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The Sanitary Control of Molluscan Shellfish. Some observations on existing methods and their possible improvement.

by

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During the period 1954-1959, reports were made to the I.C.E.S. Shellfish Committee on the methods and standards adopted by member countries for the sanitary control of molluscan shellfish. Since these reports were made, there has been active research into problems associated with the methods of examination and as a result of this, it is possible that modifications may be required to the methods now in use; some of these problems were discussed at an O.E.C.D. meeting (Anon. 1961) held in Paris in October 1961. The aim of this present report is to discuss some of the problems associated with shellfish control, in the light of recent advances which have been made in the techniques of examination.

1. Basis for control

There is some division of opinion as to whether sanitary control should be based upon the bacteriological tests of water from the growing areas, or of shellfish, or of both. In the United States, shellfish growing areas are classified as approved, restricted or prohibited on the basis of the median of the coliform counts of waters (M.P.N.'s of up to 70, 70-700, and greater than 700/100ml respectively). In addition to this, shellfish standards based on the coliform count and total plate counts are used. In France, provisional standards which have been recently adopted (Boury 1962) employ enumeration of E.coli in the shellfish, and these standards are used together with topographical and bacteriological tests of water from the shellfish - producing areas. Although no rigid standards are laid down for water, the aim is to ensure there is no reduction of the sanitary quality of water in an area, known to produce good quality shellfish. In the United Kingdom, control is based almost entirely upon the E.coli content of the shellfish, probably because local authorities responsible for this control are not able to collect representative samples of water from the shellfish growing areas on a routine basis. Holland has a system of approved and prohibited areas, based upon the E.coli content of the water and of the shellfish (Grijns 1959). It is understood that Spain is adopting a system of control similar to that used in France.

Although the examination of water provides a useful guide, it is not likely that control can be based entirely upon this method. Wide variations of the bacterial loadings of estuarine waters may result from tidal, seasonal and climatic conditions, and these are reflected in the shellfish themselves, which may also show independent variations. Kelly et al. (1960) and Wood (unpublished) have shown that the relationship between the level of pollution of oysters and water is complex, being influenced by such conditions as water temperature, suspended silt, salinity and the presence of stimulating substances in seawater (Collier et al. 1953). However, once the level of pollution of an area is known, water sampling may provide a useful index of continuing satisfactory conditions, although the ultimate and most satisfactory system of control must always rest upon bacteriological examination of the shellfish themselves. Since the examination of water is generally simpler and more rapid than that of shellfish, the examination of large numbers of water samples may be particularly useful for surveys of new areas or for checking existing areas of production. However, in order to interpret these results fully, further tests are needed to determine the shellfish/water pollution relationship over a wide range of conditions.

2. Methods of examination

(a) Shellfish. All the published methods in use for examining shellfish when harvested, are based upon the enumeration of coliforms or E.coli in individual or grouped shellfish. However, recent advances in food bacteriology have suggested that enterococci may be a more satisfactory indication of faecal pollution, because of their generally greater resistance outside the animal body (Butteaux 1959, Burman 1961) and the relative lack of specificity of existing tests for coliforms and E.coli. Butteaux (1959) recommends that the hygienic quality of drinking waters and food can best be made by an examination for both E.coli and enterococci, thus supporting the views of Wilson & McCleskey (1951a) who compared these indicators in oysters. There is increasing evidence that E.coli is less resistant than most pathogenic enterobacteria and viruses (Butteaux and Mossel 1961). Polluted oysters have been associated with infectious viral hepatitis (Christensen, 1956, Jensen, 1963) and it may be necessary to make a comparison of the viability of potential viral pathogens with several bacterial indicators, before the most suitable test can be selected.

The methods used for estimating coliforms and E.coli are basically of two sorts - those which rely upon colony counts in roll-tubes of solid media, and those employing two or more series of dilution tubes.

Roll tube method. The roll-tube count for E.coli in MacConkey agar at 44°C is used in Belgium, Holland and the United Kingdom, and although suitable in many respects (specificity, speed and economy) suffers from a lack of precision at low levels of pollution, mainly on account of the sample size.

The specificity and the total numbers of E.coli isolated by the roll-tube method were increased by pre-incubation of the roll-tubes for 2 hours at 37°C, the average increase of E.coli in 22 samples of water being 22.3% (Pretorius 1961).

Dilution tube methods. An assessment of the dilution tube techniques for examining shellfish has appeared previously (Wood 1957) but the large errors involved in these techniques should again be mentioned. The American method attempts to estimate coliforms, but because of the doubtful value of these organisms as evidence of faecal pollution (Burman 1961) attempts are being made to improve the specificity of the method using an elevated temperature (44.5°C) and a buffered bile (E.C.) medium for E.coli. (Kelly, 1962).

The phenol broth method employed in France is known to be reasonably specific for E.coli; at the Burnham laboratory, 91% of 111 indologenic tubes produced gas in brilliant green bile broth at 44°C. A serious criticism of this and the American method is the long delay before the result is obtained.

At present, no standard method is published for the examination of shellfish in Scandinavia, but tests are now being carried out by the Nordisk Metodik-Komite for Levnesmidler, Copenhagen, and it is hoped that a report on these methods will be available next year.

(b) Waters. At present, E.coli or coliforms are used as the main indicators of faecal pollution of seawater. In the United Kingdom the Public Health Laboratory uses the 15 tube 3 dilution M.P.N. test for E.coli as employed for the examination of drinking water, although the Fisheries Laboratories employ direct colony counts on MacConkey agar at 44°C. The Water Pollution Research Laboratory is now successfully using a membrane filter technique with a Teepol/lactose medium for the enumeration of coliforms in seawater, and the U.S. Shellfish Sanitation Section is testing the use of membrane filters for enumerating both coliforms and enterococci (Kelly 1962). The standard U.S. and French methods for examining water are variations of their shellfish examination techniques.

Providing specific tests can be derived for E.coli or enterococci, it would seem that the membrane filter method offers the most promise, being precise even at low levels of pollution, and economic in time and materials.

3. Standards

The British standards for shellfish are not laid down by law, but the

recommendations of Sherwood & Scott Thomson (1953), made after comparing the 44°C roll-tube colony count with the old-established Fishmonger's Co. method (Knott 1951) have been in use for some time. More recently there has been a move towards higher standards, mainly because a large proportion of oysters and mussels for human consumption now pass through some form of purification. Nowadays few local authorities would tolerate the sale of shellfish, samples of which were consistently in the region of 5 E.coli/ml (the maximum recommended for safe shellfish) and there is a tendency towards the acceptance of shellfish which are generally in the range 0-2 E.coli/ml, with only occasional samples from a particular source falling in the 5/ml region. The experience of one local authority which examined samples of Native and Portuguese oysters purified in commercially-operated ultraviolet cleansing plants from 1960-63 was as follows:-

	Total	E.coli/ml in pools of 5 oysters			
		0	1	2	2 - 4.8
Numbers of samples	707	685	15	4	3
%	100	96.8	2.1	1.1	

Recently, the standards applied to the French shellfish industry have been published (Boury 1962) and these are in general agreement with the British. It is stated

Boury (1962)		Sherwood & Scott Thomson (1953)	
Class	E.coli/ml by phenol broth method	Grade	E.coli/ml by roll-tube method
I	Less than 1	I	Up to 5
II	Between 1 and less than 5	II	Between 6 and 15
III	Between 5 and less than 15		
IV	15 or more	III	More than 15

that shellfish from a purification plant should fall into Class I, but no indication is given of the significance of the other grades, and the action taken by the controlling agency. It is emphasised that these standards are to be considered together with topographical and bacteriological tests of the waters.

In the U.S.A., methods and standards are now under revision, but in 1959 (Anon. 1959) details were given of the maximum coliform counts which could be expected to be present in several species of shellfish as harvested from approved areas /median water M.P.N.'s of 70/100ml or less/. These may be summarised as follows:-

Species	Area	Maximum coliform MPN/100ml	
		Normal	Occasional
1. Oysters (Crassostrea virginica)	North & Middle Atlantic Coast	230	2400 ±
	South & Gulf	2400 ±	-
2. Hard Clams (Venus mercenaria)	Middle Atlantic Coast	230	2400 ±
3. Soft Clams (Mya arenaria)	Canada, North & Middle Atlantic Coast	2400 ±	-
4. Mussels (Mytilus edulis)			

± Where two consecutive samples reach 2400/100 ml investigations are made.

The adoption of the two-level maxima for shellfish is similar in some respects to the U.S. water standards, where the median count must not exceed 70/100ml, and not more than 10% of the counts exceed 230/100ml. This method has the advantage of taking into account the natural variability of pollution in shellfish. It is suggested by the present author that the most realistic standard for shellfish is one which lays down the maximum number of indicator organisms which may occur in 90% and 10% of the samples from a particular source. Such a standard could be applied to current British practice by requiring that 90% of samples from a particular source did not contain more than 2 E.coli/ml, and 10% not more than 5/ml. At the maximum permitted levels of pollution, the mean maximum count would then be 2.3/ml. The apparent agreement of this figure with the American one [230/100ml] is of no significance, as the latter are based upon coliform estimations.

4. The effect of storage upon bacterial content of shellfish

In France, Holland and the United Kingdom, control is based upon the presence of E.coli, no attempt being made to estimate the numbers of other organisms present. This is probably because most of the raw shellfish are sold in the shell, and consumed within a few days of harvesting. In America, where shucking is commonplace, the coliform and total plate count are used as a means of estimating quality.

At Burnham, preliminary tests were started to compare the changes which took place in the numbers of E.coli and coliforms (roll-tubes of MacConkey agar at 44°C and 35°C) and "total" bacteria (nutrient agar at 30°C) in oysters stored out of water at various temperatures. The results obtained so far are not easily interpreted, but several points emerge. When oysters were stored for 4 days at 18°C, the numbers of E.coli showed a slight increase, followed thereafter by a decrease in numbers; at temperatures between 5°C and 14°C counts showed a steady decline. When batches of oysters were stored for 4 days at 5-19°C, the numbers of coliforms increased over twenty times to 12,500/ml. followed thereafter by a decrease in numbers. When oysters were stored at 19°C for 7 days, the "total" number of bacteria increased logarithmically to 341 x 10^4/ml, over 50 times the initial count; at lower temperatures increases of 5 times were shown.

It is evident from this single experiment that E. coli is of limited value in assessing the sanitary quality of stored shellfish, and that attention should be directed to the enumeration of other bacteria i.e. enterococci, coliforms or "total" bacteria. On the other hand Wilson & McCleskey (1951b) showed that

E.coli was a better indicator of pollution in shucked oysters stored at 4-6°C than either enterococci or coliforms. Clearly further work is required in this field.

There is evidence that the presence in oysters of bacteria other than those of faecal origin may be associated with cases of mild gastro-enteritis. Observations at Burnham have shown that oysters taken from upstream oyster grounds subjected to large amounts of land drainage, may contain considerable numbers of non-faecal organisms, even after purification, and it is known that bacteria of the Pseudomonas/Vibrio group form a major portion of the flora of fresh unpolluted oysters (Colwell & Liston 1960). The presence of substantial numbers of these or other harmless bacteria in oysters when taken from the sea or after a period of unsatisfactory storage, may be associated with these mild infections. To establish this relationship more clearly, further enquiry is necessary into the numbers and identity of non-faecal bacteria present in raw shellfish taken for routine control purposes from a variety of sources including clean areas, purification plants and at various stages of marketing before shellfish reach the consumer.

Conclusions

From this short account, it is hoped that the need for further research into problems arising from sanitary control of shellfish is established, and that shellfish bacteriologists will be stimulated to undertake and report upon current investigations in this field. A recent report by Mazieres (1963) is an important contribution to our knowledge of this field, but at the time of writing was not available for detailed study.

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