Reprinted from JOURNAL OF BACTERIOLOGY
Vol. 50, No. 2, August, 1945

THE SIGNIFICANCE OF SODIUM CHLORIDE IN STUDIES OF STAPHYLOCOCCI[1]

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Received for publication March 22, 1945

The discovery by Koch (1942) that usually only staphylococci are able to grow on agar media containing 7.5 per cent NaCl has fundamental bearings on the problem of isolating and testing staphylococci.

When 75 grams of NaCl are added to 1 liter of bacto phenol red mannitol agar and the sterilized and poured medium is inoculated with material containing staphylococci and then incubated 36 hours at 37 C, nearly all the organisms that grow luxuriantly are staphylococci that coagulate plasma, and almost all of them are surrounded by yellow zones. Nonpathogenic staphylococci, on the contrary, produce small colonies surrounded by red or purple zones.

Experiments with a small series of cultures indicated that the sensitivity and accuracy of the method are superior to those of methods previously described (e.g., Chapman, 1944c). Moreover, other bacteria are so completely inhibited that it is possible to use a considerably heavier inoculum than was possible in former methods, and enterococci, which are about the only organisms that cause interference on tellurite-treated (Chapman, 1944a) alkaline bromthymol blue lactose agar (Chapman *et al.*, 1937) rarely grow on 7.5 per cent NaCl phenol red mannitol agar.

Aside from its excellent value in isolating staphylococci, 7.5 per cent NaCl has even deeper significance in biochemical tests of this organism. If stock cultures are carried on proteose lactose agar (Difco, formula of Chapman) to which has been added 75 grams of NaCl per liter, the following phenomena will be noted.

Effect on chromogenesis. The chromogenic power of staphylococci is so enhanced that the most sensitive previous method for determining this property, viz., incubation at room temperature for 10 days on milk agar in conjunction with incubation on other media at 37 C (Chapman, 1943), rarely produces pigment with staphylococci that are not chromogenic on 7.5 per cent NaCl proteose lactose agar. However, cultures that do not produce pigment when grown 12 hours on 7.5 per cent NaCl proteose lactose agar at 37 C must still be incubated at room temperature up to 10 days on this medium. The differentiation between porcelain white and pigmented cultures is sharper than on any other medium tested, with the possible exception of milk agar, although it is advisable to gather the growth into a loop for best demonstration of the presence of pigment in the culture.

Of 100 cultures isolated from 7.5 per cent NaCl phenol red mannitol agar, usually one from each nose, throat, or feces culture, 75 were chromogenic on

[1] Aided by grants from the Ophthalmological Foundation, Inc.

incubation at 37 C for 12 hours, another 17 developed pigment after further incubation at room temperature, whereas only 8 were not chromogenic, and they were not chromogenic on milk agar either. Some of the chromogenic cultures produced deeper pigment when grown on milk agar, but this did not affect the differential value of 7.5 per cent NaCl proteose lactose agar.

Since 7.5 per cent NaCl proteose lactose agar was found to be an excellent medium for maintaining stock cultures and because it is satisfactory for determing the chromogenic power of staphylococci, it is suggested that, if these results should be confirmed on a larger series of cultures, the use of milk agar may be abandoned and replaced by 7.5 per cent NaCl proteose lactose agar. The latter medium has the additional advantage in prolonged cultivation that it does not dry out as rapidly as do media containing less NaCl.

Coagulation of plasma. The coagulation of plasma is improved when the cultures are grown on 7.5 per cent NaCl proteose lactose agar for 12 hours at 37 C. Broth enhances the clotting power of staphylococci, and the essential ingredient was found to be NaCl. When the cultures are grown on 7.5 per cent NaCl proteose lactose agar for 12 hours at 37 C and a loopful is suspended in 0.3 ml of broth containing 0.5 g of NaCl per liter, the suspension will then contain the optimum amount of NaCl. This is another reason why it is preferable to carry stock cultures of staphylococci on 7.5 per cent NaCl proteose lactose agar.

Maintaining purity of the cultures. Since almost all other bacteria fail to grow on 7.5 per cent NaCl proteose lactose agar (although many of them are not killed), this medium helps to prevent contamination of the staphylococcus cultures. Even if a heavily contaminated culture should yield cultures containing other bacteria, the NaCl content of the medium tends to prevent growth of these other bacteria, and a second purification on the same medium should eliminate them entirely.

Effect on dissociation of staphylococci. Dissociation is common in staphylococcus cultures, even after overnight incubation. As a result, dissociants lacking chromogenic or hemolytic power, may be isolated from most chromogenic and hemolytic cultures, respectively, and such dissociants are, therefore, considered "degenerate" daughter races of the parent cultures.

This tendency to degenerate is so reduced and most of the biochemical properties are so enhanced when cultures are grown on 7.5 per cent NaCl proteose lactose agar that there is considerable reduction in the proportion of cultures isolated that lack one or more of the biochemical properties characteristic of such cultures. Therefore, differentiation between the reactions of pathogenic and nonpathogenic staphylococci is much sharper than it is by usual methods. Plasma was coagulated by 91 of the 100 staphylococci isolated in these experiments, as compared with 77 per cent by previous methods (Chapman, 1944c).

The Stone reaction. The power of staphylococcus cultures to cause zoning when grown on Stone's medium (Stone, 1935) is reduced when 75 grams of NaCl are added to each liter of the medium. It is possible that many of the numerous nonenterotoxigenic cultures that ordinarily cause zoning on Stone's medium may be eliminated by the added NaCl. However, some of the enterotoxigenic cul-

tures also produce less zoning on the NaCl modification, and the diagnostic significance of this modification can only be determined by tests of a large number of *freshly isolated*, pharmacologically tested cultures.

Relative selectivity and specificity of 7.5 per cent NaCl phenol red mannitol agar. Aliquots of samples from different parts of the body were plated on alkaline bromthymol blue lactose agar (Chapman *et al.*, 1937) and on 7.5 per cent NaCl phenol red mannitol agar, and typical or suspicious colonies were tested for their power to coagulate plasma. Of a total of 208 cultures, 181 gave essentially similar results. Of the rest, there were more plasma-coagulating colonies on alkaline bromthymol blue lactose agar in 3 per cent of the cultures. There were, however, considerably more colonies on the 7.5 per cent NaCl phenol red mannitol agar medium in the other 11 per cent of the cultures, usually when the culture was heavily contaminated or when the number of staphylococci was small. Of the 38 feces cultures in the group, plasma-coagulating staphylococci were found in 11 (29 per cent), as compared with only 9.7 per cent by the tellurite bromthymol blue lactose agar method (Chapman, 1944b) and 5.8 per cent by earlier methods (Stiles and Chapman, 1940).

CONCLUSIONS

The addition of a concentration of 7.5 per cent NaCl to solid culture media effectively inhibits most bacteria other than staphylococci. Pathogenic cultures of staphylococci grow luxuriantly, whereas nonpathogenic staphylococci grow poorly.

The addition of 75 grams of NaCl to 1 liter of bacto phenol red mannitol agar provides an improved isolation medium for plasma-coagulating staphylococci.

The addition of 75 grams of NaCl to 1 liter of proteose lactose agar provides an excellent medium for stock cultures of staphylococci. It inhibits contaminating bacteria, enhances chromogenesis and power to coagulate plasma, and reduces the dissociative ("degenerative") tendency.

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