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## HERPETIC INFECTION OF THE CHORIO-ALLANTOIC MEMBRANE OF THE CHICK EMBRYO \*

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To date attempts to infect chicks and chickens with the virus of herpes simplex have been unsuccessful. Recent work in this laboratory has proved the susceptibility of the extra-embryonic membranes of the chick to the viruses of fowl-pox, cow-pox and herpes simplex.<sup>1</sup> This paper is a more detailed account of the experiments dealing with herpes simplex.

Fertile white leghorn eggs are incubated in the usual manner for 12 to 14 days. The eggs are then candled to locate the embryo and air sac. Each of these is ringed with a colored pencil. The surface of the shell overlying the embryo is carefully cleansed with green soap. By means of a cutting carborundum disc attached by a flexible shaft to a small electric motor a square window 1 to 1.5 cm. in diameter is cut in the shell over the embryo. The window is then coated with a thin layer of paraffin and gently stripped from the underlying shell membrane. Exercising diligent aseptic precautions the underlying shell membrane is removed by cutting around the edges of the window with blunt pointed scissors. This exposes the extra-embryonic membranes, the chorion being uppermost. This membrane may then be easily inoculated. By means of a transfusion syringe filled with a mixture of sterile paraffin and vaseline the edges of the window are ringed, a sterile coverslip is placed over the opening and the edges sealed with a hot needle.

The herpetic virus used in this work was the HF strain obtained from the Rockefeller Institute. Glycerinated brain was emulsified

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and inoculated intracerebrally into a rabbit which was killed 4 days after developing a typical herpetic encephalitis. The brain was removed aseptically and preserved fresh in the ice-box, as well as in glycerin. Small fragments of fresh brain (approximately 1 mm. cubes) were used for inoculation. Transplants were made after 48 hours by removing the membrane sterily and introducing fragments of the infected membrane into other eggs. It was found that this method was rather tedious, consequently an attempt was made to inoculate eggs by using ground, emulsified brain and to transplant by inoculating ground, emulsified membranes, with the result that more abundant infections developed. The virus has been carried through six generations in the chick membranes.

In experiments using tissue fragments for inoculation changes may be noted in the chorio-allantoic membranes within 12 hours. The first change is a congestion of the vessels. Within 24 hours one sees around the bit of inoculum a rather opaque, grayish red zone which spreads peripherally. Careful examination at this time reveals within and around this zone minute grayish opacities just visible at a magnification of 30 diameters. These are particularly abundant along the course of the blood vessels. At 36 hours well defined, raised, grayish opacities, some of which are more than 1 mm. in diameter, are visible to the naked eye. As the lesion progresses these smaller areas tend to coalesce so that at the end of 72 to 96 hours the whole of the exposed membrane may be involved. Inoculation of ground, emulsified material leads to similar lesions. This method, however, provokes a more diffuse reaction which develops more rapidly. By either method the lesions are focal in distribution.

In this work some of the embryos die, due presumably to trauma. A large percentage of those that survived the inoculation were sacrificed at 2, 3, or 4 days for histological study and for further transfers. A few were allowed to run their course undisturbed. In some the process involved the membranes extensively and led to the death of the embryo; in others the involvement was less extensive and the chick hatched in the usual manner. Such chicks showed no gross changes. Examination of embryos which died also showed no changes. At other times the lesions developed in the usual manner and then regressed, ending in complete gross resolution. One egg which showed typical gross lesions with subsequent complete resolution was reinoculated, resulting in another characteristic "take."

For microscopic study the membranes were fixed in Zenker's solution and stained with hematoxylin and eosin or with eosin and methylene blue. The vessels throughout the entire membrane appear congested and there is some hemorrhage. There is a marked polymorphonuclear leukocytic reaction beneath the ectoderm of the chorion. In addition one sees large mononuclear leukocytes scattered here and there. In older lesions, particularly those in which tissue fragments served as the inoculum, there are many fibroblasts. In one instance there is a marked hyperplasia of ectoderm with a minimal amount of necrosis. In another, necrosis is the important feature and there is little or no hyperplasia. In any event some of the cells which remain show the characteristic intranuclear changes of infection due to the virus of herpes simplex.

Within the nuclei of ectodermal cells one sees inclusions of different size, homogeneity and staining reaction. This type of change is usually seen in groups of cells which correspond to the focal lesions noted in the gross, but it may be observed in isolated cells. The nucleus is almost invariably swollen and the chromatic material is collected along the nuclear membrane. Within such cells one sees usually one, but sometimes two or more intranuclear masses distinct from the nucleoli. Some of these bodies are quite small, no larger than nucleoli, others are so large that they almost completely fill the greatly swollen nuclei. They are separated from the nuclear membrane by a clear zone, and are acidophilic in reaction. The smaller bodies appear to be composed of minute granules, while the larger ones are more homogeneous and hyaline in appearance. The largest inclusions stain basophilically and under high magnification appear to be composed of closely packed, minute, basophilic granules. Within other nuclei the inclusion may completely fill the nuclear space. These inclusions stain faintly and appear very finely granular. Such nuclei may become exceedingly large, sometimes attaining a volume considerably greater than that of the entire normal cell. In these nuclei there is a delicate bluish reticulum which appears to divide the nucleus into many small compartments. These compartments are filled with minute, almost invisible, lavender granules. The cytoplasm of these cells shows few if any changes. Occasionally it appears foamy, but this is not the rule.

## DISCUSSION

It is of considerable interest that one is able to infect the chorio-allantoic membranes of white leghorn chick embryos with a strain of herpes simplex virus which is innocuous to adult white leghorn chickens both by corneal and by cutaneous inoculation. The gross lesions of the herpetic membranes are entirely comparable to those of the herpetic keratitis of rabbits. Specific intranuclear inclusions are seen in the cells of the infected membranes. Both eosinophilic and basophilic inclusions are present and are characteristic of the herpetic inclusions observed in other experimental animals. The peculiar reticulum and minute lavender granules within many of the swollen nuclei constitute a very interesting change somewhat suggestive of that described by Goodpasture and King,<sup>2</sup> and again by Goodpasture and Woodruff,<sup>3</sup> in the cytoplasm of epithelial cells within the lesions of molluscum contagiosum. It has not been possible, however, to demonstrate any "elementary corpuscles" similar to those described by Borrel in fowl-pox, by Lipschütz in molluscum contagiosum, and by Paschen in vaccinia.

It has been suggested by Goodpasture and his coworkers<sup>1</sup> that the method of inoculating chick embryos may be of value in the study of various other virus diseases, including those which have not been engrafted upon a foreign host. The fact that the embryonic membranes of the chick are more susceptible than baby or adult chicks points to such a conclusion.

## CONCLUSIONS

1. The chorio-allantoic membranes of the chick embryo are susceptible to infection with a strain of herpes simplex virus which is innocuous to adult chickens of the same breed.
2. The microscopic lesions of these membranes are like those of herpetic lesions of mammals.
3. A peculiar nuclear change in ectodermal cells is described, characterized by enormous enlargement of the nucleus, and by a partitioning of it by delicate trabeculae into compartments which are filled by minute, uniform and faintly stained basophilic granules.

REFERENCES

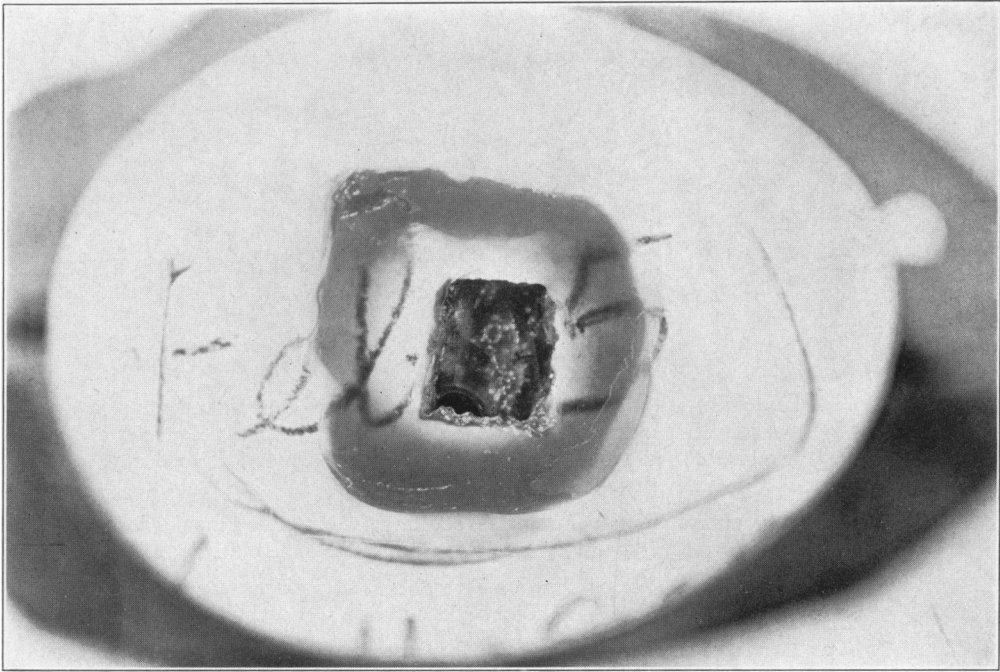
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## DESCRIPTION OF PLATES

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### PLATE I

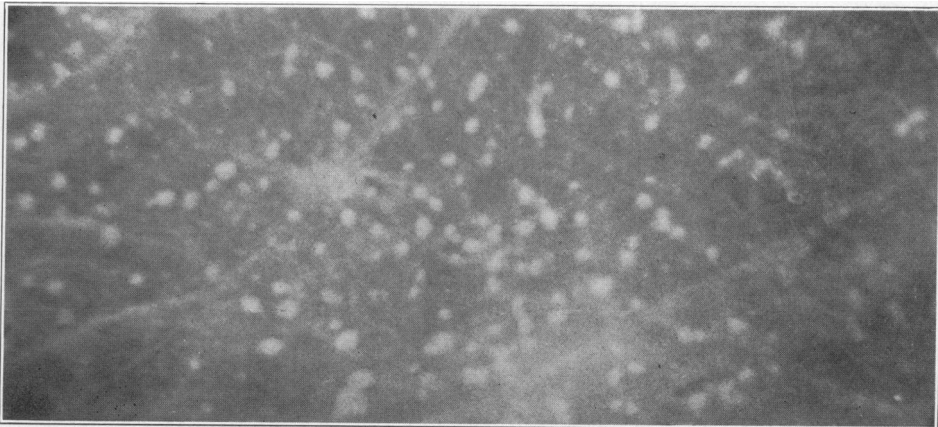
- FIG. 1. Gross photograph showing the glistening pox on the chorio-allantoic membrane of a 48 hour inoculated egg.  $\times 1.5$ .
- FIG. 2. Photograph of portion of above membrane magnified eight times.
- FIG. 3. Photomicrograph of 36 hour membrane showing the diffuse, small, pox-like eruptions.  $\times 10$ .



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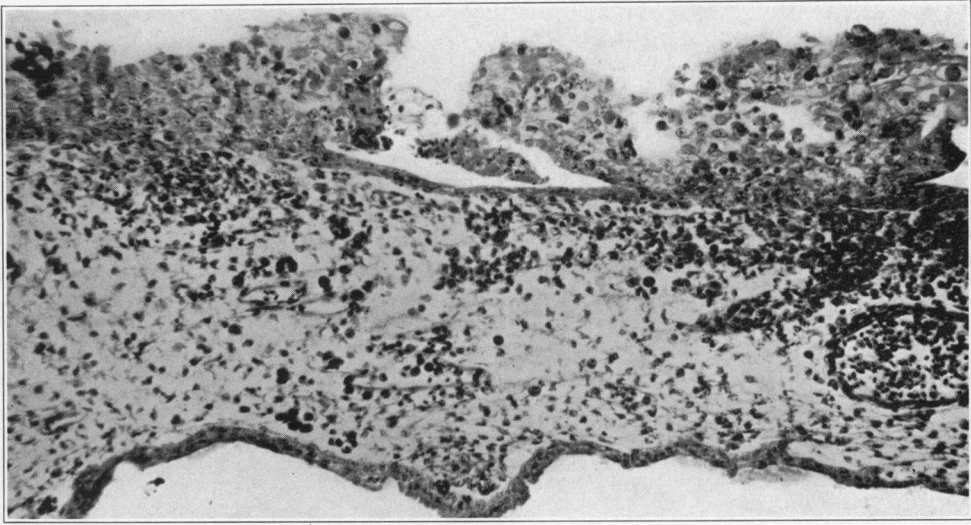
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Herpetic Infection of Chorio-Allantoic Membrane

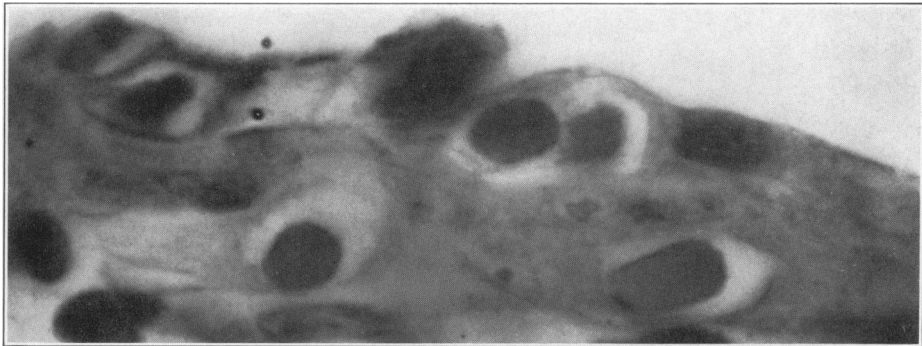
PLATE 2

- FIG. 4. Photomicrograph of entire chorio-allantoic membrane at the site of a hyperplastic lesion. Inclusion bodies may be seen within the nuclei of epithelial cells. The inflammatory exudate beneath the lesion is also illustrated. Eosin-methylene blue stain.  $\times 200$ .
- FIG. 5. Ectoderm of chorion showing several large, very finely granular inclusions which completely fill the nuclei. Eosin-methylene blue.  $\times 1800$ .
- FIG. 6. Ectoderm of chorion showing several smaller intranuclear inclusions which appear granular. Eosin-methylene blue.  $\times 1800$ .
- FIG. 7. Two large ectodermal cells from the hyperplastic chorion. The one on the right shows a poorly defined cytoplasm represented by the clear zone around the greatly enlarged nucleus. The dark staining reticulum is illustrated. The finely granular material can be seen within the compartments formed by the interlacing trabeculae. The cell on the right shows the finely granular appearance of the large inclusions without the reticular material. Eosin-methylene blue.  $\times 2300$ .
- FIG. 8. Two intranuclear inclusions in the ectodermal cells of the chorion, similar to those in Fig. 2. Eosin-methylene blue.  $\times 1800$ .

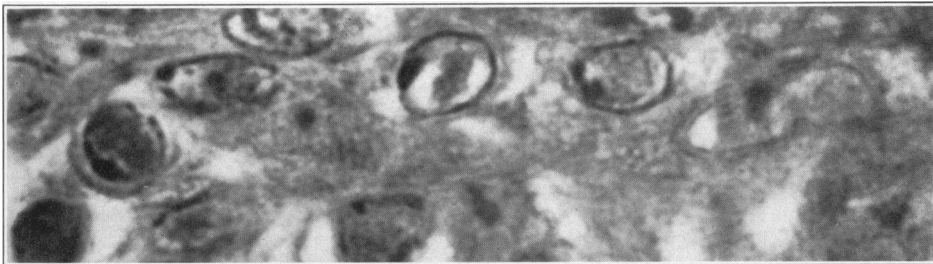




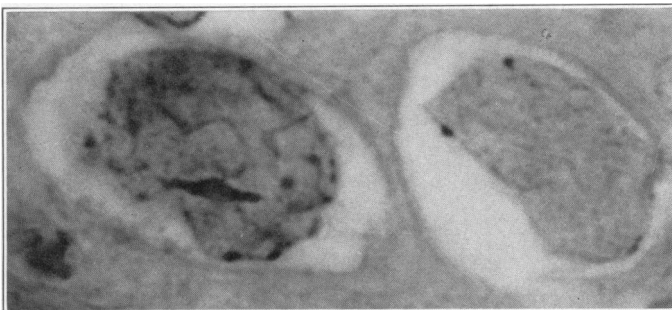
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