has recently been shown to prevail in typhus fever and in small-pox. Fisher of this laboratory, in a study still unpublished, has shown that in hemorrhagic small-pox there is a hemolytic streptococcus in the blood with characters as specific for the disease as is the *Streptococcus scarlatinae* for scarlet fever.

Still more striking in this connection is the situation with Proteus X-19 in its relation to typhus fever. Silber[1] has shown that *B. proteus vulgaris,* which does not react serologically with the serum of typhus cases, can be made to do so as the result of its *in vivo dissociation* in collodion sacs in the abdominal cavities of typhus infected guinea pigs.

As knowledge of the biologic nature of the filtrable phases of bacteria increases it becomes clearer that their designation as viruses —for example, the tuberculosis virus—is apt to be misleading. On the other hand, the validity of these filtrable phases of bacteria is not in the slightest doubt, regardless of the specious explanations that have been raised regarding leaky filters, etc.

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Mass Cultures of Streptococcus Hemolyticus in Broth.

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One of us[1] found that a small amount of glucose appeared to be essential in media to be used for the cultivation of hemolytic streptococci and pneumococci. It seems likely that by a splitting of this substance to lactic acid, these organisms are able to derive energy for their growth. The amount of growth was roughly proportional to the percent of sugar, up to the point where sufficient acid was produced to check the growth of the organisms. This use of a phosphate buffered broth to delay the culture in reaching a toxic pH, thus allowing the utilization of more glucose and the development of heavier growth, is now very common. However, even a heavily buffered medium will yield not more than 2 or 3 times the volume of bacteria that can be obtained from the ordinary unbuffered infusion broth.

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[1] Silber, L., *Z. f. Hyg. and Infekt.*, 1928, **108**, 146.

[1] Mueller, J. H., *J. Bact.*, 1922, **8**, 309.

In the preparation of cultures of these organisms for securing large quantities of bacterial sediment or products, one might perhaps, by the occasional addition of sterile NaOH solution, keeping the medium constantly at a pH of 7.2 to 7.8, obtain considerably greater yields than from buffered broth. We have found this to be the case. Ordinary meat infusion broth containing 2% glucose and 5 or 6 drops of 0.2% phenol red solution to 100 cc. is inoculated heavily with a young, plain broth culture of a hemolytic streptococcus or a pneumococcus. After a few hours, when growth commences and the color begins to change from red to yellow, sterile N/1 or stronger NaOH is added from a capillary pipette until the proper red color returns, and incubation is continued. This process is repeated as often as necessary, perhaps every 15 minutes, as long as growth continues. With only 2% glucose this is usually 5 or 6 hours. With larger quantities of glucose it may be much longer, and go more slowly, perhaps because of osmotic effects or the accumulation of metabolic products. At the end of this time, streak plates prepared from such cultures show both streptococci and pneumococci to be still alive, whereas streaks from control unneutralized 0.5% glucose cultures which have become strongly acid are frequently dead. The yields of centrifuged streptococci are very much greater from the neutralized cultures than from controls. From 100 cc. of control culture one obtains ordinarily less than 0.1 cc. of packed sediment, which is usually a dirty gray color and undoubtedly consists in part at least of material precipitated from the broth by the acid formed. From 100 cc. of neutralized broth, approximately 0.5 cc. of white sediment is obtained, made up largely of live organisms.

In the case of pneumococci, autolysis apparently proceeds rather quickly, and the centrifuged bacteria are not so bulky. However, precipitin tests with type-specific sera show much higher concentrations of soluble specific substance in the neutralized cultures than in the controls.

It is a peculiar fact that streptococcus cultures grown in this way are often largely gram negative in smear, although perfectly viable.