was made for the polysaccharide N in the precipitate. At the point of maximal precipitation the products recovered with dilute acid and with concentrated NaCl[3] were both 91% precipitable, while those recovered with dilute alkali and with alkaline calcium phosphate[4] were 86 and 85% precipitable respectively.

Since proteins are more easily degraded by alkali than by acid, it is to be expected that the antibody recovered with alkali is less pure than that recovered with acid. While the antibody recovered with concentrated salt is as pure as that recovered with dilute acid, the latter method gives a higher yield and requires less time.

Recovery of rabbit antibody from immune precipitate. This precipitate is much more soluble than that of equine origin. In the absence of NaCl, complete solution of the precipitate occurs when the pH is above 8.6 or below 4. For this reason, the recovery is low and shows no optimal point. If the precipitate is suspended in 3% NaCl instead of water, the recovery is much improved. On the acid side there is an optimum at pH 2.70 with a recovery of 64%. On the alkaline side the optimum lies between pH 9 and 10 and the recovery varies between 30 and 50%. This variation is probably due to the fact that rabbit immune precipitate is gelatinous in alkaline solution and does not reach equilibrium as easily as in acid solution.

Summary. There is an optimal pH for the recovery of antibody from immune precipitate of Type I pneumococcus by treatment with dilute acid or alkali. In the presence of NaCl, the percentage of recovery is increased. The mechanism of the recovery is discussed. The present findings furnish the basis of a method for the isolation of antibody which is better than any of the existing methods.

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Distribution of Murine Typhus Rickettsiæ in Developing Chick Embryo.

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Following the method of Goodpasture, Zia[1] cultivated successfully typhus Rickettsia of endemic and epidemic types in the chorioallantoic membrane of developing chick embryo. This was confirmed by

[3] Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1936, **64**, 161.

[4] Felton, L. D., *J. Immunol.*, 1932, **22**, 453.

[1] Zia, S. H., *Am. J. Path.*, 1934, **10**, 211.

Wenckebach[2] with endemic typhus, Bengtson and Dyer[3] with Rocky Mountain spotted fever, and Cox[4] with endemic typhus fever and Rocky Mountain spotted fever. These authors made a special study of the distribution of the Rickettsia in the infected embryo and found that whole embryo, brain, liver, chorioallantoic membrane, and yolk sac were infectious for guinea pigs, thus proving the presence of Rickettsial bodies. On the other hand Bengtson was not able to demonstrate any Rickettsia by direct smear in any of the organs. In view of the fact that various viruses have a different distribution in the fertilized egg, it was considered of interest to study the distribution of Rickettsia of the murine typhus fever by cultural methods. The procedure employed was as follows:

Growth of murine typhus Rickettsia in Maitland culture was inoculated on to the chorioallantoic membrane of fertilized eggs 10-12 days old. They were incubated at 34°C for 8-9 days after which the eggs were opened, and the membrane, liver, spleen, stomach, intestine, brain, lungs, heart, and kidney were aseptically removed and inoculated separately on to Zinsser's "tissue-agar" media[5] and Maitland flasks. Direct smear of all tissues were negative except for a few organisms occasionally found in smears made from the membranes. The cultures were examined after 12-20 days of incubation, and Macchiavello's technic of staining was employed for demonstration of Rickettsia. Sections of brain, liver, and spleen were made and stained with eosin and hematoxylin.

Result: It was found that about 80% of the 48 eggs so inoculated showed dead embryos on examination, of which approximately 10% was found to have died of bacterial contamination. The high mortality recorded may be due to several factors: spontaneous death, accidental injury during inoculation, and toxic effect of the Rickettsial infection. As far as our experience goes about 20% of the control 9-day-old fertilized eggs incubated at 34°C died spontaneously without any apparent cause. Therefore the high mortality of the embryos might be attributed to either trauma or infection. In 2 instances Rickettsia was isolated from the liver of dead embryos and in one instance from all the visceral organs and brain of a dying embryo; the growth was particularly heavy in this case. These findings suggest that some of the embryos actually died of Rickettsial infection. Of the remaining 7 surviving embryos, Rickettsia

2 Wenckebach, G. K., *Z. f. Hyg. u. Infekt.*, 1936, **117**, 358.

3 Bengtson, I. A., and Dyer, R. E., *Pub. Health Rep.*, 1935, **50**, 1489.

4 Cox, Herald R., *Pub. Health Rep.*, 1939, **53**, 2241.

5 Zinsser, H., *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 604.

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.

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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.