THE BACTERIOLOGICAL EXAMINATION OF OYSTERS AND ESTUARIAL WATERS.

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THE following paper embodies the main facts obtained during a prolonged investigation as regards the pollution of Tidal Waters and of Shell Fish, undertaken on behalf of the Royal Commission on Sewage Disposal[1].

The subject will be considered under the following five headings:—

I. *The Bacteriological Examination of the River Thames and the Thames Estuary.*

This portion of the inquiry involved the bacteriological examination of:—

(1) The water of the River Thames at Sunbury and Hampton above the intakes of some of the London Water Companies. (2) Filtered water as supplied to the consumers by the Southwark and Vauxhall and by the East London Water Works Companies. (3) The sewage effluents discharged into the Thames at Barking and Crossness. (4) The River Thames at (*a*) Barking, and at (*b*) Crossness; at (*c*) Purfleet; at (*d*) Grays; at (*e*) Mucking; and (*f*) at the Chapman

[1] Royal Commission on Sewage Disposal; Fourth Report; Vol. III; Reports by Dr Houston on Bacteriological Investigations.

Lighthouse. Also, (5) the bacteriological testing of (*a*) the Barking and Crossness chemically-precipitated sludge, and (*b*) the water in the Barrow Deep into which this sludge is discharged.

Briefly, the results may be summarised as follows:—

B. coli (or coli-like microbes) per c.c.

River Thames at	Crossness, Purfleet, Barking	About 100 to 1000 per c.c.
	Grays.	10 to 1000, but more usually 100 per c.c.
	Hampton, Sunbury	Usually 10 to 100, but more often 10 than 100 per c.c.
	Mucking.	1 to 100; more often 10 than 1; but very seldom 100 per c.c.
	Chapman, Barrow Deep	Ranged usually from 1 in 10 c.c. to 1 in 1 c.c

Spores of *B. enteritidis sporogenes* per c.c.

River Thames at	Crossness, Purfleet, Barking, Grays	Usually 1 to 10, but more often 10 than 1.
	Mucking.	1 to 10, but more often 1 than 10.
	Barrow Deep	1 per 10 c.c. to 1 per 1 c.c., but rather more often the latter.
	Sunbury, Hampton	1 per 10 c.c. to 1 per 1 c.c., but rather more often 1 per 10 c.c.
	Chapman.	Positive result with 1 c.c. only in about every fourth sample.

Crossness, Purfleet, Grays, Mucking and Chapman are about 2, 7, 12, 22, and 27 miles respectively below Barking. Sunbury and Hampton are above the intakes of some of the London Water Companies. The Barrow Deep begins about 13 miles seawards of the Nore. During 1902 the combined volume of effluent from the Barking and Crossness works amounted to a daily average of 232 million gallons. About 50,000 tons of sludge are deposited weekly in the Barrow Deep.

The chemical effluents at Barking (Northern outfall) and Crossness (Southern outfall) contained usually 100,000 *B. coli* (or coli-like microbes) and 100 to 1000 spores of *B. enteritidis sporogenes* respectively per c.c.

The chemically-precipitated sludge at Barking (Northern outfall) and Crossness (Southern outfall) contained usually 1,000,000 to

10,000,000,000 *B. coli* (or coli-like microbes) and 10,000 to 100,000 spores of *B. enteritidis sporogenes* respectively per c.c.

The filtered water as supplied to the consumers by the Southwark and Vauxhall and by the East London Water Works Companies usually contained *B. coli* (or coli-like microbes) in 100, in 10, and sometimes even in 1 c.c.

Conclusions.

The water of the River Thames at Sunbury and Hampton above the intakes of some of the London Water Companies is most unsatisfactory from the bacteriological point of view.

The Barking and Crossness "chemically-produced" effluents resemble in their biological composition raw sewage.

The bacteriological condition of the River Thames at Barking, Crossness and Purfleet is very unsatisfactory.

At Grays, the water showed some slight evidence of improvement.

At Mucking, the Thames water showed definite signs of improvement.

At the Chapman Lighthouse the Thames water was so far improved relatively, as seemingly to vie in biological purity (*qua* B. coli) with some of the samples of filtered London water.

The alleged *gross* pollution of the Essex and Kent foreshores Eastwards of a line connecting the Chapman Lighthouse with Stoke, as a result of the discharge of the Barking and Crossness effluents into the Thames, would thus appear from these data to be without sufficient warrant.

The water obtained from the Barrow Deep was found to be, under the circumstances, remarkably satisfactory. The alleged *serious* pollution of the Thames Estuary, as a result of the deposit of sludge by the London County Council in the Barrow Deep, is not supported by the results of the bacteriological analyses.

As regards the filtered London (*main*) water (Southwark and Vauxhall and East London Water Companies) it is undesirable to speak definitely. Many of the coli-like microbes were atypical in character, nevertheless, the results were unsatisfactory from the bacteriological point of view.

Speaking in general terms the results indicate :—(1) That the water of a tidal river grossly polluted in its lower estuarial reaches may after a flow of 25 miles become so far purified by sedimentation, dilution, and

the operation presumably of bactericidal agencies, as to become seemingly as little objectionable, or in some respects less objectionable, bacteriologically than certain of our public water supplies. (2) That the deposition in the sea of chemically-precipitated sludge in enormous quantities, if carried out under proper conditions, need not result necessarily in the production of nuisance or serious pollution of the surrounding water, and that such deposition may be thought of as an economical and seemingly not unsatisfactory means of disposing of this material. (3) The danger of hastily condemning waters and other materials without a wider knowledge of comparative bacteriology and of the correlation of bacteriology and epidemiology than is at present available.

II. *The Inoculation of Sewage with* B. pyocyaneus *and the subsequent isolation of this microbe from* (1 *a*) *the effluent from a continuous filter;* (2 *a*) *septic tank liquor; and* (2 *b*) *the effluent from contact beds.*

In the case of the continuous bed *B. pyocyaneus* appeared in the effluent within *less than ten minutes* from the start of the experiment and was present (at first invariably, later at irregular and increasingly rare intervals) for some considerable time afterwards (*at least* 5 hours in the first experiment; ten days in the second experiment).

In the case of the septic tank and contact beds *B. pyocyaneus* appeared in the septic tank liquor *within* $2\frac{1}{2}$ *hours* from the start of the experiment, and in the contact bed effluents at the earliest possible time, *i.e.*, the first emptying of the bed after the passage of *B. pyocyaneus* through the septic tank and into the beds. In the first experiment *B. pyocyaneus* was still present in the septic tank liquor 24 hours from the start of the experiments and in the contact bed effluent up to the third day. In the second experiment, which was carried over a longer period, *B. pyocyaneus* was isolated from the septic tank liquor and from a contact bed effluent on the ninth day.

The results indicate the inadvisability of relying on septic tanks, contact beds, and continuous filters to remove altogether the element of potential danger to health associated with the discharge of effluents from these processes of sewage treatment either into *drinking-water* streams or into estuarial waters in the neighbourhood of shell-fish layings. This statement in no way implies that these processes of

sewage treatment are not capable of yielding satisfactory effluents from the chemical and practical point of view and in relation to *non-drinking-water* streams.

III. *The Bacteriological Examination of Samples of Unpolluted Sea-water.*

Thirty-four samples of deep-sea water were collected on the North-west coast of Scotland within sight of land though remote from all possibility of contamination at separate spots along a line more than 100 miles in length.

The results showed conclusively that *B. coli* (or coli-like microbes) may be absent from as large a volume as 100 c.c. of unpolluted sea-water. The *B. enteritidis sporogenes* test likewise yielded negative results when using 10 c.c. of the water for cultural purposes.

Laboratory experiments were also carried out to ascertain the vitality of *B. coli* in *pure* water kept at a definite temperature (20° C.). Four experiments were made with sea-water and six with tap-water. The results showed that *B. coli* added to sea-water and tap-water in large amount was no longer capable of demonstration in 1 c.c. of the sample after a maximum of nine days and a minimum of three days. A negative result with 1 c.c. does not mean necessarily the absolute death of *B. coli,* but it certainly indicates the relative disappearance of this microbe from the sample. The persistence of *B. coli* in polluted water and in mud is a separate question.

At the present time the attempt to lay down absolute standards is not justifiable, but the division of waters into classes, with tentative standards for comparative purposes, is perhaps permissible. In this connection the following tabular statement may be of interest to fellow-workers (see Table I. p. 178).

Conclusions.

1. *B. coli* and the spores of *B. enteritidis sporogenes* are absent from 100 c.c. and 10 c.c. respectively of multiple samples of unpolluted sea-water.

2. *B. coli* and *B. enteritidis sporogenes* are commonly present in 1/100,000 c.c. and 1/100 to 1/1,000 c.c. respectively of sewage.

3. Taking in account both these extremes, estuarial waters,

TABLE I.

CLASS*	Standard based on numerical abundance of *B. coli* (or non-liquefying, gas-forming coli-like microbes)	Numerical standard confirmed or modified according to response of the coli-like microbes in *pure culture* to certain well-known biological tests	Provisional bacteriological conclusions confirmed or modified by topographical observations	Provisional bacteriological & topographical conclusions confirmed or modified by epidemiological & administrative considerations
I A water showing no evidence (bacteriologically) of objectionable contamination	No *B. coli* in 100 c.c.	For example :— (1) *Neutral-red broth test*—Greenish-yellow fluorescence (48 hrs. at 37° C.) (2) *Lactose peptone test*—Gas and acid production (48 hrs. at 37° C.) (3) *Indol test* — Indol in broth cultures (5 days at 37° C.) (4) *Litmus milk test*—Acid clotting of milk (5 days at 37° C.) Of course, the more tests applied the better, but the above are all tests of known value [*As regards tests* (1), (2), (3) *and* (4), *my work for the Local Government Board on the* B. coli *of recently-voided normal human faeces shows that of* 101 B. coli, 98, 92, 98, *and* 92 *per cent. respectively yielded positive results to one or other test. As regards all four tests* (*taken in conjunction*) 85 *per cent. yielded positive results.*]	For example :— Dilution; set of the tides; prevailing winds; float experiments; rate of flow; distance; time interval, etc.	For example :— Questions of practicability; whether the contaminating material is likely to have a high or a low enteric morbific value; past epidemiological experience in circumstances broadly parallel, etc. etc.
II A water showing appreciable, although slight, evidence (bacteriologically) of objectionable contamination	*B. coli* present in 100 c.c., none in 10 c.c.			
III A water showing definite signs (bacteriologically) of pollution, and therefore to be viewed with some degree of suspicion	*B. coli* present in 10 c.c., none in 1 c.c.			
IV A water showing such obvious signs (bacteriologically) of objectionable pollution as to be condemned† on the basis of results	*B. coli* present in 1 c.c., none in 0·1 c.c. (1 not 10)			
V	*B. coli* present in 0·1 c.c. None in 0·01 c.c. (10 not 100)			
VI	*B. coli* present in 0·01 c.c., none in 0·001 c.c. (100 not 1000). [Primary standard for sewage effluents; non-drinking-water streams]			
VII	*B. coli* present in 0·001 c.c., none in 0·0001 c.c. (1000 not 10,000). [Secondary standards for sewage effluents; non-drinking-water streams]			

* It must, of course, be definitely understood that I am not prepared to say that a water of Class III, II, or even I is necessarily always "safe," much less that a water of Class IV—VII has a definite "disease value."

† This does not mean necessarily administrative practical or legislative condemnation, but rather that the evidence of pollution is sufficiently defined to merit objection from the bacteriologist's point of view.

N.B. The *B. enteritidis sporogenes* test is not dealt with here. Perhaps the safest standard to suggest is that a water should yield a negative result when using 10 c.c. for cultural purposes.

sea-water, and, indeed, water and liquids in general, may be divided *broadly* into nine classes by means of the *B. coli* test[1].

4. This division of waters into classes does not involve necessarily the difficult question of absolute standards, but it may be convenient to condemn or object to a water of the fourth class (+ 1, − ·1 c.c.); regard with some degree of suspicion a water of the third class (+ 10, − 1 c.c.); consider not wholly free from evidence of probably objectionable pollution a water of the second class (+ 100, − 10 c.c.); and unconditionally on the basis of results approve a water of the first class (negative 100 c.c.).

5. *B. coli*, artificially added to unpolluted sea-water and to tap-water speedily loses its vitality or, at all events, becomes greatly diminished in number under laboratory conditions of experiment. The continued persistence of *B. coli in any number* in estuarial waters may be traced to continuous excremental pollution and the presence of unoxidised organic pabulum in the water.

6. Neither the epidemiologist, nor the topographist, nor even the bacteriologist can assign a definite "disease value" to a given pollution.

7. Conclusions as regards the *degree* of potential danger to health arising from sewage pollution must, if sound, be based consciously or unconsciously on an assumed or real knowledge of the amount of fresh sewage matters present in the water, and more particularly on the number of living microbes of *recent* intestinal origin.

8. There appear to be three ways of estimating *degrees of pollution.* The topographical or inferential method[2]; the chemical or indirect (*qua* microbes) but broadly useful method[3]; and the bacteriological or seemingly most direct method[4].

[1] Negative 100 c.c.; +100, −10 c.c.; +10, −1 c.c.; +1, −·1 c.c.; +·1, −·01 c.c.; +·01, −·001; +·001, −·0001; +·0001, −·00001; +·00001. First, second, third, fourth, fifth, sixth, seventh, eighth, and ninth classes respectively. It is obvious that these classes may be subdivided further, but whether or not this is desirable at present is a moot point. For example, we may subdivide the second class according to whether a positive result is yielded with 90, 80, 70, 60, 50, 40, 30, or 20 c.c. of the sample. Such subdivision might be expressed by the term sub-class, 1, 2, 3, 4, 5, 6, 7, 8, or 9 respectively. The same principle applies to the other classes.

[2] In the sense indicated here the topographical method theoretically is mainly speculative in character. But having regard to the breadth of its operations, it may be and indeed is actually of signal and indispensable value in practice.

[3] The chemical method is a definite and extremely accurate method, and although indirect in character, may, within certain limits and in certain directions, yield most valuable results.

[4] The bacteriological method is direct *qua* numerical estimation of intestinal microbes, but indirect as regards disease-producing bacteria. Although seemingly the best method, and extremely delicate, it is nevertheless to be thought of as of relative, not absolute, value.

9. In all but obvious cases of contamination *degrees* of pollution need to be measured by the bacteriologist, *who should interpret his results in the light of local observations and epidemiological considerations.*

10. However well-balanced the representations of the topographist may be as regards estuarial pollution, they are expressions of opinion involving in large measure the personal equation, and if uninfluenced by quantitative and qualitative bacteriological data, may be lacking in comparative value, and may therefore be inconclusive.

IV. *The Bacteriological Examination of Samples of Water and of Oysters obtained from the Helford and Penryn Rivers (Cornwall).*

This report deals with the quantitative and qualitative bacteriological examination of water and oysters obtained from two rivers in Cornwall, not far distant from each other topographically, but widely different as regards their local surroundings.

The Helford River traverses a sparsely-populated district, and ranks as one of the purest localities in England for the growth and fattening of oysters.

The Penryn River is polluted, and its oyster layings lie under the ban of suspicion.

It is beyond question that the Helford River, from the point of view of the topographist and the epidemiologist, would be approved. It is equally certain that the Penryn River, on topographical and epidemiological grounds, would be regarded with great suspicion, if not condemned.

The primary object of this investigation was to compare, bacteriologically, the water and oysters from these two rivers[1].

The results may be briefly summarised as follows:

[1] It is essential to note that in my detailed report to the Commission full details are given in each and every case of the biological characters of the coli-like microbes isolated in pure culture from both the waters and the oysters. It may be said, however, that the samples of Helford water and oysters (as well as the samples of Penryn water and oysters) were found to contain *typical B. coli* (on the basis of the tests employed) in greater or less number. The matter is largely one of proportion.

Helford Water.

B. Coli or coli-like Microbes			*B. enteritidis sporogenes*
100 c.c.	10 c.c.	1 c.c.	10 c.c.
Positive result	Positive result	Positive result	Negative result
28 %	40 %	32 %	100 %

Helford Oysters.

B. coli test:

1 out of 25 (4 %) contained 1 *B. coli* or coli-like microbes per oyster.
5 „ (20 %) „ 10 „ „ „ „
16 „ (64 %) „ 100 „ „ „ „
3 „ (12 %) „ 1000 „ „ „ „

B. enteritidis sporogenes test:

Less than 10 spores of *B. enteritidis sporogenes* per oyster, 16 out of 25, 64 per cent.
8 out of 25 contained 10 but less than 100, 32 %.
1 „ 25 „ 100 „ „ 1000, 4 %.

Penryn Water.

B. coli or coli-like microbes				*B. enteritidis sporogenes*	
10 c.c.	1 c.c.	·1 c.c.	·01 c.c.	10 c.c.	10 c.c.
Positive result	Positive result	Positive result	Positive result	Positive result	Negative result
16 %	36 %	44 %	4 %	56 %	44 %

Penryn Oysters.

B. coli test:

1 out of 25 (4 %) contained 100 *B. coli* or coli-like microbes per oyster.
13 „ (52 %) „ 1000 „ „ „ „
11 „ (44 %) „ 10,000 „ „ „ „

B. enteritidis sporogenes test:

3 out of 25 (12 %) contained 10 spores of *B. enteritidis sporogenes* per oyster.
20 „ (80 %) „ 100 „ „ „ „ „ „
2 „ (8 %) „ 1000 „ „ „ „ „ „

The following division of oysters into classes, with tentative standards for comparative purposes, may be of interest to fellow-workers (Table II. p. 182).

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TABLE II. *Bacteriological Grouping of Oysters in Classes with Tentative Standards for Comparative Purposes.*

CLASS*	Standard based on numerical abundance of *B. coli* (or non-liquefying, gas-forming coli-like microbes), in the whole contents of the oyster shell (*i.e.* liquor, body and interior juices of the oyster)	Numerical standard confirmed or modified according to response of the coli-like microbes in pure *culture* to certain well-known biological tests	Provisional bacteriological conclusions confirmed or modified by topographical observations	Provisional bacteriological and topographical conclusions confirmed or modified by epidemiological and administrative considerations
I An oyster showing no evidence (bacteriologically) of objectionable contamination	No *B. coli*	For example:— (1) *Neutral-red broth test*—Greenish-yellow fluorescence (48 hours at 37° C.) (2) *Lactose peptone test*—Gas and acid production (48 hours at 37° C.) (3) *Indol test*—Indol in broth cultures (5 days at 37° C.) (4) *Litmus milk test*—Acid clotting of milk (5 days at 37° C.) Of course, the more tests applied, the better, but the above are all known tests of value ‖	For example:— Dilution: set of the tides; prevailing winds; float experiments; time interval; distance, etc., etc.	For example:— Questions of practicability; whether the contaminating material is likely to have a high or a low enteric morbific value; past epidemiological experience in circumstances broadly parallel, etc., etc.
II An oyster showing appreciable, although slight, evidence (bacteriologically) of objectionable contamination	1 *B. coli* per oyster†			
III An oyster, showing definite signs (bacteriologically) of pollution &, therefore, possibly to be viewed with some degree of suspicion	10 *B. coli* per oyster†			
IV *Stringent standard* An oyster showing such obvious signs (bacteriologically) of pollution as to be condemned on a stringent standard	100 *B. coli* per oyster†			
V *Lenient standard* An oyster showing such unmistakeable evidence (bacteriologically) of pollution as to be condemned on the basis of results§	1000 *B. coli* per oyster†			
VI An oyster showing such *gross* evidence (bacteriologically) of contamination as to be outside the pale of recognition.	10,000 *B. coli* per oyster†			

* It must, of course, be definitely understood that it cannot be said either that oysters of Class II or even Class I are necessarily always safe, or that oysters of Class III—VI have a definite "disease value."

† To obtain approximate results *per c.c.* of oyster, divide the foregoing figures by ten.

§ This does not mean administrative, practical, or legislative condemnation, but only that the evidence of pollution is sufficiently defined to merit objection from the bacteriologist's point of view.

‖ As regards tests (1) (2) (3) and (4) my work for the Local Government Board on the *B. coli* of recently-voided normal human faeces shows that of 100 *B. coli*, 98, 92, 98, and 92 per cent. yielded positive results in each instance to one or other test. As regards all four tests (taken in conjunction) 85 per cent. yielded positive results.

As regards the *B. enteritidis sporogenes* test, a lenient standard would seem to be less than 100 spores of this anaerobe *per oyster*. A more stringent standard would be less than 10 spores *per oyster*.

Conclusions.

On the one hand, the water of an estuarial river, such as the Helford River, which, on topographical grounds, has been, and would still be considered eminently well suited for the breeding, growth, and fattening of oysters for market, may contain *B. coli* or coli-like microbes, in 100 c.c. (28 %), in 10 c.c. (40 %), or in 1 c.c. (32 %). As regards *B. enteritidis sporogenes* all the samples yielded a negative result when using for cultural purposes 10 c.c. of the water.

Oysters obtained from the Helford River contained *B. coli* or coli-like microbes in greater number than did comparable quantities of the surrounding water: 1,000 per oyster, about 100 per c.c. of oyster (12 %); 100 per oyster, about 10 per c.c. of oyster (64 %); 10 per oyster, about 1 per c.c. of oyster (20 %); 1 per oyster (4 %). As regards spores of *B. enteritidis sporogenes*, the results were:—100 per oyster, about 10 per c.c. of oyster (4 %); 10 per oyster, about 1 per c.c. of oyster (32 %); less than 10 per oyster, *i.e.*, less than 1 per c.c. of oyster (64 %).

On the other hand, the water of an estuarial river such as the Penryn River, which on topographical grounds has been rightly considered dangerous *qua* oyster layings, but which *qua* bacteriological facts does not abound in microbes of intestinal origin, yielded, as regards *B. coli* or coli-like microbes, the following results:— 100 per c.c. (4 %); 10 per c.c. (44 %); 1 per c.c. (36 %); + 10 c.c. (16 %). As regards the spores of *B. enteritidis sporogenes*, 56 % yielded a positive, and 44 % a negative result when using 10 c.c. for cultural purposes. The Penryn water was more than ten times as impure bacteriologically as the Helford water.

Oysters obtained from the Penryn River contained *B. coli* or coli-like microbes in much greater number than did comparable quantities of the surrounding water: 10,000 per oyster, about 1000 per c.c. of oyster (44 %); 1000 per oyster, about 100 per c.c. of oyster (52 %); 100 per oyster, about 10 per c.c. of oyster (4 %). As regards spores of *B. enteritidis sporogenes*, the results were:—1000 per oyster, about 100 per c.c. of oyster (8 %); 100 per oyster, about 10 per c.c. of oyster (80 %); 10 per oyster, about 1 per c.c. of oyster (12 %).

The Penryn oysters were therefore about 100 times more impure than the Helford oysters.

If the worst results from the "good" place (Helford) are to be

compared with the best results from the "bad" place (Penryn), assistance from the bacteriologist in determining the *status* of oysters or water is not to be looked for. On any other basis of comparison the contrast is most striking, and the results, in my opinion, indicate that the *quantitative* and *qualitative* bacteriological testing of oysters and estuarial waters may prove of great practical value in cases of doubtful pollution.

Neither oysters nor water are to be condemned on bacteriological grounds, unless the number of objectionable microbes exceeds what may be termed a "permissible limit of biological impurity." But where the line should be drawn remains to be determined.

Waters and oysters have been arranged in classes (*see* Table II.) according to the results of the *B. coli* test, and although this does not necessarily involve the acceptance of standards it may be convenient to adopt certain standards *solely for comparative purposes.*

For example :—To object to (1) a water containing *B. coli* in 1 c.c., or the spores of *B. enteritidis sporogenes* in 10 c.c.; and (2) to reject oysters containing 1000 (lenient standard) or 100 (stringent standard) *B. coli*; or 100 (lenient standard) or 10 (stringent standard) spores of *B. enteritidis sporogenes* respectively per oyster; *subject always to the examination of a number of samples, and to the interpretation of the results of the* B. coli *test in the light of a knowledge of the biological attributes of these microbes.*

Applying these tentative standards to the Helford and Penryn results, and including, *for the purposes of a broad summary,* "coli-like microbes," the following results are obtained :—

Water

	Penryn		Helford	
	Passed	Condemned	Passed	Condemned
B. coli or Coli-like Microbes: (*Standard* negative result, 1 c.c.)	16 %	84 %	68 %	32 %
B. enteritidis sporogenes test: (*Standard* negative result, 10 c.c.)	44 %	56 %	100 %	none

Oysters

	Penryn		Helford	
	Passed	Condemned	Passed	Condemned
B. coli or Coli-like Microbes: *Lenient Standard* (less than 1000 per oyster, or 100 per c.c. of oyster)	4 %	96 %	88 %	12 %
Stringent Standard (less than 100 per oyster, or 10 per c.c. of oyster)	none	100 %	24 %	76 %
B. enteritidis sporogenes test: *Lenient Standard* (less than 100 per oyster, or 10 per c.c. of oyster)	12 %	88 %	96 %	4 %
Stringent Standard (less than 10 per oyster, or 1 per c.c. of oyster)	none	100 %	64 %	36 %

Method adopted in Examining Oysters Bacteriologically.

Cleansing of the Oysters:—

The outside of the oyster shells was well scrubbed with soap and water, and cleansed as thoroughly as possible under clean running water; the shells were then well washed in running *main* water, and finally with sterile water.

Cleansing of the Hands:—

The hands of the experimenter were thoroughly cleansed with a hard scrubbing brush, soap, and water, then rinsed first with 1 in 1,000 corrosive sublimate solution, and finally with sterile water.

Subsequent procedure:—

The oysters were laid out upon a sterile towel, the flat shell uppermost. They were opened in this position with a sterile knife, held in the right hand, while they were held in position with a corner of the sterile cloth grasped in the left hand. Great care was taken to avoid any loss of the liquor. This liquor in the shell was poured into a sterile 1000 c.c. cylinder, the oyster was then partly cut up with sterile scissors, and the liquor thus freed also allowed to run into the cylinder; finally, the oyster was cut up into small pieces, and added to the cylinder. *Ten oysters were thus treated in each experiment.* The volume of oyster + oyster liquor was *read off*, and usually varied between 80 and 120 c.c., so that the oysters, being of *medium* size and containing a medium amount of liquor, 100 c.c. might be considered a fair average[1] of the total shell contents of ten oysters. Sterile water was then poured into the cylinder up to the 1000 c.c. mark, and the whole well stirred with a sterile rod.

The following amounts of this liquid were taken for cultural purposes (primary cultures):—

[1] This average has been used generally as a convenient basis for calculating the relation between the number of bacteria per c.c. in the oyster and the water over the oyster layings. It is, however, an under-estimate of the bulk of many oysters. It must be remembered that the bacteria are not uniformly distributed within the contents of the oyster shell. No doubt the liquor, alimentary tract and perhaps the gills harbour most of the microbes, and the tissue of the body of the oyster may be, relatively speaking, sterile.

Culture A 100 c.c. = contents of 1 oyster.
„ B 10 c.c. = „ $\frac{1}{10}$ „ multiply by 10.*
„ C 1 c.c. = „ $\frac{1}{100}$ „ „ „ 100.*
„ (1) 1 c.c. of $\frac{1}{10}$ dilution (·1 c.c.) = $\frac{1}{1000}$ oyster, multiply by 1000.*
„ (2) 1 c.c. of $\frac{1}{100}$ dilution (·01 c.c.) = $\frac{1}{10000}$ oyster, multiply by 10,000.*
„ (3) 1 c.c. of $\frac{1}{1000}$ dilution (·001 c.c.) = $\frac{1}{100000}$ oyster, multiply by 100,000.*
„ (4) 1 c.c. of $\frac{1}{10000}$ dilution (·0001 c.c.) = $\frac{1}{1000000}$ oyster, multiply by 1,000,000.*

* To obtain the number of bacteria, *e.g. B. coli, per oyster.* (*Divide the figures thus obtained by* 10, *if the results per c.c. of oysters are wanted.*)

These amounts A, B, C, 1, 2, 3, 4 were used for the examination for *B. coli* (primary cultures), and the amounts B, C, 1, 2, 3 for the *B. enteritidis sporogenes* test. Experience has shown that it is best to make the primary cultures in triplicate. Then if, as regards the *B. coli* test, a sugar medium is employed, at least two out of the three primary cultures should form acid and gas to allow of a *preliminary numerical* diagnosis being made. Similarly, in respect of the *B. enteritidis sporogenes* test, the "enteritidis" change should occur in at least two out of the three anaerobic milk cultures to merit a positive result being recorded.

The subsequent procedure, so far the *B. coli* test was concerned, was the isolation of *B. coli* or coli-like microbes from the primary cultures, by means of secondary gelatine plate cultures, followed by study of the isolated microbe in the *pure state* in following media :—

Gelatine "shake" cultures (for "gas" formation); Broth cultures (for indol formation); Litmus milk cultures (for acid and clotting); Lactose peptone cultures (for acid and gas); neutral-red broth cultures (for greenish-yellow fluorescence).

As regards *B. enteritidis sporogenes*, the inoculation of animals was not practised, so that the results must be interpreted in the sense that a positive result means the "enteritidis change" in anaerobic milk culture without proof of virulence.

The chief advantages of this method are as follows :—

1. It is a *definite quantitative* method, succeeded by qualitative records.
2. It gives the average volume of the whole contents of the oyster shell.
3. It yields results based on collective examination of ten oysters.
4. It includes the examination of the entire contents of the shell,

not of a fraction either of the liquor or the gastric or intestinal juice, or of the mixture of these liquids.

5. The results can be stated as number of bacteria, either per oyster, or per c.c. of oyster.

An alternative Quantitative Method for the Bacteriological Examination of Oysters.

An alternative method for the bacteriological examination of oysters may be given here, although the routine work was carried out by the foregoing method.

The oysters are cleaned and opened, with the precautions already noted. Then the body of the oyster is cut into small pieces with sterile scissors; this process should be carried out in such a way as to ensure the thorough mixture of the gastric juice of the oyster and the liquor. The oyster, meanwhile, is carefully held with the concave shell downwards and the flat shell bent back or altogether removed. To examine the liquid contents of the shell without this preliminary step may partake of the nature of the examination of the last sample of sea-water imbibed by the oyster before finally closing its shell. Indeed, the experiments detailed elsewhere seem to indicate that *per unit of volume* the gastric juice of the oyster may be more impure bacteriologically than the oyster liquor.

The next step is to withdraw 1 c.c. of the oyster mixture with a sterilised 1 c.c. pipette and add it to 9 c.c. of sterile water in a test tube (dilution 1). 1 c.c. of dilution (1) is used to inoculate a second tube containing 9 c.c. of sterile water (dilution 2). 1 c.c. of dilution (2) is used to inoculate a third tube containing 9 c.c. of sterile water (dilution 3), and so on to further dilutions if necessary. Primary cultures, using in each instance 1 c.c., are next made severally from dilutions (1) (2) (3), corresponding to $\frac{1}{10}$, $\frac{1}{100}$, and $\frac{1}{1000}$ c.c., respectively, of the oyster mixture.

Further, 1 c.c. of the oyster mixture direct, that is without any dilution, is used to make another primary culture. From these primary cultures, after incubation for two days at 37° C., secondary plate cultures are made, and from these plates the coli-like colonies are subsequently picked out and studied in pure culture in various media.

The above procedure applies to the *B. coli* test, but it is obvious

that the same dilutions might also be employed for the *B. enteritidis sporogenes* test.

The above method answers fairly well if the oysters contain a sufficient volume of liquor, and if conclusions are based on the examination of at least 10 oysters. Nevertheless, this method is not free from certain objections. The volume of liquor in oysters varies enormously, ranging from as little as 0·1 c.c. to over 10 c.c., and the results might naturally vary in corresponding degree. Sometimes the amount of liquor is too small to allow more than one culture being made, even if that end be achieved, at other times it is so large as to suggest that sea-water is diluting the bacterial contents of the oyster itself to a considerable extent. At all events, it is obvious that if the liquid contents of the shell may vary one hundred times, it is difficult to ensure that comparative quantitative records are always obtained.

V.

Appendices A to L.

Results of a number of separate bacteriological observations bearing on the general question of the pollution of estuarial waters and shell-fish.

Appendix A.

Results of the Bacteriological Examination of samples of Sea-Water, Estuarial Water, water over shell-fish layings, etc. Collected for the most part during the visits paid by the Commission to various centres of the Oyster Industry.

Generally speaking the bacteriological results were broadly parallel with the topographical surroundings of the places whence the samples were derived.

With a view of rendering a general survey of all the results as regards *B. coli* comparatively easy, the samples have been grouped (irrespective of the biological attributes of the coli-like microbes) in separate classes (− 10 c.c.; + 10 c.c., − 1 c.c.; + 1 c.c., − ·1 c.c.; + ·1 c.c., − ·01 c.c.; + ·01 c.c., − ·001 c.c.; + ·001 c.c., − ·0001 c.c.; + ·0001 c.c., − ·00001 c.c.; + ·00001 c.c.) as follows:—

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B. coli test.—Samples yielding a negative result with 10 *c.c. of the sample.*

No.	
5.	Gutner Creek, oyster pond.
B.	Solent, midway between Ryde and Southsea piers.
17.	Crouch River, 1 mile above Burnham.
22.	Crouch River, fattening beds at mouth of river.
25.	Roach River, Barling Creek, top of fishery.
26.	Roach River, storage pit at Poole Creek.
32.	Blackwater, Strood Channel, opposite Victory Inn.
33.	„ „ „ 100 yards higher up.
1.	Hunstanton, high water, ¼ mile from land.
2.	„ low „ „ „
3.	„ high „ ½ mile west of pier.
4.	„ low „ „ „ „
5.	„ „ „ „ from sewage outfall.
6.	„ high „ 1 mile from shore opposite sewage works.
61.	„ Sea pool over mussel beds, 1 mile N.W. pier.
62.	The Wash, about ⅔ flood tide, near Roaring Middle.
1.	Firth of Forth at Gullane, shore sample.
2.	„ „ „ „ „
3.	„ „ „ „ „
4.	„ „ „ „ „
5.	„ „ „ „ „
6.	Ribble estuary at Lytham, boat sample, high water.
36.	North Sea, opposite Harwich, some miles out to sea.
1.	„ „ off Flamboro' Head, 5 miles out.
A.	English Channel, midway between Newhaven and Dieppe.

B. coli test.—Samples yielding a positive result with 10 *c.c., but a negative result with* 1 *c.c. of the sample.*

No.	
2.	Langston Channel, flood tide, near ferry.
3.	Solent Water, shore sample, opposite Grand Hotel.
4.	North Saltern Creek, near mouth of Emsworth Channel.
A.	Langston Channel, flood tide, near ferry.
23.	Crouch River, at Holywell, 3 parts flood tide.
24.	Roach River, at White House fattening beds, high water.
29.	Pyfleet Channel, opposite packing house, ⅔ flood tide.
30.	Pyfleet oyster pit, No. 23, near packing house.
35.	Pyfleet Channel, head of channel, about high tide.
34.	West Mersea, Mr Bean's oyster pit.
34A.	„ „ but mud stirred up.
51.	Teign River under Shaldon Bridge, ⅔ flood tide.
53.	„ „ opposite gas works, ⅚ flood tide.

No.
60. Brancaster Staith, mussel pit.
6. Firth of Forth, at Gullane, shore sample.
1. Ribble estuary at Lytham, high water, 1 mile from pier.
3. „ „ „ „ „ „ ½ „ „ „
10. Bosham, oyster pit.
15. Southwick, one of Brazier's oyster ponds, low water.
63. Mouth of Witham River, dredged sample of mud and water.

The last three samples yielded a negative result with 1 c.c. of the sample, but no 10 c.c. cultures were made.

B. coli test[1].—*Samples yielding a positive result with* 1 *c.c., but a negative result with* $\frac{1}{10}$ *c.c.* (1 *not* 10 *in* 1 *c.c.*).

No.
6. Langston Channel, near high tide, near Hayling fishery.
7. Emsworth longshore oyster pit (Kennet).
11. Chichester Channel, Del Quay, about high water.
13. Southwick, one of Brazier's oyster ponds, low water.
27. Brightlingsea Creek, over private layings, 2 hours flood.
28. Colne Channel, extreme point of fishery, 2 to 3 hours flood.

B. coli test.—Samples yielding a positive result with $\frac{1}{10}$ *c.c., but a negative result with* $\frac{1}{100}$ *c.c.* (10 *not* 100 *in* 1 *c.c.*).

No.
8. Emsworth longshore oyster pit (Foster).
14. Southwick Channel, opposite Brazier's ponds, ¼ flood.
20. Burnham, pile house, Gutter Creek.
54. Teign River, between Combe Cellars and Shaldon Bridge, ⅝ flood.
56. „ „ mussel bank above gas works, ⅝ ebb tide.
58. „ „ underneath Shaldon Bridge, low water.
59. „ „ opposite Devon trading wharf, low water.
4. Ribble estuary at Lytham, off pier, low water.
5. „ „ „ „ „
16. Sea-water off Brighton, 300 yards east of Portobello outfall.

[1] According to the tentative standard of a negative result with 1 c.c. (*see* Division III.), all the foregoing samples would be passed, and all the following samples rejected bacteriologically. That is, if the numerical results, as regards *B. coli* and coli-like microbes, be considered independently of the biological attributes of such microbes.

B. coli test.—Samples yielding a positive result with $\frac{1}{100}$ *c.c., but a negative result with* $\frac{1}{1000}$ *c.c.* (100 *not* 1000 *in* 1 *c.c.*).

No.

9. Emsworth Channel, 7 yards below sewer outfall, $\frac{1}{2}$ flood tide.
12. Shoreham Channel, just above Norfolk Bridge, near low tide.
52. Teign River, about 2 miles above Shaldon Bridge by Combe Cellars, $\frac{2}{3}$ flood tide.
55. Teign River, between Combe Cellars and Shaldon Bridge, $\frac{5}{6}$ ebb tide.
57. „ „ opposite gas works, low water.
2. Ribble estuary at Lytham, $\frac{1}{2}$ mile south of pier, low water.

B. coli test.—Samples yielding a positive result with $\frac{1}{1000}$ *c.c., but a negative result with* $\frac{1}{10000}$ *c.c.* (1000 *not* 10,000 *in* 1 *c.c.*).

No. 18. Burnham, fine bed effluent.

B. coli test.—Samples yielding a positive result with $\frac{1}{10000}$ *c.c., but a negative result with* $\frac{1}{100000}$ *c.c.* (10,000 *not* 100,000 *in* 1 *c.c.*).

No samples come under this category, but the heading is retained for the sake of uniformity.

B. coli test.—Samples yielding a positive result with $\frac{1}{100000}$ *c.c.* (*at least* 100,000 *per c.c.*).

No.

19. Burnham, combined effluent.
21. Burnham, mixed effluent and sea-water at sluice.
31. Brightlingsea, tank effluent, Ives' patent.
64. Hunstanton, sewage effluent, at outfall.

APPENDIX B.

Experiments dealing with the Bacteriological Examination of Deep Sea Oysters and surface samples of Sea-Water collected over the Oysters.

The results show that in deep sea oysters derived from deep sea-water remote from sewage pollution *B. coli* and coli-like microbes and also the spores of *B. enteritidis sporogenes* are either absent or, at all events, seldom detectable. The same is true of the surface water over such oysters.

It is nevertheless true that in shallow water near the shore around our English coast, in situations practically available and well suited

for the growth and fattening of high class oysters, it may be and indeed would seem to be impossible to obtain similar bacteriological results.

Yet it is necessary to start with a basis, and the basis in the present instance is that *B. coli* and *B. enteritidis sporogenes* seemingly form no essential part of the bacterial flora of pure sea-water, and that they have no part in the economy of the oyster.

Without going into the experiments in detail it may be said that certain of the experiments indicate that water from the open sea collected about 9 miles from the Norfolk coast may be *at least one-hundred million times purer* (qua *bacteriological facts*) *than a sewage effluent of average quality; from* 10 *to* 100 *times purer than Whitstable sea-water; from* 100 *to* 10,000 *times purer than West Mersea sea-water; from* 10 *to* 1,000 *times purer than Crouch River water; from* 100 *to* 1,000 *times purer than Roach River water; and from* 1,000 *to* 10,000 *times purer than Pyfleet water derived from the neighbourhood of the oyster layings in these localities respectively.*

With Deep Sea Oysters the result was frequently totally negative as regards the presence of coli-like microbes of any sort. For example, in one experiment a total of 57 c.c. of liquor derived from five oysters contained no coli-like microbes.

Appendix C.

Results of the Bacteriological Examination of Samples of Whitstable, West Mersea, Crouch, Roach, and Pyfleet oysters and corresponding samples of water.

Two hundred oysters were examined altogether, in four separate batches of ten oysters from each of the foregoing five localities. Twenty corresponding samples of water collected over the layings were examined altogether; four from each of the above-named five localities.

The Whitstable and Pyfleet oysters are known all over the world, and the West Mersea, Crouch and Roach oysters are probably no whit inferior in quality. No outbreak of enteric fever has ever, it is believed, been traced to the consumption of oysters from these layings. At the great annual Colchester oyster feast, tens of thousands of Pyfleet oysters are consumed in one day. The general topographical surroundings of these oyster layings have been either unconditionally approved or the layings considered so far removed from *recent* sewage

pollution as to be practically safe[1]. These practical considerations should be borne in mind in scrutinising the results of the bacteriological examinations.

The chief results may be summarised briefly as follows:

Waters.

Number of coli-like microbes considered independently of their biological attributes.

[Tentative standard, none in 1 c.c.]

	+ 100 c.c. No. of samples	+ 10 c.c. No. of samples	+ 1 c.c. No. of samples	+ $\frac{1}{10}$ c.c. No. of samples
Whitstable water	1	3	—	—
West Mersea water	—	2	1	1
Crouch river	2	1	1	—
Roach river	—	2	2	—
Pyfleet water	—	—	1	3

B. enteritidis sporogenes:

[Tentative standard, negative result 10 c.c.]

	Negative 10 c.c. No. of samples	+ 10 c.c. No. of samples	+ 1 c.c. No. of samples
Whitstable water	3	1	—
West Mersea water	4	—	—
Crouch river	4	—	—
Roach river	4	—	—
Pyfleet water	2	1	1

Oysters.

Number of coli-like microbes[2] considered independently of their biological attributes.

[Tentative standards:—less than 1000 (lenient standard); less than 100 (stringent standard) per oyster.]

	10 per oyster No. of samples	100 per oyster No. of samples	1000 per oyster No. of samples
Whitstable oysters	3	1	—
West Mersea oysters	1	2	1
Crouch oysters	1	2	1
Roach oysters	—	4	—
Pyfleet oysters	—	3	1

[1] It must, of course, be understood that throughout this article no personal responsibility is incurred for topographical expressions of opinion as regards the purity or otherwise of oyster layings. The rule has been to accept the opinions of the highest authority on the subject, as if they were final, for the purposes of comparison with the bacteriological results.

[2] Within the limits of this article it is impossible to consider the "modifications of test readings" suggested in my detailed report to the Commission. It may be said, however, that the samples both of water and of oysters obtained from these layings occasionally, if not uniformly, contained *typical B. coli* (on the basis of the tests employed) in greater or less number. The matter is largely one of proportion.

B. enteritidis sporogenes test:

[Tentative standard:—less than 100 spores (lenient standard); less than 10 spores (stringent standard) per oyster.]

	Less than 10 per oyster No. of samples	10 per oyster No. of samples	100 per oyster No. of samples
Whitstable oysters	—	1	3
West Mersea oysters	3	1	—
Crouch oysters	—	3	1
Roach oysters	2	2	—
Pyfleet oysters	1	3	—

If the Whitstable, West Mersea, Crouch, Roach and Pyfleet layings are to be accepted as pure on topographical grounds it is obvious that a "certain degree of biological impurity" must be accepted as permissible if oyster culture is to be encouraged around our English shores. But where the line should be drawn is matter for conjecture.

Appendix D.

Experiments undertaken to ascertain whether *B. coli* or coli-like microbes can be practically always isolated from a mixture of ten oysters derived from layings which, judged from the topographical point of view, would be considered either above suspicion or at least reasonably secure from pollution; and also to ascertain whether such microbes can usually be isolated from each individual, or, at all events, from a majority of the ten oysters experimentally tested.

The Helford, Whitstable, West Mersea, Crouch, Roach and Pyfleet oyster layings have all been approved on topographical grounds. These layings are indeed considered to be, from the topographical point of view, among the purest (some of them perhaps *the purest*) layings around the *English Coast.*

To suggest therefore that the oysters from these layings are impure, in the sense that they are a source of danger to health, would be a grave step to take. It would be equivalent to implying that secure conditions of oyster culture in England are practically unattainable.

As a bacteriologist I offer no opinion as to the possibility or otherwise of oysters from these layings giving rise to epidemic disease.

Quantitative Results.

Estimated number of coli-like microbes *per c.c.* of oyster liquid (mixed liquid and juices of oyster).

Oyster	1	2	3	4	5	6	7	8	9	10
Helford	100	100	100	100	100	100	100	10	10	10
Whitstable	10	10	10	10	10	1	1	1	1	1
West Mersea	10	10	10	10	1	1	1	1	1	none
Crouch	10	10	1	1	1	1	1	1	1	1
Roach	10	10	10	10	10	10	1	1	1	none
Pyfleet	10	10	10	1	1	1	1	1	1	1

It is not suggested that these figures should be accepted as indicating the respective merits of the different oysters. The point of interest is that as regards 60 oysters (10 from each of six separate layings) the number of coli-like microbes corresponded:

In 7 oysters (11·6 %) to one hundred
„ 23 „ (38·3 %) „ ten
„ 28 „ (46·6 %) „ one
„ 2 „ (3·2 %) „ not any

} coli-like microbes per c.c. of oyster liquid (mixed liquor and juices of oyster).

Many of these coli-like microbes were not identical in behaviour with typical *B. coli*, but the only point emphasized here is that practically all the oysters derived from certain of the *reputedly* purest layings in England could be regarded as containing from 1 to 100 coli-like microbes per c.c. of oyster liquid (mixed liquor and body juices).

Qualitative Results.

As regards a list of the biological characters of the *B. coli* contents of individual oysters, much depends on the amount of material used for cultural purposes, and also on the "chances" involved in the speculative choice of colonies for subculture. This being understood, it is justifiable, in relation to my thesis, to select the particular batch of ten oysters from each of the six layings which, when submitted to examination, yielded the most typical *B. coli*[1].

[1] It must be understood, definitely, that I have *not*, as regards any of the layings, selected oysters from *separate* batches, so as to make up a total of ten oysters, but have chosen the particular batch of ten oysters out of a series of batches of ten oysters yielding the most typical *B. coli*.

To express the results of subcultural tests the word "flaginac" will be used in the following sense:

fl	ag	in	ac
Indicates greenish-yellow fluorescence in neutral-red broth cultures.	Indicates acid and gas in lactose peptone cultures.	Indicates indol formation in broth cultures.	Indicates acidity and clotting of litmus milk cultures.

The word "flaginac" thus indicates that a microbe was indistinguishable, as regards the tests employed, from the typical *B. coli* of the human intestine. Whenever letters are placed in brackets this indicates an incomplete reaction. The absence of a character is expressed by the omission of the letters chosen to indicate that attribute.

Biological Attributes of the most typical Coli-like Microbes derived from each of 60 *Oysters independently of their relative Abundance.*

Oysters	1	2	3	4	5	6	7	8	9	10
Helford *Exp.* I.	Flag-inac	Flag-inac	Flag-inac	Flag-inac	Flag-inac	Flag-inac	Flag-inac	Flag-inac	Flag-inac	Flag-inac
Whitstable *Exp.* III.	,,	,,	,,	,,	,,	,,	,,	,,	Flag (ac)	Agac
West Mersea *Exp.* VI.	,,	,,	,,	,,	,,	,,	,,	,,	Flag-inac	Flag-inac
Crouch *Exp.* VIII.	,,	,,	,,	,,	,,	,,	,,	,,	,,	Flag
Roach *Exp.* XI.	,,	,,	,,	,,	,,	,,	,,	,,	,,	Flagin (ac)
Pyfleet *Exp.* XIII.	,,	,,	,,	,,	,,	,,	,,	,,	,,	Flag-inac

It thus appears as regards 60 oysters (10 from each of six separate layings), all of which contained coli-like microbes, that the biological attributes of the microbes in question may be expressed as follows:—

56 oysters contained	"flaginac"	microbes	*That is,* 93 % *of the oysters contained microbes indistinguishable, as regards the tests employed, from the typical* B. coli *of the human intestine.*
1 oyster	,, flagin(ac)	,,	
1 oyster	,, flag(ac)	,,	
1 oyster	,, agac	,,	
1 oyster	,, flag	,,	

The inference is that practically all the best known English oysters are liable to contain, given a batch of ten oysters, coli-like microbes in each of the ten oysters (100 %), and that these coli-like microbes may be indistinguishable, as regards the tests employed, from the typical *B. coli* of the human intestine.

The matter may be regarded as one of *proportion*; and, in the circumstances, until the "permissible degree of biological impurity" can be defined, no English oysters should be condemned on bacteriological grounds alone, unless, indeed, the results indicate bacteriologically such *gross* pollution as to afford no possible room for doubt of their objectionable quality. This does not mean that the quantitative and qualitative bacterioscopic examination of oysters is unimportant. The contrary is my own view, but until knowledge is more complete, it would, I consider, be unjustifiable to damage an important industry by relying *exclusively* on the bacteriological facts, the *precise* interpretation to be placed on these facts remaining matter for speculation.

Meanwhile demonstration of the *mere presence* of *B. coli*, as the result of qualitative bacteriological analysis, even in 90 % of a batch of oysters from any layings around the English coast, would seem, in face of the above results, insufficient to condemn such layings.

APPENDICES E—K.

In *Appendix E* of the Report will be found the results of experiments designed to ascertain whether immersion for a considerable time of the bodies of oysters in strong germicidal solutions is to be relied on to destroy *B. coli* (or coli-like microbes); whether, that is, these microbes are or are not contained within the alimentary tract of the oyster.

APPENDIX F refers to experiments designed to ascertain whether or not prolonged washing of the bodies of oysters after removal from their shells frees such washed oysters of microbes of undesirable sort. The results show that neither prolonged washing of the bodies of polluted oysters nor immersion in germicidal solutions can be relied on to free such oysters from *B. coli* or coli-like microbes owing to the presence of these bacteria in the interior of the oysters.

APPENDIX G relates to a quantitative series of experiments to ascertain the relation between the biological composition of (1) the liquor and "washings" of the oyster and (2) the body of the oyster.

Without entering into detail it may be said that the results appear

to indicate that the "coli-yielding fraction" of the body of the oyster may contain *per unit of volume* a larger proportion of coli-like microbes than the liquor.

APPENDIX H contains: I. A series of experiments to ascertain whether *B. coli* (or coli-like microbes) are normally present in the stomach of the oyster.

II. A series of quantitative experiments to determine the number of *B. coli* (or coli-like microbes) present in the stomach juice of oysters.

Briefly stated, the results show that *B. coli* (or coli-like microbes) may not only be present in the alimentary tract of certain oysters but that the proportionate number of these undesirable bacteria may be very great.

APPENDIX I. Experiments designed to ascertain whether the *B. coli* met with within the shell of the oyster may have been derived from the exterior of the shell or from the manipulative procedure involved in the opening and examination of the shell contents.

The experiments clearly show that whatever precautions be taken as regards examination polluted oysters contain *within* their shells microbes of undesirable sort.

APPENDIX J. Experiments designed to ascertain whether or not polluted oysters re-laid in sea-water remote from sewage pollution can rid themselves within a reasonable time of microbes of undesirable sort (*e.g. B. coli*).

The results, on the whole, were disappointing but the subject merits a prolonged investigation.

APPENDIX K. Experiments designed to ascertain the length of time that *B. coli* or coli-like microbes can persist in oysters when the latter are separated from their natural environment and placed under artificial, and, if the term be admissible, "dry" conditions.

The results conclusively show that *B. coli*, originally present in oysters, *may* not lose its vitality for more than a week under the conditions of experiment. The assumption that *B. coli rapidly* perishes in oysters when the latter are separated from their natural environment is thus not borne out by the results of my analysis.

APPENDIX L.

The results of the bacteriological examination of oysters bought either in the market or at well-known fish shops or restaurants.

Anglo-Dutch, Whitstable, Portuguese, Blue Point, and Burnham

oysters were examined. They were bought at Sweeting's, Spiers and Pond's, Driver's, Scott's, and the Farringdon Market.

All the oysters contained coli-like microbes, and in the majority of instances, either in the last dilution yielding coli-like microbes, or in the last dilution-but-one microbes, were isolated, indistinguishable with regard to the tests employed from the *B. coli* of the human intestinal tract. Further, in the great majority of instances the *B. enteritidis sporogenes* test yielded positive results.

The results obtained would seem to indicate the danger of hastily condemning (on bacteriological grounds) oysters placed on the market for sale, for example:

(1) An oyster might have been derived from a relatively pure locality, but at the period when it was submitted to examination the few coli-like microbes originally present might conceivably for some reason or other have multiplied in the oyster.

(2) On the other hand, an oyster might have been derived from a polluted laying, but at the period when it was submitted to examination, the coli-like microbes originally present might conceivably for some reason or other have declined in number in the oyster.

(3) Previous to examination the oysters may have been kept under sanitary or insanitary conditions, placed in clean or dirty water, mixed or unmixed with meal or other substance.

Acknowledgement must be made of the large amount of work carried out by previous workers on this subject. Among the bacteriologists in this country who have devoted much time and attention to the subject the names of Boyce, Buchanan, Foulerton, Herdman, Hewlett, Klein, Lorrain Smith, McWeeney, Scholberg and Thresh must be mentioned. Recently Dr Eyre has published a preliminary note on the "Distribution of *B. coli*" which has an important bearing on the subject. Reference must also be made to a most instructive paper by Messrs Clark and Gage, the American bacteriologists[1]. These two observers come to the conclusion that "the ability to demonstrate clearly the presence of a specific sewage organism, such as *B. coli*, is an invaluable aid in determining the question of purity or pollution." They, however, qualify this statement in the next sentence, as follows: "In many samples from polluted sources *B. coli* has not been found in

[1] "On the value of tests for bacteria of specific types as an index of pollution," by Messrs Clark and Gage. From the Thirty-fourth Annual Report of the State Board of Health for Massachusetts for 1902.

either shell water or intestine." Their explanation is that "among the many bacteria normally present or finding lodgement in the intestine or in the shell water, the stronger and more numerous species may evidently destroy the *B. coli* before laboratory examination is possible." I do not quite agree with this explanation and believe that failure to isolate *B. coli* in these cases arose not from the real absence of this microbe but to the presence of other bacteria in such abundance as to render the isolation of *B. coli* a matter of considerable difficulty.

The authors also examined (*a*) the intestines of shell-fish, (*b*) shell-fish liquor and (*c*) sea-water, the samples of shell-fish and sea-water being collected at varying distances from a sewer outfall. In all cases (*a, b, c*) *B. coli* was found in a larger percentage of samples collected $\frac{1}{2}$ to $\frac{3}{4}$ mile from the sewer outfall than in similar samples (*a, b, c*) collected 0 to $\frac{1}{8}$ mile from the source of contamination. The explanation again suggested is that *B. coli* is destroyed in polluted samples of water and shell-fish between the time of collection and of laboratory examination.

Whatever the true interpretation of these facts may be, it will not be disputed that the more *recent* and the more *gross* the pollution the greater is the element of potential danger to health likely to be. Hence if the bacteriologist is specially apt to encounter negative results in cases of gross and recent pollution the practical utility of his tests is open to question.

The results obtained by these observers were achieved by qualitative, not by combined quantitative and qualitative methods, and there is seemingly no record in their paper of the amounts of material submitted to cultural tests. If their investigations had been carried out on a combined quantitative and qualitative basis I believe their results would have shown that, on the average, the number of *B. coli* and of coli-like microbes in shell-fish and estuarial waters runs broadly parallel with the degree of pollution.

The section devoted to shell-fish forms but a relatively speaking unimportant portion (pages 18 to 20) of the entire report (36 pages) by Messrs Clark and Gage. Speaking of the report as a whole it may be said that the investigations cover much new ground, afford a fund of useful information and suggest many new lines of inquiry of an important kind.