

embryos are not injured by the lecithin, however, as they will ultimately develop to normal plutei if left in these solutions for a sufficient time.

If cholesterol, suspended in a mixture of m/100 sodium oleate and m/2 NaCl be mixed with the lecithin in equal proportions the retarding action of the lecithin upon the development of *purpuratus* eggs is almost completely neutralized. The slight retardation which is observed in these mixtures may be due to the sodium oleate which is employed to keep the cholesterol in suspension, since sodium oleate is very toxic for sea-urchin eggs and embryos.

Cholesterol itself, when added to sea-water, has no influence upon the rate of development of the eggs. The emulsions of cholesterol are, however, coagulated by the salts in sea-water and the cholesterol is completely thrown out of suspension in the form of coarse flocculi.

79 (775)

On acid agglutination as a method of differentiation of bacteria.

By H. J. SEARS.

[*Division of Bacteriology, Department of Medicine, Stanford University.*]

Michaelis and several other workers following him have claimed for the phenomenon of acid agglutination a specificity comparable with that of specific serum agglutination. The reaction is specific, they say, in that optimum agglutination is produced in suspensions of bacteria of a single species by a definite concentration of hydrogen ions, irrespective of the acid used, and in that this concentration is, in general, different for different species. Their method has been to prepare solutions of definite hydrogen ion concentrations by using mixtures of a weak acid with its sodium salt, the concentrations being calculated from the formula

$$C_H = k \cdot \frac{C_{\text{acid}}}{C_{\text{salt}}},$$

where k represents the dissociation constant of the acid used. The differences between the constants obtained in this way for

the different species are especially marked in the case of the typhoid-colon group. Schidorsky and Reim claim to have had considerable success in the practical diagnosis of typhoid using this method.

Jaffé, working with a number of strains in the typhoid-colon group, obtained constants differing somewhat from those of Michaelis, and observed also several strains of each species which showed markedly different behavior toward the reagents from that of the general average.

In my investigations I made use of six strains of *B. typhosus* and four of *B. coli*. In two sets of experiments, using in one case, acetic acid and sodium acetate, and in the other, lactic acid and sodium lactate, the behavior of five strains of *B. typhosus* was shown to be the same toward both of these reagents. Three of the strains regularly failed to agglutinate at all even when concentrations of hydrogen ion varying between .7 and 900×10^{-5} were used. The other two strains agglutinated about equally in all the concentrations between 14 and 200×10^{-5} . Variations in the concentrations of salt present between N/40 and N/200 made no noticeable difference.

Using acetic and lactic acids without the presence of the salt, the same three strains failed to agglutinate, the other two, however, showing agglutination between the values of 60 and 300×10^{-5} . The hydrogen ion concentrations in these cases were calculated from the formula

$$C_H = \sqrt{k \cdot C_{\text{acid}}}.$$

Comparing these acids with the strong acid HCl, which can be assumed to be completely dissociated in dilutions above N/100, it was found that the three strains which showed resistance toward the weaker acids were partially agglutinated by values of C_H of from 50 to 500×10^{-5} while the other two were completely agglutinated by these concentrations. A sixth strain also showed partial agglutination by the same values.

The conclusions to be drawn from these results would seem to be, first, that the hydrogen ion concentrations necessary to bring about agglutination in suspensions of typhoid bacilli may differ considerably with the way in which these concentrations are ob-

tained; second, that equal agglutination is obtained in wide ranges of concentrations; and third, that these facts together with the existence of atypical strains of both the *B. typhosus* and the *B. coli* make the differentiation of these two species by this method extremely uncertain.