

The Egg as a Medium for Cultivation of *Mycobacterium Tuberculosis* from Tuberculous Materials.

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During the course of a comparative study of media for isolation of *Mycobacterium tuberculosis* from sputum and other infectious material, it was decided to attempt culture of this organism in eggs. This was prompted by the observation that cultural methods now used for this purpose prescribe media containing whole eggs or egg yolk and because of the successful cultivation of viruses and bacteria on the chorioallantoic membrane of the developing egg. The developing egg has been used by Brandly and Graham¹ and Bradford and Tittsler² for the culture of bacteria. The former authors obtained growth of *Brucella*, *Salmonella* and *Pasteurella* species upon the chorioallantoic membrane of developing eggs and the latter cultured *Neisseria gonorrhoea* in the developing egg. Although these authors were studying the invasive powers or pathogenicity of the inoculated organisms their observations are of interest. Bradford and Tittsler suggest the method as a means of culturing *Neisseria gonorrhoea* from sources such as joint fluid.

In the present study the primary object was cultivation of the organism from infected material in the simplest and quickest manner possible. The egg of the chicken was used with no attempt to obtain a developing embryo. As a preliminary experiment, a suspension of a pure culture of freshly isolated *Mycobacterium tuberculosis* was injected into the yolks of fresh eggs. The egg shell was sterilized with 5% phenol followed with 95% alcohol and then pierced with a sterile pin. The suspension of *Mycobacterium tuberculosis* was then inoculated ($\frac{1}{2}$ cc.) into the yolk of the egg and the pin hole covered with a drop of paraffin. The egg was then placed in the 37°C. incubator and incubated for 20 days. After incubation the egg was broken and the contents were placed in a sterile Petri dish. One might expect that eggs treated in this manner would be spoiled and quite objectionable but this was found not to be the case. The appearance of the eggs treated in this manner is quite different

¹ Brandly, C. A., and Graham, R., *Science*, 1936, **84**, 315.

² Bradford, W. L., and Tittsler, R., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 241.

from that of an uninoculated control egg. The inoculated egg contains numerous, easily visible, greyish patches in both the white and the yolk which are not present in the control. When these greyish patches are picked, crushed upon a glass slide, stained by the acid-fast procedure and examined with the microscope, countless numbers of acid-fast rods are seen in what is apparently a pure culture.

Following this successful culture of *Mycobacterium tuberculosis* in the egg, the procedure was repeated using sputum from a tuberculous patient. In this experiment both fresh eggs and hard-boiled eggs were used and the sputum was concentrated with 6% sulphuric acid, 3% hydrochloric acid, 4% sodium hydroxide, and 5% oxalic acid before inoculation. The eggs were inoculated, using the same procedure previously described, with $\frac{1}{2}$ cc. amounts of the residue after concentration. The results obtained were like those described in the pure-culture inoculations. For example, in one series of 8 raw and 8 hard-boiled eggs inoculated with concentrated sputum 7 of the 8 raw eggs and 6 of the 8 hard-boiled eggs gave very definitely positive growths. In the boiled eggs the greyish patches of discoloration were found mostly in the yolk. If the boiled eggs were inoculated through the air sac the organisms grew in the air sac with the "piled up, crumb like" growth which is so characteristic of *Mycobacterium tuberculosis*. Very seldom could one observe other organisms in the eggs. In the positive eggs the acid-fast organisms were always present in great numbers. Material from positive eggs was inoculated into guinea pigs to establish the pathogenicity of the acid-fast rods. In all cases active tuberculosis was produced in the guinea pigs. In a few cases eggs were examined after 10 days' incubation. Acid-fast organisms could be found in abundance upon microscopic examination but the greyish patches were too small to be easily observed. At the present time it is not known which method of concentration is best for this method of culture.

This procedure gives promise of being a quick and simple method for culturing *Mycobacterium tuberculosis* from tuberculous material.