolated enteritidis spores from laboratory dust. They are also present in polluted water, *e.g.*, the River Severn, but never in water supplied to the city of Liverpool. Dr. BALFOUR STEWART has recently drawn attention to the wide distribution of enteritidis sporogenes 'in quite innocent substances,'† for he found the spores from various samples of wheat, barley, oats, oatmeal, flour, rice, cornflour, etc., also from clover and various grasses. In all, 'sixty samples were examined, and forty-one gave an enteritidis-like growth in milk; of these, thirty were fatal to guinea-pigs when inoculated, and eleven were pathogenic, but not fatal.'

It appeared highly probable that enteritidis spores are also present in the atmosphere, I therefore examined the air outside the laboratory by aspirating a measured quantity through sterile plugs of cotton wool; these plugs were then transferred bodily into milk, heated and incubated anaerobically as usual. The volume of air analysed on the four occasions was 480, 2,500, 142, 960 litres. On cultivation each milk tube presented an enteritidis-like growth, the first and third cultures only were tested by inoculation and proved to be of normal virulence. Again, recently-sterilised milk exposed to the open air in sterile Petri dishes for a few hours frequently presents, after heating to 80° C. and anaerobic cultivation, changes characteristic of enteritidis. On one occasion five Petri dishes of sterile milk were exposed in the rooms of a private house for fourteen hours. After incubation three yielded a typical enteritidis-like growth, and contained the usual bacilli. Two of the cultures were inoculated, one was of normal virulence, the other pathogenic. Since, therefore, virulent enteritidis spores constantly occur in the atmosphere and in deposits of dust, the fact that they have been isolated from the surface of wheat, rice, &c., is not surprising.

As the spores of enteritidis are very resistant to heat, and 'have been found to withstand perfectly unharmed a temperature of 100° C. for several minutes, and one of 98° C. for a considerable time,'‡ it is not improbable that in some cooked dishes prepared from flour, oatmeal, &c., the spores survive. For example, STEWART

* *Medical Officer's Report*, Local Government Board, 1895-96, p. 204.
† *Thompson Yates Laboratories Report*, vol. iii, p. 31.
‡ *Medical Officers's Report*, Local Government Board, 1898-99, p. 336.

demonstrated virulent enteritidis spores in two out of two samples of rice, and ANDREWES showed that 'the temperature of a rice pudding removed hot and bubbling from the oven' averaged from '90° C. to 92° C., and nowhere exceeded 98° C.'

But do the spores of this organism ever occur in food which is eaten without cooking? I thought they might be present in such food as strawberries, which, growing near the ground, are liable to be contaminated with particles of manure and soil. Samples of ripe strawberries were therefore brought to the laboratory in paper bags, and their surface sponged with small pieces of sterile wool soaked in sterile milk, the wool was then dropped into tubes of recently sterilised litmus milk and treated as usual.

In twenty-five strawberries examined thus, obtained on three occasions from three different shops and representing about six varieties, the results of milk cultivation were the following :—

Seven tubes contained a typical and fifteen an atypical enteritidis-like growth. The whey from five of the typical tubes was inoculated into guinea-pigs in the usual proportions: two were found to be non-pathogenic, and three pathogenic but not fatal. On routine microscopical examination of the milk cultures, it was noticed that the cover-slip preparations differed from those usually obtained from milk cultures of enteritidis from evacuations, inasmuch, as in addition to the usual bacilli other organisms were constantly present; the atypical cultures contained many spores.

Of six milk cultures made from the surface of six ripe-red gooseberries, two were typical and four atypical. One typical culture only was inoculated, and proved to be of normal virulence.

Crystallised white sugar was next examined. Six samples of apparently clean sugar were bought in the ordinary way; and six samples, somewhat dusty through exposure in various shop windows, were supplied in sterile test tubes. A two per cent. solution of each sample was made with sterile milk and treated as usual.

The result of analysis and the weight of sugar tested are given below :—

DUSTY SUGAR

| No. | Weight in Grammes | Culture | Result of Inoculation |
|---|---|---|---|
| 1 | 1 | Typical | Not inoculated |
| 2 | 1 | Typical | Not inoculated |
| 3 | ·5 | Typical | Pathogenic |
| 4 | ·5 | Typical | Diminished virulence |
| 5 | ·5 | Typical | Pathogenic |
| 6 | ·5 | Typical | Not inoculated |

CLEAN SUGAR

| No. | Weight in Grammes | Culture | Result of Inoculation |
|---|---|---|---|
| 1 | 1 | Atypical | Not inoculated |
| 2 | 1 | Negative | — |
| 3 | 1 | Negative | — |
| 4 | 1 | Typical | Normal virulence |
| 5 | 1 | Atypical | Not inoculated |
| 6 | 1 | Negative | — |

Every typical culture contained the characteristic bacilli.

Enteritidis spores are not infrequently isolated from oysters and other shell-fish, which are either eaten raw or after cooking for so short a time that probably the spores survive.

I have constantly found enteritidis spores in samples of a large sausage or saveloy of doubtful antecedents, which is made, cooked, and extensively sold by the pork butchers of this city. The saveloy is not recooked after purchase, but should be eaten while fresh, which is rather fortunate, since I obtained, on making agar plates of two samples, from two thousand to three thousand colonies of various organisms per gramme of sausage. When testing for enteritidis spores two or three pieces about the size of a pea were taken from different parts of the interior of the saveloy. The number of typical and atypical milk cultures obtained from eight samples bought from different shops are set down below. Typical cultures only were inoculated.

| No. | Typical | Atypical | Result of Inoculations |
|---|---|---|---|
| 1 | 1 | 1 | Normal virulence |
| 2 | 1 | 2 | Pathogenic |
| 3 | 2 | 1 | Pathogenic |
| 4 | 2 | 1 | Not inoculated |
| 5 | 1 | 2 | Not inoculated |
| 6 | 2 | 1 | Normal virulence |
| 7 | 1 | 2 | Not inoculated |
| 8 | 1 | 2 | Non-pathogenic |

It is interesting to note that samples 1 and 2 were subsequently eaten by the laboratory attendants without ill effect.

The pork butchers unfortunately decline to give any information concerning the genesis of the saveloy. Probably, however, they are made from the intestines of the pig, and from odd scraps of meat which have been exposed to dust and dirt, and consequently the presence of enteritidis spores in them is not extraordinary.

Since the epidemic, 1895, the spores of bacillus enteritidis have been frequently discovered in milk. Klein isolated them from eight out of ten samples of milk, bought in 'various quarters' of London,* and Hewlett from eight out of fifteen samples.† Andrewes examined the milk supplied to St. Bartholomew's on six consecutive days, with positive results on five occasions.‡ Samples of milk sold in Liverpool have been systematically examined in this laboratory during the last fourteen months. About 10 c.c. of each sample were placed in sterile test tube, heated to 80° C., for a quarter of an hour and incubated anaerobically. At first the resulting enteritidis-like cultures were tested upon guinea-pigs. Out of twenty-one inoculations the virulence was normal in nine instances, and diminished in five, four were pathogenic but not fatal, and two were non-pathogenic. Thus the results of inoculation proved that enteritidis bacilli were present in eighty-six per cent. of the enteritidis-like cultures, and forty-two per cent. were of normal virulence.

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* *Medical Officer's Report*, Local Government Board, 1897-98, p. 236.
† *Transactions of the Jenner Institute of Preventive Medicine*, second series, p. 75.
‡ *Lancet*, January 1899, p. 9.

The following table gives the number of samples examined every month, and the percentage of enteritidis-like cultures; about half the samples were 'Railway' and half 'Town' milks :—

| | Number Examined | Percentage of Enteritidis-like Cultures |
|---|---|---|
| November, 1899 ... | 23 | 13 |
| December ,, ... | 42 | 30 |
| January, 1900 ... | 44 | 1 |
| February ,, ... | 50 | 8 |
| March ,, ... | 54 | 27 |
| April ,, ... | 37 | 35 |
| May ,, ... | 50 | 22 |
| June ,, ... | 48 | 10 |
| July ,, | 41 | 14 |
| August ,, ... | 47 | 11 |
| September ,, ... | 43 | 9 |
| October ,, ... | 55 | 7 |
| November ,, ... | 35 | 5 |
| December ,, ... | 39 | 5 |

It appears from these figures, probably owing to the more energetic Public Health Department, that the enteritidis spores are less frequently present in Liverpool than in London milk. I am unable to account for the high percentage of enteritidis spores during November and December, 1899. Although the available figures are not yet sufficient to establish a periodic cycle of bacillus enteritidis sporogenes, nevertheless they appear to indicate that these spores were more common in Liverpool milk during March, April, and May, 1900, when the death rate from epidemic diarrhoea was low, than in the summer months, when the death rate from epidemic diarrhoea was high. As Stewart pointed out, the existence of enteritidis spores in milk is not necessarily an indication of sewage pollution, because in Liverpool milk, at any rate, there is no relation between the presence of bacillus enteritidis sporogenes and of bacillus coli. I think, in many cases, the organism is simply an index of dust contamination.

Believing enteritidis sporogenes is non-pathogenic to the human alimentary canal, Dr Stewart suggested the following experiment.

A litre of new milk was heated for a quarter of an hour at 80° C., and incubated at 37° C. After twenty-four hours, it presented the changes typical of enteritidis. We both ate about three teaspoonfuls of the curd, which was crowded with the characteristic bacilli, and afterwards inoculated a large guinea-pig with 2 c.c. of the whey. Next day, the animal was dead with extensive oedema. The observers continued in normal health. I have repeatedly eaten the greater part of enteritidis-like milk cultures derived from food materials, which subsequently proved to be virulent in guinea-pigs, without evil results.

These experiments are not convincing, but only suggestive ; firstly, because enteritidis does not spore in fresh virulent milk cultures, and consequently the bacilli may have been killed by the gastric juice ; secondly, because healthy individuals are usually able to resist infection by micro-organisms.

The litre of new milk mentioned above, although *not* incubated anaerobically, produced a typical enteritidis culture of normal virulence. In this laboratory, typical enteritidis-like cultures have often been obtained in recently sterilised milk *without* anaerobic incubation in a Buchner's cylinder ; not because anaerobiosis is unnecessary, but because the process of sterilisation converts the milk into an anaerobic medium by driving off all or the greater part of the oxygen it has previously absorbed from exposure to the atmosphere. Klein has demonstrated that 'stale' milk, *i.e.*, milk which has not been recently sterilised is a bad anaerobic medium because it has become re-charged with oxygen ; and the oxygen may be driven off by re-sterilisation. If two tubes of recently sterilised litmus milk are simultaneously inoculated with enteritidis sporogenes, heated for one quarter of an hour at 80° C. and incubated at 37° C., the one in a Buchner's cylinder with pyrogallic acid and potash, the other aerobically, it will be found that the first sign of the growth of the bacilli is bleaching of the litmus in the lower part of *each tube*. But whereas the culture in the Buchner's cylinder will soon be totally bleached, the other becomes red at the top, and eventually when the formation of gas and whey is complete, the redness will extend in two or three days to the curd at the bottom. If now the anaerobic culture be removed from the Buchner's cylinder, its curd though bleached will also become slowly reddened by the gradual absorbtion of oxygen. That is to say, in most, if not all, instances the only apparent difference between two enteritidis cultures in recently sterilised litmus milk, incubated anaerobically and aerobically, respectively, is reddening of the litmus at the top of the aerobic culture. It would probably be found that both cultures were equally virulent to guinea-pigs.

I have found that if a stream of sterile air is passed, for a few minutes, through a milk culture after inoculation with enteritidis sporogenes, and heating for quarter of an hour at 80° C., the characteristic change takes place several hours later than in a control culture, though it is usually still typical. If the milk was completely oxygenated by this process it is probable that an atypical culture would result.

To return, I think it has been shown that bacillus enteritidis sporogenes is, as HEWLETT thought, a ubiquitous organism ; that enteritidis spores of normal virulence contaminate food which is eaten with impunity. The wide distribution of the bacillus was unknown when it was first called a 'diarrhoeal'* organism. I admit it is possible that under certain unknown conditions bacillus enteritidis sporogenes may be pathogenic on the human intestine ; but so may any other bacillus. Referring to the frequency of enteritidis spores in milk consumed without toxic effect, KLEIN suggests that 'much may depend on the strain of bacillus enteritidis which obtains entrance into the milk,' also on certain atmospheric 'chemical or other' conditions.

The diarrhoea epidemic at St. Bartholomews in 1895 'appeared referable' to the presence of enteritidis spores in certain milk. But there is no definite proof that the spores *were* present in this toxic milk, for no specimen of it was available for examination. It is true that enteritidis spores were detected in a sample obtained three days after the epidemic from one farm, which had supplied the hospital on the day of the outbreak with only about nine per cent. of the total milk consumed.

The second epidemic was also attributed to milk, which unfortunately does not appear to have been submitted to any chemical or bacteriological examination beyond the isolation from it of a particular 'ubiquitous' microbe.

Rice pudding was the incriminated diet in the third epidemic. Dr. ANDREWES states that, on microscopical examination of the pudding, not only was an enteritidis-like bacillus detected, but also other organisms, namely, 'cocci and small bacilli resembling lactic acid bacillus.' Bacillus enteritidis was isolated in a non-sporing condition from the pudding ; yet no further investigation was made into the 'nature' of the other organisms.†

But, even if the enteritidis in the rice pudding was 'the direct cause of the disease,' from what ingredient was it derived, from the rice, from the sugar, or from the milk ? And how can sanitary authorities hope to prevent epidemics of diarrhoea due to a bacillus which, though frequently present in 'quite innocent substances' and eaten with impunity, is occasionally exceedingly pathogenic ?

The third main reason for associating enteritidis with diarrhoea appears to be its pathogenicity to rodents as guinea-pigs. But even if cultures of a micro-organism grown in favourable conditions are pathogenic when inoculated under the skin of a guinea-pig, why shonld that organism necessarily, or even probably, be pathogenic to the intestine of man ? Guinea-pigs fed on cultures of enteritidis are not attacked with diarrhoea, which is truly fortunate considering the bran and oatmeal they eat frequently contain enteritidis spores of normal virulence.

* *Medical Officer's Report*, Local Government Board, 1895-96, p. 204.
† *Lancet*, January 1899, p. 9.

To sum up, the bacillus enteritidis was first discovered in connection with a specific epidemic of diarrhoea. It was thought that this bacillus probably caused the outbreak, because it was assumed to be an *uncommon* organism, and was present in the evacuations of the persons attacked and also in a sample of milk supposed to be similar to the original toxic milk. The truth of this supposition was confirmed by the occurrence at a later date of two similar epidemics, apparently also due to the same bacillus.

Meanwhile it was discovered that the organism in question has a very wide distribution, is commonly present in the air and in dust, and is frequently consumed in food *without* producing diarrhoea.

Again, it has been proved that enteritidis occurs in the evacuations from chronic diarrhoea, from diarrhoea secondary to other diseases, and from healthy individuals; it is in fact a normal inhabitant of the human intestines, and consequently its presence in the evacuations obtained in certain epidemics has *no special significance.*

But, as KLEIN pointed out, the bacillus of Gaertner, bacillus proteus vulgaris and streptococcus pyogenes, although also normal inhabitants of the intestine, may under certain unknown conditions set up violent diarrhoea.* The value of this analogy is considerably lessened, because it is highly probable, if not actually demonstrated, that cultures of these organisms are much more virulent to animals when producing diarrhoea than when they are quiescent; whereas, the spores of enteritidis, obtained from normal or diarrhoeal evacuations, are equally virulent.

I believe it is impossible to demonstrate the causal relationship between bacillus enteritidis and epidemic diarrhoea by isolating it from the evacuations and from food, or by testing its virulence upon guinea pigs.

KLEIN, however, records that the swollen lymphoid follicles in the intestine of a fatal case of cholera nostras contained, on microscopical examination, in almost pure culture, an organism 'morphologically like' bacillus enteritidis.†

So it is important to ascertain whether enteritidis sporogenes is constantly present in the inflamed intestinal walls of those who have presumably died from its activity, making sure that its presence is not the result of post-mortem invasion.

Again, does the serum of patients suffering from epidemic diarrhoea exert any agglutinating action upon enteritidis bacilli?

In conclusion, I submit, there is at present no satisfactory evidence that bacillus enteritidis sporogenes is a cause of acute, or of epidemic diarrhoea.

* *Medical Officer's Report*, Local Government Board, 1898-99. p. 332, 336.
† *Medical Officer's Report*, Local Government Board, 1897-98, p. 283.

# FURTHER NOTE ON BILE SALT LACTOSE AGAR

By ALFRED MacCONKEY

In a previous article of this Volume (p. 40) of the *Thompson Yates Laboratories Reports*, I gave an account of some rough experiments made with this medium. These experiments have been continued, and Grübler's pure salts have been used instead of the impure commercial salts. Cholalic acid, glycocholic acid, taurocholic acid, sodium glycocholate and sodium taurocholate have been experimented with, and it has been found that it is only the sodium taurocholate which gives the characteristic reaction, namely, a cloudiness or haze in the medium in the vicinity of the colonies. This salt also seems to exert a more pronounced inhibitory effect than the glycocholate. The medium was made in the following way :—

1,000 c.c. of tap water were put into a flask, and 2 per cent. peptone, and 0.5 per cent. of sodium taurocholate, and 1.5 per cent. of agar added. The flask was then put into the autoclave and kept at 105° to 110° C. for about one-and-a-half hours. The mixture was cleared with white of egg, using as little as possible, and filtered. Then 1 per cent. of lactose was added to the filtrate, the medium was distributed into test tubes, ten c.c. in each, and sterilisation effected by fifteen to twenty minutes in Koch's steriliser on each of three successive days.* The plates made were incubated at 42° C. for forty-eight hours. Organisms which produce acid with lactose in forty-eight hours give a characteristic reaction in this medium. The acid causes a precipitation of the taurocholate with the formation of a cloudiness round the colony. Thus there is a marked difference between the colonies of such organisms as the B. typhi abdominalis and those of the B. coli communis group.

*B. Typhi Abdominalis.*

Surface colonies—Small, round, raised, and semi-transparent.

Deep colonies—Lens-shaped, white, and opaque.

The medium remains quite clear.

*B. Coli Communis.*

Surface colonies—Roundish or irregular in shape with flattened top, opaque-white with a small spot of yellow or orange in

* If the medium be overheated, changes take place in the lactose and the B. typhus will give a haze.

the centre. A few have a haze round them. The size is about twice that of B. typhi abdominalis.

Deep colonies—Lens-shaped, orange white, and all have a haze round them.

This haze makes the colonies appear larger than they really are. A drop or two of a solution of ammonia placed upon the colony will cause the haze to disappear. If the plates be exposed to the light for several days it will be found that the colour of the colonies has deepened and that those on the surface have a tendency to become mucoid.

| | |
|---|---|
| *B. Enteritidis* (Gærtner)<br>*B. of Hog Cholera* (Flexner)<br>*B. of Psittacosis* (Nocard) | Colonies similar to those of B. typhi abdominalis |
| *B. Pyogenes foetidus*<br>*B. Neapolitanus* | Colonies similar to those of B. coli communis. |
| *B. fluorescens liquefaciens* | Slightly raised, flattened, roundish, translucent, greenish-white colonies with very slight fluorescence. |

*Cocci.*

Surface colonies—Round, raised, opaque-white.

Deep colonies—Usually opaque white. Under the microscope they appear black with a rough irregular hairy edge.

The following few organisms have been tried and apparently do not grow on this medium at 42° C.

B. Megatherium.

B. Subtilis.

B. Filamentosus.

Sarcina lutea.

Torula alba.

The following experiment was performed to ascertain the inhibitory power at 42° C. of this medium upon ordinary earth organisms.

About two to three grammes of garden mould were shaken up with 100 c.c. of tap water and 1 c.c. of the mixture put into a tube and poured into a plate.

At the end of forty-eight hours there were, besides many moulds, ten colonies (both surface and deep). All these colonies were subcultured, and nine gave a growth. These nine were then cultured in different media and the reaction noted. The incubation temperature was always 42° C.

The following table gives the reactions of these nine organisms :

| | | Broth | Litmus Milk | Litmus Glucose Broth | Litmus Lactose Broth | Agar Stab. | Gelatine Stab. | Potato | Indol | Gram's Method | Tauro-cholate Agar Stab |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Sluggishly-motile short bacillus | General turbidity | Acid : no clotting | Acid and gas | Acid : no gas | Moist, greyish-white growth | Filmy, opaque white | Almost invisible moist growth | (?) | — | Haze |
| 2 | „ „ „ | „ | „ | „ | „ | „ | „ | „ | „ | — | „ |
| 3 | Motile short bacillus ... ... | „ | Acid and clotting | „ | Acid and Gas | „ | „ | Moist whitish growth | + | — | Haze and Gas |
| 4 | „ „ „ ... ... | „ | „ | „ | „ | „ | „ | „ | + | — | „ |
| 5 | „ „ „ ... ... | „ | „ | „ | „ | „ | „ | „ | + | — | „ |
| 6 | „ „ „ ... ... | „ | „ | „ | „ | „ | ... | „ | + | — | „ |
| 7 | Coccus in short chains ... | Very slight general turbidity | „ | Acid | Acid | Grey-white growth | Liquefied | Invisible | — | + | No haze |
| 8 | „ „ „ ... | „ | Alkaline | „ | No change | Opaque and white | Opaque-white, scanty growth | „ | — | + | „ |
| 9 | „ „ „ ... | „ | Acid | „ | „ | „ | „ | „ | — | + | „ |

On gelatine plates there was no difference to be made out between the colonies of the various bacilli. They were all irregular-shaped, filmy, and translucent. Some were more heaped up and opaque in the centre, but that was all.

It is noteworthy that all the bacilli appear to belong to the B. coli communis group.

The glucose broth and lactose broth used were two per cent. solutions of peptone in water, to which 0·5 per cent. glucose and 1 per cent. lactose had been added respectively. Into each tube was put one of Durham's fermentation tubes.

As an ordinary nutrient agar plate made from the same earth was over-crowded, the inhibiting effect of the taurocholate medium is most marked.

It may, therefore, be recommendcd to bacteriologists as an addition to the means at their disposal for the isolation of the B. typhosus.

# NOTE ON THE STAINING OF FLAGELLA

By ALFRED MacCONKEY

It is a generally accepted idea that staining the flagella of bacteria is difficult to accomplish, and that a 'mordant' is necessary to fix the dye in the substance of the flagellum. Is this idea correct? A 'mordant' is defined as 'a substance used to fix colours in stuffs.' Is this what takes place in the process of staining flagella? At first sight this would appear to be the case, for we find that flagella will not usually stain unless a 'mordant' be used. A little consideration, however, raises doubts as to the correctness of this interpretation of the process. If the 'mordant' acts merely as a fixing agent it should not leave much effect upon the size of the cilia, which should, within limits, appear always of the same thickness; and the depth of colour should depend upon the degree of concentration of the 'mordant' and stain, upon their inter-reaction, and upon the length of time the film was subjected to their action. But, in practice, we find that this is not quite the case. The thickness of the flagella depends upon the strength of the 'mordant' and upon the length of time it is allowed to act; and the depth of colour depends upon the thickness of the substance to be stained. If the flagella are very fine, the strength of the staining fluid and the duration of its application make very little difference, for the flagella will always appear faintly stained; whereas, if the flagella be thick, a comparatively brief application of the stain will dye them a deep colour.

These facts suggest two explanations of the action of 'mordants.' In the first place, the action may be due to the deposition of a precipitate upon the outside of the flagellum, this precipitate being dyed in the subsequent staining process.

In the second place, the 'mordant' may cause the flagellum to swell and become thicker. Thus it would produce a greater depth and breadth of substance for the stain to act upon.

We know that, to be visible, a line must have a certain breadth, and it is quite conceivable, considering the size of bacteria, that their flagella may be too fine to be seen even when stained. Of these two explanations, I am inclined to favour the second for the following reasons:—

1 In all the usual methods, the flagella when stained appear to be thicker than we suppose them to be in reality, and the organisms themselves are larger than when stained in the ordinary way.

2 Certain mixtures, which, when freshly made, cause the flagella to be thick and to stain deeply, after a time lose their power. This power is not lost all at once, but only gradually, and this declination is associated with an increasing fineness of the flagella and decreasing depth of colour. They become finer and finer, and more faintly stained, until finally they are no longer visible. At the same time the bodies of the organisms also become smaller, though they stain equally well. By none of the methods I have used have I succeeded in obtaining very fine deeply-stained flagella. When fine, they have always been faintly coloured and when thick more deeply dyed.

3 If a 'mordant' acted merely as a fixing agent, subsequent treatment with acids should have some effect. After using the 'mordant,' I have kept films in contact with Acid. Sulph. twenty-five per cent., Acid. Acet. ten per cent., and Acid. Nitric. ten per cent. for several minutes without any effect upon the subsequent staining of the flagella.

If, however, spirit be used instead of the acids, the flagella can only be stained faintly, or are not visible. This later result might be used in support of the 'precipitate' theory, the precipitate being soluble in spirit. But it does not invalidate the theory of 'thickening,' as the spirit may cause shrinkage by dehydration. The method used in these experiments was the following:

(*a*) De Rossi's potash tannic acid. Tannic acid twenty-five per cent., dissolved by heat in one per cent. solution of caustic potash in water. This is saturated with fuchsin.

(*b*) Perchloride of iron ten per cent. dissolved in water + five per cent. glacial acetic acid.*

(*c*) Water + five per cent. glacial acetic acid.

The films are made in the usual way, allowed to dry in the air, and then exposed to formalin vapour for about half an hour.

Equal parts of (*a*) and (*b*) are mixed in a test-tube, and boiled. Then an equal bulk of (*c*) is added, the whole shaken up and filtered twice. This mixture is allowed to remain in contact with the film for a time, which varies with the organism used and the result required. From two to seven minutes is a good time for B. typhosus.

The cover slip is then well washed in water (treated with acid if preferred, and washed in water), and stained for about ten minutes in a warm solution of one of the ordinary dyes (an alcoholic-aqueous, one to four or weaker solution of gentian violet gives good results), well washed, dried and mounted in balsam.

The 'mordant' retains its properties for a few hours only.

In specimens stained by this process the protoplasm of the organisms is somewhat enlarged. In many cases the protoplasm appears to be surrounded by a 'capsule,' or 'thickened cell membrane,' which may or may not be stained.†

* This solution seems to lose its power after a few days.

† C.f. *Bacteriol. Diagnost.*, Lehmann and Neumann, Vol. II., p. 18.

When this 'swollen membrane' is visible the flagella appear to be prolongations of it and to have no connection with the protoplasm of the body of the organism.

4 I isolated from sewage a spirillum, which, in liquid media, developed a very long terminal flagellum, and this flagellum was plainly visible in every specimen stained in the ordinary way, *i.e.* without the use of 'mordants.' This flagellum must then have been composed of a substance quite different from that of the flagella of other organisms, a substance which absorbed ordinary dyes ; or it must have had the same composition as any other flagellum and have been visible merely because it was thicker. No adequate proof has been brought forward that the flagella of various organisms differ in their composition, and therefore the reasonable presumption is that it was its breadth which rendered this flagellum visible.

Unfortunately, circumstances prevented for a time further experiments with this organism, and it died. I have not had an opportunity of trying the effect of 'mordants' on it.

For these reasons I venture to suggest that the present ideas of flagella staining may be wrong, that flagella may stain with the ordinary dyes, but are invisible on account of their fineness, and that the so-called 'mordants' may act merely by causing the flagella to swell and thus become visible when stained.

# REPORT ON A PRIMARY MALIGNANT GROWTH OF THE KIDNEY

By KEITH MONSARRAT, F.R.C.S.E.

Assistant Surgeon, Children's Infirmary, Liverpool

The Pathological Report on this growth may, with advantage, be prefaced by a short outline of the

*Clinical History*—A boy, aged three years, was admitted to the Children's Infirmary on September 12, 1900. The family history was unimportant. About twelve months previously the mother noticed a ' small lump ' on the right side of the abdomen which she could move about easily. Since then it had become much larger. He had occasionally passed blood in his urine, but no definite statement as to how often and in what quantity could be elicited. He had latterly suffered from frequent vomiting, usually soon after food and had been very drowsy and ' feverish.'

*Anamnesis*—Child thin but not emaciated. He is drowsy and resents being roused. The thoracic organs are healthy. The abdomen is protuberant. On the right side extending round from the loin to within half an inch of the umbilicus is a large mass which fills the interval between the ribs and pelvic brim. The borders of this mass are rounded and the contour of the whole is lobulated. It is of firm consistence, and no sense of fluctuation is to be felt anywhere. Enlarged superficial veins are present over the whole of the front of the abdomen passing upwards on to the thorax; they are more marked on the right side. Urine—Acid. Contains no blood, albumin, or sugar. The amounts recorded on the four days preceding operation were twelve and a half ounces, twelve ounces, six and a half ounces, eight ounces; these amounts were not accurate, however, as a considerable quantity was passed with the faecal evacuations and not measured. When first seen the child's complexion was very dusky, and the axillae, neck, and lower abdomen were distinctly brown in colour.

*Operation*—On September 20 I removed the growth through an anterior incision. After opening the abdomen, the posterior parietal peritoneum was divided over the front of the tumour, and the ascending colon displaced inwards. The after history of the wound was complicated by a sinus in the loin, which persisted for nearly three months, but eventually healed. The left kidney was examined at the operation, and appeared healthy ; there were no secondary growths found. The amount of urine increased after the operation, the average for the seven following days being 14·7 ounces. The total urea passed in twenty-four hours was estimated on thirteen occasions after operation, the average being 128·8 grains.

The charts shew an apyrexic curve, with the exception of three unexplained and unimportant rises. The child increased in weight, and his general condition on leaving hospital was excellent. The dark colouration of the skin became distinctly more noticeable. He was seen on December 28, 1900, when to all appearance he was in robust health.

*Pathological Report*—The growth weighs thirty-eight ounces. It is oval in shape, but the outline is very irregular owing to the projection of lobes of growth, chiefly on the inner aspect ; one very prominent lobe projects forwards and upwards from the inner and anterior margin. The whole mass is covered by a fibrous capsule ; this is comparatively thinned over the prominent portions of the lobes, but strongly marked in the intervals between them. At the lower and external pole lie the remains of the renal tissue ; the upper border of this is represented by a sharp edge on the outer aspect of the mass, which can be lifted away from the growth with the handle of a knife ; the handle easily separates kidney and growth inwards and downwards for two inches ; below this they appear to be incorporated with one another. The renal tissue forms the outer and lower angle, the inferior border, and the greater part of the inner and lower angle of the tumour. The renal artery is seen between renal tissue and growth on the inner aspect, in front of a line bisecting the mass from the inner side ; it disappears under the edge of the compressed renal tissue, but by separating this from the growth, the artery can be traced between them for about an inch, when it enters the kidney substance ; the direction of the artery is downwards, forwards, outwards, and then backwards to the hilum.

The stump of the severed ureter lies behind the line bisecting the growth from within outwards ; its direction is almost directly downwards and slightly outwards, round the inferior and inner pole of the new growth to end, after about half an inch of course between kidney and growth, in the pelvis. Its termination could not of course be seen before bisecting the mass. This was done from the inner aspect and the two halves separated.

The renal tissue was then seen to be represented by a strip about 5 m.m. in thickness for the most part, but in the lower half of this a column of new growth, spherical in section, bulges into the pelvis, and is bounded on the outside by the remains of cortex, and on the inside by the pelvic lining.

This column forms a valuable clue to the exact anatomical relationship and the place of origin of the growth, as it is in direct continuity with the main mass. The pelvis on section is crescent shaped, the concavity of the crescent being occupied by the inferior border of the growth, and no renal tissue is distinguishable here outside the pelvic lining. The convex border of the crescent is formed by the above-mentioned strip of kidney tissue, except where the spherical column of new growth bulges into the pelvis.

The pelvic walls are in contact, but the extent of the lining points to there having been some dilatation. The ureter is patent.

The section of the growth shews a division into irregular lobules, but there are also septa marking off four lobes, one forming the apical portion of the mass, one the central and much the largest portion, and two smaller on the inferior and outer aspect of the central lobe. The upper lobe can be lifted off the central lobe by a slight blunt dissection on the anterior and outer parts, but posteriorly it is blended with the latter. The lower two small lobes are less perfectly differentiated. The lobes differ in naked eye structure ; the central mass appears to consist largely of fat with comparatively scanty interstitial matter ; the upper lobe is greyish in colour as contrasted with the yellow of the central lobe, and no fatty tissue is appreciable ; the same applies to the most external of the other two lobes, while the fourth resembles the central.

Distributed throughout the growth are many small cysts, the largest about the size of the head of an ordinary tin tack.

The relations of the new growth to the remains of the renal tissue enable one to decide with certainty its seat of origin.

The relation of the growth to the lining of the pelvis, and more especially the position of that out-growth which lies between pelvis and cortex at the lower pole of the growth, shows that it originated in the renal tissue itself.

The position of the artery and the ureter show that it originated above the hilum, and about centrally as regards the antero-posterior dimension. Thirdly, the position of the renal remains enables one to decide that the point of origin was on the inner face of the kidney, the growth, by extension, displacing the kidney downwards and outwards, or rather causing it to rotate through a segment of a circle, of which the hilum is the centre, the direction of rotation being from right to left. The seat of origin was therefore in the substance of the kidney, and in the upper and inner quadrant. As stated above, the upper edge of the renal tissue on the outer aspect of the growth is sharply defined, and it can be dissected off without tearing through anything except fibrous bands. This first suggested the idea that the growth was extra-renal in origin, but the other relations negatived this. Evidently the extension of the growth was first in an upward direction, subsequently, meeting the resistance of the capsule, the pressure would be exerted in an outward and downward direction.

The well-marked lobulation probably has relation to the original lobulation of the kidney. The lobe which forms the apex of the growth is so distinctly marked off, that it was thought to represent the adrenal body. Histologically, however, this was not confirmed.

*Histology*—There is a certain uniformity throughout the whole tumour in that the various elements of which it is composed are present in all parts, but they are very unequally distributed.

Central Mass—Fatty tissue predominates. Other elements appear as scattered islands and tracts in the sections. The fat is enclosed in a fine connective tissue mesh.

Strands of striped muscle cells run in all directions. The striation of many of the cells is easily seen, but in many also it cannot be distinguished. The latter cells are for the most part uninucleated and tapering, the striated cells are stouter and often shew more than one nucleus. All are evidently young cells not far advanced in development. Strands of young fibrous tissue are also present, but more scantily than the muscle tissue ; the cells are narrow, with long tapering nuclei.

These bundles of muscle and fibrous tissue can all be traced to connections with the cells of what I will for the present call the *Pseudo-cystic formations*.

The larger of these bodies are easily seen macroscopically as crateriform depressions on section ; other forms of them are represented by white dots.

The histology of these bodies is as follows :—

The smallest and youngest consist of—

1 A limiting layer of flattened cells, of connective tissue type.

2 A mass of polyhedral cells within, with large nuclei staining deeply.

From this simple structure they can be traced through a series of changes partly degenerative, partly proliferative.

In the first stage of these changes the cells centrally placed develop a squamous cell body, oval or irregular in shape, and the nuclei stain less distinctly. The cell bodies are hyaline, and between them branches a delicate reticulum (Fig. I).

It is convenient to follow these central cells in the first place, and then to consider those peripherally placed.

The squamous cells next become somewhat shrunken, the nuclear outlines are blurred and the intercellular reticulum is more distinct. The cellular layers then split up into ribbons and separate from one another, and the reticulum consists of strong fibrils circularly arranged (Fig II).

Lastly, the central parts are represented by a (Fig III) whorl-like arrangement of flaky fibrils, the cells having disappeared entirely. In some specimens even this has disappeared, and the structure is a true cyst, containing occasionally some granular debris.

These changes are of course degenerative. The evolution of the peripheral cells is, however, in the direction of proliferation. The limiting layer is usually quite indistinctly marked off from the cells immediately within ; these are in many specimens set endwise on the outer layer and are cubical in shape, but this arrangement is not constant. From these peripheral cells to a greater or less degree in all specimens, tracts and columns of cells originate and pass out in all directions into the fatty matrix.

These cells are round cells of sarcomatous type. Where they take origin

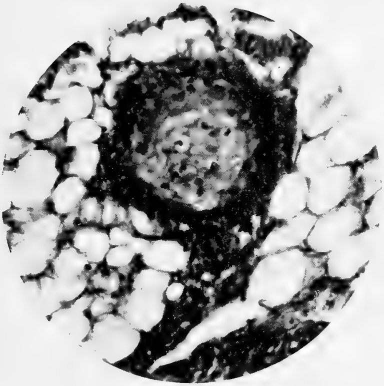

FIG. 1

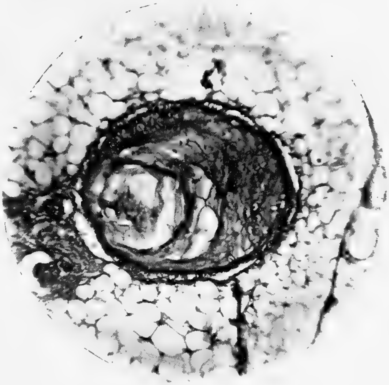

FIG. 2

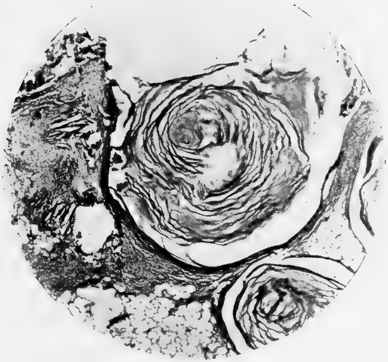

FIG. 3

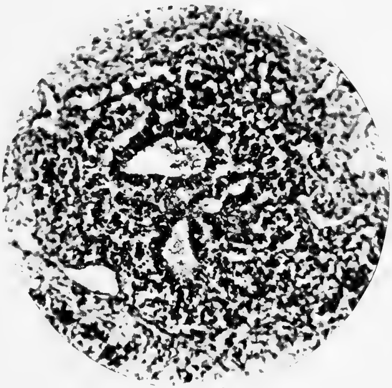

FIG. 4

FIG. 1—Shews a pseudo-cyst (Malpighian Corpuscle) in early stage. Central cells squamous or breaking up. Nuclei stain faintly. Peripheral cells proliferating. Edge of sarcomatous mass below.

FIG. 2—Shews pseudo-cyst later. Centrally squamous cells breaking up into ribbons. Fibrillation distinct. To the left a sarcomatous out-growth from the periphery.

FIG. 3—Shews complete fibrillation of interior of pseudo-cyst. Sarcomatous tissue below.

FIG. 4—Shews tubules with broken contour, from the cells of which originate, by proliferation, a mass of round or irregular cells ; the alveolar arrangement not being re-produced.

from the peripheral cells of the pseudo-cysts the outlines of the latter are interrupted (Figs I and II); specimens which are embedded in sarcomatous structure shew no definition except where the central squamous cell passes into the polyhedral type, the latter passing insensibly into the somewhat smaller and rounder sarcoma cell. In some specimens, however, even thus embedded there is a single row of cubical cells with large nuclei outside the squamous cells.

The peripheral cells of the pseudo-cystic bodies, therefore, give origin to sarcomatous elements. But the sarcomatous elements in this main central lobe which we are now considering are not exclusively derived from the pseudo-cysts. Adjacent to many of these, *Tubules* lined by cubical cells are seen, cut in various directions. Their outlines are interrupted by proliferative protrusions of these cubical cells, which, growing outwards, become sarcomatous in type. Scattered tubules, the cells of which are proliferating in this way, are also seen away from the proximity of the pseudo-cysts.

The muscle and fibrous tissue cells forming the strands, are in connection with the cells of the limiting layer of the pseudo-cysts for the most part.

Apical Lobe—This portion of the growth shews microscopically a much smaller proportion of fatty tissue than the central mass. Pseudo-cysts are seen here also, but none are degenerated to the stage of complete fibrillation, but consist centrally of large squamous cells with a fine reticulum. Sarcomatous tissue forms the main mass of this lobe, developed as before from the outer layers of the pseudo-cysts. However, tubules are more numerous here, and most of the sarcoma cells originate from the cells of these (Fig. IV). In fact the outlines of these tubules are usually quite irregular and broken. Muscle and connective tissue cells are distributed throughout the sarcomatous tissue.

Sections from the inferior lobes of the growth shew an almost uniform sarcomatous structure, with some interstitial fatty tissue. Throughout the structure the outlines of scattered and irregular tubules are seen. None of the pseudo-cystic formations were seen in the sections from this portion of the growth.

The vascularity of the different lobes varies. Where the sarcomatous cells are most numerous the vessels are many and large; on the other hand, where fatty tissue predominates they are small and very few in number.

From this description of the histology of the tumour it will be seen that the whole structure is traceable to the cells of certain tubules, and what have been provisionally termed pseudo-cysts. The significance of these two elements must now be considered. The kidney, with the exception of the collecting tubules, is developed from a portion of the intermediate cell mass, posterior to the Wolffian Body, and the first elements differentiated are the Malpighian Corpuscles and convoluted tubules. These both originate as solid masses of mesoblastic cells, and are subsequently hollowed out, the Corpuscle developing at the blind end (that not in connection with the peritoneal involution). The foetal Malpighian Corpuscle consists of a limiting layer, and a lining of epithelioid cells within, polyhedral or even cubical. Apart from the capillaries which are present in the developed glomerulus, the glomerular tuft is formed by a local proliferation of these epithelioid cells, and of the *membrana propria* or limiting layer, these both covering and passing in all directions between the capillary recticulum. The epithelioid cells become squamous in the adult state.

This structure is identical with a definite stage in the changes of the pseudo-cystic bodies. It has been seen that a few young specimens appear as solid masses of polyhedral cells, encircled by a limiting layer of flattened cells, and that later the central polyhedral cells become squamous.

The absence of the glomerular capillaries does not negative the identity, as they are a secondary in-growth. In their absence no glomerular cavity would develop, depending as it does on secretion derived from the capillaries, and instead, the squamous cells which normally form a lining and a support for the internal structure of the glomerulus, fill the whole interior of the limiting layer. The pseudo-cysts are therefore to be looked upon as Malpighian corpuscles. The structure is sufficient to decide the identity, and it is supported by the presence of the tubules which lie adjacent to and around the pseudo-cysts. Whether these tubules are in this position or more isolated, they must be considered the representatives of the primitive tubules from which the uriniferous tubules develop.

The new growth is therefore traceable to a perversion of the cellular elements from which, under normal development, Malpighian corpuscles and uriniferous tubules arise. The result of this perversion is the production of a tissue largely consisting of fat cells, but also in varying proportion of round cells, with large nuclei in a scanty stroma ; in addition young striated muscle cells and young connective tissue cells are unequally distributed throughout the structure. A name cannot be given to such a tumour on the general principle that malignant tumours arising from glandular epithelium are carcinomata, and those arising from connective tissue are sarcomata. The principle would seem to apply to growths originating from a substratum of adult cells, but in such a case as the present its application presents, among others, the following difficulties. The cells in many situations have obviously arisen as a proliferation of those of the tubular epithelium, on the other hand the resulting growth is composed of solid masses of round and irregularly shaped cells, and the structure is what is commonly called sarcomatous.

Birch-Hirschfeld* in describing a somewhat similar tumour remarks that these primary malignant growths of the kidney belong to no one group in the present classification, and gives to that under his consideration the name 'sarcomatous glandular tumour of the kidney.' Other authors have remarked on the same difficulty in nomenclature, and until the growths which apparently arise in connection with irregularities in embryological processes can be separately classified, it will be impossible to arrive at any satisfactory solution.

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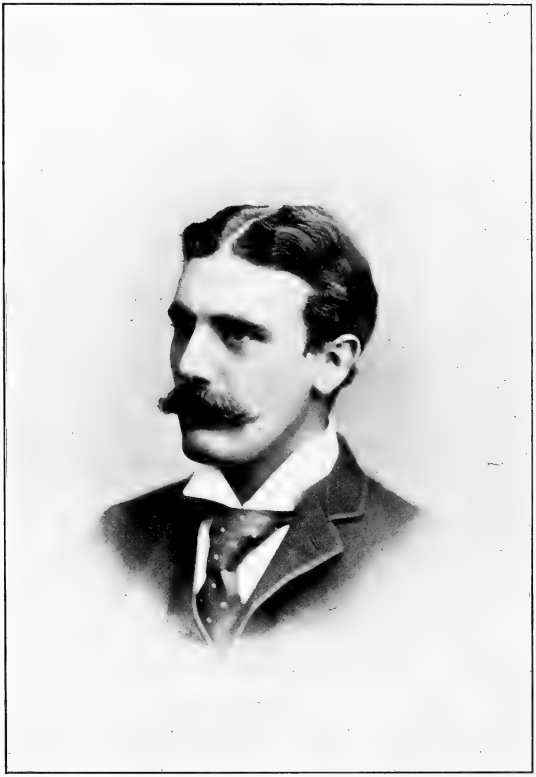
* Birch-Hirschfeld. *Ziegler's Beitr. Z. Path. Anatom.* Bd. xxiv. P. 343.



The late Dr. Myers

# In Memoriam

WALTER MYERS, M.A., M.B., B.C. Cantab., B.Sc. London, who, on a mission of the Liverpool School of Tropical Medicine to Para on the Amazon to investigate yellow fever, died of that disease in January, 1901.

'From the service of truth and humanity upon earth, in the morning of his manhood, he passed to GOD.'

---

On Sunday, the 20th of January, the Tropical School learnt by cablegram from Para that both members of the Yellow Fever Expedition had contracted yellow fever, Dr. MYERS being reported critically ill. On Monday, January 21, the sad news of his death was received.

The following two letters arrived on February 9, from Para.

Letter from His Excellency, the Governor of the State of Para, to the Secretary of the Liverpool School of Tropical Medicine :—

SIR,

It is my painful duty to inform you of the death of Dr. WALTER MYERS, who—a victim to his love of science and to humanity—has just succumbed to the terrible illness which robs us of so many precious lives, whilst himself employed in combating this terrible disease. In the name of the State of Para, and interpreting the feelings of regret with which we are all possessed, I desire hereby to express to the Liverpool School of Tropical Medicine our deep sympathy for the loss they have just suffered. To the honourable family of the deceased, who has left us a ray of light to illumine the field of science and an example of altruism and abegnation, I also desire to offer our very sincere sympathy in their bereavement.—I remain, Sir, yours sincerely.

(*Signed*) DR. JUAN PAES DE CARVALHO.

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Letter from Mr. A. J. BEALE, of the firm of Messrs. SINGLEHURST, BROCKLEHURST & Co., Para, to Mr. GEORGE BROCKLEHURST (Member of the Committee of the Liverpool School of Tropical Medicine).

DRS. DURHAM AND MYERS

It is with very great regret we have to inform you of the death of Dr. WALTER MYERS, of the Yellow Fever Commission, which occurred on the 20th instant, at 5 p.m., from yellow fever at the Yellow Fever Hospital. Dr. DURHAM is recovering rapidly, and was yesterday able to leave his room for a short time. Both doctors were attacked with the fever on the 16th instant, Dr. Durham's case declaring itself first, his colleague procuring him a carriage to convey him from the L. S. Institute to the hospital. A few hours later Dr. Myers was found to be with a high fever, and it was at once found that in his case the disease was of a malignant type. They were treated at the Yellow Fever Hospital, and everything possible was done for them. Besides the nurses, a doctor was always at the hospital, and Dr. Paes de Carvalho was with them two or three times daily. It is supposed that both doctors contracted the disease during an autopsy on a very malignant case of yellow fever, which had occurred at the hospital two or three days previous.—Yours truly,

*p. pro.* SINGLEHURST, BROCKLEHURST & CO.,
(*Signed*) A. J. BEALE.

Para, Brazil, *January* 23, 1901.

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The following obituary notice of Dr. Walter Myers appeared in the *British Medical Journal* of February 2, 1901.

Walter Myers was born twenty-nine years ago, and was the son of Mr. George Myers, a well-known Birmingham citizen. He was educated at King Edward's School. After leaving school he studied in the Biological Laboratories of Mason College from 1888 to 1890 while preparing for the Intermediate Examination for the B.Sc. Lond. degree, which he took in 1892. In the Botanical Laboratory of Mason College—where he won the Senior Botanical Prize in 1890—he developed that strong taste for microscopical study which played an important part in subsequently determining his mental bias in his purely medical studies. He proceeded to Cambridge in 1890, having won a Natural Science Scholarship at Caius College, and graduated M.B., B.C. in 1897.

Those who knew Myers and the work that he has carried on in recent years know how great a loss his death will be to the science of medicine. The late Professor Kanthack was early struck by the scientific enthusiasm and acumen displayed by Myers when he came to work in his laboratory, and before the late professor died he recommended Myers for the John Lucas Walker Scholarship on the understanding that he should continue his work on immunity, more especially with the ferments found in diseased products and in the blood.

After working for a short time at Cambridge, Myers received word that he would be allowed to continue his investigations in Professor Ehrlich's Laboratory, where he entered so thoroughly into the spirit of the place that he soon produced further excellent work on cobra poisoning, and commenced an investigation on the action of the various forms of proteids and their anti-bodies. This work, of which he had published a preliminary account before he left on the Yellow Fever Expedition, following up that already carried out by Ehrlich, is one of the most important of recent contributions to the study of immunity.

His more important papers: 'Cobra Poison in Relation to Wassermann's New Theory of Immunity' (*Lancet*, 1898, vol. ii, p. 23); 'The Action of Cobra Poison on the Blood: a Contribution to the Study of Passive Immunity' (in collaboration with Mr. J. W. W. Stephens, M.A., M.B. Cantab.) (*Journal of Pathology and Bacteriology*, 1898, vol. v, p. 279); 'On the Interaction of Toxin and Antitoxin: Illustrated by the Reaction between Cobra Lysin and its Antitoxin' (*op. cit.*, 1900, vol. vi, p. 415); Myers (and Stephens): 'Influence of Cobra Poison in the Clotting of

Blood and the Action of Calmette's Antivenomous Serum upon the Phenomenon' (*Proceedings Physiological Society*, p. I, in the *Journal of Physiology*, vol. xxiii) ; 'On Immunity against proteids' (*Lancet*, 1900, vol. ii, p. 98) ; indicate the work on which he was engaged, how much he had already done, and what promise he had given for the future.

Only those who knew him well had any idea of the kindliness of his disposition, his thoughtfulness for others, and his enthusiasm for his work. He was a Cambridge man of the best type, with a well-stored mind ; extremely fond of music and a very good musician, he was always a pleasant and interesting companion. Some idea of what was thought of him in Germany may be gathered from the following letter, received this week from Professor EHRLICH :—

[*Translation.*]

MY DEAR COLLEAGUE.—I have learnt with the greatest sorrow of the death of our common pupil and friend, MYERS. It is, indeed, a great loss—such an able, strenuous, trustworthy worker, and of so good a character. When you receive more detailed particulars from Dr. DURHAM, may I ask you to kindly communicate them to me ?

When the Liverpool School of Tropical Medicine was sending out the expedition, DURHAM and MYERS were approached to see whether they would take charge of it, and, though both of them fully appreciated the dangers to which they would be exposed and the risks they ran, they decided that they would take these risks and do the work that came to their hand. MYERS' death adds another name to the roll of martyrs to scientific investigation. His loss will be greatly felt by his many friends in Cambridge, and most by those who knew him best, for only those could appreciate to the full his fine and sterling qualities.

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Immediately on receipt of the news the Committee of the Liverpool School of Tropical Medicine called a special meeting at which the following resolution was passed :—

*Resolved—*

'That the Liverpool School of Tropical Medicine desires to place on record its heartfelt sympathy with the family of the late Dr. WALTER MYERS in the very sad loss that they have sustained by the premature death in Para of that distinguished scientist in the discharge of a noble duty.

The School wishes to acknowledge in a public manner the high courage and the unselfish spirit that prompted the late Dr. MYERS unhesitatingly to accept the invitation of the School to take part in a most dangerous expedition, the principal object of which was the investigation of yellow fever, to which fatal disease Dr. MYERS fell a victim.

The School trusts that his family may derive some consolation from the fact that Dr. MYERS lost his life on behalf of humanity and science.'

The question of raising a suitable memorial to Dr. MYERS was at once taken in hand by the Committee, the result of which will be made public later. In the meantime the Committee have made offers, both of which have been accepted, to erect a tombstone over Dr. MYERS' grave in Para, and to present to the University of Birmingham, since he began his medical studies in Birmingham, a suitable brass memorial in his memory.

# THE PREVENTION OF MALARIA IN TROPICAL AFRICA

By S. R. CHRISTOPHERS, M.B. Vict.

Since the discovery by Ross that malaria is a disease transmissible by the mosquito, many new facts bearing upon the prevention of malaria have come to light. Investigations carried on by Grassi, Celli, Bignami, and others in Italy, by Koch in New Guinea, by the Malaria Commission of the Royal Society and Colonial Office, by the expeditions of the Liverpool School of Tropical Medicine in Africa, have furnished us with a considerable amount of information concerning the life-history and distribution of mosquitoes, and on the nature of human malaria generally. As a result of these investigations many methods of combating malaria have suggested themselves, and have even been applied in several instances with success. The destruction of the larvae of anopheles, though a very obvious method of combating malaria, is by no means the only one. Other methods suggested by further researches are now acknowledged by many to be more easy of application and more effective. The conditions under which malaria occurs differ much, and under these different conditions widely different means of prevention may be found to be effective. Certainly many means, which in Europe are to be commended, must in Africa be very difficult to carry out, if not quite impossible.

The elucidation of a fact hitherto quite unexpected, namely, that the African native is not free from malaria, but is affected to an extraordinary degree, makes the prophylaxis of malaria in Africa a problem quite distinct from that of the prevention of malaria in Europe. A fundamental difference in the conditions in Africa and Europe at once presents itself. In Europe what is desired is to prevent malaria among the entire population. In Africa it is an evident impossibility to make any impression on malaria among a vast population, whose want of amenity to control can only be appreciated by those who have had actual experience of these people. In Africa what must be aimed at is the prevention of malaria among Europeans. This in itself will be a sufficiently great task to accomplish. The European is but one among many thousands of natives; it is in tiny settlements scattered over an enormous area that Europeans are for the most part to be found in Africa. Even in the few large towns the Europeans are but a few in comparison with the great bulk of natives. Thus a town of 30,000 or 40,000 inhabitants does not contain more than at most 150 Europeans.

Before discussing in detail the special methods of prevention which promise success in Africa, it will be well to briefly pass in review the main lines upon which malaria may be combated.

There are three main directions in which we may hope to influence malaria :

1 To attempt to destroy anopheles, either by doing away with the pools they breed in, or by destroying the larvae or adults.

2 To attempt to so protect men that anopheles cannot infect them, as by the use of mosquito nets or mosquito-proof houses.

3 To attempt to destroy the parasite in man himself, so that even though anopheles are present malaria is still absent.

The first and second methods appear at first most likely to lead to success. Recent researches, however, have shewn that the third method is a most effective way of dealing with the problem.

It may be pointed out that it appears to have been in this latter way that malaria has disappeared from the fen districts of England. Anopheles are still widely distributed, but there is no malaria. The probable explanation is that extensive use of quinine by the inhabitants of these districts has led to this result, and that the method Koch employed of combating malaria in New Guinea by quinine was unwittingly the cause of the disappearance of malaria in England.

## I. THE DESTRUCTION OF ANOPHELES

*The Superficial Drainage of an Area.* Such a method can evidently be applied only to very limited areas. Even in Europe it would be difficult of application apart from towns, whilst in Africa there can be no question of its application except under certain special conditions. Even in most African towns drainage would be by no means a light task. In Lagos draining or filling up possible anopheles pools would be a gigantic undertaking. In Accra, situated on porous soil with an extremely scanty rainfall, it would not be so difficult. Even in Accra, however, it would probably only be possible after a water supply had been brought from the Aburi hills, for at the present time many of the inhabitants depend for water upon the very pits and wells which act as breeding-places. In Freetown, which is built on rock, the central parts of the town might be rendered free from all pools, yet the outskirts could not be so treated without great outlay.

Surface drainage in Africa must then be considered essentially of limited application. It is, indeed, only feasible when applied to a segregated community of Europeans. Such a special use of drainage is evidently by no means the enormous operation involved in draining a town of many thousands of inhabitants.

*The Application of Culicicides.* Great expectations were at one time held as to the efficacy of this method. In Africa the result of its application has not been encouraging, and the destruction of anopheles by this means over an area of any size in Africa is by no means probable. It soon became evident that, in the rains and in swampy districts, anopheles breeding-places were so numerous, so widespread, and often so easily overlooked that little could be done under these circumstances. It still seemed possible that something might be done in the dry season, when the number of pools was often greatly reduced, to influence the number of anopheles in the ensuing rainy season. This might have been the case were it not that the life of anopheles is often a very lengthy one, and that anopheles often remain months without any possibility of their having recourse to water for breeding purposes. Late in the dry season prolonged search may be necessary before any breeding places are discovered in a district, and indeed large areas may not contain a single breeding-place. In spite of this adult anopheles are usually to be found in native huts throughout the district. The fact is that many thousands of anopheles, which have not originated in any pool existing at this time, have remained over (hibernating ?) months after the pools they have bred in have disappeared. In spite, then, of the, at first sight, promising conditions during the dry season, it is doubtful whether at this time it is possible to influence the number of anopheles in the ensuing rains.

Were it possible generally to make quite sure that *all* pools in a district were regularly treated with culicicides, and were the process kept up for six months or a year, it is probable that the results would be most pronounced, and that malaria would be much diminished. Unfortunately, in practice such thoroughness is found to be very difficult and, out of Europe, almost impossible.

*The Destruction of the Adult Insects.* The adult insects can be attacked during the day by fumigation of houses. This process carried out systematically must be a great adjunct in any scheme of destruction of anopheles. It is a method, however, which is only adapted to Europe, and very little could be done in this way in Africa.

All the methods of combating malaria by destroying anopheles form a powerful means of prophylaxis where considerable labour can be commanded and where the area to be rendered free from anopheles is not large. They are quite useless in the prevention of malaria in the most deadly of conditions, namely, the up-country stations of Africa. As a whole, methods of this class are much more suited to Europe than to Africa.

## II THE PROTECTION OF MAN FROM BITES OF ANOPHELES

As a means of prophylaxis this group is of the greatest importance.

The experiment carried out by Drs. Sambon and Lowe has shewn that enthusiastic men may, in a mosquito-proof house, so effectually exclude anopheles as to live in a malarious district without contracting the disease.

Unfortunately, the effectiveness of these means depends almost entirely upon the person employing them. The majority of men in the tropics, either because they do not appreciate the danger of being bitten, or from mere carelessness, are unwilling to take any trouble to avoid the bites of anopheles. It is, therefore, usual to see mosquito nets used in such a way that they afford but slight protection; whereas by the use, with scrupulous and unremitting attention, of a mosquito net a careful man may remain years in a malarial district without contracting malaria. It is also highly improbable that many men in Africa would so effectually exclude anopheles from a mosquito-proof house as the investigators on the Roman Campagna did. Indeed, it is highly probable that mosquito-proof houses would be as useless in the hands of most men in the tropics as mosquito nets are at present. Methods of protection from bites of anopheles are of great efficacy to those who are content to undergo some discomfort to avoid malaria, but of very little use to the average man who as yet protects himself at best only half-heartedly.

*Mosquito Nets.* As a means of protection the proper use of a mosquito net stands pre-eminently alone. Anopheles in most places do not bite by day, and as a rule are not very bold, so that they do not bite freely until the lights are out. By the careful use of a net the bites of anopheles may be almost done away with.

*Clothing.* A great deal of protection is afforded by the habitual use of certain precautions of dress. It is rare whilst a light is in the room for the face and hands to be bitten by anopheles. The parts usually bitten whilst one is still outside the net are the ankles, which are usually freely exposed, covered only by a single thickness of sock. By the use of double socks, mosquito boots, or puttees the ankles may be perfectly protected.

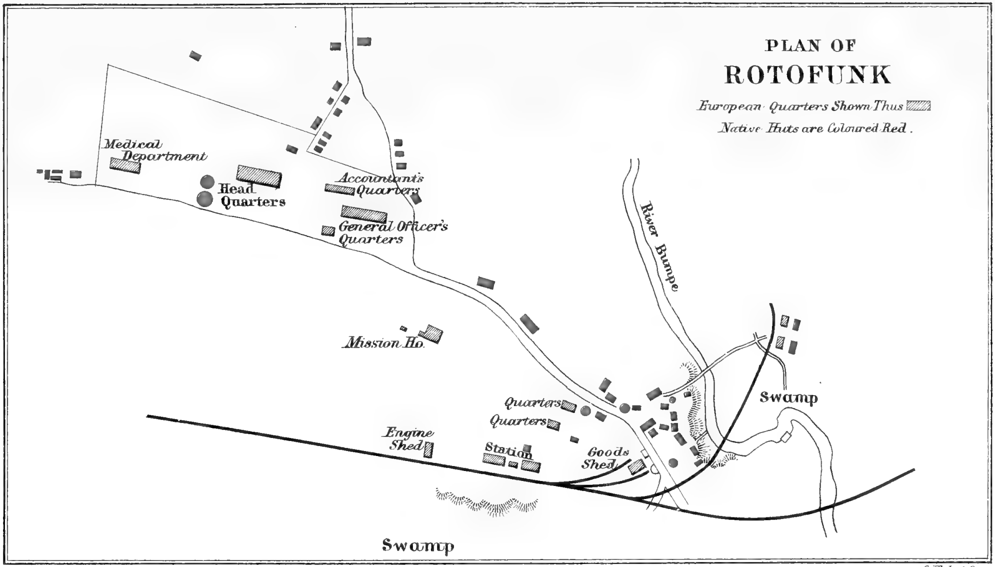
*Mosquito-Proof Houses.* As mentioned above mosquito-proof houses have been shewn to be feasible in Italy. It is, however, unlikely that such a means of protection will be largely used in the tropics. The climate in Italy and Africa are entirely different. In houses such as Drs. Sambon and Lowe occupied, life in the tropics would be most unpleasant. With the most perfect ventilation, with doors and windows thrown open, it is often so excessively hot at night that sleep is difficult. Most Europeans occupy rather large and roomy houses and spend much of their spare time on the verandah. Protection of this kind we shall see also later is by no means the most promising method of preventing malaria among Europeans in Africa.

*Repellant Bodies.* So far no repellant body of any effectiveness has been discovered.

## III. THE ELIMINATION FROM A COMMUNITY OF THE SOURCE OF INFECTION IN MAN

Two most important methods have the above result as their aim. They are:

1 The method employed with success by Professor Koch in New Guinea, *viz.*, to search out all cases of malaria (the concealed ones in particular), and to render them harmless by curing them with quinine.


PLAN OF
ROTOFUNK
European Quarters Shown Thus
Native Huts are Coloured Red.
Medical Department
Head Quarters
Accountant's Quarters
General Officer's Quarters
Mission Ho.
River Bumpe
Swamp
Quarters
Quarters
Engine Shed
Station
Goods Shed
Swamp
G. Philip & Son

In order to combat malaria he, at Stephansort, where malaria was rife, sought out all cases of malaria and persons with parasites in their blood, and treated them systematically with quinine.

By this means he was able to greatly reduce malaria in the on-coming season at Stephansort, although the climatic conditions were particularly favourable for the development of the disease. Koch, in his fifth report to the German Imperial Health Bureau, sums up as follows: 'The results of our experiment, which has lasted nearly six months, have been so uniform and unequivocal that they cannot be regarded as accidental. We may assume that it is directly owing to the measures we have adopted that malaria here has in a comparatively short time almost disappeared.'

In a community then which can be kept under observation and systematically treated with quinine, malaria can be combated very effectively. In many parts of the world this method very probably will be the way in which the desired diminution of malaria will be brought about. In Africa, however, such a method is quite inapplicable, and it would be quite impossible to carry it out under existing conditions, even in the most limited way.

A second method of getting rid of the source of infection is, however, peculiarly adapted to Africa.

2 The method first recommended by the Malaria Commission, and later strongly advocated by the expedition of the Liverpool School to Nigeria, *viz.*, segregation of Europeans from Natives.

This method is a corollary of the discovery that native children in Africa practically all contain the malaria parasite and are the source from which Europeans derive malaria.

Koch shewed in New Guinea that in most places infection was very prevalent in native children, so much so that in some villages 100 per cent. of those examined contained parasites. He also shewed that as the children increased in age immunity was produced, so that in the case of adults a marked immunity was present and malarial infection was absent.

The Malaria Commission shewed independently that a condition of universal infection existed among the children of tropical Africa, associated with an immunity of the adults. This infection in children had many remarkable characteristics. The children were in apparent health, but often contained large numbers of parasites, and a small proportion only of the children failed to shew some degree of infection. Not only were the children in every village and every hut infected, but anopheles were found which, from feeding on the children, had become infected.

It became evident that European malaria in Africa was but a mere indication of an enormous degree of infection throughout the whole of the malarious regions of Africa; and that the clue to the prevention of malaria among Europeans in Africa lay in the appreciation of this previously unsuspected and enormous source of infection.

The Liverpool School Expedition found a similar condition of affairs in all parts of Nigeria visited by them.

With a knowledge of the ubiquity of native malaria, the method of infection of Europeans becomes abundantly clear. The reputed unhealthiness or healthiness of stations is seen at once to be dependent on the proximity or non-proximity of native huts. The attack of malaria after a tour up country, the malaria at military stations like Prah-su, the abundance of malaria on railways, are all explicable when the extraordinary condition of universal native infection is appreciated.

It is evident that could Europeans avoid the close proximity of native huts they would do away with a very obvious and great source of infection. That they could avoid the neighbourhood of huts no one who has studied the conditions of life in Africa can doubt. On the other hand European houses in tropical Africa are almost always to be found with several native huts close at hand. When it is understood that each of these huts certainly contains many children with parasites in their blood, and also scores or hundreds of anopheles to carry the infection, then the frequency with which Europeans suffer from malaria is scarcely to be wondered at.

Sometimes the huts are those of the Europeans' servants, and here a mere command would be sufficient to ensure, in a day or two, freedom from such sources of infection. In other cases the huts are beyond the control of the European : but here again had the site of his house been chosen with a view to health, and situated away from huts originally, malaria would have been avoided. Throughout Africa Europeans are suffering from constant and repeated attacks of malaria, which owe their origin entirely to the presence of these huts, and which might with infinitesimal trouble be avoided.

## SUMMARY

To sum up then the application of these methods to conditions in Africa.

1 The destruction of larvae is from the magnitude of the task only applicable in special conditions.

2 The methods of protection from mosquito bites are efficient only when great personal care is used. These means necessitate the most vigilant and unremitting attention, and are therefore more a matter for the individual than a community.

3 (*a*) The seeking out of cases of malaria, or any drug treatment of the natives, is quite impossible in Africa.

(*b*) The separation of Europeans from the close neighbourhood of any native hut is a way in which Europeans may, without constant worry and trouble, protect themselves from malaria.

It is a method, moreover, which is peculiarly adaptable to the very conditions under which other methods are most futile and malaria most deadly, namely, the small settlements scattered about the African colonies.

Up to the present, segregation has not received the attention which its simplicity and practicability deserve. It is significant, however, that the two latest expeditions to Africa, namely, the Malaria Commission and the Liverpool School Expedition, are unanimous in upholding it as pre-eminently above all other schemes of prophylaxis as applied to Africa. No doubt the absence of attention is partly due to an insufficient appreciation of the condition of universal native infection, and partly to a want of knowledge of the conditions under which Europeans live in Africa.

It has been objected that segregation is impossible to the trader and commercial section of the community, and that the natives would by its application be apt to take offence. Both these objections are based upon a mistaken idea as to what it is proposed should be done to diminish malaria in Europeans by segregation.

As it is essential that the conditions of life of Europeans in Africa should be first thoroughly understood, a brief description of these conditions as most commonly seen is desirable.

1 *Up-country Stations.* Whether Government official quarters, missionary or traders' quarters: the Europeans' house may or may not be a well-built dwelling adapted to the climatic conditions. In either case there is always:

(*a*) A palm or grass hut or more pretentious building which is nominally the kitchen, but which is also used by a variable number of native servants and often by their families as a sleeping place. This structure is always within a few yards of the house.

(*b*) On some pretext, or for no assignable reason, there are always a few huts or a small village close at hand. This condition is universal, and the practice is responsible for the malaria from which Europeans in Africa suffer.

(*c*) Unlimited ground quite free from huts and easily attainable.

Here it is evident that there is nothing to prevent the European living in comfort and health.

The kitchen is essential; not so its use by all the native servants as a sleeping place. It is doubtful, indeed, whether any servant is necessary at night in most instances. Certainly many servants in the towns go to their homes and return again in the early morning. Were even one servant considered a necessity at night there need be no more, and there certainly need not be any children allowed. In a day or two the servants would build new quarters at any distance if so ordered.

The native huts of the village should have been avoided in the first instance, and, indeed, for a house to be placed close to a village can be looked upon now only as a folly. In many cases the European has it in his hands to move to a less dangerous neighbourhood, or even to get the huts themselves done away with.

By such simple means would malaria be successfully combated. By such means stations now deadly with malaria might be rendered healthy. Not only is it the case that quarters already erected may be improved, but quarters not yet erected may be made healthy or unhealthy as they are erected with the principle of segregation in view or with the principles which have hitherto prevailed.

The accompanying plan is that of a new railway settlement on the Sierra Leone railway. Miles of land free from huts exist along the line, but the close neighbourhood of native huts has been selected. At the time of building of these quarters it lay in the power of the engineers to have a malaria-free settlement. Instead of which, by the non-observance of a simple fact, the station is most malarious. In this particular instance much ingenuity has been shewn in providing each set of European quarters with plenty of malarial infection.

Before malaria is lessened among Europeans in Africa it must be generally recognised that malaria is practically an infectious disease, and that it is present in every native hut.

2 *A Town Residence.* In towns only is there any difficulty in carrying out the principle of segregation. In two instances, however, this has been carried out in towns with the result that the segregated communities of Europeans are notoriously the most healthy on the West Coast.

Even when no scheme of complete segregation can be carried out, the principle should always be borne in mind, and, whenever opportunity offers, huts should be removed and European houses built in the open. Possibly segregation will be even more beneficial in dealing with new settlements and new towns than with those already built among bad surroundings.

One thing is certain, that no scheme but segregation offers the least promise of a wide success in Africa.

# ENLARGED SPLEENS AND MALARIA

By C. W. DANIELS, M.B. Cantab., British Guiana Medical Service

Enlargement of the spleen is so much more common in malarial countries than elsewhere that the dependence of this condition on malaria can hardly be doubted. The exact relation of the two conditions is, however, obscure.

In India the 'Spleen Test,' *i.e.* the proportion of unselected persons who are found to have obvious enlargement of the spleen, has by many been relied on as *the* test of the prevalence of malaria in a district.

Elsewhere, probably, as will be shown, owing to working with a greater variety of races, difficulties have been met with in placing reliance on this view. British Guiana is certainly a more malarious country than most parts of India, yet in a series of two hundred and twelve post-mortem examinations made in the Berbice Lunatic Asylum, 1881-1883 (*Asylum Journal*, Feb., 1883), recorded by Dr. Grieve, it was shown that a larger proportion of spleens were below than above the normal range of weight. Of these two hundred and twelve, one hundred and twenty-three or 58 per cent. were between four and ten ounces, *i.e.* were about normal; fifty-five or 26 per cent. were under four ounces, or were subnormal; and only thirty-four or 16 per cent. were over ten ounces. Even of these thirty-four, ten were from ten to twelve ounces, and only five or 2·3 per cent. over twenty ounces, markedly enlarged. These patients were adults, and the majority were probably negroes, but the returns are not sub-divided according to race or age.

From 1893-96, some eighteen hundred post-mortem examinations were made, and the presence or absence of malarial pigment was determined in each case by microscopical examination.

The result, as shown in the subjoined table, up to 1895, showed that there was no fixed relation, except in cases of acute malaria, between the weight of the spleen and the presence of malarial pigment in that organ; and further, showed that great differences existed in the size of the spleen in different races, whilst there was comparatively little difference in the prevalence of pigmentation.

The results were similar in males and females; but, to avoid repetition, the tables for adult males only are given.*

*Fuller particulars of this portion of this series are given in the *British Guiana Medical Annual* for 1895, Appendix to '*Notes on a series of Post-mortem Examinations*.'

TABLE I

| Race | East Indians | | Negroes Natives B.G. | | Aboriginals | | Chinese | | Portuguese | | Immigrant Negroes | | Europeans | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Not Pigmented | Pigmented | Not Pigmented | Pigmented | Not Pigmented | Pigmented | Not Pigmented | Pigmented | Not Pigmented | Pigmented | Not Pigmented | Pigmented | Not Pigmented | Pigmented |
| Number of Cases . . . | 345 | 80 | 171 | 57 | 9 | 4 | 19 | 6 | 37 | 3 | 65 | 63 | 12 | 7 |
| Percentages, 5 ozs. & under | 4·9 | 11·2 | 33·9 | 35·2 | ... | ... | 5·2 | 16·6 | 5·4 | ... | 46·1 | 31·9 | 25·5 | ... |
| " 10 ozs. " | 18·5 | 32·5 | 47·3 | 42·6 | 22·2 | 25 | 36·8 | 33·3 | 30 | 33·3 | 32·3 | 41·2 | 41·6 | ... |
| " 15 ozs. " | 24 | 23·7 | 9·9 | 14·8 | 11·1 | ... | 15·7 | 33·3 | 16·2 | ... | 6·1 | 14·2 | 8·3 | 57·1 |
| " 20 ozs. " | 15·6 | 20 | 6·4 | 5·5 | 22·2 | 50 | 21 | 16·6 | 24·3 | ... | 12·2 | 6·3 | 16·6 | 14·2 |
| " 25 ozs. " | 12·7 | 2·5 | 1·2 | ... | 11·1 | ... | ... | ... | 8·1 | ... | ... | 4·7 | ... | 14·2 |
| " 30 ozs. " | 9·8 | 2·5 | ·58 | 1·9 | 22·2 | ... | 5·2 | ... | 8·1 | ... | ... | ... | ... | 14·2 |
| " 40 ozs. " | 5·2 | 5 | ·58 | ... | ... | ... | 15·7 | ... | 2·7 | 66·6 | ... | 1·6 | ... | ... |
| " 50 ozs. " | 5·5 | ... | ... | ... | ... | 25 | ... | ... | 2·7 | ... | 1·5 | ... | ... | ... |
| " 60 ozs. " | 2 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| " over 60 ozs. | 2 | 2·5 | ... | ... | 11·1 | ... | ... | ... | 2·7 | ... | ... | ... | ... | .. |

Of the races represented, the Aboriginal American Indians, the Chinese, and Portuguese are represented in too small numbers to be of much value in themselves, but it is seen that they correspond to the Indian type and not to the Negro.

The two last columns differ from the others in that they represent races exposed to malarial infection in adult life only, such as the Immigrant Negroes from Barbados and other non-malarial West Indian Islands.

The age incidence amongst adults is represented in the following table ; but at these ages, twenty and over, recent malarial attacks are rare as shown by the rarity of recent malarial pigmentation, and therefore this table does not show the effects of recent attacks.

TABLE II

| Race | | E. Indian | | Native Negroes | | Immigrant Negroes | |
|---|---|---|---|---|---|---|---|
| Ages | | Not Pigmented | Pigmented | Not Pigmented | Pigmented | Not Pigmented | Pigmented |
| 20-25 | 10 oz. and under | 33·3 | 49·9 | 75 | 71 | 66·6 | 47·5 |
| | Over 10 oz. | 66·6 | 50·1 | 25 | 28·9 | 33·3 | 52·5 |
| 26-45 | 10 oz. and under | 17·9 | 39·6 | 86·4 | 80 | 85·4 | 79·9 |
| | Over 10 oz. | 82·1 | 60·4 | 13·6 | 20 | 14.6 | 19·9 |
| Over 45 | 10 oz. and under | 37·4 | 60 | 81·9 | 92·2 | 74·5 | 100 |
| | Over 10 oz. | 62·6 | 40 | 18 | 7·6 | 25·5 | — |

In younger persons the pigmented organs are much heavier than the unpigmented organs; so that at these ages the spleens which show pigmentary evidence of malaria are also enlarged.

In British Central Africa post-mortem examinations were not obtainable, but clinical observations were made. Amongst the native adults I did not see a single case of enlarged spleen, though several hundred examinations were made; but I was informed by others that the condition, though rare, did occur.

In native children, however, enlarged spleen was common. The variation in the age incidence was marked, and differed also in different districts according to the liability to malarial infection as determined by the length of residence requisite for infection of susceptible newcomers. In districts represented in the first vertical column of Table III, a few of the newly-arrived Europeans escape 'fever' for a year or more; in those represented in the second column infection is usual in a month, and few persons escape much longer; whilst in those represented in the third column an exposure for a fortnight or so results in the infection of nearly 25 per cent. of the Europeans exposed.

TABLE III

ENLARGED SPLEENS IN PERCENTAGES

| | Highlands 3,000 feet or more | Lake Level 1,500 feet odd | Lower Shiré 300 feet or less |
|---|---|---|---|
| Aged two years and under - | 18·4 | 18·8 | 48·1 |
| Two to four years - - - - | 31·5 | 45·1 | 57·1 |
| Fifteen years and under - - | 26·2 | 11·5 | 13·7 |

The total number of these children was eight hundred and fifty. Of these five hundred and thirty-five were fifteen and under; one hundred and twenty-four were two to four years old; and one hundred and ninety-one were under two years. The ages in only a portion of the cases could be determined with certainty, in the others it had to be estimated from the development.

Attacks of 'fever' are common in cachectic or persons with enlarged spleens. In these attacks it is exceptional to find malaria parasites; pigment is usually absent from the organs in fatal cases, and the action of quinine is uncertain.

Persons with chronic enlarged spleens, with or without cachectic, are peculiarly liable to certain diseases, particularly lobar pneumonia.

As regards Europeans, the fact that few are in continuous residence in badly malarial places for long periods renders such questions very difficult to consider. Even with negroes there is some reason to suppose that their acquired immunity is diminished by residence in a non-malarial country. If in an ordinary attack of untreated malaria the parasites be counted in successive cycles, the number is not as a rule increased in the second or third observed cycles, and is often diminished. When working with Major Ross on *Proteosoma*, I was able to confirm his observation on birds infected by mosquitoes, to the effect that the number of parasites found at first rapidly increased, then remained stationary for two or three days, and finally rapidly diminished to such an extent that, whilst at the height of the invasion eight or nine parasites might be found in a field, later a single parasite only would be found in a slide on one day and none on another.

These observations show that there is a check on the multiplication of the parasites, as otherwise they would increase in geometrical progression. Natural cure is common, and the parasites disappear or occur in such small numbers that they are not found in blood examinations, and leave no deposit of pigment in the organs.

In many of these cases it is probable that a small residue of parasites is left, as in such cases recurrence of the parasites takes place without fresh infection. This interval between attacks is one of temporary immunity. It may be long or short, but in newcomers is usually under a month. It varies in different individuals, and in the same individual at different times.

In a place, or even in a house, where frequent fresh infections are occurring, the period of immunity may be of several months after a single attack of fever. In one station, few newcomers escaped fever for more than a month after arrival ; but the older residents did not have attacks more frequently than every three, four, or six months or more. In this place, with the older residents, infections must have been more frequent than actual attacks of fever ; so that several of the infections must have been abortive, indicating a degree of immunity in these persons.

A common history amongst the older European residents was repeated attacks of fever in the first two, three, or four years, and very little or only slight attacks subsequently. Many of these persons were not cachetic, and had no splenic enlargement. In the temporary periods of immunity the person often appears to be in robust health.

The loss of acquired immunity by change of residence to a non-malarial country would, if fully established, indicate that, though immunity to malaria is acquired by repeated invasions of the parasite (*i.e.* relapses), repeated fresh infections, however abortive, are required for its maintenance.

Under these circumstances a high degree of immunity is not likely to be attained in countries or districts where for a lengthy portion of each year fresh infections do not occur.

In the malarial countries in which I have worked, though there has been a seasonal variation, there has been no period during which fresh infections have not occurred.

As a test for the prevalence of malaria, the 'spleen test' may be worse than useless unless race and age are taken into account. In Africa, if examination be made of persons five to fifteen years of age, the least healthy districts would appear to be the least malarial, judged by the proportion of enlarged spleens. If adult natives alone were examined all districts would appear to be free from malaria.

In South America malaria would appear to be rare or common in a district according to the race, Negro or Indian, examined.

Even in a country with a population of uniform race, examined at an age at which splenic enlargement is most common, we known too little of the other factors causing enlargement of the spleen for the test to be at all an accurate one.

It must always be remembered that even such characteristic sequelae of diseases as neuritis after diphtheria, nephritis after scarlatina, and organic cardiac valvular disease after rheumatism, and even stricture after gonorrhoea are dependent on other factors as well as on the primary disease ; and the frequency of the sequela would afford no real measure of the prevalence of the primary disease. Excluding the factors, race and age, splenic enlargement can be considered as a rough inaccurate measure of the prevalence of malaria, but still a useful one.

# DIAGRAMS ILLUSTRATING THE LIFE-HISTORY OF THE PARASITES OF MALARIA*

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*With Plates*

The parasitology of the red blood-corpuscle of vertebrates was opened in 1870 by Ray Lankester's discovery of the Drepanidium ranarum. In 1880 Laveran made the important observation that somewhat similar intra-corpuscular organisms exist in the blood of human beings suffering from malarial fever. Since then Danielewsky, Kruse, Koch, Dionisi, and others have demonstrated allied parasites in the blood of reptiles, birds, bats, and monkeys, and Smith and Kilborne have shown that the disease of oxen called Texas cattle fever is due to an intra-corpuscular parasite of another kind. As the result of these observations we are now familiar with a considerable number of such organisms. All of them are usually classed among the protozoa, and in the somewhat artificial order of the sporozoa. They are generally divided into three groups, which are as follows :—

Group I The parasite of Texas cattle fever, Pyrosoma (or Apiosoma) bigeminum, Smith and Kilborne, and similar organisms found in dogs and some other mammalia (?) ; minute pear-shaped intra-corpuscular bodies, which are known to be communicated among oxen by the cattle tick, Boöphilus bovis.

Group II Organisms apparently allied to the Gregarinidae ; found in reptiles ; numerous species.

Group III Intra-corpuscular amoebae or myxopods found in man, monkeys, bats, birds, and possibly frogs. Four species are known to undergo further development in gnats.

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Reprinted from *The Quarterly Journal of Microscopical Science.*

It is this last group which claims our attention at present. The diagrams are meant to illustrate a discourse delivered by one of us at the Royal Institution (*Proceedings of the Roy. Inst.*, 1900, and also *Nature*, March 29, 1900); but we shall now give a description sufficient to enable the reader to follow the life-history from this paper alone. The figures show the appearance of the parasites as seen in unstained preparations—the cytology of some of the stages (Figs. 53-60) in the gnat not yet being sufficiently established to warrant illustration in a scheme of this kind.

We adopt the name Haemamoebidae, Wasielewsky, for the whole group. At least three species occur in human beings (producing the different varieties of malarial fever); one species in monkeys, three in bats, and two in birds. We illustrate only the species found in man and birds, those of monkeys and bats being closely similar to the human species, but, so far as we know at present, not identical. The development of four of the species has been followed in gnats. The three human species develop in gnats of the genus Anopheles, while one of the avian species (Haemamoeba relicta) lives in gnats of the Culex pipiens type. The insect hosts of the remaining species have not as yet been found.

So far as we know, the life-history of all the species is practically identical, and is as follows:—The youngest parasites are found as minute amoebulae living within or upon the red corpuscles of the vertebrate hosts. Each contains a nucleus, which stains by the Romanowsky method. Growing rapidly in size, the amoebulae convert the haemoglobin of the containing corpuscles into a varying number of brown or black granules, which are called the melanin or malarial pigment. These granules lie in the bioplasm of the parasite surrounding the nucleus. After an interval of from one to several days (according to the species concerned) the amoebulae, still contained within the corpuscle, reach maturity, and become either (*a*) sporocytes or (*b*) gametocytes. In the case of the amoebulae which become sporocytes the nucleus divides into a number of segments (varying according to the species). Each segment of the nucleus surrounds itself with a portion of the bioplasm, and becomes a spore—the process being obviously one of simple asexual propagation. Finally the corpuscle which contains the parasite, and which has now been almost entirely destroyed by it, bursts and liberates the spores, allowing them and a small nucleus de reliquat, consisting chiefly of the melanin, to fall into the liquor sanguinis. The melanin is taken up by the phagocytes of the host, while the spores attach themselves to fresh red corpuscles, become amoebulae in their turn, and thus continue the life of the organisms indefinitely within the vertebrate hosts.

In the case of the amoebulae which become gametocytes the history is quite different. It is not yet definitely known what determines a given amoebula to become either a sporocyte or a gametocyte, but the fact must be accepted. In the gamoetocytes the nucleus does not divide as in the case of the sporocytes—the parasite reaches maturity without showing any sign of spore-formation. In the majority of species (genus Haemamoeba) the gametocyte has a general form similar to that of the sporocyte before the spores are produced; but in one species (genus Haemomenas) the gametocyte has a special (crescentic) shape, which is recognisable at an early stage in its career. As their name indicates, the gametocytes are sexual forms, male and female. They possess no function within the vertebrate host, but are meant to continue the life of the organisms within a second host—a suctorial insect.

When the gametoyctes are drawn into the stomach cavity of gnats (middle intestine) they immediately undertake their sexual functions. The male gametocyte (the nucleus of which is larger than that of the female) is destined to give origin to a number of microgametes, or spermatoza; the female gametocyte develops into one macrogamete, or ovum, together with a residuum consisting chiefly of melanin. A few minutes after ingestion by the gnat both male and female gametocytes break from the enclosing corpuscle, and swell slightly. Attached to the naked parasite one can now often perceive one or two small spherical objects, which may possibly be the homologues of polar bodies. A few minutes later a quivering movement is observed in the male gametocytes, due to the emission of the microgametes. These bodies are long filaments endowed with very active powers of locomotion, and consisting of a thread of chromatin surrounded by a thin scroll of bioplasm. Breaking away from the parent cell, and leaving behind the melanin of that cell as a residuum, the microgametes travel through the liquor sanguinis contained in the stomach of the gnat in search of a macrogamete. This being found, one microgamete enters the macrogamete and unites with its nucleus, producing a zygote.

Shortly after the act of fertilisation the zygote may in some species become motile (when it is technically called a vermicule), and generally changes its shape. At all events, it travels towards the parietes of the stomach. If the insect be of an inhospitable species the zygote perishes; but if the insect be hospitable the zygote passes through the parietes and affixes itself on or just under the outer muscular coat of the stomach. Here it becomes motionless and commences to grow rapidly in size.

At first of about the size of a red corpuscle, and still containing the characteristic black granules of melanin, the zygote, after a week or so, reaches a very large size; that is, it becomes about 60 $\mu$ in diameter, or about eight times its original diameter, and about five hundred times its original bulk. As we have said, we are not satisfied regarding the nature of the nuclear changes during the growth of the zygotes, but it is clear that the parasite acquires a very distinct capsule, and that its substance divides into from eight to twelve meres, which can easily be distinguished without staining. Each mere seems finally to become a spherical blastophore, bearing on its surface a number of filamentous, or rather spindle-shaped blasts, in the manner depicted in Fig. 60; at least, it is easy, by rupturing a nearly mature zygote, to expel a number of such bodies. When the zygote reaches maturity the blastophores disappear, leaving the capsule packed with thousands of the blasts (and containing also some residual fatty globules).

The capsule now bursts spontaneously, and pours the blasts into the body-cavity of the gnat. On drying and staining the blasts are easily seen to be of about 12 to 16 $\mu$ in length, with a central nucleus, one or two clear oval areas, and tapering extremities. No definite movements have been observed in these bodies—possibly on account of the reagent (salt solution) which must be used to make them visible in fresh preparations. By some means or other, however, they find their way into remote parts of the host, and finally pierce the capsule of its salivary gland, enter the salivary cells, and lastly the salivary ducts, in all of which situations they can easily be seen with the aid of a strong salt solution. From the salivary ducts they evidently pass through the insect's middle stylet or tongue into the circulation of a fresh vertebrate host, in which it is to be presumed they at once become the amoebulae with which the life-history of the parasites commenced. At all events, numerous experiments, both on birds and on man, have demonstrated the fact that gnats whose salivary glands contain the blasts are capable of establishing infection by their bites in the appropriate vertebrate hosts.

It should be noted that this life-history is in no way a hypothetical one. Every fact has been confirmed over and over again by many capable observers. The stages in the vertebrate hosts, first established by Laveran and Golgi, have been scrupulously studied, and have given rise to a mass of literature already very large. The sexual functions of the gametocytes—which can be witnessed *in vitro*,—originally observed by MacCallum, have been seen also by Koch and Marchoux; while the facts are confirmed by the cytological studies of Bignami and others. The development in the gnat and the infection of the healthy vertebrate host by the bite of the gnat, originally established by one of us, have been confirmed by Daniels, Koch, Grassi, Bignami, Bastianelli, and many others, and are accepted by Laveran, Ray Lankester, Metschnikoff, Manson, Celli, and other distinguished men of science. A full history of the subject of the relation between malaria and gnats, together with a complete bibliography, has been written by Nuttall.*

The terminology used above was arranged in consultation with Professor Herdman, F.R.S. It has been adopted by Laveran and Manson. Grassi and others had previously employed the terms gamete and zygote, but used sporozooid for the bodies we call blasts. We use the word blast somewhat in the sense employed in embryology, and as synonymous with sexually-produced spores. Ray Lankester suggests for them the name filiform young.

It should be added that, within the capsules of mature zygotes large black bodies (Fig. 67) are often to be found. It is doubtful whether these have any real connection with the parasites.

* Nuttall, *Hygienische Rundschau*, numerous papers, 1898, 1899, 1900. Also, *On the Role of Insects . . in the Spread of Diseases*, *Johns Hopkins Hospital Reports*, Baltimore, vol. viii.

The most recent figures of stages of the malaria parasites are those published by Koch, *Zeitschrift fur Hyg. und Infect.*, 1899 (photographs); Grassi, Bignami, and Bastianelli, *Annali d'Igiene sperimentali*, 1899 (plates); *Sierra Leone Expedition, Thompson Yates Laboratories Report*, vol. ii. (photographs and drawings).

We divide the haemamoeba into two genera (*Nature*, August 3, 1899), namely :—

Genus I—Haemamoeba, Grassi and Feletti. The gametocytes have a shape like that of the sporocytes before the spores have been produced.

Genus II—Haemomenas, gen. nov. (syn. *Laverania*, Grassi and Feletti, in part). The gametocytes have a special (crescentic) shape.

## DESCRIPTION OF PLATES

Illustrating Major Ronald Ross's and Dr. R. Fielding-Ould's paper on *The Life-History of the Parasites of Malaria.*

Figs. 1-41 Illustrate the two avian species and the three human species in the blood-vessels of the vertebrate hosts.

Figs. 42-52 Illustrate the development of the gametocytes of one of the human species, selected for the purpose, in the stomach cavity of anopheles, or in a drop of blood freshly extracted from the finger (in which the parasites develop much as they do in the stomach cavity of the insects).

Figs. 53-67 Illustrate the further development of the parasites in the tissues of the gnat.

Figs. 1-7 Development of Haemamoeba Danilewskii (syn. *Halteridium Danilewskii*, Labbé) in the blood of pigeons, jays, crows, etc. The amoebula is elongated, and lies by the side of the nucleus without disturbing its position. Several varieties exist.

Fig. 1 Spore.
Figs. 2-4 Growth of amoebula.
Fig. 5 Sporocyte.
Fig. 6 The containing corpuscle has burst, leaving nucleus, melanin, and free spores.
Fig. 7 Gametocyte.

Figs. 8-14 Development of haemamoeba relicta (syn. *Proteosoma Grassii*, Labbé) in the blood of sparrows, larks, crows, etc. The amoebula lies at one end of the corpuscle, and pushes the nucleus towards the other end.

Fig. 8 Spore.
Figs. 9-11 Growth of amoebula.
Fig. 12 Sporocyte.
Fig. 13 Freed spores.
Fig. 14 Gametocyte.

Figs. 15-22 Development of haemamoeba malariae, the parasite of quartan fever of man. The containing corpuscle is a small or medium-sized one, the melanin consists of coarse dark-brown granules, the amoeboid movements are slow, the spores are about eight to twelve in number, development takes seventy-two hours.

Fig. 15 Spore.
Figs. 16-18 Growth of amoebula.
Fig. 19 First appearance of spore formation.
Fig. 20 Sporocyte.
Fig. 21 Freed spores.
Fig. 22 Gametocyte.

Figs. 23-29 Development of haemamoeba vivax, the parasite of tertian fever of man. The containing corpuscle is nearly always an exceptionally large one, the melanin consists of very fine light-brown granules, amoeboid movements are rapid, the spores are about twelve to twenty in number, development takes thirty-six hours.

Fig. 23 Spore.

Figs. 24-26 Growth of amoebula.

Fig. 27 Sporocyte.

Fig. 28 Freed spores.

Fig. 29 Gametocyte.

Figs. 30-41 Development of haemomenas praecox, the parasite of remittent, pernicious, or aestivo-autumnal fever of man. Several varieties, which are possibly distinct species. The amoebulae are small, contain only a few clumps of black melanin, and retire to the spleen, bone-marrow, etc., on approaching maturity ; the gametocytes have a special crescentic shape.

Figs. 30-34 Growth of amoebula becoming a sporocyte.

Fig. 35 Freed spores.

Figs. 36-39 Growth of amoebula becoming a gametocyte.

Fig. 40 Male gametocyte (portion of red corpuscle still present).

Fig. 41 Female gametocyte (remains of corpuscle entirely disappeared).

Figs. 42-52 Development of gametocytes of haemomenas praecox in stomach cavity of Anopheles.

Fig. 42 Male gametocyte.

Fig. 43 Female gametocyte

Figs. 44-45 Gametocytes become oval five minutes after ingestion by the gnat.

Figs. 46-47 Gametocytes become spherical a few minutes later. Polar bodies appear at the margin.

Fig. 48 Male gametocyte emitting microgametes (the so-called 'flagellate body').

Fig. 49 Female gametocyte or macrogamete awaiting fertilisation.

Fig. 50 Free microgametes.

Fig. 51 Microgamete entering macrogamete.

Fig. 52 Fertilised macrogamete, or zygote.

Figs. 53-67 Development of Haemomenas praecox in tissues of anopheles.

Fig. 53 Zygote approaching inner surface of stomach wall.

Fig. 54 Zygote piercing stomach wall.

Figs. 55, 56 Zygotes affixed to outer coat of stomach. They possess an apparently alveolar structure, and still contain granules of melanin.

Fig. 57 Zygote increases in size.

Figs. 58, 59 Zygotes increasing in size and dividing into meres, which become blastophores bearing blasts.

Fig. 60 A single blastophore bearing a number of blasts affixed to it, each by one extremity.

Fig. 61 Stomach seen by a low power, and dotted with a number of mature zygotes.

Fig. 62 A fully mature zygote packed with blasts, some of which are escaping from a rupture in the capsule.

Fig. 63 Free blasts in body cavity of anopheles.

Fig. 64 Blasts entering capsule of salivary gland ; also lying within salivary cells and duct.

Fig. 65 Junction of ducts of three lobes of salivary gland of one side, with blasts.

Fig. 66 Blasts escaping from extremity of middle stylet or tongue of anopheles.

Fig. 67 Capsule of a mature zygote containing five large black bodies, provisionally known as 'black spores.'

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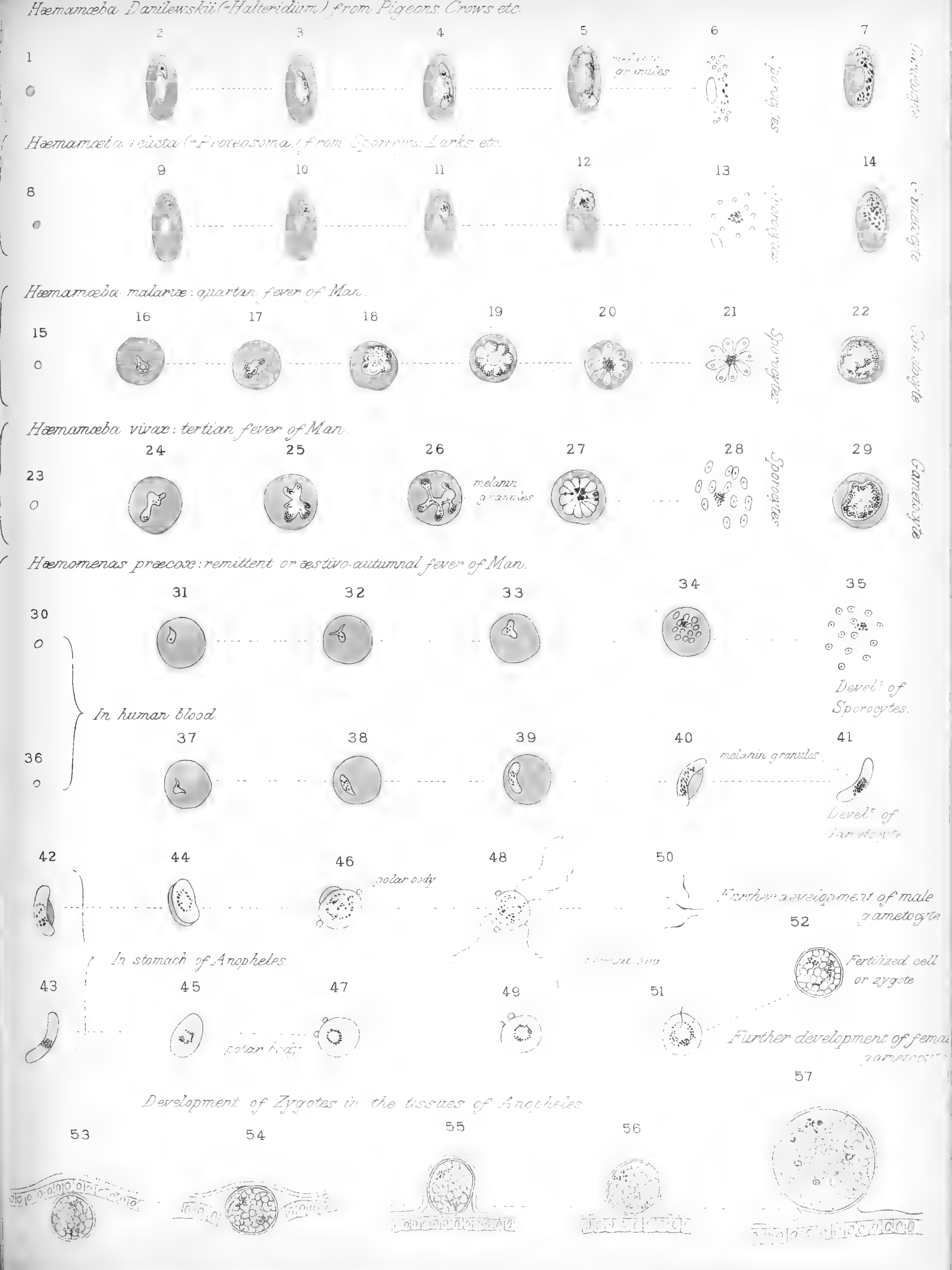
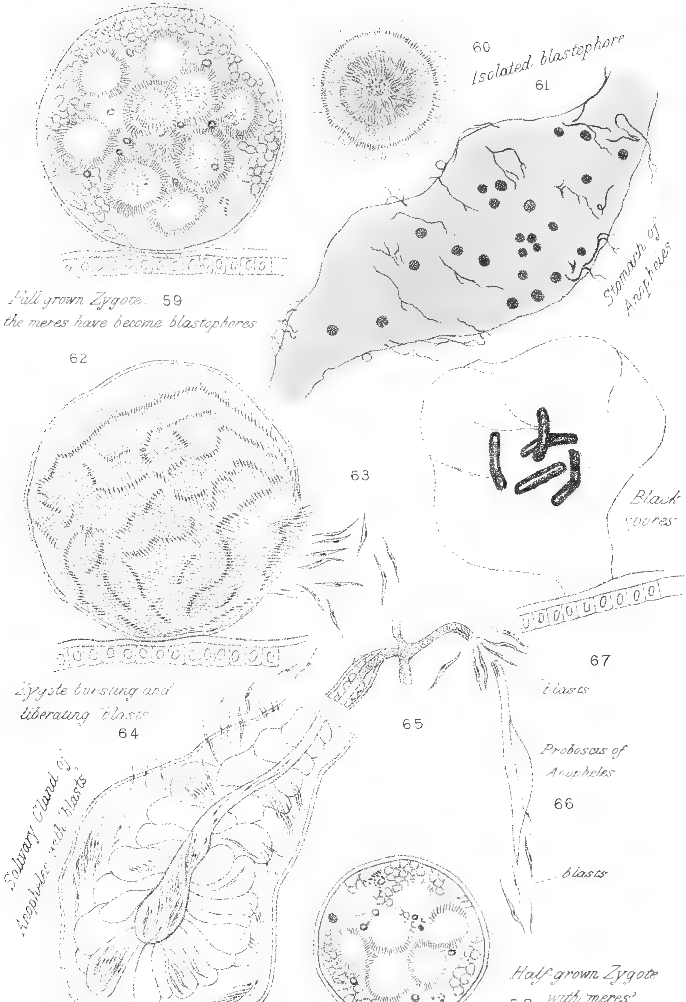
Note—We may point out that the development of the remaining human species in anopheles and of H. relicta in culex are almost identical with those of H. praecox given in the plates.

All the figures, except Fig. 61, are magnified 2500 diameters. Fig. 61 is magnified about 70 diameters, the stomach tissues being flattened and extended by the cover-glass.


Hæmamœba Danilewskii (=Halteridium) from Pigeons, Crows etc.
granules
Sporocytes
Gametocyte
Sporocytes
Gametocyte
Hæmamœba malariæ: quartan fever of Man.
Sporocytes
Gametocyte
Hæmamœba vivax: tertian fever of Man.
melanin granules
Sporocytes
Gametocyte
Hæmomenas præcox: remittent or æstivo-autumnal fever of Man.
Devel.t of Sporocytes.
In human blood.
melanin granules
Devel.t of
polar body
Further development of male gametocyte
Fertilized cell or zygote
In stomach of Anopheles
polar body
Further development of female
Development of Zygotes in the tissues of Anopheles




R. Fielding-Ould, del. LIFE HISTORY OF MALARIAL PARASITES. A.S. Huth, Lith. London

*Later stages of Zygotes in Anopheles: formation of 'blasts' or 'filiform young'.*

LIVERPOOL SCHOOL OF TROPICAL MEDICINE—MEMOIR III

# REPORT

OF THE

# MALARIA EXPEDITION TO NIGERIA

OF THE

LIVERPOOL SCHOOL OF TROPICAL MEDICINE
AND MEDICAL PARASITOLOGY

BY

H. E. ANNETT, M.B., D.P.H. (Vict.)
DEMONSTRATOR IN TROPICAL PATHOLOGY
J. EVERETT DUTTON, M.B., B.Ch. (Vict.)
AND
J. H. ELLIOTT, M.D. (Tor.)

Part I. Malarial Fever, Etc.

*WITH ILLUSTRATIONS AND PLANS*

AT THE UNIVERSITY PRESS OF LIVERPOOL 1901

TO THE

RIGHT HONOURABLE LORD LISTER, F.R.S., LL.D., D.C.L.,

THIS REPORT IS RESPECTFULLY DEDICATED

BY THE

LIVERPOOL SCHOOL OF TROPICAL MEDICINE

# ISSUED BY THE COMMITTEE

OF THE

# LIVERPOOL SCHOOL OF TROPICAL MEDICINE AND MEDICAL PARASITOLOGY

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Due acknowledgement must also be rendered for the help of the local agents of the African Association, Miller Bros. and Co.; Oil Rivers Trading and Exploration Company; and the Niger Company.

L

## DIARY OF THE EXPEDITION'S MOVEMENTS

| | |
|---|---|
| 1900 | |
| March 21 | Sailed from Liverpool per s.s. 'Olenda' (Captain Hampson). |
| April 11 | Arrived at Old Calabar, Southern Nigeria. |
| May 3 | One of the members started on a visit to Okoyong, Adiabo, and Ikoneto—a few miles up the Calabar and Cross Rivers—returned May 7. |
| " 14 | Two members visited Creek Town, on the Calabar River. |
| " 17 | Sailed for Bonny per s.s. 'Sokoto' (Captain Wyndham). |
| " 18 | Arrived at Bonny. |
| June 18 | Two members started on a tour of 'the creeks.' |
| | Arrived at Bakana (Slave Trees). |
| " 20 | Visited native town at Top Bakana. |
| | Arrived at Bugama. |
| " 21 | Arrived at Degema. |
| " 23 | Visited Egwanga, up the Opobo River. |
| " 24 | Walked through the bush to Kwatown, four miles from Egwanga. |
| " 25 | Arrived at the Consulate, Opobo. |
| " 26 | Visited native town of Opobo. |
| " 28 | Returned to Bonny. |
| July 18 | One of the members started for Okrika and Degema. |
| " 22 | Returned to Bonny. |
| Aug. 1 | One of the members sailed for the Opobo River. |
| " 2 | Left Opobo and reached Akwete. |
| " 5 | Visited Obunko and Ohumbele. |
| " 6 | Reached Azumini. |
| " 8 | Returned to Akwete. |
| " 9 | Arrived at Bonny. |
| " 10 | Dr. Elliott returned to Liverpool per s.s. 'Sokoto.' |
| " 11 | Drs. Annett and Dutton proceed to Degema. |
| " 13 | Arrived at Akassa, by steam launch through the creeks. |
| " 20 | Visited Brass. |
| " 21 | Started for Agberi, on the River Niger. |
| " 22 | Arrived at Agberi. |
| " 23 | Reached Abo. |
| " 24 | Visited Utshi—reached Asaba. |
| " 28 | Visited Onitsha. |
| Sept. 4 | Started for Lokoja. |
| " 5 | Passed Idah. |
| " 6 | Arrived at Lokoja. |

| | | |
|---|---|---|
| 1900 | | |
| Sept. | 13 | Returned down stream by Niger Company's steam launch 'Nupe.' |
| " | 14 | Arrived at Onitsha. |
| " | 20 | Visited Abutshi. |
| " | 27 | Sailed for Burutu. |
| " | 28 | Arrived at Abo. |
| " | 30 | Arrived at Ganagana. |
| " | 31 | Arrived at Burutu. |
| Oct. | 8 | Sailed for Liverpool per s.s. 'Bornu' (Captain Hele). |
| " | 28 | Arrived at Liverpool. |

# REPORT OF THE MALARIA EXPEDITION TO NIGERIA OF THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE AND MEDICAL PARASITOLOGY

## I. PRELIMINARY

I.—*Introduction.* Scientific research and investigation in the subject of the aetiology of malarial fever during the last twenty years of the past century furnished results unequalled in their important bearing, not only in the science of parasitology, but more especially on the imperial question of the colonisation of the notoriously unhealthy parts of tropical and subtropical countries.

In 1880 Laveran,[1] in Algiers, first discovered adventitious living organisms in the red blood corpuscles of patients suffering from malarial fever, which were characterised by the possession of a dark pigment, and by their capability of executing amoeboid movements.

In 1889 Golgi,[2] of Pavia, by studying the 'rosace' forms, was able to differentiate between tertian and quartan forms of fever, and to trace out the processes of maturation and sporulation of the parasites and their relations to the periodicity of these fevers.

In 1894, Manson[3] originated the idea that the malarial parasite was capable of an existence outside the human body, in which the 'flagellate' form played an important rôle. He was led to suggest that the mosquito served as a host for the further development of the parasite.

Ross, in 1897[4] and succeeding years,[5] established the truth of these ideas, and first succeeded in the cultivation of the crescent form of the aestivo-autumnal parasite (*Haemomenas praecox*) in the stomach of *Anopheles rossii*. In 1898 the life-history of a similar parasite of birds (*Proteosoma grassii*, *Haemamoeba relicta*) was worked out by him—from the formation of zygotes from the fertilised female parasite (*macrogamete*) in the stomach wall of *Culex pipiens* to the collection of the germinal rods (blasts, sporozoïtes) in the cells of the salivary glands. The complete biological cycle of the parasite was furnished by the production of infection in healthy birds by the bites of mosquitoes previously fed on infected birds. Confirmation of these researches, both in general bearing and in detail, has been afforded by the observation of the fertilisation process in the case of halteridum of birds (*Haemamoeba danilewskii*), as well as of human aestivo-autumnal parasites (*Haemomenas praecox*) by MacCallum,[6] and further of the formation of the 'vermicule' in the case of proteosoma of birds (*H. relicta*), by Koch,[7] and in the case of human malaria by Grassi,[8] who also observed by histological processes the passage of the 'vermicule' through the epithelial lining of the stomach of the mosquito.

The Italian pathologists and zoologists, Bastianelli and Bignami,[9] Celli[10] and Grassi,[11] and others having peculiar facilities and opportunities for research in the subject in Italy, have contributed many confirmatory articles, and also by considerable experimental work have brought forward evidence which exculpates mosquitoes of the genus *Culex* from any part in the transmission of human malarial fever.

The interesting experiment of Manson and Thorburn,[12] who, in London last year, allowed themselves to be bitten by mosquitoes infected from a case of tertian fever in Italy, and after fourteen days acquired a typical attack, exhibiting tertian parasites in the blood, afforded a strikingly conclusive confirmation of the work of Ross and of Italian observers.

These discoveries naturally opened up the question as to how the results obtained might be turned to practical account for the prevention of malarial fever, so that commissions and expeditions were despatched to the tropics to ascertain at what period of the life-history of the parasite in man and in the mosquito the parasite is the most vulnerable, and how the attack might best be delivered.

In 1899 there were sent out a German Commission under Professor Koch in German East Africa, a Royal Society's Commission to British Central Africa, and the expedition to West Africa from the Liverpool School of Tropical Medicine under Major Ross. In 1900, another German Commission, under Professor Koch, was despatched to the East Indies ; the Royal Society's Commission visited West Africa, and a second expedition from the Liverpool School of Tropical Medicine was sent to West Africa.

*Objects of the Expedition.*—The experience of Major Ross in India, and the consideration of the results of his work, led to the conclusion that the parasite could be most easily attacked during its life in the mosquito, and as there was considerable evidence to exclude mosquitoes of the genus *Culex* from taking any part in the transmission of malarial fever, the first expedition of the Liverpool School of Tropical Medicine, of which one of us was a member, was undertaken to study the bionomics of mosquitoes of the genus *Anopheles*, with a view to suggesting better modes of prevention of malarial fever than those hitherto known to us.[13] As to the conclusions arrived at and the methods suggested, these will be found fully described in the report of the expedition.[14]

The objects of the present expedition were as follows :—

(1) To further explore West Africa to ascertain under what varied conditions mosquitoes of the genus *Anopheles* lived and propagated, with a view of ascertaining the most feasible and practical methods of preventing malarial fever.

(2) To investigate the conditions under which malarial fever is conveyed to Europeans.

(3) To corroborate and extend recent discoveries and researches on the subject.

It was not intended to limit observations to malarial fever alone, but to study also other tropical diseases as opportunity arose, and to note in addition the general sanitary condition of the places visited.

*History of the Expedition.*—Nigeria, Northern and Southern, were chosen for the field of operations.

The expedition consisted of :—

H. E. Annett, M.D., D.P.H. (Vict.), Demonstrator in Tropical Pathology, Liverpool School of Tropical Medicine.

J. Everett Dutton, M.B., B.Ch. (Vict.)

J. H. Elliott, M.D. (Toronto).

The proceedings of the members of the expedition during the seven months in West Africa are given in the diary of the expedition's movements. The first five weeks were spent at Old Calabar, but the work of the expedition here was almost fruitless, because of the condition of the natives, who are a dull, unintelligent people, understanding almost no English. For the next three months, Bonny, a very old and well-known trading station, was made headquarters, and here most of the work of the expedition was done. Accommodation was spacious, and intercourse with the natives was easy ; English is spoken, and civilisation fairly advanced. Numerous experiments and researches were carried on here, and in view of what the later experience of the expedition proved, it was unfortunate that a longer stay was not made. From the time of leaving Bonny, the work of the expedition became more or less exploratory only. The Niger district of Southern Nigeria having only recently been taken over by the Government, the expedition found at all the places on the Niger and its delta which were visited, accommodation so scanty as to render scientific investigation impossible, in fact, in some places actual living was rough and uncomfortable. In Northern Nigeria, Lokoja alone was visited, and here, again, the accommodation which was available rendered a longer stay than a few days impossible.

## II. TOPOGRAPHY AND STATISTICS

Roughly, Nigeria may be divided in its topographical characters into three regions—the region of mangrove swamp, with but little vegetation beyond the mangrove tree, lining the coast and reaching inland to an extent varying from a few to a hundred miles; studded here and there with the low-lying, dirty, swampy towns and villages of the African negroes. Beyond this is the thickly-forested belt—the palm oil region—with much cleaner and often well-arranged towns; and natives engaged chiefly in agricultural pursuits. Further inland is the deforested tract extending to the edges of the central desert. This country is hilly and undulating, and covered with a short scrub and but few trees, and is chiefly occupied by tribes professing Mohammedism. Here and there the depressing monotony of the extent of mangrove swamp is relieved along the course of the rivers or the streams forming the delta, by the occurrence of patches of thickly forested land of small area—anticipating the character of the forested belt further inland.

*Old Calabar*, the chief town of Southern Nigeria and the seat of government administration, is situated about fifty miles up the Old Calabar river, on its left bank. The river up to here is lined with mangrove swamps and studded with islands of mangroves. The town is placed on the edge of the forested plateau which lies beyond the region of swamp. The mangrove region extends on the opposite bank still some miles further inland. The plateau is about two hundred feet high, where it here comes to the river; it terminates rather abruptly, and slopes rapidly to the water's edge. Old Calabar is built on this slope. Two short spurs jut out, so that the town is surrounded in a semi-circular fashion by the edge of the plateau. On the western half of the slope is the native town of Duketown, which is continued over the summit of the spur into the small village of Henshawtown. A creek, some swampy district, and a small valley separate this native half from what may be called the European half of the town. The European quarters are built mostly on the edge of the eastern half of the plateau; government offices and other administrative premises are dotted about on the slope below. Over the easterly spur is the small village of Oldtown, and Quatown is another small village about one-half to one mile inland on the level plateau. The factories of the various trading companies are dotted about on the water's edge from the extreme end of the native town to the end of the European half.

Proceeding up the hill on the European side of the creek from the Queen's beach and the offices of the marine department, are met with, first the customs and post-offices, and the public works department offices about half way up the hill;

The Native Town of Duketown—Old Calabar

ON THE EXTREME RIGHT AND LEFT ARE TWO EUROPEAN FACTORIES, ALMOST SURROUNDED BY NATIVE HUTS. TO THE RIGHT OF THE FACTORY, JUST SHOWING ON THE EXTREME LEFT, IS THE 'CREEK.'

then nearer the top is the Vice-consulate, and on the summit are the Consulate and Medical house. Further round are the Force house and barracks of the West African Frontier Force, the prison, the European hospital, and a Presbyterian mission station.

On the edge of the creek also on the European side of it are built the native hospital and the dwellings of native clerks, court messengers, and orderlies. It is not permitted that native houses should be built on this side of the creek, thus procuring a distance of about half a mile between the government European quarters and the native town. The few native huts which have existed there for a long time before the present European quarters were built are being gradually removed.

The vicinity of these quarters has been excellently laid out, and is kept in remarkably good order. Excellent roads, well ditched with cemented gutters, cross the hill in all directions ; the quarters are fairly well designed. The total European community at Old Calabar numbers about one hundred and twenty.

Duketown presents a marked contrast to the European half of the town. Here the native huts are crowded together, although some efforts have been made by the medical and public works officers of the district to construct wide roads and to improve the sanitary condition of the town. Narrow winding and irregularly made paths run down the hill side between the native huts which, reaching down to the beach, are crowded round the factories of the trading companies there. On the whole, however, the huts, which are of clay, are fairly clean, although the Calabar native is unintelligent, lazy, and but little influenced as yet by civilisation. Another mission station is built near the summit of this hill, surrounded by native houses.

Throughout the native town and villages on the river's edge numerous dug-out canoes, drawn up to the neighbourhood of the native huts, formed the principal breeding-places of *Anopheles*—and here and there a 'puddle' in the clay at the sides of the paths contained larvae. On the edges of the 'creek,' besides innumerable canoes, several small puddles in this swampy district were found to contain larvae also.

A narrow footpath runs along the whole length of the beach behind the trading factories, and from one factory to the next. This is badly constructed, and permits of the formation of shallow pools during the rains—we observed these several times with innumerable *Anopheles* larvae. The proximity of the native huts to the factories, and the presence of many breeding-places of *Anopheles* in their immediate vicinity, explain the prevalence of malarial fever among the Europeans here, who, moreover, from the low position of the factories on the water's edge and from the general contour of the district, are denied the enjoyment of those refreshing breezes which is secured by the Europeans in the more favourable position on the hill.

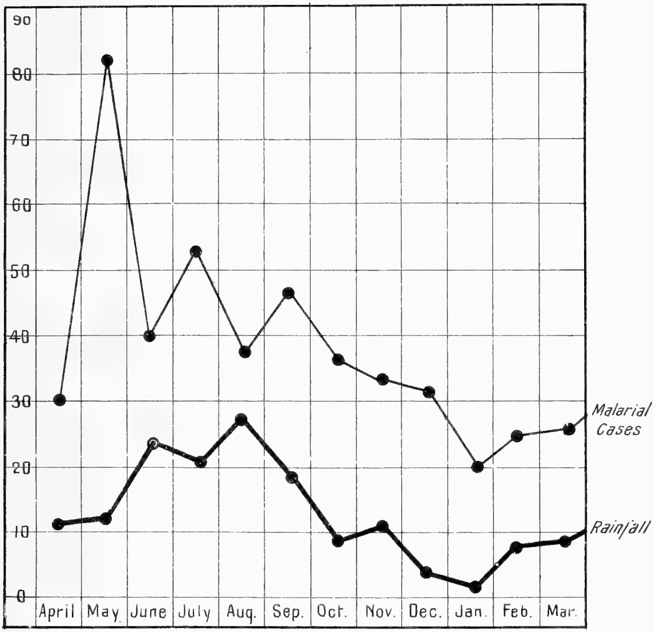
Near the quarters of the Government officials on the hill no breeding-places of *Anopheles* could be found during the period of our visit, except a small 'duck' pond near the consulate. The water of this cemented pond was so often disturbed by fowls, turkeys, and other poultry, that never more than very few larvae were found here. Since our visit it has been regularly emptied.

The water supply of the natives is a spring in the valley above the 'creek'—that of Europeans is rainwater caught from the roofs of their quarters, and stored in large iron tanks. A scheme is on foot, however, to bring water to the town from a spring about two miles distant, in the bush, and engineering operations are at present being carried out. The spring occurs at the bottom of what appears to be a natural depression some one hundred feet deep. A well has been already bricked out here to the depth of some eight feet, and the neighbourhood of this was at the time of our visit flooded over a small area around. Here innumerable larvae were found. The district is visited by many natives for drinking water, and a number of labourers are employed during the day in the structural operations. These and the occupants of a small hut on the edge of the depression are the only people within half a mile of the place. The presence of such a large number of *Anopheles* larvae was therefore remarkable.

Of the other places in Southern Nigeria visited by the members of the expedition but little description need be here given—they will be sufficiently referred to in subsequent chapters. The European communities at these places seldom exceed ten in number.

*Akassa* must, however, be particularly referred to as a place which, in our opinion, should be at once abandoned by Europeans. A vice-consulate and the engineers' quarters are the only European habitations here, and these have been built on 'made' sites in the midst of the mangrove swamp. Here are the only engineering yards of the country, which were taken over from the Niger Company by the Government at the beginning of last year. Were not the swampy nature of the district sufficient reason for abandoning the place, it has been rendered almost uninhabitable because of the prevalence of malarial fever among the Europeans—brought about by the presence of the small native village on one side and of the dwellings of the native artisans and labourers with their families on the other side of the engineering quarters, while the proximity of the barracks of the native soldiers is a perpetual menace to the health of the officials at the vice-consulate. To abandon this place and to transfer administrative quarters and engineering yards to Brass, a comparatively healthy district some twenty-five miles off, where already an excellent vice-consulate building exists, would, in our opinion, involve the expenditure of a much less sum than will be required to make Akassa inhabitable and at all healthy.

*Statistics.*—It is very desirable that fuller and more detailed accounts of the meteorological conditions occurring at the various stations of Nigeria should be kept: at present there is a deplorable dearth of instruments for these purposes throughout the colony. It is only with considerable difficulty that we have been able to gather




*It is to be noted how closely the number of Cases of Malarial fever among Europeans, follow the variations in the rainfall, and especially how the largest number of cases occur at the time when the rainy season has become fairly established.*

details for the construction of the following tables and charts illustrating the relation between the amount of rainfall and the prevalence of malarial fever.

For an estimation of the rainfall we have found it necessary to take the average fall of three stations in the colony, and for the number of cases of malarial fever we were not able to obtain the reports of all the stations.

The following table gives the average rainfall per month of three stations in Southern Nigeria (one of which is Old Calabar) and the total number of cases reported as malarial fever among Europeans. It is necessary to point out that a few of the cases among Europeans were probably not truly malarial in character.

| | Average Rainfall | Total Number of European Cases |
|---|---|---|
| 1899—April ... ... ... | 10·46 | 30 |
| „ May ... ... ... | 11·71 | 83 |
| „ June ... ... ... | 24·77 | 40 |
| „ July ... ... ... | 21·13 | 54 |
| „ August ... ... ... | 27·09 | 38 |
| „ September ... ... | 19·65 | 47 |
| „ October... ... ... | 9·08 | 37 |
| „ November ... ... | 10·40 | 33 |
| „ December ... ... | 4·58 | 31 |
| 1900—January ... ... | 2·68 | 20 |
| „ February ... ... | 8·63 | 25 |
| „ March ... ... ... | 9·43 | 26 |

(Chart I)

It is to be noted how closely the number of cases of malarial fever among Europeans follows the variations in the rainfall, and especially how the largest number of cases occur at the time when the rainy season has become fairly established. Allowing for the period of the life history of the malarial parasite in the mosquito, and for an incubation period of the disease in man of from seven to twenty days, the relation of cases to rainfall is such as would have been anticipated from a consideration of the nature of the breeding places of *Anopheles* in the districts considered.

*Lokoja* is the most important town of Northern Nigeria. It is situated at the confluence of the Niger and Benue rivers, at a distance of about four hundred miles from the sea coast. Behind the town is a hill—Mount Patti—reaching about one thousand feet in height, and running almost parallel to the direction of the river bank. The hill slopes steeply down for a distance of about seven hundred feet, from which point the slope is less steep and takes the form of four low ridges with intervening vales running at right angles to the river bank. The most southerly of these ridges is called the 'hospital hill'—on which are built the European hospital, the quarters of the medical officers and of nurses and dressers.

The next is the 'barracks' hill with the bungalows of the officers of the First West African Frontier Force. The third may be called the 'Residency' hill, having the quarters of the government resident, the post office, and behind these the houses of a number of native clerks and other government employés. On the fourth hill and on the area beyond between Mount Patti, which here approaches nearer to the river, and the bank is the native town.

A road about three miles in length starts from the neighbourhood of the hospital, and, crossing the barracks and residency hills, enters the native town. On each side of this road, as it passes down the side of the hospital hill and up the barracks hill, are grouped the huts of the native soldiers, not very far distant from the bungalows of the Europeans officers. Several other native houses are dotted about near these bungalows.

In each of the valleys between the hills runs one or more small hill streams.

Between the residency and the river, on the sides of the road already mentioned, a bank and other European quarters are in course of construction.

On the water's edge and completely surrounded on one side and at the back with native huts, which reach up almost to the very boundary rail of the compound, is the factory of the Niger Company. Here there are usually some ten or twelve Europeans employed, while at the barracks live often as many as thirty.

Soldier Town is the name given to the district near the river at the base of the ridge known as 'hospital hill,' which is occupied by the greater number of the soldiers of the force with their families.

It is not difficult when one considers the manner in which the dwellings of Europeans are everywhere surrounded by those of natives at Lokoja, to understand how the town has gained for itself a reputation of unhealthiness, and more particularly the barracks of the West African Frontier Force, which might with very little trouble be made quite healthy.

The European quarters have throughout the town been allowed to become encroached upon by the native huts. As already mentioned, the Niger Company's factory has native houses close to its boundary rails : the bungalows of the officers have a collection of soldiers' huts, and a few other scattered huts not very

PORTION OF THE BARRACK SQUARE AT LOKOJA, SHOWING A EUROPEAN OFFICER'S BUNGALOW, AND TWO OR THREE NATIVE HUTS CLOSE BY. TO THE RIGHT IS A STACK OF ANT-PROOF PILES, SIMILAR TO THOSE ON WHICH THE BUNGALOWS ARE BUILT. THEY ARE PARTIALLY OVERGROWN WITH GRASS.
THE TRAPS CONTAIN WATER WITH MANY *Anopheles* LARVAE

far distant from them, while behind the residency are the houses of native clerks. In all these cases it would not be difficult, considering the ease with which native huts can be pulled down and re-built, to remove this dangerous condition.

Moreover, the neighbourhood of the European quarters provided many places for the breeding of *Anopheles*. In the barracks square an interesting condition was met with. The bungalows are supported on ant-proof piles, that is, piles provided with a cup to contain water. In these cups when they happened to have water in them were found *Culex* larvae in great numbers. About the centre of the square was a collection of such piles which, not having been moved for some time, were partly overgrown and hidden by long grass. The cups of these piles were found to contain *Anopheles* larvae. This illustrates the peculiar habits of the *Anopheles*, for though in this undisturbed position they were numerous, in similar cups under the bungalows none could be found. The whole surroundings afforded a beautiful example, shewing the source of infection (there were several native huts scattered about close by), and the means by which the infection was easily carried by *Anopheles* bred close at hand, to the Europeans in their bungalows.

A similar example was also provided by the conditions around the factory of the Niger Company. Between the factory boundary and the native huts we discovered a typical *Anopheles* pool containing numerous larvae. The pool was a portion of a very badly constructed drain which ran through the factory's area, which in its course also afforded several other breeding-places.

Besides these, there were many other breeding places of *Anopheles*. In a subsequent chapter it will be pointed out that the construction of roads and footpaths as carried out in many parts of West Africa provides numerous such breeding places. Such occurred on a large scale at Lokoja. The road already mentioned and other roads off this were found by us so badly ditched on each side, that instead of serving to quickly remove water, they permitted of the formation of a long string of puddles on each side, containing numerous *Anopheles* larvae. In some places, in spite of the fact that there is a good natural slope down the hillsides, the bed of the ditch formed a series of steps, water lodging on each. One road in particular is to be mentioned. It runs down the 'hospital' side of the 'barracks' hill. On each side are huts of native soldiers, at the top the officers' bungalows. This road was reconstructed during our visit to Lokoja, and after the work its condition was as bad as before. These samples serve to illustrate how the unhealthy condition of a European settlement is brought about often by the Europeans themselves, by permitting the natives to settle beside them, and further by the unwitting neglect of elementary sanitary and engineering principles.

Parts of the small hill-streams already referred to as running in the shallow valleys a.,o provided *Anopheles* breeding places. These streams become torrents during heavy rain, quickly washing out any larvae; but, during gentle rains, parts of them, where they swell out into shallow pools with sluggish stream, form excellent breeding-places, and probably in the dry season, when they almost dry up, the pools last remaining perform a similar function. By wading along the course of some of these streams we are able to locate the breeding-places of *Anopheles* which they provided, always at a distance not exceeding two or three hundred yards from human habitations. Beyond this distance above the district of huts and bungalows we found no larvae.

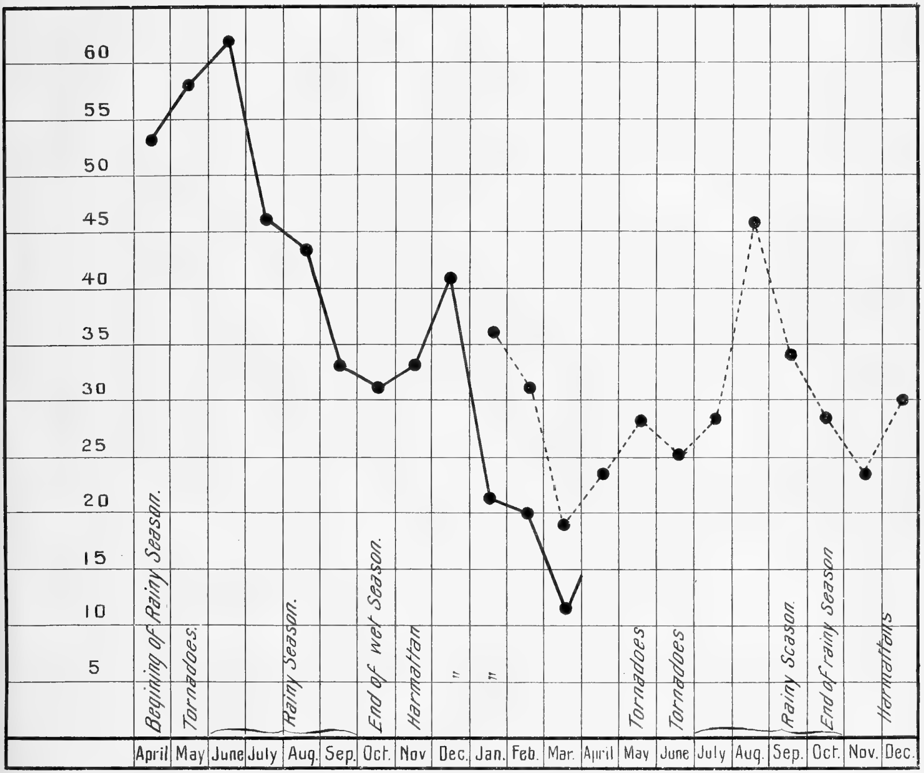
Still another condition was observed at Lokoja. At a point near the river bank we found a small area of cultivated land. We were unable to ascertain precisely the nature of the produce, but it was probably rice. The surface was dug so as to form rows of butts and furrows. One portion of the area was swampy, and water puddles were formed for a short distance along many of the furrows, and also along a narrow footpath crossing the field. In many of these puddles *Anopheles* larvae were found. Habitations in the immediate neighbourhood were scarce, but at a distance of two hundred yards was a collection of native huts.

*Statistics.*—Northern Nigeria.—The only statistics available are those given in the reports of the medical officers of the West African Frontier Force.

(Chart II)

The points of the line curve represent the percentages per month of the total European force admitted into hospital. Those of the dotted curve shew the actual number of cases admitted per month. Both curves shew a marked increase in the number of cases of malarial fever after the beginning of the rainy season, and the former also a smaller increase after the cessation of the rains. During the course of the wet season itself the breeding-places of *Anopheles* would be continually flooded out, but during its onset and cessation the insects would be able to breed continuously.

*The following table has been constructed from the reports of Dr Wordsorth Poole, Principal Medical Officer, W.A.F.F. from January 1898, to March 31st, 1899, and of Dr McDowell, Acting Principal Medical Officer, for the year ending December 31st, 1899.*



## III. HAEMAMOEBIDAE IN NIGERIA

*The Haemamoebidae in Definitive Hosts.*—The nature of the expedition and its objects did not permit of a very extensive and minute investigation into the question of the proportion of mosquitoes infected, and the nature of the infection in the numerous places visited. During our three months' stay at Bonny, however, a number of mosquitoes comprising both *Anopheles costalis* and *A. funestus* were dissected and examined microscopically. These were obtained from huts in the native town, as well as from the European quarters of the two factories at Bonny and their outhouses. A total number of two hundred and eighty-one *Anopheles* were dissected, and seven only found to be infected. Roughly, much less than half of these were obtained from the native town, and since, in the light of later observations, it is very probable that the infected insects came from the native town, it can be roughly estimated that, whereas of the total only 2·6 per cent. showed infection, considering the dilution by mosquitoes from the neighbourhood of the factories where no native children dwelt, the percentage of infected mosquitoes in the native town probably exceeded six.

The nature of the infection in the seven *Anopheles* was as follows :—The exact type of parasite—tertian, quartan, or aestivo-autumnal—was difficult to decide.

(1) One mature zygote, showing typical arrangement of zygotoblasts.
(2) One large zygote, showing a few granules of fine pigment.
(3) One mature zygote, with zygotoblasts.
(4) One zygote about half developed.
(5) One zygote about five days old.
(6) Two large zygotes, not quite mature, containing few pigment granules. Salivary glands infected.
(7) Two small zygotes with pigment.

The great rarity with which 'gametes' of any type are met with in the blood of Europeans in West Africa, and the utmost difficulty experienced in work among native children, rendered any experiments requiring the feeding of mosquitoes on infected subjects, which it had been our intention to pursue, impossible during the comparative short stays we were able to make at different stations. Such investigations would require a long stay at one place.

*Haemamoebidae in Intermediary Hosts.*—The most striking result of the recent scientific expeditions to tropical countries for the study of the aetiology of malarial fever is the discovery, first by Professor KOCH, the director of the German Malaria Expedition, that the native children of tropical towns and villages are infected to an enormous extent with the parasites of malarial fever, and that they probably constitute the only source from which the disease is conveyed, by the agency of mosquitoes of the genus *Anopheles*, to Europeans. This has been corroborated by the members of the Royal Society's Commission in West Africa, and also by us. Professor KOCH's reports[1] show that he found malarial parasites in the blood of a proportion of the native children under two years of age, to the extent, in some places, of 100 per cent.; of children between two and five, up to 46·1 per cent. ; and between five and ten, up to 23·5 per cent. were infected ; while in natives over ten, none were found to contain parasites. The proportions obtained by the Royal Society's Commission in West Africa, who also discovered the infection of native children independently, are somewhat higher than these ; at Accra[2], from 23 to 90 per cent. of 'babies' ; from 20 to 57 per cent. of children up to eight years ; from 28 to 30 per cent. up to twelve years ; and over that age infection was rare ; while at Lagos, of children under two years, from 50 to 100 per cent.; between two and five years, 40 to 75 per cent. ; and between five and ten years, 25 per cent. showed either parasites or pigmented leucocytes in their blood, the percentage varying according to the locality.

We were able at almost every town we visited to obtain a number of children for examination. The following tables show our results :—Our method of examination consisted in making a blood smear of about two inches in length and half an inch in breadth. After drying and fixing in absolute alcohol, the preparation was stained by a modification of ROMANOWSKY's method for 'chromatin' staining, and the whole smear carefully examined with a one-twelfth oil immersion objective (ZEISS). The presence of pigmented leucocytes was not specially recorded in the results, the presence of parasites alone serving as the index of infection. The number of parasites found varied from two or three per whole smear up to a large number in every field of the microscope. The ages of the children are given as nearly as could be judged from a general glance. It is possible that in some cases they may be one or two years out. Of those over ten years the majority were from ten to fourteen ; only very few between fourteen and eighteen were examined. The different places at which examinations were possible are as follows :—

(*A*) *Bonny Town.*—The native town is close to the Government Vice-consulate and European factories. Many of the specimens were obtained by a house to house visitation indiscriminately, others by visits to huts belonging to one or two Bonny chiefs.

(*B*) *Herbert Jumbo's Plantation.*—This is some five miles up the river from Bonny Town. Here lives the chief, Herbert Jumbo, in the midst of his men and boys and their families, previously 'slaves.'

(*C*) *Ju-Ju Town*, *Bonny*.—This is the name given to another plantation nearer the mouth of the river, belonging to Chief William Brown. The conditions here are similar to those above described. There are no Europeans in the district.

(*D*) *Akwete Town*.—There are no European traders here, but a Consular Court necessitates the continual presence of one or two Government officials.

(*E*) *Egwanga Town*.—The specimens were taken from children of a small native village, which practically surrounds the traders' factories here.

(*F*) *Onitsha*.—The children who furnished specimens came to the dispensary of the Church of England Missionary Society's station here, or to the school belonging to the same Society.

(*G*) *Akassa*.—Some specimens were obtained from the children of the Hausa soldiers here, who lived in huts in close proximity to the Vice-consulate ; others from children of native engineers, etc., employed in the engineering yard. In the midst of them live three or four Europeans in the Government service.

(*H*) *Asaba*.—The only children obtainable were those of the Hausa soldiers, one or two companies being stationed here.

(*I*) *Lokoja Town*.—The nominal King of Lokoja could prevail upon only very few of his people to provide us with specimens.

(*J*) Others were obtained from the barracks of the Hausa soldiers at Lokoja. The Yoruba soldiers refused absolutely.

| | A. Bonny Town | | | B. Plantation | | |
|---|---|---|---|---|---|---|
| Ages | No. Examined | No. Infected | Percentage | No. Examined | No. Infected | Percentage |
| 0-1 | 6 | 0 | ... | 2 | 0 | ... |
| 1-2 | 6 | 4 | ... | 7 | 2 | ... |
| 2-3 | 7 | 3 | ... | 4 | 2 | ... |
| 3-4 | 16 | 9 | ... | 6 | 4 | ... |
| 4-5 | 7 | 2 | ... | 6 | 3 | ... |
| 5-6 | 3 | 0 | ... | 2 | 1 | ... |
| 6-7 | 4 | 0 | ... | 4 | 1 | ... |
| 7-8 | 3 | 2 | ... | 2 | 1 | ... |
| 8-9 | 2 | 0 | ... | 6 | 2 | ... |
| 9-10 | 1 | 0 | ... | 1 | 0 | ... |
| 10+ | 12 | 1 | ... | 18 | 1 | ... |
| 0-5 | 42 | 18 | 42·8 | 25 | 11 | 44·0 |
| 5-10 | 13 | 2 | 15·3 | 15 | 5 | 33·3 |
| 10+ | 12 | 1 | 8·3 | 18 | 1 | 5·5 |
| Total... | 67 | 21 | 31·3 | 58 | 17 | 29·3 |

| | C. Ju-Ju Town | | | D. Akwete | | |
|---|---|---|---|---|---|---|
| Ages | No. Examined | No. Infected | Percentage | No. Examined | No. Infected | Percentage |
| 0-1 | 1 | 0 | ... | 10 | 1 | ... |
| 1-2 | 11 | 8 | ... | 4 | 2 | ... |
| 2-3 | 12 | 8 | ... | 8 | 3 | ... |
| 3-4 | 9 | 5 | ... | 3 | 1 | ... |
| 4-5 | 12 | 7 | ... | 4 | 2 | ... |
| 5-6 | 4 | 0 | ... | 4 | 1 | ... |
| 6-7 | 10 | 1 | ... | 2 | 0 | ... |
| 7-8 | 9 | 4 | ... | 5 | 1 | ... |
| 8-9 | 2 | 1 | ... | 1 | 0 | ... |
| 9-10 | 1 | 0 | ... | 5 | 0 | ... |
| 10+ | 1 | 0 | ... | 2 | 0 | ... |
| 0-5 | 45 | 28 | 23·0 | 29 | 9 | 31·0 |
| 5-10 | 26 | 6 | 62·2 | 17 | 2 | 11·7 |
| 10+ | 1 | 0 | ... | 2 | 0 | ... |
| Total... | 72 | 34 | 47·2 | 48 | 11 | 22·9 |

| | E. Egwanga | | | F. Onitsha | | |
|---|---|---|---|---|---|---|
| Ages | No. Examined | No. Infected | Percentage | No. Examined | No. Infected | Percentage |
| 0-1 | 3 | 1 | ... | 0 | ... | ... |
| 1-2 | 4 | 3 | ... | 4 | 2 | ... |
| 2-3 | 8 | 7 | ... | 2 | 2 | ... |
| 3-4 | 3 | 2 | ... | 5 | 1 | ... |
| 4-5 | 2 | 1 | ... | 4 | 1 | ... |
| 5-6 | 2 | 1 | ... | 4 | 2 | ... |
| 6-7 | 2 | 0 | ... | 5 | 0 | ... |
| 7-8 | 0 | ... | ... | 2 | 1 | ... |
| 8-9 | 1 | 1 | ... | 1 | 0 | ... |
| 9-10 | 0 | ... | ... | 2 | 0 | ... |
| 10+ | 0 | ... | ... | 0 | ... | ... |
| 0-5 | 20 | 14 | 70·0 | 15 | 6 | 40·0 |
| 5-10 | 5 | 2 | 40·0 | 14 | 3 | 31 0 |
| 10+ | 0 | ... | ... | 0 | ... | ... |
| Total... | 25 | 16 | 64·0 | 29 | 9 | 21·4 |

| | G. Akassa | | | H. Asaba | | |
|---|---|---|---|---|---|---|
| Ages | No. Examined | No. Infected | Percentage. | No. Examined | No. Infected | Percentage |
| 0-1 | 3 | 1 | ... | 5 | 3 | ... |
| 1-2 | 2 | 0 | ... | 1 | 1 | ... |
| 2-3 | 1 | 1 | ... | 2 | 2 | ... |
| 3-4 | 1 | 0 | ... | 3 | 2 | ... |
| 4-5 | 0 | ... | ... | 2 | 2 | ... |
| 5-6 | 2 | 2 | ... | 0 | ... | ... |
| 6-7 | 1 | 0 | ... | 2 | 0 | ... |
| 7-8 | 2 | 1 | ... | 1 | 0 | ... |
| 8-9 | 0 | ... | ... | 2 | 0 | ... |
| 9-10 | 0 | ... | ... | 2 | 2 | ... |
| 10+ | 0 | ... | ... | 0 | ... | ... |
| 0-5 | 7 | 2 | 28.5 | 13 | 10 | 76.9 |
| 5-10 | 5 | 3 | 60.0 | 7 | 2 | 28.5 |
| 10+ | 0 | ... | ... | 0 | ... | ... |
| Total... | 12 | 5 | 41·6 | 20 | 12 | 60.0 |

| | I. Lokoja Town | | | J. Lokoja Soldiers | | |
|---|---|---|---|---|---|---|
| Ages | No. Examined | No. Infected | Percentage | No. Examined | No. Infected | Percentage |
| 0-1 | 0 | ... | ... | 7 | 4 | ... |
| 1-2 | 1 | 1 | ... | 6 | 6 | ... |
| 2-3 | 1 | 0 | ... | 4 | 3 | ... |
| 3-4 | 0 | ... | ... | 1 | 0 | ... |
| 4-5 | 2 | 1 | ... | 2 | 1 | ... |
| 5-6 | 1 | 1 | ... | 1 | 0 | ... |
| 6-7 | 0 | ... | ... | 0 | ... | ... |
| 7-8 | 1 | 1 | ... | 0 | ... | ... |
| 8-9 | 1 | 0 | ... | 0 | ... | ... |
| 9-10 | 2 | 0 | ... | 0 | ... | ... |
| 10+ | 7 | 2 | ... | 0 | ... | ... |
| 0-5 | 4 | 2 | 50·0 | 20 | 14 | 70·0 |
| 5-10 | 5 | 2 | 40·0 | 1 | 0 | ... |
| 10+ | 7 | 2 | 28·5 | 0 | ... | ... |
| Total... | 16 | 6 | 37·5 | 21 | 14 | 66·6 |

These tables show that the number of infected children varies from place to place from 22·9 to 66·6 per cent.; that over ten years of age very few are infected; that children between 0 and five years are infected to a great extent.

The following table shows the total numbers and percentage of children infected at different ages throughout the whole country traversed by the expedition.

TABLE SHEWING THE TOTAL NUMBER OF CHILDREN EXAMINED AND INFECTED THROUGHOUT NIGERIA

| Ages | No. Examined | No. Infected | Percentage Infected |
|---|---|---|---|
| 0-1 | 37 | 10 | 27·3 |
| 1-2 | 46 | 29 | 63·0 |
| 2-3 | 49 | 31 | 63·0 |
| 3-4 | 47 | 24 | 51·0 |
| 4-5 | 41 | 20 | 48·8 |
| 5-6 | 23 | 8 | 34·8 |
| 6-7 | 30 | 2 | 6·6 |
| 7-8 | 25 | 11 | 27·5 |
| 8-9 | 16 | 4 | 25·0 |
| 9-10 | 14 | 2 | 14·2 |
| 10+ | 40 | 4 | 10·0 |
| | | | |
| 0-5 | 220 | 114 | 51·8 |
| 5-10 | 108 | 27 | 25·0 |
| 10+ | 40 | 4 | 10·0 |
| Total... | 363 | 145 | 39·9 |

By plotting down to scale the figures of the last table, with ages as abscissae, and percentages of children infected as ordinates, a curve is obtained shewing the relation of age to infection. This demonstrates that the number infected is greatest between the ages one and three, and is large also up to five years. The curve shows a very decided fall in the sixth year, after which there is a slight rise, and a rapid fall again during the ninth and tenth years. As to the reasons for the fall in the sixth year, nothing can be given—it may be only accidental.

(Chart III)

The curve also shews how immunity from malarial fever is acquired among the natives. There is besides a certain inherited immunity, since it is well-known that the children of Europeans are severely, and often fatally, attacked by the disease in the tropics, while the native children seem to be but little affected. A temperature of 103° F. was once noted in a child of six months, and occasionally small children in arms were met who evidently were sick and had temperatures—but beyond these, most of the children seem to suffer but little inconvenience, although an examination of their blood at the time might reveal numerous parasites in every field of the microscope. Immunity appears to be more or less completely established by the time the age of ten years is reached, in some cases, however, a longer period appears necessary.

This acquired immunity lasts for a considerable number of years, in many cases a life-time—the period really depending on the extent and frequency of infection during childhood and on individual idiocyncrasy. It has been observed that in all the places visited by us a proportion of the native children were infected, and that the proportion varied and, therefore, the chances of exposure to infection varied. It is possible that there exist in West Africa towns and villages where no malarial fever exists—the natives of such places on entering a malarious district would suffer almost as severely as Europeans. It was only occasionally that we met a native adult complaining of fever, and then only slight inconvenience was occasioned by the attack, which seldom lasted more than twenty-four hours. The returns of the Principal Medical Officer for Southern Nigeria shew a number of adult natives treated, entered as suffering from malarial fever. As microscopical examination was but very little used as a means of diagnosis, it is probable that, although some of these cases may have been true malarial fever, others are, no doubt, fevers of a different nature. In two cases of adults suffering from fever, examined by us, ring forms were found in fair number.

The actual extent and nature of the infection in individual cases at the time of examination are given below.

It will be seen that aestivo-autumnal, quartan, and tertian parasites were found, and, at the time of our visit, the majority of the cases were aestivo-autumnal, while there were also a number of quartan cases, and only a few tertian. Probably the

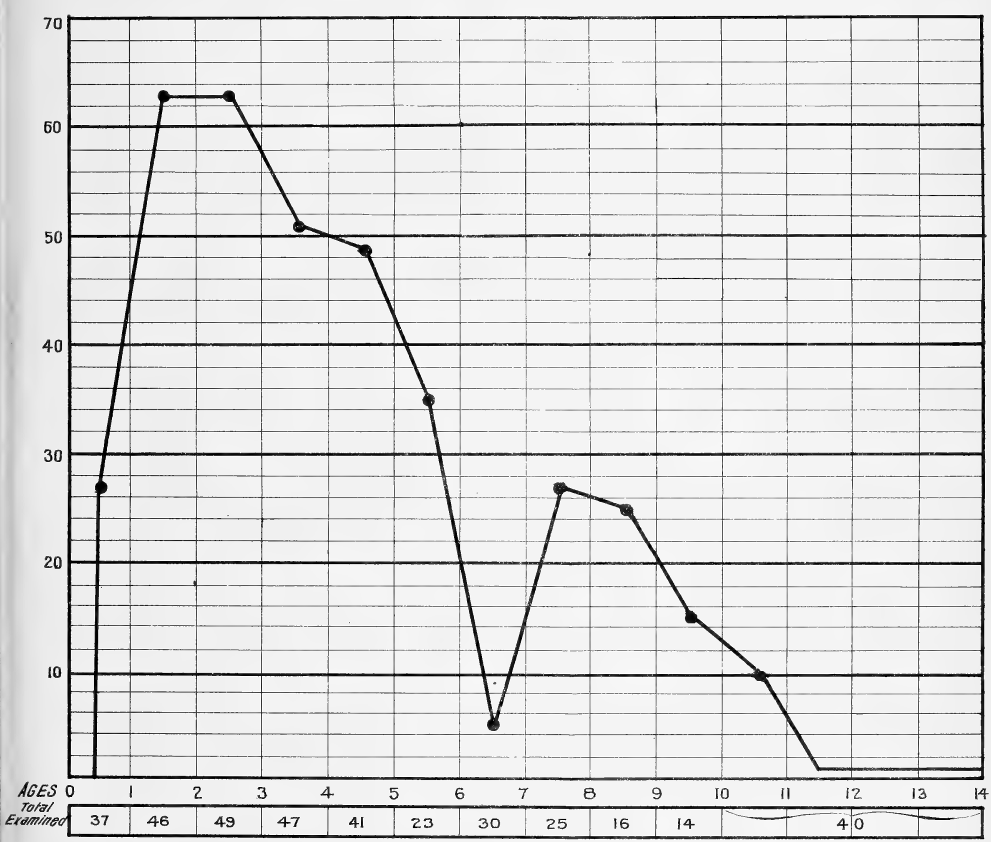



*Table shewing relation of age to Percentage of infected Children.*

proportional numbers of the cases varying according to the time of the year. It will be noted in the following list that crescents (gametocytes) were frequently met with in native blood, and often several were found in each specimen—they are seldom seen, and then only exceedingly few (one or two per blood smear) in the blood of Europeans in West Africa. In the report[1] of the Royal Society's Commission, it was stated, as the opinion of its members, that quartan and tertian parasites do not exist in West Africa, but our specimens, which exhibit all stages of these parasites, from the young forms to fully matured sporocytes and gametocytes, negative this opinion. It must be pointed out that no crescents were observed by them in the blood of native children.

### A. Children of Bonny Town

| Age | No. Examined | Result | |
|---|---|---|---|
| 0-1 | 6 | All negative | The actual ages, where accurately noted, were three, eight, six, eight and eight months respectively |
| 1-2 | 6 | 4 positive | (1) Shewed four crescents<br>(2) An occasional aestivo-autumnal ring form<br>(3) Numerous aestivo-autumnal ring forms<br>(4) One crescent ; one large and two small quartans |
| 2-3 | 7 | 3 positive | (1) An occasional ring form ; all stages of quartan parasites<br>(2) Numerous aestivo-autumnal ring forms—all stages<br>(3) " " " " " " |
| 3-4 | 16 | 9 positive | (1) Two crescents<br>(2) An occasional aestivo-autumnal ring<br>(3) Seven crescents<br>(4) Several aestivo-autumnal rings<br>(5) Few aestivo-autumnal rings<br>(6) A single crescent<br>(7) A few aestivo-autumnal rings<br>(8) Several aestivo-autumnal rings<br>(9) Numerous aestivo-autumnal rings, mostly of large size |
| 4-5 | 7 | 2 positive | (1) An occasional aestivo-autumnal ring<br>(2) Few aestivo-autumnal rings |
| 5-6 | 3 | | |
| 6-7 | 4 | | |
| 7-8 | 3 | 2 positive | (1) Three aestivo-autumnal rings seen<br>(2) Numerous aestivo-autumnal rings—all stages |
| 8-9 | 2 | | |
| 9-10 | 1 | | |
| 10+ | 12 | 1 positive | (1) Numerous aestivo-autumnal rings : actual age twelve-thirteen years |

## B. Herbert Jumbo's Plantation—Bonny

| Age | No. Examined | Result | |
|---|---|---|---|
| 0-1 | 2 | Negative | Ages : six months and unknown, respectively |
| 1-2 | 7 | 2 positive | (1) Several aestivo-autumnal rings<br>(2) Several small and one large quartan form |
| 2-3 | 4 | 2 positive | (1) Occasional ring form<br>(2) Numerous ring forms and all stages of quartan |
| 3-4 | 6 | 4 positive | (1) Few aestivo-autumnal rings<br>(2) Occasional aestivo-autumnal rings<br>(3) " " " "<br>(4) Few aestivo-autumnal rings |
| 4-5 | 6 | 3 positive | (1) Numerous ring forms and all stages of quartan<br>(2) Numerous tertian parasites<br>(3) Several quartan parasites |
| 5-6 | 2 | 1 positive | (1) Numerous aestivo-autumnal rings |
| 6-7 | 4 | 1 positive | (1) All stages of quartan parasites |
| 7-8 | 2 | 1 positive | |
| 8-9 | 6 | 2 positive | (1) Very occasional aestivo-autumnal ring<br>(2) Numerous tertian parasites |
| 9-10 | 1 | | |
| 10+ | 18 | 1 positive | (1) Several aestivo-autumnal rings : age 10-11 years |

## C. Juju Town

| Age | No. Examined | Result | |
|---|---|---|---|
| 0-1 | 1 | | |
| 1-2 | 11 | 8 positive | (1) All stages of quartan parasites<br>(2) Few aestivo-autumnal ring forms<br>(3) Numerous ,, ,, ,,<br>(4) ,, ,, ,, ,,<br>(5) ,, ,, ,, ,,<br>(6) An occasional ring form<br>(7) ,, ,, ,, ,, and a few quartan forms<br>(8) All stages of quartan |
| 2-3 | 12 | 8 positive | (1) Few aestivo-autumnal rings<br>(2) An occasional ring form<br>(3) Several young and a few mature quartan forms<br>(4) Few aestivo-autumnal rings<br>(5) Numerous aestivo-autumnal rings<br>(6) Two crescents<br>(7) Several aestivo-autumnal rings<br>(8) Several young quartan parasites—also sporulating forms |
| 3-4 | 9 | 5 positive | (1) A large, a medium sized, and a small tertian parasite<br>(2) Many aestivo-autumnal rings<br>(3) Few ,, ,, ,,<br>(4) ,, ,, ,, ,,<br>(5) ,, ,, ,, ,, |
| 4-5 | 12 | 7 positive | (1) All stages of quartan<br>(2) Very numerous aestivo-autumnal ring forms<br>(3) Several ring forms, and a few half-matured quartans<br>(4) Several aestivo-autumnal rings<br>(5) All stages of quartan<br>(6) Nine quartan parasites found—almost mature<br>(7) Few aestivo-autumnal rings |
| 5-6 | 4 | | |
| 6-7 | 10 | 1 positive | (1) An occasional ring form |
| 7-8 | 9 | 4 positive | (1) Few aestivo-autumnal rings<br>(2) Several aestivo-autumnal rings<br>(3) Very occasional ring form<br>(4) ,, ,, ,, and two crescents |
| 8-9 | 2 | 1 positive | (1) Several ring forms and a few mature quartans |
| 9-10 | 1 | | |
| 10+ | 1 | | |

## D. Akwete Children

| Age | No. Examined | Result | |
|---|---|---|---|
| 0-1 | 10 | 1 positive | The ages given were three, nine, five, four, four, six, six, eight, and seven months, respectively<br>(1) The positive case was eight months old and contained many young quartan forms, also a few mature and sporulating forms |
| 1-2 | 4 | 2 positive | (1) Few aestivo-autumnal ring forms<br>(2) " " " |
| 2-3 | 8 | 3 positive | (1) Many aestivo-autumnal rings<br>(2) Few " " " and two crescents<br>(3) Several " " " |
| 3-4 | 3 | 1 positive | (1) Few aestivo-autumnal rings |
| 4-5 | 4 | 2 positive | (1) " " " " —all stages<br>(2) Very occasional ring form |
| 5-6 | 4 | 1 positive | (1) Several aestivo-autumnal rings |
| 6-7 | 2 | | |
| 7-8 | 5 | 1 positive | (1) Few aestivo-autumnal rings |
| 8-9 | 1 | | |
| 9-10 | 5 | | |
| 10+ | 2 | | |

## E. Egwanga Children*

| Age | No. Examined | Result | |
|---|---|---|---|
| 0-1 | 3 | 1 positive | Few aestivo-autumnal rings ; age 6 months ; had a temperature of a 103° F. at time of taking blood ; negative cases, ages 2 months and 5 months |
| 1-2 | 4 | 3 positive | (1) Numerous aestivo-autumnal rings<br>(2) Few " " "<br>(3) Many quartan forms—all stages |
| 2-3 | 8 | 7 positive | (1) Few aestivo-autumnal rings<br>(2) Numerous aestivo-autumnal rings<br>(3) Very numerous aestivo-autumnal rings<br>(4) Several aestivo-autumnal rings—one crescent<br>(5) Numerous aestivo-autumnal rings<br>(6) Several aestivo-autumnal rings<br>(7) A few crescents |
| 3-4 | 3 | 2 positive | (1) Few aestivo-autumnal rings<br>(2) " " " several crescents |
| 4-5 | 2 | 1 positive | (1) Few aestivo-autumnal rings |
| 5-6 | 2 | 1 positive | (1) Few ring forms, one large quartan parasite |
| 6-7 | 2 | | |
| 7-8 | 0 | | |
| 8-9 | 1 | 1 positive | (1) Very few ring forms |
| 9+ | 0 | | |

* Since going to press Dr. Hanley, of Opobo, has forwarded more blood smears of native children at Egwanga, which have also been examined.

(1) Age about 6 years—Several ring forms
(2) " " 8 " Negative
(3) " " 5 " Negative
(4) " " 5 " Negative
(5) " " 4 " Many large ring forms
(6) " " 4 " Few ring forms
(7) " " 9 " Two ring forms seen
(8) " " 8 " Numerous quartan parasites—many sporulating
(9) " " 5 " Negative
(10) " " 5 " Many aestivo-autumnal rings
(11) " " 10 " Negative
(12) " " 6 " Numerous aestivo-autumnal rings
(13) " " 6 " Occasional ring form
(14) " " 15 " Negative
(15) " " 6 months—Few ring forms, three crescents

## F. Onitsha Children

| Age | No. Examined | Result | |
|---|---|---|---|
| 0-1 | 0 | | |
| 1-2 | 4 | 2 positive | (1) Several aestivo-autumnal rings<br>(2) Numerous aestivo-autumnal rings |
| 2-3 | 2 | 2 positive | (1) Two crescents<br>(2) Few aestivo-autumnal rings |
| 3-4 | 5 | 1 positive | (1) Many quartan parasites |
| 4-5 | 4 | 1 positive | (1) Few quartan parasites |
| 5-6 | 4 | 2 positive | (1) Occasional ring form<br>(2) " " " |
| 6-7 | 5 | | |
| 7-8 | 2 | 1 positive | (1) A single crescent |
| 8-9 | 1 | | |
| 9-10 | 2 | | |
| 10+ | 0 | | |

## G. Akassa

| Age | No. Examined | Result | |
|---|---|---|---|
| 0-1 | 3 | 1 positive | (1) Several aestivo-autumnal rings (5 months) |
| 1-2 | 2 | | |
| 2-3 | 1 | 1 positive | (1) Few aestivo-autumnal rings |
| 3-4 | 1 | | |
| 4-5 | 0 | | |
| 5-6 | 2 | 2 positive | (1) Few aestivo-autumnal rings<br>(2) " " " " |
| 6-7 | 1 | | |
| 7-8 | 2 | 1 positive | (1) Numerous aestivo-autumnal rings |

## H. Asaba—Soldiers' Children

| Age | No. Examined | Result | |
|---|---|---|---|
| 0-1 | 5 | 3 positive | (1) Very occasional ring form : age 5 months<br>(2) Numerous tertian parasites—all stages<br>(3) Few ring forms, and a few half-matured tertian forms |
| 1-2 | 1 | 1 positive | (1) An occasional ring form |
| 2-3 | 2 | 2 positive | (1) Numerous aestivo-autumnal ring forms : one crescent<br>(2) Few ,, ,, ,, ,, |
| 3-4 | 3 | 2 positive | (1) Several aestivo-autumnal ring forms<br>(2) Numerous ,, ,, ,, ,, two crescents |
| 4-5 | 2 | 2 positive | (1) Few ,, ,, ,, ,,<br>(2) ,, ,, ,, ,, ,, |
| 5-6 | 0 | | |
| 6-7 | 2 | | |
| 7-8 | 1 | | |
| 8-9 | 2 | | |
| 9-10 | 2 | 2 positive | (1) Few aestivo-autumnal rings<br>(2) A very occasional ring form |

## I. Lokoja Town

| Age | No. Examined | Result | |
|---|---|---|---|
| 0-1 | 0 | | |
| 1-2 | 1 | 1 positive | An occasional ring form |
| 2-3 | 1 | | |
| 3-4 | 0 | | |
| 4-5 | 2 | 1 positive | Two crescents |
| 5-6 | 6 | 1 positive | A single ring form seen |
| 6-7 | 0 | | |
| 7-8 | 1 | 1 positive | A single crescent |
| 8-9 | 1 | | |
| 9-10 | 2 | | |
| 10+ | 7 | 2 positive | (1) Age 12-13 years ; few large quartan forms<br>(2) Occasional ring form : age 10-11 years |

## J. Lokoja—Barracks

| Age | No. Examined | Result | |
|---|---|---|---|
| 0-1 | 7 | 4 positive | (1) Few aestivo-autumnal rings—one crescent<br>(2) Several " " "<br>(3) Numerous aestivo-autumnal rings<br>(4) An occasional ring form |
| 1-2 | 6 | 6 positive | (1) Several aestivo-autumnal rings<br>(2) Numerous " " "<br>(3) An occasional ring form<br>(4) Numerous aestivo-autumnal rings<br>(5) " " " "<br>(6) " " " " |
| 2-3 | 4 | 3 positive | (1) Few " " "<br>(2) Several " " "<br>(3) An occasional ring form |
| 3-4 | 1 | | |
| 4-5 | 2 | 1 positive | (1) Several aestivo-autumnal rings |
| 5-6 | 1 | | |

*Haemamoebidae in Europeans.* The habit of taking quinine, prevalent among Europeans in West Africa, on every occasion of indisposition, makes the examination of their blood, during an attack of malarial fever, very difficult. Occasionally we met with a case which showed numerous parasites in every field of the microscope, but more generally found very few, and often no parasites at all. It is common experience that parasites often rapidly disappear from peripheral blood on the administration of quinine.

Dr. Hanley, the District Medical Officer at Opobo, was kind enough to make specimens of blood smears for us from all the Europeans available in his district, independent of the presence of an attack of 'fever' at the time. The results, with some remarks, are given in the following table :—

| Name | Length of present stay on coast | Total time on the coast | Result and Remarks |
|---|---|---|---|
| F | 4 months | ... | Has had 7 years on the Coast, then 7 years away previous to present visit. No parasites |
| H | 10 months | ... | Negative |
| P | 16 months | ... | Negative |
| A | 2 years and 4 months | 10 years | Negative. Has had but little fever. Haemoglobin estimated at 65 per cent. |
| R | 4 months | ... | Negative. Had attack of fever 15 days after arrival on Coast |
| R (2nd specimen) | 4 months | ... | Showed a very occasional ring form. Temperature at time of taking specimen, 101° F. |
| P | 5½ months | 10 years | Negative. Haemoglobin estimated at 80 per cent. |
| H | 18 months | ... | 3 ring forms found |
| T | ... | 30 years | Has an enlarged spleen reaching midway between the umbilicus and the symphysis pubis. Has had fairly good health. Examination of blood, negative. Haemoglobin estimated at 30 and 20 per cent. on the two occasions |
| T (2nd specimen) | ... | ... | |
| G | ... | 2nd voyage | Negative |
| McI | 12 months | ... | One crescent found. Has had bad health lately. One attack of fever eight months after arrival |
| T | ... | 2nd voyage | Negative |
| B | 6 weeks | 2nd voyage | Negative |
| M | 8 months | ... | Negative |
| T | 6 months | 11 years | Negative |
| M | 2 years and 4 months | 8 years | Negative |
| D | 4 months | 6 years | Negative |
| M | 6 months | ... | Negative |
| H | 9 months | ... | Negative |

### Other Haemamoebidae

*Haemamoebidae danilewskii* was found in a large number of birds, viz. :—

| | |
|---|---|
| *Columba livia* | (The common pigeon) |
| *Turtur senegalensis* | (The wild dove) |
| *Passer diffusus* | |
| *Ceryle rudis* | (Black and white kingfisher) |
| *Cypselus affinis* | (Humming bird) |
| *Alcedo guentheri* | (Blue and red kingfisher) |

At Old Calabar a flock of pigeons (common tame pigeon), obtained from the native town, were found infected with *Halteridium* throughout, while at a European factory among pigeons which had been in the country for nine months none were found to be infected. The factory was at some distance from native dwellings, and the pigeons had never mixed with any from the native town. Similarly some pigeons from Bugama, which had been in the country some eighteen months, were not infected—they had no opportunity of contact with pigeons from the native town, which is some two miles distant.

At Lokoja a number of tame pigeons were examined—they were obtained from different sources : some were infected, others not—they had lived in contact some weeks.

Our own collection of pigeons, some of which were infected, were together in a wooden cage for some three or four months, but infection remained limited to those originally infected.

Attempts were made to cultivate *Halteridium* in mosquitoes of both genera, but they failed completely.

*Haemamoebidae relicta—Proteosoma* was never found, although hundreds of birds of many different kinds were examined.

*Other blood parasites*—Numerous domestic fowls and a number of small bats were examined, but no blood parasites were found.

## IV. SOME POINTS ON THE BIONOMICS OF *ANOPHELES*

*Breeding-places.*—Throughout such an extensive country as Nigeria, it was natural to expect considerable diversity in the conditions under which mosquitoes of this genus breed. The conditions varied roughly in the three different belts of country already described, and mixed conditions occurred where one belt emerged into the next.

In the region of mangrove swamps the native dug-out canoes are almost entirely the habitat of *Anopheles* larvae and pupae, for instance at Bonny, Okrika, Opobo, Bakana Town.

The canoes containing larvae are generally old and unused, and drawn up on to the foreshores of the rivers or the edges of the creeks into the neighbourhood of the native huts. There are, in places, a considerable number of these, which are now and then augmented by the addition of other canoes during a period of rough 'sass' weather. They are always more or less full of water according to the rainfall, and many have a green (protococcus) growth on their sides, or contain algae.

Only occasionally are 'puddles' containing larvae to be found in these mangrove districts, and it may be mentioned here that quite as often *Anopheles* larvae are found in water in the bottoms of broken gin bottles, in old calabashes, on the tops of barrels, in tubs, and old iron pots.

In the neighbourhood of European dwellings in this district, consisting mainly of those of Government officials and traders, and usually built on made sites, it is found that the breeding places of *Anopheles* have been ignorantly made by the 'white man' himself. They consisted of 'duck ponds,' cemented or tubbed in (larvae are, however, never found when ducks frequent these ponds), shallow wells, with their sides protected by a palm oil puncheon or other barrel, and occasionally uncovered rain barrels. In some places, *e.g.*, round the Consulate at Opobo, the nature of the surface is such as to favour the formation of a small fresh water marsh, in the puddles of which larvae exist.

On the small areas of fertile land, which are here and there interspersed on the river banks in the midst of the mangrove swamps, and on which the 'factories' of the various trading companies are usually built, the breeding places of *Anopheles* consist of shallow puddles, scattered here and there, permitted by the unevenness of the surface and lack of any systematic attempt at surface drainage. These 'puddles' are kept full of water during the wet season, and are frequent along the footpaths crossing the areas, and along the sides of warehouses. In fact, the presence of the enormous number of mosquitoes at the factories at Slave Trees, Bakana, and at

Portion of the Foreshore at Old Calabar

THE UNUSED DUG-OUT CANOES ARE MORE OR LESS FULL OF WATER CONTAINING *Anopheles* LARVAE
ON THE RIGHT IS THE FACTORY OF A EUROPEAN TRADING FIRM ; THE NATIVE HUTS
ARE BUILT CLOSE UP TO ITS WALLS

Bugama, which are completely surrounded by miles of mangrove swamp, can be traced to no other source. At the African Association's factory at Slave Trees, no other breeding places can exist. The small area on which the factory stands is cleared, and surrounded also by a mud swamp covered by tidal water twice a day. Here myriads of *Anopheles* were encountered.

In the region above the mangrove swamp—the forested belt—the natives are more of an agricultural than a fishing race, and consequently the 'dug-out' canoe is considerably less in evidence as a breeding place, except in towns on the river banks. This district is flat, and its surface clayey. The natives build their huts of clay, dug out of pits in the immediate neighbourhood of their huts. These often contain water in which *Anopheles* larvae are easily found. In addition, 'puddles' are also present in hollows either weathered or worn out in the clayey surface. In the large ponds and in the pools in the neighbourhood of springs from which the native obtains drinking water, and which generally contained fish, we were never able to find larvae, except in the small pieces of water locked off at the edges as the level of the water fell. These conditions obtain at Onitsha, Abutshi, Asaba, Abonnema, Degema, and Egwanga.

In the immediate neighbourhood of European dwellings in this district, bad surface drainage, or the proximity of clay pits from which the 'boys' obtain mud for huts, or of wells from which they obtain their drinking water, supply sites for *Anopheles* larvae.

In the region above the forested belt, the country merges into a tract of deforested, more or less open, country, undulating and hilly, sparsely wooded, and covered with a short scrub. Here the breeding places of *Anopheles* consist of the pools, and back eddies, and sluggish corners in the course of the small streams running between the hills, of the badly-made ditches along the sides of roughly constructed roads and footpaths; and occasionally, in swampy districts, of the furrows between the butts and drills of cultivated fields. Such conditions obtain at Lokoja, in Northern Nigeria.

During the period of the expedition's visit to the river Niger, the rainy season prevailed. This river, which, in the height of the dry season (December to March), is reduced to the condition of a small sinuous canal, only admitting of the passage of launches drawing less than three feet of water, rises during the wet season a height of forty feet, and expands into a great river, reaching in parts a breadth of one to one-and-a-half miles. During the fall of the stream in the dry season it is said that numerous pools are left on the sloping banks, which afford breeding places for innumerable *Anopheles.* In some places, such as Asaba, a most rigorous search, both along the river banks and inland during the wet season, failed to reveal any breeding places for *Anopheles.* Pits from which mud is obtained for hut-building purposes are common; almost each compound has its clay pit, partially filled with clayey water, often quickly drying up in the hot sun, occasionally so replenished by rain showers as to be for a period more or less full of water ; some, however, deep, and always containing water. They also serve in many places as duck ponds. They were examined time after time but no larvae found, although a few *Anopheles* adults could always be procured in the houses in the neighbourhood. It has been surmised that the chief breeding-places of *Anopheles* in these parts are the pools on the river banks above mentioned, and that during the greater part of the wet season *Anopheles* do not breed copiously. Whether this is so, only careful observation during the dry season can determine.

It is to be remarked that *Anopheles* larvae were never found in the bush or at any great distance from human habitation. The greatest distance observed was under half a mile. Near Old Calabar, at a point some two or three miles in the bush, is a spring, which is intended to be utilised for the water supply of Old Calabar. Engineering operations have already been commenced, and a well bricked in to the depth of some eight feet. The immediate neighbourhood of the well is flooded. Innumerable *Anopheles* larvae were found here. The spring occurs at the bottom of a natural depression in the surface, some eighty or a hundred feet deep, which is surrounded with thick forest growth, and approached only by narrow footpaths. The only habitations in the neighbourhood are a small hut on the edge of the depression, and another one about half a mile along one of the paths. Two native villages occur at a distance of about a mile. The spring is visited daily by scores of natives for drinking water, and a number of labourers are at present carrying out engineering operations. The occurrence of such an extensive breeding-place away from any large collection of natives is extremely remarkable.

The fact that *Anopheles* larvae are occasionally found in those sites which are generally occupied by *Culex*, namely, broken bottles, calabashes, iron pots, barrels, etc., tends to suggest that in places where the natural breeding-places of *Anopheles* become, either from scarcity of rain or in consequence of artificial destructive means, very scarce, *Anopheles* will make use of any available water which will last sufficiently long for the purpose of laying their eggs. The conditions above mentioned were met with principally in the middle of Bonny native town, where the usual breeding-places, the dug-out canoes, were at some distance, and any puddle which might form on the narrow footpaths and streets was continually disturbed by the trampling of passers-by.

*Breeding-places of Culex.*—These consisted of sites similar to those already described by many authors as occurring in the immediate proximity of dwelling-houses in the tropics—pots, bottles, tins, cans, calabashes, tubs, barrels, iron vessels, rain tubs, water tanks, pools, puddles, canoes, cocoanut husks, the hollows at the junction of the leaves and stems of the banana tree—and any place where water lodges for a few days. Larvae were found in fire-buckets and other vessels inside houses of Europeans.

The Old Town Spring near Old Calabar

*Anopheles* larvae in great numbers were found in the water of the bricked-out well, and of the flooded area in its immediate neighbourhood

*Ova.*—Besides the characteristic manner in which *Anopheles* ova are deposited on the surface of water, they can also be distinguished from *Culex* ova in other ways. The ovum of *A. costalis* is roughly ovoid in shape ; its length is about 0·48 millimetre, its total breadth 0·16 millimetre. The anterior end is the broader ; the superior surface is slightly concave antero-posteriorly ; this surface is broad anteriorly, narrower posteriorly, and constricted in the centre, presenting a shape somewhat resembling the sole of a boot ; it is limited on all sides by a striated border. The inferior surface, that in contact with the water, is convex.

The chitin of the lower surface is beautifully marked with hexagonal figures, which are well seen in an empty egg case. The upper surface has no such markings, but at each end has five very small rounded bosses of transparent chitin. The distinguishing feature of the *Anopheles* ovum, pointed out by Ross,[2] is the presence of two lateral wings of transparent cuticle. These have a width of about 0·02 millimetre, and are about 0·3 millimetre long. They are attached one on either side of the ovum throughout the greater part of its length. This cuticular structure has a wide, oval area of attachment, extending below in a semi-circular manner on to the inferior surface, but limited above by the serrated edge of the upper surface. It is hollowed out on its upper surface. The free outer edge is serrated, the whole surface presenting a milled appearance. Slight maceration of the ovum separates this structure from the egg case.

The larva lies with its head situated at the broader end of the egg case. The top of the anterior end of the egg case is broken off in a spiral manner to allow of the escape of the larva.

It was noted that when mosquitoes of the genus *Culex* are forced to lay their eggs on a small surface, *e.g.*, on water in a narrow test tube, the eggs are arranged in a pattern very similar to that of *Anopheles* ova.

When freshly laid, the eggs are whitish in colour, becoming black in the course of an hour.

Actual countings of the number of ova deposited at one laying gave the following numbers : 138, 145, 233, and 179.

*Larvae.*—*Anopheles* larvae were occasionally found infected with a parasite (*Brachionus*) which caused them to present a fluffy appearance and hindered their growth, so that they continued in the larval stage for an abnormally long period, and at length died.

The numbers of males and females which hatched out from a number of larvae were counted on several occasions—396 larvae produced 185 males and 211 females.

*Habits of Adult Anopheles.*—In the preceding paragraph it is seen that the numbers of male and female *Anopheles* which hatch out from a batc . of larvae are approximately equal. Nevertheless, out of a large number of *Anopheles* collected inside European and native quarters but very few males were present; from notes made at the time we counted only 22 males to 293 females, but the proportion is probably only about half that, since many females were taken without note being made of their number.

As to what becomes of *Anopheles* during the day, no exact facts have been observed, and whether the majority remain hidden in the darker parts of habitations, or hide among vegetation, is not absolutely certain. But after sunset, clouds of mosquitoes were often observed flying and hovering in characteristic flocks in the neighbourhood of native huts, about eight feet above the ground. On capturing and examining many of these, all were found to be *Anopheles* males.

In native huts it is never difficult to obtain *Anopheles* females; a large number are always found, for example, in the Kroo boys' huts, while perhaps in the European dwelling-house only an occasional one can be caught. In fact, we found it a good practice in many parts where *Anopheles* were apparently scarce in European quarters, to provide the native boys who acted as our servants with mosquito curtains (they often craved for these); a number could be obtained in this way every morning inside the curtains, which had been badly applied by the 'boys.' Many of the Kroo boy servants make themselves rough curtains of any 'cloth' they can obtain; these invariably provide a supply of *Anopheles*.

It is popularly believed that mosquitoes, and more especially those of the genus *Anopheles*, bite only during the evening and night. It is common experience that many of the genus *Culex* bite during the day time; and, as to *Anopheles*, we have often observed these insects alight on different parts of us, and feed voraciously, in broad daylight. It probably depends very considerably on circumstances. If a feed of blood cannot be obtained at evening or night time when the great majority of these insects feed, then they will bite in the day time. For instance, in offices occupied only during the day time, we were able to find *Anopheles* with distended abdomens. In fact, in the darker parts of the rooms, under tables and desks, behind chairs, etc., *Anopheles* were always to be found; and here they rest until an opportunity of feeding is presented to them, be it day or night; or maybe they are disturbed, fly about, and often attack the intruder.

Considerable evidence has now been accumulated to prove that the distance which is traversed by a mosquito is never very great, and extremely rarely reaches so much as half a mile. The fact that their breeding places are always within a short distance of some dwelling, and have only very rarely been found at a distance even of half a mile, negatives the probability of a long flight. The outbreaks of malarial fever on board ships whose crews had never left the ship, were explained when mosquitoes of the genus *Anopheles* were collected on board ships in malarious districts; and cases occurred which, from their long period of incubation, required as an explanation that these insects should be carried on board for a considerable time after leaving the malarious district. The experience of the expedition afforded proof of the truth of this surmise. In making the tour of the 'creeks' behind Bonny, on board the s.s. 'Sobo,' the first stopping place was Slave Trees, Bakana. This place is renowned in those parts for the number of its mosquitoes, which, as has been previously mentioned, are almost all of the genus *Anopheles*. The ship anchored about a quarter of a mile from the shore; at night mosquitoes came on board in large numbers. In the cabins and saloons were great numbers of them. One night only was spent here. But for a week afterwards *Anopheles* could be observed in the darker corners of the saloons, and even ten days afterwards an occasional one could be caught, although the ship has been out to sea again in the meantime, in fact, had reached Opobo River. Probably in the smaller and closer quarters of the sailors they would have been found still later.

In the report of the expedition to Sierra Leone, the following observations of the propagation of *Anopheles* occur :—

'We also observed that while naturally-fed gnats invariably laid eggs after two or three days, those which had been bred from the larvae in captivity, and had then been isolated and fed in test tubes, *never* did so, although before being isolated they had long been in company with males. The inference is that fertilisation takes place only after the female has been fed. We noted also that, in a cage where many male and female gnats, both *Culex* and *Anopheles*, were kept together for weeks, eggs were never laid—although the insects were fed, as described, on bananas, and the cage contained water for them to lay their eggs in. It seems, then, that a meal of blood is necessary before fertilisation. Lastly, we observed that previously fed and fertilised insects would lay a second batch of eggs after a second meal of blood, without a second fertilisation; but never laid a second batch of eggs without a second meal of blood. That is, one fertilisation suffices for several batches of eggs, but one meal of blood for only one batch of eggs.'

This is summed up in the following sentence—'Although these gnats (*Culicidae* which feed on men) can live indefinitely on fruit, and perhaps juices of plants, the female requires a meal of blood, both for fertilisation and for the development of her ova. In other words, *the insects need blood for the propagation of their species*.'

Proof of the truth of these inferences is afforded by a series of experiments which we carried out, chiefly at Bonny.

*Experiment I*—Four male *Anopheles* and five females hatched from pupae were placed together in a cage with a small pool of water and fresh banana: all died in four or five days, having laid no eggs; on dissection, the ovaries were found undeveloped.

*Experiment II*—Repeated the experiment with thirty to forty mosquitoes of each sex. It was noted that many, both males and females, fed on the banana immediately on introduction. All died in nine or ten days; no ova laid.

*Experiment III*—A number of male and female *Anopheles* were introduced into a cage with water and banana. The females were all fed on blood *once* only, before introduction ; all died within four days—no ova in water or in insect.

*Experiment IV*—Repeated this experiment with a large number of males and females. The females fed once on blood, afterwards on banana and water. Some few lived twelve days, all were dead on fourteenth ; no ova found. Ovaries undeveloped ; spermathecae empty.

*Experiment V*—Introduced into a cage a number of females and males, the females had not been in contact with males before feeding : they were fed only once on blood. Five days afterwards, no eggs having been laid, a further number of males and females were introduced, the females having been in contact with males since birth—and having had one feed of blood. Banana and water were kept freshly supplied daily in the cage. All died within eighteen days from the first, thirteen days from the second introduction ; no eggs having been laid.

*Experiment VI*—A number of *Culex* males and females were placed in a cage with banana and water ; they had no blood ; but lived for sixteen days—laying no eggs.

*Experiment VII*—A number of *Culex* males and females introduced with banana and water. The females were fed on blood on introduction and again seven days afterwards. Eggs were laid on the ninth day of the experiment, two days after the last feed of blood.

These experiments were performed in small cages, made of mosquito netting, of size about twelve inches long, twelve inches deep, and six inches wide.

Among mosquitoes of the genus *Culex*, copulation was often observed in the small cages of our experiments, but with *Anopheles* never. It was thought possible that these latter required a longer flight than could be obtained in cages of the dimensions given, so that our next experiments were made with a large cage eight feet long by six deep by four wide, into which were introduced a small iron tank and a soil puddle in an earthenware vessel containing water and a number of peeled bananas which were daily replenished.

*Experiment VIII*

June 8 Thirteen newly-hatched males and twelve newly-hatched females (*Anopheles*) were introduced into the large cage, the females have been fed on the blood of one of us.

„ 9 Introduced some males caught in native quarters and four newly-hatched, blood-fed females.

„ 10 Introduced eight blood-fed females and a number of males, all newly-hatched out.

„ 13 Re-fed thirteen of the above females, some would not feed, others had died.

June 15 Re-fed nine of the above females; introduced ten more freshly-hatched, blood-fed females and ten males, three of which were caught in the native town; three females were found dead on the water.

„ 17 Re-fed thirteen females; one found dead on the water; showed undeveloped ovaries and empty spermatheca.

„ 19 Re-fed eleven females; introduced three caught males from the native town.

„ 21 Re-fed ten females; only one living male can be seen. First noticed the ovaries beginning to swell as a greyish patch on upper surface of abdomen.

„ 23 Re-fed eight females.

„ 24 Re-fed six females; no males can be seen.

„ 26 Re-fed eight females; one female was accidentally killed while feeding. Ova were found fully developed enclosed in their usual chitinous ridged capsule. No spermatozoa in the spermatheca.

„ 28 Re-fed nine females—all except one shewed distended abdomens

„ 30 Introduced three females caught in native town. Re-fed nine females.

July 2 Re-fed eight females.

„ 5 Re-fed six females; eggs were found on the surface of the water in the small iron tank. All the females were apparently full of eggs except one, presumably the one which had laid the eggs. This mosquito was caught and kept by herself in a small cage.

| | | |
|---|---|---|
| July 7 | Only four females remain in the large cage, two of which would not feed—also two males present. | The isolated female was re-fed on blood. |
| „ 9 | Re-fed four females in large cage. | This female laid more eggs; she was again re-fed. The ova of the 5th had not hatched out. |
| „ 11 | Re-fed four females. | More eggs laid; re-fed with blood; eggs of 5th and 7th had not hatched out. |
| „ 12 | The four females and two males remaining in the large cage were removed and placed in a small cage. | Few more eggs laid. None of the previous eggs hatched. |

| | | |
|---|---|---|
| July 13 | One female found dead on the surface of the water with developed ova and empty spermatheca. Others re-fed on blood. | Re-fed on blood ; abdomen swelling. |
| „ 16 | One female dead on water ; contained developed ova. Other two females re-fed. | Laid more eggs. Was re-fed on blood. |
| „ 18 | Re-fed ; no ova laid. | Re-fed. |
| „ 20 | Re-fed ; no ova laid. | Re-fed ; more eggs had been laid. None of the previous eggs had hatched. |
| „ 21 | Added eight males to this cage. | Added four males to this cage. |
| „ 23 | One of the two females had died ; contained ova with transparent cuticle ; empty spermatheca. | Six eggs laid. Re-fed on blood. |
| „ 25 | The remaining female died. | Re-fed. Abdomen again swelling. |
| „ 27 | | Few eggs laid. Re-fed on blood. |
| „ 29 | | Found struggling on the surface of the water ; had lost one leg ; was killed. Ovaries contained thirty-three developed ova with chitinous covering ; empty spermatheca. None of the eggs laid hatched out. |

This experiment was repeated in a small cage entirely.

*Experiment IX*

June 26 Ten females (*Anopheles*) hatched out from pupae, were fed on blood and introduced with a number of hatched-out males, into a small cage with banana and water.

„ 28 Eight of the females were re-fed on blood ; four males caught in the native town were added.

„ 30 Eight again re-fed ; three more town males introduced ; abdomen beginning to swell.

July 2 Re-fed eight females.

„ 5 Re-fed seven females ; two males found dead on water ; eggs laid.

„ 6 Eggs of yesterday hatched out.

July 7 More eggs laid; re-fed four of the five remaining females, the other would not feed.
" 9 Eggs of seventh hatched out. Re-fed four females; one would not feed.
" 11 Few more eggs laid. Re-fed two females; three would not feed.
" 12 Eggs of yesterday hatched out.
" 13 Re-fed five females; no more eggs laid.
" 16 Re-fed three females; numerous eggs laid; one female dead on the water; two appeared thin.
" 17 Eggs of 16th hatched out.
" 18 Re-fed four females; one would not feed; no ova.
" 20 Re-fed three females; eggs laid.
" 21 Placed more males in cage. Eggs of yesterday hatched.
" 23 Re-fed one female; others escaped.
" 25 The remaining female would not feed; more eggs laid.
" 26 Eggs of yesterday hatched out.
" 27 Remaining female dead on water.

Thus our surmise that a long flight was required for copulation proved false, since the ova in the second of the two experiments just detailed had been fertilised and hatched out. Why fertilisation did not take place in the larger cage is difficult to explain, unless owing to the comparative small number of mosquitoes, both males and females, in so large a space, the opposite sexes seldom met. It is to be noted that in this experiment eggs were laid in water in an iron tank preferably to that in an artificial soil puddle, and further, that although fertilisation did not take place still the ovaries developed apparently normal ova, which, however, did not produce larvae.

It is further to be remarked that, with a regular supply of blood (every other day), eggs are laid within eight days of the birth of the adult female from the pupae; and that they continue to be deposited every second or third day afterwards, if water is available, until the death of the insect.

In these experiments it is seen that females in confinement in cages may live and be fertile for a period of at least seven weeks; from which it may be surmised that under natural conditions they might live much longer.

The following experiment shows that even in the absence of males, the ovaries develop ova.

*Experiment X*

July 17 Introduced five females hatched from pupae; no males added. The females were fed on blood.

July 19 Re-fed the females.
,, 22 Re-fed the females.
,, 25 Five females re-fed.
,, 27 Re-fed two females.
,, 29 Re-fed two females ; ovaries beginning to swell.
,, 31 Re-fed the two females.
Aug. 2 Re-fed the two females ; swelling of abdomen more pronounced
,, 4 Again re-fed.
,, 7 Re-fed.
,, 9 Re-fed.
,, 11 Would not feed.
,, 13 One had disappeared ; the other would not feed.
,, 15 Re-fed both females ; abdomen very swollen ; no eggs laid.
,, 19 Both had disappeared. (Probably eaten by ants).

In order to show that blood is necessary for the development of the ovaries and the formation of developed ova, the following experiment was performed. The females were not fed on blood.

*Experiment XI*

July 8 Ten females introduced into a small cage with a number of males caught in the native town ; no blood was given ; banana and water also placed in cage.
,, 16 Ten females still alive ; the banana was replenished every day.
,, 18 Eight females alive ; no eggs ; one dead female examined showed undeveloped ovaries.
,, 21 More males caught in native town introduced.
,, 25 One female dead on the water ; showed undeveloped ovaries.
,, 27 Only one female left ; no eggs laid.
,, 29 All dead ; no eggs laid.

Thus, without feeding on blood, female *Anopheles* may live at least twenty-two days on vegetable juices alone, but the ovaries remain undeveloped, although many males may be present.

The next experiment indicates that a regular and frequent blood feed is necessary—as often as every other day, every fourth day being insufficient—for the development of ova.

*Experiment XII*

July 18 Ten female *Anopheles*, bred from pupae, introduced into a small cage with six males caught in native town.
,, 20 Two females had died.

July 21 More caught males were added.

„ 23 Re-fed five females ; one would not feed ; others had died.

„ 27 Re-fed three females ; two dead on the water showed undeveloped ovaries, and empty spermathicae. Added five more males from native town.

„ 29 All had died.

The following three experiments were undertaken to ascertain whether males from a certain batch of eggs from one adult female could fertilise the females of the same batch ; Experiments XIII and XIV seem to contradict one another in their results. They also serve to show that the males directly after hatching reach sexual maturity very quickly, and are able to fertilise.

*Experiment XIII*

July 1 Nine females developed from pupae were fed on blood of one of us and introduced into a small cage together with banana and water.

„ 3 Nine females re-fed.

„ 5 Re-fed eight females.

„ 7 Re-fed six females ; one dead on water ; abdomens swelling.

„ 9 Re-fed five females ; one would not feed ; no eggs laid.

„ 11 Re-fed six females ; one egg found on water.

„ 13 Re-fed five. One would not feed.

„ 15 Re-fed five. Few eggs laid irregularly on water ; one female dead on water shewed fully developed ova with chitinous covering.

„ 18 Re-fed two females ; three would not feed ; single egg of 11th had disappeared ; those of 15th had not hatched out.

„ 20 Re-fed four females ; one would not feed ; more eggs laid ; those of 15th had not hatched.

„ 23 Re-fed three females ; one dead ; more eggs laid.

„ 25 Females would not feed.

„ 27 Re-fed three females ; no males present ; more eggs laid.

„ 29 Females would not feed.

„ 31 Two females dead ; one only fed ; no more eggs. Introduced six freshly-hatched males into cage.

Aug. 2 Some eggs on water ; pregnant female dead—ovaries contained eggs with chitinous covering. Eggs of 27th not hatched.

„ 4 Eggs of 2nd not hatched.

*Experiment XIV*

Aug. 7 Placed in small cage twelve females fed on blood, with a number of males hatched from the same batch of eggs of an adult female *Anopheles*.
,, 9 Re-fed.
,, 11 Re-fed.
,, 13 Re-fed.
,, 15 Re-fed.
,, 17 Re-fed.
,, 19 Re-fed; eggs laid, but accidentally lost.
,, 21 Re-fed; more eggs laid.
,, 23 Re-fed two of the remaining three females; the other would not feed. The eggs of 21st hatched out.
,, 25 Re-fed two females.
,, 27 Re-fed two females.
,, 29 Re-fed two females.
Sept. 1 One dead; the other would not feed—very pregnant.
,, 3 Re-fed the remaining females.
,, 5 Dead; no eggs laid.

*Experiment XV*

Aug. 7 Thirteen females fed on blood of one of us, and introduced into cage with a number of males, hatched from a different batch of eggs.
,, 9 Re-fed.
,, 11 Re-fed.
,, 13 Re-fed.
,, 15 Re-fed.
,, 17 Re-fed.
,, 19 One only remaining; re-fed.
,, 21 Re-fed the single female.
,, 23 Eggs laid; the mosquito dead.
,, 24 Eggs of 23rd hatched out.

Thus it does not matter whether the males and females are developed from the eggs of a single female or from different females, fertilisation occurs in both cases.

It was intended to continue these experiments after leaving Bonny, but this proved impossible under the circumstances already referred to.

They prove, however, the following points :—

(1) That a purely vegetative existence is insufficient for the propagation of mosquitoes of the genus *Anopheles*.

(2) That blood is necessary for the development of ova.

(3) That the blood must be available regularly—at least every two days—for the development of ova.

(4) That the power of propagation of the species is acquired in a very short time after the production of the *imago*, and is extremely vigorous during the whole life of the insect if feeds of blood are available.

(5) That one act of fertilisation by the male suffices for a considerable period of ova production.

(6) That even in spite of fertilisation not having occurred, ova develop if regular feeds of blood are procurable, and may be deposited on water.

(7) That unfertilised, fully-developed ova may be carried by the female for a considerable period (four weeks in our experiments).

## V. THE PREVENTION OF MALARIAL FEVER

At the commencement of this chapter, which will treat of the methods to be adopted for the prevention of malarial fever, the members of the expedition wish very strongly to indicate that the suggestions given are based on their own experiences and observations, and, as other scientific workers have as a result of their researches in other parts of the world suggested somewhat different lines of procedure with the same object in view, we consider the recommendations hereafter suggested as the most suitable, in fact the only possible, if malarial fever in the country of Nigeria is to be successfully combated. Whether they can be satisfactorily adopted in other parts of West Africa we are not, from personal observation, able to say, but it is noteworthy that the members of the only other malaria expedition which has made a long stay on the Coast—namely, the Royal Society's Commission in the districts of Sierra Leone, Accra, and Lagos, have recommended absolutely the same methods.

With a view as to the possibility of preventing malarial fever, it is to be noted that during the life history of the malarial parasite in the bodies of its two hosts—man and the mosquito (*Anopheles*)—the parasite may be attacked or avoided,

*A* in its intermediary host—man,

(i) during the incubation period of the disease; and

(ii) during the course of the disease.

*B* in its definitive host—the mosquito of the genus *Anopheles* by

(i) preventing inoculation, that is, their bites;

(ii) the destruction of the insect in any of its stages of development—as ovum, larva, or adult.

*A.* The only means of attacking the parasite during its life history in the intermediary host—man—at present known is by the action of quinine, either as a prophylactic during the incubation period of the disease, or as a curative measure during the course of the disease.

Professor Koch, as a result of his researches in the East Indies, has recommended the wholesale administration of quinine to Europeans and natives as the most practical method for the prevention of malarial fever in those parts. However practicable it may appear as a preventive measure in the parts visited by him, it is absolutely impracticable in West Africa. Manson[1] also suggested this as one of a number of methods for adoption throughout West Africa. There are a number of conditions which strongly militate against such a course of procedure. It has been shewn in a previous chapter that a large percentage of native children under ten years of age, and almost all children under five years, are infected with malarial parasites, often in large numbers, and that their blood frequently contains the parasites in that stage—'gametes'—in which they are naturally fitted for the further development of their life history in the mosquito. It is evident that the mosquito, while serving as the definitive host for the malarial parasite, carries the infecting agent from the native children to other natives and to Europeans. As it is the custom throughout the whole of Nigeria and, indeed, throughout the whole of West Africa, almost universally, for the European to dwell in close proximity to the native, the children constitute continually an eminently dangerous source of malarial fever for the European. It is, therefore, evident that if the method suggested—the universal administration of quinine—be adopted, the native must be also treated—as Professor Koch suggested, and apparently carried out successfully in parts of the German East Indies. It is not sufficient that the European alone should use quinine—and, moreover, it is with difficulty that the majority of Europeans on the Coast can be prevailed upon to use quinine regularly and intelligently.

Professor Koch qualifies the possibility of the adoption of this method by the supposition that the people to be treated are an 'intelligent and obedient community.' Exceedingly few of the natives of West Africa, and especially of Nigeria, can be brought under this description—in fact it can be safely stated that throughout the whole of Nigeria, we never met with a community which could be in anyway classed as 'obedient and intelligent.' The native of Old Calabar—the seat of government in Southern Nigeria—is stupid, unintelligent, and indifferent; those of the Bonny and Opobo districts, who have been longer in contact with Europeans, are just feeling the effects of civilisation, but they still look upon any new procedure on the part of the 'white man' with superstitious distrust and perplexity. The natives of other parts of the delta and of the Niger banks are mostly uncivilised, and often run away at the sight of a European: while there are towns in the interior only occasionally visited by 'white men,' or which are absolutely unopened. It is true that the native chiefs are often intelligent and educated men, but these are exceedingly few. It is extremely doubtful whether in such towns as Sierra Leone, Accra, Cape Coast Castle, and Lagos, where civilisation is fairly advanced, the introduction of any such practice as Professor Koch suggests, is at all possible. It is evidently absolutely impossible for Nigeria, even if the cost and freightage of the immense quantities which would be necessary for the purpose, did not put it completely out of question—for there are numbers of towns of population exceeding 5,000, some even reaching 30,000 and 50,000, and even more.

Among Europeans in West Africa, the usual practice as to the taking of quinine as a prophylactic, is to take five grains every day, or five to ten grains when they feel a little indisposed, 'out of sorts,' or when they think of it. Of the inefficiency of the latter as a preventative measure there can be no doubt, and it is very doubtful whether, by the cultivation of a habit, the former is of any value. Furthermore, it is even questionable whether, in many cases, a larger dose (fifteen grains) at intervals as suggested, has not deleterious effects when taken when in apparent health. We have observed startling effects in some cases after a single administration to healthy persons of even ten grains.

*B.* It is by attacking the definitive host that the best results in the prevention of malaria fever have been, up to the present, anticipated.

### I. Prevention of Biting by Anopheles

(*a*) *Culicifuges and Fumigation.*—The many substances which have been put forward as culicifuges to be smeared on the exposed parts of the body, or to be used as perfumes for the purpose of preventing the bites of mosquitoes, are not only obnoxious in their use but absolutely useless. Moreover, fumigation of premises can be of no practical value. The 'wily' *Anopheles* deserves its appellation to an extent little expected, and such subterfuges as smearing with kerosene, or the use of lavender and other substances can have but little effect, while fumigation is more likely to expel the European rather than the mosquitoes.

(*b*) *The use of mosquito-proof houses and of mosquito curtains.* Manson[2] advises the extensive use of these conveniences, together with a universal dosing with quinine for a period for prevention of malarial fever in West Afriea. It is not evident, from the report of his suggestions available, whether the use is to be limited to Europeans only, or is to be extended to the natives; if the latter case, then the scheme is evidently impracticable, and if the former it is inefficient.

The mosquito curtain is astonishingly misused by Europeans on the West Coast of Africa. We very rarely met with one who used the curtains in a careful and proper manner. Almost all are so placed as to hang outside the bedposts and reach on to the ground, being either free or weighted. This is an improper way of hanging the curtains, which thus act as a trap for those mosquitoes which have taken shelter during the day-time under the bed—as very commonly happens. The majority of the nets were sometimes so torn as to be of no protective use whatever, others had a few holes. All these were practically useless—the persistent *Anopheles* will discover the smallest hole capable of affording its body admission in the search for blood. It was common to hear considerable surprise expressed at the presence of gorged mosquitoes inside these nets regularly every morning.

Further, it is hardly to be expected that persons who neglect the proper use of the simple mosquito curtain, will attend to the nets at the doors and windows of a mosquito-proof house. In fact, we met with such a house in a district where mosquitoes were very numerous, and found the nets in a condition very similar to that of the mosquito curtains. Apart from the impediment to ventilation which would be produced by the use of nets at the doors and windows of a mosquito-proof house—and this is a serious consideration in climates such as that of West Africa—the habits of the European in that country expose him repeatedly to the bites of *Anopheles* at times of the day when it would be impossible for him to be inside a mosquito-proof house. The offices of the government and the warehouses and shops of the traders it would be impossible to protect in this way, while innumerable people continually enter and leave—and here many *Anopheles* are often very troublesome. Similarly, work being done for the day, it is a common practice with all the Europeans to sit out after sunset in their verandahs, or in the open, enjoying the cool of the evening—exposed to the attack of mosquitoes, which become active at this time of day.

### Destruction of the Insect

(*a*) *Of adults.* This evidently can be executed to a very small extent only, and is not to be considered as of any material help to prevention. The ease with which they can be caught is, however, noteworthy—native boys soon recognised the difference between *Anopheles* and *Culex*, and brought us often as many as fifty or more of the former every day, either in test tubes or even in bottles—large numbers in each.

(*b*) *Of larvae—and the extirpation of breeding places of Anopheles.* This presents a very large field of operations against the mosquito. The extermination of the definitive host will naturally occasion the destruction of the malarial parasite.

The chief result of the previous expedition to West Africa (Sierra Leone) was to establish the fact that breeding-places of *Anopheles* are often easy of destruction or of prevention, and that this procedure would, to a large extent, prevent malarial fever in districts where it was thoroughly carried out. Two courses were suggested.

1 Efficient drainage—and the construction of proper roads—and the filling up of pools and puddles of water to prevent the formation of breeding-places.

2 The use of 'culicicides,' to be added to existing breeding-places regularly and intelligently, to kill larvae present, and to prevent future use of the water for the purpose of breeding.

Although the first method was suggested as the only really efficient measure, the second was offered as a temporary measure until the financial condition of the colony would permit of the expenditure necessary for the carrying out of the first, and also for adoption in those rarer circumstances where the more efficient method could not be applied.

The material changes which have been brought about in the prevalence of malarial fever in some towns in tropical and sub-tropical countries, especially in India and Southern Africa, by the introduction of a good drainage scheme, were previously described as the results of general sanitary improvement, and it was decidedly established, before the part played by mosquitoes in malarial infection was even hinted at, that the adoption of good drainage methods was often immediately followed by a more or less complete disappearance of malarial fever in the district. Now, of course, such improvement must be ascribed to the destruction of the breeding-places of *Anopheles*.

As has already been shewn in previous chapters of this report, the conditions met with in Nigeria are extremely varied. Districts may be very roughly classified, according to the nature of their more common breeding-places for *Anopheles*, as follows :—

(*a*) Those in which native dug-out canoes, and occasionally shallow surface puddles are usual—for example, at Old Calabar, Bonny, Opobo, Okrika, etc. etc.

(*b*) Those with shallow surface puddles only—the areas of many of the factories of the various trading companies.

(*c*) Those having mud and clay pits, wells and pools, and few surface puddles.—Abonnema, Egwanga, Okoyong, etc.

(*d*) Those with 'made' roads, having ditches at the side and small hill streams—Lokoja.

(*e*) The neighbourhood of fresh-water marsh districts in cultivated and occasionally uncultivated areas.—Lokoja, vice-consulate at Opobo.

(*f*) Towns on the banks of rivers which fall considerably during the dry season.—Agberi, Asaba, and many others.

It must be distinctly understood that these do not include all the breeding-places of *Anopheles*, and we are of opinion that it would be extremely difficult, even in a small district, to indicate all the collections of water which might from time to time serve as breeding-places. For we urge that the destruction of the usual breeding-places of *Anopheles* would be followed by the adoption of any piece of water that might be presented if of sufficient duration, and that at length the various unlimited breeding-places usually frequented by *Culex* would be resorted to. Such a condition, however, would be rendered difficult under the circumstances of modern European domestic life.

In almost all of the classes into which districts have been placed according to the conditions under which *Anopheles* breed, an efficient surface drainage is more or less applicable. Many of the compounds of the trading companies' factories (*b*) are roughly kept, and present irregular unlevelled surfaces permitting the formation of numerous shallow puddles in the rainy season. The careful construction of a few gutters and ditches would obviate the evil, and thus destroy almost the whole of *Anopheles* breeding-places in the immediate vicinity of the factories—which often are the only source of *Anopheles*.

The numerous clay and mud pits of districts (*c*) which occur in almost every native town outside the mangrove swamp region, would be difficult to treat by drainage. From them the natives obtain mud or clay wherewith to build their huts and

ROAD LEADING UP THE 'BARRACKS' HILL TO THE OFFICERS' QUARTERS AT LOKOJA.

RE-CONSTRUCTED DURING THE VISIT OF THE EXPEDITION. SHEWS *Anopheles* PUDDLES ALONG THE DITCH AT THE ROADSIDE. AN OFFICER'S BUNGALOW AND THE ADJACENT NATIVE HUTS ARE SEEN ON THE RIGHT NEAR THE TOP new ones are continually being made—the old ones remaining unfilled. Those in the neighbourhood of European quarters could, however, be easily filled up with earth and refuse.

(*d*) The art of making good roads and footpaths seems to have been lost by the Europeans of the West Coast. They are made with such evident lack of engineering skill as to permit of the rain-water lodging in shallow puddles along their course, or are so badly ditched on each side that water lodges in the gutters as a series of small pools. These are conditions encountered in many parts of the Protectorates, and especially at Lokoja, in Northern Nigeria, as already referred to. Further, after being once constructed, the roads seldom obtain any further attention—the ditches are permitted to partially fill up or become overgrown with weeds. They invariably form excellent breeding places for *Anopheles* : we were always able to find numerous larvae in them.

It is essential that public works and engineering departments in Nigeria should bestow considerably more attention to the construction of properly 'battered' and 'ditched' roads and footpaths, especially in the neighbourhood of the European quarters—both goverment and traders—and thereby remove circumstances which are often a dangerous factor in conditions conducive to ill-health among Europeans in these districts.

To the small hill streams again principles of drainage are applicable. In the hilly regions of Northern and Southern Nigeria hill streams are numerous, gorged and rapid during the wet season, small and sluggish in the dry. During the latter season they afford numerous places for the breeding of *Anopheles*, especially at the points where they are near human habitations. Here and there they swell out into shallow pools from which the water flows very slowly : here larvae are often found, as well as in the sluggish corners and back eddies of the stream. It is evident that a deepening of the stream in parts, or a reconstruction of the channel in others, with occasional attention to see that the channel is clear, would annihilate the undesirable conditions.

(*e*) These conditions have been already described, and, in the places where they were observed by us, it was by no means difficult by judicious drainage to remedy them. In places where these conditions are extensive—reaching over a considerable area of country—drainage might prove very expensive and even impossible.

(*f*) We had no experience of these conditions, and as to whether they really occur we are not certain, having only hearsay evidence of their existence from others who have experienced a dry season on the River Niger. As previously stated, it is very possible for such conditions to arise, but actual observation is necessary to arrive at any decision as to their extent and how they might be treated, if at all.

There is no doubt that thorough surface drainage in very many parts of Nigeria would go a long way towards preventing malarial fever among Europeans, but it is essential that it be very efficiently and thoroughly carried out; and that the breeding-places should be intelligently searched for.

Further, there are, no doubt, other conditions to which a drainage system could be applied, which have not been included in these remarks, and perhaps others which have not been encountered by us.

## II. Destruction of Larvae by the Use of Culicicides

The general use of 'culicicides' as a really efficient method is impracticable. From observations already made, it is clear that the very varying conditions under which *Anopheles* breed do not permit of the general application of any substance which will destroy larvae or prevent their development—applicable either regularly to the surface, as kerosene, tar, etc., or as a substance, such as lime, as has been suggested, which, once introduced, might render puddle, pool, or other collection of water useless for breeding purposes in the future.

Such a duty—in the carrying out of a general practical measure—as the discovery and treatment of *Anopheles* breeding-places, could not be relegated to a native official or number of officials, even under the supervision of a European sanitary officer. The varying and unlimited conditions under which *Anopheles* do and may breed, require the constant attention of one with some special knowledge and previous experience.

In the report of the previous expedition, the use of 'culicicides' was advocated for extensive trial in Sierra Leone, as a result of the observations of the expedition during their visit. And this serves as a striking example of the variability of the conditions at different seasons of the year, for on the visit of the members of the Royal Society's Commission in the dry season, those conditions for which the treatment was suggested had disappeared, and a new set, producing innumerable breeding-places, had arisen for which, in the opinion of the members of the commission, the operations previously suggested were not applicable.

Under certain rare circumstances, apart from any general consideration, the use of 'culicicides' would have to be resorted to as the only method of treatment.

Kerosene regularly applied appears to be the most reliable of a number of proposed culicicidal re-agents: the cheap ideal substance which will have the effect of rendering pools permanently uninhabitable for the larvae is still undiscovered. From a number of experiments performed by us in West Africa with various substances, we are not able to recommend any such substance with the desired properties; further, we deem lime and gas lime, which have been suggested as a possible ideal re-agent, of no value for the purpose.

Our attention having been directed to a statement in the medical journals[3] that in the country in the neighbourhood of Lake Chad, no mosquitoes occur, and no fever prevails, led us to try the action of the native potash as a 'culicicide.' Native potash is a substance occurring in large quantities as an efflorescence in the country referred to, and to its action of rendering water, in which the potash is dissolved, distasteful to *Anopheles*, is ascribed the freedom of the district from mosquitoes. From our experiments we are unable to infer any decided culicicidal effects (its action was much inferior to that of lime); we are, therefore, inclined to consider the freedom of the district from mosquitoes, if it be really so, as not due to the presence of this substance.

## RECOMMENDATIONS

Above we have discussed at some length the practicability of some of the suggestions which have been advanced, either singly or together, as general methods for the prevention of malarial fever in a European community. We have shewn that in a great measure they are unsuitable for adoption on a large scale in Nigeria—partly on account of unreliability and inefficiency—partly because of the enormous expense. But it must not be understood that we wish to discourage in any way the use of the more reasonable of them as measures of some value under certain circumstances, but they are throughout to be considered very subservient to the measures to be suggested; and though by their employment the chances of infection of malarial fever would be more or less diminished, absolute protection against the disease could never be obtained.

### I. Segregation of Europeans at a Distance of about half-a-mile from Natives

The fact that the native children especially constitute a perpetual source of danger to the European, as being naturally the source from which he is infected with the parasites of malarial fever carried from the native by the mosquito of the genus *Anopheles*, suggests very pointedly that the safest and surest plan on the part of the European to guard himself absolutely from any chance of infection would be to live at a distance from the native.

This would have been unnecessary, of course, had the possibility of the suggestion of the use of quinine wholesale among natives been feasible, but the enormous quantity of the drug that would have been required, and the more or less uncivilised condition of West African natives, have been shewn to render the plan impossible.

Similarly, it having been shewn how a total and universal destruction of the insects by any practical means is impossible, and, moreover, since mosquito-proof houses are not at all capable of protecting Europeans from the bites of *Anopheles* at all times of the day and in all the varying circumstances of European life in West Africa—since, indeed, they are impracticable on a large scale—the adoption of the measure of segregation from the native is still more strongly indicated

Segregation of Europeans at a distance from all natives offers itself now as the only measure by which absolute freedom from the disease can be guaranteed, and all the scientific evidence that has been collected respecting the cause of malarial fever and the manner in which infection among Europeans is brought about, markedly

AN *Anopheles* POOL—A PORTION OF A BADLY-CONSTRUCTED DRAIN—AT LOKOJA. THE RAILINGS BOUND THE AREA OF THE NIGER CO.'S FACTORY—THE HUTS OF THE NATIVE TOWN APPROACH ALSO UP TO THE RAILINGS points to the adoption of segregation principles as the only way in which absolute protection from the disease can be assured.

As has already been pointed out several times, it is almost universally the rule in West Africa to find European houses built round by native quarters, a practice which long experience in India has taught Europeans to carefully avoid. The degree of proximity of the native huts varies in different places. For example, at Old Calabar, many of the factories are almost surrounded, except in front, by native habitations ; similarly at Egwanga, the small native town is built by the side and back of one of the factories, the huts abutting on to the boundary walls. Also at the Niger Company's factory at Lokoja the native houses are very close up to the Company's boundary railings. At other places a small collection of native huts in the vicinity of European quarters serves as a continual source of infection—for example, at Akassa, the barracks of the native soldiers are close to the government vice-consulate, and the small native town near to the quarters of the engineering staff.

It is not essential that the children are of natives of the district, those from other parts of the Coast are equally dangerous. Akassa engineers' quarters may be again mentioned as an example where the engineering artisans, chiefly natives of Lagos, Accra, and Sierra Leone, are housed with their families alongside to the European house. A large proportion of these native children were found by us to contain malarial parasites. Similarly, also, at Asaba, the proximity of the barracks of the Hausa soldiers, who have their wives and children with them, is a dangerous menace to the health of the officers at the Force house.

Examples of the opposite condition of affairs might also be given, for instance, at Old Calabar the Government offices and consulate, vice-consulate, and medical house, are comparatively free from malarial fever, it having been established that the natives shall not build on the European side of the creek separating the two slopes on which the native town and European quarters are built. This creek is at a distance of about half-a-mile from the houses mentioned. The nearer proximity of the barracks of native soldiers to the Force house may possibly account for some cases of fever which occur there.

Further, the factories at Slave Trees, Bakana, and Bugama are at some considerable distance from any natives, who dwell on the opposite side of the river ; the Europeans here enjoy a comparative freedom from fever—although the condition of the surface permits of the production of myriads of *Anopheles* on the spot.

Native servants living in the neighbourhood of Europeans form a source of danger which might in a great measure be prevented. Although they themselves cannot be regarded as an at all common source of infection—indeed only a very rare one—their presence round the European becomes dangerous in two ways. It is the custom to have a number of native servants sleeping in or about the quarters of Europeans.

There is no doubt that natives attract *Anopheles* more than Europeans, and their presence under the circumstances indicated, attracts infected *Anopheles* from other native huts. Although then, a European house may be well segregated, and there may be also no frequenting of the servants' quarters by native children capable of infecting the otherwise harmless *Anopheles* there, yet the native servants form a route along which infected *Anopheles*, from quarters containing children in the neighbourhood, may pass and gain access to Europeans. Further, the native habits permit of the constant frequenting of the native servants' quarters by all sorts of natives from the neighbourhood, and especially of women and children. Some chance of the infection of the otherwise harmless *Anopheles* of the servants' quarters is thus possible. The ideal arrangement for the quarters of native servants is then such as will permit of one or two of them sleeping close to the Europeans—to be within call and to render assistance if necessary—and the others to be relegated to special huts of their own at a distance of about half-a-mile. From our observations in West Africa this could be easily done without occasioning the slightest inconvenience.

Among the trading community, even if properly 'segregated,' there is, perhaps, a further chance of infection among those assistants whose duty requires their presence for a number of hours each day in those 'stores' or 'canteens' of the factory at which the natives of the neighbourhood are retail customers. Here, a number of native women are continually passing in and out, staying often some minutes, with babies on their backs or children at their sides. These latter may be a source from which the otherwise uninfected *Anopheles*, usually so abundant in the shady parts of such warehouses, may become infected, and hence some chance of European infection arise. But, in our opinion, such chances are extremely small—the period of each visit is short, everyone is usually on the move, and, altogether, the prospects of *Anopheles* obtaining a feed of blood from the few native children around, infinitesimally small. Such chances might, however, be negatived by individual precautions against the attacks of the insects by the assistants themselves.

These illustrations of the conditions under which Europeans contract malarial fever in West Africa will also serve for many others than those mentioned. The government administrative commissioner, who visits many places where no Europeans dwell, has to rely on the native huts for his lodgment—and there is usually provided for him one in the midst of the native town. In a few days after his return to headquarters he has an attack of fever contracted during his visit.

Similarly the trading agent, in the interests of his firm, visits a native chief up country (the middleman) and elects to stay one or two days with him at his house in the midst of a crowd of native huts—or may be an assistant at the week end spends his Saturday afternoon and Sunday in a native town, with the result—an attack of fever some days afterwards.

The explorer or traveller visits a native town and is entertained by the chief or other leading man, and passes on; perhaps reaches a swamp or passes through districts where, it is said, mosquitoes do not occur: here, may be, he has an attack of fever contracted in the native town last visited, and in no way connected with the swamp which is usually blamed; nor upsetting the mosquito theory because of the supposed absence of the insects in certain districts.

The frequency of malarial fever among missionaries is no doubt explained by the manner in which they are continually exposed to infection in quarters frequented by native children—huts, schools, churches, etc.

The possibilities of the adoption of the method of separation of the living quarters of Europeans from those of the natives in Nigeria are varied.

In new stations at present in course of construction, or about to be constructed, the adoption of such a principle would at once stamp those stations for a healthy prospect. It is in this direction that the greatest amount of benefit is expected to arise. In Northern Nigeria, especially, and in Southern Nigeria, in the near future, when country at present commercially inaccessible to Europeans has been opened up, it cannot be too strongly urged how great is the opportunity for the trial of such an experiment—indeed, it can be hardly called an experiment, since, apart from the consideration of the immediate cause of infection by malarial fever by the agency of the mosquito, it is only common reason that the practice of surrounding oneself with a crowd of uncivilised or semi-civilised natives is an unhealthy principle. Now that malarial fever has been completely traced to this practice, no doubt it will be utterly abandoned.

It seems to us quite as easy now to establish stations with a little care and judgment exercised in the selection of their sites, which shall be healthy, as it was previously, in ignorance, to create unhealthy ones. It must, however, be given as a caution that once established on the improved lines, that is, apart from native quarters, the vicinity of European dwellings must be kept strictly free from the encroachment of native huts—as such is from our observation the usual course of events, the presence of a European immediately attracts the native to settle close beside him—indeed, in some places this is exactly how the present evil conditions have been brought about.

In Nigeria, the stations already established are with two or three exceptions only small. The European community seldom exceeds twelve in number. They consist of administrative, trading, and missionary stations. Many of the government quarters can be segregated with but little difficulty—at Bonny, for example, by removing the nearest native huts and re-arranging the accommodation for the 'Kroo' boys and native servants—and at many places on the river Niger, where the present conditions of living are only temporary, more suitable and healthy sites could easily be acquired. With the trading stations, however, there is more difficulty since they consist of large warehouses and stores erected at considerable expense. Mission stations also generally present similar undesirable conditions.

In the larger towns, such as Old Calabar, the difficulties become almost unsurmountable. Removal of European business premises is entirely out of the question, but the exigencies of trade and administrative work would permit of the occupation of healthier sites during the evening and night, as has been suggested by the medical and public works officers of that town.

But in all such cases which are difficult to deal with, probably a great deal might be done to remove the natives from their present positions near factories. We do not suggest as feasible a total destruction of such native huts at once, but we see no objection why such control over the neighbouring land should not be gradually acquired, so as to bring about the removal of these native huts, and thus remove a dangerous menace to health. These native huts are of but little worth, and are easy to construct in a very short time. It will become a subject for government policy to enable Europeans to proceed in this direction, involving, perhaps, slight compensation to the natives affected, and it is trusted that, after consideration of all the advantages and the economy which would accrue upon the prevention of malarial fever among a community of Europeans, both engaged in their employ and in trade, the government will in the near future adopt such a policy as will help and render easy the adoption of the principle of segregation.

## II. The Surface Drainage of Areas round European Quarters

It has already been pointed out that one way in which infected *Anopheles* may filter through from native childrens' quarters to Europeans at a distance of even half-a-mile from them is by means of the quarters of native servants lodged at different points in the intermediate space.

Yet another way is by means of such collections of water as might serve for breeding places. It is easy to perceive that a number of infected *Anopheles* from childrens' quarters in search of water on which to lay their eggs, might have to choose a collection near to European quarters and from it fly to the nearest European habitation.

Complete drainage of the surface and the destruction of all such collections of water as might serve as breeding-places for *Anopheles* must then necessarily accompany the adoption of the measure of 'segregation,' if the community is to continue completely protected from malarial fever attacks, and it is our opinion that such a combination of the practice of segregation and efficient surface drainage would be followed by complete freedom from the disease among Europeans. The details of the various conditions under which *Anopheles* have been observed to breed in Nigeria

PORTION OF A CULTIVATED AREA AT LOKOJA, SHOWING BUTTS AND FURROWS, IN THE LATTER OF WHICH *Anopheles* PUDDLES OFTEN OCCUR

have already been given, and it only remains to once again emphasise that an efficiently and intelligently executed system of extirpation is necessary.

Under circumstances where at present 'segregation' is impossible, the destruction of breeding-places of *Anopheles* by drainage and other means already referred to would prevent the multiplication of the insect to some extent, and thus render the chances of infection much less. But it is to be remembered that so long as the mosquito is able to obtain feeds of blood she can live for very long periods, during which she is continually on the watch for a suitable opportunity to deposit her eggs, and that the most unlikely and unusual sites would be utilised for that purpose. This shews the necessity for a thorough destruction of water collections and of the great difficulty of eradicating all, especially in native surroundings.

But the creating and maintaining of breeding-places by such means as the construction of bad roads and footpaths, wells, unnecessarily uncovered, and so on, is inviting disaster. Half-a-mile has been fixed as the distance which should separate European and native quarters from a consideration of the habits of *Anopheles*. There is some considerable evidence for the assertion that these insects do not fly far and that they spend their lives very close to the spot at which they were hatched from larvae. It is extremely rare also that a breeding place for *Anopheles* is found at a greater distance than half-a-mile from the dwellings of man or from spots frequented by man.

*Objections raised against the principle of segregation.* The only objections which we have become cognisant of, have arisen chiefly among the West African merchants. Excluding any reference to expense, the chief objection has been that such a segregation as that suggested would seriously affect trade. It must be remembered that a distance of half-a-mile only has been fixed. We are totally unable to perceive how in any part of Nigeria such a procedure could in any way affect the trade at any of the factories we have visited. We urge that the principal trade is done, not with natives in the immediate neighbourhood of a factory, but with influential chiefs and middlemen, who generally dwell some distance up the rivers. The small retail trade done with natives living close to factories would be quite uninfluenced if they were removed to a distance of half-a-mile. In fact, some factories are situated at more than half-a-mile from any native town, and do excellent trade—to walk such a distance and carry back their wares is a mere nothing to a native.

The only other objection heard of refers to social conditions. It must have arisen from a misunderstanding of what was suggested, probably from the roughest idea of what 'segregation' might mean,—a wholesale removal of Europeans from among the natives, who were to be regarded as unfit for any intercourse or dealing. Such aggravated ideas are evidently absurd, and it is not clear how the adoption of the principle of 'segregation,' as described above, can in any way influence the present social relations between European and native.

In concluding these recommendations for the prevention of malarial fever, it has been considered necessary to more particularly point out in what way they can be adopted in individual cases. Presuming, beforehand, that attention be paid in every case to precautions exercisable by every individual—namely, the careful use of mosquito curtains and of suitable clothing to prevent the bites of mosquitoes, as well as the use of mosquito-proof houses in certain circumstances, and the intelligent and regular administration of quinine, a certain amount of care might be exercised by some individuals who meet unusual circumstances.

We have stated that, for a community of Europeans, segregation and drainage are to be relied upon, while the use of mosquito-proof houses, mosquito curtains, quinine, and other measures are subservient to these, and more applicable by individuals ; also that, in a segregated community, the servants must also be properly placed.

For military officers in permanent camp, it is advised that particular care be bestowed on the disposition of the huts of the native soldiers—which should be placed at a distance of half-a-mile from officers' quarters, and those of soldiers with their families at a still greater distance, if possible. In temporary camp, choice of a site on 'segregation' principles is advised, that is, away from the native huts.

Commissioners and other government officials, as well as travellers and explorers, should avoid sleeping in native huts. It is advisable to pitch tents for themselves and their servants and followers for sleeping purposes away from native towns and villages.

Agents and assistants at trading factories will be careful in the choice of sites for new stations, and will as far as possible re-arrange their compounds so as to place their servants and labourers at as great a distance as the extent of their areas permit and, moreover, to ensure that no collection of surface water on the area can exist.

Similar advice might be given to those Europeans engaged at mission stations.

With those engaged on surveying parties or on the construction of railways, the exercise of the principle of segregation is very easy. It is again the custom at each little station to find Europeans surrounded by natives of all sorts and ages. It is evident that at such places where absolute control over the disposition of all houses, tents, and huts is easy, to so place all such native followers and labourers as not to constitute a danger, presents no difficulty whatever. The construction of railways in West Africa has always been associated with many cases of malarial fever, which were formerly attributed to 'the turning over of the soil.' Now they are easily explained by the formation of numerous breeding-places for *Anopheles* created by turning over the soil in the construction of the railway—thus supplying the carrier of the disease; and the practise of permitting labourers and servants with their families to live close to European quarters—thus supplying the

THE ROAD LEADING INTO THE NATIVE TOWN OF LOKOJA.

ALONG ITS SIDES ARE DITCHES FORMING A STRING OF PUDDLES CONTAINING *Anopheles* LARVAE. TO THE LEFT, BEYOND THE PORTION TAKEN IN THE PHOTOGRAPH, A BANK AND OTHER EUROPEAN QUARTERS ARE IN COURSE OF CONSTRUCTION.

source of the infection. Such undertakings ought to continue absolutely free from malarial fever, if the above precautions were practised.

For the prevention of malarial fever among European crews on board ship, the regular administration of quinine, according to the suggestions of Professor Koch, seems to us very feasible. We have shown how *Anopheles* come on board and remain often a considerable time. There is also a possibility of their becoming infected from the few children which are carried from port to port on the West Coast of Africa. The chief source of their infection is, of course, the children of the native towns opposite which the vessels anchor. Professor Koch suggests that the administration of quinine be undertaken in the following manner, and there is no reason why this should not be regularly carried out by the ships' doctors, after the outward-bound vessels have reached the coast. Fifteen grains of quinine are to be administered on each of two successive days, preferably in the early morning ; then a period of seven days without the drug is allowed, and the administration of fifteen grains again continued daily for two days, and then another seven days' interval, and so on, for the whole voyage.

# APPENDIX

## AN UNCLASSIFIED WEST AFRICAN FEVER

In the *British Medical Journal* of January 26, 1901, S. W. Thompstone, F.R.C.S. and R. A. Bennett, M.B., M.R.C.S., district medical officers at Old Calabar, gave a preliminary note on an unclassified type of West African fever—to which they proposed to give the name 'hyperpyrexial fever.'

The cases occurred during the stay of the members of the expedition at Old Calabar, and we were able to watch the course of the disease in both cases.

By the kind permission of Drs. Thompstone and Bennett we are allowed to reproduce the short account of their cases here—and we are indebted to Dr. Shekleton of Bonny for the earlier part of the temperature chart of the second case, which occurred at Bonny. Because of lack of hospital accommodation and the impossibility of affording such constant attention as such a case demanded, this patient was shipped to Old Calabar and treated at the European hospital there.

We now give the remarks of the authors of the communications to the *British Medical Journal.*

### 'Clinical Features of Hyperpyrexial Fever

This fever is generally ushered in by a slight rise of temperature followed by profuse perspiration and a fall in the temperature to about 99° F. After a period of apyrexia of perhaps twenty-four hours' duration the temperature begins again to rise, slowly at first, but when 105° is passed with alarming rapidity, one degree in ten minutes having been frequently observed, and it may reach 107° on the second day. For fourteen or even for thirty days subsequently there is absolutely no tendency for it to fall. The skin acts either very slightly or not at all, and all antipyretic drugs fail.

The tongue is at first furred on the dorsum and red at the edges and tip ; late in the disease it becomes dry and shrivelled. There is no enlargement of the liver or spleen. The urine is of normal character and abundant ; the bowels are regular or inclined to looseness. The conjunctivae are injected and the pupils contracted. The mind remains remarkably clear in most cases, except when the temperature is at its highest, but constant symptoms in the early days are great anxiety and restlessness.

With regard to the examination of the blood, no plasmodia nor pigmented leucocytes have ever been discovered, but in two of the later cases it was noticed that the blood tended to coagulate the moment it was exposed to the air, so that it was only with great difficulty that satisfactory films could be obtained.

### Typical Examples of the Disease

Case I. This patient had been on the Coast for about five months, and had had no fever until the present attack. During the last month he had lived in a tent at a quarry some twenty miles up the Akwayafe river, and, feeling ill four days before admission, got into a canoe and found his way down to the mouth of the river, where he was picked up by a launch and brought to the hospital. On admission, his temperature was only 99·6° F., and he was apparently going on satisfactorily when, as in the previous cases, his temperature began slowly to creep up—the beginning of a constant tendency towards hyperpyrexia. He was treated by baths, as will be subsequently described. After his fourth bath he was put into a cold pack, which was changed every hour for four days. The temperature then steadied, and eventually he left for home practically well.

CASE II. This patient was sent from an out-station on the fourteenth day of his illness, and was still under treatment when the record was made. So marked was the tendency to hyperpyrexia that on two occasions no fewer than two cold baths were necessary in the twenty-four hours, and it was not until the twenty-second day of the disease that it was possible to substitute the continuous pack for the cold bath. From that time until now the temperature has averaged 102°. The patient lies helpless in bed, occasionally showing some glimmering of intelligence, but for the most part remaining unconscious of his surroundings, taking nourishment well, but passing urine and faeces under him. This condition, as far as we could tell, may continue indefinitely, The temperature shows no tendency to assume alarming proportions, and the heart and lungs are acting well.

### CHARTS

### TREATMENT OF THE CONDITION

This may be summed up in one word—baths. The patient is put into a cold bath, and his temperature brought down to 100° or 99°. He sleeps for about an hour, and then feels fairly comfortable, but the temperature at once begins to creep up again, and in eighteen or twenty-four hours it is back at 106°, or at the point at which it is considered advisable to check it. Quinine and other drugs have been systematically tried without influencing the course of the disease ; the coal-tar antipyretics are absolutely useless. Cold sponging and packs are incapable by themselves of reducing the temperature, although in a recent case which recovered it was found that by reducing the temperature by means of the cold bath to 101° or 102° it was possible to keep it there by means of cold packs renewed at hourly intervals.

### PROGRESS OF THE CASES

It has been observed that, if the patient is to recover at all, some change for the better is to be looked for about the end of the third week. The temperature, which up to this time would constantly rise at once after the bath, might remain at 102° or 103° for several hours, and perhaps in the course of the next week two or more days might pass without the necessity for a bath. Convalescence is gradual, and it may be six weeks after the onset of the fever before the temperature finally assumes its normal course. On the other hand, in 50 per cent. of the cases which have come under observation a fatal issue has occurred.'

Several examinations of the blood of both cases were made by Drs. THOMPSTONE and BENNETT and by ourselves, but neither malarial parasites nor pigmented leucocytes were observed. Several times on the day of the arrival of the second case at Old Calabar, and on many subsequent occasions, we carefully examined the blood both by fresh and stained specimens.

Dr. SHEKLETON, of Bonny, states that he found the ring forms of aestivo-autumnal fever in the blood of his patient on the first day of the attack, but none subsequently.

On the sixteenth day of the disease (two days after arrival at Old Calabar) an estimation of the number of corpuscles gave 4,384,000 red, and 15,000 white per C.M., by the THOMAS ZEISS apparatus ; and the haemoglobin was estimated at 90 per cent. Three attempts to obtain micro-organismal cultivations from the blood on agar-agar and serum failed. A peculiar tendency of the blood to rapid coagulation was noted. Dr. THOMPSTONE states he has never been able to find malarial parasites in other cases of this disease which have come under his observation. It is to be pointed out that these cases occur during the dry season, at a time when the number of cases of malarial fever is smallest. There is often a history of exposure to the sun's heat, rough conditions of life, and hard work.

The onset, symptoms, and course of the disease are quite atypical of malarial fever : there is absence of rigors, shivering, vomiting, severe headache, etc., etc. ; the liver and spleen are not enlarged nor tender : consciousness is preserved often to the end. The disease may last about a week, or be extended to three or four. There is a continual tendencv to hyperpyrexia, only checked by cold baths, by which means the temperature is easily reduced, but it gradually creeps up again directly the cold applications are removed. After a hyperpyrexial stage of one to three weeks' duration, there is always a very long period during which the temperature continues above the normal—a very extended lysis—varying during the day not more than a degree, and falling gradually degree by degree every four or five days.

Drugs, including quinine and phenacetin, seem to have no effect on the course of the disease.

Lung trouble caused the death of the second case on the evening of the day previous to our departure from Old Calabar, and, through the kindness of Dr. THOMPSTONE, we were able to hold a post-mortem examination on the case two hours after death, the details of which we are able to record :—

No rigor mortis : but little wasting : no jaundice : small clean sore over sacrum : no trace of syphilis.

*Abdomen*—No fluid in peritoneal cavity : serous membrane appeared normal, excepting two small patches, one of old, one of recent perihepatitis, on the upper surface of the right lobe of the liver.

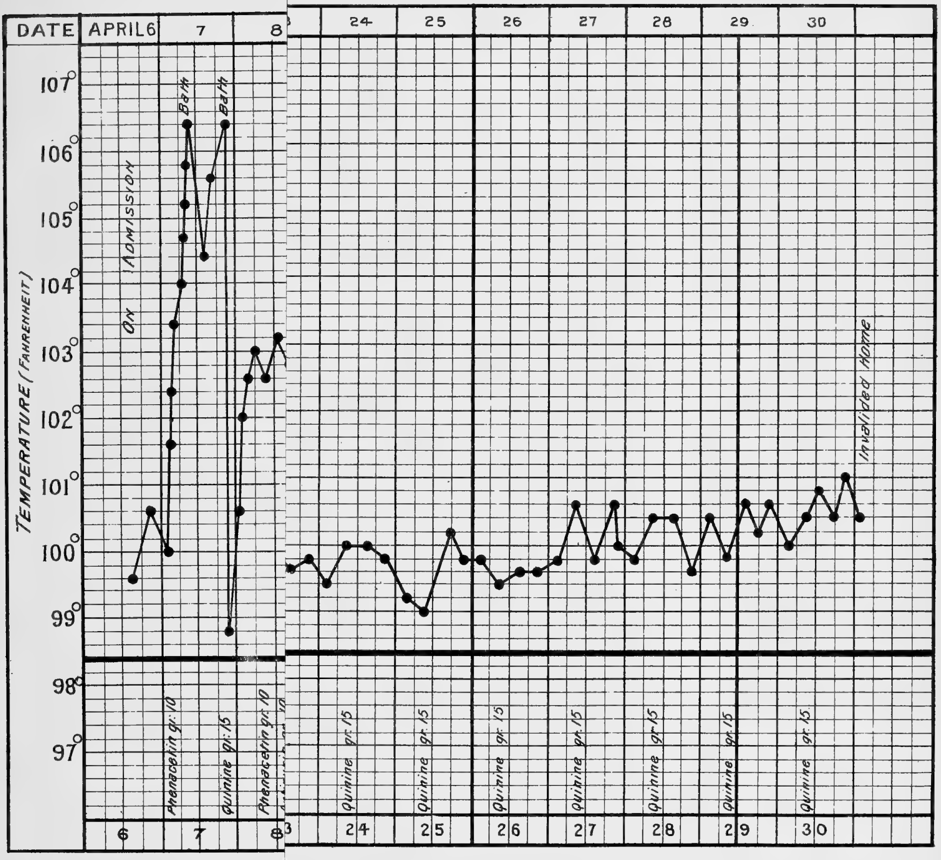
*Pancreas*—Somewhat large and firm.

*Spleen*— Did not extend below the costal margin—about the size of one's hand ; firm in texture—externally of slaty appearance ; on section, firm, dark in colour.

*Liver*—Two small patches of perihepatitis on upper surface of right lobe. Somewhat enlarged and firm ; on section, firm and dark in colour : no sign of fatty degeneration nor cloudy swelling.

*Kidneys*—Large—capsule stripped uniformly, somewhat adherent all over. On section, red and granular—'large red granular kidney.'

*Stomach*—Contained a quantity of gruesome, blackish-brown stained mucus in flakes between the rugae. Mucous membrane pale, shewed very small petechial areas to which the blackish shreds were attached.



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TEMPERATURE (FAHRENHEIT)
107°
106°
105°
104°
103°
102°
101°
100°
99°
98°
97°
On Admission
Bath
Bath
Invalided Home
Phenacetin gr. 10
Quinine gr. 15
Phenacetin gr. 10
Quinine gr. 15
Quinine gr. 15
Quinine gr. 15
Quinine gr. 15
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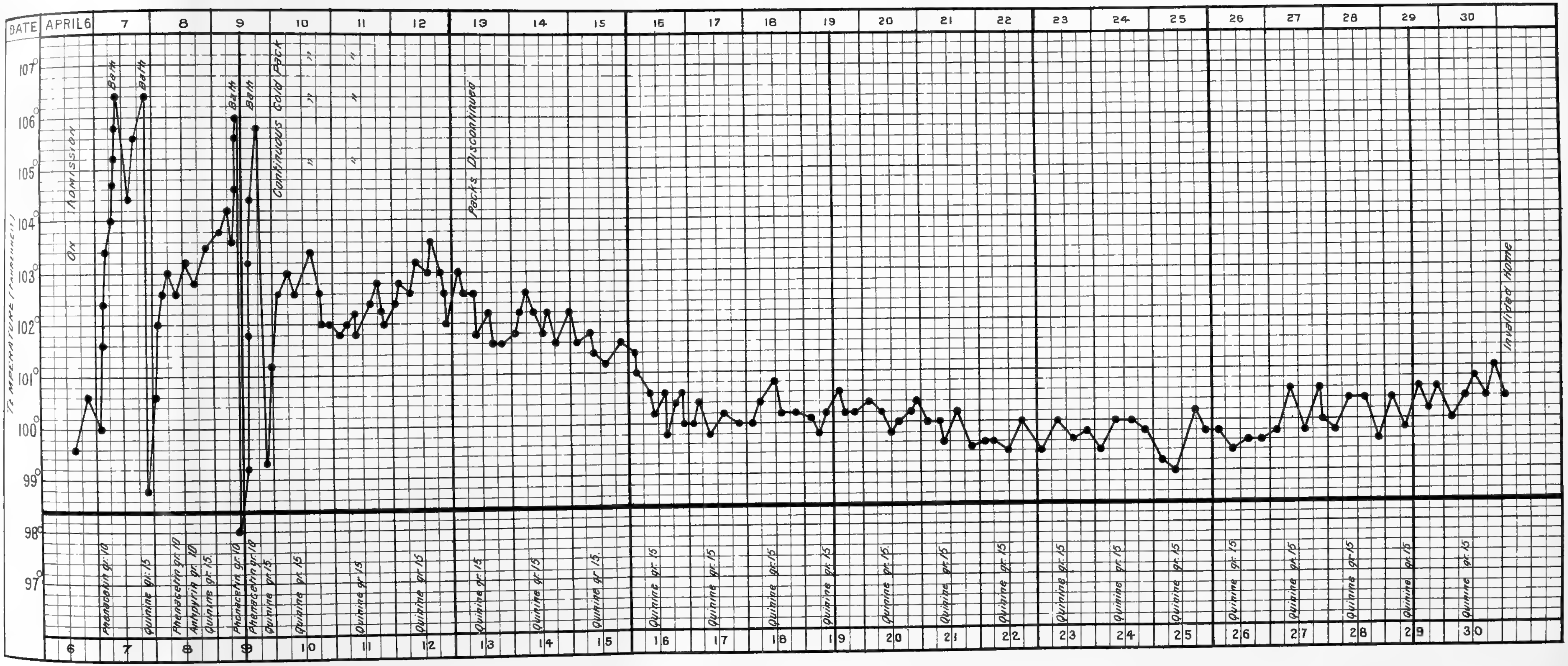

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100°
99°
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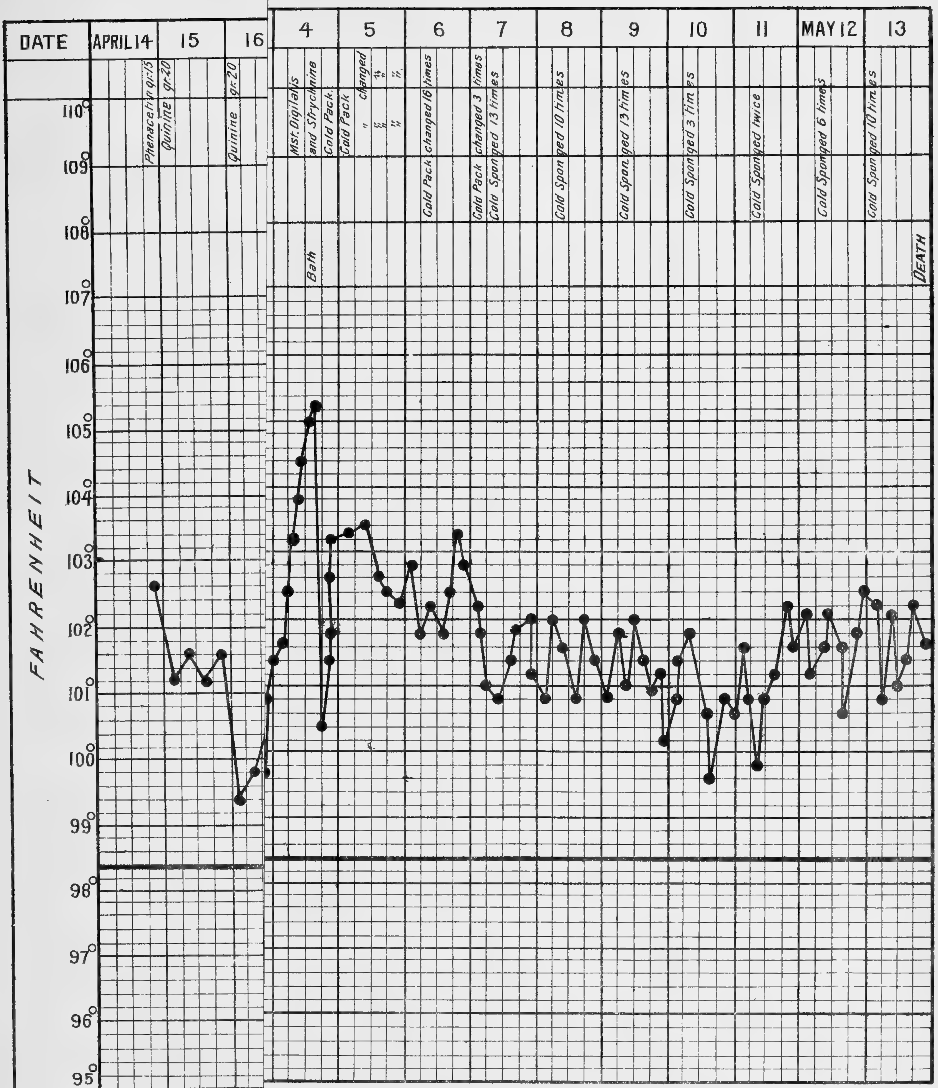
97°
On Admission
Bath
Bath
Bath
Bath
Continuous Cold Pack
Packs Discontinued
Invalided Home
Phenacetin gr. 10
Quinine gr. 15
Phenacetin gr. 10
Antipyrin gr. 10
Quinine gr. 15.
Phenacetin gr. 10
Phenacetin gr. 10
Quinine gr. 15.



DATE
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MAY 12
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Phenacetin gr. 15
Quinine gr. 20
Quinine gr. 20
Mst. Digitalis and Strychnine
Bath
Cold Pack.
Cold Pack changed
Cold Pack changed 16 times
Cold Pack changed 3 times
Cold Sponged 13 times
Cold Sponged 10 times
Cold Sponged 13 times
Cold Sponged 3 times
Cold Sponged twice
Cold Sponged 6 times
Cold Sponged 10 times
DEATH
FAHRENHEIT
110°
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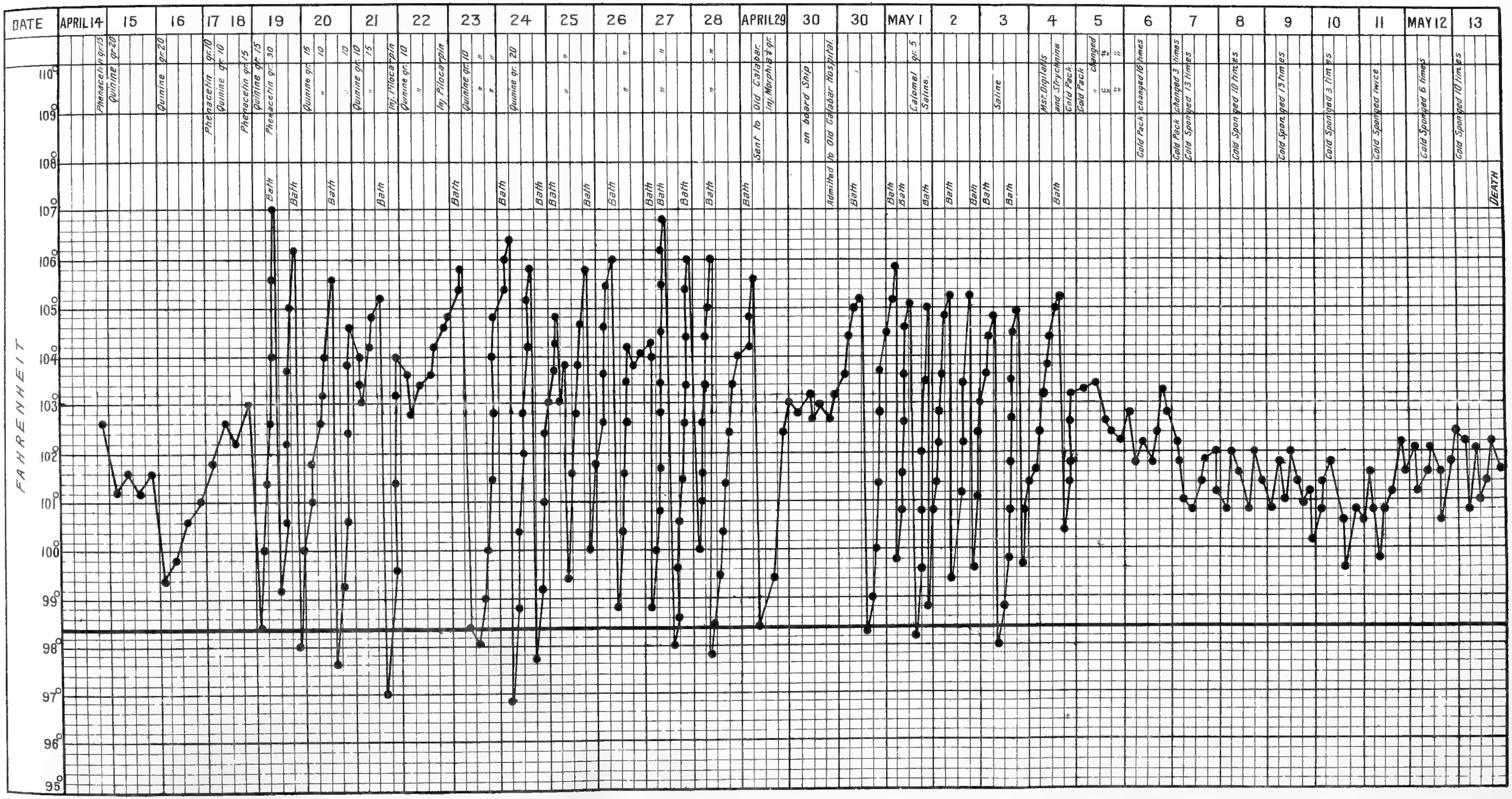

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Phenacetin gr. 15
Quinine gr. 20
Quinine gr. 20
Phenacetin gr. 10
Quinine gr. 10
Phenacetin gr. 15
Quinine gr. 15
Phenacetin gr. 30
Quinine gr. 15
Quinine gr. 10
Inj. Pilocarpin
Quinine gr. 10
Inj. Pilocarpin
Quinine gr. 10
Quinine gr. 20
Sent to Old Calabar
on board Ship
Admitted to Old Calabar Hospital
Calomel gr. 5
Saline.
Saline
Mist. Digitalis
and Strychnine
Cold Pack
Cold Pack
changed
Cold Pack changed 16 times
Cold Pack changed 3 times
Cold Sponged 13 times
Cold Sponged 10 times
Cold Sponged 13 times
Cold Sponged 3 times
Cold Sponged twice
Cold Sponged 6 times
Cold Sponged 10 times
Bath
DEATH

*Duodenum and Small Intestine* were stained a dull, greyish-brown colour. About nine inches from duodenum to a few inches from the coecum, the gut containing frothy, blackish-brown, mucous material staining the mucous membrane. No petechial nor haemorrhagic spots. Mucous membrane otherwise normal.

*Coecum and large Intestine* appeared normal.

*Suprarenals and Bladder*—normal.

*Thorax*—No pleuritic fluid—slight old adhesion at the base of the right lung. No periodical fluid.

*Heart*—Left ventricular wall hypertrophied. Cardiac muscle firm. Valves healthy. Slight early atheroma of the aorta just above the valves.

*Lungs*—Some old pigmented fibroid patches at both apices. Patch of old pleuritis at right base adhering to the diaphragm. Bronchi contained a quantity of yellowish muco-pus. This condition extended down to the small bronchioles. On section of the lung, muco-pus welled from numerous points. No broncho-pneumonia. No hypostatic congestion.

*Oesophagus*—Normal.

*Trachea*—Contained a little muco-pus.

*Epiglottis*—Two very small ulcers at the base size of pinhead.

*Pharynx and Thryroid Gland*—Normal.

*Brain*—Rather wet and oedematous, firm, otherwise appeared normal; numerous small pachionian bodies.

## Morbid Histology

*Liver*—Sections of the liver present very little pathological change. There is a slight increase of fibrous tissue around the portal canals, with a slight thickening of 'Glysson's' capsule. Slight congestion of the hepatic zone. The liver cells are sharply defined—the nucleus staining well with Haematein, the cell protoplasm shews a very slightly granular appearance. Slight fatty infiltration occurs in the portal zones of some of the liver lobules. Marked yellow pigmentary deposit is observed in the liver cells throughout the whole lobule. No recent malarial pigment can be made out in the capillary endothelial cells, and traces of old pigment are only very occasionally seen. The liver cells throughout the lobules give a marked reaction of iron with pure haematoxylin, and with potassium ferrocyanide and hydrochloric acid.

*Spleen*—Sections shew an increase of fibrous tissue. The trabeculae are thickened and the splenic pulp appears overgrown with fibrous tissue. The small arteries of the Malpighian corpuscles, which are inconspicuous, are greatly thickened both throughout their internal and external coats. The splenic capsule is thickened. Malarial pigment only occurs at very rare intervals throughout the sections. With ferrocyanide of potash and hydrochloric acid, and also with pure haematoxylin no very marked iron reaction is brought out. Masses of haematoidin pigment were scattered throughout the sections.

*Kidney*—Sections again shew fibroid changes in the thickened capsule of the organ, with a tendency to spread into the kidney substance. Some of Bowman's capsules are thickened—and there is some thickening of the arteries. The renal cells of the tubules are well defined—nuclei stain well—the protoplasm has a slightly granular appearance, and in places there is a slight epithelial desquamation, with a granular deposit in the tubules. There is some congestion of the stellate veins and vessels of the boundary layer. No pigment present.

*Lung*—Sections shew a condition of marked emphysema. Round some of the bronchi the alveoli are filled with a catarrhal exudation, and a few golden corpuscles are present. The bronchi shew catarrhal inflammatory changes.

*Brain*—In sections of the cerebral cortex, and of the corpus striatum,* no pathological changes can at present be made out. No malarial pigment is present.

The interesting features to be noted in the results of this examination are the absence of recent malarial pigment from the positions in which it is generally found in cases of malarial fever—and secondly, the absence of cloudy swelling of the parenchymatous epithelium of the organs, and of marked fatty degeneration such as one would naturally have expected as a result of a period of some four weeks' high temperature. It must be stated that the patient had been several years on the West Coast of Africa, and had had several attacks of malarial fever, thus accounting for the presence of a little old pigment.

The absence of cloudy swelling and fatty degeneration changes in the organs points not only to the non-malarial nature of the disease but, we take it, also that a 'toxic' substance produced in the course of a specific infective disease was also absent. It has been suggested that the characteristics of this disease—the peculiar absence of local symptoms, the manner in which the temperature is uninfluenced by drugs, but easily affected by cold applications, and the long period through which it gradually becomes normal, as well as the conditions found on pathological examination—might be all explained by some profound disturbance in the process of heat regulation.

In this connection it may be pointed out, on the West Coast of Africa a temperature of about 130° F. and an atmospheric humidity of between 90 and 100 per cent. are often associated, and that these conditions have considerable influence on the health of Europeans in those parts—accounting for some few of those cases treated as malarial fever cases. From our own observations such cases occur, from which the typical signs and symptoms of malarial fever are absent. It has been noted that no cases of insolation, siriasis, sunstroke, sun or heat fever are found mentioned in the medical reports of Southern Nigeria. It was our experience at Old Calabar that the climatic conditions often produced a slight rise of temperature, and for experimental purposes we occasionally undertook rather severe exercise, such as hard walking (provided with sun helmet or shade) in the open, and almost invariably noted a rise of temperature after an hour or so, although perspiring profusely at the time. Temperatures reaching up to 102·2° F. were observed—normal being reached in the course of a few hours. It is easy to imagine that an exaggeration of such conditions, together with a constitutional condition in which from any cause the dissipation of the heat of the body does not balance the production might, perhaps, lead to such an overthrow of the process of heat regulation as to occasion conditions similar to those of the so-called 'hyperpyrexial' fever cases described.

*Further investigations in the pathological histology of the corpus striatum in this case are being undertaken.

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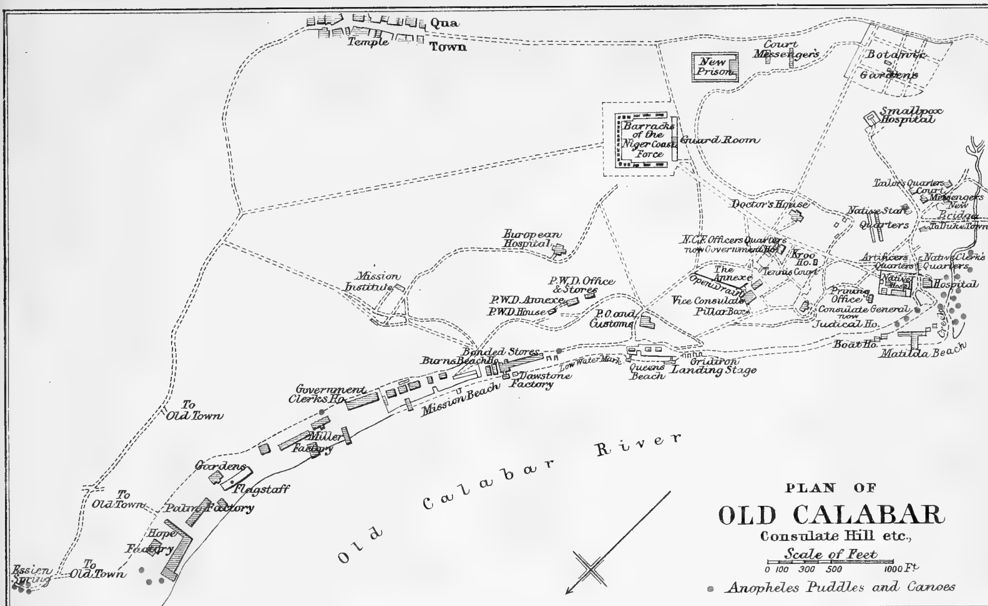
The accompanying plans of the Consulate Hill, Old Calabar, and of part of Duke Town, shew the positions of the breeding-places (canoes, puddles, and spring) of *Anopheles*. We are indebted to H. M. Bradford, Esq., Acting Chief Officer of the Public Works Department for these, the only available plans. The breeding-places are marked in red, and it is to be noted how they are most abundant generally in the neighbourhood of those factories which are more or less surrounded by native huts. The plan of part of Duke Town also shews how efforts are being made to relieve the crowded and badly arranged condition of the native town by the construction of wide roads and streets.

DONALD FRASER, PRINTER TO THE UNIVERSITY PRESS OF LIVERPOOL

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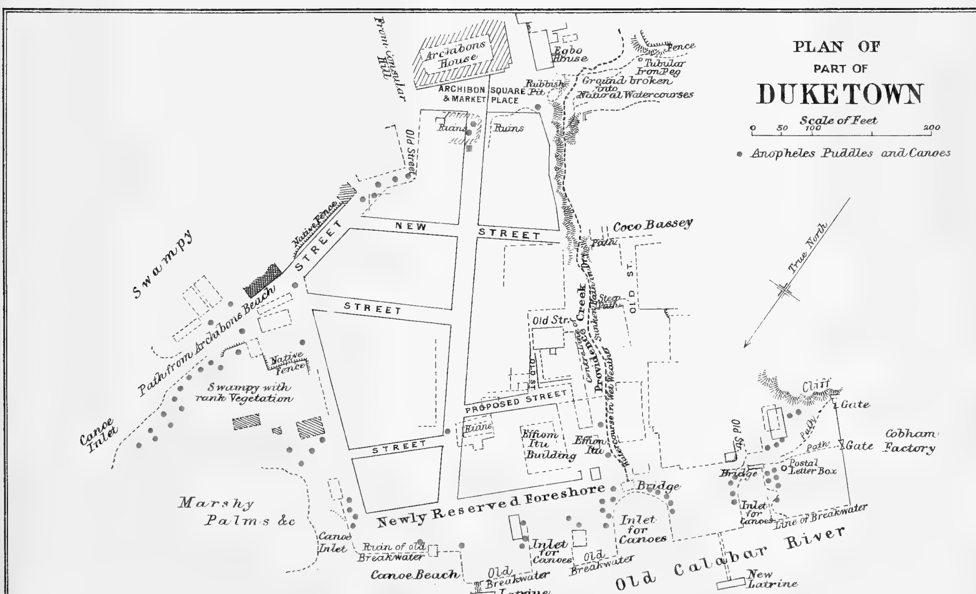


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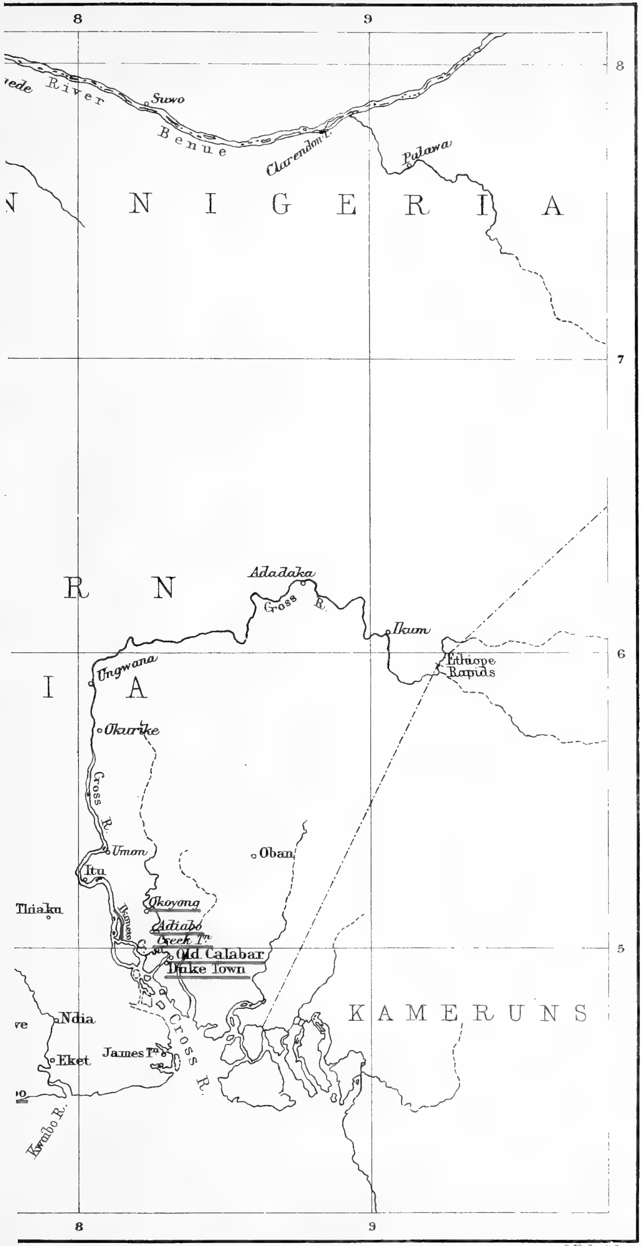
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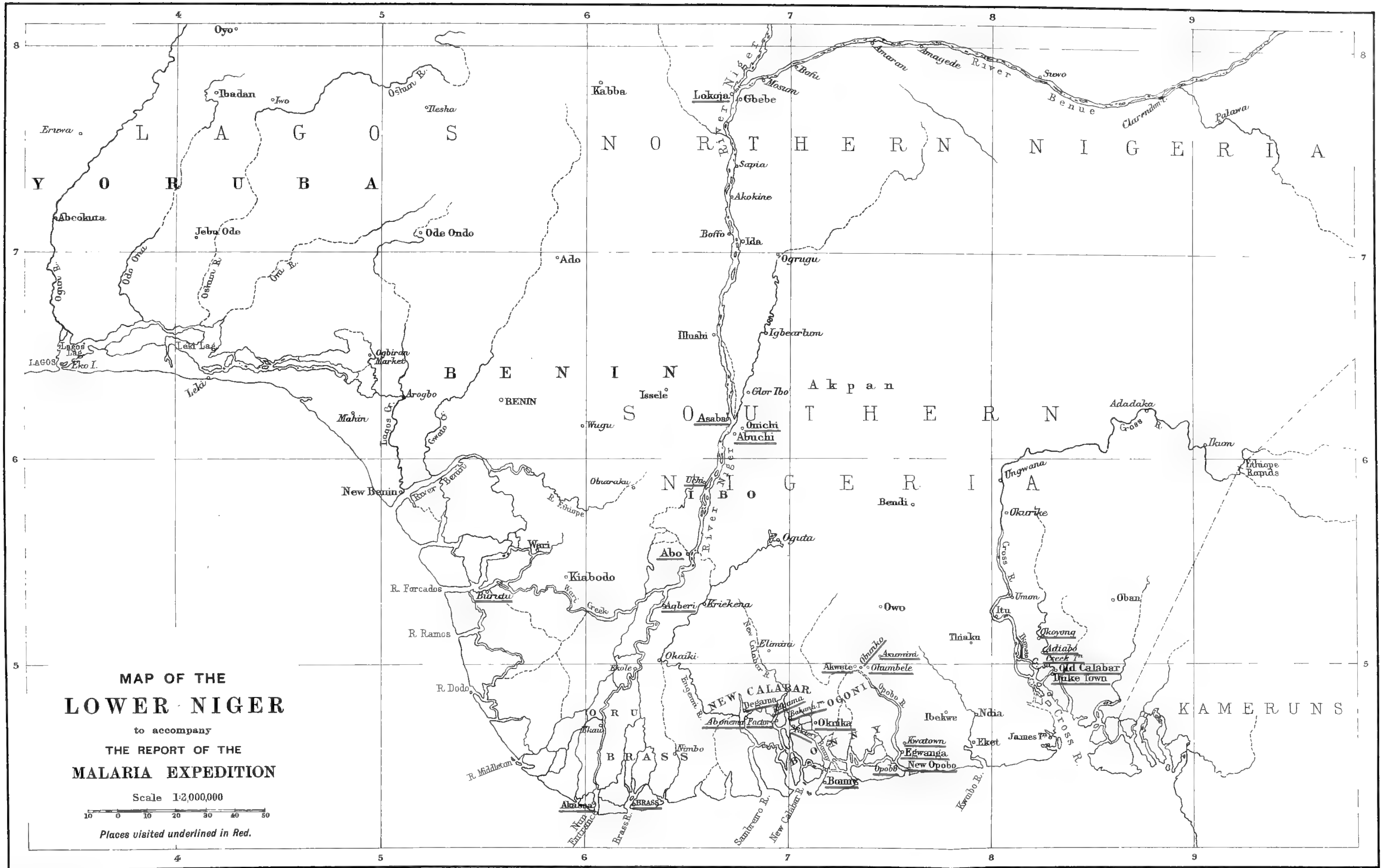


**MAP OF THE**

**LOWER NIGER**

to accompany

THE REPORT OF THE

**MALARIA EXPEDITION**

Scale 1:2,000,000

10 0 10 20 30 40 50

*Places visited underlined in Red.*

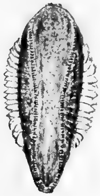

FIG. 1

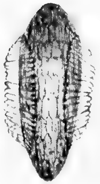

FIG. 2

FIG. 3

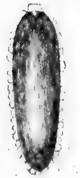

FIG. 4

FIG. 5

FIG. 1. *Anopheles* ovum—superior surface
FIG. 2. ,, ,, —inferior ,,
FIG. 3. ,, ,, —side view
FIG. 4. *Culex* ovum
FIG. 5. *Anopheles* ovum—empty egg case
FIG. 6. The 'float'—upper surface
FIG. 7 ,, ,, —lower ,,

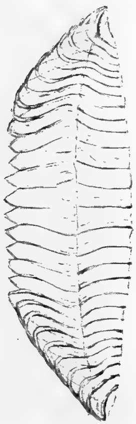

FIG. 6

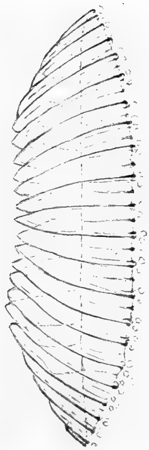

FIG. 7

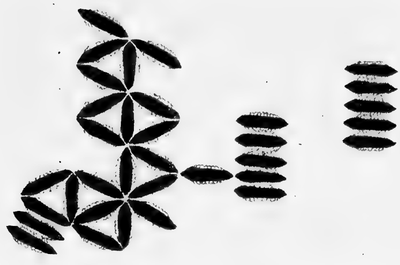

Fig. 1. The characteristic pattern in which *Anopheles* ova are deposited on the surface of water

Fig. 2. Showing larvae in several stages of their escape from the ova

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LIVERPOOL

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