ROUTINE METHODS OF SHELLFISH EXAMINATION WITH REFERENCE TO SEWAGE POLLUTION.

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It is unnecessary to introduce the observations which I propose to make by any reference to the gravity of the problem of the sewage contamination of edible shellfish, in its relation to the public health: this side of the question is already familiar to readers of the *Journal of Hygiene*. But the fact that some forms of disease are conveyed—perhaps perpetuated to some extent—by the consumption of such articles of food is one which is now of the greatest possible importance to those engaged in the administration of the sea fisheries; and this aspect of the question is in some danger of being ignored. About half a dozen years ago the Royal Commission on Sewage Disposal invited evidence from representatives of the Fishery Authorities, and it was then foreseen that the question was likely to become one of very great practical importance; and some of the Fishery Committees began to accumulate information with regard to the pollution of the layings under their control.

The Lancashire and Western Sea Fisheries Committee which has jurisdiction over the largest shellfish producing areas in the British Isles began to examine into the condition of the shellfish beds about 1904, and since then a great amount of information has been obtained. Every natural bed and laying has been surveyed and charted; every sewer outfall has been examined with respect to its influence on adjacent layings; while systematic bacteriological analyses have been carried on ever since. It was soon seen that the condition of some of the shellfish beds constituted a most serious menace to the public health; but it was also discovered that the Fishery Authorities had no power to prevent the marketing of even such dangerously polluted molluscs. Repeated representations were made to successive Ministers for legislation designed to confer this power on some authority; but so far these representations have been unsuccessful. During the last year particularly the danger foreseen in 1904 has been realised and the industry is now suffering from the effects of periodic "scares."

This in itself is sufficient reason for legislation. It is also unfortunate that the Fishery Committees are placed in the difficult position of being expected by the Health Authorities to take steps to prevent the export of polluted shellfish. There is no coordination between the two sets of Committees, and the Public Health Officers are not usually aware of the legal incapacity of the Fishery Authorities to stop the evil[1]. It has happened, in the proceedings of Public Authorities, that the blame of the distribution of disease by means of sewage-contaminated mussels has practically been laid on the Fishery Committees: the fact is, of course, that the latter are absolutely powerless to deal with the matter.

In England and Wales the control of the shellfisheries lies entirely with the Fishery Committees, except where exclusive rights of fishing belong to some person or corporation. With respect to public fisheries the Committees have power to prohibit entirely, or restrict in any manner desirable, the methods, or seasons of taking mussels, cockles, or other shellfish. Thus there are "close seasons"; illegal methods and instruments of fishing; and size limits below which shellfish may not be taken. The Committees have power to prohibit the discharge into the sea of any substance "detrimental to sea-fish or sea-fishing"; and they

[1] There is the less reason for this since a particularly clear statement of the law with regard to the pollution of tidal waters is contained in the 4th Report of the Royal Commission on Sewage Disposal (1904); while the Proceedings of the Fishery Committees are accessible to public officers who wish to consult them.

have power to spend public money in stocking or restocking shellfisheries; in transplanting these animals; in artificial cultivation; and in making scientific experiments for these purposes.

Apparently these powers might be used so as practically to prevent shellfish which are undesirably polluted from reaching the public markets: in practice they are entirely useless. Thus a Fishery Committee may close against fishing any part of the sea, or foreshore, within territorial limits, from which shellfish are being taken, *but this closure must be in the interest of the fishing industry.* A byelaw prescribing a regulation can only be suggested by the local fishery authority: it is enacted by the central authority, in this case the Board of Agriculture and Fisheries. Before confirming the byelaw the Board enquire into the reasons alleged for the institution of the regulation. A Fishery Committee may obtain a byelaw in the interest of the industry, but not in that of the public health. In 1904 the Lancashire Committee sought power to enforce a regulation prohibiting the taking of mussels from a certain part of the shore within the area under their jurisdiction. The mussel bed in question had long possessed an evil reputation. Several large sewer outfalls opened almost directly on to it, so that the shore on which the mussels were growing was grossly polluted by faecal matter. There was direct epidemiological evidence of the transmission of disease by means of these shellfish; and the results of bacteriological analyses were most unequivocal. The local Medical Officer of Health, recognising the dangers of the laying, obtained permission from his Committee to exhibit notices enjoining the fishermen not to take the mussels, but this prohibition could not, of course, be enforced. Finally the Fishery Committee drafted a byelaw closing the fishery, and submitted this to the Board of Agriculture and Fisheries for approval. The Board refused to confirm the byelaw, pointing out that the powers possessed by a local Fisheries Committee "did not extend to the making of byelaws for the closure of a mussel bed or other fishery for shellfish for the purpose of protection of public health." The mussel bed therefore still remains as an occasional focus of infection, for the Fishery Committee, which has power to close it, cannot do so in the interest of the public health; while the local Health Committee which can act for the protection of the public health cannot close a mussel bed.

There are two important limitations of the powers nominally enjoyed by the Fishery Committees with reference to the discharge of objectionable substances into the sea. If the discharge is sewage, and if it is

made by a local sanitary authority in virtue of power conferred on it by a local or general Act of Parliament, or by a Provisional Order confirmed by Parliament, the Section of the Sea-Fisheries Regulation Act (2, *e*) of 1888 which would otherwise enable the Committee to prohibit the discharge is nullified. Now it is apparently the case that the Public Health Acts have been regarded as conferring such powers upon the local health authorities. The latter are therefore enabled to discharge their sewage at any convenient spot into the sea, with, or without, regard to the situation of any local shellfish beds. It is true that the Local Government Board now takes steps to consult the Fishery Committees when such new sewer outfalls are being planned; and proper attention is paid to the question of the possible fouling of shellfish beds before the Board sanction the proposed works. But it is still the case that the majority of sewer outfalls have been planned in the past without sufficient regard for the shellfisheries. The second limitation is an even more serious one. The discharge must be "detrimental to sea-fish"—the latter term includes shellfish. Therefore in attempting to restrain a sanitary authority from discharging sewage in the neighbourhood of a shellfish bed the Fishery Committee would have to prove that sewage is detrimental to the molluscs. Now this is impossible, for the greater the amount—up to a certain high limit—of sewage reaching mussels, the better do the latter grow. We find therefore that mussels are always situated in such places where they receive drainage from the land. It is therefore impossible to prohibit the discharge of sewage near a shellfish bed on the score that injury results to the animals. The position of affairs was put very concisely by Lord Onslow—then President of the Board of Agriculture and Fisheries—at a meeting of representatives of Sea-Fisheries Authorities held in London in 1904: "If," he said, "anything is done to threaten the valuable life of the mussel I can step in and take the necessary steps to protect it, but if the mussel, in the enjoyment of crude sewage, should threaten the life of a human being I am absolutely powerless to interfere in the matter."

Neither do the Rivers Pollution Prevention Acts afford a remedy, for tidal waters—in which shellfish beds are situated—are excluded from the operation of the Acts. It is true that the Local Government Board may make an Order declaring a tidal water to be a "stream" within the meaning of the Acts, but this power has been reluctantly used.

Finally the powers possessed by the Fishery Committees under the Shellfish Act of 1895 vanish whenever it is attempted to utilise them so as to provide against the evil of polluted mussels or other shell-

fish reaching the public markets. Under this Act the Committees may spend public money in transplanting shellfish from places where they grow badly to places where they would grow well; or they may store shellfish so as to provide spawning reserves; or they may replenish an exhausted fishery. But they may not remove mussels from a polluted to an unpolluted area; nor may they provide storage ponds for the reception of the shellfish while undergoing a period of quarantine. To do these things would require the expenditure of public money "for the purpose of the protection of public health." And the fear of a surcharge by the Local Government Board Auditor weighs heavily upon the Local Fishery Committees.

Thus the position of the Committees is one of detachment with regard to the general question of the dissemination of disease by means of polluted molluscs. It is true that a good deal of local investigation has been carried out with the object of ascertaining the bearings of the question on the industry; but in regard to active measures for the safeguarding of the public the Committees, who might be expected to know most about the whole thing, are in a position of absolute legal impotence —an *impasse* produced by the lack of coordination between the two series of authorities. It appears to me that with the great exceptions of the work of Dr A. C. Houston (1904, *a*) and of Dr H. T. Bulstrode (1895), both of whom have investigated the question with regard to the sea-fisheries, the public health authorities have generally treated the question rather apart from the natural conditions under which shellfish are produced, and the practicability of taking steps to deal with polluted shellfish without necessarily interfering unduly with the fishing industry. So far as my own experience goes the Public Health Committees have not sought assistance from the fishery authorities, and have not paid attention—to the extent that is desirable—to the important interests involved: those of the livelihood of the shell-fishermen.

From this point of view therefore—that the general question is one that affects the fishery, just as much as it does the public health—it would seem useful to give some account of the experience gained in the investigation of the conditions as they obtain on the west coast of England and Wales.

Methods of Sampling.

A shellfish bed is usually a fairly considerable area. In the case of one west coast mussel fishery the total productive area is about 550 acres. Not all of this sea-bottom is fished at one time, for circumstances

usually dictate that the fishing is carried on at some one part of the whole district, and then after a period is shifted to some other part. In the district to which I refer a sub-area of about 25 acres is often the scene of a busy fishery. If the conditions with regard to pollution were uniform over all these 25 acres one sample taken from any spot would afford a reliable indication of the degree of pollution.

But the conditions are seldom so uniform. A mussel bed is usually a raised part of the sea-bottom—a "scar"; or it is the bottom and slopes of an estuary or channel; or perhaps the sides of a wall, or embankment. In most cases the shellfish are covered by water for a variable fraction of the whole twenty-four hours and are then laid bare by the tide. In most cases the shellfish at different parts of the same general area are exposed to varying degrees of pollution. An ordinary case is that of a channel of no great width, into which there discharge one or more sewer outfalls. Perhaps the engineer who designed the sewerage system provided for an intermittent discharge so that the effluent might be carried away by the ebb-tide; but it is usually safe to assume that the discharge is crude sewage; that it is continuous; and that the purification plant, if there is one, is not worked as efficiently as was contemplated in the designing. On the bottom and slopes of such a channel, and on one or more banks in the middle, are mussels. The tidal rise and fall is often considerable—it may be put at 15 to 25 feet, and it varies from day to day. At the time of high water of flood-tide the channel is filled with water which has come in from the open sea, and this is relatively, or perhaps practically, unpolluted.

All the time, during both flowing and ebbing tides, the sewers are discharging, unless the head of water due to the rise of the tide should bank back the effluent in the outfall pipes. But at the time of low tide the volume of water in the channel is minimal, and therefore the proportion of contained sewage is greatest then. Comparative cultures of similar volumes of water at different states of the tide give usually very different results. As a rule 1 c.c. of water from the channel at the time of high tide should be sterile to media demonstrating the existence of intestinal microbes only; while a similar volume of water taken when the tide is at its lowest will contain a significant number of such organisms: about 5 to 50 per c.c. is a likely range. In May 1908 I made such comparative cultures of samples of water taken from the Barrow Channel opposite to the Fisheries Laboratory. Two c.c. of water were taken every two hours and were plated in about twenty c.c. of neutral-red, bile salt, lactose agar. The results were as follows:

Nos. of intestinal bacteria in 2 c.c. of water from Barrow Channel.

Flood-tide water		Ebb-tide water	
5 hours before high water	0	6¼ hours before low water	0
3 ,, ,, ,,	1	4¼ ,, ,, ,,	0
1 ,, ,, ,,	0	2¼ ,, ,, ,,	200
		¼ ,, ,, ,,	+1000*

* Counting was impossible because of the fusion of the very numerous colonies.

Thus the water at the time when the tide is lowest contains very many more intestinal organisms than when the tide is highest. The numbers of organisms contained in the unit volume—say one c.c.—varies with the proximity to the sewer outfalls. In 1906 I made a series of cultures of the water in the estuary of the river Conway. Samples of the flood-tide water were sterile (with regard to the particular medium mentioned above) but the numbers of organisms isolated from one c.c. were greatest at the upper extremity of the estuary, and least at the opening into the sea. The numbers varied from 0 to 77.

Analyses of water from the estuary of the river Conway.

(1 *c.c. of water inoculated in neutral-red, bile salt, lactose agar, and incubated for* 20 *hours at* 41·5° *C.*)

26 Oct. 1906. Low water.

Source of water	Nos. of intestinal bacteria per c.c.*
1. Pools near high water mark on beach at mouth of estuary	0
2. Mid-channel, near mouth of estuary	5
3. Mid-channel, higher up estuary	27
4. Mid-channel, opposite Conway	70

* Averages of counts from two plates.

3 Dec. 1908. Low water.

Source of water	Nos. of intestinal bacteria per c.c.*	Physical condition of the water	
		Chlorine %	Salinity %
1. Mid-channel, in estuary just below Conway	38	5·81	10·52
2. Mid-channel, opposite Conway	36	1·88	3·42
3. Mid-channel, above Conway	20	0·53	0·95

* Averages of counts from 4 plates.

Sometimes quite irregular results are obtained when an attempt is made to demonstrate the increase in the bacterial contents of the water of such an estuary with approach to the origin of the pollution, and

these are to be traced to unusual conditions of wind and tide causing eddies and surface drifts of water[1].

Consider a part of a mussel scar which is exposed to the atmosphere for about two hours near low water of spring tides. As the tide ebbs it comes to contain an increasing proportion of sewage, and if the velocity of the stream is not too great the latter floats at the surface by reason of its lower density. Therefore just about the time when our hypothetical mussel bed is being laid bare by the tide it is being bathed in water in which the pollution is maximal; and in extreme cases the liquid flowing over it may be practically undiluted sewage. Now contrast the conditions with regard to risk of pollution obtaining on such a bed, with those encountered by mussels which are situated on a scar, or on the sides of a channel, so that they are only covered by the tide during two hours before and two hours after high water of neap tides. While the shellfish are being bathed with sea-water the pollution of the latter is minimal, and by the time that the ebbing tide has come to contain a significant proportion of sewage the mussels are laid bare and are no longer in contact with the polluted liquid.

Two such mussel beds may be so close together as to be known by the same name. Yet one might be highly polluted while the other might be so slightly contaminated as to be practically clean.

If there are mussels at the bottom of a relatively deep channel—say ten to twenty feet deep at low water of spring tides—and if sewers discharge into this channel between high and low water marks, it may nevertheless be the case that the shellfish are not so highly polluted as might be supposed. The sewage floats at the surface of the sea and may not come into contact with the shellfish until it is greatly diluted. But during high and strong spring tides the velocity of the stream may be sufficient to produce a mixture of the water which may bring about a greater degree of pollution. Also certain conditions of wind prevailing during the time of spring tides may cause the level of low water to be several feet lower than is normal, and this too may be a cause of increased pollution.

It is clear that much may depend on the precise conditions under which the sample is taken, and upon the precise spot. If this is so then

[1] It is necessary to bear in mind that such conditions may produce quite unexpected results. Thus a normally clean foreshore may become very foul for a short time as the result of drifts caused by unusual winds; and the same causes are likely to affect the drift of surface floats when these are employed to ascertain the direction that may be taken by a sewage effluent.

great caution is necessary in applying the results of analysis of a sample of shellfish purchased from a market stall or shop, *to the general locality from which the molluscs are said to have been taken.* Not only so but the Report must, in justice to the fisherman, consider the length of time which has elapsed since the shellfish were taken from the sea, and the conditions under which they have been stored. If water is taken from a well, or shellfish from the shore, for the purpose of analysis, care is usually taken to pack the samples in sterile vessels, and if inoculations cannot immediately be made the samples are usually stored in a refrigerator. What then is to be said of the interpretation of the results of the analysis of shellfish which may have been taken from the sea some six days before the date of the sampling, and which may have been stored in insanitary conditions in the meantime? The discovery of pathogenic organisms in such shellfish might indeed be conclusive proof of the origin of a disease or epidemic, but the tracing of the latter to the part of the sea from which the shellfish were alleged to have come might be erroneous. It is surely unfair to condemn a locality on the results of such an analysis made perhaps on moribund animals in which partial decomposition may already have begun.

The fullest possible information relating to the circumstances of collection of the sample, and the conditions under which the consignment of shellfish were stored *after removal from the fishery,* is absolutely essential. In one case which came within my own experience mussels were gathered from scars which, though not free from pollution, were still relatively free from significant contamination. But these scars were some distance from the nearest railway station, and the fishermen were obliged to bring them in their boats to a point on the beach near to the station, and to wash, sort, and pack them there. Occasionally the men were unable to send away the fish on the day of collection, and in these circumstances the bags containing them were stored overnight on the beach, where they were just covered with the tide. Unfortunately a sewer discharged a few yards away from the place where the shellfish were thus stored, and so the mussels, originally fairly clean, became effectually contaminated. It is not surprising therefore that a Medical Officer of Health, reporting on a sample of such mussels, said that they contained *Bacillus coli,* and that the bags, when opened, emitted an odour of sewage. But the interpretation which might have been put upon the results of this analysis—that the mussels generally which came from this locality were significantly polluted—would not have been justified.

It is therefore necessary to examine a number of samples from the same general locality in order to guard against the undue influence of special or accidental local conditions. If, for instance, there is a difference of ten feet in the level of a laying over which shellfish are situated care should certainly be taken to choose the molluscs from every part of the bed, and it is always desirable so to conduct the analysis that every one of the shellfish so sampled should be separately analysed.

Isolation of intestinal bacteria.

In all analyses made by myself the fish were examined on the day after that on which they were collected, and it was generally necessary to store them overnight in a refrigerator. All moribund molluscs (indicated by the gaping of the shells) were rejected. Isolation was always carried out by plating on the surface of the neutral-red, bile salt, lactose agar medium suggested by A. S. Grünbaum and E. H. Hume (1902). The mussels were washed under the tap and were opened so that the adductor muscles of the shell, the pedal muscles, and the muscles of the mantle, were alone cut through. The soft parts of the mollusc were retained in the right-hand valve of the shell. Sterilised knives were prepared, two for each shellfish. About 10 to 15 c.c. of the medium were previously poured into Petri capsules and allowed to set. Pipettes were made by drawing out quill glass tubing of about $\frac{1}{4}$ inch in diameter and were sterilised, and a rubber teat attached to each. With care it is possible to make these little pipettes so that they may deliver approximately the same volume of liquid in each case. A slit was then made in the body of the animal immediately over the stomach, and through the dark-green "digestive gland"—really an extension of the lumen of the stomach—and the sample quantity of the stomach juices was withdrawn and placed on the centre of the plate, and evenly distributed over the surface of the latter by means of a wide platinum loop. The volume of fluid taken amounted to about 0·1 c.c. Usually a mussel contains enough to make two or three separate inoculations. It was found useful to dry the plates after inoculation by exposing them in the incubator with the lids slightly tilted up for about ten minutes. The colonies were counted after 20—24 hours' incubation.

Counts so made are only relative to each other and the absolute numbers of bacteria per shellfish cannot be deduced from them. It is

nevertheless probable that the majority of the contaminating organisms are contained in the cavities of the stomach and digestive gland. The method is inapplicable in the case of the cockle since the small size of the latter mollusc renders dissection very difficult. When cockles were examined, and when it was desired to make an estimate of the total numbers of bacteria per mollusc, either cockles or mussels, the method suggested by Dr A. C. Houston (1904, *e*) for oysters was adopted. Five mussels were opened so that the soft parts of each lay in one valve, and as much as possible of the fluid contents of these was poured into a small sterilised porcelain mortar: the water contained in the shell had also been drained into the mortar. The body of the mussel was then cut up into as fine pieces as possible with scissors and the pulp was poured into the mortar and rubbed up with the pestle so as to obtain as uniform an emulsion as possible. The emulsion was then put into a 250 c.c. CO_2 flask and the latter was filled up to the mark with sterile water. After mixing as thoroughly as possible one c.c. was plated by mixing with 10 c.c. neutral-red agar fluid at 39° C. A sample of cockles consisted of ten animals made up to 100 c.c. One c.c. of the mussel mixture contains 0·02, and one c.c. of the cockle mixture contains 0·1 animal. With such quantities there is seldom any difficulty in counting the colonies.

Three principal categories of colonies grow on such a plate: (1) Large rapidly growing red colonies varying in tint from deep crimson to pale pink. The deep colonies are lenticular in shape and grow in the direction of least resistance. They are usually surrounded by a slight opacity, or haze. (2) Small deep-red colonies. (3) Medium-sized colourless translucent surface colonies, often surrounded by a clear ring due to the discharge of the colour of the medium. Colonies of the first category are those regarded as produced by "intestinal bacteria."

Occasionally the liquid contained in the shell cavity was also examined, but I think that little is to be gained by this procedure. The pallial liquid is only the last portion of sea-water taken into the shell before the latter was closed. It is not likely that multiplication of bacteria takes place on the film of mucus covering the body of the animal, for the latter is ciliated and the mucus is rapidly removed and taken into the mouth. If there is an excess of bacteria in the water of the shell over that in the sea covering the laying this is probably due to the discharge of the excreta of the animal into the shell cavity after the latter has been closed.

The precision of the counts.

The numbers of colonies contained on the surface plates made by the first method may vary greatly, but this is due to the individual variation in the bacterial contents of the stomach fluid in different mussels; to the varying degree of concentration of the fluid; and to the error involved in the construction and use of the pipettes. It would be possible to make the latter strictly uniform in capacity but it is hardly worth while. If the numbers of colonies do not differ greatly it may be assumed that the conditions are nearly uniform over the area from which the sample was taken; but if they differ very much further sampling may be necessary. If, however, in the practice of the second method several plates be made from the same emulsion, using precisely the same volumes of liquid for inoculation, a somewhat large variation in the number of the resulting colonies may be observed; and this is due to errors of experiment. Thus five mussels were made up to 250 c.c. and one c.c. of the emulsion was plated in each of ten capsules. The emulsion had been very carefully made and the flask containing it had been allowed to stand until the heavier solids had settled to the bottom. The counts were: 210, 258, 274, 277, 302, 305, 352, 375, 453 and 730. One of these values, 730, is very great and may be rejected. If however the others be plotted they can be made to fit a normal curve of frequency error with modulus 96·14. The error of mean square is 67·97. The true value is therefore just as likely to be 244 or 380 as the average, which is 312. The probable error of the average itself is $\pm 15{\cdot}58$, and since the precision of an average varies with the square root of the number of observations a fairly large number of plates would have to be made to reduce the error greatly. Quantitative bacteriological analyses are often regarded as comparable in accuracy to the analogous modes of procedure employed by volumetric chemists, but it would appear that such methods are rude and inaccurate when compared with those of the chemists. I do not see how the method indicated in the last few paragraphs is to be greatly improved. The bacteria inhabiting the bodies of shellfish are mostly contained in the cavities of the alimentary canal and digestive gland and the juices of these cavities can only be set free by mechanical disintegration of the body of the animal. Sometimes there is comparatively little fluid in the alimentary canal, and it may be thick and viscid. If the whole of the soft parts of an

oyster or mussel were cut up and the liquid allowed to drain away it is probable that a variable amount would still adhere to the walls of the intestine, and in the tubules of the gland. An uniform emulsion can hardly be prepared; for the harder parts of the body, such as the muscle bundles, break up with great difficulty. These parts must be allowed to settle so as to bring the mixture into a form in which it can be manipulated with pipettes. The error in the analysis must therefore be considered and care should be taken that it is always less than the range of values which it is desired to bring into comparison.

Characters of the organisms isolated.

Organisms which divide rapidly when cultivated on neutral-red, bile salt, lactose agar, forming colonies after twenty to twenty-four hours' incubation at 42° C.; and which are about 1 to 2 mm. in diameter if they grow on the surface, or about ½ mm. in diameter if they grow in the deep, have been regarded as "colon-like" or "intestinal" bacteria. It seems probable that the majority of the organisms growing in this manner on the medium are such as find their normal habitat in the intestinal canals of man and the domesticated animals, but it is, of course, necessary to examine the truth of this postulate. If relative counts be made of the numbers of colonies produced as the result of the cultures of similar fractional parts of the bodies of shellfish, or samples of sea-water, taken from localities known to present varying conditions as regards liability to pollution, it will generally be found that the less likely the chances of pollution, the fewer are the numbers of "colon-like" bacteria isolated by means of the medium in question. I have already referred to the comparative cultures of water from the Barrow Channel, and it seems to me that these prove the truth of the postulate. The water that comes in from the sea on the flood-tide must be regarded as practically free from pollution. It contains some colon bacilli, of course, but these are seldom present in one c.c. The tidal streams surge out and in from this channel so that twice in every twenty-four hours mixing and enormous dilution must take place. On the other hand the channel at the time of low water is comparatively narrow, and it receives the crude sewage of Barrow-in-Furness, Dalton, and some other communities. It must therefore contain an appreciable proportion of sewage. So also with the case of the Conway Estuary.

A comparison of the results of cultures of mussels and other shellfish, taken from regions presenting very different conditions with regard to the possibility of pollution, leads to analogous conclusions. The worst-polluted mussels that have come into my hands were some taken from the shores of the Mersey Estuary near to the Egremont Landing Stage. A sewer discharged directly on the mussel bed, and four others were situated within a distance of about one mile, and in such positions that the first of the flood-tide must have carried the effluent almost on to the shellfish. The bed was very foul and accumulations of faecal matter, water-closet paper, and other debris were scattered over it. There was also direct evidence of the communication of disease by means of these particular shellfish. Ten mussels were examined and the numbers of colonies counted on ten plates, each inoculated with about 0·1 to 0·2 c.c. of the stomach contents, were 250, 250, 300, 600, 900, 1000, 1000, and in three plates counting was impossible because of the fusion together of the exceedingly numerous colonies.

In this case the topographical evidence showed that the pollution was gross, and the results of the bacteriological analysis were strictly concordant. Compare these results with those of an analysis of shellfish brought in from the open sea. Mussels are not found at sea at a considerable distance from the land, but oysters are, and the latter may be used for the purposes of comparison. In 1904 I obtained two samples of the latter molluscs by dredging (A) near the Liverpool North-West Light Vessel, 12 miles from land, and (B) from the Morecambe Bay Light Vessel, about 16 miles from land. The sea at A is not entirely without the range of land pollution since the hoppers carrying dredged material deposit their loads in the neighbourhood; old boots, crockery, and similar refuse may be found when trawling thereabouts. The sea at B may be regarded as quite outside the reach of ordinary contaminating influences. Six oysters were taken from each locality, and a slit being made in the body over the stomach of each, about 0·25 c.c. of the stomach fluid was plated on the surface of the neutral-red agar. The same volume of fluid was also used for inoculations in previously boiled litmus milk, which was then heated to 75° C. for 20 minutes and incubated anaerobically. Four of the plates made were sterile and two gave each one colon-like colony. The organisms from one of these colonies did not, however, ferment lactose. All six oysters gave a typical *enteritidis* reaction. The oysters taken from locality B yielded sterile plates only, although about 0·5 c.c. of stomach liquid was used for the inoculations, and a period of 48 hours

was allowed for the incubation. Four of the six milk tubes were sterile after incubation while in the other two the milk was rendered acid, and a slight atypical clot was produced. This is the only analysis I have made in which all of the samples failed to give a typical *enteritidis* reaction. It has been pointed out that the value of such a negative result is very great and my experience confirms this statement[1].

It seems probable then that the numbers of colonies growing on neutral-red, bile salt, lactose agar give reliable indications (in the majority of cases) of the grade of pollution. The reaction with this medium may therefore be regarded as a simple but satisfactory test for organisms of the *Bacillus coli* category. Nevertheless we are not absolved from the necessity of further examining the reactions of the organisms isolated.

Cultural reactions of organisms of the Bacillus coli group.

No pathologist is likely to have any difficulty in identifying organisms of the above group, but it is not an easy matter for anyone whose daily task is not bacteriological work to satisfy himself as to what small series of reactions he ought to apply, as a matter of routine, in the identification of *Bacillus coli*. This difficulty appears to me to be all the greater since those bacteriologists in this country who have had most experience in the examination of shellfish do not employ precisely the same series of reactions for the identification of *Bacillus coli*. The Table on p. 427 summarises the main tests employed by the bacteriologists who have had most experience of this work.

From these series of tests, and others which have been adopted by other workers, it should be possible to determine with certainty whether a particular organism is the typical *Bacillus coli communis*, or some closely allied form. But the large number of reactions which have to be applied render routine work difficult and tedious.

When I began this work I made use of the Table published by MacConkey (1901) for the identification of the organisms isolated from primary cultures in bile salt agar. The Table included the employment of glycerine, peptone, and litmus broth as a means of distinguishing *Bacillus coli* from some other nearly related bacteria, but it appeared later that there was some inconstancy in the reaction obtained with this medium;

[1] The experiments made by Dr Houston (1905) with regard to the occurrence of *Bacillus coli* in deep-sea oysters will be familiar to most readers. I have obtained similar results.

and it seemed that slight differences in the precise manner of preparation determined in some cases whether or not fermentation occurred. Further, it was necessary to wait for six days before it was certain that the broth would not ferment. Usually the majority of the tubes contained no gas after twenty-four hours incubation, but in about one-half the reaction occurred after four or five days. Although in most instances it is possible to determine whether or not an organism is motile, cases frequently arise where it is very uncertain that motility is not really exhibited. For these reasons both the glycerine fermentation and motility tests were abandoned. With regard to other cultural

Reactions employed	Workers: Houston (1904 *a*)	Klein (1905)	McWeeney (1904)	MacConkey (1901, 1906)
1. Formation of indole in peptone broth	+	+	+	+
2. Fluorescence in neutral-red broth ...	+	+	+	...
3. Acid and gas in lactose broth ...	+	+	+	+
4. Acid and gas in milk	+	+	+	...
5. Acid and gas in bile salt broth ...	+	+	...	+
6. Non-liquefaction of gelatine ...	+	+	+	+
7. Gas bubbles in gelatine "shake" cultures	...	+	...	...
8. Non-retention of Gram's stain ...	...	...	...	+
9. Growth in phenolated media ...	...	+	...	...
10. Acid and gas in glucose broth ...	...	...	...	+
11. Acid and gas in mannite broth ...	...	...	...	+
12. Acid and gas in dulcite broth ...	...	...	...	+
13. No reaction in inulin broth ...	...	...	...	+
14. No reaction in adonite broth ...	...	...	...	+
15. Voges' and Proskauer's reaction negative	...	...	...	+
16. Ratio of H/CO_2 in glucose fermentations = 2/1	...	...	...	+
17. Motility exhibited	...	...	...	+

The + signs indicate that the reaction in Col. 1 is employed.

reactions there is some doubt as to their applicability in the precise diagnosis of *Bacillus coli*. The formation of gas bubbles in gelatine appears to depend on the exact nature of the gelatine employed—perhaps on the purity of the other constituents of the medium. In one case about twenty tubes of this medium all failed to react, nevertheless the same organisms bubbled gelatine which had been kept for about ten months, and which had been prepared in the same laboratory and apparently from the same formula. The difference was possibly due to the greater concentration of the medium, for the jelly in each tube had shrunk up to the extent of about one fifth of the original

volume; but it was more probably due to some slight difference in the constitution of the medium. The formation of indole; the growth in phenolated media; the production of fluorescence in neutral-red broth; and the clotting and reddening of litmus milk, all appear to be general reactions which may be exhibited by organisms allied to the typical *Bacillus coli*. It would appear then that we are compelled to resort to fermentation reactions in pure sugars if we wish to identify the organisms

TABLE I.

Reactions of 153 *organisms isolated from Cultures on Neutral-Red, Bile Salt, Lactose Agar.*

	Bile salt broth	Glucose broth	Lactose broth	Mannite broth	Cane sugar broth	Litmus milk	Total	%
Coli-like organisms	ag	ag	ag	ag	0	ac	85	55
	ag	ag	ag	ag	ag	ac	36	23
Not fermenting mannite	ag	ag	ag	a	0	ac	1	
Not fermenting glucose	ag	0	ag	ag	0	ac	1	
Fermenting lactose but not milk	ag	ag	ag	ag	ag	a	1	2·5
	ag	ag	ag	ag	0	a	1	
	ag	ag	ag	ag	0	0	1	
	ag	ag	ag	ag	ag	0	1	
Not fermenting lactose	ag	ag	0	ag	ag	a	2	
	ag	ag	0	ag	0	0	2	
	ag	ag	a	ag	a	a	1	
	ag	ag	0	a	a	a	1	
	ag	a	0	a	a	a	1	
	ag	a	a	a	a	ac	1	
	ag	ag	0	ag	0	ac	1	
	ag	ag	a	ag	0	ac	1	
Other aberrant organisms	a	a	a	a	a	ac	1	10
	a	a	a	a	a	a	1	
	a	a	a	a	a	0	1	
	a	a	a	a	0	ac	1	
	a	a	a	a	0	a	1	
	a	a	a	0	0	ac	1	
	a	a	a	0	a	a	2	
	a	a	0	a	a	a	1	
	a	a	0	0	a	a	1	
	a	a	0	0	0	ac	2	
	a	a	0	0	0	0	2	
	0	a	a	0	a	ac	1	
	0	0	0	0	0	ac	1	

ag = acid and gas, ac = acid and clot, a = acid, 0 = no reaction.

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studied. In this connection it may be of interest to give here the reactions of 225 bacteria isolated from cultures of shellfish in neutral-red, bile salt, lactose agar. These are all of which I have kept the records. After plating out, several colonies were selected from each plate, and these were inoculated on the surface of nutrient agar contained in slant tubes. The pure subcultures were incubated for 24 hours and then the tertiary subcultures were made. The latter were incubated for 48 hours, but dulcite cultures were usually kept for a week, and sometimes were kept at room temperature after incubation at 39° C. Cultures tested for the Voges and Proskauer reaction were kept at room temperature for a week.

Most of the reactions included in MacConkey's Table have been included in the methods of differentiation of these organisms. Apparently we may regard the fermentation of cane-sugar as non-essential in the identification of *Bacillus coli*. We find therefore that about 75% of the organisms cultivated produce acid and gas in (1) bile salt broth, (2) glucose broth, (3) lactose broth, (4) mannite broth, and (5) clot and acidify litmus milk. About 5% did not ferment lactose and were certainly not *B. coli*. A few gave the equivocal result of fermenting lactose but not milk, and *vice versa*. The majority, however, prove to be forms nearly allied to the typical colon bacillus.

In later analyses I employed the other fermentation tests recommended by MacConkey (1906) but with less satisfactory results. Table II gives the results of 72 series of reactions, most of which are those suggested in MacConkey's paper. The gas-ratio and the motility were not systematically observed.

Approximately the same percentage of the organisms subcultured conform to the principal characters given in MacConkey's first paper (1901). But if we regard the fermentation of dulcite, and the non-fermentation of inulin and adonite, as essential characters of the colon bacillus, only about 18% of the organisms isolated and identified provisionally as such can be so diagnosed. This may be possibly the real proportion, but the results are in other respects less satisfactory, and productive of some confusion.

Among these 72 organisms there are no less than 17 distinct categories—assuming for the moment that every combination of all or some of the reactions possible is indicative of a distinct species of organism. With the exception of those in the two top lines, and those fermenting dulcite, but not inulin nor adonite, there is considerable difficulty in classifying the forms according to the cultural reactions

displayed. Nevertheless the distribution of the reaction-frequencies appears to be determined by some manner of grouping of the organisms according to their biological characters, for there are two main categories—1, 2 and 9, 10—and the frequencies of these and the other groups do not conform to the normal law of error.

TABLE II.

Reactions of 72 organisms isolated from Cultures on Neutral-Red, Bile Salt, Lactose Agar.

	Bile salt broth	Glucose broth	Lactose broth	Mannite broth	Cane sugar broth	Dulcite broth	Inulin broth	Adonite broth	Voges & Proskauer		Total	%
									Milk	reaction		
1	ag	ag	ag	ag	0	0	0	0	ac	0	17	28·6
2	ag	ag	ag	ag	0	0	a	0	ac	0	11	15·3
3	ag	ag	ag	ag	0	0	0	a	ac	0	1	
4	ag	ag	ag	ag	0	0	a	a	ac	0	1	
5	ag	ag	ag	ag	ag	0	0	0	ac	0	1	
6	ag	ag	ag	ag	ag	0	a	ag	ac	0	3	4
7	ag	ag	ag	ag	0	0	0	ag	ac	0	3	4
8	ag	ag	ag	ag	0	0	a	ag	ac	0	3	4
9	ag	ag	ag	ag	0	ag	a	0	ac	0	7	9·7
10	ag	ag	ag	ag	ag	ag	0	0	ac	0	6	8·3
11	ag	ag	ag	ag	ag	ag	a	ag	ac	0	1	
12	ag	ag	ag	ag	0	ag	a	ag	ac	0	2	
13	ag	ag	ag	ag	ag	ag	ag	0	ac	0	1	
14	ag	ag	ag	ag	ag	ag	a	0	ac	0	1	
15	ag	ag	ag	ag	ag	a	a	0	ac	0	1	
16	ag	ag	ag	ag	a	a	a	0	ac	0	1	
17	ag	ag	ag	a	a	a	a	0	ac	0	1	
18	ag	ag	ag	ag	0	a	a	a	ac	0	1	
19	ag	ag	ag	ag	0	a	a	ag	ac	0	1	
20	ag	ag	a	ag	ag	ag	a	0	0	+	1	
21	ag	ag	a	ag	a	0	0	ag	a	+	1	
22	ag	ag	a	ag	ag	a	a	0	0	0	1	
23	a	a	ag	ag	ag	0	a	a	ac	0	2	
24	a	a	ag	ag	0	0	0	0	ac	0	1	
25	a	a	a	a	a	0	0	ag	ac	0	1	
26	a	a	a	a	a	0	a	a	0	0	1	
27	a	a	a	a	ag	0	a	0	ac	0	1	

Obviously such results as these place us on the horns of a dilemma. It is absolutely necessary, in justice to the interests involved, that the approval or condemnation of a shellfish laying, or a large consignment of fish, should be based on the results of examination of a fairly large number of individual specimens—and probably also of a number of

separate samples, taken *in situ*: that is, if the bacteriological results are held to be sufficient evidence of the grade of pollution. A certain number of bacteria resembling the colon bacillus are estimated as being present in each shellfish, and a fraction of these must be subcultured in order that this provisional identification may be confirmed. In the analyses made by myself ten mussels, or other shellfish, usually formed a sample. Each mollusc was examined individually so as to get an idea of the range of variability, and often a strictly quantitative estimation of the numbers of bacteria of the intestinal group present in the whole body of a shellfish was made by method two (see p. 422). Then a certain number—usually ten—of the colonies provisionally identified as *B. coli* were subcultured and examined in detail. Often it was quite essential to take several samples so as to study the influence of season, winds, tides, or other conditions. If, say, five of the ten sample colonies answered to the tests for *B. coli* it was assumed that about half of all those isolated in the primary cultures, and identified as "intestinal bacteria," were really colon bacilli. The labour of such an investigation is considerable and one is almost compelled to adopt the minimum number of tests necessary for the diagnosis of *B. coli*. On the other hand it appears that more tests are necessary than was formerly supposed for this end; and if one hesitates to make use of them he runs the risk of identifying as the colon bacillus organisms which do not possess the significance of this form; and in applying the conclusions deducible from this diagnosis with consequences detrimental to the shellfish industry.

Variability of reaction.

It might be expected that the multiplication of the reactions employed in the identification of organisms of the intestinal group would lead to a greater differentiation of species. But the trouble is that the number of apparently distinct forms becomes large—so large as to appear to make it *a priori* improbable that they can all be separate species. The difficulty is analogous to that which has occasionally arisen in purely zoological investigation as the result of the work of systematists endowed with more than the average analytical powers. The "splitters" have burdened the literature with a host of names which have come to possess only historical interest; and have called forth the "lumpers" whose tendency has been to confuse together well-defined species. In later days science has been rescued

from both by the mathematical study of variation. Now it appears to an outsider who has to study bacteriological literature that there has been a tendency towards the creation of ill-defined species of bacteria by the pathologists, and that a number of those described have really no separate identity, in the meaning of the term "specific identity" as employed by the systematic zoologists and botanists. On the other hand there appears to be a tendency towards the confusion of probably separate organisms on the part of bacteriologists who have to employ easy routine methods of identification of organisms of economic significance.

If one were to isolate faecal *Bacillus coli*, taking great care to secure a number of colonies resulting from the division of one original organism, and then proceed to cultivate these separately, using identical series of tests for each, would one obtain precisely the same series of results for each of the colonies studied? I think it is very doubtful. In that case one would prove the existence of metabolic variability in the species studied—a result which is indeed more than probable. If a great number of organisms isolated from different strains of *Bacillus coli* were so studied[1] we should be able to form "frequency curves" expressing the probability of any particular series of reactions being associated with an organism of the type of *Bacillus coli*; and we should be able to say what particular deviation from this general series of reactions should be regarded as removing the organism from this category of bacteria. It appears not improbable that such is the only method by which we should be able to devise a series of tests which might be applied with reasonable probability to the identification of the bacteria obtained from polluted sea-water or shellfish.

It seems to be clearly proved that organisms of the typhoid-coli type do not normally inhabit sea-water, or the tissues of marine shellfish—a conclusion which emerges from the experimental work of Klein (1905), Herdman and Boyce (1899), and others. Placed in clean sea-water both *B. typhosus* and *coli* cease to reproduce and soon disappear. If this is the case with mammalian intestinal organisms in general when they enter the sea it is probable that "loss of attribute"—that is, changes in metabolic activity leading to the failure to produce one or other fermentation reactions—should precede this ultimate dissolution of the bacteria. As the result of such changes a certain proportion of the organisms sampled—a proportion variable with the precise conditions—will fail to respond to one or more of the tests applied. I have

[1] This has already been done, to some extent, by Dr Houston (1904, 1906).

noticed—though I have no extensive series of results to quote—that there is sometimes a general similarity in reaction between the bacteria isolated from one particular sample, when compared with the results of other samples taken under different conditions. One may explain this on the hypothesis that the differences in reaction were due to a longer or shorter sojourn in the sea; and consequent loss of fermentation powers. It is universally recognised that recent pollution is far more significant than pollution of remote date. It is very difficult to isolate *Bacillus typhosus* from shellfish, though the employment of such media as that of Drigalski and Conrad (1902), or the neutral-red, bile salt, lactose agar used in the present investigations, renders the separation of the organisms in question by no means difficult[1].

Would a known strain of faecal *Bacillus coli* multiply in a sterile medium resembling as much as possible the juices of the alimentary canal of the mussel or oyster? Probably it might do so for a short time, but it is not certain that the organisms thus produced would give all the reactions exhibited by the original strain of bacillus. The ideal way to carry out the experiment would be to infect shellfish known to be perfectly clean and sterile (so far as sewage organisms are concerned). But it appears that bacteria of intestinal origin rapidly disappear when

[1] I have only succeeded once in isolating what appears to have been *Bacillus typhosus* from shellfish (1907). The laying from which the mussels were obtained was situated immediately round a sewer outfall which continuously discharged crude sewage. The sewer served a seaside resort having a large fluctuating holiday population. The time was June. The reactions of the organism were as follows:

It formed a round, slightly raised, translucent, colourless colony of about two mm. in diameter, on neutral-red, bile salt, lactose agar after twenty-four hours' incubation at 41° C.

It was very motile.

It formed acid and gas in bile salt glucose broth;
acid only in glucose litmus broth;
a slight discoloration in lactose litmus broth;
acid only in mannite broth;
acid only in milk.

And it gave no reaction with cane sugar litmus broth.

It agglutinated—in a dilution of one in thirty—in a serum which gave a positive reaction with a known strain of *Bacillus typhosus*. (This test was made for me by Mr Lewis, of the Pathological Department in the University of Liverpool.)

The local Public Health Officers denied the existence of enteric fever in the locality, a condition which proves nothing, since convalescents or "carriers" may have been resident there and have been unknown.

It is however very probable that *B. typhosus* may have been present in several samples of shellfish examined by me, but the labour of isolating and examining all the colonies likely to have been produced by this organism is too great to admit of its being carried out as a matter of routine practice.

inoculated in shellfish or sea-water. If loss of attribute could be shown to ensue as a consequence of such procedure it is clear that the principle of "revivifying" microbes isolated from shellfish, by repeated subculture in media resembling as closely as possible those in which they have their natural habitat, should be applied. This has been suggested, but the counsel appears to be one of perfection, and probably not applicable as a matter of routine practice.

Standards of permissible impurity.

Responsible bacteriologists who have discussed the institution of such standards have generally hesitated to suggest them, except in a tentative manner. Nevertheless most of those who have had much experience in the analysis of shellfish must—unconsciously perhaps—have set up some sort of standard in relation to their own work; for it is only with reference to some criterion of impurity, or by the statement of some remedial measures, that the results of such investigations can be expressed in a form suitable for administrative purposes.

Topographical and epidemiological evidence are the only guides in the application of bacteriological results towards the erection of a standard of a permissible impurity. If a laying is evidently grossly polluted, and if faecal matter and sewage debris are found among the shellfish, no analysis is necessary; but a knowledge of the bacterial contents of the shellfish gives information which can be applied to the interpretation of bacteriological results in cases where the topographical conditions are unknown; as when, for instance, the shellfish have been taken from a shop, and the place of origin cannot be traced; and generally in cases where the report of the analysis is the only evidence obtainable. Epidemiological facts furnish a standard, for if a certain number of cases of illness can be traced year by year to a definite locality, and if the approximate numbers of *Bacillus coli* present in the shellfish taken from this locality are known, it is evident that such knowledge may be applied (with all due caution of course) to other localities where the distribution of disease by the shellfish has not been studied. It would give at all events *a priori* reasons for suspicion.

Bacteriological results if they are to be applied to the approval or rejection of shellfish must obviously be quantitative ones, for shellfish like the mussel nearly always contain bacteria which are of intestinal origin. My own experience has been that no sample of ten or more mussels can be examined without finding *Bacillus coli*, or at least some

organisms resembling this form. This statement applies, of course, to the layings of the west coast of England. Mussels are almost always found in creeks, or estuaries, where fresh water flows down from the land, and fishermen say that it is the "fresh" which is favourable to the growth of the molluscs. Certainly remarkable results have been obtained, in the way of the cultivation of these shellfish, by relaying them in such situations where they may obtain a plentiful supply of water of moderate salinity. It is, however, the substances contained in the water draining down from the land that are the factors producing the more rapid growth of the shellfish. The contained soluble carbon and nitrogen compounds may, in themselves, provide a source of food that can be utilised directly by the mussels; or these food stuffs may provide the pabulum for the diatoms or other protista—after resolution into inorganic compounds by fermentation and nitrifying bacteria—and the protista may then serve as a source of food for the molluscs. However this may be it seems to be generally the case that the largest mussels are those which have been grown where there is a certain proportion of sewage matters in the sea-water flowing over them[1]. The sea-water which is in contact with a mussel laying must therefore contain sewage bacteria, even although the laying may not be in immediate proximity to a sewer outfall. It may not always be possible to demonstrate the existence of such bacteria in one c.c. of the water but they will generally be found in larger volumes.

There are generally more of such organisms in the body of a mussel, or other shellfish, than are to be found in the same volume of the surrounding sea-water, as may be proved by making comparative cultures from the stomach contents, and from the water in the pallial cavity. Yet it appears that *Bacillus coli*, or its congeners, do not multiply in sea-water. Probably there is an initial multiplication in the tissues of the shellfish, after which the intestinal organisms begin to undergo loss of attribute, and their growth becomes inhibited. If the shellfish taken from a polluted place were put into perfectly clean sea-water and kept for a sufficient time it is probable that intestinal organisms would disappear entirely from their tissues. But in natural conditions there must be a continual reinfection of the molluscs.

Shellfish taken from layings which may be supposed to be outside the influence of sewer outfalls may contain appreciable numbers of sewage bacteria. I may refer to the case of a mussel bed at Roosebeck, in Morecambe Bay, which I examined and reported upon in 1906 (1907).

[1] A statement which has not, however, universal application.

This laying is situated immediately to the north of the mouth of Barrow Channel, and the direction of the tidal streams, together with the existence of training walls, renders it very improbable that any of the polluted water passing down the Channel from Barrow-in-Furness can come near to the mussels. It is situated about eight miles from Ulverston which appears to be the only community from which sewage might possibly come into contact with the laying. But it is very probable that before the effluent from the outfalls at Ulverston could reach Roosebeck the sewage would be so largely diluted as to render the contamination of little significance. There are one or two small outfalls on the shore about a mile from the laying, but these may be neglected. Considering the topographical evidence one would say that the shellfish were, in all probability, quite clean. Yet I found that the numbers of intestinal bacteria isolated from about 0·2 c.c. of the stomach juices were in the cases of ten mussels 40, 65, 9, 9, 2, 64, 28, 13, 3, and 9—average 24·2.

Cleansing of polluted shellfish.

If polluted mussels be supplied with clean sea-water there is a rapid partial disappearance of the intestinal bacteria contained in their tissues. This is a direct inference from the work of Klein (1905) and others. It appears that oysters cleanse themselves, in such circumstances, more rapidly than mussels, and mussels more rapidly than cockles. In the summer of the present year I made some experiments designed to ascertain the period in which this partial cleansing of polluted mussels might be expected to take place. The shellfish were taken from an undoubtedly polluted area—one with regard to which there was direct epidemiological evidence of the transmission of enteric fever by means of the mussels taken therefrom. The topographical conditions were quite in accord with the meaning of the epidemiological results. These polluted mussels contained on the average 1900 intestinal bacteria per shellfish[1]. They were put into large wooden boxes which were then deposited on the beach in a situation where they were half a mile from the nearest sewer outfall: further, they were placed about half-way up the beach so that they were uncovered when the tide had ebbed to the extent of about one half of its usual period. The water which they received, while not unpolluted, was reasonably clean. The

[1] The plates also contained about 25 colourless colonies each. None of these colonies appeared on the plates made from the relaid shellfish.

experiment was not made with the object of bringing about a complete disappearance of intestinal bacteria, but was intended to suggest some practical means, with reference to the particular locality, of storing the shellfish taken from the polluted beds in the vicinity, for such a time as would enable them to eliminate the greater proportion of the contained sewage bacteria. After four complete days a sample of the mussels was taken and it was found that the number of contained bacteria had been reduced to about 150 per shellfish—a reduction of about 93 per cent. They were left for about three times this period, but it was found that the further reduction of the contained bacteria was slight. For all practical purposes the cleansing had taken place during the first four days during which the shellfish had been relaid. In the course of the experiment a short gale sprung up and one of the boxes containing the mussels went adrift, with the result that it sailed into highly polluted water and the shellfish became reinfected to about their original degree. The box was replaced in the same place and it was again found that the bacteria were eliminated in four days to the same extent as before.

These two series of results indicate the possibility of setting up a standard of bacteriological impurity which may be regarded as of little importance from the point of view of the public health. It is extremely unlikely that the Roosebeck mussels are contaminated to such a degree as need cause any apprehension of disease as the result of their use as human food. No cases of disease have ever been traced to the use of these shellfish. In the case of the cleansing experiments a residue of intestinal bacteria remained after about twelve days' sojourn in reasonably clean sea-water, which amounted to about 150 per mussel. Now Klein (1905, pp. 50—53) showed that mussels containing the enormous number of six millions of *Bacillus typhosus* per shellfish were cleaned to the extent that one mollusc contained about 14,000 bacilli after seven days, simply by a daily change of the sea-water in which the shellfish were contained. The numbers of this bacillus that could possibly be taken up by a mussel or oyster in natural conditions could not be expected ever to reach the number of millions; and if even the rate of cleansing experienced in Klein's experiments were to hold good in the sea, under the conditions of the experiments referred to above, it may be expected that any pathogenic bacteria imbibed by the molluscs would be eliminated. We have seen that even after the short period of four days the reduction was considerable.

But it appears that the institution of a standard depends on the consideration of both topographical and epidemiological evidence; and that the results of bacteriological analyses are to be interpreted in the light of such information. This makes it doubtful whether we are ever quite justified in applying the results of analyses alone in administrative routine. It would be very desirable if a local authority were able to reject or approve a consignment of shellfish on the evidence of a report by a bacteriologist, for a good deal of trouble would thus be avoided. Unfortunately the adoption of such procedure would in many cases result in hardship to the fishermen, while the real source of pollution might not always be traced.

Summary and Conclusions.

(1) At present no public authority possesses legal power to deal with the question of the contamination of shellfish.

(2) It is not sufficient to test shellfish exposed for sale in a market or shop. These may have been contaminated subsequent to removal from the fishery; and multiplication of the contained bacteria may have taken place. The results of such analyses may lead to unjustifiable condemnation of a laying. It is essential that a topographical examination should be made and that samples for analysis should be taken from the laying itself.

(3) In the case of natural shellfish beds there is so much variability in the conditions with regard to the susceptibility to pollution that a fairly large number of the animals must be examined. The labour of the analyses is therefore so great that the development of some simple routine test for faecal contamination is most desirable. Since most natural shellfish layings are situated within the "sewage zone," and therefore contain *B. coli*, quantitative results are essential.

(4) There are considerable differences in practical routine work in regard to the methods of isolation of intestinal organisms from shellfish; and also with respect to the number and nature of the reactions necessary for the identification of *B. coli*. It is desirable that some generally recognised series of tests should be uniformly adopted by bacteriologists engaged in such work. Further, different micro-organisms, possibly of varying degrees of significance as indicators of faecal contamination, may have been confused. There is possibly some variation in cultural characters in *B. coli*, and investigation of

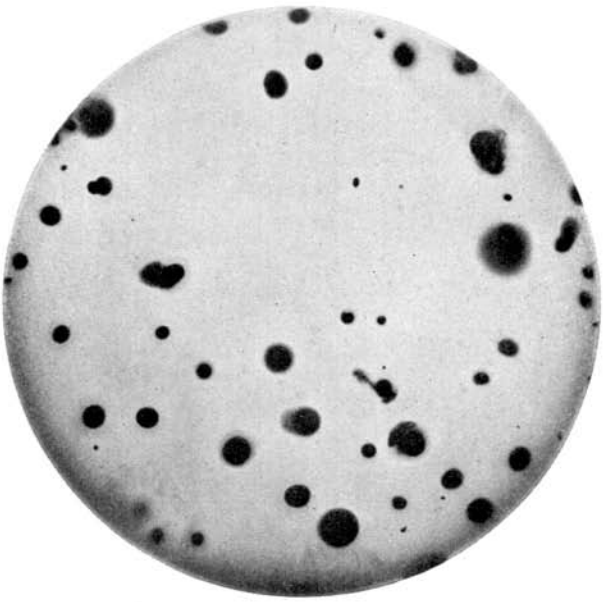

Fig. 1.

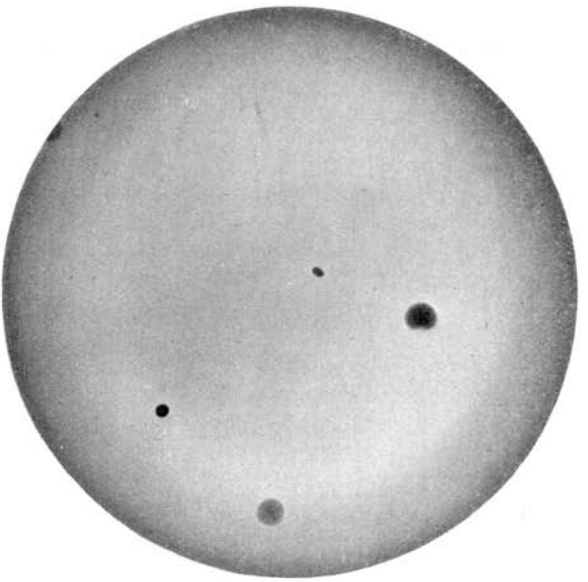

Fig. 2.

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this variability is desirable. Investigation of the changes in cultural reactions undergone by intestinal organisms when entering the sea, or the tissues of marine shellfish, is also very desirable.

(5) Remedial measures other than the simple closure of a contaminated laying might be suggested. It is possible to subject the shellfish to treatment which will cause them to clean themselves of contained sewage bacteria. The source of the pollution may be removed; and sterilisation of the shellfish may be practised.

EXPLANATION OF PLATE II.

Fig. 1. Culture from 1 c.c. of an emulsion of the bodies of 5 mussels made up to 250 c.c. (=0·02 mussel). Neutral-red, bile salt, lactose agar was used for isolation. Incubated for 20 hours at 42° C. and kept for three days at room temperature before being photographed. These mussels were badly polluted.

Fig. 2. A similar culture (0·02 mussel) made precisely as above from five of the same lot of mussels after they had been kept in unpolluted sea-water, in the open, for four days. The plates represent fairly the difference in bacterial contents that may be expected from such treatment.

(The photographs are by my colleague, Mr A. Scott.)

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