BACTERIAL INDICES OF POLLUTION IN OYSTER PRODUCING AREAS IN PRINCE EDWARD ISLAND

By

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A Thesis

Submitted to the Faculty of Graduate Studies and Research, McGill University, in partial fulfilment of the requirements for the Degree of Doctor of Philosophy

April, 1955

Mobile Laboratory of the Laboratory of Hygiene, Department of National Health and Welfare

ACKNOWLEDGEMENTS

Experiments were conducted during the 1952 and 1953 oyster investigations in the Mobile Laboratory of the Department of National Health and Welfare. The author is indebted to Mr. I.E. Erdman and Mr. J.M. Kelly of the Mobile Laboratory Service for technical assistance during the field investigations.

The author wishes to thank Professor P.H.H. Gray for his continued interest in the problem and for his generous assistance. He is also grateful to Dr. E.T. Bynoe, Chief, Bacteriological Laboratory Services, Laboratory of Hygiene, Department of National Health and Welfare, Ottawa, for his helpful suggestions and advice, and for reviewing the manuscript.

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INTRODUCTION

The need for sanitary control of molluscan shellfish resulted from the use of rivers, streams, and the sea for the disposal of sewage. Sewage discharged into the sea is rapidly and enormously diluted, but many shellfish beds of commercial importance are situated within long inlets or estuaries where dilution is less effective. In such places molluscs, by virture of their mode of feeding, can become polluted to an extent which make them unsafe to eat uncooked.

The Canadian Atlantic Maritime Provinces constitute the northern limit of the range of the Atlantic oyster (Ostrea virginica). Needler (1932) points out that the greatest production of oysters on the Atlantic Coast of North America is south of New York, and that there are practically no oyster beds north of Boston except in the relatively shallow and protected tidal waters of Prince Edward Island and northern New Brunswick, and in the salt Bras d'Or Lakes of Nova Scotia. Control of the production and marketing of Canadian Atlantic oysters has become a problem of major importance to public health authorities because most of the producing oyster beds are found in tidal bays, estuaries, and rivers, many of which are subject to pollution from sewage and land drainage.

A bacteriological examination to separate polluted from unpolluted shellfish might at first appear possible, but the bacteriological examination of shellfish, like the testing of water, is only part of the total evidence which should be taken into account. Topographical surveys of the beds from which the shellfish have been gathered are also important, and meteorologic and hydrographic conditions at the time of fishing must

be considered. The interpretation of bacterial counts in terms of safety must necessarily be empirical. Not until the epidemiological results of eating shellfish of different degrees of bacteriological purity have been studied for many years will it be possible to relate bacterial counts to the degree of risk associated with eating polluted shellfish.

Because the direct search for the presence of specific pathogenic bacteria in water or shellfish is unlikely to prove of value for routine control purposes, attention has been directed to the demonstration of bacterial species of known excretal origin, particularly organisms of the "coliform" group, faecal streptococci, and Clostridium welchii. Since these organisms are constantly present in the human intestine, usually in numbers greatly exceeding those of pathogenic intestinal bacilli, and since their death rate in water is rather slower than that of organisms of the enteric group, it follows that whenever typhoid or paratyphoid bacilli, for example, gain access to a water supply through excretal pollution, they are always accompanied by the natural organisms inhabiting the intestine.

The coliform group, according to the American Public Health Association "Standard Methods for the Bacteriological Examination of Shellfish and Shellfish Waters" (1947), is considered to include all bacteria which: "Upon transfer from a positive presumptive test (gas positive in standard lactose broth), show fermentation with gas formation in a lactose medium containing 0.00133 per cent of brilliant green and 2.0 per cent of bile (brilliant green lactose bile broth)." In all discussion in this thesis of the coliform group this definition will be used.

The finding of coliform bacteria in water shows, therefore, that recent excretal pollution has probably occurred, and, though not constituting of itself conclusive evidence of danger, is nevertheless sufficient to indicate that the water is potentially dangerous. Attention became more and more directed to the coliform group rather than to faecal streptococci and Cl. welchii, partly because of the greater ease with which coliform bacteria could be demonstrated, and partly because Cl. welchii was found to survive considerably longer in water than members of the coliform and typhoid-paratyphoid groups.

The decimal dilution method has been extensively used for estimating the numbers of coliform bacteria in samples of water and shellfish. A test utilizing a selective medium of growth, lactose broth, has been found superior to the plate count method for the enumeration of coliform bacteria in a mixed bacterial population, particularly when few coliform bacteria are present. As recommended in "Standard Methods for the Bacteriological Examination of Shellfish and Shellfish Waters" (1942), the test consists of the inoculation of five tubes of plain lactose broth with three or more decimal quantities or dilutions of the sample and observation of the inoculated tubes to determine the relative numbers in which fermentation with the production of gas occurs. Incubation is at 35.5° - 37° C. The number of coliform bacteria present in the sample may be estimated from the relative numbers of positive and negative tubes by the Most Probable Number Method of Hoskins (1933, 1934). Coliform densities are expressed as Most Probable Numbers (M.P.N.'s) per 100 ml. of water or shellfish.

The Standard Methods test for coliform bacteria in water and shellfish has been used in routine bacteriological surveys which have been made periodically in most of the shellfish-producing areas in the Canadian Atlantic Maritime Provinces by the Department of National Health and Welfare. All surveys have been made during the summer months (May to October, 1939 to 1954). Individual area surveys have been of one to four weeks' duration. The data obtained from these studies have been used to establish the boundaries between areas open to the taking of shellfish and those areas which, for reasons of potentially dangerous pollution, must be closed to fishing. Shellfish growing waters which contain more than 50 coliform bacteria per 100 ml. expressed in terms of most probable numbers are considered to be so polluted as to be unfit for the growing of oysters for human consumption. Similarly, oysters with M.P.N. 's of coliform bacteria of more than 230 per 100 ml. are considered to be polluted.

Between those shellfish which are grossly polluted, and those which are practically free from coliform bacteria, there are those shellfish upon which suspicion of pollution must fall. The definition of this group is difficult. It is believed, however, that the bacteriological results and sanitary engineering observations have led to sound and realistic closure recommendations in a great majority of the areas surveyed.

Since 1948, however, a number of bacteriological surveys have provided data which have been decidedly at variance with the results of previous bacteriological studies and with sanitary engineering observations (Tennant, 1948; Tennant and Erdman, 1951). In each instance,

large numbers of coliform bacteria were isolated from seawater and oyster specimens taken throughout entire river and bay systems, and these results could not be correlated with faecal pollution as evaluated by sanitary engineering studies. This condition has been encountered only during periods of heavy rain, with resultant heavy run-off from farm and pasture lands to the shellfish growing areas.

While much of the faecal material washed into the water at a time of increased run-off is probably of animal origin, it would be unwise to conclude that such pollution is without public health significance. The Standard Methods coliform test does not indicate the origin of coliform bacteria; oysters containing excessive numbers of coliform bacteria would be subject to rejection upon bacteriological examination at the market regardless of the source of the pollution. The fishing industry has expressed dissatisfaction with the bacteriological methods presently used for the detection of domestic pollution. It has been claimed that the Standard Methods test for coliform bacteria may indicate that a growing area is unfit for the production of shellfish for human consumption even when there is no other evidence to indicate that this was so.

It was evident that a comprehensive bacteriological study should be initiated in a representative oyster producing area. An investigation of the sanitary bacteriology of two river, estuary, and bay systems in Queens County, Prince Edward Island, was therefore made during 1952 and 1953. This study served as the basis for an assessment of the value, (1) of the coliform bacteria and the faecal streptococci (enterococci)

content of seawater as indices of the sanitary quality of an oyster producing area under varying seasonal and tidal conditions, and (2) of the routine bacteriological survey procedures in the sanitary control of oyster production.

REVIEW OF LITERATURE

Bacteriologists in the last quarter of the Nineteenth Century demonstrated the relationship of pathogenic organisms in water to enteric disease. Their work laid the foundation for the modern concept of "indicator organisms" as a measure of the bacterial quality of water. The presence of indicator organisms can be interpreted as the possibility that pathogens may be present and the probability that, at times, the water will be dangerous. It is assumed that the pollution is of faecal origin, and that pathogenic bacteria will be associated with the indicator organisms.

Escherichia coli (Bacterium coli) and the other coliform bacteria are the indicator organisms most often used as indices of the bacteriological quality of water. In 1893, Theobald Smith, referring to Bacterium coli, wrote as follows: "It is safer to infer that any organism which is so uniformly present in the intestinal tract, and which possesses to a slight degree pathogenic properties, really belongs there, and that its presence outside the intestines in soil and water may be regarded as due to their contamination with faecal discharges of men and animals." Thus the role of E. coli as an indicator of excretal pollution is as old as the bacteriology of water itself; the majority of modern methods, are founded on the common belief that coliform bacteria may be used as a satisfactory index of public health hazard in water supplies.

Because E. coli and the coliform bacteria are abundant in the intestine and rare in environments which have not been polluted with faecal material, Houston (1912) concluded that the presence of such bacteria is adequate evidence of faecal pollution, and that water containing Bact. coli should be declared unfit for human consumption.

Coliform Types and their Differentiation and Significance

Escherich (1885) recognized the existence of two distinct types of coliform bacteria, and described a variety to which he applied the name B. lactis aerogenes. Contemporary workers (Laruelle, 1899; Levy and Bruns, 1899) advocated the use of pathogenicity tests for distinguishing between the species isolated from water, but Savage (1903) showed that such tests, as a measure of water pollution, yielded no important information. For a time it was believed that Escherich's bacilli represented the perfect indicators of faecal pollution, but it was not long before a number of observers were able to establish that bacilli apparently indistinguishable from those described by Escherich were widely distributed in nature. Such organisms were isolated in typical or atypical forms from the faeces of various animals (Dyar and Keith, 1894) grain, cereals, and tubers (Laurent, 1899; Klein and Houston, 1899; Prescott, 1902), from soil and water where the possibility of faecal pollution appeared to be remote (Jordan, 1903; Houston, 1912; MacConkey, 1905), and from the dust of streets, grass, trees, plants, and the washers of pumps and wells (Schobl and Ramirez, 1925). The behaviour of the coli-typhoid organisms in soil attracted the attention of other workers (Savage, 1907; Mair, 1908; Laybour, 1920), but beyond establishing that these organisms could live for some time outside the body, these investigators learned nothing of special value for the purposes of differentiation. Savage (1907) noted that some alteration of character occurred in B. coli in soil; he believed, however, that there was no evidence to show that aberrant types represented typical B. coli in an unfavourable environment. Savage found that a large proportion of coliform bacteria from surface wells

were atypical in one or more characteristics. In discussing whether aberrant forms represented "decadent" B. coli in a non-intestinal habitat, or were merely the survivors under more suitable conditions of the few atypical coliform bacteria normally present in excreta, he wrote: "The more nearly an organism isolated resembles an 'excretal' B. coli the greater its significance as an indicator of pollution. Consequently the fewer required to condemn a sample of water in which they occur. Stated as a working proposition, the more the characters of the coli-like organisms deviate from that which for convenience may be spoken of as the typical form, the greater the proportionate number of them required to condemn the water."

The finding of coliform bacteria where excretal pollution appeared to be absent or remote strengthened the belief that the coliform group was saprophytic and widely distributed in nature. The observations of Savage were, however, a definite step in the direction of separating species more in terms of their natural history than of their cultural characteristics; his belief that atypical B. coli was of less sanitary significance than typical B. coli was the basis on which many of the more modern concepts were founded.

The production of indole from peptone by some coliform types was studied by a number of workers (Ehrlich and Boehme, 1905; Gore, 1921; Gnezda, 1899; Kovacs, 1928). Many authorities found, however, that the production of acid from carbohydrates and the production of indole from peptone provided inadequate means of differentiating the coliform bacteria. Their value for the purpose of classification is undoubted, but their apparent inconstancy precludes their use as a sole means of distinguishing members of the coliform group.

Two other biochemical methods were found to be helpful in the classification of coliform bacteria. The Methyl Red Test was developed in 1915 by Clark and Lubs, who pointed out that in a suitable medium coliform bacteria differed in their production of acid; "coli" types produced a high acidity which was constant, while coliform bacteria of the "aerogenes" type produced a much less acid reaction, and on continued incubation became more alkaline. This difference in the acidity produced in the cultivation of "coli" and "aerogenes" coliform types could be recognized by the addition of the indicator methyl red. Closely associated with the Methyl Red Test is a test described by Voges and Proskauer (1898). These workers noted that a colour reaction took place if certain cultures in a suitable medium were treated with potassium hydroxide and allowed to stand for some time; a pink colour reaction was found to be due to the presence of acetyl-methyl-carbinol. Levine (1916) recommended that the term "Voges-Proskauer Reaction" be restricted to designate the formation of acetyl-methyl-carbinol from dextrose.

Wood (1920) summarized the then current opinion as follows: "The results of various investigators show that the lactose-fermenting bacilli can be divided into two main groups by the methyl-red and Voges-Proskauer tests, that the MR- VP+ type is rare in the faeces of man and animals, is more common in surface water and sewage, and is the predominant type in grains and soils. These findings are in favour of the view that they are either the natural survivors of the lactose fermenters present in excretal matter, or are derived from soil or possibly grain, and consequently their presence in water or food products is to be regarded as of less sanitary significance than the presence of excretal B. coli."

Unfortunately, discrepancies were found which could not be explained by this hypothesis. Koser (1923) found that 80 per cent of cultures from polluted waters, and 73 per cent from unpolluted waters, were MR+ VP-. This type of result was also reported by Chen and Rettger (1920), Perry and Monfort (1921), Pawan (1925), and Hicks (1927). Winslow and Cohen (1918) found the content of MR+, VP-, coliform bacteria to be practically the same in polluted and unpolluted water; they concluded that the agreement between the two tests was imperfect, and that some modification of interpretation was required.

Koser (1918) found that B. lactis aerogenes was able to grow profusely in a medium containing uric acid as the sole source of nitrogen, and that B. coli would not grow in this medium. Other workers (Chen and Rettger, 1920; Perry and Monfort, 1921) found that a test for uric acid utilization gave a better indication of the source of coliform bacteria than did the Methyl-Red and Voges-Proskauer tests; Bardsley (1926) was unable to confirm this in the case of coliform bacteria isolated from water.

Brown (1921) observed that B. coli was unable to grow in a citrated broth, while B. aerogenes grew more luxuriantly in a citrated broth than in plain nutrient broth. Koser (1923) confirmed Brown's findings, and showed that B. coli was incapable of using citric acid or sodium citrate as its only source of carbon; he concluded that the ability to utilize citrate was a definite and constant character of B. aerogenes.

Koser (1923) reported a close correlation between the citrate, methyl-red, and Voges-Proskauer reactions in the case of coliform bacteria isolated from faeces; he found, however, that coliform bacteria isolated from soil were often judged to be "faecal" on the basis of the MR-VP tests,

and "non-faecal" on the basis of the citrate test. Koser concluded that not all methyl-red positive, Voges-Proskauer negative coliform types were of faecal origin, and that the faecal and soil types could be differentiated by the citrate test; he believed that the character of citrate utilization was stable, and in no way altered by unfavourable conditions of environment.

The liquid citrate medium developed by Koser (1923) had the disadvantage of appearing turbid when large inocula were used, even when no growth ensued. This observation led Simmons (1926) to devise a solid medium; the addition of agar and brom-thymol blue to Koser's citrate broth obviated the disadvantage of turbidity as a criterion of growth.

Koser (1923) examined 104 coliform cultures isolated from soil supposedly remote from faecal pollution, and 33 cultures from frequently contaminated soils; he found that 67.3 per cent of the former were of the "aerogenes" type (MR-, VP+, Citrate +), while 63.6 per cent of the latter were of the "coli" type. A number of intermediate strains (MR+, Citrate +), which Koser considered to be more closely allied to the "aerogenes" than to the "coli" group, were also found.

Pawan (1925) reported that, while the use of the MR-VP tests reduced considerably the number of strains from non-faecal sources to be regarded as "excretal B. coli", the citrate test almost eliminated them. Hicks (1927) found that the citrate and indole tests, used conjointly, were of considerable value in differentiating "faecal" and "non-faecal" coliform types.

It has been suggested by Koser (1924, 1926, and 1927) that strains of B. coli which are of non-faecal origin are usually able to use citrates as a sole source of carbon, and that, like B. aerogenes, such strains

should probably not be considered as indicating faecal pollution, since faecal strains are usually citrate negative. Burke-Gaffney (1932) found that only two per cent of 278 coliform strains isolated from faeces were citrate positive. On the other hand, Brown and Skinner (1930), Tonney and Noble (1931), Gray (1932), Skinner and Brudnoy (1932), and Taylor (1942) found that faecal strains of B. coli are by no means all citrate negative; they concluded that the correlation described by Koser between habitat and citrate utilization was not confirmed. Skinner and Brudnoy (1932) concluded that, if citrate-utilizing coliform strains are not to be considered as indicating faecal pollution, it should first be shown that they are not often found in faeces.

In support of their view that "aerogenes" organisms may be true faecal types, Raghavachari and Iyer (1940) quote Minkewitsch and Rabinovitch (1936) for the opinion that all soil strains of coliform bacteria are derived by adaptation from faecal B. coli, and Horwood and Webster (1937) for the suggestion that "aerogenes" types form the normal flora of the small intestine, but are transformed into B. coli upon arrival in the large intestine.

The finding of B. aerogenes and the intermediates as the predominant types in infected urine by Burke-Gaffney (1932) caused him to delve further into the relation of these organisms to excretal B. coli. He writes: "It is difficult to believe that faecal colon bacilli could reach the urine in anything like large numbers from any source other than the faeces, in which it is agreed that B. aerogenes are few. If, then, the organisms are derived directly from faeces, how is this factor to be reconciled with the cultural reactions already described? Hill et al. (1929) suggest that in the urine the aerogenes respond to some

selective action during their sojourn in the urinary tract, or that they survive the coli type and therefore ultimately outnumber them. In the case under review, the cultures were not all typical B. aerogenes. An unusually high percentage of them produced indole, and nine per cent were of the intermediate type. What then is the position of these intermediate organisms? In the present experiments they were entirely absent from unpolluted soil, rare in faeces, and relatively common in sewage and polluted soil. If one adds to this the fact that in samples from polluted sources, both this intermediate group and the aerogenes group retained the power to produce indole, it would seem that a definite chain of events was taking place. Is one entitled to assume that the power to utilize citrate, the retention of indole-producing capacity, and the occasional production of a positive methyl-red reaction are all cultural manifestations of an environmental change? It is suggested that the farther it is removed from its normal intestinal habitat, the less does B. coli retain its faecal characteristics, and that non-faecal characters are developed before the latter are entirely lost. Does B. coli in fact, when placed in unfavourable surroundings, adopt a wider metabolic activity as a biological necessity, and in the urine, divorced from its natural habitat, has the transition already begun? Such a theory might explain the occurrence of the intermediate group, and the true significance of the aerogenes type."

Houston (1925) sounded a note of warning which cannot be lightly disregarded: "Some bacteriologists are a little too eager to deny recognition to the aerogenes group because they are apt to be associated with the washings from grain and soils. Yet it is in times of flood when all sorts of unchartable pollutions are swept into watercourse that these

soil microbes may be perhaps specially noticeable, and few will deny that floods are periods of epidemiological danger."

Apparently the intermediates are found as ubiquitously as are any of the other coliform bacteria. The first reports of such intermediate types were made by Koser (1924, 1926, 1927), who isolated a number of strains from soil, water, and human and animal faeces. Intermediates have been found in faeces by Kline (1930) Skinner and Brudnoy (1932), Parr (1934), and Tittsler and Sandholzer (1935). The latter authors also studied strains isolated from urine, water, and soil. Kline (1930) isolated intermediates from milk and other dairy products, Hajna and Perry (1936) obtained intermediate strains from the intestinal contents of various cold-blooded animals, and Griffiths and Fuller (1936) found intermediates in commercial fish and fillets. Intermediates have also been reported in water by Ruchhoft et al. (1931), Gray (1932), Poe (1932, 1934), France (1933), Parr and Caldwell (1933), and Tennant (1949). Parr and Caldwell (1933) found that 8.4 per cent of 1,407 samples of a well water contained intermediate coliform bacteria, and in no case where coliform intermediates occurred could the possibility of faecal pollution be excluded. Kline (1935) reported that the intermediate strains studied were comparatively stable in pure culture, and did not fluctuate in regard to basic differential characters in short periods even though their environment was altered, nor did they dissociate to new types, although they showed marked changes in colony morphology. Kline concluded that no natural environment is exclusively the abode of any single type of coliform organism, and that no single type of these organisms is found exclusively in any natural environment.

Vaughn and Levine (1942) emphasize that the use of inadequate methods of determining the Voges-Proskauer reaction and the use of 37° C. rather than 30° C. for incubation accounted for most of the usual characteristic irregularities in most intermediate cultures studied. It is important to note that true Escherichia coli type I cultures have been diagnosed as intermediates because Simmons citrate agar made with tap water had been used for differential purposes.

Most observers are agreed that the "IMViC" biochemical tests form a reliable means of subdividing coliform bacteria into the various types. Levine (1941) reported that certain coliform types appeared to be associated with specific sources, and that there may be a high degree of correlation between the incidence of certain coliform types and hygienic significance.

Buttiaux et al. (1948) regard the presence of E. coli as evidence of recent pollution, and the presence of other coliform types as evidence of "remote" or intermittent pollution - a view held by many others.

On the other hand, Neumann (1950) considers that the validity of classifying bacteria of faecal and non-faecal origin within the coliform group is questionable. Clark (1952) concluded that true faecal pollution is characterized by a representation of all major coliform types, and that the presence of only one coliform type in water or shellfish would constitute a decidedly abnormal situation.

44° - 46° C. Tests for Escherichia Coli

Eijkman (1904) made the first attempt to devise a test which would be specific for B. coli; he claimed that only faecal B. coli of warm-blooded animals was capable of fermenting glucose at a temperature of 46° C. Opinions on the value of Eijkman's test were divided; Konrich (1910) and Hehewerth (1911) both found a number of strains of faecal B. coli which failed to develop in glucose broth at 46° C. With this criticism of his work Eijkman (1912) himself agreed; he stated that a negative result could not be regarded as proof of the absence of B. coli, and that the test was valid only in its positive aspect. Flu (1915) considered the test a valuable indication of recent contamination, while de Graaff (1922) found that incubation at 46° C. inhibited "aerogenes" organisms. Wilson (1929) incubated MacConkey plates at 45° C. and found that a positive result invariably indicated the presence of B. coli, but that negative results did not have the opposite significance. Leiter (1929) supported the use of the 46° C. Eijkman test, reporting that the "aerogenes-cloacae" group grew just as well as B. coli at temperatures below 45° C., and that even at 49° C. fifty per cent of strains of B. coli could be isolated in almost pure culture by this test.

Perry (1929) found that, while B. coli was invariably Eijkman-positive, a great many methyl-red-negative coliform bacteria behaved in the same way.

Brown and Skinner (1930) found that only a small percentage of human faecal B. coli fermented glucose at 46° C.; many true B. coli failed to grow, and a number of "aerogenes" and atypical coliform strains gave a positive Eijkman test. They noted that positive cultures after 48 hours

contained few or no viable organisms. Ruchhoft et al. (1931) also found that some "aerogenes" strains grew well at 46° C., and considered that a positive result from a water sample at this temperature did not indicate the presence of faecal B. coli from warm-blooded animals alone.

Taylor and Goyle (1931), working in the tropics, had to postulate the survival of faecal B. coli in soil to justify the positive Eijkman results they obtained from water samples which came from areas considered to be free from obvious human and animal pollution. They then showed that B. coli had in fact a considerable survival period in soil at a temperature of 80° F. They used the test on water samples, and found that the results related to a remarkable degree with the probable sanitary quality of the water, and were of far more value than those obtained at the usual incubation temperature of 37° C.

Burke-Gaffney (1932) found the 46° C. test very unreliable in the tropics, as more organisms of the B. aeregenes than of the B. coli type developed. In this finding he was in agreement with the work of Brewster (1929) and of Brown and Skinner (1930), but it is difficult to understand such results in the light of more recent experience, since even a temperature of 44° C. is found today to inhibit "aerogenes" strains almost completely.

Williams, Weaver, and Scherago (1933), and Perry and Hajna (1933), suspected that excessive acid formation in the Eijkman medium was causing some strains of B. coli to fail to grow at 46° C., and developed new media in which the concentration of glucose was considerably reduced. They concluded that Eijkman's fundamental observations were correct when a suitable medium and a carefully controlled incubation temperature were used.

Skinner and Brown (1934) draw attention to the fact that the use of small tubes and water-bath incubation were the only means of securing a controlled and uniform temperature in the medium. Lack of uniformity in these details may explain the divergent results in the literature. These authors concluded that the Eijkman test did not differentiate reliably between B. coli from warm-blooded and from cold-blooded animals, and that 48 hour incubation was necessary for the test.

Webster and Raghavachari (1934) found that the Eijkman test sometimes failed to reveal pollution, that it was insufficiently sensitive, and that it added no useful information to the results of routine tests at 37° C.

Hitherto the question of the optimum temperature for the test had not been given the attention it deserved, but with the work of Levine, Epstein, and Vaughn (1934) it was carefully studied. They found that all Escherichia strains grew well in Eijkman's broth and in standard lactose broth at 43° - 44° C., but that these strains were markedly inhibited as regards gas production at 45° - 46° C. The Aerobacter strains were almost entirely inhibited at the lower temperature.

Wilson et al. (1935) found that some strains of B. coli could not ferment glucose at 46° C. At 44° C., however, practically all indole-positive strains of B. coli grew well and produced gas; no strain of B. aerogenes produced gas at this temperature, and very few grew at all. Wilson and his associates stressed the need for using a water bath in preference to an incubator for 44° C. incubation. The correlation between their modified Eijkman test and the indole test was striking, as was the negative correlation between the Eijkman test and growth in

citrate. They observed that gas production was better in MacConkey broth at 44° C. than in the modified Eijkman medium of Williams et al. (1933). The classification of the various coliform types suggested by Wilson and his associates is presented in Table XV.

Topley and Wilson (1936) summed up the position at the time by advocating exact standardization of the water-bath temperature at 43° - - 45° C. They considered that the value of the test was greatly enhanced by the replacement in the medium of glucose by lactose, that MacConkey's broth was the best medium, and that the test was better than any other single test for the isolation of typical B. coli.

Dodgson (1937) noted that a large number of coliform strains in oysters were killed at 44° C., and that the test was very promising for the detection of B. coli in mussels.

MacKenzie and Hilton-Sergeant (1938) made comparative studies of faeces, using incubation temperatures of 37° and 44° C. They formed a high opinion of the modified Eijkman test, and concluded that the isolation of B. coli type I subsequent to incubation at 44° C. is more certain than by selecting colonies from plates prepared after incubating in MacConkey broth at 37° C. They were of the opinion that 48 hour incubation should be allowed before reporting a negative result; they also believed that the percentage of false positives was negligible, and that the test indicated the absence of dangerous pollution with greater certainty than did the 37° C. presumptive test.

The findings of Wilson et al. (1935) were confirmed by Bardsley (1938), although Bardsley pointed out that numerical estimations of

B. coli type I would be upset if Irregular type II (the only coliform type of which she considered to be a threat to the specificity of the test) were present. She considered, however, that the occurrence of Irregular type II was so rare that it would be of little account in comparison with the degree of experimental error of the method.

Dodgson (1938) found that at a temperature of 44° C. all citrate-positive coliform bacteria in shellfish failed to produce gas, while the majority of typical B. coli were able to do so. Clegg and Sherwood (1939) concluded that, owing to its high degree of specificity for B. coli, the 44° C. test was of considerable value in the examination of shellfish. They reported a close negative relationship between the Eijkman and citrate tests, and observed that temperatures above 44° C. were detrimental to the growth of B. coli.

Raghavachari and Iyer (1939) reported that some strains of B. aerogenes isolated from Indian waters produced acid and gas in MacConkey broth at 44° C. This was confirmed by Professor Wilson, who reported that their behaviour was the same in his hands.

Perry (1939), held that the greatest value of the Eijkman test was in the examination of samples with a high content of Aerobacter strains, because these were apt to overgrow B. coli at 37° C. Hajna and Perry (1939) found that many B. aerogenes strains grew at 44° C., but that MacConkey's medium strongly inhibited them.

Raven, Peden, and Wright (1940) found a large number of instances in which citrate utilizers grew at 44° C. Harding (1940) also isolated many "aerogenes" strains which gave positive results at 44° C.

Raghavachari and Iyer (1940) concluded that the 44° C. test could be considered specific for distinguishing between faecal and non-faecal types only if it could be proved that 44° C. positive "aerogenes-like" organisms found in water were normal intestinal organisms. It should be noted, however, that this applies to Indian waters,where so many of these "recalcitrant" strains have been found. These workers quote Sen (1937) as reporting that many strains of B. aerogenes isolated from human faeces gave a positive Eijkman reaction. They also report that, of 478 organisms studied, 31 were of the B. aerogenes I type, and no less than 24 of these were Eijkman-positive. These strains were frequently retested at 44° C. and all were consistently positive; similarly, the seven Eijkman-negative strains remained negative. The authors concluded that all coliforms may be faecal in origin, and that they may change their properties when removed from their normal habitat.

Taylor (1941) found that, of 30 cultures of B. aerogenes, six were 44° C. positive; after retesting these cultures, Taylor concluded that there is no doubt that strains of B. aerogenes which are positive at 44° C. do exist.

Batty-Smith (1942) isolated three cultures of Intermediate type I and two of B. aerogenes type I which were capable of fermenting lactose at 44° C. Stuart et al. (1942) reported that the more-primitive members of the Family Enterobacteriaceae, Serratia and Erwinia, were frequently killed by a temperature of 45.5° C. in 24 hours, even in cultures heavily inoculated, while Aerobacter was not. In their Eijkman characteristics the intermediates were much more closely related to Aerobacter than to Escherichia because Aerobacter and intermediates seldom produced gas from lactose at 45.5° C. while Escherichia seldom failed to do so.

The large proportion of 44° C. positive B. aerogenes and intermediate types found by Taylor (1941), as compared with other workers, argues that the distribution of such types is localized; Raven et al. (1940) and Harding (1940) have also expressed this opinion.

Hajna and Perry (1943) reported the development of an EC Medium (buffered tryptose lactose bile salt broth) for the isolation of coliform bacteria at 37° C. and of Escherichia coli at 45.5° C. In the examination of 147 samples of drinking water of various types, not a single false presumptive test was encountered among 1,176 gas-positive tubes while 58.5 per cent of false presumptive tests were obtained with standard lactose broth.

Further work by Perry and Hajna (1944) indicated that both lauryl sulphate tryptose broth and EC medium are highly sensitive and specific media for the isolation of coliform bacteria from water, shellfish, and sewage. Wattie (1948), however, found that the incubation of presumptive cultures in EC medium at 45.5° C. or even at 44° C. may not be expected to detect more than about 25 per cent of the coliform group, or about 50 per cent of the E. coli present in the samples. Unpublished data from investigations conducted by the Sanitary Engineering Division of the Los Angeles Department of Water and Power indicate that there is some repression of E. coli with EC medium and with Eijkman broth.

It may be concluded that a considerable number of bacteriologists, particularly in Great Britain, regard Escherichia coli as the best available index of faecal pollution; the majority of North American investigators, however, now agree that the presence of coliform bacteria of the Aerobacter-intermediate group can provide information of equal importance.

That being so, it is unlikely that the Eijkman test for E. coli can supplant the Standard Methods Presumptive coliform test; the two tests may have value when used in conjunction. It has been suggested that two sets of standards be laid down, one to indicate the allowable number of Escherichia coli per 100 ml., and a more lenient standard for the number of "intermediate-aerogenes-cloacae" (I.A.C.) organisms per 100 ml. of specimen.

Coliform Bacteria in Faeces

In view of the extent of the work on the coliform bacteria in faeces it is disappointing to find that very few quantitative examinations have been made. All investigators are agreed that E. coli type I is by far the most abundant coliform type in faeces of man and other animals including birds, and that the proportion of the different coliform types is less certain.

Hill et al. (1929) summarised the results of many different workers and showed that the numbers of B. aerogenes and intermediates reported varied from zero to 16.0 per cent of the total. Ruchhoft et al. (1931) examined 32 samples of faeces from 11 persons and 10 animals; only four coliform types were represented: B. coli type I, 83.2 per cent; B. coli type II, 11.5 per cent; Intermediate type I, 2.0 per cent; and B. aerogenes type I, 3.3 per cent. The presence of intermediates in faeces was thus confirmed. Bardsley (1934) also found B. aerogenes type I and intermediates in relatively small numbers in faeces. Bardsley (1938), by the use of primary incubation in MacConkey broth with subsequent inoculation from positive tubes into citrate media, found intermediates or B. aerogenes in

sixty-one of one hundred samples of human faeces examined. In eight cases the numbers were equal to or greater than those of B. coli. B. aerogenes were reported in approximately fifty per cent of faecal samples studied by Dulaney and Smith (1939). B. aerogenes or intermediates were found in 87 per cent of 253 samples of faeces examined by Reedy and Puncochar (1940); in 166 samples B. aerogenes types, together with intermediates, were present in numbers of at least 10,000 per gram. Parr (1938) found B. aerogenes in 33 per cent and intermediates in 31 per cent of the faeces samples analysed. Carpenter and Fulton (1937) found citrate-utilizing coliform bacteria in 49.9 per cent of 466 samples of human faeces. 13.3 per cent of the samples contained intermediates; such coliform bacteria were characteristically present in certain individuals. Carpenter and Fulton concluded that coliform bacteria of the intermediate group are of sanitary significance.

There is some evidence to show that the distribution of the various coliform types is affected by climatic conditions. Clemesha (1912) showed that the bacterial flora of human and animal faeces in India not only differed from those in England in the relative proportions of the organisms which they contained, but that they were subject to definite local and seasonal variations. In this connection he referred to the comparative rarity of true B. coli communis (Escherichia coli) in India, even in faeces, a point which had already been discussed by Castellani (1910) in Ceylon.

The resistance of coliform bacteria to drying has received the attention of a number of investigators. Kon (1933) found E. coli and B. aerogenes to occur equally in milk (58:42), but noted that B. coli

predominated (44:1) in bovine faeces. This was confirmed by Cohn (1950), who stated that while E. coli predominates in cow faeces, raw milk and utensils are often contaminated by other coliform types. The relative rarity of E. coli in milk is due to its slight resistance to drying. Cohn found that dried faeces on the udder rarely contained E. coli; in thin faeces films E. coli died in two to three hours. The ascendency of Aerobacter and intermediates over E. coli in stored faeces was also reported by Parr (1938).

On the other hand, Bardsley (1948) reported that periodic sampling of cow faeces left to dry on the open meadow showed that reduction in coliform count was very gradual and largely dependent on the moisture content, and that E. coli type I was still overwhelmingly dominant three to seven weeks after the samples were voided. Laboratory experiments in which cow faeces were stored in moist and dry chambers, gave similar results.

Sears, Brownlee, and Uchiyama (1950, 1952) consider that there is ample evidence that man invariably acquires Escherichia coli during the first day or two of life if not, indeed, even before birth, and that he is never thereafter without it. The faeces of a number of persons were examined repeatedly over a period of two and one-half years with the object of studying the changes in the coliform flora. The flora tended to have a fairly constant pattern, consisting of organisms of rarely more four antigenic types. Of these one or two "residents" were dominant types, firmly established and persisting for long periods, and the other "transients" were found for a few weeks at most. Attempts to alter the coliform flora by feeding with large amounts of cultures of new strains

did not result in more than a transient appearance of the new strain in the faeces.

It has been shown by numerous authorities that, apart from the intestinal flora of the larger domestic animals, B. coli may be present in the faeces of frogs (Raju, 1922), gulls and fish (Houston, 1923), and of small birds (Emmel, Minkewitsch, 1930), in sufficient quantities to produce positive results in the waters which may contain them.

In general, there is overwhelming evidence to show that E. coli type I is the dominant type of the coliform bacteria in faeces; it is, however, doubtful if it is also true, as is often stated, that A. aerogenes and the intermediate types are rare or few in number. The figures obtained by various workers suggest that of the coliform cultures isolated from faeces, five per cent or more of the total were types other than E. coli. Expressed numerically this may sometimes represent a million or more cells per gram of faeces, numbers which can scarcely be considered to be without significance. The question of the significance of A. aerogenes, according to Taylor (1942), is clearly whether they are more prevalent in any habitat other than faeces.

It may also be concluded that the results of different workers show that storage of faeces causes a more rapid disappearance of E. coli than of intermediate and Aerobacter types, but that little is known of the relative importance of different factors - light, pH, and availability of oxygen-on the rate of change.

Coliform Bacteria in Soil, and in Plant Materials

The behaviour of coliform bacteria in soil has attracted the attention of a fairly large number of investigators. Houston (1902) reported that B. coli may persist in soil for months after it gains access, but does not multiply there. Savage (1905) studied the effect of tidal mud on the viability of B. coli, and reported that the organisms were found in the proportions of one per gram for three months after the initial pollution. Savage (1907) noted that some alteration of character occurred in B. coli in soil, but concluded that there was no evidence to show that aberrant types represented typical B. coli in an unfavourable environment. Young and Greenfield (1923) controlled the moisture content of soil samples after seeding with B. coli; they reported that these bacteria survived for as long as seven years. Skinner and Murray (1926) reported that B. coli from fresh cow faeces or pure culture disappeared from soil in 200 days after inoculation. Gray (1932) examined six samples of soil which in his opinion had not been contaminated by human or animal faecal matter, and found that all samples contained A. aerogenes and that five yielded B. coli. Kulp (1932) reported that coliform bacteria were viable three years and seven months after the initial seeding in soil. Taylor and Goyle (1931), working in the tropics, showed that B. coli had a considerable survival period in soil at a temperature of 80° F.

Sterilized soil which was held in a thre-inch stovepipe twenty feet in length was inoculated with a carefully purified culture of B. coli by Kline (1935). Sterile water was allowed to seep slowly through

the soil for 410 days. Of 726 cultures isolated at various sampling points during the period, 560 cultures (77.1 per cent) retained the same characters as the original culture. The remaining 166 cultures varied in respect to the methyl-red, citrate, or Eijkman tests, but no variations occurred before the forty-seventh day. Seventy-five cultures (10.3 per cent) isolated after the ninety-fifth day were able to utilize citrate as the sole source of carbon.

Koser (1923) examined 104 cultures isolated from soil supposedly remote from faecal pollution, and 33 cultures from frequently-contaminated soils. He found that 67.3 per cent of cultures from the first were of the "aerogenes" type, while 63.6 per cent of the second were of the "coli" type.

Soil plots low in organic matter were watered with cultures of E. coli type I, Intermediate type I, or A. aerogenes type I, and sampled at intervals by Bardsley (1948). All three types tended to die out gradually, and no profound changes were apparent in the biochemical reactions of the three types as a result of a prolonged sojourn in soil. Soil which had been heavily contaminated with cow manure three years previously still contained large numbers of viable E. coli type I. Washings from soil holding accumulated animal faeces may be the source of large numbers of coliform bacteria long after the land has ceased to be used as pasture.

A survey of the density and types of coliform bacteria in soils from a number of widely-spaced localities in the United Kingdom and elsewhere by Taylor (1951) has shown that such organisms are not present in very large numbers. Of the soils examined, 27 per cent contained no coliform bacteria in 15.5 grams of soil; of the soils which contained coliform

types, only 17 per cent had presumptive counts greater than 180 per gram, and only 11 per cent had confiremd counts greater than 100 per gram. The number of coliform organisms was closely related to the probable animal population; numbers were greatest in pasture lands and least in soils from remote hills. These results confirm the work of Bardsley (1934) on acid moorland soils, and of Koser (1926) and Minkewitsch (1930) on a variety of soils. Application of farmyard manure to the Rothamsted plots produced no permanently large population of coliform organisms. The presence of relatively large numbers of these organisms in soils from wooded areas remote from domestic animals and man suggests appreciable contamination from small wild animals. The predominant coliform type isolated in these studies by Taylor was E. coli type I, whatever the location or vegetation type of the soil. Intermediate type I, A. aerogenes type I, and E. coli type II were the only other coliform types of numerical significance; Intermediate type I was more prevalent in arable and pasture soils than in any other locations. Taylor considers that the evidence supports the view that soil is not the natural habitat of either the intermediate types or E. coli type II; he finds this strange in view of the fact that crude sewage, improperly purified sewage effluents, liquors from septic tanks, or other materials of faecal origin may contain very high populations of "intermediate" or "aerogenes" organisms. Taylor concludes that it seems likely that the most important habitat of all major coliform types is in faeces.

Taylor (1951) reports that, in such soils as are subject to intermittent excretal contamination, there appear to be fluctuating populations

of Gram negative rods with characteristic coliform reactions to the "IMViC" tests, but whose reactions on lactose at 37° C. vary between the production of slight concentrations of acid to vigorous and rapid formation of gas. The evidence obtained suggests that most of these types producing little or no gas are either "lactose-degraded" coliform bacteria or paracolon bacteria, and that their reactions are largely those of the Intermediate type I and A. aerogenes type I variety. Such coliform bacteria with weak lactose-fermenting powers have been termed Paracolobactrum by Bergey (1948). Further, though it may well be that E. coli type I does not persist as long in sewage or soil as other coliform bacteria, Taylor's data suggest that in soil this may be masked by the loss of lactose-germenting ability by the intermediate-aerogenes types, which are consequently not usually detected at 37° C. Taylor concluded that false positive reactions, particularly from arable and pasture soils, are due to coliform types that produce very small amounts of gas in the tubes of the presumptive test but negligible amounts when isolated and tested in pure culture.

The study of the survival of coliform bacteria in soil has been complicated by the contention of some investigators and reviewers that the Aerobacter-intermediate group is a part of the normal flora of soils, grains, and grasses. Topley and Wilson (1931) state, "And there seems little doubt that the B. aerogenes-B. cloacae group, so demarcated, consists of bacilli which live normally on plants or in the soil." Authoritative manuals and text-books on water examination (Ministry of Health, 1939; E.W. Taylor, 1949) still state that soil is a probable normal habitat of E. coli type II and of the intermediate types, and that veget-

ation is the normal habitat of A. aerogenes types I and II. It thus seems that some bacteriologists have come to regard the intermediate types and A. aerogenes as indicative of pollution from soil and plants and not from human and animal sources; it would follow that these workers consider that these bacteria can multiply and survive indefinitely in soil. In the light of the many investigations wherein it has been found that the Aerobacter-intermediate group may sometimes constitute a major, and occasionally the dominant, portion of the coliform flora of human and animal faeces, the present reviewer considers the opinion summarised by Topley and Wilson to be open to serious question.

From different papers on the coliform content of grasses and grains certain points seem clear. First and foremost is the finding that enormous numbers of lactose-fermenting bacteria can be detected on plant material if an incubation temperature of 30° C. is employed, but that only negligible numbers of these bacteria can be isolated when the temperature of incubation is 37° C. (Allen and Harrison, 1936). With incubation at 37° C., Wilson et al. (1935) found coliform bacteria in only half of the ninety miscellaneous samples of straw, hay, grass, decaying leaves, grains, meals, and feeding cakes which they examined. The samples which by nature of habitat had been in contact with soil, and hence possibly with manure, gave relatively large numbers of B. coli; the remainder of the specimens were marked by the prevalence of B. aerogenes types. It still remains to be shown whether the various coliform types can normally multiply in soils, grasses, or grains, and whether they are found in those habitats in greater numbers than in the faeces of man and animals. Taylor (1951) considers that it does appear probable that, in the decomposition of organic matter when a temperature approaching body heat is

attained, coliform bacteria can multiply, possibly to as great an extent as in the human body. This problem offers a large field for research.

Taylor (1942) concludes that it is incorrect to say that any soil is beyond doubt free from all pollution, as some workers have described their samples, for every portion of the globe is open to pollution from faeces of birds and wild animals, or the washings therefrom, and it is well known that such faeces may contain several millions of coliforms per gram. The most noticeable point concerning the coliform content of the soil is the rarity of coliform bacteria in soils not obviously polluted. It thus appears that where there has been no pollution there are no coliform bacteria.

Where pollution of the soil has been recent it has been generally found that E. coli types have been greatly in excess of other coliform types; after the initial pollution of the soil a gradual mortality of all types occurs, but the evidence seems overwhelming that E. coli types are the first to die and that intermediate and Aerobacter types may survive much longer. The relative proportions of these types may vary according to the time which has elapsed since pollution occurred, the climate, and the type of soil and its reaction.

Taylor (1951) concludes that there is now sufficient experimental evidence to discredit statements that soil is the natural habitat of A. aerogenes or intermediate types of coliform bacteria.

Mallmann and Litsky (1951) conclude that the only organisms, other than coliform bacteria, that could be used as indicators of faecal contamination in soil were the enterococci; these organisms died out rapidly

in soil, while the coliform bacteria used in their tests persisted in soil for long periods. Virulent typhoid bacteria died out in soil much more rapidly than did the enterococci. Mair (1908) previously reported that Salmonella typhosa could survive in natural soils in large numbers for about twenty days, and in small numbers for seventy to eighty days. Melick (1917) found that the longevity of the typhoid bacillus in soil depended on the source and the strain, and Creel (1912) reported that, under ideal conditions, the typhoid bacillus survived thirty-one days in soil. Mallmann and Litsky (1951) found that the longevity of the coliform organisms, the typhoid bacilli, and the enterococci in soil was prolonged by an increase in the organic content of the soil.

Viability of Adventitious Pathogens in Sea Water

It is the consensus that there are no autochthonous marine bacteria capable of infecting man, but the literature is replete with contradictory accounts of the viability of adventitious pathogens in seawater. Some workers claim that seawater is highly lethal for bacteria from land-dwelling animals, while others have presented data which indicate that such bacteria can live almost indefinitely in the sea. Several factors which influence the survival of bacteria in seawater must be taken into consideration to reconcile these divergent views. The biological properties of seawater may vary widely; the synthetic, diluted, or autoclaved seawater used in many of the experiments may not have been the same as natural seawater.

DeGiaxia (1889) observed that enteric bacteria perish quickly in the sea. He found more than 100,000 bacteria per ml. of seawater fifty

meters from a sewage outfall in the Gulf of Naples, 26,000 at a distance of 350 meters, and fewer than 100 per ml. 3,000 meters from the sewage outfall. Controlled experiments showed that Bacillus anthracis and Vibrio comma were unable to compete with saprophytes in polluted seawater; Salmonella typhosa and pathogenic species of Staphylococcus were even less resistant. The period of survival was a function of the organic content and bacterial population of the water. In grossly polluted water these organisms survived for less than 24 hours.

In a summary of the experiments of various authors, Frankland and Frankland (1894) concluded that, in general, human pathogens do not survive as long in seawater as in fresh water. Soper (1909), however, found that the virulence of Salmonella typhosa was not reduced by exposure to seawater in two or three weeks.

Beard and Meadowcroft (1935) designed experiments to simulate natural conditions in polluted seawater, and noted a rapid diminution in numbers of both Salmonella typhosa and Escherichia coli, although some of each survived for more than a month. The test bacteria were suspended in freshly collected, unfiltered water from San Francisco Bay in semi-permeable membrane cells, which were immersed in the bay. The death rate of the bacteria was invariably higher in unfiltered water which was sterilized by passage through an L-3 Chamberland candle.

Zobell (1936) noted that, although 99.9 per cent of the sewage organisms were killed after two days suspension in seawater, a few survived for nearly a month.

Carpenter, et al. (1938) found that 80 per cent of the organisms in sewage were killed by natural seawater within thirty minutes. Waksman and Hotchkiss (1937) noted that fresh seawater exerted a marked

destructive effect upon the cells of marine bacteria; this effect followed an initial rise in numbers. Seawater sterilized by heat or by filtration through a Berkefeld filter, and prepared salt water, had no destructive effect upon the bacteria. Krassilnikov (1938) found that water from the Black Sea was germicidal for terrestrial bacteria until boiled. He stressed the importance of the organic content of the water as a factor affecting the survival of bacteria.

Stryszak (1949), whose previous work indicated that protozoa played an important part in the destruction of Salmonella organisms in the Gulf of Gdansk, Poland, reported that the protozoa were relatively inactive at a temperature below 3.5° C. At these temperatures the count of S. enteritidis, S. typhi, and S. paratyphi B added to seawater rose during the first 48 hours, whereas at temperatures between 5.5° C. and 18.5° C. there was in most cases a rapid fall. Typhoid epidemics arising from polluted water are said to occur most often in the cold season. Stryszak also observed that the greater the amount of organic matter and of saprophytic bacteria in a water, the larger were the numbers of protozoa and the quicker was the elimination of the pathogens.

A report by Peso et al. (1949) contains an historical survey of the search for pathogenic intestinal bacteria in sewage and water. In the early days of this century successful isolations were obtainable only from sewage, but in more recent times, as bacteriological media have become more efficient, there are more records of the recovery of these organisms from river water and seawater. Findings are not all in agreement, but Peso and his associates conclude that, in general,

practically all Salmonella typhi organisms disappear within four to six days, but that they survive for a longer period at a low temperature and when other microorganisms, particularly protozoa, are not present. Nevertheless, according to some reports this organism has been isolated from such an environment after several weeks and even months. Little experimental work has been done with other species of Salmonella, but it is suggested by Peso et al. that as they are more robust, for the most part, than S. typhi they may survive in water and sewage longer than the latter organism. Reported outbreaks of water-borne dysentery prove that Shigella can survive in sewage and water for a sufficiently long time to be a danger to health.

Survival of Coliform Bacteria in Seawater

Zobell (1946) considers that the sanitary significance of the coliform bacteria in seawater depends up satisfactory answers to two important questions. Firstly, do the intestinal tracts of marine animals harbour coliform organisms, and secondly, how long do coliform bacteria of terrestrial origin survive in the sea?

Browne (1917) reported that E. coli was absent in seawater specimens from Buzzard's Bay and Vineyard Sound, Massachusetts; E. coli was found in only 39.8 per cent of 93 fish which he examined, and the presence of these bacteria in the fishes' intestines seemed to be a function of the amount and type of food present. Gibbons (1934) concluded that coliform bacteria are present only fish from contaminated waters. Griffiths and Fuller (1936) detected only a few coliform bacteria in commercial fish, and concluded that E. coli is not a normal intestinal inhabitant of marine fishes.

ZoBell (1941) failed to find coliform bacteria in any of 961 samples of seawater collected at stations remote from any possibility of terrigenous contamination. Large numbers of coliform bacteria were found in polluted bays and estuaries. E. coli was found only in "feedy" fishes taken relatively near shore. In the opinion of ZoBell, coliform bacteria do not constitute part of the normal bacterial flora of marine fishes, although such bacteria may survive for considerable periods of time if ingested.

Land drainage, and raw and partially-treated sewage, contribute huge numbers of coliform bacteria to the sea. Warren and Rawn (1938) estimate from their data that enough coliform bacteria are discharged by sewage effluents along the west coast of the United States each day to give more than a hundred for every liter of water in the North Pacific Ocean, if evenly distributed. Comparable sanitary conditions were found on the east coast of the United States by Weston (1938). In fact, however, such organisms are found only in bays, harbours, and tide water, which are often heavily polluted.

Data obtained during extensive surveys around the Hyperion outfall, summarized by Knowlton (1929), show the rapidity with which coliform bacteria succumb to the sea. The Hyperion outfall carries raw sewage from nearly two million inhabitants of Los Angeles a mile into the ocean. Millions of coliform bacteria per ml. are found near the sewer outlet, but the number decreases with distance from the outlet much more rapidly than can be explained by dilution. Coliform bacteria were never traced more than a mile or two from sewer outfalls in the open ocean. A commission appointed by the California State Bureau of Sanitary Engineering

(1943) reported that only in solids and greases were coliform bacteria able to survive for long periods of time in the sea. Dienert and Guillerd (1940) conducted similar experiments in Europe; they concluded that, while seawater is neither antiseptic nor inimical to E. coli, sewage discharged into the sea is rapidly purified by predatory organisms, sedimentation, and dilution. Vaccaro et al. (1950) came to essentially the same conclusions. They also found that bactericidal activity was most marked during the warm summer months, and that this activity was abolished if the water was autoclaved.

Doudoroff (1940) demonstrated that a fairly constant fraction of the total number of bacteria in a fresh-water culture of E. coli could reproduce on direct transfer to a saline medium.

Weiss (1951) reported that a study of the adsorption of E. coli by river and estuarine silts led to the conclusion that, in the range of turbidities usually encountered in natural waters, E. coli is adsorbed to the particulate matter, and the rate of sedimentation is increased. Silts suspended in sea water generally showed a decreased capacity to adsorb.

Suckling (1943) stated that B. coli type I does not remain viable as long in water as does Intermediate type I or B. aerogenes type I. Taylor (1952), however, concluded that B. coli type I appears to survive much longer in water than was originally thought.

The Coliform Index as a Measure of the Pollution of Shellfish and Shellfish Growing Waters

Oysters, in common with other molluscan shellfish, are liable to pollution with sewage. If such polluted shellfish be eaten, in an uncooked or partially cooked condition, by human beings, serious or even fatal illness may, and from time to time, does ensue. It is therefore obvious that the consumption of such shellfish is highly undesirable, not only in the interests of the individual consumer who may contract disease from the shellfish, but also in the interests of the general public, amongst whom the disease may indirectly be spread.

A mass of epidemiological evidence has been accumulated, especially since 1893, which places the causal relationship between polluted shellfish and the incidence of certain outbreaks of disease beyond reasonable doubt. The disease predominantly associated with the consumption of polluted shellfish is typhoid or enterid fever. It must be borne in mind, however, that polluted shellfish are potentially liable to convey any infection which may exist in sewage and which can produce disease in man if introduced by mouth. Thus shellfish may be, and frequently are, a dangerous food; the importance of limiting their fishing to areas shown, by bacteriological and sanitary engineering surveys, to be free from sewage contamination cannot be over-emphasized.

Considerable difference of opinion has arisen over the validity of the Standard Methods test for the presence of members of the coliform group as an index of faecal pollution in shellfish and in shellfish growing waters. Many of those who have studied shellfish pollution problems

in more southern waters have long been convinced that high scores based on the entire coliform group may have little value as indicators of faecal pollution of shellfish, especially where water temperatures rise rapidly to a high level during the summer months. Hunter (1939) states that, while the coliform bacteria have long been considered the only bacterial group which can be used with even a reasonable degree of accuracy as a measure of pollution, if environmental factors tend to permit or promote multiplication of the normal flora of organisms, together with the coliform contaminants, an interpretation of the sanitary significance of the coliform group becomes difficult.

Eliot (1926) reported that B. cloacae were often found in oysters when B. coli and B. aerogenes were absent, and considers that the presence of this coliform type in market oysters can be disregarded.

Perry and Bayliss (1936) correlated B. coli and coliform determinations with sanitary survey information in a comprehensive survey of oyster-producing areas in Chesapeake Bay, Maryland. No significant correlation between the concentration of coliforms in oysters and various degrees of pollution was found. There was a fair correlation between the concentration of coliform bacteria in seawater, and pollution. During the summer months there was a large increase in the numbers of coliform bacteria in both seawater and oysters; enormous increases in the coliform content of oysters were noted when the wster temperature rose above 70° F., in both grossly-polluted and relatively-unpolluted areas. At such times many oysters from waters of unquestionable purity were judged as grossly-polluted. A close correlation was, however, found between the concentration of E. coli in both oysters and water and the amount of pollution.

Perry and Bayliss considered that the observed increase in the coliform group in both the water and the oysters may indicate that self-purification of the oysters was taking place. They concluded that the coliform group tends to distort and magnify the picture of real pollution in water when temperatures are high, while in oysters it may indicate gross pollution when in reality such does not exist.

The investigations of Perry (1939) indicate that many coliform bacteria, particularly of the B. cloacae type, are present in shell oysters and shucked market oysters when the temperature exceeds 60° F.; Perry concludes that these bacteria are without significance as indices of pollution.

Tennant (1949) found that there was a close relationship between water temperature and the coliform bacterial content of soft shell clams; when water and mud temperatures remained low, the Standard Methods test for coliform bacteria provided a significant index of sewage contamination, but when water temperatures reached a critical threshold level, multiplication of coliform bacteria within the clams occurred.

On the other hand, other investigators state that any content of coliform bacteria, however, atypical, in a water or food sample indicates faecal pollution. Gard and Nilssen (1942) particularly stress this viewpoint. Data presented by Parr (1938), Bardsley (1948), Taylor (1942, 1951), and other investigators previously cited in the present review, all support this conclusion.

Wattie, Arcisz, and Dallas (1949) report that an increase in the coliform index of seawater is followed by an increase in the coliform bacterial content of the shellfish, usually within 24 hours; this response

appeared to be independent of the water temperature at temperatures below 20° C. The per cent increase in coliform density in seawater was followed in many instances by a similar per cent.increase in the numbers of coliform bacteria in the shellfish. A decrease in the coliform density of the water was followed by a drop in the coliform content of the shellfish within 48 to 72 hours and, in some instances, within 8 hours.

Because of the diversity of opinion regarding the validity of the Standard Methods test for coliform bacteria as an index of sewage pollution, it was deemed most important that further experiments to clarify the issue be carried out.

Enteric Pathogens in Water, Sewage and Shellfish

During the period of establishment of the coliform group as an indicator of polluted seawater and shellfish, considerable effort was devoted to the isolation of enteric pathogens, particularly Salmonella typhosa, and to the relative rates of decrease of coliform bacteria and enteric pathogens under various conditions. The introduction, in 1927, by Wilson and Blair of their bismuth sulphite agar medium stimulated a number of investigators during the next ten years to study the isolation of certain enteric pathogens. These workers were successful in isolating S. typhosa from sewage and polluted waters, using various modifications of the Wilson-Blair medium.

Each annual report of the London Metropolitan Board Laboratories from 1927 to 1938 contains some reference to the isolation of enteric pathogens from water supplies. Wilson (1929), and Wilson and Blair (1931) reported numerous isolations of S. typhosa from polluted waters

and shellfish. Green and Beard (1938) reported the isolation of S. typhosa from Palo Alto sewage in nine of fifty-one one ml. samples; Ruchhoft (1934) reported isolations in two 0.1 ml. samples of Chicago activated sludge, while Heukelekian and Schuloff (1935) reported the failure to isolate S. typhosa from the sewage of fifteen municipalities in 0.1 ml. amounts. Hajna (1935) isolated S. typhosa from six of twenty-two samples of crude sewage from Baltimore. Mom and Schaeffer (1940) reported an extensive series of isolations from sewage and from river water at Bandoeng, Dutch East Indies, where the morbidity rates for typhoid are approximately thirty cases per thousand per year. Mom and Schaeffer, and Wilson (1933) stress the relationship between the typhoid morbidity rate and the concentration of S. typhosa found in the sewage of the community.

Dodgson (1938) cites several epidemiological instances of the prolonged survival of virulent typhoid bacilli in mussels and oysters. His experimental evidence indicates that S. typhosa survived in oysters, mussels, and other shellfish in seawater for more than three weeks. Hunter and Harrison (1928) related several instances of the demonstration of typhoid bacilli in shellfish. They record evidence for the survival of the typhoid bacilli in oysters for from nine to forty-two days, and of E. coli for from seven to seventeen days. Tonney and White (1925) found that the longevity of S. typhosa in the shell liquor of both shucked and shell oysters in storage varies with the temperature at which they are kept. In general, the temperature best suited for the preservation of the shellfish tends to prolong the life of the typhoid bacilli in the oysters. At 45° F., S. typhosa survived for twenty-two days in shucked oysters, and for sixty days in shell oysters.

Shell oysters artificially contaminated with typhoid bacilli by floating for one hour in sea water to which typhoid bacilli had been added, and then placed at ice-box temperature were found to contain living typhoid bacilli for as long as twenty-four days by Jordan (1925); there was no evidence of multiplication, and after the first days a diminution in numbers was plainly apparent.

An account of eight cases of typhoid fever, caused by eating raw clams dug from the Thames River not far from a sewer outlet, appears in the Annual Report of the Connecticut State Board of Health for 1927. Clams dug from a posted area along the shore of a New Haven harbour caused the first recorded death from typhoid fever in Connecticut for 1948 (2). One case of typhoid fever from infected oysters, five cases from mussels, and one hundred and six cases from clams were finally considered to have been infected by eating shellfish originating from condemned areas in the harbour of the city of New York from 1933 to the end of 1948; these cases are exclusive of typhoid fever cases from shellfish which were obtained from peddlers or other persons whose source of supply could not be traced, or investigations which could not be thoroughly completed. An unestimated number of food poisonings and other gastro-inestinal illnesses traceable to eating of shellfish from uncertified areas is known to have occurred during this period, but statistics could not be assembled as many such cases were not reported to the Public Health authorities (123).

The studies made by Kehr and Butterfield (1943) of the available data in the literature emphasize the basic value of the coliform test as an index of the possible presence of pathogens in seawater and shellfish

and indicate that a very real danger may exist when coliform bacteria are present in even moderately high concentrations. The factor of safety provided by the ratio of a million or so coliforms present in faeces and sewage for each S. typhosa would, it is believed, take care of the usual fluctuations in the ratio of S. typhosa to the coliforms, provided the density of coliform bacteria in the ingested food be kept low or eliminated entirely by methods which reduce the general bacterial population.

While it has generally been accepted that pollution of animal origin was not so potentially dangerous as pollution of human origin, the literature is quite extensive on the presence of members of the Salmonella group in ducks and duck droppings. Edwards et al. (1948) report on fifty-six outbreaks of salmonellosis in ducks, from which 13 Salmonella types were isolated; all of these types are recorded as having been associated with outbreaks of salmonellosis in humans. Pathogenicity of Salmonella from ducks is more directly demonstrated by Mallam (1946), Scott (1930), and Snapper (1944), who cite cases of human salmonellosis resulting from the ingestion of duck eggs or food prepared from them.

Of particular significance from the standpoint of pollution is the method of infection of these eggs. Both Mallam and Scott agree that the prime origin of Salmonella in eggs is faecal material.

Hilbert (1939) reports occasional S. anatum infections among Long Island ducks, while Hansen (1942) and Clarenberg (1939) report Salmonella infections ranging from one to five per cent in ducks in Europe and the United States. These reports indicate that it is quite

common for ducks to be heavily infected with Salmonella. Edwards et al. (1948) state that birds constitute the greatest reservoir of paratyphoid infection among the domestic animals.

Viable pathogenic types of Salmonella were isolated from the discharges of duck farms and from the waters immediately adjacent to these farms by Bidwell and Kelly (1950). They also found it possible to recover Salmonella bacteria one-quarter mile from the nearest duck farm. Perhaps their most significant observation was the recovery of Salmonella typhi-murium from a sample of oysters taken from an oyster bed considered to be of satisfactory sanitary quality. This area received duck farm wastes equivalent to the discharge of raw sewage from a town of more than twenty thousand persons. Coliform bacterial numbers were excessive in this same area.

Slow Lactose Fermentation by Coliform Bacteria

Borman et al. (1944) write that, "Avidity for lactose is a primary differentiating character which has the weight of tradition behind it. In addition, it is the basis for many practical procedures in the fields of sanitary and of diagnostic bacteriology. Procedures of practical and theoretical importance require consideration of more than the simple ability to ferment lactose or the lack of it. The production of gas and even the rate of its production are thus magnified in importance. Examination of the characteristics of the Enterobacteriaceae as a whole suggests that loss or retardation of the ability to split lactose marks one of the way-stations along the evolutionary path of these organisms - one of the

transition points between commensal and parasitic existence. Some pathogens (including the Sonne dysentery organism and perhaps some of the paracolon group) retain a certain degree of their original lactose-splitting ability, and other pathogens, notably the Salmonella types, occasionally yield lactose-fermenting variants which apparently have regained a lost ancestral capacity. Conversely, the generally accepted view that an organism which rapidly attacks lactose is non-pathogenic has proved to be so nearly correct that it is seldom questioned."

Parr (1936, 1938) has made an extensive study of slow lactose fermenters from faeces, and reports that they frequently appear at times of crisis in the faecal flora. From that point of view they are admittedly a sign of potential danger. On the other hand, Griffin and Stuart (1940) encountered few aberrant strains from faecal material, and report that their incidence in non-faecal sources is extremely high; they prefer to consider the anaerogenic strains of Aerobacter and intermediates as possible links in a putative evolutionary chain. One strain of A. aerogenes type II was maintained for a period of two years during which time it was frequently checked for constancy of reaction. At the end of this period an anaerogenic variant appeared which was serologically and biochemically identical with the parent strain except in gas production from carbohydrates.

It is thus apparent that delayed or weak fermentation of lactose by coliform organisms has long been recognized, yet the sanitary significance of such organisms in water and in milk and other foods has been extremely puzzling. Stuart et al. (1940) report that many water laboratories express doubt of the sanitary significance of coliforms producing less than ten per

cent gas in presumptive tests, on the grounds that these organisms were usually found in waters where the sanitary survey indicated that the water was of good hygienic quality. On the other hand, these writers consider that some significance could be attached to these organisms on the ground that they may represent attenuated cultures that might be easily rejuvenated under natural conditions. It is frequently postulated that environmental factors, such as chlorination, storage, and the presence or predominance of extraneous organisms, account for the slow or weak fermentation of lactose by coliform bacteria. When pure cultures react in this manner they are considered by Stuart and his associates to be attenuated, degraded, or devitalized with respect to lactose, especially when the ability to ferment lactose rapidly is acquired after serial transplanting in lactose broth. In some cases it is true that the culture as a whole becomes a rapid fermenter through the restoration of a temporarily-lost adaptive enzyme (lactase). The fact that slow fermentation of lactose by a coliform strain may be as inherent and fixed a property in that strain as is rapid fermentation in another strain, is overlooked by many bacteriologists. Consequently, Stuart and his associates conclude that to disregard slow or weak lactose fermenting bacteria, particularly in the presumptive test, may be dangerous.

In routine water examinations coliform bacteria must compete in the lactose broth tubes with other bacteria which usually outnumber them by about 100 to 1, and, although growing, they may not attain the density necessary for the production of gas. In a large number of tests in lactose broth, Chambers (1950) found that the minimum density of coliform bacteria with which gas production was first observed was 40,000,000 per

ml.; the mean and median values were about 170 million per ml. These densities were about forty per cent less than were required when brilliant green bile broth was used. The production of even a small amount of gas in the presumptive test appears to be significant.

The Membrane Filter Method in Sanitary Bacteriology

The removal of bacteria from liquids by filtration through a membrane prepared from a derivative of cellulose is not a new process. Reviews by Bigelow and Gemberling (1907), Pierce (1927), and Ferry (1936) credit Fick in 1855 with the application of collodion membranes in biological investigations. Numerous modifications for the improvement of the membranes were suggested by the various investigators.

Zsigmondy and Bachman (1918) developed a method for the preparation of a membrane that could readily be adapted to commercial production. Filtration procedures using the Zsigmondy membranes were suggested for the determination of total bacterial counts, for coliform bacteria determinations, and for the isolation of pathogenic bacteria, from water and other fluids. Such procedures were used extensively in Germany.

Goetz and Tsuneishi (1951), during cooperative investigations in Germany and the United States of America, designed filtration equipment for holding the membranes during filtration, and developed an improved membrane; these investigators then applied the molecular filter membrane method to the bacteriological analysis of water.

Clark et al. (1951), Clark and Kabler (1952), and Kabler and Clark (1952) reported on a series of studies in which the Goetz Membrane Filter Method was used for the estimation of coliform bacteria in water

and sewage. Selective media for the enumeration and differentiation of the coliform group and Salmonella typhosa were described. Clark and his associates report that their improved membrane filter technique allows the quantitative removal of bacteria from river and well water, and from sewage; large quantities can be easily and routinely examined. Methods of cultivation are relatively rapid and simple; coliform determinations require approximately 18 hours in place of the 3 or 4 days required by the Standard Method. In general, Clark et al. contend that the membrane filtration method provides a substantial reduction in time, material, equipment, labour, and space required for the bacteriological analysis of water and, at the same time, that the method will be more certain and precise in results than are the methods now in use.

Woodward (1952) found that, although the membrane filter method gives coliform results of the same order of magnitude as the dilution method, the differences between the results by the two methods frequently is greater than can be explained by the inherent variability of the determinations. On the basis of the average coefficient of variation of the membrane filter results, the precision of a one-membrane filter determination is roughly the same as that of a thirty-tube Most Probable Number determination.

Enterococci as an Index of Pollution

Because of dissatisfaction with the coliform index of pollution, a number of investigators have engaged in a search for a more reliable criterion of pollution than the coliform group.

Some workers consider that the enterococci (faecal streptococci) may prove to be the solution to the problem of a more certain index of faecal pollution. This group of organisms was first reported by Lawes and Andrews (1894). Six years later, Houston (1900) reported that faecal streptococci were readily found in polluted waters and seemingly absent in non-polluted water samples. The work of these and other British workers led Suckling in 1943 to state:

"Streptococci are used as an indicator of pollution on the same grounds as Bacterium coli, namely:

1. They are present in faeces and sewage and are found in known polluted waters.

2. They are not found in pure waters, virgin soil, and sites outside of contact with human and animal life.

3. They do not multiply outside the animal body (except in such media as milk)."

In the United States, Winslow and Hunnewell made the first report on these organisms in 1902. Since then, several methods of isolation have been suggested, and in 1939 Darby and Mallman suggested the use of selective media which would permit the enterococci to grow without competition from certain other bacteria. Four years later, Hajna and Perry (1943) proposed their "SF Medium", which they reported to be highly selective for enterococci if the incubation temperature was 45° C.

In 1944, White and Sherman indicated that a medium containing penicillin was highly selective for this group of organisms. The possibilities of such a medium for routine use in sanitation bacteriology led Winter and Sandholzer (1946) to investigate it in detail; they found

that faecal streptococci were present in all of the samples of human, domestic-animal, and wild-animal faeces that were tested, and that soils of virgin wooded areas did not contain enterococci, whereas the soil of pasture land did. In faecal samples, enterococci were outnumbered approximately a hundred times by coliform organisms. It is of interest, however, that samples of polluted water taken right at the source of pollution had an average enterococci count twenty times that of the average coliform count; then, as the distance from the point of pollution increased, numbers of enterococci decreased more rapidly than did coliform numbers, so that beyond a hundred yards or more the coliforms were greater in number. These workers concluded that the enterococci may serve as a more specific and reliable index of faecal pollution than the coliform bacteria, even though the former may be present in fewer numbers than the latter in polluted areas.

Data obtained by Ostrolenk, Kramer, and Cleverdon, (1947) show excellent correlation to exist between sanitation and recovery of both E. coli and enterococci. Enterococci in artificially contaminated soils and in normal faeces appeared to survive longer than E. coli under identical conditions.

Investigations by Ostrolenk and Hunter (1946) demonstrated that faecal streptococci are common in the excreta of ten animal species, and that they occur in significant numbers. These authors consider that, although the faecal streptococci are generally outnumbered by the coliform bacteria, their resistance to chemical agents, and possibly to other environmental factors, makes them of sanitary significance as indices of faecal pollution. Brown and Gibbons (1949) report that the

examination of 162 egg powders from various sources revealed that one hundred per cent contained enterococci in relatively large numbers, while 26.5 per cent were negative for E. coli, and 25.9 per cent contained less than one per gram. Brown and Gibbons concluded that, in egg powder, enterococci seem to furnish a better index of the number of organisms of faecal origin in the liquid egg than E. coli or the coliforms. Clark, Geldreich, and Jeter (1949) report that the enterococcus content of stored raw sewage showed a greater percentage reduction in any given period than the coliform bacteria. They suggested that the presence of enterococci in water indicates more recent sewage pollution than if only coliform organisms are present.

Geldrich, Reading, and Malaney (1950), in a comparative study of the confirmed coliform test, the EC reaction, and the enterococcus test, concluded that, in the daily examination of a water supply which varied in pollution density, the confirmed coliform test is the most sensitive and the enterococcus test the least sensitive of the three indicators. Lattanzi and Mood (1951) compared enterococci and E. coli as indices of water pollution. They found that a positive correlation existed between the two tests, and that a high E. coli index will usually predict a correspondingly high enterococci index. Density of E. coli will approximate sixty-three times that of enterococci. More skill is needed in carrying out the enterococcus test, but the time required for its completion is less than for the E. coli test.

Clark, Reading, and Kabler (1950) found that the elimination of the staining procedure in the positive confirmatory test for the enterococci did not change the Most Probable Number of 322 samples examined.

They concluded that the increased expenditure of time and materials for the preparation of the Gram-stained smears from the slant-broth culture tubes is not warranted, and that the slight error introduced is without practical significance. Cremer and Southgate (1950) found that growth with formation of acid in sodium azide broth incubated at 45° C. was an almost specific test for enterococci.

Several outbreaks of food poisoning of a mild type in which enterococci were the predominant organisms isolated from the suspected foods are described in a paper by Buchbinder et al. (1948). The literature was discussed in some detail, and it was pointed out that, in several instances in which the organisms were closely identified, they were found to be enterococci.

The relatively small numbers of food poisoning outbreaks reported as caused by Streptococcus faecalis is, however, in sharp contrast to the widespread occurrence of this organism in nature. Some success in producing experimental Streptococcus faecalis food poisoning in man is presented in a paper by Osler et al. (1948); symptoms of acute gastric or intestinal disturbance, or of both, were produced in six, or possibly seven, of twenty-six human volunteers who ate foods in which strains of S. faecalis had grown for five hours. These workers concluded that these results confirmed the etiological role of S. faecalis in naturally-occurring outbreaks of food poisoning. Dangler and Steffen (1948) consider that the enterococci may be as important as the staphylococci as causal organisms in outbreaks of food poisoning.

Land-wash Pollution of Shellfish Growing Areas

It has been pointed out by Koser (1927) that surface washings may be undesirable even though strictly "faecal" organisms are not found, and that the presence of coliform bacteria beyond certain limits, irrespective of type, shows the existence of undesirable conditions. On the other hand, there are a number of reports of the finding of coliform bacteria, sometimes in fairly large numbers, where no source of contamination could be found and where the water was judged to be good sanitary quality. In many instances these reports have been dismissed with the assumption of undiscovered sources of contamination; it is probable in some cases that periodic landwash pollution was responsible for the large coliform counts recorded. Koser suggests that differential tests might aid in the interpretation of such results.

The initial sanitary quality of the run-off water from any catchment area depends upon the extent to which the surface area is polluted. Gainey and Lord (1952) state that there is never any run-off from frost or dew; also, precipitation in the form of snow often melts so slowly that all the moisture either vaporizes or seeps into the soil with no run-off, whereas an equivalent quantity of water falling as rain over the same period of time may result in significant land-wash. A tenth of an inch of rain falling within a few minutes often results in no run-off, while two inches falling over the same period may be largely lost as run-off. Two inches of rain falling as a slow drizzle over a period of twenty-four hours might be readily absorbed by the soil, whereas if it fell within an hour most of it would run off.

Gainey and Lord point out that the steeper the grade, the larger will be the percentage of rain lost as run-off. In a forest or grass-land area where there is a heavy stand of vegetation either living or dead, the surface covering offers great resistance to the run-off of wster, even though the amount and rapidity of rainfall and contour of surface are conducive to a rapid run-off. On the other hand, when the land is under cultivation, and where during a large portion of the year the land is bare, the conditions are almost ideal for maximum land-wash; in the case of heavy rains, almost one hundred per cent of the water may be lost in this way if the surface is of a hard, tight clay structure. It has been estimated that under average conditions the run-off water equals one-sixth of the precipitation.

Following heavy rains, the number of bacteria gaining entrance from surface washings from cultivated soil may overshadow all other sources. Soil usually contains one to one hundred million bacteria per gram capable of growing aerobically on nutrient agar. A suspension of one gram of soil per liter of water would thus contain from one to one hundred thousand bacteria per ml. Gainey and Lord (1952) point out that an ordinary muddy river water may contain in excess of this amount of soil. Furthermore, where there is much faecal material on the surface of the soil, this is carried along with the soil into streams, lakes and harbours. A billion coliform bacteria per gram of faeces is not exceptional (Gainey and Lord, 1952); assuming that one gram of bird faeces containing this number was uniformly distributed in a reservoir of 12,000,000 gallons of water, and that the organisms remained viable until

tested for, they could be readily detected by the standard procedure, and such a water would contain twice as many coliform bacteria as is tolerated by most drinking water standards.

EXPERIMENTAL

A. Methods

Selection of Oyster Producing Areas for Study

Two areas in Prince Edward Island, Vernon River (including Orwell River and Orwell Bay), and Pinette River, were selected for study (Fig. IIa). Both areas contain river, estuary, and bay systems surrounded by farm and pasture lands and scattered wood lots. The only known potentially-dangerous foci of human pollution are at and above Vernon Bridge, on Vernon River. Serious land-wash pollution was noted in both areas during surveys made by the author in 1951 (190). Conflicting bacteriological and sanitary engineering observations have made the definition of closed and open areas difficult. Both areas include many features which could be considered typical of most oyster-producing areas in Prince Edward Island, New Brunswick, and Nova Scotia.

Establishment of Fixed Sampling Stations

Seventy-two fixed water-sampling stations were established in the Vernon River area, and thirty-seven similar stations were established in Pinette River. The sampling stations were selected in a manner calculated to provide adequate coverage for all sectors in the study areas (see Figs. I and II). All stations were fixed on the maps by means of landmarks.

Oysters from fixed stations on each of three oyster beds in each of the test areas were sampled periodically during the study period.

Six soil sampling stations (A - F inclusive) were established on the Vernon River watershed (see Fig. I).

FIGURE I - VERNON RIVER AREA, QUEENS COUNTY, PRINCE EDWARD ISLAND

SEAWATER SAMPLING STATIONS 1-72
OYSTER SAMPLING STATIONS 48, 66, 68

SEAWATER SAMPLING STATIONS
(SALINITY DETERMINATIONS) 18, 25, 31, 41, 54, 71

SCALE (APPROXIMATE) - 1,000 feet to 3/8 inch

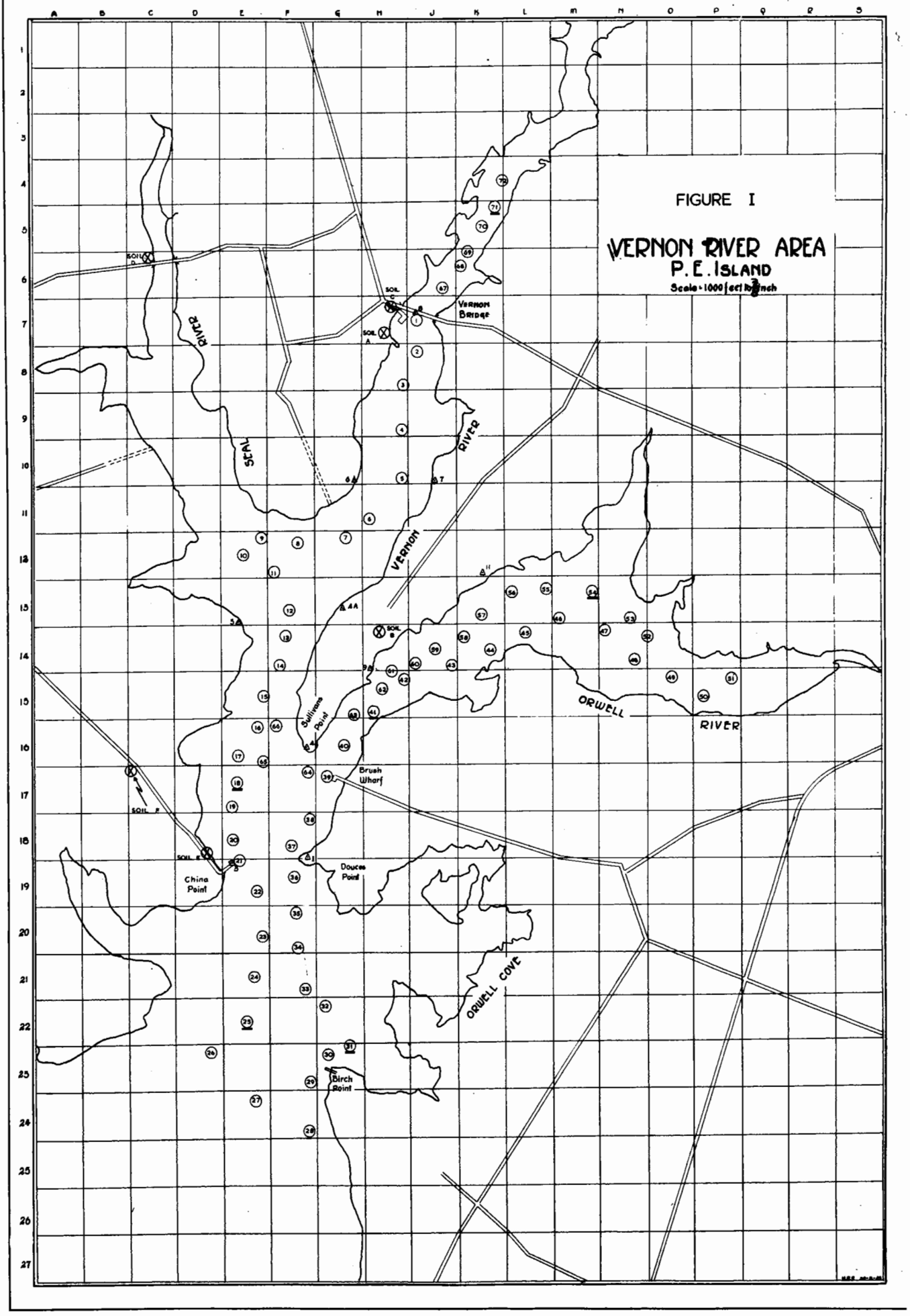


FIGURE I
VERNON RIVER AREA
P.E. ISLAND
Scale - 1000 feet to inch
SEAL RIVER
VERNON RIVER
ORWELL RIVER
ORWELL COVE
Vernon Bridge
Sullivans Point
Brush Wharf
Doucas Point
China Point
Birch Point
SOIL A
SOIL B
SOIL C
SOIL D
SOIL E
SOIL F

FIGURE II - PINETTE RIVER AREA, QUEENS COUNTY, PRINCE EDWARD ISLAND

SEAWATER SAMPLING STATIONS 1-37
OYSTER SAMPLING STATIONS 7, 8, 16

SEAWATER SAMPLING STATIONS
(SALINITY DETERMINATIONS) 7, 9, 19, 25, 37

SCALE (APPROXIMATE) - 1,000 feet to 7/16 inch

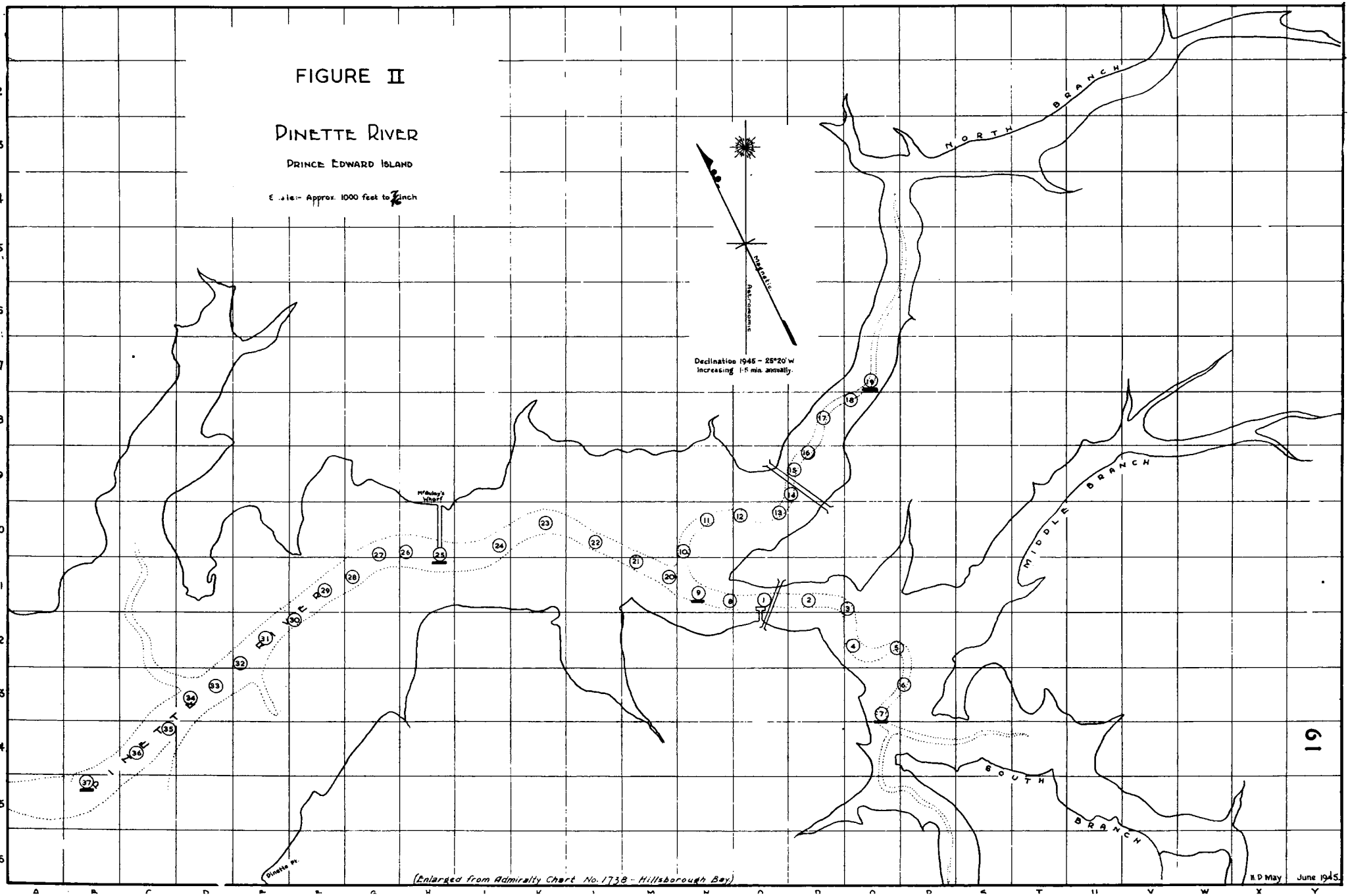
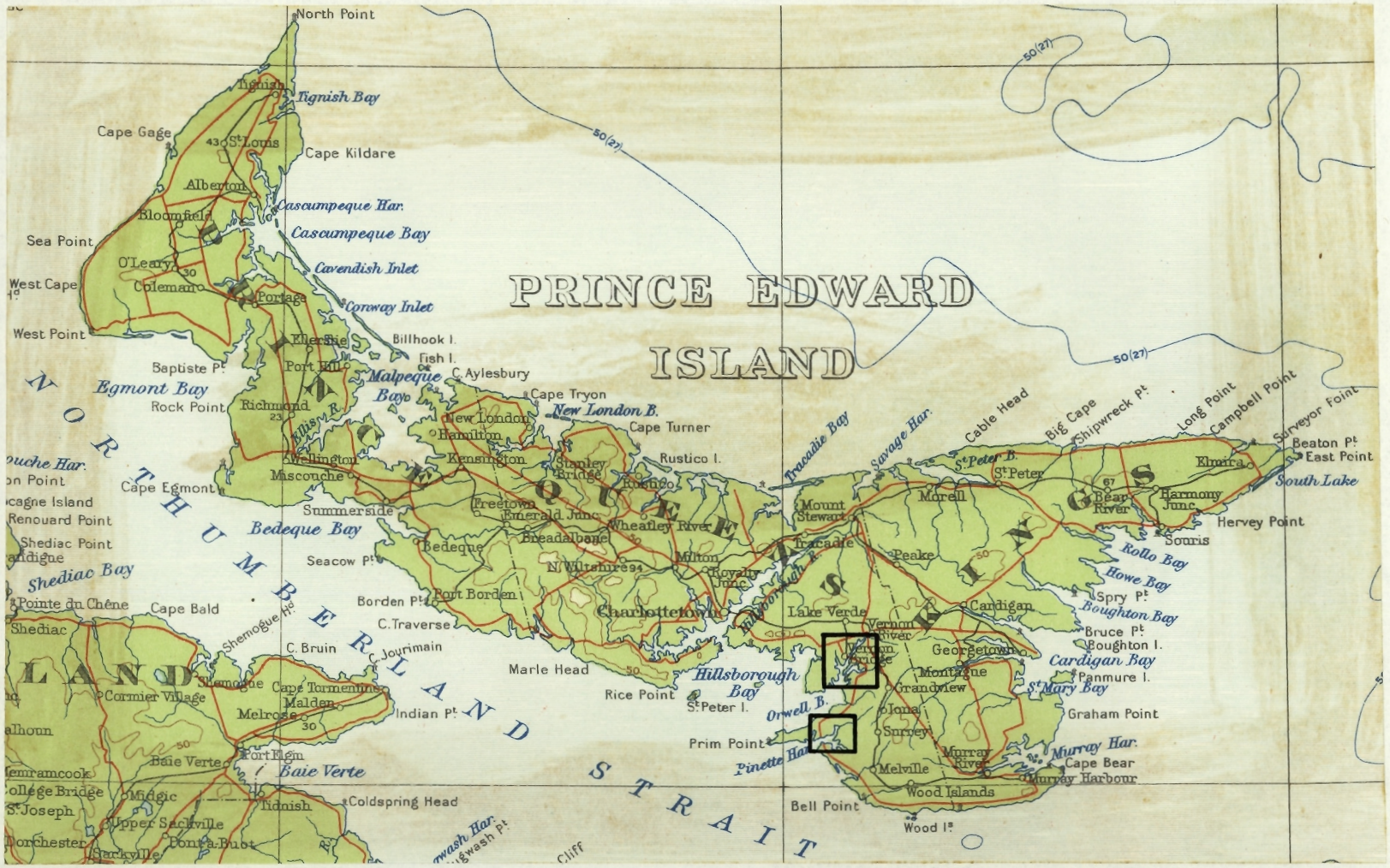


FIGURE II
PINETTE RIVER
PRINCE EDWARD ISLAND
Scale:- Approx. 1000 feet to 1 inch
Magnetic
Astronomic
Declination 1945 - 25°20' W
Increasing 1·5 min. annually.
NORTH BRANCH
MIDDLE BRANCH
SOUTH BRANCH
INNER TOP
RIVER
McAuley's Wharf
Pinette Pt.
(Enlarged from Admiralty Chart No. 1738 - Hillsborough Bay)
N.D. May June 1945.
61

FIGURE II (a) - MAP OF PRINCE EDWARD ISLAND



Scale 1:1,000,000

Bacteriological Examination of Shellfish Growing Waters

a. Collection and Transportation of Samples

Samples of seawater from fixed sampling stations in the study areas were collected at low tide in sterile glass bottles. Samples were kept at a temperature of 10° C. or lower, and were examined bacteriologically as soon as possible after collection. All samples were tested within six hours of the time of collection.

b. Determination of Most Probable Numbers of Coliform Bacteria

Water samples were taken from each of the fixed sampling stations in the two study areas periodically during the investigation. Examination for coliform bacteria was made according to the Confirmed Test Procedure presented in the American Public Health Association Standard Methods for the Bacteriological Examination of Shellfish and Shellfish Waters (1947), with five-tube series of Bacto-Lactose Broth in at least three dilutions, and confirmation in Bacto-Brilliant Green Lactose Bile (2%) Broth. The numbers of coliform bacteria were expressed as the most probable numbers (M.P.N.'s) per 100 ml. of sample. M.P.N.'s were determined from the table of most probable numbers given by Hoskins (1934). Thirteen surveys were made in the Vernon - Orwell area, and ten in Pinette River (for results, see Tables III to VI inclusive).

c. Salinity and Rainfall Determinations

Water samples were taken periodically during the study from six sampling stations in the Vernon - Orwell area, and from five sampling stations in Pinette River, for salinity tests.

Salinities were determined with a standard salinometer, and the readings were corrected for temperature. Corrected salinity values are reported in Table II (a and b).

Daily rainfall data for Queens County, Prince Edward Island, as recorded by the Experimental Farm Service at Charlottetown, are reported in Table I.

d. Determination of Most Probable Numbers of Enterococci

The test developed by Winter and Sandholzer (1946) for the enumeration of enterococci was used during the present investigation. The test includes:

1. A presumptive test in which the production of acid, and growth turbidity, in a sodium azide enrichment medium after incubation at 45° C. was interpreted as the presence of enterococci.

2. The positive presumptive tests were confirmed by inoculating a slant-broth preparation of a penicillin-sodium azide confirmation medium. Pin point colonies on the slant, and growth sediment in the broth, were interpreted as confirmed positive evidence of the presence of enterococci.

Preparation of Media: Dehydrated prepared media supplied by Difco Laboratories, Detroit, U.S.A., was used.

1. Bacto-Enterococci Presumptive Broth (normal strength).

0.5% Dextrose

0.5% Tryptone

0.5% Yeast Extract

0.04% Sodium Azide

0.0032% Brom Thymol Blue

The medium was tubed in 7 ml. amounts, and autoclaved at 15 pounds pressure for 15 minutes.

2. Bacto-Enterococci Presumptive Broth (double strength).

A concentrated medium, containing the same ingredients as the normal strength medium, was prepared by increasing the percentage of each ingredient twofold. The concentrated medium was tubed in 10 ml. amounts, and autoclaved at 15 pounds pressure for 15 minutes.

3. Bacto-Enterococci Confirmatory Agar and Broth.

(i) Agar slants

0.5% Dextrose

0.5% Tryptone

0.5% Yeast Extract

0.04% Sodium Azide

0.001% Methylene Blue

1.5% Agar

The medium was tubed in amounts for long-surfaced slants, autoclaved at 15 pounds for 15 minutes, and slanted.

(ii) Broth

The broth medium differs from the slant medium in having no agar, and in having 6.5 per cent sodium chloride added. The medium was autoclaved at 15 pounds for 15 minutes in flasks, and cooled to room temperature. 650 units of penicillin per liter of medium were added. Sufficient broth to cover one-half of the slant was added aseptically to each slant preparation.

Procedure: Replicate water samples taken from a number of the fixed sampling stations in the study areas (twenty-three in the Vernon -

Orwell area, nineteen in Pinette River) during each survey were examined for enterococci. Tubes of the presumptive enrichment broth were inoculated with the same sample dilutions in the same manner as in the Standard Methods Presumptive Tests for coliform bacteria (177). The inoculated tubes were incubated at 45° C. in a water bath, and observed periodically after eight hours for the production of acid, as shown by a yellow colour in the broth, and for growth, as indicated by turbidity. Negative tubes were discarded after incubation for 24 hours. The production of acid and turbidity was interpreted as a positive presumptive test.

As soon as a positive reaction was noted, a loopful of the sediment at the bottom of the tube was transferred to the broth of the slant-broth preparation and the loop zig-zagged on the surface of the slant as the loop was withdrawn. The inoculated Confirmatory cultures were then incubated at 37° C., and examined after incubation for 24 hours for pin point colonies on the slant and for sediment growth in the broth. The numbers of enterococci were expressed as most probable numbers (M.P.N.'s) per 100 ml. of sample. M.P.N.'s were determined from the table of most probable numbers given by Hoskins (1934). Enterococci M.P.N.'s are recorded in Table VI.

e. Determination of Coliform Numbers by Membrane Filtration

The membrane filter method for the enumeration of coliform bacteria in water, developed at the United States Public Health Service Environmental Health Service (EHC), Cincinnati, was used during the present investigation. The procedure has been described by Clark and Kabler (1952).

The stainless steel filter apparatus used during the study was manufactured by the Leslie R. Burt Company, Arcadia, California. It consisted of a funnel and a receptacle.

Filter membranes manufactured by the Lovell Chemical Company, Watertown, Massachusetts (Millipore Filters, Hydrosol Assay Type, 48 mm., grid marked), and absorbent pads made by Schleicher and Schuell, Keene, New Hampshire (No. 470, 48 mm.), were used during the investigation.

Media: The examination of water for coliform bacteria by the membrane filter method was performed in two steps with two media. Double-strength medium with 0.5 per cent lactose was used for enrichment. The formula for this nutrient is:

Bacto-Peptone	40 gm.
Bacto-Yeast Autolysate	6 gm.
Dipotassium hydrogen phosphate	3 gm.
Sodium chloride	5 gm.
Lactose	5 gm.
Distilled water	1,000 ml.

The pH was adjusted to 7.0 with potassium hydroxide, and the medium was tubed in 30 ml. quantities and sterilized in the autoclave for 15 minutes at 121° C.

The Endo broth used during the present study consisted of a basal medium and two solutions (Clark et al., 1951). The basal medium contained:

Bacto-Lactose	20 gm.
Bacto-Neopeptone	20 gm.
Potassium dihydrogen phosphate	7 gm.
Distilled water	1,000 ml.

The pH was adjusted to 7.0 with potassium hydroxide, and the medium was tubed in 30 ml. quantities and sterilized in the autoclave for 15 minutes at 121° C.

The Endo medium was prepared by mixing 30 ml. of basal broth, 1 ml. of sterile 9 per cent sodium sulphite solution, and a sufficient quantity of fuchsin solution. Basic fuchsin solution was prepared by dissolving 3 grams of basic fuchsin in 50 ml. of alcohol and adding sufficient water to make 100 ml. The fuchsin-sulphite ratio is critical in the finished medium. Each new lot of dye solution was titrated to determine the exact quantity to be used. This titration consisted of adding 1 ml. of sulphite to each of a series of the basal broth tubes. Starting with 0.7 ml. of the fuchsin solution in the first tube, the quantity added was increased by 0.1 ml. in each tube of the series until 2 ml. were added to the last tube. Membranes having a constant number of coliform and non-coliform organisms were cultivated on medium from each tube. The optimum dye concentration was based on maximum recovery of coliform bacteria with best differentiation.

Procedure: All material and equipment used in the membrane filter technique was sterilized. Membranes were sterilized by gentle boiling in water. All other equipment was sterilized in an autoclave for 15 minutes at 121° C. The filtration apparatus was wrapped in heavy kraft paper and sterilized at the end of each day; Clark and Kabler (1952)

have shown that it is sufficient to rinse the funnel with 30 to 50 ml. of sterile dilution water between individual filtrations, leaving the membrane in place. Sterilization of the funnel between filtrations was therefore not necessary.

The size of the seawater sample examined depended on the expected bacterial density and on the amount of silt present in the water. The volumes examined ranged from 25 ml. to 100 ml.

The sterile funnel base was mounted on a filter flask connected to a water tap aspirator. The sterile MF membrane was placed, grid side up, on the porous carbon disk. The funnel was then assembled by means of a bayonet locking device.

The sample of water to be tested was poured into the funnel, and a vacuum was applied to the system. The sample filtered very rapidly unless it contained a high concentration of suspended solids. When the membrane appeared dry, the funnel was removed, and the membrane was picked up with sterile forceps and placed on a previously prepared nutrient pad.

Sterile absorbent pads were placed in the sterile bottom half of a 60 x 15 mm. Petri dish. 2.2 ml. of the Albimi M enrichment medium was pipetted into each dish, so that the pad became completely saturated with medium, and a slight excess of the liquid was visible around the periphery of the pad. The membrane was carefully removed from the funnel base with sterile forceps and placed, with the grid side up, on the wet nutrient pad. Care was taken to ensure that no air was trapped between the membrane and the pad. The sterile Petri dish top was placed over the bottom of the dish, and the assembly was incubated for two hours at 37° C. in an atmosphere of saturated humidity.

At the end of the two-hour enrichment period, the membrane was transferred to a sterile Petri dish containing an absorbent pad saturated with 2.2 ml. of Endo broth. Incubation was continued for 12 to 15 hours in the same incubator. Coliform colonies appeared on the purplish-red membrane as dark red colonies with a golden metallic reflection (sheen). Colonies of other organisms that were not completely inhibited by the fuchsin-sulphite medium appeared without sheen.

Water samples taken on various dates between August, 1952 and July, 1953, from the same fixed sampling stations (twenty-three in the Vernon-Orwell area, nineteen in Pinette River) selected for enterococci determinations, were examined for coliform bacteria by the membrane filtration method. All colonies with a typical metallic sheen were counted as coliform bacteria. Colony counts are reported, as MF coliform counts per 100 ml. of sample, in Table VIII (a and b).

f. Confirmation of Positive Lactose Broth Cultures in Eijkman Broth

Positive lactose broth cultures obtained from Standard Methods Presumptive Coliform Tests made on water samples from selected sampling stations in the Vernon-Orwell area during the 1953 study were transferred to Bacto-Eijkman Broth. Inoculated Eijkman broth fermentation tube cultures were incubated at 44° C. for 24 hours. Fermentation with the production of gas was recorded. Most probable numbers of Eijkman-positive coliform bacteria were determined for each sample, for comparison with coliform most probable numbers obtained by Standard Methods confirmation of the same positive lactose broth cultures in Bacto-Brillian Green Bile (2%) Broth. Eijkman most probable numbers appear in Table XXVI (a and b).

Bacteriological Examination of Oyster Specimens

a. Collection and Transportation of Specimens

Specimens of oysters from Stations 48, 66, and 68 in the Vernon-Orwell area, and from Stations 7, 8, and 16 in Pinette River, were collected at low tide. Eight to ten oysters constituted a sample. The oysters were fished from the oyster bed with an oyster rake, and immediately placed in heavy kraft paper, wax-lined bags for transportation to the laboratory. All specimens were tested within six hours of the time of collection.

b. Preparation of Oyster Samples

All oysters were thoroughly scrubbed with stiff-bristled brushes under a stream of water of known bacteriological purity. The brushes were sterilized in an autoclave, or by immersion for at least five minutes in boiling water. Cleaned oysters were placed on clean paper towels and allowed to dry in the air of the laboratory.

Oysters were shucked directly into a tared sterilized Waring Blendor jar; sufficient oysters were shucked to provide 200 grams of oyster meats and shell liquor for each sample. Commercial oyster shucking knives sterilized in the autoclave were used to open the oysters. 200 grams of sterilized one per cent saline solution were weighed into the Blendor jar to make a 1 to 2 dilution. The jar was placed on the Waring Blendor, and the contents ground for one minute at the standard motor speed. The resulting homogeneous mixture, and decimal dilutions in one per cent saline, were used to inoculate the test media.

c. Determination of Coliform Most Probable numbers

Examination for coliform bacteria was made according to the Confirmed Test Procedure presented in the American Public Health Association, "Standard Methods for the Bacteriological Examination of Shellfish and Shellfish Waters" (1947), with five-tube series of Bacto-Lactose Broth in at least three dilutions, and confirmation in Bacto-Brilliant Green Lactose Bile (2%) Broth. The numbers of coliform bacteria were expressed as the most probable numbers (M.P.N.'S) per 100 grams of sample. M.P.N.'s were determined from the table of most probable numbers given by Hoskins (1934). M.P.N.'s are recorded in Tables X and XI.

d. Determination of Enterococci Most Probable Numbers

Oyster samples were tested for enterococci by the Winter-Sandholzer procedure outlined in Bacteriological Examination of Shellfish Growing Waters (D). The numbers of enterococci were expressed as M.P.N.'s per 100 grams of sample; these are recorded in Tables X and XI.

e. Determination of Coliform Plate Counts

Duplicate 2 ml. portions of a 1 to 2 dilution of the oyster sample were plated in sterile Petri dishes with sterile Violet Red Bile Agar (Difco). The agar was thoroughly mixed with the sample by gently tilting and rotating the plates. The mixture was allowed to solidify at room temperature. A thin layer of the same agar medium was then poured over the surface and allowed to solidify. The plates were then inverted, and incubated at 37° C. for 24 hours. Typical dark-red subsurface colonies were counted under a Quebec colony counter. Results were expressed as coliform bacteria per gram of sample. Coliform plate counts obtained from oyster specimens appear in Tables X and XI.

f. Confirmation of Positive Lactose Broth Cultures in Eijkman Broth

Positive lactose broth cultures obtained from Standard Methods Presumptive Coliform Tests made on oyster specimens during the 1953 study were confirmed in Eijkman Broth (Difco), as described in Bacteriological Examination of Shellfish Growing Waters (F). Most probable numbers of Eijkman-positive coliform bacteria were determined for each sample; M.P.N. values appear in Table XI.

Bacteriological Examination of Soil Specimens

Soil specimens were collected periodically from six fixed sampling stations (A - F inclusive) on the Vernon River watershed. Specimens were taken from the top one-inch of the soil surface with sterile spoons, and were transferred to sterile wide-mouth glass jars for transportation to the laboratory.

Ten-gram samples of soil were weighed into tared dilution bottles containing 90 ml. of sterile water, and thoroughly shaken. A series of decimal dilutions of the initial 1 to 10 dilution was prepared, and duplicate 1 ml. portions of each dilution were plated in sterile Petri dishes with sterile Violet Red Bile Agar (Difco). The agar was thoroughly mixed with the inoculum by gently tilting and rotating the plates. The mixture was allowed to solidify at room temperature. A thin layer of the same agar medium was then poured over the surface and allowed to solidify. The plates were then inverted, and incubated at 37° C. for 24 hours. Typical dark-red subsurface colonies were counted under a Quebec colony counter; results were expressed as coliform bacteria per gram of sample.

Twenty-gram portions of the same soil specimens were weighed on tared metal weighing dishes, dried to constant weight in an oven, and

reweighed. The moisture content of each sample was determined. The observed coliform plate counts were corrected for the per cent moisture content of the soil samples, and are reported, on a dry weight basis, in Table XIII.

Bacteriological Examination of Faeces Specimens

Specimens of seagull, chicken, horse, swine, cattle, and human faeces were collected from the Vernon River watershed. A total of forty faeces samples, and one sample of sewer sludge, were collected and examined during the investigation.

Specimens were transferred from the collection site to sterile glass bottles by means of sterile spoons.

Ten-gram samples of faecal material were weighed into tared dilution bottles containing 90 ml. of sterile water, and thoroughly shaken. A series of decimal dilutions of the initial 1 to 10 dilution was prepared, and duplicate 1 ml. portions of each dilution were planted in sterile Petri dishes with Violet Red Bile Agar (Difco). The agar was thoroughly mixed with the inoculum by gently tilting and rotating the plates. The mixture was allowed to solidify at room temperature. A thin layer of the same agar medium was then poured over the surface and allowed to solidify. The plates were then inverted, and incubated at 37° C. for 24 hours. Typical dark-red subsurface colonies were counted under a Quebec colony counter; results were expressed as coliform bacteria per gram of sample.

Twenty-gram portions of the same faeces specimens were weighed on tared metal weighing dishes, dried to constant weight in an oven, and reweighed. The moisture content of each sample was determined. The

observed coliform plate counts were corrected for the per cent moisture content of the faeces samples, and are reported, on a dry weight basis, in Table XIV.

Isolation and Classification of Coliform Bacteria

Coliform bacteria isolated from water, oyster, soil, and faeces specimens by the procedures outlined above were classified according to their biochemical reactions. All strains were subjected to the following tests:

a. Indole Test (Kovacs)

Bacto-Tryptone Broth in a 1 per cent concentration was tubed in 5 ml. quantities and sterilized in the autoclave for 15 minutes at 121° C. Inoculated tubes were incubated at 35° to 37° C. for 24 ± 2 hours.

The Kovacs test reagent was prepared by dissolving 5 grams of paradimethyl-amino benzaldehyde in 75 ml. of amyl alcohol, and adding 25 ml. of concentrated hydrochloric acid. 0.3 ml. of the reagent was added to each tryptone broth culture, and the tube allowed to stand for ten minutes. A dark red colour in the amyl alcohol surface layer constituted a positive indole test; retention of the original colour of the amyl alcohol reagent constituted a negative test.

b. Methyl Red Test

M.R. - V.P. Medium was prepared by dissolving 17 grams of dehydrated Bacto-M.R. - V.P. Medium in 1,000 ml. of distilled water. The medium was tubed in 6 ml. quantities and sterilized in the autoclave for 15 minutes at 121° C. Inoculated tubes were incubated for 5 days at 30° C.

Five drops of methyl red solution (prepared by dissolving 0.1 gram of Bacto-Methyl Red in 300 ml. of 95 per cent methyl alcohol and diluting to 500 ml. with distilled water) was added to 5 ml. of culture. A positive (acid) reaction was indicated by a distinct red colour, while a negative reaction was indicated by a yellow colour.

c. Voges-Proskauer Test

One ml. of the M.R. - V.P. Medium culture prepared in (B) above was transferred aseptically with a sterile one ml. pipette after the culture had been incubated for 48 hours. To 1 ml. of culture were added 0.6 ml. of 5 per cent a-naphthol in absolute ethyl alcohol, and 0.2 ml. of 40 per cent potassium hydroxide. The development of a crimson to ruby colour in the mixture from 2 to 4 hours after the addition of the reagents constituted a positive test for acetyl-methyl-carbinol. Results were read not later than four hours after the addition of the reagents.

d. Citrate Test

Bacto-Simmons Citrate Agar was prepared by dissolving 24.2 grams of the dehydrated medium in 1,000 ml. of distilled water. The medium was tubed in 7 ml. quantities, sterilized in the autoclave for 15 minutes at 121° C., and allowed to cool in a slanting position. The agar slopes were lightly inoculated with the test cultures by means of a standard loop, and incubated at 35° to 37° C. for 72 hours. Growth was indicated by colony formation and a change in the colour of the medium from its initial green to a deep blue.

e. Gelatin Liqufaction Test

Bacto-Nutrient Gelatin was prepared by dissolving 128 grams of the dehydrated medium in 1,000 ml. of distilled water. The medium was

tubed in 5 ml. quantities and sterilized in the autoclave for 15 minutes at 121° C. Stab inoculations with the test organisms were made, and the inoculated tubes were incubated at 35° - 37°C. for seven days. After incubation the tubes were placed in a refrigerator to determine whether or not the gelatin was still capable of solidifying.

f. MacConkey Broth 44° C. Test

Bacto-MacConkey Broth was prepared by dissolving 35 grams of the dehydrated medium in 1,000 ml. of distilled water. The medium was dispensed in fermentation tubes in 7 ml. quantities and sterilized in the autoclave for 15 minutes at 121° C. A fermentation tube of the medium was inoculated with a pure coliform culture and incubated in a carefully regulated water bath maintained at a temperature of 44 ± 0.5° for 48 hours. Production of gas constituted a positive result. Production of acid was demonstrated by the colour of the medium changing to yellow.

Before the coliform strains were subjected to any of the above tests they were purified by re-plating on Bacto-Tryptone Glucose Extract Agar. Single, discrete colonies were picked to Tryptone Glucose Extract Agar (Difco) slopes before transfer to the test media.

Survival of Coliform Types in Synthetic Seawater

Synthetic seawater was prepared as follows (ZoBell, 1946):

Distilled water (H_2O)	1,000.00 grams
Sodium chloride (NaCl)	24.32 "
Magnesium chloride ($MgCl_2.6H_2O$)	10.99 "
Sodium sulphate (Na_2SO_4)	4.06 "
Calcium chloride ($CaCl_2.6H_2O$)	2.25 "
Potassium chloride (KCl)	0.69 "

Sodium bicarbonate ($NaHCO_3$)	0.20 grams
Potassium bromide (KBr)	0.10 "
Strontium chloride ($SrCl_2.6H_2O$)	0.042 "
Boric acid (H_3BO_3)	0.027 "

The distilled water was sterilized in the autoclave for 15 minutes at 120 C. and cooled to room temperature before the addition of the reagent grade chemicals. After the chemicals had been dissolved by gentle heating and thorough mixing, the synthetic seawater was dispensed aseptically in sterile 500 ml. Erlenmeyer flasks (250 ml. per flask). Sterile foil-covered rubber stoppers were used to seal the flasks. Control tests indicated that coliform bacteria were absent in 100 ml. of the synthetic seawater.

Each flask was inoculated with 1 ml. of a 24 hour nutrient broth culture of a purified coliform strain isolated during field studies in Prince Edward Island. One ml. quantities of each synthetic seawater culture, and decimal dilutions, were plated in duplicate on Bacto-Violet Red Bile Agar. All plates were incubated at 35.5° to 37° C. for 24 hours and examined by transmitted light. Purplish red coliform colonies were counted from the dilution giving 30 to 300 colonies per plate, where possible. The coliform counts from duplicate plates were averaged, and the results were expressed as coliform bacteria per ml. of synthetic seawater.

The flasks were placed on a machine shaker in a cold room (temperature 38° to 40° C.); mechanical shaking provided an undetermined amount of aeration of the cultures.

Portions of culture were removed aseptically from each flask periodically during the duration of the experiment, and tested in the manner outlined above; tests were continued until coliform bacteria were absent in 4 ml. of synthetic seawater for two consecutive tests. The mean percentage survival of coliform types were calculated for each test interval. Two separate experiments, in which a total of 16 strains were tested, were conducted; results are reported in Tables XXVIII to XXXI inclusive, and in Figures V (a) and V (b).

B. Observations and Results

1. Rainfall

Rainfall in Queen's County, Prince Edward Island, as recorded by the Dominion Experimental Farm Service at Charlottetown, is tabulated in Table I. Heavy local precipitation on the Vernon River watershed on May 20, 1953, was not recorded at the Charlottetown station.

So little rain fell during July, August, and September, 1952, that land-wash pollution from farmlands to the study areas was negligible. A considerable amount of precipitation occurred in all Queen's County, P.E.I., areas in April, 1953, but during May and June, 1953, very little rain fell. The soil remained moist during May, however, and a local rainstorm over the Vernon River watershed on May 20, 1953, caused moderate land-wash pollution in the Vernon River system.

2. Salinity Determinations

Salinity values determined for Vernon River and Pinette River seawater specimens during the investigations are recorded in Tables II (a) and II (b).

3. Coliform Most Probable Number (MPN) Determinations from Seawater Specimens

a. Vernon River (including Orwell River)

For purposes of discussion, the Vernon River system was divided into four sectors, A, B, C, D, (Table III). Tables IV (a) and IV (b) reveal that, during the July 24 to October 9, 1952, period, a considerable variation in low-tide coliform M.P.N.'s occurred in water samples. Only negligible numbers of coliform bacteria could be isolated from water samples taken from all sectors of the river system on July 24 and July 29.

TABLE I - RAINFALL, CHARLOTTETOWN, PRINCE EDWARD ISLAND, PORTIONS OF 1951, 1952, AND 1953. RAINFALL IN INCHES

DATE	RAINFALL
June, 1951	
1	.17
3	.31
4	.14
7	.38
8	.23
9	.13
15	.12
16	.26
17	.02
24	.20
25	.78
26	.31
July, 1951	
1	.65
2	1.53
3	.04
5	.49
6	1.20
12	.27
13	.45
14	.01
20	1.60
29	.02
30	.02
August, 1951	
1	.82
2	.46
6	.02
9	.62
14	.48
15	.03
17	.18
18	.35
19	.03
22	2.40
23	.12
28	.38
30	.18
31	.21
Sept., 1951	
1	.83
3	.30
4	.21
5	.01
7	.33
16	.47
17	.10
23	.30
24	.16
26	.25
28	.07
29	.02
October, 1951	
5	.01
8	.30
9	.07
12	.14
13	.06
20	.04
25	.47
June, 1952	
2	.60
3	.92
4	.02
7	.64
8	.18
9	.01
10	.39
11	.06
13	.01
15	.26
16	.07
18	.41
21	.04
22	.05
23	.01
25	.03
26	.02
27	.14
July, 1952	
5	.16
11	1.19
13	.16
15	.06
19	.01
20	.04
21	.03
29	.83
August, 1952	
1	.05
3	.15
6	.20
7	.07
8	.03
13	.28
17	.15
18	1.96
22	.01
23	.13
24	.13
30	.18
Sept., 1952	
5	.15
8	.16
11	.14
12	.01
13	.02
15	.35
16	.19
17	.14
20	.37
22	.08
October, 1952	
3	.57
4	.17
8	.47
14	.02
16	.61
19	.03
20	.41
21	.10
May, 1953	
5	.01
6	.05
12	.09
13	.18
14	.10
15	.51
17	.16
18	.76
19	.05
*20	.03
22	.02
23	.43
24	.26
29	.13
June, 1953	
2	.05
7	.43
8	.03
11	.04
14	.25
15	.17
22	.23
26	.06

TOTALS:	1951	1952	1953
May			2.78
June	3.05	3.86	1.26
July	6.28	2.48	
August	6.28	3.34	
September	3.05	1.61	
October	1.09	2.38	

* Heavy local precipitation in the Vernon River Area on May 20, 1953

TABLE II (a) - VERNON RIVER SALINITY DATA

Sampling date	Salinities (Grams per kilogram of Seawater)						
	Sampling Station						
	18	25	31	41	54	71	Average
1952							
July 24	26.2	26.2	27.7	26.3	26.5	19.6	25.4
July 29	27.9	27.9	29.1	26.3	25.2	18.4	25.8
Aug. 5	27.3	27.18	28.4	24.3	21.7	10.9	23.3
Aug. 21	26.3	26.3	27.5	26.2	26.0	15.4	24.6
Sept. 9	25.9	27.0	26.8	25.7	21.8	16.7	24.0
Sept. 25	26.6	27.8	27.7	26.5	25.7	14.8	24.9
Oct. 9	27.4	27.6	27.7	24.9	23.7	13.0	24.1
Mean 1952 Salinities	26.8	27.1	27.8	25.7	24.4	15.5	24.6
1953							
May 15	26.7	26.8	27.7	26.3	27.0	12.3	24.5
May 18	26.1	26.8	28.1	26.2	25.5	15.5	24.7
May 21	25.7	26.2	28.3	25.7	24.3	12.4	23.8
June 4	27.1	26.8	27.0	26.3	25.5	15.7	24.7
June 9	25.0	27.0	16.3	24.9	20.7	9.5	20.6
Mean 1953 Salinities	26.1	26.7	25.5	25.9	24.6	13.1	23.7
Area Sector	B	D	D	C	C	A	-

TABLE II (b) - PINETTE RIVER SALINITY DATA

Sampling date	Salinities (Grams per kilogram of Seawater)					
	Sampling Station					
	7	9	19	25	37	Average
1952						
July 26	23.3	25.8	19.2	27.1	27.2	24.5
July 31	19.1	23.0	16.5	25.8	27.1	22.3
Aug. 6	24.2	25.5	21.5	26.8	28.0	25.2
Aug. 14	19.6	24.7	17.9	25.9	27.3	23.1
Aug. 27	14.0	23.4	14.0	23.4	26.0	20.2
Sept. 10	17.0	23.4	16.8	24.7	25.9	21.6
Sept. 24	15.8	22.3	15.8	23.7	26.5	20.8
Oct. 14	24.7	26.1	18.3	27.4	27.5	24.8
Mean 1952 Salinities	19.7	24.3	17.5	25.6	26.9	22.8
1953						
June 16	22.8	25.9	22.1	26.0	26.7	24.7
July 1	25.2	26.4	23.0	26.4	28.4	26.0
Mean 1953 Salinities	24.0	26.2	22.6	26.2	27.6	25.5

TABLE III - VERNON RIVER AREA

Section	Water Sampling Stations	Description
A	1 - 5 incl. 67 - 72 incl. (11)	Vernon River, upstream from a straight line drawn between Oyster Monuments 6 and 7.
B	6 - 21 incl. 37 - 39 incl. 64 - 66 incl. (22)	Remainder of Vernon River, upstream from a straight line drawn between Oyster Monuments 1 and 3, and <u>not</u> including Orwell River upstream from a straight line drawn between Oyster Monument 4 and Brush Wharf.
C	40 - 63 incl. (24)	Orwell River, upstream from a straight line drawn between Oyster Monument 4 and Brush Wharf.
D	22 - 36 incl. (15)	Orwell Bay, to the seaward side of a straight line drawn between Oyster Monuments 1 and 3.

TABLE IV (a) - SEAWATER SPECIMENS, SECTORS A AND B, VERNON RIVER AREA. COLIFORM DATA

SECTOR	STA.	MOST PROBABLE NUMBER OF COLIFORM BACTERIA PER 100 ML. SEAWATER														
		June 21, 1951	July 24, 1952	July 29, 1952	Aug. 5, 1952	Aug. 21, 1952	Sept. 9, 1952	Sept. 25, 1952	Oct. 9, 1952	Mean MPNs, 1952	May 15, 1953	May 18, 1953	May 21, 1953	June 3, 1953	June 4, 1953	June 9, 1953	Mean MPNs, 1953
A	1	920	26	<1.8	240	79	33	23	49	64.3	95	220	79	350	14	170	154.7
	2	240	17	14	540	79	49	17	70	107.9	33	33	79	220	7.8	130	83.8
	3	350	13	17	240	49	23	17	130	69.7	34	33	33	540	22	70	222.0
	4	540	17	13	49	21	33	17	70	31.4	26	110	46	22	7.8	33	40.8
	5	170	14	4.5	280	49	33	22	46	64.1	23	7.8	240	70	4.5	22	61.2
	67	350	7.8	17	170	110	49	33	540	132.5	1600	350	350	130	4.5	350	464.1
	68	280	14	4.5	350	130	110	33	220	123.1	1600	110	540	110	11	130	488.5
	69	920	49	11	350	140	110	33	120	116.4	540	110	240	920	23	350	363.8
	70	350	6.8	2	350	170	110	139	170	135.4	350	220	1600	350	33	170	453.8
	71	1600	33	33	540	170	110	95	170	164.4	180	540	140	170	79	1600	451.5
	72	540	130	46	920	240	79	79	240	247.7	540	240	350	350	70	920	411.6
B	6	350	11	<1.8	49	11	13	4.5	110	28.4	23	15	240	14	2	33	54.5
	7	140	4	2	17	23	13	2	49	15.7	11	33	220	17	2	13	49.3
	8	130	4.5	<1.8	110	7.8	33	11	79	35.0	11	23	130	23	4.5	110	50.3
	9	110	2	<1.8	23	2	13	11	130	25.9	31	22	350	17	2	33	75.8
	10	110	4.5	2	13	4	13	4	130	24.4	13	14	240	22	4	49	57.0
	11	64	4.5	<1.8	33	7.8	11	4.5	46	15.3	17	4.5	130	13	<1.8	13	29.6
	12	140	4	<1.8	70	1.8	12	7.8	70	23.7	33	11	130	17	2	23	36.0
	13	49	2	2	26	13	7.8	4.5	23	11.2	33	4.5	540	-	<1.8	7.8	117.1
	14	170	4	<1.8	46	7.8	6.8	2	23	12.8	1.8	11	240	-	<1.8	14	53.4
	15	49	2	2	49	21	7.8	<1.8	14	13.7	13	7.8	70	-	2	13	21.2
	16	170	2	<1.8	33	2	6.8	4.5	33	11.6	13	17	220	-	<1.8	7.8	51.6
	17	79	<1.8	1.8	33	13	4.5	1.8	17	10.2	4.5	22	170	-	<1.8	13	41.9
	18	23	<1.8	<1.8	4.5	2	11	4.5	9.3	4.5	7.8	7.8	33	-	2	6.8	11.5
	19	17	<1.8	<1.8	7.8	4.5	4.5	4.5	7.8	4.2	17	23	170	-	<1.8	11	44.2
	20	20	<1.8	<1.8	29	7.8	4	<1.8	13	3.5	7.8	22	170	-	<1.8	13	42.6
	21	11	<1.8	9.3	<1.8	4.5	2	<1.8	4	2.8	7.8	7.8	110	-	2	4.5	26.4
	37	70	<1.8	<1.8	4	4.5	4	1.8	4.5	2.7	2	7.8	33	-	4	4.5	10.3
	38	33	2	<1.8	4.5	2	<1.8	2	7.8	2.6	7.8	2	11	-	<1.8	4.5	5.1
	39	49	4	<1.8	<1.8	1.8	4.5	2	21	4.5	11	4	79	-	2	2	19.6
	64	23	<1.8	2	6.8	2	4.5	4.5	23	6.1	2	17	49	-	2	2	14.4
	65	240	<1.8	1.8	4.5	4	4.5	2	7.8	3.5	5.6	33	49	-	2	33	24.5
	66	920	<1.8	<1.8	33	7.8	3.7	<1.8	7.8	7.5	2	2	49	-	2	11	13.2

TABLE IV (b) - SEAWATER SPECIMENS, SECTORS C AND D, VERNON RIVER AREA. COLIFORM DATA

SECTOR	STATION	MOST PROBABLE NUMBER OF COLIFORM BACTERIA PER 100 ML. SEAWATER														
		June 21, 1951	July 24, 1952	July 29, 1952	Aug. 5, 1952	Aug. 21, 1952	Sept. 9, 1952	Sept. 25, 1952	Oct. 9, 1952	Mean MPNs, 1952	May 15, 1953	May 18, 1953	May 21, 1953	June 4, 1953	June 9, 1953	Mean MPNs, 1953
C	40	79	<1.8	<1.8	4.5	2	9.3	4.5	33	7.6	23	11	79	<1.8	2	23.0
C	41	49	2	<1.8	13	2	2	1.8	110	18.7	17	14	79	2	<1.8	22.4
C	42	70	2	2	27	1.8	4.5	9.2	22	9.8	7.8	4.5	13	<1.8	2	5.5
C	43	49	2	2	130	13	14	6.8	22	27.1	17	4.5	17	2	17	11.5
C	44	110	2	4.5	240	9.2	49	26	130	65.8	13	<1.8	13	4.5	4.5	7.0
C	45	49	2	12	130	9.3	70	49	41	44.8	22	11	46	2	17	19.6
C	46	46	4.5	2	47	11	79	4.5	41	27.0	7.8	17	49	<1.8	-	18.5
C	47	79	<1.8	2	-	11	130	14	49	38.4	13	33	46	2	-	23.5
C	48	170	2	4.5	-	11	170	4.5	-	32.0	13	11	23	4.5	-	12.9
C	49	33	2	4.5	-	6.8	-	2	-	3.8	-	21	130	4.5	-	51.8
C	50	49	11	6.8	-	4.5	-	13	-	8.8	-	13	140	2	-	51.7
C	51	130	4.5	33	-	13	-	33	-	20.9	-	23	240	46	-	103.0
C	52	95	49	6.8	-	4.5	26	4.5	-	18.2	-	31	110	<1.8	-	47.6
C	53	130	<1.8	6.8	-	13	46	11	-	15.4	11	11	170	<1.8	-	48.0
C	54	170	<1.8	7.8	-	7.8	17	6.8	110	24.9	11	9.3	23	2	-	11.3
C	55	240	2	6.8	350	4.5	95	17	49	74.9	7.8	49	79	1.8	-	34.4
C	56	110	2	<1.8	350	4.5	33	21	22	61.8	17	33	13	<1.8	-	15.8
C	57	49	4.5	<1.8	350	<1.8	33	4.5	27	59.9	2	13	17	<1.8	-	8.0
C	58	33	4.5	<1.8	140	2	7.8	6.8	33	27.7	2	17	17	2	17	11.0
C	59	33	2	<1.8	350	7.8	13	11	33	59.5	4.5	33	49	<1.8	7.8	18.9
C	60	13	4.5	<1.8	240	4.5	14	4.5	27	42.1	17	49	46	4.5	2	23.7
C	61	140	7.8	<1.8	64	<1.8	4.5	7.8	49	19.0	33	23	9.3	2	7.8	15.0
C	62	23	6.8	2	49	2	7.8	6.1	33	15.2	9.3	13	13	1.8	2	7.8
C	63	49	<1.8	2	49	2	6.8	6.8	17	11.9	6.8	7.8	23	2	4.5	8.8
D	22	33	4.5	<1.8	2	<1.8	4.5	<1.8	6.1	2.4	6.8	14	280	<1.8	13	62.8
D	23	23	2	<1.8	4.5	4.5	2	<1.8	4	2.4	4	6.1	130	1.8	11	30.6
D	24	33	<1.8	<1.8	2	14	2	<1.8	2	2.9	4.5	7.8	240	4.5	<1.8	51.4
D	25	13	<1.8	<1.8	2	1.8	2	2	4.5	1.8	<1.8	1.8	170	<1.8	4.5	35.3
D	26	12	<1.8	4	7.8	4.5	<1.8	2	2	2.9	6.8	6.8	49	2	2	13.3
D	27	17	2	2	2	4.5	2	<1.8	6.1	2.7	4	4.5	170	2	<1.8	36.1
D	28	2	<1.8	<1.8	<1.8	<1.8	4	<1.8	2	0.9	2	<1.8	2	<1.8	<1.8	<1.8
D	29	2	<1.8	<1.8	<1.8	<1.8	2	2	1.8	0.8	<1.8	<1.8	4.5	<1.8	<1.8	<1.8
D	30	7.8	2	<1.8	1.8	1.8	2	<1.8	4.5	1.7	<1.8	2	<1.8	<1.8	<1.8	<1.8
D	31	6.8	<1.8	<1.8	<1.8	4.5	<1.8	4	4.5	1.9	33	<1.8	4.5	<1.8	2	7.9
D	32	7.8	<1.8	<1.8	<1.8	<1.8	2	<1.8	6.8	1.3	7.8	4.5	1.8	<1.8	<1.8	2.8
D	33	70	<1.8	<1.8	2	<1.8	<1.8	<1.8	4.5	0.9	17	<1.8	13	<1.8	<1.8	6.0
D	34	13	4.5	<1.8	2	4.5	2	2	4	2.7	11	<1.8	7.8	<1.8	2	4.2
D	35	49	<1.8	<1.8	4.5	4	<1.8	4.5	11	3.4	2	6.1	49	2	<1.8	11.8
D	36	13	<1.8	<1.8	4.5	1.8	3.7	1.8	7.8	2.8	7.8	7.8	27	<1.8	<1.8	8.5

The coliform content of samples taken from Sectors A and C during periods of spring tides on August 5 and October 9, 1952, however, reached a significantly high level; on these dates the coliform M.P.N.'s of seawater samples from these two sectors approximated those obtained during the survey of June 21, 1951, when moderate land-wash pollution occurred. Coliform M.P.N.'s from water samples taken on three other dates (August 21, September 9, and September 25, 1952) are between these extremes; results obtained on August 21 approximate a mean value which would appear to substantiate the sanitary engineering evaluation of the area.

During 1953, there was a considerable variation in coliform M.P.N.'s from seawater specimens taken at low tide in all sectors of the Vernon River system. On June 4, seawater from all four sectors contained negligible numbers of coliform bacteria; coliform M.P.N.'s ranged from less than 1.8 to 79, and only two samples had M.P.N. values of more than 50. M.P.N.'s from water samples taken in Sector A on May 15, May 18, June 3, and June 9 ranged from 7.8 to 1,600, and 30 of 44 specimens gave M.P.N.'s of more than 50; of 174 seawater specimens taken from Sectors B, C, and D on these same dates, one specimen gave a coliform M.P.N. of 110, while the remaining 173 samples gave M.P.N.'s ranging from less than 1.8 to 49. Heavy local rainfall on May 20, 1953, caused considerable land-wash on the Vernon River watershed on May 21; water samples taken in all four sectors on that date had coliform M.P.N.'s ranging from 9.3 to 1,600, and 38 of 72 specimens had coliform M.P.N.'s of more than 50.

Twelve water samples were taken during the 1953 study from the Vernon River at or above tidewater, and from streams flowing from 22 to more than 1,600, and only three samples had coliform M.P.N.'s of less than 50 (Table V).

b. Pinette River

The coliform M.P.N.'s obtained from low-tide seawater samples taken from 37 Stations in Pinette River during twelve sampling surveys made in 1951, 1952, and 1953, appear in Tables VI (a) and VI (b).

Samples taken on September 24, 1952, had coliform M.P.N.'s ranging from less than 1.8 to 280, and included ten samples with coliform M.P.N.'s of more than 50; these results were similar to those obtained during the routine survey of July 2, 1951, when moderate land-wash pollution occurred. In contrast, coliform M.P.N.'s obtained from water samples taken during seven other surveys made during the 1952 study period ranged from less than 1.8 to 79, and only five (of 259) samples gave M.P.N.'s of more than 50. Mean coliform M.P.N.'s calculated for the 37 sampling stations ranged from less than 1.8 to 51.5, and in only one instance was the mean coliform M.P.N. value more than 50.

Coliform M.P.N.'s from specimens taken on June 16 and July 1, 1953, ranged from less than 1.8 to 170, and only 4 of 74 seawater specimens gave coliform M.P.N.'s of more than 50. Mean coliform M.P.N. values calculated for the 37 sampling stations ranged from 1.0 to 93.5; only two stations had mean coliform M.P.N. values greater than 50.

4. Enterococci Most Probable Number (MPN) Determinations from Seawater

The results obtained from the examination of specimens of seawater for their enterococci content appear in Table VII (a and b).

Very low enterococci M.P.N.'s were obtained from seawater samples taken in 1952 during eight surveys of 19 sampling stations in Pinette River and seven surveys of 23 stations in the Vernon River system; no close relationship with the coliform M.P.N.'s obtained from the same samples is evident.

TABLE V - STREAM WATER SAMPLES, VERNON RIVER, 1953

Specimen Number	Date of Sampling	Source (Vernon River)	Coliform M.P.N. per 100 ml.	Enterococci M.P.N. per 100 ml.
1	May 11	Above Road Bridge	49	4.5
2	May 11	Below Road Bridge	>1,600	110
3	May 12	At Vernon Mill	22	<1.8
4	May 12	At waterfall, head of Tidewater	46	<1.8
5	May 12	Mill pond, below waterfall	>1,600	130
6	May 12	Below Road Bridge	140	4.5
7	May 29	At Waterfall, head of Tidewater	540	4.5
8	May 29	Below Road Bridge	540	4
9	May 29	Mouth of first tributary Creek, Vernon Tidewater	350	4
10	May 29	Mill pond, below waterfall	540	2
11	May 29	Tributary Creek entering Vernon River 1 mile above Vernon Bridge	540	<1.8
12	May 29	Seal River, at Source	>1,600	2

TABLE VI (a) - PINETTE RIVER COLIFORM DATA. SEAWATER SPECIMENS, STATIONS 1-19

STA.	MOST PROBABLE NUMBER OF COLIFORM BACTERIA PER 100 ML. SEAWATER												
	July 2; 1951	July 26, 1952	July 31, 1952	Aug. 6, 1952	Aug. 14, 1952	Aug. 27, 1952	Sept. 10, 1952	Sept. 24, 1952	Oct. 14, 1952	Mean MPNs, 1952	June 16, 1953	July 1, 1953	Mean MPNs, 1953
1	23	6.8	<1.8	7.8	23	2	4.5	23	<1.8	8.4	23	23	23
2	70	7.8	1.8	6.8	2	1.8	7.8	13	<1.8	5.1	2	13	7.5
3	17	2	7.8	6.8	17	7.8	7.8	23	4	9.5	13	49	31
4	31	4.5	4.5	7.3	14	7.8	4.5	79	7.8	16.2	13	33	23
5	49	2	4	79	14	11	4.5	49	<1.8	20.4	22	21	21.5
6	70	4	23	6.8	14	11	9.3	79	4.5	19.0	22	22	22
7	49	7.8	33	17	11	46	4.5	280	13	51.5	23	17	20
8	13	4.5	2	4.5	2	6.8	6.8	17	<1.8	5.5	7.8	7.8	7.8
9	17	2	2	13	1.8	2	4.5	13	4.5	5.4	23	7.8	15.4
10	49	<1.8	4.5	7.8	4.5	7.8	11	13	4.5	6.6	4.5	33	18.8
11	140	2	13	13	13	6.8	4	33	4.5	15.1	23	23	23
12	33	4.5	7.8	14	6.8	4.5	2	46	<1.8	10.7	13	23	18
13	46	7.8	14	13	14	70	4	140	7.8	33.8	23	13	18
14	180	2	17	12	49	4.5	22	130	13	31.2	33	27	30
15	49	7.8	17	7.8	17	33	7.8	170	4.5	33.1	17	95	56
16	110	4.5	33	49	27	33	49	79	23	37.2	11	170	90.5
17	33	23	33	17	17	21	23	70	13	27.1	7.8	17	12.4
18	79	4	17	46	31	17	17	110	23	33.1	170	17	93.5
19	110	7.8	11	79	17	70	33	79	70	45.9	70	22	46

TABLE VI (b) - PINETTE RIVER COLIFORM DATA. SEAWATER SPECIMENS, STATIONS 20-37

STA.	MOST PROBABLE NUMBER OF COLIFORM BACTERIA PER 100 ML. SEAWATER												
	July 2, 1951	July 26, 1952	July 31, 1952	Aug. 6, 1952	Aug. 14, 1952	Aug. 27, 1952	Sept. 10, 1952	Sept. 24, 1952	Oct. 14, 1952	Mean MPNs, 1952	June 16, 1953	July 1, 1953	Mean MPNs, 1953
20	26	4.5	2	13	9.3	6.8	4.5	33	2	7.9	11	17	14.0
21	110	13	2	13	<1.8	4	2	13	2	6.1	7.8	11	9.4
22	23	2	2	7.8	2	2	4.5	7.8	4.5	4.1	4.5	21	12.8
23	23	4.5	2	6.8	2	<1.8	<1.8	7.8	<1.8	2.9	4	17	10.5
24	46	2	4.5	<1.8	11	1.8	4	4.5	<1.8	3.5	4.5	13	8.8
25	8	7.8	2	13	<1.8	2	<1.8	7.8	2	2.9	2	23	12.5
26	23	4.5	<1.8	2	<1.8	4.5	23	2	4.5	4.5	<1.8	23	11.5
27	11	<1.8	<1.8	4.5	4.5	<1.8	2	7.8	2	2.6	4.5	13	8.8
28	23	4.5	<1.8	2	2	<1.8	4	23	<1.8	4.4	2	2	2.0
29	14	4.5	2	<1.8	7.8	<1.8	2	4.5	<1.8	2.6	2	13	7.5
30	7	11	<1.8	4.5	<1.8	<1.8	<1.8	2	4	2.7	2	7.8	4.9
31	13	<1.8	11	2	4.5	1.8	2	2	4.5	3.5	4.5	4.5	4.5
32	2	2	2	2	2	2	<1.8	4.5	<1.8	1.8	2	2	2.0
33	13	4.5	<1.8	1.8	<1.8	<1.8	2	6.1	2	2.1	2	7.8	4.9
34	8	4.5	<1.8	<1.8	<1.8	<1.8	4.5	4.5	6.8	2.5	4.5	2	3.3
35	13	<1.8	2	33	<1.8	<1.8	2	<1.8	2	4.9	4.5	4	4.3
36	4.5	7.8	<1.8	49	<1.8	2	<1.8	<1.8	2	7.6	7.8	<1.8	3.9
37	4.5	<1.8	4.5	2	<1.8	<1.8	2	4.5	<1.8	<1.8	2	<1.8	1.0

FIGURE III (a) - MEAN COLIFORM M.P.N. VALUES

FROM VERNON RIVER SEAWATER

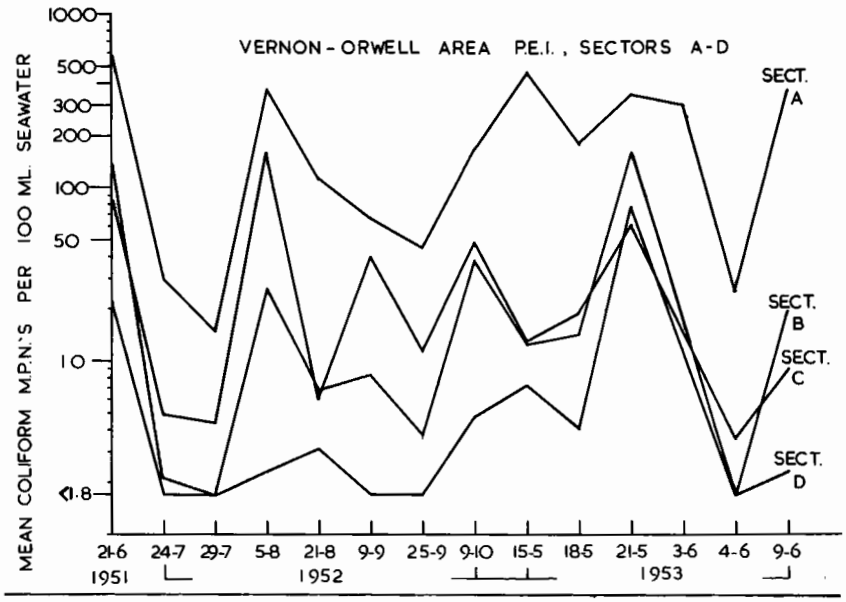


FIGURE III (b) - MEAN COLIFORM M.P.N. VALUES

FROM PINETTE RIVER SEAWATER

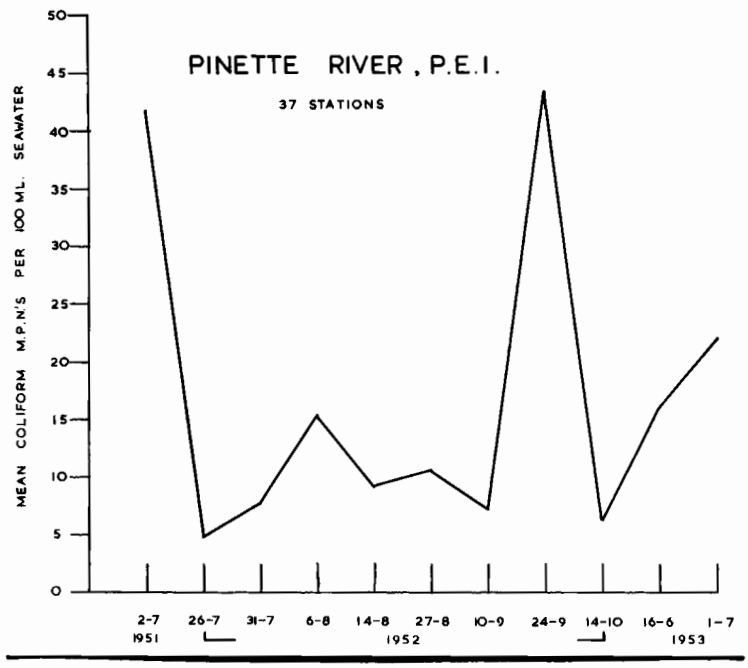


TABLE VII (a) - VERNON RIVER ENTEROCOCCI DATA. SEAWATER SPECIMENS, STATIONS 1-71

STA.	MOST PROBABLE NUMBER OF ENTEROCOCCI PER 100 ML. SEAWATER													
	July 24, 1952	July 29, 1952	Aug. 5, 1952	Aug. 21, 1952	Sept. 9, 1952	Sept. 25, 1952	Oct. 9, 1952	May 15, 1953	May 18, 1953	May 21, 1953	June 3, 1953	June 4, 1953	June 9, 1953	Mean MPNs
1	4.5	4.5	<1.8	4.5	14	4.5	17	15	11	4	2	<1.8	<1.8	6.2
4	4.5	2	<1.8	2	17	7.8	33	4	3.7	4.5	<1.8	2	1.8	6.2
7	<1.8	<1.8	<1.8	2	6.8	1.8	2	2	4.5	2	<1.8	<1.8	<1.8	<1.8
9	<1.8	<1.8	<1.8	2	4.5	<1.8	4.5	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8
12	2	<1.8	<1.8	<1.8	22	<1.8	<1.8	<1.8	<1.8	1.8	2	<1.8	<1.8	2.0
15	<1.8	<1.8	<1.8	<1.8	4.5	<1.8	4.5	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8
18	<1.8	<1.8	<1.8	<1.8	6.8	2	2	<1.8	<1.8	<1.8	-	<1.8	<1.8	<1.8
21	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	-	<1.8	<1.8	<1.8
25	<1.8	<1.8	<1.8	<1.8	2	<1.8	<1.8	<1.8	<1.8	1.8	-	<1.8	<1.8	<1.8
28	<1.8	<1.8	<1.8	<1.8	1.8	<1.8	<1.8	<1.8	<1.8	<1.8	-	<1.8	<1.8	<1.8
31	<1.8	<1.8	<1.8	<1.8	2	1.8	<1.8	<1.8	<1.8	<1.8	-	<1.8	<1.8	<1.8
35	2.2	<1.8	<1.8	<1.8	2	1.8	<1.8	<1.8	<1.8	<1.8	-	<1.8	<1.8	<1.8
37	<1.8	2	<1.8	<1.8	9.2	1.8	<1.8	<1.8	<1.8	<1.8	-	<1.8	<1.8	<1.8
41	<1.8	<1.8	<1.8	<1.8	4.5	<1.8	<1.8	<1.8	<1.8	1.8	-	<1.8	<1.8	<1.8
44	4	2	<1.8	<1.8	6.8	<1.8	<1.8	<1.8	<1.8	<1.8	-	<1.8	<1.8	<1.8
47	4.5	4.5	<1.8	<1.8	7.8	2	1.8	<1.8	<1.8	<1.8	-	<1.8	-	1.9
51	<1.8	<1.8	<1.8	<1.8	-	-	-	<1.8	<1.8	<1.8	-	<1.8	-	<1.8
54	<1.8	<1.8	<1.8	2	14	2	<1.8	<1.8	<1.8	<1.8	-	<1.8	-	<1.8
60	<1.8	<1.8	<1.8	<1.8	4	4.5	<1.8	<1.8	<1.8	<1.8	-	<1.8	<1.8	<1.8
64	<1.8	<1.8	<1.8	2	2	<1.8	<1.8	<1.8	<1.8	<1.8	-	<1.8	1.8	<1.8
66	<1.8	2	<1.8	<1.8	2	<1.8	<1.8	<1.8	<1.8	<1.8	-	<1.8	<1.8	<1.8
68	4.5	7.8	<1.8	6.8	17	1.8	9.2	4.5	2	<1.8	4	4.5	2	4.9
71	7.8	4.5	2	14	11	11	4.5	4.5	6.1	4	6.8	6.8	6.8	7.5

TABLE VII (b) - PINETTE RIVER ENTEROCOCCI DATA. SEAWATER SPECIMENS, STATIONS 1-37

STA.	MOST PROBABLE NUMBER OF ENTEROCOCCI PER 100 ML. SEAWATER									
	July 31, 1952	Aug. 6, 1952	Aug. 14, 1952	Aug. 27, 1952	Sept. 10, 1952	Sept. 24, 1952	Oct. 14, 1952	June 16, 1953	July 1, 1953	Mean MPNs
1	<1.8	2	<1.8	2	4	6.8	<1.8	<1.8	<1.8	<1.8
3	<1.8	<1.8	<1.8	<1.8	1.8	4.5	<1.8	<1.8	<1.8	<1.8
5	<1.8	<1.8	<1.8	<1.8	11	27	<1.8	<1.8	2.0	4.5
7	<1.8	2	<1.8	<1.8	7.8	7.8	2	<1.8	<1.8	2.2
9	<1.8	2	<1.8	<1.8	<1.8	4.5	<1.8	<1.8	<1.8	<1.8
11	<1.8	2	2	<1.8	2	<1.8	<1.8	<1.8	4.5	<1.8
13	<1.8	2	2	23	<1.8	7.8	2	<1.8	<1.8	4.1
15	<1.8	11	<1.8	23	22	22	<1.8	<1.8	1.8	8.8
17	<1.8	1.8	<1.8	13	13	6.8	<1.8	2	2	4.3
19	<1.8	2	1.8	2	13	11	4	<1.8	2	3.8
21	<1.8	<1.8	<1.8	<1.8	2	<1.8	<1.8	<1.8	<1.8	<1.8
23	2	<1.8	<1.8	<1.8	<1.8	2	<1.8	1.8	<1.8	<1.8
25	<1.8	2	<1.8	2	2	4	<1.8	<1.8	<1.8	<1.8
27	<1.8	2	<1.8	<1.8	2	2	<1.8	<1.8	<1.8	<1.8
29	<1.8	<1.8	<1.8	<1.8	<1.8	2	<1.8	<1.8	<1.8	<1.8
31	<1.8	<1.8	<1.8	<1.8	<1.8	2	<1.8	<1.8	2	<1.8
33	<1.8	<1.8	<1.8	2	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8
35	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8
37	<1.8	<1.8	<1.8	2	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8

One hundred and four of 148 seawater samples taken during 1952 from all Vernon River sectors had enterococci M.P.N.'s of less than 1.8, while the remainder had M.P.N.'s ranging from 1.8 to 22; mean enterococci M.P.N. values for samples from the 23 stations ranged from less than 1.8 to 9.5. Similarly, 87 of 119 seawater samples taken from the same stations during six sampling surveys in 1953 had enterococci M.P.N.'s of less than 1.8. The remainder gave enterococci M.P.N.'s ranging from 1.8 to 15; mean M.P.N. values for samples from the 23 stations ranged from less than 1.8 to 5.5. The land-wash following heavy rainfall on May 20, 1953, did not result in higher enterococci M.P.N.'s in seawater samples taken on May 21, 1953.

Ninety-five of 152 seawater samples taken during 1952 from Pinette River gave enterococci M.P.N.'s of less than 1.8, while the remainder gave M.P.N.'s ranging from 1.8 to 27; mean enterococci M.P.N. values for seawater samples from the 19 sampling stations ranged from less than 1.8 to 11.1. Thirty of 38 seawater samples taken from the same stations during two sampling surveys in 1953 gave enterococci M.P.N.'s of less than 1.8, while the remainder gave M.P.N.'s ranging from 1.8 to 4.5.

The very low (spring) tides of August 5 and October 9 had no apparent effect on the enterococci content of seawater samples taken on those dates.

5. Membrane Filter (MF) Coliform Counts from Seawater

Tables VIII (a and b) show the results obtained from membrane filter (MF) coliform counts from seawater specimens.

TABLE VIII (a) - MEMBRANE FILTER (MF) COLIFORM COUNTS. SEAWATER SPECIMENS, VERNON RIVER AREA

STA.	COLIFORM MF COUNT PER 100 ML. SEAWATER										
	Aug. 21, 1952	Sept. 9, 1952	Sept. 25 1952	Oct. 9, 1952	May 15, 1953	May 18, 1953	May 21, 1953	June 3, 1953	June 4, 1953	June 9, 1953	Mean MF Counts, 1953
1	110	15	20	-	63	180	180	130	30	125	118.0
4	20	14	12	11	63	62	255	50	15	80	87.5
7	10	4	5	4	53	67	120	26	12	60	56.3
9	0	7	8	10	21	28	96	14	9	36	34.0
12	3	10	-	-	12	29	95	20	7	37	33.3
15	0	1	0	4	5	28	76	-	6	17	26.4
18	2	4	1	0	7	20	29	-	3	4	12.6
21	1	0	0	0	5	6	49	-	4	4	13.6
31	1	0	1	0	8	0	0	-	1	0	1.8
35	1	1	2	1	8	6	16	-	3	5	7.6
37	1	0	0	0	8	14	28	-	6	6	12.4
41	0	0	2	3	30	45	17	-	4	14	22.0
44	0	15	2	1	20	54	29	-	8	40	30.2
47	10	10	14	17	16	26	11	-	6	-	14.8
51	10	-	-	-	-	95	24	-	70	-	63.0
54	10	12	7	-	8	25	15	-	2	-	12.5
60	0	7	5	4	13	59	23	-	4	14	22.6
64	3	0	1	0	35	13	46	-	5	0	19.8
66	1	0	2	1	4	11	56	-	8	8	17.4
68	60	11	18	10	13	126	1590	190	80	175	362.3
71	160	200	22	58	29	250	1310	270	200	370	404.8

TABLE VIII (b) - MEMBRANE FILTER (MF) COUNTS, WATER SAMPLES, PINETTE RIVER

Station	Coliform MF Count per 100 ml. Seawater							
	Aug. 6, 1952	Aug. 14, 1952	Aug. 27, 1952	Sept. 10, 1952	Sept. 24, 1952	Oct. 9, 1952	June 16, 1953	July 1, 1953
1	11	1	2	2	17	-	19	27
3	4	3	3	-	9	1	23	64
5	0	0	8	0	28	0	30	73
7	7	2	8	0	8	1	25	94
9	0	1	6	0	9	1	14	35
11	0	2	11	3	3	1	10	42
13	0	3	7	0	18	1	14	71
15	11	4	10	0	45	6	28	78
17	6	2	6	4	6	7	64	98
19	11	3	21	8	7	7	120	145
21	-	-	-	-	-	-	3	30
23	3	0	3	5	5	1	3	24
25	0	0	3	0	11	1	1	10
27	0	0	0	0	2	0	2	10
29	0	0	1	16	4	0	2	7
31	0	0	1	0	0	0	2	6
33	0	0	0	0	2	0	1	8
35	0	0	0	0	2	0	2	1
37	-	-	0	0	0	0	3	0

It became evident, after preliminary membrane filtrations had been made in July, 1952, that modifications of the Environmental Health Center Endo's Broth Medium would be necessary before the procedure could be used for the estimation of coliform bacteria from seawater specimens because heavy concentrations of silt suspended in the seawater were deposited on the membrane filters during filtration. When a two-hour nutrient broth enrichment period preceded transfer of the membranes to Endo's Broth, non-lactose-fermenting bacteria developed profusely; coliform bacteria did not always develop their characteristic "sheen." On the other hand, if preliminary enrichment of the membranes was eliminated or if the enrichment period was reduced, the incidence of non-lactose-fermenting bacteria was reduced and coliform bacteria developed their characteristic "sheen", but the recovery of coliform bacteria was materially reduced.

A suitable modification of the Endo's Broth sulphite-fuchsin complex was discovered late in July, 1952. It was found necessary to increase the basic fuchsin content of the E.H.C. Endo's Broth six-fold. The recovery and differentiation of coliform bacteria on this modified Endo's Broth was much improved, and MF coliform counts agreed more closely with the coliform M.P.N. determinations on replicate water samples. Satisfactory results could not be obtained with dehydrated Endo's Broth prepared by Difco Laboratories, even when additional basic fuchsin was added.

The modified Endo's Broth developed in 1952 was used with considerable success during the 1953 study, although the adsorption of basic fuchsin by silt deposits remained a problem. It was found to be preferable to filter 25 ml. of the seawater specimen through each of four membranes to filtering 100 ml. through a single membrane.

Mean coliform MF counts and M.P.N. values from seawater specimens are compared in Tables IX (a) and IX (b). The ratios of coliform M.P.N.'s to MF counts for specimens of seawater from Vernon River and from Pinette River were 1 to 0.43 and 1 to 0.25 respectively in 1952, and 1 to 0.85 and 1 to 1.81 in 1953. The mean ratio of coliform M.P.N.'s to MF counts for all seawater samples examined in 1953 was 1 to 1.13.

6. Bacteriological Examination of Oysters

Coliform M.P.N. values, coliform plate counts (Violet Red Bile Agar), and enterococci M.P.N. values obtained from the analysis of oyster specimens from producing beds in Vernon River and Pinette River are recorded in Tables X (a and b) and XI (a and b).

During the 1952 study, only 4 of 14 oyster specimens taken from three sampling stations in Vernon River had coliform M.P.N.'s of 230 or less; mean coliform M.P.N. values, (omitting the very high values recorded for specimens taken on September 25, 1952), were 850, 358, and 893 respectively for specimens from Stations 48, 66, and 68. Oyster specimens taken from these same stations on May 21, 1953, had coliform M.P.N.'s of 330, 5,400, and 3,500 respectively. Of 10 oyster specimens taken on other dates in 1953 from the same stations, 5 had coliform M.P.N.'s of less than 230, and the mean values for specimens from stations 48, 66, and 68 were 179.3, 69.9, and 920 respectively.

The enterococci M.P.N.'s obtained in 1952 from 14 oyster specimens from Vernon River ranged from less than 18 to 91; 6 specimens had enterococci M.P.N.'s of more than 20. In 1953, enterococci M.P.N.'s obtained from 13 oyster specimens from the same stations ranged from less than 18 to 78; only 1 specimen gave an enterococci M.P.N. of more than 20.

TABLE IX (a) - COMPARISON OF MEAN COLIFORM MOST PROBABLE NUMBERS AND MF. COUNTS, VERNON RIVER AREA, P.E.I.

	1952		1953	
Sampling Station	Mean Coliform M.P.N.'s per 100 ml. seawater (4 Determinations)	Mean Coliform MF Counts for 100 ml. seawater (4 Determinations)	Mean Coliform M.P.N.'s per 100 ml. sea water (6 Determinations)	Mean Coliform MF Counts per 100 ml. seawater (6 Determinations)
1	46.0	48.3	169.8	118.0
4	35.3	14.3	40.8	87.5
7	21.8	5.8	49.3	56.3
9	39.0	6.3	75.8	34.0
12	22.9	6.5	36.0	33.3
15	10.7	1.3	21.2	26.4
18	6.7	1.8	11.5	12.6
21	2.6	0.2	26.4	13.6
31	3.3	0.5	7.9	1.8
35	4.9	1.3	4.2	7.6
37	3.7	0.2	10.3	12.4
41	1.9	1.3	22.4	22.0
44	28.6	4.5	7.0	30.2
47	51.0	12.8	23.5	14.8
51	23.0	10.0	103.0	63.0
54	35.4	9.7	11.3	12.5
60	12.5	4.0	23.7	22.6
64	8.5	1.0	14.4	19.8
66	4.8	1.0	13.2	17.4
68	123.5	25.0	488.5	362.3
71	136.3	110.0	451.5	404.8
Average	29.6	12.7	76.7	65.4
No. Samples	84	84	126	126

TABLE IX (b) - COMPARISON OF MEAN COLIFORM MOST PROBABLE NUMBERS AND MF COUNTS, PINETTE RIVER, P.E.I.

	1952		1953	
Sampling Station	Mean Coliform M.P.N.'s per 100 ml. seawater (6 Determinations)	Mean Coliform MF Counts per 100 ml. seawater (6 Determinations)	Mean Coliform M.P.N.'s per 100 ml. seawater (2 Determinations)	Mean Coliform MF Counts per 100 ml. seawater (2 Determinations)
1	10.5	6.6	23.0	23.0
3	11.8	4.0	31.0	43.5
5	26.3	6.0	21.5	51.5
7	18.3	4.4	20.0	59.5
9	6.5	2.8	15.4	24.5
11	12.4	3.3	23.0	27.0
13	41.5	4.8	18.0	42.5
15	40.0	11.8	56.0	53.0
17	27.0	5.1	12.4	81.0
19	58.0	9.5	46.0	132.5
23	2.8	2.8	10.5	13.5
25	4.1	2.5	12.5	5.5
27	3.5	0.5	8.8	6.0
29	2.4	3.5	7.5	4.5
31	2.8	0.2	4.5	4.0
33	2.0	0.3	4.9	4.5
35	1.0	0.3	4.3	1.5
Average	15.9	4.0	18.8	34.0
No. Samples	102	102	34	34

Page 102 was omitted due to a

typographical error in pagination.

TABLE X (a) - OYSTER SPECIMENS, VERNON RIVER AREA, 1952

Dates of Sampling	Coliform MPN per 100 ml.			Enterococci MPN per 100 ml.			Coliform Plate Count per ml.		
	Sampling Station Number								
	48	66	68	48	66	68	48	66	68
July 29	1300	20	-	<18	<18	-	10	1	-
Aug. 5	-	130	1100	-	<18	<18	-	2	10
Aug. 21	790	110	790	18	<18	45	3	2	4
Sept. 9	460	230	790	20	20	78	5	1	7
Sept.25	24000	13000	3500	91	78	91	160	160	50
Oct. 9	-	1300	-	-	<18	-	-	2	-
Mean Values*	850	358	893	<18	<18	41	6	1.6	7

*Omitting Sept. 25 data.

TABLE X (b) - OYSTER SPECIMENS, VERNON RIVER AREA, 1953. SAMPLING STATIONS 48, 66, 68

DATE OF SAMPLING	COLIFORM M.P.N. PER 100 ML.			ENTEROCOCCI M.P.N. PER 100 ML.			COLIFORM PLATE COUNT PER ML.			EIJKMAN M.P.N. PER 100 ML.		
	STA. 48	STA. 66	STA. 68	STA. 48	STA. 66	STA. 68	STA. 48	STA. 66	STA. 68	STA. 48	STA. 66	STA. 68
May 15	-	-	1,300	-	-	<18	-	-	76	-	-	20
May 18	130	110	490	<18	<18	78	27	11	81	78	45	170
May 21	330	5,400	3,500	20	20	20	25	173	400	78	130	330
June 4	78	20	-	20	<18	-	42	7	-	45	20	-
June 9	330	78	1,100	<18	<18	20	21	9	74	68	20	460
June 23	-	-	790	-	-	<18	-	-	34	-	-	790
Mean Values	217	1,400	1,400	<18	<18	23	29	50	133	67	54	354

TABLE XI (a) - OYSTER SPECIMENS, PINETTE RIVER, 1952

Dates of Sampling	Coliform MPN per 100 ml.			Enterococci MPN per 100 ml.			Coliform Plate Count per ml.		
	Sampling Station Number								
	7	8	16	7	8	16	7	8	16
July 31	16000	230	230	20	<18	20	TNC	2	2
Aug. 6	-	490	700	-	20	20	-	4	10
Aug. 14	1700	1300	580	20	20	18	15	12	2
Aug. 27	490	330	2400	<18	<18	18	2	<1	TNC
Sept.10	110	45	78	20	<18	<18	<1	3	<1
Sept.24	1300	170	490	68	<18	20	1	<1	<1
Oct. 14	1300	-	230	130	-	<18	8	-	2
Mean Values	3483	428	673	43	<18	<18	-	3.5	-

TABLE XI (b) - OYSTER SPECIMENS, PINETTE RIVER, 1953

Dates of Sampling	Coliform MPN per 100 ml.			Enterococci MPN per 100 ml.			Coliform Plate Count per ml.			Eijkman MPN per 100 ml.		
	Sampling Station Number											
	7	8	16	7	8	16	7	8	16	7	8	16
June 23	2,400	130	1,700	<18	<18	20	14	23	30	330	20	310
July 1	230	-	1,300	<18	-	40	68	-	52	78	-	230
Mean Values	1,315	-	1,500	< 18	-	30	41	-	41	204	-	270

Of 19 oyster specimens taken from Pinette River during the 1952 study, 12 had coliform M.P.N.'s of more than 230; the mean coliform M.P.N. values for oyster specimens from sampling stations 7, 8, and 16 were 3, 483, 428, and 673 respectively. There was a marked week-to-week-variation in the coliform M.P.N. of oysters taken from these stations. Three of 5 oyster specimens taken from these same stations in 1953 had coliform M.P.N.'s of more than 230. Of the 24 oyster specimens taken from Pinette River during the investigation, only 3 had enterococci M.P.N.'s of more than 20.

While the direct isolation of coliform bacteria for classification was the primary reason for plating all oyster specimens on Violet Red Bile Agar (Difco), coliform plate counts were recorded. A fairly close correlation between coliform (V.R.B. Agar) plate counts and coliform M.P.N.'s was observed during the 1952 study, but the ratio of coliform M.P.N.'s to coliform (V.R.B. Agar) plate counts for all oyster specimens examined during the 1953 study was 1 to 5.7.

7. Coliform Bacteria in Soil

Coliform plate counts (Violet Red Bile Agar) were made on 65 specimens of soil from 6 sampling stations on the Vernon River watershed (Fig.I). A description of the soil sampling sites is given in Table XII. Coliform plate counts are recorded in Table XIII.

Coliform plate counts for soil specimens ranged from less than 10 to 34,000 per gram; no coliform bacteria were isolated in 14 of 65 soil specimens, while 23 other soil specimens had coliform plate counts of less than 100 per gram. Soil specimens from Stations A, B, and F yielded more coliform bacteria than did speciments from Stations C, D, and E,

although there was a considerable variation in the numbers of coliform bacteri isolated from soil specimens from the same stations on the different sampling dates.

8. Coliform Bacteria and Enterococci in Faeces

Coliform plate counts (Violet Red Bile Agar, Difco) were made on 40 specimens of faeces (cattle, horse, swine, chicken, seagull, and human) collected on the Vernon River watershed. One specimen of sewer sludge from a sewer at Vernon Bridge on the Vernon River was also tested for coliform bacteria. Enterococci M.P.N.'s were determined for 23 of the faeces specimens. Results are tabulated in Table XIV (a and B).

Coliform plate counts from faeces specimens ranged from 170 to 2,400,000,000 per gram of faeces. No coliform bacteria could be isolated from 4 faeces specimens in the dilutions used. The coliform content of dried faeces was markedly lower than that of fresh faeces.

More coliform bacteria than enterococci were isolated from specimens of fresh faeces. The coliform to enterococci ratios, for faeces specimens with definitive coliform counts and enterococci M.P.N.'s, were 1 to 0.52 for human faeces (5 specimens), and 1 to 0.03 for animal and bird faeces (9 specimens); for 3 specimens of dried animal faeces, however, the coliform to enterococci ratio was 1 to 5.4.

TABLE XII - SOIL SAMPLING STATIONS VERNON RIVER AREA

Station	Description
A	West shore Vernon River, Near Vernon Bridge; hayfield, sloping to river; sampling site approximately 250 ft. from water; never pastured during study period.
B	West shore Orwell River; potato field, sloping to river, sampling site approximately 500 ft. from water; surrounded by pasture lands.
C	West shore Vernon River, at Vernon Bridge; bridge causeway; sampling site approximately 25 ft. from water.
D	West shore Seal River, at Sea River Bridge; pasture land; sampling site approximately 100 ft. from water; direct drainage to river.
E	West shore, China Point; 500 ft. upstream from wharf; on shore, below hayfield.
F	West side China Point Road; pasture land, draining directly to stream; sampling site approximately 1,000 ft. from stream.

TABLE XIII - SOIL SPECIMENS, VERNON RIVER AREA, COLIFORM PLATE COUNTS (V.R.B. AGAR)

Date of Sampling	Coliform Plate Count per gram of soil					
	Soil Sampling Station					
	A	B	C	D	E	F
1952						
July 25	<10	34,000	<10	10	<10	40
Aug. 19	5	120	70	50	<10	80
Sept. 4	<10	100	<10	<10	20	10
Sept.17	20	<10	20	<10	<10	1,200
Oct. 3	500	800	20	30	<10	70
Oct. 16	15	700	10	40	<10	8,000
1953						
May 10	185	60	225	14	<10	1,350
May 16	6,700	480	230	45	-	-
May 20	1,300	7,500	80	320	170	-
May 27	2,100	790	440	1,000	-	-
June 12	290	1,300	260	40	-	-
June 18	110	200	54	85	< 10	300

TABLE XIV (a) - FAECES SPECIMENS, 1952 COLIFORM PLATE COUNTS (V.R.B. AGAR)

Faeces Specimen	Condition	Coliform Plate Count per gram (Thousands)
Cattle # 1	Fresh	10,000
Cattle # 2	Dry	<10
Cattle # 3	Dry	<10
Cattle # 4	Dry	0.2
Cattle # 5	Fresh	38,000
Cattle # 6	Dry	0.55
Seagull # 1	Fresh	12,000
Seagull # 2	Fresh	270,000
Seagull # 3	Dry	<10
Horse	Fresh	100,000
Swine	Fresh	6,000
Chicken	Fresh	75,000
Sewer Sludge	Fresh	20,000

TABLE XIV (b) - COLIFORM PLATE COUNTS AND ENTEROCOCCI

MOST PROBABLE NUMBERS FROM FAECES, 1953

FAECES SPECIMEN	CONDITION	COLIFORM PLATE COUNT PER GRAM (Thousands)	ENTEROCOCCI MPN PER GRAM (Thousands)
Cattle # 1	Fresh	200	2
Cattle # 2	Fresh	1,800	<1.8
Cattle # 3	Fresh	18,000	<1.8
Cattle # 4	Fresh	100	<1.8
Cattle # 5	Fresh	400	2
Cattle # 6	Dry	75	-
Cattle # 7	Fresh	7,800	-
Cattle # 8	Fresh	3,800	-
Cattle # 9	Dry	<10	4.5
Cattle # 10	Fresh	18,000	0.9
Cattle # 11	Dry	2	22
Horse # 1	Dry	6	2.3
Horse # 2	Dry	10	7.8
Horse # 3	Fresh	980	160
Horse # 4	Fresh	2,500	160
Horse # 5	Fresh	580	9.2
Chicken # 1	Fresh	10,000	1,600
Chicken # 2	Fresh	9,000	>160
Chicken # 3	Fresh	23,000	>160
Seagull # 1	Fresh	450,000	1,600
Seagull # 2	Fresh	410,000	1,600
Seagull # 3	Fresh	15,000	-
Seagull # 4	Fresh	50,000	-
Human # 1	Fresh	160,000	920
Human # 2	Fresh	360,000	1,600
Human # 3	Fresh	140,000	240,000
Human # 4	Fresh	780,000	160,000
Human # 5	Fresh	2,400,000	1,600,000

9. Classification of Coliform Bacteria Isolated from Water, Oysters, Soil, and Faeces

One thousand, eight hundred and ninety-one coliform bacteria isolated from specimens of seawater, stream water, oysters, soil, and faeces from the study areas were classified according to their biochemical reactions (Table XV). Data obtained from the classification of these bacteria appear in Tables XVI to XX inclusive. Thirteen distinct coliform types were isolated; these were grouped into three major categories, "coli", "intermediate", and "aerogenes" types (see Table XXI).

During the 1952 study, coliform bacteria of the "coli" type were predominant in specimens of seawater, soil, and faeces. Three hundred and ninety-nine (80.1 per cent) of the 498 coliform bacteria isolated from seawater were "coli" types, while only 34 (6.8 per cent) and 65 (13.1 per cent) of the isolated strains proved to be "intermediate" and "aerogenes" types respectively; similarly, 80 (86.0 per cent) of 93 coliform strains isolated from faeces specimens were "coli" types, as were 60 (66.7 per cent) of 90 coliform bacteria isolated from soil specimens. It is evident that "coli" types constituted the major coliform bacterial flora present on the watersheds and in the seawater of the study areas during the 1952 portion of the investigation. It is therefore surprising to note that only 33 (31.1 per cent) of 106 coliform strains isolated from 33 oyster specimens from the study areas were "coli" types; 3 (2.8 per cent) were "intermediate" types, and 70 (66.1 per cent) were "aerogenes" types.

TABLE XV - CLASSIFICATION OF COLIFORM BACTERIA

Type	44° C.	Indol	M.R.	V.P.	Cit.	Gel.
E. coli Type I	+	+	+	-	-	-
E. coli Type II	-	-	+	-	-	-
Irregular I	-	+	+	-	-	-
Irregular II	+	-	+	-	-	-
Irregular III	-	-	+	-	-	+
Intermediate Type I	-	-	+	-	+	-
Intermediate Type II	-	+	+	-	+	-
Irregular IV	-	-	+	-	+	+
A. aerogenes Type I	-	-	-	+	+	-
A. aerogenes Type II	-	+	-	+	+	-
A. cloacae	-	-	-	+	+	+
Irregular V	-	-	-	+	-	-
Irregular VI	+	-	-	+	+	-

TABLE XVI (a) - CLASSIFICATION OF COLIFORM BACTERIA ISOLATED FROM SEAWATER, VERNON RIVER AREA, STATIONS 1-71, 1952

Coliform types isolated 1952	Sector A		Sector B		Sector C		Sector D	
	No.	%	No.	%	No.	%	No.	%
E. coli Type I	122	(75.8)	39	(72.2)	62	(69.7)	6	(100)
E. coli Type II	1	(0.6)	1	(1.85)		-		-
Irregular I	2	(1.2)	1	(1.85)		-		-
Irregular II	4	(2.5)	1	(1.85)	4	(4.5)		-
Intermediate Type I	9	(5.6)	1	(1.85)	7	(7.9)		-
Intermediate Type II		-	1	(1.85)		-		-
Irregular IV		-		-	1	(1.1)		-
A. aerogenes Type I	13	(8.1)	6	(11.1)	12	(13.5)		-
A. aerogenes Type II	8	(5.0)	4	(7.4)	1	(1.1)		-
A. cloacae	1	(0.6)		-	1	(1.1)		-
Irregular VI	1	(0.6)		-	1	(1.1)		-
Totals	161		54		89		6	

TABLE XVI (b) - CLASSIFICATION OF COLIFORM BACTERIA ISOLATED FROM SEAWATER, VERNON RIVER AREA, STATIONS 1-71, 1953

Coliform types isolated 1953	Sector A		Sector B		Sector C		Sector D	
	No.	%	No.	%	No.	%	No.	%
E. coli Type I	32	(28.3)	16	(10.3)	6	(7.9)	2	(11.8)
E. coli Type II	9	(8.0)	27	(17.4)	16	(21.1)	2	(11.8)
Irregular I	4	(3.5)		-	1	(1.3)		-
All "coli" Types	45	(39.8)	43	(27.7)	23	(30.3)	4	(23.5)
Intermediate Type I	23	(20.4)	27	(17.4)	8	(10.5)	2	(11.8)
Intermediate Type II	2	(1.8)	3	(1.9)		-		-
Irregular IV	1	(0.9)	1	(0.6)	1	(1.3)		-
All "intermediate" Types	26	(23.0)	31	(20.0)	9	(11.8)	2	(11.8)
A. aerogenes Type I	25	(22.1)	53	(34.2)	22	(28.9)	8	(47.1)
A. aerogenes Type II	9	(8.0)	9	(5.8)	9	(11.8)	1	(5.9)
A. cloacae	7	(6.2)	14	(9.0)	7	(9.2)		-
Irregular V	1	(0.9)	5	(3.2)	6	(7.9)	2	(11.8)
All "aerogenes Types	42	(37.2)	81	(52.3)	44	(57.9)	11	(64.7)
Totals	113		155		76		17	

TABLE XVII (a) - CLASSIFICATION OF COLIFORM BACTERIA ISOLATED FROM SEA-WATER AND OYSTERS, PINETTE RIVER, STATIONS 1-37, 1952

Coliform types isolated 1952	Seawater, Stations 1 - 37		Oysters, Station 7		Oysters, Station 8		Oysters, Station 16	
	No.	%	No.	%	No.	%	No.	%
E. coli Type I	135	(71.8)	2	(18.2)	7	(38.9)	3	(25.0)
Irregular I	3	(1.6)	-		-		1	(8.3)
Irregular II	17	(9.1)	-		-		-	
Irregular III	1	(0.5)	-		-		-	
Intermediate I	11	(5.9)	-		-		-	
Intermediate II	3	(1.6)	-		-		-	
Irregular IV	1	(0.5)	-		-		-	
A. aerogenes I	10	(5.3)	6	(54.5)	9	(50.0)	7	(58.4)
A. aerogenes II	6	(3.2)	3	(27.3)	1	(5.55)	-	
A. cloacae	-		-		-		1	(8.3)
Irregular VI	1	(0.5)	-		1	(5.55)	-	
Totals	188		11		18		12	

TABLE XVII (b) - CLASSIFICATION OF COLIFORM BACTERIA ISOLATED FROM SEA-WATER AND OYSTERS, PINETTE RIVER, STATIONS 1-37, 1953

Coliform types isolated 1953	Seawater, Stations 1 - 37		Oysters, Station 7		Oysters, Station 8		Oysters Station 16	
	No.	%	No.	%	No.	%	No.	%
E. coli Type I	18	(20.9)	-		-		-	
E. coli Type II	4	(4.7)	-		-		-	
Irregular I	3	(3.5)	-		-		-	
Irregular II	1	(1.2)	-		-		-	
All "coli" Types	26	(30.2)	-		-		-	
Intermediate Type I	11	(12.8)	6	(100.0)	1	(33.3)	2	(66.7)
Intermediate Type II	6	(7.0)	-		-		-	
All "intermediate" Types	17	(19.8)	6	(100.0)	1	(33.3)	2	(66.7)
A. aerogenes Type I	16	(18.6)	-		-		-	
A. aerogenes Type II	19	(22.1)	-		-		-	
A. cloacae	3	(3.5)	-		-		1	(33.3)
Irregular V	2	(2.3)	-		-		-	
Irregular VI	3	(3.5)	-		2	(66.7)	-	
All "aerogenes" Types	43	(50.0)	-		2	(66.7)	1	(33.3)
Totals	86		6		3		3	

TABLE XVIII - CLASSIFICATION OF COLIFORM BACTERIA ISOLATED FROM OYSTERS, VERNON RIVER AREA

Coliform types isolated, 1952 and 1953	Station 48		66		68	
	No.	%	No.	%	No.	%
(a) 1952						
E. coli Type I	4	(25.0)	7	(31.8)	7	(25.9)
Irregular I	-		1	(4.55)	1	(3.7)
Intermediate Type I	1	(6.25)	1	(4.55)	1	(3.7)
A. aerogenes Type I	11	(68.75)	13	(59.1)	12	(44.5)
A. aerogenes Type II	-		-		4	(14.8)
A. cloacae	-		-		2	(7.4)
Totals	16		22		27	
(b) 1953						
E. coli Type I	-		-		7	(23.3)
Intermediate Type I	-		2	(20.0)	7	(23.3)
A. aerogenes Type I	-		7	(70.0)	12	(40.0)
A. aerogenes Type II	-		1	(10.0)	1	(3.3)
A. cloacae	1	(100.0)	-		3	(10.0)
All "aerogenes Types	1	(100.0)	8	(80.0)	16	(53.3)
Totals	1		10		30	

TABLE XIX - CLASSIFICATION OF COLIFORM BACTERIA ISOLATED FROM SOIL SPECIMENS, VERNON RIVER AREA, 1952 AND 1953

SOIL STATIONS	NUMBERS AND PERCENTAGES OF COLIFORM TYPES ISOLATED																				Totals
	E. coli I		E. coli II		Irregular II		Intermed. I		Irregular IV		A. aerogenes I		A. aerogenes II		A. cloacae		Irregular V				
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%			
(a) 1952																					
A	-	-	-	-	-	-	-	-	-	-	5	55.6	1	11.1	3	33.3	-	-			9
B	16	55.2	-	-	2	6.9	-	-	-	-	9	31.0	2	6.9	-	-	-	-			29
C	11	78.6	-	-	-	-	-	-	-	-	1	7.1	2	14.3	-	-	-	-			14
D	2	28.5	-	-	-	-	1	14.3	1	14.3	3	42.9	-	-	-	-	-	-			7
E	-	-	-	-	-	-	-	-	-	-	1	100.0	-	-	-	-	-	-			1
F	24	80.0	2	6.7	3	10.0	-	-	1	3.3	-	-	-	-	-	-	-	-			30
TOTALS	53	58.9	2	2.2	5	5.6	1	1.1	2	2.2	19	21.1	5	5.6	3	3.3	-	-			90
(b) 1953																					
A	-	-	1	2.0	-	-	21	42.0	-	-	25	50.0	-	-	3	6.0	-	-			50
B	-	-	-	-	-	-	19	33.9	2	3.6	32	57.1	-	-	1	1.8	2	3.6			56
C	11	19.0	-	-	-	-	11	19.0	1	1.7	25	43.3	2	3.4	7	12.1	1	1.7			58
D	1	6.3	-	-	-	-	1	6.3	-	-	5	31.3	-	-	9	56.3	-	-			16
E	-	-	4	57.1	-	-	2	28.6	-	-	1	14.3	-	-	-	-	-	-			7
F	-	-	-	-	-	-	-	-	-	-	20	90.0	-	-	1	5.0	1	5.0			22
TOTALS	12	5.7	5	2.4	-	-	54	25.8	3	1.4	108	51.7	2	0.9	21	10.0	4	1.9			209

TABLE XX - CLASSIFICATION OF COLIFORM BACTERIA ISOLATED FROM FAECES SPECIMENS, VERNON RIVER AREA, 1952 AND 1953

FAECES	NUMBERS AND PERCENTAGES OF COLIFORM TYPES ISOLATED																			
	E. coli I		E. coli II		Irregular I		Irregular II		Intermed. I		Intermed. II		A. aerogenes I		A. aerogenes II		A. cloacae		Irregular VI		Totals
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
(a) 1952																					
CATTLE (4)	28	87.5	-	-	1	3.1	-	-	-	-	3	9.4	-	-	-	-	-	-	-	-	32
SWINE (1)	10	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10
HORSE (1)	8	88.9	1	11.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9
SEAGULL (2)	19	86.5	-	-	-	-	1	4.5	-	-	-	-	-	-	-	-	-	-	2	9.0	22
CHICKEN (1)	10	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10
SEWER SLUDGE (1)	2	20.0	-	-	-	-	-	-	-	-	1	10.0	-	-	7	70.0	-	-	-	-	10
TOTALS	77	82.7	1	1.1	1	1.1	1	1.1	-	-	4	4.3	-	-	7	7.5	-	-	2	2.2	93
(b) 1953																					
CATTLE (11)	82	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	82
HORSE (5)	52	98.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1.9	-	-	53
MANURE (1)	6	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
CHICKEN (3)	38	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	38
SEAGULL (4)	28	35.0	1	1.3	20	25.0	3	3.8	8	10.0	3	3.8	6	7.5	8	10.0	3	3.8	-	-	80
HUMAN (5)	60	89.6	-	-	-	-	-	-	2	3.0	-	-	4	6.0	1	1.5	-	-	-	-	67
TOTALS	266	81.6	1	0.3	20	6.1	3	0.9	10	3.1	3	0.9	10	3.1	9	2.8	4	1.2	-	-	326

TABLE XXI (a) - CLASSIFICATION OF ALL COLIFORM BACTERIA ISOLATED IN 1952

Source	Totals	All "coli" Types		All "Intermed." Types		All "aerogenes" Types	
		No.	%	No.	%	No.	%
All Faeces Specimens	93	80	86.0	4	4.3	9	9.7
All Soil Specimens	90	60	66.7	3	3.3	27	30.0
Seawater, Vernon River Area	310	243	78.4	19	6.1	48	15.5
Seawater, Pinette River	188	156	83.0	15	8.0	17	9.0
All Seawater Specimens	498	399	80.1	34	6.8	65	13.1
Oysters, Vernon River Area	65	20	30.8	3	4.6	42	64.6
Oysters, Pinette River	41	13	31.7	0	-	28	68.3
All Oyster Specimens	106	33	31.1	3	2.8	70	66.1
Totals	787	572	72.7	44	5.6	171	21.7

TABLE XXI (b) - CLASSIFICATION OF ALL COLIFORM BACTERIA ISOLATED IN 1953

Source	Totals	All "coli" Types		All "Intermed." Types		All "aerogenes" Types	
		No.	%	No.	%	No.	%
All Faeces Specimens	326	290	89.0	13	4.0	23	7.0
All Soil Specimens	209	17	8.1	57	27.3	135	64.6
Seawater, Vernon River Area	361	115	31.9	68	18.8	178	49.3
Seawater, Pinette River	86	26	30.2	17	19.8	43	50.0
All Seawater Specimens	447	141	31.5	85	19.0	221	49.4
Oysters, Vernon River Area	41	7	17.1	9	22.0	25	60.9
Oysters, Pinette River	12	0	-	9	75.0	3	25.0
All Oyster Specimens	53	7	13.2	18	34.0	28	52.8
Vernon Stream Samples	69	30	43.5	6	8.7	33	47.8
Totals	1,104	485	43.9	179	16.2	440	39.9

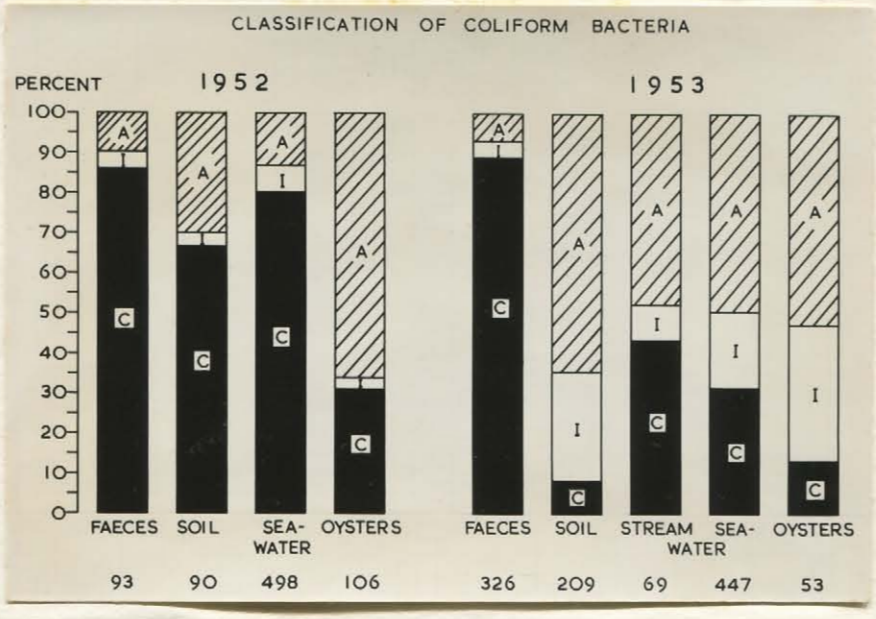
Escherichia coli type I was the dominant "coli" type isolated during the 1952 study;Aerobacter aerogenes type I was the dominant "aerogenes" type. Six (4.8 per cent) of 124 A. aerogenes type I strains produced gas in MacConkey's Broth at 44° C., and were classified as Irregular VI.

During the 1953 study, coliform bacteria of the "coli" type were the dominant coliform type in specimens of faeces; in specimens of soil, water, and oysters, however, "aerogenes" types were predominant. Two hundred and twenty-one (49.4 per cent) of 447 coliform bacteria isolated from seawater during the 1953 study were "aerogenes" types, while 85 (19.0 per cent) and 141 (31.5 per cent) of the coliforms were "intermediate" and "aerogenes" types respectively. Similarly, 135 (64.6 per cent) of 209 coliform bacteria isolated from soil specimens were "aerogenes" types, as were 28 (52.8 per cent) of 53 coliform bacteria isolated from oyster specimens. Two hundred and ninety (89.0 per cent) of 326 coliform bacteria isolated from faeces specimens were "coli" types.

E. coli type I was the dominant "coli" type, and A. aerogenes type I was the dominant "aerogenes" type. Five (1.9 per cent) of 266 A.aerogenes type I strains produced acid and gas in MacConkey's Broth at 44° C., and were classified as Irregular VI.

The distribution of the coliform types in the various sources is shown in Figure IV.

FIGURE IV - THE INCIDENCE OF COLIFORM BACTERIAL TYPES IN SPECIMENS OF FAECES, SOIL, WATER, AND OYSTERS, VERNON RIVER AND PINETTE RIVER, P.E.I., 1952 AND 1953



A: "aerogenes" coliform types

I: "intermediate"coliform types

C: "coli" coliform types

10. Isolation and Classification of Slow-Lactose-Fermenting Bacteria

In addition to the 1,891 coliform bacteria isolated from specimens of water, soil, oysters, and faeces by the Membrane Filter and Violet Red Bile Agar Plate Count techniques, 1,229 other Gram negative bacteria were isolated from the same membranes and plates.. One hundred and thirty of these strains were isolated during the 1952 study, and 1,099 were isolated in 1953. All of the 1,229 strains were isolated from colonies which were identical in appearance with colonies formed by typical coliform strains, and they were counted as coliform bacteria when plate and MF counts were determined; all, however, failed to ferment lactose with the production of acid and gas within 48 hours at 37° C. Seventeen of the 130 cultures isolated during the 1952 study produced slight amounts of gas from standard lactose broth after incubation at 37° C. for 3 to 21 days. Eight-five of the 1,099 strains isolated in 1953 fermented lactose with the production of acid and gas in 3 - 7 days at 37° C., 274 produced acid and gas from lactose broth after incubation at 37° C. for 8 - 21 days, and 373 strains fermented lactose, with the production of acid only, within the 21-day incubation period; the remaining 367 strains failed to ferment lactose in 21 days incubation at 37° C.

Since the 1,229 non-lactose-fermenting strains closely resembled coliform bacteria in morphology and biochemical reactions, they were classified in the same manner as the coliform bacteria previously identified. Results of the classification of all Gram negative bacteria which fermented standard lactose broth slowly or not at all appear in Tables XXII to XXV inclusive.

TABLE XXII (a) - CLASSIFICATION OF COLIFORM BACTERIA WHICH FAIL TO FERMENT LACTOSE WITH THE PRODUCTION OF GAS AT 37° C., IN 48 HOURS, 1952

Coliform Type	Source							
	All Sources		Soil		Seawater		Oysters	
	No.	%	No.	%	No.	%	No.	%
E. coli I	1	0.8	-		1	1.0	-	
E. coli II	1	0.8	-		1	1.0	-	
Irregular I	3	2.3	-		3	3.1	-	
Intermediate I	13	10.0	4	19.0	7	7.3	2	15.4
Intermediate II	7	5.3	-		7	7.3	-	
A. aerogenes I	56	43.1	16	76.2	36	37.6	4	30.8
A. aerogenes II	21	16.2	-		17	17.7	4	30.8
A. cloacae	28	21.5	1	4.8	24	25.0	3	23.0
Totals	130		21		96		13	

TABLE XXII (b) - CLASSIFICATION OF COLIFORM BACTERIA WHICH FAIL TO FERMENT LACTOSE WITH THE PRODUCTION OF GAS AT 37° C., IN 48 HOURS, 1952

	All Types	All "coli" Types		All "Intermed." Types		All "aerogenes" Types	
Source	Totals	No.	%	No.	%	No.	%
All Faeces Specimens	1	-		-		1	100.0
All Soil Specimens	21	-		4	19.0	17	81.0
Seawater, Vernon River Area	70	4	5.7	9	12.8	57	81.5
Seawater, Pinette River	26	1	3.8	5	19.2	20	77.0
All Seawater Specimens	96	5	5.2	14	14.6	77	80.2
Oysters, Vernon River Area	7	-		-		7	100.0
Oysters, Pinette River	6	-		2	33.3	4	66.7
All Oyster Specimens	13			2	15.4	11	84.6
Total Isolations	131	5	3.8	20	15.3	106	80.9

TABLE XXIII (a)-CLASSIFICATION OF COLIFORM BACTERIA WHICH FAIL TO FERMENT LACTOSE WITH THE PRODUCTION OF GAS AT 37° C., IN 48 HOURS, ISOLATED FROM SEAWATER SPECIMENS, VERNON RIVER AREA, STATIONS 1-71, 1953

Type Classification	Sector A		Sector B		Sector C		Sector D	
	No.	%	No.	%	No.	%	No.	%
E. coli Type II	4	(2.8)	6	(2.5)	1	(0.6)	-	
Irregular I	16	(11.1)	1	(0.4)	8	(4.8)	1	(3.3)
Irregular II	3	(2.0)	5	(2.1)	-		-	
All "coli" Types	23	(16.0)	12	(5.1)	9	(5.5)	1	(3.3)
Intermediate Type I	11	(7.6)	10	(4.2)	11	(6.7)	1	(3.3)
Intermediate Type II	5	(3.5)	2	(0.8)	-		-	
Irregular IV	-		2	(0.8)	-		-	
All "intermediate" Types	16	(11.1)	14	(5.9)	11	(6.7)	1	(3.3)
A. aerogenes Type I	24	(16.7)	53	(22.4)	47	(28.5)	7	(23.3)
A. aerogenes Type II	33	(22.9)	49	(20.7)	38	(23.0)	8	(26.7)
A. cloacae	25	(17.4)	61	(25.7)	35	(21.2)	9	(30.0)
Irregular V	23	(16.0)	48	(20.3)	25	(15.2)	4	(13.3)
All "aerogenes" Types	105	(72.9)	211	(89.0)	145	(87.9)	28	(93.3)
Totals	144		237		165		30	

TABLE XXIII (b) - CLASSIFICATION OF COLIFORM BACTERIA WHICH FAIL TO FERMENT LACTOSE WITH THE PRODUCTION OF GAS AT 37° C., IN 48 HOURS, ISOLATED FROM SEAWATER AND OYSTER SPECIMENS, PINETTE RIVER, STATIONS 1 - 37, 1953

Type Classification	Seawater Stations 1 - 37		Oysters, Station 7		Oysters, Station 8		Oysters, Station 16	
	No.	%	No.	%	No.	%	No.	%
E. coli Type II	1	(0.5)	1	(7.1)	6	(54.5)	2	(7 .4)
Irregular I	3	(1.6)	-		-		-	
All "coli" Types	4	(2.1)	1	(7.1)	6	(54.5)	2	(7.4)
Intermediate Type I	6	(3.2)	-		-		1	(3.7)
Intermediate Type II	8	(4.2)	-		-		-	
All "intermediate" Types	14	(7.4)	-		-		1	(3.7)
A. aerogenes Type I	18	(9.5)	3	(21.4)	5	(45.5)	1	(3.7)
A. aerogenes Type II	121	(64.0)	9	(64.3)	-		17	(63.0)
A. cloacae	29	(15.3)	1	(7.1)	-		6	(35.3)
Irregular V	3	(1.6)	-		-		-	
All "aerogenes" Types	171	(90.5)	13	(92.9)	5	(45.5)	24	(88.9)
Totals	189		14		11		27	

TABLE XXIII(c) - CLASSIFICATION OF COLIFORM BACTERIA WHICH FAIL TO FERMENT LACTOSE WITH THE PRODUCTION OF GAS AT 37° C., IN 48 HOURS, ISOLATED FROM OYSTER SPECIMENS, VERNON RIVER AREA, 1953

Type Classification	Station 48		Station 66		Station 68	
	No.	%	No.	%	No.	%
E. coli Type II	2	(4.2)	18	(45.0)	13	(22.8)
Irregular II	-		-		1	(1.8)
All "coli" Types	2	(4.2)	18	(45.0)	14	(24.6)
Intermediate Type I	3	(6.3)	4	(10.0)	5	(8.8)
Intermediate Type II	-		-		1	(1.8)
All "intermediate" Types	3	(6.3)	4	(10.0)	6	(10.5)
A. aerogenes Type I	27	(56.3)	10	(25.0)	6	(10.5)
A. aerogenes Type II	3	(6.3)	5	(12.5)	14	(24.6)
Irregular V	1	(2.1)	1	(2.5)	10	(17.5)
A. cloacae	12	(25.0)	2	(5.0)	7	(12.3)
All "aerogenes" Types	43	(89.6)	18	(45.0)	37	(64.9)
Totals	48		40		57	

TABLE XXIV - CLASSIFICATION OF COLIFORM BACTERIA WHICH FAIL TO FERMENT LACTOSE WITH THE PRODUCTION OF GAS AT 37°C. IN 48 HOURS, ISOLATED FROM SOIL SPECIMENS, VERNON RIVER AREA, 1953

SOIL STATIONS	NUMBERS AND PERCENTAGES OF COLIFORM TYPES ISOLATED (LACTOSE-NEGATIVE, 37°C., 48 HOURS)																	
	E. coli II		Irregular II		Intermed. I		Intermed. II		Irregular IV		A. aerogenes I		A. aerogenes II		A. cloacae		Irregular V		Totals
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
A	2	8.0	-	-	1	4.0	-	-	-	-	19	76.0	1	4.0	1	4.0	1	4.0	25
B	-	-	-	-	5	15.6	-	-	-	-	10	31.3	-	-	17	53.1	-	-	32
C	-	-	2	13.3	2	13.3	1	6.7	-	-	7	46.7	2	13.3	-	-	1	6.7	15
D	5	25.0	-	-	3	15.0	-	-	1	5.0	6	30.0	-	-	4	20.0	1	5.0	20
E	1	14.3	-	-	1	14.3	1	14.3	-	-	1	14.3	-	-	3	42.9	-	-	7
TOTALS	8	8.1	2	2.0	12	12.1	2	2.0	1	1.0	43	43.4	3	3.0	25	25.3	3	3.0	99

TABLE XXV - CLASSIFICATION OF COLIFORM BACTERIA WHICH FAIL TO FERMENT LACTOSE WITH THE PRODUCTION OF GAS AT 37° C., IN 48 HOURS, 1953

Source	Totals	All "coli" Types		All "Intermed." Types		All "aerogenes" Types	
		No.	%	No.	%	No.	%
All Faeces Specimens	17	2	11.8	11	64.7	4	23.5
All Soil Specimens	99	10	10.1	15	15.2	74	74.7
Seawater, Vernon River	576	45	7.8	42	7.3	489	84.9
Seawater, Pinette River	189	4	2.1	14	7.4	171	90.5
All Seawater Specimens	765	49	6.4	56	7.3	660	86.3
Oysters, Vernon River	145	34	23.4	13	9.0	98	67.6
Oysters, Pinette River	52	9	17.3	1	1.9	42	80.8
All Oyster Specimens	197	43	21.8	14	7.1	140	71.1
Stream Samples, Vernon River	21	2	9.5	2	9.5	17	81.0
Totals	1099	106	9.6	98	8.9	895	81.5

The incidence of slow- or non-lactose-fermenting strains in the various test specimens is given in Table XXVI.

TABLE XXVI - INCIDENCE OF COLIFORM BACTERIA WHICH FAIL TO FERMENT LACTOSE WITH THE PRODUCTION OF GAS AT 37° C. IN 48 HOURS

	Non-Lactose-Fermenters (48 Hours, 37° C.) Percentage of All Isolations	
Source	1952	1953
Faeces	0.0	5.0
Stream Water	-	23.3
Soil	19.0	32.1
Seawater	16.2	63.1
Oysters	10.9	78.8

11. Confirmation of Positive Presumptive Lactose Broth Cultures in Eijkman Lactose Medium (Difco), with Incubation at 44° C. for 48 Hours

During the 1953 study, all positive presumptive standard lactose broth cultures from Standard Methods tests for coliform bacteria in selected seawater and oyster specimens were confirmed by transfer to Eijkman Lactose Medium (Difco) tubes. The Eijkman Broth cultures were then incubated for 48 hours at 44° C., and the production of gas recorded as a positive test. Eijkman Most Probable Numbers (M.P.N.'s) were determined, for comparison with the coliform M.P.N.'s obtained from the Standard Methods Confirmed Test. Results are reported in Tables XXVI (a) and XXVI (b).

The mean Eijkman M.P.N. values for (1) Pinette River seawater specimens, (2) Vernon River seawater specimens, and (3) all oyster specimens,, were 4.1, 9.0, and 178.6 per 100 ml. respectively. Expressed as percentages of the mean coliform M.P.N.'s for the same specimens, the incidence of Eijkman-positive coliform strains was 21.8 per cent, 11.7 per cent, and 16.4 per cent, for (1), (2), and (3) respectively. There was a close correlation between the per cent incidence of Eijkman-positive coliform bacteria and the observed incidence of Escherichia coli type I and Irregular types II and VI strains as determined by the direct isolation and identification of coliform bacteria from the same specimens (Table XXVII).

TABLE XXVI (a) - VERNON RIVER EIJKMAN DATA, WATER SAMPLES, STATIONS 1 - 71

Sta.	E. coli Type I M.P.N.'s per 100 ml. (Confirmation in Eijkman Broth, 44° C.)						
	May 15	May 18	May 21	June 3	June 4	June 9	Mean 1953 MPNs
1	33	17	79	17	4.5	70	36.8
4	17	9.3	21	7.8	2	23	13.4
7	2	17	17	2	<1.8	4.5	7.1
9	4	4.5	11	2	<1.8	23	7.4
12	<1.8	4.5	13	<1.8	<1.8	13	5.4
15	<1.8	4.5	13	-	<1.8	7.8	5.1
18	2	2	4.5	-	<1.8	4	2.5
21	<1.8	4.5	11	-	<1.8	<1.8	3.1
25	-	1.8	2	-	<1.8	<1.8	1.0
28	-	-	2	-	-	<1.8	1.0
31	<1.8	-	4.5	-	-	<1.8	1.5
35	<1.8	1.8	<1.8	-	<1.8	<1.8	0.4
37	<1.8	2	4.5	-	<1.8	4.5	2.2
41	2	2	6.8	-	<1.8	<1.8	2.2
44	<1.8	-	13	-	<1.8	4.5	4.4
47	4.5	4.5	4	-	2	-	3.8
51	-	7.8	<1.8	-	4.5	-	4.1
54	<1.8	<1.8	<1.8	-	<1.8	-	<1.8
60	<1.8	<1.8	1.8	-	<1.8	<1.8	0.4
64	<1.8	<1.8	2	-	<1.8	2	0.8
66	<1.8	<1.8	6.8	-	<1.8	2	1.8
68	110	33	79	23	4.5	27	46.1
71	46	70	79	23	49	70	56.1
						Average.....	9.0

TABLE XXVI (b) - PINETTE RIVER EIJKMAN DATA, WATER SAMPLES, STATIONS 1 - 37

Sta.	E. coli type I M.P.N.'s per 100 ml. (Confirmation in Eijkman Broth, 44° C.) June 16	July 1	Mean 1953 MPNs
1	2	7.8	4.9
3	2	7.8	4.9
5	1.8	13	7.4
7	7.8	6.8	7.3
9	7.8	2	4.9
11	2	7.8	4.9
13	<1.8	2	1.0
15	11	14	12.5
17	2	7.8	4.9
19	2	14	8.0
21	<1.8	2	1.0
23	1.8	2	1.9
25	<1.8	4.5	2.3
27	2	2	2.0
29	2	2	2.0
31	2	4.5	3.3
33	<1.8	7.8	3.9
35	<1.8	1.8	0.9
37	<1.8	<1.8	<1.8
		Average	4.1

TABLE XXVII - INCIDENCE OF E. COLI TYPE I AND IRREGULAR II AND VI IN SEAWATER AND OYSTER SPECIMENS AS DETERMINED BY (1) EIJKMAN CONFIRMATION, AND (2) DIRECT ISOLATION OF COLIFORM BACTERIA (IMViC TEST)

Source	Mean Eijkman M.P.N.'s per 100 ml.	Mean Coliform M.P.N.'s per 100 ml.	Incidence of Eijkman-Positive Coliform Bacteria	Incidence of *E. coli* I and Irregular II and VI (Direct Isolation)
Pinette Seawater	4.1	18.8	21.8%	24.4%
Vernon River Seawater	9.0	76.6	11.7%	15.5%
All Oyster Specimens	178.6	1,089.8	16.4%	17.0%

12. Survival of Coliform Strains in Synthetic Seawater

The survival time and the death rate of various coliform strains in synthetic seawater (ZoBell, 1946) were determined in two separate experiments; results are given in Tables XXVIII to XXXI inclusive, and are reported graphically in Figures V (a) and V (b).

Twelve coliform strains (5 "coli" types, 3 "intermediate" types, and 4 "aerogenes" types), all isolated during the 1952 field investigations, were tested in the first experiment. The temperature of incubation was 40° F. "Coli" types survived for a much longer period than the "intermediate" and "aerogenes" types; "coli" types were isolated from the synthetic seawater after 168 hours, while only one of the other coliform types survived longer than 80 hours.

Eleven of the twelve coliform strains were re-tested in the second experiment; five other coliform strains were tested at the same time. The temperature of incubation was 38° F. One "coli" strain survived for 204 hours, one survived for 216 hours, and the remaining five "coli" strains survived for 228 hours in the synthetic seawater. In comparison, the mean survival times for "intermediate" and "aerogenes" coliform strains were 180 hours and 144 hours respectively.

In both experiments the rate of death was much less rapid for "coli" types than for "intermediate" and "aerogenes" coliform types.

When less than ten colonies appeared on the Violet Red Bile Agar plates prepared from synthetic seawater at the various test times in the latter portion of the experiments, all of the colonies growing on the plates were isolated and their pertinent biochemical characteristics

determined. One hundred and ninety-six cultures were tested in this manner. All such isolations gave biochemical reactions identical with those given by the original strain used to inoculate the synthetic seawater.

TABLE XXVIII - NUMBERS OF COLIFORM BACTERIA IN SYNTHETIC SEAWATER AT 40°F.

COLIFORM CULTURE	COLIFORM BACTERIA: PLATE COUNTS PER ML. OF SYNTHETIC SEAWATER																	
	0 Hours	4 Hours	8 Hours	16 Hours	24 Hours	32 Hours	48 Hours	56 Hours	64 Hours	72 Hours	80 Hours	96 Hours	104 Hours	120 Hours	144 Hours	168 Hours	176 Hours	192 Hours
407 *E. coli* I	62,000	45,000	31,000	33,000	26,000	24,000	22,700	22,500	19,600	19,000	10,400	4,600	2,800	260	140	15	*0	*0
707 *E. coli* I	19,900	17,200	14,200	17,000	12,000	10,000	7,900	9,100	6,800	5,100	4,000	2,500	1,800	180	93	13	*0	*0
348 *E. coli* II	52,000	26,600	16,500	21,000	13,000	11,000	7,400	8,300	7,200	7,200	2,000	1,950	760	270	104	8	*0	*0
384 Irregular I	44,000	23,700	13,100	17,000	11,000	10,000	6,700	6,900	5,500	6,100	3,200	2,500	560	50	24	1	*0	*0
917 Irregular II	49,000	41,000	34,000	32,000	14,600	14,000	12,600	14,000	8,600	8,200	2,800	3,300	660	100	27	2	*0	*0
392 Intermediate I	15,000	7,000	590	1,700	2,000	460	260	110	96	63	8	*0	*0					
117 Intermediate I	24,500	12,400	3,900	8,600	4,200	1,800	330	240	200	68	16	*0	*0					
404 Intermediate II	4,200	1,600	340	520	340	150	20	8	3	1	*0	*0	-					
375 *A. aerogenes* I	24,400	5,600	3,100	5,300	2,500	410	90	71	45	25	7	2	*0					
857 *A. aerogenes* I	13,000	2,600	1,300	3,000	1,700	460	400	140	82	32	7	*0	*0					
961 *A. aerogenes* II	32,000	3,900	1,160	2,100	800	580	150	87	30	16	2	*0	*0					
995 *A. cloacae*	17,900	3,900	270	1,100	600	145	40	27	18	9	2	*0	*0					

*0 = Absent in 4.0 ml.

TABLE XXIX - PERCENTAGE SURVIVAL OF COLIFORM BACTERIA IN SYNTHETIC SEAWATER AT 40 °F.

COLIFORM CULTURE	PERCENTAGE SURVIVAL OF COLIFORM BACTERIA IN SYNTHETIC SEAWATER															
	4 Hrs.	8 Hrs.	16 Hrs.	24 Hrs.	32 Hrs.	48 Hrs.	56 Hrs.	64 Hrs.	72 Hrs.	80 Hrs.	96 Hrs.	104 Hrs.	120 Hrs.	144 Hrs.	168 Hrs.	176 Hrs.
407 *E. coli* I	72.6	50.0	53.3	41.9	38.7	36.6	36.3	31.6	30.6	16.8	7.4	4.5	0.4	0.2	0.002	0
707 *E. coli* I	86.4	71.4	85.4	60.3	50.3	39.7	45.7	34.2	25.6	20.1	12.6	9.0	0.9	0.5	0.06	0
348 *E. coli* II	51.2	31.7	40.4	25.0	21.2	14.2	16.0	13.8	13.8	3.9	3.8	1.5	0.5	0.2	0.02	0
384 Irregular I	53.9	30.0	38.6	25.0	22.7	15.2	15.7	12.5	13.9	7.3	5.7	1.3	0.1	0.05	0.002	0
917 Irregular II	83.7	69.4	65.3	30.0	28.6	25.7	28.6	17.6	16.7	5.7	6.7	1.4	0.2	0.06	0.004	0
392 Intermediate I	46.7	3.9	11.3	13.3	3.1	1.7	0.8	0.6	0.4	0.05	0	-	-	-	-	-
117 Intermediate I	50.6	15.9	35.1	17.1	7.3	1.3	1.3	1.0	0.8	0.3	0.07	0	-	-	-	-
404 Intermediate II	37.1	8.1	12.4	8.1	3.6	0.5	0.2	0.07	0.02	0	-	-	-	-	-	-
375 *A. aerogenes* I	23.0	12.5	21.7	10.2	1.7	0.4	0.4	0.3	0.2	0.1	0.03	0.006	0	-	-	-
857 *A. aerogenes* I	20.0	10.0	23.1	13.1	3.5	3.1	1.0	0.6	0.2	0.005	0	-	-	-	-	-
961 *A. aerogenes* II	12.2	3.6	6.6	2.5	1.8	0.5	0.3	0.1	0.05	0.006	0	-	-	-	-	-
995 *A. cloacae*	21.8	1.5	6.1	3.4	0.8	0.2	0.15	0.1	0.05	0.01	0	-	-	-	-	-

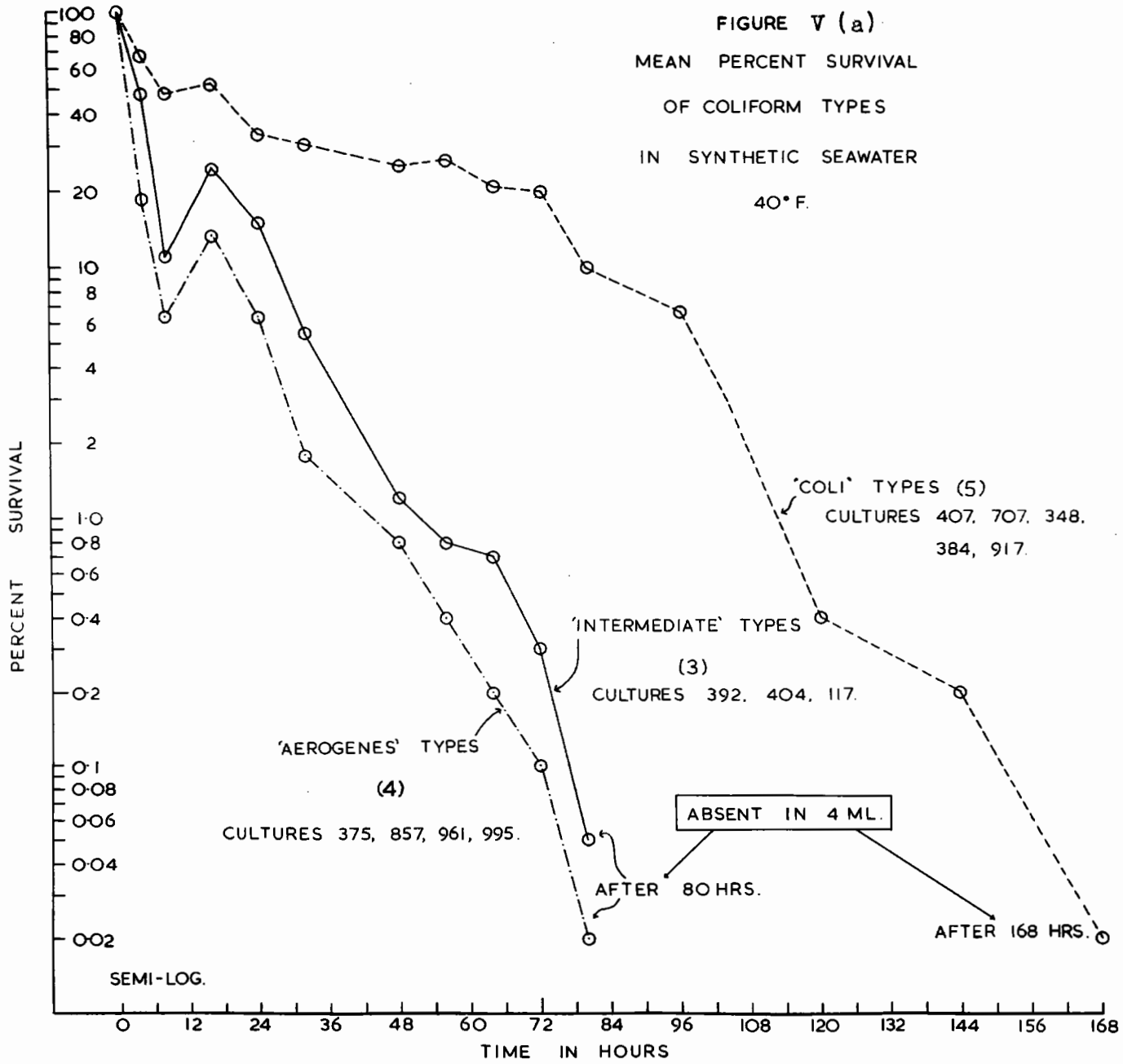


FIGURE V (a)
MEAN PERCENT SURVIVAL
OF COLIFORM TYPES
IN SYNTHETIC SEAWATER
40° F.
PERCENT SURVIVAL
100
80
60
40
20
10
8
6
4
2
1·0
0·8
0·6
0·4
0·2
0·1
0·08
0·06
0·04
0·002
SEMI-LOG.
'COLI' TYPES (5)
CULTURES 407, 707, 348,
384, 917.
'INTERMEDIATE' TYPES
(3)
CULTURES 392, 404, 117.
'AEROGENES' TYPES
(4)
CULTURES 375, 857, 961, 995.
ABSENT IN 4 ML.
AFTER 80 HRS.
AFTER 168 HRS.
0
12
24
36
48
60
72
84
96
108
120
132
144
156
168
TIME IN HOURS

TABLE XXX - NUMBERS OF COLIFORM BACTERIA IN SYNTHETIC SEAWATER AT 38°F.

COLIFORM CULTURE	COLIFORM BACTERIA: PLATE COUNTS PER ML. OF SYNTHETIC SEAWATER																			
	0 Hours	6 Hours	12 Hours	28 Hours	36 Hours	48 Hours	54 Hours	72 Hours	84 Hours	96 Hours	120 Hours	132 Hours	144 Hours	156 Hours	168 Hours	180 Hours	192 Hours	204 Hours	216 Hours	228 Hours	240 Hours	248 Hours
407 *E. coli* I	27,500	11,300	9,700	8,300	7,800	7,100	5,100	4,300	4,300	5,300	2,750	1,250	630	270	145	83	64	34	7	2	*0	*0
707 *E. coli* I	35,500	33,100	32,800	32,600	31,200	25,700	22,200	17,700	13,400	14,000	9,450	6,700	5,600	3,000	2,600	1,120	680	310	19	1	*0	*0
351 *E. coli* I	18,500	14,500	17,900	18,600	17,900	16,400	8,300	7,500	7,100	5,500	3,400	1,300	870	340	294	43	24	6	1	*0	*0	-
699 *E. coli* I	29,000	14,600	12,600	9,900	9,600	9,700	4,100	3,500	2,300	1,540	780	490	270	120	59	14	10	2	*0	*0	-	-
348 *E. coli* II	22,000	10,100	13,700	10,800	9,900	9,200	5,600	4,700	5,100	3,800	1,900	1,400	310	250	165	35	30	8	2	1	*0	*0
384 Irregular I	26,500	21,100	23,800	23,000	22,000	22,000	21,400	15,800	13,900	13,100	6,900	4,200	3,100	1,030	530	300	248	144	5	2	*0	*0
917 Irregular II	21,000	16,400	16,300	14,400	14,100	14,400	10,100	8,000	7,000	5,900	5,600	4,600	3,200	1,100	1,050	550	740	560	110	41	2	*0
392 Intermediate I	62,000	16,800	9,100	8,200	7,900	7,100	2,100	680	330	325	246	39	6	1	2	*0	*0	-				
117 Intermediate I	69,000	15,000	6,200	5,900	5,600	5,300	5,200	4,500	2,400	2,400	1,900	780	630	260	210	30	*0	*0				
153 Intermediate I	5,000	1,750	420	410	410	380	113	51	28	5	1	*0	*0	*0	-	-						
330 Intermediate I	5,500	1,700	1,400	1,650	1,560	1,500	740	375	155	87	4	*0	*0	*0	-							
375 *A. aerogenes* I	23,000	1,300	700	620	430	390	57	52	46	34	18	8	7	5	*0							
857 *A. aerogenes* I	32,000	18,500	9,700	6,500	5,200	4,800	3,600	1,140	480	430	180	22	9	1	*0							
410 *A. aerogenes* I	11,300	300	310	320	300	320	75	47	39	10	1	*0	*0	-								
961 *A. aerogenes* II	19,000	1,400	800	710	670	630	510	390	126	59	2	*0	*0	-								
995 *A. cloacae*	17,000	5,600	4,900	890	760	610	106	24	4	3	*0	*0	-	-								

*0 = Absent in 4.0 ml.

TABLE XXXI - PERCENTAGE SURVIVAL OF COLIFORM BACTERIA IN SYNTHETIC SEAWATER AT 38°F.

COLIFORM CULTURE	PERCENTAGE SURVIVAL OF COLIFORM BACTERIA IN SYNTHETIC SEAWATER																			
	6 Hrs.	12 Hrs.	28 Hrs.	36 Hrs.	48 Hrs.	54 Hrs.	72 Hrs.	84 Hrs.	96 Hrs.	120 Hrs.	132 Hrs.	144 Hrs.	156 Hrs.	168 Hrs.	180 Hrs.	192 Hrs.	204 Hrs.	216 Hrs.	228 Hrs.	240 Hrs.	248 Hrs.
407 E. coli I	41.1	35.3	30.2	28.4	27.6	25.8	18.4	15.6	19.3	10.0	4.5	2.3	1.0	0.5	0.3	0.2	0.1	0.03	0.007	0	-
707 E. coli I	93.2	92.4	91.8	87.9	72.4	62.5	49.9	37.7	39.4	26.6	18.9	15.8	8.4	7.4	3.2	1.9	0.9	0.05	0.003	0	-
351 E. coli I	78.4	96.8	100.5	96.8	88.6	44.9	40.5	38.4	29.7	18.1	6.8	4.7	1.8	1.6	0.2	0.1	0.03	0.005	0	-	-
699 E. coli I	50.3	43.4	34.1	33.1	33.4	14.1	12.1	7.9	5.3	2.7	1.7	0.9	0.4	0.2	0.05	0.03	0.007	0	-	-	-
348 E. coli II	45.9	62.3	49.1	45.0	41.8	25.5	21.4	23.2	17.0	8.6	6.2	1.4	1.1	0.8	0.2	0.14	0.04	0.007	0.005	0	-
384 Irregular I	79.6	89.8	86.8	83.0	83.0	80.8	59.4	52.5	49.4	26.0	15.9	11.7	3.9	2.0	1.1	0.9	0.5	0.02	0.008	0	-
917 Irregular II	78.1	77.6	68.6	67.1	68.6	48.1	37.9	33.3	28.1	26.7	21.9	15.2	5.2	5.0	2.6	3.5	2.7	0.5	0.2	0.01	0
392 Intermediate I	27.1	14.7	13.2	12.7	11.5	4.4	1.1	0.5	0.5	0.4	0.06	0.01	0.002	0.004	0	-					
117 Intermediate I	21.7	9.0	8.6	8.1	7.8	7.5	6.5	3.5	3.4	2.8	1.1	0.9	0.4	0.4	0.04	0					
153 Intermediate I	35.0	8.4	8.2	8.2	7.6	2.3	1.0	0.5	0.1	0.02	0										
330 Intermediate I	30.9	25.5	30.0	28.4	27.3	13.5	6.8	2.8	1.6	0.07	0										
375 A. aerogenes I	5.7	3.0	2.7	1.9	1.7	0.24	0.22	0.20	0.15	0.08	0.03	0.03	0.02	0							
857 A. aerogenes I	57.8	30.3	20.3	16.3	14.8	11.3	3.6	1.5	1.3	0.6	0.07	0.03	0.003	0							
410 A. aerogenes I	2.7	2.7	2.8	2.7	2.8	0.7	0.4	0.3	0.09	0.009	0										
961 A. aerogenes II	7.4	4.2	3.7	3.5	3.3	2.7	2.1	0.7	0.3	0.01	0										
995 A. cloacae	32.9	28.8	5.2	4.5	3.6	0.6	0.14	0.023	0.017	0											

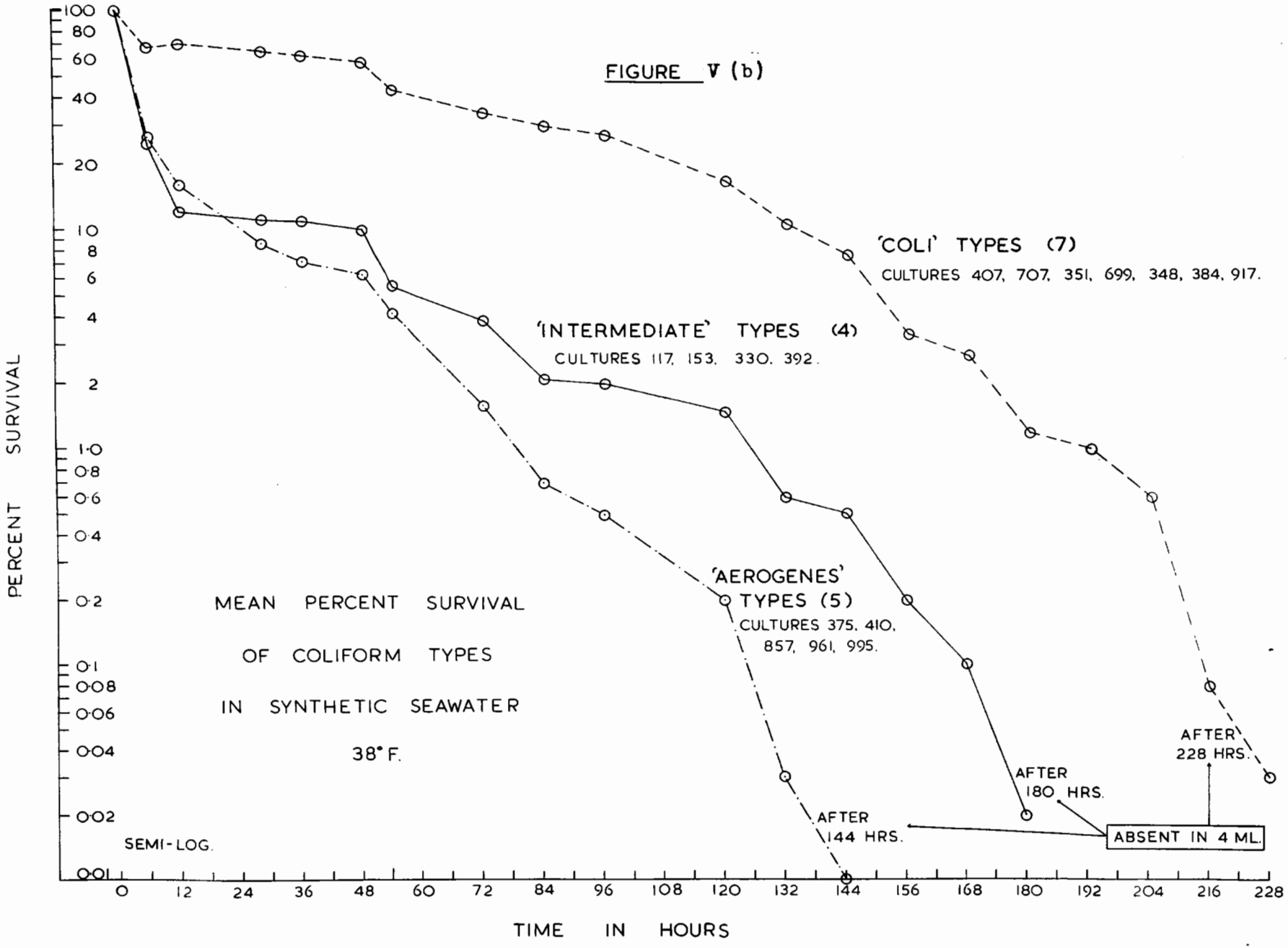


FIGURE V (b)
PERCENT SURVIVAL
100
80
60
40
20
10
8
6
4
2
1·0
0·8
0·6
0·4
0·2
0·1
0·08
0·06
0·04
0·02
0·01
SEMI-LOG.
0
12
24
36
48
60
72
84
96
108
120
132
144
156
168
180
192
204
216
228
TIME IN HOURS
'COLI' TYPES (7)
CULTURES 407, 707, 351, 699, 348, 384, 917.
'INTERMEDIATE' TYPES (4)
CULTURES 117, 153, 330, 392.
'AEROGENES' TYPES (5)
CULTURES 375, 410, 857, 961, 995.
MEAN PERCENT SURVIVAL
OF COLIFORM TYPES
IN SYNTHETIC SEAWATER
38° F.
AFTER 144 HRS.
AFTER 180 HRS.
AFTER 228 HRS.
ABSENT IN 4 ML.

GENERAL DISCUSSION

Rainfall in Queens County, Prince Edward Island, during the July to October, 1952, and May and June 1953, periods was considerably less than had been anticipated. The farmlands on the watersheds of the study areas became so dry during the summer of 1952 that even the heavy rains of August 18 did not result in any significant land-wash pollution. It would thus appear that serious land-wash pollution does not occur unless heavy rains fall on soil already saturated by previous precipitation. Significant land-wash pollution did occur in the Vernon River system on May 21, 1953, following a heavy rainstorm. On this date coliform M.P.N. values for water samples taken in all sectors of the Vernon River area were greatly in excess of the mean coliform values determined during the remainder of the investigation.

A considerable week to week variation in the coliform content of seawater from fixed sampling stations was noted. For example, surveys of the Vernon River system made on July 24, July 29, and September 25, 1952, and June 4, 1953, provided results which indicated that the entire area was free from serious pollution; on the other hand, surveys made on June 21, 1951, and on May 21, 1953, indicated that the entire Vernon River area was subject to significant pollution. When the data obtained from all surveys of seawater sampling stations is considered, however, it is evident that Sector A seawater contained excessive numbers of coliform bacteria most of the time, and that Sectors B, C, and D were free from significant numbers of coliform bacteria except when heavy rainfall caused increased run-off from farmlands on the watershed.

The marked effect of very low spring tides on the bacterial content of narrow tidal river channels has not previously been reported. In the Vernon River system, where many productive oyster beds are located in the Vernon and Orwell Rivers, dilution becomes a major factor in determining the sanitary quality of oysters. If we can assume that the numbers of coliform bacteria entering the rivers during periods of dry weather are relatively constant, the removal of most of the water from the river channels by spring tides will automatically result in greatly increased coliform numbers per unit volume. The data reported in Tables X and XI indicate that oysters, under these conditions, will filter out and concentrate within their bodies large numbers of coliform bacteria from the water, and that these will persist in the oysters for some appreciable time.

Where sources of direct human or animal pollution are few in number, or absent, as in the Pinette and Orwell Rivers, most of the coliform bacteria found in seawater have been washed from farm and pasture lands on the watershed. Essentially, the data obtained from bacteriological surveys provide a means of measuring the amount of land drainage reaching the shellfish growing waters, and the capacity of the body of water to dilute the pollution. In shallow river, estuary, and bay systems, where the inflow of fresh water results in salinities of less than 20 grams per kilogram of seawater, the addition of even moderate amounts of land drainage, especially during spring tides, may result in coliform M.P.N.'s of more than 50; in systems containing larger volumes of water, the dilution factor may be the significant agent in reducing the number of coliform bacteria per unit volume of seawater.

The many complicating factors may make a short-term assessment of the bacteriological quality of a shellfish producing area impossible; provision should be made, where limitations in time and equipment permit, for bacteriological investigations to extend over a two- or three-month period in important shellfish areas. It may be possible to establish a mobile laboratory unit at some central point, and to make periodic bacteriological examinations of specimens from as many as six or seven shellfish producing areas during the investigation. Such a program would provide information regarding seasonal variations in the coliform content of seawater, and lead to a greater understanding of the basic problems affecting shellfish sanitation.

A fairly close relationship was found between coliform M.P.N.'s from oyster and seawater specimens from the same sampling stations; with one exception, coliform M.P.N.'s from oyster specimens were less than 230 when the mean coliform M.P.N.'s for seawater specimens from the same sampling stations were less than 50.

The enterococci M.P.N.'s obtained from seawater specimens during the investigation were much lower than the coliform M.P.N.'s obtained from the same specimens; in 1952 the coliform to enterococci ratio for all specimens of seawater was approximately 13 to 1, as compared to 87.5 to 1 for specimens from the same sampling stations in 1953. The enterococci content of seawater from the Vernon River system was not increased by the spring tides of August 5 and October 9, 1952, or by the increased land drainage noted on May 21, 1953. It may be argued that the small number of enterococci continuously present in seawater from the study areas is indicative of direct,

recent, pollution, and that the marked increase in the numbers of coliform bacteria which follows increased land-wash on the watershed indicates remote pollution of minor public health significance. On the other hand, surface washings may be undesirable even though strictly "faecal" bacteria are not found, and the presence of coliform bacteria beyond certain limits, irrespective of type, may show the existence of undesirable conditions. The coliform to enterococci ratio is so large that standards for shellfish producing waters, based on the Most Probable Number of enterococci per 100 ml. of seawater, would require almost complete freedom from these organisms. The data obtained during the present investigation may not justify the inclusion of the enterococci test in routine shellfish surveys; it is probable, however, that a test for enterococci will have value under conditions which cause the results of the Standard Methods coliform test to be inconclusive.

The Membrane Filter method was found to be readily adaptable to the examination of seawater for coliform bacteria. It was necessary, however, to reduce the volume of sample to be filtered, and to supply sufficient basic fuchsin in the medium to inhibit non-coliform bacteria and to satisfy the dye-adsorption requirements of the silt present in the seawater. It is unfortunate that preliminary tests could not have been conducted prior to the initiation of the investigation, because a portion of the 1952 MF coliform count data was obtained with imperfect media. Modifications of the MF Endo's Broth medium will have to be made, by trial and error, when the MF technique is first used in each shellfish producing area; the silt content of seawater may vary widely from area to area and even from time to time.

While the coliform M.P.N. to coliform MF count ratio for specimens of seawater taken from the study areas in 1953 was 1 to 1.13, it should be noted that only 37.9 per cent of the coliform-like colonies isolated in pure culture from MF membranes produced gas from standard lactose broth at 37° C. in 48 hours. Thus the coliform M.P.N. to lactose-fermenting-bacteria MF count ratio was 1 to 0.43; less than half of the coliform bacteria enumerated by the Standard Methods Confirmed Test were detected by the Membrane Filtration technique. The seawater specimens tested during the present investigation contained relatively small numbers of coliform bacteria, and relatively large amounts of silt, organic matter, and other bacteria; under such conditions, more coliform bacteria will be isolated by the use of the Standard Methods Confirmed Test than by membrane filtration. It is evident that the Standard Methods Confirmed Test for coliform bacteria and the MF Endo's Broth technique do not measure precisely the same organisms. It would therefore appear that the MF technique should not replace the Standard Methods Confirmed Test in shellfish investigations; the MF technique will, however, be useful in special studies.

Many workers and reviewers have advanced the theory that E. coli, and related "coli" types, are true indices of faecal pollution, and that "intermediate" and "aerogenes" coliform strains are indigenous to soil, grasses, and grains, and at best may be considered as indices of remote pollution. There is considerable evidence to show that E. coli type I is the dominant coliform strain in faeces, but it is doubtful if it is also true, as is often stated, that A. aerogenes and the intermediate coliform strains are rare or few in number in faeces. Data

obtained during the present investigation, and those reported by other workers, suggest that ten per cent or more of the coliform strains isolated from faeces are types other than E. coli. Expressed numerically, a million or more "intermediate" or "aerogenes" coliform strains may be present per gram of faeces, and these strains may therefore have considerable significance as indices of faecal pollution. If, on the other hand, "intermediate" and "aerogenes" strains represent only ten per cent of the coliform bacteria in faeces, nine times as many "coli" types should be present in seawater and shellfish subject to faecal pollution.

Where pollution of soil has been recent, coliform bacteria of the "coli" type have generally been found to be present in greater numbers than other coliform types; a similar distribution of coliform strains could be expected in seawater receiving direct drainage from the soil. During the 1952 study, "coli" types were the dominant coliform strains in faeces (86.0 per cent), soil (66.7 per cent), and seawater (80.1 per cent). It is most interesting to note, therefore, that a complete reversal of coliform type distribution was found in oyster specimens from the same areas; 66.1 per cent of coliform bacteria isolated from oyster specimens during the 1952 study were "aerogenes" types. Thus the "aerogenes" minority strains in seawater gained ascendency over the "coli" strains upon entry into oysters; this may indicate that "aerogenes" coliform strains are capable of longer survival than other coliform types in the oyster, or that the biochemical reactions of some "coli" types are altered upon ingestion and retention by oysters. In 1953, despite the much higher proportional incidence of "intermediate" and "aerogenes" types in seawater, there was further evidence that these coliform types are favoured over "coli" types within the body of the oyster.

In 1953, 89.0 per cent of the coliform bacteria isolated from faeces were "coli" types, but in soil (8.1 per cent), seawater (31.5 per cent), and oysters (13.2 per cent) "coli"types were in the minority. Thus, while coliform M.P.N.'s from seawater and oyster specimens were somewhat higher in the early-summer study period of 1953 than in the late-summer period of 1952, probably because of heavier land drainage, "aerogenes" and "intermediate" coliform strains accounted for the increase. It is possible that the increased incidence of "aerogenes" and "intermediate" coliform strains in soil, seawater, and oyster specimens may be attributed to a longer survival period in these media. The present studies, however, of the survival of coliform strains in synthetic seawater, held at temperatures approximating seawater temperatures in the study areas, provided date which indicate that "coli" types survive approximately twice as long as other coliform strains under these conditions. Other factors, such as sedimentation and the antagonistic action of other organisms, which will affect the survival of coliform bacteria in natural seawater were not considered in these experiments.

The increased incidence of "intermediate " and "aerogenes" coliform types observed in seawater and oyster specimens in the 1953 study cannot be related to direct faecal pollution as defined by sanitary engineering studies; Vernon River Sector A is the only portion of the study areas exposed to direct human faecal pollution. These data therefore support the hypothesis stated by many British workers that "intermediate" and "aerogenes" coliform types are indices of remote pollution.

Three thousand, one hundred and twenty Gram negative bacteria were isolated from Violet Red Bile Agar plates and MF preparations during the present investigation. During the 1952 and 1953 studies, 16.5 and 49.9 per cent respectively failed to ferment lactose, in pure culture, at 37° C., or fermented lactose slowly or weakly; these organisms were provisionally classified as paracolon bacteria (Paracolobactrum, Bergey, 1948), although their serology was not investigated. Other workers have isolated paracolon bacteria from faeces, soil, and water, but never, to the author's knowledge, in the numbers observed during the 1953 study.

Of the paracolon bacteria isolated in 1953, 81.5 per cent were classified as "aerogenes" types on the basis of their biochemical reactions, and only 9.6 per cent could be classified as "coli" types. The predominance of Paracolobactrum aerogenoides (Bergey, 1948) strains is in accordance with the findings of Borman et al. (1944). The higher incidence of paracolon bacteria in specimens examined in 1953 than in those analysed in 1952 suggests a relationship between paracolon bacteria in seawater and oysters and increased land-wash.

It must be remembered that these paracolon strains attacked lactose on primary isolation; colony formation on both Violet Red Bile Agar and MF Endo's Broth was, in every instance, typical of true coliform bacteria, and such colony formation is dependent on the ability of the bacteria to break down lactose. Evidently these strains had an insufficient capacity for gas production to give clearly positive reactions in pure culture.

It is significant that the incidence of paracolon bacteria increased in direct proportion with the distance from the primary pollution source; during the 1953 study, 5.0, 32.1, 63.1, and 78.8 per cent of all isolations from faeces, soil, seawater, and oyster specimens respectively were paracolon bacteria. These data tend to support the hypothesis of Stuart *et al.* (1940) that coliform bacteria may become attenuated with respect to the fermentation of lactose after exposure to unfavourable environmental conditions. This hypothesis would explain the gradual increase in the incidence of paracolon bacteria as faecal material is washed from the primary source on the watershed to the seawater and finally to the oyster.

Paracolon bacteria have been implicated as the causative organisms in enteric disease. Pathogenic paracolon bacteria are generally considered to be organisms of a slightly lower scale of virulence which have evolved from the *Salmonellae*. The paracolon bacteria isolated during the present investigation appear, however, to be strains of coliform bacteria which have become attenuated or "degraded" with respect to the fermentation of lactose. Stuart *et al.* (1940) and Taylor (1951) consider that such "degraded" coliform strains have little hygienic significance, although these authors suggest that further study is needed.

Coliform type Irregular VI, a variation of *A. aerogenes* type I, is the only "aerogenes" or "intermediate" coliform type which produces acid and gas in Eijkman Lactose Medium at 44° C.; of the *A. aerogenes* type I strains isolated, only 4.8 per cent in 1952 and 1.9 per cent in 1953 were classified as Irregular VI. Thus an Eijkman Test for lactose fermentation at 44° C. was almost specific for two "coli" types, *E. coli*

type I and Irregular II. The routine Eijkman test requires the inoculation of five-tube series of Eijkman Lactose Medium in at least three dilutions, in a manner similar to the inoculation of Lactose Broth in the Standard Methods Presumptive Test for coliform bacteria. The Eijkman procedure requires a considerable expenditure of time and materials; it was considered possible that an Eijkman Confirmation Test, in which positive presumptive lactose broth cultures from Standard Methods coliform determinations were "confirmed" by direct transfer to tubes of Eijkman Lactose Medium with incubation at 44° C. might provide essentially the same information. There was a fairly close correlation between Most Probable Numbers of Eijkman-positive coliform bacteria, as determined by this test, and the observed incidence of E. coli type I and Irregular II and VI coliform strains as determined by the direct isolation and identification of coliform bacteria from the same specimens. It was possible, therefore, by an Eijkman Confirmation Test, to obtain a rough approximation of the incidence of E. coli type I in seawater and oyster specimens with a minor expenditure of time and materials.

The time has come for a reassessment of the criteria used in the bacteriological control of shellfish and shellfish producing waters. Where pollution of a producing area is either completely absent, or heavy and direct, the Standard Methods Confirmed Test for coliform bacteria may provide a means for the accurate delineation of pollution limits. It must be recognized, however, that in shallow river and bay systems the simple determination of coliform bacteria may not be related at all times to sanitary conditions. Data obtained during the present

investigation suggest that, under abnormal meteorological and tidal conditions, tests for E. coli or for enterococci may provide a more precise measure of the hygienic quality of oysters and other shellfish.

SUMMARY OF RESULTS

1. An investigation of the sanitary bacteriology of two representative oyster producing areas in Prince Edward Island, Vernon River and Pinette River, was conducted on June 21, 1951, and during the July to October, 1952, and May and June, 1953, periods.

2. Heavy rainfall caused land-wash pollution in the Vernon River system on June 21, 1951, and on May 21, 1953; on those dates, coliform Most Probable Numbers from seawater and oyster specimens taken from fixed sampling stations in the study area were much higher than during the remainder of the study period.

3. Very low spring tides caused a marked increase in the number of coliform bacteria per unit volume of seawater. In narrow river channels, where salinities of less than 20 grams per kilogram of seawater were recorded, the addition of even moderate amounts of land drainage during spring tides resulted in coliform M.P.N.' per 100 ml. of more than 50 in the seawater.

4. Because of changing weather and tidal conditions, there was a considerable variation in the coliform content of seawater and oyster specimens taken from the same sampling stations on different dates during the investigation. Coliform M.P.N.'s from specimens taken from the Vernon River on July 24, July 29, and September 25, 1952, and June 4, 1953, were so low as to indicate that all portions of the Vernon River system were free from significant pollution; coliform data obtained from surveys made on June 21, 1951, and May 21, 1953, indicated that the

entire Vernon River area was subject to serious pollution. When all data, from fourteen surveys in Vernon River and eleven surveys in Pinette River, were considered, however, it was evident that seawater from Sector A of the Vernon River contained excessive numbers of coliform bacteria most of the time, and that the remainder of Vernon River (Sectors B, C, and D) and all of Pinette River were free from significant numbers of coliform bacteria except when heavy rainfall caused increased run-off from farmlands on the watershed.

5. A fairly close relation was found between coliform M.P.N.'s from oyster and seawater specimens from the same sampling stations; with one exception, coliform M.P.N.'s from oyster specimens were less than 230 when the mean coliform M.P.N.'s for seawater specimens from the same sampling stations were less than 50.

6. The ratio of coliform bacteria to enterococci for all specimens of seawater tested in 1952 was approximately 13 to 1, as compared to 87.5 to 1 for all seawater specimens from the same sampling stations in 1953. The enterococci content of seawater and oyster specimens taken from the Vernon River system was not increased by the spring tides of August 5 and October 9, 1952, or by the increased land drainage noted on May 21, 1953.

7. The Membrane Filter technique for the enumeration of coliform bacteria was found to be readily adaptable for the examination of seawater specimens. It was necessary, however, to reduce the volume of sample to be filtered, and to add sufficient

basic fuchsin to the medium to inhibit non-coliform bacteria and to satisfy the dye-adsorption requirements of the silt present in the water.

8. The coliform M.P.N. to MF count ratios for specimens of seawater from Vernon River and from Pinette River were 1 to 0.43 and 1 to 0.25 respectively in 1952, and 1 to 0.85 and 1 to 1.81 respectively in 1953. The meam coliform M.P.N. to MF count ratio for all seawater specimens tested in 1953 was 1 to 1.13. Only 37.9 per cent, however, of the coliform strains isolated from the MF preparations during 1953 fermented lactose broth with the production of acid and gas (37° C., 48 hours) when isolated in pure culture. These results indicate that the two test procedures do not measure precisely the same group of organisms.

9. Coliform plate counts for soil specimens from the Vernon River watershed ranged from less than 10 to 34,000 per gram; no coliform bacteria were isolated from 14 of 65 soil specimens, while 23 specimens had coliform counts of less than 100 per gram. There was a considerable variation in the numbers of coliform bacteria isolated from soil specimens from the six sampling stations on the different sampling dates.

10. Coliform plate counts from forty faeces specimens ranged from 170 to 2,400,000,000 per gram of faeces. Coliform plate counts from dry faeces specimens were much lower than those from fresh faeces specimens. More coliform bacteria than enterococci were isolated from specimens of fresh faeces; ratios of coliform

bacteria to enterococci were 1 to 0.52 for human faeces specimens, and 1 to 0.03 for animal and bird faeces specimens. For three specimens of dried animal faeces, however, the ratio of coliform bacteria to enterococci was 1 to 5.4.

11. One thousand, eight hundred and ninety-one coliform bacteria isolated from specimens of seawater, stream water, oysters, soil, and faeces from the study areas were classified according to their biochemical reactions.

In 1952, coliform bacteria of the "coli" type were predominant in specimens of soil, faeces, and seawater; in oyster specimens, however, only 31.1 per cent of the coliform strains isolated were "coli" types. In 1953, "coli" types predominated in specimens of faeces, but in specimens of soil, water, and oysters "aerogenes" types were predominant. Thus, while coliform M.P.N.'s from seawater and oyster specimens were somewhat higher in the early-summer study period of 1953 than in the late-summer period 1952, "aerogenes" and "intermediate" coliform strains accounted for the increase.

12. In addition to the 1,891 coliform strains isolated from specimens of water, oysters, soil and faeces during the investigation, 1,229 other Gram negative strains were isolated from the same specimens; although their primary isolation was dependent on the fermentation of lactose, these strains, when isolated in pure culture, had an insufficient capacity for gas production in lactose broth to give clearly positive reactions at 37° C. within 48 hours, and were tentatively classified as paracolon bacteria.

13. Of the 1,099 paracolon strains isolated in 1953, 81.5 per cent were classified as "aerogenes" types on the basis of their biochemical reactions; only 9.6 per cent could be classified as "coli" types. The higher incidence of paracolon bacteria in specimens examined in 1953 than in those analysed in 1952 suggests a relationship between paracolon bacteria in seawater and oysters and increased land-wash.

14. In 1953, the incidence of paracolon bacteria increased in direct proportion with the theoretical distance from the primary pollution source; 5.0, 32.1, 63.1, and 78.8 per cent of all bacteria isolated from faeces, soil, seawater, and oyster specimens respectively were paracolon strains.

15. An "Eijkman Broth Confirmation Test" is described, in which positive presumptive lactose broth cultures from Standard Methods coliform determinations were "confirmed" by transfer to tubes of Eijkman Lactose Medium, with incubation at 44° C. for 48 hours. There was a fairly close correlation between Most Probable Numbers of Eijkman-positive coliform bacteria, as determined by this method, and the incidence of E. coli type I and Irregular II and VI coliform strains as determined by the direct isolation and identification of coliform bacteria from the same specimens of seawater and oysters.

16. The survival time and the death rate of sixteen coliform strains in synthetic seawater at 38° to 40° F. were determined in two separate experiments. Under the experimental conditions, "coli" types survived for a much longer time than "intermediate" and "aerogenes" types, and the rate of death was much less rapid for "coli" types than for other coliform types.

CONCLUSIONS

1. Where sources of direct human or animal pollution are few in number, or absent, most of the coliform bacteria found in seawater, and oyster specimens from oyster growing areas are washed from farm and pasture lands on the watershed. The data obtained from bacteriological surveys provide a means of measuring the amount of land drainage reaching oyster growing waters, and the capacity of the body of water to dilute this pollution.

2. Significant land-wash pollution of oyster producing areas does not occur unless heavy rains fall on soil already saturated by previous precipitation.

3. Very low (spring) tides cause a marked increase, in narrow river channels, in the numbers of coliform bacteria per unit volume of seawater and oysters.

4. The numbers of coliform bacteria in seawater and oyster specimens are not related at all times to hygienic conditions as determined by sanitary engineering surveys. Under abnormal meteorological and tidal conditions, tests for Escherichia coli or for enterococci may provide a more precise measure of the sanitary quality of oysters and other shellfish.

5. An Eijkman Lactose Medium Confirmation Test was shown to be of practical value for the estimation of Escherichia coli type I in seawater and oyster specimens. This test should be included in bacteriological surveys of oyster producing areas.

6. The increased incidence of Aerobacter and intermediate coliform types observed in seawater and oysters during the 1953 study was attributable to increased run-off pollution. Aerobacter and intermediate coliform types were the dominant coliform strains in oysters, regardless of coliform type distribution in seawater specimens taken from the same sampling stations. The data obtained during the present investigation support the hypothesis stated by some British workers that Aerobacter and intermediate coliform types are indices of remote pollution, and are of only slight hygienic significance.

7. Escherichia coli type I and other coliform strains of the "coli" type survived for a longer period of time in synthetic seawater at 38° to 40° F. than did Aerobacter and intermediate coliform types.

8. The American Public Health Association Standard Methods Confirmed Test for coliform bacteria and the Environmental Health Center Membrane Filter Endo's Broth technique do not measure precisely the same group of organisms.

9. Coliform bacteria may become attenuated with respect to the fermentation of lactose after exposure to unfavourable environmental conditions. Large numbers of such paracolon bacteria were present in soil, seawater, and oysters from the study areas in 1953. There was a direct relationship between the incidence of paracolon bacteria and increased land-wash pollution. The incidence of paracolon bacteria increased in direct proportion with the theoretical distance from the primary

pollution source; the paracolon group was therefore an index of remote pollution of only slight hygienic significance.

10. The many complicating factors may make a short-term assessment of the bacteriological quality of a shellfish producing area impossible. Bacteriological investigations in important shellfish producing areas should extend over two-or three-month periods.

CLAIM OF ORIGINAL WORK OR CONTRIBUTION TO KNOWLEDGE

The present investigation is entirely original in the sense that the experiments were planned and conducted by the author. This investigation is unique because it is the first comprehensive bacteriological study to be conducted in Canadian oyster producing areas. To the author's knowledge there is no reference in the literature to similar studies in other countries. A number of contributions to knowledge have been made:

1. Very low (spring) tides, and land-wash pollution following heavy rains, cause a marked increase in the numbers of coliform bacteria per unit volume of seawater and oysters.

2. It has been almost universally accepted that the bacterial content of shellfish reflects that of the overlying seawater. It has been shown, however, in this study that Aerobacter and intermediate coliform types are the dominant coliform strains in oysters from the study areas regardless of the coliform type distribution in seawater from the same sampling stations.

3. The relative public health significance of the various coliform types has been the subject of considerable contention. While the British favour the hypothesis that Aerobacter and intermediate coliform strains are indices of remote pollution of only slight sanitary significance, workers in Canada and in the northern United States of America generally consider that all coliform types are of equal hygienic significance. The data obtained from the present investigation support the British hypothesis.

4. A modified Escherichia coli Confirmation Test using Eijkman Lactose Medium has been shown to have practical value for the assessment of the sanitary quality of seawater and oysters.

5. Little or no attention has been paid by governmental control agencies to the public health significance of coliform bacteria attenuated with respect to the fermentation of lactose. The incidence of such paracolon bacteria in soil, seawater, and oysters during the present investigation was much higher than has previously been reported. There was a direct relationship between the incidence of paracolon bacteria and increased land-wash pollution. The incidence of paracolon bacteria increased in direct proportion with the theoretical distance from the primary pollution source; it was concluded that the paracolon group was therefore an index of remote pollution of slight hygienic significance.

6. The American Public Health Association Standard Methods Confirmed Test for coliform bacteria and the Environmental Health Center Membrane Filter Method do not measure precisely the same group of organisms.

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