Evidence for Increased Serum Glutamic Oxalacetic Transaminase (SGO-T) Activity Following Graded Myocardial Infarcts in Dogs

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Elevation in serum concentration of the enzyme, glutamic oxalacetic transaminase, (SGO-T), is an accurate index of myocardial infarction in dogs. The enzyme rises in a rough proportion to the extent of myocardial necrosis. The test is a sensitive guide to myocardial injury. Infarcts less than 1 Gm. in weight result in a significant alteration in serum concentration of the enzyme. Concentrations of the enzyme in infarcted muscle are markedly lower than in normal muscle, suggesting that seepage through damaged cells causes the rise in serum concentration. Experimental and clinical myocardial injury result in similar abnormalities.

GLUTAMIC oxalacetic transaminase (GO-T) has been found to be present in all human sera that have been tested. Comparable concentrations are found whether the more laborious chromatographic method is used or whether the relatively simple and rapid spectrophotometric assay is employed. When serum is added to excesses of aspartic acid and alpha ketoglutaric acid buffered at optimal pH in the presence of coenzyme 1 (DPNH+) and malic dehydrogenase, serum glutamic oxalacetic transaminase can be measured in a spectrophotometer by the decrease in optical density resulting from the oxidation of reduced diphosphopyridine nucleotide (DPNH+) to the oxidized form of the enzyme (DPN). (See fig. 1.) Other workers have been measuring the concentration of glutamic oxalacetic transaminase (GO-T) in various animal tissues and have found the enzyme in high concentration in heart muscle, skeletal muscle, brain, liver, and kidney in decreasing order. This property led us to study serum levels following acute myocardial infarction in human subjects. Serum glutamic oxalacetic transaminase (SGO-T) rises 2 to 20 times above the upper limits of normal within 6 to 72 hours after infarction.4

Dogs subjected to experimental myocardial infarction by injection of plastic microspheres into the coronary circulation invariably exhibit a rise in serum transaminase 6 to 48 hours after injury, which is apparently roughly proportional to the amount of myocardial necrosis. The enzyme has also been found elevated when myocardial necrosis was produced in rabbits by the intravenous injection of papain.5 11 The first of these methods subjects the dogs to prolonged anesthesia and possibly secondary liver damage. In the second, the possibility of the effect of papain upon other transaminase-rich tissues cannot be excluded. Striking elevations in serum glutamic oxalacetic transaminase follow acute liver damage, and lesser rises occur after acute renal or skeletal muscle damage.5 8 In order to exclude serum transaminase elevation due to injury to hepatic, renal or other transaminase sources, we used the method de-
developed by LeRoy and associates for inducing acute myocardial necrosis experimentally.9

The purpose of these studies was to answer the following questions, if possible. 1. Does the serum concentration of glutamic oxalacetic transaminase invariably rise following myocardial necrosis? 2. What is the smallest infarction that would elevate the serum level of transaminase? 3. Will the serum glutamic oxalacetic transaminase rise without infarction but after ischemia? 4. Will the operative procedure employed affect the level in the serum? 5. Is glutamic oxalacetic transaminase released from necrotic muscle or is it produced elsewhere as a nonspecific response? 6. What is the mechanism of serum glutamic oxalacetic transaminase elevation following experimental myocardial infarction?

METHODS

Measurement of Enzyme

The serum levels of glutamic oxalacetic transaminase were analyzed by a spectrophotometric method.1 The reaction is based on a double enzyme system with all substrates present in excess; the limiting factor is the concentration of glutamic oxalacetic transaminase in the serum.

The only actual measurement in the assay of the rate of this reaction is the spectrophotometric analysis of the decrease in optical density as oxidized diphosphopyridine nucleotide (DPN, coenzyme 1) is formed from the reduced enzyme (DPNH+). The rate is dependent on the concentration of serum transaminase. One unit is designated as a change in optical density of .001 per milliliter per minute at wave length 340 m. The normal range is 8 to 40 units per milliliter per minute (fig. 1).

Fig. 1. Shows the chemical reactions involved in measuring the serum glutamic oxalacetic transaminase.

Production of Myocardial Infarction

* The techinic used was that of LeRoy and co-workers.9 Intravenous sodium pentobarbital anesthesia was administered and respiration sustained by intermittent positive pressure oxygen through tracheal intubation. No dog was anesthetized for more than three hours. A left lateral chest incision was made, the fourth rib resected, and nooses of braided silk loosely placed around chosen branches of coronary arteries. The ends of the tie were brought out through stab wounds in the chest at right angles to the artery and buried subcutaneously (fig. 2). The advantage of this method is that the myocardial infarct can be produced at any chosen interval subsequent to the operation at a time when the serum glutamic oxalacetic transaminase has returned to normal limits. In this way we separate the alteration of serum concentration of transaminase which results from surgery from that due to coronary ligation.

Before infarction, the dogs were given morphine sulphate subcutaneously (up to 30 mg; are usually necessary to abolish pain), 0.1 mg of atropine sulphate intravenously per kilogram of body weight and 15 mg of aminophylline intravenously per kilogram of body weight. The appropriate ties were then drawn tight, occluding the coronary artery, and serial venous bloods and electrocardiograms were obtained.* The observations of LeRoy and associates on the diminution of mortality following this premedication were substantially confirmed in our experiments.

Homogenates

Homogenates of infarcted and normal areas of dog heart were prepared in an ice-water bath as soon after death of the animal as possible. When tissues were not homogenized at once, they were quick frozen and stored in dry ice. Homogenization of saline suspensions of minced muscle was complete

* All electrocardiograms were recorded with the Poly-Viso (Sanborn), a direct writing machine, usually at a paper speed of 50 mm. per minute.
RESULTS

Sixteen dogs had 18 operations with an immediate operative mortality of three and a postoperative mortality of two (total 28 per cent). In the 11 remaining dogs 10 infarcts were produced in nine dogs with a mortality of 10 per cent. One dog failed to develop infarction after coronary ligation. One animal (control) had a thoracotomy without disturbing the heart or opening the pericardium.

In the one dog with thoracotomy alone, the serum glutamic oxalacetic transaminase rose to 60 units within 12 hours and fell to normal in four days. In another a second rise up to 40 units occurred on the seventh and eighth days, thereafter falling and remaining below 40 units. The first rise we attribute to skeletal muscle damage at operation with consequent release of transaminase and the second, possibly to liver dysfunction as a result of anesthesia and other causes. These early transaminase elevations were noted following every operation done in this study whether or not the pericardium was entered.

Figure 3 shows the postoperative elevation until the fourth day. On the fifth day the ligature previously placed about the left coronary artery was pulled tight. Five hours later the serum glutamic oxalacetic transaminase was 86 units; at seven hours post ligation the level was 120 units and within 17 hours had risen to 274 units, returning to normal 72 hours after ligation. At autopsy fresh infarction of the anterior wall and anterior portion of the interventricular septum was found. (See fig. 4.)

Figure 5 shows the serum levels of glutamic oxalacetic transaminase following a minute apical infarction. On the first day of the experiment, a “blank” operation (interruption of the pectorals and removal of a rib) was performed. The level rose following operation. On the fourteenth day, the chest was re-entered and ties were loosely placed around a terminal branch of the left anterior descending coronary artery. The level again rose following operation. On the twenty-first day after the original operation, the ligature was pulled tight and an apical myocardial infarct produced. The serum transaminase rose to 120 units, falling to normal limits within 24 hours. The cause of the

![Figure 3](http://circ.ahajournals.org/)

**Fig. 3.** Shows the level of transaminase following placement of a ligature about the left coronary artery and after ligation of this vessel with the resulting myocardial infarction shown in figure 4.

![Figure 4](http://circ.ahajournals.org/)

**Fig. 4.** Infarct produced by procedure described in legend of figure 3.
surprising, considering the duration of infarction, transaminase levels, and that the heart of this dog contained a fresh apical infarct weighing less than 1 Gm. (See fig. 6.)

Table 1 shows the relationship between the size of the myocardial infarct and the peak level of the serum glutamic oxalacetic transaminase as well as the duration of the rise. In general, the larger the size of the myocardial infarct, the higher the maximum rise of serum transaminase and the longer the duration of elevation. That we did not see an absolutely linear relationship between the size of the infarct, as seen at autopsy, and the peak or duration of transaminase abnormality is not surprising, considering the many variables that might influence this curve, i.e., variations in blood volume (size of "diluting medium") in different sized animals, rapidity of necrosis, completeness of necrosis, rate of removal and breakdown of the enzyme, original concentrations of enzyme in the individual normal heart muscle, and other factors. It was unexpected that as close a relationship existed as reported.

Figure 7 describes an unusual experiment.
On day zero a thoracotomy was done and the pericardium opened. This was followed by elevation of the serum glutamic oxalacetic transaminase to 180 units which fell to normal on the eighth day. Then the chest was re-entered through scar tissue and the ligature placed about a main branch of the left coronary artery. No blood samples were obtained for three days following this operation. On day 21 the tie was tightened and the artery completely occluded. The level remained below 40 units for the eight days until the animal was sacrificed. At autopsy, although the ligature was completely occluding the main left circumflex artery, no gross or microscopic evidence of infarction was present.

The electrocardiograms in the upper row (fig. 8) show the ST-T wave changes of pericarditis following the two operations on the heart. The tracing returned to normal before the main left circumflex artery was occluded. The electrocardiograms in the lower row show progressive S-T elevation in lead III with reciprocal depression in V4 and T-wave changes. The tracing returned toward normal at 30 minutes and became completely normal within 45 minutes. The different form of the T wave at two and one-half hours may be attributed to the change in heart rate. No Q wave is seen.

In this experiment it was planned to produce a large posterior myocardial infarction. However, despite the fact that the animal was prepared in the routine fashion and the tie was proved at autopsy to have completely occluded the main trunk of the left circumflex coronary artery, only S-T and T-wave changes were seen on the electrocardiogram which spontaneously

Fig. 8. Shows the electrocardiographic changes seen in the dog whose transaminase levels are described in figure 7.
This table compares the glutamic oxalacetic transaminase content of normal heart muscle with that of necrotic heart muscle of dogs (each dog being his own control) at varying times after infarction. Dog X-14 showed evidence of very early infarction on microscopic examination only, having died two hours following coronary ligation.

<table>
<thead>
<tr>
<th>Dog</th>
<th>Normal Muscle</th>
<th>Infarcted Muscle</th>
<th>Age of Infarct</th>
<th>Ratio Concentration Normal to Infarcted Myocardium</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-49</td>
<td>200,000</td>
<td>—</td>
<td>—</td>
<td>1.3/1</td>
</tr>
<tr>
<td>X-14</td>
<td>305,400</td>
<td>233,400</td>
<td>2 hours</td>
<td>2/1</td>
</tr>
<tr>
<td>X-33</td>
<td>451,000</td>
<td>218,630</td>
<td>20 hours</td>
<td>11/1</td>
</tr>
<tr>
<td>X-110</td>
<td>465,000</td>
<td>23,000</td>
<td>7 days</td>
<td>20/1</td>
</tr>
<tr>
<td>X-79</td>
<td>348,000</td>
<td>33,200</td>
<td>7 days</td>
<td>45/1</td>
</tr>
<tr>
<td>X-2</td>
<td>250,000</td>
<td>5,630</td>
<td>9 days</td>
<td></td>
</tr>
</tbody>
</table>

The reasons why no myocardial infarction was produced are not apparent, unless the atropine-aminophylline premedication completely protected the area of myocardium supplied by this artery. We believe that this combination of findings indicates that a rise in the serum glutamic oxalacetic transaminase may result from leakage of enzyme through severely damaged cell membranes and does not occur after ischemia of short duration.

Transaminase Concentration in Normal and Infarcted Heart Muscle

Further support for the concept that transaminase is released into the blood stream only from a necrotic area of heart muscle is provided by the observations on the concentration of this enzyme in normal and infarcted muscle.

If serum enzyme levels remain normal following reversible functional abnormalities in the heart, one might expect that in situations of irreversible damage (i.e., necrosis) a rise in serum enzyme concentration is the result of loss of this enzyme into the blood stream from the necrotic area. Indirect evidence for this is presented in table 2. It can be seen that there is a much smaller concentration of enzyme in the necrotic heart muscle than in normal heart muscle from the same animal. In addition, it would appear that the older infarcts have released more transaminase than the recent ones, and thus the loss of enzyme is a function of the duration as well as the amount of necrosis. The ratio of enzyme in normal to infarcted muscle as high as 45:1 (dog X-2) is especially striking.

**Discussion**

The serum glutamic oxalacetic transaminase was invariably elevated following myocardial infarction in the dogs studied. The height of the rise of the enzyme in the sera as well as the duration of the rise was roughly proportional to the amount of infarcted heart muscle. These findings were strikingly similar to those reported following transmural myocardial infarction in man. In the latter the height and duration of transaminase activity also appeared to be correlated with the size of the infarct. The sensitivity of the test is attested to by the fact that infarcts less than 1 Gm. in size resulted in significant, but short, elevations of the serum transaminase (dog X-54, X-52 and X-17).

Thoracotomy, per se, results in elevation of the serum glutamic oxalacetic transaminase, presumably by release from damaged pectoral and intercostal muscles, and this must be taken into consideration in the evaluation of the test following major surgery.

Myocardial ischemia of 45 minutes duration seen in dog X-84 who failed to develop infarction after ligation of the left circumflex coronary artery did not result in increased transaminase activity. This is analogous to the usual absence of increased levels following angina pectoris and coronary insufficiency in man associated with reversible S-T and T-wave changes.
These problems are at present under further study.

The fact that the activity of the serum glutamic oxalacetic transaminase in infarcted muscle is only 2 to 10 per cent of that in the normal muscle of the same heart, together with the observation that the transaminase activity in infarcted muscle diminishes proportionately to the age of the infarct, strongly suggests that the mechanism of elevation of transaminase activity is simply one of release of the enzyme into the blood stream following death or increase in cellular membrane permeability. The routes of excretion and degradation of the enzyme are not yet known but are under study.

The spectrophotometric method of assay of serum glutamic oxalacetic transaminase is relatively simple, rapid, and inexpensive. In our hands its accuracy is highly reproducible, and we have used the method to investigate damage of heart and skeletal muscle and liver. It is apparent that a rise in transaminase activity is a useful index of the degree of necrosis of transaminase-rich tissue and does not in any way represent a nonspecific measurement of tissue inflammation.

Conclusions

Myocardial infarction due to ligation of coronary arteries in the dog is invariably followed by an increase in the serum glutamic oxalacetic transaminase activity.

The increase in activity occurs within six hours following ligation and both the degree and duration of enzyme abnormality appear to be proportional to the size of the infarct.

Myocardial ischemia of 45 minutes duration did not influence the serum level of glutamic oxalacetic transaminase.

Since the enzyme activity of infarcted muscle is only 2 to 10 per cent of that of normal muscle, the mechanism of elevation of transaminase activity is probably through release of the enzyme into the blood stream following an increase in the permeability of the injured heart muscle cell.

Acknowledgments

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We are indebted to the technical help of Martin Podgainy, Alfred Friedman and Patricia Van Dawson.

Conclusiones in Interlingua

Infarcimento myocardial causate per ligation del arterias coronari in canes es invariablemente sequite per un augmento del activitate de transaminase oxalacetic glutamic le sero. 

Iste augmento occurre intra 6 horas post le ligation. Le grado e le duration del anormalitate enzymic pare esser proportional al dimensiones del infarcimento.

Ischemia myocardial durante 45 minutas non influentia le nivello de transaminase.

Proque le activate enzymic del musculo infarcte amonta a solo inter 2 e 10 pro cento del norma, le mechanismo del elevation in le activitate de transaminase depende probablemente del augmentate permeabilitate cellular del musculo cardiac e un consequente disbucamiento del enzyma a in le fluxo sanguine.

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