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EXPERIMENTS AND OBSERVATIONS

ON THE

VITALITY

OF THE

Bacillus of Typhoid Fever

AND OF

Sewage Microbes in Oysters and other Shellfish.

INVESTIGATIONS

ON BEHALF OF

The Worshipful Company of Fishmongers, London,

BY

E. KLEIN, M.D., F.R.S.

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EXPERIMENTS ON OYSTERS AND OTHER SHELLFISH.

My Committee has charged Professor Klein with conducting experiments on oysters and other shellfish in order to ascertain the vitality of the typhoid bacillus and other sewage microbes in them. The results obtained with oysters, cockles, and mussels are herewith published by the Fishmongers' Company.

J. Wrench Towse,

Clerk of the Worshipful Company of Fishmongers, London.

May, 1905.

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Report of Experiments and Observations on the Vitality of the Bacillus of Typhoid Fever and of Sewage Microbes in Oysters and other Shellfish.

By E. KLEIN, M.D., F.R.S.,

Lecturer on Advanced Bacteriology in the Medical School of St. Bartholomew's Hospital, London.

ALREADY in 1893 the then Medical Officer of the Local Government Board, Sir Richard Thorne, in his Summary (Reports and Papers on Cholera in England in 1893, Local Government Board) makes the following trenchant remarks on page 29, in reference to a number of cholera attacks in which the history pointed to infection by means of oysters and shellfish which had been procured from, and specifically fouled at Cleethorpes and Grimsby, *viz.*:—"But one thing is certain, oysters and shellfish, both at the mouth of the Humber and at other points along the English coastline, are at times so grown and stored that they must of necessity be periodically bathed in sewage more or less dilute; oysters have more than once appeared to serve as the medium for communicating disease, such as enteric fever, to man; and so long as conditions exist such as those with which the oyster trade of Cleethorpes and Grimsby is shown to be associated, conditions which may at any time involve risk of the fouling of such shellfish with the excreta of persons suffering from diseases of the type of cholera and enteric fever, so long will it be impossible to assert that their use as an article of diet is not concerned in the production of disease of the class in question."

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In the subsequent two or three years several outbreaks of typhoid or enteric fever having been demonstrated to be caused by the consumption of oysters derived from sewage-polluted layings in America, in France, and in England (see Report of the M.O., "Oyster Culture and Disease," 1894–1895, Appendix 3 and 4), the Local Government Board had instituted in 1894–1895 a careful survey of all oyster beds and oyster ponds along the whole coast of England and Wales; the results of those investigations were published in 1896 by the Medical Department of the Board as a separate volume ("Oyster Culture and Disease"), and it will be seen therein to what a large extent oysters are laid down and stored in several places in England in a manner which must be considered not only objectionable *quâ* cleanliness *per se*, but also must be instrumental in conveying occasionally dangerous infection to those consuming them.

Without intending to cite all those cases of typhoid fever in which in single instances and in a small group of individuals who had partaken of oysters, mussels, or cockles derived from polluted localities (such as have been described by various health officers—Dr. Newsholme, Dr. Thresh, Dr. Nash, Dr. Allen, and others), typhoid fever has been demonstrated to have been caused by such shellfish, I will mention the two instances only in which in recent years, to wit, November 9, 1902, infection with typhoid fever by polluted oysters has manifested itself in a somewhat dramatic fashion and on a considerable scale—I refer to the now historic mayoral banquets at Winchester and Southampton. The demonstration of this infection, of the derivation of the typhoid oysters from sewage-polluted ponds at Emsworth, are well known; they have been well described by Dr. Bulstrode in the annual report of the Medical Officer of the Local Government Board, 1902–1903, pp. 129–189. Even subsequent to these outbreaks, *viz.*, during 1903 and 1904, cases of enteric fever have been traced in numerous single instances to the consumption of polluted oysters or polluted cockles (Dr. Collingridge, Dr. Allen, Dr. Buchanan, and others), and it is common knowledge that, with the

exception of a few isolated instances in which since 1896 an improvement in oyster layings and oyster storage has been effected, the general system obtaining in a good many instances, *viz.*, of exposing oysters to "be periodically bathed in sewage more or less dilute," is still the same as it was in 1895, that is to say, "conditions which may at any time involve risk of the fouling of such shellfish with the excreta of persons suffering from diseases of the type of cholera and enteric fever." As a matter of fact, I have in several instances discovered the B. typhosus in shellfish coming from polluted sources. These are the instances:—

1. In a sample of oysters derived from Grimsby in 1895.
2. In a sample of oysters brought over direct from America, 1903.
3. In a sample of mussels gathered from a polluted place on the shore of Southend-on-Sea, 1904.
4. In a sample of oysters gathered from a place in Langstone Harbour, about 600 yards distance from the Portsmouth sewer outfall, 1904.

I shall have presently an opportunity of showing that the identification of this microbe in such shellfish is a matter of no small difficulty, owing to such shellfish always containing a large amount, and in preponderance, of sewage microbes, greatly impeding the identification. In order to detect the B. typhosus in shellfish or other materials (water, milk) exposed to sewage pollution, and therefore harbouring sewage microbes, the former must be present in appreciable numbers, and if found would *à priori* conclusively indicate that the specific pollution (*i.e.*, with typhoid excreta) must have been considerable. Until the last few years such identification was an almost hopeless task, but at present the task has been facilitated to a considerable degree by the discovery by Drigalski and Conradi of a culture medium, on which the presence of the B. typhosus can be easier detected than by the former methods. I say easier, although I should not omit to add that also by this method its presence must be in fair proportion. The first case in which the typhoid bacillus was found in the Grimsby oysters was described in the Report of

the Medical Officer of the Local Government Board ("Oyster Culture in its Relation to Disease," 1894–1895, pp. 114 and 115), and it was discovered by the older method: phenolated broth and phenolated gelatine, so that in this instance it must have been present in very considerable numbers. In the other instances (2, 3, and 4) above mentioned the identification was somewhat easier, *viz.*, by the Drigalski-Conradi medium, of which presently more will be said. In the last instance, *viz.*, oysters from Langstone Harbour, the typhoid bacilli were met with in one oyster to the amount of several dozen per oyster. When I speak of the B. typhosus having been identified, I mean the microbe had responded to all and every test—of which there are many, as will presently be described—which denote the known characters of the microbe of typhoid or enteric fever.

From the foregoing it will appear interesting to inquire whether and to what extent under the circumstances of actual specific fouling of shellfish, such as must occasionally occur in estuaries and on the shores of the coast, where oysterlayings and oysterponds, mussels and cockles, are exposed to almost continuous sewage pollution, the obnoxious and dangerous microbes which have found entrance into the shellfish are readily, and by what methods, removable from such shellfish; or whether having once gained entrance remain in it and make as it were therein a home for themselves. It is clear that if the latter should be the case no remedy would be available to render such shellfish—principally oysters—fit for consumption; whereas, if the former should be the case a remedy would be available. With reference to oysters this question is of greater importance, inasmuch as the great majority of these shellfish are consumed in raw state, whereas in the case of mussels and cockles—leaving out the instances in which the enthusiastic gatherers eat them raw—some kind of heating process, though as we shall see this is not always effective and reliable, is employed previous to their being eaten, and therefore the majority of dangerous microbes presumably are in many cases devitalised.

The dangerous microbe in shellfish with which we are chiefly concerned is, of course, the Bacillus typhosus. It is not, as we shall see later, the only dangerous microbe, but it certainly is the chief one, because infection with it, as mentioned above, has hitherto been of somewhat conspicuous frequency, and it is chiefly this microbe, *i.e.*, the microbe of typhoid or enteric fever, that need occupy us here.

In the experiments which I conducted for the Local Government Board, "Oyster Culture in Relation to Disease," 1894–1895 (pp. 116–120), oysters were kept in sea water infected with the B. typhosus, and it was found that this microbe was recovered from the interior of some of the oysters as late as 18 days after infection.

Professor Herdman in 1895 states (Report on the Lancashire Sea Fisheries) that in the case of oysters grown in water infected with B. typhosus it was found that there was no apparent increase of the organisms, but that they could still be identified in cultures taken from the water of the pallial cavity and rectum 14 days after infection.

Dr. Chantemesse (Proceedings of the Academie de Médicine of Paris, June (?) 1896) placed oysters for 24 hours in sea water intentionally infected with B. typhosus, then kept them for 24 hours out of this water; examining them after the lapse of this time, he found in the liquor and in their bodies B. typhosus.

Herdman and Boyce in a series of experiments conducted with oysters infected with B. typhosus (Oysters and Disease, Thompson Yates Laboratories, Vol. II, 1898–1899, Lancashire Sea Fisheries, Memo. No. 1) summarise, p. 54, their results thus: "In our experimental oysters inoculated with typhoid we were able to recover the organism from the body of the oyster up to the tenth day. We show that the typhoid bacillus does not increase in the body or in the tissues of the oyster, and our figures indicate that the bacilli perish in the intestine."

Most observers are agreed that the typhoid bacillus does not multiply within the oyster, and is gradually destroyed or eliminated when the oyster is placed in clean sea water,

although as to the time required for this process of cleansing the various observers are not agreed; thus, while my experiments in 1894–1895 would indicate the duration of vitality of the B. typhosus in the oysters to be three weeks, Chantemesse at first recommended about the same period (*quelques semaines*), later he reduced the time to 8 days (l.c. 9 June, 1896). Professors Herdman and Boyce, l.c. p. 54, say: "In our experiments in washing infected oysters in a stream of clean sea water .... there was a great diminution or total disappearance of the typhoid bacilli in from one to seven days."

In order more accurately to determine the vitality of the typhoid bacilli in oysters, and to ascertain how and in what period a given number of B. typhosus introduced in or ingested by oysters, disappears from their (oysters) interior under conditions resembling those obtaining in nature more or less, the Worshipful Company of Fishmongers have charged me with undertaking the required experiments, not only with oysters, but also with mussels and cockles. Such numerical determination is at present possible, and a matter comparatively easy to achieve, as will presently appear when describing the method used.

It must be obvious that as regards oysters the problem resolves itself into the following questions, *viz.*: 1. Given clean oysters, *i.e.*, oysters which are laid down in clean water, what power, if any, have such oysters to destroy or eliminate a definite number of B. typhosus injected into or ingested by them, and further in what time can they do this, supposing that they are afterwards kept under conditions best for such destruction or elimination, *i.e.*, kept in clean water daily renewed? The answer to this question would demonstrate in an absolute way whether or not such power is inherent in the oyster. 2. Is there any difference in these respects between oysters which are derived from clean layings and oysters derived from initially sewage polluted beds? 3. Is there any difference, and what, between oysters previously infected with the B. typhosus, which are then kept in clean sea water continually changed, and such as having been infected are not placed under these favourable conditions?

The first part of this Report deals with experiments capable of furnishing definite answers to these questions, and these answers will enable us to draw conclusions with something like exactness as to the means required for dealing with oysters presumably dangerously polluted. The second part of this Report deals in similar fashion with mussels and cockles. In a third part observations and experiments are described which deal with the general question of identification in oysters of microbes derived from sewage, a question which at present is still imperfectly understood, and on account of this not unfrequently misinterpreted.

Series A.—Experiments with the B. typhosus in Oysters.

Before we enter on a description of the details of these experiments and the methods by which the experiments were carried out, it may not be out of place to give in a general way a summary of the present knowledge concerning this microbe.

The typhoid bacillus—Bacillus typhosus—is the essential cause of typhoid or enteric fever; that is to say, when introduced into a susceptible individual—generally by way of the digestive organs—it is capable of setting up, after an incubation period of from 10 to 14 days, the clinically and pathologically well-defined acute febrile disease known as enteric or typhoid fever.

The microbe is found in large numbers—multiplying readily—in the interior of the ileum and in its swollen mucous membrane, Peyer's glands; it is found in great abundance in the swollen and inflamed mesenteric glands, and in the swollen spleen. In the intestine, and also in the typhoid stools, its demonstration is somewhat made difficult by the simultaneous presence—generally in predominating numbers—of other bacteria similar to it, but not the same, to wit Bacillus coli (see below), but it has been shown, and by some of the most modern methods it has become more easily to do so, particularly during the second and third week of the illness,

viz., that in the contents of the ileum and in the fluid and semi-fluid (typical pea soup) typhoid stools the typhoid bacillus does occur in great numbers. In several instances of cultivation by the Drigalski-Conradi medium it was possible to show that in the fluid typhoid stools the B. typhosus was present to the number of one to three millions per one cubic centimetre, that is to say, about one to two hundred thousand per drop (minim) of the stool. As regards the mesenteric glands and the spleen of a case of typhoid fever, the demonstration by the microscope and particularly by culture of the B. typhosus can be demonstrated readily, and in culture made with a trace of a particle of these organs crowds of typhoid bacilli—generally in pure culture—can be obtained. It has been further shown that in some cases even several months after convalescence has set in, typhoid bacilli are still present in the stools of the patient, although as a rule a few weeks after convalescence has commenced they have practically disappeared from the bowels. It has further been shown that in localised inflammations following upon the acute stages of the disease—particularly in the lung—the typhoid bacilli may be, and sometimes are, present in large numbers. One of the most important results of research that has been brought to light is this, that typhoid bacilli pass out of the body by way of the kidney and urine in enormous numbers during convalescence, so much so that it has been calculated that in at least 25 per cent. of cases (according to more recent observations over 25 per cent.) the convalescent voids typhoid bacilli by way of the urine (Bacilluria), this fluid being in some marked instances turbid by the number of typhoid bacilli—up to 6000 millions per one cubic centimetre (Dr. Horton Smith, Gulstonian lectures before the Royal College of Physicians, 1900). It must be obvious that these results are of the utmost importance to public health, and I will for the sake of illustrating this importance take the following into consideration: Up to the time and before the above fact concerning the copious presence of B. typhosus in the urine of a large percentage of convalescents was elucidated, the attention of sanitarians, physicians, and nurses was chiefly

directed to the stools of typhoid patients as being capable of conveying the disease germs during the first four to six weeks, that is during the active phases of the disease and the early stages of convalescence, the urine not being specially attended to. But since we now know that the patient, weeks after convalescence has set in, voids typhoid bacilli by the urine, the presence in any locality of a convalescent from typhoid fever, in whom the stool has perhaps ceased to be infective, remains, nevertheless, a fruitful source of typhoid bacilli. We may have a seaside place in whose population no typhoid fever cases have occurred, but to which seaside place a person convalescent from typhoid fever has been taken for recuperating; the sewage of this seaside place—ostensibly free from typhoid fever—would nevertheless contain plenty of typhoid bacilli which might find access to shellfish laid down or kept on or near the shore of such a place.

The typhoid bacillus belongs to a large group of microbes —coli-typhoid group—which in morphological, cultural and physiological respects possess certain characters in common, but the individual species constituting the group differ, nevertheless, from one another in definite manner. As to the B. typhosus its essential differential character is that it is found, as described above, in definite distribution in typhoid fever and in this disease only, and that its introduction into the alimentary canal under suitable conditions, and its multiplication within the infected person, sets up the specific disease typhoid fever, as has now amply been demonstrated by indirect epidemio-logical evidence, as also unfortunately in several direct instances amongst those who have worked in the laboratory with cultures of the typhoid bacillus. None of the other species belonging to the coli-typhoid group are connected, as cause, with typhoid fever, although some, like the Bacillus Gaertner, some virulent coli-like organisms, the Bacillus paratyphosus and Bacillus dysenteriæ, are connected with other acute intestinal diseases, but not of the nature of true enteric fever.

The morphological and cultural characters by which the

B. typhosus is distinguished from other coli-like microbes of the coli-typhoid group (all of which fail to liquefy gelatine and are gram negative in staining) are these:—(*a*) Morphological: motile, cylindrical bacilli, multiflagellated, the thin, long, wavy flagella distributed over the whole body; the bacilli in culture are capable of forming shorter or longer threads; like other coli-bacilli they grow well at all temperatures up to 38° or 40° C. (*b*) Cultural: on gelatine colonies angular discs, with thicker centre, filmy margin, finely granular; on gelatine streak translucent, filmy, dry-looking band, slightly thicker in centre, irregular margin; in ordinary, as also in sugar gelatine shake culture, no gas formation, colonies uniformly distributed throughout the gelatine; grows always slower than B. coli; on agar not characteristic, except that the growth is slower and more translucent than that of most B. coli; in litmus milk acid production, slower than that of most B. coli, milk remains fluid; in phenol broth good growth, no gas formation; in ordinary broth rapid growth and uniform turbidity, no indol formation; in neutral red broth no change of colour; on potato colourless, filmy growth; on potato gelatine colonies smaller round and more translucent than those of most B. coli; on potato agar and in urine gelatine grows more filamentous than B. coli; in Proskauer and Capaldi medium I negative, in Proskauer and Capaldi medium II positive—B. coli gives the reverse test; on Drigalski-Conradi medium the colonies are characteristically bluish in laterally reflected light, violet-blue in directly reflected light; translucent, filmy, violet margin, thicker more or less acuminated centre, finely granular; the bacilli composing the colonies are oval to cylindrical, motile; in MacConkey fluid (litmus glucose taurocholate of soda, peptone) acid formation but no gas; in litmus lactose peptone growth, litmus becomes bleached, no acid or alkali, no gas; blood serum of typhoid patients (Widal's test), or blood serum of an animal previously injected (prepared) with typhoid culture, acting on broth culture, or emulsion of gelatine or agar culture of B. typhosus, the bacilli become arrested in their motility and agglutinated into large more or less

dense clumps in marked and rapid manner (Bordet-Gruber test in vitro), and in higher dilutions than other allied microbes.

Small doses of a recent culture of B. typhosus intraperitoneally injected cause death of guinea-pigs from acute peritonitis in a short time, 20 to 36 hours, according to size of dose, though it has to be remembered that when subcutaneously injected it acts locally only, except very virulent strains in fair doses produce sometimes general infection and death. The virulence differs with different strains; an animal previously prepared with subfatal doses of culture of B. typhosus (of either living or dead culture) is immunised and protected against an otherwise fatal intraperitoneal dose of virulent B. typhosus, the animal in proportion to its previous preparation suffers no ill effects, the peritoneally injected bacilli rapidly undergoing granular degeneration and change into granules and globules of dead matter (Pfeiffer's phenomenon or test *in corpore*). Assuming that in hanging drop and in staining a microbe shows the characters above mentioned, produces in litmus milk acid but no coagulation, gelatine streak and shake positive, neutral red negative, in phenol broth or MacConkey fluid good growth, no indol formation, in litmus lactose peptone negative, litmus bleached, and further in Drigalski and Conradi plates, in Proskauer and Capaldi medium I and II, in flagella stained specimens, and in agglutination test in high dilution with typhoid serum (of man, better of typhoid immunised animals), it answers in positive fashion, we would consider these sufficient to establish the identity of the microbe in question with the B. typhosus.

From the foregoing it will have been gathered that, in order to definitely identify a particular microbe as the B. typhosus, a number of tests, morphological, cultural and experimental, have to be employed, and it will also be readily understood that if in any material, subjected to analysis, the typhoid bacillus should be associated with other microbes belonging to the coli-typhoid group, the difficulty of isolation of the B. typhosus out of the mixture must be

correspondingly greatly increased. And it is precisely materials which contain such mixtures (water, milk, shellfish fouled by filth and excremental matters) that we are often called upon to analyse for the presence of the B. typhosus. In ordinary domestic sewage the number of B. coli communis alone amounts to between 100,000 and one million, or even more, per 1 c.c.; in ordinary normal fœcal matter B. coli communis alone amounts to something between 40 or 50 millions and 400 to 1000 millions per one gram, in the fluid typhoid stool (pea soup stool) the number of B. coli amounts to something like 14 to 20 millions per 1 c.c. The B. coli communis, as also other coli-like microbes belonging to the coli-typhoid group, grow in all the media in which the B. typhosus is capable of growing, and unfortunately with greater ease and rapidity; but there is no medium known in which the reverse is the case, and it will therefore be readily understood that by cultivation—the only method which as the first step in the analysis can be resorted to—the isolation of the B. typhosus from amongst a number —generally an overwhelming number—of coli-bacteria and other microbes in a given mixture must be, in the nature of things, an extremely difficult matter, unless the B. typhosus should happen to be present in very large numbers. Add to this the well-recognised fact that, taking the above tests for differentiation of the B. typhosus from the other species of the coli-typhoid group, the differences are small, and some of them more or less those of degree only, and a negative result *quâ* isolation of the B. typhosus from the polluted materials can be easily understood, although the polluted material (water, shellfish, milk) had been proved to be specifically polluted with typhoid excreta, having produced typhoid fever in the consumers.

Under these circumstances, any method by which even the favouring growth and the rapid recognition by culture of bacteria belonging to the coli-typhoid group could be effected is of advantage, though it is only a first small step; this is achieved by Parietti's method (adding a certain amount, 0·05 per cent., of phenol to the

culture medium), by which other bacteria not belonging to the coli-typhoid group are kept back, while the bacteria of the latter group grow undisturbed. The same, to a large extent, is the case with Elsner potato gelatine. But it must be obvious from what has been said above that such inhibition (for a time, at any rate) of other bacteria not belonging to the coli-typhoid group does not carry us very much further, because the difficulty about the coli-bacteria amongst themselves is still present. MacConkey's fluid is a further step in advance, because in this medium the rapid production of acid—reddening of the litmus fluid—denotes already in 24 hours or so, the presence of acid-forming bacteria, most probably belonging to the coli-typhoid group, and possibly including the B. typhosus. This medium keeps back non-acid producers to a large extent, and, owing to the easily perceived change in colour of the litmus to red, the presence of an acid-producing microbe is at once made out. Unfortunately, all coli-bacteria forming acid grow well in this medium, and, therefore, it does not carry us much further in the isolation of the B. typhosus than did Parietti's method. The same remarks apply to all other media that have been hitherto described, with the notable exception of the Drigalski-Conradi medium, for by this medium used for surface plates, as described by Drigalski-Conradi (" Zeitschrft f. Hygiene," vol. 39, p. 283) (Nutrose, lactose, litmus, crystal violet, agar), we are at once placed into this advantageous position, that we can not only keep back or exclude (by the use of the crystal violet) bacteria other than coli-typhoid, but we can in positive fashion and in isolated aspect recognise at once those colonies which are not B. typhosus. Any colony which after 24–36 hours' incubation at 37° C. appears on the medium red in colour surrounded by a red halo (reddening of the litmus constituent of the medium due to rapid acid production from, *i.e.*, fermentation of the lactose by the bacteria constituting the said colony), cannot be one of B. typhosus, but must be one of B. coli, probably B. coli communis; if the medium did nothing else than this, it alone would for obvious reasons be a great help, for we could from

our further study and search for the B. typhosus safely exclude and disregard those red-haloed red colonies. But the medium does more than this, for every red colony in general, and every colony which is neutral in colour, *i.e.*, neither red nor blue, can likewise be excluded as being certainly not a colony of B. typhosus, because the colonies of B. typhosus after one, two, or, better, three days at 37° C. appear bluish or more or less violet-blue. As will presently be further stated, not every "blue" colony is necessarily one of B. typhosus, but if we find in our plate colonies of blue or violet-blue colour, we have a guide to which colonies we have to direct our attention in our further study and tests. How important a step in advance of all others this method of Drigalski-Conradi is, can best be estimated by the following illustration: if, for instance, we are working with a given material (water, milk, shellfish), in which by previous analysis by means of Drigalski plates we have ascertained the number of B. coli and the absence of blue colonies in a definite amount of that material, we can, after adding to the material a trace of typhoid culture, without any difficulty ascertain by Drigalski plates the number of B. typhosus in any given amount of the material by merely counting the blue or violet-blue colonies which have made their appearance in the plates. Of course we would also be able, if necessary, to make from these blue colonies the further tests for B. typhosus. In the experiments to be presently described, the enumeration by Drigalski plates of the number of B. typhosus introduced into oysters or taken up by them while in sea water, to which a small amount of a pure culture of B. typhosus had been added, was easily carried out; indeed, such exact determination, as will presently be shown, was made possible because we had this method at our disposal.

The oysters, with the exception of those in Experiment IV, selected for our experiments, as also the mussels and cockles, were all clean, containing no microbes of the coli-typhoid type. The sea water used for the experiments was clean, and free of any microbes of the coli-typhoid type—in fact, the sea water had been previously sterilised. The infecting material

was a pure culture of our laboratory B. typhosus. Under these conditions, therefore, the presence of any B. typhosus in the oysters or in the sea water could be determined readily and at once numerically by means of Drigalski plates, since all colonies of the colour and appearance resembling those of B. typhosus could, without hesitation, be declared as those of B. typhosus. The other tests: microscopic examination in the hanging drop, agglutination with typhoid serum, subcultures in MacConkey fluid, neutral red broth, litmus milk, streak and shake gelatine culture, would fully confirm the diagnosis. We shall have later an opportunity to describe experiments with oysters of a certain locality—experiments made not with B. typhosus but with B. coli—in which the Drigalski plates revealed the presence of microbes whose colonies in their blue colour and general appearance bore a great resemblance to those of B. typhosus, but which by microscopic tests and by subculture in the various media could be recognised as different; but in our experiments (except Experiment IV) of testing the vitality of the B. typhosus in oysters, cockles, and mussels, no such disturbing microbes were present at starting, and under the conditions above stated none could have been afterwards present to disturb the simplicity of the procedure. This can in no way interfere with the general results obtained, since our object was to determine how far and to what degree and in what manner living oysters, cockles, and mussels as such have the power to deal with the B. typhosus that have had access to them. Whether other microbes are present in the oysters or whether other additional microbes are introduced with the B. typhosus are questions which do not materially alter the simple and fundamental problem, *viz.*—can, and to what degree do shellfish deal with the B. typhosus?—and, therefore, the simpler the conditions for elucidating it, the more accurate, it may be expected, will be the result. There is one further point which, at the outset, has to be stated here—this is the character of the B. typhosus on Drigalski medium in surface plates. We mentioned above that it was by this method that we analysed the shell-

fish and the water, and that by this method we were enabled to determine the number of B. typhosus introduced into the shellfish or into the surrounding water.

Now, what are the characters by which the B. typhosus can be readily recognised by the Drigalski-Conradi plate method?

A given small amount of water or of substance of shellfish—up to 0·1 c.c.*—containing a limited number of B. typhosus is uniformly rubbed, after the Drigalski method, over the surface of the medium (Nutrose, litmus, lactose, crystal violet, agar), previously set—about quarter-inch depth—in a flat plate dish—the plate dish which I use is four-and-a-half inches in internal diameter and seven-eighths of an inch deep—and the plate is then placed in the incubator at 37° C.

Inspecting the plate after 24 hours, the typhoid colonies are at once recognised as isolated round translucent blue dots; when inspected with a glass in semi-reflected light, they are violet-blue, and well differentiated from the purple medium; the colonies are moist looking, thin at their margin, a little thicker in the centre; after 48 hours, and better still after 72 hours, the colonies are several millimetres in breadth, bluish in the middle, violet at the *thin* margin, which latter at the same time has lost its regularly circular outline, being slightly irregular; in transmitted light the substance is distinctly but *finely* granular, and, owing to the prominent thicker centre, the colonies look more or less like limpets, being low conical. When a trace of the colony is distributed in a little sterile bouillon, the bacilli which constitute the substance of the colony are seen to be shorter or longer cylindrical rods, many of them actively motile. When tested according to Koch-Drigalski's method, by mixing a few drops of the bouillon emulsion with a trace—a small platinum loop—of blood serum of a typhoid-prepared animal, it will be seen that arrest of motility and distinct agglutination into large

* This amount can be easily spread out and rubbed over the plate surface, without leaving any excess fluid—even 0·15 c.c. can be so dealt with; more than that cannot be satisfactorily managed.

compact clumps occur within a minute or two. As we shall point out later in detail, colonies may be blue or bluish or blue-violet, without being those of B. typhosus, but the above differential characters, *viz.*, *conical* in shape, with *prominent centre, flat thin margin*, violet-blue in the middle part, *violet* in the margin when viewed in reflected light on black ground, *finely granular, moist or glistening* in aspect; the individual bacilli short cylindrical in shape (not filamentous and not in chains), motile and quickly clumping and in marked manner with typhoid serum, are sufficient presumptive indications * of the colonies being those of B. typhosus. Subcultures in the different media are made as a matter of routine, so as to confirm the diagnosis.

We proceed now to describe in detail the experiments which we made with oysters, cockles, and mussels.

In all our experiments with oysters, the method used was this: the oyster, after the outside of the shell had been thoroughly washed and brushed under the tap, was opened with a sterile knife, the liquor was drained off as completely as possible, the body of the fish with its mantle and branchiæ was then transferred to a sterile glass dish and herein cut up (minced) with sterile scissors as finely as possible; after thoroughly mixing the minced material, the fluid (thick turbid) is removed with sterile glass pipette and measured. From this fluid a definite amount, in no case more than 0·1 c.c. or 0·15 c.c. (generally the former quantity), was either directly transferred to a Drigalski plate, or, as in those cases in which the presence of a large number of B. typhosus in the oysters could be supposed, $\frac{1}{10}$ c.c. of the oyster-mince was first diluted by a measured amount of sterile sea water, and of this dilution $\frac{1}{10}$ c.c. was used and dealt with on the Drigalski medium in the plate. If the number of

* Flat colonies, deep blue in reflected light, fringed at margin, dry looking, are not B. typhosus; colonies bluish or pale blue in reflected light, with greenish margin, uniformly raised, moist looking, are not B. typhosus; colonies bluish violet, strongly granular, with thin margin, but composed of filamentous bacilli, are not B. typhosus, they do not grow on Drigalski plate at 37° C. Very small, blue, uniformly-raised, colonies may be those of streptococci or vibrios.

B. typhosus in the oyster could be expected to be small, as for instance in the later oysters of a series, more than one Drigalski plate was made directly with the fluid of the minced oyster, each plate receiving 0·1 c.c. After having, by means of the sterile bent glass rod, carefully and thoroughly and uniformly rubbed the material over the surface of the dry medium (all previous moisture having been previously removed by allowing the plates to evaporate it spontaneously for 2–3 hours in the incubator), the plates are transferred to the incubator at 37° C.

As mentioned above, the typhoid colonies are noticeable already after 24 hours, and a preliminary counting can now be made, but it has to be controlled after the plate has been placed back in the incubator for at least another day, generally two more days, because the character of the colonies can by this time be considered fully established. By this time, colonies, which after the first 24 hours' incubation might be doubtful typhoid colonies, can with certainty be declared to be or not to be typhoid, and all those which show the above differential characters in the same manner may be taken to be typhoid colonies; agglutination test and subcultures on gelatine, and if necessary in other media, are made, selecting at random from different quarters of the plate one colony for the purpose. Since after two, or better, three, days' incubation at 37° C. the character of all the colonies in the plate—typhoid and not typhoid—is fully established, and since the typhoid colonies are distinct and different from all others by colour, size, general aspect, and shape, there is no difficulty in at once diagnosing them and to recognise their identity,* and it is not therefore necessary to test more than a few of them for agglutination and subculture on gelatine. The growth on this gelatine subculture (after 24 hours' incubation at 20° C.) is inspected, examined in the hanging drop and tested for agglutination, and must, if B. typhosus, comply with the required tests: on gelatine translucent filmy growth, composed of cylindrical motile bacilli, agglutinating markedly and instantaneously with a

* See photograms accompanying this Report.

trace of typhoid serum of a prepared rabbit, just like a similar gelatine culture of the laboratory B. typhosus kept and tested for control.

Subcultures from the Drigalski plate in the different media (neutral red broth, litmus milk, MacConkey fluid, lactose litmus peptone, shake gelatine, phenol-broth, Proskauer and Capaldi I and II) are made; if there were any doubt about a colony, further similar subcultures are made in each series of oysters from the Drigalski plates of the first and last oyster, and occasionally in addition from one or the other in the middle of the series. Intraperitoneal injection of guinea-pigs with a given dose ($\frac{1}{6}$–$\frac{1}{10}$ or less) of a 24 hours' old agar culture was practised for testing the virulence of the microbe, generally only of the first and last oyster of the series from which the microbe was recovered, and in all cases it was found to have retained its full virulence.

EXPERIMENT I.

Clean Burnham Oysters.—These were obtained from one of the foremost oyster shops in the City. The oysters were thoroughly cleaned on the outside of the shell, and one was used for a preliminary test by means of a Drigalski plate for the presence of B. coli and for that of blue colonies, the rest were placed in a clean wooden tub * in 2000 c.c. of clean sea water. The tubs used were oval in shape and could hold easily 12–16 natives in one layer; the sea water was always sterilised by heat (90°–100° C.), and after cooling well and repeatedly shaken up with air. As a matter of routine we always prepared the 2000 c.c. of sterile sea water the day previous to using it. Having ascertained after 24 hours' incubation at 37° C. by the Drigalski plate that the test oyster was clean, we now proceeded to infect twelve of the

* Each tub after having been used was well brushed under the hot water tap, and was then kept filled with the hot water 80° C. for some time (hours). This process was, as a rule, repeated on two or even three subsequent days. In the later experiments the tubs were even "steamed" before being used again.

oysters which had been in the sterile sea water for the 24 hours, in the following manner: a thick zinc wire, bent at right angle at the last inch, is carefully inserted between the two halves of the shell, the oysters in the sea water having their shells spontaneously opened; immediately as the wire end is inserted the shell closes so tightly on it that the oyster can now be lifted out of the water on to a glass plate; the end of the cannula of a hypodermic syringe filled with turbid emulsion of B. typhosus is easily introduced close to the wedged-in wire, and the desired amount—(in our case 1 c.c.) —of culture slowly injected. When finished the cannula of the syringe is withdrawn—the cannula being thinner than the wedge—and by gentle action the wire is removed; the shell immediately closes again tightly. The whole proceeding need not and does not occupy more than a few minutes, and, as subsequent observation showed, no harm had thereby been done to any part of the oysters. In the above manner 12 oysters were injected with the B. typhosus, each with 1 c.c. of the emulsion.

The emulsion injected was obtained by distributing in sterile sea water the growth from the surface of a pure agar culture 24–48 hours old of our laboratory B. typhosus. Before using the emulsion for the injection, a Drigalski plate was made with a definite amount of a definite dilution of it, and after 48 hours' incubation at 37° C. the number of colonies of B. typhosus was counted, and thereby their number per 1 c.c. of emulsion ascertained. It was thus found that the number of B. typhosus injected into each oyster was 162,500,000.

The determination was made thus: 0·1 c.c. of the typhoid emulsion was added to 100 c.c. sterile sea water, well shaken; of this dilution 0·01 c.c. was carefully and well rubbed over the surface of Drigalski plate. After incubation for 48 hours the Drigalski plate showed numerous colonies of B. typhosus and no others, all being of the same colour and aspect; a careful count showed 1625 colonies in the plate, that is to say, $1625 \times 100 \times 1000 = 162{,}500{,}000$ per 1 c.c.

Although the amount actually injected within the cavity

of the shell was 1 c.c., it has to be mentioned that while the injection was proceeding a little fluid, about the same quantity that was being injected, was escaping from the oyster near the lock, so that although this escaped fluid appeared to be the clean water from within the shell, and although the injection was made fairly gradually, we cannot suppose that none of the injected bacilli escaped with the water. At any rate, the above amount of B. typhosus was injected into the shell of each of 12 oysters. Of these six were put back into the sterile sea water, the other six were transferred to a plate and placed in the cold chest. The first six will be designated as "wet oysters," the other six as "dry oysters." The analyses of the latter would show whether any, and what, changes took place in the number of B. typhosus as compared with the wet oysters. It is well known that oysters after they are removed from their ground are, in many instances, not consumed at once, but are occasionally kept for days at the wholesale dealer's or the retailer's in a "dry" state, in barrels, bags and the like; in fact, oysters imported from a distance must of necessity be so kept. As regards the "wet" oysters the sea water (2000 c.c.) was changed after 24, 48, 72, 96 and 144 hours, that is to say, after one, two, three, four and six days.

The oysters analysed were taken in this order: Oyster 1, wet, after having been one day in clean water; oyster 2, dry, having been kept dry one day; oyster 3, wet, after two days in clean sea water; oyster 4 having been kept dry for two days, and so on.

Oyster 1 was taken out of the sea water after one day, its outer surface well brushed under the tap, then dried with a clean cloth, opened with sterile knife, the liquor drained off as well as possible, then minced with sterile scissors in a sterile glass dish, well mixed and the turbid fluid measured. It amounted to just one cubic centimetre. From this fluid made two Drigalski plates, each with $\frac{1}{100}$ c.c., *i.e.*, 10 cubic millimetres.

After incubation for 48 hours the colonies, all of the colour, aspect, and nature of B. typhosus—there were no others—

were carefully counted. They were recounted after three days' incubation, and were found to amount to the average of 700 per $\frac{1}{100}$ c.c., that is to say, the whole oyster contained 70,000 B. typhosus. Exactly the same procedure was followed with oyster 2 dry. The amount of fluid was also just 1 c.c. The average number of typhoid colonies present in the two Drigalski plates (each inoculated with $\frac{1}{100}$ c.c.) amounted to 1,200,000 B. typhosus for the whole oyster. Of the subsequent wet oysters the amount of fluid, after mincing, was practically the same, *viz.*, just 1 c.c. Of the dry oysters the amount was less, but it was always brought up to just 1 c.c. by adding sterile water.

Oyster 3, wet—2 days in clean sea water showed 9100 B. typhosus per oyster.
„ 4, dry—2 days dry showed 175,000 B. typhosus per oyster.
„ 5, wet—3 days in clean sea water showed 1100 B. typhosus per oyster.

Seeing from the result in oyster 5 that the number of B. t. is rapidly diminishing, I used for the Drigalski plate of oyster 7 and oyster 9 $\frac{1}{10}$ c.c. of the oyster, and in the case of oyster 11 I made three plates, each with $\frac{1}{10}$ c.c. of the oyster.

Oyster 6, dry—3 days dry showed 42,000 B. typhosus per oyster.
„ 7, wet—4 days in clean sea water showed 320 B. typhosus per oyster.
„ 8, dry—4 days dry showed 3700 B. typhosus per oyster.
„ 9, wet—6 days in clean sea water showed 0 B. typhosus in $\frac{1}{10}$ part of oyster.
„ 10, dry—6 days dry showed 40,000 B. typhosus per oyster.
„ 11, wet—7 days in clean sea water showed 0 B. typhosus per $\frac{3}{10}$ of oyster.
„ 12, dry—7 days dry showed 1220 B. typhosus per oyster.

All the oysters had their shell well and tightly closed, and on opening were found to be quite normal in appearance, plump and juicy.

In all the preceding experiments the Drigalski plates contained practically no other colonies except those of

B. typhosus, and these, after 48 hours and 72 hours, could readily be identified as such. It should also be added that the counting of the colonies, except in the case of dry oyster 2, presented no difficulties, and was always repeated to control the first counting. As was mentioned already, in all instances an accurately measured quantity of the turbid fluid part of the minced oyster, $\frac{1}{100}$ or $\frac{1}{10}$ c.c. as the case required, was used for Drigalski plates, and the total quantity of the minced oyster was kept at just 1 c.c. Where originally deficient, it was brought up to 1 c.c. by the addition of sterile sea water.

In all plates the colonies were found isolated, not in fused groups, thus proving that the bacilli were fairly uniformly distributed in the fluid of the minced oyster, and had not formed nests, as it were, in the oyster tissues ; that is to say, had not multiplied and made aggregations within the tissues of the oyster.

As was mentioned on a former page, colonies were taken at random, and the required tests—examination in the hanging drop, agglutination test, subculture on gelatine, agglutination of this, and ultimately, if required, in other media—were carried out. After a little practice the recognition of the typhoid colonies on Drigalski plates in all these and the subsequent experiments was merely a matter of patient examination under a magnifying glass.

Tabulating the results of the preceding Experiment I. we obtain this :—

TABLE I.

Oysters injected with 160 millions B. typhosus each :

WET—*i.e.*, kept in clean sea water frequently changed.

Oyster 1—	after 1	day	in water,	70,000	B. typhosus	per oyster.
„ 3—	„ 2	days	„	9100	„	„
„ 5—	„ 3	„	„	1100	„	„
„ 7—	„ 4	„	„	320	„	„
„ 9—	„ 6	„	„	0	„	per $\frac{1}{10}$ part of oyster.
„ 11—	„ 7	„	„	0	„	per $\frac{3}{10}$ part of oyster.

DRY—*i.e.*, kept out of sea water.

Oyster 2—	after 1 day	. .	1,200,000	B. typhosus	per oyster.
„ 4—	„ 2 days	. .	175,000	„	„
„ 6—	„ 3 „	. .	42,000	„	„
„ 8—	„ 4 „	. .	3700	„	„
„ 10—	„ 6 „	. .	40,000	„	„
„ 12—	„ 7 „	. .	1220	„	„

We learn from this experiment that oysters infected with huge numbers of B. typhosus, then kept in clean sea water changed frequently—practically every day—were able to clean themselves and to get rid of them in a comparatively short space of time; in four days the number of B. typhosus decreased to an enormous extent (320), and after six days none could be found in $\frac{1}{10}$ part of the oyster, that is to say, less than 10, if any, in the whole body of the fish. At the same time we learn the important fact that oysters of the same kind kept out of the water retained the injected B. typhosus to a markedly greater extent (40,000 after six days), although also under these conditions their number considerably decreased. This part of the experiment, while pointing out the danger attached to specifically infected oysters being kept out of the water, shows at the same time that the body of the oyster *per se* is not a soil in which the typhoid bacillus is capable of multiplying; on the contrary, the tissues of the oyster distinctly acting inimically on the microbe. Those oysters which were kept after infection in fresh sea water might, one would perhaps be inclined to conclude, have cleaned themselves of the extraneous B. typhosus—extraneous to the oyster—on account of being kept in changing water, but this evidently does not apply to those oysters that were kept out of the water; consequently we are justified in, in fact are driven to, concluding that the tissues of the oysters *per se* are endowed with the faculty of devitalising this microbe. Considering that we started with 160 millions of B. typhosus per oyster we come down to 1220 in the course of seven days, during which time the oysters were left entirely to themselves and without any influence the surroundings could have exerted on them.

EXPERIMENT II.

Clean Colchester Oysters.—One oyster well brushed on outside and prepared in the manner already described, $\frac{1}{10}$ part of the oyster contained no microbes capable of growing at 37° C. on Drigalski medium, that is, it contained no B. coli or any microbes forming blue colonies on that medium. The rest (25) had been also thoroughly brushed on the outside under the tap and then placed in clean tub in sea water (4000 c.c.) to which previously, while sterile, emulsion of a pure culture of B. typhosus had been added to the extent that each cubic centimetre contained 744,000 B. typhosus. This determination was made in the following manner: to the 4000 c.c. of sterile sea water were added 4 c.c. of a turbid emulsion of B. typhosus, prepared by well shaking up the growth covering the surface of 48 hours' old agar culture of B. typhosus with 10 c.c. of sterile sea water. Immediately after the addition of the 4 c.c. of the typhoid emulsion to the 4000 c.c. of sterile sea water and well shaking it up, 1 c.c of the infected water was added to 99 c.c. of sterile distilled water, and of this dilution $\frac{1}{10}$ c.c. was used for one Drigalski plate. After 48 hours' incubation the colonies, all of B. typhosus, were carefully counted and found to amount to 744, so that 1 c.c. of the infected sea water contained $744 \times 10 \times 100 = 744{,}000$ B. typhosus.

The oysters having been kept in the infected sea water for 24 hours were taken out, well rinsed on the outside and drained, were divided in two lots, one lot (12) were placed in the cool chest dry, the other lot (12) were transferred to a clean tub sterilised by steam, supplied with 2000 c.c. of sterile sea water, and the remaining oyster 1 was, after well brushing it under the tap, used for analysis. The oyster having been opened with a sterile knife, and the fluid drained off as carefully and as well as possible, the whole fish was finely minced with sterile scissors. Total amount of turbid fluid drained off of the minced material was 1·75 c.c.; from this made two Drigalski plates each with 150 cubic millimetres. After incubation the enumeration of the two plates,

that is, 0·3 c.c. of the oyster, showed, as near as could be counted, 6400 colonies, that is, about 40,000 B. typhosus for the whole fish. As regards the oysters kept in sea water in the tub, the tub and the sterile sea water were changed after one, two, three, five, six, and seven days; oysters of the wet lot were analysed one day after change, two, five, six, seven, and nine days; of the dry lot we analysed after one day dry, two, three, five, six, seven, and nine days dry.

One day after change, oyster 3 (wet)—total amount of fluid 3 c.c., $\frac{1}{10}$ c.c. for one Drigalski plate, contains 46 colonies of B. typhosus—this would amount to 46 × 30 = 1380 B. typhosus per whole oyster.

One day dry, oyster 2—total amount of fluid 4 c.c., $\frac{1}{10}$ c.c. for one Drigalski plate, contains 1000 colonies of B. typhosus—this would amount to 1000 × 40 = 40,000 B. typhosus per whole oyster.

Two days after change in sterile sea water, oyster 5 (wet) —total fluid 2 c.c.; two days, oyster 4 (dry)—total fluid 1 c.c.; of each oyster $\frac{1}{10}$ c.c. for one Drigalski plate.

Total number of colonies of B. typhosus in Drigalski plate of oyster 5 were 22, that is, 22 × 20 = 440 B. typhosus per whole oyster; Drigalski plate of oyster 4 contained 377 colonies of B. typhosus—this amounts to 3770 B. typhosus for the whole oyster.

Three days dry, oyster 6—total fluid 1 c.c.; $\frac{1}{10}$ c.c. was used for one Drigalski plate, which developed 70 colonies of B. typhosus—this amounts to 700 B. typhosus for the whole oyster.

Five days after change in sterile sea water, oyster 7— total fluid a little under 2·8 c.c., of this made two Drigalski plates each with $\frac{1}{10}$ c.c.

Total number of colonies of B. typhosus in the two plates was six (two in one, four in the second plate)—this would amount to about 82 B. typhosus for the whole oyster.

Five days dry, oyster 8—total amount 1·5 c.c.; $\frac{1}{10}$ c.c. produced in a Drigalski plate 10 colonies of B. typhosus— this would amount to 150 B. typhosus per whole oyster.

Six days after change in sterile sea water, oyster 9—total

amount 3·3 c.c.; made two Drigalski plates, each with 150 cubic millimetres (0·15 c.c.). Both plates together had 4 colonies (three in one, one in the other) of B. typhosus—this would be 44 B. typhosus for the whole oyster.

Six days dry, oyster 10—total amount 0·4 c.c.; $\frac{1}{10}$ c.c. produced in a Drigalski plate 70 colonies of B. typhosus—amounts to 280 B. typhosus per whole oyster.

Seven days after change in sterile sea water, oyster 11—total amount 3 c.c.; $\frac{1}{10}$ c.c. produced in a Drigalski plate 0 colonies of B. typhosus.

Seven days dry, oyster 12—total amount of fluid 1·5 c.c.; $\frac{1}{10}$ c.c. produced in a Drigalski plate 34 colonies of B. typhosus—this is equal to 510 B. typhosus for the whole oyster.

Nine days after change in sterile sea water, oyster 13—total amount 2 c.c.; $\frac{1}{10}$ c.c. produced in a Drigalski plate 0 colonies of B. typhosus.

Nine days dry, oyster 14—total amount 1·5 c.c.; $\frac{1}{10}$ c.c. produced in a Drigalski plate six colonies of B. typhosus—this amounts to 90 B. typhosus per oyster.

Tabulating the results of Experiment II we find thus :—

TABLE II.

Oyster 1—after 24 hours in typhoid-infected sea water contained	40,000	B. typhosus.
„ 3—after 1 day in clean sea water	1380	B. typhosus.
„ 5— „ 2 days „ „	440	„
„ 7— „ 5 „ „ „	82	„
„ 9— „ 6 „ „ „	44	„
„ 11— „ 7 „ „ „	0	„
„ 13— „ 9 „ „ „	0	„
Oyster 2—after 1 day dry	40,000	B. typhosus.
„ 4— „ 2 days „	3700	„
„ 6— „ 3 „ „	700	„
„ 8— „ 5 „ „	150	„
„ 10— „ 6 „ „	280	„
„ 12— „ 7 „ „	510	„
„ 14— „ 9 „ „	90	„

Allowing for the much greater number of B. typhosus introduced into the oysters of the first experiment, the results of Experiment II harmonise well with those obtained in Experiment I, namely: the rapid decrease and equally rapid total disappearance of B. typhosus from the oysters which, after infection, were kept in clean sea water repeatedly changed; while in oysters at the same time and manner infected, but afterwards kept out of the water (dry), the decrease, though taking place, is much slower: after the first day dry (oyster 2) no decrease being noticeable, the oyster containing the same number of B. typhosus as the oyster 1 immediately after infection, *viz.*, 40,000; whereas in oyster 3 that had been kept 24 hours in clean sea water the number had decreased considerably—to 1380.

Also from this experiment we are justified in concluding that the decrease of the B. typhosus in the oysters could not have been due to a simple "washing out" process, but must be due to the capability of the oyster to directly devitalise the B. typhosus, being something alien to the tissues of the oysters and not capable of maintaining its existence therein; the dry oysters are clear proof for this conclusion.

That this function of the destruction of the B. typhosus by the tissues of the oysters *per se* would be more marked and extensive in those that were kept after infection in clean sea water constantly changed than in those kept out of the water is to be expected, since in the former the ordinary processes of the tissues would go on unabated and in normal fashion, which could not be the case in oysters kept out of the water.

All the oysters of this experiment were, on opening, found in all respects normal, plump and juicy, their shell well closed.

EXPERIMENT III.

This experiment is in reality a continuation of Experiment II in this sense, that several oysters of the same batches left over from Experiment II were subjected to reinfection and analysis; at the same time the sea water into which after infection they were transferred, and which was frequently

(every 24 hours) changed, was analysed for B. typhosus, in order to obtain an insight into the problem whether and to what extent the decrease of B. typhosus is referable to a "washing-out" process. The experiment was made in the following manner:—

Of Experiment II six oysters of the wet lot and four oysters of the dry lot were left over; they were transferred to a fresh sterile tub into 2000 c.c. of sterile sea water; the tub and the 2000 c.c. sterile sea water were changed every day for three days. Seeing that the previously wet oysters were already free of B. typhosus at the termination of Experiment II, and seeing that in the previously dry oysters the number of B. typhosus had, by the end of Experiment II, come down to 90 per oyster, it was quite in accordance with fact to suppose that if these previously dry oysters are placed for further three days in clean sea water they would be free of the microbe. I am referring to the ascertained fact that in clean sea water the previously wet oysters had in two days from 82 B. typhosus per oyster come down to 0; three days in clean sea water would therefore, in all probability, bring down the number 90 B. typhosus (oyster 14) to 0 B. typhosus. At any rate, whether or no some stray B. typhosus are left in the oyster, it would not fundamentally alter the nature of the Experiment III, in which the remaining oysters were placed in sea water reinfected with a large number of B. typhosus.

To 2000 c.c. of sterile sea water in a flask, emulsion of B. typhosus, made by distributing in sterile sea water a 48 hours old pure agar culture of B. typhosus, was added. 1 c.c. of the infected sea water of the flask was added to 99 c.c. sterile water; of this dilution a Drigalski plate was made with $\frac{1}{200}$ c.c. The infected sea water was then poured over the above 10 oysters (two batches) in a clean sterile tub. These two batches were kept well separated in the tub, and will be described as "previously wet" and "previously dry" oysters, both batches, however, being kept, now and afterwards, in the sea water in the tub, as will be presently described.

The Drigalski plate yielded on incubation 118 colonies of

B. typhosus, that is, 118 × 200 × 100 = 2,360,000 B. typhosus per 1 c.c., or a little over 2¼ millions.

Of the oysters having been kept for 24 hours in the infected sea water, one of the "previously wet" lot and one of the "previously dry" lot were taken for analysis, as also a certain amount—same manner of dilution as above—of the sea water in the tub; the remaining oysters were all taken out of the infected water—keeping the two batches separate—well rinsed under the tap on the outside, and transferred to a fresh clean tub with fresh sterile sea water.

Of the sea water in tub infected with B. typhosus 24 hours previously, 1 c.c. was added to 99 c.c. of sterile water; of this dilution a Drigalski plate was made with $\frac{1}{20}$ c.c. This plate on incubation yielded 63 colonies of B. typhosus; this would mean that the infected sea water contained 126,000 B. typhosus per 1 c.c.

Oyster 15 (previously wet), after having been in the infected sea water for 24 hours—total amount of fluid of the minced oyster, 3 c.c.; $\frac{1}{10}$ c.c. of this added to 10 c.c. sterile sea water, and of this dilution took $\frac{1}{10}$ c.c. for one Drigalski plate. This plate yielded a pure culture of B. typhosus—28 colonies; this would mean that oyster 15 contained 28 × 3000 = 84,000 B. typhosus per oyster.

Oyster 16 (previously dry), after having been in infected sea water for 24 hours, was opened; total amount of fluid of the minced oyster, 2 c.c. Of this added $\frac{1}{10}$ c.c. to 10 c.c. sterile water; with $\frac{1}{10}$ c.c. of this dilution made one Drigalski plate, which yielded a pure culture of B. typhosus —659 colonies; this would mean 659 × 2000 = 1,318,000 B. typhosus per whole oyster. If we suppose that each of the "previously wet" and the "previously dry" oysters took out of the infected sea water the same number of B. typhosus as oyster 15 and 16 respectively, we would get 84,000 × 6 for the "previously wet" lot and 1,318,000 × 4 for the "previously dry" lot, that is—

504,000 B. typhosus for the six of the former,
5,272,000 „ „ four of the latter.
5,776,000 Total.

The sea water had been infected with B. typhosus to the amount of 2,360,000 B. typhosus per 1 c.c., and as there were 2000 c.c. of the sea water, we have then 2,360,000 × 2000.

4,720,000,000 B. typhosus had been originally present in the 2000 c.c. of the sea water in the tub in which the oysters were placed; the ten oysters had therefore removed in 24 hours from this total only 5,776,000 B. typhosus, so that there should have remained in the tub after 24 hours—nothing else happening—a little over 4660 millions of B. typhosus. But according to our analysis the sea water in the tub after 24 hours contained only 126,000 B. typhosus per 1 c.c., that is, 126,000 × 2000 for the total; that is to say, the total sea water now—24 hours after infection—contained only 252 millions of B. typhosus. The number of B. typhosus in the sea water in the tub must have suffered a decrease from 4660 millions to 252 millions, that is, not more than $\frac{1}{18}$ part of the original number were left. There are no data as to an active destruction of B. typhosus going on in the oysters during the same 24 hours, but we may, without much danger of error, assume that the chief destruction of the microbes was going on in the sea water itself. The sea water was, before being infected, sterile sea water; the oysters yielded no microbes except B. typhosus taken in from the infected water. It follows from this that the just named rapid destruction in the sea water in the tub could not have been caused by the presence of other microbes, but must be referred to an inimical action of the sea water itself. This was proved directly by experiment; 100 c.c. of sterile sea water were infected with a given number of the same B. typhosus and kept for 24 hours. The analysis showed that the diminution amounted to $\frac{1}{17}$, against the figure $\frac{1}{18}$ found in the above experiment. To show that this inimical effect of our sterile sea water was not due to the sterility of the sea water, but to the sea water as such, the experiment was made by comparing in a parallel series the effect of non-sterile sea water exactly as it had been received from the same portion of the sea (Lowestoft) from which all our sea water was obtained—that

is to say, at the same time that the 100 c.c. of the sterile sea water were infected with a given number of B. typhosus, 100 c.c. of the non-sterile sea water were infected with the same amount of the same culture of B. typhosus; 24 hours after, a Drigalski plate was made with a definite amount and the number of typhoid colonies ascertained. It turned out that in the non-sterile sea water the decrease of the number of the B. typhosus was practically the same, *viz.*, between $\frac{1}{16}$ and $\frac{1}{17}$. It is not necessary to enter here into the details of these experiments, since they were not strictly within the scope of the shellfish inquiry, but the result is clear, *viz.*, that the sea water *per se* had a powerful destructive action on the B. typhosus. By saying this I do not intend to omit another important fact, *viz.*, that although sea water is capable of materially reducing already in 24 hours the number of B. typhosus, the reduction does not go on at the same great rate every subsequent 24 hours, for it has been experimentally shown by myself, Professor Herdman, Boyce, and others that in sea water infected with B. typhosus some living individuals can be recovered from large amounts of the water even after many days and weeks.

We proceed now with our original analyses.

As mentioned above, the oysters after having been removed from the infected water were placed for 24 hours in sterile sea water in fresh tub; and this change, both of tub and sterile sea water, was effected every 24 hours. Analyses of the sea water and of one oyster of the "previously wet" and one oyster of the "previously dry" lot were made every 24 hours after change of the sea water, with the following results:—

Sea water of tub one day after change—$\frac{1}{10}$ c.c. was used directly for one Drigalski plate; this yielded a pure culture of B. typhosus, 25 colonies; that is to say, this sea water contained 250 B. typhosus per 1 c.c., or for the total amount (2000 c.c.) 500,000 B. typhosus. This half-million of B. typhosus in the water in the tub could have been derived solely from the eight infected oysters in it, the sea water having been sterile and the tub having been well

brushed and steamed before use. This would indicate that of the eight oysters a number of living B. typhosus had actually passed out of their interior.

Oyster 17, "previously wet," opened 24 hours after change into sterile sea water—total amount of fluid, 2·75 c.c.; with $\frac{1}{20}$ c.c. of this fluid made one Drigalski plate; this yielded 17 colonies of B. typhosus (no other colonies)—this amounts to 935 B. typhosus per whole oyster.

Oyster 18, "previously dry," 24 hours after change into sterile sea water—total amount of fluid, 2·75 c.c.; $\frac{1}{10}$ c.c. of this fluid was added to 0·9 c.c. of sterile sea water; of this took $\frac{1}{10}$ c.c. for one Drigalski plate. Plate yielded a number of B. typhosus amounting for the whole oyster to 1900.

Assuming all the previously wet oysters and all the previously dry oysters contained at the same date the same number of B. typhosus respectively, we had before the change into the sterile sea water a total stock of B. typhosus in the eight oysters of 4,374,000 B. typhosus. At the end of 24 hours in sterile water they would represent a stock of 10,375 B. typhosus only, so that a large margin is here offered for discharge of B. typhosus by the oysters into the sea water. The presence of the 500,000 B. typhosus in this sea water (2000 c.c.) as above found at that stage would, therefore, be readily explained, although it must be evident that the difference between 4,374,000 and 10,375 is too large to permit of ascribing to the discharge of the half-million in the 2000 c.c. of the sea water, the entire cause of this great reduction of the B. typhosus in the oysters, and we are justified in concluding that besides a comparatively small discharge of living B. typhosus from the infected oysters into the surrounding sea water—even accepting the destruction of B. typhosus going on in the water—the chief cause of the great reduction in the number of B. typhosus within the oysters is due to inimical action by the oysters themselves. This would be in harmony with what we found in the "dry" oysters of Experiment II.

Sea water after second change was analysed, $\frac{1}{10}$ c.c. direct

being used for one Drigalski plate. The plate remained free of any colonies.

Oyster 19, previously wet, after two days' changes—total amount of fluid, 3·5 c.c.; $\frac{1}{10}$ c.c. yielded three colonies of B. typhosus—this amounts to 105 B. typhosus per oyster.

Oyster 20, previously dry, after two days' changes—total amount of fluid, 3·5 c.c.; $\frac{1}{10}$ c.c. yielded 19 colonies of B. typhosus—this amounts to 646 B. typhosus for the whole oyster.

Sea water after third change yielded likewise no B. typhosus per $\frac{1}{10}$ c.c.

Oyster 21, previously wet, after three days' changes—total amount, 2·6 c.c.; $\frac{1}{10}$ part of oyster yielded no colonies of B. typhosus.

Oyster 22, previously dry, after three days' changes—total amount, 2·3 c.c.; $\frac{1}{10}$ c.c. yielded 31 colonies of B. typhosus—this amounts to 713 B. typhosus for the whole oyster.

The sea water was analysed after fourth and sixth changes; $\frac{1}{10}$ c.c. direct yielded no colonies.

Of the oysters only two were left of the previously wet lot, *viz.*, oyster 23 and 25; neither of them yielded any colonies of B. typhosus in $\frac{1}{10}$ part of oyster.

It will be noticed in this series that two days after change the sea water per $\frac{1}{10}$ c.c. did not contain any B. typhosus; at this stage there were six oysters—four previously wet, two previously dry—in the water; assuming that all previously wet and dry oysters contained, when placed in this sea water 24 hours previously, the same number of B. typhosus, *viz.*, 935 and 1900 respectively, the total number of B. typhosus assumed to be in these six oysters would only have amounted to 7540, so that in 2000 c.c. of the surrounding water, even assuming that the whole of B. typhosus were passed out into the water, it would have only amounted to between three and four B. typhosus per 1 c.c.; in $\frac{1}{10}$ c.c., therefore, none would have been detected.

Tabulating the results of this Experiment III:

Table III.

Sea Water.

Immediately after infection .	2,360,000	B. typhosus	per 1 c.c.
1 day „ „ .	126,000	„	„
1 day after change . . .	250	„	„
2 days „ „ . . .	0	„	per $\frac{1}{10}$ c.c.
3 „ „ „ . . .	0	„	„
4 „ „ „ . . .	0	„	„
6 „ „ „ . . .	0	„	„

Previously Wet Oysters.

Oyster 15—1 day after infection	84,000	B. typhosus	per oyster.
„ 17—1 day after change	935	„	„
„ 19—2 days „ „	105	„	„
„ 21—3 „ „ „	0	„	per $\frac{1}{10}$ c.c.
„ 23—6 „ „ „	0	„	„
„ 25—7 „ „ „	0	„	„

Previously Dry Oysters.

Oyster 16—1 day after infection	1,318,000	B. typhosus	per oyster.
„ 18—1 day after change	1900	„	„
„ 20—2 days „ „	646	„	„
„ 22—3 „ „ „	713	„	„

From this Table III it will be seen that the previously wet oysters cleared themselves of the B. typhosus of the re-infection in a remarkably short period, from 84,000 24 hours after infection to 105 after two days' (*i.e.*, twice) change of the water, and no colonies of B. typhosus could be obtained from $\frac{1}{10}$ c.c.—that is, if any, they must have been less than 10—after three days' changes of the sea water; in other words, the oysters were even more successful in dealing with the B. typhosus now than they were after the first infection (Experiment II), for on looking back to Table II it will be seen that from oysters originally infected with 40,000 B. typhosus, even after six days in clean sea water, changed

every 24 hours, there were still B. typhosus recovered, whereas in Experiment III we started with 84,000 per oyster—*i.e.*, more than twice the number—after reinfection, and could discover no B. typhosus three days after change to clean water.

The previously dry oysters, on the other hand, do not seem to have acquired this power of dealing rapidly with the ingested B. typhosus during the first 24 hours (1,318,000 after 24 hours in infected water), and it took them an appreciably longer time to clean themselves although kept in clean water; this can be easily understood if we remember that these oysters had, before reinfection, been kept out of the water for nine days, that is, under abnormal conditions. This may well have detracted from the power of their tissues to regain their full activity when replaced in clean water.

All the oysters of this Experiment III, like those of Experiment II, on opening, looked quite normal, plump and juicy, and their shells well and tightly closed.

EXPERIMENT IV.

By this experiment it was sought to ascertain whether oysters at starting sewage-polluted, that is, coming from distinctly sewage-polluted beds, behaved in the same or different way in regard to B. typhosus. For this purpose oysters were taken from the foreshore of Southend, which, as also other shellfish of the same locality, Dr. Nash, the Medical Officer of Health for Southend, had distinctly declared as sewage-polluted and dangerous, and against the consumption of which he gave emphatic warning by public placard.

Most of these Southend oysters (natives) were very small —some not bigger than the size of a penny—and on the outside extremely dirty. They were well scraped and brushed under the tap till from all parts all mud had been removed as carefully as possible. They were then placed in a clean tub and covered with sterile sea water (2000 c.c.), to

which just previously of an emulsion of B. typhosus so much had been added that each cubic centimetre contained 2,470,000 B. typhosus (for method, see previous experiment).

The oysters were kept in this typhoid-infected water for 24 hours, and after retaining for analysis one of the smallest oysters (No. 1), the rest were taken out, well rinsed on outer surface, and separated into two lots, each lot containing about the same proportion of "small" and "full-sized" oysters. Lot 1 ("wet oysters") was then transferred to fresh clean tub and covered with 2000 c.c. sterile sea water. The other lot ("dry oysters") was laid out on a plate and placed in cool chamber. The small oyster No. 1 yielded on analysis 95,800 B. typhosus and 900 B. coli communis. It has to be remembered that this oyster was a very small one, the shell not larger than the size of a penny. In this case one-hundredth part of the minced body of the oyster yielded on a Drigalski plate 958 colonies of B. typhosus and 9 colonies of B. coli communis.

It is not necessary to detail all the procedure in the analysis of the sea water and the two lots of these oysters, since they were the same as were described in the previous experiments, and we can at once proceed to give the summary of the results:

The sterile sea water as also the tub for the wet oysters were changed every day.

Sea water immediately after infection				2,470,000	B. typh.	per 1 c.c.
„	of tub, 24 hours	„	„	1,530,000	„	„
„	„	1 day after change		13,180	„	„
„	„	2 days „	„	10,580	„	„
„	„	4 „	„ „	20	„	„
„	„	6 „	„ „	0	„	per $\frac{1}{10}$ c.c.
„	„	8 „	„ „	0	„	„

It will be seen from this that one day after having been changed the sea water still contained 13,180 B. typhosus per 1 c.c., which could only have been derived from the interior of the oysters, since the sea water had been sterile when added to the infected oysters, and the tub well brushed and

cleaned; and the same applies also to the sea water two days after change (10,580 B. typhosus per 1 c.c.), *i.e.*, the second lot of originally sterile sea water. It will presently appear that the oysters of this lot still contained at this period an enormous number of B. typhosus in the interior, and therefore the conclusion is obvious, *viz.*, that the above B. typhosus in the sea water after change had been passed out by the infected oysters. This is confirmed by the further fact that as soon as the number of B. typhosus in the oysters markedly decreased (see below), no B. typhosus could be discovered in $\frac{1}{10}$ c.c. of the surrounding sea water.

The analysis of the wet oysters showed :—

Oyster 1 (very small)—after 1 day in infected water contained 95,800 B. typhosus per oyster ; 900 B. coli com.
„ 3 (small)—after 2 days in clean water, 752,800 B. typhosus per oyster ; no B. coli com.
„ 5 (very small)—after 4 days in clean water, 1200 B. typhosus per oyster ; no B. coli com.
„ 7 (very small)—after 6 days in clean water, 200 B. typhosus per oyster ; no B. coli com.
„ 9 (medium sized)—after 7 days in clean water, 378 B. typhosus per oyster ; no B. coli com.
„ 11 (full sized)—after 8 days in clean water, 56 B. typhosus per oyster ; no B. coli com.
„ 13 (small size)—after 9 days in clean water, 390 B. typhosus per oyster ; no B. coli com.
„ 15 (full sized)—after 11 days in clean water, 0 B. typhosus per $\frac{1}{8}$ part of oyster.

It appears, therefore, from these analyses that the oysters cleared themselves of the B. typhosus decidedly less rapidly than previously clean oysters (Experiments I, II, and III), which under similar conditions in the course of four to six days had practically cleaned themselves of this microbe. It will be, however, noticed that the polluted oysters cleaned themselves very rapidly of the B. coli communis, for thus must be interpreted the fact that the very small oysters, containing originally at least 900 B. coli communis, were free of

this microbe after two days (two changes) in clean sea water.

The analysis of the oysters of the dry lot showed :—

Oyster 2—	after	2	days	dry	contained	58,700 B. typhosus per oyster ; no B. coli com.
„ 4—	„	4	„	„	„	17,400 B. typhosus per oyster ; no B. coli com.
„ 6—	„	6	„	„	„	37,900 B. typhosus per oyster ; no B. coli com.
„ 8—	„	7	„	„	„	1300 B. typhosus per oyster ; no B. coli com.
„ 10—	„	11	„	„	„	innumerable B. typhosus, very large number of B. coli com.

This oyster always looked weak, it did not close its shell promptly ; when opened, eleven days dry, it had no liquor in the shell, and it looked abnormal, brownish. This, therefore, must be considered as an abnormal case, in which the activity of the oyster tissues was unhealthy and in abeyance, and this would explain the inability of the fish to deal with either the B. typhosus or the B. coli, both these microbes having been capable of multiplying in the oyster.

Omitting this abnormal oyster, we see, then, that also in this experiment the dry oysters did not clean themselves in anything like the same extent as did their wet companions : this is in agreement with the results of the previous experiments, in which clean oysters were used. It is noteworthy that also the dry oysters were able to effectually deal with the B. coli communis in two days, which clearly points to the conclusion that the tissues and activities of the normal oyster *per se* are as inimical to the B. coli communis as to the B. typhosus, both being as regards the oyster aliens, and therefore when found in the oyster must have been derived from the surroundings.

Table IV gives the summary of this Experiment IV.

Table IV.

Sea Water.

Immediately after infection .	2,470,000	B. typhosus per 1 c.c.
24 hours „ „ .	1,530,000	„ „
1 day after change . . .	13,180	„ „
2 days „ „ . . .	10,580	„ „
4 „ „ „ . . .	20	„ „
6 „ „ „ . . .	0	„ per $\frac{1}{10}$ c.c.
8 „ „ „ . . .	0	„ „

Wet Oysters.

Oyster 1—after 1 day in infected water,	95,800	B. typh. per oyster.
„ 3—after 2 days in clean water,	752,800	„ „
„ 5— „ 4 „ „ „	1200	„ „
„ 7— „ 6 „ „ „	200	„ „
„ 9— „ 7 „ „ „	378	„ „
„ 11— „ 8 „ „ „	56	„ „
„ 13— „ 9 „ „ „	390	„ „
„ 15— „ 11 „ „ „	0 per $\frac{1}{8}$ part of oyster.	

Dry Oysters.

Oyster 2—after 2 days dry . .	58,700 B. typhosus per oyster.
„ 4— „ 4 „ „ . .	17,400 „ „
„ 6— „ 6 „ „ . .	37,900 „ „
„ 8— „ 7 „ „ . .	1,300 „ „
„ 10— „ 11 „ „ . .	Innumerable; abnormal.

The foregoing experiments, confirmatory as they have been to one another, all point in the same direction, and are, I think, without further repetition, sufficient for drawing some general conclusions.

In the first place, it is clearly shown that oysters during the period these experiments were carried out, *viz.*, September, October, and November—that is, when oysters are in a fit state for consumption—and there is no reason why the same should not be applied to the oysters during the rest

of the season (December till April)—are perfectly capable of living in sterile sea water and to retain their normal character and aspect in perfect condition. In the second place, the oysters after infection with even large numbers of the B. typhosus remain to the eye indistinguishable in all respects from non-infected normal oysters. This latter point is of course important from a practical point of view, inasmuch as oysters which are so infected would in the ordinary course of things remain undetected. So long as the oyster shell is well closed and the oyster on opening would present the normal appearance of colour, juiciness and plumpness, it would naturally pass as "of good quality."

As has been pointed out on a former page, the longer persistence of the B. typhosus in oysters out of the water makes such oysters dangerous to a higher degree than when they are kept in the water. Now, it is common knowledge that on many occasions oysters when taken from an infected laying—or, at any rate, from a polluted locality—are packed and kept in barrels, tubs, or the like, sometimes for short, sometimes for long periods. This applies, of course, in a conspicuous degree to oysters coming into England from distant countries—America, France, Holland—but it applies also to many oysters coming from distant localities in England into London or other large towns, *viz.*, they are kept out of the water, *i.e.*, in "dry" state, sometimes for several days before they reach the consumer. From the experiments we have described it must be obvious that this practice should be done away with, for there is no difficulty whatever in any part of England or Holland to keep oysters in clean sea water, which can be frequently changed; if we can do so at a very small cost indeed in a laboratory in London, I do not see that the same thing should not be possible in seaside and other places; all that seems required is a sufficiently large receptacle, which can be thoroughly brushed out and scalded with boiling water, and a sufficient supply of clean sea water. We get here delivered in the laboratory five gallons of sea water (23 litres) at the price of sixpence, that is to say, sufficient water to give to each four

dozen oysters at least eight changes, or a change of nearly three litres of fresh sea water for eight or ten consecutive days—surely more than enough for the purpose. All that is therefore required is a small primary outlay, insignificant as compared with the price charged for oysters to the consumer.

Our experiments have further shown that even when oysters are infected with large numbers of B. typhosus, incomparably larger than would be the case under ordinary natural conditions, they clean themselves in a comparatively short time if kept in clean sea water; under laboratory conditions even the at first polluted oysters, having been infected each with between 95,000 and 800,000 B. typhosus, had done so in less than twelve days.

Although, as pointed out on a former page, the typical (fluid) typhoid stool during the third week and the typhoid urine during convalescence contain enormous numbers of B. typhosus—amounting to many millions per each cubic centimetre—sewage as it flows out of the sewers, and as even in the worst places it might directly bathe oyster layings or oyster ponds, would in no case contain such great numbers of B. typhosus as were used in our experiments. It will be remembered that ordinary domestic sewage contains human dejecta in a highly-diluted state, and therefore unless typhoid stool or typhoid urine as such are directly allowed to bathe the oysters, the number of typhoid bacilli in the sewer outfalls would be under the worst conditions comparatively small. So much more advantageous that the remedy against the consumption of typhoid-infected oysters, being simple, would be capable of readier application. The remedy would be this: Place the oysters after removal from the polluted layings in tanks or ponds receiving no other than clean sea water. As far as I can see, to obtain the necessary amount of clean sea water from outside the range of the polluted area, and to have this frequently changed in the tanks or ponds, is a simple matter of arrangement, which after a first outlay would not involve more than a trifling expenditure, ludicrously small if compared with the large interests at stake, the high prices paid for good and safe oysters, and the big profits that

would and do accrue from the sale of oysters which would rightly be considered as perfectly safe.

Not that I would recommend any relaxation in insisting that oyster beds should be as far removed as possible from sewage and other pollution; but in those instances in which oyster layings are unfortunately for one reason or another uncontrolled and established in localities accessible to pollution or actually polluted, the remedy for rendering these oysters clean and safe seems to me simple and well worth trying, in the interest of the owners whose property at present is greatly depreciated, unless surreptitiously made active, and above all in the interest of the public, who in the majority of instances have to rely on the mere statement of interested parties to the effect that particular oysters are supposed to be derived from clean beds.

SERIES B.

Experiments with the B. typhosus in Cockles and Mussels.

Infection with typhoid fever through cockles or mussels is, in the nature of things, of less extensive occurrence than through oysters, since cockles and mussels are incomparably less frequently eaten in a raw state than oysters. Although the methods generally employed of preparing either cockles or mussels for consumption are open to criticism in respect of destroying by those methods the infective agent, if present, the general method is nevertheless capable in some degree of achieving this. As is well known, both cockles and mussels are in bulk subjected to a process that is designated as "cooking," consisting in either plunging a mass of these shellfish in boiling water, and taking them out as soon as the water again commences to bubble, generally sooner, or in heating the water till it commences to bubble. By either process the end in view is to expose the shellfish to heat for a sufficient time till their shell opens, so as to separate the fish from the shell by simple agitation; the fish, although

coagulated on the outside, nevertheless retains its juicy soft quality, is not tough or too much shrunk, the latter condition making them unsaleable. Now, I have shown experimentally (*see* Report of the Medical Officer of the Local Government Board, 1900–1901, p. 570) that pouring boiling water over a heap of cockles, these at once all open their shells, although the temperature in the course of very few minutes falls below 65° C.; and, as a matter of fact, I have shown that if cockles previously infected with the B. typhosus are thus treated in a heap, the B. typhosus can readily be recovered from the interior of the fish from the middle of the heap, although of such cockles the shell is widely open and the outside of the fish is coagulated. It must be obvious that, if in dealing in practice with these shellfish the object in view is what it generally is, *viz.*, merely to get the fish readily out of the shell, and to obtain the former in a juicy, not shrivelled, condition, then we must expect that many a consignment of the so-called "cooked" shellfish cannot be considered safe if they happen to be previously contaminated, since the amount of "cooking" as generally practised does not ensure destruction of infective germs. Some time ago, at the request of the Fishmongers' Company, experiments were carried out by me on the premises of the Fishmongers' Company, as also at the instance of Dr. Collingridge at Leadenhall Market, and we have shown that mussels and cockles *en masse* can be safely "*steamed under pressure*" without injuring in the slightest degree the proper aspect and condition of the fish, and that, treated in this way, few minutes (three to five minutes) suffice to make them sterile of all infective germs. At Leigh, I understand this method is followed with success, and at no greater cost than formerly by the haphazard methods. Unfortunately, both mussels and cockles are occasionally eaten in a raw state, as, for instance, by tourists, excursionists, and children, and as both mussels and cockles are "dirty feeders," and being found at or near the foreshore, which in some places is well exposed to sewage pollution, it is readily understood that

infection with typhoid fever through eating raw mussels, and particularly raw cockles, is an occurrence of which several instances are on record. I would refer amongst others to the reports of Dr. Nash, Dr. Thresh, and Dr. Allen.

It is, therefore, important to see in what way the cockle and mussel are capable of dealing with the B. typhosus which happen to have access to them.

EXPERIMENT V.

A batch of fine cockles was received direct from Leigh-on-Sea; they arrived in sand. After well rinsing several dozen of the cockles on the outside they were transferred to a clean tub, and 2000 c.c. of sea water infected with B. typhosus to the amount of four millions per 1 c.c. were poured over them; in this infected sea water they were left for 24 hours. They were then taken out, well rinsed on the outside with clean sea water, and two cockles being retained for immediate analysis, the rest were placed in clean sand wetted with sterile sea water. This procedure, namely, rinsing them with clean sea water and then keeping them in clean sand wetted with a little sterile sea water, was found to be the safest way of keeping them alive. Keeping them in sea water alone—which was tried with a portion of the infected cockles—failed, because they soon died; but keeping them in wet sand succeeded, as it is the nearest approach to the way in which cockles naturally live.

The fresh sand wetted with sterile sea water was again changed after two, four, six, nine, and eleven days. As stated just now, two of the cockles (1 and 1a) were analysed after having been 24 hours in the typhoid infected sea water. In this, as also in all the subsequent analyses, both of cockles and mussels, exactly the same methods were followed as have been described in the experiments with the oysters, and it is not necessary to repeat the details of the entire procedure again, except to state that after opening the cockles or mussels the liquor within the shell was drained off as much

and as carefully as possible, the fish finely minced and well mixed, the amount of the resulting fluid carefully measured, and of this $\frac{1}{10}$ c.c. was used for cultivation by Drigalski plate after definite dilution. The description of the analysis of the first two test cockles will suffice.

Cockle 1.—Total amount of fluid, 0·8 c.c.; $\frac{1}{10}$ c.c. of this was diluted with 10 c.c. sterile sea water, and of this dilution $\frac{1}{10}$ c.c. was used for one Drigalski plate.

Cockle 1a.—Total amount of fluid, 0·85 c.c.; same procedure as above.

The result was: Cockle 1 contained 474,560 B. typhosus; cockle 1a contained a little over 520,000 B. typhosus. This would average about half a million B. typhosus per cockle.

In order to ascertain the number of B. coli communis, $\frac{1}{40}$ part of the total fluid, *i.e.*, $\frac{1}{50}$ c.c. of cockle 1, was used direct for one Drigalski plate, and in it were found three colonies of B. coli communis, readily recognised by their bright red colour and bright red halo. This would mean that in the whole cockle something like 120 B. coli communis had been present.

Cockle 2 was taken out and analysed 1 day after change into clean sand and sterile water; it contained 153,000 B. typhosus, no B. coli communis.

„ 4* was taken out and analysed 2 days after change; it contained 382,000 B. typhosus, no B. coli.

„ 6, after 5 days' change, contained 358,000 B. typhosus.

„ 8, „ 6 „ „ „ 1,541,000 „

„ 10, „ 7 „ „ „ 138,600 „

„ 12, „ 9 „ „ „ 69,000 „

„ 12a, „ 9 „ „ „ 111,000 „

„ 14, „ 10 „ „ „ 1,600 „

„ 14a, „ 10 „ „ „ 69,000 „

„ 14 was not quite normal, for its shell was not closed and the body was somewhat discoloured and shrunk.

* In this experiment the cockles of analysis are all marked by even numbers; the reason is that I meant to keep some (uneven numbers) in sea water only without sand, but after a day or two they all had died.

It appears then, from this series, that the cockles embody readily a large number of the B. typhosus from the infected sea water, larger in proportion than oysters. In Experiment II, after 24 hours in infected water, the proportion of B. typhosus in the oyster and 1 c.c. of sea water was as 40 to 744 (or about 1 : 18); in Experiment III it was as 84 to 2250 (or about 1 : 26); in Experiment IV it was as 95 to 2470 (or 1 : 28) whereas in the case of the cockles of Experiment V it was as 500 to 4000 or 1 : 8.

Another striking fact is the persistence of B. typhosus in large numbers in the cockles even after ten days' change; 24 hours after infection the cockle examined had half a million; after ten days the normal cockle 14a still contained 69,000 B. typhosus.

The abnormal cockle, on the other hand, had a greatly reduced number, *viz.*, 1600 B. typhosus, so that as compared with the abnormal oyster 10 of Experiment IV the reverse condition obtained, for we found that in this abnormal oyster the B. typhosus had considerably increased, whereas in the abnormal cockle 14 we found the smallest number of B. typhosus in the whole series. This would suggest that the B. typhosus thrives in the cockle well so long as this animal is in a normal state—a suggestion which is borne out in a decided manner by the analysis mentioned in the foregoing Experiment V, for we find that in the cockles after two, five, and particularly after six days' change the number of B. typhosus had gradually risen. After one day's change their number had fallen from 500,000 to 153,000; then it rose, till after six days' change it had surpassed by more than threefold the number of B. typhosus in the first cockle, having risen from 500,000 to 1,540,000. In a former report (l.c.) I had already observed this phenomenon of increase of B. typhosus in the cockle as time went on; here we have definite proof by numerical evidence.

The cockle, then, differs in a dangerous way from the oyster, inasmuch as not only is the cockle not capable of dealing so well with the ingested B. typhosus as the oyster, but it appears to offer to the B. typhosus even facilities for

increase; and for these reasons alone, cockles coming from a polluted locality require, for their being rendered safe, a thorough and careful disinfection by heat such as was indicated above, *viz.*, *steaming under pressure* for at least three minutes.

Table V gives the summary of Experiment V.

TABLE V.

Cockles kept in sea water infected with B. typhosus to the amount of four millions per 1 c.c.

Cockle 1—24 hours in infected sea water, contained about 500,000 B. typhosus.

„ 2—after 1 day's change in clean wet sand, contained about 153,000 B. typhosus.

„ 4—after 2 days' change in clean wet sand, contained about 382,000 B. typhosus.

„ 6—after 5 days' change in clean wet sand, contained about 358,000 B. typhosus.

„ 8—after 6 days' change in clean wet sand, contained about 1,541,000 B. typhosus.

„ 10—after 7 days' change in clean wet sand, contained about 138,000 B. typhosus.

„ 12—after 9 days' change in clean wet sand, contained about 69,300 B. typhosus.

„ 12a—after 9 days' change in clean wet sand, contained about 111,000 B. typhosus.

„ 14—after 10 days' change in clean wet sand, contained about 1600 B. typhosus; abnormal.

„ 14a—after 10 days' change in clean wet sand, contained about 69,000 B. typhosus.

EXPERIMENT VI.

Several dozen fresh mussels were well cleaned under the tap and were then placed in sterile sea water in a clean tub, to which an emulsion of pure culture B. typhosus was added to the amount of 5,170,000 B. typhosus per

1 c.c. After having been kept herein for 24 hours they were taken out, well washed on the outside, and, except one mussel which was used immediately for analysis, the others were transferred to a clean tub, were covered with sterile sea water and clean well washed seaweed. After six hours the water was poured off except as was sufficient to keep the mussels in a wet condition. Under natural conditions the mussels are covered with water at the flood tide and uncovered with the ebb. The above procedure, *viz.*, clean tub, sterile sea water and weeds, was repeated every 24 hours, and each time, *i.e.*, each 24 hours, the excess of the water was only allowed to cover the mussels for six hours. In this way the majority of the mussels could be kept alive and in normal condition for seven days after they were taken out of the infected water. All those that were examined after opening looked perfectly normal in every respect, and contained a considerable amount of liquor. As with the oysters and cockles after opening, the fluid within the shell was drained off as completely and as carefully as possible, then the whole fish was taken out, finely minced and well mixed, and of the fluid a definite amount was used for cultivation in a Drigalski plate. The mussels were chosen just as they came to hand, some being large, some medium sized.

Mussel 1 (large), kept in infected sea water for 24 hours—total amount of fluid a little over 2 c.c.; added $\frac{1}{10}$ of this to 10 c.c. sterile sea water; of this dilution used $\frac{1}{100}$ c.c. for one Drigalski plate. This plate yielded 300 colonies; this would mean a little over 6 millions B. typhosus per mussel, *viz.*, $300 \times 100 \times 100 \times 2 = 6{,}000{,}000$.

Mussel 2 (medium size), total amount of fluid 0·4 c.c., after 1 day's change contained 74,800 B. typhosus per mussel.

„ 3 (large), total amount of fluid 2·4 c.c., after 2 days' change contained 628,660 B. typhosus per mussel.

„ 4 (medium size), total amount of fluid 1·8 c.c., after 3 days' change contained 36,000 B. typhosus per mussel.

„ 5 (medium size), total amount of fluid 2·5 c.c., after 5 days' change contained 58,000 B. typhosus per mussel.

Mussel 6 (medium size), total amount of fluid 2·5 c.c., after 6 days' change contained 6,250 B. typhosus per mussel.

„ 7 (medium size), total amount of fluid 0·5 c.c., after 7 days' change contained 14,200 B. typhosus per mussel.

This experiment could not, unfortunately, be continued, because the remaining mussels could not be kept alive. But as far as it goes it shows that mussels take up the B. typhosus from the surrounding water with great ease, the first mussel having taken up in 24 hours from the infected water the B. typhosus to the enormous amount of over six millions—greater in proportion than what was observed with oysters or cockles. Notwithstanding the daily change of sterile sea water during five days, there were still discovered in the mussels B. typhosus in considerable numbers. But on the whole there may be said to have been going on a distinct decrease of the B. typhosus in the animal—slower than was the case with the oysters, quicker than with the cockles. No increase of the microbe, as in the case of the cockles, was noticed; and therefore it seems justifiable to say that in respect of dealing with the ingested B. typhosus, the mussel stands between the oyster and the cockle—that is, it is capable of ingesting the B. typhosus from the surrounding water in greater proportion than either the oyster or the cockle, and the B. typhosus does not undergo increase within the mussel.

Table VI gives the summary.

Mussels in sea water infected with B. typhosus to the amount of over 5 millions per 1 c.c.

Mussel 1 (large), kept 24 hours in infected sea water, contained over 6,000,000 B. typhosus.

„ 2 (medium), 1 day after change, contained over 74,000 B. typhosus.

„ 3 (large), 2 days after change, contained over 628,660 B. typhosus.

„ 4 (medium), 3 days after change, contained over 36,000 B. typhosus.

Mussel 5 (medium), 5 days after change, contained over 58,000 B. typhosus.

„ 6 (medium), 6 days after change, contained over 6000 B. typhosus.

„ 7 (medium), 7 days after change, contained 14,200 B. typhosus.

SERIES C.

Experiments with Oysters kept in Sterile Sea water infected with Human Fæcal Matter.

In the foregoing experiments (Experiments IV and V), we had the opportunity of showing that, just like the B. typhosus, so also the B. coli communis when originally present rapidly disappears from the oysters and cockles if these be kept in clean surroundings. As this question of the presence of B. coli has recently received a great deal of attention, and caused a radical divergence of opinion, we propose to discuss it somewhat in detail, but first wish to record some experiments made expressly to determine in exact manner how oysters are capable of dealing with the B. coli communis, derived from human fæcal matter. This determination by means of Drigalski plates is extremely simple, since the colonies of this microbe are already after 24 hours at 37° C. conspicuous by their size, by their red colour and marked red halo.

EXPERIMENT VII.

One gram of fæcal matter of a healthy man was shaken up in 2000 c.c. sterile sea water, a determination was made by means of a Drigalski plate with $\frac{1}{10000}$ part of a cubic centimetre (1 c.c. of the sea water emulsion was added to 99 c.c. sterile water: dilution 1; of this 1 c.c. was added to 9 c.c. sterile water: dilution 2; $\frac{1}{10}$ c.c. of this dilution 2 was used for the Drigalski plate). This plate showed, after incubation at 37° C., 23 colonies of B. coli communis. These

were visible next day as good-sized red colonies with distinct red halo; they were all of the same kind and aspect, *viz.*, typical B. coli communis; this would mean 230,000 B. coli communis per 1 c.c. of the infected sea water, or 460 millions of this microbe per 1 gram of fæcal matter.

Thirteen Whitstable oysters were kept for 48 hours in sterile sea water, and two of them on analysis by Drigalski plates having been proved to contain no B. coli communis or any other B. coli, the remaining 11 oysters were placed in a clean tub into the above fæcally infected sea water. Here they remained for 24 hours. They were now taken out, well rinsed under the tap, and divided into lots—six oysters were transferred to a fresh clean tub and supplied with sterile sea water, which was repeated every 24 hours as long as any of these "wet" oysters remained; four oysters were placed "dry" in cool chamber, and the remaining eleventh oyster was used for analysis. At the same time of the above infected sea water, *i.e.*, kept for the 24 hours, analysis by Drigalski plate was made. Of the sea water, 24 hours after infection with fæcal matter, $\frac{1}{1000}$ part of a cubic centimetre yielded 177 colonies of B. coli communis, or 177,000 B. coli communis per 1 c.c.

Oyster 1—after 24 hours in infected sea water—treated in the usual manner, yielded 77 colonies of B. coli communis per $\frac{1}{600}$ part of the body—that is, 46,200 B. coli communis for the whole oyster.

Sea water which had been changed and in which five of the infected oysters had been placed was analysed 24 hours after the change, using $\frac{1}{10}$ c.c. direct for one Drigalski plate. This yielded 65 colonies of B. coli communis; this means 650 B. coli communis per 1 c.c.

Oyster 2, taken from the "dry" lot, one day after removal from infected sea water yielded 8575 B. coli communis—343 colonies per $\frac{1}{25}$ part of body.

Oyster 3, taken from the "wet" lot, one day after change yielded 2325 B. coli communis per whole oyster—31 colonies per $\frac{1}{75}$ part of body.

The sea water in the tub was analysed—having been changed 24 hours previously, as well as the tub into which

the five remaining "wet" oysters had been transferred—it yielded 50 B. coli communis per 1 c.c.

Oyster 4, "dry"—having been kept two days dry, yielded 7970 B. coli communis.

„ 5, "wet"—having been two days in (twice) changed sterile sea water and fresh tub, yielded 1305 B. coli communis.

The sea water, which contained the remaining four wet oysters, was analysed after three changes; it yielded 0 B coli communis per $\frac{1}{10}$ c.c.

Oyster 6, "dry"—kept three days dry, yielded 5790 B. coli communis.

„ 7, "wet"—kept three days in (thrice) changed sterile sea water, yielded 216 B. coli communis.

„ 8, "dry"—kept four days dry, yielded 2625 B. coli communis.

„ 9, "wet"—kept four days in (four times) changed sterile sea water, yielded 11 B. coli communis.

„ 11, "wet"—kept seven days in (five times) changed sterile sea water, yielded 18 B. coli communis.

„ 13, "wet"—kept eight days in (six times) changed sea water, contained no B. coli communis per $\frac{2}{3}$ part of oyster.

Summarising the above results in tabular form, we have:—

TABLE VII.

SEA WATER INFECTED WITH NORMAL FÆCAL MATTER.

SEA WATER.

Immediately after infection . .	230,000	B coli	per 1 c.c.
After 24 hours	177,000	„	„
„ 1 day change	650	„	„
„ 2 days' „	50	„	„
„ 3 „ „	0	„	per $\frac{1}{10}$ c.c.

Wet Oysters.

Oyster 1	after 24 hrs.	in infected water,	. . .	46,200	B. coli	per oyster.
„ 3	„ 1 day	change	. . .	2325	„	„
„ 5	„ 2 days'	„	. . .	1305	„	„
„ 7	„ 3 „	„	. . .	216	„	„
„ 9	„ 4 „	„	. . .	11	„	„
„ 11	„ 7 „	„	. . .	18	„	„
„ 13	„ 8 „	„	. . .	0	„	per $\frac{2}{3}$ oyster.

Dry Oysters.

Oyster 2	1 day dry	. .	8575	B. coli communis	per oyster.
„ 4	2 days dry	. .	7940	„ „	„
„ 6	3 „ „	. .	5796	„ „	„
„ 8	4 „ „	. .	2625	„ „	„

Starting, then, with 230,000 B. coli communis per 1 c.c., the sea water had diminished them in 24 hours down to 177,000; but then we should not forget that there have been 11 oysters in the water; taking all these 11 oysters as having contained, or rather having withdrawn from the water, the same number of B. coli communis, *i.e.*, 11 times 46,200, there would have been on account of this withdrawal only an insignificant number (half a million) removed from the 460,000,000 originally added to the water. From this it follows that the number of B. coli communis had actually been reduced by the sea water from the original 460,000,000 to 353,500,000 (177,000 × 2000 c.c., minus 500,000 for the eleven oysters). This reduction does not, therefore, compare with that observed in connection with the B. typhosus, and this coincides with what is known as to the greater hardiness and greater resistance of the B. coli communis over the B. typhosus. We notice, however, that in the oysters kept in clean sea water, frequently changed, the reduction of the B. coli communis was rapid and marked. By three days' changes the number of B. coli communis had been brought down to 216 (from the original 46,200), and after eight days' changes no B. coli communis could be discovered any longer.

Whereas in the oysters not kept in sea water the decrease, though progressing, was distinctly slower; after four days dry the oyster still contained more B. coli communis (2625) than the wet oyster after one day change in clean water.

There can, then, be no question about the fact that the oyster *per se* is capable of dealing with the B. coli communis in the same manner as with the B. typhosus, *viz.*, that also the B. coli communis does not multiply in the oyster; when taken in from the surroundings by oysters clean at starting the number of B. coli communis decreases, and if the oysters are kept in clean water the microbe rapidly disappears.

I consider this additional definite proof that the B. coli communis is as foreign to the oyster as the B. typhosus, and that therefore when B. coli communis is found in oysters it is derived from the surroundings, and must be of fairly recent importation.

EXPERIMENT VIII.

In order to obtain further confirmation, the next experiment was made in the same manner, but this time with native oysters of a different kind, *viz.*, Colchester oysters.

One of these oysters (1), immediately on receiving them, was well brushed and cleaned on the outside and then used in the usual manner for analysis, $\frac{1}{15}$ part of the body of the oyster being used for a Drigalski plate. The others were placed in sterile sea water in a fresh tub. No colonies of B. coli came up in the plate of oyster (1).

After 24 hours in clean sea water we took out a further oyster (2), $\frac{1}{13}$ part of the oyster body being used for a Drigalski plate. No colonies of B. coli came up in this plate.

We now infected 2000 c.c. of sterile sea water with one gram of fæcal matter of a healthy person. By making a Drigalski plate on the same plan of dilution as in Experiment VII, it was ascertained that each c.c. of this water contained 22,000 colonies of B. coli communis—that is to

say, 44 millions per 1 gram of fæces—a considerably smaller number than in Experiment VII; in fact, barely the tenth part of the number used in the latter experiment.

Into this infected sea water were placed the remaining ten oysters, and they remained herein for 48 hours. After this period the infected sea water as also one oyster (3) were analysed, the remainder were well rinsed under the tap and then transferred to a fresh tub and fresh sterile water, and this procedure was repeated after a further day, also after two, four, six and seven days; at each of these periods of change, oyster or oysters were taken out and analysed.

The sea water, 48 hours after infection, contained 4110 B. coli communis per 1 c.c. The sea water in tub one day after change contained 40 B. coli communis per 1 c.c.—that is, the originally sterile sea water in the tub to which the infected oysters had been transferred. The sea water twice changed contained 0 B. coli per $\frac{1}{10}$ c.c.

Oyster 3—having been kept 48 hours in the fæcally infected sea water, contained in its body 650 B. coli communis.
„ 4—having been kept 1 day in sterile sea water, contained 84 B. coli communis.
„ 5—having been kept 2 days in sterile sea water, contained 600 B. coli communis.
„ 6—having been kept 3 days in sterile sea water, contained 484 B. coli communis.
„ 7—having been kept 5 days in sterile sea water, contained 52 B. coli communis.
„ 8—having been kept 7 days in sterile sea water, contained 48 B. coli communis.
„ 9—having been kept 7 days in sterile sea water, contained 0 B. coli communis per $\frac{1}{8}$ oyster.
„ 10—having been kept 8 days in sterile sea water, contained 18 B. coli communis.
„ 11—having been kept 8 days in sterile sea water, contained 0 B. coli communis per $\frac{1}{8}$ oyster.

One of the oysters, not analysed, died two days after the commencement of the experiment.

From this we learn that by using for infection of the sea water a considerably smaller number of B. coli communis, and therefore the number initially ingested by the oysters being relatively small, it made no appreciable difference in the manner and time in which the oysters became clear of the B. coli communis, as compared with the oysters of the preceding experiment in which the initial number in the sea water was ten times greater.

Table VIII gives a summary of the results of Experiment VIII, and for comparison we repeat Table VII as Table IX, giving the analysis both of the wet oysters of Experiment VIII and of the oysters of previous Experiment VII.

Table VIII.

Sea Water Infected with Normal Fæcal Matter.

Sea Water.

Immediately after infection.	22,000	B. coli communis	per 1 c.c.
After 48 hours	440	,,	,, ,,
After 1 day's change . .	40	,,	,, ,,
After 2 days' change . .	0	,,	,, per $\frac{1}{10}$ c.c.

Oysters.

Oyster 3—48 hours in infected sea water, contained 650 B. coli communis per oyster.
,, 4—after 1 day's change, contained 84 B. coli com. per oyster.
,, 5— ,, 2 days' ,, ,, 600 ,, ,, ,,
,, 6— ,, 3 ,, ,, ,, 484 ,, ,, ,,
,, 7— ,, 5 ,, ,, ,, 52 ,, ,, ,,
,, 8— ,, 7 ,, ,, ,, 48 ,, ,, ,,
,, 9— ,, 7 ,, ,, ,, 0 ,, ,, per $\frac{1}{8}$ part of oyster.
,, 10— ,, 8 ,, ,, ,, 18 ,, ,, per oyster.
,, 11— ,, 8 ,, ,, ,, 0 ,, ,, per $\frac{1}{8}$ part of oyster.

Table IX.

Preceding Experiment VII.—Sea Water Infected with 230,000 B. coli communis per 1 c.c.

Oyster,	24 hours in infected sea water				46,200	B. coli	communis.
„	1 day's change				2325	„	„
„	2 days'	„			1305	„	„
„	3	„	„		216	„	„
„	4	„	„		11	„	„
„	7	„	„		18	„	„
„	8	„	„		0	„	„

This comparison emphasises, therefore, the fact that even when the initial number of the ingested B. coli communis is very large, the oyster is capable of dealing with it successfully, and it cannot be a question of mere "washing out" by the water of the ingested B. coli, but must depend on the activity of the tissues of the oysters in dealing with the foreign intruder.

The difference observed here may, it is true, be due to the two samples of oysters coming from different localities, but I hardly think this a satisfactory explanation. If there be an extraneous cause it is more likely to be due to the sample of the oysters used in Experiment VIII not having been quite so good or fresh as those of Experiment VII; one oyster dying early in the experiment would point in that direction. At any rate, about the fact that even when the initial number of the B. coli communis is very great, the normal oyster is capable in clean water of rapidly clearing itself of this microbe. This fact would, then, once for all set at rest the implied suggestion by the Royal Commission on Sewage Disposal, *viz.*, that the B. coli communis is a normal inhabitant of the body of the oyster. If there is one thing clear, it certainly is the fact contrary to that suggestion, *viz.*, it is a fact conclusively proved that oysters from clean places and oysters kept in clean water are free of B. coli communis; and further, that if they should happen to have imbibed them from the surrounding water, they, by being again placed in clean water, rapidly clean themselves of the intruder.

EXPERIMENT IX.

This experiment was undertaken to test the behaviour of oysters towards the B. coli communis of ordinary domestic sewage. One dozen "small Dutch oysters" were obtained from a first-class shop in the City—the oysters had just arrived direct from Holland. The oysters were carefully brushed under the tap and placed in a clean tank in 2000 c.c. of sterile sea water. Twenty-four hours after, two were taken out for analysis by Drigalski plates—two plates for each oyster. The result was that neither of them contained in $\frac{1}{10}$ and $\frac{1}{5}$ part of the body of the fish any B. coli communis—that is to say, in none of the four plates were any red colonies with red halo to be found.

The test by Drigalski plates, as I have already explained, is undoubtedly the best that we have, and for this reason: that only B. coli communis produces on these plates already after 24 hours at 37° C. round distinctly red colonies, several millimetres in breadth with red halo; and if no such colonies, *viz.*, largish red with red halo, make their appearance in 48 hours, according to my experience—extending now over a considerable number of analyses—the conclusion is justified, and is confirmed by other culture tests, that no B. coli communis is present in the material analysed. I must insist on this, for the reason that all other methods used for preliminary diagnosis are decidedly inferior, because colonies of the above kind invariably respond to all tests of B. coli communis, whereas the colonies of other microbic species, unlike the above red colonies with red halo, on the *complete* series of tests being made do not answer to the true B. coli communis. Although in several of the tests they may simulate the B. coli, if the tests are completed, they can be proved to be not the true B. coli communis, but to be different from this, the typical and constant microbe of excremental matters. I have for years past—now more than 12 years—insisted on a distinction being drawn between the typical B. coli communis of excremental matters and what, owing to one or the other similar character, may be

designated as a coli-like microbe. I have repeatedly drawn attention to this, that whereas the B. coli communis is the typical microbe of fæcal matter, the derivation and distribution of many coli-like microbes is at present not sufficiently known and cannot therefore be used for diagnostic purposes. It is therefore satisfactory to find that the American observers* draw the same sharp distinction; they find that oysters coming from clean, not sewage-polluted, layings have no B. coli communis, although they may on first tests show microbes which are coli-like,† and that in proportion to the pollution of the layings by sewage they contain the B. coli communis.

Messrs. Clark and Gage give the following table, based on analyses of shellfish and water during three years:—

SHELLFISH AND SHELL WATER CONTAINING THE TRUE B. COLI COMMUNIS.

Character of Source.	Number of Sources.	Percentage of Samples Positive.		
		Shellfish.		Sea Water.
		Shell Water.	Intestine.	
Not polluted . .	15	0	0	0
Doubtful . . .	22	8	6	17
Polluted . . .	8	41	35	44

Statements such as have been repeatedly made (see the Report of the Bacteriologist of the Sewage Commission; Dr. Foulerton's paper read at the Folkestone Meeting 1904 of the Royal Institute of Public Health, in the Bacteriology

* Thirty-fourth Annual Report of the State Board of Health of Massachusetts for 1902, p. 18.

† Of 58 species which gave the "presumptive" tests, only 12 were found to be B. coli communis (l.c. p. 20).

section), *viz.*, that oysters derived from "clean" layings, or sea water taken many miles away from the shore, contained large numbers of the true B. coli communis, are to me perfectly unintelligible. I must confess I have not succeeded in verifying this. I have not found, for instance, that oysters coming from layings which are miles away from any source of sewage or manure pollution, *e.g.*, some layings in Halford River, some layings in Hayling Island, "contain 1000 B. coli communis per oyster" (see Report of Sewage Commission); in fact, I have not found anything approaching such a condition even in oysters directly from sewage-bathed ponds.* I have quite recently had the opportunity to examine oysters which came from the mouth of Langston Harbour, only 600 yards distant from the principal sewer outfall of Portsmouth, and what I found was that of nine oysters examined all contained B. coli communis. But in what numbers? Three were specially tested by Drigalski plates each made with one-fiftieth part of the body, and they were found to contain: one 200 but not 300 B. coli communis *per oyster*, a second one 150 but not 200 B. coli communis *per oyster*, and a third 50 only. A sample of oysters derived from layings several miles away from the above showed B. coli communis only in one out of nine oysters,

* The culture tests for B. coli communis of fæcal matter of man and of ordinary domestic sewage are these :—

1. B. coli communis forms on Drigalski medium at 37° C., after 24–36 hours, colonies several millimetres in size, distinctly red, with distinct red halo when viewed in transmitted light.
2. In ordinary nutrient gelatine shake culture it forms colonies all through the medium with numerous gas bubbles already in 24 hours at 20° C.; in gelatine streak it forms at 20° C. a rapidly spreading dry band with irregular margin; no liquefaction of gelatine at any time.
3. Neutral red broth at 37° C. is changed in 24–36 hours from cherry red to greenish fluorescent.
4. It turns MacConkey fluid (litmus, glucose, taurocholate of soda, peptone) red, forming acid with numerous gas bubbles.
5. It grows well in phenol broth at 37° C., making it turbid in 24 hours with copious gas formation.
6. It produces indol in nutrient broth at 37° C. in 3–5 days.
7. It turns lactose peptone litmus in 24–36 hours at 37° C. red (acid production) with copious gas formation.
8. Litmus milk at 37° C. becomes red in 24 hours, due to acid production, the milk becoming clotted in 1–3 days.

and in that one there was only one colony of B. coli communis per one-tenth part of the body of the oyster. And it is precisely on account of the ready and reliable manner in which B. coli communis can be identified by the method of Drigalski plates that the greatest importance attaches itself to this method.

Having ascertained, then, that our small Dutch natives contain no B. coli communis, they were divided in two lots: one (five oysters) was kept in sterile sea water (2000 c.c.) without any addition, the other lot (five oysters) was transferred to a fresh tub with sterile sea water (2000 c.c.) to which 5 c.c. of crude sewage (of St. Bartholomew's Hospital) were added. Analysis of this sewage made by Drigalski plate at the same time showed that it contained 220,000 B. coli communis per 1 c.c.—that is to say, each cubic centimetre of the infected sea water contained 550 B. coli communis. The first lot of oysters, *viz.*, in clean tub with clean sea water, will be mentioned here as "clean lot," the second lot, *viz.*, in sewage polluted sea water, will be mentioned as "polluted lot."

Oyster 1 (clean lot)—kept 1 day in sea water, contained no B. coli communis.

„ 2 (polluted lot)—kept 1 day in sewage polluted water, contained 800 B. coli communis.

„ 3 (clean lot)—kept 2 days in clean sea water, contained no B. coli communis.

„ 4 (polluted lot)—kept 2 days in polluted sea water, contained 150 B. coli communis.

The polluted lot were taken out after having been kept 48 hours in the polluted water, well rinsed under the tap and transferred to clean tub and 2000 c.c. clean (sterile) sea water. The clean lot received 2000 c.c. fresh sterile sea water, and this procedure was repeated on each of the following two days.

Oyster 5 (clean lot)—kept 3 days in clean sea water, contained no B. coli communis.

Oyster 6 (polluted lot)—kept 1 day in clean water, contained 100 B. coli communis.

„ 7 (clean lot)—kept 4 days in clean water, contained no B. coli communis.

„ 8 (polluted lot)—kept 2 days in clean water, contained no B. coli communis per $\frac{1}{10}$ part of body.

„ 9 (clean lot)—kept 5 days in clean water, contained no B. coli communis.

„ 10 (polluted lot)—kept 3 days in clean water, contained no B. coli communis.

Tabulating the results of this experiment:

TABLE X.

Sea water infected with crude sewage from St. Bartholomew's Hospital to the amount of 550 B. coli communis per 1 c.c. sea water (220,000 B. coli communis per 1 c.c. crude sewage).

CLEAN LOT.

Oyster 1—after 1 day,* contained no B. coli communis.
„ 3— „ 2 days, „ „ „
„ 5— „ 3 „ „ „ „
„ 7— „ 4 „ „ „ „
„ 9— „ 5 „ „ „ „

POLLUTED LOT.

Oyster 2—kept 1 day in polluted sea water, 800 B. coli communis.
„ 4— „ 2 days „ „ 150 „ „
„ 6—kept 1 day in clean sea water, 100 „ „
„ 8— „ 2 days „ „ 0 „ „ per $\frac{1}{10}$ part of oyster.
„ 10— „ 3 „ „ „ 0 B. coli communis per $\frac{1}{10}$ part of oyster.

Starting, then, with clean oysters and placing them in sewage-polluted sea water—polluted with sewage B. coli communis to the amount of 550 per 1 c.c.—we find that after

* This will be understood as the time when the comparison between the two lots commenced.

24 hours in this polluted water the oyster had taken in 800 B. coli communis. After a further day only 150 were found in the next oyster, and after having been transferred to clean water they practically cleared themselves in two further days. The previous experiment has shown us that the sea water *per se* is capable of materially reducing in 48 hours the number of B. coli communis, and, therefore, the reduction of this microbe in the oysters after 48 hours from 800 to 150 is what we might expect. This experiment is in so far interesting, as it appears to point to this, *viz.*, that clean oysters are capable of dealing promptly with the B. coli communis of sewage, seemingly more promptly than with the B. coli communis directly derived from human fæcal matter.

SERIES D.

In the following series of observations, an attempt was made to differentiate those microbes of sewage and of fæcal matter which in Drigalski plates are capable of forming "blue" colonies—that is, colonies that might interfere with and aggravate the diagnosis and recognition of the colonies of B. typhosus and similar microbes of a pathogenic character like the B. enteritidis Gaertner, both of which would indicate *specific* pollution; the former being derived from the typhoid patient (bowel discharge, urine), the latter from the bowel discharges of a person affected with certain forms of acute gastro-enteritis, and most probably also with the acute ailment called paratyphoid, recognised now as different from typhoid fever. The microbe of this disease, viz., the Bacillus paratyphosus appears from all accounts to closely resemble the B. Gaertner, being possibly a variety of this latter.

The B. Gaertner forms blue-violet colonies on Drigalski medium at 37° C., but they grow slower, coming up slower and remaining smaller than those of B. typhosus, and are marked also from the latter by showing a central opaque spot. By microscopic examination in the hanging drop, by subculture in neutral red broth and in litmus milk, in MacConkey fluid, and by the agglutination test with typhoid serum, the differential

diagnosis is readily established. For B. Gaertner is shorter than B. typhosus; B. Gaertner gives positive neutral red broth test, it turns the litmus milk at first slightly acid (red), but after two or three days gradually alkaline (blue and slate colour), and it produces acid and gas in MacConkey fluid. It does not agglutinate with typhoid in anything like the high dilution that B. typhosus does—that is to say, it is by all the above tests easily distinguished from the B. typhosus (see a former page). There would be, therefore, none but a preliminary difficulty in differentiating between the two microbes. B. Gaertner in small doses is highly virulent to rodents, both after subcutaneous injection, as also by feeding, and in these respects differs from the B. typhosus. We have already indicated that all colonies of B. coli communis can by means of the Drigalski plates at 37° C. be recognised already in 24 hours, they being several millimetres in diameter, being red and surrounded by a distinct red halo; other acid-forming (red) coli-like microbes are slower in developing, and are much smaller. These are always numerous in sewage, but being small and slow in coming, although red, and even some with indication of red halo, can at once be neglected, as far as the search for specific microbes is concerned.

The B. dysenteriæ forms neither red nor blue colonies, being neutral, like many other microbes not belonging to the coli-typhoid group. These, therefore, do not offer any basis for further inquiry by means of the Drigalski medium.

But there are a number of species of microbes of sewage and of fæcal matters which, on account of their forming blue or bluish colonies in the Drigalski medium, require special considerations in reference to shellfish, in order to differentiate them from B. typhosus and from B. Gaertner.

I.—Microbes of Sewage forming "Blue" Colonies on Drigalski Medium.

(*a.*) Amongst the many clean oysters which I have examined, I have not found any as yet which contain bacteria

which, on Drigalski medium incubated at 37° C., are capable of yielding blue colonies like B. typhosus. I have examined a considerable number of Burnham oysters, of Colchester oysters, of clean Dutch oysters, of Whitstable oysters, and others; and I have not found in them any bacteria which, incubated on Drigalski medium at 37° C. for 24–72 hours, form such blue colonies. On three occasions when examining clean oysters, I have been, however, greatly puzzled by the appearance in the Drigalski plates of blue colonies with violet margin, which, by their size, by their slightly irregular contour, and their more or less conical form, and uniformly granular appearance, looked very like those of B. typhosus. And the difficulty became considerably enhanced by noticing that an emulsion of the bacilli—which were very motile—of these colonies, subjected to agglutination test with typhoid serum, gave a strikingly positive test. When, after the method of Koch-Drigalski, a loop of typhoid serum was added to several big drops of the emulsion—made by distributing a trace of such a blue colony in sterile beef broth—arrest of movement and agglomeration into large conspicuous clumps took place within a a few minutes, the process of agglutination being quite complete within five or six minutes.

This occurred with quite a series of clean Whitstable oysters, derived from Whitstable and from Langstone channel. The difficulty and puzzle was, however, soon solved, *viz.*, these blue colonies appeared only after the plates had been taken out of the hot incubator, *i.e.*, at 37° C., and were then kept for several days either at 20° C., or at the ordinary temperature of the laboratory. So long as the plates had been kept in the hot incubator, *i.e.*, at 37° C., there was no trace of these blue colonies, but they gradually made their appearance after they had been kept for several days at the lower temperature.

Another fact noticed about these typhoid-like blue colonies was this, that the bacilli composing them were thinner than the B. typhosus and decidedly more cylindrical, and even filamentous, but they showed active mobility just like the B. typhosus. Sub-cultures in the different media estab-

lished the differences in a striking manner. (1) They failed to grow in all media, if these are incubated at 37° C.; there was no perceptible growth at this temperature either on agar, or in broth, or in milk. (2) They completely failed to alter MacConkey fluid at 37° C.; B. typhosus turns it red, but forms no gas. (3) They failed to cause any change in litmus milk; B. typhosus forms gradually acid (red) without altering the fluid character of the milk. (4) They produced no growth either in neutral red broth or in phenol broth.

In addition to these differences the microbe grew very slowly on gelatine: it took two to three days before any distinct growth could be noticed, and then it was very transparent, unlike that produced by B. typhosus; and, lastly, in shake gelatine it did not form its colonies in the depth of the medium like the B. typhosus, but, besides being much slower in its growth, it formed colonies only or principally near the free surface of the medium.

It is therefore clear that this microbe need cause no difficulty in the search for the B. typhosus on Drigalski plates kept at 37° C., being a microbe not capable of growing at that temperature.

(*b.*) Of the most frequent "blue" or "violet" colonies found in Drigalski plates inoculated with sewage or fæcal matter, with sewage or fæcal matter polluted shellfish—oysters, mussels, and cockles—are those of streptococci. These appear already after 24 hours at 37° C.; better and more conspicuously later as violet-blue small round dots, uniformly raised and moist looking. A particle of a colony emulsified and looked at under the microscope is at once recognised as a compound of diplococci and short streptococci. They possess the additional character of staining with gram. Owing to their small size, their violet-blue colour, and their appearance under the microscope, *viz.*, being cocci, they need not further offer any difficulty in respect of being mistaken for anything else.

(*c.*) Repeatedly I have come across in Drigalski plates at 37° C., that had been inoculated with sewage, *bright blue* small colonies, more or less conical in shape. They are composed of vibrios or comma bacilli; they resemble in size, shape,

and staining more or less the well-known vibrio of cholera, but they differ from this latter in this essential respect, that they do not liquefy gelatine at any period of their growth, forming on this medium non-liquefying translucent, more or less angular colonies. In peptone salt they grow feebly, do not form any marked pellicle on the surface of this fluid, and do not produce nitroso-indol: sufficiently distinct differences from the vibrio of cholera. Besides, this non-liquefying sewage vibrio, when injected, even in large doses, intra-peritoneally into a guinea-pig, causes no disease. This same vibrio was found on Drigalski plates inoculated with the fluid of a mussel derived from a polluted locality, and twice in oysters which had been distinctly polluted with sewage.

I attribute, therefore, to the presence of this vibrio in Drigalski plates, inoculated from oysters or other shellfish, an important diagnostic value, because this vibrio appears present in sewage in small numbers only, and when, therefore, present in shellfish is *à fortiori* strong presumptive evidence of sewage pollution.

All vibrios form on Drigalski plates bright blue, small, moist, round colonies, and in respect of this marked colour and small size can readily be recognised; a simple microscopic specimen in the hanging drop shows the actively motile comma-shaped, S-shaped, and shorter or longer spirillar forms.

From a cockle derived from a particularly polluted locality—black mud on the Upper Orwell—I have isolated by the Drigalski plate at 37° C. a motile vibrio which differs from the above non-liquefying sewage vibrio in the following respects: its colonies are bright blue, moist, round, small, raised in the centre—that is, conical. After two to three days the colonies reach the diameter of several millimetres. This vibrio liquefies gelatine a little faster than the cholera vibrio, but slower than the vibrio finkler. It grows well in peptone salt water, and forms thereon in several days a pellicle composed of matted masses of wavy or spiral threads; it does not produce nitroso-indol; it grows well in litmus milk, which becomes distinctly reddened (acid pro-

duction) by the growth; the milk remains fluid for about a week, after that date it becomes firmly clotted. The vibrio when injected intraperitoneally into the guinea-pig in very minute doses—a loopful of a recent agar surface growth —causes acute peritonitis and death, the turbid peritoneal exudation being crowded with the vibrio, and the intestines much inflamed.

As stated above, the vibrio is motile; it is distinctly shorter than the cholera vibrio, and is possessed of one or two short terminal flagella. It does not become agglutinated with blood serum of an animal protected by cholera vibrio. Owing to its having been obtained from a cockle, I have named it vibrio cardii* (cardium edule—the common cockle). As I have not had opportunity of making further analysis in this direction of cockles of other localities, I am at present unable to attribute to this vibrio any particular diagnostic value.

(*d.*) A third group of microbes forming "blue" or "bluish" colonies on Drigalski medium, which can be isolated from ordinary crude sewage and from human fæcal matter, and which are not to be met with in clean oysters, comprises various species, but none of them are to be mistaken for either B. typhosus or B. Gaertner, as will presently be described. Some of these species are such as occur in filth of various kinds, and, therefore, cannot be considered as diagnostic for sewage or fæcals, but we will consider them here nevertheless, because they are not microbes of shellfish *per se.*

(1.) First and foremost are bright blue colonies, which already after 24 hours at 37° C. can be noticed with the unaided eye as distinctly blue, flat, roundish patches; after another 24 hours their margin becomes fringed, filmy, and rapidly spreading. Examined in the hanging drop, they appear as rapidly motile cylindrical bacilli, but they give no sign of agglutination with typhoid blood serum. Making a sub-culture on gelatine they are seen to rapidly liquefy the gelatine; they are, in fact, the common Proteus vulgaris.

* "Centralblatt f. Bakteriologie," 1905.

(2.) Another microbe of common and copious occurrence in sewage, and forming pale blue colonies on Drigalski medium, is one of which the colonies appear rounded, uniformly raised, and in two or three days show in reflected light a distinctly greenish peripheral portion contrasting markedly with the violet centre. The uniformly raised condition, the pale blue colour, and the greenish margin are sufficiently distinct to differentiate them from the typhoid or Gaertner colonies. They are composed of short motile bacilli, and show no sign of agglutination with typhoid blood serum.

(3.) A further cylindrical motile microbe, forming deep blue round colonies, is one which can be readily distinguished by an ordinary gelatine sub-culture, for in this it forms here a distinct bluish-green fluorescence, without liquefying the gelatine, being, in fact, the common Bacillus fluorescens putidus, *viz.*, a microbe common in filth of all kinds.*

(4.) One of the most frequent microbes both of sewage and of fæcal matter, which forms round, bluish or blue-violet colonies, raised in centre, flat at the periphery, and on inspection of the Drigalski plates in some respects resembles the colonies of B. typhosus or Gaertner, is one which is represented by short cylindrical motile bacilli. On account of the blue colour of the colonies with violet marginal part, on account of their raised condition, and on account of their being made up of cylindrical motile bacilli, they could be easily mistaken for B. typhosus, the more so since they show distinct agglutination with a highly potent typhoid serum, although in high dilution no agglutination takes place. But the above characters possessed by the colonies as they appear on the Drigalski plate might be presumptive of B. typhosus or B. Gaertner. More careful observation and sub-cultures, however, soon show that they are altogether different. In the first place, these blue colonies are slower in their development, *i.e.*, smaller than those of B. typhosus or B. Gaertner. This difference is, how-

* A microbe frequently met with in Drigalski plates infected with sewage, forming bright blue colonies, is conspicuous by its forming rounded, very flat, dry, scaly colonies; it need not trouble us here in our diagnosis of fæcal microbes specific or non-specific.

ever, obviously only of secondary value, since in a plate in which colonies of B. typhosus or B. Gaertner are numerous those that are in more crowded position are always much smaller, although not slower in coming up, than where the colonies are more isolated.

This last fact applies not only to Drigalski plates, or to the microbes at present under consideration, but it applies to all kinds of microbes, and to all kinds of cultures, *viz.*, where isolated and sufficiently apart from one another the colonies are much larger than in places where they are more crowded.

A more important difference is the fact that in the hanging drop made with a small particle of a colony many of the bacilli are seen to be arranged in longer or shorter chains, a fact not observed in similar colonies (from Drigalski medium) of B. typhosus or B. Gaertner.

Sub-cultures made in the different media soon decide the separate position of the microbe under consideration, for on the gelatine surface it forms a slow-growing translucent film; in gelatine shake culture colonies do not appear in the deeper parts, but are all crowded on and near the surface of the gelatine, and it does not form gas in glucose gelatine; in litmus milk (at 37° C.) it forms strong alkalescence (blue) after some days; neutral red broth (at 37° C.) after some days is turned orange, with slight fluorescence; MacConkey fluid (at 37° C.) remains unaltered; it grows only very feebly in phenol broth at 37° C.; no agglutination is observed with typhoid blood serum or with Gaertner blood serum even in moderately high dilutions (*e.g.*, 1 in 100) if a particle of a recent gelatine culture is submitted to the test.

As stated above, this microbe is of constant and of fairly numerous occurrence in sewage and in fæcal matter; its colonies on Drigalski medium resemble by their colour and general aspect those of B. typhosus, the bacilli are short cylindrical, very motile and forming chains, and, as just detailed, are in all media in sub-culture distinctly and easily differentiated from B. typhosus and from B. Gaertner. It is further to be remembered that unlike the typhoid-like colonies not capable of growing at 37° C., mentioned on a

former page as having been met with in certain oysters, the sewage microbe at present under consideration grows well at 37° C., and has not been met with in any of the numerous Drigalski plates from clean shellfish which I have had before me; and therefore, without attributing at present to it any special derivation, I can with confidence say that it is foreign to the shellfish *per se*, and when found in it may with probability be taken to be derived from sewage or similar filth. In order to be able to refer to it, I propose to name it *Bacillus streptoides*, on account of its tendency to form chains.

(5.) As a last and important microbe which I have met with, once in Drigalski plates inoculated with a trace of typical fluid typhoid stool and twice out of five samples of sewage of St. Bartholomew's Hospital, is a species which in all morphological and cultural characters coincides with the Bacillus fæcalis (alkaligenes), first isolated by Petruschki from typhoid stools. The microbe which we isolated produced on Drigalski plates distinctly blue colonies, which in their growth, form, and aspect might be mistaken for those of B. typhosus or of B. Gaertner; examined in the hanging drop the component bacilli were cylindrical, motile, and multiflagellated, and therefore not different from the B. typhosus or B. Gaertner; but they failed to become agglutinated with typhoid serum or with Gaertner serum in moderate dilution (1 : 20). On gelatine surface and in gelatine shake culture the growth quite resembles that of B. typhosus, but here the similarity ends, for the microbe in question produces in litmus milk (at 37° C.) forthwith distinct alkali, the milk retaining its fluid character; it turns MacConkey fluid (at 37° C.) blue, and it produces no gas in glucose media; injected into guinea-pigs subcutaneously it exerted no pathogenic action.

Dr. Durham* has given it as his opinion that the Bacillus fæcalis (alkaligenes) of Petruschki is identical with B. Gaertner. As stated just now, and as has been well known to Petruschki, it has certain characters in common both with the B. typhosus as also the B. Gaertner.

* Brit. Med. Journal, December 17, 1898.

It appears to me that Durham's statement seems to rely, besides the morphological characters of the microbe in question, chiefly on the fact that it produces alkali in litmus milk. But this will barely be considered a sufficient reason, since, in the first place, other alkali-producing microbes do the same, and, in the second place, the manner of the alkali production is distinctly different for the B. fæcalis and the B. Gaertner. If the B. fæcalis is planted in litmus milk, and this is kept at 37° C., it will be noticed that the litmus milk from the outset becomes more and more blue, whereas with the B. Gaertner under the same conditions the litmus milk for the first 24–48 hours shows a tinge of redness, that is, slight acid production, and this gradually gives way to a change into slate colour and later into deep blue. It requires only sub-cultures in glucose gelatine, in MacConkey fluid and in neutral red broth to establish marked differences, for, as mentioned on a former page :

B. Gaertner turns neutral red broth greenish fluorescent;
„ „ MacConkey fluid red, and forms slowly gas therein ;
„ ferments glucose gelatine, forming gas therein ;

whereas the action of the B. fæcalis is in these respects quite negative.

The presence of blue colonies looking like those of B. typhosus or B. Gaertner, composed of motile cylindrical bacilli, and answering to the tests characteristic of the B. fæcalis (alkaligenes), is therefore of an important diagnostic value, inasmuch as they have not been at present found in any but human excremental matter; unfortunately, their occurrence in highly diluted matter, such as is employed for making a Drigalski plate, is rather rare, but if present it is of so much greater importance.

On a former page we mentioned an experiment (IX) in which one lot of Dutch oysters were placed in sewage polluted sea water, the other lot being kept in sterile sea water for control. After 24 hours one oyster of each lot, as described in Experiment IX, was analysed by Drigalski

plates. In the plates of both oysters there came up blue colonies of the following characters.

Drigalski plate of oyster 1, from clean sea water, contained three blue colonies, that is, 150 per oyster; these proved to be colonies of a motile rapidly liquefying vibrio, in many respects similar to vibrio finkler.

Drigalski plate of oyster 2, from polluted water, contained crowds of beautifully blue colonies, slightly raised, homogeneous and watery. On microscopic examination the majority proved to be motile vibrios, rapidly liquefying gelatine like vibrio finkler.

Drigalski plate of oyster 5, kept three days in clean water, had no blue colonies.

Drigalski plate of oyster 6, two days in polluted water, one day in clean water, had two bright blue colonies. This would make 100 per oyster. These were shown to be the same, *viz.*, small watery colonies as above, *viz.*, vibrio, but non-liquefying, and in sub-culture of the same characters as those mentioned as occurring in the sewage.

Oyster 4, having been kept for 48 hours in sewage polluted sea water, when examined showed in a Drigalski plate (prepared with $\frac{1}{50}$ part of the oyster) two watery blue colonies. This would amount to 100 per oyster. Sub-cultures of both these blue colonies proved them to be of the nature of the motile Bacillus streptoides described as isolated from the sewage.

From this experiment we learn, then, that an oyster not placed in the sewage polluted water (oyster 1) contained a liquefying vibrio (probably similar to vibrio finkler), the same as oyster 2, which had been 24 hours in sewage polluted water; further, that in another oyster (6), which had been 48 hours in sewage polluted and one day afterwards in clean water, we identified the non-liquefying vibrio isolated from the sewage; and in an oyster (4) which had been 48 hours in sewage polluted water we isolated the motile B. streptoides, the same as was found in the sewage.

From these facts it appears, then, justifiable to conclude that the oysters placed in sewage polluted water had imbibed

from that sewage the non-liquefying vibrio and the motile B. streptoides, both forming blue colonies but of different aspect, size, and constitution, and therefore capable of being at once recognised by means of the Drigalski plates. In the analysis of oysters for sewage microbes the presence in Drigalski plates of these two species may therefore be of diagnostic value.

We append here a number of photographic representations of the appearances in Drigalski plates made with typhoid materials; although the colonies are not coloured in these photograms, it is not difficult to recognise their character.

These photograms are reduced to half the actual size; all colonies, except when specially mentioned, appeared blue, and of the uniform character and aspect of the typhoid colonies, as described in the text; there was no difficulty when inspecting them under a glass of ascertaining their nature, which was also confirmed by agglutination test and sub-cultures in the various media made of various colonies indiscriminately chosen.

Series I.

Fig. 1.—Plate charged with $\frac{1}{10000}$ part of a cubic centimetre of sea water immediately after infection of the sterile water with culture of B. typhosus (see Experiment IV). This plate contained 247 colonies of B. typhosus and no others; this amounts to 2,470,000 B. typhosus per 1 c.c.

Fig. 2.—Same sea water, $\frac{1}{10000}$ part of a cubic centimetre 24 hours after infection; the plate contains 153 colonies of B. typhosus; in addition one large (opaque) and one small (dot-like) colony not blue and not B. typhosus; this would therefore amount to 1,530,000 B. typhosus per 1 c.c.

Fig. 3.—The sea water in which the infected oysters had been kept had been changed 24 hours previously; $\frac{1}{10}$ c.c. of it now examined yielded 1318 blue colonies of B. typhosus,

and in addition two stray, not typhoid, colonies: one on the left middle, one near the upper left part of margin. The sea water therefore contained 13,180 B. typhosus per 1 c.c.

Series II.

Figs. 1, 2, 3, and 4 represent plates made from wet oysters of same lot as Experiment IV.

Fig. 1.—Oyster 1, kept for 24 hours in typhoid-infected water—the plate was made with $\frac{1}{100}$ part of the oyster; it yielded 958 blue colonies of B. typhosus, and 9 colonies of B. coli communis, seen in the photo as large, white, opaque colonies. There were in addition three or four other nondescript colonies; the above result would amount to 95,800 B. typhosus and 900 B. coli communis per oyster.

Fig. 2.—From same lot, oyster 3, 24 hours in typhoid-infected water, two days afterwards in sterile water; $\frac{1}{100}$ part of the oyster yielded 7520 blue colonies of B. typhosus, calculated by counting as carefully as possible two separate $\frac{1}{16}$ sections of the plate; there were no colonies of B. coli in the plate. This would mean 752,000 B. typhosus per oyster.

Fig. 3.—From same lot, oyster 5, 24 hours in typhoid-infected water, four days afterwards in sterile water; $\frac{1}{100}$ part of the oyster was used for the plate; it yielded 12 (blue) colonies of B. typhosus, three large and ten minute neutral nondescript colonies. This would mean 1200 B. typhosus per oyster.

Fig. 4.—From same lot, oyster 9, 24 hours in typhoid-infected water, seven days afterwards in sterile sea water; $\frac{1}{6}$ part of the oyster yielded 63 (blue) colonies of B. typhosus. This amounts to 378 B. typhosus per oyster.

Series III.

Figs. 1, 2, and 3 represent plates made from oysters of dry lot, of same Experiment IV.

Fig. 1.—Oyster 2, 24 hours in typhoid-infected water, and two days afterwards kept dry; $\frac{1}{100}$ part of the oyster

was used for the plate; this yielded 587 (blue) colonies of B. typhosus. This amounts to 58,700 B. typhosus per oyster.

Fig. 2.—Oyster 4, 24 hours in typhoid-infected water, four days afterwards kept dry; $\frac{1}{100}$ part of the oyster was used for the plate; this yielded 174 (blue) colonies of B. typhosus. This amounts to 17,400 B. typhosus per oyster.

Fig. 3.—Oyster 8, 24 hours in typhoid-infected water, seven days afterwards kept dry; $\frac{1}{100}$ part of the oyster was used for the plate; this yielded 13 (blue) colonies of B. typhosus. This amounts to 1300 B. typhosus per oyster.

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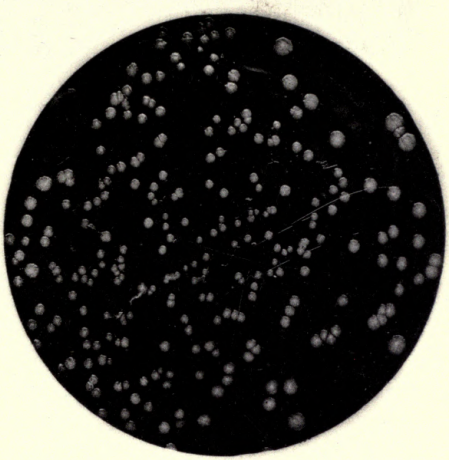


FIG. 1.

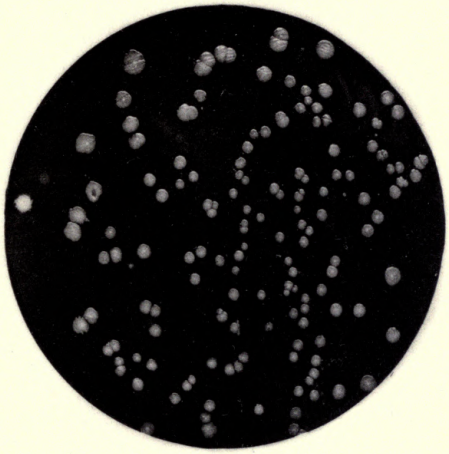
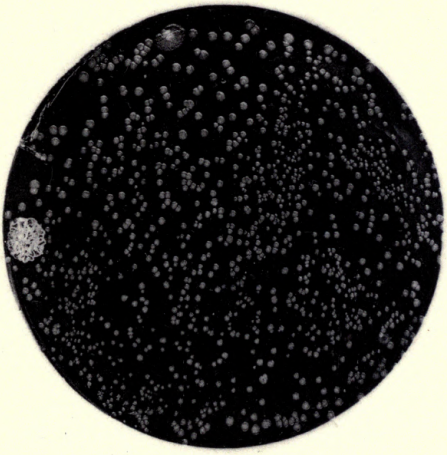


FIG. 2.



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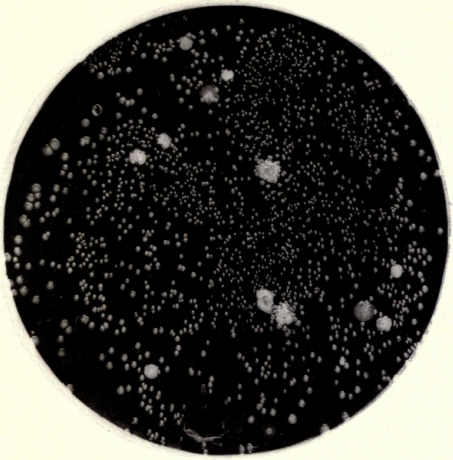

FIG. 1.

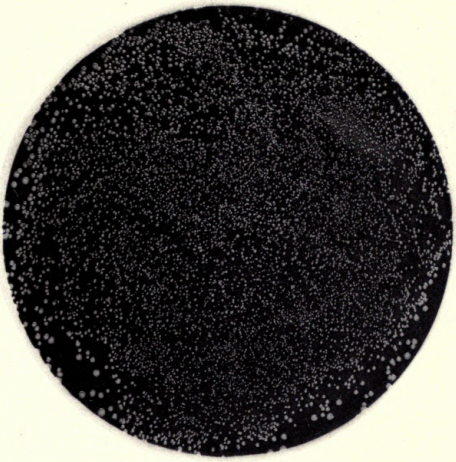

FIG. 2.

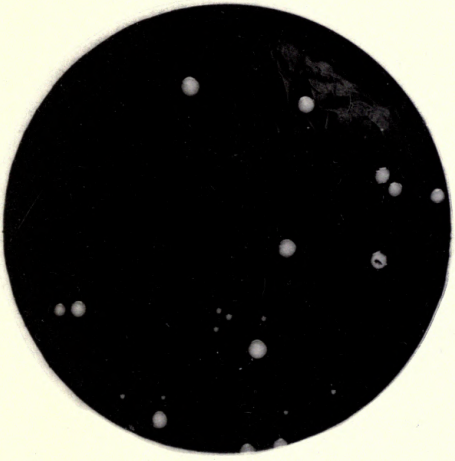

FIG. 3.

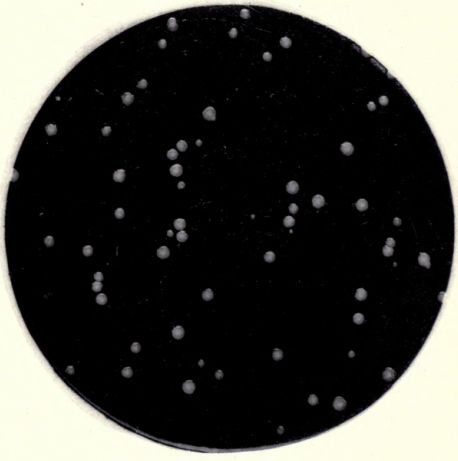

FIG. 4.

SERIES III.

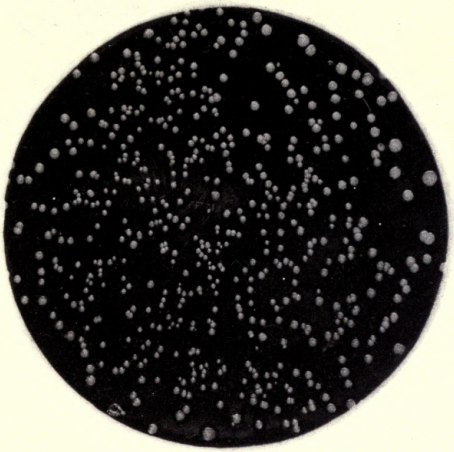

FIG. 1.

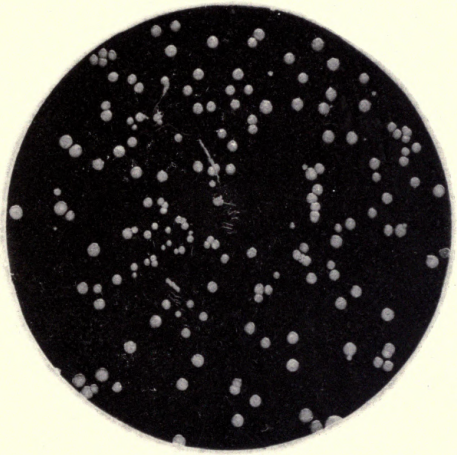

FIG. 2.

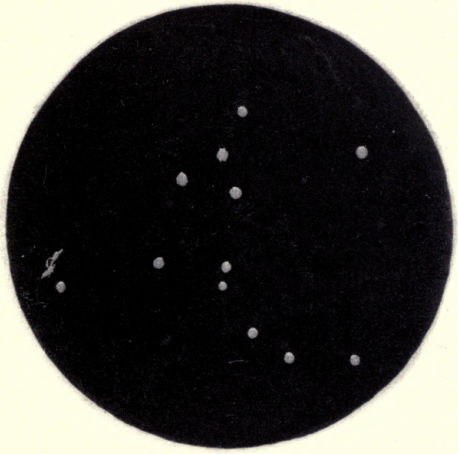

FIG. 3.

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