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8521 C\*

**Bactericidal Action of Sea-Water.**

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It has been shown by the author<sup>1</sup> that sea-water inhibits the multiplication of freshwater bacteria while marine bacteria require sea-water media for their initial isolation, although these differences rapidly disappear following the laboratory cultivation of the bacteria. Similar observations on the selective bacteriostatic action of sea-water have been reported by Berkeley,<sup>2</sup> Lipman,<sup>3</sup> and Korinek.<sup>4</sup>

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\* P represents a preliminary, C a complete manuscript.

<sup>1</sup> ZoBell, C. E., and Feltham, C. B., *Proc. Fifth Pac. Sci. Congress*, 1933, **3**, 2097.

<sup>2</sup> Berkeley, C., *Trans. Roy. Soc. Canada*, 1919, **13**, 15.

<sup>3</sup> Lipman, C. B., *J. Bact.*, 1926, **12**, 311.

<sup>4</sup> Korinek, J., *Centralbl. f. Bakt.*, Abt. II, 1927, **71**, 73.

It is generally known that intestinal and other bacteria of public health interest do not survive very long when emptied directly into the sea unless there is appreciable organic matter present or considerable freshwater dilution. Colon bacilli are rarely recovered from the open sea and they occur far less frequently in the vicinity of sewage effluents than can be accounted for by dilution or oceanic circulation. The failure of freshwater bacteria to survive in the sea has been attributed by various investigators to predacious protozoa, lack of nutrients, bacteriophage,<sup>5</sup> or to the lethal effect of sunlight. The following experiments reveal that sea-water *per se* is bactericidal.

In the first series of experiments sewage was appropriately diluted with "formula C"† and 1.0 cc. quantities of the resultant dilutions were transferred to 99 cc. water blanks consisting of different kinds of sea-water as well as a "formula C" control. After thorough mixing by shaking, 1.0 cc. quantities of the final dilutions were plated with nutrient agar. About one minute elapsed from the time the final dilutions were made in sea-water until the agar was poured. Additional 1.0 cc. samples were plated after 15 minutes and again after 30 minutes, and at 30-minute intervals thereafter. The plates were incubated 4 days at 37°C. and the colonies counted. Table I summarizes typical findings on the death-rate of sewage bacteria in sea-water dilution waters. The results are expressed as ratios on a basis of the number of bacteria developing from the "formula C" control being 100.

It will be observed from Table I that sea-water rapidly kills sewage bacteria although 5 to 10% sea-water favors their prolonged survival. Similar results have been obtained with the autochthonous microflora of milk, washings from the hands and buccal cavity, tap-

TABLE I.  
Relative Numbers of Sewage Bacteria Which Survived in the Various Dilution Waters for the Stated Period of Time.

Dilution water	Time in Minutes					
	1	15	30	60	90	120
"Formula C" control	100	96	99	102	100	106
Autoclaved sea-water	85	76	51	43	42	34
5 % "	100	98	103	106	99	103
10 % "	102	101	109	106	102	104
25 % "	96	86	82	73	70	72
50 % "	88	81	72	68	69	60
75 % "	81	70	58	45	42	39
Berkefeld-filtered sea-water	81	47	29	18	14	11

<sup>5</sup> Fernand, A., Sempe and Chavanne, *Bull. Acad. de Med.*, 1925, **93**, 184.

† "Formula C" is a balanced salt solution found to be superior to other dilution waters tried by Butterfield, *J. Bact.*, 1932, **23**, 355.

water, and lakes. Likewise stock cultures of various freshwater bacteria are gradually killed when suspended in sea-water but the death-rate of laboratory acclimatized cultures is much slower than that of similar bacteria which occur in nature. Thus results obtained by suspending stock cultures of *Bact. coli* and human pathogens in sea-water have little public health significance.

Moreover, sea-water which has been autoclaved is less bactericidal than sea-water which has been sterilized by Berkefeld filtration. This is attributed to the thermolability of certain germicidal properties of sea-water, because Berkefeld-filtered sea-water which has been heated behaves as ordinary autoclaved sea-water. Kiribayashi and Aida<sup>6</sup> noted that cholera vibrios live longer in boiled than in raw sea-water but they attributed it to harmful saprophytes.

Evidence that unaltered sea-water has a greater germicidal potency than either heated or Berkefeld-filtered sea-water was obtained by submerging the bacteria in the sea confined in Coors porous porcelain filter tubes impregnated with collodion. Different kinds of freshwater bacteria were suspended in autoclaved sea-water and immediately transferred to the semi-permeable tubes. After being stoppered, some of the tubes were lowered into the sea at the end of the Scripps Institution pier, some were submerged in autoclaved sea-water and some in Berkefeld-filtered sea-water. All were held at the same temperature. At 30-minute intervals the tubes were opened aseptically and 1.0 cc. samples were plated. Table II summarizes representative data. The results are expressed as ratios on a basis of 100 representing the number of bacteria which were recovered from the "formula C" control in semi-permeable tubes.

TABLE II.  
Relative Numbers of Sewage Bacteria Which Survived in Sea-water in Semi-permeable Tubes Submerged in Natural, Autoclaved and Berkefeld-filtered Sea-Water.

Treatment	Time in Minutes			
	30	60	90	120
"Formula C" control	100	91	82	97
Natural sea-water	47	31	15	3
Autoclaved "	56	38	27	36
Berkefeld-filtered sea-water	39	20	23	19

Within the limits of the experimental error the results obtained with autoclaved sea-water and Berkefeld-filtered sea-water under these conditions are not unlike the findings in the previous experiment. However, after the first hour the death rate of freshwater

<sup>6</sup> Kiribayashi, S., and Aida, T., *U. S. Pub. Health Eng. Abst.*, 1934, 14, 61.

bacteria was much greater when submerged in the sea than when submerged in autoclaved or Berkefeld-filtered sea-water. Giaxa<sup>7</sup> observed that pathogens soon perish in raw sea-water although they may survive indefinitely in sterilized sea-water.

This seems to indicate that raw sea-water possesses some constituent which exerts a bactericidal effect besides its salts. Further proof was forthcoming by suspending bacteria in synthetic sea-water and in isotonic NaCl solutions. It was found that the death-rate was somewhat greater in the 3.0% NaCl solutions than in synthetic or in autoclaved sea-water, probably due to a better mineral balance in the latter, but in Berkefeld-filtered sea-water and more particularly in raw sea-water (submergence experiments) the death rate was greater than in the NaCl solution. In fact, sewage bacteria survived longer in 6.0% NaCl (approximately twice the salt content of sea-water) than they did in raw sea-water. It is believed that the bactericidal potency of raw sea-water is even greater than is indicated by these experiments in which the bacteria were surrounded by the semi-permeable walls of the retaining tubes.

Corroborating the conclusions of Burke and Baird,<sup>8</sup> it has been demonstrated that most freshwater bacteria can be acclimatized to tolerate and actually to grow in sea-water media although many of the bacteria undergo other physiological and in some cases morphological transformations. The addition of organic matter reduces the bactericidal potency of sea-water.

## 8522 P

### **Influence of Posterior Pituitary Extracts on Sodium Balance in Normal Subject and in Patient with Diabetes Insipidus.**

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In the normal subject anti-diuretic extracts of the posterior pituitary cause an increase in the excretion of sodium and chlorine with the result that the balance for these elements becomes negative.<sup>1, 2</sup> In Fig. 1 we have presented data on a normal subject (male, 25

<sup>7</sup> Giaxa, D., *Z. f. Hyg.*, 1889, **6**, 162.

<sup>8</sup> Burke, V., and Baird, L. A., *J. Bact.*, 1931, **21**, 287.

<sup>1</sup> Manchester, R. C., *PROC. SOC. EXP. BIOL. AND MED.*, 1931, **29**, 717.

<sup>2</sup> Engel, R., McQuarrie I., und Ziegler, M., *Arch. f. Exp. Path. u. Pharm.*, 1933, **173**, 248.