cork cells of cork-silica cell pairs are probably devoid of silica.

Analyses were made to determine the amount of silica present in cork-silica cell pairs in immature and mature internodes. In an immature 6-cm internode, where the uppermost centimeter was exposed to the atmosphere (sheath absent), no silica could be detected with the electron microprobe in silica cells, except in the uppermost centimeter of the internode. The cork cells had no silica present; even the cork cells at the top of the internode had silica present at a level only slightly above background. In a mature 9-cm internode, where again only the uppermost centimeter was exposed to the atmosphere, silica was present in significant amounts in silica cells in the uppermost 5 cm with none detectable in the lowermost 4 cm. The amount of silica in cork cells was insignificant. Silica in silica cells in these upper levels of the mature internode, compared with a sample of pure SiO_2 (776 count/sec), averaged 83 percent silica. Both sets of data indicate that silica deposition in silica cells must take place quite rapidly, because at one level, silica is totally absent, and at the next highest level, it occurs in significant amounts ~ 500 to 600 count/sec. The data also indicate that silica is accumulated by silica cells in both exposed and ensheathed portions of the internode. Thus, exposure of the internode to the atmosphere by its emergence from the sheath, together with the expected increase in amount of transpiration from the exposed portion of the internode, may not be essential for the silica accumulation process.

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β-Hydroxybutyrate Dehydrogenase: Lack in Ruminant Liver Mitochondria

Abstract. *The enzyme* D(−)-β-*hydroxybutyrate dehydrogenase has been reported to occur in all mitochondria isolated from mammalian tissues and is especially high in liver. By contrast only a very low concentration of the enzyme is detectable in mitochondria from bovine and sheep liver. The amount of the enzyme in mitochondria from other ruminant tissues such as kidney and heart are comparable with those from mammalian sources. The very low activity of* β-*hydroxybutyrate dehydrogenase is not referable to the inability of substrates to penetrate mitochondria or to decreased electron transfer activity but rather to a lack of the enzyme in ruminant liver mitochondria.*

The enzyme D(−)-β-hydroxybutyrate dehydrogenase (BDH) (E.C. 1.1.1.30) catalyzes the reversible NAD-linked oxidation (*1*) of D(−)-β-hydroxybutyrate (BOH) to acetoacetate (*2*). The enzyme is stereospecific for the D(−)-stereoisomer, has a specific and absolute requirement for lecithin (*3*), and is found tightly bound to the mitochondrial membrane (*4*). The enzyme can be released from the membrane by treatment of mitochondria with phospholipase A (*5*). Earlier studies concerning the distribution of this dehydrogenase in animal tissues have shown that it is present in all tissues examined (*4*); the activity is usually greatest in liver. However, we find that liver mitochondria from ruminants, both beef and sheep, have very low BDH acivity. The BDH activity in heart and kidney mitochondria from the same two ruminant sources is in the same range as that from nonruminants (20 to 25 times higher than that of ruminant liver).

Bovine, rat, and sheep mitochondria were isolated as described (*6*). The bovine heart mitochondria were prepared on a large scale as reported (*6*), except that 0.25*M* sucrose containing 0.01*M* HEPES (*1*), *p*H 7.55 (sucrose-HEPES), was substituted for the "sucrose-Tris" buffer used originally. The preparation of mitochondria from rat, sheep, and bovine liver and kidney cortex was, however, scaled down so that 200 g of tissue could be processed within approximately 2 hours. The tissue was trimmed free of connective tissue, minced with scissors, and then suspended in three volumes (volume to weight) of cold sucrose-HEPES buffer. The minced tissue was homogenized with a glass homogenizer of the Potter-Elvehjem type. Two loose fitting pestles were used sequentially with the clearance between the pestle and the homogenizer wall being 0.026 and 0.018 inch, respectively. The pestles were rotated at 1000 rev/min. The homogenate was sedimented for 10 minutes at 1650 rev/min in a refrigerated centrifuge (MSE Mistral 6L) in a six place swinging bucket rotor (Cat. No. 62301). The resulting supernatant was decanted through a double layer of cheesecloth and then sedimented for 10 minutes at 18,000 rev/min in a Spinco preparative centrifuge (No. 30 rotor). Two distinct layers were obtained. The upper layer was discarded after being separated mechanically from the lower layer with a glass rod. The lower layer, which was a rich brown color, contained the purified mitochondria. The purified mitochondria were suspended and centrifuged twice more, the first time in one-half and the second in one-fourth of the original volume of buffer. Each time any residual upper layer was discarded.

Total mitochondrial protein was analyzed using a modification of the method of Lowry (*7*). Crystalline bovine serum albumin served as the protein standard for the biuret method (*8*). Mitochondria, whose protein content had previously been determined by the biuret method, served as a secondary standard for the Lowry procedure.

Livers from frog, cat, monkey, and a variety of rodents all have been shown to have substantial BDH activity (*4*). In agreement with these observations, we also found that rat liver mitochondria have high BDH activity (Table 1). Bovine heart and kidney mitochondria also had a substantial amount of the dehydrogenase activity. Consequently, it was surprising when a very low BDH activity was observed in bovine liver mitochondria. Moreover, the BDH activity of bovine liver mitochondria was so low that it was necessary to use the more sensitive assay reaction, β(−)-hydroxybutyrate to cytochrome *c* reductase, to obtain an accurate measurement of BOH oxidation (Table 1). Because the low BDH activity in the bovine liver mitochondria suggested that ruminant liver might be exceptional in this respect, mitochondria were prepared from calf and sheep liver. Mitochondria from these two sources also had very low BDH activity.

Table 1. Comparison of several enzymatic activities of mitochondria isolated from various tissues. All activities are represented as micromoles of substrate utilized per minute per milligram of protein.

Enzyme	Rat heart	Bovine heart	Bovine kidney	Rat liver	Bovine liver	Calf liver	Sheep liver
Succinate-cytochrome *c* reductase*	1.02	1.06	1.03	0.46	0.50	0.43	0.47
NADH-Cytochrome *c* reductase*	1.05	1.51	0.85	0.31	0.33	0.37	0.30
β-Hydroxybutyric dehydrogenase†	0.090	0.102	0.094	0.212	<.005‡	<.005‡	<.005‡
β-Hydroxybutyrate-cytochrome *c* reductase§	0.095	0.117	0.098	0.200	0.004	0.002	0.001

* Measured at 32°C in the presence of mitochondrial phospholipid (*16*). † Measured at 38°C as described (*17*). ‡ In the ruminant liver mitochondria, the detection of BDH activity was at the limits of resolution of the instrumentation employed. Consequently, the rates obtained were variable. § Measured at 38°C. The reaction mixture contained in 1 ml: 40 m*M* tris-acetate, *p*H 7.4, 0.2 m*M* EDTA, 50 μg of cytochrome *c*, 30 μg of micellar mitochondrial phospholipid (*5*), 1 m*M* NAD, and about 50 μg of mitochondrial protein. The reaction suspension was incubated 15 minutes at 38°C before the reaction was initiated by the serial addition of 20 μg of KCN and 20 mmole of DL-β-hydroxybutyrate (Na salt). Specific activities were calculated with a millimolar extinction coefficient for reduced cytochrome *c* of 9.25 per centimeter per millimolar. Since two molecules of cytochrome *c* are reduced per molecule of BOH oxidized, this assay is about six times more sensitive than the BDH assay.

To show convincingly that mitochondria contain very low BDH activity, we used techniques which enhance accessibility of the substrate to the dehydrogenase. In no instance was it possible to increase the BDH activity of ruminant liver mitochondria (Table 2). Therefore, ruminant liver mitochondria have very low BDH activity when compared to the liver mitochondria of non-ruminants or to mitochondria from extrahepatic tissue in general. Moreover, since calf liver mitochondria also have very low BDH activity, the absence of the enzyme appears not to be correlated with the development of a functional rumen. The very low BDH activity observed here for liver mitochondria of ruminants provides a rational explanation for the report that BDH activity in bovine liver tissue is 30 times smaller than that of rat liver tissue (*9*).

Our preparations used in these studies were of high purity and possessed good morphology as judged by electron microscopy. The high degree of purity was further confirmed by the very low amounts of glucose-6-phosphatase activity (E.C. 3.1.3.9) in our mitochondria. Determination of glucose-6-phosphatase indicated that there was less than 2 percent contamination by microsomes.

The mitochondrial preparations were capable of oxidative phosphorylation. For these determinations, phosphate incorporated into ATP (*1*) was trapped in the form of glucose-6-phosphate with the use of hexokinase and glucose. The oxygen consumption was measured manometrically, and the phosphate esterified was determined by the method of Martin and Doty as modified by Lindberg and Ernster (*10*). The number of moles of ATP formed for each gram atom of oxygen consumed (P/O ratio) by the beef liver mitochondria ranged from 1.8 to 2.0 when succinate was used as substrate, and from 2.3 to 2.5 when a mixture of malate and pyruvate was used. The BDH activity was measured immediately after the mitochondria were shown to possess optimal P/O ratios. The low BDH activity in ruminant liver mitochondria remained stable long after the P/O ratio decreased.

The electron-transport capacities of mitochondria from the various ruminant tissues were compared by measuring the succinate-cytochrome *c* reductase (E.C. 1.3.99.1) and the NADH-cytochrome *c* reductase (E.C. 1.6.99.3) activities. These studies revealed that the activities of both branches of the mitochondrial electron-transport chain were higher in both the bovine heart and kidney preparations than in the liver preparations (Table 1) as has been reported earlier (*6*). The electron-transport activities of calf and sheep liver mitochondria were similar to those of adult bovine liver mitochondria. Thus, the electron-transport activity in liver mitochondria from different ruminants was similar, and gross changes in the amount of electron-transport activity do not occur as the animal matures. The rate of succinate or NADH oxidation in beef liver mitochondria was comparable to that in rat liver mitochondria, and thus electron transport is not rate limiting. The low BDH activities of beef and sheep liver mitochondria are therefore due to lower amounts of β-hydroxybutyric dehydrogenase in the mitochondrion.

Table 2. Effect of various treatments on the bovine liver mitochondrial β-hydroxybutyric dehydrogenase activity. The NAD was measured in the presence of total mitochondrial phospholipid (*17*).

Treatment of mitochondria	NAD (μmole reduced min^{-1} mg^{-1} of protein)
Fresh mitochondria	0.004
Frozen and thawed (three times)	0.004
Deoxycholate treatment*	0.004
Phospholipase A†	0.003
Sonication‡	0.002

* Concentrated mitochondrial suspension (25 mg of protein per milliliter) was treated with 0.2 mg of deoxycholate per milligram of mitochondrial protein for 30 seconds at 3°C. Deoxycholate was then diluted out by adding 5 μl of the sample in a final volume of 1.0 milliliter of the assay suspension. Assay was performed by observing the formation of NADH at 340 nm (*17*). † Mitochondria were treated with 4 μg of *Naja Naja* venom (phospholipase A) per milligram of mitochondrial protein at 37°C. The venom was prepared and used in the manner described earlier (*5*). ‡ Sonication was performed at 3°C for 15 seconds on a 20-ml suspension [mitochondrial protein (5 mg/ml) in 0.25*M* sucrose-HEPES, *p*H 7.55] with a Branson Sonifier at an output of 3.25 amp.

In non-ruminants, ketone bodies are produced almost exclusively in the liver where BDH activity is higher than in the extrahepatic tissues (*11*). By contrast, ruminant liver BDH activity is very small in comparison to that in the extrahepatic tissue. Perhaps in these animals, ketone bodies are normally produced by the rumen rather than by the liver. This might account for the low BDH activity we observe in ruminant liver mitochondria.

Katz and Bergman (*12*) have proposed two principal sites for ketogenesis in the adult ruminant. The amount of BOH released from the liver of fed, non-ketotic ewes is very small in comparison to that released into the blood draining the portal viscera. However, during starvation or ketosis, the amount of BOH in the blood that is contributed by the liver increases significantly. Thus, in the case of the adequately fed, non-ketotic animal, the portal bed which drains from the rumen appears to be the principal source of ketone bodies. In the ketotic animal a sizable proportion of the blood ketone bodies are produced by the liver.

The animals in our studies were non-ketotic. The small BDH activity of ruminant liver mitochondria may account for the small BOH release from the liver of non-ketotic animals (*12*). However, it would seem difficult to explain the increased release of BOH from the ketotic liver because the BDH activity of the mitochondria is so low. It is possible that an induction of liver BDH occurs as the animal becomes ketotic. In this respect, BDH activity has been reported to be induced in brain tissue from starved rats (*13*). A similar situation may exist in ruminant liver which would allow the animal to adapt to changing amounts of metabolites during starvation. Alternatively, a new mechanism

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			