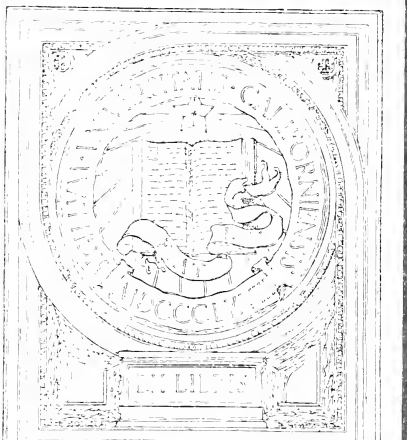
RA
602
09R4

1914

UC-NRLF

B 3 653 787



BIOLOGY
LIBRARY
G

State of Rhode Island and Providence Plantations

CONTRIBUTIONS

TO THE

Bacteriology of the Oyster

THE RESULTS OF EXPERIMENTS AND OBSERVATIONS MADE WHILE CONDUCTING AN INVESTIGATION DIRECTED AND AUTHORIZED BY THE COMMISSIONERS OF SHELL FISHERIES OF THE STATE OF RHODE ISLAND.

BY

LESTER A. ROUND, PH. D.

PROVIDENCE:
E. L. FREEMAN CO., STATE PRINTERS,
1914

UNIV. OF
CALIFORNIA

State of Rhode Island and Providence Plantations

CONTRIBUTIONS

TO THE

Bacteriology of the Oyster

THE RESULTS OF EXPERIMENTS AND OBSERVATIONS MADE WHILE CONDUCTING AN INVESTIGATION DIRECTED AND AUTHORIZED BY THE COMMISSIONERS OF SHELL FISHERIES OF THE STATE OF RHODE ISLAND.

BY

LESTER A. ROUND, PH. D.

PROVIDENCE:
E. L. FREEMAN CO., STATE PRINTERS,
1914

PUBLIC
HEALTH
LIBRARY

TABLE OF CONTENTS.

BIOLOGY
LIBRARY
G

PREFACE.

In March, 1910, the Commissioners of Shell Fisheries authorized an examination of the sanitary condition of the waters of Narragansett Bay and its tributaries, relative to the growing of oysters. They placed Prof. F. P. Gorham, head of the Department of Bacteriology of Brown University, in charge of this work, with the writer as an assistant.

On beginning this work it was found that there were many problems that would require more study than could be given while performing the routine bacteriological examinations of water, mud, shellfish, etc. To a great extent this work was new and the method of procedure had to be worked out as the investigation progressed. While there had been much work performed upon shellfish examinations, both abroad and in this country, there were still many problems which had not been solved and it was deemed advisable by the Commission that some of these problems which were of great importance to the shellfish industry should be given special attention.

The writer was early assigned to conduct a series of experiments and investigations along the lines that had been found would apparently prove of the greatest advantage to the oyster industry. The results of some of these investigations is published in this booklet.

The writer wishes to take this opportunity to express his sincerest thanks to Prof. F. P. Gorham, head of the Bepartment of Dacteriology of Brown University, whose valuable advice and criticisms have been exceedingly helpful and under whose direction the work herein reported has been done; to Drs. A. D. Mead, H. E. Walter and P. H. Mitchell, who have made valuable suggestions and criticisms on different points in the work; to the members of the Narragansett Bay Oyster Company, the American Oyster Company, the Wickford Oyster Company and the Beacon Oyster Company, and to Captain William B. Welden, all of whom have rendered valuable aid in carrying out many of the experiments; also to Mr. W. B. Mason of The Merchants' Cold Storage and Warehouse Company who has given free use of the company's cold storage rooms for the experiments on hibernation.

L. A. R.

Brown University,
May 1, 1914.

D11408

THE BACTERIOLOGY OF THE CLOACAL AND GILLS CHAMBERS OF THE OYSTER.

In describing the anatomy of the gills of the oyster, Kellogg in his book on "Shellfish Industries" makes the following statement: "Behind the body the four gills unite so as to separate a space above the cloacal chamber, from the large mantle chamber below." From this statement it has been assumed at times that the two chambers were entirely distinct and so constructed that bacteria could not pass from one chamber to the other, and that for this reason the bacterial content of the two chambers would differ. Anyone familar with the anatomy of the oyster knows that every day several gallons of water are filtered through the gills into the cloacal chamber. While it is probable that most of the protozoa and algæ are caught in the mucus which the gills secrete, it is also probable that a great many of the bacteria escape, being entrapped by the mucus and pass on into the cloacal chamber. But even though the gill-filter were proven to be bacteria-proof no one has demonstrated that bacteria cannot pass along the space between the mantle and the shell, or around the edge of the shell, between the flaps of the mantle and so pass from one chamber to the other. While it seems very probable from the structure of the oyster that bacteria can pass fron one chamber to the other without difficulty, properly conducted experiments are necessary to prove it. In order thus to prove that bacteria can and do pass, from one chamber to the other, the following experiments were tried.

SERIES 1.

Exp. 1. A well shaped mature oyster about four inches long and two broad was selected. Care was taken to obtain an oyster with a flat right valve. The oyster was placed in a frame with the left valve down so that the right valve was level and was then clamped firmly to the table. A hole was bored through the right valve into the gill chamber quite close to the edge and another into the cloacal

UNIV. OF
CALIFORNIA

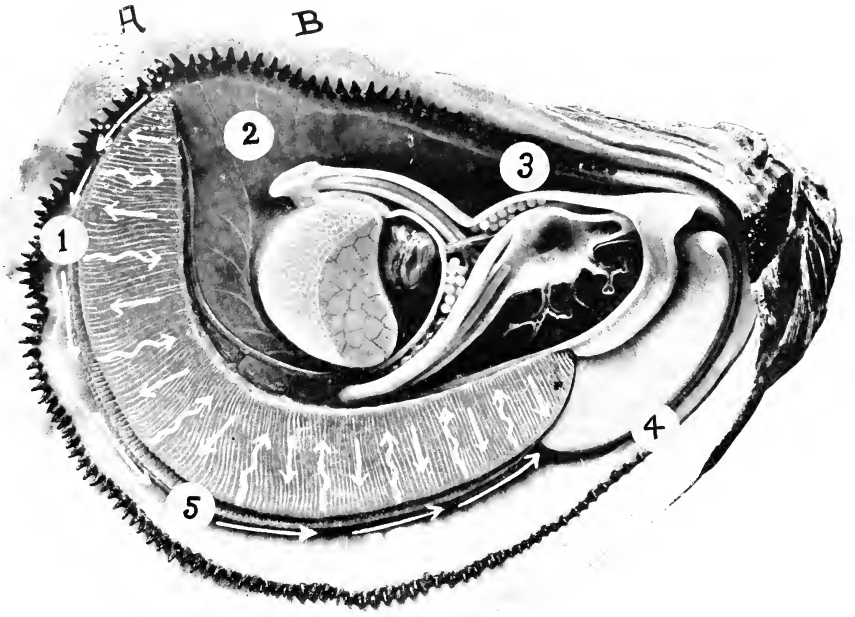


Plate Showing Position of Holes Bored in Shell of Oyster and Arrows Showing Direction of Ciliary Currents.

Photo by kindness of Mass. Fish and Game Commission.

chamber, at the anal orifice. This latter opening was $\frac{1}{2}$ to $\frac{3}{4}$ of an inch above the lower end of the partition which separated the two chambers. A loopful of B. prodigiosus was then placed in the gill chamber, and loopfuls were removed from the cloacal chamber and plated at intervals of ten minutes for one hour, and then at the end of two hours and three hours. B. prodigiosus is a non-motile organism and was chosen because of its ease of identification and because in all our work extending over four years we have never isolated it from oysters.

No red colonies were found in the two control samples from the two chambers, but every plate made from the cloacal chamber after the introduction of the B. prodigiosus into the gill chamber showed colonies of B. prodigiosus.

Exp. 2. The above experiment was repeated with another oyster and B. prodigiosus was again found in the cloacal chamber ten minutes after its introduction into the gill chamber.

SERIES II.

Exp. 1. In another set of experiments four oysters were used. In these oysters five holes were bored as indicated in the plate shown on opposite page. Three of these holes opened into the gill chamber (1, 4, 5). Another hole (2) was made into the cloacal chamber near the anal orifice, and the last hole (3) opened on the edge of the mantle about an inch above the anal orifice.

All four of these oysters were inoculated in hole No. 5 with a loopful of B. prodigiosus. Loopfuls from the other holes were inoculated upon agar slants at two minute intervals, for ten minutes and then every five minutes, for twenty minutes, making a total of 30 minutes in each case. The result is seen in the following table:

TABLE NO. 1.

Showing the time at which B. prodigiosus was isolated from the different holes after inoculation of the gill chamber at hole No. 5.

Oyster No.	1				2				3				4			
Hole No.	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2 min	0	+	0	+	0	0	0	+	0	+	0	0	0	0	0	+
4 "	+	+	+	+	+	+	+	+	0	+	+	+	+	+	0	+
6 "	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+
8 "	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+
10 "	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+
15 "	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20 "	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+
25 "	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30 "	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+

+ = presence of B. prodigiosus. 0 = absence of B. prodigiosus.

From this table it is seen that in oysters Nos. 1 and 2 B. prodigiosus was isolated from all the holes at the end of four minutes; in oyster No. 3 at the end of six minutes, and in oyster No. 4 not until the end of fifteen minutes. Oyster No. 4 was a long narrow oyster and hole No. 3 in this case was necessarily moved further into the middle of the oysters, so that the opening came nearly over the stomach, and so did not reach the cloacal chamber. In the other cases, hole No 3 was made nearer the edge of the oyster and close to the free edge of the mantle, so that there was much greater chance of bacteria reaching the hole from the liquor between the free edges of the mantle, for the edges of the mantle are everywhere free except, at the "head" end, where the the edges fuse and form a hood, which is attached to the body by a flap of tissue. Between the free edges of the mantle and between the hood and the body there is a space which extends around the whole oyster and forms a kind of moat or trench filled with the liquor in which bacteria can and do move by the currents set up by the ciliary mechanism of the oyster, which will be described a little later. It will also be noticed that in every oyster of this series B. prodigiosus was isolated from hole No. 4 before they were from hole No. 1. although the distance between holes Nos. 4 and 5 were nearly twice as far as

between Nos. 1 and 5. It will further be noted that in oysters Nos. 1 and 3 B. prodigiosus was isolated from the cloacal chamber (hole No. 2) before it was found in hole No. 1, although the distance between holes Nos. 5 and 2 was also about twice as far as between 5 and 1. In no case did B. prodigiosus appear at hole No. 1 before it did at No. 2.

SERIES III.

A third set of experiments were now performed in order to show that bacteria can pass from the cloacal chamber to the gill chamber and to ascertain, if possible, the avenue through which this takes place. In this experiment two oysters were used and secured to the bench in the same manner as in the previous experiments. Three holes were bored into the branchial chamber as indicated by the Nos. 1, 5 and 4 in the plate. One hole was bored into the cloacal chamber as indicated by No. 2 in the plate. Control inoculations were made as before from these four holes. These showed no colonies of B. prodigiosus. A loopful of B. prodigiosus was placed in hole No. 2, and loopfuls were taken from holes Nos. 1, 5 and 4 at intervals of two minutes for fourteen minutes. The results are shown in table No. 2.

TABLE NO. 2.

Showing the time at which B. prodigiosus was recovered from holes Nos. 1, 5 and 4 after inoculation of the cloacal chamber at hole No. 2.

Oyster No.	1			2		
Hole No.	1	5	4	1	5	4
Control	0	0	0	0	0	0
2 minutes	0	0	0	+	0	0
4 "	0	0	0	+	+	+
6 "	+	0	0	+	+	+
8 "	+	+	0	+	+	+
10 "	+	+	+	+	+	+
12 "	+	+	+	+	+	+
14 "	+	+	+	+	+	+

+ = presence of B. prodigiosus. 0 = absence of B. prodigiosus.

From this table it can be seen that B. prodigiosus was isolated from all the holes in oyster No. 1 at the end of ten minutes and in oyster No. 2 at the end of four minutes. It is further seen that in oyster No. 1 B. prodigiosus appeared first at hole No. 1, two minutes later at hole No. 5, and after another interval of two minutes at hole No. 4. In oyster No. 2 the bacillus appeared first at hole No. 1 and two minutes later at holes Nos. 5 and 4. In neither case did B. prodigiosus appear at holes 5 or 4 before it appeared at hole No. 1, nor in either case at hole No. 4 before hole No. 5.

To understand the reason for these results a description of the ciliary mechanism of the oyster is necessary.

When one opens an oyster without mutilating it, there is found between the two flaps of the mantle four folds of tissue which are the gills. These folds appear solid, but are really flaps folded back upon themselves and attached by the edges to the body so that really each gill is V shaped in cross section and the four gills form a double W (WW). With the unaided eye it can be seen that there are fine striations running vertically across each gill. These are the gill filaments. If we examine these filaments with a microscope we will see innumerable hairs or cilia about 1–500th of an inch long or less, waving vigorously back and forth. If we examine the cilia closely we find that they lash vigorously in one direction, recover themselves slowly and repeat the vigorous stroke. The movement is quite comparable to a man rowing a boat. He pulls vigorously in one direction, recovers himself and repeats the stroke. Now if we consider the boat fastened so that it could not move, the oarsman's efforts would send the water past the boat instead of propelling the boat through the water. Here we have the exact condition in the oyster. As Brooks (The Oyster, 1906) says, these little hairs "set up a current in the water. Each one is so small that its individual effect is inconceivably minute, but the innumerable multitude causes a vigorous circulation and each one is set at such a position that it drives the water before it from the gill chamber into one of the water pores and so into one of the water tubes inside the gill. As these are filled they overflow into the cloacal chamber and fill that." This set of cilia are located on the edges of the filaments and force the water through the gills from the branchial into the cloacal chamber. There is another set of cilia which wave in the opposite direction and by means of the mucus which is secreted by the mucus cells, they collect and entangle the micro-organisms and carry them over to the free edge of the gill

where a third set of cilia located on the very edge of the gill conveys the entangled organisms on the mouth. The arrangement of these last two sets of cilia can be seen in the plate. In this diagram the bent arrows show the course of the water through the gills into the cloacal chamber. The straight arrows indicate the course of the mucus and the entangled micro-organisms to the mouth.

When the valves of the oyster are open the current induced by the cilia is carried out of the oyster between the points "A" and "B." When the valves of the oyster are closed, however, the cilia keep waving as vigorously as before, because the oyster has no control over their movement, but in this case the current cannot pass out between the valves and we have what might be called a closed circulation. Instead of going out between the points "A" and "B," as is the case when the valves are open, the current must neccessarily return to the gill chamber around point "A," for a study of the currents induced by the cilia and taking the direction indicated by the arrows shows that no other course is possible. All the cilia of the cloacal chamber direct their motion towards point "A" and "B." All the currents in the branchial chamber are either through the gills into the cloacal chamber or along the edge of the gills to the mouth. As water is driven through the gills to the cloacal chamber water from the cloacal chamber must necessarily take its place. As point "A" is the point of least resistance the water necessarily passes from the cloacal chamber to the gill chamber around that point and further not only is there nothing to obstruct this current, but the current induced by the cilia on the edge of the gills is such that it would draw the water from the cloacal chamber into the gill chamber around this point. Hence we see that in the oyster we have a complete cycle of currents induced by ciliary motion. The result is that all the water in the oyster is filtered through the gills many times in an hour and the process is repeated every few minutes.

It happens that when bacteria enter the gill or branchial chamber, two courses are open. They may follow the currents through the gills into the cloacal chamber or they may become entangled in the mucus of the gills and be conveyed along the edge of the gills to the mouth. The chances of a bacterium going in either of these courses are about equal and if many bacteria are present some may go by one course and some by the other.

A study of table No. 1 will show that the B. prodigiosus followed both of these courses, some were entangled in the mucus and were

carried to the mouth (hole No. 4) while others escaped the mucus and passed through the gills into the cloacal chamber (hole No. 2). A further study of table No. 1 will show that the bacteria passed with the currents for this particular bacterium was non-motile and so could not have reached the different points by its own activity. Moreover, the interval of time which separated the inoculation of the branchial chamber and the subsequent recovery of the bacterium from the different holes in series II was only 4 minutes in all, except two cases when it was six and fifteen minutes. The distance between holes 5 and 4, 5 and 2, and 5 and 3, in all cases was at least an inch, in most cases, more. In series III the bacterium was recovered from all the holes in four minutes in one case and ten minutes in the other. The rate of travel of bacteria varies with the species, temperature, etc., but it is inconceivable that a bacterium of the speediest variety could move a distance of over an inch in four minutes by its own activity. In the case in hand, *i. e.* a non-motile bacterium, it is out of the question.

It is also seen in table No. 1 that in two cases B. prodigiosus was isolated from hole No. 2 before it was recovered from hole No. 1. In the two other cases they were recovered at the same time. While this is not conclusive it leads the writer to believe that the bacteria isolated at hole No. 1 had previously passed through the gills and the cloacal chamber and back into the branchial chamber by the return current. The results of the experiments in series III lend support to this view. The bacteria did not go directly from hole 5 to hole 1 because the currents along the edge of the gills is too strong to allow a bacterium to pass in that direction. An examination of this current under the microscope will convince anyone that a bacterium could not travel in that direction.

A study of table No. 2, which shows the appearance of B. prodigiosus in the gill chamber after the inoculation of the cloacal chamber, shows that the organisms appeared at hole No. 1 and later at hole Nos. 5 and 4. This is the order of time in which a current from the cloacal chamber and taking the direction of the arrows of the edges of the gills would appear at holes Nos. 1, 5 and 4, in the branchial chamber.

From the foregoing facts it is plain that the gills are not bacteria proof; that bacteria can and do pass from the gill chamber to the cloacal chamber through the gills and moreover, that bacteria may pass from the cloacal chamber to the gill chamber without passing through the gills. It is seen that we have a complete circle of currents

within the closed shell of the oyster which, under the conditions of the experiments, makes a complete circuit several times in an hour, and thus ensures a thorough mixing of the water and the bacterial content of the two chambers. In the conditions of the experiments the complete circuit was made in at least six minutes and in three cases in so short a period as four minutes. It naturally follows that any difference of bacterial count between the two chambers is not to be expected and such differences as are observed are within the limits of experimental error.

METHODS OF SHELLFISH EXAMINATION.

As soon as sufficient epidemiological evidence had been accumulated to show conclusively that oysters are under certain circumstances contributing factors in the spread of typhoid, Asiatic cholera and other gastro-enteric disturbances, it was but natural that bacteriologists should look for the specific cause of these diseases in the oysters themselves. If the typhoid bacillus and the spirillum of Asiatic cholera could be found in oysters, that would be evidence which no one could dispute. Although diligent search has been made for the typhoid bacillus in oysters on numerous occasions since 1893, it is interesting to note that there are on record four instances only in which B. typhosus has been reported to have been isolated from oysters. The first instance was reported by Klein.[1] Regarding this finding Klein says:

"In view of the importance likely to be attached to the finding of this bacillus in such numbers in one of these East Coast oysters, particular care has been exercised in subjecting it to every possible test . . . As a result, in all and every one of its characters it coincides with the typhoid bacillus obtained from the spleen of a typical case of typhoid fever, and for this reason I am prepared to affirm that this bacillus obtained from the "Deep Sea" oyster is the typhoid bacillus." Besides the cultural tests used, the Bordet-Durham reaction (macroscopic agglutination with immune serum 1:100) and Pfeiffer's phenomenon were also used and both proved positive while the controls in both instances were negative. In this instance the evidence seems quite sufficient to support Klein's assertion.

[1]Report of the Medical Officer to the Local Government Board, 1894-5. Supplement, Appendix No. 2, p. 115.

The second instance is cited by Fuller:[1] In 1902 at a meeting of physicians at Pera, Turkey, it was reported that a large percentage of the typhoid cases occurring in Constantinople could be traced to the consumption of polluted oysters and an examination of the oysters in a few instances showed the presence of B. typhosus. The writer has not been able to obtain the reference to this paper and the characteristics of the species have not been studied. As a result no definite comment can be made upon the findings in this instance.

The third instance is reported by Johnstone.[2] There is not so good evidence to support the identity of this bacillus as in the case reported by Klein. Johnstone's bacillus "formed acid and gas in bile salt glucose broth" and a "a slight discoloration in lactose litmus broth" and "agglutinated—in a dilution of one to thirty—in a serum which gave a positive reaction with a known strain of bacillus typhosus." All authorities are agreed that the typhoid bacillus produces no gas in any sugar medium. In regard to the agglutination in a dilution of one to thirty, the writer is inclined to question the specificity of so low a dilution. The report referred to above does not say what the titre of the serum was with any known strain of typhoid, nor whether one to thirty was the highest dilution that would give a positive reaction, though we are led to suspect that this was the case. A dilution of one to thirty cannot be relied upon explicitly, for other organisms closely related to typhoid as some strains of B. coli will agglutinate in a dilution of one to thirty and in the case of a strong serum in one to one hundred.[3]

In 1908 Stiles[4] isolated four organisms from oysters obtained from Jamaica Bay, Long Island which "resembled B. typhosus biologically, but did not agglutinate typhoid immune serum." In 1911, while investigating an epidemic of typhoid following a banquet given October 5, 1911, at the Music Hall, Goshen, N. Y., Stiles again examined oysters from Jamaica Bay, where the oysters were obtained for the banquet and in this instance he was able to isolate two strains of B. typhosus from oysters "which had been allowed to 'drink' under an oyster house at Inwood, Long Island." Besides

[1]The Distribution of Sewage in the waters of Narragansett Bay, with Especial Reference to the Contamination of the Oyster Beds, App. to Rep. of Commissioner of Fisheries for year ending June 30, 1904.

[2]Routine methods of Shellfish Examination with Reference to Sewage Pollution, Journal of Hygiene, IX. 1909, 433.

[3]Hiss & Zinsser: Text Book of Bacteriology, 1912, p. 42.

[4]Bureau of Chemistry, Bulletin No. 136.

showing all the cultural characteristics of the typhoid bacillus, it also agglutinated in five minutes in a 1:1000 dilution of typhoid immune serum. This organism was isolated from the oysters seven days after they were taken from the water. Later oysters from the same lot were examined after they had been out of the water twenty-one days and kept at 39° F. An organism was isolated which resembled typhoid in all its cultural characteristics and agglutinated macroscopically in a dilution of 1:1000. This test was confirmed by hanging drop preparations in dilutions of 1:200.

There can be no possible doubt that the organisms isolated by Stiles are true typhoid bacilli, while little can be desired to confirm the identity of the organism isolated by Klein.

An interesting feature of the work of Stiles is that he demonstrated the typhoid bacillus in oysters which had been infected under natural condition and which had been kept out of water for three weeks. Klein,[1] Foote[2] Herdman and Boyce,[3] and others have reported instances in which typhoid bacilli have been isolated after varying lengths of time up to 18 days after infection from oysters artificially infected with large numbers of typhoid bacilli in pure cultures or from typhoid stools and kept in sea water in the laboratory. So far as the writer is aware Stiles is the first one to show that oysters infected under normal circumstances with sewage containing typhoid bacilli and kept under favorable conditions can still harbor B. typhosus after 21 days. The condition here are somewhat different from laboratory experiments in that in sewage along with the typhoid bacilli are other bacteria whose influence is exceedingly hostile to the growth of B. typhosus.[4]

It is interesting to see that this organism has been isolated so few times, in spite of the abundant epidemological evidence in so many instances which points conclusively to the infection of oysters and other shellfish with typhoid bacilli. The reason for this, however, is quite readily understandable when we consider the number of typhoid bacilli which could be found in the sewage of any town or city in comparison with the number of other organisms found in that same sewage. It would be a case of searching for the proverbial

[1]Loc. cit.

[2]A Bacteriological study of Oysters, with Special Reference to them as a source of Typhoid Infection," 18th Ann. Report Com. State Board of Health.

[3]Oysters and Disease, Thompson Yates Laboratory Report, 1–2.

[4]Jordan, Russell & Zeit, Journal of Infectious Diseases I, 1904, 641.

needle in the hay stack. Moreover the incubation period of typhoid varies from two to three weeks and this would make the period of infection two or three weeks before suspicion would be thrown on the oysters. That is, two or three weeks would elapse after infection, before we began to look for the organism. During this space of time the other oysters of the same laying would, in all probability, have time to rid themselves of the organisms, provided they too were infected. In the case of an epidemic of typhoid due to eating raw oysters, the time to examine the oysters for typhoid bacilli would be at the moment they were eaten. We may be quite sure from the history of the cases that the oysters which were consumed did contain B. typhosus, but we have no assurance that all the oysters of that particular bed contained the organism. There is great variation in the number of sewage organisms contained in the individual oysters of the same bed. This individual variation will be still greater if the bed is large and the amount of sewage small, tho highly infected with B. typhosus and other sewage organisms. Sewage does not ordinarily contain typhoid bacilli in constant numbers at any time and unless there is an extensive epidemic, B. typhosus would appear only intermittently and then in comparatively small numbers. In view of these facts the wonder is, considering that B. typhosus die off rapidly, both in sea water and in oysters, that typhoid bacilli have ever been found at all.

The spread of cholera through infected oysters has not attached so much attention as the transmission of typhoid. The latter is distributed much more widely throughout the world and the opportunity for such transmission is much greater. Occasionally, however, there has appeared references to the spread of cholera through infected oysters. In 1849 there was a small epidemic of cholera in England which was attributed to eating oysters. In 1893 Sir Richard Thorne attributed a number of scattered cases of cholera in England to the consumption of oysters. Recently it has been reported that a large extent of oyster beds in Italy have been destroyed because they were thought to be a menace to the public health on account of the danger of the cholera infection.

In most, if not all epidemics of typhoid from infected oysters or other articles of food, there have been a greater number of cases of gastro intestinal disturbances which have not developed into

typhoid.[1] We cannot tell the exact cause of these intestinal upsets. It may be due to bacteria other than typhoid or it may be due to chemical or ptomaine poisons which appear in the sewage as the end products of bacterial metabolism. Whatever the cause we are led to expect these disturbances as concomitants of any outbreak of typhoid due to an infected food.

Since one can rely so little upon the finding of the specific disease organism in sewage and in oysters, it was but natural that an index of greater reliability should be sought. Klein[2] at the beginning of his experimental work as well as in some previous investigations ascertained that B. coli and other intestinal bacteria form no part of the flora of oysters grown in non-polluted water and for this reason used B. coli as an index of pollution. Klein's observations in regard to the bacterial content of oysters grown in water free from sewage has been confirmed by Houston,[3] Ferguson [4] Fuller[5] and others. The presence of B. coli as an indication of sewage pollution has been adopted by all workers in this field and is the index used to-day to determine bacteriologically the presence of fecal matter.

In examining oysters, however, we have quite a different problem from the examination of water, for we have not only the juice, but the body of the oyster, the mucus covering the body, the alimentary canal, etc., to consider. It is interesting to see how the methods of examination have changed as our knowledge of the bacteriology of the different parts of the oyster has increased.

Perhaps the first person to make an extended study of the bacteriology of the oyster was Klein, who in 1893,[6] made a study of the "Relation of Oysters and Disease" for the Local Government Board. Klein describes his method of analysis as follows:—

"Each oyster was carefully washed and brushed in a small quantity of sterile water, with a view to collect therein any microbes adhering to its shell. Next, the oyster, after a further cleansing under a water tap and drying with a clean cloth was opened with a sterile knife,

[1]As an illustration, the reader is referred to the following reports: H. T. Bulstrode, in local Government Board, 32d Annual Report, 1902-1903, Suppl. App. A. pp. 129-189; H. W. Conn, The "Oyster Epidemic" of typhoid fever at Wesleyan University, Medical Record, 46,1894, 743-6; G. W. Stiles, Sewage Polluted Oysters as a Cause of Typhoid and other Gastro-intestinal Disturbances, Bureau of Chemistry, Bulletin 136, 1912.

[2]Loc. cit.

[3]4th Rep. Royal Sewage Commission, 1904.

[4]Bull. Virginia State Board of Health, May, 1909.

[5]Loc. cit.

[6]Supplement to Report of Medical Officer to Local Government Board, Appendix No. 2, pp. 109 and 117.

and its body mashed up with the liquor contained in the shell . . . and about ¼ to ½ c.c. of the liquor and the oyster tissue was removed by means of a freshly made capillary pipette and introduced into a phenolated broth tube which was incubated at 37° C for 24 hours." If growth occurred the culture was plated and the suspicious colonies fished and studied in pure culture. This method allowed no comparison between the bacterial content of the shell liquor and the "oyster tissue." Besides it did not allow a determination of the number of colon bacilli in the whole oyster nor per unit volume. Moreover, we have no evidence that any part of the oyster tissue except the epithelium of the outside of the body and the lining of the alimentary tract contain bacteria and this large amount (in comparison to the amount of shell liquor) of finely divided tissue—for it must have been finely divided to have been taken up in a capillary pipette—would interfere greatly, if one tried to obtain an accurate count.

Chantemesse, in June, 1896, reported to the Académie de Medicine, Paris, his observations on the relation of oysters to disease. In the article presented at this meeting he does not give the details of his technique, but says the shell liquor and the bodies of the oysters were submitted to a bacteriological examination and B. coli were found.

The next important investigation after that of Klein is the work of Herdmann and Boyce.[1] A great number of experiments were performed on the chemistry and biology and also on the bacteriology of the oyster. Only a small part of their work related to the presence of B. coli in normal oysters. For this work the stomach contents were used. The following is quoted from their report:—

"The method of analysis consisted in first cauterizing the mantle over the region of the stomach and then inserting a fine sterilized glass pipette, the pipette was moved about and when sufficient of the contents of the stomach and the juice had risen in the pipette, the latter was removed and its contents transferred to liquified agar, ordinary gelatine or sea-water gelatine and plate cultivations made."

Apparently no attempt was made to determine the number of colon bacilli either per unit quantity or in the contents of the stomach as a whole.

The next important investigation we have noted is the work of Dr. Houston.[2] Dr. Houston's method of analysis is as follows:—

---

[1](1) Lancashire Sea Fisheries Memoir No. 1. (2) Proceedings of Royal Society, 1899. (3) Thompson Yates Lab. Rpt. 1-2.

[2]Fourth Report of Royal Sewage Commission. Vol. III, 1904

Cleaning of Oysters:—

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

Cleansing of the Hands:—

"The hands of the experimenter were thoroughly cleansed with a hard scrubbing brush, soap and water, then rinsed first with 1:1000 corrosive sublimate solution, and finally with sterile water.

Subsequent Procedure:—

"The oysters were laid out upon a sterile towel, the flat shell uppermost. They were opened in this position with a sterile knife, held in the right hand, while they were held in this position with a corner of the sterile towel grasped in the left hand. Great care was taken to avoid any loss of liquor in the shell. This liquor was poured into a sterile 100 c. c. cylinder, the oyster was then partly cut with sterile scissors and the liquor thus freed allowed to run into the cylinder. Ten oysters were thus treated in each experiment. The volume of the oyster plus the oyster liquor was read off, and usually varied bewteen 80 and 120 c.c., so that the oysters, being of medium size and containing a medium amount of liquor, 100 c.c. might be considered a fair average of the total shell contents of the ten oysters. Sterile water was then poured into the cylinder up to the 1,000 c.c. mark, and the whole well stirred with a sterile rod.

"An Alternative Quantative Method for the Bacteriological Examination of Oysters.

"An alternative method for the bacteriological examination of oysters may be given here, although the routine work, except where otherwise stated, has been carried out by the foregoing method.

"The oysters are cleansed and opened, with the same precautions already noted. Then the body of the oyster is cut into small pieces with sterile scissors: this process should be carried out in such a way as to insure the thorough mixture of the gastric juice of the oyster and the liquor. The oyster, meanwhile, is carefully held with the concave shell downwards and the flat shell bent back or altogether removed. To examine the liquid contents of the shell without this

preliminary step may partake of the nature of the examination of the last sample of sea water imbibed by the oyster before finally closing the shell. Indeed, the experiments detailed elsewhere seem to indicate that per unit volume the gastric juice of the oyster may be more impure bacteriologically than the oyster liquor.

"For cultural purposes the following quantities were made by proper dilutions:—100 c.c., 10 c.c., 1 c.c. 1–10 c.c., 1–100 c.c., 1–1000 c.c."

It appears that this was the first attempt to determine the number of B. coil or coli-like organisms within the oyster. The supposition was that the supernatant liquid above the oysters contained in an even distribution all the bacteria that were present in the shell liquor, the juices of the body, and on the outside of the oyster. Whether this assumption is true or not will be discussed later when the writer takes up his own experiments. Houston also performed "a series of experiments to ascertain the relation between the biological (bacteriological L. A. R.) composition of (1) the shell liquor and surface "washings" of the oyster, and (2) the "washed bodies of the oysters." In this series of experiments, four in number, by rapid fire calculation and assumptions, Houston arrives at some very startling conclusions.[1] From these experiments he states that volume for volume the stomach of the oyster contains more bacteria than any other part of the oyster. The method of conducting the experiments and the premises assumed and conclusions drawn will be discussed more at length when the writer takes up similar experiments of his own.

Fuller in the article cited above describes his method as follows:—

"In the examination, inoculations were made from the liquor contained between the shells, from the contents of the intestines, stomach, and rectum, and in some cases from portions of the visceral mass. In order to obtain samples of the juice from an oyster under aseptic conditions, the specimens to be examined were scrubbed thoroughly in tap water with a stiff brush, washed off in running sterile water, and dried on a sterile towel, after which they were opened with a sterile knife. To obtain cultures from the stomach, the top of the mantle covering the interior end of the oyster was slit open and the large palps on either dide of the mouth pushed aside; the mouth region was sterilized by passing a hot scalpel over these parts and a portion of the stomach contents was drawn out by means of a fine pipette or platinum loop introduced through the mouth opening. Cultures from the intestines were made in the following

[1]See page 27.

manner: After opening the shell, the oyster was removed from the shell and dried between filter papers. A hot spatula was then passed upon the surface of the mollusk directly over that portion of the intestine which it was desired to reach, and the tube was then opened with a sterile scalpel. Through this opening a portion of the contents was drawn out by means of a pipette or platinum loop. Portions of the visceral mass were obtained by cutting out cubes of flesh from that portion of the body after sterilizing the surface with a hot scalpel."

McWeeney[1] in his examination of oysters on the Irish Coast used the shell liquor alone, if abundant. But in cases where the amount of shell liquor was small he supplemented the small quantity of liquid "with a block of tissue cut from the animal itself so as to include portion of the alimentary canal."

The next worker to do a great deal of routine and experimental work in the examination of shellfish was H. W. Clark. In a preliminary report published in 1902[2] Clark describes his method of analysis as follows:—

"To determine the presence of B. coli in the juice on the shell, the clams, oysters, etc., were washed with sterilie water, then opened, and this juice inoculated into bouillon."

"To determine whether the germ was present in the bodies of the clams, oysters, etc., they were opened after washing with sterile water, and the intestine, after maceration with sterile water, was inoculated into phenol dextrose bouillon.

In 1905, Clark[3] in a report covering his experimental work for the previous five and one-half years makes the following statement in regard to the "Examination of Raw Oysters:"—"The shell liquor and the crushed body of the oyster were examined together by inserting the entire mass in a fermentation tube, and if fermentation was obtained, carrying out the cultural tests."

In determining the presence of B. coli in the body of the oyster as detailed in his first report it appears that Clark disected out the alimentary tract. This is not stated as part of the procedure, but it is implied from the above quotation. This procedure would be rather cumbersome if one attempted to use it on a large scale in routine exam-

[1]Report on the Bacteriosceopic Examination of Samples taken from Shellfish Layings on the Irish Coast, Local Government Board for Ireland, 1904

[2]Senate Document 336, State of Mass., 1902.

[3]Report Mass. State Board of Health, 1905, 427.

inations. Moreover, Clark apparently assumes that the bacteria isolated in this manner all came from the intestinal tract and that no contaminating organisms from the mucus on the outside of the body entered into the bacterial flora of the macerated intestine. The writer in some experiments to be given in detail later has shown that on the average there are more—often many times more—bacteria in the mucus on the body of the oyster than in the total amount of shell liquor and further that volume for volume the contents of the stomach do not contain so many bacteria as the shell liquor. Since the stomach contains more liquid on the whole than the rest of the intestinal tract, it is but natural that it should contain more B. coli than the remainder of the intestinal tract. This would be all the more evident when it is understood that B. coli do not grow in oysters, but probably diminish as they pass through the intestinal tract.[1]

In his second article cited above, the whole contents of the oyster shell, "the shell water and the crushed body of the oyster were examined together by inserting the entire mass in a fermentation tube." Obviously this would allow of no comparison between the bacterial flora of the shell liquor and the body of the oyster. Yet in a following paragraph and also in a table he gives the results of the analysis in "Per cent. of Samples Giving Positive Tests," in "Shell Water, Intestine" and "Mash." Obviously there is some discrepancy, for if he followed out the method described it would be impossible to make such a differentiation. It is possible, however, that Clark was using a combination of the technique as stated in the two reports. The shell water and the "intestinal content" were examined as stated in his report of 1902, and his "mash" consisted of the shell liquor and crushed body, the entire mass of which was inserted into the fermentation tube. It would appear, however, that in order to carry out a combination of these two pieces of technique, two oysters would be necessary, one for the shell liquor and intestine and another for the "shell water and the crushed body." If this were true the individual variation of course, would allow of no definite comparison between all the parts tested. It may mean that the remains of the body tissue after dissecting out the intestine and the unused portion of the shell liquor were mixed and constituted the shell water and crushed body. But, in whatever manner we try to explain the matter, the fact remains that the method as described is insufficient to account

[1]Hardman & Boyce, loc. cit.

for the results obtained. But, as the results are expressed in the text and again in more detail in a table, we can feel quite certain that the method of analysis in the second report is not given in sufficient detail and the results expressed in the table are accurate so far as his methods would allow.

From the table referred to above it is seen that in the examination of one hundred and forty-five oysters approximately fifty per. cent of them gave positive tests for B. coli in the shell liquor, seventeen per cent in the "mash" and between seven and eight per cent. in intestine.

In three following tables is given the results of the analysis of shell liquor and intestine of 265 other oysters, making a total of 410 oysters examined in all. A comparison of the percentage of positive results in the shell liquor and intestine shows that B. coli were found nearly four times—50 to 14— as often in the shell liquor as in the intestine. From these experiments it seems apparently beyond question that the greatest number of B. coli are in the shell liquor of the oyster and that the body of the oyster should be disregarded in our search for the colon bacillus.

Stiles[1] describes his method of analysis as follows:

"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 oysters, 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."

Gage[2] discribes his methods as follows:—

"The upper shell being removed, a portion of the liquor in the lower shell is now transferred to a fermentation tube with a sterile pipette, or a portion of this shell-water may be carefully poured directly from the shell into the tube. The latter method is much simpler than the use of pipettes, but requires that the shell be so handled in the previous operation that the lip over which the liquor is poured has not been contaminated. The body is now washed with sterile water, then while held with the fingers of the left hand, an incision is made with a sterile scalpel and a portion of the intestine

[1]Shellfish Contamination from Sewage-Polluted Waters and from other Sources, Bureau of Chemistry, Bulletin 136, April 11, 1911.

[2]Methods of Testing Shellfish for Pollution, Jour. or Infectious Deseases, 1910, VII, 78.

transferred with sterile forceps to another fermentation tube, care being taken not to touch the parts where the incision is made with the fingers or to contaminate it in any way. This procedure is repeated until 10 individuals have been tested from each sample jar."

It would appear that the work of Clark has had wide influence in determining the method of shellfish analysis now in use in this country. So far as the writer is aware and so far as the literature at hand shows, the only part of the oyster used for bacteriological analysis for some years has been the shell liquor. The "Committee on Standard Methods of Shellfish examination" appointed by the American Public Health Association has recommended the use of the shell liquor only. So far as a perusal of the recent literature is concerned no one has questioned the advisability and propriety of using the shell liquor alone for analytical purposes except Gorham[1] upon results obtained by the writer in the laboratory of Brown University.

It will be noticed in all the work cited in which parts of the intestine have been used for analysis, except in the case of Fuller, no mention has been made of trying to avoid taking bacteria from the outside of the oyster as well. In the writer's opinion a great many of the bacteria alleged to have been found in the intestinal tract have come from the mucus on the outside of the body. There is no doubt that the intestine of the oyster does contain bacteria of sewage origin, but the mucus on the outside of the body is much more likely to contain such bacteria.

BACTERIOLOGY OF THE SHELL LIQUOR AND "WASHINGS" FROM THE BODY OF THE OYSTER.

A matter of great interest to the writer is that in all the work done upon oysters experimentally and otherwise no one has mentioned the mucus of the oyster or apparently realized that it plays any part in the bacteriology of the oyster.

The matter of the mucus in the oyster juice and on the oyster's body appears so self-evident that it seems impossible that it should have been entirely neglected. This mucus serves at least two purposes. (1) It acts as a protection to the body of the oyster and protects it from the deleterious effects of sea water in just the same way as the mucus of the dog fish and other selachians protects their skin from

[1]Report of Commissioners of Shell Fisheries, State of R. I., 1914.

the action of the sea water. (2) The other and more important function from the bacteriological point of view is that it serves as a net for the entrapping of the food of the oyster which consists largely of diatoms and algæ, but is made up of all sorts of microscopic particles, living or dead, organic or inorganic. As a consequence, the bacteria as well as the other microscopic organisms get entangled in this mucus.

When one opens an oyster and collects the juice, usually a great many particles of mucus, some particles very large comparatively speaking, are seen in the liquid. If one handles an oyster after opening, it is found covered with a vicid, slimy substance which does not wash off the hands easily If the bodies of the opened oysters are allowed to stand for sometime there rises to the surface long strings and flakes of this greenish yellow mucus. In shucking houses it is customary to allow opened oysters to lie for some time in large vats filled with water and with occasional stirring allow the mucus to rise to the surface of the water and run over the edge, if running water is used, or if not, it is skimmed off with a perforated dipper. This mucus often collects in "ropes" two, three, or more inches long and sometimes in large flakes the size of a half dollar.

If one examines the liquor of the oyster he has just opened, it usually contains a great number of particles of mucus, some large, some small. If one collects the liquor in a bottle and allows it to stand over night it will be found to have separated into two distinct layers, a heavy, thick, viscous layer on the bottom and a clear, more limpid layer on the top. The bottom layer is the mucus which has precipitated out. Standard Methods of Water Analysis requires a water sample to be shaken twenty-five times before the analysis commences, in order to break up any clumps of bacteria. The second Progress Report of the Committee on Standard Methods of Shellfish Examination[1] recommends that "bacterial counts shall be made of a composite sample of each lot obtained by mixing the shell liquor of five oysters. Agar shall be used for the culture medium and in general the procedure shall be in accordance with the method recommended by the committee on Standard Methods of Water Analysis of the American Public Health Association." It can be inferred from the last sentence of the quotation that it includes shaking the sample. In draining the liquid from the oyster the water runs out of the shell not at a single point, but over a considerable part of the edge of the shell.

[1]Jour. Am. Pub. Health, II, 1912, 34.

For this reason the mouth of the ordinary water sample bottle is not large enough to collect all the juice and so in most laboratories a sterile petri dish is used for the purpose. This would preclude the possibility of shaking. Now if shaking of a water sample, which to the eye is perfectly clear, is advisable to break up the clumps of bacteria and give a more even distribution of bacteria, what can be said of the juice of the oyster which has a decided milky appearance and which usually contains strings and flakes of mucus large enough to be seen several feet away? If one plates a cubic centimeter of this mixture without shaking, the flakes will appear in the solid medium as irregular, opaque particles. The probabilities are from the writer's experience that the flakes of mucus carry a large number of bacteria and we have a large confluent mass of colonies developing around each mucus flake. Even if flakes are not present, large confluent masses of colonies from the size of a penny to the size of a quarter develop, which render counting impossible. Usually, however, only bile tubes are used for the presumptive test for B. coli and no plates made so that this clumping is not noticeable except where bile tubes do not duplicate or where one gets a positive test in the 1–10th c.c. or 1–100th c.c. dilution and not in the 1 c.c. or a positive presumptive test in the 1–100th c.c. dilution and not in the 1 c.c. or 1–10th c.c. dilution. In a study of about 2,000 tubes in the presumptive test the writer found that they duplicated only about two-thirds of the time and that in one set one might get a positive presumptive test in the 1–100th c.c. dilution and in the duplicate set only in the 1 c.c. dilution or not at all.

Aside from the part played by the mucus in oyster juice the part played by the mucus left upon the body of the oyster is, generally speaking, much more important. Often much more mucus is left upon the body of the oyster than is found in the oyster juice. As the mucus is the part which catches the bacteria and holds them, it follows that often more bacteria are left upon the oyster's body than are found in the oyster juice. Hence, it follows that, if we only examine the juice of the oyster we are only finding a fraction of the bacteria really present in the oyster. These facts will be brought out more clearly when the experimental work upon which these statements are based, is taken up.

The idea of comparing the number of bacteria found in the shell liquor with the number that can be "washed" from the body of the

oyster is not new. [1]Houston performed a series of experiments on this point and his technique and results are given in some detail.

EXPERIMENT "A."

September 9th, 1903.

"The oysters utilized for this experiment were gathered in the Helford River, at low tide, on September 8th. They were cleansed in the manner described elsewhere, before being opened with a sterile knife. Each oyster was carefully detached from the two valves of its shell, with as little injury as possible, and washed in the manner about to be described.

"A sterilized funnel was placed in a sterile, 1,000 c.c. measuring cylinder as shown in the accompanying figure. The liquor in the oyster shell was poured into the cylinder before the oyster was completely detached, and then the oyster was removed from the shell with sterile forceps, held over the funnel, well washed with sterile water, and allowed to rest in the funnel. Ten oysters were treated severally in this manner, and then allowed to drain in the funnel.

1. LIQUOR.

"The total amount of sterile water employed for washing purposes was..... 810 c.c.

The total volume of liquid (oyster liquor and "washings") in the measuring cylinder was..... 840 c.c.

Therefore the volume of oyster liquor for 10 oysters was..... 30 c.c.
or 3 c.c. liquor per oyster.

"The funnel containing the oysters was then lifted into a second sterile cylinder, and sterile water was poured into the first cylinder up to the 1,000 c.c. mark.

"The cultures were then carried out in the ordinary way described elsewhere.

Results of the Examination of the Liquor.

"Coli-like microbes were isolated in pure culture from 1 c.c. and 0.1 c.c. of the litre of mixed oyster liquor and sterile water.

[1]Loc. cit.

This result indicates that the litre consisting of oyster liquor + sterile water contained coli-like (apart from slow liquefaction of gelatine) microbes in amount corresponding to about 10 per c.c. The whole litre could thus be considered to contain about 10,000 coli-like microbes derived from 30 c.c. of oyster liquor.

Hence, if 10 oysters yield 30 c.c. of liquor containing 10,000 coli-like microbes, taking the average liquid contents of each oyster as 3 c.c., this works out at 1,000 coli-like microbes in the liquid contents of each oyster, or about 330 coli-like microbes per c.c. of oyster liquor.

II. OYSTERS.

"The oysters were one by one removed from the funnel, cut up with sterile scissors, and placed in the second sterile cylinder. A known quantity of sterile water (100 c.c.) was then added, the total volume read off, and hence after deducting 100 c.c. the volume of the oysters was obtained. It was found to be 90 c.c. Sterile water was then added to the cylinder until the volume of the liquid was equal to 1,000 c.c.

"The cultures were then carried out in the ordinary way described elsewhere.

Results of the Examinations of the Oysters' Bodies.

'Coli-like microbes were respectively isolated from 10 c.c. and 1 c.c. of the litre consisting of a mixture of washed oyster bodies and sterile water.

"The litre consisting of sterile water+macerated oysters might be considered to contain about 1,000 coli-like microbes derived from the bodies of 10 oysters. Therefore, each oyster body (deprived as far as possible of its natural liquor) would contain coli-like microbes corresponding in number to about 100.

"The total volume of oyster bodies being 90 c.c., the volume of each of the 10 oysters averaged 9 c.c.; each 9 c.c. of oyster body could be considered to contain 100 coli-like microbes, or, roughly speaking, 11 coli-like microbes per c.c. of body bulk. The contrast is very striking when this number is compared with that of 330 coli-like microbes per c.c. of oyster liquor, *i. e.*, volume for volume the oyster liquor contains about 30 times as many coli-like microbes as the oyster body.

"But the liquid contents of the oyster's stomach are certainly much less than 1 c.c., probably about 0.1 c.c. It is probably that the coli-like microbes isolated from the macerated oyster bodies in the foregoing experiment were totally, or in great part, derived from the contents of the stomach and intestinal tract. In fact, it is conceivable that the 100 coli-like microbes, which each washed oyster was found to contain, were all, or to a great extent, derived from the stomach juice which, for comparative purposes, may be assumed to be about 0.1 c.c. But if the body volume of each oyster be taken as 9 c.c., the volume of the stomach contents on the above assumption is only about one-ninetieth of the total bulk.

"This view alters considerably the complexion of affairs. For the ratio between the number, per unit of volume, of coli-like microbes present, respectively, in the oyster liquor and stomach juice, would then be 33:100. In other words, acting on this assumption the coli-like microbes were three times more numerous per unit of volume in the stomach or intestinal juice then in the oyster liquor.

1	2	3	4	5	6	7
Experiment.	Volume of oyster liquor per oyster in c.c.	Volume of oyster body per oyster in c.c.	Number of coli-like microbes per cc. of oyster liquor.	Number of coli-like microbes per cc. of oyster body.	Ratio of columns 4 and 5.	Ratio as regards coli-like microbes of oyster liquor to oyster body per unit of volume.*
A.	3	9	330	11	33:1	33:100
			about	about	about	about
B.	7.5	9.5	1.3	1	1.3:1	1:100
C.	3	8	330	125	2.64:1	3.3:100
D.	6	10	166	100	1.66:1	1.66:100

*On the assumption that all the coli-like microbes obtained from the macerated bodies of the oysters were derived from the stomach juice (taking the volume of the stomach juice as 0.1c.c. and the volume of the oyster apart from its liquor as 10c.c.)."

From these experiments and the conclusions drawn it is clear that Dr. Houston thought that all the bacteria on the outside of the oysters were washed off with the sterile water used to wash the bodies of the oysters and that all the organisms found in the minced oysters came from the stomach. Whether we can accept Dr. Houston's supposition or not will be discussed later under the writer's own experiments in this connection.

Stiles in the bulletin referred to above made some analyses showing the relative numbers of bacteria in the shell liquor and meat of oysters. He concludes: "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 cubid centimeter as the minced meat."

Stiles does not give his method of determining the number of bacteria in the minced body of the oyster. It may well be that his results actually do show the relative numbers of bacteria in the two parts of the oyster. His experiments included the results of only fifteen analyses, and the results uniformally show a greater number of bacteria in the shell liquor than in the minced body meat. In the light of the writer's results of similar analyses, however, we are led to believe that the method of analysis is not adequate to demonstrate the relative number of bacteria in the two parts of the oyster. It is conceded by all that the tissues of the oyster are sterile. It is only the outside of the body and the alimentary tract which normally harbor bacteria. It is easy to understand how so much minced tissue will interfere with accurate results. Secondly no mention is made of how the bacteria were separated from the minced meat. An immense amount of shaking would be necessary to make an even suspension of bacteria if one tried to wash them from the minced particles of the oyster meat. The bacteria are attached to the body of the oyster by the mucus which is not easily removed. Even though the minced oysters were shaken vigorously in water or salt solution, the particles would quickly settle out and being more or less entangled in the mucus a coagulum would be formed which settling out rapidly would take a great many if not most of the bacteria out of suspension. This is purely suppositional since the method of analysis is not given, but this is a perfectly logical method of procedure and a very probable explanation of the results. The temperature of the water from which the oysters were taken is not given. In the writer's opinion this is

an important matter, for the temperature of the water will influence the metabolism of the mucus secreting cells and will determine the amount of mucus present on the body of the oyster. This matter will be discussed further in another connection.

When the writer began his experiments, he did not know of Houston's work and so the experiments were not carried out in exactly the same manner, but, nevertheless, the experiments throw considerable light on the work just cited. The idea that the mucus of the oyster played a part as yet unappreciated led the writer to perform the following series of experiments.

Experiment I.

September 29, 1913, ten oysters were taken to the laboratory and analyzed as follows: The oysters were opened according to "Standard Methods" and the liquor drained into a small bottle graduated in two cubic centimeter divisions. The oysters were allowed to drain until a drop would not come away at least every five seconds. The amount of liquor was then read off and an equal volume of sterile salt solution added and the whole shaken vigorously one hundred times. The body of the oyster was removed from the shell and placed in a sterile jar and a quantity of sterile salt solution added equal to the volume of the shell liquor. The jars were covered and allowed to stand for a short time while the oyster juice was being inoculated into plates and bile tubes. The jars containing salt solution and oyster meat were then stirred vigorously with a sterile pipette and an attempt made to remove with the pipette as much mucus as possible from the body of the oyster. Then one cubic centimeter of the solution and dilutions thereof were inoculated into plain agar plates and lactose-peptone-bile in the same manner as in the case of oyster juice. A careful record was kept of the number of cubic centimeters of juice obtained from each oyster and the amount of salt solution used in washing each oyster in order to make a comparison of the bacterial content of all the shell liquor with the total number of bacteria washed from the oyster. This would show which part contained the greater number of bacteria.

Experiment II.

The above experiment was repeated on oysters obtained October 7, 1913. The total number of bacteria found in the shell liquor and the

washings from the bodies of the oysters in each of the two experiments is shown in the following table:

Table Showing the Total Number of Bacteria in the Shell Liquor of each Sample and the Total Number Washed from the Bodies of the Oysters Without Shaking.

Date.		20 Count.	B. coli Count.
Sept. 29.	Shell Liquor	330,000	7,400
	"Washings"	48,000	1,700
Oct. 7.	Shell Liquor	480,000	5,900
	"Washings"	50,000	850

The detailed results are shown in the two following tables:

Date.	No. of Oyster.	C.C. of Oyster Liquor.	20°C Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings
Sept. 29, 1913	1	8	27,000	1,900	1,600	800	200	300
" "	2	4	4,000	1,600	80	400	20	120
" "	3	3	2,900	2,400	60	30	20	30
" "	4	10	51,000	2,400	200	10	20	21
" "	5	11	13,000	5,500	220	11	20	21
" "	6	10	14,000	4,800	200	100	20	30
" "	7	9	76,000	14,000	1,800	90	200	210
" "	8	13	14,000	4,700	260	130	20	30
" "	9	5	75,000	6,000	1,000	50	200	210
" "	10	10	58,000	4,600	2,000	100	200	210
Totals		83	334,900	47,900	7,420	1,721	920	1,182

Date.	No. of Oyster.	C.C. of Oyster Liquor.	20°C Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
October 7, 1913	1	10	40,000	1,000	200	100	20	30
" "	2	20	20,000	3,700	2,000	10	100	101
" "	3	12	54,000	20,000	240	10	20	21
" "	4	10	70,000	300	20	10	2	3
" "	5	18	13,500	1,200	18	100	1	61
" "	6	14	126,000	12,000	2,800	200	200	214
" "	7	18	73,800	5,000	180	200	10	21
" "	8	10	12,000	3,200	200	200	20	20
" "	9	12	72,100	3,400	240	0	20	20
" "	*10	.....	.....	.....	.....	.....	.....	.....
Totals.....	.....	.....	481,300	49,800	5,898	830	393	511

*Not examined.

It will be noticed that in the last two columns of the table is given the score based upon the shell liquor alone and upon the shell liquor and the "washings" from the oyster combined. The method of scoring is based upon the same principal as the method of scoring recommended by "Standard Methods," but it works out a little differently for the method of analysis followed by the writer is not strictly in accordance with "Standard Methods." In the latter method 1 c.c., 1–10 c.c. and 1–100 c.c. quantities of the shell liquor are inoculated into lactose-peptone-bile. If the presumptive test shows B. coli in 1 c.c. dilution and not in the 1–10 c.c. and the 1–100 c.c. then the score of this oyster is one. If it shows B. coli in 1–10 c.c. and not in 1–100 c.c., the score is ten; if in 1–100 c.c. the score is 100. In other words, the score of the oyster equals the number of B. coli found in one cubic centimeter of the shell liquor. In the writer's experiments the shell liquor was carefully measured and diluted with an equal volume of one per cent. NaCl solution. One cubic centimeter of this mixture was used to make the various dilutions. The result is that the various dilutions contained ½ c.c., ⅒ c.c. and

1/200 c.c. of the shell liquor. Gas appearing in these respective dilutions would indicate two, twenty and two hundred B. coli per cubic centimeter instead of one, ten and one hundred as in the procedure of "Standard Methods."

The last column gives the combined score, in other words, the score based upon the number of B. coli in both the shell liquor and in the washings. The number of B. coli in each is added together and divided by the number of cubic centimeters of shell liquor. This method makes no allowance for the amount of mucus present on the body of the oyster. This quantity would not exceed one cubic centimeter on the average, for many of the oysters were small. It is more convenient and just as accurate for comparative purposes to ignore this quantity, while it is much more convenient in dividing the total number of B. coli found in the oyster by the quantity of shell liquor. It avoids fractions much more often than would be the case if we added one to the number of cubic centimeters of shell liquor. Occasionally, however, in the combined score, the quotient is not an even number and so the score is made the whole number next above or below depending whether the fraction was less than or more than one-half. Thus if the score came 20.4 it would be called 20; if the fraciton were .5 or more it would be called 21.

It would seem from these experiments that there is no question that the shell liquor contains many more bacteria than are left on the body of the oyster and that in analysis we could ignore entirely the bacteria left on the body of the oyster.

These results did not equal the writer's expectation and it was thought that perhaps the treatment of the oyster's body was not sufficient to remove all the mucus and bacteria present. Accordingly the following method of analysis was adopted for the subsequent experiments: The oyster liquor was collected and diluted in the same manner as before. It was shaken vigorously one hundred times before inoculating into agar and bile. The body of the oyster after draining was transferred to a sterile large mouthed, glass stoppered bottle and covered with twenty cubic centimeters of one per cent. NaCl solution. The oyster and salt solution were shaken fairly vigorously one hundred times and the solution of salt and mucus was removed by the pipette or poured into a smaller glass bottle and again shaken vigorously one hundred times. This mixture was then inoculated into the bile tubes and the agar plates. At first one per cent. sodium carbonate

solution was used with the hope that it would cut the mucus more readily, but later the salt solution was found just as effective. The shaking appeared to be the important feature.

It was found that a great deal of shaking was necessary to break up the clumps of bacteria and separate them from the mucus. If not thoroughly shaken the resulting plates would be found to contain large areas of confluent colonies which rendered counting impossible. Every bit of mucus would be found to be a nucleus around which would be a large confluent ring of colonies. After a thorough shaking, however, the flakes of mucus would in nearly all cases remain sterile and the bacteria would be found in well separated colonies evenly distributed in the medium.

Table Showing the Total Number of Bacteria in the Shell Liquor of Each Sample and the Total Number Washed from the Bodies of the Oysters by Shaking.

	No. of Experiment.		20°C. Count.	37°C. Count.	"Red" Count.	B. coli Count.	Score.
October 13	1	Shell Liquor	650,000			4,800	560
		"Washings"	500,000			14,000	*1,952
October 23	2	Shell Liquor	190,000			178	26
		"Washings"	45,000			500	101
October 27	3	Shell Liquor	200,000			113	8
		"Washings"	100,000			2,080	111
October 31	4	Shell Liquor	50,000			2,500	313
		"Washings"	24,000			3,200	720
November 3	5	Shell Liquor	78,000			2,000	248
		"Washings"	15,000			2,600	599
November 8	6	Shell Liquor	1,050,000			17,000	1,410
		"Washings"	140,000			8,800	2,327
December 6	7	Shell Liquor	100,000	39,000	10,000	4,000	284
		"Washings"	15,500	6,900	1,500	4,400	656
December 19	8	Shell Liquor	130,000	30,000	3,000	850	74
		"Washings"	35,000	3,000	800	275	84

*The score opposite the "washings" is the score based upon the number of B. coli in the shell liquor and in the "washings" from the body of the oysters as described in the text.

Table No. 1.—Showing Results of First Experiment.

Temperature of water 16° C.

Date.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		37°C. Count.		Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
October 13, 1913	1	13	130,000	47,000	.....	.....	.....	.....	260	1,000	20	97
" "	2	8	57,000	34,000	.....	.....	.....	.....	160	2,000	20	270
" "	3	9	22,000	53,000	.....	.....	.....	.....	180	200	20	42
" "	4	9	65,000	60,000	.....	.....	.....	.....	180	2,000	20	242
" "	5	8	22,000	29,000	.....	.....	.....	.....	160	200	20	45
" "	6	13	79,000	34,000	.....	.....	.....	.....	260	2,000	20	174
" "	7	12	77,000	40,000	.....	.....	.....	.....	240	2,000	20	187
" "	8	8	74,000	40,000	.....	.....	.....	.....	1,600	200	200	225
" "	9	9	88,000	136,000	.....	.....	.....	.....	1,600	2,000	200	450
" "	10	10	60,000	56,000	.....	.....	.....	.....	200	2,000	20	220
Totals	.....	.....	674,000	529,000	.....	.....	.....	.....	4,840	13,600	560	1,952

TABLE No. II.—*Showing Results of Second Experiment.*

Temperature of water 13.5°C.

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		37°C. Count.*		Red Count.†		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
October 23, 1913	1	10	13,000	2,200	.....	.....	.....	.....	0	0	0	0
" "	2	7	13,000	4,200	.....	.....	.....	.....	14	200	2	31
" "	3	13	17,000	4,000	.....	.....	.....	.....	26	0	2	2
" "	4	6	35,000	2,500	.....	.....	.....	.....	120	20	20	24
" "	5	9	27,000	10,000	.....	.....	.....	.....	0	20	0	2
" "	6	9	21,000	5,200	.....	.....	.....	.....	18	20	2	4
" "	7	11	23,000	3,600	.....	.....	.....	.....	0	20	0	2
" "	8	8	10,000	5,000	.....	.....	.....	.....	0	0	0	0
" "	9	7	20,000	5,600	.....	.....	.....	.....	0	20	0	3
" "	10	6	7,000	3,800	.....	.....	.....	.....	0	200	0	33
Totals	.....	.....	186,000	46,100	.....	.....	.....	.....	178	500	26	101

*Not made. †Not made.

TABLE No. III.—*Showing Results of Third Experiment.*

Temperature of Water, 16°C.

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		37°C. Count.*		Red Count.†		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
October 27, 1913	1	21	31,500	29,000	.....	.....	.....	.....	21	2,000	1	96
" "	2	20	8,000	9,000	.....	.....	.....	.....	20	20	1	2
" "	3	16	10,000	7,000	.....	.....	.....	.....	32	0	2	2
" "	4	12	19,200	10,000	.....	.....	.....	.....	24	20	2	4
" "	5	10	9,000	9,000	.....	.....	.....	.....	0	0	0	0
" "	6	12	64,000	11,000	.....	.....	.....	.....	0	20	0	2
" "	7	8	24,000	8,000	.....	.....	.....	.....	16	20	2	5
" "	8	14	15,000	9,000	.....	.....	.....	.....	0	0	0	0
" "	9	12	4,000	9,000	.....	.....	.....	.....	0	0	0	0
" "	10	13	12,000	2,400	.....	.....	.....	.....	0	0	0	0
Total			196,700	103,400	.....	.....	.....	.....	113	2,080	8	111

*Not made. †Not made.

TABLE No. IV.—*Showing Results of Fourth Experiment.*

Temperature of Water, 13°C.

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		37°C. Count.		Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
October 31, 1913	1	7	2,000	2,200					14	200	2	31
" "	2	8	2,400	1,400					160	20	20	23
" "	3	8	7,000	5,200					0	200	0	25
" "	4	20	12,000	1,800					200	200	10	20
" "	5	10	5,000	2,100					200	200	20	40
" "	6	19	5,500	1,200					19	0	1	1
" "	7	7	2,100	1,200					140	2,000	20	289
" "	8	8	5,500	4,400					160	0	20	20
" "	9	9	5,000	2,800					180	200	20	42
" "	10	7	1,700	1,200					1,400	200	200	229
Total			48,200	23,500					2,473	3,220	313	720

TABLE NO. V.—*Showing Results of Fifth Experiment.*

Temperature of Water, 12°C.

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		*37°C. Count.		†Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
November 3, 1913	1	12	14,000	800	.....	.....	.....	.....	24	0	2	2
" "	2	8	2,500	1,200	.....	.....	.....	.....	1,600	0	200	200
" "	3	18	2,800	1,200	.....	.....	.....	.....	28	200	2	13
" "	4	7	11,500	5,600	.....	.....	.....	.....	140	2,000	20	306
" "	5	12	5,000	600	.....	.....	.....	.....	0	0	0	0
" "	6	11	900	1,600	.....	.....	.....	.....	220	0	20	22
" "	7	9	11,000	1,200	.....	.....	.....	.....	18	20	2	4
" "	8	11	25,000	800	.....	.....	.....	.....	0	0	0	0
" "	9	8	3,500	800	.....	.....	.....	.....	16	200	2	27
" "	10	8	2,000	800	.....	.....	.....	.....	0	200	0	25
Totals	...	...	78,200	14,600	.....	.....	.....	.....	.....	.....	.....	.....

*No count made. †No count made.

TABLE No. VI.—*Showing Results of Sixth Experiment.*

Temperature of Water, 12°C.

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		*37°C. Count.		†Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
November 8, 1913	1	7	38,000	21,000					140	2,000	20	306
" "	2	12	216,000	8,000					2,400	0	220	200
" "	3	12	150,000	20,000					2,400	2,000	220	367
" "	4	12	60,000	1,600					2,400	200	220	217
" "	5	24	122,000	15,000					2,400	200	100	108
" "	6	8	320,000	46,000					1,600	2,000	250	450
" "	7	15	29,000	1,000					3,000	20	200	201
" "	8	11	10,000	14,000					220	220	20	38
" "	9	10	80,000	12,000					2,000	2,000	200	220
" "	10	10	15,000	2,800					200	200	20	220
Total			1,040,000						16,760	8,820	1,410	2,327

*No count made. †No count made.

TABLE No. VII.—*Showing Results of Seventh Experiment.*

Temperature of Water, 8°C.

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		37°C. Count.		Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor. and Washings.
December 6, 1913	1	24	67,000	7,000	16,000	2,800	4,320	800	480	2,000	20	103
" "	2	10	3,200	300	3,000	0	400	0	20	0	2	2
" "	3	14	10,000	1,400	5,600	800	1,000	0	2,800	200	200	214
" "	4	8	1,400	4,200	1,600	2,000	500	200	160	2,000	20	270
" "	5	9	14,000	1,200	5,500	500	550	100	180	200	20	42
" "	6	17	3,700	800	5,000	600	2,500	0	340	20	20	21
" "	7	10	4,600	700	2,400	200	800	400	20	20	2	4
" "												
" "												
" "												
Total			103,900	15,600	39,100	6,900	10,070	1,500	4,000	4,440	284	656

TABLE No. VIII.—*Showing Results of Sample Number Eight.*

Temperature of Water, 6°C.

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		37°C. Count.		Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
December 19, 1913.....	1	18	25,000	1,600	6,300	220	550	80	36	20	2	3
" "	2	14	19,000	450	5,600	200	850	60	280	20	20	21
" "	3	7	17,000	1,110	7,000	240	1,800	40	14	0	2	2
" "	4	18	4,000	380	700	100	100	80	36	0	2	2
" "	5	9	4,500	1,000	1,500	160	150	0	180	200	20	24
" "	6	10	24,000	1,500	1,700	360	120	20	20	0	2	2
" "	7	8	4,500	500	200	60	120	20	16	0	2	2
" "	8	12	15,000	7,000	1,600	240	100	80	240	20	20	22
" "	9	12	12,000	7,000	550	200	50	60	24	0	2	2
" "	10	11	7,500	14,000	600	1,200	65	380	22	20	2	4
Total.....	...	...	132,500	34,530	25,750	2,980	3,905	820	868	280	74	84

In comparing the total number of bacteria in the shell liquor of all the oysters in each of the experiments with the total number washed from the bodies of these oysters it is seen that the total number of bacteria in the shell liquor of all the oysters was greater than the number washed from the bodies of the oysters. In the first experiment the numbers are nearly equal, but in the subsequent experiments there is a great difference. If we consider the individual oysters in all the experiments, we find that in only ten of the oysters out of seventy-seven was there a greater number of bacteria washed from the body than was found in the shell liquor of the corresponding oyster. In one instance the numbers were equal. In the remaining sixty-six oysters there were more bacteria in the shell liquor that were washed from the bodies of the oysters. The 37° C. count and the "red count" were made on only seventeen oysters and in only two instances did the number of bacteria washed from the bodies of the oysters exceed the number found in the shell liquor, while the total number from all the oysters of the two experiments showed that there were on the average a great many more in the shell liquor than were washed from the bodies of the oysters.

When we consider the number of B. coli found in the shell liquor and the number washed from the body of the same oyster we find the relative numbers quite different. It will be seen in six out of the eight experiments the total number of B. coli washed from the bodies of all the oysters of the experiment exceeded the total number in the shell liquor. In the first two experiments the difference is especially marked. If we consider the individual oysters we find that in thirty-three instances there were more B. coli on the body of the oyster than were in the shell liquor; in thirty oysters the number in the shell liquor exceeded the number washed from the body; in fourteen instances the numbers were equal. But if we consider the total number of B. coli found in the "washings" with the total number found in the shell liquor of all the oysters examined in this series of experiments we find there were on the average more B. coli in the "washings" than there were in the shell liquor.

We have no reason at present to suppose that B. coli should be distributed other than equally among the other bacteria in the oyster, yet there seems to be a concentration of B. coli in the mucus on the outside of the body of the oyster. The amount of shell liquor in the oysters averaged about ten cubic centimeters. If we consider that there was left upon the body of the oyster one cubic centimeter of

mucus, we find that there were volume for volume more than ten times as many B. coli on the body of the oyster as there were in the shell liquor. The question arises at once as to whether this unequal distribution of B. coli among the other bacteria in these two parts of the oyster is real or only apparent. It may be due to the difference in methods of analysis. With our present knowledge of the bacteriology of the oyster the writer is led to believe that this relation does not actually exist, but is due to the difference in methods used to determine the total number of bacteria and the number of B. coli.

Another point which appears interesting to the writer is that there is apparently a direct relation between the temperature of the water from which the oysters are taken and the relative number of B. coli found in the shell liquor and on the body of the oyster. It will be noticed that in the first three experiments there were a great many more B. coli on the body of the oyster than in the shell liquor, but this proportion is gradually reduced and in the sixth and eighth experiments there were more B. coli in the shell liquor than were found on the bodies of the oysters. The ratio of the total number of bacteria in the shell liquor to the total number washed from the bodies of the oysters in each sample is shown in the following table:

Table Arranged According to Temperature Showing the Approximate Ratio of the Total Number of Bacteria in the Shell Liquor to the Number in the Washings from the Bodies of the Oysters in each Sample.

Temperature.	Date.	20°C. Count.	37°C. Count.	"Red" Count.	B. coli Count.
16° C.	Oct. 13	1:3:1	.....	.....	1:3
16° C.	" 27	2:1	.....	.....	1:18
13.5° C.	" 23	4:2:1	.....	.....	1:2.8
13° C.	" 31	2:1	.....	.....	1:1.3
12° C.	Nov. 3	5:2:1	.....	.....	1:3:1
12° C.	" 8	7:5:1	.....	.....	2:1
8° C.	Dec. 6	6:5:1	6:1	7:1	1:1.1
6° C.	" 19	3:7:1	10:1	4:7:1	3:1

A long series of experiments necessitating the examination of a great number of oysters and extending over a whole year would be required to establish this relationship. However, this supposition is not so different from what we might expect when we consider the

biology of the oyster. The optimum temperature for the growth of the oyster is, probably between 20° C. and 25° C. At this temperature the cells of the oyster are most active. The mucus sells will secrete a larger amount of mucus than at decidedly lower temperatures. The more mucus secreted the more will remain clinging to the body of the oyster. Generally speaking the greater the amount of mucus the greater the number of bacteria we would expect to find in the mucus on the outside of the body. As the temperature of the water lowers, the metabolic processes of the oysters are correspondingly slowed and a smaller amount of mucus and for this reason fewer bacteria will be found on the body of the oyster. For this reason it seems fair to assume that the apparent relation between the temperature of the water and the proportion of B. coli on the outside of the oyster and the shell liquor is real and not accidental.

These two sets of experiments throw light on the findings of Houston cited above. It is easily seen that simply pouring water over the body of the oyster is not sufficient to remove all the bacteria. The experiments of the writer on the comparison of the bacterial content of the stomach and shell liquor shows that per unit volume the shell liquor contains on the average over twenty times as many bacteria as the stomach juices. Evidence from all sides shows that Houston's assumption that all the bacteria were washed from the body of the oyster by simply pouring water over the oyster and further that the bacteria found in the minced meat of the oysters so treated came entirely from the stomach are not in accordance with the facts.

These experiments show the necessity of examining not only the shell liquor, but also the mucus on the outside of the body of the oyster. This is especially true during the warmer months. At this time there are on the average many more B. coli on the body of the oyster than is contained in the shell liquor. It is perfectly legitimate to consider the mucus on the body of the oyster as part of the oyster juice. If we so consider the mucus, it makes a very decided difference in the score of the oyster. In one instance the combined score of one oyster was ninety-six times the score based upon the shell liquor alone. The combined score is never less and often many times more than the score based upon the shell liquor. If there were any constant relation between the B. coli content of the shell liquor and the mucus removed from the body of the oyster, the examination of the shell liquor alone would be sufficient. But as no such relation exists the necessity of examining both the shell liquor and the mucus is at once apparent.

COMPARISON OF THE BACTERIAL CONTENT OF THE STOMACH AND OF THE SHELL LIQUOR OF OYSTERS.

Houston in his report to the local Government Board, 1904, makes the following statement: "The experiments detailed elsewhere seem to indicate that per unit of volume the gastric juice of the oyster is more impure bacteriologically than the oyster liquor." The experiments upon which this statement is based are taken up in some detail under "Bacteriology of the Shell Liquor and 'Washings' from the Body of the Oyster," and so it is not necessary to take up these experiments in this connection. The writer has shown that these experiments and the conclusions drawn are not based upon sound assumptions and so these results are not to be relied upon.

Clark,[1] in a long series of experiments has shown that in both clams and oysters the shell liquor is much more likely to yield B. coli or sewage streptococci than either the stomach, intestine or rectum.

Both of these workers studied the B. coli content of the different parts of the oyster. The writer could not obtain any badly polluted oysters at the time of year during which the experiments were conducted and so he examined the shell liquor and stomach contents for the total number of bacteria which each part contained. It would have been possible to infect oysters artificially with the colon bacillus, but it is not certain that one could simulate natural conditions exactly and consequently wrong conclusions might be drawn. We have no reason to suppose that B. coli are distributed other than equally among the other bacteria in the oyster and so a comparison of the total quantity per unit volume ought to show the relative frequency with which one would expect to find any particular bacterium in either part of the oyster.

In this series of experiments forty-one oysters were used. The method of examination was as follows:—The juice of each oyster was collected in a small glass-stoppered bottle which was calibrated in two cubic centimeter divisions. The amount of shell liquor was read off in cubic centimeters and diluted with an equal amount of one per cent. sodium chloride solution. The shell liquor and the sodium chloride solution were shaken vigorously one hundred times and one cubic centimeter of this mixture was transferred to a tube containing nine cubic centimeters of one per cent. sodium chloride solution and

[1] Report State Board of Health of Mass. 1905, 428.

one cubic centimeter of this dilution was plated in agar. Also a cubic centimeter from this tube was transferred to another tube in nine cubic centimeters of salt solution. A cubic centimeter of this mixture was also plated. By this method of dilution the plates contained respectfully one twentieth and one two hundredth of a cubic centimeter of the original shell liquor. The plates were made in duplicate. After the oyster had been drained of its liquor the flat valve was removed and the other valve containing the body of the oyster was set on the edge and allowed to drain for several minutes. The excess of liquor was then removed with a piece of blotting paper and the region over the stomach was seared with a hot spatula and an incision made into the stomach with a sterile scalpel. With a graduated pipette one-twentieth of a cubic centimeter of the stomach contents was removed and plated another twentieth of a cubic centimeter was transferred to a tube containing nine cubic centimeters of salt solution and 1 cubic centimeter of this mixture plated. These plates contained respectfully one-twentieth and one-two hundredth of a cubic centimeter. The plates were also made in duplicate and in all cases the average of the two plates was taken as the count for each oyster. The counts given in the table below are for one-twentieth of a cubic centimeter of the oyster juice and the stomach contents.

Table Comparing the Number of Bacteria in One-Twentieth of a Cubic Centimeter of the Shell Liquor with the Number of an Equal Quantity of the Stomach Contents.

No. of Oyster.	No. of bacteria in shell liquor of oyster.	No. of bacteria in stomach contents of oyster.
1	20	1
2	220	5
3	8	1
4	130	6
5	16	1
6	1	11
7	50	3
8	90	2
9	20	8
10	110	13
11	3	9
12	85	6
13	12	0
14	17	4
15	430	3
16	55	4
17	1,000	1
18	95	1
19	500	2
20	260	1
21	340	1
22	120	1
23	340	1
24	155	0
25	1,000	2
26	725	1
27	38	6
28	2	2
29	1,000	20
30	1,000	3
31	90	12
32	19	14
33	50,000	470
34	10,000	280
35	100,000	150
36	15,000	10,000
37	10,000	10
38	24,000	30
39	10,000	10
40	50,000	2,500
41	50,000	25
Totals	304,351	13,620

From the table it is seen that in only two oysters, numbers six and eleven, out of the forty-one examined, was the number of bacteria per unit volume greater in the stomach contents than in the shell liquor. In oyster, twenty-eight the numbers were equal. In the remaining thirty-eight oysters there were more bacteria per unit volume in the shell liquor than in the stomach contents. In these thirty-eight oysters the ratio per unit quantity of the number of bacteria in the shell liquor to the number in the contents of the stomach varied from 3 to 2 in oyster Nos. 37 to 2000 to 1 in oyster No. 41. The ratio of the total number of bacteria per unit volume in the shell liquor of the forty-one oysters to the total number of bacteria in an equal quantity of the stomach contents was as 21.6 to 1. That is, a comparison of the average number of bacteria found in shell liquor with the number of bacteria in the stomach contents shows that per unit quantity there were more than twenty times as many bacteria in the shell liquor as in the stomach juice.

LENGTH OF TIME NECESSARY FOR BACTERIA TO PASS THROUGH THE INTESTINAL TRACT OF THE OYSTER.

So far as the writer is aware no one has ever made any determination of the rate at which food passess through the alimentary tract of the oyster. While it is difficult to determine this matter directly, it seemed possible to inoculate the shell liquor of oysters with some bacterium not found in oysters and trace its progress through the intestinal canal. B. prodigiosus was chosen because of its ease of identification and because the writer has never found it in oysters, and so far as he is aware it has never been reported as occurring in oysters.

In the first experiment twelve oysters were inoculated by sawing off a piece of the lip of the shell and inserting a loopful of a culture of B. prodigiosus into the branchial chamber. The oysters were layed very carefully upon cotton thoroughly saturated with water and covered with a glass dish to prevent evaporation. They were kept at the laboratory temperature which is about 20°C. At various intervals, as shown in the tables, three oysters were removed and examined. The examination was made as follows:—The right valve of the oyster was removed and the gills and mantle carefully dissected away. The remaining part of the body was then washed for several minutes in running tap water. The left valve containing the oyster was then

set on edge and allowed to drain thoroughly. The surplus water was removed with filter paper. The oyster was then seared with a hot spatula over the stomach, over the intestine, where it bends sharply upon itself on the ventral side and on the rectum just above the anus. An incision was then made into these three parts of the alimentary tract with sterile scalpels and a sterile capillary pipette inserted and a portion of the contents removed and plated upon agar which was grown at room temperature for two days and examined for red colonies. Control samples of the shell liquor were plated before the inoculation with B. prodigiosus and these were negative in all cases. In the first experiment the time of examination after inoculation ranged from thirteen hours to twenty-seven hours. In the second experiment the time varies from five hours to seventy-four hours.

Table Showing Length of Time at which B. Prodigiosus was Isolated from Different Parts of the Alimentary Tract after the Inoculation of the Gill Chamber.

No. of Oyster.	Hours after inoculation.	Stomach.	Intestine.	Rectum.
Experiment I.				
1	13	+	0	0
2	13	+	0	0
3	13	0	+	0
4	18	0	0	0
5	18	+	0	0
6	18	0	+	+
7	23	0	0	0
8	23	0	0	0
9	23	0	0	+
10	27	0	0	0
11	27	0	0	0
12	27	+	0	+
Experiment II.				
1	5	0	0	0
2	5	0	0	0
3	5	+	0	0
4	22	0	0	0
5	22	0	0	0
6	22	+	0	0
7	48	0	0	0
8	48	0	0	0
9	48	0	0	+
10	74	0	0	+
11	74	0	+	+
12	74	0	0	0

From these tables it can be seen that the first appearance of the bacteria in the intestine was thirteen hours after inoculation and in the rectum five hours later. We would expect to find the organisms in the stomach within a very short time after inoculation of the shell liquor. Since these experiments are few in number one must necessarily be conservative in the conclusion drawn.

THE BACTERIAL CONTENT OF OYSTERS DURING STORAGE.

The change in the bacterial content of oysters during storage at a temperature at which they are kept in oyster houses and during transportation is a matter of very great importance from the point of view of the public health. The oyster is a living organism capable of maintaining itself for a long period when removed from its natural element. It is possible that the digestive juices or the phagocytic cells of the oyster might materially decrease the number of bacteria in the oyster. On the other hand, even if the digestive secretions and the phagocytic cells were bactericidal, it is possible that the rapid multiplication of the bacteria in the shell liquor might be sufficient to maintain or increase the number of bacteria in the oyster as a whole. In order to observe the change in the bacterial content of oysters during storage the writer carried out the following experiment:

About a bushel of polluted oysters were taken from the Providence River December 5, 1913, and put into storage in the Laboratory at an average temperature of 10°C. The temperature was fairly constant and did not rise above 11°C., although for a short time during a period of exceptionally cold weather the temperature fell to 8°C., but it soon rose again to 10°C. The oysters were put into storage in the bag just as they were brought to the laboratory. No attempt was made to clean them in any way. As soon as they arrived a sample of ten oysters was taken from the bag and put on ice and examined the following day. At intervals other samples of ten oysters were removed and examined. The method of examination was the same as that described under "The Bacteriology of the Shell Liquors and the Washings from the Bodies of the Oysters." In all except two instances a 20°C. count, a 37°C. count, a "red" count, and a B. coli count were made. The detailed analysis of each oyster and the bacterial content of each sample as a whole is shown in the following table:

Table Showing Change in Bacterial Content of Oysters During Storage at 10°C.

Date.	Length of Storage.		20°C. Count.	37°C. Count.	"Red" Count.	Colon Count.	Total Colon Count.
December 6	0 Days	Shell Liquor	104,000	39,000	10,000	4,000	
		"Washings"	15,600	6,900	1,500	4,440	8,400
December 9	4 "	Shell Liquor	950,000	78,000	10,000	1,400	
		"Washings"	110,000	13,000	6,500	3,200	4,600
December 12	7 "	Shell Liquor	1,150,000	84,000	14,000	5,400	
		"Washings"	1,300,000	140,000	46,000	3,100	8,500
December 16	11 "	Shell Liquor	6,200,000	180,000	73,000	2,500	
		"Washings"	460,000	45,000	3,700	900	3,400
December 22	17 "	Shell Liquor	5,700,000	500,000	2,800	3,600	
		"Washings"	1,800,000	42,000	1,200	900	4,500
December 30	25 "	Shell Liquor	3,600,000	850,000	29,000	3,100	
		"Washings"	700,000	160,000	36,500	325	3,425
January 5	31 "	Shell Liquor	1,000,000	Not made	Not made	3,000	
		"Washings"	210,000			650	3,650
January 27	53 "	Shell Liquor	2,200,000	18,500	1,900	1,300	
		"Washings"	4,200,000	97,000	60,000	450	1,750
February 23	80 "	Shell Liquor	Not made	Not made	Not made	4,300	
		"Washings"				550	4,850

The Following Tables Show the Change in Bacterial Content of Oysters During Storage at 10°C.

TABLE NO. I.—*Showing Bacterial Content at Beginning of Storage.*

DATE.	No. of Oyster.	C. C. of Oyster Liquor.	20°C. Count.		37°C. Count.		Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
December 6, 1913	1	24	67,000	7,000	16,000	2,800	4,300	800	480	2,000	20	103
" "	2	10	3,200	300	3,000	0	400	0	20	0	2	2
" "	3	14	10,000	1,400	5,600	800	1,000	0	2,800	200	200	214
" "	4	8	1,500	4,200	1,600	2,000	500	200	160	2,000	20	270
" "	5	9	14,000	1,200	5,400	500	550	100	180	200	20	42
" "	6	17	3,700	800	5,100	600	2,600	0	340	20	20	21
" "	7	10	4,600	700	2,400	200	800	400	20	20	2	4
Total			104,000	15,600	39,100	6,900	10,150	1,500	4,000	4,440	284	655

TABLE NO. II.—*Showing Bacterial Content After Four Days Storage.*

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		37°C. Count.		Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
December 9, 1913	1	7	112,000	8,500	1,550	1,450	770	1,000	140	200	20	49
" "	2	6	45,000	5,500	400	500	200	160	120	20	20	24
" "	3	10	100,000	2,000	2,000	1,200	500	600	200	0	20	20
" "	4	8	112,000	5,000	9,000	750	190	60	160	200	20	45
" "	5	9	125,000	6,000	3,500	750	160	20	180	200	20	42
" "	6	12	190,000	24,000	16,000	1,300	1,550	280	240	200	20	37
" "	7	7	105,000	7,000	13,000	800	125	200	14	200	2	31
" "	8	5	30,000	30,000	28,000	4,000	1,200	3,400	10	20	2	6
" "	9	8	80,000	2,400	2,700	1,300	3,800	450	160	200	20	45
" "	10	9	50,000	17,000	2,200	850	1,400	240	180	2,000	20	242
Total	...	...	949,000	107,000	78,350	12,900	9,895	6,510	1,404	3,240	164	541

TABLE No. III.—*Showing Bacterial Content After Seven Days Storage.*

DATE.	No. of Oyster.	C C. of Oyster Liquor.	20°C. Count.		37°C. Count.		Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
December 12, 1913	1	13	1,040,000	300,000	14,000	80,000	1,000	40,000	26	200	2	17
" "	2	6	210,000	240,000	3,800	1,200	50	20	120	20	20	23
" "	3	10	800,000	200,000	11,000	9,000	5,500	40	200	200	20	40
" "	4	11	880,000	160,000	15,000	11,000	700	500	2,200	2,000	200	382
" "	5	12	480,000	90,000	4,000	17,000	1,211	60	240	20	20	22
" "	6	14	84,000	8,500	1,700	600	700	40	280	200	20	34
" "	7	6	120,000	90,000	11,000	7,000	4,200	5,000	12	200	2	35
" "	8	7	210,000	16,000	5,000	2,600	70	40	140	20	20	23
" "	9	5	150,000	22,000	9,000	5,500	250	120	1,000	20	200	204
" "	10	6	180,000	200,000	9,500	4,500	480	320	1,200	200	200	233
Total			1,154,000	1,326,000	84,000	138,000	14,150	46,140	5,418	3,080	704	1,013

TABLE No. IV.—*Showing the Bacterial Content After Eleven Days Storage.*

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		37°C. Count.		Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
December 16, 1913	1	11	180,000	20,000	8,500	3,000	250	200	280	200	20	34
" "	2	6	570,000	70,000	5,500	600	70	20	1,200	200	200	233
" "	3	9	110,000	28,000	8,000	12,500	2,700	1,300	18	200	2	24
" "	4	10	160,000	21,000	1,700	3,400	400	240	200	200	20	40
" "	5	10	2,800,000	160,000	9,000	800	300	20	200	0	20	20
" "	6	10	130,000	4,500	29,000	4,500	140	1,500	200	0	20	20
" "	7	10	180,000	45,000	300	160	40	20	20	20	2	4
" "	8	10	480,000	12,000	96,000	18,000	68,000	300	20	20	2	4
" "	9	10	80,000	23,000	5,000	1,200	800	60	200	20	20	22
" "	10	10	1,500,000	80,000	15,000	650	140	60	200	20	20	20
Total			6,190,000	463,500	178,000	44,810	72,840	3,720	2,538	880	326	423

TABLE NO. V.—*Showing the Bacterial Content After Seventeen Days Storage.*

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		37°C. Count.		Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
December 22, 1913	1	8	74,000	13,000	4,000	1,200	65	0	16	0	2	2
" "	2	7	850,000	44,000	7,000	9,500	300	340	140	200	20	49
" "	3	14	25,000	22,000	1,500	2,000	110	100	280	20	20	22
" "	4	8	50,900	5,000	1,300	1,000	500	0	16	20	2	5
" "	5	6	21,000	230,000	1,400	2,600	12	100	0	200	0	33
" "	6	9	100,000	8,500	2,300	1,600	500	20	180	200	20	42
" "	7	1	2,055,000	300,000	36,000	1,400	24	0	2,400	20	200	202
" "	8	12	2,900,000	230,000	190,000	6,000	1,100	520	240	20	20	22
" "	9	6	395,000	400,000	9,000	11,000	180	20	12	20	2	5
" "	10	15	250,000	500,000	250,000	5,000	0	60	300	200	20	33
Total	...	...	5,720,000	1,752,500	502,500	41,300	2,791	1,160	3,584	900	306	415

TABLE No. VI.—*Showing Bacterial Content After Twenty-Five Days Storage.*

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		37°C. Count.		Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
December 30, 1913	1	17	1,000,000	14,000	140,000	5,000	7,500	140	34	20	2	3
" "	2	5	125,000	8,000	65,000	32,000	7,500	34,000	10	0	2	2
" "	3	13	100,000	3,600	155,000	2,200	2,100	900	260	200	20	35
" "	4	10	110,000	45,000	70,000	9,000	4,000	300	2,000	20	200	202
" "	5	11	120,000	230,000	175,000	2,800	900	240	220	20	20	22
" "	6	8	240,000	17,000	8,000	20,000	1,400	0	16	20	2	5
" "	7	12	575,000	70,000	85,000	40,000	1,700	0	240	20	20	22
" "	8	10	800,000	35,000	35,000	8,000	0	0	20	0	2	2
" "	9	13	26,000	8,500	14,000	2,4000	26	100	260	0	20	20
" "	10	13	450,000	270,000	90,000	7,500	3,600	800	26	20	2	4
Total			3,606,000	701,000	837,000	155,500	28,726	36,480	3,086	320	296	317

TABLE No. VII.—*Showing Bacterial Content After Thirty-One Days Storage.*

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		37°C. Count.		Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor. and Washings.
January 5, 1914	1	8	280,000	42,000	.....	.....	.....	.....	16	0	2	2
" "	2	9	34,000	20,000	.....	.....	.....	.....	360	20	40	42
" "	3	10	52,000	13,000	.....	.....	.....	.....	20	0	2	2
" "	4	12	145,000	22,000	.....	.....	.....	.....	2,400	0	200	200
" "	5	7	36,000	14,000	.....	.....	.....	.....	0	0	0	0
" "	6	8	140,000	48,000	.....	.....	.....	.....	160	20	20	23
" "	7	10	70,000	15,000	.....	.....	.....	.....	20	200	2	22
" "	8	16	160,000	20,000	.....	.....	.....	.....	0	200	0	13
" "	9	6	48,000	6,500	.....	.....	.....	.....	12	0	2	2
" "	10	11	50,000	13,000	.....	.....	.....	.....	22	200	2	20
Total	.....	.....	1,015,000	213,500	.....	.....	.....	.....	3,010	640	370	326

TABLE NO. VIII.—*Showing Bacterial Content After Fifty-Three Days Storage.*

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		37°C. Count.		Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
January 27, 1914	1	14	450,000	37,000	4,800	1,500	550	300	28	0	2	2
" "	2	3	25,000	26,000	1,900	1,600	240	500	60	200	20	87
" "	3	11	400,000	36,000	2,400	42,000	110	30,000	220	20	20	22
" "	4	6	12,000	1,200	850	3,000	0	100	12	0	2	2
" "	5	10	160,000	11,000	4,000	0	100	0	200	0	20	20
" "	6	6	38,000	22,000	240	0	0	0	120	0	20	20
" "	7	11	310,000	15,000	1,300	1,200	660	200	220	20	20	22
" "	8	10	25,000	2,000	1,400	2,800	100	2,200	20	20	2	4
" "	9	9	320,000	4,000,000	90	40,000	0	24,000	180	0	20	20
" "	10	11	450,000	38,000	1,500	4,800	110	2,800	220	220	20	38
Total			2,190,000	4,188,200	18,480	96,900	1,870	60,100	1,280	460	146	237

TABLE No. IX.—*Showing Bacterial Content After Eighty Days.*

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	*20°C. Count.		*37°C. Count.		*Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
February 23, 1914	1	6	.....	.....	.....	.....	.....	.....	120	20	20	23
" "	2	6	.....	.....	.....	.....	.....	.....	120	20	20	23
" "	3	8	.....	.....	.....	.....	.....	.....	16	200	2	27
" "	4	7	.....	.....	.....	.....	.....	.....	140	20	20	23
" "	5	11	.....	.....	.....	.....	.....	.....	22	20	2	4
" "	6	10	.....	.....	.....	.....	.....	.....	2,000	20	200	202
" "	7	9	.....	.....	.....	.....	.....	.....	1,800	0	200	200
" "	8	6	.....	.....	.....	.....	.....	.....	12	20	2	5
" "	9	4	.....	.....	.....	.....	.....	.....	80	20	20	24
" "	10	1	.....	.....	.....	.....	.....	.....	6	200	6	206
Totals			.....	.....	.....	.....	.....	.....	4,316	540	492	737

* Not made.

The results are so irregular that we can draw no very specific conclusions. It appears that in the first two weeks there is no initial decrease but rather a steady increase in the total number of bacteria present. This increase is also apparent in the 37°C. count and the "red" count. On January 27, fifty-three days after the beginning of the experiment there was a remarkable change in the proportion of bacteria in the "washing" as compared with the shell liquor in all except the B. coli count. The detailed analsyis for this date shows that oyster number nine is responsible for this marked change. It is very probably that this oyster had died and decomposition was taking place.

The B. coli count shows a decrease on the fourth day, but this decrease is not particularly marked and may well be due to variations in the oysters and not to an actual decrease. This is all the more likely when it is found that on the seventh day the number of B. coli is approximately the same as on the first day. The subsequent examinations show that the number of B. coli is about one-half the initial number and remains fairly constant throughout the experiment. In the last analysis made, eighty days after the beginning of the experiment, all the bile tubes showing gas after twenty-four hours incubation were tested for B. welchii by inoculating a cubic centimeter of the bile into freshly sterilized milk tubes and incubating anærobically. No visible change took place in the milk after eighteen hours incubation. It was a noticeable fact that not over ten per cent. of the tubes showing gas after twenty-four hours incubation had one hundred per cent. of gas, the amount said to be characteristic of B. welchii. Most of the tubes had about fifty per cent. gas. From these facts it appears that the fermentation was caused by some member of the B. coli group and not by B. welchii. It is not surprising to find that B. coli should live eighty days in oysters under such conditions for Clark[1] has shown that B. coli will live in ten per cent. sewage eighty-four days and in fifty per cent. sewage one hundred and sixty-six days. Unpublished results from this laboratory show that B. coli will live in sea water for one hundred and eighty days. The writer has shown in the experiments on the hibernation of the oyster that B. coli will live in oysters kept at 1.5°C. for at least one hundred days.

[1]Report of State Board of Health of Mass., 1905, p. 455.

The important conclusion to be drawn from this series of experiments is that under the conditions of the experiment bacteria of the B. coli group do not materially increase or decrease in oysters in the shell during storage.

CLEANSING OF POLLUTED OYSTERS.

As soon as the etiological connection between oysters and certain epidemics of typhoid and gastro-enteritis was firmly established, the question at once arose as to how long a time it would take oysters known to be polluted to free themselves from sewage organisms after they had been removed to water free from sewage contamination.

Klein[1] put oysters into tanks in the laboratory and infected them with B. typhosus. About one-third of the water was removed every day and replaced with clean sea water. Oysters were removed at various intervals and examined for B. typhosus. The experiments were repeated several times and B. typhosus was isolated at the end of the experiment in every case. The various experiments were concluded on the seventh, ninth, fourteenth, sixteenth, seventeenth and eighteenth day after infection. The bacilli were isolated from the sea water twenty-one days after the beginning of the experiment. Of course, these experiments did not approximate natural conditions and so we can draw no definite conclusions from them regarding the length of time necessary for oysters to rid themselves of these bacteria when taken from polluted areas and re-layed in water free from pollution.

Herdmann and Boyce[2] tried the experiment of infecting oysters artifically with large numbers of B. typhosus and then subjecting them "to a running stream of pure clean sea water." Eighteen oysters were infected and examined at different intervals varying from one to seven days. Only the stomach contents were examined and considerable allowance must be made for this, for the writer has shown in another part of this paper that the number of bacteria contained in the stomach are quite insignificant compared with the number in the shell liquor and on the body of the oyster.

In three of the eighteen oysters examined which had washed for three, five and seven days, respectfully, no typhoid bacilli were found. In the other fifteen oysters examined B. typhosus was

[1]Relation of Oysters and Disease, Supplement to the Report of the Medical Officer to the Local Government Board, 1893.

[2]Loc. cit.

found in varying numbers. Herdmann and Boyce sum up the matter as follows: "The result was definite and uniform; there was a great diminution or total disappearance of B. typhosus in from one to seven days."

Johnstone[1] took oysters known to be polluted and transferred to the purest water available. He found under the conditions of the experiment that four days was a sufficient period of quarantine, since after that time no further cleansing took place, because the water of the locality was not entirely free from sewage contamination.

Phelps in this country[2] found that only two to four days was necessary for polluted oysters to cleanse themselves when transferred to clean water.

In 1913, Fabre-Domergue[3] read a paper before the Académie de Medicine in which he recommended the placing of polluted oysters in basins fed by filtered water and removed often enough to insure complete evacuation of the liquid contained in the shells and in the digestive tract. From his results he considers it an established fact that this procedure eliminates all pathogenic bacteria from the molluscs in six or seven days.

Field[4] says: "These (oysters) get bacteria from the waters filled with waste and sewage, and it takes them at least seventy-two hours to free themselves from these impurities that they have taken in from the waters of the different harbors." Field does not say upon what evidence, if any, this statement is based. But he adds that in Massachusetts a law has been passed requiring such polluted oysters to be transferred to clean water and allowed to remain for four weeks before offered for sale.

The writer's own experiments on the cleansing of polluted oysters confirm in part the work cited above. It appears that the rapidity with which sewage bacteria are eliminated is influenced to quite a large extent by the temperature. If the water is warm, say around 20°—25 C. the oysters remain open probably most of the time. As this is about the optimum temperature for the most rapid growth and development of the oyster, it is also the temperature at which the oyster is most active. The ciliary motion is more rapid than at lower temperatures which would increase the amount of water

[1]Jour. of Hyg IX, 1909.
[2]Jour. Am. Public Health Assn., Vol. 1, 1911, 305.
[3]Cited in Jour. Am. Med. Asso., LXI, 1913, 134.
[4]Report of Proceedings of 3rd Am. Convention of Nat. Asso. of Shellfish Commission, p. 34.

filtered through the gills and so increase the amount of "wash water" for carrying away the bacteria. Also the ciliary motion in the alimentary canal would be hastened and so the organisms contained therein would be more quickly disposed of. Further, the capacity of the oyster to digest and assimulate bacteria would be at its height at a temperature at which the cells are most active. Hence, the optimum temperature for the growth and development of the oyster we would expect to be the period at which all contaminating organisms would be eliminated most rapidly. As the temperature lowers the activities of the oyster lessen accordingly. Further, while above 20°C. the oyster has its valves open most of the time, as the temperature is lowered the oyster is more and more inclined to keep its valves closed for longer and longer periods. This would prevent the mechanical effect of the filtered water in carrying away the bacteria. This mechanical effect is very important for the writer has shown in another part of this paper that bacteria pass through the gills with the filtered water very rapidly. Further, the activity of the cells concerned in the digestion of bacteria would also be less active and also the antagonism between different species of bacteria would be lessened. So it is seen that at lower temperature the tendency would be for oysters to eliminate bacteria more slowly than at higher temperatures. Various opinions have been expressed regarding the temperature at which oysters "hibernate" or close their shells and remain closed due to the low temperature of the water. The theory of "hibernation" of the oyster was first proposed by Gorham[1] to explain the results obtained in his investigation of the sanitary conditions of the oyster beds of Narragansett Bay. The temperature at which this phenomenon is supposed to take place is a little above 0°C. So far as the writer is aware no experimental work of an exact nature has been done to substantiate or disprove this theory, but from personal observation the writer is led to suspect that the temperature at which the oyster closes its shell for a relatively long period is considerably higher as will appear from one of the experiments detailed below.

On the other hand, experiments to be detailed later under the hibernation of oysters seem to show that oysters do open and are active at temperatures only one to two degrees above 0°C. It appears that when the temperature is low oysters will close their shell

[1](1) Rep. of Commissioners of Shell Fisheries of R. I., 1910. (2) Seasonal Variation in the Bacterial Content of Oysters, Jour. Am. Pub. Health, Jan., 1912.

for sometime, but not for indefinite periods. It also appears that the closure of the shell is not due to cold rigor or loss of control of the adductor muscle, for at a temperature of 1.5°C. the oyster can open and close its shell with the same ease as at higher temperatures.

In the following experiments only the shell liquor was used. The method of examination of the oysters was the procedure recommended by the Second Progress Report of the Committee on Standard Methods of Shellfish Examination of the American Public Health Association. The medium used was lactose-peptone bile and the tubes were inoculated in duplicate. The tubes were examined every twenty-four hours for three days. If ten per cent. or more of gas appeared during this time, it was considered to show the presence of intestinal bacteria. Unfortunately in the first experiment the investigation had to be discontinued after November 29th, so that we have only the results extending over 12 days.

Experiment 1.

November 16, 1912, about a bushel of polluted oysters were taken from the Providence River and the following day were transferred to Wickford Harbor. They were laid upon clean sandy bottom on the edge of the channel and were well separated to allow free access of water. The temperature of the water at the time of taking the oysters was 14°C. The average of the maximum and minimum temperature at Wickford for November 16 and 17 was 6.5°C. A sample of the oysters was taken at the time they were placed in Wickford Harbor and the analysis showed a score on fifteen oysters of 870. Samples were shipped to the laboratory every day until November 29th. These were analyzed immediately so that only three or four hours elapsed between the time of collecting the sample and the time of analysis. The following table shows the results of the analysis of fifteen oysters on the different days. The temperature is the average of the maximum and minimum temperature as recorded at the lobster hatchery of the Inland Fish Commission which was located nearby.

Tables Showing the Results of Analysis of Fifteen Oysters Taken from a lot of Polluted Oysters which had been put into Unpolluted Water at Wickford on November 17, 1912.

Date, November 17, 1912.

Dilution of Shell Liquor.	Average Temperature 14°C.																													
	No. of Oyster.																													
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
1 c. c.	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1–10 c. c.	+	+	+	+	+	0	0	0	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	0	+	+	+	+	+	0
1–100 c. c.	+	0	0		+	0	0	0	0	0	+	0	+	0	0	0	+	0	+	+	+	0	0	0	+	0	+	0	0	0

Score, 870.

Date, November 21, 1912.

Dilution of Shell Liquor.	Average Temperature 5.2°C.																													
	No. of Oyster.																													
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
1 c. c.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1–10 c. c.	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1–100 c. c.	0	0	+	+	+	0	+	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	+	0	+	0	0	0

Score, 690

Date, November 22, 1912.

Dilution of Shell Liquor.	Average Temperature 6.4°C.														
	No. of Oyster.														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 c. c.	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
1–10 c. c.	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ 0
1–100 c. c.	+ +	+ +	+ 0	+ +	+ +	+ 0	+ +	+ +	+ +	+ +	+ +	+ 0	+ +	+ +	+ 0

Score, 1,500.

Date, November 23, 1912.

Dilution of Shell Liquor.	Average Temperature 7.2°C.														
	No. of Oyster.														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 c. c.	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
1–10 c. c.	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
1–100 c. c.	0 0	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +

Score, 1,410.

Date, November 24, 1912.

Dilution of Shell Liquor.	Average Temperature 6.1°C.																													
	No. of Oyster.																													
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
1 c. c.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1–10 c. c.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1–100 c. c.	+	+	+	+	+	+	+	0	+	0	+	0	+	+	+	0	0	0	+	0	+	+	0	0	+	0	+	0	+	0

Score, 1,320.

Date, November 25, 1912.

Dilution of Shell Liquor.	Average Temperature 6.1°C.																													
	No. of Oyster.																													
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
1 c. c.	+	0	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1–10 c. c.	+	0	+	0	0	0	+	+	+	0	+	+	+	+	0	0	+	+	+	0	+	+	+	+	+	+	+	+	+	+
1–100 c. c.	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	+	0	0	0	+	0	+	0

Score, 420.

Date, November 26, 1912.

Dilution of Shell Liquor.	Average Temperature 5°C.																											
	No. of Oyster.																											
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
1 c. c.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1–10 c. c.	+	+	+	+	+	+	+	0	+	+	+	+	+	0	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+
1–100 c. c.	+	+	+	0	+	0	0	0	+	+	0	0	0	0	+	+	+	0	+	+	0	0	+	+	+	+	+	0	+	+

Score, 1,140.

Date, November 28, 1912.

Dilution of Shell Liquor.	Average Temperature 6.1°C																											
	No. of Oyster.																											
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
1 c. c.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	+	0	+	+	+	+	+	+	+	+	+	+	+	+
1–10 c. c.	0	0	+	+	+	0	+	+	+	+	+	+	+	0	+	+	+	0	+	0	+	+	+	+	+	+	+	+	+	+
1–100 c. c.	0	0	+	+	+	0	+	+	+	0	+	+	0	0	+	+	0	0	0	0	0	0	0	0	+	0	0	0	+	+

Score, 780.

Date, November 29, 1913.

Dilution of Shell Liquor	Average Temperature 4.4°C.																											
	No. of Oyster.																											
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
1 c. c.	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0
1–10 c. c.	+	+	+	+	+	0	0	0	0	0	0	0	+	+	0	0	+	+	+	+	+	0	+	0	+	0	0	0	0	0
1–100 c. c.	+	0	+	0	0	0	0	0	0	0	0	0	+	0	+	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0

Score, 132.

+ = positive presumptive test for B. coli.
0 = negative presumptive test for B. coli.

Unfortunately the experiments could not be continued and so we cannot say whether the apparent cleansing, which appeared on the last two days, especially on the last day, was due to a fortunate selection of oysters or was the indication of a real elimination of the intestinal bacteria. The writer is led to believe that the oysters had just begun to open and so allowed the bacteria to be washed out. The low temperature of the water slowed the metabolic processes of the oyster and so, as food and oxygen were not needed in so great quantities, an oyster could maintain itself for sometime without renewing its supply. As soon as the supply was exhausted, however, the oyster opened its shell.

This investigation shows that under the conditions of the experiment with a temperature between 7.2°C. and 5°C. a period of twelve days is not sufficient to allow oysters to free themselves from intestinal bacteria.

Experiment II.

May 13, 1913, about a bushel of polluted oysters were taken from Providence River and transferred to the same location in Wickford Harbor as in the previous experiment.

The water of Wickford Harbor at the place where the oysters were put down was tested by the lactose-peptone-bile presumptive test and no sewage organisms were found. The methods and conditions of the experiment were the same as in the previous experiment except that ten oysters were used instead of fifteen. The sanitary condition of the oysters at the time of transplantation and on two subsequent occasions is shown in the following table:

Tables Showing Results of Analysis of Ten Oysters from a lot of Polluted Oysters which had been put into Relatively Unpolluted Water at Wickford, May 13, 1913.

Date, May 13, 1913.

Dilution of Shell Liquor.	Average Temperature.																	
	No. of Oyster.																	
	1		2		3		4		5		6		7		8		9		10	
1c.c.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1–10c.c.	+	+	+	+	+	+	0	0	+	+	+	+	+	+	+	+	+	0	0	0
1–100c.c.	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	0	0	0	0	0

Score, 460.

Date, May 17, 1913.

Dilution of Shell Liquor.	Average Temperature, 12.7°C.																	
	No. of Oyster.																	
	1		2		3		4		5		6		7		8		9		10	
1c.c.	+	+	+	+	+	+	+	+	+	+	+	0	+	0	+	+	+	+	+	+
1–10c.c.	+	0	0	0	+	+	+	+	0	0	+	0	0	0	+	0	+	+	0	0
1–100c.c.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0

Score, 73.

Date, May 22, 1913.

Dilution of Shell Liquor.	Average Temperature 14.7°C.																	
	No. of Oyster.																	
	1		2		3		4		5		6		7		8		9		10	
1c.c.	+	0	+	+	+	+	+	+	+	+	+	+	0	0	+	+	+	+	+	+
1–10c.c.	+	0	+	0	0	0	0	0	+	+	0	0	0	0	0	0	0	0	0	0
1–100c.c.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Score, 28.

From the table it is seen that at the beginning of the experiment there were on the average forty-six B. coli per cubic centimeter of oyster juice. After a period of four days this number had dropped to an average of 7.3 per cubic centimeter and after a period of nine days the number had still further decreased so that there were on the average only 2.8 B. coli per c.c. of the oyster juice. This shows that under the conditions of the experiment, oysters which contained 46 B. coli per cubic centimeter can in nine days free themselves from B. coli to such an extent that there remains only 2.8 B. coli pr cubic centimeter. This is well within the standard adopted by the Bureau of Chemistry which allows oysters to be shipped in interstate commerce which contain 4.6 B. coli per cubic centimeter of shell liquor.

Experiment III.

On November 8, 1913 a bushel of oysters were taken from Providence River and transplanted to Wickford. These oysters were put into two galvanized iron baskets and hung into the water from the floor of the Beacon Oyster Co. These oysters were suspended in the water near the edge of the channel and located only a few yards from the place where the oysters in the two previous experiments were placed. A sample of ten oysters was taken from this lot and carried to the laboratory for analysis. These ten oysters were found to be badly polluted and had a score of 640. Samples were sent to the laboratory and analyzed on November 10, 12, 14, 17, 19, 21 and 24. The methods of analysis were the same as in the previous experiments with two exceptions. The 1–10 c.c. and 1–100 c.c. dilutions were made in duplicate, while the one cubic centimeter samples were only inoculated singly. The oyster liquor was drained into glass-stoppered bottles which were graduated so that the amount of liquor could be read off in cubic centimeters. An equal amount of sterile one per cent. sodium chloride solution was added and the bottle shaken vigorously one hundred times. One cubic centimeter of this mixture was used for the first inoculation and to make the proper dilutions. As a result the quantities as given in the table are for the mixture of shell liquor and salt solution. The amount of shell liquor in the dilutions is not 1 c.c., 1–10 c.c. and 1–100 c.c., but ½ c.c., 1–20 c.c. and 1–200 c.c. But as ten oysters were used the result equals an analysis of five oysters where 1 c.c., 1–10 c.c. and 1–100 c.c. samples of the shell liquor were used. For comparative results,

however, it does not matter what quantity we use provided we use the same amount every time. The following table shows the results of the examination on the different days.

Table Showing the Results of Analysis of Polluted Oysters which were put into Comparatively Uncontaminated Water at Wickford, November 8, 1913.

Date, November 8, 1913.

Quantity of Shell Liquor and Salt Solution.	Average Temperature.									
	No. of Oyster.									
	1	2	3	4	5	6	7	8	9	10
1c.c.	+	+	+	+	+	+	+	+	+	+
1–10c.c.	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
1–100c.c.	0 0	+ +	+ +	+ 0	+ 0	+ +	+ 0	0 0	+ 0	0 0

Score, 730.

Date, November 10, 1913.

Quantity of Shell Liquor and Salt Solution.	Average Temperature 11.1°C.									
	No. of Oyster.									
	1	2	3	4	5	6	7	8	9	10
1c.c.	+	0	+	+	+	+	+	+	+	+
1–10c.c.	+ 0	+ 0	+ 0	0 0	+ +	+ +	+ +	+ +	+ 0	+ 0
1–100c.c.	0 0	0 0	0 0	0 0	+ 0	0 0	0 0	0 0	0 0	+ 0

Score, 190.

Date, November 12, 1913.

Quantity of Shell Liquor and Salt Solution.	Average Temperature 8.8°C.									
	No. of Oyster.									
	1	2	3	4	5	6	7	8	9	10
1c.c.	0	0	0	0	+	+	+	+	+	+
1–10c.c.	0 0	0 0	0 0	+ 0	0 0	0 0	+ 0	0 0	+ 0	+ 0
1–100c.c.	0 0	+ 0	0 0	0 0	0 0	+ 0	0 0	0 0	+ 0	0 0

Score, 37.

Date, November 4, 1913.

Quantity of Shell Liquor and Salt Solution.	Average Temperature 9.7°C.									
	No. of Oyster.									
	1	2	3	4	5	6	7	8	9	10
1c.c.	+	+	+	0	0	+	+	+	+	+
1–10c.c.	0 0	+ 0	0 0	0 0	+ 0	0 0	0 0	0 0	0 0	0 0
1–100c.c.	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0

Score, 10.

Date, November 17, 1913.

Quantity of Shell Liquor and Salt Solution.	Average Temperature 7.7°C.									
	No. of Oyster.									
	1	2	3	4	5	5	7	8	9	10
1c.c.	+	+	+	+	+	+	+	+	+	+
1–10c.c.	0 0	0 0	0 0	+ 0	0 0	+ 0	0 0	0 0	0 0	0 0
1–100c.c.	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0

Score, 10.

Date, November 19, 1913.

Quantity of Shell Liquor and Salt Solution.	Average Temperature 7.3°C.									
	No. of Oyster.									
	1	2	3	4	5	6	7	8	9	10
1c.c.	+	+	+	+	0	+	+	0	+	0
1–10c.c.	+ 0	0 0	+ 0	0 0	0 0	+ +	0 0	0 0	0 0	+ 0
1–100c.c.	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0

Score, 19.

Date, November 21, 1913.

Quantity of Shell Liquor and Salt Solution.	Average Temperature 10.5°C.									
	No. of Oyster.									
	1	2	3	4	5	6	7	8	9	10
1c.c.	+	+	+	0	+	+	+	+	+	+
1–10c.c.	+ 0	0 0	0 0	0 0	0 0	+ 0	0 0	0 0	0 0	0 0
1–100c.c.	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0

Score, 19.

Date, November 24, 1913.

Quantity of Shell Liquor and Salt Solution.	Average Temperature 10.5°C.									
	No. of Oyster.									
	1	2	3	4	5	6	7	8	9	10
1c.c.	+	0	+	+	+	+	0	+	+	+
1–10c.c.	0 0	0 0	+ +	0 0	+ 0	0 0	0 0	+ +	0 0	0 0
1–100c.c.	0 0	0 0	0 0	0 0	0 0	0 0	0 0	+ 0	0 0	0 0

Score, 28.

+ = positive presumptive test for B. coli.
0 = negative presumptive test for B. coli.

It is seen from the tables that the oysters cleaned themselves as much in six days as at any time. The samples taken on the day of transporting showed on the average twenty-three B. coli per cubic centimeter of oyster juice. Six days later the sample showed only one B. coli per cubic centimeter and they showed no further cleansing after ten more days. The fact that the water at this place is not entirely free from sewage contamination probably explains why the oysters did not show any further elimination of B. coli, apparently there were enough B. coli in the water to maintain a small number in the oyster at all times.

Conclusions.

In one experiment with a temperature averaging 9.7°C. over the period of the investigation the oysters showed an elimination of B.

coli from 73 per cubic centimeter to one per cubic centimeter in six days. In another experiment with an average temperature of 13°C. during the period of investigation the oysters showed an elimination of B. coli from an average of 46 B. coli per cubic centimeter to 7.3 B. coli per cubic centimeter in four days and to 2.8 per cubic centimeter of shell liquor in nine days. As no examination was made between the fourth and ninth day, it is quite possible that the limit of possible elimination was reached sometime before the ninth day. No doubt an examination on the sixth or seventh day would have shown a B. coli content sufficiently low to pass the standard set by the Bureau of Chemistry of the Federal Government.

In another experiment in November, 1912, with an average temperature 5.4°C. twelve days was not sufficient to eliminate B. coli to any appreciable extent. The examination on the twelfth day showed a very marked decrease in the number of B. coli, but as no subsequent examinations were made it is not possible to say with authority whether this was the beginning of an elimination process or not, though the writer is led to believe such was the case. The interesting feature of this experiment is that no elimination took place in nine days, while in the other two experiments a very marked reduction took place in six days in one case and in five and nine days in the other case.

These sets of experiments seem to throw some light upon the so-called hibernation of the oyster. With an average temperature of 13°C. in one case and 9.7°C. in the other the oysters opened and began to eliminate B. coli almost immediately, but in the first experiment with an average temperature of 5.4°C. no reduction in B. coli was found until the twelfth day. These experiments lead the writer to believe that when the temperature of the water is somewhere between 9°C. and 5°C. oysters close their shells for a longer or shorter period. But from experiments detailed elsewhere, the writer believes that there is no time above 0°C. when oysters close their shells for an indefinite period. The length of time that oysters remain closed is in inverse proportion to the temperature which determines the rapidity of the metabolic processes going on within the oyster.

EXPERIMENTS ON THE HIBERNATION OF THE OYSTER.

The so-called hibernation of oysters has attracted much attention during the last four years. The theory that oysters close their shells

when the temperature of the water approaches 0°C. was first put forward by Gorham in 1910;[1] to explain certain bacteriological findings in Providence River oysters. It was found that during the warmer months the oysters in certain parts of the river were badly polluted, but in January, with the temperature of the water around 0°C., the oysters were found free from colon bacilli. In order to explain this phenomenon Gorham advanced the theory that when the temperature of the water approaches 0°C. the oyster closes its shell and remains closed until the temperature of the water begins to rise and then it opens its shell and resumes its normal activity. This period was called its "Hibernation Period." A little later Pease,[2] Field, of the Massachusetts Fish and Game Commission, and others advanced a similar idea. So far as the writer is aware, however, no experiments have been tried to confirm or deny this theory. The experiments of the writer cited elsewhere on the cleansing of polluted oysters seem to show that oysters do remain closed for several days with a temperature of about 5°C. But in order to throw further light upon the matter the following experiments were tried.

Experiment I.

January 12, fourteen oysters were placed in sea water which had been inoculated with a pure culture of B. coli. The oysters were left in the sea water a day and a night. They were removed January 13th, and the outside of the shells scrubbed thoroughly with a stiff brush and running tap water and were then put into a strong solution of calcium hypochlorite for one-half hour and stirred up about once a minute. They were then put into 7% formalin for the same length of time and stirred with a glass rod for a few seconds at about one minute intervals. They were then washed for a considerable time in fast running tap water, temperatures between 7°C. and 8°C., and stirred at intervals of two or three minutes. The oysters were then taken (Jan. 13), to a cold storage room of the Merchant's Cold Storage and Warehouse Co., Providence, and put into storage at 34°F. (about 1.1°C.) The temperature of the room is maintained constant throughout the year and is never allowed to vary more than .5°F. The next day sterile sea water which had been kept in the

[1](1) Report of Commissioners of Shell Fisheries of R. I., 1910. (2) Seasonal Variation in the Bacterial Content of Oysters, Am. Jour. Pub. Health, II, 1910, 24.

[2]Some Bacteriological Problems in the Oyster Industry, The Fishing Gazette, 28, 1911, 865, July 15.

room for several days was poured into the dishes until it covered the oysters. Immediately after the oysters were covered five samples of two cubic centimeters each were taken from each dish and inoculated into bile tubes and incubated at 37°C. for 18 hours. Every tube showed gas. January 22, the oysters were examined in the dishes and it was found that four were closed tightly, five were open widely enough to be seen as they lay in the dishes and the other seven were found to be slightly open. The opening of these last seven was not perceptible to the eye, but upon taking them out and squeezing them one could hear a "squashy" sound, showing that they were not firmly closed. Apparently the five oysters that were open had lost their sensitiveness, for they would not remain closed when the valves were pressed together. The mechanical stimulation of the gills and mantle was not tried. The oysters were observed on several days until February 2nd and it was found that some of the oysters that had been firmly closed at first had opened and vice versa.

The oysters were not observed again until March 23. It was found that two of the oysters in one dish were open and dead. Two others were wide open but closed immediately when touched. These two oysters were brought to the laboratory and put into a dish of sterile sea water and observed for several days. They were just as active as oysters freshly brought from the beds. They were then tested for B. coli. Both oysters showed gas in 1–100 c.c. of shell liquor. These tubes were plated in litmus-lactose-agar and typical colon colonies were found in the plates from one oyster, but not from the other. This showed that B. coli can live under such condition for at least sixty-nine days.

The remaining oysters were again examined April 24, one hundred days after they were put into storage. Five of the oysters were apparently living, while the others were dead. These five were brought to the laboratory and examined. It was found that three were closed tightly, while the other two appeared a little "weak." One of the tightly closed oysters was put into a dish of sea water and it soon opened like an oyster removed only recently from its natural element. When the shell was touched it would close immediately, though its movements were not so vigorous as those of an oyster taken directly from the water. When the gills and mantle were touched with a wire it did not respond readily. Apparently its tactile sensations were not very acute, although after repeated stimulations it closed and gripped the wire so that it took considerable

strength to pull it out. The writer has noticed that oysters which have been removed from sea water for some time require a great deal of stimulation to make them close again, though after they have been open for a time they react immediately. It may be that the tango-receptors are very much dulled or that the desire for oxygen is stronger than the sense of self-protection.

The other four oysters were opened with the proper precautions and the mixed shell liquor and the "washings" from the body were inoculated into bile tubes. Two of the oysters were normal in appearance and exceptionally plump. The other two showed slight evidences of decomposition. All the tubes from three of the oysters showed gas and typical B. coli colonies were isolated on litmus-lactose-agar plates. Further identification was not regarded as necessary. The tubes inoculated from the third oyster showed no gas after three days incubation.

Experiment II.

Seven oysters were obtained fresh from the water and impregnated with a solution of azolitmin in sea water. They were then washed thoroughly with a stiff brush in running water and immersed in chromic acid for a few seconds and then washed again. All the color was removed in this manner. January 29 they were placed in tumblers and put into cold storage at 34°F. They were left over night to acquire the same temperature as the room and then the tumblers were filled with sea water. The dishes were watched to see if any color had escaped from the oysters. February 2 a slight coloration was found in the bottom of two of the tumblers, but this did not appear to increase for several days. The oysters were not examined again until March 23rd. The color had disappeared from the two tumblers that had previously been discolored. It was observed, however, that the water in the tumblers was not entirely clear. There was a sediment in the bottom of the tumblers that resembled the bits of mucus thrown off by oysters. One of the oysters was taken to the laboratory and placed in sea water. It soon opened, but did not contain any color. It was as active as a normal oyster. Some of the mucus thrown out by the oyster had a purplish color which had been stained with azolitmin.

April 17 the remaining six oysters were examined. It was found that three of the oysters were open and the other three closed. Covers

were fitted to all the dishes and during the process two of the oysters closed. The other one remained open even after reaching the laboratory. After the cover was removed and the shell touched with a glass rod it closed immediately. A heavy precipitate was found on the bottom of each of the dishes and a great deal of mucus was seen in suspension. This matter could not have come from the outside of the oyster, because they were thoroughly cleaned before the experiment began. There is no question but what the oyster had opened; three were found open and two of them closed immediately upon being agitated. The other three must have opened in order to discharge so much mucus, but had closed again of their own accord at 34°F.

Two oysters were infected with B. coli and put into dishes in cold storage January 20. The dishes were later found to be cracked and the water leaked out. These two oysters were brought to the laboratory April 17. One was put into a dish of sea water, while the other was opened and two cubic centimeters of the juice was inoculated into each of four bile tubes. Gas appeared in each tube and typical B. coli was isolated on litmus-lactose-agar plates. This was eighty-seven days after infection. The other oyster opened before morning, but was apparently dead for it would not respond to a mechanical stimulation of its gills and mantle. When opened both oysters appeared plump and in prime condition. From their appearance they could not have been told from oysters freshly caught.

From these experiments the writer believes that oysters do close their shells for varying periods, depending upon the temperature. Whether they close their shells under natural conditions when the temperature falls around 0°C. no one has determined. That they do not lose control of their adductor muscles is demonstrated in both experiments. The writer is lead to believe that there is no definite period at which this phenomenon can be said to begin. Mitchell in an unpublished observation states that with a temperature below 20°C. oysters get "nervous" and will close upon the slightest provocation and remain closed for fairly long periods. It appears that at this temperature the irritability of the oyster is much increased.

These experiments lead one to conclude that the so-called period of hibernation of the oyster is a relative term. The length of time that they remain closed depends upon the temperature which determines the rapidity of the oxidative and other metabolic processes of the oyster. An oyster will remain closed as long as its supply of

food and oxygen remains sufficient and the lower the temperature the longer this period will be. The oyster does not close on account of "rigor frigoris," for the control of the adductor muscle is still very marked at a temperature of 1.1°C., and is scarcely distinguishable from normal.

Mitchell,[1] in an extended study of the oxygen requirements of shellfish states as one of his conclusions that "oysters of medium sizes, at temperatures between 19° and 28°C., used from 7 to 35 decimilligrams of oxygen per hour per 100 grams of entire weight. The amount varies with the temperature, so far as experiments show, according to simple relationship, so that the curve approximates a straight line." . . . "The common clam (Mya Arenaria) shows a higher oxygen requirement than the oyster."

The theory of hibernation which the writer has advanced appears to be in harmony with the experiments of Mitchell on the oxygen requirements of oysters. The lower the temperature the less the amount of oxygen used. But no matter what the temperature so long as the oyster is living it needs a certain amount of oxygen to carry on its oxidative processes. When the amount available within its shell is exhausted, it will open to renew its supply.

The statements of practical oyster growers also leads to the same conclusion. It is said that oysters from Narragansett Bay in February cannot be shipped very far in the shell, because, as the oyster men say, they will "cluck," that is, open their shells and allow the shell liquor to run out. The explanation no doubt is that during the "zero weather" of January, the oysters are closed and as their oxygen requirements under the circumstances are small they can remain closed for sometime without exhausting the supply available in the shell liquor. The period of cold weather, however, is sufficiently long perhaps to allow the oysters, even with their small requirements, to nearly, if not quite exhaust the available supply of oxygen within their closed shells. The result is that in February when they are removed to the opening house or express car which has relatively a much higher temperature than the water from which they were taken, the metabolic processes of the oyster are greatly increased and there is a demand for more oxygen. The supply within the shell, which has already been greatly reduced, is quickly used up, and consequently the oyster opens to renew its supply.

[1]The Oxygen Requirements of Shellfish, Bull. U. S. Bureau of Fisheries, XXXII, 1912, 209

It is said that the soft-shelled clam does not hibernate during the winter. The second quotation from Mitchell's paper, namely, that the oxygen requirement in the common clam is higher than in the oyster may account for this phenomenon. The sooner the available quantity of oxygen is used up, the more quickly will the mollusc open to renew its supply.

SUGGESTED CHANGES IN STANDARD METHODS OF SHELLFISH EXAMINATION.

The Second Progress Report of the Committee on Standard Methods of Shellfish Examination recommends that "twelve oysters of the average size of the lot under examination, with deep bowls, short lips and shells tightly closed, shall be picked out by hand and prepared for transportation to the laboratory." . . .

"Bacterial counts shall be made of a composite sample of each lot obtained by mixing the shell liquor of five oysters." . . .

Under the heading of "Methods of Rating Oysters for B. coli," the following statement is made: "The following values shall be assigned to the presence of bacteria of the B. coli group in each of the five oysters examined." Then follows a statement and illustration of the method of scoring as adopted by the American Public Health Association. It is clear at once that if we mixed the shell liquor of the five oysters and examined it as a composite sample, it would be impossible to assign values "to the presence of bacteria of the B. coli group in each of the five oysters examined," for the composite sample must be treated as the juice of a single oyster. It is evident that a composite sample is not what is intended, but rather that each oyster shall be examined separately.

Some workers have based their analysis upon several composite samples of five oysters each, while others have used five, ten or fifteen oysters separately. There is great variation in the bacterial content of oysters from the same lot. In one oyster there may be one hundred B. coli per cubic centimeter of the shell liquor, while in another oyster from the same sample they may be entirely absent. The important consideration in the examination of oysters is the average number of B. coli in the oysters as a whole and not the number in any individual oyster. For this reason the larger the sample, within reasonable limits, the more accurate the results as an indication

of the B. coli content of the oysters of any particular area. Smith,[1] in the analysis of one hundred and twenty-five oysters in each of a series of samples, came to the conclusion that not less than fifteen oysters should be used. The use of too small a sample may account in part for the wide variation in results obtained by different analysts in the examination of the same oyster bed at approximately the same time. In the writer's opinion twenty-five oysters is not too large a sample to be used in any analysis.

The changes which the writer would suggest in "Standard Methods of Shellfish Examination," are as follows:

The size of the sample should be at least twenty-five oysters. After reaching the laboratory the oysters should be scrubbed thoroughly with a stiff brush in water free from B. coli and dried. When ready for examination the oyster should be held between the thumb and the fore-finger and the lip of the shell flamed in the bunsen burner or burned off with alcohol. The opening should be done with an oyster knife which has previously been burned with alcohol. The method of drilling a hole through the shell and pipetting out the oyster juice should never be substituted as an alternative method.

The shell liquor of the five oysters of each of the five composite samples should be collected in sterile, graduated, glass-stoppered bottles and the bodies of the five oysters should be placed in a wide-mouth, glass-stoppered bottle. The amount of shell liquor should be read off and an equal amount of sterile one per cent. salt solution or sea water added to the bottle containing the bodies of the oysters. The stopper should be replaced and the bottle shaken at least one hundred times. (The writer's experience has been that, if the oysters are opened carefully so as to avoid mutilation, the bodies of the oysters are damaged but very little by this procedure unless the shaking is especially vigorous.) The salt solution and mucus should then be decanted into the bottle containing the shell liquor and the whole shaken vigorously one hundred times to break up any clumps of bacteria and to separate as far as possible the bacteria from the bits of mucus. The five sets of oysters should be treated in this manner, making five samples of five oysters each. If the operation is conducted properly there should be an equal quantity of shell liquor and salt solution in each of the five composite samples.

[1]Size of the Sample Necessary for the Accurate Determination of the Sanitary Quality of Shell Oysters, American Journal of Public Health, III, 1913, 705.

The subsequent procedure should be the same as that recommended by "Standard Methods" and the method of scoring should be the same except that the score as obtained by this method should be multiplied by two, because we are using ½ c.c. 1–20 c.c. and 1–200 c.c. of the original shell liquor instead of 1 c.c., 1–10 c.c. and 1–100 c.c. as recommended by "Standard Methods."

The advantages of this method are that we are basing our examination upon twenty-five oysters instead of five and the result will be much nearer the true bacterial content of the sample.

Another point worthy of consideration by the Committee on "Standard Methods" is the number of bile tubes to be used in the different dilutions. The writer in all the work reported in this paper and for a long time previous has used duplicate tubes. An interesting feature of this method is that both tubes from each dilution show gas only approximately two-thirds of the time. The writer has regarded gas in either of the two duplicate tubes as positive for the dilution and has assigned it the value as recommended by "Standard Methods." By using this method approximately thirty-three per cent. more B. coli are found than would be the case if only one tube were used.

"Standard Methods" under "Illustration of the Application of the Method of Rating Oysters for B. coli" recommends the transferring of a positive result in a high dilution in one oyster to a lower dilution in another oyster, if in the latter oyster the B. coli test is negative in the lower dilution. Below is an illustrated case from "Standard Methods:"

Case C. Results of B. Coli Tests in Dilutions Indicated.

Oyster.	1.0c.c	0.1c.c	0.11c.c	Numerical value.
1	+	+	0	10 (not 100)
2	+	+	0	10
3	+	+	+	100
4	+	+	+	10 (not 100)
5	+	0	0	10 (not 1)

140 = rating.

But suppose in this sample the tubes have been inoculated in duplicate instead of one tube for each dilution. The following table shows a not unexpected result:

Results of B. Coli Test in Duplicate Tubes in Dilutions Indicated.

Oyster	1 c.c.	0.1c.c	0.001c.c
1	+ +	+ 0	0 0
2	+ +	+ 0	0 0
3	+ +	+ +	+ 0
4	+ +	+ 0	+ 0
5	+ 0	0 0	0 0

The question now arises as to what numerical value we shall assign to the dilutions which are positive in one tube and not in the other. Is it proper to assign full value to these dilutions? Would it not be fairer to assign one-half the value recommended by "Standard Methods" to these dilutions, because basing our calculation upon both tubes there are only one-half the B. coli present that would be indicated by the positive result alone?

Suppose now we wanted to transfer the positive result in the 1–100 c.c. dilution of oyster No. 4. Should it be transferred to the negative tube in oyster No. 5, or to the 1–10 c.c. dilution of the same oyster? Further, what shall we do with the positive result in the 1–100 c.c. dilution of oyster No. 3. Shall it remain where it is or shall it be transferred to the negative tube in the 1–10 c.c. dilution of oyster No. 1 or 2? Again suppose in oyster No. 3 in the 1–100 c.c. dilution both the tubes should be positive and there were only one tube negative in the 1–10 c.c. dilution of any of the oysters, should these two positive tubes be separated and transferred to the negative tubes in two of the other oysters?

But whatever method we use for transferring, shall we assign the full value recommended by "Standard Methods" to the dilutions which are positive in one dilution and negative in the other, or shall we assign the better value, *i. e.*, one-half the value recommended by "Standard Methods?" By taking advantage of the various possibilities we can obtain ratings varying between thirty, the lowest possible, and one hundred and forty, the highest possible. In the first case the oysters would be very near the permissible standard, while in the other, the oysters would be considered badly polluted. If we use three tubes in each dilution the matter is still further complicated.

Another possibility would be to regard each set of tubes separately and average the results. This would be the simplest method, but it would not give so low a result as would be possible by one of the other methods. In the case in hand the rating would be seventy-seven as against thirty, the rating obtained by one of the other methods.

The writer has a case in mind in which the rating on one set of tubes was three, which showed the oysters to be in a high state of purity, while the duplicate set showed a rating of thirty-two, which would condemn the oysters on the strict application of the standard set by the Bureau of Chemistry. Obviously it would be unjust to base our rating on either of the two sets of tubes alone.

In the writer's opinion the standard set by the Bureau of Chemistry of twenty-three as the highest permissible rating is very stringent and every opportunity should be given the oyster growers to avail themselves of a method of oyster analysis which will be more accurate in its results and a method of rating that will more nearly represent the sanitary condition of their product.

THIS BOOK IS DUE ON THE LAST DATE STAMPED BELOW

AN INITIAL FINE OF 25 CENTS

WILL BE ASSESSED FOR FAILURE TO RETURN THIS BOOK ON THE DATE DUE. THE PENALTY WILL INCREASE TO 50 CENTS ON THE FOURTH DAY AND TO $1.00 ON THE SEVENTH DAY OVERDUE.

PUBLIC HEALTH LIBRARY

JAN 3 1957

MAY 1 1957

LD 21-100m-12,'43 (8796s)

Photomount
Pamphlet
Binder
Gaylord Bros., Inc.
Makers
Stockton, Calif.
PAT. JAN. 21, 1908

U.C. BERKELEY LIBRARIES
C029382763