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Intracellular and Extracellular Mineral Crystal Formation Induced by Viral Infection of Cell Cultures[1]

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Intracellular mineral crystals were observed in cell cultures infected with one or more feline viruses. One was a feline syncytium-forming virus, and the other was a new feline virus which produced intranuclear inclusions in cell cultures. Crystals were noted both in autogenous cell cultures from trypsinization of kidneys and urinary bladders of infected cats (three passages) and in subcultures of culture fluids from these, made in a stable feline kidney cell line. Free mineral crystals were also noted in the fluids of the cell cultures. In two bladder and two kidney infected autogenous cultures, structures resembling the urinary calculi observed in obstructed cats were seen. These consisted of mineral crystals in a matrix of cellular debris.

During investigations of virus-induced urolithiasis, a feline picornavirus (Manx strain) was isolated and found to induce urolithiasis in experimental male cats (3; Rich, Fabricant, and Gillespie, Cornell Vet., *in press*). The study of the pathogenesis was complicated by the isolation of feline syncytium-forming virus (myxo-like virus) from the kidney cultures of 12 cats in which urinary obstruction was induced experimentally after inoculation or exposure to the fourth cell culture passage of the Manx virus strain. The syncytium-forming virus was also isolated from all of the five bladder explant cultures initiated from these cats (1).

In microbiological studies of eight spontaneous cases of urolithiasis, syncytium-forming virus was isolated from kidney and bladder cultures of each cat (Fabricant and Rich, J. Amer. Vet. Med. Ass., *in press*).

Recently an apparently new feline virus was isolated from the kidney pool of two 5-week-old littermate kittens. It produced an adenovirus-like cytopathic effect in cell cultures and intranuclear inclusions. Preliminary studies indicated that this new isolate produced type B intranuclear inclusions and appeared to be chloroform resistant (Fabricant and Gillespie, *unpublished data*). The same virus was isolated from first transfer cultures of a 3.5-month-old cat (no. 779) which died 11 days after showing respiratory disease signs. Prior to death, picornavirus isolation was made from pharyngeal swabs. The cat had pneumonia and was extremely emaciated. Syncytium-forming virus isolations were made from primary autogenous cell cultures of the lungs, bladder, and kidney of this cat. More recently, this new virus was also isolated from bladder and kidney cell cultures of a littermate (no. 780) with spontaneous urolithiasis. As in the previous case, respiratory disease signs had been observed and picornavirus had been isolated from pharyngeal swabs 1 month prior to its death from urolithiasis (*unpublished data*).

This paper deals with the induction of intracytoplasmic and extracellular mineral crystal formation in cell cultures infected with two feline viruses. One of the viruses is the syncytium-forming virus previously reported (1); the other virus is a newly isolated one which produced intranuclear inclusions (*unpublished data*).

MATERIALS AND METHODS

Preparation of autogenous cell cultures. Cat kidney cortex and urinary bladders were minced and treated with a trypsin-ethylenediaminetetraacetic acid mixture in the usual manner for preparation of cell monolayer cultures. The cell suspensions were placed in 250-ml disposable tissue culture flasks (Falcon) with 40 ml of an equal mixture of Leibowitz and McCoy's media containing 20% fetal calf serum. The *p*H of the growth medium was adjusted with sodium bicarbonate to 7.3 to 7.4. As soon as growth was confluent (2 to 7 days), the cell monolayers were trypsinized and first

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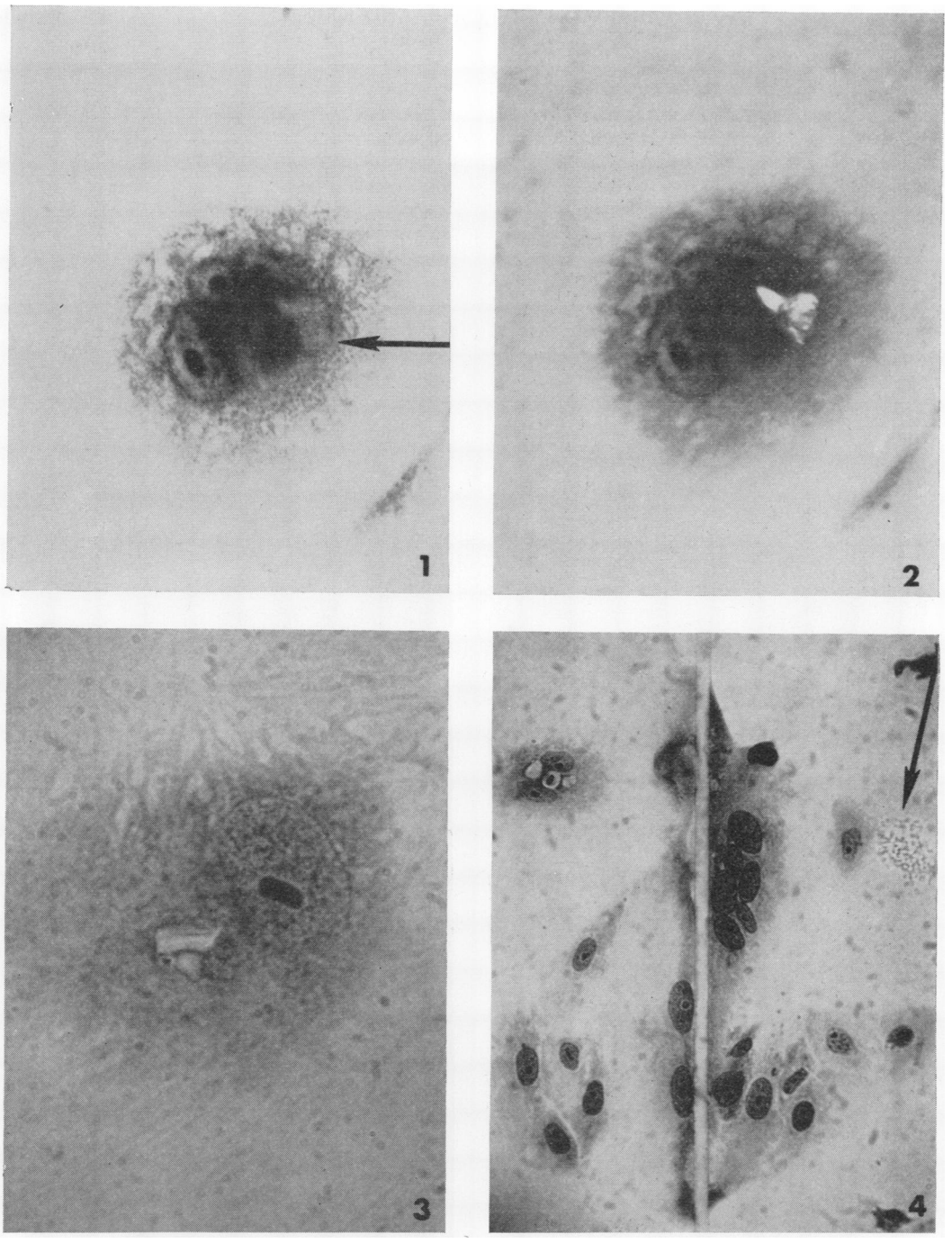

FIG. 1. *Numbers 1 to 3 were photographed from a first transfer cat kidney cell culture infected with adeno-like virus (from cat no. 779). Number 2 is the same cell as number 1, but the intracytoplasmic mineral is clearly revealed by use of polarized light. The number 3 cell shows vacuolated cytoplasm of cell containing two crystals. (Numbers 1 to 3 oil immersion, ×720.) Number 4 was taken from the second transfer cat kidney cell culture infected with syncytium-forming virus (from experimental cat with urolithiasis). It shows one very large syncytium with a crystal extending beyond both ends of the cell. A smaller, two-nucleated cell has two definite crystals and a basophilic granule in a vacuole. In number 4 an arrow points to microextracellular crystals (×250). Numbers 1 to 4 were taken from Leighton tube cover slips stained with May-Grünwald-Giemsa stain. Both cell cultures are autogenous (derived from infected cats).*

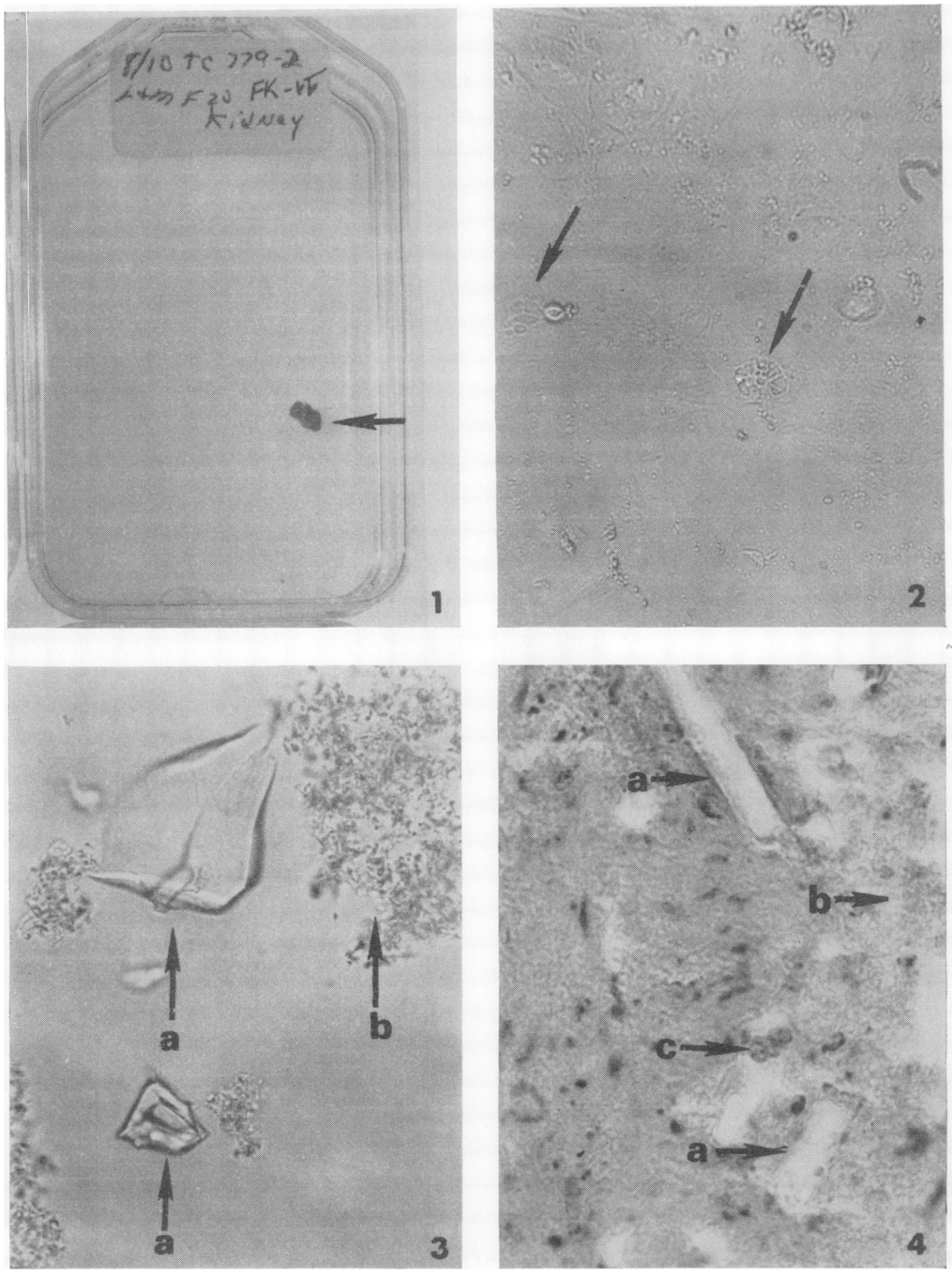

FIG. 2. *Numbers 1 to 4 were obtained from a first transfer (autogenous) cat kidney culture (cat no. 779). Syncytium-forming virus was isolated from the primary culture. Number 1 shows a tissue culture calculus which closely resembles a urinary calculus. Number 2 represents the monolayer in the flask. The arrows point to a type of vacuole seen in the cells. These vacuoles often contain crystals (×100). Number 3 is an unstained preparation from the tissue culture calculus. The (a) arrows point to larger crystals and the (b) arrows indicate microcrystals (×400). Number 4 shows a cross-section of the tissue culture calculus stained with Delafield stain, arrows (a) are directed to the larger cylindrical crystals, (b) arrows to the microcrystals and free protein (?), and arrow (c) to a dead cell superimposed on another crystal (×400).*

transfer cultures were made to other flasks. Leighton tube cultures were also made. Second transfer cultures were made in the same manner as the first. Lung, tonsil, and other organs were also cultured in the same manner or in explant cultures previously described (1).

Crandell feline kidney cell line cultures (CRFK; 1) were also inoculated with fluids from different passages of the autogenous cultures. Leighton tube cover slips were examined unstained and stained with May-Grünwald-Giemsa, hematoxylin-eosin, and Wright's stains.

The tissue culture structures resembling the feline urinary calculi (2) were examined unstained and also with Delafield stain after fixation with absolute alcohol, embedding, and sectioning.

CRFK cells and media components were monitored for mycoplasmal contamination and passenger viruses (1).

RESULTS

Examination of unstained autogenous cultures (primary, first, and second transfer cultures) and subcultures to the CRFK cells infected with either syncytium-forming or adeno-like virus revealed highly refractile intracytoplasmic inclusions. The stained Leighton tube cover slip preparations disclosed that the intracytoplasmic inclusions were mineral crystals (Fig. 1). In some of the stained autogenous cultures, cytopathic changes indicated that both syncytium-forming virus and the adeno-like virus may have been present because both syncytial cells and cells with intranuclear inclusions were observed. No mineral crystalline formations were noted in uninoculated CRFK cells.

After 16 days of culture and incubation in a carbon dioxide incubator at 35 C, four autogenous first transfer cultures (two urinary bladder and two kidney) from cat no. 779 contained "tissue culture calculi" floating in the supernatant fluids. These tissue culture calculi closely resembled the calculi removed from the bladders and urethra of cats with spontaneous or experimentally induced urolithiasis (Fig. 2). One of the tissue culture calculi was removed and measured 7 mm long, 3 to 5 mm wide, and 3 mm thick. The wet weight was 79.2 mg.

The culture flasks contained confluent cell monolayers. A large number of cells showed various stages of cytopathic changes with highly refractile intracytoplasmic inclusions (Fig. 2). A portion of one unstained tissue culture calculus was examined microscopically. Large mineral crystals were seen in this preparation with masses of microcrystals (Fig. 2). Another tissue culture calculus was fixed, sectioned, and stained with Delafield stain. The cross section revealed many large crystals with numerous microcrystals, cellular debris, and a number of dead cells (Fig. 2).

Tissue culture calculi have also been observed in first transfer bladder and kidney cultures from the littermate cat no. 780 which died from urolithiasis 20 days after cat no. 779.

Studies are now in progress to identify the intracytoplasmic mineral crystals and the mineral crystals in the tissue culture calculi.

DISCUSSION

Although mineral crystalline formation induced by viruses is a new finding, other microorganisms, notably fungi, have been used commercially to produce citric acid (4).

The implications of these observations in the pathogenesis of viral-induced urolithiasis are clear. If two viruses can induce mineral crystal formation in vitro in cell culture, the possibility exists that the same phenomenon may occur in vivo.

In view of these observations, the induction of urolithiasis in cats after injection or exposure to a feline picornavirus and consistent isolation of syncytium-forming virus from obstructed cats (spontaneous cases or experimental) would indicate a more complex etiology of the disease than originally envisioned. The feline picornavirus infection may either (i) activate latent viral infections of the urinary tract or (ii) act synergistically with the syncytium-forming virus to produce urolithiasis.

The fact that a recently isolated adeno-like virus also induces intracytoplasmic mineral crystals suggests further that under similar or other conditions this virus may also have the capability of causing urinary obstruction in cats.

ACKNOWLEDGMENTS

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ADDENDUM IN PROOF

Since this manuscript was submitted a "tissue culture culculus" was observed in an autogenous heart cell culture derived from cat no. 780 which had spontaneous urolithiasis.

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