laboratory (*15*), and Sephadex LH-20 was purchased from Pharmacia, Piscataway, New Jersey.

Rats on the low phosphorus diet develop severe hypophosphatemia, which is partially corrected by the administration of 25-OHD_3 (Table 1). These animals have marked intestinal calcium transport activity, even more marked than that observed in rats on low calcium diets (*16*). Rats fed a normal amount of phosphorus (0.3 percent) show a hypophosphatemia that is corrected by the 25-OHD_3 treatment. Although calcium transport is high, it is approximately half that of rats on the low phosphorus diet. Thus, in agreement with Morrissey and Wasserman (*10*), low phosphorus diets result in high rates of calcium absorption in rats as well as in chicks.

Because low phosphorus diets that result in hypophosphatemia have been shown to result in increased biosynthesis of 1,25-$(OH)_2D_3$ (*9*), the vitamin D–derived hormone responsible for the initiation of intestinal calcium absorption, it was of interest to determine if the increased transport of calcium could be correlated with the production of 1,25-$(OH)_2D_3$ and its accumulation in the intestine. The low phosphorus diet stimulates the accumulation of 1,25-$(OH)_2D_3$ in blood and intestine (Fig. 1); there is more than twice as much 1,25-$(OH)_2D_3$ in the intestine and blood of rats on the low phosphorus diet than in those of animals on the normal phosphorus diet (Table 1). More 24,25-dihydroxyvitamin D_3 [24,25-$(OH)_2D_3$] is observed in the chromatographic profiles of rats on the 0.3 percent phosphorus diet than in those from rats on the 0.1 percent phosphorus diet.

Dietary phosphorus does not affect the accumulation of radioactivity in blood serum, kidney, and intestine, whereas it markedly affects the amount of that radioactivity appearing as 1,25-$(OH)_2D_3$ (Table 2). Thus, low phosphorus diets and the resulting hypophosphatemia stimulate 1,25-$(OH)_2D_3$ production, which is probably responsible for increased calcium transport and may contribute further to the hypercalcemia. The increased accumulation of 1,25-$(OH)_2D_3$ under conditions of hypercalcemia and hypophosphatemia, in which there should be little or no parathyroid secretion, demonstrates that parathyroid hormone is not necessary for 1,25-$(OH)_2D_3$ production in the hypophosphatemic animal. It also provides support for the idea that the inorganic phosphate concentration in renal cells is an important determinant of 1,25-$(OH)_2D_3$ production.

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22 February 1973

Virus-Induced Cholesterol Crystals

Abstract. *One of the crystal types induced in cell cultures by a new feline herpesvirus was identified as cholesterol by crystal structure, polarized light microscopy, and mass spectroscopy.*

During our studies on virus-induced feline urolithiasis, we isolated a virus which produced cytopathic effects of the adenovirus type in cell cultures (*1*). This virus has been identified as a new feline herpesvirus. Intracellular and extracellular chemical crystals were observed in cell cultures infected with this virus. The crystals varied in size, shape, and structure, and some were birefringent when examined in polarized light (*1*).

The crystal formations were first observed in cell cultures derived from tissues of spontaneously obstructed cats. Similar formations have been seen in cell cultures of the stable Crandell feline kidney (CRFK) cell line infected with this herpesvirus. The crystals have not been observed in the uninfected control CRFK cells. The initial demonstration of the various crystal types in infected cells made with a microscope with either conventional or polarized light was confirmed by examination of ultrathin sections of similar cells with an electron microscope.

During the examination of unstained, living cell cultures derived from kidneys, bladders, and hearts of cats with either spontaneous or experimental cases of urolithiasis, many fat globules were observed in supernatant fluids within 24 hours after the initiation of culture. These fat globules persisted. On occasion a cell was seen to release fat globules into the culture fluids. Many cells in these cultures contained

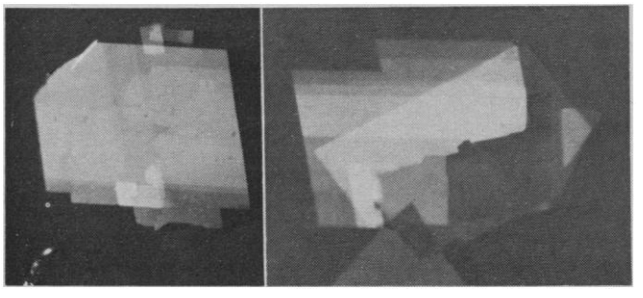
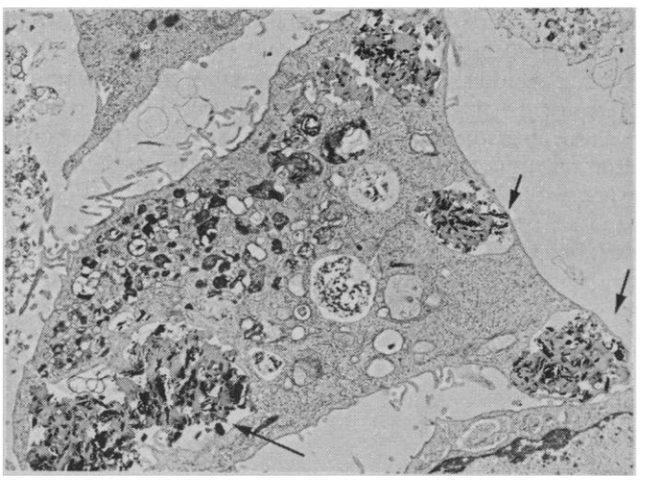


Fig. 1. Cholesterol crystals from a cell culture infected with a new feline herpesvirus photographed with polarized light (× 310).



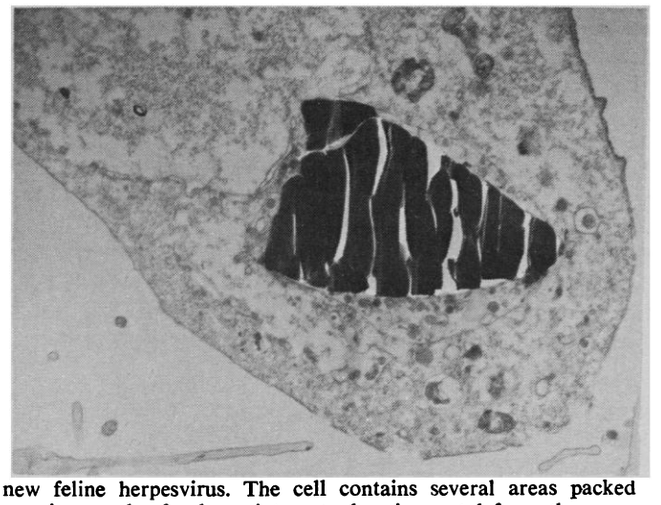


Fig. 2 (left). Electron micrograph of a cell infected with the new feline herpesvirus. The cell contains several areas packed with different crystal types (× 450). Fig. 3 (right). Electron micrograph of a large intracytoplasmic crystal from the same culture as in Fig. 2 (× 9500).

numerous round refractile cytoplasmic "vacuoles" which on closer examination appeared to be fat globules. This observation was confirmed on infected cells grown in Leighton culture tubes with cover slips and then stained with oil red-O. Similar observations were made on infected CRFK cell cultures.

Monolayers of the cell cultures infected with herpesvirus were found to remain attached for months in the culture flasks. Periodic examinations of these cultures were made during the prolonged incubation. A number were found to contain appreciable amounts of cholesterol-like crystals after periods of incubation varying from 1 to 16 weeks. The cholesterol-like crystals were not observed in uninfected cell cultures during the 1-month period (occasionally 2 months) in which these monolayers remained intact. These monolayers tend to detach soon after the logarithmic growth phase.

The crystals observed with polarized light were found to be birefringent. One of the cell culture fluids containing a large amount of the cholesterol-like crystals (Fig. 1) was filtered through a sterile Swinny (cellulose acetate) Millipore filter with a pore size of 45 nm. The filtrate was discarded, and a few milliliters of chloroform were passed through the filter to dissolve the crystals. The chloroform filtrate was collected, evaporated to dryness in a special glass cell, and analyzed by mass spectroscopy.

The mass spectra of the cell culture sample and of an authentic cholesterol sample were determined on the same mass spectrometer (*2*). The major peaks in the mass spectrum of the cell culture sample correspond with those in the mass spectrum of the cholesterol sample.

The previous failure to identify any of the crystal types (Figs. 2 and 3) as cholesterol may be related to the use of electron diffraction (*3*). Organic crystals such as cholesterol are unstable when subjected to electron beams even for short periods.

We have previously reported that infection with a virus of the adenovirus type (now identified as a new feline herpesvirus) in cell cultures of an autogenous or a stable cell line will induce the formation of different types of intracellular and extracellular chemical crystals in these cultures (*1*, *4*). The identification of cholesterol as one of these crystal types indicates that such viral infections may have a role in the etiology not only of urolithiasis, but also of degenerative vascular diseases.

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5. Supported by the Morris Animal Foundation and by PHS grants PR 05462-09 and PR 05462-10 from the Division of Research Facilities and Resources, National Institutes of Health. We thank Prof. J. Fabricant for his continued advice and encouragement.

28 March 1973

Methylmercury as Percentage of Total Mercury in Flesh and Viscera of Salmon and Sea Trout of Various Ages

Abstract. *The proportion of methylmercury to total mercury in the flesh of salmon* (Salmo salar) *1 to 7 years old and sea trout* (Salmo ocla) *1 and 2 years old was found to average 93 percent with a range of 81 to 98 percent, and to be independent of the age of the fish. In salmon and sea trout 1 and 2 years old, methylmercury constituted 26 to 67 percent of the total visceral mercury, without age dependence.*

Bache *et al.* (*1*) reported an increasing proportion of methylmercury to total mercury (34 to 93 percent) with increasing age in lake trout (*Salvelinus namaycush*) in Cayuga Lake, Ithaca, New York. In large-scale surveys in Sweden, mostly on pike, such a trend could not be observed (*2*, *3*). In the work that is reported here, the proportion of methylmercury to total mercury has been studied in salmon (*Salmo salar*) and sea trout (*Salmo ocla*) of various ages. No age dependence is found.