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Isolation of a Feline Virus Associated with Intranuclear Inclusion Bodies. (23783)

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During the summer of 1957, an outbreak of an upper respiratory disease occurred in a group of 5-10-week-old kittens. It was acute, infectious and characterized by conjunctivitis with lacrimation, and nasal discharge accompanied by coughing and sneezing. A viral agent, cytopathogenic for feline kidney cells in tissue culture, was isolated at the time the kittens first exhibited signs of sickness. This paper is a report on the isolation of this agent with a description of the inclusion bodies in tissue cultures and in the cat.

Materials and methods. Tissue cultures of feline kidney cells were prepared in tubes by the trypsin digestion method described by Madin(1). The cells were grown in a nutrient medium consisting of 0.5% lactalbumin hydrolysate in modified Hanks solution with 10% lamb serum. The maintenance fluid used at the time of inoculation was lactalbumin hydrolysate with the addition of 5% lamb serum. Streptomycin (0.5 mg/ml), penicillin (500 u/ml) and mycostatin (100 u/ml) were incorporated in all media. *Isolation technics*: Primary isolation of the agent was made by swabbing the throat and conjunctiva with cotton swabs which were then placed in feline kidney tissue cultures. After 3 hours incubation at 35°C the nutrient fluid was removed and the monolayer rinsed twice and covered with 1 ml of medium prior to additional incubation. Titrations of this agent were made by inoculation of 0.1 ml of decimal dilutions into each of 4 tubes of feline kidney tissue culture. The 50% cytopathogenic end point ($TCID_{50}$) was calculated by the method of Reed and Muench(2). Replicate titrations of virus-containing fluid of various tissue culture passages showed $TCID_{50}$ of 10^{-6} to $10^{-6.6}$ per 0.1 ml. Serial 2-fold dilutions of the inactivated sera were mixed with equal volumes of tissue culture

fluid containing approximately 200 $TCID_{50}$/0.1 of the virus. The serum-virus mixtures were incubated at room temperature for 1 hour. Then 0.1 ml of each mixture was inoculated into each of 4 tubes of feline kidney tissue culture. The presence of neutralizing antibodies was indicated by the absence of cytopathogenic changes. Both tissue cultures and necropsy material were fixed in Bouin's fluid and stained with either hematoxylin and eosin or Giemsa. Histological preparations of tissue culture were made employing either coverslips or the collodion technic(3).

Results. Small foci of degeneration, characterized by rounding of the cells, were first observed after 24 hours of incubation (Fig. 1 and 2). At this time intranuclear inclusion bodies were demonstrated in stained preparations (Fig. 3). These inclusions, when well developed, were homogeneous, acidophilic, usually spherical or oval and separated from the nuclear membrane by a clear halo. The

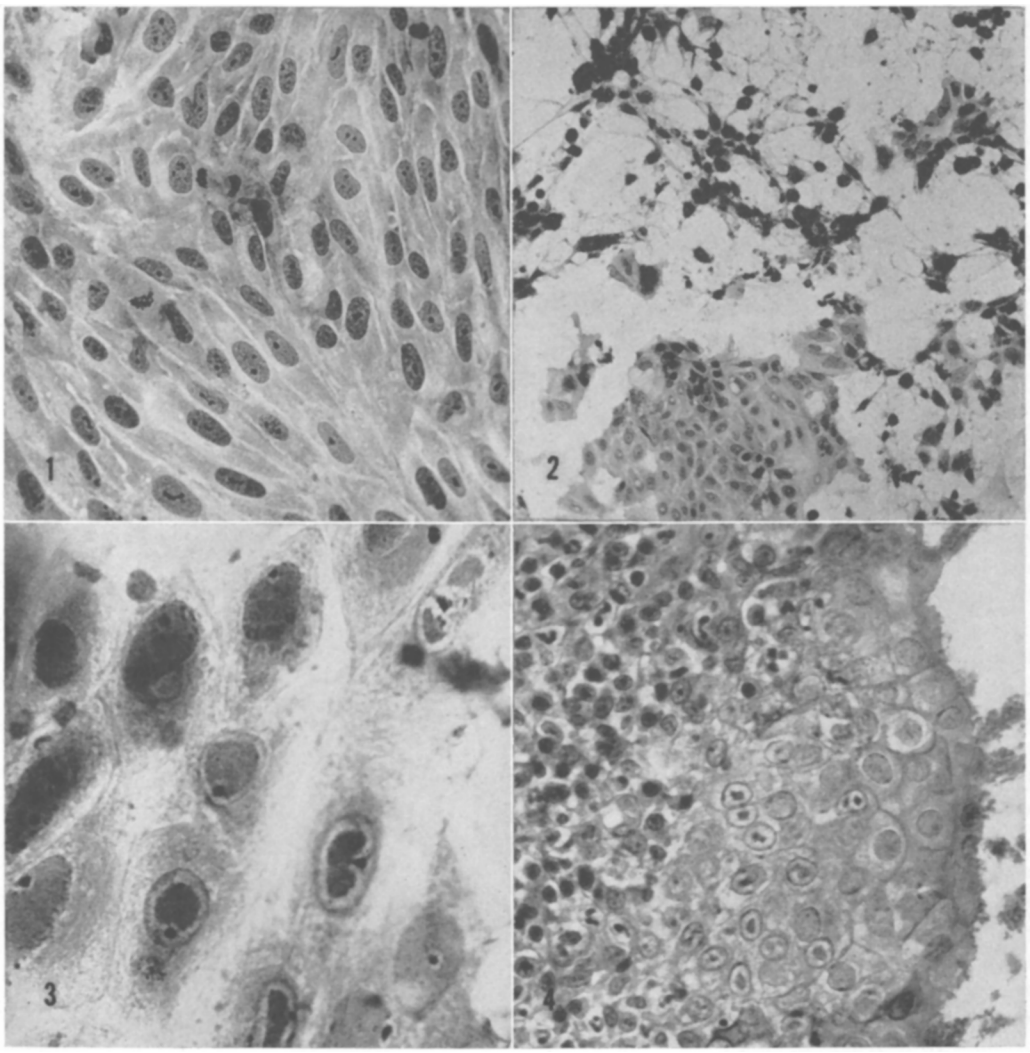

FIG. 1. Uninoculated tissue culture of feline kidney. Giemsa ×265, AFIP-MIS 57-14274.
FIG. 2. Cytopathogenic changes in tissue culture infected with feline virus. H & E ×100, AFIP-MIS 57-14275.
FIG. 3. Intranuclear inclusions in culture of feline kidney with feline virus. H & E ×755, AFIP-MIS 57-14287.
FIG. 4. Intranuclear inclusions in epithelial cells of tonsil in the domestic cat. H & E ×400, AFIP-MIS 57-14343.

nucleoli usually persisted at the edge of the inclusion. These inclusions still were observed in the 12th and last tissue culture passage made to date. All attempts to culture the agent on bacteriological and PPLO media have been unsuccessful. The agent was given the laboratory designation of C-27 virus.

Four kittens were inoculated intranasally with 0.5 ml of tissue culture fluid from the 3rd passage. One uninoculated kitten was placed in an adjoining room as a control. Pyrexia, lacrimation and nasal discharge with coughing and sneezing developed in the 4 kittens, 1 to 3 days after inoculation. The control kitten remained normal throughout the experiment.

Both preinoculation and postinoculation sera from the experimental kittens were tested for specific neutralizing antibodies against the virus. All preinoculation sera failed to prevent cytopathogenic changes. No neutralizing antibody was demonstrated in the postinoculation sera from the 2 kittens sacrificed at 48 and 72 hours. In the animal sacrificed 17 days after inoculation the appearance of cytopathogenic changes was delayed. However, the serum from the remaining kitten, drawn 21 days after inoculation, neutralized the virus in a 1:4 dilution. Serological studies are being continued to confirm this immune response to the agent.

Tissues from the 2 kittens sacrificed at 48 hours and 72 hours were fixed in Bouin's fluid and sections were stained with hematoxylin and eosin. Intranuclear inclusions similar to those in tissue culture were demonstrated in the epithelium of the turbinate, tonsil, nictitating membrane and trachea (Fig. 4 and 5).

In the experimental kittens inclusions were most numerous in the pseudostratified columnar and columnar epithelium of the upper respiratory tract. The apparent sequence of inclusion development, as suggested by the stages in cell variation from normal to those with well developed inclusions, is as follows: The normal columnar epithelial cell stained with hematoxylin and eosin has an acidophilic cytoplasm and basophilic nucleus with a distinct nucleolus and fine, evenly distributed chromatin. Affected cells show margination of the nucleolus

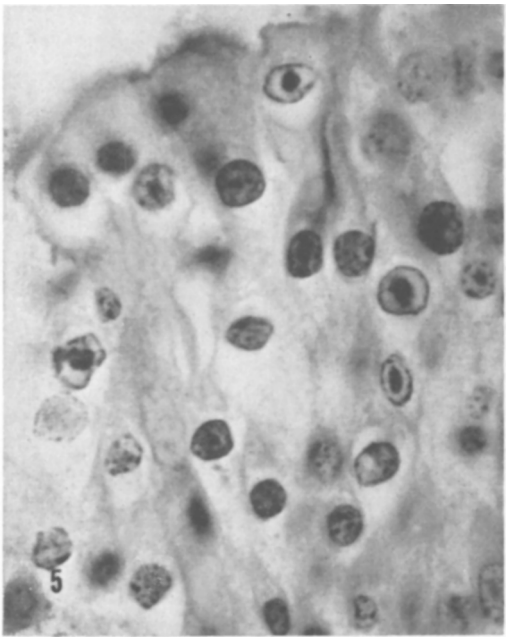

FIG. 5. Intranuclear inclusion in epithelial cells of turbinates in the domestic cat. H&E ×850, AFIP-MIS 57-14339.

and the chromatin, with eventual disappearance of the nucleolus. The center of the nucleus becomes eosinophilic. The homogeneous acidophilic material filling the nucleus contracts, leaving a clear halo around the now spherical inclusion. Well formed inclusions occupy from one-third to one-half of the otherwise clear space within the often shrunken but intact nuclear membrane. Presence of the intranuclear inclusions is usually associated with degenerative changes in the cytoplasm. The cytoplasm becomes pale, later forming a clear zone around one side of the nucleus. In addition to the usual clearing near the nucleus, the cytoplasm may become ballooned, granular and necrotic, losing its distinct outline with consequent disruption of the superficial mucosa. When this occurs localized infiltration of polymorphonuclear cells with submucosal congestion and edema may be seen.

The virus in tissue culture fluid (pH 7.4) survived storage at −60°C for at least 3 months. It was successfully lyophilized.

To determine something of the host range of the virus, 5 day embryonated eggs, weanling mice and rabbits were inoculated. The

agent was not recovered from eggs 5 days after inoculation via the chorioallantoic membrane. No deaths or illness occurred in mice inoculated intracranially or in rabbits inoculated by corneal scarification, both observed for the following 21 days.

Discussion. The 3 feline viruses to which this agent (C-27) was compared were those of feline pneumonitis(4), and panleucopenia (5,6) and the kidney cell degenerating virus (KCD)(7). Comparison was made of clinical symptoms, histopathologic features of necropsy material and the cultural characteristics of the virus in a tissue culture host.

The C-27 virus involves predominantly the upper respiratory system, producing symptoms similar to those of feline pneumonitis. In infections with C-27 virus intranuclear inclusions are observed in epithelial cells of the upper respiratory tract and in tissue cultures; intranuclear inclusions are not known to occur in feline pneumonitis. Furthermore, feline pneumonitis virus in suitable hosts forms elementary bodies(8) characteristic of the psittacosis-lymphogranuloma group and it has not been propagated in tissue culture systems.

Leucopenia, thirst and enteritis, recognized symptoms of feline panleucopenia, are not observed in the disease described here. Intranuclear inclusions in panleucopenia have been reported(6,9), but only in the intestinal mucosa, and recent study(10) indicates that they are not demonstrable consistently.

Although Fastier's(7) KCD virus produced cytopathogenic changes in feline kidney cell cultures, it did not form inclusions or elementary bodies, nor did it cause clinical disease in cats. Fastier considered it an orphan virus.

The evidence presented suggests that C-27 is a previously unrecognized viral agent pathogenic to the domestic cat.

Additional studies are in progress to characterize and identify the virus and the disease more fully.

Summary. 1) A viral agent cytopathogenic for feline kidney tissue culture was isolated from a cat with a respiratory infection. It has been shown to be capable of reproducing the illness in susceptible kittens with the development of specific neutralizing antibodies. Intranuclear inclusions have been demonstrated in both tissue culture preparations and kittens in which the disease was reproduced. 2) The agent has survived lyophilization and storage in tissue culture fluid for at least 3 months at −60°C.

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