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# Nutritional Factors Concerned with Colony Development of *C. Diphtheriae.*

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One of us[1] has observed that certain strains of the diphtheria bacillus fail to grow from light inocula on plates prepared from a medium consisting of agar, acid-hydrolyzed casein, nicotinic and pimelic acids, beta-alanine, inorganic salts and a carbon source. Growth sometimes occurs after a lag of 2 or 3 days, sooner as the size of the inoculum increases. Such a medium without the agar, inoculated with a loopful of culture, supports rapid and abundant growth. The addition of horse serum to the agar medium improves it markedly, so that single colonies, (presumably resulting from single organisms or small clumps), appear within 24 hours and at the end of 2 days' growth, attain a considerable size. The horse serum can be diluted, acidified and boiled, and the coagulum removed, the filtrate still possessing the original ability to stimulate prompt growth.

It seems possible that some growth accessory material is involved in the phenomenon which is formed by the organisms themselves, once growth is started. It is carried over in sufficient quantities in large inocula to serve for the initiation of growth, after which, more is formed and development proceeds normally. With a light inoculum this is not the case, and unless the material is supplied in the medium, growth fails.

Further experiments have indicated that the sera of different species of animals do not behave alike. Thus, horse serum can be satisfactorily replaced by ox serum, but not by that of the hog, or by human serum.

If the horse or ox serum be freed as completely as possible from coagulable protein and the clear solution subjected to dialysis through cellophane, or ultrafiltration through collodion membranes, the lag-preventing factor is retained by all but the membranes of relatively large pore size. This fact directed attention to the residual non-coagulable proteins, serum mucoid and the like, but it has not thus far been possible to show any close relationship between these substances and the accessory factor.

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1 Mueller, J. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 632.

It was then found that when active solutions were concentrated somewhat in vacuum and precipitated by acetone, a separation appeared to take place into two components, neither of which alone was adequate for colony development. When the separate fractions were combined in the original proportions and added to the basic medium, normal growth resulted.

The constant presence of both materials in ox serum suggested the possibility that milk might be found to be a readily available source. Experiment proved this to be the case, and milk appears to be even richer in the factors than serum. Here, however, the behavior is somewhat different in that all the active material is precipitated with the casein upon simple acidification.

Further work on the characterization of the factors is to be reported.[2]

*Summary.* Rapid development of single colonies of a number of strains of *C. diphtheriae* on a "synthetic medium" agar occurs only in the presence of certain constituents of the blood serum of the horse and ox. Human and swine sera are ineffective. Activity is not impaired by removal of the protein by coagulation. The active material does not dialyze and evidently consists of at least two substances, one of which is soluble in acetone. Cow's milk is rich in these agents, which will be further characterized in subsequent reports.

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## Oleic Acid in Colony Development of *C. Diphtheriae.*

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In a preceding note[1] certain properties are described of substances concerned with rapid colony development of strains of *C. diphtheriae.* These occur in horse and ox, but not in human or swine serum. At least 2 compounds are involved, one of which is acetone soluble. Cow's milk is rich in both factors, which are precipitated together with casein upon slight acidification.

[2] Cohen, S., and Mueller, J. H., PROC. SOC. EXP. BIOL. AND MED., in press.

* Aided by a grant from the Commonwealth Fund.

[1] Snyder, John C., and Mueller, J. H., PROC. SOC. EXP. BIOL. AND MED., in press.