was isolated from various organs, chorioallantoic membrane, liver, spleen, stomach, intestine, brain, lungs, heart, and kidney. The growth of Rickettsia was particularly abundant and regular from liver, while those from the membrane and the brain were poor. In this respect, murine typhus Rickettsia simulates louping ill and influenza virus[6] in producing both a local lesion on the chorioallantoic membrane and a systemic effect. Apart from the increase of fibrous tissue and proliferation of cells in the ectodermal layer of the membrane previously noted, there was evidence of fatty degeneration in the liver and massive eosinophilic infiltration of the spleen, but no pathological change was found in the brain. All these seem to suggest that typhus Rickettsia of the murine type is able to produce a local lesion on the chorioallantoic membrane as well as a generalized infection which often kills the chick embryos.

10600

Cultivation of Gonococcus in Tyrode-Serum Mixture.

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In the preparation of gonococcus polysaccharides, we have encountered much difficulty in growing this organism in large quantities. While several liquid and semisolid media[1-4] have been used to grow this organism in large amounts and to keep its viability for a limited period, none was found to serve our purpose satisfactorily. The best medium is probably that of Singh[5] but its preparation is rather complicated. In the course of our investigation, we have found the following medium to be simple and efficient, not only for mass-production of gonococcus but also for preserving its viability for a long period.

The medium was prepared by simply mixing 4 parts of Tyrode solution and one part of horse or human serum. When properly

6 Burnet, F. M., *Gt. Brit. Med. Res. Council, Rep. Series No.* 220, 7, 1936.

1 Clark, L. T., and Ferry, V. S., *J. Immunol.*, 1931, **21**, 233.

2 Carbus, B. C., *J. A. M. A.*, 1932, **98**, 532.

3 Warden, C. C., *J. Inf. Dis.*, 1913, **12**, 93.

4 Torrey, J. C., and Buckell, G. T., *J. Inf. Dis.*, 1922, **31**, 125.

5 Singh, N., *Ind. J. Med. Res.*, 1934, **21**, 769.

prepared this mixture always gives a pH of 7.8. It was sterilized by filtration through Seitz filter, after which it was put into sterile flasks plugged with sterile rubber stoppers and finally sealed with paraffin before incubation at 37°C for 48 hours to ensure sterility.

The ability of this medium to support the growth of gonococcus was tested with 7 freshly isolated strains and one stock strain of gonococcus. Following inoculation with a suspension of gonococcus derived from these strains and upon incubation at 37°C diffuse granular growth began to appear after 48 hours. On further incubation a considerable portion of the growth appeared to have settled down at the bottom of the flasks. The maximal growth, however, was not reached until the end of a week's incubation. The growths of 7-10 days' incubation at 37°C were found to be rich enough to yield approximately 1 mg of crude gonococcus polysaccharides per 100 cc of the culture. In our experience a similar yield of gonococcal polysaccharides would require the growth from 15 petri dishes (10 cm in diameter) of nutrient agar pH 7.4 containing 5% horse blood. Thus it is well illustrated that the Tyrode-serum medium is suitable for growing the gonococcus in large amounts especially for purpose of preparing gonococcal vaccines, nucleoprotein, and polysaccharides.

The viability of gonococcus at 37°C in this medium contained in sealed flasks was determined by plating on blood-agar medium (pH 7.4, 5% horse blood) at weekly intervals. In 6 of the 7 freshly isolated strains studied, growth on plates was regularly present as long as 2½ months, beyond which no viable organism could be recovered. In the remaining strain the organism was apparently viable even at the end of 3½ months. No change in the morphological character of the organism or colony has been noted during this period of observation. This, therefore, demonstrates clearly that gonococcus can survive in this medium at 37°C for a much longer period than that ordinarily observed with use of other media.

It is to be noted that sealed flasks have been used. The amount of free air contained in these flasks seemed to have an effect on the quantity of the resultant growth. The mechanism involved in this regard is not entirely clear to us, but it is likely that the amount of free CO_2 present may have something to do with the maintenance of an optimal pH for profuse growth.

From these observations it can be said that the Tyrode-serum medium described is a simple and efficient medium for the mass-cultivation of gonococcus and the preservation of its viability at 37°C.