

of a rabbit has been used to initiate the infection.² When transfers are to be made from infected membranes the cover-glass is removed from the window, and the upper portion of the membrane is exposed as widely as possible by breaking off surrounding egg shell with aseptic precautions. The infected membrane is then cut out and placed in a Petri dish containing sterile .9 per cent. saline solution. A small piece of tissue from the thickened and hemorrhagic area is removed with scissors and smears from this are stained by Morosow's method to determine the presence of Paschen bodies.³ These bodies appear in enormous numbers in the infected membranes although they are difficult to demonstrate in smears from the skin and cornea of chickens infected with vaccinia. Their presence in the smear is diagnostic of infection. Other smears are stained with Loeffler's methylene blue to determine the presence of bacteria. If Paschen bodies are abundant and no bacteria are demonstrable a piece of infected membrane measuring from .5 to 1 mm in diameter is inoculated upon the membrane of each of five or six embryos. We have found twelve day embryos to be perhaps most satisfactory. Pieces of the remaining infected membrane are then inoculated into culture media and others are placed in 50 per cent. glycerol in .9 per cent. saline and in fixatives for histological study.

Sections of infected membranes fixed twenty-four hours in Zenker's solution and stained in a 2 per cent. solution of acid fuchsin for ten to thirty minutes, then counter-stained with Loeffler's methylene blue and differentiated in absolute alcohol, present a most instructive picture. Guarneri bodies are found in all stages of development, finally filling and replacing almost completely the cytoplasm of the cell. It is to be observed that Guarneri bodies occur not only in the epithelial cells of ectoderm and endoderm, but in great abundance, reaching a relatively large size, in capillary and venous endothelium, in fibroblasts and possibly in the smooth muscle cells of blood-vessels. It is to be noted that the inclusions develop in great numbers and to a large size before there is any necrosis or cellular infiltration, although inflammatory exudate is abundant in older necrotizing areas. The effect of the infection on capillary endothelium is of interest. The endothelial cells are frequently stimulated to an active multiplication, so as to form small nodules and cords completely obliterating the vascular lumens. Petechial hemorrhages particularly from capillaries are numerous. The ectodermal cells of the membrane are affected first and apparently most severely, although there is

often a more widely spread effect upon the mesoderm. Entoderm is affected least.

Smears made from infected membrane frequently show masses of partially dispersed Paschen bodies within the cytoplasm of cells; and pieces of membrane examined in distilled water show round or oval masses in which minute bodies are to be seen in rapid Brownian motion, just like those seen in the inclusions of fowl-pox and *Molluscum contagiosum*. Studies are being made upon the relation of the Paschen bodies to the structure of Guarneri bodies. The abundance of both these elements and the unusually large size of the latter make this tissue especially adaptable to such an investigation.

Virus passed through eight generations in chick embryos has been applied to the skin of two or three day old chicks after plucking the down. Macroscopic nodules about 1 mm in diameter appear at the site of inoculation within three days. Remaining apparently stationary for two or three days they rapidly recede. Chicks recovered from vaccinia were infected two weeks later with fowl-pox virus and the lesions evolved exactly like those of fowl-pox in normal chicks. Vaccinia in the chick induces no immunity to fowl-pox.

Our observations suggest that the chick embryo might be used advantageously for cultivating bacteria-free vaccine on a large scale for human vaccination.

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² A strain of Levaditi neurovaccine was kindly supplied by Dr. T. M. Rivers.

³ M. A. Morosow, *Centralbl. f. Bakteriol.*, Pt. I, Orig., 1926, 100, 385.