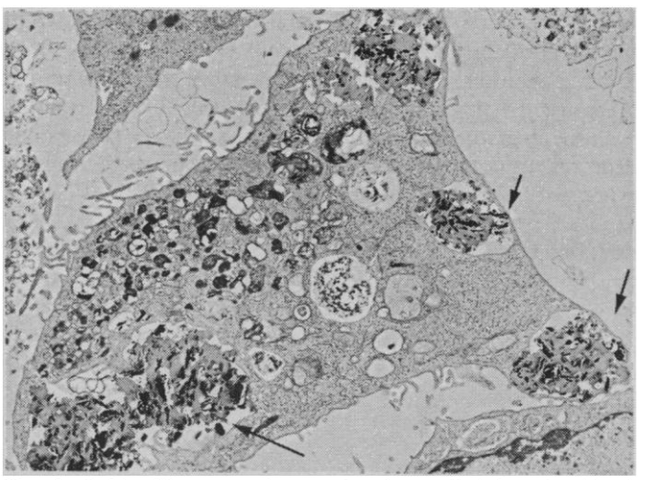

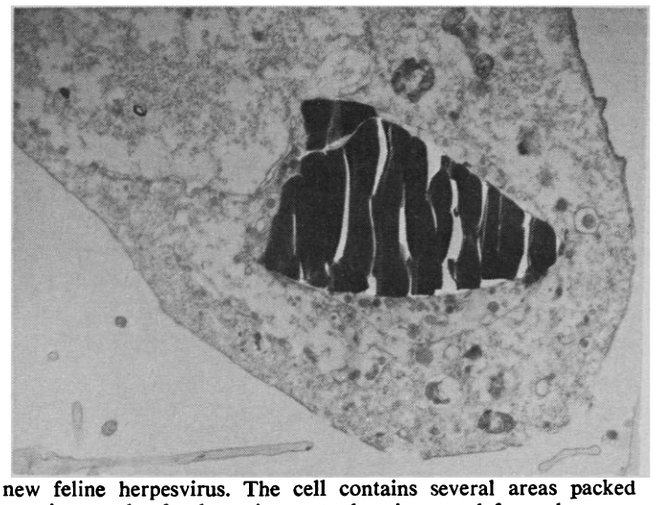

Fig. 2 (left). Electron micrograph of a cell infected with the new feline herpesvirus. The cell contains several areas packed with different crystal types (× 450). Fig. 3 (right). Electron micrograph of a large intracytoplasmic crystal from the same culture as in Fig. 2 (× 9500).

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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2. Analyzed on model AEI-MS-902 (Associated Electrical Industries, Manchester, England) at the NIH High Resolution Mass Spectrometry Laboratory at Cornell University by Dr. J. W. Serum.
3. The electron diffraction studies were made by P. Kunsman at Cornell University.
4. C. G. Fabricant and J. H. Gillespie, in preparation.
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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.

The muscles and viscera of salmon and sea trout of various ages were analyzed for total mercury by neutron activation analysis (*4*) and for methylmercury by gas chromatography (*3*, *5*). The results are shown in Table 1. Most of the salmon had been cultured in ponds in the Dalälven River and were obtained from the salmon research institute at Älvkarleö. The age of these fish was exactly known. Some of the 1- and 2-year-old salmon and all of the sea trout were game fish and had been caught in the Mörrumsån River. Their ages were estimated from their weights and lengths and from the annual rings of their scales.

Table 1 shows that the mercury concentration in the flesh of salmon increases roughly with the age of the fish and that practically all the mercury in the flesh in salmon and sea trout is in the form of methylmercury, irrespective of age (average, 93 percent; range, 81 to 98 percent). Table 1 also shows that in 1- and 2-year-old salmon and sea trout the methylmercury in the viscera constituted 26 to 67 percent of the total visceral mercury, with no age dependence. The viscera make up only 10 to 25 percent of the weight of the fish, and thus cannot depress the methylmercury percentage of any of the fish samples below 67 percent. I observed no increase in the proportion of methylmercury to total mercury with the age of the salmon or sea trout (muscles plus viscera).

A few samples of muscle and viscera of 1-year-old pike from Vassbotten, a part of Lake Vänern, were also studied. The average percentage of total mercury as methylmercury in the muscles of four pike was 88 percent (with a range of 83 to 92 percent), which does not differ significantly from the methylmercury percentage in older pike (average 94 percent with a standard deviation of 9 percent) (*3*). In one sample of viscera (homogenate from five pike) 86 percent of the total mercury was methylmercury.

When Bache *et al.* (*1*) reported an increasing proportion of methylmercury to total mercury with increasing age in Cayuga lake trout, they were discussing fish that had not been eviscerated. Table 1 shows that not even in the viscera of 1- and 2-year-old Swedish salmon from ponds in the Dalälven River was the methylmercury percentage (51 to 67 percent) as low as that reported by Bache *et al.* for noneviscerated lake trout of the same age (31 to 43 percent). However, fish meal containing 77 percent of the total mercury as methylmercury had been fed to the salmon. In Swedish game salmon and sea trout from the Mörrumsån River the methylmercury percentage in the viscera was 26 to 59 percent.

The recovery by Bache *et al.* of methylmercury added to fish samples was 55 percent. Without correction for partition coefficients, the method used in the investigation reported here gives 79 percent recovery of methylmercury added to fish flesh. With correction for partition coefficients, the recovery is 98 percent with a standard deviation of 3 percent.

Table 1. Concentrations of total mercury and methylmercury (calculated in terms of mercury) in flesh and viscera of salmon (*Salmo salar*) and sea trout (*Salmo ocla*) of various ages. *N*, number of fish in homogenate.

Species and catching place	Age (yr)	Avg. length (cm)	Avg. weight (g)	*N*	Total mercury (mg/kg)	Methylmercury: Calculated in terms of mercury (mg/kg)	Methylmercury: Percentage of total mercury	
				Flesh				
Salmon from Dalälven	1	11	14	9	0.068	0.068	100	94
	1	13	27	9	.063	.055	87	
	2	21	78	4	.106	.097	92	93
	2	22	100	1	.110	.096	87	
	2	20	74	1	.108	.096	89	
	2	27	197	1	.109	.104	95	
	2	26	170	1	.100	.100	100	
	3	39	366	3	.091	.086	95	98
	3	30	241	1	.122	.114	93	
	3	34	367	1	.102	.104	102	
	3	37	606	1	.105	.106	101	
	4	33	342	1	.162	.158	98	97
	4	40	688	1	.106	.102	96	
	5	48	1240	1	.224	.192	86	86
	5	47	767	1	.309	.256	83	
	5	45	1109	1	.185	.165	89	
	6	49	1280	1	.226	.209	92	
	7	55	1600	1	.280	.251	90	
					Average for ages 1 to 7		93	
Salmon from Mörrumsån	1	7	4	6	0.250	0.240	96	
	2	14	22	4	.321	.291	91	92
	2	14	23	4	.293	.276	94	
					Average for ages 1 and 2		94	
Sea trout from Mörrumsån	1	8	5	15	0.296	0.240	81	
	2	16	39	3	.272	.258	95	94
	2	17	46	3	.259	.232	90	
	2	16	45	3	.218	.207	95	
	2	16	42	3	.242	.230	95	
					Average for ages 1 and 2		91	
				Viscera				
Salmon from Dalälven	1	12	20	18	0.061	0.041	67	
	2	21	78	4	.070	.036	51	
	2	24	135	4	.070	.044	63	
Salmon from Mörrumsån	1	7	4	6	0.165	0.098	59	
	2	14	22	8	.375	.114	30	
Sea trout from Mörrumsån	1	8	5	30	0.469	0.121	26	
	2	16	42	6	.305	.104	34	
	2	16	44	6	.257	.079	31	

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