FATE OF FRESH WATER BACTERIA IN THE SEA

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Fresh water bacteria[1] are continually carried into the sea by streams, leachings from the shore, dust and migrating animals. Salt water bacteria are also carried into fresh water. The interchange of bacteria between fresh water and the sea is so extensive and continuous that unless the environmental change is inhibitory we may expect to find many species common to both environments.

The fate of fresh water bacteria in the sea is of considerable importance. The classification of marine bacteria would be greatly simplified if it were established that fresh water bacteria do not maintain themselves in the sea. If we must consider that any bacteria taken from the sea may be identical with fresh water forms the identification becomes more difficult. The higher forms of animals and plants are mainly distinct. To what degree this is true of the simpler forms, particularly bacteria, remains to be determined.

The fate of the fresh water bacteria in the sea is of interest in connection with the general biology of that region. If the fresh water bacteria survive and carry on their activities as in fresh water we may apply much that we know of fresh water bacteriology to an analysis of changes occurring in the sea. The renewal of the food supply of the ocean as of the land depends, to a large extent, on bacterial decomposition. The fresh water and soil bacteria are responsible for the formation of the nitrates and other decomposition products favorable to plant life washed into the sea from the land. Do they also take part in the decom-

[1] By the term fresh water bacteria is meant all bacteria carried into the sea from the land.

position occurring in the sea? The sea differs from the land in that it could support life in so far as the food supply is concerned without decomposition processes because of additions from the land. Decomposition occurs in the sea, however, and is so vitally important to the fertility of that region that an exact knowledge of the organisms involved and the stage in the process for which each is responsible is important.

The main change in the environment to which fresh water bacteria are exposed upon entering the sea is an increase in the concentration of salts, mainly sodium chloride. There are other changes associated with depth but these apply only to parts of the sea. It appears to us that if fresh water bacteria can survive exposure to the salt concentration of the sea they should survive and carry on their activities when carried into the sea to the same extent as when carried into large bodies of fresh water such as lakes.

A number of factors such as organic matter, temperature, light, biological antagonism, number of organisms, age, hydrogen ion concentration, and amount of oxygen affect bacterial life in the sea but these are common to fresh water and probably are not determining factors in the survival of fresh water forms in the sea. For an analysis of factors affecting survival in water and saline suspension the reader is referred to Ballantyne (1930).

The experiments described in this paper deal with (1) the survival of river bacteria in sea water, (2) their ability to carry on their activities, (3) their ability to adapt themselves to concentrations of salt greater than those present in the sea.

EXPERIMENTAL

Materials

Standard beef extract broth and agar with a pH of 7.1 to 7.3 unless otherwise stated, were used for culture and plating work. Six per cent glycerol was added to the agar slants. The sea water was obtained in the middle of the Strait of San Juan de Fuca at full tide and sterilized by heat. Twenty-one organisms were isolated from the Palouse River. These consisted of cocci, bacilli, actinomyces, including aerobes and facultative anaerobes. Not all were used in each experiment.

Experiment 1. To compare the survival of fresh water bacteria in sea water and fresh water

Twenty-four-hour broth cultures of 9 river organisms were prepared and inoculated into sea water, pH 7.7; tap water, pH 7.5; and broth, pH 7.5. After shaking, 1 cc. amounts were plated out to serve as a standard for determing the death curve. The tubes were incubated at 20° to 22°C., for seventy days. A similar set of tubes was prepared and incubated at 7° to 12°C. At intervals of from one to five days, 1 cc. amounts were plated out and compared with the standard.

In the cultures held at 20° to 22°C., an immediate and fairly comparable decline in numbers occurred in the sea and tap water with an increase in the number of organisms in the broth. All the cultures in both the sea water and tap water remained viable for forty-five days. In general the decline in numbers was somewhat greater in sea water. Between the forty-fifth and seventieth day, 3 of the cultures in sea water died out. All the cultures in tap water survived. In the broth the number increased up to twenty to twenty-five days and then remained constant or decreased. All were viable at seventy days.

In the cultures held at 7° to 12°C., the death rate was more rapid. Four cultures in sea water and 3 in tap water died out by the fortieth day. Three cultures in sea water and 3 in tap water survived seventy days, when the experiment was discontinued. In the broth the drop in numbers began earlier, about the fifteenth day, and two of the cultures failed to survive the seventy days.

The results obtained indicate that fresh water bacteria survive in the sea for a considerable time. In general, fresh water bacteria do not survive as long in sea water as in the tap water used. They survive longer at 20° to 22°C., than at 7° to 12°C. The results also indicate that some of the species at least will survive longer in sea water at 20° to 22°C., than in tap water at 7° to 12°C.

The results obtained with the broth suggest that the organism survives as long and possibly longer in the presence of organic matter. Ballantyne and Winslow and associates have shown that organic matter is a factor in survival. Undoubtedly there was more organic matter in the sea water than in the tap water. The tap water was from a deep well and practically free from organic matter. As a possible off-set to the effect of such organic matter as may have been present in the sea water the pH was 7.7 as compared with pH 7.5 of the tap water. An experiment made to determine the effect of pH on the toxicity of salt demonstrated an increase in toxicity with an increase in pH. This is slight within a range of pH 0.2 and apparently less in the presence of organic matter.

For bacteria to multiply and survive indefinitely in either fresh or sea water organic matter must be present. Experiment 1 demonstrated the ability of fresh water bacteria to survive for a considerable time in sea water in the presence of such organic matter as was there. Apparently no multiplication took place. It seemed advisable to determine whether the organisms would multiply and carry on their activities as in fresh water. Their ability to do so would indicate that the very young cells can resist sea water. In general, bacteria have a better chance to survive exposure to bactericidal and bacteriostatic agents in the presence of organic matter.

Experiment 2. To determine whether fresh water bacteria can decompose organic matter in the sea

Meat tubes were prepared by adding canned salmon to sea water. Two series of such tubes were inoculated from forty-eight-hour cultures of 20 river organisms. One series of tubes was incubated at 7° to 12°C., and the second series at 37°C. The tubes were examined at ten and twenty days.

Of the 20 cultures held at 7° to 12°C., eleven showed no growth. In the remaining nine cultures there was growth and decomposition to some extent, varying with the organism. Growth occurred in all the tubes incubated at 37°C. The amount of growth and decomposition of the salmon varied with the organism. In 4 tubes the growth was slight. Three of the organisms grew nearly as well at 7° to 12°C., as at 37°C. The optimum temperature for growth for some of the organisms may have been between those used.

The results obtained indicate that many fresh water bacteria can grow in sea water in the presence of organic matter. It follows that the young cells can survive exposure to salt water under the conditions of the experiment.

Since fresh water bacteria were found to survive and multiply in sea water it was decided to determine how long they would survive in presence of organic matter in salt concentrations greater than those present in sea water. Results obtained under such conditions should not be compared with results obtained with sea water due to other factors present, but they do indicate the effect of sodium chloride on the organisms and this salt appears to be the dominant factor affecting the survival of fresh water and land organisms in the sea.

Experiment 3. To determine the tolerance of fresh water bacteria for sodium chloride concentrations greater than those found in the sea

To plain broth, pH 8.0 was added sufficient NaCl to make molar concentrations of 0.3, 0.48, 0.7, 0.9, 1.5, 2.0. The sea has a salt concentration varying around 0.48 M. Each series of tubes was inoculated with a twenty-four-hour culture of 1 of 9 river organisms. Plain broth was used as control. The tubes were incubated at 7° to 12°C. Transfers at three-day intervals were made to determine viability. The experiment terminated at seventy days.

Of the 9 organisms 3 were spore-bearers. All of these survived exposure to salt concentrations greater than that found in the sea but none survived the seventy days in a 2.0 M concentration. All three survived fifty days in all concentrations. The length of survival of each varied with the salt concentration.

Of the 6 non-spore-bearing organisms one survived seventy days in concentration equal to or slightly greater than that found in the sea. This one did not survive as long as the spore-bearers in the greater concentrations of salt. The other 5 non-spore-bearing organisms in salt concentrations of 0.7 molar survived from sixteen to forty-four days, in 2.0 molar concentrations two to twenty-five days. It is evident that bacteria in the presence of organic matter will survive for a considerable time in salt concentrations greater than that found in the sea. However, salt in concentration of 0.3 M, which is less than that of the sea, reduces viability. Comparative survival of the organism in salt water, salt broth, and sea water with equal concentrations of NaCl was not determined. Undoubtedly organic matter is a factor in survival. The work of Ballantyne and others supports these views. Our experiment was not conducted long enough to determine to what degree organic matter contributed to survival in the presence of salt.

We have assumed that in so far as the fate of fresh water bacteria in the sea is concerned the main factor is the sodium chloride concentration. Other factors effect bacterial life but they are common to both sea and fresh water. Other salts are present in sea water but they are not in sufficient concentration in themselves to inhibit fresh water bacteria. The possibility of their increasing or decreasing the toxic action of salt was considered. An experiment was made to determine this point by adding the salts of KCl, $MgSO_4$, $CaCl_2$, and K_2SO_4, to a salt solution in concentrations approximating those reported for sea water. The toxic action of the sodium chloride was not increased and apparently in one case was decreased. The combined action of salts on bacteria has been critically investigated by Winslow and Dolloff (1928). Apparently salts other than sodium chloride are not determining factors in the survival of fresh water bacteria in the sea. Gases probably do not affect bacterial survival to any greater extent in the sea than in fresh water.

The preceding experiments deal mainly with the new factors encountered which might affect bacterial survival in the sea. The following experiments deal with certain factors affecting bacterial life in any environment but which have significance in the interpretation of experimental results on the effect of salts on survival.

Bacteria of all ages and conditions of dormancy enter the sea. In previous experiments we used twenty-four-hour cultures. The following experiment was made to determine the effect of age on survival. Experiment 2 demonstrated that young cells can survive in the presence of organic matter, but did not indicate their comparative resistance. All cells must be young before they be come old. If the young cells are less resistant this may lead to a disappearance of the species in the sea.

Experiment 4. To determine the comparative resistance to salt of young and old bacterial cells

Tubes of plain broth containing a 4 molar concentration of NaCl were inoculated with 14 river organisms. A series was prepared for each species from cultures aged 4, 12, 24, 48, 72, 120, 168, and 192 hours. Incubation was at room temperature. Transfers were made at one-half hour intervals to determine the death time.

The results obtained indicate that the young cells are much more sensitive to the action of salt than older cells. The cells reached their greatest resistance at twenty-four to forty-eight hours. Cultures older than forty-eight hours either retained their tolerance for salt or showed a slight reduction up to one hundred ninety-two hours.

The bacteriostatic and bactericidal action of most if not all substances is in general increased with an increase in temperature. An experiment was made to determine the effect of heat on the bactericidal action of a strong sodium chloride solution. A 4-molar concentration at 7° to 12°C., room temperature and 45°C. was used. The organisms survived longer at the lower temperatures. In weaker salt concentrations, i.e., in ordinary sea water with a molar concentration of about 0.485 the organisms survived longer at 20° to 22°C. than at 7° to 12°C. These results are in accordance with what we know of the effect of heat on antiseptics and the survival of organisms in cultures. They suggest that the chance of fresh water bacteria to survive in the sea may vary somewhat with the temperature.

The fate of an organism in a new and unfavorable environment depends to some extent upon adaptation. All cells have the power of adaptation. It has been demonstrated that bacteria can adapt themselves to both physical and chemical factors affecting life. Burke, Ulrich and Hendrie (1928) demonstrated that *Staphylococcus albus* can develop increased tolerance for acriflavine, one of the antiseptic dyes, within six hours. Since the salt in the sea is in sufficient concentration to affect the survival of fresh water bacteria the ability of the organism to develop increased tolerance for salt becomes a factor affecting their existence.

Experiment 5. Bacterial adaptation to sodium chloride

Saline broth was prepared with molar concentrations of 0.45, 0.5, 0.55, 0.6, 0.65, and 0.7 of sodium chloride. Tubes with a 0.45 molar concentration were inoculated with 20 river bacteria. As controls, cultures in plain broth were made each forty-eight hours. After forty-eight hours incubation at 37°C., transplants were made to broth containing a 0.5 molar concentration. This procedure was repeated until the broth containing the 0.7 molar concentration of salt was used. Fourteen of the organisms survived this treatment. As a means of detecting increased tolerance for salt, transfer of the 14 organisms and their controls of the same age were made into broth containing a 4 molar concentration of salt. This was kept at room temperature. At the beginning and at thirty-minute intervals up to seven hours transplants to agar slants were made. The results were read after twenty-four hours incubation at 37°C., and twenty-four hours at room temperature.

Two of the organisms showed decreased tolerance for salt, 2 had the same tolerance as the controls and 10 showed increased tolerance, some living more than twice as long as the controls. This experiment demonstrated that a large percentage of fresh water bacteria can develop increased resistance to salt, starting in a concentration in excess of that frequently encountered in brackish water about the mouths of streams.

It has been demonstrated in other experiments in this laboratory that injurious effects of exposure to an unfavorable environment may be transmitted. An organism after exposure may be less rather than more resistant. This depends on the concentration. In demonstrating increased tolerance or adaptation to any agent it is advisable not to expose to too great a concentration. We are of the opinion that the reason the 4 organisms did not adapt themselves is that conditions were not favorable. This applied also to the six strains that died out. The following experiment favors this view.

In the former experiment we utilized the process of exposure and transplants to greater concentrations of salt in broth. In the following experiment we used exposure to salt in agar as a means of selecting the most resistant organisms each time and using them for subsequent exposure. This represents a process of exposure and rigorous selection to alter the cultures.

Salt agar plates were prepared by adding sodium chloride in molar concentrations of 0.8, 0.85, 0.9, 1.0, 1.5, and 2.0. The 21 river organisms were streaked on these plates. The plates were incubated twenty-four hours at 37°C. and twenty-four hours at room temperature. From colonies on the greatest concentration of salt agar streaks were made on agar containing greater salt concentration. This was done four times. All the 21 organisms at first grew on salt agar containing 0.85 molar concentration. The majority grew on a 1.0-molar concentration in agar, a concentration about twice that occurring in the sea.

As the result of 4 transfers on salt agar all the organisms including those that failed to acquire tolerance in the preceding experiment in which salt broth was used, acquired greater tolerance for salt. All the organisms except 1 developed resistance to salt twice that tolerated at first and by the control, or from 4 to 5 times that occurring in the sea. It should be mentioned that a bacteriostatic agent is not as effective in agar as in broth or water.

DISCUSSION

The fate of fresh water bacteria in the sea, i.e., their ability to maintain themselves, will depend on how long they can survive in the absence of organic matter, whether they can carry on their activities and multiply in the presence of organic matter and to what degree they can adapt themselves to the new factors affecting their survival. Our experiments suggest that many fresh water species of bacteria can under favorable conditions or in favorable regions maintain themselves in the sea.

In our experiments we have assumed that the main distinctive factor affecting the survival and growth of fresh water bacteria in the sea is the concentration of sodium chloride and that if this salt does not destroy these organisms they will be able to maintain themselves in the sea to the same extent as in large bodies of fresh water.

Other factors affecting bacterial life in general are common to the sea and large lakes. They vary in different areas of the sea as in fresh water. We can exclude gases as interfering in the sea to any greater extent than in fresh water. These vary with depth, animal and plant life and sunshine. Salts other than sodium chloride are present in small concentration and are probably not a determining factor in the sea. Our experiments demonstrated that they do not increase the toxic action of sodium chloride. In the open sea the water is slightly on the alkaline side of neutrality, about pH 8. In tide pools it becomes more alkaline. In the great depths it is less alkaline but never neutral. It is possible that these factors vary sufficiently so that some areas are less favorable than others to the survival of fresh water bacteria.

Bacteria entering the sea at the mouths of large rivers are exposed to brackish water for some time. Adaptation to increased salt concentration may be established before exposure to pure sea water occurs. This is possibly a factor affecting survival.

Certain other evidence has a bearing on the fate of fresh water bacteria in the sea. It is well known that intestinal organisms survive exposure to brackish water. Typhoid and other diseases are spread by sewage contaminating oyster beds. Bacteriological examination of fish canneries and their products suggests survival of fresh water bacteria near shore. For purposes of isolation and identification bacteria are sometimes exposed to or grown in salt concentrations greater than those present in the sea. Hill and White (1929) have shown that cocci may be isolated in agar containing 6 to 15 per cent salt. Their work shows that many bacteria can be grown in media containing salt greatly in excess of that found in the sea.

Korineck (1926) found that bacteria from various sources other than the sea grow on media containing sea water. He believes however that in the sea they probably exist in a dormant state and do not take part in the decomposition of organic matter.

The death rate of fresh water bacteria in the sea is undoubtedly high. It is high in large bodies of fresh water where the water is so greatly in excess of organic matter that there is no multiplication. Sunshine at the surface, sedimentation, and antagonisms account for the destruction of great numbers. Bacterial populations vary as these factors vary. We believe, however, that many fresh water bacteria carried into the sea can maintain themselves and carry on their activities as in fresh water and contribute much to the biology of the sea. This possibility must be given consideration in the solution of some of the remaining unsolved problems connected with marine biology. It appears probable that the great fertility of the tide area is due in part to the fresh water bacteria surviving there. Even though they do not survive in the ocean they must still be considered as dominant factors in the formation of the nitrates washed into the sea from the land. These nitrates as well as other decomposition products resulting from bacterial activity are factors in the maintenance of a rich littoral flora and fauna. Furthermore, the bacterial cells serve as a food supply for certain protozoa.

The fate of marine bacteria reaching fresh water remains to be determined and is under investigation. The work of Korineck (1926) and of Lipman (1926) suggests that at least some of the marine bacteria have little chance to survive in fresh water.

CONCLUSIONS

1. Fresh water bacteria survive in sea water nearly as long as in tap water. They survive longer at 20° to 22°C., than at 7° to 12°C. Some survive longer in sea water at 20° to 22°C., than in tap water at 7° to 12°C. They probably survive longer in the presence of organic matter.
2. Some fresh water bacteria survive for a considerable time in broth containing salt in concentration 2 to 4 times that found in the sea.
3. Many fresh water bacteria can develop increased tolerance for salt twice that shown originally and grow in concentration in excess of that found in the sea.
4. Salts in the sea other than sodium chloride do not reduce viability of fresh water bacteria.

5. Cells of young cultures are more susceptible to salt than the cells of older cultures. The greatest resistance is reached in twenty-four-to forty-eight-hour cultures.

6. Fresh water bacteria can decompose organic matter in the sea.

REFERENCES

BALLANTYNE, E. N. 1930 On certain factors influencing the survival of bacteria in water and saline solutions. Jour. Bact., **19**, 303.

BURKE, VICTOR, ULRICH, CATHERINE, AND HENDRIE, DON 1928 Bacterial adaptation to acriflavine. Jour. Inf. Dis., **43**, 126.

HILL, JUSTINA, H., AND WHITE, EDWIN C. 1929 Sodium chloride media for the separation of certain gram-positive cocci from Gram-negative bacilli. Jour. Bact., **18**, 43.

KORINECK, T. 1926 Über susswasserbakterien im meere. Cent., Bakt., 2 Abt., 66 (2424) 500.

LIPMAN, CHARLES B. 1926 The concentration of sea water as affecting its bacterial population. Jour. Bact., **12**, 311.

WINSLOW, C. E. A., AND DOLLOFF, A. F. 1928 Relative importance of additive and antagonistic effects of cations upon bacterial viability. Jour. Bact., **15**, 68.