Shellfish producers and regulatory agencies in the United States and Canada have a common interest in this study which shows a high increase in bacteria count during the shucking operation—suggesting that the shucking stage is the most likely source for contamination of oysters during processing and marketing.

Bacteriological Control of Oysters During Processing and Marketing

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I NTELLIGENT GUIDANCE of the oyster industry by regulatory officials has been hampered for some time by a lack of fundamental knowledge of the changes in bacterial content of oysters as they proceed through shucking, packing, and transportation to the market. Without this background of information, it has been difficult to evaluate the regulatory program and to make reasonable interpretation of bacteriological results obtained at the market level. The importance of the problem has recently been manifested by reports of high bacteriological results obtained on shipments made to certain consuming

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The results of this study are also being published in the Proceedings of the Joint Meeting of the Oyster Institute with the National Shellfisheries Association. centers. These difficulties have been recognized by Federal and State regulatory agencies, and, as a result, the Robert A. Taft Sanitary Engineering Center of the Public Health Service was requested to undertake a study to determine some of the factors involved. The study was conducted by the center's Shellfish Sanitation Laboratory, when it was located at Woods Hole, Mass.

The Sampling Program

It was considered of first importance to determine the bacterial changes that should normally be expected in oysters during harvesting, shucking, washing, packing, and transportation to the market. To determine this, it was quite evident that there should be designed a carefully controlled program of sampling in representative shucking houses and related establishments, tracing as nearly as possible a single lot of oysters from the time of delivery as shell stock to the shucking house to receipt of the product in the wholesale market. The eastern oyster, *Crassostrea virginica*, was the species studied throughout this project.

The program required the cooperative efforts of interested agencies in the producing and receiving States involved and of interested agencies of the Canadian Department of National Health and Welfare for the actual work of inspection, sampling, and laboratory examination. It was considered that adequate data could be obtained if an inspection and a sampling of each plant were conducted at least once a month.

The following four sampling stations were established at each plant for tracing a single lot of oysters:

1. "Shell oysters"—A composite sample was taken of shell stock on shuckers' benches.

2. "As shucked"—A composite sample was taken of shucked oysters as they were shucked. For this sample, each shucker shucked 1 oyster directly into a sterile 1-pint mason jar. Random sampling was conducted in the larger plants where 1 oyster collected from each shucker would provide more than 1 pint of sample.

3. "First skimmer"—A composite sample of shucked oysters was collected from at least 5 shuckers' pots as the oysters were delivered to the packing room and after preliminary washing on the first skimmer.

4. "As packed"—A sample was collected from a commercial can immediately after packing. The sample represented the lots previously taken as samples at the three preceding sampling stations. If the pack was intended for the market, two 1-pint cans or one $\frac{1}{2}$ -gallon can were also taken for shipment to the Shellfish Sanitation Laboratory at Woods Hole, Mass. If the lot was intended for repacking, the container was marked for identification when it reached the repacking house.

Arrangements were made for the interception of commercial shipments at marketing centers. Notification was made by telegram to the control agencies in the marketing city, giving destination, expected time of arrival, and approximate size of shipment.

To assure that an examination of the packed oysters would be conducted at the market level, a pilot shipment of each such lot was made to the Shellfish Sanitation Laboratory at Woods Hole. Two 1-pint cans or one $\frac{1}{2}$ -gallon can of the lot being shipped to market on that day were shipped, refrigerated in ice, by railway express to Woods Hole. The time in transit of both shipments was approximately the same. The oysters, on arrival, usually had sufficient ice to maintain the temperature of the samples below 50° F. There was no way to determine whether the samples had been refrigerated continuously at this temperature since on some occasions re-icing was necessary in transit.

Methods of Examination

As nearly as possible, bacteriological procedures were similar in all participating laboratories. They were conducted according to an outline submitted to the laboratories before the beginning of the study. The outline described the procedure somewhat more in detail than is contained in the procedure recommended by the American Public Health Association for the bacteriological examination of shellfish and shellfish waters (1).

Results

During the 1950-51 and 1951-52 seasons, some 45 inspections and samplings were made in the shucking houses selected. Of these, 42 samplings were followed through pilot shipment to Woods Hole. The 3 series not included were incomplete.

Results of bacteriological examinations are shown in the accompanying table and in figures 1 and 2. The results were grouped according to the following classifications:

	Coliform MPN's per 100 ml.	Standard plate count per ml.				
Group 1	Less than 230	1–1,500.				
Group 2	230-2,400	1,600-10,000.				
Group 3	2,401-24,000	11,000-50,000.				
Group 4	24,001-less than	51,000-less than				
	160,000.	1,000,000.				
Group 5	160,000 or more	1,000,000 or more.				

These values are not intended to be suggested as control standards. Some of the values, however, are limits suggested by the Public Health Service and the Canadian Department of Health and Welfare, on an interim basis, for use in evaluating the bacteriological quality of shellfish as taken from the growing area or as sampled in the processing plant or in the market. Source materials for some of these values are listed below:

1. The United States Public Health Service

Values	Shell		As shucked		First skimmer		As packed		Woods Hole		Market		
	Num- ber	Per- cent	Num- ber	Per- cent	Num- ber	Per- cent	Num- ber	Per- cent	Num- ber	Per- cent	Num- ber	Per- cent	
	Coliform MPN												
Less than 230 230-2,400 2,401-24,000 24,001-<160,000 160,000 or more Number of samples.	27 10 8 0 0 45	60 22 18 0 0	0 12 25 5 1 43	$\begin{array}{r} 0 \\ 28 \\ 58 \\ 12 \\ 2 \\ \hline \\ \end{array}$	3 19 17 3 1 43	7 44 40 7 2	6 17 19 0 0 42	15 40 45 0 0	4 14 9 6 9 42	10 33 21 14 21	0 16 10 6 3 35	0 46 28.5 17 8.5	
	Standard plate count												
1-1,500 1,600-10,000 11,000-50,000 51,000-<1,000,000 1,000,000 or more Number of samples.	22 14 7 1 0 44	50 32 16 2 0	5 21 16 0 0 42	12 50 38 0 0	3 24 16 0 0 43	7 56 37 0 0	6 26 9 2 0 43	14 60 21 5 0	18 8 7 7 2 42	43 19 16. 5 16. 5 5	4 19 5 7 0 35	11 54 14 20 0	

in the Manual of Recommended Practice for Sanitary Control of the Shellfish Industry (2)suggests a limiting coliform MPN (most probable number) of 230 per 100 ml. in oyster shell stock sampled at the growing area or in shell stock or shucked oysters at the point of shucking. Coliform MPN's of that value or greater should be interpreted as indicative of unfavorable conditions or practices surrounding the production and handling of the product. The manual also suggests that, in occasional samples, a coliform MPN value of 2,400 may be tolerated.

2. The Interdepartmental Shellfish Committee of Canada, at a meeting in Ottawa in March 1950, suggested the following classifications of results for shucked oysters from the United States, as received in the Canadian market. Since that time, these limits have actually been applied on an interim basis in reviewing shipments from the United States.

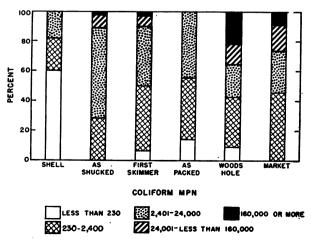
Class 1. Acceptable. Shellfish with most probable numbers (MPN) of coliform bacteria of not more than 2,400 per 100 ml. and/or a standard plate count of not more than 50,000 per ml.

Class 2. Acceptable on Condition. Shellfish with a coliform MPN of less than 160,000 per

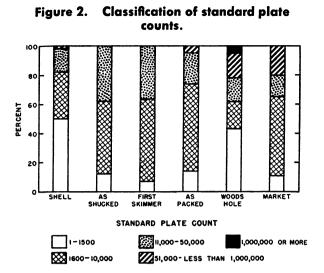
100 ml. and/or a standard plate count of less than 1,000,000 per ml.

(The United States Public Health Service is notified of the receipt of these shipments falling in class 2. The oysters are accepted on the condition that the State authority concerned will conduct immediate investigations of the producer's plant and operations, and a report of such investigations will be submitted by the Public Health Service to the Canadian Department of National Health and Welfare. On the basis of this report, Canada will reject or permit

Figure 1. Classification of coliform MPN results.



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further shipments from the producer in question.)

Class 3. Rejectable. Shellfish with a coliform MPN of 160,000 or more per 100 ml. and/or a standard plate count of 1,000,000 or more per ml.

A classification of the coliform results obtained is given in figure 1. Of particular significance is the difference between the shell stock and samples as shucked. In comparing the results obtained at these sampling points, it must be borne in mind that in the laboratory examination of shell stock the exterior surfaces of the shell are thoroughly scrubbed under running water to remove adhering mud and detritus. Of the shell stock samples, 60 percent (27 of 45 samples) showed coliform MPN's less than 230, and no samples showed coliform MPN's in excess of 24,000. Comparing these results with the product as shucked, none of the samples of ovsters as shucked showed coliform MPN's of less than 230, and approximately 75 percent (31 of 43 samples) of the samples showed coliform MPN's in excess of 2,400. A significant number-approximately 15 percent (6 of 43 samples)-showed coliform MPN's in excess of 24,000.

Suggestions concerning the cause of the higher bacterial content after shucking can be gathered from a supplementary investigation in which an additional sampling station was interposed. To eliminate the possibility of contamination from the shucking equipment, samples were collected from a shucker who had previously sanitized his equipment and had thoroughly scrubbed his hands. A series of 18 such samplings was conducted. Comparison of results obtained on samples from this series with those obtained on the same lots regularly commercially shucked showed little difference in either coliform MPN or standard plate count. There then remains only the factor of the bacterial content of the mud and detritus on the exterior surfaces of the shells and the possibility of incorporation of this material with the oyster meats during the shucking operation.

Some reduction in coliforms was accomplished in the remaining stages of processing. The number of "as packed" samples showing coliform MPN's of 2,400 or less increased from 28 to approximately 50 percent (23 of 42 samples) of the total number of samples examined. There were no samples showing coliform MPN's in excess of 24,000.

The results obtained on the Woods Hole samples show some increase in coliform bacteria. The greatest difference between the product as packed in the shucking house and as received at Woods Hole is in the number of samples showing coliform MPN's in excess of 24,000. About 35 percent (15 of 42 samples) of the samples were in this group although there was no significant change in the percentage showing coliform MPN's of 2,400 or less.

For comparative purposes, there is included in figure 1 an analysis of results of examinations made by the Canadian Department of National Health and Welfare on shipments to Montreal (see "Market," fig. 1) from the same producing State, collected during the same period, but not necessarily of the same lots. Approximately the same number of samples (35) were examined. The similarity to the Woods Hole samples is quite close. It will be seen that an almost equal percentage (43 and 46 percent) showed coliform MPN's of 2,400 or less. No "market" samples showed coliform MPN's of less than 230, while a significant number-approximately 10 percent (4 of 42 samples)-of the Woods Hole samples were in that category.

Results of agar plate counts (tryptone glucose extract agar, 48 hours, 37° C.) are classified in figure 2.

The standard plate count has been mentioned (1) as a useful index of general sanitation and Determinations of standard refrigeration. plate count were made on all samples examined bacteriologically. The results indicate, generally, little difference between the standard plate count and coliform MPN. The increase in number of bacteria, as indicated by the standard plate count, between the "shell" and "as shucked" samples, is practically of the same order as the increase in coliform bacteria. The same degree of recovery in the product as packed will be noted, as well as the increase during transportation to Woods Hole or to the market.

Conclusions and Recommendations

The bacteriological results obtained reveal that the increase in bacterial content of oysters during processing occurred in the shucking operation. Examination of the shell stock showed that the meats were usually of good bacteriological quality. However, the same oysters shucked in commercial practice showed a significant increase in bacteria—both the coliform MPN and the standard plate counts were higher.

The exact cause of the increase in bacteria cannot be attributed entirely to contamination as a result of sanitary deficiencies in the shucking room. Oysters shucked under controlled commercial conditions known to be clean showed little difference in bacteriological results from those shucked under regular commercial practice. The more serious source of contamination is therefore apparently the mud and detritus adhering to the exterior of the shells.

Since incorporation of such material during the shucking operation is unavoidable if the oysters are muddy, contamination from this source would be eliminated only by thorough washing of the shell stock at the time of harvesting and before transfer to the shucking bins.

In the plants under investigation, there generally was observed a reduction in bacteria as the oysters proceeded through the processes of washing and packing although return to the level of the shell stock was not accomplished.

REFERENCES

- Recommended procedure for the bacteriological examination of shellfish and shellfish waters. [Revision of recommended methods of procedure. Report of the Standard Methods Committee for the Examination of Shellfish of the American Public Health Association.] Am. J. Pub. Health 37: 1121-1127 (1947).
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PHS Staff Announcement

Dr. Henry van Zile Hyde, chief, Division of International Health, Public Health Service, was elected chairman by the Executive Board of the World Health Organization on May 27, 1954. Dr. Hyde was first appointed by the President as the United States member of the board in 1948 and reappointed in 1953. During the war, he served in the Middle East with the Foreign Economic Administration and later as chief of the Middle East office of the United Nations Relief and Rehabilitation Administration. He has also served as chief of health and sanitation for the Institute of Inter-American Affairs and for the Technical Cooperation Administration.