Tissue Enzymes

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The transaminase enzyme was first discovered by Braunstein and Kritzman in 1938 in pigeon breast muscle. This enzyme was later purified by Cohnen in 1940. In 1941, Cohnen (4) showed that the enzyme transaminase was particularly abundant in cardiac and skeletal muscle. It was this observation that led La Due (13) and his associates to estimate serum levels of transaminase following myocardial infarction with the idea that the necrotic myocardium would be likely to liberate aminopherase into the blood stream. They also discovered that hepatitis and skeletal muscle necrosis were responsible for elevated serum levels of the enzyme.

The first determinations of transaminase levels were tedious and inaccurate until 1951 when Cammarata and Cohnen devised a photometric determination. In 1954 Karmen, Wroblewski and La Due described an accurate, reproducable method employing paper chromatography but this was still a long procedure requiring over 36 hours for one determination. At the same time Karmen (9) and workers described a photometric method that was simple, inexpensive, accurate, extremely reproducable and required one hour to run. The Sigma methods of transaminase determinations are modifications of the Karmen technique and they can be run with any spectrophotometer or any colorimeter which is fitted with a light filter in the range of 490 to 520 m u.

These initial observations made by La Due, Wroblewski and Karmen (13) have been confirmed both in man (3,10,14,15, 18 & 19) and under experimental conditions in animals. (1,9,15,16,18 & 20) It has also been demonstrated that infarctions produced by ligation of the pulmonary, renal, splenic and mesenteric arteries may produce elevated serum transaminase levels.

Most of the work done with tissue enzymes has been with serum glutamic oxaloacetic transaminase (SOG-T) and its importance in myocardial infarcts. As a result of this work, other important enzymes have been discovered such as: serum aldolase (SA), lactic dehydrogenase (LD), serum glutamic pyruvic transaminase (SGP-T), serum malic dehydrogenase (SMD), phosphohexose isomerase (PI), adenosine triphosphatase and lipoprotein lipase. Serum aldolase and lactic dehydrogenase are effective in the diagnosis of myocardial infarction. Serum glutamic pyruvic transaminase is very good in aiding in the diagnosis of hepatitis, but is less effective in the diagnosis of cardiac necrosis (2). More work needs to be done on serum malic dehydrogenase and phosphohexose isomerase before their true value will be known. It was found that adenosine triphosphatase and lipoprotein lipase were of no clinical value (19).

Besides showing elevated serum levels with myocardial infarction, SGO-T shows a similar high serum level with a number of disease conditions in man and animals (12). Some of these conditions are: cardiac arrhythmias with ventricular rates of 160 or more, acute hepatitis, hepatic cirrhosis, obstructive jaundice, 50% of the pancreatic cases, 75% of the jaundice cases, muscle crush injuries, extremity embolus, pulmonary emoblism, surgical trauma, prolonged shock, hemolytic crisis, dermatomyositis and experimental ligation of renal, cardiac, mesenteric, splenic and pulmonary vessels. SGO-T was normal in conditions such as neoplastic diseases, acute febrile and chronic infections, uremia, pulmonary infarctions, stress, metabolic diseases and congenital diseases unless there was heart, liver, muscle or kidney necrosis (13).

Clinical Use of Enzymes

In man the diagnosis of acute myocardial infarction can in most instances be established by a correlation of clinical,

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laboratory and electrocardiagraphic evidence. There is nevertheless a significant number of cases in which an unequivocal diagnosis is not possible. The elevation of serum enzymes following myocardial infarction appears to offer a new objective test for establishing this diagnosis. In certain cases Ostrow (17) and his workers felt it was more useful than the EKG. Two examples were in the left bundle-branch blocks where the electrocardiographic picture was masked or distorted and in the extension of a myocardial infarct. La Due and Wroblewski (12) found a significant correlation between the SGO-T level and the EKG estimated size of the infarct.

In experimental work with dogs, Cornelius *et al.* (5,6,7) and Crawley (8) have done the most work. Cornelius' work indicated that the SGO-T enzyme should be of diagnostic value for canine and feline myocardial necrosis and SGP-T should be of value as an indicator of liver necrosis in both species. Crawley stated that SGO-T was of value in diagnosing cardiac necrosis, but was of no positive value in cases of epicarditis or valvular insufficiency.

The serum enzyme levels were found to rise roughly in proportion to the amount of infarcted tissue while the concentration of the enzyme in the tissue decreased in proportion to the age of the infarct. The enzyme levels rose 2 to 20 times their normal serum levels. The peak of the serum enzyme level was reached in 36 to 48 hours and the level was usually back to normal in three to five days. An infarction of as little as 5 to 10% of the total myocardium or liver was associated with significant rises in transaminase levels.

Serial determinations of serum enzyme levels are the most satisfactory. The first check should be begun as soon as possible after the infarction. Then two samples should be drawn at 12 hour intervals and two more at 24 hour intervals for a total of five samples in 72 hours. If only one test is possible, it should be taken somewhere between 24 and 48 hours after the onset of symptoms.

In clinical cases (11), there did not appear to be any consistent relationship between the enzyme level and the blood sedimentation rate, leucocyte count, protein or fibrinogen content of the serum, location of infarct, use of anticoagulants, digitalis, antibiotics, sedatives or pressor agents. Kattus *et al.* (10) showed that the SGO-T peak was about two days ahead of the temperature, leucocyte count and blood sedimentation rate peaks. La Due and Wroblewski (12) were unable to find any correlation between the height of the SGO-T level and the age, sex, color, weight, temperature, leucocyte count, blood sedimentation rate, presence of shock, blood pressure, presence of heart failure, location of infarct or mortality rate of the patient. They also found that although the brain was high in SGO-T, brain damage failed to cause a serum elevation, probably because of a blood brain barrier.

The enzymes indicate not only the severity of the disease but because of their rapid increase in the serum, they frequently suggest the presence of acute infarction prior to the occurrence of diagnostic electrocardiographic changes and liver function tests. However, they still leave much to be desired since they return to normal levels on about the fifth day of the disease and give no information as to the further course of the illness, unless an extension of the initial infarction occurs (21).

Current Clinical Methods for Analysis of Enzymes

For a veterinarian in practice, a colorimetric test such as the kits made by Sigma* or Omni** can be used. If preferred and available, serum samples can be submitted to a local medical laboratory for enzyme analysis. In either case the blood for these enzyme checks must be drawn carefully to avoid hemolysis which affects the readings. The temperature must be watched closely and the testing should be done at a uniform temperature. The serum can be separated from the cells and stored at refrigerator temperatures for four days before being analyzed. The enzymes are stable at refrigerator temperatures for at least four days and they lose very little activity in 30 days.

* Sigma Chemical Company, 3500 DeKalb Street, St. Louis 18, Missouri.
** Omni Tech, Sun Valley, California.

Normal Canine Enzyme Levels

The units given below were compiled from the blood enzyme level determinations from 25 apparently healthy dogs that were given a physical examination coupled with clinical laboratory tests. The laboratory work consisted of an erythrocyte count, leucocyte count, leucocyte differential count, hematocrit reading, hemoglobin reading, blood sedimentation rate, urine specific gravity, urine pH, urine albumin, urine sugar, urine sediment and methylene blue liver function test.

Employing the Sigma Kit and a number 401 A Lumetron Colorimeter* with a 490 mu light filter, the SGO-T units ranged from 14 to 48 Sigma-Frankel units with an average of 26 units. The SGP-T units ranged from 4 to 30 Sigma-Frankel units with an average of 19 units. With the Omni Test Kit, the SGO-T units ranged from 16 to 45 units with an average of 25 units. The SGP-T units ranged from 6 to 28 units with an average of 18 units. In all of these one unit is equal to the amount of transaminase activity necessary to produce a decrease in the optical density at 340 mu of 0.001 per minute per ml. of serum under the conditions of Karmen (9) at 25 C per cm. of light path.

Summary

As with all other clinical laboratory tests, tissue enzyme levels should only be used as an aid to the diagnosis. Unless the entire picture including the symptoms, history and other laboratory tests are considered, the full value of the tissue enzymes will not be realized. Although there is still much to be understood about tissue enzymes in canine clinical work, it is now known that SGO-T can be of definite value in the clinical diagnosis of myocardial infarction and SGP-T can be useful in the clinical diagnosis of liver infarction.

* Photovolt Corporation, 1115 Broadway, New York 10, N. Y.

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TRICHINOSIS

1962 was the first year since 1956 that no cases of human trichinosis were reported in Iowa. Typically, trichinosis is reported in small groups of cases that have developed in persons who have eaten improperly cooked pork. For example, the 19 cases that were reported in 1961 were all from one source of infection. The offending food was home-made, uncooked, smoked pork sausage. While the rate of trichinosis in hogs is declining, the infection still exists.

Pork is a highly nutritious, tasty food, but it always should be cooked well done before it is eaten.