LACTATE DEHYDROGENASE ISOENZMES IN THE DOG

by

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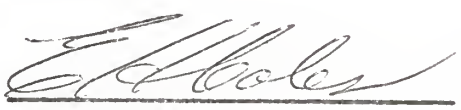

Major Professor

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INTRODUCTION

Determination of serum enzyme activities has been a useful tool in clinical diagnosis for nearly two decades. The knowledge that certain enzymes are present in high concentration in certain tissues or groups of tissues is utilized by determining levels of circulating enzymes in plasma. Changes in serum enzyme activities have generally been regarded as a reflection of damage to the tissues of origin. Actually, a more complete consideration of the subject suggests that serum enzyme levels may be elevated for any or all of several reasons:

1. An increased metabolic requirement may bring about increased biosynthesis of an enzyme. Thus, if intracellular levels are increased, serum levels will be elevated even if cellular destruction is not elevated above normal turnover rate.

2. Any enzyme must be removed from plasma by a specific mechanism. If this mechanism is blocked, the serum enzyme level will increase even though cellular turnover rate remains normal.

3. Increased cell death with liberation of normal levels of enzyme will result in increased serum activities.

4. Some enzyme molecules may leach through the wall of an essentially intact cell.

From the above considerations it is obvious that serum enzyme levels may be elevated while the tissue(s) of origin appears normal when examined under the light microscope. This may be due to leaching or it may be due to increased biosynthesis or inhibited catabolism of the enzyme. The fact that serum enzyme activities and histopathologic changes have not always coincided has caused some confusion. However, the principles mentioned above make this a matter of common sense and predictable. It also is apparent that the validity of a serum enzyme determination is largely dependent on its correlation with clinical findings and other laboratory data.

In addition to potential confusion attending these basic features of enzymology, many serum enzyme determinations lack adequate tissue specificity. While some enzymes are relatively tissue specific, many are present in several major parenchymatous organs. To gain specific information by total serum enzyme determinations an entire battery of tests would be necessary. For this reason analysis of lactate dehydrogenase (LDH) isoenzymes was of interest. Through this technique, several tissues can be rather specifically assessed for current cellular damage. In order to adapt this test to clinical use in the dog it was necessary to know the isoenzyme distribution in each major tissue, the nature of the normal serum zymogram the effects of accidental hemolysis of a serum sample, and the behavior of the isoenzymes when injected intravenously. These were the major objectives of this study.

REVIEW OF THE LITERATURE

Total serum lactate dehydrogenase (LDH) activities have been a helpful, although nospecific, aid to clinical diagnosis in man and animals for more than a decade (Freedland et al., 1965; Goldman and Kaplan, 1963; Hauss and Lepplman, 1958; Levitan et al., 1960; Taylor and Preston, 1965; Wacker et al., 1956; Wroblewski, 1958). According to these authors altered LDH levels have been considered significant in myocardial infarction, hepatitis, and neoplastic disease. The dog has been used in the study of LDH activities in myocardial infarction (Wroblewski et al., 1961). Wroblewski (1958 and 1961) also reported the following total LDH activities in tissues of man and dog: (reported in LDH units/wet gram tissue)

	Dog	Man
Kidney	640,000	640,000
Sk. Muscle	600,000	600,000
Liver	390,000	390,000
Heart	240,000	240,000
Pancreas	150,000	150,000
Spleen	140,000	140,000
Brain	130,000	130,000
Lung	25,000	25,000
Serum	---	400

Although these values appeared in different reports they were identical for dog and man. This seems quite remarkable.

The suggestion was made, however, that heart, liver, and skeletal muscle contribute enough activity to serum LDH that disease changes would be reflected in serum levels. In addition to myocardial and liver disease, Levitan et al. (1960) reported on the value of total LDH in the diagnosis of polycythemia and myelofibrosis. Several authors reported on changes in total LDH in neoplastic disease (Dioguardi et al., 1962-1963; Freedland et al., 1965; Goldman et al., 1963 and 1964; Ng et al., 1966). Total LDH was elevated with malignant neoplasms, but not benign neoplasms. Total LDH levels have also been studied in diseases of muscle (Dawson and Romanul, 1964).

Total serum LDH lacks specificity since the enzyme is present in high concentration in several major tissues. It was demonstrated in man that serum LDH could be electrophoretically separated into 5 enzymatically active subunits called isoenzymes (Cann, 1958; Hill, 1958; Markert and Moller, 1959; Markert, 1963) and that the distribution of these isoenzymes had clinical significance (Barnett, 1962; Dioguardi et al., 1966; Freeman and Opher, 1965; Lance, 1966; Preston et al., 1965; Starkweather et al., 1966; Wright et al., 1966). Markert (1963) and Markert and Moller (1959) demonstrated that the LDH molecule was a tetramere composed of four monomeres. Two basic monomeres types were found. Heart muscle contained a monomere (H) apparently associated with aerobic metabolism while liver and most types skeletal muscle contained a monomere (M) associated with anaerobic metabolism. When equal parts of heart (HHHH) tetra-

mere and muscle (MMMM) tetramere were mixed in saline and repeatedly frozen and thawed, five isoenzymes (HHHH, HHHM, HHMM, HMMM, MMMM) appeared in 1:4:6:4:1 distribution. Other studies (Appela and Markert, 1961; Markert and Massaro, 1968; Fritz and Jacobson, 1965) furnished additional evidence that LDH had a tetrameric structure which could be hydrolyzed into monomeres by freezing and thawing, and by addition of various salt solutions or guanidine. Appela and Markert (1961) provided evidence that hydrogen bonding was essential to the enzymatically active form of LDH. They also suggested that only the tetrameric form of the enzyme was active. The enzyme was irreversibly inactivated by urea and guanidine hydrochloride.

Several authors (Cahn et al., 1962; Fine et al., 1963; Fieldhouse and Masters, 1966; Hinks and Masters, 1964; Vesell and Philip, 1963) reported on the developmental changes in isoenzyme distribution. In essence, maturation resulted in a decrease in anaerobic (M) and an increase in aerobic (H) LDH.

Ng and Gregory (1966) reported that antibody against LDH had an inhibitory effect on the enzyme and slowed the growth of tumor cells in tissue culture. Such studies were made possible by improved methods of purifying the enzyme such as that described by Reeves and Fimognari (1963).

Genetic control of H and M biosynthesis presents confusing features. In man, 2 loci may be responsible for production of the 2 monomere types. However, some species of fish have more than 5 isoenzymes (Markert and Faulhaber) suggesting

more than 2 loci. Also, several reports (Buta et al., 1966; Fritz and Jacobson, 1963; Ressler and Tuttle, 1966) described further subfractionation of the 5 basic isoenzymes. When electrophoresed in starch gel or polyarcrylamide gel LDH_2 may be separated into 2 subunits, LDH_3 into 3 subunits, LDH_4 into 4 subunits and LDH_5 into 5 subunits. Two hypothesis have been proposed to explain this phenomenon. Fritz and Jacobson (1965) suggested some biochemical compound such as nicotine adenonine dinucleotide (NAD) may attach to 2 of the M monomeres but not H monomeres at any given time. They recognized the possibility, however, that a third genetic locus could produce a second type of M monomere, creating the same effect. Whatever the difference, it must be one of molecular size and shape rather than electrical charge, since the isoenzymes are only subfractionated in media which exert a sieving effect. This phenomenon raises a question about the number of genetic loci responsible for biosynthesis of LDH isoenzymes.

Rate of clearance of the enzyme from plasma is an important consideration. Wroblewski and LaDue (1955) studied the clearance rate of heart LDH from the dog. They injected LDH intravenously (approximately 240,000 units) into normal dogs. Serum LDH levels returned to normal in 2.5 hours and dropped to below preinoculation levels for 3 to 4 hours, thereafter. Fleisher and Wakim (1963) and Wakim and Fleisher (1963) also studied clearance and/or catabolism rates of various serum enzymes in dogs. They provided evidence that blockade of

the reticuloendothelial system prolonged the clearance time of serum glutamic oxalacetic transaminase (SGOT) and LDH but not serum glutamic pyruvic transaminase (SGPT). It was also suggested that different forms of SGOT and LDH were cleared from plasma at different rates. Boyd (1967) studied the comparative rate of disappearance from plasma for heart LDH and muscle LDH. He found that muscle LDH disappeared from plasma faster than heart LDH.

While much literature exists regarding the clinical determination of LDH isoenzymes in man, there is a dearth of such information in animals. However, several pilot studies have been made in animals. Studies of LDH isoenzyme activities have been reported in the rat (Buta et al., 1966), the horse (Carper and Henson, 1967; Coffman et al., 1967; Coffman et al., 1969; Gerber, 1966), ruminants (Hinks and Masters, 1964) cats and guinea pigs (Hinks and Masters, 1966), fish (Markert and Faulhaber, 1965), sheep (Paulson, et al., 1966), and peafowl (Rose and Wilson, 1966). All mammalian species have 5 isoenzymes although they may be difficult to visualize in normal individuals.

The only clinical studies in which the serum isoenzyme distribution was correlated with disease in animals have been in the horse. Coffman et al., (1967) reported an increase in LDH_1 in myocarditis, LDH_5 in hepatitis, LDH_1 and LDH_2 in hemolytic disease and an increase in all isoenzymes in a case of neurofibrosarcoma. Gerber (1966) reported changes in se-

rum isoenzyme in paralytic myoglobinuria in the horse. Carper and Henson (1967) reported LDH isoenzyme distribution in several disease processes. However, Carper and Henson's isoenzyme determinations were made by heat lability rather than electrophoresis which did not coincide with electrophoretic results. Reports of electrophoretic LDH zymograms in horses are very promising.

MATERIALS AND METHODS

Experimental Animals

Young adult mongrel dogs were used throughout the study. Dogs which were considered healthy on the basis of a physical examination were deemed suitable. One dog used for determination of isoenzyme clearance rates was euthanatized following the study and lung, spleen, lymph node, kidney, and liver were examined for histopathologic changes. Tissue extracts were made from dogs which were euthanatized at the request of the owner or the pound, and not because of any clinically apparent illness.

Preparation of Zymograms

All zymograms were done according to the method of Wright *et al.*, (1966) or a modification thereof. There were no differences in the results obtained with the original method and the modification. The only difference was the support medium used in the electrophoretic separation. The method described by Wright *et al.*, (1966) was as follows:

Stock reagent solutions included:

Solution 1:

Phosphate buffer	40 ml
Sodium cyanide	24 mg
$MgCl_2$	8 mg
Lithium lactate	272 mg

Solution 2:

Phenazin methosulfate 1 mg/1 ml distilled H_2O

Solution 2 was prepared daily.

A 15 cm length of 35 mm filmstrip was covered with 1% agarose solution in 0.05 M buffer, pH 7.5. Before the agarose gelled, three 1 cm lengths of microhematocrit tubing were placed 3 cm apart in the agarose. The first piece of tubing was laid 3 cm from either end of the strip. When the gel had hardened, the tubing was removed, leaving discreet throughs for application of the samples.

The filmstrip was placed in a Gelman* electrophoresis chamber containing 0.5 M phosphate buffer pH 7.5. Fifteen lambda of serum or tissue extract were placed in the troughs using a Hamilton syringe, and the sample was electrophoresed at 12 ma per strip. Three samples were run on each strip. Excessive chamber temperature was prevented by the addition of cubes of frozen buffer. Migration of the proteins was monitored by placing a drop of ovalbumin stained with brom thymol blue at the points of application. The stained ovalbumin was allowed to migrate 2-2.5 cm.

Following electrophoresis, reagent agar consisting of 1 ml of 1% aqueous agarose, 1 ml solution 1, 0.03 ml solution 2, 1.5 mg NAD, and 0.6 mg P-nitro blue tetrazolium per sample was placed on the strip. Following application of reagent agar the

* Gelman Instrument Company, Ann Arbor, Michigan

strips were incubated in a high moisture tray for one hour at 37 C then soaked for one hour in 5% acetic acid to remove buffer salts, and dried by hanging in front of a hot air blower.

The following modification of the method of Wright et al., (1966) was also employed. Sepraphore III cellulose polyacetate strips* were soaked in .05 M phosphate buffer, pH 8.6. Following thorough saturation, the strips were placed on an absorbent paper and the excess buffer removed by blotting. Approximately 20 lambda of serum or extract was applied to the strip using 2 applications with a Beckman applicator.** The strips were placed in a Gelman electrophoresis chamber containing .05 M phosphate buffer, pH 8.6 and electrophoresed at 255 volts for one hour.

With this modified method, the reagent agar was applied differently. Strips of 3M type 628 dry photo projection transparency material*** were cut to correspond in size to the Sepraphore III strips. A 5 cm area was marked off in the center of the 3M strip with wax pencil. Two ml of aqeuous agarose-reagent solution was then pipetted onto the pencilled area and allowed to gel. The electrophoresed Sepraphore III strips were then carefully laid over the gel so that the isoenzymes would contact reagents. The strips were incubated in a high moisture tray for one hour at 37 C, and then soaked for 1 hour in 5% acetic acid and dried. The agarose adhered to the

* Gelman Instrument Company, Ann Arbor, Michigan
** Beckman and Company, Des Moines, Iowa
*** 3M Company, Topeka, Kansas

Sepraphore III and the 3M plastic was carefully peeled off. The Sepraphore III was cleared by soaking for 5 minutes in absolute methanol, followed by soaking in 15% acetic acid in 95% ethanol for 20-30 seconds. The strips were then smoothed out on a glass plate to dry and clear. In some instances, repeated painting of the cellulose acetate strips with clearing solution was required.

Zymograms run on filmstrip and on Sepraphore III were integrated for isoenzyme quantitation on a Beckman Analytrol.*

Technique for Total Lactate Dehydrogenase Determinations

Total LDH was determined by the NAD·2H to NAD, pyruvate to lactate method, using a Coleman Hatachi model No. 101 spectrophotometer.** Pyruvate excess was used. A mixture of serum, buffer and NAD·2H was incubated 20 minutes at 37 C to accommodate endogenous reactions. The test reaction was initiated with pyruvate. Readings were made at 30 second intervals and a minimum of 3 linear readings used to establish the rate of reaction.

Technique of Total Protein Determination

Total protein was determined by the standard biuret method.

* Beckman and Company, Des Moines, Iowa
** Scientific Products, Kansas City, Missouri

Preparation of Tissue Homogenates

The dogs used as a source of tissue were euthanatized with sodium pentabarbital intravenously. A serum sample and a whole blood sample were taken prior to euthanasia. Samples of skeletal (gluteal) muscle, liver, spleen, lung, myocardium, renal cortex, and renal medulla were removed in pieces approximating 1-1.5 cubic centimeters and dropped into iced tap water. One gram of tissue was minced into pieces approximating 2-3 cubic millimeters. The minced tissue was washed in saline 3 to 4 times, until the supernate was free of hemolyzed erythrocytes. The washed tissue was suspended in 9 ml of 0.1M phosphate buffer, pH 7.4 and sonified with a Branson sonifier.* The sonified tissue was removed by centrifugation and the supernate used for assay. Sonification was carried out in stainless steel centrifuge tubes immersed in ice water to prevent loss of enzyme activity due to excess heat. Tissue extractions for injection were carried out in like manner, except that equal parts buffer and minced tissue were homogenated in a Waring blender.

Hemolysates were prepared by removing the erythrocytes from whole blood by centrifugation and washing 3 times with physiologic saline. Four ml of 1:4 suspension of erythrocytes in physiologic saline were mixed with 1 ml of toluene and gently rotated. Erythrocyte stroma was removed by centrifugation and the resultant lipid layer removed by suction. The remaining

* Bronson Instruments, Standford, Connecticut

supernate was used for assay. Total LDH was not assayed in erythrocyte hemolysate because the spectrophotometer would not accommodate the dense red color.

Evaluation of the Effects of Hemolysis on Normal Serum Zymograms

Accidental hemolysis of serum: Serum samples were drawn from 11 dogs for electrophoretic separation of LDH isoenzymes. Varying degrees of hemolysis were present in 6 of the 10 samples. Four samples were judged to be free of hemolysis according to observation with the naked eye. Those samples which were hemolyzed were graded 1- through 4- with 4- representing the greatest degree of hemolysis. A whole blood sample was taken from each dog contributing a hemolyzed blood sample and erythrocyte hemolysate was made of each as described above. Zymograms were performed on each serum sample and on each hemolysate.

Comparison of accidental hemolysis with addition of toluene hemolysate to serum and intentional hemolysis by trauma of erythrocytes in saline and serum: A sample of unhemolyzed canine serum was obtained. A whole blood sample was obtained from the same dog and a toluene hemolysate was made of part of the erythrocytes. Washed erythrocytes were also suspended 1:4 in physiologic saline and serum. The erythrocytes in saline and in serum were hemolyzed by vigorously shaking the tubes. Toluene hemolysate was added to the unhemolyzed serum until the color matched that of the saline and serum in which erythrocytes were hemolyzed by trauma. Zymograms were performed on unhemolyzed serum, serum with toluene hemolysate added, serum con-

taining erythrocytes hemolyzed by trauma and saline containing erythrocytes hemolyzed by trauma. This was to determine whether or not the effect of hemolysis was the same if hemolysate was made, allowed to stand, and then added to serum which had been allowed to stand.

Evaluation of Tissue Contribution to the Serum Zymogram

Samples of the major tissues were removed fresh from 2 dogs euthanatized with sodium pentabarbitol and extracts were made as described above. Zymograms, total LDH, and total protein determinations were performed on each sample from each dog as described above. The modification of the method of Wright et al., (1966) was used for tissue zymograms.

Evaluation of Effects of Isoenzyme Clearance from Plasma on the Serum Zymogram

Tissue extracts were prepared for injection as described. Extracts used in the first 3 parts of this study were stored at -20 C prior to use. Purified extracts of LDH_1, LDH_2 (from beef heart) and LDH_5 (from rabbit skeletal muscle) were obtained and diluted in physiologic saline to approximately 10 ml per injection. This series of experiments was subdived as described below. Mongrel dogs weighing approximately 20 pounds were used for these experiments.

Comparative disappearance rates of rabbit muscle LDH and beef heart LDH from dog serum: two dogs were obtained and confined in stainless steel cages. One dog was given 312,000

International Units activity of LDH from rabbit skeletal muscle, and the other dog 312,000 International Units activity of LDH from beef heart. Preinoculation sample and post inoculation samples were taken and serial bloedings were made 30, 60, 90, 120, 150, 180 minutes and 360 minutes following injection. After serum levels had returned to preinoculation levels, the same dogs were inoculated in reverse after a 24 hour waiting period. Bleeding intervals were the same as before, and the two dogs were averaged together. Only total serum LDH was determined in this experiment. Zymograms were not performed.

Effect of reticuloendothelial blockade with india ink on disappearance rate of LDH from plasma: extracts of canine gluteal muscle were prepared for injection as described and equal parts (levels of activity) were mixed, (approximately 312,000 International Units for each of two experimental dogs). The volume for each dog was 18 ml. Prior to injection of the enzyme, one dog received 10 ml india ink intravenously as a reticuloendothelial blocking agent. Preinoculation, post-inoculation, 0.5 hour, 1.5 hour, 2.5 hour, 3.5 hour, 4.5 hour, 5.5 hour, 6.5 hour, 7.5 hour, and 8.5 hour samples were drawn. Following the experiment the dog which received the india ink was euthanatized with sodium pentabarbitol and necropsied. Only total serum LDH was determined on these samples.

The above experiment was repeated in which zymograms as well as total LDH were performed on each sample.

Four additional experimental clearance studies were completed using extracts that had not been frozen.

1. Canine heart extract (312,000 International Units) was injected into a dog. Preinoculation, postinoculation, and 1, 2, 3, 4 and 5 hour samples were drawn. Zymograms and total LDH were performed.

2. The above experiment was repeated using an extract from canine gluteal muscle.

3. Experiment 1 was repeated, using equal parts heart and gluteal muscle extract.

4. The dog used in the above study was injected with 10 ml of a 50% suspension of red blood cell stroma and connective tissue particles from muscle, to block the reticuloendothelial system and experiment 3 was repeated.

Determination of Km Values of Canine Heart Muscle Extract and Canine Skeletal Muscle Extract LDH for Lactate

As zymograms involved the reaction lactate to pyruvate and the reaction was stopped at a set time rather than at equilibrium, it appeared worthwhile to determine if either heart LDH or gluteal muscle LDH had a greater affinity for the substrate. Therefore, Km values were determined for extracts of both tissues for lactate, according to the method of Lineweaver and Burke (Mohler and Corder, 1966). These values were determined on the two tissues separately and mixed together suspended in plasma.

RESULTS AND DISCUSSION

Zymograms

Advantages and disadvantages were noted for both methods of performing zymograms. When filmstrip base was used, it was not necessary to clear the strip prior to quantitation. However, background color, spotting, and retention of buffer salts were minor problems. The cellulose acetate method gave a more discreet separation with sharper peaks on the densitometer. However, streaking of the bands along the margins of the strip occurred during incubation. The main objection to the cellulose acetate strips was difficulty in clearing the portion of the strip beneath the agar.

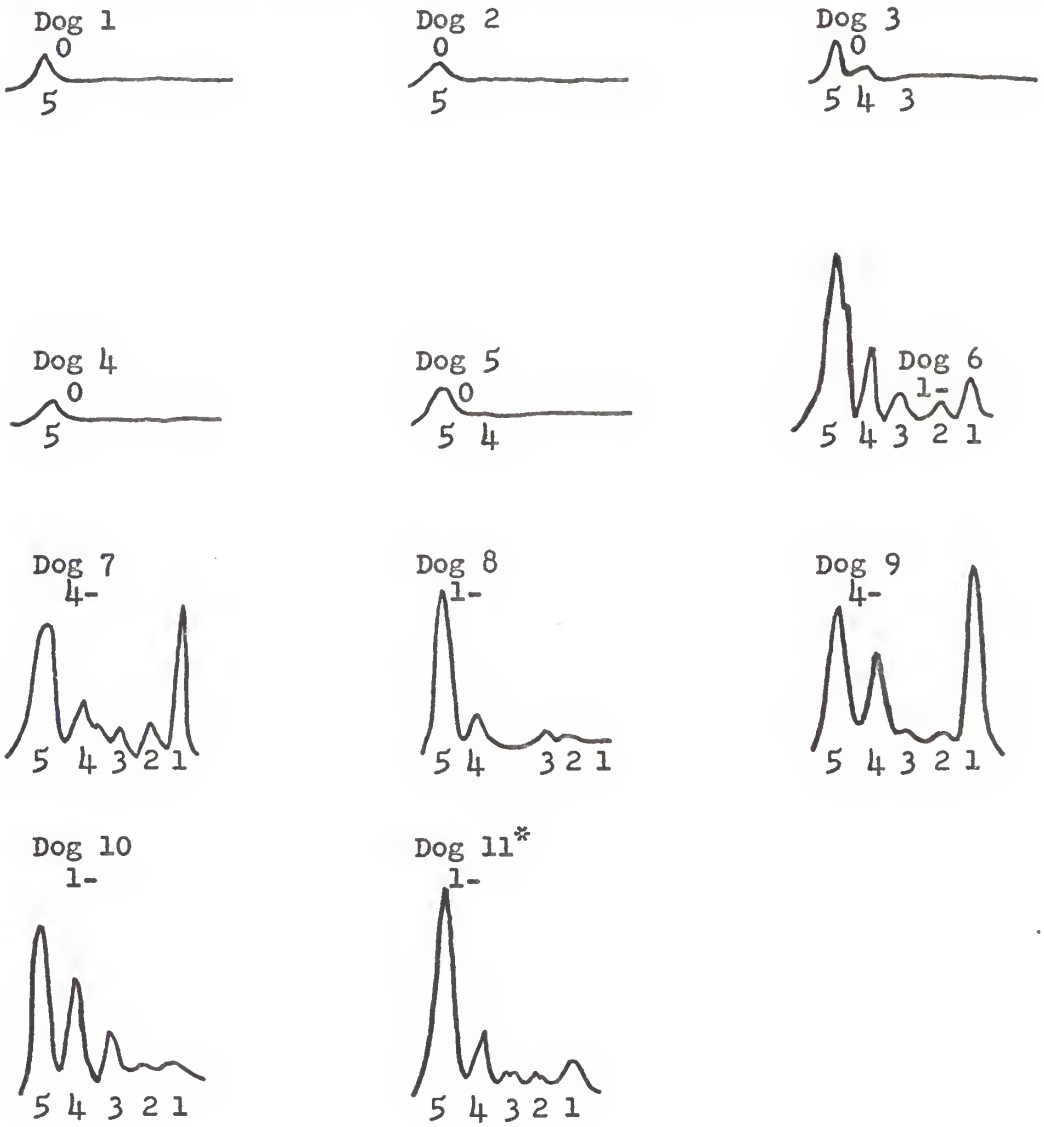
It was originally assumed that reagent agar could be pipetted onto the Sepraphore III as it was onto the agar gel. However, when this was attempted the liquid agar washed the enzyme protein into random streaks. In agar gel the protein migrated through the gel and was stable when subjected to a liquid. Migration on cellulose acetate was a surface phenomenon, however, and protein was washed away if not properly fixed.

Evaluation of the Effects of Hemolysis on Normal Serum Zymograms

Accidental Hemolysis of Serum: Serum zymograms from 11 dogs are shown in Figure 1. Samples 1 through 5 were free of visible hemolysis. Samples 6 through 11 were hemolyzed. In the nonhemolyzed samples only LDH_5 was clearly visible. This

Figure 1

The degree of hemolysis is graded 0 through 4⁻ in 11 sera, 6 of which were accidentally hemolyzed at exsanquination.



* LDH_2 and LDH_3 are each split into 2 subunits.

was typical of samples from normal dogs. Hemolysis had a marked effect on the zymogram, as there was an increase in all bands. The major change was a predominance of LDH_1 and LDH_2. This was expected, as these two bands predominated in hemolysate. However, hemolysis also made LDH_5 more prominent, and caused discernible increase in LDH_3 and LDH_4.

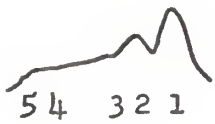
Comparison of accidental hemolysis with addition of toluene hemolysate to serum and intentional hemolysis by trauma of erythrocytes in saline and serum: The zymogram of a toluene hemolysate produced the same zymogram as hemolysate produced by trauma. All bands were visible on the zymogram when erythrocytes were hemolyzed in normal serum. LDH_1 was predominant, LDH_5 was increased in intensity and $LDH_{3,4}$ were visible but of low intensity.

All 5 bands were visible in the zymogram when a toluene hemolysate was added to unhemolyzed serum. In this case the intensity of the bands decreased from LDH_1 through LDH_5. Thus, there was an increase in the intenstiy of LDH_5 when erythrocytes were hemolyzed in serum rather than hemolyzed separately and added to serum. This is noteworthy as hemolysate contained only LDH_1 and LDH_2. Zymograms from unhemolyzed serum, hemolysate, serum plus toluene hemolysate and serum which was intentionally hemolyzed when drawn are presented in Figure 2. All samples are from the same dog.

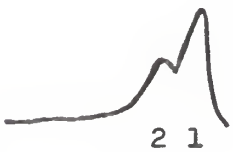
Figure 2

Comparison of accidental hemolysis with addition of toluene hemolysate to serum and intentional trauma of erythrocytes in saline and serum.

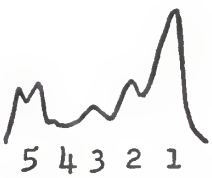
unhemolyzed serum



serum plus hemolysate



hemolysate (toluene and saline)



hemolyzed serum

The potentiation of LDH_5 when erythrocytes were disrupted in the presence of serum (Figure 1 and 2) but not when hemolysate was added to serum, suggested two possibilities.

1. Dimere and trimere forms as well as the active tetramere form of LDH must have been present in serum (i.e. MM, MMM).

2. At least part of the LDH released from the erythrocyte must have been released as monomeres which repolymerized.

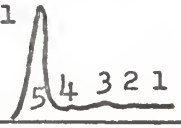
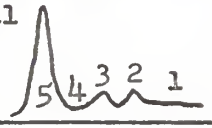
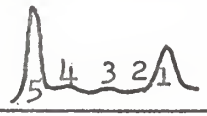
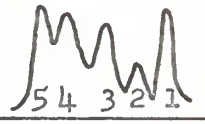
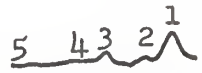
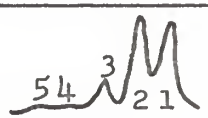
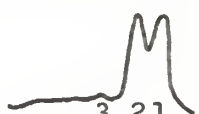
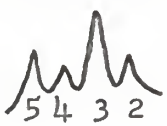
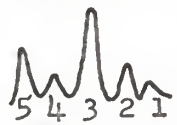
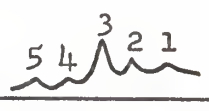
Since both LDH_1 and LDH_2 were demonstrated in erythrocytes, some M monomere would be available. Thus, if HHHH, and HHHM were hydrolyzed and released in M, MM, and MMMM, it is logical that LDH_5 could have been potentiated, which it was. While monomeric release of enzyme was not proven, the possibility was raised by the observations noted above.

Evaluation of Tissue Contribution to the Serum Zymogram

Results of isoenzyme electrophoresis, and LDH activity expressed in international units per mg protein for two dogs presented in Table I.

Zymograms were highly reproducible between the tissues of the two dogs. This favors the possible use of the test clinically, as cardiac muscle and erythrocytes presented zymograms entirely different from liver and skeletal muscle. These tissues would be of primary clinical importance.

LDH activity per mg. protein and isoenzyme distribution are given for each major tissue in 2 dogs. The fast (anodic) isoenzyme is numbered LDH_1.

Tissue	Dog I	Dog II
Skeletal Muscle	2.43* 	7.34 
Renal Cortex	4.25 	5.86 
Renal Medulla	1.44 	4.66 
Heart	4.08 	3.27 
Liver	2.17 	4.10 
Spleen	1.72 	2.06 
Lung	1.54 	1.92 

* LDH activity (International Units) per mg protein

Zymograms of the kidney were of interest as there was a predominence of LDH_5 in the renal cortex and a predominance of LDH_1 in the medullary extract.

There was a predominance of one isoenzyme in most tissue zymograms. Spleen, however, did not conform to this principle. High concentrations of LDH_3 and LDH_5 were noted, with other bands visible but of low intensity. This probably reflects the difference in age of the cell population (Wright et al., 1966).

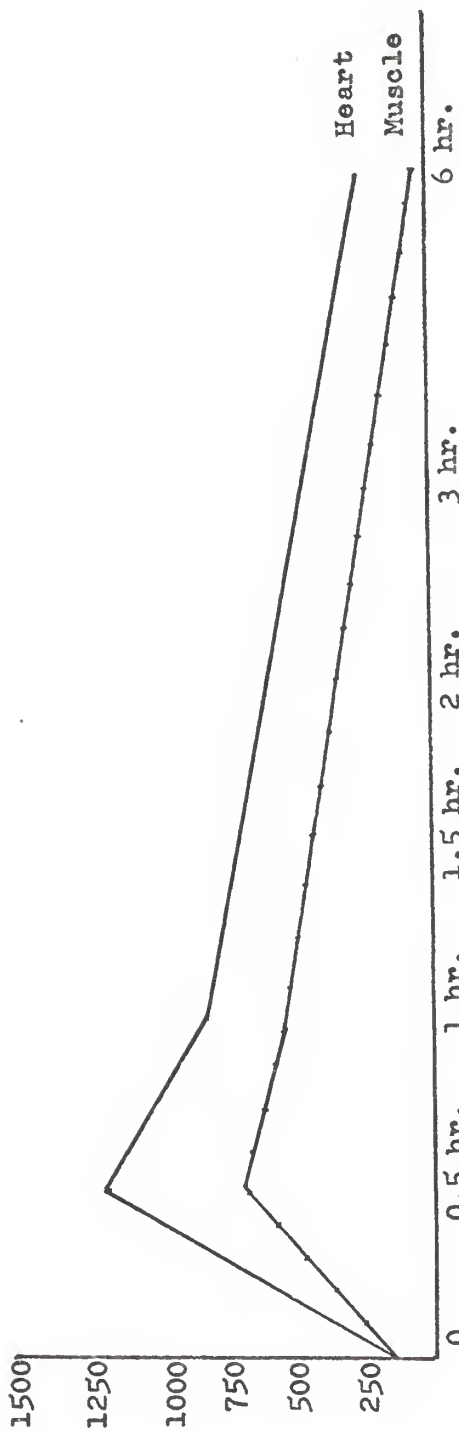
Specific activity in the various tissues was not reproducible between the two dogs. This would probably not interfere with clinical use of the test, however, as liver, skeletal muscle, cordiac muscle, renal cortex, and spleen all contained sufficiently high levels to influence the serum zymogram under clinical conditions. The difference in specific activities between the two dogs is difficult to explain. Probably it is due to genetic influence or induced changes due to metabolic needs or both.

Evaluation of Effects of Isoenzyme Clearance from Plasma on the Serum Zymogram

Comparative disappearance rates of rabbit muscle LDH and beef heart LDH from dog serum: the rate of disappearance of rabbit skeletal muscle LDH and beef heart LDH from plasma is presented in Figure 3. The clearance rates were similar for both types of LDH. This experiment had several disadvantages. A purified enzyme was used which did not represent

Figure 3

Comparative disappearance rates of rabbit muscle LDH and beef heart LDH from dog serum.



circumstances which would prevail when a tissue dies in vivo. Also, both types of LDH were heterologous and could, therefore, be regarded as foreign protein. These factors could have distorted the results of the study.

Effect of reticuloendothelial blockade with india ink on disappearance rate of canine LDH from plasma: results of this experiment are given in Figure 4. In this instance, dog tissue homogenates were used rather than purified enzyme. This overcame the problems of foreign protein and more nearly reproduced actual tissue necrosis.

When dogs were injected with homogenate in phosphate buffer, a brief period of syncope was noted. This could have been due to the effects of K- on heart muscle or the sudden injection of 18 ml volume or both.

The dog injected with india ink had a higher peak level of LDH and was slower to clear the enzyme. At 5.5 hours post-injection enzyme activities dropped below preinoculation levels and rose to preinoculation levels by 8.5 hours. Both dogs were clinically normal at the end of the trial period.

Effect of reticuloendothelial blockade with india ink on disappearance rate of isoenzymes from plasma: serial total serum LDH values for this study are given in Figure 5, page 28. Serum zymograms of the dog injected with india ink are presented in Figure 6, page 29, and for the control dog in Figure 7, page 30.

Figure 4

Effect of reticuloendothelial blockade with india ink on disappearance rate of canine LDH from plasma. (1 = india ink)

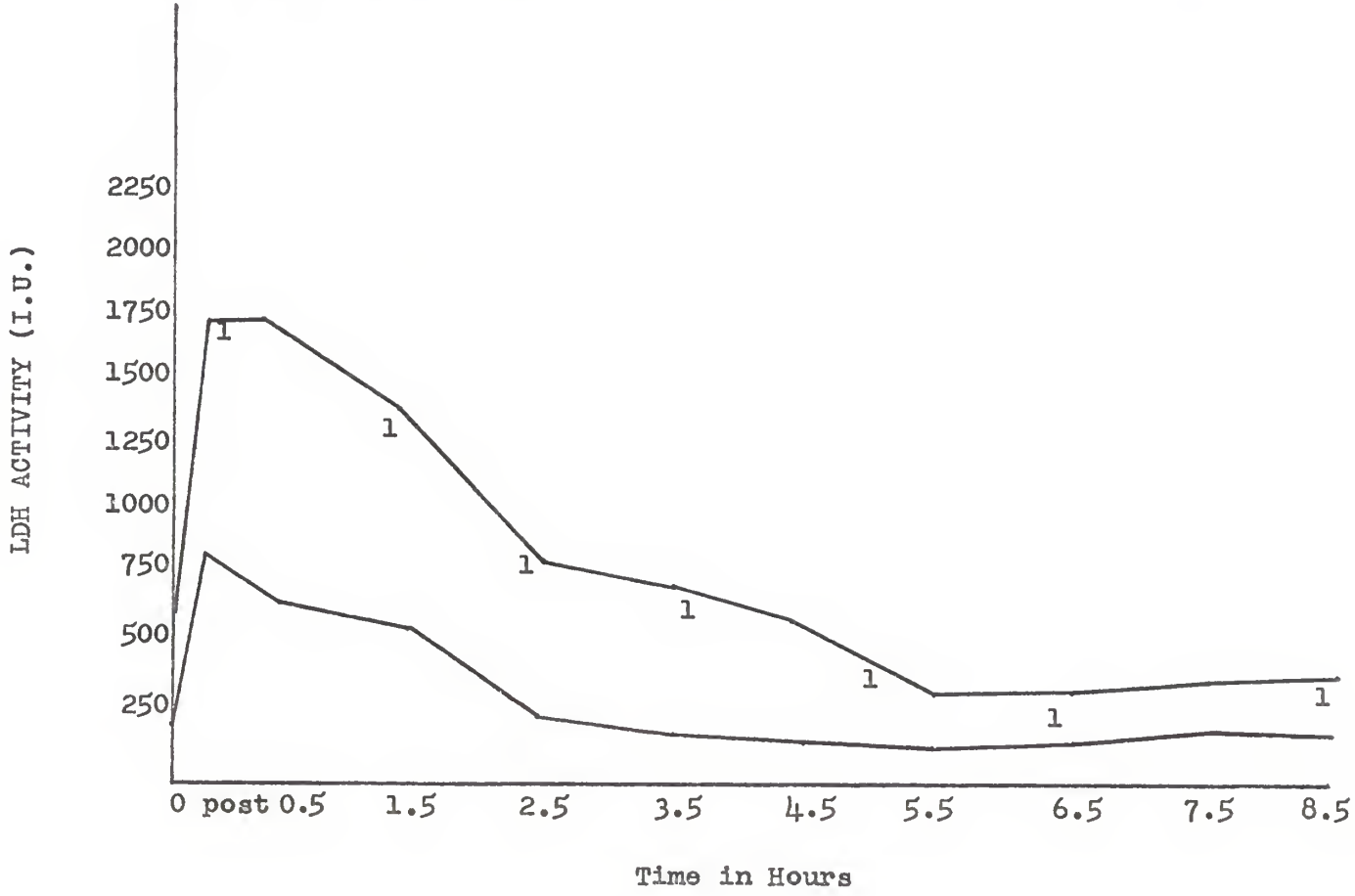


Figure 5

Effect of reticuloendothelial blockade with india ink on disappearance rate of canine LDH from plasma. Zymograms were also performed on these sera (Figures 6 and 7). (1 = india ink)

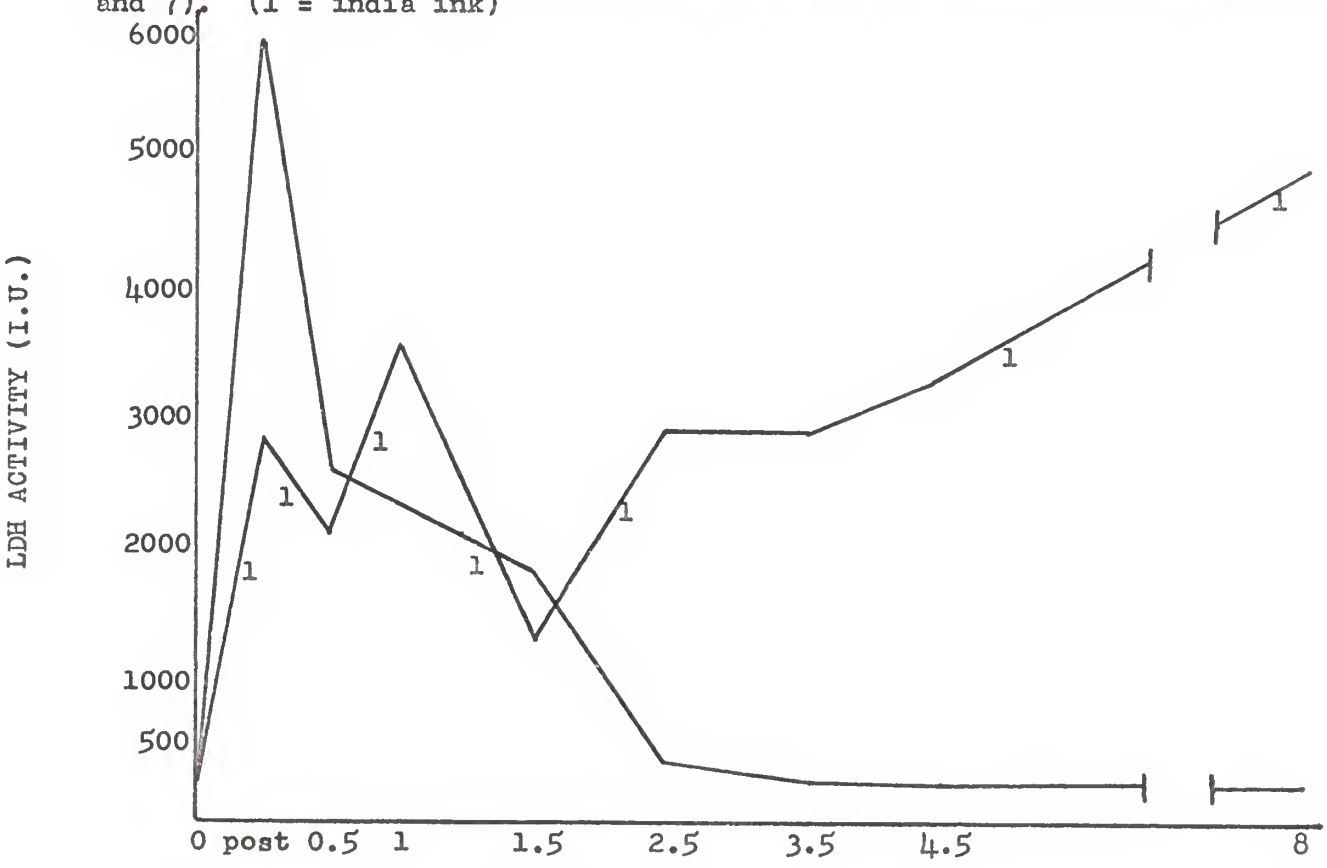
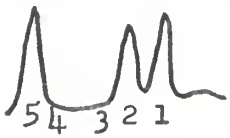


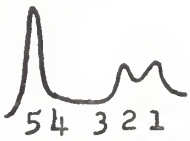
Figure 6

Zymograms from dog (Figure 5) receiving india ink, followed by 312,000 International Units of combined cardiac and muscle LDH.

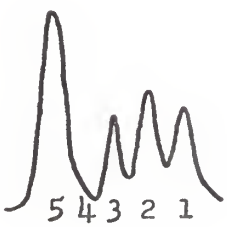
pre



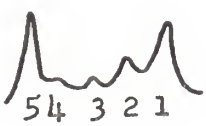
post



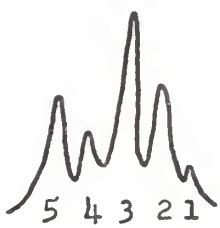
1/2 hour



1 hour



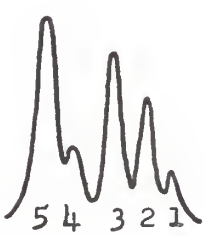
1 1/2 hour



2 1/2 hour

3 1/2 hour

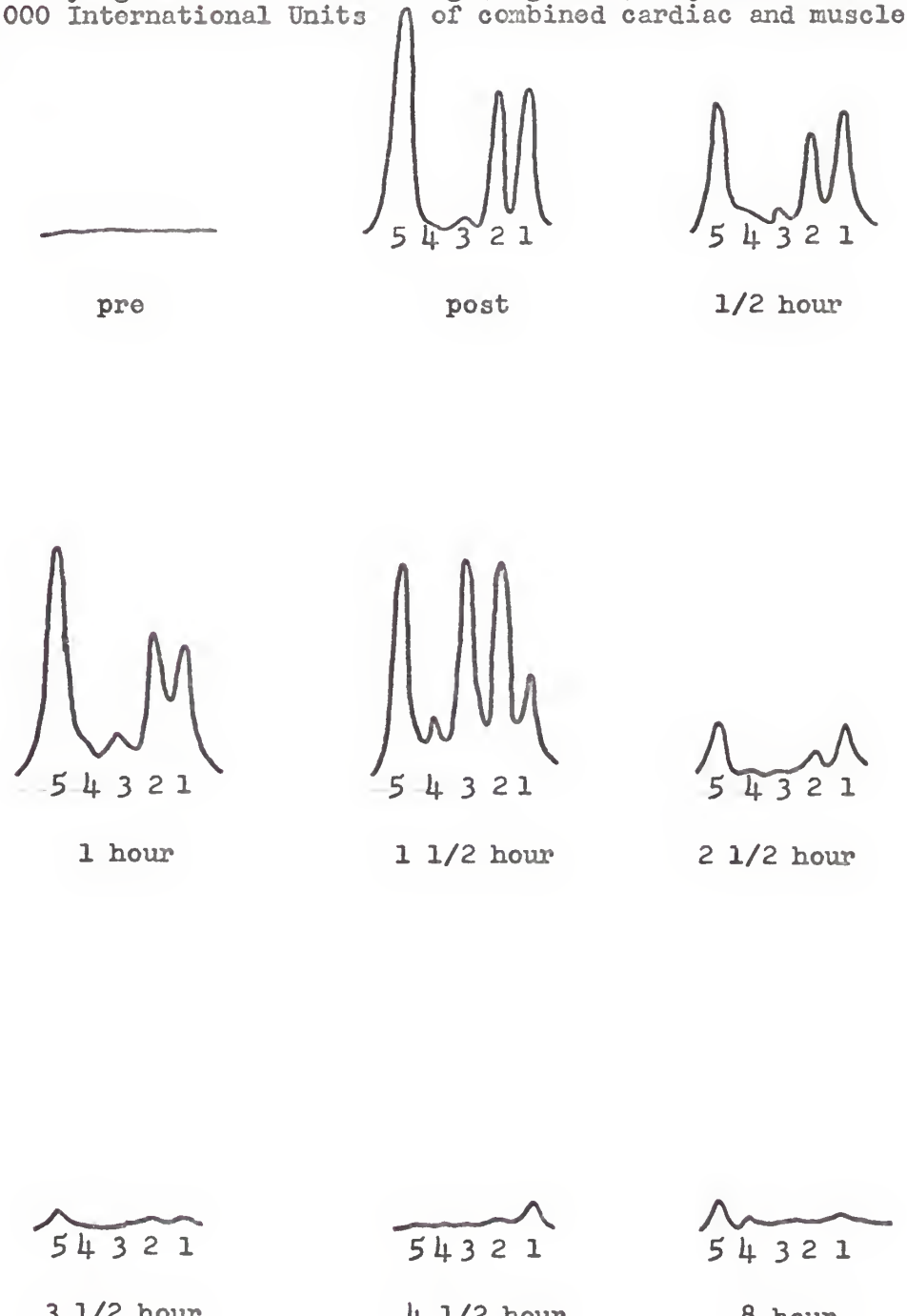
4 1/2 hour



8 hour

Figure 7

Zymograms from control dog (Figure 5) injected with 312,000 International Units of combined cardiac and muscle LDH.



Following injection of the mixed extracts into the control dog, LDH_1, LDH_2 and LDH_5 increased and decreased concurrently. However, LDH_5 was removed somewhat faster than LDH_1, a fact that was particularly apparent at 4.5 hours postinjection. Prior to injection, LDH_5 was visible, as were traces of LDH_4 and LDH_3. LDH_1 and LDH_2 were not visible. At 4.5 hours, LDH_1 and LDH_2 were readily visible and LDH_5 could hardly be seen. Near the end of the test the concentration of the bands was not sufficient to allow accurate quantitation. At 1.5 hours postinjection LDH_3 abruptly appeared in heavy concentration and then subsided. This was quite unexpected. A logical explanation would be that in vivo hybridization of H and M had occurred. This would support the possibility of circulating monomere, dimere and trimere forms noted in the hemolysis studies.

A markedly different pattern was seen in the dog injected with india ink. Isoenzyme LDH_3 appeared at 2.5 hours, and persisted through the study. In addition, LDH_1 decreased in intensity while LDH_5 increased in intensity.

In the control dog, total serum LDH values steadily returned to preinoculation values. In the dog receiving india ink, however, total values declined to half the postinjection level 1.5 hours postinjection, then rapidly rose to a level which exceeded postinjection levels.

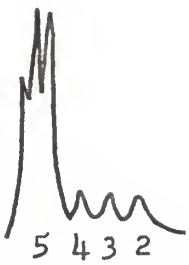
As the dog injected with india ink was comatose at the end of the trial, it was euthanatized with sodium pentabarbitol

and necropsied. Microscopic examination of the tissues was performed. The lungs were extensively congested and there was diffuse distribution of macrophages which were filled with pigment. These cells were primarily located along the alveolar wall but occasionally a single macrophage was observed in large blood vessels. Some of the large cells free in alveoli, however, did not contain pigment. The Malpighian bodies in the spleen were surrounded by macrophages which were filled with pigment. In addition, there was a diffuse distribution of cells in the spleen which contained variable amounts of pigment. Some of the younger reticuloendothelial cells did not contain pigment. Comparatively few cells in the lymph nodes contained this pigment and these cells appeared to be large macrophages. There was also evidence of local areas of neutrophilic response in the spleen. Pigment was lacking in the kidney except for a few cells which were located in the blood vessels. The greatest amount of phagocytized material was found in the liver. Virtually all Kupffer cells contained phagocytized material.

Comparative disappearance rate of the isoenzymes and the effect of reticuloendothelial blockade with hemologous cellular debris on the respective disappearance rates: zymograms of the heart and muscle extracts used are given in Figure 8. Zymograms and total LDH values of the clearance series for heart are shown in Figure 9, page 34. Some heart LDH was still present at 5 hours. Slight increase in LDH_5 accompanied the clearance

Figure 8

Zymograms of heart and muscle extract used in comparing disappearance rates of the isoenzymes before and after reticuloendothelial blockade with homologous cellular debris.

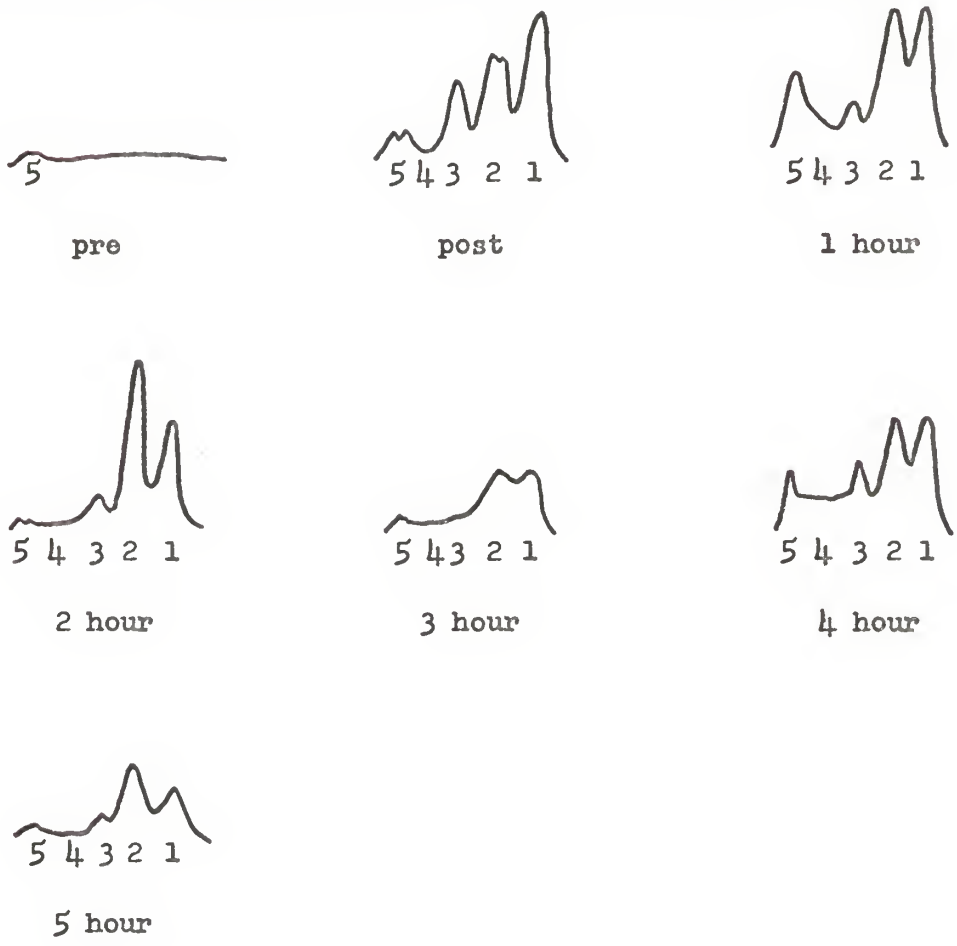


muscle extract

heart extract

Figure 9

Zymograms and total activities for clearance of dog heart LDH. 312,000 International Units of activity were injected.



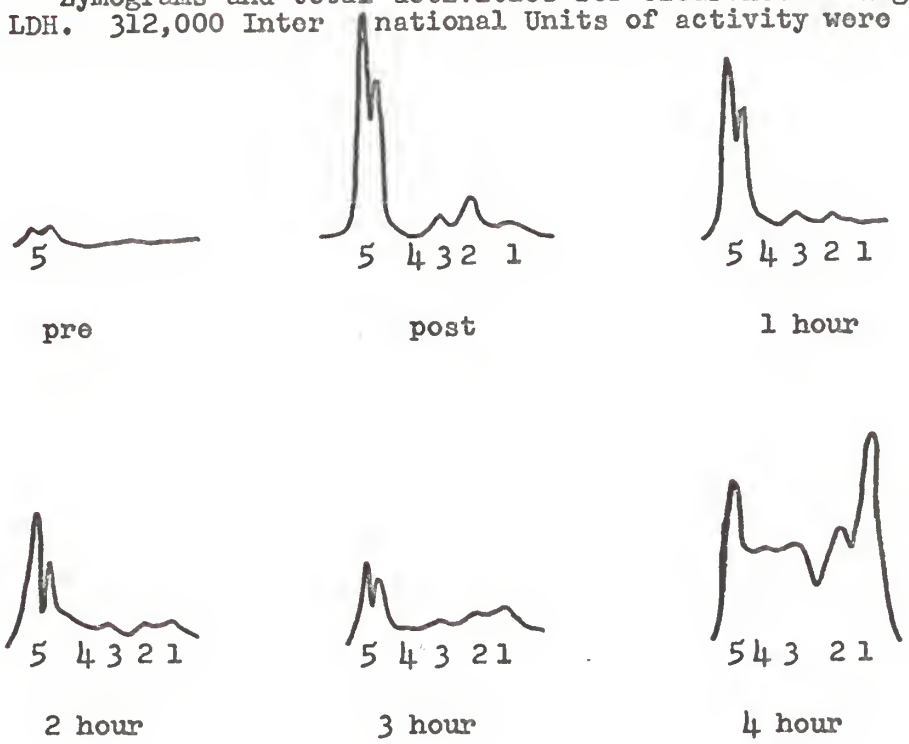
of heart LDH. Zymogram and total LDH values of the muscle LDH clearance series are given in Figure 10. The postinjection zymogram almost duplicated the zymogram of muscle extract. However, as the clearance process proceeded, LDH_1 and LDH_2 increased markedly.

Injection of muscle extract caused a more marked increase in LDH_1 and LDH_2 than heart extract caused in LDH_5. Total LDH activities also demonstrated that muscle LDH disappeared from plasma faster than heart LDH. This suggested that LDH_5 cleared preferentially and the normal clearance of LDH_1 and LDH_2 was blocked. This would support previous studies in sheep (Boyd, 1967).

Zymograms and total serum LDH values of the clearance series for combined heart and muscle are given in Figure 11, page 37. In this study LDH_3 was never prominent. Ninety minute samples were not drawn in this study, so it is possible that this phenomenon was missed. These extracts had not been frozen and it is also possible that freezing weakened the intermolecular bonding in the tetramere structure. Thus, appearance of LDH_3 was not a constant finding. By 3 hours postinjection LDH_5 was hardly visible while LDH_1 and LDH_2 were prominent. It was obvious that LDH_5 was cleared much more rapidly than LDH_1 and LDH_2. When heart and muscle were injected together total LDH activities decreased faster than when the extracts were injected separately.

Figure 10

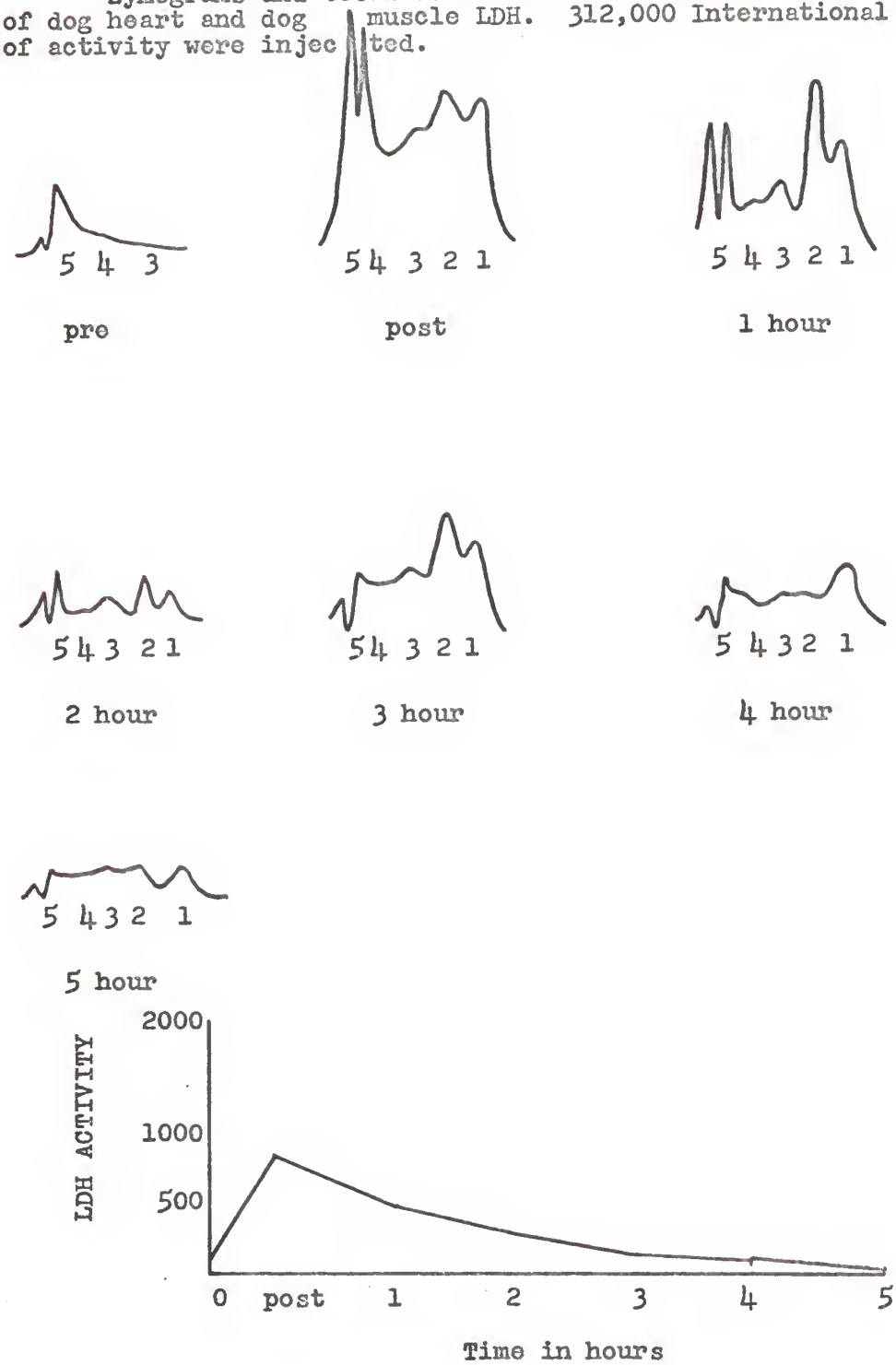
Zymograms and total activities for clearance of dog muscle LDH. 312,000 International Units of activity were injected.



*hemolyzed

Figure 11

Zymograms and total activities for simultaneous clearance of dog heart and dog muscle LDH. 312,000 International Units of activity were injected.



When heart and muscle extract were injected simultaneously, preinoculation levels were reached in 3 hours. When 5 grams of homologous cellular debris was injected prior to the enzyme, preinoculation levels were also reached in 3 hours, (Figure 12). This suggested that the debris entering circulation during a disease process would not decrease disappearance rate of LDH in the dog. LDH_5 rapidly returned to an intensity comparable to preinjection levels. LDH_1 and LDH_2 were not visible on the preinjection zymogram, but were still prominent at 5 hours. Thus, this clearance series provided further evidence that LDH_5 was cleared from circulation preferentially to LDH_1 and LDH_2.

Determination of Km Values of Heart Muscle Extract and Skeletal Muscle Extract LDH for Lactate

Lineweaver, Burke plots for heart LDH and muscle LDH were completed separately in buffer and mixed together in plasma. The Km values for lactate were:

Heart . . . . . 5.3×10^{-2}

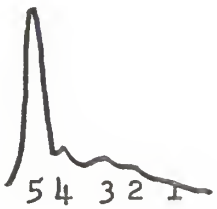
Muscle . . . . . 7.1×10^{-2}

Heart and Muscle in plasma . . 6.2×10^{-2}

These data indicate that heart LDH had a greater affinity for lactate than did muscle LDH. Therefore, Km values could not be the reason that LDH_5 was the only prominent isozyme on a normal zymogram. Also, these data suggest that competitive in-

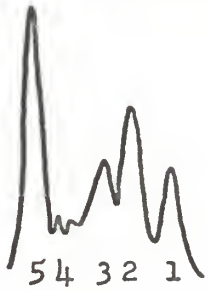
Figure 12

Zymograms and total activities for simultaneous clearance of dog heart and dog muscle following pretrial injection of red blood cell and muscle cell stroma. The tissue extract was injected 5 minutes after the cellular debris. 312,000 International Units of activity were injected.



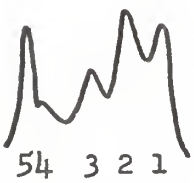
pre

post

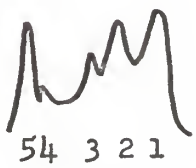


1 hour

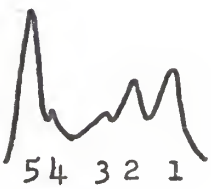
2 hour



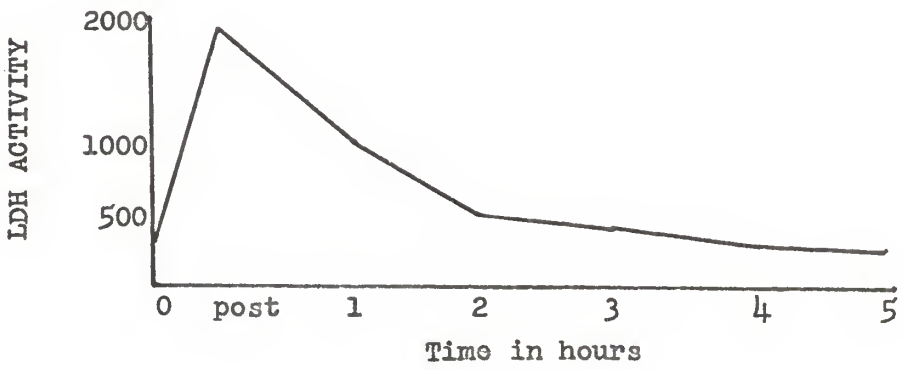
3 hour



4 hour



5 hour



hibition was not involved in either extract or in plasma since the apparent Km fell exactly between 5.3×10^{-2} and 7.1×10^{-2} when the two were mixed in the presence of plasma. This also supported the accuracy of the individual determinations.

GENERAL DISCUSSION

It was hoped that once basic characteristics were better established the LDH zymogram would be a useful aid in clinical diagnosis of myocardial infarction, myocarditis, hepatitis, erythrocyte dyscrasia, and neoplastic diseases (particularly the leukemias). The purpose of this study was to provide a basic overview of some characteristics of LDH isoenzymes in the dog. Several questions were answered and several more raised which may deserve further study.

Hemolyzed serum samples were virtually worthless for LDH zymograms. As was expected, accidental hemolysis grossly distorted the apparent distribution of the LDH isoenzymes. This represented a major problem, as collection of a completely hemolysis free sample is difficult in small dogs. It is worth noting, however, that many enzyme tests are falsely elevated by hemolysis. This fact is far too commonly disregarded. It is suggested that for best results, the sample be drawn by jugular venipuncture with a sillconized syringe and needle and centrifuged in a siliconized tube prior to clotting. The largest gauge needle which can reasonably be employed should be used. The needle should be removed from the syringe prior to carefully running the blood down the side of the tube. The process may be simplified by using a vacutainer tube* in animals which will not be stressed by loss of 10 ml blood.

* Becton-Dickinson Company

The serum zymogram of the normal dog was bizare as only LDH_5 was clearly visible. It was postulated that the Km of LDH_5 for lactate might be enough lower than that of the other isoenzymes that that band of color developed over LDH_5 much more readily than over the other isoenzyme. Determination of skeletal muscle Km (lactate) for LDH and heart muscle Km (lactate) for LDH showed that this was impossible. Actually, the Km for lactate of heart LDH was lower than the Km of muscle LDH. Therefore, the more intense development of color over LDH_5 could not possibly be due to Km difference. Since the concentration of substrate was the same over each isoenzyme, the greater intensity of color over LDH_5 must be a true reflection of greater enzyme concentration. It was also thought that LDH_1 may be present in lower concentration as it was cleared from circulation faster than LDH_5. This was also disproved by this study as was cleared faster than LDH_1. Since clearance studies showed that LDH_5 was removed from plasma faster than LDH_1 and LDH_2, much more LDH_5 must be contributed from tissues. This is logical since skeletal muscle is rich in LDH_5 and is present in greater mass than other tissue studied. This is also true in man and horse, however, and LDH_5 is barely visible in the normal serum zymogram of those species (Wright et al., 1966; Coffman et al., 1968).

Efforts to determine the effect of reticuloendothelial blockade were inconclusive. India ink markedly prolonged the

clearance of LDH_1 but there was a marked increase in LDH_5, suggesting that the liver was damaged by the india ink, although histopathology revealed no necrosis. Blockade with homologous cellular stroma had virtually no effect on clearance time. Results of the clearance studies suggested that metabolites and macromolecules were more likely to interfere with disappearance of LDH isoenzymes than was cellular stroma.

Distribution of the isoenzymes in tissues appeared to be reproducible enough to encourage clinical adaptation of the serum zymogram. Total concentration in various tissues was variable. However, this should make no difference as long as total activities in heart, skeletal muscle, liver, and red blood cells are high enough to be detected when a gram of wet tissue is lysed and made available to general circulation. This would appear to be the case.

This study has raised one question which would definitely warrant further investigation. In two clearance study sequences when heart and muscle LDH was injected simultaneously, LDH_3 spontaneously appeared. This could be the result of storing the tissue extract or some unrealized deviation in technique. It could also be a mechanism which occurs in nature and not previously recognized. The immediate clinical importance of this phenomenon is obscure. However, it warrants further consideration as it involves the basic concept of LDH structure and behavior.

The LDH zymogram appears to have promise as a diagnostic aid in canine medicine. Furthermore, the dog is well suited to experimental study of this enzyme because only LDH_5 is visible in normal serum and this is of low intensity. Also, because of the tissue specifity afforded by this test, it may have promise as an aid in pathogenesis studies.

BIBLIOGRAPHY

Appela, Ettore and Markert, C. L., 1961: Dissociation of of Lactate Dehydrogenase into Subunit with Guanidine Hydrochloride. Biochem. and Biphy. Res. Comm. 6(3): 171-176.

Barnett, H., 1962: Electrophoretic Separation of Lactate Dehydrogenase Isoenzymes on Cellulose Acetate. J. Biochem., 84: 83-84.

Boyd, J. W., 1967: The Rates of Disappearance of L-Lactate Dehydrogenase Isoenzymes from Plasma. Biochem. Biophys. Acta, 132: 221-231.

Buta, J. L., Conklin, J. L., and Dewey, M. M., 1966: Subfractions of Lactate Dehydrogenase of the Rat. J. Histochem. and Cytochem., 14(9): 658-662.

Cahn, R. D., Kaplan, N. O., Levine, L. and Zwilling, E., 1962: Nature and Development of Lactic Dehydrogenases. Science, 136: 962-969.

Cann, John R., 1968: Recent Advances in the Theory and Practice of Electrophoresis. Immunochemistry, 5: 107-134.

Carper, H. A. and Henson, J. B., 1967: Lactic Dehydrogenase Isoenzymes in the Horse. Am. J. Vet. Clin. Path., 1: 18-19.

Coffman, J. R., Cawley, L. P. and Mussman, Harry, 1967: Clinical Application of the LDH Zymogram in the Horse. Proc. Am. Assoc. Eq. Prac., 109-122.

Coffman, J. R., Cawley, L. P. and Mussman, Harry, 1969: Lactate Dehydrogenase Isoenzymes in Equine Infectious Anemia. Cornell Vet., (In Press).

Dioguardi, N., Agostoni, A., and Fiorelli, G., 1962/63: Characterization of Lactic Dehydrogenase in Cells of Myeloid Leukemia. Enzymol. Biol. Clin., 2: 116-126.

Dioguardi, N., Ideo, G., Mannucci, P. M., Fiorelli, G., and Agostoni, A., 1966: Multiple Molecular Forms of Lactatedehydrogenase (LDH) of Normal and Leukemic Cells of the Myeloid Line. Enzym. Biol. Clin., 6: 1-9.

Fieldhouse, Beryl, and Masters, C. J., 1966: Developmental Redistributions of Porcine Lactate Dehydrogenase. Biochemistry, Biophys. Acta, 118: 538-548.

Fleisher, G. A. and Wakin, K. G., 1963: The Fate of Enzymes in Body Fluids--An Experimental Study III, Disappearance Rates of Glutamic Oxalacetic Transaminase II under Various Conditions. J. L. and Clin. Med., 61(1): 98-106.

Freedland, R. A., Theis, J. H. and Cornelius, C. E., 1965: Blood Enzymes in Bovine Lymphosarcoma. N. Y. Acad. Sci. (Annals): 1313-1320.

Freeman, Irving, and Opher, A. W., 1965: Lactic Dehydrogenase Isoenzymes in Myocardial Infarction. Am. J. Med. Sci., 49/131 - 54/336.

Fritz, P. J. and Jacobson, K. B., 1963: Lactic Dehydrogenases: Subfractionation of Isozymes. Science, 140: 64-65.

Fritz, P. J. and Jacobson, K. B., 1965: Multiple Molecular Forms of Lactate Dehydrogenase. Biochemistry, 4(2): 282-289.

Gerber, H., 1966: Activity Estimations of Serum Enzymes in Veterinary Medicine III F. LDH Isoenzymes in Some Organs and in Serum of Diseased and Healthy Horses. Translated Title. Schweiz. Archiv. Tierheil., 108, 33.

Goldman, Robert D. and Kaplan, N. O., 1963: Alterations of Tissue Lactate Dehydrogenase in Human Neoplasms. Biochem. Biophys. Acta., 77: 515-518.

Goldman, R. D., Kaplan, N. O. and Hall, T. C., 1964: Lactic Dehydrogenase in Human Neoplastic Tissues. Cancer Res., 24: 289-399.

Hauss, W. H. and Leppelmann, H. J., 1958: Reactive Changes of Enzyme Activities in Serum and Liver as Symptoms of "Acute Syndrome". N. Y. Acad. Sci., (Annals), 74: 250-259.

Hill, B. R., 1958: Further Studies of the Fractionation of Lactic Dehydrogenase of Blood. N. Y. Acad. Sci. (Annals), 75: 304-310.

Hinks, Mary and Masters, C. J., 1964: Dévelopmental Changes in Ruminant Lactate Dehydrogenase. Biochemistry, 3(11): 1789-1791.

Hinks, Mary and Masters, C. J., 1966: The Ontagenetic Variformity of Lactate Dehydrogenase in Feline and Cavian Tissues. Biochem. Biophys. Acta., 130: 458-468.

Lance, B. Marcille, 1966: A Method for Separation and Quantitation of LDH Isoenzymes on Cellulose Acetate. Technical Bulletin Reg. Med. Tech., 36(8): 207-210.

Levitan, Ruven, Wasserman, L. R. and Wroblewski, Felix, 1960: Serum Enzyme Activities in Patients with Polycythemia and Myelofibrosis. Cancer, 13: 1218-1220.

Markert, C. L. and Massaro, E. J., 1968: Lactate Dehydrogenase Isozymes: Dissociation and Denaturation by Dilution. Science, 162: 695-697.

Markert, C. L. and Faulhaber, Ilse, 1965: Lactate Dehydrogenase Isozyme Patterns of Fish. J. Exp. Zool. 159: 319-332.

Markert, C. L. and Moller, F., 1959: Multiple Forms of Enzymes: Tissue, Ontogentic and Species Specific Patterns. Proc. Nat. Acad. Sc., 45: 753-763.

Markert, C. L., 1963: Lactic Dehydrogenase Isozymes: Dissociation and Recombination of Subunits. Science, 140: 1329-1330.

Mohler, H. R. and Corder, E. H., 1966: Biological Chemistry, 1st Edition, Harper and Row, New York and London: 228-231.

Ng, C. W. and Gregory, K. F., 1966: Antibody to Lactate Dehydrogenase II. Inhibition of Glycolysis and Growth of Tumor Cells. Biochem. Biophys. Acta. 130: 477-485.

Paulson, G. D., Pope, A. L. and Baumann, C. A., 1966: Lactic Dehydrogenase Isoenzymes in Tissues and Serum of Normal and Dystrophic Lambs. Proc. Soc. Exp. Bio. and Med. 122(2): 321-324.

Preston, J. A., Briere, R. O. and Batsakis, J. G., 1965: Rapid Electrophoretic Separation of Lactate Dehydrogenase Isoenzymes on Cellulose Acetate. Am. J. Clin. Path. 43(3): 256-260.

Reeves, W. J. and Fimognari, G. M., 1963: An Improved Method for the Preparation of Crystalline Lactic Dehydrogenase from Hog Heart. J. Biol. Chem. 238(12): 3853-3858.

Ressler, N. and Tuttle, C., 1966: Significance of Sub-bands of Lactic Dehydrogenase Isozymes. Science. 210: 1268-1270.

Rose, R. G. and Wilson, A. C., 1966: Peafowl Lactate Dehydrogenase Problem of Isoenzyme Identification. Science. 153: 1411-1413.

Starkweather, W. H., Spencer, H. H., Schwarz, E. L., and Schock, H. K., 1966: The Electrophoretic Separation of Lactate Dehydrogenase Isoenzymes and their Evaluation in Clinical Medicine. J. Lab. and Clin. Med. 329-343.

Taylor, R. V. and Preston, J. A., 1965: Evaluation of Serum Enzyme Tests and Electrocardiographic Changes in Diagnosis of Acute Myocardial Infarction. Michigan Medicine. 751-755.

Vesell, E. S. and Bearn, A. G., 1958: The Heterogeneity of Lactic and Malic Dehydrogenase. N. Y. Acad. Sci. (Annals) 75: 286-291.

Vesell, E. S. and Philip, John, 1963: Isozymes of Lactic Dehydrogenase: Sequential Alterations during Development. N. Y. Acad. Sci. (Annals): 243-256.

Wacker, W. E. C., Ulmer, D. D. and Vallee, B. L., 1956: Metalloenzymes and Myocardial Infarction. N. E. J. Med. 255(10): 449-456.

Wakim, K. G. and Fleisher, G. A., 1963: The Fate of Enzymes in Body Fluids--An Experimental Study II. Disappearance Rates of Glutamic Oxalacetic Transaminase I under Various Conditions. J. Lab. and Clinical Med. 61(1): 86-97.

Wakim, K. G. and Fleisher, G. A., 1963: The Fate of Enzymes in Body Fluids--An Experimental Study IV. Relationship of the Reticuloendothelial System to Activities and Disappearance Rates of Various Enzymes. J. Lab. and Clin. Med. 61(1): 107-119.

Wright, E. J., Cawley, L. P. and Eberhardt, Lucile, 1966: Clinical Application and Interpretation of the Serum Lactic Dehydrogenase Zymogram. Am. J. Clin. Path. 45(6): 737-745.

Wroblewski, Felix, and LaDue, J. S., 1955: Lactate Dehydrogenase Activity in Blood. P.S.E.B.M., 90: 210-213.

Wroblewski, Felix, 1958: The Mechanisms of Alteration in Lactic Dehydrogenase Activity of Body Fluids. N. Y. Acad. Sci. (Annals), 75: 322-338.

Wroblewski, Felix and Gregory, Kenneth F., 1961: Lactic Dehydrogenase Isozymes and their Distribution in Normal Tissues and Plasma and in Disease States. N. Y. Acad. Sci. (Annals), 94: 912-932.

LACTATE DEHYDROGENASE ISOENZYMES IN THE DOG

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ABSTRACT

Lactate Dehydrogenase (LDH) isoenzymes have been separated by electrophoresis of serum of man, horse, cow, cat, guinea pig, fish, rat, mouse and dog. In man and horse, the LDH electrophoretogram (zymogram) has been helpful in clinical diagnosis because of tissue specificity reflected by changes in distribution of serum isoenzymes. Myocardial infarction, hepatitis, myositis, hemolysis and neoplastic disease produce characteristic changes in the serum zymogram. A study was conducted to determine the normal serum zymogram of the dog, the behavior of isoenzymes in dog serum and to explore the dog as a possible model for investigation of isoenzyme catabolism.

Electrophoretic separation of the LDH isoenzymes was accomplished on cellulose acetate and agar gel. Tissues for enzyme extraction were obtained from healthy dogs immediately following euthanasia with sodium pentabarbitol. The tissues were removed, placed in iced water, weighed, minced, washed in distilled water, suspended in phosphate buffer and sonified. Following centrifugation the supernate was removed and used for assay. Total LDH activity and isoenzymes were measured on the heart, skeletal muscle, liver, spleen, lung, renal cortex, renal medulla, and erythrocyte hemolysate.

The effect of hemolysis on the serum zymogram was studied, as was the comparative disappearance rate of rabbit muscle LDH vs. beef heart LDH and canine heart LDH vs. canine skeletal

muscle LDH from plasma. The effect of reticuloendothelial (RE) blockade on isoenzyme catabolism was also studied.

Hemolysis caused an increase in all 5 LDH isoenzymes although only LDH_1 and LDH_2 were present in erthrocyte hemolysate. Isoenzyme distribution in tissue extracts were similar to those reported for man and horse. In vivo recombination of the isoenzymes was observed in one catabolism study. LDH_5 was catabolized faster than LDH_1. The dog holds promise as a model for LDH isoenzyme catabolism studies because in normal serum, either no LDH is visible or only a trace of LDH_5 is visible.