Serum Enzymes in Bovine Practice

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Introduction

Disease is a complicated state in which there is an impairment of the normal body function. Signs, the objective evidence of disease, are not totally specific and for the veterinarian to make an accurate diagnosis he sometimes requires auxiliary help. Clinical chemistry, more specifically enzymology, is a new and yet useful adjunct that can be used by the veterinary practitioner.

Cows that become sick following parturition and have only vague clinical signs will be diagnosed at times as metabolic liver disease or reticuloperitonitis. The recommendations to the owner, regiment of treatment, and prognosis could be completely different and are dependent upon an accurate diagnosis. The pathogenesis of many diseases, i.e. liver disease, is based on the development of biochemical lesions and as such affect, directly or indirectly, the enzyme systems of the body.

Enzymes appear in the circulation with acute or progressive disease and as such offer many advantages over conventional diagnostic methods. Palpation of an enlarged liver per-rectum in the bovine generally reflects chronicity. The transaminase activity (serum glutamic-oxaloacetic - SGOT) may have been elevated several weeks previously while in chronic liver disease the transaminase levels may be normal. Diagnosis and treatment of liver disease in acute stages may be lifesaving while little or nothing may benefit the chronic, severely damaged liver.

Origin of Enzymes

With cell death or alteration of cell permiability, enzyme leakage occurs. The activity of any serum enzyme is dependent on the rate of release of the enzyme from the cell, and the rate of its removal from the circulation. Thus, an enzyme's activity will increase if more enzyme is presented to the circulation or if its removal is hindered.

Specificities of Enzymes

Ideally, an enzyme that is present in only one tissue would offer great diagnostic advantages. Any alteration of the concentration of a particular enzyme would then specifically locate the lesion. Unfortunately, an enzyme is usually present in many tissues, at least to a small degree. However, some enzymes are present in one tissue at such a high level that its elevation in the serum greatly narrows down the lesion location. For example, creatine phosphokinase (CPK) in the bovine is present in skeletal muscle, cardiac muscle and brain (Table 1). Hence, marked elevation of serum CPK localizes the lesion either to skeletal or heart muscle. Table 1 lists various enzymes and their major tissue sources.

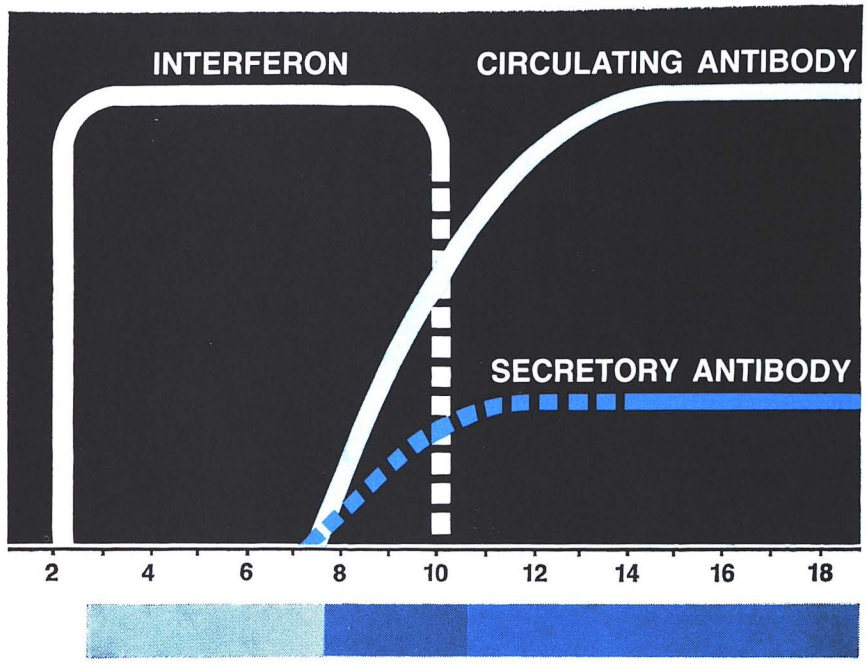
TABLE 1

Enzymes and Their Major Tissue Sources[3]

Enzyme	Source
Aldolase (ALD)	Skeletal muscle
Alkaline phosphatase (SAP)	Bone, liver, kidney, intestinal mucosa, spleen
Creatine phosphokinase (CPK)	Skeletal muscle, brain, myocardium
Glutamic dehydrogenase (GLDH)	Liver
Glutamic oxalocetic transaminase (SGOT)	Liver, skeletal muscle, pancreas
Glutamic pyruvic transaminase (SGPT)	Skeletal muscle, liver (small amts.)
Lactate dehydrogenase (LDH)	All tissues
Ornithine carbamyl transferase (CT)	Liver
Sorbitol dehydrogenase (SDH)	Liver

Methods of Reporting Enzyme Results

There are many methods by which laboratories report enzyme activity. Clinicians must be certain that any reported enzyme value is indicative of normal or elevated activity. The recommendation of the International Union of Biochemistry Committee on Nomenclature is that a unit of



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activity be defined as the quantity of enzyme that will cause the conversion of a u mole (micromole) of substrate or the production of a u mole of product per minute. This unit is referred to as the International Unit (IU) and may be expressed as milli-units per ml (m U/ml). The universal acceptance of international units would lessen much of the prevailing confusion regarding the use of serum enzymes.

Table 2 illustrates the importance of knowing the normal values and which method was used to determine serum activity. For example, a serum SGOT activity of 200 in Sigma-Frankel units/ml would be twice normal, while if reported by the SMA system* it would only be slightly above normal. If normal control values are unavailable for a particular enzyme procedure that you are using or if you lack confidence in the normal values, determine the enzyme level on at least one control animal as well as on the abnormal animal. If possible, select the control animal of the same breed, age, and sex as the abnormal animal (see example used in Table 3).

TABLE 2

Normal Enzyme Values for Bovine

Enzyme	Method	Values	References
Aldolase (ALD)			
	BMC mU/ml*	3-16	Dunavant & Rich
	International Units	28.2 ± 7.4	Zimmerman et.al.
Alkaline phosphatase (SAP)			
	H.I.U.**	17.3 ± 13.0	Laird
	King-Armstrong units/ 100 ml	0.3-114	Allcroft & Folley
	SMA-12/60 mU/ml***	52-400	Van Kampen
Creatine phosphokinase (CPK)			
	BMC mU/ml*	0-50	Dunavant & Rich
	H.I.U.**	4.98 ± 31.3	Laird
Glutamic dehyrdogenase (GLDH)			
	BMC mU/ml*	0-3	Dunavant & Rich
Lactate dehydrogenase (LDH)			
	BMC mU/ml*	217-517	Dunavant & Rich
	H.I.U.**	400 (x)	Laird
	International units	473 ± 97	Zimmerman et.al.
	SMA-12/60 mU/ml***	19-320	Van Kampen
Glutamic oxaloacetic transaminae (SGOT)			
	BMC mU/ml*	13-40	Dunavant & Rich
	H.I.U.**	143.6 ± 26.1	Laird
	Karmen	33 ± 8	Zimmerman et.al.
	Sigma-Frankel units/ml (S-F units)	36 - 95	Dunavant & Rich
	SMA-12/60 mU/ml***	47-189	Van Kampen
Glutamic pyruvic transaminase (SGPT)			
	H.I.U.**	75 ± 9.7	Laird
	Karmen	19 ± 3	Zimmerman et.al.
	Sigma-Frankel units/ml (S-F units)	8-24	Kaneko
Ornithine carbamyl transferase (OCT)			
		5.1 ± 2	Hansen
	1 uM/hr/ml	0.284 ± .02	Kaneko
Sorbitol dehydrogenase (SDH)			
	BMC mU/ml*	0.2-4	Dunavant & Rich
	mU/100 ml	0.58-1.53	Kaneko

*Determinations done using Boehringer Mannheim Corporation (BMC) reagents and Teco-Meter R.
**Hycel International Unit, (H.I.U.) Hycel, Inc., Houston, Texas.
***Technicon Corporation, Tarrytown, New York.

TABLE 3

Enzyme Changes Associated with Locoweed Poisoning

	CPK*	SGOT	SGPT	SDH	GLDH
Control female calf	38	90	40	1.9	4.0
Loco female calf	39	205	8	43.3	13.5
Control male calf	32	100	38	3.0	5.0
Loco male calf	32	182	18	21.7	17.5

*CPK, SDH and GLDH determinations done using Boehringer Mannheim Corporation reagents and Teco-Meter R, results in International units (mU/ml). SGOT and SGPT were measured in Sigma-Frankel units.

Clinical Significance of Enzymes in Bovine Practice

1. *Myopathies, Myositis and Myocardial Degeneration*

Several enzymes are useful in diagnosing muscle injury of cattle. Increases in serum aldolase (ALD), creatine phosphokinase (CPK), serum glutamic oxaloacetate transaminase (SGOT), or lactate dehydrogenase (LDH) activity are expected in acute or progressive muscle disease. CPK is more specific than the latter two enzymes (Table 1) hence, increase in CPK activity support the clinical diagnosis of skeletal muscle or myocardial injury. SGOT elevations in the bovine, in the absence of liver disease, are indicative of muscle damage. Aldolase, in our limited experience, is a good enzyme test for diagnosing muscle injury. LDH has wide normal fluctuations, hence very high levels can only be viewed as significant. In veterinary medicine, total LDH is considered to be of limited dianostic use. This enzyme is present in all tissues and as such lack specificity. Hemolysis causes many enzymes to be released from red blood cells, particularly LDH. Lactate dehydrogenase isoenzymes in the bovine, also have have limited uses.

Nutritional myopathies (White Muscle Disease) are associated with marked elevations of CPK, SGOT and LDH activities. Treatment with selenium has shown a reduction in clinical signs of White Muscle Disease, as well as a reduction in enzyme levels (10).

The bovine practitioner sees numerous cases of parturient paresis. Many of these do not respond to calcium therapy and are than called the "downer cow." This syndrome includes many different

Reg. T.M. - Technicon Instrument Corp., Tarrytown, New York.

disorders. It is important to differentiate these conditions and clinical enzymology can help us do so. Some of these downer cows, in our experience, may be suffering from metabolic liver disease or muscle disease. A profile of enzyme tests will help localize the lesion or lesions. A complete liver-muscle profile would include CPK, SGOT, sorbitol dehydrogenase SDH), and glutamic dehydrogenase (GLDH). Let's assume that the cow has been down for two days and has muscular injuries as a result of attempting to rise. Clinically muscular injury is difficult to ascertain. Enzymologically the CPK and SGOT levels would be significantly elevated (greater than two x above normal) while the SDH and GLDH (liver enzymes) would be within the normal range. On the other hand if SGOT, SDH and GLDH were elevated the cow undoubtedly has liver injury. If all four enzymes were increased, the cow would have both muscle and liver degeneration. This example illustrates how a diagnosis can be arrived at through enzyme determinations. Prognosis can likewise be attained by serial interval determinations of enzymes. For example, serum CPK values return to normal within two to three days following muscle injury, while SGOT rises slower but remains elevated for several days (Figure 1). If

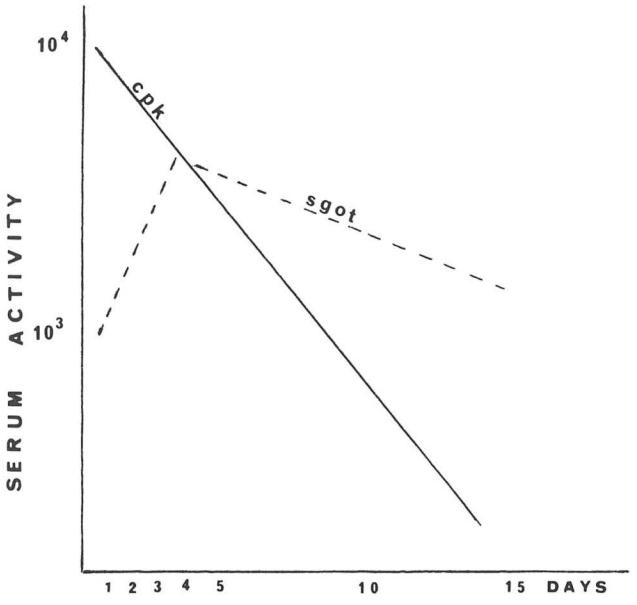

Figure 1: A case of surgically induced myositis in the horse. CPK was at its peak activity (7,000 m U/ml) six hours following surgery; however, SGOT did not reach its peak until the fourth day, then slowly dropped off. This horse fully recovered. CPK = Boehringer Mannheim Corporation international units (m U/ml). SGOT = Sigma-Frankel units/ml. (S-F units).

the CPK is returning to normal, yet the SGOT is elevated, there has only been a single insult and the animal is recovering. If the CPK level remains high over a period of four to five days, the condition is progressive and the prognosis is guarded.

Acute and/or progressive muscular dystrophies, endocarditis and myocarditis, foreign body pericarditis, myositis that occurs as part of a generalized disease including blackleg, would undoubtedly cause elevations in SLD, CPK and SGOT activities in serum.

Elevations of the muscle enzymes would help eliminate diseases of bone and nerves from consideration.

Osteopathies

Bone contains a considerable amount of alkaline phosphatase (SAP). Serum alkaline phosphatase should be elevated in conditions where there is increased osteocytic activity or in diseases of liver that prevent SAP from being eliminated by the biliary system. Hence, healing fractures, rickets, osteomyelitis, liver tumor, any obstruction of biliary system could cause elevations in SAP. Disease of bone in the bovine, in our limited experience, does not generally cause increases in SAP. SAP will increase in the rare case of liver abscesses in which the abscess is causing biliary stasis. Immature animals have higher normal values than mature animals. Generally, SAP in the bovine is of limited value because of its lack of specificity and because of the wide range in normal values (Table 2).

Liver Disease

Liver disease is always a disease possibility in any sick cow, however, confirmation of this diagnosis has been difficult. The SGOT enzyme procedure has been available for many years, yet many cows suspected of having liver disease have had normal SGOT activities. Additional serum enzymes are now available for evaluating the liver's condition. Yet, no single enzyme can completely evaluate the liver. As previously discussed, the SGOT may take a few days before it reaches peak serum activity, therefore, its value is limited in very acute liver degenerations. Conversely, the SDH and GLDH rise rapidly in serum but return to normal levels in a few days because of their short serum half-lives. Thus, we need not think of a single, specific liver enzyme but of a profile of liver enzymes. Serum enzymes which are relatively specific for hepatocellular damage in cattle are SDH or GLDH. In our experience, GLDH in cattle seems to be more sensitive than SDH. The recommended profile to evaluate both liver and muscle function in the bovine should include CPK, SGOT, SDH and GLDH (discussed under myopathies).

Hepatocellular degeneration in the bovine is produced by toxic hepatitis-phosphorus, arsenic, carbon tetrachloride, hexachlorethane, poisonous plants; infectious hepatitis-leptospirosis, bacteria; and parasitic hepatitis-flukes, ascarids. In our university practice, liver degeneration is diagnosed in cattle secondary to plant toxicities, liver abscesses due to penetrating wire, or metabolic diseases associated with downer cow syndrome, acetonemia, mastitis or metritis.

Recently, locoweed poisoning was diagnosed in several, young black Angus calves from southern Colorado. Hence, this herd will serve as an example of how liver disease can be diagnosed by enzyme tests. Two loco calves were presented for examination. Biochemical changes in blood are reported in Table 3 for four Angus calves approximately six months of age. Two of these are control Angus calves and two are the loco calves.

Note that the CPK's and SGPT's are normal in the loco calves; however, the SGOT, SDH, and GLDH's are markedly elevated. The muscle is apparently uninvolved (normal CPK) and the SGOT activity is coming from a diseased liver (elevated SDH and GLDH). In this particular example, either a profile of CPK and SGOT or an individual test such as SDH or GLDH, would have told us that the calves had severe liver disease.

The second example concerns the enzyme changes associated with hepatosis in a six-year-old Holstein cow. This cow had calved three weeks prior to hospital admittance and was treated for retained placenta two days after calving. She became very weak, went down, and was unable to rise one day prior to entry. The enzyme activities taken on the first day were as follows: SGOT - 210 S-F units (normal less than 95 - Table 2), SDH - 12.8 m U/ml (normal less than four), GLDH - 19.1 m U/ml (normal less than three).** All of these enzymes were markedly elevated indicating severe liver degeneration. A diagnosis of left displaced absomasum (LDA) and liver degeneration was made. Surgery was done for the LDA, the cow did not improve and died one week following surgery. On necropsy the liver was enlarged and yellow. Histologically there was marked fatty vacuolization with retention of bile. This type of hepatic lipidosis is seen secondary to many primary diseases in the bovine. An inadequate liver function makes it more difficult for the cow to recover from diseases like displaced abomasum, toxic mastitis or metritis, and acetonemia.

Neuropathies

As shown in Table 1, the CPK content of the brain is second to that found in skeletal muscle. Thus, attempts have been made to use serum CPK values as means for detecting neuropathies in sheep and cattle. Serum CPK values have been reported to be elevated in polioencephalomalacia and focal symmetrical encephalomalacia (8) in sheep. A few cases of listeriosis in both sheep and cattle have had elevated serum CPK values (personal experience). To eliminate the possible complications of muscle degeneration one should always determine the SGOT activity. A normal SGOT and an elevated CPK, in the bovine that has been ill for three to four days, supports a diagnosis of brain degenerations.

Bovine polioencephalomalacia may be produced by thiamine deficiency (6). A diagnosis of thiamine deficiency can be confirmed by measuring the transketolase content of heparinized blood and the effect of thiamine pyrophosphate addition in vitro (TPP-effect). Thiamine is a co-factor for the enzyme transketolase and its activity is depressed in thiamine deficiency. The TTP effect is measured by adding thiamine pyrophosphate to the system and measuring the formation of end product (sedoheptulose-7-phosphate) (7). Either a low transketolase level (below 60) or a markedly increased TPP effect (100%) suggests a diagnosis of thiamine deficiency or polioencephalomalacia in the bovine (11).

The uses of serum enzymes in diagnosis of central nervous disorders is a new concept, one that has not been completely studied and evaluated. We await further studies.

For summation purposes, Table 4 presents our suggested enzyme profiles for diagnosis of muscle, liver and central nervous degeneration in the bovine.

TABLE 4

Recommended Enzyme Profiles in Diagnosing Bovine Diseases

Degeneration	Enzyme Profile
Skeletal Muscle, Myocardium	ALD, CPK, SGOT
Liver	SGOT, SDH, GLDH
Central Nervous System	CPK, SGOT

Practicality of Enzyme Tests

Now that you are convinced that the determination of serum enzymes is invaluable in bovine medicine, how and where do you have the determinations done. Two approaches are open to you. The first is that you develop or expand your

(Continued on page 68)

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(Continued from page 12)

own laboratory to perform these tests, the second that you send the sample to a commercial laboratory. Each of these has advantages and disadvantages and the selection of one over the other depends on your own situation. If you choose to do the former, you will need a spectrophotometer which measures wavelengths as low as 340 nm. Acceptable spectrophotometers include B & L Spectronic "20" - cost approximately $375; Coleman, Junior 11 - cost $625.00; Coleman, Junior 11A - cost $750.00; Turner Model 330 - cost $600.00. This is only a partial list, many more expensive spectrophotometers are available. However, these instruments lack sensitivity for very accurate enzyme determinations, therefore we recommend the use of the Teco-Meter R***, cost approximately $650.00. This instrument is designed to offer any laboratory an opportunity to perform UV determinations (366nm) and a few selected colorimetric procedures at economical prices. A single enzyme test performed on the Teco-Meter R using available kits cost $.50 to a $1.00 and generally takes 10-15 minutes before the results are available. Commercial laboratories will do many of these enzyme tests at a cost of $4 to $5 per test but it takes 24 to 48 hours before you receive the results. The Teco-Meter or similar instruments will not be outdated; new, rapid tests are continually being adopted to their use.

Table 5 lists commonly available equipment and the enzyme tests that each will presently perform.

TABLE 5

Correlation of Commonly Available Equipment with Enzyme Tests

	Bio-Dynamics*	Ames BMI**	Teco-Meter R***	Spectrophotometer+	SMA†
ALD			X	X	
SAP	X	X	X	X	X
CPK	X		X	X	±
GLDH			X		
LDH	X		X	X	X
SGOT	X	X	X	X	X
SGPT	X	X	X	X	±
SDH			X	X	

*Unimeter 250, Bio-Dynamics, Inc., Indianapolis, Indiana.

**Ames/BMI Blood Analyzer, Ames Company, Elkhart, Indiana.

***Teco-Meter R is an ultraviolet photometer designed to measure absorption at a wavelength of 366 nm. Boehringer Mannheim Corporation, New York, New York.

+Spectrophotometer, numerous manufactures, which measures wavelengths as low as 340 nm. By using Sigma Chemical Co. diagnostic kits, all tests but GLDH can be performed.

†An autoanalyzer that performs 12 biochemical serum tests. Technicon Corporation, Tarryton, New York. Machine usually not set up to do CPK or SGPT.

Conclusion

The measurement of many serum enzymes can aid the veterinary clinician in making accurate diagnoses and predicting the outcome of disease. However, to use these aids properly, the clinician must know what units and how the enzyme values are reported and some basic pathophysiology so that enzyme values can be correctly interpreted. The determinations of enzymes are here to stay; in fact, new, low cost, simplified kits are being commercially prepared at a rapid rate.

Only a few enzymes, those considered possible for a commercial laboratory or veterinary hospital laboratory to determine and those more practically orientated, have been discussed in this paper. Further findings will lengthen the numbers of useful diagnostic enzymes that can be used by the bovine practitioner.

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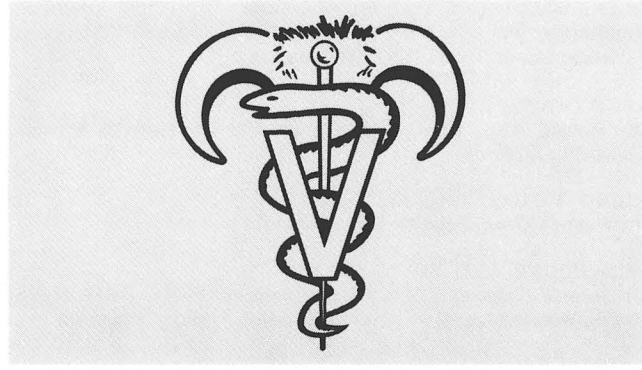

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