A STUDY OF THE MULTIPLICATION OF THE AVIAN ENCEPHALOMYELITIS VIRUS IN CHICK PANCREATIC CELL CULTURES

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follicles. By electron microscopy, herpes-type unenveloped virus particles were found in the nuclei and the cytoplasm of these cells, and enveloped particles were rarely present in the perinuclear and intercellular spaces.

In the contact exposure group, C-form lesions were observed and involuted changes were also found in the later stages.

In the field group of MD, C-form lesions were predominant and B-form lesions were also seen.

The histopathogenesis of the necrosis and disappearance of the follicles in the BF of chickens infected with MDV was discussed. By electron microscopy, the characteristics of the pathologic involution of the BF and morphological relationship between inclusions and herpes-type virus particles could be demonstrated.

A STUDY OF THE MULTIPLICATION OF THE AVIAN ENCEPHALOMYELITIS VIRUS IN CHICK PANCREATIC CELL CULTURES

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1) Cells obtained by collagenase treatment from chick pancreases were cultured, and then monolayers were made about 10 days after cultivation. The monolayers consisted of some sorts of epithelioid cells and fibroblasts at a low percentage.

2) The maximum virus titer of the cell-culture fluid ($10^{2.9}$ EID<sub>50</sub>/ml) was obtained 8 days after having been inoculated with $10^{5.0}$ EID<sub>50</sub> of an embryo-adapted AEV (AEV-VR strain). The virus titers of the cell phase did not rise in parallel with those of the culture fluids. The infected cells maintained by medium changes gave virus titers of $10^{3.7}$ EID<sub>50</sub>/ml in the cell-culture fluid 17 days after inoculation (AI). Virus multiplication was evidently observed in the inoculum of $10^{5.0}$ EID<sub>50</sub>, but not in case of $10^{1.0}$ EID<sub>50</sub>.

3) A chick-pancreas-passed AEV (UP strain, inoculum of pancreas suspension of $10^{3.6}$ EID<sub>50</sub>) and a field isolate of AEV (K-71, inoculum of brain suspension of $10^{2.7}$ EID<sub>50</sub>) multiplied in the cell cultures. The virus titers of the cell-culture fluids were $10^{3.9}$ and $10^{2.1}$ EID<sub>50</sub>/ml respectively 8 days AI. The infected cell cultures maintained by medium changes for 15~20 days revealed virus titers of $10^{3.2}$ and $10^{2.9}$ EID<sub>50</sub>/ml respectively. The UP strain multiplied more rapidly
compared with K-71 isolate.

4) Other cell cultures were made using pancreases from chicks which had been infected with UP strain or K-71 isolate. The virus titers of the cell-culture fluid of these viruses decreased for several days after cultivation and thereafter increased rapidly ($10^{3.7}$ EID$_{50}$/ml on the 14th day of cultivation in UP strain; $10^{2.8}$ EID$_{50}$/ml on the 10th day in K-71 isolate). The relatively high virus titers persisted till at least the 24th or the 35th day. On the other hand, the relatively high titers of the cell phase virus lasted from the beginning to the end of the cultivation ($10^{2.6} - 10^{3.4}$ EID$_{50}$/ml in UP strain; $10^{2.5} - 10^{2.9}$ EID$_{50}$/ml in K-71 isolate).

5) Neither a cytopathic effect nor an inclusion body was observed in the cell cultures infected with AEVs. No AEV-antigen-positive cell was detected by the direct fluorescent antibody technique.

PATHOLOGICAL OBSERVATIONS OF THE THYMUS IN MAREK'S DISEASE

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The morphologic changes occurring in the thymuses of chickens infected with Marek's disease virus (MDV-JM strain) were studied by light and electron microscopy. One hundred and four birds of 4 groups were examined: They consisted of 22 birds of an inoculated group (on the 1st to 56th day postinoculation), 22 birds of a contact exposure group (on the 1st to 163rd day postinoculation), 16 birds of a field group (103 to 186 days old) of Marek's disease (MD) and 44 birds of a normal control group (4 to 157 days old).

In the normal control group, the thymus grew gradually with age and the maximum size was attained between 92 to 127 days after hatching. Involuted changes (physiologic involution) were first observed in female chickens on 157 days old after the beginning of egg-laying.

In the inoculated group, the thymus showed acute involution in the early stage of infection (on the 6-8th day postinoculation). The involuted thymuses were characterized by generalized depletion of lymphocytes, marked atrophy of the parenchyma, replacement by swollen reticulum cells, macrophages and multinucleate syncytia, coincided with herpesvirus particles and probably viral antigen (fluorescent antibody techniques). All birds of the inoculated group were negative for precipitating antibodies to MDV antigens and showed overwhelming infection.