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.

We have found that ordinary commercial casein provides an abundant source of both substances. One cc of a 10% solution of casein in dilute NaOH added to 15 cc of the agar base† induces rapid growth of colonies with our test strain. The two fractions may be separated by prolonged extraction of the dry casein with hot 96% alcohol, or better, by dissolving the casein in dilute NaOH, reprecipitating it with acid, and repeatedly extracting the moist casein with cold acetone. The present report concerns only the nature of the alcohol and acetone soluble fraction.

Butter is extremely rich in the acetone soluble active material, a few milligrams emulsified in dilute NaOH giving maximal colony development. Saponification of the butter yields a fatty acid fraction containing all the activity. The resulting acids have been esterified and distilled fractionally in vacuum as their methyl esters. When hydrolyzed and subsequently tested, the activity tends to be greatest in those fractions containing the unsaturated acids, particularly oleic acid.

All commercial preparations of oleic acid which we have examined possess activity of about the same order of magnitude as that of the fractionally distilled methyl ester fraction, namely, heaviest growth with about 1 mg per 15 cc of medium. The direct proof of the identity of the growth factor with oleic acid, that is, the use of synthetic material, has not been possible, since the synthesis of oleic acid appears never to have been carried out, and evidently would present considerable experimental difficulty. Certain other evidence, indirect, but chemically fairly conclusive, has been obtained that this acid, rather than some accompanying impurity, is the active agent. These experiments will be detailed in our complete report.

Summary. One of the 2 (or more) factors concerned with colony development, (growth from small inocula) of *C. diphtheriae* appears to be oleic acid. The maximal effect is obtained with about 1 mg in 15 cc of basic medium. The other factor, present in the serum of certain species, in milk, and in commercial casein, is being further investigated.

† The basic medium contains an acid hydrolysate of casein, beta-alanine, nicotinic and pimelic acids, a carbon source and inorganic salts.