abdominal approach is preferred. The cervical approach was generally accompanied by respiratory distress after more than one hour of lymph collection.

Table I also presents a comparison of data obtained from studies made on paired mice in which thoracic duct lymph was collected from either the neck or abdomen at same time of day. Mean values for lymph flow, lymphocyte content, and total lymphocyte output did not differ. Although mean values were similar for abdominal and for cervical lymphocyte output for paired animals, individual cell outputs collected simultaneously in paired animals on same day were closer to each other than in collections made at similar times but on different days.

On the basis of the mean total thoracic duct lymphocyte output of 1.5 cells/hr x 10^6 for anesthetized or unanesthetized mice, it can be calculated that at least 35 million cells enter the blood from this duct in a 24-hour period. This is 3.3 times the total calculated number of circulating lymphocytes.

Summary. 1. The mouse is a suitable animal for collection of thoracic duct lymph in the partially-restrained unanesthetized state. 2. Initial values for lymph flow and lymphocyte output did not vary significantly in the unanesthetized as compared to the anesthetized mouse. 3. A continuous decrease in lymph flow and lymphocyte output from the thoracic duct occurred over a 24-hour period in the unanesthetized mouse. 4. Values for thoracic duct lymph flow and lymphocyte output did not vary significantly, whether collected from the neck or abdomen.

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Cytopathology of Feline Viral Rhinotracheitis Virus in Tissue Cultures of Feline Renal Cells. (24993)

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In the original report(1) on isolation of a feline virus associated with intranuclear inclusion bodies the agent was designated as C-27 virus. We now propose the name "feline viral rhinotracheitis" for the disease entity induced by this agent. The virus in this and future communications will be referred to as feline viral rhinotracheitis (FVR) virus. In this paper we describe the sequence of morphological changes with formation of multinucleated giant cells or syncytia in cell cultures infected with FVR virus, and report our results from the use of different methods of fixation for demonstration of inclusions.

Materials and methods. The original FVR virus obtained from the throat of a cat exhibiting clinical signs of upper respiratory infection was isolated in cultures of feline renal cells. The virus used was from 5th and 6th tissue culture passages. Primary cultures of feline renal cells were prepared on 11 x 22 mm coverslips in Leighton tubes. Tissue culture methods employed were those of Madin *et al.* (2). The cells were grown in nutrient medium consisting of 0.5% lactalbumin hydrolysate in modified Hank's salt solution containing 0.01 M Tris (Hydroxymethyl aminomethane), adjusted to pH of 7.4, and 10% lamb serum. The maintenance fluid used at time of inoculation was lactalbumin hydrolysate

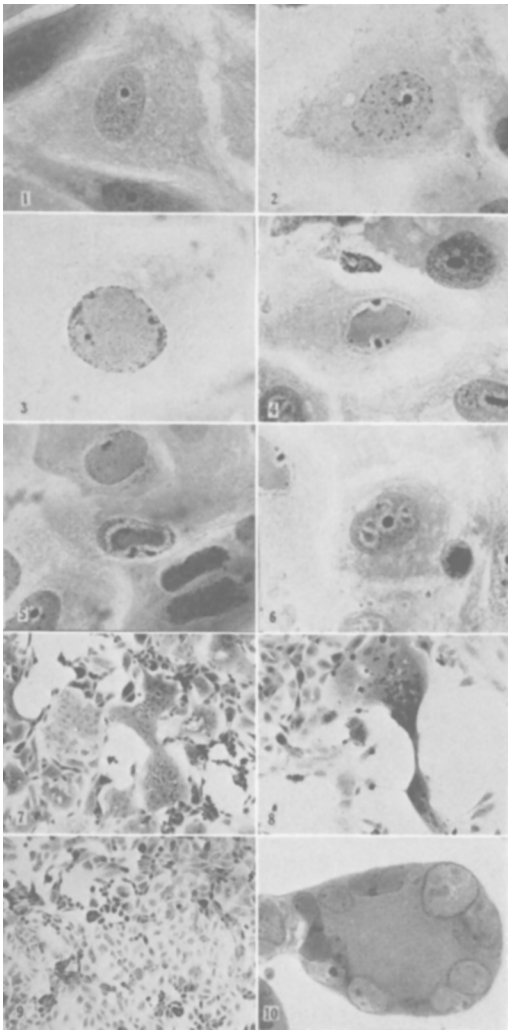

FIG. 1. Control. Cell from uninoculated culture of feline renal cells. H & E stain, × 500. AFIP-MIS 59-1154.

FIG. 2. Cell soon after inoculation with feline virus, showing stippling of nuclear chromatin material. H & E stain, × 500. AFIP-MIS 59-1157.

FIG. 3. Cell from same culture showing beginning of chromatin margination. H & E stain, × 500. AFIP-MIS 59-1161.

FIG. 4. Cell in later stage of infection, with early condensation of nuclear material. H & E stain, × 500. AFIP-MIS 59-1158.

FIG. 5. Cell with a well-formed intranuclear inclusion body. Note shrunken but intact nuclear membrane. H & E stain, × 500. AFIP-MIS 59-1160.

FIG. 6. Cell from infected culture containing aggregates of nuclear material. H & E stain, × 500. AFIP-MIS 59-1156.

FIG. 7. Cultures of feline renal cells infected with the virus, showing early formation of syncytium. H & E stain, × 35. AFIP-MIS 59-1150.

FIG. 8. Same culture as Fig. 7, with later stage of syncytium development. H & E stain, × 35. AFIP-MIS 59-1150.

FIG. 9. Early lesion seen in tissue culture of feline renal cells infected with feline virus. Note giant cells. H & E stain, × 35. AFIP-MIS 59-1151.

FIG. 10. Well-developed giant cell in feline tissue culture infected with the virus. H & E stain, × 433. AFIP-MIS 59-1148.

with addition of 3% lamb serum. Streptomycin (0.5 mg/ml), penicillin (500 u/ml), and mycostatin (100 u/ml) were incorporated in all media. Cultures were inoculated with 0.1 ml of tissue culture fluid containing a calculated 100 $TCID_{50}$. Following inoculation, all cultures were incubated at 37°C and observed with a light microscope at magnification of 100 for presence of cytopathogenic change. The coverslips for studying sequence of inclusion formation were fixed in Bouin's fluid for 30 minutes, then kept in 70% alcohol until stained with hematoxylin and eosin (H & E). To determine best method of fixation for H & E stained preparations, the following solutions were used: Bouin's, Zenker's acetic, methanol, acetone, and 10% neutral formalin.

Results. Cytopathology. In unstained preparations the first detectable lesions were seen at 40-48 hours. At this time small foci involving 10 to 12 cells were present. These foci consisted of cells which were either rounded or elongated in shape and were joined together by protoplasmic bands. The affected cells were more refractile than normal cells. Between 52 and 72 hours these foci increased in size and number, and at 96 hours they were present throughout entire monolayer. The entire culture was involved at 120 hours, and the cells were all released from the glass surface at end of 144 hours.

Normal cells stained with H & E have an acidophilic cytoplasm, basophilic nuclei with distinct nucleolus, and fine, evenly distributed chromatin (Fig. 1). The sequence of inclusion development, as suggested by stages in cell variation from early nuclear alteration to well-developed inclusions are as follows:

The earliest discernible changes are seen primarily in nuclei of cells stained after 36 hours of incubation. At first there is enlargement of nuclei and stippling of basophilic nuclear chromatin. Chromatin granules and

nucleoli fragment into fine particles dispersed on light-staining background of nuclear substrate (Fig. 2). These granules then marginate, leaving a central area of homogeneous nuclear material (Fig. 3). Later this nuclear material becomes acidophilic and contracts, forming a well-developed inclusion surrounded by clear halo (Figs. 4 and 5). Outside the halo the basophilic nuclear membrane remains intact though often somewhat shrunken. In a few cells which retain their nucleoli, 2 or more acidophilic intranuclear inclusions with small individual halos have been observed (Fig. 6).

Characteristic cytoplasmic change is formation of multinucleated giant cells or syncytia. Individual cell loses its distinct cellular membrane and fuses with adjacent cells. In early stages the cytoplasm of cells in the syncytia is paler than in other cells (Fig. 7). These syncytia contract and eventually break away from the cellular mass to form separate multinucleated giant cells (Fig. 8). In the final stage of development, these giant cells are round or club shaped and have a granular, intense eosinophilic cytoplasm with nuclei about the periphery (Figs. 9 and 10). In these nuclei are inclusions at various stages of development. No specific cytoplasmic inclusions were found.

Discussion. Although intranuclear inclusions were demonstrated at the time this virus was first isolated, the significance of syncytium formation was not initially recognized as a feature of its cytopathogenicity. This belatedly recognized cytopathic change was suggestive of a possible mutation of our original isolate or the introduction of another agent. Upon reexamination of H & E preparations of our earlier passages, we observed occasional small giant cells with 2 or more nucleoli. Only one type of plaque was produced in cultures by the method described by Dulbecco and Vogt(3). All of these cytopathogenic changes, now considered characteristic of FVR virus, were inhibited by homologous immune rabbit serum.

It appeared unlikely that the tendency to formation of giant cells was a nonspecific alteration in the feline cultures, since primary cultures had been used in all our studies. In addition, we used cultures of feline renal cells for several years and never previously experienced this type of cytopathogenic change in uninfected cultures. Development of typical intranuclear inclusions is observed in these giant cells. In later tissue culture passages, these multinucleated giant cells are larger and occur with greater frequency. We observed nuclear changes and well-developed inclusion bodies in necropsy material from cats experimentally infected with the virus. However, giant cells similar to those found in tissue cultures have not been observed in the disease.

In this work, metabolism of cultures was measured by acid production in the media. Comparison of hydrogen ion concentration of fluids from noninfected and infected cultures indicate that there is a slight metabolic inhibition in the infected cells. Reduction in pH of fluids is less for infected cultures than for normal. Although there is a pH differential between fluids of infected and noninfected cultures, it is not sufficient to apply the color test for infectivity in the tissue culture system studied. Robbins *et al.*(4) reported that metabolic activity of cultures infected with polio virus was less than that of normal control cultures. In tissue cultures infected with adenovirus(5) and the virus of infectious bovine rhinotracheitis(6), the reverse is seen. With these latter viruses, infection results in a decrease in pH of culture fluids. Recognition of these basic differences characteristic of these viruses leads to a better understanding of the virus-cell relationship.

Of fixatives studied, inclusion bodies were demonstrated in H & E preparations which were fixed in Bouin's fluid, Zenker's acetic, methanol, and acetone. Bouin's fixation consistently gave the best results and is used routinely in our laboratory. Formalin-fixed tissue culture preparations were unsatisfactory for studying the cytopathology, and inclusion bodies were not demonstrable when such preparations were used.

The virus was propagated in primary cultures of feline lung and testicle, resulting in development of cytopathogenic changes and inclusions similar to those occurring in cultures of feline renal cells. Cytopathogenic changes were not demonstrated in cultures of

bovine kidney, Chang liver, human amnion, monkey kidney, human conjunctiva, and "L" cells.

Summary. Cytological changes occurring in cultures of feline renal cells infected with virus of feline viral rhinotracheitis are described. The features of cytopathogenicity of this virus are (1) development of intranuclear inclusion bodies; (2) formation of giant cells; and (3) a slight reduction in metabolic rate of infected cells.

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Effect of Midbrain Lesions on Ovulation and Adrenal Response to Stress in Female Rats.* (24994)

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As part of a continuing study attempting to identify brain areas essential for normal pituitary function effects of localized brain lesions on stress-induced corticosterone release and adrenal ascorbic acid decrease have been investigated. Earlier experiments revealed a dichotomy between adrenal ascorbic acid decrease and corticosterone release following surgical stress in rats with electrolytic lesions placed in different parts of the hypothalamus(1). In the present study adrenal responsiveness has been measured in rats with lesions in the diencephalon, midbrain and at the level of rostral pons. In addition, since lesions in the midbrain have been shown to inhibit ovulation(2) changes in ovulatory cycle were investigated in some animals.

Materials and methods. Coagulating brain lesions were made in 28 adult female Sprague-Dawley rats (225-300 g) from our breeding colony. The coagulating current (2.7 milliamperes/sec.) was delivered for 5-10 sec. from a Birtcher Blendtome surgical unit through paired stainless steel insect pin electrodes 1 mm apart, insulated except for 1 mm at tip. Midline lesions were made at various levels between posterior border of mammillary body and posterior end of the interpeduncular nucleus 1 to 3 mm from base of brain. In control rats electrodes were inserted but no current applied. Rats were maintained postoperatively in individual cages on Purina lab chow or force-fed 3-5 times daily with milk-5% glucose solution. Constant room temperature (37-38°C) and controlled lighting conditions were maintained(2). Four days after lesioning, the left adrenal vein was cannulated and blood collected for 30 minutes. Pre- and post-stress adrenal ascorbic acid levels were determined immediately after cannulation ("resting" levels) and at end of collection(3), using the method of Roe and Kuether(4). Corticosterone was analyzed as noted earlier(5). When necessary, anesthesia was induced by pentobarbital sodium (3 mg/100 g, i.p.). Nine rats were studied for changes in ovulatory cycles by method described by Critchlow(2). In these rats, unilateral ovariectomy was performed on first or second day post-lesioning. No rat was included in the series if microscopic examination of pituitary revealed damage from lesioning.

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