

to about 90° by bringing the water in the jacket to the boil, and the tubes should be exposed to this heat for half an hour each day on three or four successive days, when, on placing them in the incubator in the usual manner, all but a few will be found to remain perfectly sterile. If Loeffler's serum is required, the serum is mixed with one-fourth its volume of Loeffler's bouillon before filling into the tubes. This addition does not interfere with the property of coagulating and remaining transparent. If blood serum is employed, care should be taken in collecting it that the clot is allowed to form before transporting the jars of blood. After standing forty-eight hours in a refrigerator or in a cool cellar, an abundant supply of clear serum can be obtained. The presence of small traces of hæmoglobin in the serum does not much impair its translucency, certainly not enough to render it unsuitable for the isolation of diphtheria bacilli.

The serum tubes could be prepared and kept in stock by druggists if the method ever comes into general use, which seems unlikely. The examination may be made from material taken direct from the throat by scraping the membrane, or, preferably, a piece of membrane may be detached by a pair of forceps or a swab of cotton wool. If the membrane has to be transported, it may be put into a clean, dry test-tube or folded up in blotting paper. To examine, it need only be moistened by a drop of sterilized water. A microscopical examination can be made by smearing the piece of membrane over the surface of a cover-glass, passing it three times through a flame. After drying and staining with a drop of any aniline stain, but preferably by Gram's method, the diphtheritic bacilli are seen as short thick rods (about the same length as tubercle bacilli), lying in little groups. These bacilli are present in enormous numbers in the early stages of diphtheria, but diminish rapidly in number as the membrane softens. The highly characteristic involution forms, which assume comma or club shapes with swollen ends, and present a protoplasm broken up into small granules, are only recognizable with a good immersion lens.

The cultures are made by drawing the infected needle in parallel lines along the surface of the serum. By treating two