TABLE II.—*Samples collected outside the turpentine-producing States*—Continued.

DEALERS' SAMPLES—Continued.

L. and P. No.	Sampled at—	Sample labeled or sold as—	Specific gravity.	Refractive index.	Color.	Initial distilling temperature.	Per cent distilling below 165° C.	Per cent distilling below 170° C.	Polymerization.		Minimum per cent of mineral oil present.
									Per cent residue.	Refractive index of residue.	
					mm.	*° C.*					
12710	Clarksville, Tenn	Pure turpentine	.8660	1.4705					19.4	1.4500	19.2.
12712	Washington, D. C	Turpentine	.8535	1.4645					18.6	1.4505	18.6.
12713		do							1.2	1.5050	None.
12875	Washington, D. C	do							1.0	1.5090	Do.
12940	do	Pure turpentine	.8639	1.4700					.8		Do.
13033		do	.8650	1.4708					.8	1.5150	Do.
13130	Danville, Ky	Spirits of turpentine	.8777	1.4713	160				.4	1.5115	Do.
13131	Cincinnati, Ohio	Pure brand turpentine	.8673	1.4712	80				.8	1.5140	Do.
13167	Washington, D. C	Pure turpentine							.4	1.5150	Do.
13168	do	do	.8641			159	90.7	96.7	.5	1.5146	Do.
13205	Springfield, Mass	Pure spirits of turpentine.	.8613	1.4682	80				8.4	1.4715	8.4.
13529	Douglas, Ariz	Pure turpentine	.8646	1.4698	65				1.2	1.5100	None.
13672	Waterbury, Conn	Pure spirits of turpentine.	.8596	1.4680	120				5.6	1.4600	5.6.
13673	Washington, D. C	Turpentine	.8637	1.4700	200				1.0	1.5120	None.

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U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF CHEMISTRY—BULLETIN No. 136.

H. W. WILEY, Chief of Bureau.

SHELLFISH CONTAMINATION FROM SEWAGE-POLLUTED WATERS AND FROM OTHER SOURCES.

By

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WASHINGTON:

GOVERNMENT PRINTING OFFICE.

1911.

LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Chemistry,
Washington, D. C., August 26, 1910.

Sir: I have the honor to submit for your approval a report prepared by G. W. Stiles, jr., bacteriological chemist of this Bureau, on the contamination of shellfish by sewage-polluted water. The data reported are based on an extensive and painstaking investigation during which many localities have been inspected, the conditions carefully studied, and all the bacteriological data possible obtained. The latter were found to corroborate the observations made during inspection. To no one, not even the consuming public, will this report be of greater interest and benefit than to the industries concerned. That the danger noted exists to some degree in many localities no one can deny, and that the danger is one that will increase unless intelligent and efficient measures are taken to control it is equally obvious. The investigations presented in this manuscript are of direct importance to a proper consideration of such steps as may be indicated under the food and drugs act to protect the public health, and to avoid deception. The report is in no sense intended to discredit the valuable industries concerned, but rather to point the way in which the products of these industries may be accepted with greater confidence by the public. It is believed that this report will assist in the furtherance of this purpose, and I recommend that it be published as Bulletin 136 of the Bureau of Chemistry.

Respectfully,

H. W. Wiley,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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SHELLFISH CONTAMINATION FROM SEWAGE-POLLUTED WATERS AND FROM OTHER SOURCES.

INTRODUCTION.

The contamination of shellfish from sewage-polluted waters presents a sanitary problem of increasing importance to those interested in the production of pure-food supplies. Until comparatively recently there has been but little apprehension in this country regarding the injury to oysters and other shellfish from this source, but food officials and sanitarians are now awakening to the fact that either sewage must not be promiscuously emptied into our natural bodies of water or the shellfish industries must in many cases be removed to points far distant from their present locations. Thorne[67] says:[a] "It is only within recent years that the need of protecting oyster fisheries against sewage pollution has forced itself on the attention of those who have the responsibility for protecting the public health."

When the great cities of to-day were mere villages, and what are now villages were green meadows, the wastes of man's activities were comparatively insignificant, but conditions are now very different, and with the present rapid increase in population the situation will become more and more serious. In fact, cities and villages in the past did not require complicated means of sewage disposal, yet this problem to-day has grown to such an extent that many city and State health officials are taking active steps to remedy the evils already arising from present conditions. In the light of present sanitary knowledge and in consideration of the results obtained from investigations made in this Department and elsewhere, it is known that sewage-polluted water is a menace to the shellfish industries. Such insanitary conditions can not continue to exist without increasing the probability of disease dissemination through the agency of infected oysters and other shellfish when used as food, especially when consumed raw.

The problem of sewage disposal is of national importance, and is not confined to one locality nor to a single industry. The health

[a] Reference numbers refer to bibliography, see p. 50.

of every man, woman, and child is influenced by the properly or improperly cared-for wastes of their own community. Since the seriousness of the situation and the need of better regulations is realized, every city and village should adopt proper methods of sewage disposal, so as to prevent further pollution of the natural waters; not alone to protect the extensive sea-fish industries, but as a sanitary precaution to safeguard the health and lives of millions of people.

In speaking before the Conference of Sanitary Officers at Albany, N. Y., Dr. Sedgwick[60] touched the vital point when he expressed the need of better sanitary regulations in the following terms:

> The modern sanitarian looks upon dirt not merely nor even chiefly as esthetically objectionable. He sees in it rather the vehicle of many of the worst diseases that afflict the race.
>
> Moreover dirt is not always dry or solid but often wet, fluid, and liquid. Nor is it always concentrated but often dilute, and some of the worst forms of dirt are little streams of sewage which find their way into drinking water, and, unseen and unsuspected, attack and destroy their victims.
>
> We hear much nowadays of pure-food supplies, pure-water supplies, and pure-air supplies; but the removal of the wastes and refuse from our cities, towns, villages, and farmhouses is equally important. For it is with the social organism, the municipality, the village, the family, very much as it is with the human organism; to retain putrefying wastes within their borders is an evil similar in character to that which arises in the human body from undue retention of urine or bowel contents. Poisoning ensues in the one case almost as certainly as in the other. * * *
>
> But we have learned our lesson. In the hard school of experience we have learned that hundreds of epidemics of typhoid fever and Asiatic cholera have come from the use of drinking water tainted with sewage, barely stained it may be with little trickling streams of water soiled with human excrements. And this it is which has given rise to the great problem of sewerage and sewage disposal. This it is which has caused numerous commissions, especially in western Europe and America, to study elaborately the pollution and purification of rivers.

The shellfish interests which are engaged in the solution of this problem rank among the most important industries of the country. It is estimated that in 1904 there were more than 25,000,000 bushels of oysters marketed in this country, valued at nearly $20,000,000. The operations of the various shellfish industries extend from Maine to the Gulf of Mexico on the Atlantic and over a considerable territory along the Pacific coast. Vast areas of sea bottoms are utilized for the purpose of growing oysters, and many men are engaged in the various branches of the industry. An extensive business is also being developed in maturing seed oysters taken from the Atlantic coast and transplanted in the colder waters of the Pacific, notably in the region of Puget Sound, where the conditions are unfavorable for spawning and development.

It is apparent that a grave danger threatens a valuable industry as well as the public health, and from this point of view the investigation was undertaken to determine the nature and extent of the danger and to suggest, if possible, methods of meeting and overcoming it.

EXPERIMENTAL INVESTIGATIONS.

PLAN OF WORK.

This investigation is concerned primarily with the possibility of oysters and clams becoming contaminated when grown or "floated" in waters polluted from sewage. Similar examinations of oysters from localities comparatively free from sewage are considered as standards in connection with those examined from suspicious sources. Practically all of the samples, including oysters, clams, and water, were collected by the writer from their original sources. At the time of dredging or tonging the shellfish from their natural or artificial beds, samples of water were collected in sterile bottles for bacteriological examination. When oysters were allowed to "drink," "fatten," or "float" in brackish regions, samples of the water from the floats were also collected to compare with the oysters thus treated. These investigations were carried on at different points along the Atlantic coast and the Gulf of Mexico during the oyster seasons of 1908, 1909, and 1910.

PROCEDURE FOR BACTERIOLOGICAL EXAMINATION.

COLLECTION AND SHIPMENT OF SAMPLES.

In order to obtain the samples it was necessary to secure the cooperation and services of practical oystermen actually engaged in the business. Boats properly equipped with dredging and tonging facilities were used, and at the time of collection careful notes were made as to probable sources of pollution, depth of water, direction of winds, conditions of tide, etc.; in fact any information which was thought to be pertinent was recorded.

During the first part of the investigations water samples were collected in 2-ounce, glass-stoppered bottles protected by metal-covered cylinders, the whole package having been sterilized at 160°–170° C. for at least one hour. Later, 4-ounce, glass-stoppered salt-mouth bottles, protected by aluminum cases, were used. Each half of these aluminum cases is numbered and the top and the bottom screw firmly together, making a water-tight package. The numbers facilitate the making of records and they also prevent the separation of properly fitted tops and bottoms. This particular kind of container was devised in this laboratory and is admirably adapted for shipping perishable materials.

Six of these metal cases are inclosed within a rectangular galvanized iron box, 6 by 8 by 5 inches, outside measurement, the cover being fastened with a clasp in order that the package may be sealed. This metal box is inclosed within a wooden box of sufficient size to permit a 3-inch air space to surround it on all sides, top, and bottom. Heavy, grooved, upright pieces in each corner strengthen the box and at the same time hold the metal box firmly in place. On the top of each wooden upright piece a button keeps the box from slipping up and down should the package be turned bottom upward. Cracked ice and sawdust, or ice alone, is packed in the air space for refrigeration. The lid of the box may be left unlocked to permit re-icing during long shipments, provided the inner box is sealed.

The earlier samples of water and shellfish were shipped to the laboratory in ice-cream freezers during moderately warm weather, but when the weather was extremely cold no ice was used in shipping short distances. Both water and shellfish samples were protected from melting ice during shipment by being placed in water-tight containers surrounded by ice.

Deep-water samples were taken by the aid of a heavy cylindrical jacket made of lead, arranged in such a manner as to hold the bottle in place, at the same time permitting the stopper to be lifted a given distance by a string or wire without removing it the full distance out of the neck of the bottle. A rubber band attached to each side of the clamp grasping the stopper caused it to fly back in place when the attached string was released. Surface samples were taken as near 1 foot under surface as was practicable.

Oysters and clams were fished from their beds either by tongs or by dredges, each sample being properly labeled for future identification. Generally about 12 to 15 medium-sized oysters or 6 clams constituted a sample.

PREPARATION OF SAMPLES OF SHELLFISH.

(1) Clean shells by thorough scrubbing with a brush in running tap water, rinse in sterile water, dry between folds of a sterile towel.

(2) Cleanse the hands thoroughly after scrubbing the oyster shells, select five cleaned oysters, slightly flame the lips of each shell before opening, and open with a sterile oyster knife, observing aseptic precautions, keeping deep shell downward. Either draw off liquor with sterile pipette or decant into sterile flasks.

A summary of the chief advantages of examining the whole oyster and liquor is given by Houston [33] 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 examinations 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 the mixture of these liquids.
(5) The results can be stated as number of bacteria either per oyster or per cubic centimeter of oyster.

According to the observations of the writer the total quantity of oyster liquor and body range from about 8 to 20 cc, averaging decidedly higher than 10 cc per oyster, the average recorded by Houston. In some cases this average was as high as 15 cc per oyster, including both liquor and body meat. The quantity of course varied according to the size and shape of the oyster shell.

Some preliminary work was done in order to decide upon the best and most practical method to follow. Individual oysters from the same lot were treated in various ways. Oysters obtained from clean, hard bottoms showed little difference in results when compared with those from the same source thoroughly scrubbed. Oysters from muddy bottoms showed the greatest necessity of cleaning before being opened. Practically the same method with slight modifications was used during the entire work. Only sound, representative stock was considered.

The examination of composite samples of five or more oysters was supplemented by inoculating media with the liquor from single oysters to determine the presence of *Bacillus coli* in each. It was also decided to use only the liquor bathing the oyster, instead of both meat and liquor, as the latter represents the character of the whole contents of the shell sufficiently well to determine the presence of pollution.

MEDIA EMPLOYED.

Plain agar and nutrient beef broth.—Prepared according to the standard methods recommended by the American Public Health Association.[56]

Bile salt agar.—Prepared after the formula of MacConkey [41] by adding 0.5 per cent sodium taurocholate, 2 per cent peptone, 1 per cent lactose, and 1.5 per cent powdered agar to a liter of water. To this mixture a sufficient quantity of a 1 per cent solution of neutral red is added to give a light red color to the finished medium. The bile salt agar is used as a differential medium for the isolation of *B.-coli*-like colonies. Plates are incubated from twenty-four to forty-eight hours, when the colon bacilli, if present, will appear as smooth, round, raised, entire, glistening, pink colonies.

Litmus milk fermentation tubes.—Used for the determination of the presence of *B. enteritidis sporogenes.*

In addition to the media already mentioned, the following were also tried, but because of unsatisfactory results their use was not continued: Gelatin, lactose agar, litmus lactose agar, Endo's medium, Hiss's agar, and plain agar without the addition of salt.

PLATING SAMPLES.

WATER.

Solid cultures.—In the routine water work the following cultures are made for each sample:

(*a*) Plate 1 cc, 0.1 cc, 0.01 cc, and 0.001 cc on plain agar at 25° C.
(*b*) Same dilutions on plain agar at 37° C.
(*c*) Same dilutions on bile salt agar incubated at 37° C.

Plates are incubated from two to four days, according to temperature.

Liquid cultures.—In order to determine the presence of gas-producing organisms, bile containing 1 per cent of peptone and 2 per cent of lactose is used, or 2 per cent dextrose fermentation tubes are inoculated with 10, 5, 1, 0.1, 0.01, and 0.001 cc quantities of the sample, provided no unusual pollution is suspected. With water from polluted or questionable sources 1 cc quantities are first used and then higher dilutions than are ordinarily employed. Fermentation tubes of the old style are being replaced by small inverted tubes, with one end closed. These are placed within a large test tube containing a fermentable medium. Tubes of this form require less space, and as a whole are more convenient for routine work. When desired, a large number of such tubes containing the ox-bile medium can be carried from place to place in making presumptive tests for colon organisms. By using 1 cc pipettes, graduated in tenths, the above-mentioned ox-bile medium may be inoculated with 1 cc and 0.1 cc quantities of water or oyster liquor. These tubes can be incubated over a radiator and the general character of the material determined with a fair degree of accuracy by noting the presence of fermenting organisms in this medium. Such a test is of course only tentative, but is of service in fieldwork.

OYSTERS.

The liquor removed from shell oysters is cultured in the same manner as samples of water, except that 10 and 5 cc quantities are not used. With market shucked oysters, in which the bacterial count is likely to be much higher than in the shell stock, higher dilutions are generally necessary.

Dilutions of water and oyster liquor are made by adding 1 cc of the sample to 9 cc of sterile water in a test tube or small Erlenmeyer flask, thus giving a dilution of 1:10. Dilutions of 1:100, 1:1000, etc., can be made by taking 1 cc of each lower dilution and adding to other flasks containing 9 cc of sterile water. Sterile normal salt solution is preferred by some workers. After making the dilutions each flask should be thoroughly agitated (twenty-five times) in order to break up masses of bacteria. With semisolid substances sterile glass shot may be added to the liquid for this purpose.

IDENTIFICATION OF ORGANISMS.

PURE CULTURES.

The classifications of Chester [12] and of Miquel [46] were followed in identifying species herein described. Well-isolated colonies on bile salt agar were selected from plates containing colon-like organisms and subcultures made in fermentation tubes. These cultures had been incubated for from twenty-four to forty-eight hours when examined, and, if gas-producing, each culture was sown in the following differential media: (1) Plain agar slants, (2) peptonized beef broth, (3) alkaline litmus milk, (4) potato, (5) Dunham's peptone solution, (6) 2 per cent dextrose bouillon, (7) 2 per cent lactose bouillon, (8) 2 per cent saccharose bouillon, (9) nitrate broth, and (10) gelatin, all prepared according to standard methods. Cultures were observed from one to three weeks, and all except gelatin incubated at 37° C.

Morphology and staining.—In connection with the biological characters on culture media the staining and morphological properties were observed with each culture under consideration.

Motility: Determined in freshly prepared liquid gelatin, or slant agar cultures.

Staining: Reaction to Gram's stain, methylene blue, fuchsin, etc.—also stained for flagella when convenient.

Morphology: Form, arrangement, size, involution forms, spores, etc.

Some bacteriologists consider the great amount of work involved in identifying the individual species of organisms as useless, but the experience of this laboratory is quite to the contrary. Undoubtedly more reliance can be placed upon given bacteriological results if a more exact knowledge of the contained organism is ascertained than if such a detailed study is omitted. Little is known concerning the specific action of the ordinary organisms such as molds, yeasts, and common saprophytes which are encountered in routine work on food bacteriology, and all information along this line is of value. A thorough study of the chemical nature of the bacterial products elaborated by the growth of saprophytic as well as pathogenic bacteria would also add materially to the value of the results obtained from the biological side of the investigations. These results should be further confirmed if possible by animal inoculations with the isolated bacteria or their toxins.

BACILLUS ENTERITIDIS SPOROGENES DETERMINATION.

From a number of samples of water and oyster liquor 10 cc quantities were heated for fifteen minutes at 80° C., and from each sample thus treated 1 cc and 0.1 cc were inoculated into alkaline litmus milk fermentation tubes to determine the presence of the *Bacillus enteritidis sporogenes.* This organism was recorded as being present when the medium coagulated with abundant gas formation within forty-eight hours. Smear preparations from old cultures showed the presence of numerous spore-bearing organisms in the closed portion of the tube.

DESCRIPTION OF ISOLATED ORGANISMS RESEMBLING BACILLUS TYPHOSUS.

Four different strains of motile organisms, somewhat resembling *Bacillus typhosus,* were isolated from samples of water and oysters collected during this investigation. The following general description illustrates the character of these organisms:

Morphology and staining properties.—Gram-negative, more or less actively motile bacilli, rods usually straight with slightly rounded ends, varying in size from about 0.4 to 0.8 by 1.5 to 4 microns, generally somewhat longer and more slender than *Bacillus coli.* No chains, spores, or capsules observed.

Biological characters.—Plain agar: Soft, grayish white, moderately abundant growth.

Bile salt agar: Small reddish, slowly developing colonies.

Peptonized beef broth: Rendered uniformly turbid with no ring or membrane.

Potato: No visible growth.

Alkaline litmus milk: Unchanged or faintly alkalinized after a slight initial acidity.

Sugar solutions: No gas production in dextrose, lactose, saccharose, levulose, maltose, nutrose, and inulin.

Nitrate solution: Nitrates absent in three cases, heavy trace in fourth.

Dunham's solution: Indol absent or present in slight trace.

Gelatin: Not liquefied, more or less circular flat, whitish growth on surface, filiform stab.

Agglutination: Negative in two cases with 1:50 dilutions after one hour, control stock culture agglutinated with 1:300 dilution of typhoid immune serum after fifteen minutes. Owing to difficulty in securing serum, the first two cultures were not tested. The typhoid serum was furnished by the Hygienic Laboratory of the Public Health and Marine-Hospital Service.

These cultures can not be classified as true types of *Bacillus typhosus,* although biologically they closely resemble this general group of organisms.

GENERAL CHARACTERISTICS OF THE BACILLUS COLI ORGANISMS ISOLATED.

Morphology and staining properties.—Gram-negative, feebly or actively motile, rods generally short and ovoid with rounded ends; size varying from 0.4 to 0.9 by 1 to 2.5 microns, with filaments occasionally much longer. No spores, chains, or capsules observed.

Biological characters.—Plain agar: Abundant, soft, whitish-gray growth.

Bile salt agar: Plates usually show moderate-sized, circular, yellowish-pink colonies.

Peptonized beef broth: Densely turbid, usually a slight membrane with ring and heavy sediment.

Potato: Brownish, abundant patch, darkening with age.

Alkaline litmus milk: Coagulated generally after two days at 37° C.

Rosalic acid solution: Unchanged.

Sugars: Gas in dextrose, lactose, and saccharose, usually from 30 to 50 per cent, the ratio of CO_2 to H equals 1 to 2. Acidity varying from 3 to 6 per cent.

Dunham's solution: Indol always present, either marked or in trace.

Nitrates: Usually present, generally marked reaction.

Gelatin: Flat, whitish, irregular surface growth with filiform stab. Commonly a few gas bubbles were seen in depths of medium without liquefaction.

Agglutination: Not determined.

SOURCES OF CONTAMINATION.

LOCATION OF OYSTER BEDS.

The proper control and location of oyster beds in relation to public health should be a matter of great concern to those engaged in the industry. In selecting these it is not only essential that oystermen

FIG. 1.—One of three main sewers emptying untreated sewage into the mouth of a river. This sewage combines with that from several other large cities and flows over extensive shellfish grounds.

should consider the localities best adapted to the growth, flavor, size, and appearance of their oysters, but they must also consider the possibilities of sewage contamination. Close proximity to any habitation paves the way to possible pollution, and with the multiplication of dwellings the chance of dangerous contamination increases.

Because of insanitary methods of sewage disposal large areas of once valuable oyster grounds are at the present time subjected to conditions which render the shellfish taken from them wholly unfit for food purposes. (See Tables 1, 2, 3, and 4.) On the other hand, it will be observed that the investigations show extensive oyster layings to be free from serious contamination, yet in these very localities summer cottages are springing into existence, the nearest villages and cities are rapidly encroaching upon the oyster territory, and the community does not concern itself especially about the disposal of sewage. Even the oystermen themselves are guilty of contributing their quota of contamination by dumping wastes overboard from boats or by depositing them in near-by waters through the medium of privy vaults located in their shops. These closets are almost invariably placed where nature is depended upon to remove the accumulating wastes, which are later carried out by the waves to some oyster float or bed near by.

BACTERIOLOGICAL RESULTS ON SHELLFISH AND SEA WATER.

The following tables show the bacteriological results obtained on oysters and clams in the shell and on the sea water covering the beds from which they were taken:

TABLE 1.—*Results of the bacteriological examination of shell oysters.*

COLLECTED FROM LOCALITIES REASONABLY FREE FROM EVIDENCE OF POLLUTION.

Sample No.	Bacteria per cubic centimeter (plain agar incubated four days at 25° C.).	B. coli per cubic centimeter.	B. enteritidis sporogenes.
1	6,300	0	0
2	11,000	0	0
3	1,000	0	0
4	1,000	0	0
5	4,500	0	0
6	800	0	0
7	6,000	0	0
Approximate average	4,300	0	0

COLLECTED FROM GROUNDS SHOWING PROBABLE POLLUTION.

Sample No.	Bacteria per cubic centimeter (plain agar incubated four days at 25° C.).	B. coli per cubic centimeter.	B. enteritidis sporogenes.
1	1,500	10	1
2	10,000	10	10
3	400	1	0
4	500	10	1
5	5,000	10	10
6	400	1	0
7	100,000	10	10
8	7,000	1	1
9	27,000	1	1
10	17,000	10	10
11	20,000	10	10
12	8,000	1	1
13	5,000	1	1
14	1,500	1	0
15	9,000	1	0
16	1,000	1	10
17	9,000	10	1
18	4,000	10	1
Approximate average	12,500	5.5	3.7

TABLE 2.—*Results of the bacteriological examination of hard clams (quahaugs) in the shell.*

COLLECTED FROM GROUNDS WHERE INSPECTION SHOWED NO SERIOUS POLLUTION.

Sample number.	Bacteria per cubic centimeter (plain agar incubated four days at 25° C.).	B. coli per cubic centimeter.
1	300	0
2	2,700	0
3	32,000	0
4	150,000	0
5	4,000	0
6	4,000	1
Approximate average	32,000	0.16

COLLECTED FROM GROUNDS SHOWING VERY SERIOUS POLLUTION UPON INSPECTION.

Sample number.	Bacteria per cubic centimeter (plain agar incubated four days at 25° C.).	B. coli per cubic centimeter.
1	13,000	10
2	11,000	1,000
3	1,122,000	1,000
4	2,108,000	10
5	100,000	100
6	12,000	100
7	60,000	1,000
Approximate average	489,000	460

Clams appear to contain a larger number of bacteria in their liquor and body contents than do oysters. This may be explained in part by the fact that the clams in the latter table were collected from localities where extreme pollution was indicated. Each sample generally represented at least five clams, which constituted a composite sample. In a few instances only one or two large clams were obtained from a given locality for examination.

TABLE 3.—*Results of the bacteriological examination of sea water over oyster beds.*

COLLECTED FROM LOCALITIES WHERE INSPECTION SHOWED NO SERIOUS POLLUTION.

Sample number.	Surface water.			Deep water.		
	Bacteria per cubic centimeter (plain agar four days at 25° C.).	B. coli per cubic centimeter.	B. enteritidis sporogenes per cubic centimeter.	Bacteria per cubic centimeter (plain agar four days at 25° C.).	B. coli per cubic centimeter.	B. enteritidis sporogenes per cubic centimeter.
1	30	0	0	130	0	0
2	100	0	0	40	0	0
3	20	0	0	30	0	0
4	500	1	0	700	1	0
5	100	0	0	100	1	0
6	200	1	0	300	1	0
7	20	0	0	70	0	0
8	15	0	0	40	0	0
Approximate average	120	0.2	0	170	0.4	0

TABLE 3.—*Results of the bacteriological examination of sea water over oyster beds*—Cont'd.

COLLECTED FROM LOCALITIES SHOWING PROBABLE POLLUTION.

Sample number.	Surface water. Bacteria per cubic centimeter (plain agar four days at 25° C.).	Surface water. B. coli per cubic centimeter.	Surface water. B. enteritidis sporogenes per cubic centimeter.	Deep water. Bacteria per cubic centimeter (plain agar four days at 25° C.).	Deep water. B. coli per cubic centimeter.	Deep water. B. enteritidis sporogenes per cubic centimeter.
1	50	1	0	40	1	0
2	60	1	0	60	1	0
3	10,000	1	1	2,600	10	0
4	7,000	1	1	4,000	1	10
5	2,000	1	10	2,000	1	10
6	600	0	1	4,000	1	1
7	400	0	0	200	1	1
8	300	0	1	100	1	1
9	2,300	0	1	500	1	1
Approximate average	2,500	0.6	1.7	1,500	2	2.7

These results, as a whole, indicate that in this case the deep water was more polluted as regards *B. coli* and *B. enteritidis sporogenes* than the surface water over the same locality.

TABLE 4.—*Bacteriological examination of water collected over a distance of about 200 miles, a portion of which covers extensive shellfish grounds. (September, 1909.)*

OVER OYSTER BEDS.

Sample number.	Time.	Bacteria per cubic centimeter (plain agar incubated three days). 25° C.	37° C.	B. coli per cubic centimeter.	Salt.	Remarks.
	P. M.				*Per cent.*	
1	7.30	400	50	1	2.19	Near boat landing.
2	8.00	200	100	0	2.27	Out in channel.
3	8.30	150	40	0	2.21	No gas in 1 cc.
4	9.00	100	30	0	2.19	
5	9.30	90	40	0	2.02	
6	10.00	800	200	0	2.00	Gas in 10 cc, none in 5 cc.
7	10.30	150	90	0	1.95	No gas in 10 cc.
8	11.00	150	20	0	1.92	
9	11.30	100	50	0	1.70	
10	12.00	100	40	0	1.58	
	A. M.					
11	12.30	90	40	0	1.57	Gas in 10 cc, none in 5 cc.
12	1.00	60	60	0	1.59	
13	1.30	100	40	0	1.45	
14	2.00	50	30	[a] B	1.26	
15	2.30	30	50	0	1.08	Gas in 10 cc.
16	3.00	400	100	1	.97	Gas in 5 cc.

UPPER LIMIT OF OYSTER BEDS.

Sample number.	Time.	25° C.	37° C.	B. coli per cubic centimeter.	Salt.	Remarks.
17	3.30	50	30	[a] B	0.80	Gas in 5 cc, bubble in 1 cc.
18	4.00	40	100	0	.54	Gas in 5 cc.
19	4.30	400	200	1	.36	Bubble in 0.1 cc.
20	5.00	50	40	1	.08	Bubble in 0.1 cc.
21	5.30	200	100	10	.06	About 30 miles below city.
22	6.00	500	150	1	Trace.	About 20 miles below city.
23	6.30	10,000	8,000	100	Trace.	Near landing below city.
24	7.30	160,000	90,000	1,000	Trace.	Landing near sewer.

[a] Bubble.

These samples of water were collected with difficulty from the right lower deck, aft, on a steamboat while the wind was blowing a gale and the sea was greatly agitated. They were taken in 4-ounce sterile bottles hung at the end of a weighted line. At the upper limit at which oysters grew to maturity the water had a saline content of from 0.97 to 1.08 per cent. Seed is said to set above this point, but it does not mature well.

COMPARISON OF RESULTS OBTAINED ON SAMPLES FROM KNOWN SANITARY AND INSANITARY GROUNDS.

The New York City Board of Health[52] has demonstrated that the beds from which oysters are taken for consumption in that city often lie in grossly polluted waters. It is not the depredations of the starfish, borers, drumfish, etc., that threaten the life of the shellfish industry, but the contamination by wastes. These practical facts should stimulate every oysterman to see that his grounds are in a fit sanitary condition and that they are kept fully protected from subsequent contamination. Oystermen should not deceive themselves by believing that their grounds are free from pollution, in the absence of definite evidence to the contrary. If oyster growers can not themselves determine the sanitary conditions of their layings, they should appeal to their National organizations, or enlist the aid and cooperation of their State shellfish commissions and boards of health. A thorough sanitary survey of every oyster bed should be made, and this should be supplemented by repeated bacteriological examinations of both the water and shell stock taken at regular intervals under varying conditions.

There are many factors which may influence the degree and extent of sea-water pollution, and before passing judgment on the sanitary condition of a particular oyster laying all the facts in the case should be considered. The influence of tidal change, percentage of sunlight, amount of rainfall, seasonal variations, prevailing winds and currents, the depth, and the amount of salt in the water must all be considered, though of course the proximity to sources of possible contamination is the most important item. Oysters offered for sale from polluted beds are in constant danger of seizure and condemnation by health officials. The occurrence of sewage matter in oysters can not be tolerated from a public health point of view.

Where oyster beds are proved to be polluted or located in questionable territory, and it is desired to continue their use, there would seem to be no objection to raising seed stock on such grounds for transplanting purposes only. Oysters grown on such holdings should under no circumstances be offered for sale to be consumed either raw or cooked. Numerous experiments and the opinion of practical oystermen indicate that oysters taken from polluted beds "cleanse" themselves in a few days when placed in pure sea water.

The length of time required for this cleansing process to be thoroughly accomplished undoubtedly varies according to local conditions. At least one month should always be allowed for this change to take place, although an entire season would be preferable.

Tables 5 and 6 show the sharp contrast between the bacteriological findings on water and oysters from sanitary and insanitary grounds.

TABLE 5.—*Bacteriological examination of water and shellfish collected from grounds below the sewer shown in figure 1.*

SHELLFISH.

Sample No.	Bacteria per cubic centimeter (plain agar incubated four days).		B. coli per cubic centimeter.	Salt.	Remarks.
	25° C.	37° C.			
				Per cent.	
1.......	6,000	6,000	100	....	Polluted.
2.......	40,000	30,000	1,000	....	Badly polluted.

WATER.

1.......	900	700	10	1.19	1 mile from sewer.
2.......	6,800	2,000	1,000	.13	100 yards from sewer.
3.......	1,800	700	1,000	.10	300 yards from sewer.

Extensive industries are being operated from the locality in which these samples were taken, and the products are widely distributed. The data given in Table 5 should be compared with the following results obtained on shellfish and water taken from clean grounds:

TABLE 6.—*Bacteriological examination of water and shellfish collected from clean grounds.*

SHELLFISH.

Sample No.	Bacteria per cubic centimeter (plain agar incubated four days).		B. coli per cubic centimeter.	Salt.	Remarks.
	25° C.	37° C.			
				Per cent.	
1.......	8,000	300	0	....	No pollution.
2.......	8,600	5,000	0	....	Do.
3.......	6,800	3,000	0	....	Do.
4.......	500	50	0	....	Do.

WATER.

1.......	300	100	0	2.84	16 miles from land.
2.......	1,200	500	0	2.86	3 miles from land.
3.......	300	20	0	2.88	5 miles from land.

FLOATING OYSTERS IN POLLUTED WATER.

INSPECTION DATA.

When shellfish grounds are properly located with regard to sewage disposal, it is not an uncommon practice to nullify the cleanly results obtained by taking oysters from beds free from pollution and float-

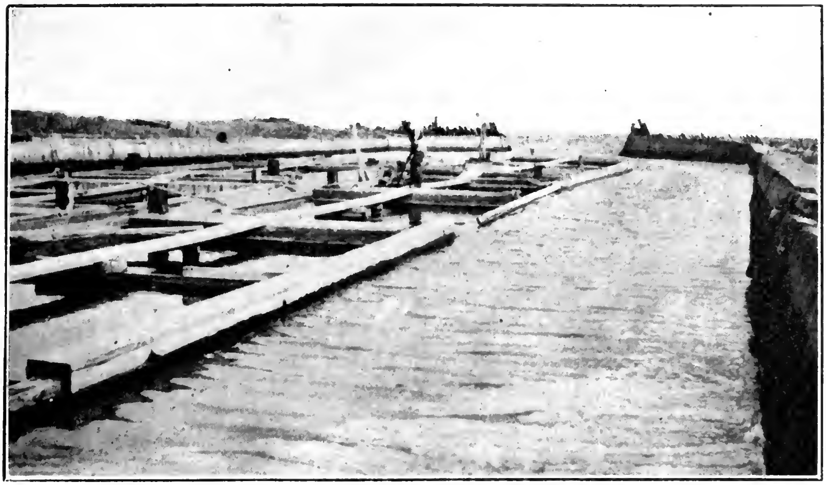

Fig. 2.—Oyster floats in sheltered artificially constructed inlets from the bay. The water of the sea does not have the same cleansing action on coves of this kind as where action of the waves and currents is unobstructed.

ing them for varying periods of time in water of a questionable character before marketing. Fortunately this custom is being discouraged or prohibited in some localities. Generally speaking, however, these floats are located for convenience rather than safety. They are constructed with slatted bottoms having an area of about 20 by 40 feet, the sides being $1\frac{1}{2}$ feet in depth, and are usually found anchored near the oyster establishment, where water is of a brackish or moderately fresh character, and where the chances of sewage pollution are greater than in the places where the oysters normally grow to maturity.

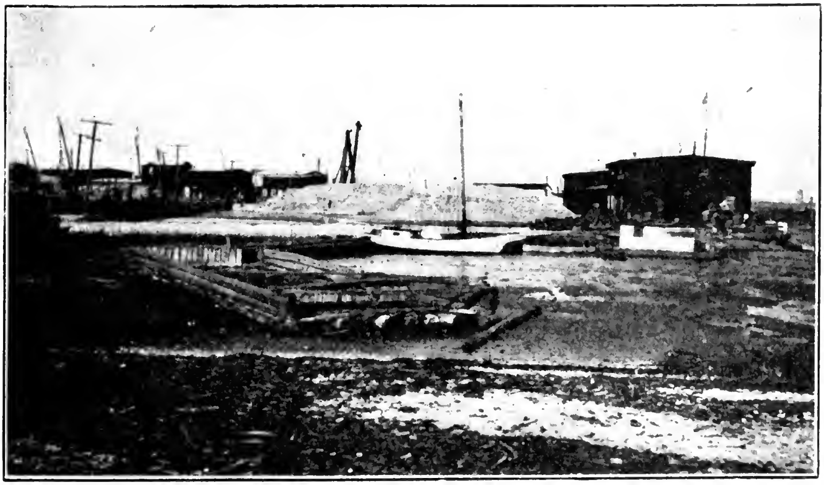

Fig. 3.—An abandoned oyster float.

This process of treating oysters in the shell is variously described as "fattening," "feeding," "freshening," "floating," "bleaching," "drinking," or "plumping." By careful observation one can see oysters on these floats open and close their shells, especially at the beginning of flood tide. The least sudden jar or visible shadow will cause them to close their shells immediately.

The method of floating oysters as ordinarily practiced is not a true fattening process, but is only a means of increasing the bulk of the oyster by adding to the water content and gives it a lighter color. These changes are brought about by osmotic action caused by the oyster being removed from comparatively salt water to a fresher medium, and they will not occur when the oyster is floated in water of the same saline content as that in which it was grown. Should the water in which these floats are placed be polluted with sewage

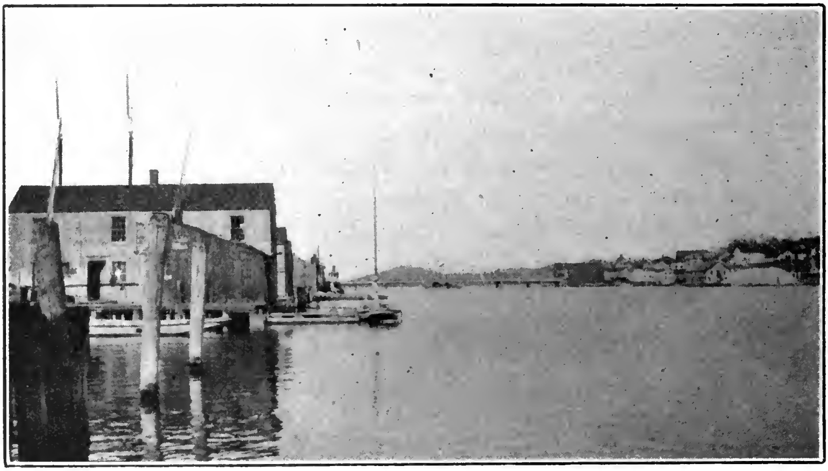

FIG. 4.—Two oyster floats anchored in the rear of oysterhouses. Privy vaults are located in the rear of these buildings, refuse being dumped directly into the river. It is a crime punishable by $100 fine to float oysters in this river. An epidemic of typhoid occurred some years ago from oysters floated in this place.

it is easy to see how the oyster may also become contaminated. Oysters may increase in bulk from 10 to 25 per cent or more when floated or when washed sufficiently long in running fresh water after shucking. This variable increase in bulk depends upon the salinity of the water from which the oyster was previously removed, the length of time that the floating or washing is continued, and the character of the water in which they are floated or washed. Shucked oysters from salt water increase in bulk the same as when in the shell if soaked in a plentiful supply of fresh water. On the other hand, plumped oysters, taken from "brackish water," when placed in comparatively salt water, will decrease in bulk according to the saltiness of the water and previous treatment.

In the course of this investigation many localities were visited where oyster floats were in operation, and in only a very few instances were the sanitary conditions satisfactory. There are hundreds of oyster floats in use during the oyster season which are located in waters of a more or less questionable nature, where the environment makes contamination possible from one or more of the following sources: From private or public sewers, privies, house drains, decaying shell heaps, stable yards, chicken yards, dead animals, manure piles on tilled land, refuse and garbage dumps of cities, etc. Figs. 2 to 10 and the data in Tables 7 and 8 show the facts on which this statement is based.

The wastes from the oyster boats are usually dumped directly overboard into the water, which subsequently bathes the growing oysters. If these boats are numerous it will be seen that the amount of pollution may be large. It is not a single source of pollution which necessarily condemns the water as being in an unfit sanitary condition, but it is the sum total of all the sources of contamination which

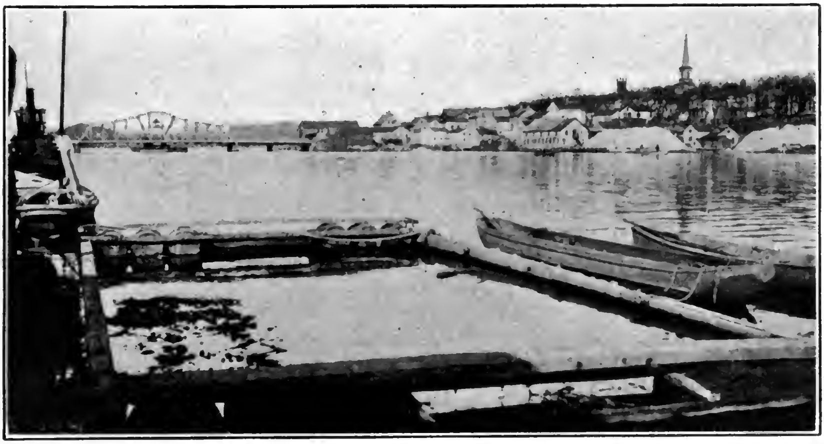

FIG. 5.—A closer view of the upper oyster floats shown in fig. 4. Note the pile of oysters in the float. Picture taken at low tide; about two hours later these same oysters were found in the adjoining oyster-house ready for sale. Oysters drink best at the beginning of flood tide and are "plumpest" about one or two hours afterwards. The main sewer of the city empties under the bridge above.

gives the bacteriologist his final results. Oyster floats are nearly always located in sheltered localities (see figs. 2–5), and in such places the chances for contamination are greatest. The full sweep of the ocean as it plays over the oyster beds under normal conditions does not take place so readily where oyster floats are anchored, and thus this factor of water purification is reduced in efficiency.

It has been proved by observations and experiments made in the course of this investigation that oysters when floated under the usual conditions—that is, in water that is only brackish—deteriorate more rapidly than the same stock unfloated. On this point Nelson[48] says that freshening oysters increases very rapidly the rate of weakening and decay, the life period being reduced one-half. This may be due in part to the decrease in the salt content of the oyster, which naturally acts as an antiseptic, or to the increased bacterial content of the water in which the shellfish are floated.

LABORATORY RESULTS ON FLOATED OYSTERS.

Some results of laboratory experiments on the vitality of *B. coli* and *B. typhosus* in salt solutions of various strength are as follows:

Saturated salt solution (c. p. sodium chlorid) inhibited growth of *B. typhosus* after three hours exposure, *B. coli* and *M. aureus* after six hours.

A 25 per cent solution of salt was sufficient to retard development of the typhoid bacillus, *B. coli*, and *M. aureus* in nutrient beef broth. Growth occurred with lower dilutions.

From the investigations made it appears that oysters grow best to maturity in water containing from about 1 to 3 per cent sea salt. Above or below this amount they do not thrive so readily; in fact they perish

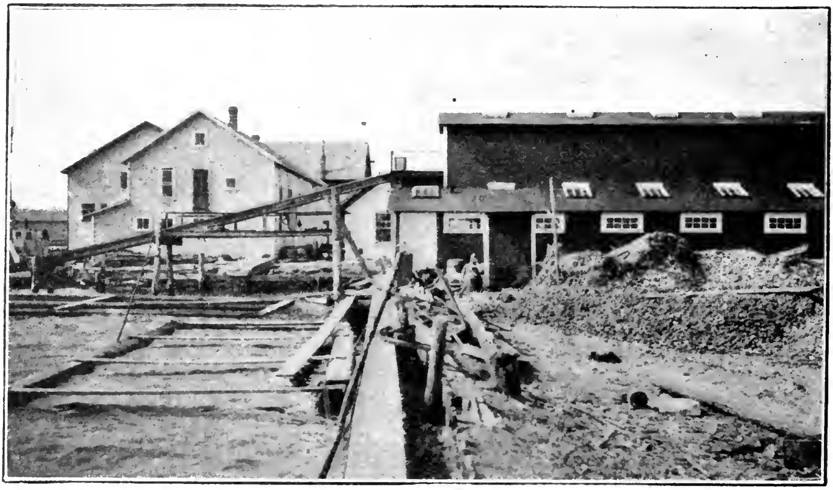

FIG. 6.—Oyster floats located near a shucking establishment. The small building in the foreground is a privy used by 100 to 150 men. The drainage from this vault seeps through the loose soil and wooden wall into the water which bathes the floats. Drainage from decaying shell heaps and the washings from these oysterhouses likewise flow into this same water. Analysis of this water and oysters on the floats showed contamination

when kept for any length of time in waters showing an appreciable variation from these figures. On this point Nelson,[49] of the New Jersey experiment station, says that the period of viability is greatest in water having a saline content of about 2 per cent, but for old oysters the figure is higher. He further says that oystermen should not be required to freshen oysters, but that this should be done by the caterer just before cooking.

There seems to be no objection to drinking oysters in waters of the same saline content as those in which they will grow to maturity, provided there is no possible source of contamination.

One of the greatest dangers arising from the practice of floating oysters is the fact that they are often consumed raw, and if polluted, they become active factors of disease dissemination. As is shown in the quotations from the literature on this subject (page 40), many of the known epidemics of typhoid fever due to eating infected shellfish were traceable to those which had become polluted during the process of drinking. There appears to be no necessity for floating oysters, and, as practiced at present, it is often exceedingly dangerous to public health.

The New York City Health Department Report on Typhoid Fever[52] shows many illustrations of floats located in waters subject to sources of pollution and the conclusion reached is as follows: "The process of 'freshening,' 'fattening,' or 'drinking,' often performed as it is in small streams, badly contaminated with sewage, is a most dangerous practice and should be discontinued." The following data on floated and unfloated oysters confirm these views:

Table 7.—*Bacteriological examination of condemned shellfish taken from floats located in polluted waters.*

COMPOSITE SAMPLES OF FIVE OYSTERS EACH.

Sample No.	Bacteria per cubic centimeter (plain agar incubated four days).		B. coli per cubic centimeter.	Salt.	Remarks.
	25° C.	37° C.			
				Per cent.	
1	7,000	3,000	10	....	Polluted.
2	19,000	1,800	10	....	Do.
3	12,000	1,000	100	....	Badly polluted.

WATER IN REGION OF FLOATS.

Sample No.	25° C.	37° C.	B. coli per cubic centimeter.	Salt.	Remarks.
1	7,000	2,500	1,000	0.40	Near sewer above float.
2	6,000	900	1	1.67	In channel above float.
3	4,600	200	1	2.06	In channel opposite floats.
4	3,700	370	10	1.96	Over float.
5	1,700	200	10	2.10	Below floats in channel.
6	600	180	10	2.17	Still farther below floats.

These results show that all the samples of shellfish and water taken from this locality were contaminated. In this case the water contained sufficient salt for the normal growth of oysters, but it was badly polluted.

Table 8.—*Bacteriological results on unfloated and floated oysters and on water over floats.*

OYSTERS.

Bacteria per cubic centimeter (plain agar, three days).		B. coli per cubic centimeter.	Salt.	Remarks.
25° C.	37° C.			
			Per cent.	
5,000	1,800	1	....	Unfloated, 4 out of 5 show gas in 1 cc only.
59,000	5,000	100	....	Floated over night, 3 out of 5 show gas in 0.01 cc of liquor.

TABLE 8.—*Bacteriological results on unfloated and floated oysters and on water over floats*—Continued.

WATER.

Bacteria per cubic centimeter (plain agar, three days).		B. coli per cubic centimeter.	Salt.	Remarks.
25° C.	37° C.			
			Per cent.	
29,000	400	1	0.59	Lower float, low tide.
2,100	600	1	.24	Middle float, low tide.
13,000	2,000	0	.14	Do.
2,000	600	10	.10	Upper float, beginning flood.
9,000	1,200	1	.10	Upper float.
420	110	0	.84	Mouth of river near oyster beds.

DISCUSSION.

The experiments were conducted on the lower float. The results of these analyses show the unfloated oysters to be moderately free from pollution, but when oysters from this same lot were floated overnight in water proved to be polluted they became dangerously contaminated. The water taken from the mouth of the river contains less salt than the water over the oyster beds farther out in the channel, where they grow to maturity. The experiments were made in a locality where about 500 oyster boats and floats operate at a distance of one-half to 1 mile above the mouth of the river (figs. 9 and 10). About 60 carloads of shell oysters are shipped daily from this point during active season. They are all passed through water of the above character for "cleansing" and shipped in the shell, presumably to be consumed raw.

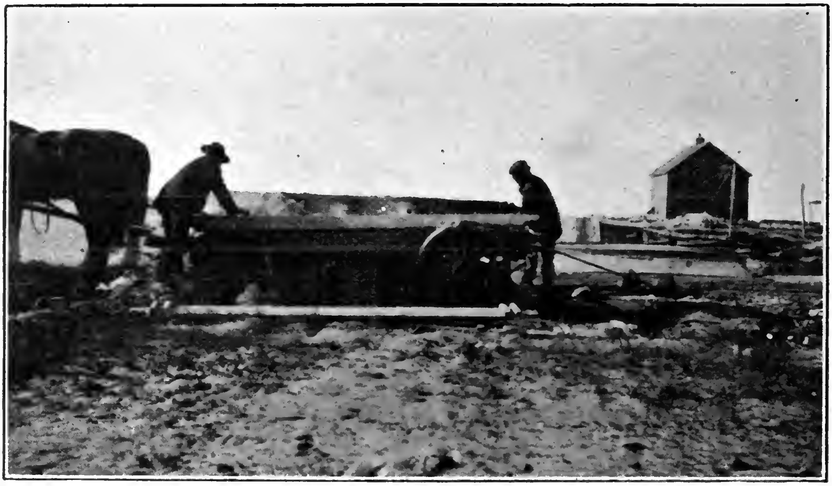

FIG. 7.—Dumping city refuse to fill a depression within 50 yards of oyster floats. Washings from this material as well as drainage from piles of manure on fertilized land drain directly into the cove where oysters are floated.

Field[21] says:

In conclusion it would seem as though the only method to protect the public would be to forbid the sale of fattened oysters, and to enforce it; also to see that oyster beds were not subject to contamination from the streams used for sewage purposes, the most important being the prevention of the process of "fattening" when the water was or could be contaminated.

The Connecticut State Board of Health[4] says:

The chief damage from oysters, which are an admitted means of conveying certain infectious diseases, comes from the custom of "floating" or "drinking" them in the brackish and generally sewage-contaminated waters of rivers and harbors immediately before they are placed on the market. * * *

There is a widespread belief that the process is actually a fattening one induced by the fresher water or by the greater abundance of food which often occurs in the places chosen. Numerous observations have, however, shown that it is not a fattening or a

Fig. 8.—View at low tide showing dead hog covered at high tide by water washing oysters on a float within 150 feet. Water and oysters were found contaminated from the float; the pollution, however, did not all come from this source.

growing process, as those terms are generally understood. An oyster used as a food contains no more nutriment after the process than before. It is plumper because it contains more water, but it is no more fattened by the absorption of the fresher water for a day or so than the calf is fattened when induced to drink large quantities of water just before being sold to the butcher—a process well known to make the animal look plumper to the eye. Floated oysters are, however, fresher to the taste, and some persons prefer this taste; others prefer the saltier flavor of those oysters marketed directly from the saltier waters. It is, however, probable that the floated oysters are more attractive to the average buyer, whether he be the consumer or the retail dealer. But the places where the oysters are floated are more liable to sewage pollution than the localities where they are grown, and hence the danger of the process unless the streams or harbors when the floating is done are free from sewage pollution. * * * The committee again strongly recommends that the "floating" be entirely discontinued, both as a measure for diminishing the typhoid fever and also in the interest of the oyster-growing industry in this State.

STORAGE OF OYSTERS.

In a report on the "Preservation of fishery products for food," Stevenson [65] has shown that the best temperature for cold storage of oysters is between 38° and 40° F. When stored in good condition they may be kept at this temperature for six weeks. As an experiment they have been kept for ten weeks, but storage for that length of time is not advisable.

Some experimental results on keeping shell oysters in this laboratory at low temperatures (about 35° F.) showed that they still remained in good condition after five weeks, but at the end of twelve weeks nearly all showed from their physical condition alone that they were unfit for food. When necessary to keep oysters in storage during the winter they should be kept under good sanitary conditions. Freshly caught oysters are preferable to those which have

FIG. 9.—About 500 boats and as many floats in operation in river. From three to five men operate each boat, and refuse is generally dumped overboard. Water showed pollution.

aged in the shop. The wet mud on the outside of oyster shells in piles, when contaminated, may pollute the oysters at the bottom of the heap by means of infected matter dripping down from the layers above. Oysters shipped in unclean freight cars may become contaminated en route, or this may occur during insanitary storage in bins at oyster houses.

SPOILAGE DUE TO LENGTH OF TIME OUT OF WATER.

Stale oysters are without question a dangerous article of food. This kind of material has undoubtedly produced serious gastro-intestinal disturbances and possibly death when consumed by individuals susceptible to ptomain poisoning. In fact any highly perishable food product of this character may quickly spoil and be injurious to health if not properly refrigerated under good sanitary conditions. As just stated, oysters may be kept for a period of six weeks under proper conditions, yet, even though they were taken from unpolluted beds and stored under good sanitary conditions, mere length of time alone may cause them to become unfit for use. No oyster should be used when the shells show the least gaping, nor when the liquor is practically all gone.

DISEASED AND GREEN OYSTERS.

In their study of the "Oyster and disease," Herdman and Boyce [28] elaborately treat the subject of green oysters. They show that cer-

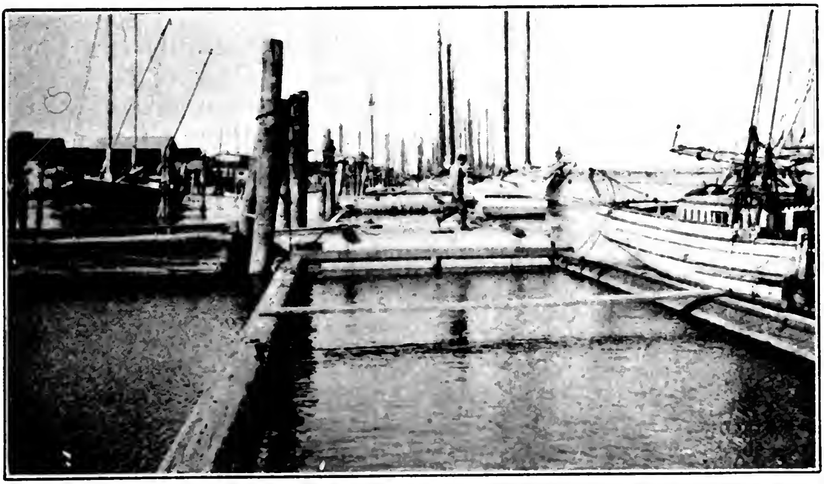

FIG. 10.—Oyster floats between the row of oyster boats (fig. 9) and the shore. Anchored from piles. Both oysters and water from this locality were found to be contaminated. Sixty carloads of oysters, mostly eaten raw, are shipped from this place daily in winter. Beds on which they grow are probably uncontaminated.

tain of the green oysters are healthy, while others are not so considered. Some forms of greenness are described as a "leucocytosis," which may be associated with an excessive amount of copper. Other species may have no copper present, but a special pigment, "marennin," is found with a certain amount of iron. In the present investigations the green oyster was frequently encountered and amounts of copper varying from 150 to 714 mg per kilogram [a] were found, while from oysters not showing the green coloration amounts varying from 14 to 40 mg per kilogram were obtained. Appreciable quantities of copper were also found in the water and mud [b] taken

[a] Analysis made by W. C. Taber, Food Inspection Laboratory.
[b] Analysis made by W. W. Skinner, Water Laboratory, Miscellaneous Division.

from the localities where the green oysters had grown. It was demonstrated that the copper contamination undoubtedly came from smelter factories located on the water front, from which the wastes and effluent drained into the harbor bathing the oyster grounds. The oysters showing the highest amount of copper were collected nearest the factories, and they possessed a distinctly metallic taste. This subject is receiving further study in the Bureau of Chemistry. It is said that certain European varieties of green oysters are cultivated as a luxury and sold at a premium because of their color. Oysters may die from sewage wastes in badly contaminated waters. In one locality it has been estimated that four out of five oysters on certain beds were destroyed from this condition of affairs during one season.

HANDLING OF SHUCKED OYSTERS.

The initial pollution of shucked oysters may be due to the shell stock being taken from sewage-polluted grounds, but in the liquor surrounding the meat and in the intestinal contents of all shellfish there are some bacteria. When the kind of bacteria are such as normally occur in unpolluted sea water, and are present in small numbers, they are considered harmless and their presence is disregarded. On the other hand, when *B. coli* and similar forms of germ life representing the presence of sewage matter are found the bacteriologist is forced to take a different view of the matter. The *B. coli* group of organisms may be present in numbers not sufficient to condemn the oysters when fresh, yet when shucked and allowed to stand improperly iced under bad sanitary conditions these few organisms may multiply and produce millions before the oysters are consumed. While it is essential that oyster beds should be free from pollution in order to be assured that the shucked stock has not been contaminated from this source, dirty methods of shucking alone may account for contamination. Oysters grown on soft muddy bottoms are generally covered with a coating of mud, and unless this is removed from the shell before shucking some of the mud is likely to get into the opened stock. The oystermen claim that this is one of the reasons for floating oysters. If it were done for this purpose alone, in pure water of the proper salt content, there would be no objection to the process; in fact it would be beneficial, in that it would allow the oyster to free itself of the sand and grit contained within the gills and at the same time cleanse the outside of the shell.

The following results illustrate the relative bacterial content of shell and market-shucked oysters:

TABLE 9.—*Comparison of bacterial content of shell clams and oysters with shucked market oysters.*

Data determined.	36 miscellaneous samples of shell oysters.	20 samples of hard-shell clams.[a]	33 samples of shucked market oysters.
Bacteria per cubic centimeter after 4 days:			
Average—			
Plain agar, 25° C.	6,000	275,000	867,000
Plain agar, 37° C.	1,000	18,000	268,000
Bile salt agar, 37° C.	200	3,000	45,000
Maximum (25° C.)	26,000	4,300,000	4,750,000
Minimum (25° C.)	150	2,000	15,000
B. coli:			
Average	7+	180	74,000
Maximum	100	1,000	1,000,000
Minimum	0	0	0

[a] Many of these clams were from badly contaminated waters.

From the results given in the table it is seen that these 33 samples of shucked oysters, probably representing fairly those ordinarily found in markets and retail stores, have greatly increased their bacterial content. This increase is largely due to negligence and unclean methods of handling.

Various opening methods are employed by oyster shuckers in different localities. The hands of the average opener are generally unclean, and the proper facilities for keeping them in a better condition are lacking in most establishments. In nearly every method employed the dirty hand of the shucker comes in contact with the opened oyster while transferring it from the shell to the bucket. Oystermen should keep their hands out of the cans of shucked oysters, especially after they are washed and prepared in containers ready for market. The average man's hands are unclean from a bacteriological point of view. A few colon organisms introduced in this way may result in the condemnation of goods shipped for a long distance, or they may spoil, and the colon bacilli unduly increase if allowed to stand some time before consumption under conditions favorable to rapid bacterial growth. Marked improvement must be made in the present manner of opening oysters before they will be satisfactory from a sanitary point of view. Washing the oyster shells, cleansing the hands from time to time, and bringing only the knife in contact with the oyster when transferring the oyster from the shell to the bucket would contribute to more sanitary results.

INSANITARY OYSTERHOUSES.

The average oysterhouse is not sanitary either in its construction or in its maintenance. Buildings of wood, with inadequate ventilating and lighting facilities, wooden floors, ceilings and walls covered with cobwebs, and accumulated dust are the conditions generally found in making inspections of these places. The modern

FIG. 11.—Shucking oysters. Each man stands at a stall where the oysters are opened and put into small buckets. General sanitary conditions very bad. In this place there were numerous long cobwebs dangling from the ceiling, with dust and filth everywhere noticeable.

food factory is not only built of fireproof material, but is carefully arranged as regards ventilation, toilet facilities, proper location, drainage, etc. Concrete floors and walls can be flushed daily with water, and with the installation of these and other sanitary measures there is no reason why any oysterhouse could not be kept in a clean, wholesome condition. Oysters from pure beds could hardly escape pollution by the time they had passed through an unclean oyster establishment. Aside from insanitary premises, the question of securing workmen who are cleanly is one of the practical difficulties

FIG. 12.—Another shucking establishment showing insanitary conditions.

with which the oystermen have to contend. Regulations should be enforced requiring better care of the hands and more personal cleanliness in general on the part of oyster shuckers. To this end, the necessary toilet facilities should be provided for their welfare and comfort.

Bacteriological tests made by exposing freshly prepared agar plates 4 inches in diameter where oysters were being shucked show the relative bacterial content of the air in such places. One set of such experiments gave the following figures:

A one-minute exposure resulted in the growth of 130 colonies; two minutes, 180 colonies; three minutes, 220; four minutes, 350; and five minutes, 430 colonies. The organisms consisted of molds, yeasts, spore-bearing and various chromogenic and other colonies.

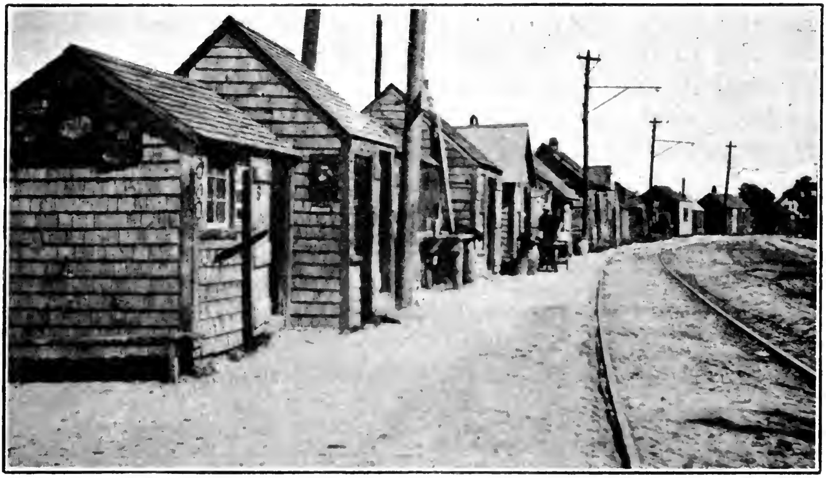

FIG. 13.—Clam diggers' huts, where shucking is done. Sanitary conditions on the interior generally very unsatisfactory.

Is it strange that under such conditions the oystermen have difficulty in keeping opened oysters, especially when tubs and containers are not covered? Contrast these results with a similar set of plates exposed in the bottling room of a clean dairy where sanitary conditions obtained: One-minute exposure, 9 colonies; two minutes, 15 colonies; three minutes, 20; four minutes, 24; and five minutes, 30 colonies.

WASHING OYSTERS.

It is essential that both the water and ice used in washing and cooling shucked oysters should be free from pollution. Natural ice harvested from polluted sources should not be used for this purpose, and even artificial ice may become contaminated by careless handling. A number of samples of water used for washing oysters were examined, and some proved to be satisfactory, while others were unfit for such purposes. The bacteriological condition of water can only be determined by making the necessary examinations. Most large cities have records of the bacterial content of their water supply, and if such waters were being used for washing shellfish the oystermen could inform themselves as to its purity from the records of the city health office.

That the liquor bathing the meat of the oyster contains more bacteria per given volume than does an equal volume of minced oyster meat is shown in Tables 10 and 11. A brief, brisk washing, not to exceed 3 or 5 minutes in duration, is usually sufficient to remove this liquor, and also the adhering sand and grit which may be clinging to the gills of the oyster. It is not necessary to soak oysters over night in order to wash them. Soaked oysters, like floated oysters, deteriorate more rapidly than do those which have not been so treated. Oysters may be washed two or three times before finally reaching the consumer. The total length of all the combined washings should not exceed 30 minutes, and pure iced water should be used. The efficiency of any washing device or method depends largely on its mechanical points, and the amount of soakage taking place will vary with the manner of washing, the relative quantity of oysters and water used, and the temperature of the wash water.

Tables 10 and 11 illustrate the relative bacterial content of the meat and liquor of shellfish, Table 11 being considered with special reference to the development in plain and in saltless agar.

TABLE 10.—*Bacterial content of oyster meat and oyster liquor compared.*

Sample No.	Oyster meats. Bacteria per cubic centimeter after four days' incubation. Plain agar at 25° C.	Oyster meats. Bacteria per cubic centimeter after four days' incubation. Bile salt agar at 37° C.	Oyster meats. B. coli per cubic centimeter.	Oyster liquor. Bacteria per cubic centimeter after four days' incubation. Plain agar at 25° C.	Oyster liquor. Bacteria per cubic centimeter after four days' incubation. Bile salt agar at 37° C.	Oyster liquor. B. coli per cubic centimeter.
1.......	2,800	30	1	55,000	34,000	10
2.......	10,900	90	1	13,500	1,200	10
3.......	3,700	2	1	93,600	30	10
4.......	1,200	6	1	4,200	70	10
5.......	2,000	20	1	3,800	300	1
6.......	7,500	....	0	44,000	....	0
7.......	2,000	....	0	7,500	....	0
Approximate average....	4,300	30	0.7	31,000	7,120	5.9

The results show that the oyster liquor in these samples contained more than seven times as many organisms per given volume as did the minced meat and body contents of the same oysters. The results further show that the liquor contained eight times as many *B. coli* per cubic centimeter as the minced meat.

TABLE 11.—*The relative number of organisms in oyster and clam meat and liquor samples on plain nutrient and on saltless agar at 25° and 37° C.*

Sample No.	Description of sample.	Organisms per cubic centimeter after four days' incubation.				Approximate average.	
		Plain agar 25° C.	Saltless agar 25° C.	Plain agar 37° C.	Saltless agar 37° C.	25° C.	37° C.
1	Oyster meat	23,000	12,000	7,000	21,000	17,500	14,000
2	Oyster liquor	230,000	190,000	60,000	43,000	210,000	51,500
3	Oyster meat	70,000	41,000	26,000	13,000	55,500	19,500
4	Oyster liquor	150,000	60,000	70,000	14,000	105,000	42,000
5	Clam meat	7,000	1,000	3,000	1,000	4,000	2,000
6	Clam liquor	21,000	6,000	2,000	3,000	13,500	2,500
7	Clam meat	110,000	100,000	20,000	11,000	105,000	15,500
8	Clam liquor	180,000	170,000	70,000	85,000	175,000	77,500
	Approximate average	99,000	72,500	32,000	24,000	85,700	28,000

Approximate average of oyster meat at both temperatures..........26,000 organisms per cubic centimeter.
Approximate average of oyster liquor at both temperatures........102,000 organisms per cubic centimeter.
Approximate average of clam meat at both temperatures...........31,000 organisms per cubic centimeter.
Approximate average of clam liquor at both temperatures..........67,000 organisms per cubic centimeter.

The general averages show nearly three times as many organisms per given volume for the liquor of oysters and of clams as for the same volume of meat substance. This explains the fact claimed by practical oystermen that the liquor spoils before the meat of shellfish.

CHARACTER OF CONTAINERS.

The proper cleansing and sterilization of utensils and containers is essential to insure freedom from contamination. Single-shipment cans properly handled are less liable to harbor objectionable bacteria than are those packages used for more than one shipment; however, there is no objection to using the latter if they are free from bacteria. Cans are often returned to the shipper in very bad condition, and unless well scrubbed and sterilized they may become a source of danger. By the use of single-shipment packages and a plentiful supply of boiling water there should be no difficulty experienced from unclean vessels. After cleansing and sterilization cans should be inverted or otherwise protected against the entrance of dust, which nullifies the good done by sterilization.

Progressive oystermen now agree that the best method of shipping oysters is in sealed packages with no ice in contact with the goods. This method prevents contamination from impure ice and protects the oysters in a better manner during shipment than did the old style of tub with ice in contact. The series of shipping experiments made fully demonstrated the superiority of the more modern method.

INSUFFICIENT COOLING.

The proper cooling and subsequent refrigeration of any perishable food product is highly essential to prevent rapid decay from bacterial activity. The results obtained in a series of experiments on the keeping qualities of oysters demonstrate that the proper refrigeration of shucked oysters is necessary to retard deterioration. Even when such oysters are kept at low temperatures above the freezing point there is some bacterial development taking place, but this is reduced to the minimum by a plentiful supply of ice.

SOURING DUE TO AGE.

The souring of oysters may occur because of the length of time they are allowed to stand after shucking. Such oysters may have

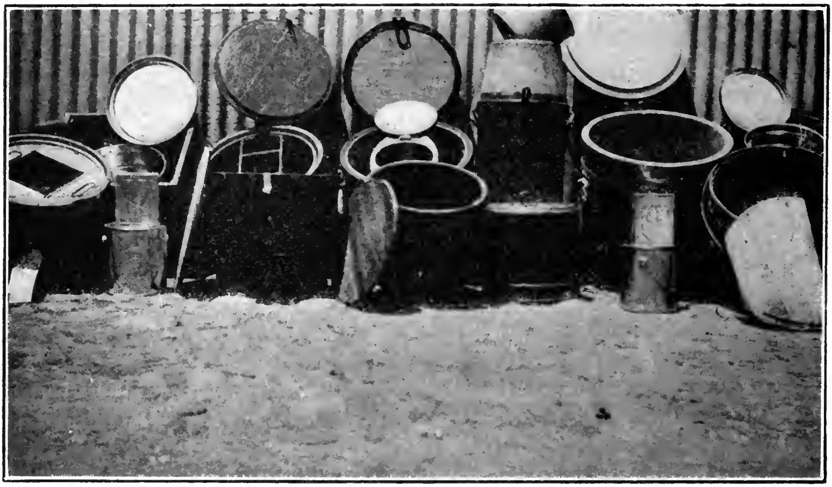

FIG. 14.—Various types of containers for shipping oysters, as used by the trade.

contained but few organisms originally when freshly opened, but because of age alone they become unfit for use. There are always enough bacteria present in oysters even when opened under the best of sanitary conditions to cause spoilage after a certain interval of standing. This length of time will depend upon the number and kind of bacteria present and the temperature at which the oysters are stored. This fact is well illustrated in fig. 15, showing the results of two methods of keeping opened oysters.

Shucked oysters are best when freshly opened, and they should be consumed at the earliest possible moment after shucking, although oysters grown on sanitary beds and handled under proper conditions remain edible for several weeks, when unshucked, but their quality does not improve with the length of time out of the shell. Illness of a serious nature may result from eating stale shellfish.

GRAPHIC PRESENTATION OF RESULTS BY TWO METHODS OF HANDLING.

The graphic chart shown in fig. 15 is a striking illustration of the widely differing results obtained by two different methods of handling the same oysters. Fourteen gallons of oysters from the same lot of shell stock were shucked by the same man, under identical conditions, washed for from three to five minutes in ice water having 16 bacteria per cubic centimeter, divided into two lots, and handled as follows: One lot was placed in uncovered cans with ice in contact and handled in the usual manner by the oysterman on whose premises the work was done, while the other lot was put in clean covered cans and placed in the ice box surrounded by cracked ice which was not in contact with the oysters. The results given in fig. 15 show that the

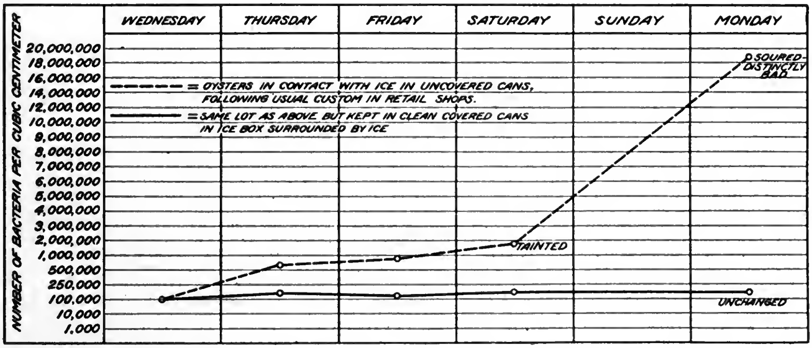

FIG. 15.—Comparison of results obtained by handling the same lot of oysters in two ways. Plotted results are averages of duplicate samples plated on plain agar at 25° C. and on plain agar and bile salt agar at 37° C., for three days.

oysters when kept clean and cold did not decompose or sour within the period of observation, while those not thus treated quickly spoiled and were soon unfit for food.

It subsequently developed that the oysters used were taken from polluted beds, thus partly accounting for the high *B. coli* content, but in spite of this fact the oysters kept under good sanitary conditions showed no appreciable change after five days' storage. Had good oysters been used in the beginning even better results would have been obtained. As it was, even with 10,000 *B. coli* present per cubic centimeter practically no increase took place in the organisms present in the oysters kept in the ice box, while in the other lot they increased to 100,000 per cubic centimeter in five days.

COOKING TESTS.

Five sets of experiments were conducted to demonstrate the value of cooking oysters and clams and the time required to destroy the organisms present by heat. All of the tests were made by exposing the shellfish to live steam (98° to 99° C.) in a steam sterilizer for periods varying from 2 to 30 minutes. About 1 quart of oysters in an Erlenmeyer flask was used for each of the tests on shucked oysters, while about 3 dozen medium-sized oysters and hard clams in the shell were laid on the shelves of the sterilizer for the other experiments. The steamer was full of live steam when the samples were introduced. Tables Nos. 12 to 15, inclusive, show the results of these tests.

TABLE 12.—*The destruction, by steaming, of bacteria in shucked oysters artificially infected with* B. coli.

Sample.	Time of cooking.	Organisms per cubic centimeter (plain agar four days, at 25° C.).	Per cent gas in dextrose fermentation tubes (four days, at 37° C.).						Per cent killed.
			0.1 cc	0.01 cc	0.001 cc	0.0001 cc	0.00001 cc	0.000001 cc	
	Minutes.								
A.......	Uncooked.	80,000,000	....	35	35	30	40	40	0.00
B.......	5	725,000	45	40	45	45	0	0	99.09
C.......	10	20,500	45	40	45	35	40	0	99.90
D.......	15	170	0	0	0	0	0	0	99.99+
E.......	20	100	0	0	0	0	0	0	99.99+
F.......	25	65	0	0	0	0	0	0	99.99+
G.......	30	0	0	0	0	0	0	0	100.00

These results show that all *B. coli* were destroyed during from 10 to 15 minutes' exposure to live steam. In the following experiment on steaming shucked oysters, naturally infected, all *B. coli* types failed to develop when exposed to live steam for from 5 to 10 minutes.

TABLE 13.—*Bacteriological results showing effect of steaming shucked oysters naturally infected.*

Sample No.	Time of cooking.	Bacteria per cubic centimeter (plain agar at 25° C. for three days).	Per cent gas in dextrose three days, at 37° C.			Per cent killed.	Remarks.
			0.5 cc	0.1 cc	0.01 cc		
	Minutes.						
1.......	Uncooked.	716,000	35	20	0	0.00	Variety of colonies on plates.
2.......	5	13,000	50	0	0	98.10+	Less variety of colonies on plates.
3.......	10	2	0	0	0	99.99+	Both colonies spore bearing.
4.......	15	10	0	0	0	99.99+	Spore-bearing colonies on plates.
5.......	20	10	0	0	0	99.99+	Do.
6.......	25	9	0	0	0	99.99+	Do.
7.......	30	5	0	0	0	99.99+	Do.

TABLE 14.—*Bacteriological results showing the effect of steaming oysters in the shell.*

Sample No.	Time of cooking.	Bacteria per cubic centimeter (plain agar at 25° C. for four days).	Per cent gas in dextrose four days at 37° C.			Per cent killed.	Remarks.
			0.1 cc	0.01 cc	0.001 cc		
	Minutes.						
1.......	Uncooked.	15,000	20	5	0	0.00	Variety of colonies on plates.
2.......	5	6,700	1	0	0	55.33	Less variety of colonies on plates.
3.......	10	270	0	0	0	98.20	Colonies largely spore bearing.
4.......	15	250	0	0	0	98.33	Colonies all spore bearing.
5.......	20	40	0	0	0	99.73	Do.
6.......	25	20	0	0	0	99.80	Do.
7.......	30	20	0	0	0	99.80	Do.

These results show that in this instance all gas-producing organisms were destroyed by from 5 to 10 minutes' exposure to live steam. In Table 15 a similar experiment on hard clams in the shell shows that gas-producing organisms survived and were recovered in 5 cc quantities of liquor from clams exposed 20 minutes to live steam. After 5 minutes' exposure, however, 91.11 per cent of the total organisms were killed. The dense shells of hard clams may account for this delayed destruction of bacteria.

TABLE 15.—*Bacteriological results showing the effect of steaming quahaugs (hard clams) in the shell for varying periods.*

No.	Time of cooking.	Organisms per cubic centimeter (plain agar at 25° C. for five days).	Per cent gas in dextrose five days at 37° C.					Per cent killed.	Remarks.
			5 cc	1 cc	0.5 cc	0.1 cc	0.01 cc		
Series A:[a]	*Minutes.*								
1.....	0	4,500	60	1	0	0	0	0.00	Variety of colonies.
2.....	5	400	25	0	0	0	0	91.11	Do.
3.....	10	200	50	0	0	0	0	95.60	Less variety of colonies.
4.....	15	60	60	0	0	0	0	98.67	Largely spore bearing.
5.....	20	40	20	0	0	0	0	99.12	Spore-bearing colonies.
6.....	25	30	0	0	0	0	0	99.34	Do.
7.....	30	20	0	0	0	0	0	99.56	Do.
Series B:									
1.....	0	106,000	65	50	35	35	0	.00	
2.....	2	53,000	20	20	20	0	0	50.00	
3.....	4	2,400	30	0	0	0	0	97.73	
4.....	6	2,000	80	0	0	0	0	98.20	
5.....	8	1,500	0	0	0	0	0	98.70	
6.....	10	1,000	58	0	0	0	0	99.06	

[a] Thirty clams were exposed to live steam in a sterilizer and five were removed for each sample at intervals of five minutes.

These results indicate that 2 minutes' exposure to live steam destroys 50 per cent of the bacteria, but *B. coli* types remained after 10 minutes' exposure, although these germs failed to grow in the sample consisting of five clams removed after 8 minutes' exposure. It appears from these experiments that at least 10 or 15 minutes are required to destroy *B. coli* in small quantities of ordinary market oysters and clams; therefore, the usual methods of cooking shellfish will not remove the danger of infection from disease-producing organisms should they be present. When larger quantities of shellfish are cooked at one time in the same container a sufficient temperature may not be reached within the interior of the mass to destroy the germs thus protected from the action of the heat. In such cases it would be advisable to maintain a high temperature for a longer time. Herdman and Boyce[41] say:

Shellfish must not be taken as a food from grounds where there is any possibility of sewage contamination; after removal from the sea, while in transit, in store, or in market, they should be carefully protected from any possibility of insanitary environment; they should not be kept longer than is absolutely necessary in shops, cellars, etc., in towns, where even if not running the risk of fresh contamination they are under conditions favorable to the reduction of their vitality and growth of their bacterial contents; the fresher they are from the sea the more healthy they are likely to be; finally, only absolutely fresh shellfish should be eaten uncooked, and those that are cooked must be *sufficiently cooked*, raised to boiling point and kept there for at least 10 minutes.

COLLATED OPINIONS ON SEWAGE CONTAMINATION.

TRANSMISSION OF DISEASE BY INFECTED SHELLFISH.

Many workers in this country and in Europe have shown by their researches that shellfish may become contaminated from polluted waters and that serious consequences follow ingestion of such food when consumed uncooked. In this connection it is a noteworthy fact that many of the recorded epidemics of typhoid fever arising from consuming infected shellfish were from oysters which had been subjected to the process of "floating" or "drinking" before being offered for sale.

In his report on shellfish pollution, Fuller [25] credits Dr. Pasquier, a French physician, in 1816, as having first reported an epidemic of typhoid fever due to eating oysters which had been laid down in an old citadel where sewage had been discharged for centuries. Fuller cites other cases as follows:

In Great Britain during the cholera epidemic in 1849 an outbreak of this disease occurred which was considered due to the consumption of condemned oysters; they were, nevertheless, given to school children.

All the members of a supper party of seven at Truro, England (1897), became ill either from typhoid or gastroenteritis due to eating oysters taken from a source known to be polluted.

At St. Andre de Sangonis, France, Dr. Chantemesse reported 14 cases of typhoid and gastroenteritis from six families who ate sewage-polluted oysters.

From 1894 to 1902, Dr. Newsholme, Brighton, England, investigated 643 cases of typhoid fever. He found 158 cases directly ascribable to the consumption of oysters from sources subsequently proven to be polluted.

At Manchester, England, from 1897 to 1902, Dr. Niven ascribed 118 cases from a total of 2,664 cases of typhoid to oysters and mussels, and 156 more cases were associated with the consumption of other shellfish.

The Atlantic City epidemic of typhoid during the summer of 1902 was traceable to oysters and clams "freshened" in sewage-polluted waters.

The investigations of Dr. Soper in 1904 showed that two-thirds of 31 cases of typhoid reported at Lawrence, L. I., were traceable to shellfish taken from polluted sources.

One of the most important and widely known outbreaks of typhoid in recent years due to eating infected oysters occurred at the Wesleyan University, October 12, 1894, at Middletown, Conn. The evidence presented by Professor Conn[15] in his report on this outbreak showed most conclusively that the 23 cases of typhoid which appeared among the 100 students and guests at their fraternity banquets were due to eating infected oysters which had been "fattened" within 300 feet of the outlets of private sewers. He concludes his report by saying:

Doubtless many cases of mysterious typhoid have been due to such a cause. To trace these is a matter of extreme difficulty. The peculiar conditions which have occurred here have been such, however, as to bring the matter into clear light, and to throw with certainty blame of typhoid distribution upon a source which has for some time been suspected, but not demonstrated. That the practice of fattening oysters in the mouths of rivers in the vicinity of sewers is dangerous to the public health is beyond question shown by the combination of conditions which have made it possible to trace the Wesleyan typhoid outbreak to the eating of a lot of infected raw oysters.

In reporting the typhoid epidemics of the mayoralty banquets at Winchester and Southampton, England, November 10, 1902, Dr. Bulstrode[11] cites 21 cases of typhoid and 118 cases of gastroenteritis from a total number of 267 guests who ate raw oysters. The oysters in question were imported from France and "laid down" for a few days in sewage-polluted "drinking" grounds at Emsworth. One patient who developed a fatal case of typhoid only ate *one* infected oyster, while certain of the guests ate only two of their three oysters.

The health officials of New York City,[51] in making a study of 1,786 cases of typhoid fever reported during 1904, in the Borough of Manhattan, obtained data on 1,322 cases. Of this number 22 cases, or 1.6 per cent, were habitual consumers of raw oysters, while 44 cases, or 3.3 per cent, habitually consumed both raw oysters and raw milk.

Dr. Fraser,[22] in speaking of the public health of Portsmouth, England, for 1907, where there was reported 233 cases of typhoid fever, says:

The one article of diet which in this town has a special relationship to typhoid fever is shellfish, and during last year no fewer than 80 persons, or 34 per cent of the total number attacked, contracted typhoid from this source. * * *

It seems that the only thing that can be depended upon to stop this loss of life is legislation, making it illegal to collect shellfish from any places certified by local medical officers to be subject to sewage pollution. Otherwise men are sure to collect and hawk the fish from such places, and the public purchases them not knowing, nor apparently caring, what their previous history has been.

In summarizing his work on shellfish pollution Fuller[25] concludes by saying:

There are those who still believe that polluted shellfish cut very little figure, generally speaking, as regards the public health. Some of these persons appear to have formulated their views without knowledge as to general experiences or the evidence upon the subject. Others for commercial reasons attempt to minimize the evidence, and class it as a whole with some statements and conclusions which are obviously of questionable accuracy. There has been a substantial harmony in the conclusions reached by all who have investigated the subject carefully.

The evidence already presented leaves no room for reasonable doubt that to a limited degree typhoid fever is transmitted by oysters, clams, and some other shellfish which become infected in sewage-polluted waters.

While scientific or medical literature contains little or no evidence to disprove the theory of disease transmission through the agency of infected sea food, it is true that sometimes disease and death are attributed to this source without just cause. For example, in April, 1908, there appeared in many of the New York papers notices of death from typhoid occurring in a family named Bendt residing in Newark, N. J., and the infection was attributed to eating bad oysters. The Department of Public Health of Newark, N. J., stated that the report was absolutely false and the death certificate gave the cause as "cerebrospinal fever." Further inquiry by the food inspector into these cases showed that the victims had not eaten oysters at all. Such reports as these, containing not the least foundation of truth, undoubtedly inflict an undeserved hardship upon an industry of much importance, and every precaution should be taken to substantiate such statements before they are made public.

PRESENCE OF BACILLUS COLI AND BACILLUS TYPHOSUS IN OYSTERS.

The present investigations disclose no reason, biological, anatomical, or otherwise, why oysters and other shellfish can not become contaminated when exposed to sewage-polluted waters, and the following references upheld the conclusion that this occurs.

Klein [38] reports: "*Bacillus coli* (typical) was found in 5 out of 8 cockles." Houston [33] concludes his report on the bacteriological examination of deep-sea oysters by saying: "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 surface water over such oysters."

Smith [62] found *B. coli*, *B. enteritidis sporogenes*, and streptococci in fluid from shellfish grown on grounds suspected to be polluted, but failed to find these germs from areas free from sewage.

Hewlett [30] says: "From my observations I have no hesitation, therefore, in concluding that oysters from water uncontaminated with sewage do not normally contain the colon or allied bacilli or the *Bacillus enteritidis sporogenes*." He examined 32 oysters from different sources, and, with the exception of 2, not one of them contained *B. coli* or *B. enteritidis sporogenes*.

In making an examination of Charles River clams, Dr. Hill [32] says:

These clams contain within their intestines at least three species of bacteria characteristic of sewage. These organisms were not found in the intestines of clams or oysters from less contaminated or uncontaminated waters. The general proposition is accepted, therefore, that food which may be eaten raw should never be exposed to untreated sewage containing the typhoid bacillus nor to uninfected sewage unless the food is of such a character that it can be thoroughly cleansed before it is eaten.

Beale [2] in his work on clams says:

The results of this examination proved that the clams were grossly polluted with sewage, inasmuch as the *B. coli communis* could be detected in $\frac{1}{600}$ and the *B. enteritidis sporogenes* in $\frac{1}{300}$ part of the clam. It is especially noteworthy that even after boiling 15 minutes the *Bacillus communis* could be recovered from the bodies of the clams.

Ewart [19] concludes that mussels can be obtained free from all evidence of sewage pollution, and states further—

That the number of *Bacillus coli* found in the mussels corresponds closely to the environment, hence the mussel can not be regarded as a filter accumulating harmful organisms. * * *

That broadly speaking, a fall in the ratio between organisms growing at 20° C. and those at 37° C. corresponds with sewage pollution. The same may be said with regard to spore-bearing forms.

After completing their investigations, the Virginia State Board of Health[69] reports: "We conclude that the colon bacillus is not found as a normal inhabitant of the oyster, either of the natural fluid of the shell nor of the intestine."

Every one of 34 samples of deep unpolluted sea water, according to Houston,[33] failed to show the presence of *B. coli* or *coli*-like organisms in quantities as large as 100 cc of the sample.

Evidence is produced from Fuller's[25] report showing that fish from unpolluted water do not harbor the colon bacillus, while to the contrary where water is known to be contaminated this bacillus is found in the intestines of fish. The influence of birds, boats, and shoreline railroads is also discussed in this report, which concludes as follows: "Generally speaking we may say that deep sea water distant from local sources is unpolluted according to *Bacillus coli* tests."

Dr. Soper,[63] in reporting the Lawrence, Long Island, outbreak, found that while 20 per cent of oysters were certainly polluted on the inside, as many as 70 per cent were polluted on the outside. Dr. Savage[59] says that "mud samples yield more reliable bacteriological evidence of the degree of contamination of a tidal river than either water or oyster samples. Muds which show high relative purity are safe for oysters."

Professor Huxley is quoted as saying in the report of the Royal Commission on Sewage Disposal:

I do not see how it can be doubted that oysters taken from a bed irrigated with sewage and eaten uncooked would be dangerous articles of diet. Does anybody pretend that it would be safe to take drinking water (unfiltered and otherwise unpurified) from a body of fresh water, of similar dimensions to any estuary which may be under consideration, at a point equally near a sewage discharge? If such a proceeding is safe, our sanitary authorities are taking a great deal of trouble in vain; if it is not safe neither is it desirable to eat oysters the juices of which are impregnated with sewage in however dilute a condition.

This commission further recommends that each Government should require a guarantee that all oysters or other shellfish imported into their country for human consumption had been procured from localities where they were not liable to contamination by sewage or other objectionable filth.

In their work on the study of shellfish Clark and Gage[14] state:

Enough study has been made by many investigators to show that *B. coli* is not a normal inhabitant of the intestines of clams or oysters, and that its presence in the intestines or juice in the shell must be due to contamination, either by drainage and sewage flowing over the clam and oyster beds, or by careless and uncleanly handling of the shellfish between the time of collecting and placing upon the market. In this work, therefore, 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."

Fuller [23] concludes his work on oysters from the Narragansett Bay by saying:

The results obtained in these experiments indicate that *B. coli* is not normally found in sea water or in common edible shellfish, and that the presence of this organism in oysters, clams, mussels, and similar shellfish is an indication of sewage pollution.

VITALITY OF BACILLUS COLI AND BACILLUS TYPHOSUS IN SEWAGE-POLLUTED WATER AND IN SHELLFISH.

The results obtained by different investigators on this point vary somewhat according to the conditions under which the experiments were conducted: the vital fact, however, is that *B. coli*, *B. typhosus*, and other organisms do survive sufficiently long, under favorable conditions, in sewage-polluted waters to transmit disease when such water is consumed, whether directly, or indirectly by such a medium as shellfish.

Savage [59] says:

Owing to the enormous difficulties inherent to the isolation of the typhoid bacillus from bacteriologically complex substances such as highly polluted tidal mud it would be rash to draw sweeping deductions from negative results, but * * * it seems justifiable to infer that typhoid bacilli can survive in polluted muds for at least two weeks, and this fairly readily, but that after about two weeks they may rapidly decrease, although they may, and probably do, persist under favorable conditions for some little longer, but in vastly diminished numbers. Experiment (3) seems to definitely show that they may survive for at least three weeks.

McNaught [42] concludes that "the duration of life of *B. coli* in unsterilized water varied greatly in waters from different sources. The purer the water the longer did *B. coli* survive in it." He further says:

In unsterilized sewage *B. coli* only survived for three weeks, while it survived over eight months in the same sewage sterilized. After six months' growth in sterilized water and eight months' growth in sterilized sewage *B. coli* retained all its original characters except that possibly its power of indol production was weakened.

The Lancet,[37] in reviewing some experiments and observations on the vitality of *B. typhosus* in oysters made by Klein, says:

The actual results detailed in the report, though valuable, are not particularly novel. They definitely settle the question as to whether the *Bacillus typhosus* will live in the oysters. It is demonstrated that there is destruction of the *Bacillus typhosus* both in the body of the oyster and in sea water; that an oyster infected with large numbers of typhoid bacilli "cleans" itself in about from 9 to 12 days when placed in clean water which is frequently changed; and that oysters kept in the dry state, though capable of destroying the bacillus, yet remain polluted for a much longer period than oysters placed in constantly changed clean water.

The investigations of Dr. Buchan [10] show that typhoid bacilli survived in mussels for at least 26 days where the organisms were found abundantly; he concludes by saying: "This experiment emphasizes the need of protecting mussel layings from all possibility of sewage contamination."

Herdman and Boyce [28] found that 14 days was about the average duration of the life of the typhoid bacillus in sea water incubated at 35° C.; when kept in the cold, their presence was demonstrated on the twenty-first day. They further state:

The bacillus probably does perish in a short time in the sea, just as it does in sea water in the laboratory, but we have no evidence before us to show how it is so in the case of the mud upon which the oysters may be laid; hence it is possible that the bacillus might be capable of a saprophytic existence. * * *

In our experiments in washing infected oysters in a stream of clean sea water the results were definite and uniform; there was great diminution or total disappearance of the typhoid bacilli in from one to seven days.

Martin [43] shows that typhoid bacilli survive for at least 12 days in unsterilized soils when kept in a moderately dry condition at a temperature ranging from 2° C. to 12° C.

Klein [39] states: "At the end of three weeks, a sample from the *Bacillus coli* sewage flask still yielded on culture innumerable colonies, whereas a sample from the flask inoculated with the typhoid bacillus yielded 12 colonies."

Houston [34] in a recent report shows that 99 per cent of the typhoid bacilli added to water died within one week. In a majority of the experiments, however, a few typhoid bacilli remained alive for one or two months.

The results obtained in this laboratory on the vitality of *B. coli* in unsterilized, but practically sterile, spring water at room and ice-box temperature showed that this organism survived only for about one month at room temperature, but remained alive after two and a half months at ice-box temperature (about 15° C.). The experiment was conducted by inoculating quart bottles of a high grade unsterilized spring water containing practically no organisms with 0.1 cc of a 24-hour bouillon culture and pouring plates at frequent intervals. The bottles during this time were kept unexposed to light.

SIGNIFICANCE OF SEWAGE ORGANISMS IN FOOD SUPPLIES.

In the light of our present sanitary knowledge the presence of appreciable numbers of *B. coli* in any water, food, or drug product is looked upon as positive evidence of fecal contamination. It is true that various workers have reported finding this germ from many sources other than the excrementitious matter of animals. Aside from this investigation there has been isolated in pure culture the colon bacillus from the following materials: Sewage, water, ice, milk, cream, ice cream, butter, buttermilk, sour milk tablets, oysters, clams, flour, corn meal, wheat, oats, eggs (dried and frozen), wormy peanuts, and moldy dried fruits. In nearly every instance definite information was obtained by inspection showing that the product had been exposed to conditions favorable to fecal contamination. Occasionally data in regard to the probable sources of pollution of a given food product can not be obtained, but as the work progresses and opportunity is afforded to study these problems from various points of view, it becomes more apparent that the colon test is invaluable as an index of pollution

Aside from being a mere indication of existing danger from sewage pollution, the colon bacillus is fast assuming the rôle of a pathogenic organism. Various intestinal derangements and other visceral disorders are now ascribed to *B. coli* infection. In a recent article Thompson [66] presents evidence substantiating the relationship of *B. coli* to certain pathological processes of the abdomen, such as appendicitis. Under normal conditions the colon bacillus appears to exist as a harmless organism in the intestinal tract, but in the presence of irritating substances or under abnormal putrefactive and fermentative conditions this bacillus seems to become an active factor in the causation of disease. The author has recovered a virulent strain of the colon bacillus and reproduced fatal infections in dogs, cats, guinea pigs, and pigeons. These results usually resulted from inoculating the animals with small quantities of a 24-hour bouillon culture, although in some cases infection was brought about by feeding material infected with the organism. The colon bacillus probably plays a further rôle as a pathogenic organism by producing toxins in albuminous food materials of a highly perishable nature. In such a product as shucked oysters, if there is a high bacterial count with thousands of *B. coli* present per cubic centimeter of the oyster liquor, it is highly probable that toxic substances have already been elaborated. With the consumption of such material gastroenteritis to some degree must invariably follow. Undoubtedly many obscure cases of ptomain poisoning and other intestinal disorders of lesser magnitude could be ascribed to eating tainted food, especially if the substances are rich in albumin and bacterial activity has appreciably developed.

In summarizing her work on the chemistry of the colon bacillus, Leach [40] gives the following analysis, showing the presence of toxins together with other complex substances in the cell of the colon bacillus:

Elementary analyses show that age, conditions of growth, and especially the composition of the nutrient medium cause bacteria of the same strain to differ widely in elementary composition. Proteid, nucleo-proteid, nucleic acid, protamin, fat, wax, lecithin, glycogen, and other carbohydrates have all been reported as obtained from the bacterial cell in varying degrees of purity. Cellulose seems to be present in certain species, but by no means in all. Besides the preparations mentioned above, crystalline compounds have been prepared and purified, proving the presence in the cell of xanthin bases, pentose, fatty acids, and perhaps thymin and uracil, toxins, enzyms, and agglutinins have been split off from the cell, but more progress has been made in determining their physiological action than their chemical nature.

In regard to *B. coli* as evidence of sewage pollution, quotations from the following authors are cited:

Mason [44] says:

Water which persistently shows *B. coli* in 1 cc sowings is of very questionable character, and, should similar results be found when operating with sowings of 0.1 cc, the water should be condemned.

Regarding the colon bacillus, Dr. Abbott [1] says:

In the normal intestinal tract of human beings and domestic animals, as well as associated with the specific disease-producing bacillus in the intestines of typhoid-fever patients, is an organism that is frequently found in polluted drinking waters, and whose presence is indicative of pollution by either normal or diseased intestinal contents; and though efforts may result in failure to detect the specific bacillus of typhoid fever, the finding of the other organism, *Bacillus coli*, justifies one in concluding that the water under consideration has been polluted by intestinal evacuations from either human beings or animals. Waters so exposed as to be liable to such pollution should never be considered as other than a continuous source of danger to those using them.

In their work on water analysis, under this subject Prescott and Winslow [56] conclude as follows:

Although the evidence is quite conclusive that the absence of *B. coli* demonstrates the harmlessness of water as far as bacteriology can prove it, that when present its numbers form a reasonably close index of the amount of pollution the authors above quoted have proved beyond reasonable cavil. It may safely be said that when the colon bacillus, as defined by the tests above, is found in such abundance as to be isolated in a large proportion of cases from 1 cc of water, it is reasonable proof of the presence of serious pollution.

In speaking of the bacterial content of drinking water, Jordan [36] says:

The most widely used and, by general consensus, the most valuable of these tests is the "colon test." This is based upon the circumstances that the colon bacillus, *B. coli*, is a common inhabitant of the human intestine, and is found in great abundance in sewage.

McNaught [42] states that—

The detection of *B. coli* in a small quantity of a drinking water is a sign of danger because it indicates excretal contamination, and where excretal contamination occurs there is a risk that the excreta may contain specific germs of disease.

According to the views of Houston [33]:

The continued persistence of *B. coli* in any number in estuarial water may be traced by continuous excremental pollution and the presence of the unoxidized organic pabulum in the water.

In his bacteriological report, Connolly [16] says:

Regarding the presence of *B. coli* in water used for drinking purposes, we have learned to look with suspicion upon water which contains this bacillus in quantities of $\frac{1}{5}$ of a cubic centimeter, or less, even though the source of the water is apparently above suspicion. This applies to surface waters, such as are usually collected in sparsely inhabited watersheds, when there is a greater possibility of colon pollution from animals than from man. In deep well water the presence of *B. coli* to any extent should positively condemn the supply.

The examination made of water and food samples for *B. coli* has resulted in diagnosing the presence of this organism only after isolation and making a thorough study of its morphological and biological characters; even under these conditions many minor differences are found as to the quantity and character of gas produced, the time required for coagulating milk, the amount of indol and nitrites formed, its reaction on various culture media, and other detailed considerations.

Unless isolated and carefully studied in pure culture, no reliable means are yet available for distinguishing the true colon bacillus, by presumptive tests alone, from other gas-forming organisms, such as *B. lactis aerogenes*, *B. cloacæ*, etc. However, the presence of any of these organisms in water is indicative of fecal matter.

There seems to be no question regarding the undesirability of the presence of the colon bacillus and allied organisms in water used in any way for food purposes. The relation of this germ to the pollution or purity of water supplies seems to be now pretty well established and there can be no doubt that water harboring this germ may be a constant source of great danger, for where *B. coli* is found the specific cause of enteric fever, namely, *B. typhosus*, and also the cholera spirillum, etc., may be present in sufficient numbers to produce infection when introduced into susceptible individuals.

SUMMARY.

(1) There is undisputed evidence to show that shellfish become contaminated when placed in sewage-polluted water, and that *B. coli* and *B. typhosus* will survive for variable lengths of time in the liquor and the body contents of such shellfish after their removal from infected water.

(2) The presence of sewage organisms in oysters and other shellfish, even in small numbers, may be indicative of great danger; for, where such organisms exist, the specific cause of enteric fever and allied disorders may also be found.

(3) The results of many investigators show that sewage-polluted shellfish have been responsible for the production of typhoid fever and other intestinal diseases. The most noteworthy cases appear to have occurred from eating oysters which had been floated in sewage-polluted water, although instances are cited where shellfish infected by polluted water, either in their natural or artificial beds, have also been the vehicle of disease transmission.

(4) The shellfish industries of this country are extensive and important, comparing favorably with other industries concerned with the production of food materials. A valuable article of food is furnished to millions of people by these industries, and thousands of

individuals find profitable employment in developing and carrying on this business in all its phases.

(5) The indiscriminate introduction of sewage into our natural bodies of water is now the greatest enemy to the shellfish industries. In order to correct this evil it will be necessary to prevent further pollution of our waters, or else to remove the shellfish industries from the grounds subject to pollution.

(6) Oyster beds should be protected from every possible source of contamination, and they should be located in water proven to be pure by repeated examinations. These examinations should consist of careful bacteriological and chemical analyses of both the water and oysters from oyster layings. The laboratory findings should also be supplemented by systematic inspection of all the territory which could in any wise affect the condition of the water flowing over the oyster beds.

(7) The practice of floating oysters in water of questionable purity should be absolutely prohibited because of the probability of sewage contamination. When it is desired to remove the gross filth from the exterior of the shell, oysters may be floated and allowed "to cleanse themselves" in suitably constructed devices in waters free from pollution, and containing no less salt than the water in which they will grow to maturity.

(8) Like other perishable food products, oysters may become unfit for use if stored or kept under insanitary conditions. This spoilage, however, may take place wholly from the length of time out of water.

(9) Oysters removed from pure beds may become contaminated during the process of shucking or preparation for the market in insanitary shucking establishments. These places should be constructed in a sanitary manner and provided with satisfactory appliances for the proper cleansing and sterilization of utensils used for shipping oysters. Without such devices it is almost impossible to prepare packages in a sanitary manner. This is particularly true when cans, barrels, or containers of any kind are used a second time without proper cleansing and sterilization. When contaminated these unsterile vessels may become active agents for the dissemination of disease-producing organisms.

(10) The liquor in the shell surrounding the oysters contains more bacteria than does an equal volume of meat from the same oyster. This liquor, together with any sand in the gills of the oyster, can be removed and the meat chilled at the same time by the use of pure ice and water. This washing process can be done efficiently within 3 to 10 minutes, depending upon the method employed. Oysters should not be allowed to soak in fresh water, as they increase in volume, change in appearance and flavor, and decompose more rapidly than those not soaked.

(11) Steaming contaminated oysters and clams in the shell, or cooking them after shucking for 15 minutes at boiling temperature, practically destroys all organisms of a questionable character, but since in practice shellfish are never cooked for this length of time, cooking can not be depended upon to remove this danger.

(12) Oysters intended to be eaten on the half shell, above all others, should be produced from beds of unquestionable purity, and they should be consumed preferably while fresh from the beds; although if properly kept at cool temperatures under sanitary surroundings shell oysters may remain wholesome and in good condition for several weeks after dredging.

(13) The investigations show that vast areas of valuable shellfish grounds in this country are now reasonably free from sewage pollution, but this territory will gradually diminish in size if sewage is not properly cared for in the future. Comparatively speaking only a small acreage is now subject to serious pollution. Active steps are now being taken in some instances to overcome this difficulty; however, it is not the satisfactory conditions which require regulation and future protection, but those places which are polluted at the present time, and yet are being used for the cultivation and sale of shellfish. The presentation of these facts should stimulate every citizen and health official alike to see that the wastes under their own jurisdiction are not adding to the difficulty of keeping our natural bodies of water and the shellfish therein free from contamination.

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