one part of the material to 7 parts of absolute alcohol, yields an excellent antigen for complement fixation in amebiasis. The extraction is conducted in an incubator at 45° C. for 15 days, the flask containing the material being thoroughly shaken several times a day during that time. After extraction, the mixture is filtered, diluted with from 3 to 5 parts of normal saline and tested for its hemolytic, anticomplementary and antigenic properties.

Antigenic extracts prepared in this simple manner appear to be more active than when the acetone insoluble lipoids are either used alone or added to alcoholic extracts of cultures of *Endamoeba histolytica* or of the mucoid material. It has been found that the dilution mentioned can generally be used with excellent results, whereas in our experience most of the lipoid antigens gave poor results when diluted.

Alcoholic extracts of the mucoid material obtained from the intestine of dogs suffering from amebic dysentery gave fully as good results as extracts made from cultures of *E. histolytica*. Owing to the difficulty experienced by many in the cultivation of this organism, the preparation of antigens from cultures has been abandoned by many laboratories. It has been shown by Faust that dogs are comparatively easily infected with *E. histolytica* and that the infection can be maintained in the laboratory by transmission from animal to animal. Sufficient mucoid material for extraction may easily be obtained from the intestine, in an acutely infected dog, and as this material is usually much richer in the amebæ than are cultures, and much more free from bacteria, it is believed that it furnishes an excellent source of antigen for use in complement fixation in the diagnosis of amebiasis.

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Effect of Temperature of Storage on Bacteria in Water Samples.

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Bacteriological control of water supplies frequently involves examination of samples which have been shipped considerable distances. All editions of Standard Methods for the Examination of Water and Sewage provide for icing samples during shipment. Berry,[1] Jordan and Irons,[2], Hale and Melia,[3] Albert, Hinman and

Jordan,[4] Eijken,[5] Huss,[6] and Rector and Daube[7] reached conclusions which are not in agreement. Berry found little difference in the *Escherichia coli* count of iced and uniced samples and no appreciable multiplication of this group in stored samples. Boruff and Buswell[8] reported that a sample of water analyzed immediately showed more viable bacteria than after shipment iced about 100 miles (1-2 days in transit). Warm polluted water iced even for 12 hours underwent a distinct decrease in count. Their work would seem to indicate that the type of sample would largely determine the changes which would take place. Icing a sample of warm polluted water might cause more profound changes than icing a sample of cold water in the winter.

The work here reported involved 5 different types of water: (1) polluted surface water; (2) water to which soil had been added; (3) diluted sewage; (4) sterile distilled water inoculated with pure cultures; and (5) tap water inoculated with pure cultures. The technic in general was to divide the sample into 3 portions for storage at 3 different temperatures—in the refrigerator at about 4° C., at room temperature from 20.5 to 21.8° C., and in the 37° C. incubator. These incubation temperatures cover about the range to which samples might be subjected between time of collection and analysis.

1. The total count of polluted surface water increased on storage at room temperature and 37° C. for 24 hours. The total count on the portion stored in the ice box, however, showed little change, only a slight increase being noticed. In this case the advantage of icing is indicated.

2. Samples of water to which soil was added showed little change in the total counts of portions stored under the 3 storage temperatures mentioned above.

3. Counts on sample of diluted sewage increased rapidly on the portion stored at 37° C. Two other portions stored at 37° C., and in the refrigerator showed little change in the number of bacteria.

4. Sterile distilled water was inoculated with *Escherichia coli, Aerobacter aerogenes, Eberthella typhi,* and 2 organisms isolated

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1 Berry, F., *Am. J. Pub. Health,* 1926, **16**, 700.

2 Jordan, E. O., and Irons, E. E., *Am. Pub. Health Assn. Papers and Reports,* 1899, **25**, 564.

3 Hale, F. E., and Melia, T. W., *J. Inf. Dis.,* 1910, **7**, 587.

4 Albert, H., Hinman, J. J., and Jordan, G., *Am. J. Pub. Health,* 1916, **7**, 2, 1010.

5 Eijken, P. A. A., *Chem. Weekblad,* 1918, **15**, 1519.

6 Huss, H., *Wasser u. Abwasser,* 1922, **17**, 244.

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8 Boruff, C. S., and Buswell, A. M., *Illinois State Water Survey,* Bulletin 28.

from sewage. All were strains which had not been carried on laboratory media. The strain of *Escherichia coli* died out rapidly when stored in distilled water at the 3 temperatures of storage; death was especially rapid at 37° C. Our results agree quite well with those reported by Hale and Melia. In one case icing seemed to show slight advantage. *Acrobacter aerogenes* died almost completely in 16 hours at all temperatures of storage in one test and within 3 hours in a second. In a third experiment somewhat longer survival periods were indicated. The 2 members of the Escherichia-Aerobacter group isolated from sewage died out quite rapidly except in one portion stored in the refrigerator. *Eberthella typhi* decreased rapidly in distilled water stored at 37° C. and room temperature, while at 3-5° C., significant decrease was not noticed after 48 hours, a period quite beyond that for which a water sample would be stored before analysis.

5. Tap water after sterilization was inoculated with 2 strains of *Eberthella typhi*, 3 strains of *Salmonella paratyphi*, 2 strains of *Salmonella Schottmülleri*, 3 strains of *Shigella dysenteriae*, and 2 strains of *Escherichia coli*. These species possess some significance for sanitary bacteriologists.

Iced samples of tap water inoculated with *Escherichia coli* showed less change in count than did the portions kept at room temperature and 37° C. With one strain of *Eberthella typhi*, however, the counts fell off somewhat in the iced portion but increased in the portion stored at room temperature. The other strain gave inconsistent results indicating that strain differences may affect the results. One strain of *Salmonella paratyphi* died out within 6 hours in iced tap water; another maintained practically the same count under these conditions; the third strain showed distinct decrease under all conditions. Two strains of *Salmonella Schottmülleri* showed little change in number when stored in the refrigerator. Icing was decidedly advantageous, however, with three strains of *Shigella dysenteriae*.

Lactose fermentation was observed after 48 hours in iced samples stored at room temperature and at 37° C. Tap water to which soil had been added, contained lactose fermenting bacteria in dilutions of 1-100,000 after 72 hours in the portions stored at all temperatures; in higher dilutions, however, gas was produced only by iced portions. Other experiments showed that lactose fermenting bacteria disappeared quite rapidly from portions of water stored at the higher temperatures.

The total count (24 hours) of polluted surface water increased upon storage at room temperature and at 37° C. In the ice box, however, the counts increased but slightly, results on analysis at the end of 24 and 48 hours, comparing favorably with those from immediate analysis. Advantage of icing this particular surface water is thus indicated. Two samples of water to which soil had been added showed slight differences in counts obtained at the 3 temperatures.

In one sample of diluted sewage stored at 37° C., marked increase in count was observed. In a second sample the 37° C. and ice box counts were not widely divergent.

A strain of *Escherichia coli* inoculated into sterile distilled water died out rapidly at the 3 temperatures of storage particularly at 37° C. This is in agreement with results reported by Hale and Melia. In one case icing appeared to be slightly advantageous.

A suspension of *Aerobacter aerogenes* in sterile distilled water died almost completely within 16 hours at all temperatures in one test and within 3 hours in a second. In another the count was not greatly affected in 24 hours, decreased markedly in 96 hours in the portion stored at room temperature, and in 144 hours in the ice box and 37° C. portions. This was the only sterile distilled water sample which did not show sudden and marked decrease in count within 48 hours.

Counts in sterile distilled water samples containing organisms 2 and 5 (isolated from sewage on Endo's agar and shown to be members of the *colon-aerogenes* group) decreased within 3 hours. In 72 hours the count was low (1-2 colonies per cc.) except in the ice box sample containing organism 5.

In sterile water stored at 37° C. and at room temperature *Eberthella typhi* (strain 340) decreased from a high initial count (greater than 300 colonies per plate) to a low count within 20 hours, while at 3°-5° C. a marked decrease was not observed after 48 hours.

Lack of food materials in sterile distilled water may have caused the high death rate of most of the organisms studied in this medium. It probably cannot be attributed to traces of copper because frequent tests indicated absence of this metal. Since tap water contains more food material and is not sterile, it affords conditions more nearly like those normally encountered in natural waters and for this reason was used in the remaining experiments.

Counts in the iced samples of tap water inoculated with *Escherichia coli* compared more favorably with original counts at the end of 24 and 48 hours than did either the sample stored at room temperature or at 37° C.

An iced portion of tap water containing *Eberthella typhi* gave counts at the end of 24 hours which were slightly lower than the original. The room temperature portion had increased in count. At the end of 48 hours; however, the samples incubated at room temperature had increased in count and the ice box sample had decreased.

The counts obtained at the end of 24 hours on tap water inoculated with *Eberthella typhi* packed in cracked ice were much nearer the original than were those obtained from the water stored at room temperature and 37° C. The sample was packed in cracked ice to simulate actual conditions used in practice. This method was discarded, however, since the cracked ice melted rapidly.

It is difficult to reach conclusions from the data obtained with 2 strains of *Eberthella typhi* in tap water. One did not behave consistently. In the first experiment distinct increases in number of cells were obtained after incubation for 144 hours when stored under the three temperatures used in all of the experiments. It is significant to point out, however, that distinct decreases in number of viable cells were obtained after storage for 24 hours. This is perhaps the period of time over which a sample would be held under actual conditions of shipment. The next experiment with this strain gave different results. After 144 hours' storage at the 3 temperatures used, a decrease in viable cells was secured. However, after 24 hours it is probable that the number of viable cells had begun to increase because the 48 count, the first one which was made, was much higher than the initial count. It is difficult to explain the results secured in these 2 experiments. They probably indicate that the actual conditions which happen to exist in the culture during storage have much to do with the behavior of the organisms contained therein. In other words, the conditions which obtain in the sample bottle may be more important than those existing around it.

One strain of *Salmonella paratyphi* died out within 6 hours when stored in tap water on ice; another maintained practically the same count under these conditions, and a third decreased in count. Results with the 3 strains at the end of 24 and 48 hours varied widely. It is probable that these differences in reaction to storage temperatures may be attributed to differences in the strains themselves.

Tap water containing 2 strains of *Salmonella schottmülleri* stored in the ice box increased in count in 24 hours, but the 48 hour counts were about identical with the originals. The 48 hour count on a sample containing a third strain of *Salmonella schottmülleri* compared well with the original. In some cases storage at room temperature and 37° C. resulted in an increase and in others a decrease in number of viable organisms.

According to the results secured with 3 strains of *Shigella dysenteriae,* icing was advantageous in maintaining the viable cells over the period of the experiments.

Fermentation in lactose broth was observed at the end of 48 hours in polluted surface water stored in the ice box and in the 37° C. incubator but not in samples held at room temperature. In another case gas was observed at the end of 144 hours in only the ice box and room temperature portions. Advantage of icing is again indicated.

Water to which soil had been added contained lactose fermenting organisms in the 1-100,000 dilutions after 72 hours at all 3 temperatures, but in the 1-1,000,000 dilutions gas was produced only at low temperatures.

Gas was observed in lactose broth cultures at the end of 144 hours in water containing *Escherichia coli* at room and ice box temperatures but not at 37° C. In another experiment on the same organism gas was evident in the ice box and 37° C. samples but not at room temperature.

At the end of 96 hours sterile water containing *Escherichia coli* fermented lactose only after storage at low temperatures. The same results were observed at the end of 24 hours with an organism isolated from sewage and at the end of 120 hours with an organism from the same source. The strains of *Escherichia coli* studied died out more readily at 37° C. in most cases.

Conclusions.—1. The characteristics of the particular strains of bacteria present in the water sample have much to do with their behavior during storage. 2. It is difficult to secure comparable counts and perhaps the slight differences up and down which were observed in some of the samples were due to difficulties in sampling. 3. The number of viable organisms present in water after storage for 48 hours in the refrigerator (0°-7° C.) varied but slightly from the original. 4. The ability to ferment lactose is not inhibited by low temperatures of storage but seemed to be maintained over longer periods in iced specimens. 5. The data collected in this investigation indicate in practically all cases that water samples should be kept cold between collection and analysis. This would indicate that icing of samples as provided in Standard Methods for the Examination of Water and Sewage is desirable.