

Infection of the Developing Chick Embryo with Dysentery Bacilli.

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The experimental approach to the problems connected with microorganisms of the *Shigella* group is greatly hampered by the lack of a convenient laboratory animal sensitive to this type of infection. The purpose of this paper is to report on our experiences with the infection of chick embryos with dysentery bacilli, which we hope may contribute toward the alleviation of this difficulty.

Goodpasture and his coworkers have studied bacterial infection in the chick embryo and have investigated the reactions of this new host.^{1, 2, 3} There are also some reports from other laboratories on applications of this method to problems of a similar scope.⁴⁻⁷

For our study we have used 2 strains of *Shigella dysenteriae* ("Shiga bacillus"), 7 of *Shigella paradysenteriae* ("Flexner bacillus"), and 7 of *Shigella sonnei*. All were carefully controlled for their microscopic, cultural and serological properties.

We found fertile eggs incubated 9 to 10 days most favorable for our purposes. We followed, in the main, Goodpasture's procedure.^{8, 9} If the usual precautions of bacteriological work are applied, the danger of contamination of the eggs is certainly not greater than that of agar plates.

The number of viable microorganisms introduced was checked by plate count of 10^{-7} dilutions of the broth culture. On the average the broth cultures contained 500,000,000 microorganisms per ml. The eggs were infected by dropping 0.1 ml amounts of broth diluted serially 10-fold with saline solution upon the chorio-allantoic membrane. The infected eggs were incubated at 37.5°C.

¹ Goodpasture, E. W., and Anderson, K., *Am. J. Path.*, 1937, **13**, 149.

² Goodpasture, E. W., *Am. J. Hyg.*, 1938, **28**, 111.

³ Buddingh, G. J., and Polk, A. D., *J. Exp. Med.*, 1939, **70**, 485, 489, 511.

⁴ Morrow, G., and Berry, G. P., *J. Bact.*, 1938, **38**, 38.

⁵ Morrow, G., Syverton, J. T., Stiles, W. W., and Berry, G. P., *Science*, 1938, **88**, 385.

⁶ Moore, M., *Science*, 1939, **89**, 1939.

⁷ Sterzi, G., and Staudacher, V., *Giorn. ital. di dermat. e sif.*, 1939, **17**, 4.

⁸ Goodpasture, E. W., and Buddingh, G. J., *Am. J. Hyg.*, 1935, **24**, 319.

⁹ Burnet, F. M., *Med. Res. Council, Spec. Rep. Ser.* 220, London, 1936.

All 3 types of *Shigella* mentioned above grew very well on the egg membrane. From one to 10 microorganisms were found to be enough to obtain growth on the membrane. If the growth was only scarce, no gross changes were seen on the membrane. If infection was intense, infiltrates varying from small greyish spots to purulent exudates, sometimes also small hemorrhages, were seen. The degree of this reaction was roughly proportional to the amount of bacteria implanted.

Generalized infection could be produced with each strain. The amounts necessary for this purpose varied from 50 to 50,000 microorganisms. They were fairly constant for each strain. Where generalized infection took place, the microorganisms could be recovered from the heart blood and the organs of the embryo, and the embryos died in 24 to 72 hours, exceptionally also on the fourth day after infection.

Smooth and partially rough cultures were infective to the same degree. However, perfectly rough variants were found to be devoid of invasive power, and thus did not kill the embryos. With our Flexner strains, there were several observations of the reversion from partially rough to smooth after egg passage. No such reversion was observed with Sonne strains.

In many cases quick disintegration of the contents of the eggs followed death. This was especially marked in cases infected with Sonne strains. Where the dead embryos were well preserved, no characteristic findings were noted upon inspection.

We are planning a histological study of both membranes and embryos in order to obtain information on the host's reaction and on possible special localizations of the infective agent.

It was found also possible to cause a lethal infection by injection of dysentery bacilli into the yolk sac of the embryo. In this case, generalized infection takes place within 6 hours.