EFFECTS OF TAENIA SAGINATA CYSTICERCOSIS ON MYOCARDIAL AND OTHER TISSUES OF BOVINE

by

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ABSTRACT

This study was designed to determine pathological distress in bovine tissues caused by Taenia saginata. Methods of analysis included spectrophotometric enzyme quantitation for glutamic-pyruvic transaminase, glutamic-oxalacetic transaminase, lactic dehydrogenase, and creatine phosphokinase. Lactate dehydrogenase isoenzymes and creatine phosphokinase isoenzymes were separated by electrophoresis prior to scanning by fluorometric procedures. Additional methods of analysis were used in blood cell studies which included packed cell volume, cyanmethemoglobin, total white blood cell and differential white blood cell counts.

Holstein calves, 6 to 7 months of age, were infected with the eggs of Taenia saginata followed by 30 consecutive days of laboratory analysis of blood samples. Following the research, the infected animals were sacrificed so that an approximation of cysticerci per unit tissue could be obtained.

Findings appear to indicate that Taenia saginata cysticercosis result in significant increases in serum glutamic-oxalacetic transaminase, lactate dehydrogenase and lactate dehydrogenase isoenzymes 3, 4 and 5. Significant increases in eosinophils and monocytes also occurred which seem to indicate a correlation between their elevation and the continual development of the cysticerci.

INTRODUCTION

Taenia saginata cysticercus is the larval stage of the human adult tapeworm Taenia saginata. The primary host for Taenia saginata cysticercus is domestic cattle, although according to Abuladze (1970), cases of Taenia saginata cysticercosis have been found in reindeer. These animals, in addition to rare cases involving wild mammals as discussed by Nelson, Pester and Richman (1965), serve as the intermediary host in the life cycle of Taenia saginata.

The host for the adult Taenia saginata is considerably more restricted than that of the cysticerci. A study by Nelson, Pester and Richman (1965) involving wild primates supports the fact than man is the only definitive host for the tapeworm Taenia saginata. However, an inconclusive case involving proglottid production by a goat was described by Southwell (1921).

Pawlowski and Schultz (1972) state that consumption of raw or improperly cooked beef by humans is the primary source of infection by the cysticerci of Taenia saginata. The scolex of the developing tapeworm commonly attaches to the small intestine of the host. A study by Penfold (1937) established the time for complete development (from ingestion of a cysticercus to an adult Taenia saginata) to be approximately 100 days. After the complete developmental stage in the human host, Taenia saginata assumes the production of proglottides on an irregular cycle.

Penfold (1937) determined that 80,000 ova are contained in a single active proglottis. As a result of infection, it is therefore possible for an infected human to expel, on the average, 720,000 ova per day. In research done by Silverman (1954), it was determined that ova of a gravid-expelled proglottis of Taenia saginata are found to be 50% mature, 40% immature and 10% infertile. It was also found that it is possible for some of the immature ova to mature within 2 weeks outside the host.

As discussed by Pawlowski and Schultz (1972), the greatest damage caused by Taenia saginata is economic. Although infections involving cysticercus seldom result in the death of the host, the high rate of cysticercosis in cattle of underdeveloped countries is of great concern to the livestock industry who depend on export trade. Other losses, aside from those of cysticercosis, are due to the medical complications resulting from the infection of humans with the adult tapeworm Taenia saginata.

In a summary of the literature involving over 3,000 cases of taeniasis, Pawlowski and Schultz (1972) found that patients suffered from abdominal pain, nausea, weakness, loss of weight, increased appetite and a diversity of other less characterized symptoms. It is also recognized that except for the expelling of proglottides, the symptoms resulting from Taenia saginata infection in humans may be completely absent.

Epizootiology

Sprent (1969) describes a Taenia saginata infection as a true zoonosis in which man acts as the definitive host and vertebrate

animals, primarily domestic bovine, act as the intermediate host. Pawlowski and Schultz (1972) state that cysticercosis can occur when either the feed, water or soil used in the maintenance of domestic bovine becomes contaminated with mature Taenia saginata ova. Other means of Taenia saginata infection between man and cattle includes sewage contamination, bird feces contamination as reported by Silverman and Griffiths (1955) and egg transmission by flies as reported by Round (1961).

Taenia saginata Cysticercus

The physical and physiological changes that result in the development of Taenia saginata cysticercus are discussed by Silverman (1954), Silverman and Maneely (1955), McIntosh and Miller (1960), Weinmann (1965, 1969), Wikerhauser, Zukovic and Džokula (1970) and Pawlowski and Schultz (1972). The developmental process includes hatching of the ingested Taenia saginata eggs, infection by the oncosphere and growth of the cysticercus.

Hatching

As discussed by Silverman (1954), the ingested eggs of Taenia saginata enter the gastrointestinal tract of the animal where the hatching process occurs. The hatching of the ova is divided into two stages by Silverman. The first stage involves the passive digestion of the striated embryosphore, with the aid of pepsin. The second stage is characterized by the rupture of the oncospheral membrane by the hexocanth embryo.

Silverman (1954) used the following artificial intestinal juice as a standard means of ova activation:

Pancreatin	500 milligrams
Cholesterol	10 to 20 milligrams
Sodium tauro-glycocholate	500 milligrams
Trypsin	20 milligrams
Sodium bicarbonate	500 milligrams
Distilled water	50 milliliters

The efficiency of the procedure is increased by adding 1 part whole fresh bovine bile and 2 parts artificial intestinal juice, as reported by Gallie and Sewell (1970).

Infection

A substance, described by Silverman and Maneely (1955) as a secretion of the penetration glands and the hexocanth hooks aids the activated oncosphere in penetrating the intestinal mucosa of the host. At this point, it is thought by Weinmann (1965, 1969) that the pre-encystment larval stage results in the first apparent host reaction to the infection. The secretion from the penetration gland is presumed to be responsible for the immunological response by the host to the hexocanth embryo.

As pointed out by Pawlowski and Schultz (1972), the means by which the onchosphere of Taenia saginata migrates to the intermediary host tissue has not yet been discovered. Reference is made to an experiment involving the intravenous injection of onchospheres into young calves which did not result in cysticercosis. Wikerhauser, Zukovic and Džokula (1970) have administered onchospheres subcutaneously, intramuscularly and intraperitoneally resulting in infection without migration from the site of innoculation.

Development of Cysticercus

Work by McIntosh and Miller (1960) involving the postencystment stages of Taenia saginata cysticercosis describes the development of cysticerci within its connective tissue cyst. It was discovered that cysticercus with a diameter of 2.5 millimeters could be found 11 days after the infection date. Table 1, from the research by McIntosh and Miller (1960, p. 171), shows the increase in both connective tissue cysts and the cysticercus in relation to the exposure day.

McIntosh and Miller found that 2 weeks after infection, the connective tissue cysts and the cysticercus had increased in size relative to day 11. However, the cysticerci were characterized as being thin and ovate at this point. Three weeks after infection, connective tissue had doubled in size while the cysticerci were increased to 10 times. Four week old cysts had not changed in appearance from the previous week, but it was noted that the area involved with scolex development had begun to invaginate.

Table 1. Correlation Between Morphological Development of Cysticercus Bovis and Age of Infection.* (Measurements in millimeters)

Age of Infection (days)	Connective Tissue Cyst		Cysticercus
	Length	Width	
11	3	2	0.13-0.15 x 0.09-0.13
14	4	3	0.16-0.17 x 0.1-0.13
21	7	6	1.3 x 0.875
	9	8	1.65 x 1.38
	10	7	1.3 x 0.9
28	6	5	1.15 x 0.82
	8	4	0.95 x 0.95
	7	5	1.18 x 1.1

*McIntosh and Miller (1960, p. 171)

Taenia saginata Cysticercosis

As the cysts continue to develop and increase in size, it is logical that some physical tissue stress might occur. The pathology involving the tissues of an animal infected with cysticercosis is discussed by Smith, Jones and Hunt (1972). They found that the tissues involved and the degree of parasitism determine the effect upon the intermediary host of Taenia saginata. As a rule, the histopathology of cysticercosis appears as displacement of normal tissue cells and a slight inflammatory reaction incompassing the active cysticerci. They also found that cysticercosis in cattle is characterized by few symptoms, but a massive invasion of critical organs such as the liver, heart and brain may cause distress followed by death. A dense connective tissue encapsulation has also been noted that results in a scar after death of the cysticercus.

If tissue damage by cysticercosis is great enough, it is possible that the serum enzyme levels would increase. This is seen in Figure 1 by Baron (1963, p. 174).

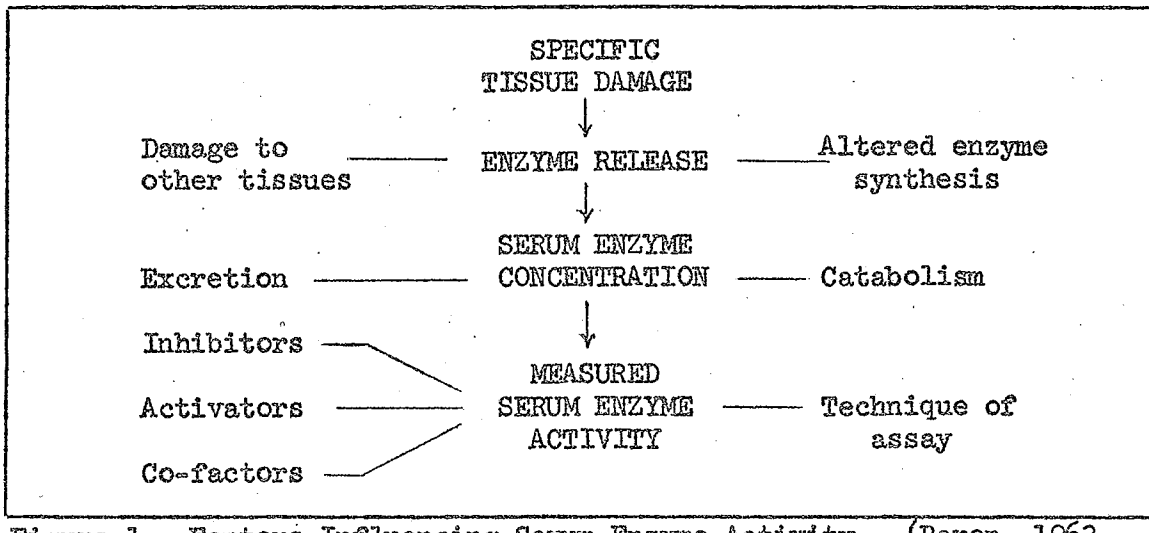

Figure 1. Factors Influencing Serum Enzyme Activity. (Baron, 1963, p. 174)

Clinical Enzymology

As discussed by Batsokis and Briere (1967), clinical enzymology is based upon methods of assay which determine the serum enzyme concentration by means of their catalytic activity. Enzymologists are able to distinguish specific tissue structure by the enzyme fractions released upon cytolysis. Some of the common conditions which can be confirmed by clinical enzymology are cardiovascular distress, hepatitis, neurological disease, skeletal muscle necrosis, bone disease, hematologic disorders and normal and complicated pregnancy.

Batsokis and Briere (1967) stated that the origin of enzyme activity in normal serum results from the constant state of tissue turnover. This makes it possible to establish a normal enzyme activity level, or baseline, for each organism. Although enzymes are not totally organ specific, an increase above the normal enzyme concentration can be used in confirming a diagnosis of pathological distress.

LaDue (1958) discusses the methods that have been developed to study enzyme activity. These methods involve chromatography, spectrophotometry and colorimetry. More recent technicological developments in the study of enzymes, as discussed by Elevitch (1973), include electrophoresis, immunoelectrophoresis, gel filtration, heat inactivation and fluorometry.

Enzymology

Some enzymes that are used in the clinical studies of myocardial infarctions, as discussed by Zimmerman and Henry (1974), are as follows: glutamic-oxalacetic transaminase (GOT); glutamic-pyruvic transaminase (GPT); lactic dehydrogenase (LDH) and its isoenzymes; and creatine

phosphokinase (CPK) and its isoenzymes. Although glutamic-pyruvic transaminase can be used for the diagnosis of myocardial infarction, it is more commonly associated with the detection of liver necrosis.

Principles of Glutamic-Pyruvic Transaminase

Glutamic-pyruvic transaminase (GPT), also known as alanine aminotransferase, is an enzyme that catalyzes the following reaction (Wroblewski and LaDue 1956, p. 169):

$$\text{Alanine} + \alpha\text{-ketoglutarate} \underset{\longleftarrow}{\overset{(GPT)}{\longrightarrow}} \text{glutamate} + \text{pyruvate}$$

Serum glutamic-pyruvic transaminase (SGPT) functions in the transfer of an amino (NH_2) group from alanine to form glutamate. As described by Karmen, Wroblewski and LaDue (1955) this reaction, by means of paper chromatographic procedures (Awapara 1949), can result in the quantitation of the end products. Wroblewski and LaDue (1956) and Henry et al. (1960) modified GPT catalytic reaction in the development of a revised spectrophotometric method of analysis. The modification involved the addition of nicotinamide adenine dinucleotide ($NADH_2$) and lactic acid dehydrogenase (LDH) to the reaction as shown here (Wroblewski and LaDue 1956, p. 569):

$$\text{Pyruvate} + NADH_2 \underset{\longleftarrow}{\overset{(LDH)}{\longrightarrow}} \text{lactate} + NAD$$

In this coupled reaction, lactic dehydrogenase (LDH) is not rate limiting because it is added in excess. The oxidation of $NADH_2$, dependent upon the concentration of pyruvate, is followed at 340 nanometers in a spectrophotometer and is proportional to GOT activity.

GPT in Human Tissues. Glutamic-pyruvic transaminase (GPT) concentrations in human tissues were found by Wroblewski and LaDue (1956, p. 570) to be greater in hepatic than other tissues. Values for each tissue type, after being converted to percentages of the total GPT activity, are revealed in Table 2. Their results also show that the concentration of GPT in the heart ranks third of the tissues tested. From their findings it was hypothesized that since myocardial GPT activity is minimal, the use of GPT in diagnosing hepatocellular damage is made more confirmative.

GPT in Bovine Tissues. The distribution of GPT in bovine tissues was reported by Cornelius and his associates (1959, p. 124). Their results, after conversion to percentages of the total GPT activity are revealed in Table 2. Unlike GPT activity in humans, they found that in bovine the heart has the highest GPT activity and considerably more than that of the liver.

Table 2. GPT Found in Human and Bovine Tissues*

Tissues	Percent of Total	
	Human	Bovine
Heart	9.0	45.8
Liver	55.8	2.0
Skeletal muscle	6.1	23.4
Kidney	24.1	5.8
Pancreas	2.5	20.6
Spleen	1.5	2.4
Lung	0.9	-
Serum	0.1	-

*Wroblewski and LaDue (1956, p. 570)

Diagnostic Importance of GPT. The clinical importance of GPT as a means of diagnosing hepatocellular necrosis was suggested by Wroblewski and LaDue (1956), was supported by Wroblewski (1957), and by Zimmerman and Henry (1974). Wilkenson (1968, p. 73) states that "Hepatocellular damage leads to the release into the serum of a number of enzymes, of which SGOT and SGPT are the most generally useful for diagnostic purposes. Elevated levels of SGOT are found in diseases of several other organs but the SGPT is relatively specific for liver damage."

From a report by Wroblewski (1957, p. 692) the increase in serum glutamic-pyruvic transaminase and serum glutamic-oxalacetic transaminase as the result of tissue destruction in relation to infectious hepatitis is noted (Figure 2). The study shows elevations of GPT greater than GOT in all instances. Values for both transaminases "peaked" at 4 days post-infection and decreased sharply over the next 3 days.

The clinical significance of SGPT in cardiac disorders is reported by LaDue (1958) and West et al. (1961). LaDue (1958, p. 310) caused an acute transmural myocardial infarction in 10 dogs by injecting plastic spheres into the coronary sinus (Figure 3). A 1 to 3 fold increase in GPT was noted, which appeared over a period of 4 days post-infarction. LaDue explained that the values for GOT and LDH showed greater increases than those of GPT following tissue damage because of their relatively greater enzyme concentration in cardiac tissue.

A report by West et al. (1961) revealed that GPT elevation in humans occurred in cases where right-sided heart failure was evident. In patients where left-sided failure was manifested, only 1 out of 39

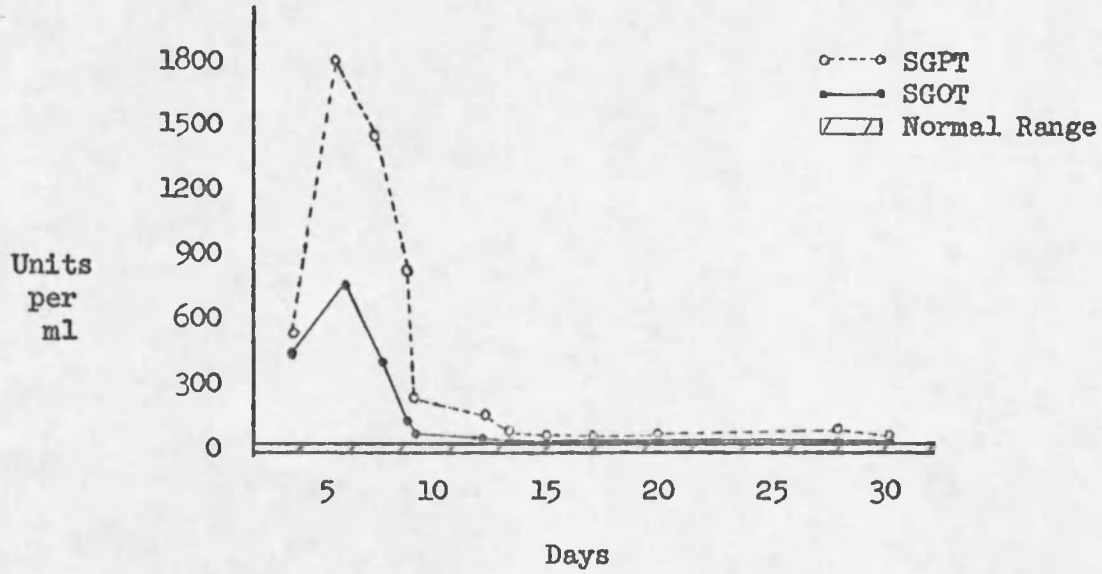

Figure 2. Alterations of Serum Enzyme Activities. (Wroblewski 1957, p. 692)

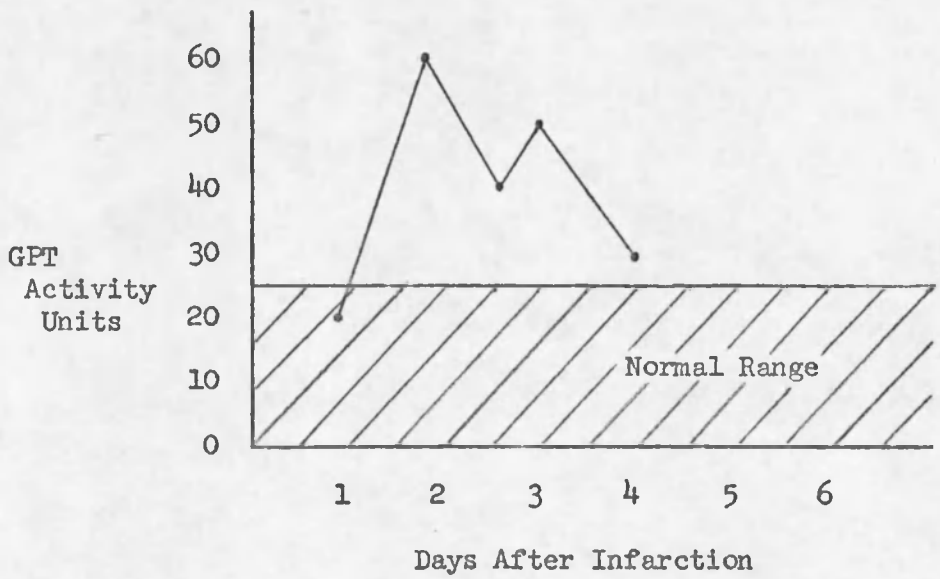

Figure 3. SGPT Elevations Following Cardiac Disorders. (LaDue 1958, p. 310)

cases showed increased GPT activity in the serum. This finding by West shows that although the concentration of GPT in cardiac tissue is less than in hepatic tissue, the diagnostic specificity of GPT in cardiac tissue is comparable to the diagnostic specificity of glutamic-pyruvate transaminase in hepatic tissue.

Principles of Glutamic-Oxalacetic Transaminase

Glutamic-oxalacetic transaminase (GOT), also known as aspartate aminotransferase, catalyzes the following reaction (Karmen, Wroblewski and LaDue 1955, p. 126):

$$\text{Aspartate} + \alpha\text{-ketoglutarate} \underset{\leftarrow}{\overset{\text{GOT}}{\rightleftharpoons}} \text{glutamate} + \text{oxalacetate}$$

Glutamic-oxalacetic transaminase (GOT) functions in the transfer of an amino (NH_2) group from aspartate to form glutamate. In the same report, Karmen and associates (1955, p. 131) also coupled the following reaction to enable the use of a spectrophotometer in analysis.

$$\text{Oxalacetate} + DPNH_2 \underset{\leftarrow}{\overset{\text{MDH}}{\rightleftharpoons}} \text{malate} + DPN$$

The spectrophotometric assay reaction required the addition of diphosphopyridine nucleotide ($DPNH_2$) and malic dehydrogenase (MDH). The malic dehydrogenase (MDH) is added in excess so that it is not rate limiting.

GOT in Human Tissues. The distribution of glutamic-oxalacetic transaminase (GOT) in human tissues was determined by LaDue (1958, p. 308). His findings for each tissue type, after being converted to

percentages of total GOT activity, are revealed in Table 3. The research by LaDue reveals that GOT is found in the greatest quantities in heart tissue and in the least quantities in serum, under normal conditions.

GOT in Bovine Tissues. The distribution of Glutamic-oxalacetic transaminase (GOT) in bovine tissues was determined by Cornelius et al. (1959, p. 124). Their findings, after being converted to percentages of the total GPT activity, are also shown in Table 3. The research by Cornelius and coworkers reveals that GOT is found in the greatest quantities in heart tissue and in the least quantities in the spleen, under normal physiological conditions.

Diagnostic Importance of GOT. The greater concentrations of GOT in heart muscle of mammals led LaDue, Wroblewski and Karmen (1954) to investigate its level in human serum after acute myocarditis. Their results reveal that GOT activity increases in the serum of patients with acute transmural myocardial infarction. LaDue and others (1954, p. 498) found elevated serum glutamic-oxalacetic transaminase (SGOT) levels in a patient after myocarditis (Figure 4). SGOT increased 2 to 20 times the normal level within 24 hours and returned to normal within 3 to 6 days after the infarction. Since this discovery, the clinical significance of GOT in heart disease has been supported by Steinberg and Ostrow (1955), Denney et al. (1956), Shabetal, Iglauer and Anderson (1957), West, Eshchor and Zimmerman (1966), Coodley (1970), Zimmerman and Henry (1974) and others.

Table 3. GOT Found in Human and Bovine Tissues*

Tissues	Percent of Total	
	Human	Bovine
Heart	30.4	33.5
Liver	27.8	16.4
Skeletal muscle	19.4	15.7
Adrenal	-	15.4
Kidney	17.8	9.3
Spleen	2.6	3.5
Pancreas	2.0	6.1
Serum	0.0	-

*LaDue (1958, p. 308)

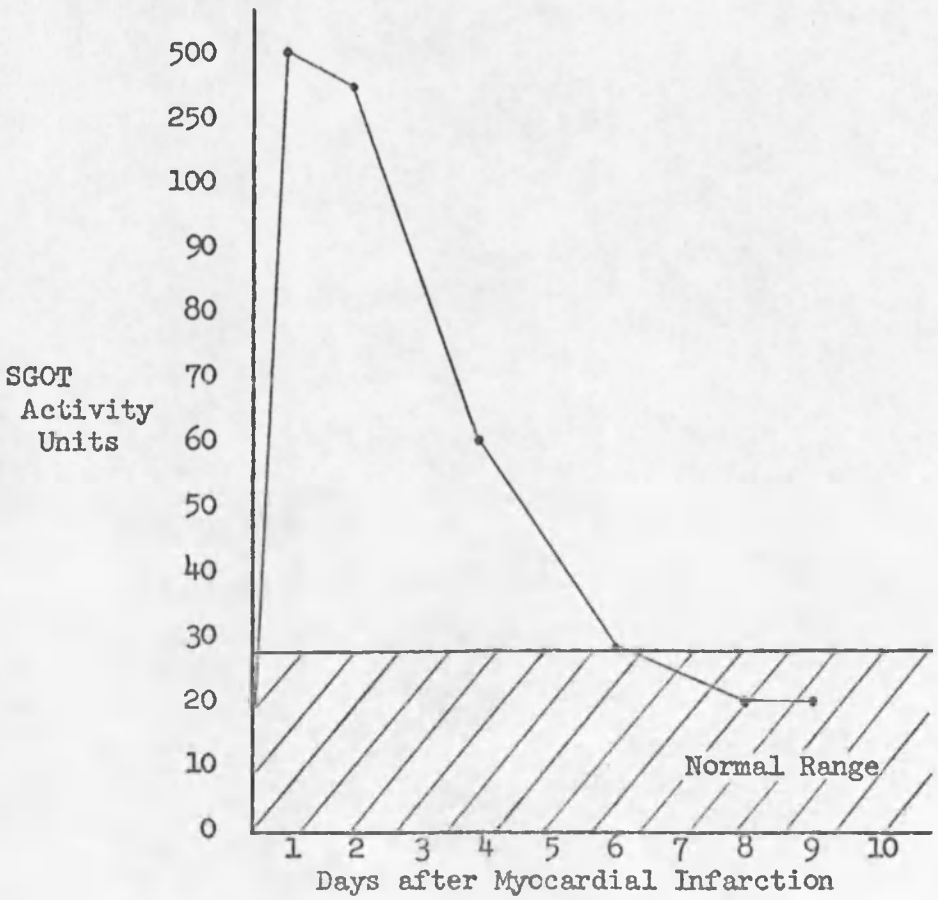

Figure 4. SGPT Levels Following Myocardial Infarction. (LaDue, Wroblewski and Karmen 1954, p. 498)

The clinical significance of SGOT in diagnosing liver disease as reported by Wroblewski and LaDue (1956) has been discussed to some degree earlier in relation to SGPT. Zimmerman (1974) discusses the clinical value of the SGOT and SGPT ratio in distinguishing a specific hepatic disorder from other pathological conditions. Their findings for hepatic disorders, myocardial infarction and progressive muscular dystrophy are shown in Figure 5 (Zimmerman 1974, p. 828).

Principles of Lactate Dehydrogenase

Lactate Dehydrogenase (LDH) catalyzes the hydrogen transfer involving the reduction of pyruvate to lactate in the following reaction (Meister 1949, p. 528):

$$\text{Pyruvate} + \text{NADH} + H^+ \underset{\leftarrow}{\overset{\text{LDH}}{\rightarrow}} \text{Lactate} + \text{NAD}^+$$

This reversible reaction, also discussed by Wroblewski and LaDue (1956), favors the production of lactate and NAD^+ in most instances. If LDH is supplied with pyruvate and NADH, the resulting enzyme activity is proportional to the oxidation of the NADH. This reaction, as described by Wacker, Ulmur and Vallee (1956), has a narrow range of pyruvate and NADH for maximal enzyme activity. Because of this, Wacker and his associates (1956, p. 453) proposed the use of the reverse reaction:

$$\text{Lactate} + \text{NAD} \underset{\leftarrow}{\overset{\text{LDH}}{\rightarrow}} \text{Pyruvate} + \text{NADH} + H^+$$

In the presence of a sufficiently alkaline media, with excess quantities of lactate and NAD provided, the reaction favors the reduction of NAD to $NADH_2$ and the formation of pyruvate. LDH activity can then be quantitated by the rate $NADH_2$ is produced.

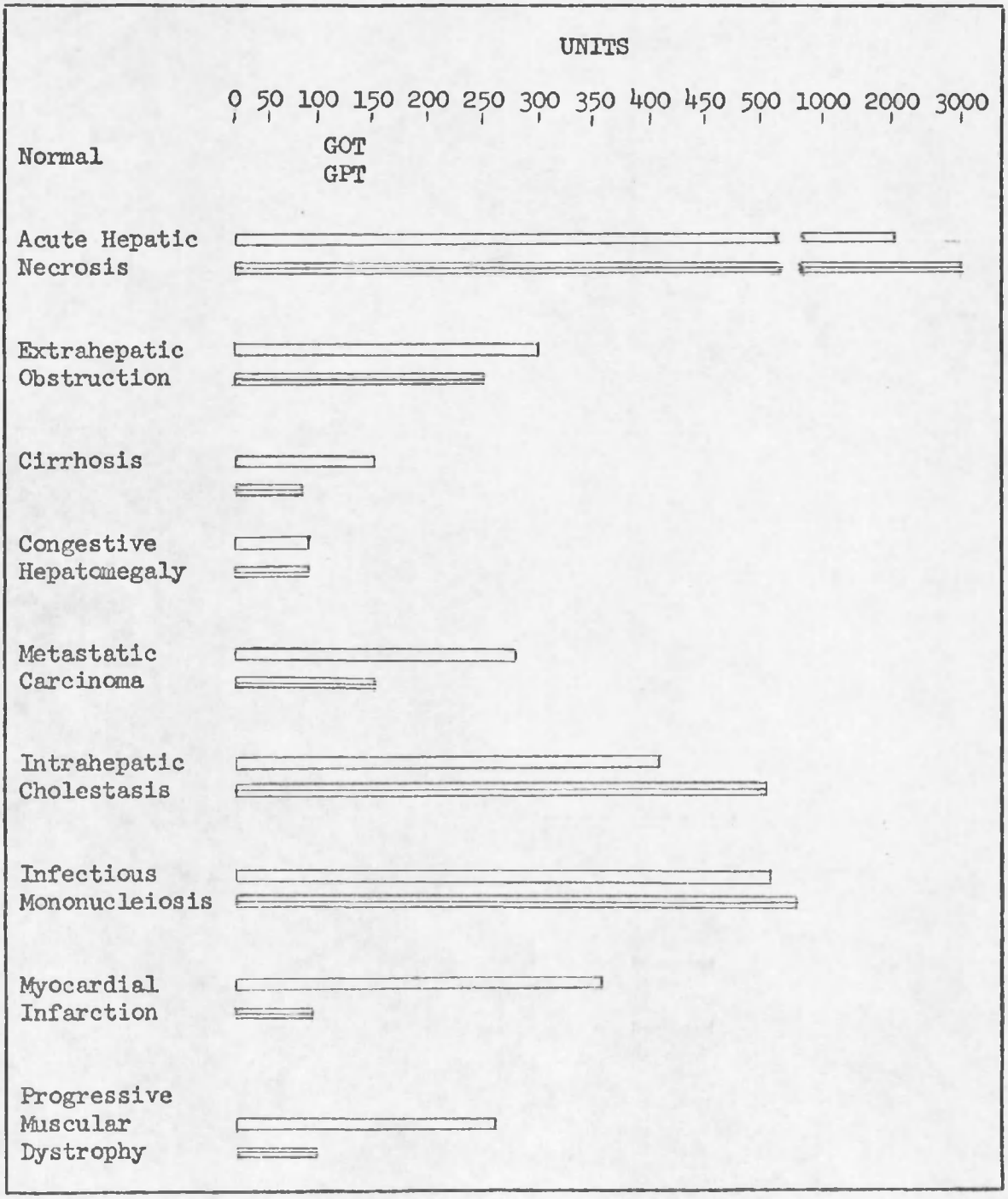

Figure 5. Relative Levels of Glutamic-Oxalacetic Transaminase and Glutamic-Pyruvic Transaminase in Patients with Hepatic, Myocardial and other Diseases. (Zimmerman 1974, p. 828)

LDH in Human Tissues. The distribution of lactate dehydrogenase (LDH) in human tissues was determined by LaDue (1958, p. 308). His data is shown in Table 4. LaDue's results show that kidney and heart have the greatest concentration of LDH while serum has the least concentration of LDH.

LDH in Bovine Tissues. The distribution of lactate dehydrogenase (LDH) in bovine tissues was determined by Cardeilhac and Cardeilhac (1962, p. 294). Their findings are shown in Table 4. These data were taken from a cow with leukemia and do not represent the precise normal level for the species.

Diagnostic Importance of LDH. The clinical significance of LDH began when Wacker and coworkers (1956) discovered that the level of zinc and the activity of zinc enzymes changed markedly after myocardial distress. Their study revealed that an increase in lactate dehydrogenase, a zinc enzyme, was related to a significant decrease in zinc concentration occuring in human serum after myocardial necrosis. Their results were so conclusive that Wacker and associates (1956, p. 449) stated that "these changes occur early in the course of the disease and are so characteristic and marked that they have proved to be valuable adjuvants to the diagnosis of myocardial infarction."

The enzymatic activity that Wacker and coworkers (1956, p. 451) reported for two "typical" patients with acute myocardial infarction are shown in Figure 6. They found that serum lactate dehydrogenase activity in patients with proven myocarditis was elevated 2 to 10 times normal levels. They also found that although the activity values

Table 4. LDH Found in Human and Bovine Tissues*

Tissues	Normal Human Tissue (Units per gram of wet tissue)	Bovine with Leukemia (Units per gram of wet tissue)
Heart	221,600	480,000
Liver	94,700	320,000
Skeletal muscle	160,200	400,000
Kidney	281,800	494,000
Spleen	108,800	102,000
Lung	73,600	-
Pancreas	131,200	-
Serum	470	-

*LaDue (1958, p. 308)

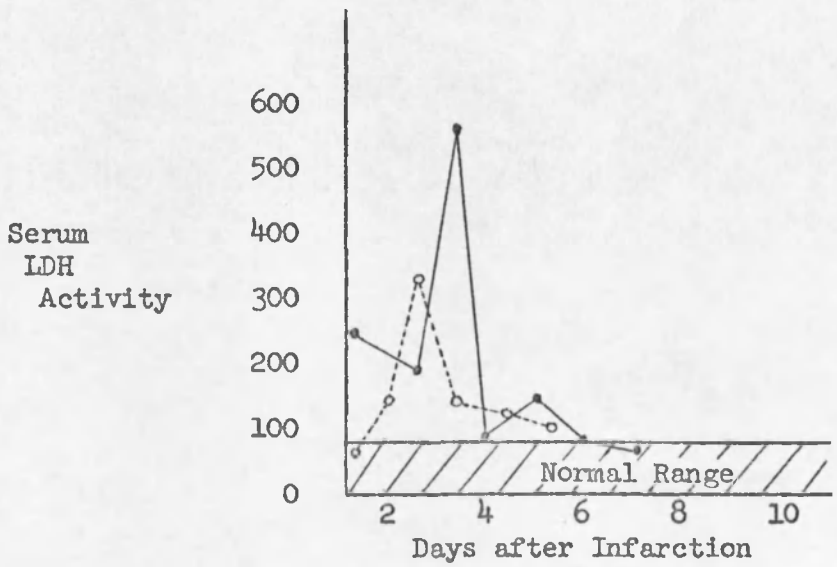

Figure 6. LDH Activity in Two "Typical" Patients after Myocardial Infarction. (Wacker et al. 1956, p. 449)

remain above normal for periods of 1 to 2 weeks, maximal lactate dehydrogenase activity occurred on the second or third day after the infarction.

Research by Ruegsegger et al. (1959) involving graded myocardial infarction in dogs, supports the findings of Wacker and associates (1956). Ruegsegger and coworkers (1959) placed silk sutures around branches of the coronary artery of mongrel dogs. After the enzyme levels due to the surgery had subsided, the sutures were tightened causing pulmonary artery ligation followed by myocardial trauma. Their results revealed that the total LDH levels in the serum increased within 6 to 10 hours after occlusion of the arteries and remained elevated for 3 days. This information and the data collected after autopsy, which compared grams of tissue per infarction to elevation of enzyme activity per infarction, supports tissue leakage as the cause of increased serum enzyme levels.

Principles of Lactate Dehydrogenase Isoenzymes

Lactate dehydrogenase "isoenzymes" as named by Wroblewski, Ross and Gregory (1960) or "isozyme" as named by Markert and Moller (1959) consist of 5 fractions (Vesell and Bearn, 1961). Cohn (1967) discusses the separation of the different molecular forms of the same enzyme and states that separation can be demonstrated immunologically, kinetically, chromatographically and electrophoretically. Cohn also relates that isoenzyme separation is simplified by means of electrophoresis which results in zones of enzymatic activity that can be quantitated by optical means. Elevitch (1973) agrees with Cohn (1967) that electrophoresis is the method of choice for separating isoenzymes,

particularily those of LDH. He also states that rapid technicological developments have slowed the acceptance of isoenzymes as a diagnostic aid.

Elevitch (1973) discusses the principle of the reaction which enables quantitation of the LDH fractions that have been separated into 5 isoenzymes by agarose film electrophoresis. The following reaction, by Elevitch et al. (1966) and shown by Elevitch (1973, p. 229) is the reversal of the reaction reported by Wroblewski and LaDue (1956) and Henry et al. (1960).

$$\text{Lactate} + NAD^{+} \underset{\text{LDH-Iso}}{\rightleftharpoons} \text{Pyruvate} + NADH + H^{+}$$

As described by Elevitch, LDH isoenzymes act as a catalyst between the reaction of lactate and the nonfluorescent NAD^{+} which yields fluorescent NADH. His findings show that the amount of fluorescent NADH produced is proportional to the amount of lactate converted to pyruvate. Because the LDH activity is also proportional to the amount of lactate converted to pyruvate, quantitation of the isoenzyme activity is possible by fluorometric scanning of NADH.

LDH Isoenzymes in Human Tissues. The heterogenic nature of lactic dehydrogenase (LDH) activity in human serum was observed by Vesell and Bearn (1961) and Sayre and Hill (1957). Their original studies indicated that there were only 3 LDH isoenzyme fractions, but with new techniques, the actual number of LDH isoenzymes was found to be 5 (Wroblewski, Ross and Gregory, 1960). Additional research by Vesell and Bearn (1961, p. 587) determined the isoenzyme concentration

in a number of human tissues. Their values, as shown in Table 5, are average percentages of total LDH activity for each tissue indicated.

LDH Isoenzymes in Bovine Tissues. Aside from the heart, many of the LDH isoenzyme levels in bovine tissues have not been reported. Research by Appella and Markert (1961), using beef heart, found that LDH isoenzymes could be separated into 4 very similar polypeptide subunits. Using the nomenclautre proposed by Cahn et al. (1962), these 4 subunits are represented by a combination such as HHMM or HMMM. Appella and Markert also noted that the subunits could be separated into 2 classes on the basis of charge, as indicated by Cahn, et al. as HHHH and MMMM. They also stated that sorting the subunits of the 2 classes into all possible combinations of 4, would result in 5 isoenzymes, which during electrophoresis are separated by charge. They also stated that the combination of 5 isoenzymes of LDH is the number commonly analyzed in several mammals.

Cahn et al. (1962) used H to indicate the subunits of LDH primarily found in the heart tissue and M to indicate the subunit of LDH primarily found in the skeletal muscle tissue. Their report also included the 5 possible LDH isoenzyme combinations discussed by Appella and Markert to be as follows: HHHH, HHHM, HHMM, HMMM, and MMMM. Figure 7 represents data by Cahn and associates (1962, p. 967) involving the separation of LDH isoenzymes from serum for 6 species, 4 of which show 5 LDH isoenzyme combinations.

Diagnostic Importance of LDH Isoenzymes. Vesell and Bearn (1961) along with Sayre and Hill (1957) hypothesized that if each of the

Table 5. Lactate Dehydrogenase Isoenzyme Activity in Human Tissues*

Tissue	Percent total activity				
	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5
Heart	73	24	3		
Kidney	42	45	11	2	
Liver	4	8	27	24	37
Skeletal muscle			5	17	78
Hemolysate	43	44	12	1	
White blood cells	12	49	33	6	

*Vesell and Bearn (1961, p. 587)

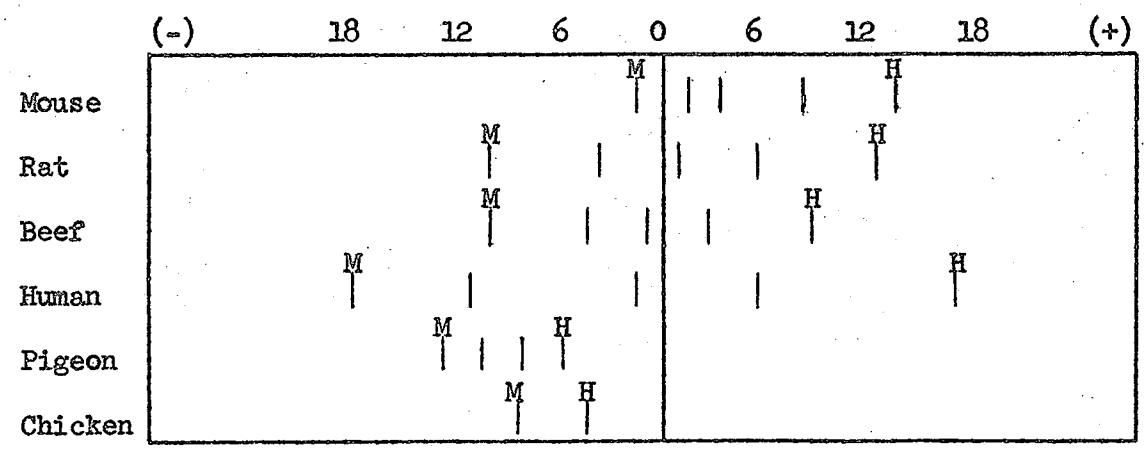

Figure 7. LDH Isoenzyme Electrophoresis for Six Species. (Cahn et al. 1962, p. 967)

electrophoretically demonstrable isoenzymes were derived from different tissues, then disease states involving a single organ would result in elevation of the isoenzyme derived from that tissue. This hypothesis was supported by the analysis of blood serum from patients with myocardial infarction and leukemia by Vesell and Bearn (1961). A summary by Zimmerman and Henry (1974, p. 855) of the diagnostic specificity of LDH isoenzymes under both American and European nomenclature is shown in Table 6. As is indicated by the "+" under both isoenzymes LDH_5 and LDH_4 (American nomenclature) or LDH_1 and LDH_2 (European nomenclature), a myocardial infarction results in a moderate elevation of the 2 isoenzymes in the serum of the patient.

Principles of Creatine Phosphokinase

Creatine phosphokinase (CPK), also known as creatine kinase (CK), adenosine triphosphate (ATP)-creatine phosphorase and ATP-creatine phosphotransferase, has been determined by several methods. Cho, Haslett and Jenden (1960) measured CPK activity by means of a titrimetric method which quantitated the hydrogen ion liberation rate in the conversion of adenosine triphosphate (ATP) to adenosine diphosphate (ADP). Hughes (1962) estimated CPK activity using colorimetric procedures which involved the determination of color intensity after a reaction involving creatine. Sax and Moore (1965) developed a method for determining CPK activity by fluorometric procedures involving a reaction between creatine phosphate and ninhydrin to form a fluophor which has fluorescense. Both the colorimetric and fluorometric methods measure the quantity of creatine and creatine phosphate formed per unit of time. Oliver (1955) proposed a spectrophotometric method of

Table 6. Nomenclature of LDH Isoenzymes with Corresponding Increases for Various Diseases*

Nomenclature					
American	LDH_5	LDH_4	LDH_3	LDH_2	LDH_1
European	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
Myocardial infarction	X	X			
Pulmonary infarction**				X	X
Congestive heart failure				X	X
Viral hepatitis				X	X
Toxic hepatitis				X	X
Cirrhosis				X	X
Leukemia granulocytic		X	X		
Pancreatitis		X	X		
Carcinomatosis (extensive)		X	X		
Megaloblastic anemia	X	X			
Hemolytic anemia	X	X			
Muscular dystrophy	X	X			

*Zimmerman and Henry (1974, p. 855)
**In pulmonary infarction, LDH_3 may be elevated

measuring oxidized nicotinamide adenine dinucleotide phosphate ($NADP^+$) linked by a reaction to CPK concentration. A similar spectrophotometric procedure proposed by Tanzer and Gilvarg (1959), involved measuring the reduced nicotinamide adenine dinucleotide (NADH) which is also linked by a reaction to the CPK concentration.

A need for improved sensitivity in the spectrophotometric measurement of CPK led Hess, Murdock and Natho (1968) to develop a new procedure. Their procedure, which follows, is based on the technique proposed by Oliver (1955) and is a result of the modification of the work reported by Nielson and Ludvigsen (1963) and Fleisher, McConakey and Pankow (1965).

The method reported by Hess, Murdock and Natho (1968) involves the use of CPK to initiate the stepwise reaction. The following reactions shown by Hess et al. (1968, p. 89) results in a spectrophotometric analysis of the CPK activity.

$$\text{ADP + creatine phosphate} \xrightleftharpoons{\text{CPK}} \text{ATP + Creatine} \quad R(1)$$

$$\text{ATP + glucose} \xrightleftharpoons{\text{hexokinase}} \text{ADP + G-6-P} \quad R(2)$$

$$\text{G-6-P + NADP} \xrightleftharpoons{\text{G-6-PDH}} \text{6-phosphogluconate + } NADPH_2 \quad R(3)$$

Where ATP = adenosine triphosphate, ADP = adenosine diphosphate, G-6-P = glucose 6-phosphate, G-6-PDH = glucose 6-phosphate dehydrogenase, NADP = oxidized nicotinamide adenine dinucleotide phosphate and NADPH = reduced nicotinamide adenine dinucleotide phosphate.

The NADPH that is produced to terminate the reaction has an absorbancy at 340 nanometers, as reported by Hess et al. (1968), which

can be linked to the catalyzing activity of CPK for quantitation. This is possible only because the ATP (R1) formed is proportional to the CPK present and the enzymes, hexokinase and G-6-PDH, follow in a like manner to form G-6-P (R2) and $NADPH_2$ (R3) respectively.

CPK in Human Tissues. In a report by Hess et al. (1964), that was aimed at defining more clearly the clinical applications and limitations of serum CPK activity, the human tissue CPK activity levels were determined. The values, which are indicated in Table 7 from Hess et al. (1964, p. 1023), show that skeletal muscle, heart and cerebral cortex contain the greatest concentration of CPK activity. A similar report by Dawson and Fine (1967) also includes human tissue CPK activity levels that support the findings of Hess et al. (1964).

CPK in Canine Tissues. Because of the relatively recent development of CPK determination methods, the concentration of CPK activity in bovine tissues has not been reported. The report by Hess et al. (1964) does include a determination for CPK activity in tissues of the canine species. From their data in Table 7, it is possible to see that human and canine tissue levels for CPK activity are the highest in skeletal muscle and heart, followed by brain and smooth muscle. It can be noted that both species have negligible amounts of CPK activity in the liver, kidney, lung, etc. A similarity between species in relation to the corresponding tissue levels, is supported by these data and the previously discussed correlations between bovine and human tissues. Upon analysis, bovine tissues would presumably contain similar concentrations of CPK activity as compared to canine and human tissues.

Table 7. A Comparison Between Human and Canine Tissue CPK Activity*

Tissues	Mean Activity per Milligram of Soluble Protein ($\times 10^{-4}$ International Units)	
	Human	Canine
Skeletal muscle	18,405	30,443
Heart	5,543	13,344
Brain	3,394	3,514
Uterus	759	-
Gallbladder	555	763
Pylorus	192	-
Lung	0.5	0
Spleen	-	263
Kidney	-	0.16
Liver	-	0.03

*Hess et al. (1964, p. 1023)

Diagnostic Importance of CPK. Serum CPK was introduced as a specific diagnostic aid by Ebaski et al. (1959) for progressive muscular dystrophy patients. A second report from the same group, Okinaka et al. (1961), supported the earlier findings in addition to stating the importance of CPK in diagnosing neuromuscular diseases. The first indication that CPK activity was elevated as a result of myocardial infarction and skeletal muscle disease came from Hess and MacDonald (1963). Their findings were supported by a second report by Hess et al. (1964). They found that serum CPK activity, 8 hours after the onset of chest pain, related to myocardial distress. The maximal serum CPK activity occurred at approximately 36 hours and then rapidly decreased to a nearly normal level at 72 hours after the onset of pain. This pattern of serum CPK activity following myocarditis, is shown in Figure 8 from Hess et al. (1964, p. 1019). A summary by Zimmerman and Henry (1974, p. 860) of the diagnostic significance of serum CPK following myocardial infarction supports the early studies by Hess and associates.

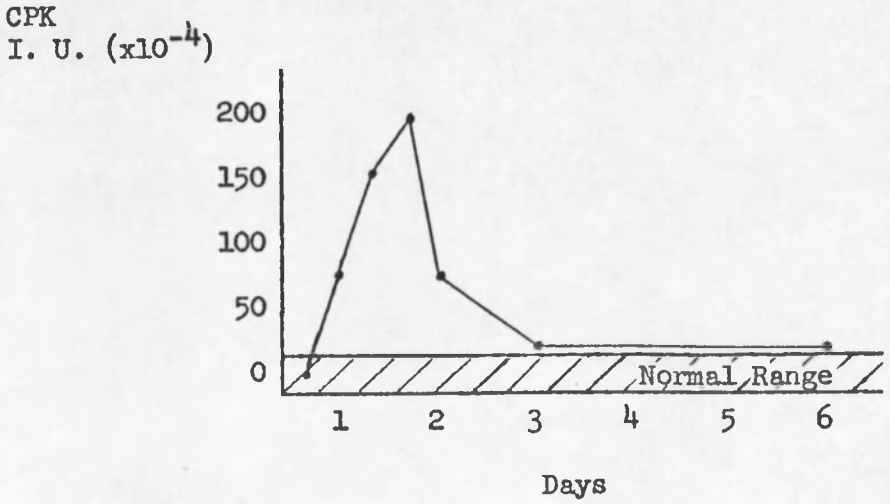

Figure 8. Serum CPK Determinations Following Acute Myocardial Infarction. (Hess et al. 1964, p. 1019)

Principles of CPK Isoenzymes

CPK isoenzymes were first separated by Sjövall and Voight (1964). Methods of separation included starch gel electrophoresis as discussed by Smithies (1959) and agar electrophoresis according to Wieme (1959). Their results indicated that CPK from extracts of human muscle and brain could be separated into 4 bands. Rosalki (1965) used cellulose acetate membranes as discussed by Cohn (1967) to separate CPK from the human heart into 2 distinct bands. In 1966, Van der Veen and Willebrands used agar gel electrophoresis (Wieme 1959) and applied nitroblue tetrozolium (NBT) for a colorimetric determination of the existence of 3 CPK isoenzymes in extracts of human and rat tissues.

One of the latest developments in the separation and measuring of the isoenzymes of CPK is the application of electrophoresis on agarose gel and scanning by fluorometric procedures. The technique, as proposed by Elevitch and Brownlaw in Elevitch (1973, p. 244) is a combination of the LDH isoenzyme fluorometric procedure (Elevitch 1973) and the application of the CPK spectrophotometric procedure (Hess, Murdock and Natho 1968). The reaction for CPK isoenzyme separation is as follows:

$$\text{Creatine phosphate} + \text{ADP} \xrightleftharpoons[MgCl_2]{\text{CPK-Iso}} \text{creatine} + \text{ATP} \quad (R_1)$$

$$\text{ATP} + \text{glucose} \xrightleftharpoons{\text{Hexokinase}} \text{G-6-P} + \text{ADP} \quad (R_2)$$

$$\text{G-6-P} + \text{NAD} \xrightleftharpoons{\text{G-6-PDH}} \text{NADH} + \text{6-phosphogluconate} \quad (R_3)$$

Where ATP = adenosine triphosphate, ADP = adenosine diphosphate, G-6-P = glucose 6-phosphate, G-6-PDH = glucose 6-phosphate dehydrogenase, NAD^+ =

oxidized nicotinamide adenine dinucleotide and NADH = reduced nicotinamide adenine dinucleotide. In this reaction, the substitution of NAD for NADP in R_3 is made because the activity of NAD has a lower degree of nonspecific activity and a higher degree of fluorescence and linearity than NADP.

CPK Isoenzymes in Human Tissues. The creatine phosphokinase isoenzymes in human tissues has been reported by Dawson and Fine (1967, p. 176). Their findings include a comparison of the tissues from an infant to those of an adult (Table 8).

CPK Isoenzymes in Various Mammalian Tissues. CPK isoenzymes (CPK_1, CPK_2 and CPK_3) have been reported by Van der Veen and Willebrands (1966). Their report indicates a strong similarity between species in the distribution and activity of CPK isoenzymes in specific organs. The data represented in Table 9 from Van der Veen and Willebrands (1966, p. 314) shows the distribution of CPK isoenzymes in the rat. Additional information, not represented in the table, but stated by Van der Veen and Willebrands as "extracts of dog and human muscles show the same CPK isoenzyme pattern as rat muscle extract except that human muscle contains a very small amount of fraction II."

A comparison of Tables 8 and 9 show that CPK isoenzymes in the human heart (CPK_2 and CPK_3) are the same 2 isoenzymes found in greatest abundance in the rat heart. Based on these data, the analysis of normal bovine tissues would presumably reveal similar concentrations of CPK isoenzyme activity.

Table 8. CPK Isoenzyme Distribution in Humans*

Tissues	CPK_1	CPK_2	CPK_3
Muscle, Adult	-	++	++
Heart, Adult	-	++	++
Muscle, Infant	-	++	++
Brain, Adult	++++	-	-
Bladder	++++	-	-
Thyroid	++++	-	-
Kidney	++++	-	-

*Dawson and Fine (1967, p. 176)

Table 9. CPK Isoenzyme Distribution in Rats*

Tissues	CPK_1	CPK_2	CPK_3
Heart muscle	±	++	++
Skeletal muscle	-	-	++++
Brain	++++	-	±
Liver	+	-	-
Kidney	+	-	-
Lung	+	-	-
Spleen	+	-	-
Red blood cells	±	-	-

*Van der Veen and Willebrands (1966, p. 314)

Diagnostic Importance of CPK Isoenzymes. The report by Van der Veen and Willebrands (1966) was the first to include evidence that CPK isoenzyme patterns were altered as a result of myocardial infarction. Their findings revealed that CPK_2 and CPK_3 increased as a result of myocarditis in humans. Konttinen and Somer (1972) report measuring the CPK isoenzymes in 21 patients with uncomplicated myocarditis. Their findings, like those of Van der Veen and Willebrands, revealed that the total serum CPK elevation after myocardial infarction is the result of increases in CPK_2 and CPK_3. Some added information from the report by Konttinen and Somer shows that even though both isoenzymes were above the normal level, the increase in CPK_3 was greater than that of CPK_2.

Blood Cell Studies

A report by Davidsohn and Nelson (1974) summarizes the employment of hematologic examination as being an integral element in the clinical description of practically every disease. Some of the tests used in hematology are packed cell volume (PCV), cyanmethemoglobin, total white blood cell (WBC) counts and differential white blood cell counts.

Principles of PCV and Cyanmethemoglobin

Davidsohn and Nelson (1974, p. 115) refer to packed cell volume (PCV) as hematocrit and define it as "the volume of erythrocytes expressed as a percentage of the volume of whole blood in a sample." A colorimetric method of measuring oxyhemoglobin, known as cyanmethemoglobin, is reported by Davidsohn and Nelson. The hemoglobin is converted

to a derivative and then quantitated by both transmittance and optical density.

Diagnostic Importance of PCV and Cyanmethemoglobin

The clinical importance of PCV and cyanmethemoglobin are discussed by Robbins (1974). These procedures are primarily used for diagnosing a loss of red blood cells, which is termed anemia.

Robbins (1974) reports the symptoms resulting from the loss or destruction of red blood cells to include weakness, malaise and easy fatiguability. Because RBC's and hemoglobin are related, it has been noted that dyspnea commonly follows mild exertion in anemia, due to the decreased oxygen content of the circulating blood.

Principles of White Blood Cells and Differential White Blood Cells

Total white blood counts (WBC), also referred to as a total leukocyte count, was reported by Davidsohn and Nelson (1974). The total number of WBC's are calculated per cubic millimeter of blood.

Differential leukocyte counting, by means of blood films, was discussed by Seiverd (1964). The WBC's are counted and divided into basophils, eosinophils, nautrophils, lymphocytes and monocytes.

Diagnostic Importance of Total WBC and Differential WBC Counts

The clinical importance of WBC's was discussed by Davidsohn and Nelson (1974). The study of leukocytes is undertaken, primarily to help establish a diagnosis, although they may also be used to study the course of a disease.

Leukocytosis refers to an increase in the total WBC count. An increase in each of the leukocytes is termed basophilia, eosinophilia, neutrophilia, lymphocytosis and monocytosis.

Basophilia is seen most frequently in allergic reactions, chronic granulocytic leukemia and myeloid metaplasia. Basophilia may be present in chronic hemolytic anemia and appears with eosinophilia in some infections.

Eosinophila is seen in allergic diseases, skin disorders, blood diseases, infectious diseases and parasitic infestations. Davidson and Nelson note that eosinophilia is more pronounced if tissues are invaded by the parasites, as in trichomoniasis. They also report that increased eosinophil counts have been demonstrated in relation to Taenia saginata cysticercosis.

Neutrophilia (an elevated neutrophil count) is primarily influenced by the rate of cell formation and release from the bone marrow versus the rate of neutrophil destruction. Physiologically, the cells increase in number as the result of conditions that do not involve severe tissue damage. These conditions include exercise, hypoxia and stress.

Lymphocytosis occurs in relation to a decrease in neutrophils. Increased numbers are also present in several infections, but primarily in the later stages of the disease. Some of the disorders where lymphocytosis is evident include examthemas (such as rubella), brucellosis, secondary syphilis and congenital syphilis.

Monocytosis generally occurs during recovery from acute infections, but may appear with subacute bacterial endocarditis. It may also be present in mycotic, rickettsial, protozoal and viral infections.

Discussion

The clinical importance of serum enzymes, SGPT, SGOT, LDH, LDH isoenzymes, CPK and CPK isoenzymes, has been discussed in relation to the diagnosis of myocardial distress in mammals. It is with this knowledge that the following research project, involving the analysis of the effects of Taenia saginata cysticercis on bovine myocardial tissue by the determination of enzymatic activities and blood cell studies, has been undertaken.

METHODS AND MATERIALS

This problem involves the daily analysis of levels of serum glutamic-pyruvic transaminase (GPT), glutamic-oxalacetic transaminase (GOT), lactate dehydrogenase (LDH), lactate dehydrogenase isoenzymes, creatine phosphokinase (CPK) and creatine phosphokinase isoenzymes, in calves in association with Taenia saginata cysticercosis. Daily blood cell studies, including packed cell volume (PCV), cyanmethemoglobin, total white blood cell counts (WBC) and differential white blood cell counts, were also undertaken to further ascertain the effect of the infection on the experimental animals.

The experiment began on January 3, 1974 and continued for 38 days. Bleeding of each animal, followed by completion of all analyses (GPT, GOT, PCV, WBC, etc.) within 10 hours after the sampling. Infection occurred on January 11, 1974 providing the study with baseline values over an 8 day period. The study was terminated on February 9, 1974, 30 days after infection. Termination of the experiment also marked the end of 38 days of "normal" values from the control animals.

Research Animals

Number, Age and Sex

Four male and 4 female Holstein calves, ranging in age from 6 to 7 months, were used to make up 2 groups. The males were used as experimental animals while the females served as controls.

Confinement Facilities

The experimental animals were maintained in separate pens at the Veterinary Science Isolation Facility located at the University of Arizona's Campbell Avenue Farm. The control animals were maintained in a single confinement at the University of Arizona's Dairy Farm. Environmental temperature could not be controlled. The daily highs and lows throughout the research period are reported in Appendix B.

Nutrition

Both groups of animals received daily rations of dairy calf starter, purchased from Arizona Feeds, which contained 18.75 milligrams of chlortetracycline per pound. In addition, the female control group received a ration of alfalfa hay daily. Both groups were also supplied with mineral blocks and water *ad libitum*.

Equipment and Handling

Three weeks prior to the experimental period, each calf was supplied with a halter which remained in place until the research terminated. During this conditioning period, with the use of a lead rope and halter, each animal was acclimated daily to the equipment used in the research. This equipment included a squeeze chute for males and a stanchion for females to confine animals while procuring blood samples.

Infection Procedures

Artificial Hatching

Prior to infection of the experimental animals, a percentage of the *Taenia saginata* eggs to be used was determined to be mature. The

procedure employed to evaluate the viability of the eggs involved the artificial hatching process as follows:

1. An aliquot of eggs was washed by placing them in a conical centrifuge tube containing normal saline. After centrifuging for 3 minutes at 1000 revolutions per minute, the saline was decanted.

2. Fresh normal saline was used to suspend the eggs, to which 15 milliliters of 1 percent acid pepsin solution (1 milliliter concentration HCl and 1 gram of pepsin in 100 milliliters of distilled water) was added.

3. After covering tightly, this suspension was placed in a 39 to 42 degree Centigrade water bath for approximately 2 hours. Frequent shaking during this period assisted in unsheathing the ova, as observed by microscopic examination.

4. After incubation and shaking, the eggs were centrifuged at 1000 revolutions per minute for 2 minutes, allowing the acid pepsin to be removed.

5. A 10 milliliter sample of artificial intestinal juice, made from the following chemicals, is thoroughly mixed with the eggs.

Pancreatin	500 milligrams
Cholesterol	10 milligrams
Sodium taurocholate	500 milligrams
Trypsin	20 milligrams
Sodium bicarbonate	500 milligrams
Distilled water	50 milliliters

6. Five milliliters of fresh bovine bile was added to the artificial intestinal juice to increase the efficiency of the hatching.

7. After covering tightly, the eggs were incubated at 39 to 42 degrees Centigrade, with frequent shaking, until motility of the embryo determined viability.

Innoculation

Two intraruminal innoculations of Taenia saginata eggs were given, injecting 1/2 of the eggs each time. The first innoculation occurred immediately after feeding. The second innoculation occurred 4 hours later when the animals had emptied their rumen and prior to the next feeding. A 16 gauge 4 inch needle was used in giving a total of 750,000 Taenia saginata eggs to each of the 4 experimental animals.

Sampling Procedures

Bleeding

Female controls were bled first, with the use of a stanchion, followed by bleeding of male experimental animals, in a squeeze chute. Blood samples were taken from the jugular veins, alternating the site of puncture from high to low and right to left sides of the neck. Collections were made using both 10 and 4 milliliter vacutainers by means of a 20 gauge 1 inch vacutainer needle.

The 10 milliliter vacutainers, containing the collected plasma and no chemicals, were allowed to coagulate before they were centrifuged at 1800 revolutions per minute for 20 minutes. The separated serum was drawn off using Pasteur pipettes and maintained at 8 degrees Centigrade until used. These serum samples were used solely for enzyme analysis where stability only becomes a factor after 24 hours.

The 4 milliliter vacutainers, containing 0.04 milliliters of 15 percent EDTA, as an anticoagulant and 0.008 milligrams of potassium sorbate as an antimycoltic agent, plus the collected plasma, were used only for the blood cell studies. These include packed cell volume (PCV), cyanmethemoglobin, total white blood cell counts and differential white blood cell counts where stability is normally not a factor when completed within 2 hours of procurement.

Enzyme Studies

Substrates used were Pfizer Nzyme reagent kits for ultraviolet (UV) kinetic determination of serum enzymes, glutamic-pyruvic transaminase (GPT), glutamic-oxalacetic transaminase (GOT), lactate dehydrogenase (LDH), and creatine phosphokinase (CPK). All changes in optical density for each enzyme were measured in 1 centimeter quartz cuvettes by a Zeiss M4A III spectrophotometer at 340 nanometers. This equipment was not equipped to maintain a constant temperature in the reaction chamber. As a substitution step, a circulating water bath was instituted in which temperatures to the nearest 0.1 degree Centigrade were taken immediately prior to density readings. Five readings from the spectrophotometer were taken at 30 second intervals against a blank which enabled readings to be taken on the linear part of the indicator scale. Calculations of GPT, GOT, LDH and CPK activity were made using a 1 minute period where the optical density readings were constant.

Pipetting equipment used in reconstitution of reagents and spectrophotometric procedures included glass serological pipettes and Eppendorf automatic pipettes. Serological pipettes were used primarily

for the measurement of substrate fractions while the Eppendorf pipettes were basically used for serum allocation.

Serum GPT and GOT

Pfizer (1973c) credits the glutamic-pyruvic transaminase reaction to Wroblewski and LaDue (1956); in their 1973b Bulletin, Karmen, Wroblewski and LaDue (1955) are noted for the glutamic-oxalacetic transaminase reaction. These principles are utilized by Pfizer in preparation of Pfizer Nzyme Substrates for use with a spectrophotometer.

Pfizer Nzyme GPT and GOT substrates were reconstituted by adding 15 milliliters of deionized water to each 5 determination reagent vial. The serological test procedures are identical for both GPT and GOT and were completed as follows:

1. After sufficient time for both substrate and cuvette to adjust to water bath temperature, 2.5 milliliters of substrate and 0.2 milliliters of serum were placed in the acclimated cuvette and mixed by gentle inversion.

2. The cuvette was immediately returned to the water bath for approximately 1 minute incubation time.

3. Following incubation, the outside of the cuvette was dried before placing it in the spectrophotometer cell holder and readings taken. The temperature of the water bath was recorded immediately following removal of the cuvette from the bath.

Serum LDH

Pfizer (1973d) employed the use of lactate dehydrogenase reactions proposed by Meister (1949) and Wacker, Ulmer and Vallee (1956)

in establishing Nzyme Reagent Procedures for LDH quantitation by spectrophotometric methods. Pfizer Nzyme LDH substrate was reconstituted by adding 15 milliliters of deionized water to each 5 determination reagent vial. The serological test procedure for determining LDH activity was as follows:

1. After adequate time for both substrate and cuvette to adjust to water bath temperature, 0.05 milliliters of serum was added to the acclimated cuvette which contained 2.65 milliliters of substrate.

2. After mixing by gentle inversion and placing in a cell holder, optical density readings were immediately taken. The temperature of the water bath was recorded to the nearest 0.1 degree Centigrade, immediately following removal of the cuvette from the bath.

Absorbance changes of LDH that are greater than 0.100 per minute at 340 nanometers in a spectrophotometer indicate high activity and can result in non-linear readings. When this occurs, as in bovine with its high LDH concentration, serum sample volume is decreased. Normally, 2.5 milliliters of substrate and 0.2 milliliters of serum results in accurate readings, but if this ratio is too high, a mixture of 2.65 milliliters of substrate and 0.05 milliliters of serum can be used. The final results are multiplied by a factor of 4.

Serum CPK

To obtain spectrophotometric analysis of CPK, Pfizer (1973a) applied the principles stated by Oliver (1955) and modified by Hess, Murdock and Natho (1968). Pfizer Nzyme CPK substrate was reconstituted by adding 15 milliliters of deionized water to each 5 determination

reagent vial. The serological test procedure for determining CPK activity was as follows:

1. After sufficient time for both substrate and cuvette to adjust to water bath temperature, 2.5 milliliters of substrate and 0.1 milliliters of serum were placed in the acclimated cuvette and mixed by gentle inversion.

2. The cuvette was then returned to the water bath for approximately 5 minutes incubation time.

3. Immediately after incubation, the outside of the cuvette was dried, followed by placing the cuvette in the cell holder of the spectrophotometer where readings were taken. The temperature of the bath was also recorded following removal of the cuvette from the water.

Control Methods Used in Enzyme Determination

Reconstituted normal and abnormal Metrix Clinical Chemistry Control Serums were used daily to check enzyme analysis procedures. This was completed prior to quantitation of each spectrophotometrically determined enzyme.

Isoenzymes

Analytic Chemists, Inc. (ACI) fluorometric reagent kits and agarose film were used in the determination of serum lactate dehydrogenase isoenzymes and serum creatine phosphokinase isoenzymes. The electrophoresis cell was of double chambered construction made of plastic with metallic electrodes that served as anode (+) and cathode (-). This unit was charged with the use of a Heathkit power supply

equipped with both variable voltage and amperage. All fluorometric scanning was done using a Turner fluorometer.

LDH Isoenzymes. Analytical Chemists, Inc. (1972b) attributed the method of lactate dehydrogenase isoenzyme separation, by electrophoresis, and quantitation, by fluorometric procedures, to Elevitch et al. (1966). The procedure for the separation of LDH isoenzymes by electrophoresis was as follows:

1. Using a Hamilton syringe and disposable tip, 1 microliter (μl) of each of the 8 serum samples was placed respectively in the 8 wells of a preformed ACI Agarose Universal electrophoresis film.

2. The film was electrophoresed at 120 volts, 12 to 16 milliamperes for 1 hour and 45 minutes using Bechman's Buffer B-2 at pH 8.8. This buffer contained 0.35 percent EDTA.

3. A 5 milliliter serological pipette was used to disperse the ACI reagent evenly over the film. This was accomplished by dispensing the reagent along the anode (+) edge of the film in front of a pipette placed horizontally on the film. The pipette was rolled to the cathode (-) edge in a single smooth motion.

4. The film was incubated in a humid chamber for 20 minutes at 38 degrees Centigrade followed by drying for 20 minutes in a hot air oven at 65 degrees Centigrade.

5. After drying, the film was cut into 8 strips, each containing the LDH isoenzymes from a single sample. These were immediately taped on the turning drum of the fluorometer and scanned.

CPK Isoenzymes. Elevitch and Brownlow (published in Elevitch 1973) developed the enzyme catalyzed reactions used by Analytical Chemists, Inc. (1972a) to report the following electrophoretic separation and fluorometric quantitation of creatine phosphokinase isoenzymes. Some aspects of the procedure were identical to those of LDH isoenzyme determination

1. Using a Hamilton syringe and disposable tip, 1 microliter (µl) of each 8 serum samples was placed respectively in the 8 wells of a preformed ACI Agarose Special Purpose electrophoresis film.

2. The film was electrophoresed at 90 volts, 4 to 7 milliamperes for 42 minutes at pH 8.8 using Bechman's Buffer B-2 without EDTA.

Steps 3 through 5 for CPK isoenzyme electrophoresis and scanning are identical to those listed under LDH isoenzymes.

Calculations

The calculation of GPT, GOT, LDH and CPK activity required the use of the following equation:

$$\frac{\Delta A/\text{minute} \times \text{assay volume} \times 1000}{\text{extinction coefficient} \times \text{lightpath (cm)} \times \text{sample volume}} = \text{mU/ml sample}$$

ΔA equals the change in absorbance, extinction coefficient of NADH equals 6.22 cm^2/ mole at 340 nanometers, lightpath equals 1 centimeter and 1 milliunit (mU) equals 0.001 international units (U). Because water bath temperatures varied, calculation of GPT, GOT, LDH and CPK activity at a constant temperature of 25 degrees Centigrade required

the multiplication of the equation results by the factors for the recorded temperature. These factors for Nyzme GPT, GOT, LDH and CPK are shown in Tables 10 and 11.

The calculation procedure used for LDH isoenzymes and CPK isoenzymes was developed during the course of this project. This procedure (Appendix A) enables the use of a map measurer scaled in centimeters (cm).

Blood Studies

PCV

The procedure used in determining the packed cell volume is as follows:

1. Unheparinized capillary hematocrit tubes were filled more than half way with mixed whole blood.

2. The end used in filling was then sealed with clay before placing the capillary tubes in a microcentrifuge.

3. After centrifuging for 5 minutes, the PCV was determined by a hematocrit scale.

Cyanmethemoglobin

Hycel reagents were used to determine the oxyhemoglobin percentage for each sample, by cyanmethemoglobin procedures. The steps employed in colorimetric determination of cyanmethemoglobin are as follows:

1. A solution, known as Drabkin's Diluent, was made containing the following chemicals, which were dissolved in 1000 milliliters of distilled water.

Table 10. Temperature Conversions for GPT and GOT*

Temperature	Factor for GPT	Factor for GOT
25° C	1.00	1.00
26° C	.93	.93
27° C	.86	.86
28° C	.80	.80
29° C	.74	.75
30° C	.69	.70
31° C	.65	.66
32° C	.60	.61
33° C	.57	.58
34° C	.53	.54
35° C	.50	.51
36° C	.47	.48
37° C	.44	.45

*Pfizer, Inc. 1973c and 1973b respectively

Table 11. Temperature Conversions for LDH and CPK*

Temperature	Factor for LDH	Factor for CPK
25° C	1.00	1.00
26° C	.90	.94
27° C	.83	.88
28° C	.76	.82
29° C	.70	.77
30° C	.65	.72
31° C	.61	.67
32° C	.57	.62
33° C	.54	.57
34° C	.51	.53
35° C	.48	.49
36° C	.45	.45
37° C	.42	.42

*Pfizer, Inc. 1973d and 1973a respectively

Sodium bicarbonate	1.0 grams
Potassium cyanide	0.05 grams
Potassium ferricyanide	0.20 grams

2. Using a hemoglobin pipette, 20 cubic millimeters (cmm) of whole plasma was added to a test tube containing 5 milliliters of Drabkin's Diluent.

3. After a 10 minute incubation period, the optical density was read at 540 nanometers with a spectrophotometer, using a blank containing only Drabkin's Diluent.

4. The following formula was used to calculate the gram percent hemoglobin.

Hemoglobin OD x K = Gm % Hemoglobin

OD = optical density, K = 36.796 and Gm = grams.

Total WBC

White blood cell counts were completed with the use of hemocytometers. This process was as follows:

1. A 1 to 20 dilution of whole plasma to solution was made with the use of a white blood cell pipette. The solution used in diluting the plasma was made as follows:

Glacial acetic acid	2 milliliters
1% aqueous Gentian violet	1 milliliter
Distilled water	100 milliliters

2. The pipette was rotated to mix the contents for 3 minutes.

3. Several drops of diluting fluid were discharged from the pipette before counting chambers of the hemacytometer were filled.

4. The leukocytes were counted in each of the 4 large corner squares, of the hemocytometer. Each of the large squares was divided into 16 smaller squares.

5. Calculation of the leukocyte count (WBC's per cubic milliliter) was determined by using the following equation:

$$\text{Leukocyte count (cells/cmm)} \quad \frac{cc}{Iso} \times d \times 10$$

Where cc = cells counted, d = the dilution factor, 10 = the factor transforming surface area of square millimeter to the volume in cmm.

WBC Differential Counts

Differential white blood cell counts, by means of blood films, were discussed by Seiverd (1964). This method involves the use of glass microscopic slides on which a blood smear, defined as having a tailing, has been made. The procedure for the differential white cell count follows:

1. A blood smear was made and allowed to dry on a microscopic slide.

2. Wright's stain was applied to the slide for 3 minutes, then diluted with deionized water for 6 minutes.

3. Distilled water is used to rinse excess stain from the slides. The slides were allowed to air dry.

4. Using a microscope with an oil immersion lens, 100 WBC's were counted and divided into basophils, eosinophils, neutrophils, lymphocytes and monocytes.

Cysticercus Counting Procedure

Following termination of the research period, the 4 infected experimental animals were sacrificed. The carcass, head, tongue, diaphragm, hanging tenderloin, esophagus, heart and liver were weighed and refrigerated in a freezer at 0 to 2 degrees Centigrade.

During the following 7 days, all muscular tissue of the 4 carcasses was boned by quarters and weighed. From each quarter, a 15 percent sample was taken in which all cysticerci were counted. This procedure enabled the approximation of the cysticerci per pound of muscle tissue. A complete counting of the cysticerci in the preserved organs terminated the analysis of the infection.

Methods of Data Analysis

Initially, raw data from the experimental and control groups were tabulated in bound record books. With the use of a calculator, all recorded enzyme optical density readings were converted to milliunits (mu) of enzymatic activity. These data, including blood cell studies, were entered on IBM data sheets for key punching.

Analysis of the data was completed in 2 forms. The first graphed all enzyme activity by days, to provide a gross means of examination. The second was an extensive analysis of variance of all data by means of a Nova 45 computer program.

Analysis of variance included 3 different approaches to the data. The first was a comparison of sex difference, by mean values, between the 2 groups over the initial 8 day baseline period. This correlation included a comparison of treatment versus control values over the entire 30 day infection period. The second approach divided the 30 day

experimental period into 3, 10 day intervals in which mean values for each variable were determined. The third approach involved breaking the entire 38 day project into 5, 7 day periods by eliminating day 1, 9 and 38. This provided the project with 5 periods of normal values from the control group and 1 period of baseline values followed by 4 periods of treatment values from the experimental group. Mean values for all variables were determined for each period.

These methods of analysis enabled a comparison of baseline to experimental values, baseline to control values and experimental to control values from the research animals. These procedures allow the presentation of the findings acquired throughout this study.

RESULTS

Number of Cysticerci Found in Bovine Tissues

Data from the calves infected with Taenia saginata eggs revealed that all examined tissues were infected with cysticerci. These findings, as reported in Table 12, are averages obtained from the total number of cysts found in the 4 experimental animals. Other tissues in which Taenia saginata cysticerci were noted, but not counted, included lung, kidney and brain.

Cysticerci in Heart and Liver

The greatest number of cysticerci found in any single tissue were located in the hearts of the infected animals. An example of the physical appearance of Taenia saginata cysticercosis in heart tissue is shown in Figure 9. Other tissues, such as the liver and esophagus, were observed to contain less than 1/10th the number of cysticeri found in the heart. Figure 10 shows the appearance of Taenia saginata cysticercosis in liver tissue. In tissue, cysticerci appear as white ovoid structures.

Enzyme and Blood Cell Mean Values for Male and Female Holstein Calves

Normal Enzyme Values for Males and Females

Normal enzyme values for serum glutamic-pyruvic transaminase (GPT), glutamic-oxalacetic transaminase (GOT), lactate dehydrogenase

Table 12. Average Number of Taenia saginata Cysticerci in Various Tissues

Tissue	Average Meat Weights in Pounds	Average Number of Cysticerci	Average Number of Cysticerci per pound
Heart	3.1	345	111
Hanging tenderloin	0.7	21	30
Diaphragm	1.1	28	26
Tongue	1.1	26	24
Head	2.0	47	23
Liver	6.0	60	10
Esophagus	0.6	6	10
Right Fore Quarter	40.3	398	10
Left Fore Quarter	40.3	392	10
Right Hind Quarter	43.8	360	8
Left Hind Quarter	42.6	313	7
Total Carcass	182	1995	11

Figure 9. Bovine Heart Infected with Taenia saginata Cysticerci

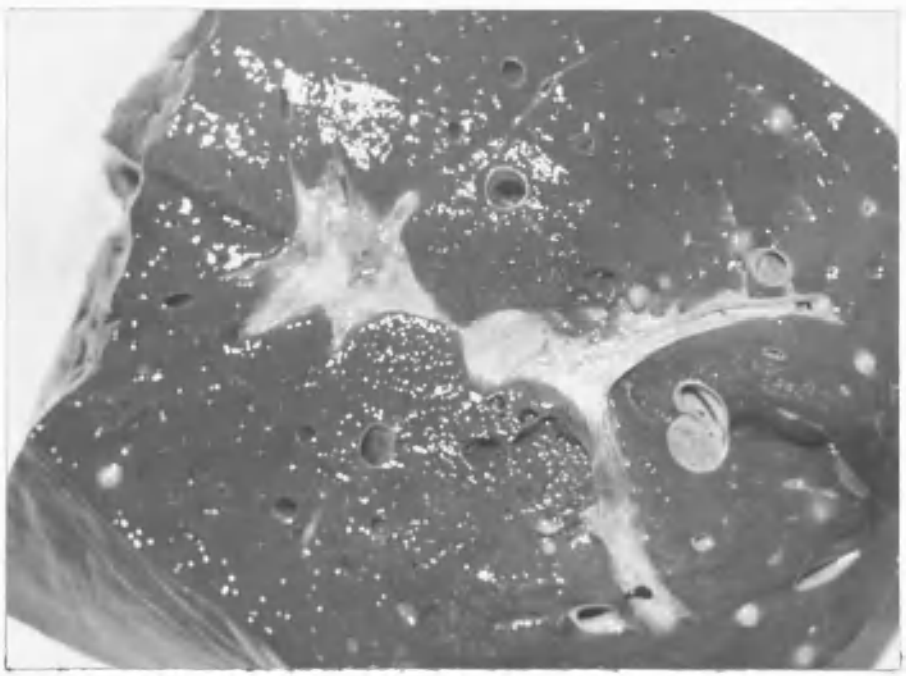

Figure 10. Bovine Liver Infected with Taenia saginata Cysticerci

(LDH) and creatine phosphokinase (CPK) are shown in Table 13. Mean values, expressed in percentages, for the isoenzymes of lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) are reported by European nomenclature in Table 14.

Examples of the separation patterns for LDH isoenzymes and CPK isoenzymes are shown in Figures 11 and 12, respectively. These figures are only "typical" and do not represent the mean values of isoenzyme separation for Holstein calves.

Analysis determined that several enzymes investigated demonstrated a significant difference between sexes. (The significant level is 0.05 unless otherwise indicated). As indicated by the astericks in Tables 13 and 14, these enzymes are GPT, CPK and isoenzymes CPK_2 and CPK_3.

Normal Blood Cell Values for Males and Females

Normal blood cell values for packed cell volume (PCV), cyanmethemoglobin, and total white blood cells (WBC) are shown in Table 15. The mean values for white blood cell differential counts are reported in Table 16.

Analysis determined that packed cell volume (PCV) and cyanmethemoglobin values were significantly different between sexes. These results are marked by astericks in Table 15.

Increased Significance after Infection in Variables Having Sex Significant Differences

Several variables, in both enzyme and blood cell studies, demonstrated sex significant differences. These differences appear to

Table 13. Normal Mean Activity Values (in milliunits) for GPT, GOT, LDH and CPK in Male and Female Holstein Calves

Enzymes	I.U./ml x 10^{-3} Male	Female
GPT	7.812	10.030*
GOT	20.025	22.021
LDH	212.427	221.691
CPK	15.802	24.621*

*indicates significant difference between sexes

Table 14. Normal Mean Activity Percentage Values for LDH Isoenzymes and CPK Isoenzymes in Male and Female Holstein Calves

	Peak number 1	2	3	4	5
Male- LDH isoenzymes	36.2	27.6	16.9	10.0	9.3
Female- LDH isoenzymes	35.2	27.3	17.1	10.5	9.3
Male- CPK isoenzymes	30.1	29.7	40.2	-	-
Female- CPK isoenzymes	27.7	24.8*	47.5*	-	-

*indicates significant difference between sexes

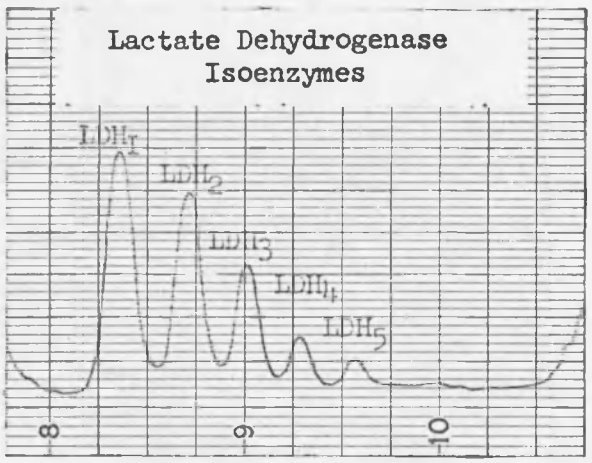

Figure 11. "Typical" LDH Isoenzyme Separation for Bovine

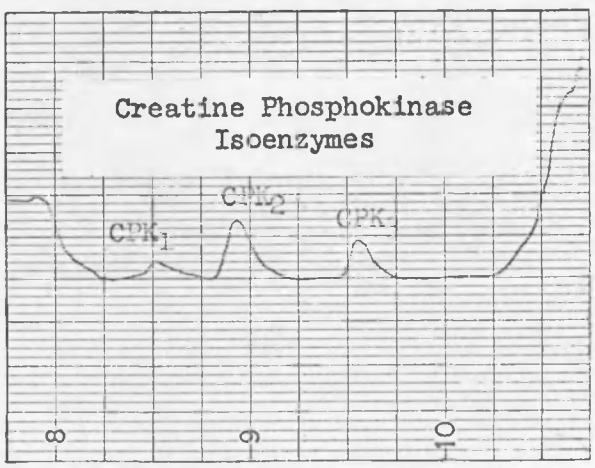

Figure 12. "Typical" CPK Isoenzyme Separation for Bovine

increase after the infection with Taenia saginata eggs. GPT, CPK_2, CPK_3 and cyanmethemoglobin changed in significance from the 0.05 to 0.01 level of probability.

Significant Differences as a Result of Taenia saginata Infection

Analysis of the data obtained from calves infected with Taenia saginata cysticerci revealed significant alterations in both enzyme and blood cell activities. Significant increases in serum enzymes glutamic-oxalacetic transaminase (GOT), lactate dehydrogenase (LDH), LDH isoenzyme 3, LDH isoenzyme 4 and LDH isoenzyme 5 were accompanied by significant increases in eosinophils and monocytes (Table 17).

Discussion of Results

The high rate of Taenia saginata cysticercosis experienced by the infected experimental animals suggests pathological distress as indicated by the results. These alternations, which occurred after the infection, were noted in both enzyme and blood cell studies.

Enzyme Alterations

Increased levels of serum glutamic-oxalacetic transaminase (GOT) occurred over a period of 1 to 7 days and 14 to 21 days following infection with Taenia saginata eggs. The first increase appears to be correlated with the migration and establishment of the hexocanth embryo. A lesser alteration, during the second increase, seems to be interrelated with the advanced growth during the larval stage.

A surge in serum lactate dehydrogenase (LDH) activity during the 7 to 21 day period following infection seems to confirm a correlation

Table 15. Normal Mean Blood Cell Values for PCV, Cyanmethemoglobin and WBC in Male and Female Holstein Calves

Blood Cell Studies	Male	Female
Packed Cell Volume (PCV) (percent of total plasma)	34.0	30.0*
Cyanmethemoglobin (gram % hemoglobin)	12.46	10.65*
White Blood Cells (WBC) (per milliliter)	11846.0	10723.0

*indicates significant difference between sexes

Table 16. Normal Mean Blood Cell Percentage Values for White Blood Cell (WBC) Differential Counts in Male and Female Holstein Calves

White Blood Cells	Male	Female
Basophils	0	0
Eosinophils	3	1
Neutrophils		
Stabs	0	0
Segs	25	23
Lymphocytes	69	73
Monocytes	3	3

Table 17. Significant Alternations as a Result of Taenia saginata Infection

Variables	Baseline	Post-Infection with Taenia saginata eggs			
	Period 1	Period 2	Period 3	Period 4	Period 5
SGOT		XX		X	
LDH			XX	XX	
LDH_3			XX	XXX	XX
LDH_4		XX		XX	
LDH_5				XX	
Eosinophils			XX		XXX
Monocytes				X	XX
		(Infection)			

X = Significance at the 0.10 level
XX = Significance at the 0.05 level
XXX = Significance at the 0.01 level
Period = 7 days

between cysticerci developments and tissue necrosis. This post-infection interval has been reported as the fastest period of growth for the Taenia saginata cysticerci and its associated encapsulation.

A rise in isoenzymes LDH_3, LDH_4 and LDH_5 (European nomenclature) also occurred as a possible result of the infection. These increases were demonstrated at various periods throughout the 1 to 30 day post-innoculation interval.

Increases in LDH isoenzymes are useful in confirming pathological conditions. Reports reveal that increases in LDH isoenzymes 4 and 5 appear in correlation with congestive heart failure, viral and toxic hepatitis as well as liver cirrhosis. It has also been noted that a surge in LDH isoenzymes 3, 4 and 5 are interrelated to pulmonary infarction or embolism. An increase in LDH isoenzymes 1 and 2, which did not occur, would have been confirming evidence for myocardial infarction due to the massive invasion of cardiac tissue by the cysticerci of Taenia saginata.

Blood Cell Alterations

Increased levels for both eosinophils and monocytes were noted during the post-infection period. These conditions, referred to as eosinophilia and monocytosis, appeared to progress as the Taenia saginata cysticerci continued to develop.

It has been reported that eosinophilia occurs as a result of parasitic infestation, blood diseases, infectious diseases and cysticercosis. Monocytosis which generally occurs in acute infections, has also been noted in subacute bacterial endocarditis as well as protozoal and viral infections.

Summary

Glutamic-pyruvic transaminase, glutamic-oxalacetic transaminase, lactate dehydrogenase and its isoenzymes, creatine phosphokinase and its isoenzymes, packed cell volume, cyanmethemoglobin, total white blood cell counts and differential white blood cell counts were employed to determine the effect of Taenia saginata cysticercosis on bovine myocardial and other tissues. These determinations were conducted daily over a 38 day period, which was divided into a 7 day baseline and a 30 day post-infection period.

Blood samples, from 8 Holstein calves, were collected from the jugular vein by means of vacutainer tubes and needles. Serum for enzyme evaluation was obtained by centrifuging the samples at 1800 revolutions per minute for 20 minutes.

Methodology used throughout the research required the use of the following equipment. A Zeiss M4Q III enabled kinetic determination for spectrophotometric enzyme quantitations. An electrophoresis cell and Turner fluorometer were used for isoenzyme separation and fluorometric scanning. Capillary tubes containing whole plasma were centrifuged to determine the packed cell volume. Darbkin's Diluent through colorimetric procedures enabled hemoglobin approximation. Hemocytometers were used to report the total white blood cell count and blood smears, prepared with Wright's stain, enabled differential white blood cell counts.

Data for all variables was first analyzed by graphing procedures for overall examination. Secondly, a Nova 45 computer program was

utilized to calculate mean values through which significant alterations were determined.

Findings appear to indicate significant enzyme increases for glutamic-oxalacetic transaminase, lactate dehydrogenase and lactate dehydrogenase isoenzymes 3, 4 and 5. Results also suggest a significant eosinophilia and monocytosis coinciding with development of Taenia saginata cysticerci.

Suggestions to aid in further study include a larger, heterogeneous group of research animals, longer experimental periods, environmental temperature controls and a more explicit evaluation of the histopathology of various tissues involved with Taenia saginata cysticerci. Institution of α-hydroxybutyric dehydrogenase enzyme analysis by spectrofluorometric procedures might aid in assessing bovine myocardial distress as a result of cysticercosis.

APPENDIX A

CALCULATION OF ISOENZYME FRACTIONS

Due to the uncertainty commonly associated with determining small areas with a planimeter, the following procedure was developed to measure the isoenzyme fractions in this study.

1. Graph paper on which the fluorometer traced isoenzyme fractions was attached to typing paper and Xeroxed.

2. From the Xeroxed copies, transparencies were produced.

3. With the use of an overhead projector, the isoenzyme fractions were projected onto a blackboard, from a constant distance. The enlargement factor was thereby constant, eliminating image distance as a variable.

4. Isoenzyme fractions were traced and then quantitated with a map measurer, scaled in centimeters.

5. Mean values were calculated from the percentages of each isoenzyme.

APPENDIX B

TEMPERATURE VARIATIONS

Date	Research Date	Temperature	
		High	Low
1/3/74	1	57	28
1/4/74	2	56	34
1/5/74	3	58	46
1/6/74	4	62	37
1/7/74	5	58	43
1/8/74	6	62	41
1/9/74	7	52	45
1/10/74	8	58	40
1/11/74	9 Infection	62	28
1/12/74	10	70	28
1/13/74	11	71	30
1/14/74	12	74	30
1/15/74	13	81	30
1/16/74	14	80	34
1/17/74	15	76	36
1/18/74	16	72	35
1/19/74	17	73	33

Date	Research Date	Temperature	
		High	Low
1/20/74	18	76	38
1/21/74	19	63	35
1/22/74	20	63	29
1/23/74	21	59	24
1/24/74	22	62	27
1/25/74	23	66	28
1/26/74	24	68	31
1/27/74	25	56	39
1/28/74	26	61	24
1/29/74	27	67	23
1/30/74	28	74	29
1/31/74	29	74	26
2/1/74	30	73	30
2/2/74	31	71	29
2/3/74	32	68	24
2/4/74	33	75	25
2/5/74	34	75	29
2/6/74	35	62	28
2/7/74	36	56	21
2/8/74	37	56	17
2/9/74	38	70	19

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