EFFECT OF STORAGE AND CHANGING SEA WATER ON CONTAMINATED OYSTERS

CHARLES KRUMWIEDE, M.D., Fellow A.P.H.A., WILLIAM H. PARK, M.D., Fellow A.P.H.A., GEORGIA COOPER, MARIE GRUND, M.D., CHARLES H. TYLER and CAROLYN ROSENSTEIN

Bureau of Laboratories, Department of Health, New York City

IN THE February issue of the Journal, our experiments on the effect of chlorination on oysters contaminated with typhoid bacilli were reported. Through the exigencies of time and available Journal space the collateral experiments on the effect of changing sea water alone were not presented. These as well as observations on the effect of dry storage were held for report at this time.

When the increase of typhoid fever occurred during the latter part of 1924, it was noticed coincidently in inland as well as seaboard cities. When investigations showed that this increase of typhoid fever was attributable to contaminated oysters it indicated that *B. typhosus* must persist in dry, stored oysters for some time. Experiments were undertaken, therefore, to determine the time of survival of *B. typhosus* under storage conditions.

Oysters for these observations were dredged at West Sayville, Long Island, and sent immediately to the laboratory. The water was obtained from Great South Bay, Long Island.

Two lots of about 80 oysters were contaminated by suspending the oysters in wire crates in tanks containing about 8 gallons of contaminated sea water: Lot A, shown in Table I, in sea water contaminated by the addition of a suspension of feces containing *B. typhosus;* Lot B, shown in Table II, in water contaminated with freshly isolated cultures of *B. typhosus.* The tanks were kept overnight at room temperature (18 to 22° C.). The oysters drank actively. They were then removed from the tank for storage. The storage conditions approximated in temperature that of the colder months (February and March) of the oyster season. The oysters were kept out-of-doors in a small shed on the north side of a building which was covered with snow. When snow was no longer available they were transferred to an icebox.

TABLE I

Survival of B. Typhosus on or in Oysters, Contaminated with Feces, during Storage. First Examination February 4, 1925

Days of Storage	Estimated number of B. typhosus per c.c. of Liquor	c.c. of Body Emulsion	Shell
0	254	Not exam.	Not exam.
1	62	110	Not exam.
5	23	10	Not exam.
7	10.4	0.9	3111
9	20.4	1.85	194
10	2.5	+(<0.08)	142
14	2.5	1.6	Neg.
21	7.6	1.5	Neg.
28	1.3	+(<0.08)	Neg.
35	30.6	0.25	Neg.
41	1.5	+(<0.08)	Neg.
49	0.25	0.25	Neg.

TABLE II

Survival of B. Typhosus on or in Oysters (Contaminated with Freshly Isolated Cultures of B. Typhosus) During Storage. First Examination February 4, 1925

Days of Storage	Estimated number of B. typhosus per c.c. of Liquor	c.c. of Body Emulsion	Shell
0	37,700	2,118	Not exam.
1	13,870	1,260	Not exam.
5	10,333	1,077	Not exam.
7	7,107	450	Not exam.
9	4,514	473	2,733
14	1,245	515	2,750
21	5	100	Neg.
28	1.6	0.6	100
35	1.4	12	Neg.[1]
41	103	32	Neg.
49[2]	45	26	Neg.

1. Oysters removed on this date and placed in fresh sea water then showed 160 *B. typhosus* per shell, irregular distribution.
2. Oysters dead.

The materials used for bacteriological examination were: washings from the shell, using 10 c.c. of broth per oyster,

the removal of the contaminated material being assisted by rubbing with cotton swab; the liquor; and the body of the oyster emulsified in 5 c.c. of broth. The number of *B. typhosus* was estimated from the number of colonies on the plates or from the smallest quantity which when placed in an enriching brilliant green broth yielded *B. typhosus* on subsequent plating.

The effect of dry storage on the numbers of *B. typhosus* on or in contaminated oysters is summarized in Tables I and II. The number of typhoid bacilli as shown in Tables I and II was greatly reduced, but bacilli still could be isolated from both liquor and body after 49 days of storage. Before this the oysters had begun to die and those examined on the last day of the experiment were unfit for food. The number of *B. typhosus* per shell was very low after 2 to 4 weeks' storage; the shells of the more lightly contaminated oysters being negative after 2 weeks, whereas with the more heavily contaminated ones the last positive finding was after 5 weeks' storage.

TABLE III

SURVIVAL OF B. COLI IN OYSTERS, CONTAMINATED WITH B. COLI, DURING STORAGE. FIRST EXAMINATION FEBRUARY 4, 1925

Days of Storage	Estimated number of B. coli per c.c. Liquor	c.c. Oyster Body Emulsion
0	82,000,000	200,000
1	425,000,000	25,500,000
6	183,000,000	250,000
8	685,000	25,000
10	550	50
21	5,050	505
28	50	5,050
35	50	550
41	1	5.5
49[1]	10	1

[1] Oysters dead.

A parallel series of observations on the persistence of *B. coli* was also carried out using the presumptive broth fermentation tube method. The results of these observations are illustrated in Table III. In this table as in Tables I and II all of the examinations are not recorded but only a sufficient number to clearly show the rate of decline in numbers of *B. typhosus* or *B. coli*. In the observations on the survival of *B. coli* only one lactose broth tube was used for each dilution tested. Evidently the actual numbers may have been the estimated numbers given or somewhere between this figure and one-tenth of this figure. Although the contamination with *B. coli* was very heavy and considerable irregularities are noticeable in the results in Table III, there is a general parallelism in the rate of reduction of *B. typhosus* and *B. coli* on dry storage.

Another observation on the survival of *B. typhosus* in dry stored oysters was made with some oysters which had been subjected to chlorination. Before chlorination (less than 1.5 p.p.m. of chlorine) the number of *B. typhosus* was 103 per c.c. of liquor and 19 per c.c. of oyster body emulsion. After storage for 8 weeks (refrigerator) 1 c.c. of liquor contained 1.2 typhoid bacilli and 1 c.c. of oyster body emulsion gave 0.19 *B. typhosus*.

These experiments show that *B. typhosus* may persist in oysters for a period of time beyond any time of storage which occurs in the trade.

The death of oysters during the period of storage led naturally to the question as to whether a dead oyster was a favorable culture medium for the typhoid bacillus. Killed contaminated oysters were stored at room and icebox temperature and approximately equal portions of body substance were ground in 2 c.c. of broth and cultured. In oysters contaminated with pure cultures and held at room temperature there was evidence of a slow multiplication approximating 15 times after 7 days, followed by a more rapid rise to approximately 1,000 times after 10 days. At icebox temperature there was a moderate increase to the 7th day, then a slight decrease, the number being approximately the same on the 10th day as on the 1st. In oysters contaminated with stool suspensions, at room temperature the overgrowth of other organisms prevented the isolation of *B. typhosus* by the direct plating method after the 1st day. By enrichment methods *B. typhosus* was recovered up to the 10th day. At icebox temperature *B. typhosus* were isolated up to the 10th day, the last examination made. There was no noticeable increase

during this time. These observations indicate the possibility of recontamination by the drip from dead oysters, which contamination would be greater if the temperature were such as to allow growth.

As was to be expected, other workers were impressed by the occurrence of oyster-borne typhoid fever in inland cities and started to investigate the viability of *B. typhosus* on or in oysters.

Kinyoun reports that oysters heavily contaminated with stock cultures of *B. typhosus* yielded *B. typhosus* up to the 15th day, when the observations were terminated.

Jordan likewise contaminated oysters with culture strains of *B. typhosus* and stored the oysters at 5° to 8° C. He isolated *B. typhosus* from the interior of the oyster up to the 24th day. After this the results were negative and the experiment was terminated on the 30th day.

Tonney and White also contaminated oysters with stock cultures of *B. typhosus*. The contamination was extremely heavy. The results with those stored at 45° F. only are quoted. The oysters were opened in the ordinary way, up to the 17th day, so that the typhoid bacilli isolated up to this day may have been from the shell or from the liquor. After the 22nd day the liquor was collected in a way to avoid contamination of the shell. In the case of dead oysters washings were examined. The results were positive to the 60th day, negative thereafter. The oysters remaining after the 39th day were all dead.

Of the earlier observers, Field working in this laboratory showed the persistence of *B. typhosus* for the longest time, *viz.*, 42 days, when the experiment was terminated.

The question of the rate of cleansing which occurs when contaminated oysters drink in successive changes of sea water has been the subject of earlier investigations. We undertook a reinvestigation of this subject because we felt that the means of isolation of *B. typhosus* at our disposal would possibly demonstrate a longer persistence of *B. typhosus* than was indicated by earlier observations. This has been the case, but the conditions of the experiment were not completely satisfactory for the longevity of the oyster and death of the oysters terminated the observations.

TABLE IV

EFFECT OF CHANGING SEA WATER ON THE NUMBER OF B. TYPHOSUS ON OR IN OYSTERS

Days after contamination	No. of changes of sea water	Estimated number of B. typhosus per c.c. Liquor	Oyster body emulsion	Estimated number of B. typhosus per shell	B. typhosus per c.c. of sea water in tank
0	0	2,675	213	40,400	12,300
1	1	424	68	9,500	550
3	2	64	42	980	800
4	3	2	0.5	75	Neg.[1]
7	6	8.6	0.5	64	Neg.
11[3]	9	4	+<0.1	84	Neg.[2]
15[4]	12	7	1.5	74	0.75
21	16	1	2.2	1.2	0.25
24[5]	18	1.6	2.2	3.7	Neg.

[1] Negative in this column means *B. typhosus* not isolated from 20 c.c. of water.
[2] Positive on the 8th and 10th days, 7th and 8th change.
[3] Still drinking grayish slime developing on shells.
[4] Same condition, one dead oyster found.
[5] Remaining oysters dead, slime increased water had disagreeable odor.

The 50 oysters for this experiment were contaminated with typhoid feces and then placed in 4 gallons of fresh sea water. The water was changed 18 times in a period of 24 days. The temperature in the shed where the tank was kept was from 60° to 70° C. and just prior to each change of water samples were taken for examination. Table IV gives the results of a sufficient number of these observations to indicate the rate of decline in the number of *B. typhosus* present.

The results indicate that 3 successive drinkings in fresh sea water will result in a reduction of about 99 per cent of the typhoid bacilli on or in contaminated oysters. This degree of reduction compares favorably with that which was obtained (see previous number of this JOURNAL) with successive treatments with chlorinated sea water and intermediate drinking. There was a difference however; with simple changes of plain sea water *B. typhosus* were isolated from the tank water almost to the end of the experiment. This continued contamina-

tion was evidently due to the rinse from contaminated oyster shells and the excretion of contaminating *B. typhosus* from within the oysters. With chlorination, however, we usually failed to isolate *B. typhosus* from the tank water. The exceptions were with heavy contamination in which case the chlorine added was unable to destroy all the typhoid bacilli in the tank water.

The failure to keep oysters alive for a sufficient time in the previous experiment was apparently due to eventual fouling of the water and reduced oxidation of the sea water which was stored in barrels. Permits are granted during the closed season for the removal of oysters from condemned beds to uncontaminated waters for cleansing. It was the intent of the above experiment to give some indication in this regard. Although the indication is that even 3 weeks is insufficient, it seemed desirable to repeat this experiment under conditions more favorable to the oysters' activity. As cold weather had supervened this could not be done, but we plan to carry out such an experiment as soon as the natural waters have warmed sufficiently to insure active drinking.

TABLE V

PERSISTENCE OF B. TYPHOSUS IN CONTAMINATED OYSTERS RETURNED TO NATURAL WATERS, 10 OYSTERS EXAMINED AT EACH TEST

No. of days in bay	Date	Temperature of water	Number positive for B. typhosus Shells	Liquors	Bodies	Pooled Gills[1]
0	12–2	.....	10	10	9	Pos.
1	12–5	Not taken	0	1	3	Pos.
4	12–9	43° F.	0	1	1	Pos.
10	12–14	36° F.	0	1	1	Pos.
17	12–21	41° F.	0	0	1	Neg.
24[2]	12–28[2]	39° F.	0	0	1	Neg.
31[2]	1–4[2]	39° F.	0	0	2	Neg.
39	1–12	30° F.	0	0	0	Neg.
51	1–26	36° F.	0	0	0	Pos.

[1] Gills from the 10 oysters put in brilliant green broth.
[2] Five control oysters (not contaminated but placed in same waters) examined on each date gave negative results.

In the meantime we have investigated the length of time that *B. typhosus* will survive in oysters during the winter months when the oysters are returned to natural waters. The place selected for this experiment was in a bay near a series of condemned oyster beds. The oysters for the experiment were secured from these beds so that the factor of change to different waters might not enter. These oysters were contaminated with typhoid feces. The contamination was comparatively light, 100 *B. typhosus* per shell, 3 per c.c. of liquor and less than 0.4 per c.c. of body emulsion. The oysters were submerged in the bay water in lots in wire crates and then brought to the laboratory at intervals for examination.* The results of these examinations are given in Table V.

The temperature of the water was taken as noted. As a further guide to the probable temperature of the water during the intermediate times we quote the following from the Weather Bureau report:

MEAN DAILY TEMPERATURE (FAHRENHEIT)

December, 1925

1	2	3	4	5	6	7	8	9
38°	43°	44°	48°	50°	50°	43°	40°	36°
10	11	12	13	14	15	16	17	18
30°	32°	41°	40°	32°	31°	32°	32°	31°
19	20	21	22	23	24	25	26	27
34°	41°	40°	37°	28°	32°	32°	20°	10°
28	29	30	31					
23°	28°	26°	26°					

January, 1926

1	2	3	4	5	6	7	8	9
33°	38°	39°	42°	42°	44°	30°	25°	27°
10	11	12	13	14	15	16	17	18
28°	28°	28°	20°	24°	32°	34°	34°	46°
19	20	21	22	23	24	25	26	27
44°	42°	46°	30°	22°	26°	23°	28°	32°
28	29	30	31					
22°	14°	30°	39°					

It is probable from the data given that little drinking took place. These observations indicate therefore that should oysters become contaminated during their more inactive period, *B. typhosus* can persist in them for at least 51 days. It was a surprise to us that we should succeed in isolating *B. typhosus* after this length of time considering how light the initial contamination was. The length of time of persistence was equally surprising as there was no reason to believe that *B. typhosus* would survive for this time even in inactive oysters. On the other hand, the conditions might be con-

* We are indebted to Dr. Rowland G. Freeman for taking care of these details.

sidered as comparable to dry storage because of the inactivity, and *B. typhosus* will persist for this time in dry stored oysters.

It might be argued that the bacilli isolated came from the relatively unsafe waters in which the oysters were placed. This we believe was not the case as shown by the control (uncontaminated) oysters. The numbers of control oysters were small due to accidental loss of some crates through breaking of the tie-ropes. Even so, we believe that the results indicate that the *B. typhosus* isolated were from the artificial contamination.

Some of these oysters were examined for *B. coli*. A score of *zero* was obtained on oysters yielding *B. typhosus*. It has not infrequently been noted in our experiments that *B. coli* colonies were very few in number or apparently absent on plate media which showed the presence of colonies of *B. typhosus*. Such observations emphasize what one tends to forget—a high *B. coli* score is an indication of danger, a low *B. coli* score is not necessarily an index of safety.

In the above work and in that previously reported both the Endo medium and brilliant green agar (Krumwiede) were employed. As was to be expected, the latter frequently yielded positive results, where the former was negative. In addition, a brilliant green broth was used for enrichment. As we knew that the oyster liquor and expecially the oyster body emulsion would reduce the activity of the dye, preliminary tests of this factor were carried out. As it would not be feasible to use several dilutions of dye with each of the many samples so as to strike the optimum in each instance, an average optimum was selected. For culturing the oyster body emulsion 1 c.c. of a 1–1000 solution of brilliant green was added to 50 c.c. of broth. For the liquor, 0.25 c.c. of the dye solution was employed. The washings from the shell were cultured in 50 c.c. of broth to which 0.25 to 0.5 c.c. of dye solution (1–1000) was added, the amount varying with the quantity of dirt washed from the shells. Veal broth of a reaction of pH.7.2 was employed.

The tables have shown how efficient this procedure has been in isolating the residual few *B. typhosus*. The maximum efficiency of the procedure was not reached, however, till the latter half of our work, that is, until we found how successful repeated platings from the enrichment broth might be. To illustrate this we have tabulated the results of a series of successful enrichments according to the day on which positive results were obtained (Table VI). The 40 positive results are those obtained in 82 attempts at enrichment of *B. typhosus* in the last chlorination experiment we carried out. The table shows how the number of positive results increased on each succeeding examination of the broth.

TABLE VI

POSITIVE FINDINGS FROM ENRICHMENT BROTH (40 SAMPLES) ACCORDING TO DAY OF POSITIVE RESULTS

Days of incubation of brilliant green broth	Specimens grouped according to days of positive findings
1	12+ 28—
3	10+ 18—
5	10+ 8—
7	6+ 2—
10[1]	2—

[1] Observations continued until the 21st day but no more samples became positive.

SUMMARY

B. typhosus persisted in shell oysters stored at refrigerator temperatures for as long as the oysters lived, a period of 41 days. The oysters dying after this time were examined on the 49th day of storage and also yielded *B. typhosus*. The *B. typhosus* on the shells of stored oysters were apparently dead after 14 to 40 days, the length of persistence depending on the degree of contamination. A dead oyster serves as a culture medium and the number of *B. typhosus* will increase if the temperature is satisfactory for multiplication. Repeated changes of fresh sea water resulted in a rapid dimi-

nution of the number of *B. typhosus* on or in oysters, the diminution reaching about 99 per cent after 3 changes of water. *B. typhosus* was isolated up to 21 days, during which time the water was changed 16 times, after which the remaining oysters died. The degree of purification following 3 changes of fresh sea water approximated that observed with a similar number of treatments of chlorinated sea water. There is a difference, however; in most instances the *B. typhosus* in the chlorinated water was apparently killed. Contaminated oysters were placed in natural waters the temperature of which was low enough to largely inhibit drinking. The oysters contained *B. typhosus* after 51 days in these waters, the last observation made. In some of these experiments as well as in some of those described in our previous report, note should be made of the prolonged persistence of minimal numbers of *B. typhosus* under different conditions. This prolonged persistence emphasizes the fact that apparently the only safe oyster is one which has been protected from any contamination with fecal pathogens for at least some months prior to harvesting. The actual time can only be determined by further careful experiments.

BIBLIOGRAPHY

Krumwiede, Park, Cooper, Grund, Tyler and Rosenstein. *A. J. P. H.*, 14, 2:142, 1926.
Kinyoun. *Pub. Health Rep.*, 40, 17, 1925.
Jordan. *Jour. A. M. A.*, 84:1402, 1925.
Tonney and White. *Jour. A. M. A.*, 84:1403, 1925.
Field. Collected Studies of Research Lab., New York City Dept. of Health, 1:194, 1905.
Krumwiede, Kohn, Kutner and Schumm. *J. Infect. Dis.*, 23:275, 1918.

SOURCES OF MATERIAL*

EVART G. ROUTZAHN, FELLOW A.P.H.A.
Russell Sage Foundation, New York City

HERE ARE a few samples of questions which public school teachers are likely to ask:

"Where can I find motion pictures about health?" "Who gets out rhymes, songs and games about the care of teeth?" "Where can I obtain a simple and readable book about nutrition for use in school work?"

The purpose of this paper is to start discussion of the problem confronting teachers of health in finding out what teaching material there is, and where and how it can be secured. As revealed in the summaries of the Chicago Health Education Conference,[1] the materials needed by the teacher are of many kinds. They include information about teaching methods; material for the children to look at, and use; and information for the parents. The itinerant teacher, the school nurse or other teacher who comes into a class room at weekly or other periodic intervals, also needs to know the sources of supply for the books, pamphlets, motion pictures, posters, slides, suit case theaters and other materials used in getting children interested in health.

My own interest in this problem is chiefly of two kinds. In the first place, I am one of those who frequently receive requests for material which, because of mistakes in reference lists, I am supposed to have; and in the second place, I am interested in the whole problem of making

* Read at the Joint Session on Health Education in the Class Room of the American Public Health Association at the Fifty-fourth Annual Meeting at St. Louis, Mo., October 22, 1926.

Although this paper is written in terms of the teacher and the school the discussion applies generally to the making and using of source lists.