Gamma-Glutamyl-Transpeptidase in Myocardial Infarction
Clinical and Experimental Studies

BY KURT G. RAVENS, M.D., SIGMUNDUR GUDBJARNASON, PH.D., CHARLES M. COWAN, M.D., AND RICHARD J. BING, M.D.

SUMMARY
The activity of γ-glutamyl-transpeptidase was determined serially in patients with acute myocardial infarction up to 32 days after the onset of chest pain. Peak enzyme activity was found in all patients between the eighth and eleventh day after infarction. Some patients showed a rise in serum activity 48 hr after infarction. In 33 mongrel dogs with experimental myocardial infarction, γ-glutamyl-transpeptidase activity was estimated in the homogenate of the normal and necrotic heart muscle as soluble and particle-bound enzyme activity. The changes of free and particle-bound activity showed different patterns. The soluble enzyme activity was highest during the first 4 days and approached normal level after 8 days. Particle-bound activity was significantly decreased after 24 hr and showed thereafter a continuous rise to 10 times the control values 10 days after the coronary artery occlusion.

These studies indicate that several determinations of γ-glutamyl-transpeptidase activity in serum of patients with myocardial infarction may reflect the reparative processes taking place in the infarcted tissue.

Additional Indexing Words:
Lysosomal enzymes

Changes in serum activity of several enzymes such as lactate-dehydrogenase (LDH), glutamate-oxaloacetic acid-transaminase (GOT), glutamate-pyruvic acid-transaminase (GPT), and alpha-hydroxy-butyric acid-dehydrogenase (HBDH) play an important role in the clinical diagnosis of acute myocardial infarction.1-3 These enzyme changes reflect the release of intracellular enzymes from ischemic and necrotic muscle into the serum and are, therefore, most pronounced during the early phase following coronary occlusion. Recently, several investigators4,5 have described changes in the activity of gamma-glutamyl-transpeptidase (γ-GTP) in patients following myocardial infarction. In contrast to the above-mentioned enzymes, γ-GTP appeared to reach maximum activity about the tenth day after coronary artery occlusion. This might be of considerable importance in the diagnosis of myocardial infarction at that late stage.

The purpose of this study was to examine the correlation between the serum changes in the activity of γ-GTP in patients with acute myocardial infarction and the corresponding changes in the activity of this enzyme in necrotic cardiac muscle of dogs with experimental myocardial infarction.

Methods
Serum γ-GTP activity was determined by a method described by Orłowski and Szewczuk.6,7 The synthetic compound γ-glutamyl-β-naphthylamine was used as a substrate and β-naphthylamine liberated during enzyme-incubation was determined after diazotation and conversion to an azo-dye by modification of the Bratton-Marshall
procedure. The amount of $\beta$-naphthylamine was calculated from a standard curve (fig. 1). A unit of enzyme activity is defined as follows:

For serum: $1 \text{ unit} = \frac{\mu\text{mole } \beta\text{-naphthylamine}}{\text{hour} \times 100 \text{ ml of serum}}$

For tissue homogenate: $1 \text{ unit} = \frac{\mu\text{mole } \beta\text{-naphthylamine}}{\text{hour} \times \text{mg of protein}}$

The enzyme assay was performed in the following way: 0.3 ml of physiologic NaCl and 0.5 ml of substrate solution (containing 5 $\mu$moles of $\gamma$-glutamyl-$\beta$-naphthylamine in 0.1 M Tris buffer pH 8.5) were pipetted into test tubes. These were placed in a water bath at 37 C and upon temperature equilibrium 0.2 ml of serum was added. The incubation was carried out for 2 hr, at which time the reaction was stopped by addition of 1.0 ml of 25% trichloroacetic acid (TCA). A blank was prepared in a similar fashion, but the serum was added after addition of TCA. The test tubes were centrifuged and 1.0 ml of the clear supernatant was used for the color reaction. The diazotation was accomplished by adding 1.0 ml of 0.1% sodium nitrite (NaNO$_2$) at room temperature. Three minutes later 1.0 ml of 0.5% ammonium sulfamate was added into the test tubes in order to destroy excess nitrite. The mixture was shaken well and allowed to stand for 2 min before 2.0 ml of the coupling reagent N-(1-naphthyl)-ethylene-diamine-diHCl (0.5% solution in 95% ethanol) was added. The tubes were subsequently placed into a water bath at 37 C. The optical density of the developing azo-dye reached its maximal intensity after 1 hr and was stable for at least 8 hr. The optical density was measured in a spectrophotometer (Beckman DU) against the blank at a wavelength of 578 m$\mu$.

The substrate $\gamma$-glutamyl-$\beta$-naphthylamine* is poorly soluble at pH 8.5. For this reason, it was not possible to prepare a clear solution of 5 $\mu$moles substrate per 0.5 ml of buffer. The experiments were therefore, carried out in a saturated substrate solution. The results obtained were reproducible with increasing enzyme (serum)-concentration in the range from 0 to 0.200 and were linear in relation to optical density (fig. 2). With further increase in enzyme activity this relationship became nonlinear, probably due to an increasing disproportion between substrate utilization and substrate availability. At higher levels of enzyme activity the number of dissolved substrate molecules reacting with the enzyme exceed the number of substrate molecules going into solution per unit of time; the result is a

*Available through Sigma, St. Louis, Missouri.

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TRANSEPTIDASE IN MYOCARDIAL INFARCTION

Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Localization of infarction</th>
<th>Special observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>G.F.</td>
<td>54</td>
<td>M</td>
<td>Inferolateral</td>
<td></td>
</tr>
<tr>
<td>N.A.</td>
<td>55</td>
<td>F</td>
<td>Anterolateral</td>
<td>Shock, congestive heart failure in the beginning</td>
</tr>
<tr>
<td>L.J.</td>
<td>23</td>
<td>M</td>
<td>Anterior wall</td>
<td>Multiple malformations</td>
</tr>
<tr>
<td>J.S.</td>
<td>67</td>
<td>M</td>
<td>Inferior wall</td>
<td></td>
</tr>
<tr>
<td>R.E.</td>
<td>60</td>
<td>F</td>
<td>Posterior wall</td>
<td>Pulmonary edema, congestive heart failure in the beginning</td>
</tr>
<tr>
<td>A.J.</td>
<td>60</td>
<td>F</td>
<td>Posterolateral</td>
<td>Shock, pacemaker temporarily</td>
</tr>
<tr>
<td>J.D.</td>
<td>58</td>
<td>F</td>
<td>Inferolateral</td>
<td></td>
</tr>
<tr>
<td>J.C.</td>
<td>68</td>
<td>M</td>
<td>Inferolateral</td>
<td>Reinfarction after 10 days</td>
</tr>
<tr>
<td>T.M.</td>
<td>75</td>
<td>F</td>
<td>Anteroseptal</td>
<td>Left cerebral infarction</td>
</tr>
<tr>
<td>M.N.</td>
<td>66</td>
<td>M</td>
<td>Posterolateral</td>
<td></td>
</tr>
<tr>
<td>H.J.</td>
<td>58</td>
<td>M</td>
<td>Acute inf.</td>
<td></td>
</tr>
<tr>
<td>R.B.</td>
<td>67</td>
<td>F</td>
<td>Anterolateral</td>
<td></td>
</tr>
<tr>
<td>K.D.</td>
<td>72</td>
<td>F</td>
<td>Subendocardial</td>
<td></td>
</tr>
<tr>
<td>M.A.</td>
<td>81</td>
<td>F</td>
<td>Anteroseptal</td>
<td>Shock</td>
</tr>
<tr>
<td>Z.H.</td>
<td>64</td>
<td>M</td>
<td>Anteroseptal</td>
<td></td>
</tr>
<tr>
<td>B.G.</td>
<td>62</td>
<td>M</td>
<td>Anterolateral</td>
<td></td>
</tr>
<tr>
<td>Z.J.</td>
<td>60</td>
<td>M</td>
<td>Posterior wall</td>
<td></td>
</tr>
<tr>
<td>P.R.</td>
<td>47</td>
<td>M</td>
<td>Posterior wall</td>
<td></td>
</tr>
<tr>
<td>U.F.</td>
<td>47</td>
<td>F</td>
<td>Anteroseptal</td>
<td></td>
</tr>
<tr>
<td>R.P.</td>
<td>62</td>
<td>M</td>
<td>Inferolateral</td>
<td>Shock</td>
</tr>
<tr>
<td>L.L.</td>
<td>43</td>
<td>M</td>
<td>Posterior wall</td>
<td>Reinfarction after 3 wk</td>
</tr>
<tr>
<td>D.B.</td>
<td>47</td>
<td>F</td>
<td>Inferolateral</td>
<td></td>
</tr>
</tbody>
</table>

relative decrease in substrate availability and reaction rate. This difficulty could be avoided by making appropriate serum dilutions. Duplicate determinations were performed for every assay.

Serum levels of γ-GTP activity were determined in 13 normal individuals serving as controls, ranging in age from 25 to 75 years. Serum γ-GTP activity was determined serially in 23 patients with acute myocardial infarction syndrome. There were 13 males (age range, 23 to 67 years) and 10 females (age range, 47 to 81 years) (table 1). Only patients who had definite clinical and laboratory evidence of severe myocardial necrosis were chosen for this study. Patients with definite liver, kidney, pancreatic, or neoplastic diseases, in which elevated serum activity of γ-GTP is known to occur were excluded.

In the experiments with dogs, myocardial infarction was produced in 33 mongrel dogs by ligation of several branches of the left circumflex and descending coronary arteries, as described previously. The dogs were sacrificed at different intervals up to 10 days after the operation.

The heart was quickly removed under barbital anesthesia, placed on crushed ice, and the coronary arteries were perfused for a short period with buffered 0.25 M sucrose solution (pH 7.4) to cleanse the vessels of blood. A piece of necrotic tissue from the infarcted area of the left ventricular wall and a piece of muscle from the nonischemic septum were cut off and weighed (normally between 5 and 8 g). The tissue was minced and homogenized in a Waring blender (for 45 sec) in a solution of 0.25 M sucrose buffered to pH 7.4 with 0.1 M Tris buffer to give a 1:10 dilution of the homogenate. The γ-GTP was estimated in two fractions of the heart muscle homogenate, in other words, the free and particle-bound activity. The homogenate was prepared at 4°C in a cold room. The homogenate was centrifuged at 1,000 x g in order to remove all cell debris and myofibrils. The supernatant was centrifuged at 15,000 x g. The supernatant was called "S-fraction" and the activities of the soluble enzyme were determined in this fraction. The resuspended precipitate of this centrifugation contained the particle-bound enzyme. This fraction was thawed and frozen eight to 10 times in order to release the enzyme from the particles. The fractionation was performed in a Spinco Beckman ultracentrifuge M2. The enzyme activities were determined on the day of preparation. The assay for estimating the γ-GTP in these fractions was the same as described for human
serum. The protein concentration of the enzyme solution did vary between 1.2 mg/ml and 0.8 mg/ml. The relationship between enzyme activity and enzyme-concentration or pH was established for both fractions and confirmed the reproducibility of this enzyme assay. The γ-GTP activity in the fractions of the septum from all animals served as control.

Results

Patients

The patients with acute myocardial infarction examined in this study are listed in Table 1. All patients suffered from severe chest pain prior to admission to the hospital. All had electrocardiographic changes typical of myocardial infarction. There were eight anterior, six posterior, and seven inferior wall infarctions. One patient had a subendocardial wall infarction. The serum activity of glutamate-oxaloacetate-transaminase (GOT) and lactate-dehydrogenase (LDH) showed an increase during the first 48 hr after the acute onset of severe chest pain. The serum activities of these enzymes decreased quickly on subsequent days. After 5 to 7 days the enzyme activities had declined to normal levels in most patients. One patient (P. R.) had high enzyme values even after 10 days. Patient J. C. had another rise in serum enzyme activities after 10 days, which was considered to be due to reinfarction. Two patients had severe congestive heart failure in the early stage after the myocardial infarction (N. A. and R. E., Table 1).

The activity of γ-GTP in serum of normal individuals without myocardial infarction was 19.3 ± 3.6 units (Fig. 3). A marked rise in the serum activity of γ-GTP was seen in 12 patients during the first 48 hr after admission (Fig. 3). There was no evidence that these patients had experienced another episode of severe chest pain several days before hospital admission. Figures 3 and 4 show that 4 days after coronary occlusion, a further rise in the activity of γ-GTP occurred in the serum of all patients, regardless of whether they had an initial increase in serum levels; maximum activity was observed between the eighth and eleventh days after coronary occlusion (average, 10 days). Thereafter the enzyme activity declined gradually in the serum. Nine patients had values two to fourfold higher than normal even after 20 days. In two patients, (J. C. and J. S., Table 1), we could detect elevated serum activity even after 32 days. In two patients (R. E. and N. A., Table 1), with severe congestive heart failure and palpable liver, an initial increase of the γ-GTP activity was followed by a slight decrease which coincided with clinical improvement of heart failure. (Table 1).
The different patterns of the changes in the serum activity of LDH, GOT, and γ-GTP in patients with acute myocardial infarction.

failure. Thereafter the typical late elevation with maximum activity about the tenth day after myocardial infarction was observed (fig. 5). No correlation could be seen between the increase of serum γ-GTP levels and the patient's age, sex, or the localization of myocardial necrosis. There was also no correlation between the duration of chest pain and the supposed extent of the area of myocardial infarction.

Experimental Animals

The changes in activity of free and particle-bound γ-GTP in the homogenate of canine myocardiums following coronary occlusion are illustrated in figure 6. The activity of free, nonparticle-bound γ-GTP (S-fraction) was $0.18 \times 10^{-2} \pm 0.03 \times 10^{-2}$ units in noninfarcted muscle. The particle-bound enzyme activity was $0.38 \times 10^{-2} \pm 0.1 \times 10^{-2}$ units which was statistically significantly higher (table 2). These values were determined in the nonischemic septum of dogs with myocardial infarction. In a control study, the γ-GTP activity in the septum was compared to that of the normal left ventricular muscle. The γ-GTP activity in the left ventricular muscle was on the average slightly higher than in the septum, but this difference was not statistically significant (table 2). As shown in figure 6 and table 2 there was an immediate increase in the activity on the second day. Thereafter, the free activity declined slowly and approached normal values after 8 days. The activities of particle-bound γ-GTP changed markedly following coronary occlusion (fig. 6, table 2). After 24 hr, there was a decrease to 53.4% of control values ($P < 0.001$) followed by a rapid rise in activity. After 2 days, the activity of particle-bound γ-GTP was fourfold higher in the infarcted area than in the control tissue. Two to six days after coronary occlusion, the particle-bound activity in the infarcted area increased at a relatively slow rate followed by a rapid rise in activity. Ten days after coronary artery occlusion the activity of γ-GTP had increased ten fold in the necrotic area compared to the normal tissue (fig. 6).

Changes in the serum activity of LDH, GOT, and γ-GTP in acute myocardial infarction with heart failure and liver enlargement (patient N.A.). The initial high γ-GTP activity declined temporarily after clinical improvement of heart failure.
Table 2

Activity of γ-Glutamyl-Transpeptidase (γ-GTP) in Normal and Necrotic Heart Muscle

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>SA × 10⁻²</th>
<th>SE × 10⁻²</th>
<th>% of control (septum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Normal heart muscle of the septum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free enzyme (S-fraction)</td>
<td>14</td>
<td>0.18</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Particle-bound enzyme (P-fraction)</td>
<td>14</td>
<td>0.38</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>B. Normal heart muscle of the left ventricular wall</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free enzyme (S-fraction)</td>
<td>5</td>
<td>0.20</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Particle-bound enzyme (P-fraction)</td>
<td>5</td>
<td>0.41</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>C. Necrotic muscle of the left ventricular wall (see below)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: N = number of experiments; SA = specific activity (mean value) in units—
μ mole β-naphthylamine

hour × mg protein; SE = standard error.

* Level P < 0.001.
† Level P < 0.05.
‡ Not significant.

The serum activity of γ-GTP could not be related to the tissue levels of heart muscle in these dogs with surgical myocardial infarction; this is because of the extensive thoracotomy wound and tissue damage which also cause a rise in serum activity of the enzyme.

Discussion

This study correlated the serum activity of γ-glutamyl-transpeptidase (γ-GTP) with the clinical course of acute myocardial infarction. In addition, the behavior of γ-GTP activity in the homogenate of the myocardium of dogs with experimental myocardial infarction was studied.

Our normal values of γ-GTP activity in serum as well as the range of the increase of enzyme activity after myocardial infarction are in agreement with the data reported by Agostoni and associates⁴ and Hedworth-Whitty and co-workers.⁵ Direct comparison with the work of Goldberg’s group⁶ is not possible because of the different substrate, N(DL-α-glutamyl) aniline, used. The changes in serum γ-GTP activity in patients with acute myocardial infarction differ markedly from those observed in serum lactate-dehydrogenase (LDH) and glutamate-oxaloacetate-transaminase (GOT) which reach a maximum during the first few days after infarction.¹ ²

In acute myocardial infarction, the activity of γ-GTP increased slowly reaching maximum activity 10 days after the onset of chest pain (fig. 7) followed by electrocardiographic changes, compatible with myocardial necrosis. About 50% of our patients showed a considerable rise of serum enzyme activity during the first 48 hr after the acute event. This observation is in accord with the findings of Hedworth-Whitty and associates.⁶ On the other hand, Agostoni and co-workers⁴ found no increase of γ-GTP activity in the first 4 days after acute myocardial infarction. It is likely that this early increase might be due in part to enzyme release from the liver, which is known to contain high amounts of γ-GTP.¹¹ ¹²
Activity of free and particle-bound γ-GTP in the infarcted heart muscle of dogs with experimental myocardial infarction.

It could be demonstrated in two patients (fig. 5) with initial heart failure and liver engorge-ment that after clinical improvement of cardiac performance, the enzyme values declined temporarily, followed by the usual rise of γ-GTP activity about the tenth day after onset of myocardial infarction.

Goldberg's group9 reported high serum activity of γ-GTP in several diseases, such as neoplastic hepatobiliary tract and pancreatic disorders. Villa and associates11 and Szczeklik and co-workers12 made similar observations in liver diseases. Our preliminary data indicate that sequential determination of changes in γ-GTP activity in serum of patients with acute myocardial infarction may detect maximal activity 10 days after the onset of myocardial necrosis.

The changes in the activity of γ-GTP in the serum of patients with myocardial infarction can be explained by the reported alterations in the activity of γ-GTP in necrotic heart muscle of animals with experimental myocardial infarction. As demonstrated in figure 6, there is an early rise of free γ-GTP which is probably due to a release of particle-bound enzymes into the cytoplasmic compartments of the myocardial cell. This is reflected by a temporary decrease in the activity of the particle-bound enzyme in the heart muscle 1 day after coronary occlusion. Thereafter the particle-bound enzyme increases continuously. It is possible that the decrease of free enzyme activity in heart muscle after 4 days is due to a washout of soluble enzyme, whereas the particle-bound fraction is not released. This would explain the late increase of γ-GTP in serum of patients with myocardial infarction.

The results show that most of the γ-GTP is particle bound and can be released by conditions which destroy or damage subcellular particle membranes of the lysosomes;13 the enzyme γ-GTP appears to belong to the lysosomal enzymes. Most of these are acid hydrolases bound to subcellular organelles.13, 14 The enzyme γ-GTP does not belong to the group of acid hydrolases, but catalyzes the transfer of glutamyl residue from one peptide to another. Studies with purified γ-GTP have

**Figure 6**

Changes in the serum activity of LDH, GOT, and γ-GTP in a patient (M.N.) with acute myocardial infarction without palpable liver enlargement.
indicated that the substrate glutamyl-β-naphthylamine can also serve as an acceptor for γ-glutamyl, which is split from another substrate molecule. This led to the conclusion that the specificity of the enzyme is only dependent upon the glutamyl residue.

Recently, Kottmeier and Wheat have demonstrated the presence of lysosomes in human and dog heart muscle by electronmicroscopic studies. Ricciutti's group examined early changes in enzyme activity of acid hydrolyases in the homogenate of canine myocardium after coronary artery occlusion. The changes in the activity of acid phosphatase reported by Ricciutti's group during the early phase of myocardial infarction are similar to the changes of γ-GTP activity found in this study.

The subsequent late increase in particle-bound γ-GTP might be attributed to the infiltration of leukocytes followed by proliferation of mesenchymal cells. These cells are rich in lysosomes and have an important function in the removal of necrotic muscle and tissue repair.

The changes in serum γ-GTP in acute myocardial infarction reported in this study characterize γ-GTP as an enzyme which reflects the reactive reparative processes in necrotic myocardium, in contrast to the enzymes (LDH and GOT), which indicate acute myocardial cell necrosis.

References

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