PRELIMINARY STUDIES ON THE VIABILITY AND DISPERSAL OF COLIFORM BACTERIA IN THE SEA*

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THE DISPOSAL of sewage in the sea is widespread and increasing. There is, however, little information to indicate how much sewage a given body of water will accommodate and even less on the fate of the pollution bacteria in the sea. The economic effects of polluted estuaries are already evident, since large areas have been closed for the taking of shellfish and many beaches throughout the country have been posted as unsatisfactory. These problems will probably become more acute, because the disposal of sewage is essential and the sea provides an efficient means of dispersion.

Harbors and estuaries frequently contain many thousands of bacteria per ml, a large proportion of which may be enteric species. In the open sea, however, the bacterial counts normally range from 50-200 per ml, and the coliform bacteria are never found in open, unpolluted sea water. This tremendous decrease in numbers occurs within a short distance from the mouth of the harbor or estuary (Calif. State Dept. Pub. Health 1943; Knowlton 1929; Mass. Dept. Pub. Health 1936; Warren and Rawn 1938; Weston 1938; Winslow and Moxon 1928). It is clear, therefore, that the introduced bacteria do not persist for extended periods in the sea. The relative importance of dilution of the polluted water by sea water, of the death of the coliform bacteria, of sedimentation and predation by animals has never been clearly assessed in the marine environment.

Our studies on this problem have included laboratory investigations of the viability of *Escherichia coli* in sea water and surveys of some polluted areas selected in the hope that the various factors in the disappearance of pollution bacteria could be evaluated. The results described here must be considered of a preliminary nature.

The results of our laboratory investigations of the death rate of *Escherichia coli* in sea water will be described first. Previous investigations of this problem have given widely divergent results, varying from death rates much more rapid than are found in fresh waters (Calif. State Dept. Pub. Health 1943; Carpenter, Setter and Weinberg 1938; ZoBell 1936, 1946) to the conclusion that sea water is neither antiseptic nor inimical to enteric bacteria (Dienert and Guillerd 1940). We have found that the laboratory treatment of the sea water influences the results greatly. The use of artificial, synthetic or diluted sea water cannot be expected to give results which will correspond to the natural phenomenon.

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When natural, unpolluted sea water is brought into the laboratory and stored in the dark a tremendous growth of the bacterial population takes place. This growth and the effect of adding *E. coli* to the water are shown in Figure 1.* The total population of the raw sea water increases to a maximum in three days, then decreases to a more or less uniform population of about a million cells per ml. A slight initial increase in bacterial numbers is also detected in the water to which the coliforms were added. This period of growth of the mixed population is followed by a decrease in numbers so that the final populations are approximately the same whether *E. coli* were added or not.

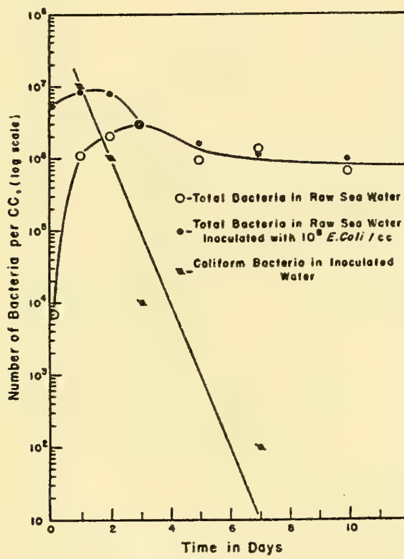

Fig. 1. Growth of marine bacteria and the viability of *Escherichia coli* in untreated sea water.

The concentrations of *E. coli* were estimated independently in this experiment by inoculating lactose broth fermentation tubes. The indicated numbers of *E. coli* thus obtained are also shown in Figure 1. They decrease regularly from the initial inoculum of 10^8 cells/ml and after approximately seven days only a millionth of this population persists. Clearly, the conditions which are suitable for the growth of marine bacteria are inimical to the growth or persistence of the coliforms. It appears that the final populations in both the raw sea water and in the water inoculated with *E. coli* consist of the normal sea water organisms.

If, instead of using untreated sea water, *Escherichia coli* are intro-

* The numbers were determined by plate counts after 7 days' growth on a medium containing 1 gm glucose, 1 gm peptone, 0.05 gm NaH_2PO_4, 15 gm agar in 1 liter of aged sea water.

duced into water sterilized by autoclaving or by boiling, the results shown in Figure 2 are obtained. In the autoclaved water the death rate of the coliform bacteria is very slow. The maximum decrease observed was to one-fifth of the total population in a period of seven days. In the boiled water the death rate is more rapid and is similar to the rate found in the untreated sea water. It is clear from these results that the bactericidal action of sea water is destroyed by the heat of autoclaving, but is unaffected by the milder boiling treatment.

Another observation shown in this figure is that a second or subsequent inoculum of *E. coli* dies off more rapidly than the first. In these experi-

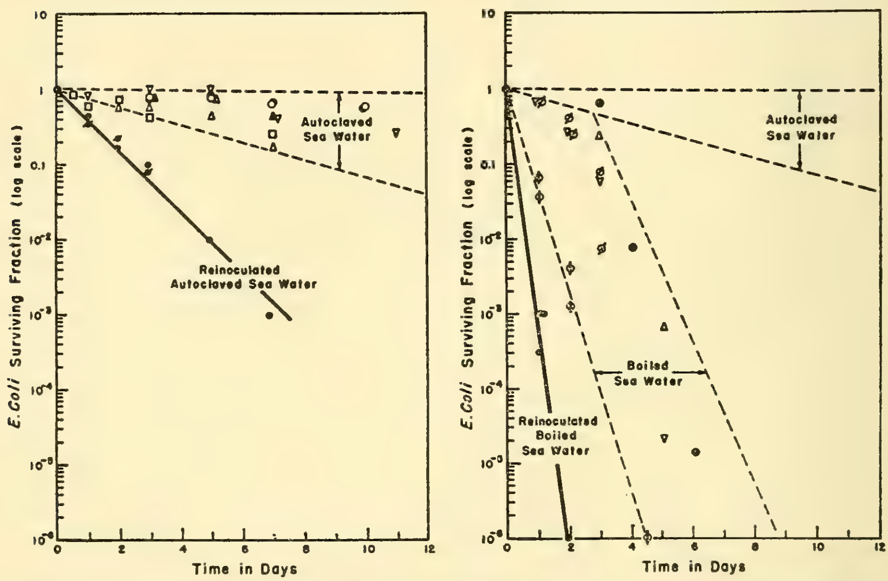

FIG. 2. The viability of *Escherichia coli* in autoclaved and in boiled sea water. The original inoculation of E. coli provided a population between $5x10^7$ and $5x10^8$ cells/ml. The surviving fraction is plotted. Different symbols represent different experiments.

ments the water receiving the first inoculum was stored until no viable cells were found. This took about 20 days for the autoclaved sea water, 5 to 10 days for the boiled water. When another inoculum of *E. coli* was added to the water, the bacterial counts decreased as shown by the heavy lines. The death rate in the reinoculated autoclaved water is eight times as great as the rate observed for the first inoculum. A threefold increase in rate was observed with the boiled water. It may be presumed that the greater death rates observed with reinoculated water correspond to what would be obtained in polluted estuaries.

The coefficients of death rate, as shown in Table I, summarize the results of these laboratory investigations. This coefficient is the reciprocal of the time (in days) for the population to decrease to one-tenth its original

TABLE I

AVERAGE COEFFICIENTS OF DEATH RATE OF ESCHERICHIA COLI IN SEA WATER TREATED IN VARIOUS WAYS

Treatment	Coefficient of Death Rate (k)	
	First inoculum days^{-1}	Subsequent inocula days^{-1}
None	1.00	
Boiled 5 min.	1.15	3.48
Autoclaved 10-15 min.	0.04	0.35

value. Thus, a coefficient of 1.0 means that a tenth of the population dies daily; the coefficient 0.04 indicates that 20 days are required for an equivalent mortality. These experiments show that sea water has a potent bactericidal action. The activity is decreased greatly by autoclaving, but not by boiling the sea water. It is increased by previous "pollution" of the water with *E. coli.*

Further investigations are necessary to determine how the bactericidal activity of sea water varies with natural conditions. Is there a seasonal variation which might be correlated with variations of the normal population of the sea? Is the bactericidal activity greater in polluted harbors than in the open sea, and what are the effects of dissolved organic matter, oxygen supply and other variables associated with pollution? What is the identity or nature of the bactericidal activity?

Some of our experiments suggest that antibiotic substances produced by marine organisms may be responsible for the death of the pollution bacteria. They do not exclude, however, the possibilities that bacteriophage or autolytic or degenerative products of the coliforms themselves may also be involved. It is possible that all three contribute to the final action. It is significant, however, that Rosenfeld and ZoBell (1947) have recently described the production of antibiotic substances by several species of marine bacteria. None of these antibiotics was inimical to gram negative species and *E. coli* was not included among their test organisms. In our experiments pour plates of the normal sea water bacteria were made and the population allowed to develop for 48 hours. The surface of one of each pair of plates was then flooded by a suspension of *E. coli,* the excess being poured off. After a total time of four days the plates were inspected and clear areas were found surrounding some of the sea water bacteria. These results suggest that some of the sea water forms produce substances inimical to *E. coli.*

It is pertinent to inquire whether the results of these laboratory experiments bear any relation to the phenomena which occur in nature. In the sea the mortality of the bacteria may be completely obscured by the circulation and mixing of water masses. The dilution of the polluted water with sea water disperses the bacteria, and this effect must be accounted for in order to observe the death rate. In complicated estuarine situations, where there are several sources of contamination, it becomes especially difficult to differentiate between mortality and dilution. The recent development of an instrument for the continuous recording of salinity and temperature, however, has made it possible to conduct more rapid and accurate surveys of hydrographic conditions. From the data thus obtained the rate of dilution of the introduced contaminated water can be estimated. It is this possibility which has stimulated our interest in the field.

A comparatively simple picture of the fate of sewage bacteria in sea water may be found at the Sewer Outfall at New Bedford, Massachusetts. Here the sewage is introduced from a seven-foot diameter outfall pipe at a depth of 30 feet, 1100 yards offshore into water that is relatively uncontaminated. During the rising tide the flow of sewage is decreased and sometimes stops completely. During the falling tide the sewage wells

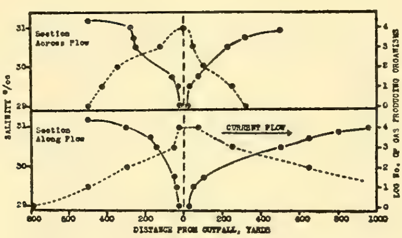

Fig. 3. The salinity and the indicated numbers of organisms producing gas from lactose broth at various distances from the New Bedford Sewer outfall.

out of the pipe in sufficient volume so that it is readily detectable. It is, indeed, difficult to obtain samples directly in the upwelling column since the boat's course is deflected by the rising and spreading current. A tidal current flow of about half a knot sweeps past the location of the outfall, and, on the ebb, carries the polluted water seaward. Several series of observations on the distribution of salinity and coliform bacteria have been made at this location.

The surface distribution of salinity and bacteria along the axis and across the axis of the tidal current are both shown in Figure 3. As would be expected, the salinity of the outfall water is low compared to the surrounding sea water of Buzzards Bay. The introduced sewage is diluted with 13-14 volumes of sea water by the time it appears at the surface. In crossing the axis of current in the neighborhood of the outfall, the bacterial numbers increase to a maximum at a location near the outfall and then decrease again on the other side. The distribution of salinity and of bacteria along the axis are similar except that the downstream distances required to reach a given concentration are greater.

The bacterial numbers are, however, always lower than would be predicted on the basis of dilution alone. To illustrate this point the dilution, expressed as percent outfall water in the sample,* and the surviving percentage of bacteria in the sections across and along the axis of current flow are plotted in Figure 4. If the bacterial count decreased only because of the dilution of the water the two sets of data would be superimposable. The surviving percentage of bacteria, however, is always less than the percent dilution of the water.

The extent by which the numbers of bacteria are less than the expected numbers is shown in Figure 5, where the numbers of bacteria found are

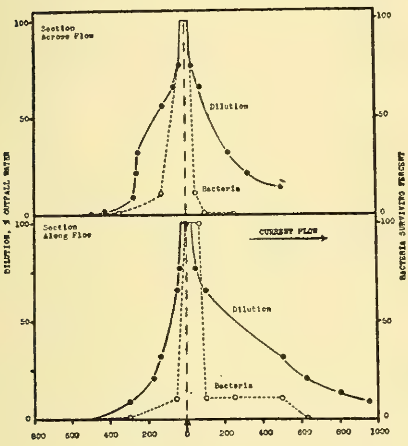

FIG. 4. The dilution of the outfall water, as calculated from the salinity change, and the percentage of bacteria surviving at various distances from the New Bedford Sewer outfall.

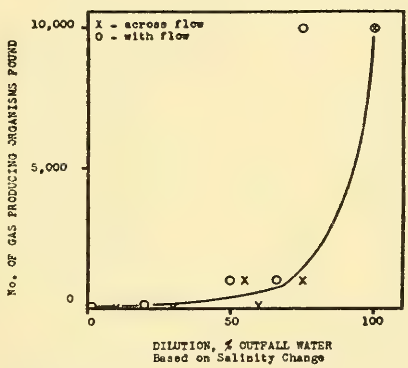

FIG. 5. The number of gas-producing organisms found plotted against the dilution of outfall water as calculated from the salinity change.

plotted against the dilution of the water. If the bacteria diminished in direct proportion to the dilution of the water a straight-line correlation would be expected since dilution of the water by 50% would lead one to expect half of the original bacterial population. The bacterial numbers are, however, substantially lower than can be accounted for by dilution alone. In the simplified situation at the New Bedford Sewer outfall, therefore, it is clear that the coliform population disappears much more rapidly than would be expected on the basis of simple dilution by sea water.

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* Each sample of water is considered a mixture of the polluted, fresher water with sea water, i.e.

$$XS_0 + (1-X)S = S_1$$

in which X is the fraction of polluted water of salinity S_0, and S and S_1 are the salinities of the sea water and of the diluted water sample.

A more complicated picture is found in polluted estuaries where the circulation of the water masses play a more important role. The water exchange in an estuary includes a net outward movement of surface water contributed by rivers at the head of the bay and a net inward movement of the denser more saline sea water at mid-depth or near the bottom. Along the length of the estuary vertical mixing tends to increase the salinity and the volume of the outflowing surface waters with the result that the total surface outflow at the mouth of the estuary is greater than the flow into the estuary from the rivers. An imporant corollary of this generalization is that introduced pollution can be removed only in the surface waters, since any material which sinks to the deeper water moves in a net "upstream" direction.

It may be pointed out that the float tests, which have long been standard techniques in the study of such situations, give only a small part

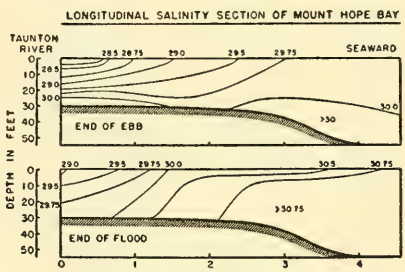

Fig. 6. The distribution of salinity in a longitudinal section of Mount Hope Bay at the end of the ebb and flood tides.

of this complicated picture. The floats show only the net flow of the surface waters, and give no information concerning the rate of vertical mixing. In studying the effects of various wind conditions the use of floats is especially deceptive. The wind drives the float more rapidly than it drives the water. The same wind, furthermore, increases vertical turbulence to such an extent that the pollution is dissipated more rapidly instead of being carried farther as suggested by the float results. Measurements of the salinity of the water provide the most useful tool in studying the exchanges and dilution of various water masses.

Typical salinity contours in Mount Hope Bay, below Fall River, Massachusetts, at the end of the ebb and flood tides are given in Figure 6. These data, collected by the continuous salinity-temperature recorder, illustrate the general principles of estuarine circulation described in the Survey of the River Tees (1931, 1935, 1936). The picture at low tide shows relatively flat, elongated salinity contours demonstrating the greater surface flow of the less saline and consequently lighter river water. The deep water retains much of its dense, high salinity character. Following the flood tide all of the contours are shifted upstream, and the gradients of salinity distribution with depth are more gradual.

An important contribution of water circulation to the disposal of pollution is the rate of dilution of the contaminated water. This occurs,

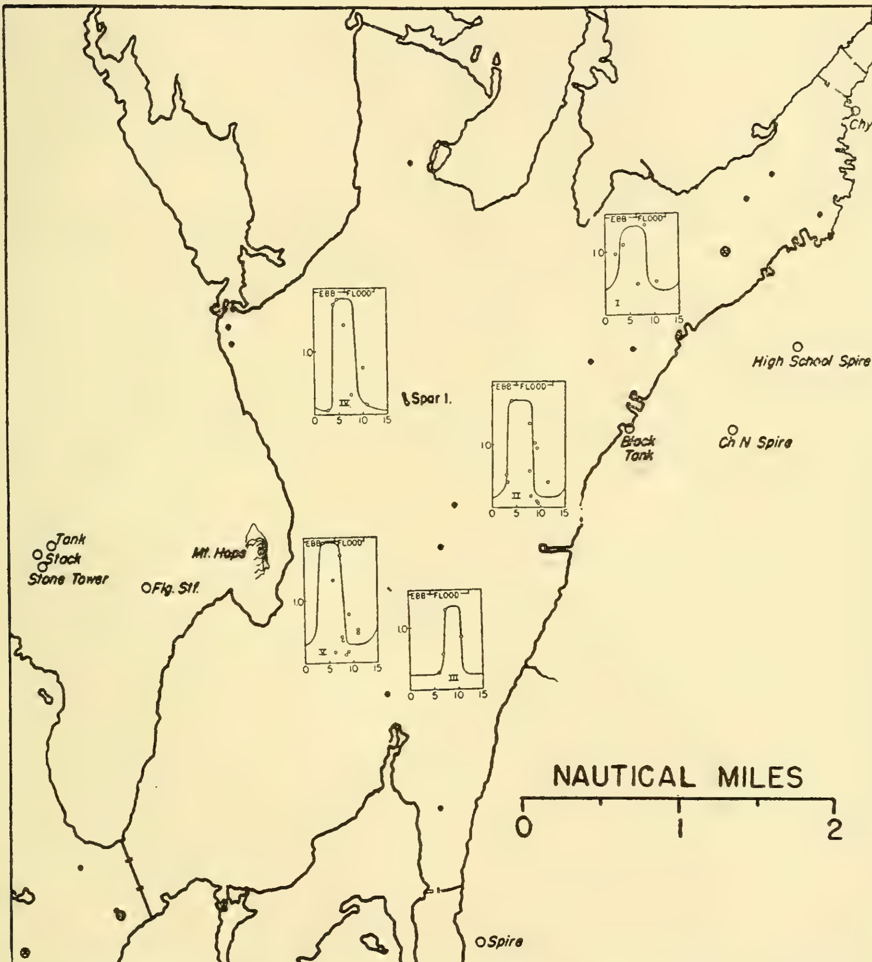

Fig. 7. The ratio between the observed and the expected bacterial counts at various locations in Mount Hope Bay at different stages of the tide. The expected numbers are computed by correcting the numbers introduced in the Taunton River (N.E. corner of chart) by the degree of dilution with sea water.

not only by horizontal mixing, but also by the vertical mixing of the surface water with the more saline deeper water. The rate of this mixing is increased as the density gradient between the surface and deeper waters decreases. As shown by the salinity contours in Figure 6 vertical mixing will be most rapid at the end of the flood tide, since at this time the vertical distribution of salinity and density is most uniform. Any increase in wind force will increase the rate of this mixing, which will tend to diminish the salinity gradients still further.

The distribution of bacteria in relation to the phase of the tide in Mount Hope Bay is shown in Figure 7. In this figure the bacteria are represented by a ratio between the number actually found and the number expected. The number expected is calculated by correcting the number introduced in the river water at the head of the bay by the degree to which this water has been diluted by mixing with sea water. The ratio thus obtained is plotted against the time after high water at each of the locations shown in this figure. If dilution were the only factor operative in this area all of these curves would be flat and show no variation with the tide. The fact that all of them increase above unity indicates that there are contributions of bacteria from sources other than the river mouth. This is to be expected since the area is densely populated and several sewerage systems empty directly into the bay. The interesting fact, however, is that in spite of this additional pollution, the numbers at each location fall far below the expected numbers during the period of high tide. Since the effect of dilution has been cancelled out it is clear that this diminution is the result of other processes which deplete the bacterial population.

To summarize, our investigations have indicated that the coliform bacteria disappear rapidly from normal sea water under laboratory conditions. This disappearance is not related in a simple way to the chemical content of sea water since autoclaving the water eliminates its bactericidal activity. The lethal factors, or substances, are apparently organic in nature and heat labile.

That the bactericidal property of sea water is important under natural conditions is indicated by the fact that the disappearance of coliform bacteria in the sea is much more rapid than can be accounted for by the dilution of contaminated water with sea water. It is fortunate indeed that this is the case, else our heavily polluted harbors would be unbearable.

Several additional problems must be studied in order to complete the picture. It would, of course, be desirable to determine the nature of the bactericidal substance or factor in sea water. The distribution of bactericidal activity in waters and muds collected from polluted areas and at varying distances from shore should aid in identifying its character. The potency of a given body of water may be expected to have an important relation to its ability to accommodate introduced pollution. Finally, considerably more fundamental information is needed concerning the principles which govern the mixing of water masses. When this information is available it should be possible to plan marine outfalls so that the introduction of pollution will lead to the minimum interference with the fisheries and economy of the area.

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