for the synthesis of BOH may be operative in ruminant liver.

In mammals, the formation of acetoacetate occurs under special conditions such as starvation. Acetoacetate can then be converted to BOH by way of BDH. The BOH must then be converted back to the acetoacetate before it can be used in fatty acid oxidation. The advantage for interconverting BOH and acetoacetate and hence the physiological role of BDH is not understood. One suggestion is that the reaction serves to shuttle electrons from extramitochondrial NADH to intramitochondrial NAD^+ (*14*). Another proposal is that the ratio of BOH to acetoacetate released into the circulating blood by the liver may reflect the redox state of the liver mitochondria (*15*); the ratios of BOH to acetoacetate in the blood are thought to coordinate the redox state of extrahepatic mitochondria with those of the liver. If these two proposed metabolic functions are valid, they do not seem to be vital to ruminant survival.

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1. Abbreviations are: NAD, nicotinamide adenine dinucleotide; NADH, reduced NAD; tris, tris-(hydroxymethyl)aminomethane; HEPES, *N*-2-hydroxyethylpiperazine - *N'* - 2 - ethanesulfonic acid; EDTA, ethylenediaminetetraacetate; and ATP, adenosine triphosphate.
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Feline Leukemia Virus: Occurrence of Viral Antigen in the Tissues of Cats with Lymphosarcoma and Other Diseases

Abstract. *Feline leukemia virus antigen is demonstrable by immunodiffusion with rabbit precipitating antiserum to purified feline leukemia virus. The feline leukemia virus antigen was found in the tissues of 25 of 33 cats with lymphosarcoma and of 5 of 13 cats with infectious peritonitis. Its presence was correlated with the occurrence of feline leukemia virus demonstrable by electron microscopy. The one clinically normal cat giving a positive test for feline leukemia virus antigen belonged to a household in which two cats had developed lymphosarcoma. With the exception of a dog with lymphosarcoma induced by feline leukemia virus, the antigen was absent from lymphosarcoma and nonlymphomatous tumors of other species (man, dog, cow, goat, or pig).*

That leukemia in the cat is commonly virus-induced is indicated by experimental transmission with subcellular material (*1*, *2*) and by the frequent presence of virus indistinguishable from the murine and avian leukemia viruses in primary cases (*2–4*). In immunodiffusion tests with antiserum to murine leukemia virus we identified at least five viral antigens (*5*), one of which is shared with the feline leukemia virus (FeLV) (*4*). Continuing this study, we have prepared rabbit antiserum to FeLV by the procedures used for preparing rabbit antiserum to group-specific antigen of murine leukemia virus (*6*). The source of FeLV for immunodiffusion tests was the pleural fluid and tissue washings of a male domestic cat (No. 169), aged 7 months, with lymphosarcoma (LSA). After the intact cells and large particles were removed, virus was sedimented (Spinco model L-2, 24,000 rev/min, SW-25 rotor, 1 hour), and then resuspended and centrifuged on a potassium tartrate density gradient (15 to 40 percent; 0.1*M* tris-HCl buffer, *p*H 8.0) (*6*). The band at approximately 1.16 g/ml, as seen by electron microscopy, consisted mainly of characteristic C-type FeLV. Before being used for immunization, the isolated FeLV was treated with ether. Like avian leukemia virus (*7*) and myxoviruses (*8*), intact murine leukemia virus elicits group-specific antibody poorly if at all in the rabbit (*6*). Hence it is necessary to disrupt the virion with ether or by freezing. One rabbit,

Table 1. Occurrence of FeLV antigen in lymphosarcoma and other diseases of cats.

Diagnosis	Cases (No.)	FeLV antigen present	Tissue extracts and body fluids of FeLV antigen-positive cases		
			Source	Cases (No.)	Antigen +
Lymphosarcoma (spontaneous)	33	25	Lymph node	20	19
			Thoracic fluid	11	9
			Spleen	9	9
			Liver	9	7
			Plasma	8	6
			Kidney	8	4
			Urine	4	0
			Brain	3	0
			Blood clot	2	2
			Bone marrow	2	1
			Salivary gland	1	1
Lymphosarcoma (experimentally induced)	3	3	Lymph node	1	1
			Bone marrow	2	2
Infectious peritonitis	13	5	Lymph node	4	3
			Spleen	3	3
			Liver	3	2
			Ascitic fluid	2	0
			Kidney	1	0
Normal tissue	11	1	Lymph node	1	1
Other tumors					
Mammary carcinoma	3				
Hepatic carcinoma	3				
Fibrosarcoma	2	0			
Hemangiosarcoma	2				
Pancreatic carcinoma	1				
Leiomyosarcoma	1				
Other nonneoplastic conditions	10	0			

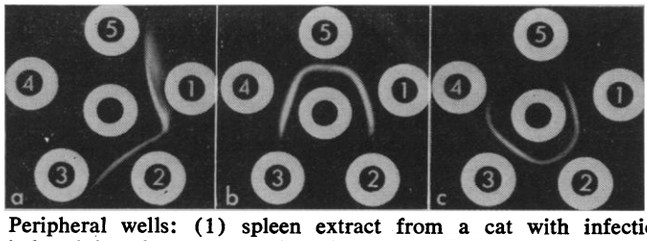

Fig. 1. Immunodiffusion tests. (a) Center well: ether-treated FeLV. Peripheral wells: (1) rabbit antiserum to FeLV; (2) rat group-specific antiserum to murine leukemia virus; (3) rabbit antiserum to mammary tumor virus; (4) rabbit antiserum to Lucké virus; (5) rat antiserum to Sendai virus. (b) Center well: rabbit antiserum to FeLV. Peripheral wells: Lymph node extracts from cats with lymphosarcoma; (1) cat No. 169; (2) cat No. 120; (3) cat No. 452; (4) cat No. 430; and (5) ether-treated FeLV. (c) Center well: rabbit antiserum to FeLV. Peripheral wells: (1) spleen extract from a cat with infectious peritonitis; (2) lymph node extract from a dog with FeLV-induced lymphosarcoma; (3) ether-treated FeLV; (4) lymph node extract from a clinically normal cat (from a household in which two cases of feline lymphosarcoma had occurred); (5) lymph node extract from a dog with spontaneous lymphosarcoma.

one dog, one cat, and two rats (all females) received three inoculations of ether-treated FeLV subcutaneously at weekly intervals, the first inoculation being with complete Freund's adjuvant and the last two with incomplete adjuvant. Serums taken 1 week after the last inoculation were absorbed as required with lyophilized feline plasma and tissue to abolish reactivity with normal feline antigens. Only the rabbit and rat serums had precipitating antibody to FeLV. Because the rabbit serum was the more active, it was selected for testing. Immunodiffusion with this rabbit antiserum was performed in 2 percent Noble agar on slides [a standard procedure we have used in defining several soluble antigens of tumor-associated viruses (*4*, *5*, *9*)]. Tissue extracts for immunodiffusion tests were prepared as described (*5*). Plasma, ascitic and pleural fluid, and urine were concentrated by vacuum dialysis or by lyophilization.

Rabbit antiserum to FeLV reacted with components released from the virion by freezing and thawing or by ether. One of these soluble antigens is the component (*4*) previously detected by antiserum to murine leukemia virus (reaction of relatedness in Fig. 1a). The FeLV viral antigen was present also in tissue extracts of lymphomatous cats (Fig. 1b and Table 1), another similarity to murine leukemia virus (*5*, *6*). Of the 33 lymphomatous cats examined, 25 (75 percent) were positive for FeLV antigen; in these it was always present in spleen and frequently in other tissues (lymph node, liver, kidney, bone marrow, clotted blood) and in plasma and effusions (*10*).

The FeLV antigen was found in the tissues of three cats with LSA induced by cell free filtrates (Table 1) (*11*).

Electron microscopy showed FeLV in five of nine cats with spontaneous lymphosarcoma (Table 2). Tumor tissue from all cats with FeLV was antigen-positive, and from one cat in which FeLV was not seen, an indication of the greater sensitivity of the serological test. Of the antigen-negative tissues examined by electron microscopy, none showed FeLV.

Tissue extracts were prepared from each of 46 normal cats and cats with diseases other than lymphosarcoma (Table 1). Of the positively reacting tissues in this control group, one came from a lymph node biopsy of an ostensibly healthy cat, shown to be infected with FeLV by electron microscopy (*12*); this cat came from a household in which two other cats developed LSA (described below) and is being kept under observation. The other positively reacting tissues came from five of a group of 13 cases of infectious peritonitis, an invariably fatal disease of cats (*13*).

The finding of FeLV in nonlymphomatous cats is not unexpected, for this is characteristic of virus of similar type in mice and chickens. It is a still-unsolved problem, therefore, whether the finding of leukemia virus in association with another disease, in this instance infectious peritonitis, indicates an etiological connection or an activation of latent leukemia virus (*14*). In the case of leukemia itself, there is other evidence for the causative role of viruses of this type (*14*).

The FeLV antigen was not demonstrable in extracts of naturally occurring LSA or nonlymphomatous tumors of other species. The following cases were examined.

Dog: lymphosarcoma, 17; melanoma, 2; hepatic adenocarcinoma, 2; chondrosarcoma, 1; mast cell tumor, 1; neurofibrosarcoma, 1; squamous cell carcinoma, 1; ovarian cystadenocarcinoma, 1.
Cow: lymphosarcoma, 7.
Pig: lymphosarcoma, 1.
Goat: lymphosarcoma, 1.
Man: lymphoid tumors (including leukemia), 20; sarcoma, 6; carcinoma, 50; benign tumors, 9; specimens of normal adult tissue, 29; fetal tissue, 3.

The tissues of one dog that contracted LSA after receiving FeLV at birth (*15*) had FeLV antigen and budding virus indistinguishable from FeLV, strongly indicating induction by FeLV rather than spontaneous canine LSA, a disease in which we have neither seen virus of this type (Table 2) nor found FeLV antigen.

Although immunodiffusion is not a sensitive test for naturally occurring antibody, precipitating antibody to indigenous leukemia virus is demonstrable in the chicken (*16*). In the case of the FeLV antigen we have not found precipitating antibody in serums from the following sources.

Cats: LSA, 24; nonlymphomatous malignancies, 11; infectious peritonitis, 15; nonneoplastic diseases, 34; normal adults, 57.
Cows: LSA, 25; normal adults, 25.
Man: LSA, 42; acute lymphoblastic leukemias, 59; chronic lymphocytic leukemias, 34; Hodgkins disease, 45; nonlymphoid malignancies, 42; normal subjects, 61.

Table 2. Electron microscopy of feline and canine tissues.

Diagnosis	Tissues examined	Cases (No.)	FeLV present (No. cases)
	Feline		
Lymphosarcoma (spontaneous)	Mediastinal and mesenteric lymph nodes	9	5
Infectious peritonitis	Mesenteric lymph nodes	8	1
Nonlymphomatous tumors*	Tumor	7	0
Nonneoplastic conditions	Peripheral lymph node and liver	2	0
	Canine		
Lymphosarcoma (spontaneous)	Peripheral lymph nodes	11	0
Nonlymphomatous tumors†	Tumor	7	0

* Includes mammary carcinoma, 4; hepatic carcinoma, 1; leiomyosarcoma, 1; osteosarcoma, 1.
† Includes osteosarcomas, 4; mastocytoma, 1; venereal cell sarcoma, 1; oral papillomatosis, 1.

A complement fixation test with this rabbit antiserum, comparable to the complement fixation tests for avian (*17*) and murine (*18*) leukemia viruses has now been developed (*19*).

With regard to the natural transmission of viruses of this type, the most important mode is probably vertical (*20*), that is, from one generation of hosts to the next, although horizontal transmission (between unrelated individuals) is also prominent in chickens (*21*) and has been observed in mice (*22*).

In this context the salivary gland was positive for FeLV antigen in the one LSA case tested (Table 1). Because soluble antigen of murine leukemia virus occurs in the milk of carrier mice (*23*), we looked for FeLV antigen in the milk of 27 normal lactating cats; but none was found in unconcentrated milk.

Reports of clustering of LSA cases among unrelated cats of single households speak for case-to-case transmission (*24*). In one household of ten cats under study, two littermates and two unrelated cats developed LSA. In the four cases FeLV antigen was present. Another cluster comprised two unrelated Abyssinian cats with LSA; a lymph node biopsy from a third apparently healthy Abyssinian in the same household (above) had FeLV antigen and characteristic C-type particles.

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10. The breed incidence of feline lymphosarcoma in our series is as follows:

Breed	No. cases	FeLV antigen-positive cases
Domestic	18	13
Siamese	10	8
Persian	2	2
Abyssinian	1	1
Burmese	1	1
Maltese	1	0

Fifty-five percent of the LSA cases occurred in cats that were not inbred (domestic), while 45 percent occurred in inbred (pedigreed) cats. The latter group, however, certainly does not represent 45 percent of the population at risk, suggesting that inbreeding may render the cat more susceptible to viral LSA. This is another similarity to the murine and avian leukemia viruses. Our figures and those of others [C. R. Dorn, D. O. N. Taylor, H. H. Hibbard, *Amer. J. Vet. Res.* **28**, 993 (1967)], after correction for the frequency of breeds in the population at risk, show that Siamese cats have the highest incidence of LSA.
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13. There are strong indications that feline infectious peritonitis has a viral etiology. The disease has been transmitted by cell-free filtrates [B. C. Zook, N. W. King, R. L. Robison, H. L. McCombs, *Pathol. Vet.* **5**, 91 (1968); J. M. Ward, R. J. Munn, D. H. Gribble, D. L. Dungworth, *Vet. Rec.* **83**, 416 (1968); W. D. Hardy, Jr., unpublished observation]. Electron microscopy of tissues from diseased cats (Zook *et al.* and Ward *et al.*) has shown the presence of virus particles ranging from 70 to 100 nm occurring within the cisternae of the endoplasmic reticulum. The particles we observed in the one out of eight cats with infectious peritonitis (Table 2) form by budding at the plasma membrane and bear a close resemblance to FeLV. The presence of FeLV antigen in this case (Table 1) adds strength to this conclusion.
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Ribonucleic Acid Metabolism of a Single Neuron: Correlation with Electrical Activity

Abstract. *The giant neuron of the abdominal ganglion of* Aplysia californica *incorporates tritiated uridine into RNA at a constant rate at rest. This rate increases under synaptic stimulation, the increase being directly proportional to the number of action potentials produced by the neuron. Multineuronal samples from stimulated ganglia failed to show an increase in incorporation.*

The relationship between the electrical activity of nervous tissue and its RNA metabolism has been a major problem of neurochemistry, and with recent evidence tending to correlate changes in neuronal RNA metabolism with learning, this relationship takes on added significance (*1*). The majority of investigations in this area have found increased RNA production following electrical activity, but the results are somewhat ambiguous, and the relationship has not been precisely quantified (*2*).

Single neuron preparations would seem to be ideal for such studies, since they allow precise monitoring of electrical activity and minimizing of errors due to the production of RNA by nonneuronal elements. However, so far these preparations have also given conflicting results; Fischer *et al.* have re-