"C"-Type Viral Particles in Plasma of Cats with Feline Leukemia

Abstract. *Linear sucrose-density gradient was used to detect and isolate typical "C"-type viral particles in plasma of cats with spontaneous and experimentally induced leukemia. The density of the agent is similar to known murine leukemia virus (1.15 to 1.17 grams per cubic centimeter). In the electron microscope the virus showed typical "C"-type particle morphology with various maturation stages. The maximum diameter of the mature viral particles in plasma was 115 millimicrons, a diameter slightly larger than budding particles observed in tissue. Leukemia was transmitted with cellular and cell-free inoculum after a 5-week period of latency.*

Transmission of feline leukemia (lymphosarcoma) was first reported by Jarrett *et al.* (*1*), who also demonstrated "C"-type viral particles in tissue of cats with spontaneous leukemia. The same result was obtained recently by Rickard *et al.* (*2*).

In establishing criteria for selection of the most suitable leukemic donors for studies of the transmission of bovine leukemia, density gradients of plasma from leukemic cows were studied for the possible detection of a viremic state. Because of the period of latency in the development of bovine leukemia (*3*) a parallel study of feline leukemia was initiated in order to study techniques capable of providing results more rapidly.

We now report (i) the characterization of "C"-type viral particles from the plasma of cats with leukemia, (ii) the transmission of leukemia to kittens by either whole cells or cell-free material, and (iii) the demonstration of the agent in the plasma and tissue of animals with experimentally induced leukemia.

At the terminal stage of illness and just before death, 60 ml of blood from a 3-year-old castrated cat with spontaneous disease was collected, treated with an equal volume of chilled 0.306*M* potassium citrate solution (*p*H 7.0) and centrifuged as described by Moloney (*4*). The pellets were suspended in 1 ml of tris-ethylenediaminetetraacetate-saline buffer at *p*H 7.0. The suspension was then layered on a linear sucrose gradient (1.06 to 1.30 g/cm^3 at 20°C) prepared essentially as described by Martin and Ames (*5*). The crude viral suspension was centrifuged for 4 hours at 175,000*g* at 3°C. The bands were examined under indirect lighting and photographed. The specific band in the area of 1.15 to 1.17 g/cm^3 was isolated and prepared for electron microscopic examination. Specimens for negative staining were fixed in glutaraldehyde and stained with 1 percent phosphotungstic acid (*p*H 6.3). Unsupported thin sections were double-stained with uranyl acetate and lead citrate (*6*). All specimens were examined in a Philips EM-200 electron microscope at 80 kv.

Analyses of results from the density gradient of plasma from a cat with spontaneous leukemia showed a definite banding in the vicinity of 1.15 to 1.17 g/cm^3 which was comparable to the banding of Rauscher leukemia virus (*7*). Similar bands were found in the plasma of three kittens with the experimentally induced disease but not in the plasma of a normal uninoculated kitten. Density-gradient bands of plasma samples from spontaneous and experimentally induced feline leukemia contained a definite band at a position of comparable density (Fig. 1).

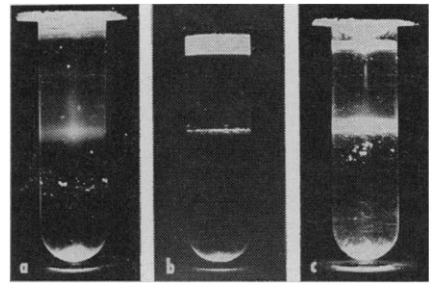

Fig. 1. Sucrose density gradient analyses of plasma from (a) a cat with spontaneous leukemia (25 ml); (b) a cat with experimentally induced leukemia (4 ml); and (c) a rat with experimentally induced Rauscher virus leukemia (15 ml).

Electron microscopic examination of the viral band from the plasma of cats with spontaneous and experimentally induced leukemia showed maturation stages of typical "C"-type viral particles. Mature particles were predominant (Fig. 2a), but occasional immature forms were observed (Fig. 2b). The mature particles had an outer envelope with a uniform electron dense nucleoid. The average maximum outer diameter of these particles was approximately 115 mμ with a nucleoid measuring 90 mμ. The immature particles consisted of an outer envelope and two concentric inner shells. The entire virion, including the outer envelope measured 115 mμ, while the outer and inner shells measured 90 and 65 mμ in diameter, respectively. Virus was easily demonstrated by negatively staining the viral band from a gradient preparation from the plasma of a kitten with experimentally induced leukemia.

In order to evaluate the biological activity of the isolated particles, we have conducted transmission experiments in two litters. Nine kittens were inoculated intraperitoneally with either cellular or cell-free inoculum: the first

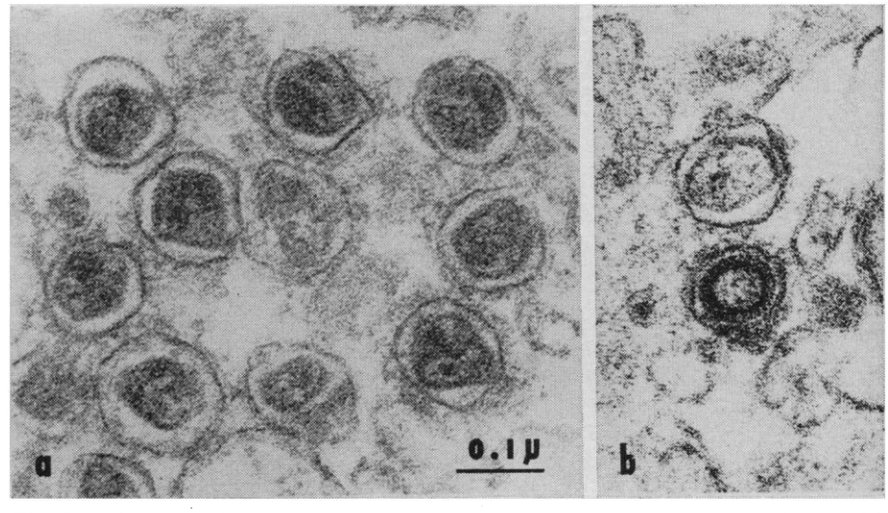

Fig. 2. Electron micrographs of "C"-type viral particles from plasma of cat with spontaneous feline leukemia; (a) mature particles and (b) immature particle.

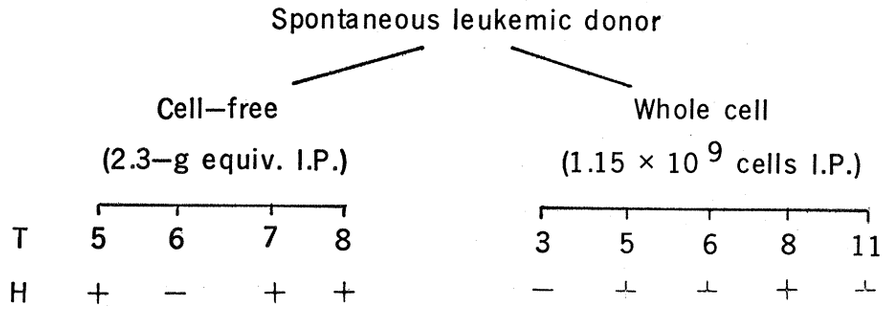

Fig. 3. Transmission of feline leukemia scheme showing the latent period in weeks (T) and histological analyses (H).

litter of five 4-day-old kittens received 1.15×10^9 whole leukemic cells; and the second litter of four 1-day-old kittens received 2.3 g equivalents of cell-free suspension prepared by techniques of differential centrifugation (Fig. 3) (*4*). Lymphosarcoma was histologically proven in three of the four kittens inoculated with cell-free suspension within 8 weeks of inoculation. Three of five kittens that were definitely leukemic, and one kitten that was questionably so—all inoculated with whole-cell suspension—also were proved to be histologically leukemic within 11 weeks after inoculation.

The "C"-type particles isolated from the plasma of cats with both spontaneous and experimentally induced leukemia showed morphological and physical characteristics that are very similar to murine leukemic viruses, particularly the virus described by Rauscher (*8*). Since the morphology and maturation of these two different animal leukemic viruses are very similar, it is not surprising that their densities are similar.

This is the first description of the maturation stages of the "C"-type viral particles in plasma of cats with leukemia. Our study suggests that feline leukemia viral particles undergo maturation stages similar to those found in mice, and, like the murine leukemia virus, mature forms are predominant in the plasma (*9*). The size of the particles in plasma was slightly larger than those reported by Jarrett *et al.* and by Rickard *et al.*; however, the size of budding particles observed in tissue was in the range described by Rickard *et al.*

Although transmission of feline leukemia was reported by Jarrett *et al.*, they observed a prolonged period of latency of 9 months (*10*). The present transmission study which utilized both cellular and cell-free inoculum demonstrates that feline leukemia can be induced as soon as 5 weeks after inoculation. This short period of latency in the recipients may be a reflection of the viremic state of the donor and perhaps of the large amount of virus inoculated.

Density-gradient analyses of plasma in conjunction with electron microscopic examination can be an effective means to detect the viremic state in the cat, and, in turn, it may be useful for selecting the best leukemic donors among populations of other animals, including man. This method appears to be a promising approach for the detection of the ideal donor where the natural period of latency may exceed 5 years or more, as it does in several animal species.

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Tiliqua scincoides: Temperature-Sensitive Units in Lizard Brain

Abstract. *Extracellular action potentials were recorded from units in the preoptic area of the brain of the Australian blue-tongued skink* (Tiliqua scincoides) *during periods of local heating and cooling of the brain (20° to 36°C) with water-perfused thermodes. In this temperature range most spontaneously firing neurons were temperature-insensitive, but eight showed sensitivity to the thermal stimulus. Five warm neurons increased their activity when the brain temperature was raised, and three cold neurons showed increased activity with fall in temperature.*

The existence of sensitivity to temperature in a reptilian brain was first described by Rodbard *et al.* in 1950 (*1*), who changed the brain temperature in turtles and observed cardiovascular responses. Only recently was this sensitivity related to thermoregulation by Hammel *et al.* (*2*), who showed that local heating or cooling of the anterior brain could affect thermoregulatory behavior of the Australian lizard *Tiliqua scincoides*. Since the presence has been shown (*3*) of temperature-sensitive neurons in the hypothalamus of warm-blooded species such as the cat, it was of interest to seek, in the reptilian brain, neurons responding to temperature even though Hammel *et al.* could find no physiological thermoregulatory response in these lizards.

Again we used the Australian blue-tongued skink. All surgery was performed under ether anesthesia or cold lethargy; the animals were then allowed to recover, and the experiments proceeded without further anesthesia. With the head immobilized in a specially built holder, a U-shaped, water-perfused thermode was implanted in the brain, aimed more or less at the preoptic region (Fig. 1)—that is, perpendicularly to the skull surface, 2 to 4 m rostral from the pineal eye, on