Myocardial Healing and Repair after Experimental Infarction in the Rabbit


SUMMARY. Adequacy of healing after acute myocardial infarction may determine the incidence of postmyocardial infarction rupture and ventricular aneurysm. Accordingly, in 36 rabbits, from 1 to 8 days after coronary ligation, and in 18 shams, we measured collagen formation and mechanical resistance of the infarcted left ventricle to stretch and rupture. Prolyl hydroxyxylanase, an intracellular enzyme of collagen synthesis, increased from control activity of 3970 ± 431 to 9224 ± 643 counts/min per mg (cpm/mg) extractable protein (P < 0.01) at 48 hours and was nearly maximal at 3 days postmyocardial infarction (14518 ± 2030 cpm/mg; P < 0.01). Lysyl oxidase, an extracellular collagen cross-linking enzyme, increased from control activity of 29.6 ± 4.8 to 74.7 ± 18.8 cpm/mg extractable protein (P < 0.01) at 72 hours and peaked at 121.5 ± 7.3 (P < 0.01) 4–6 days postmyocardial infarction. Hydroxyproline, a measure of collagen content, increased from control of 2.8 ± 0.2 to 5.3 ± 0.6 mg/g dry weight (P < 0.05) at 72 hours and continued to increase at 8 days postmyocardial infarction (14.5 ± 1.7 mg/g dry weight; P < 0.01). When enzyme activities and hydroxyproline content were expressed relative to other reference bases, including DNA, tissue protein, dry weight, and total left ventricle, similar results were obtained. The mechanical properties of the infarcted left ventricle were determined by filling a balloon in the excised left ventricle until rupture. The rupture threshold in the normal left ventricle, [664 ± 43 mm Hg (n = 16)], was not significantly different from that of the infarcted left ventricle on days 1–8 postmyocardial infarction. However, left ventricular rupture occurred more often through the myocardial infarction on days 1–4 postmyocardial infarction (59%) than on days 6 and 8 (18%; P = 0.03) when collagen content had significantly increased. Wall stress at the point of rupture in left ventricles from shams and normals was 30 ± 2 g/mm²; tensile strength in isolated left ventricle muscle strips was 25 ± 4 g/mm² and in isolated scar strips at 7 days postmyocardial infarction was 59 ± 7 g/mm². The passive stiffness of the infarcted left ventricle increased from control of 61 ± 5 to 94 ± 6 mm Hg/100 μL (P < 0.05) at 4 days and 100 ± 7 mm Hg/100 μL (P < 0.01) at 6 days postmyocardial infarction. Stiffness correlated with hydroxyproline content over the 8 days postmyocardial infarction (r = 0.599; P < 0.001). Thus, the acutely infarcted ventricle was highly resistant to rupture during the initial 48 hours postmyocardial infarction, before any increase in collagen occurred. This result suggests that the preinfarction collagen content has an important role in preventing rupture. After 72 hours postmyocardial infarction, collagen synthesis appeared to be a determinant of infarct stiffness and resistance of the infarcted ventricle to rupture. (Circ Res 53: 378–388, 1983)
few studies have addressed the post-MI healing process itself, despite its potential importance.

Accordingly, this study was designed to investigate the natural history of the reparative processes following experimentally induced MI in terms of both biochemical parameters of collagen synthesis and mechanical measures of resistance of the infarcted ventricle to stretch and rupture.

**Methods**

New Zealand albino male rabbits weighing 1.2—2.0 kg, received a uniform laboratory diet of Purina Rabbit Chow and water ad libitum. The animals underwent thoracotomy and coronary artery ligation to induce MI. At days 1, 2, 3, 4, 6, and 8 post-MI, different groups were killed and their infarcted left ventricles (LVs) were assessed for parameters of collagen metabolism and scar stiffness and strength. Six animals were studied at each post-MI interval.

In another group of New Zealand albino male rabbits subjected to coronary ligation and killed at 7 days post-MI, determinations were made of the stress required for rupture of excised post-MI scar strips. In addition, the stress required for rupture of isolated LV muscle strips from LVs of animals not subjected to surgery was also measured.

Animals (n = 52) underwent tracheostomy, thoracotomy, and coronary ligation according to the following technique. The animals were anesthetized with an intravenous dose of 54 ± 2 mg/kg of sodium thiopental, the trachea was exposed, the chest wall opened, and 2-0 silk purse-string sutures were placed through the muscular layers of the chest to allow rapid closure following ligation. The trachea was intubated with a 14-gauge Angiocath (The Deseret Company), and the animal was placed on a Harvard Apparatus Small Animal Respirator. Using an intercostal approach, we opened the chest cavity, incised the pericardium, and ligated the large marginal branch of the circumflex coronary artery with 4-0 silk halfway between the circumference of the heart below the mitral valve ring and outflow tract (Fig. 1). A measurement of infarct size on the epicardial surface was made with 0.25 ml in the balloon. The balloon was filled at a constant rate (4.3 ml/min) with a syringe pump (Harvard Instrument Company) until LV rupture occurred. The passive pressure-volume relationship was recorded using an Electronics For Medicine oscillographic recorder and Statham P23Db pressure transducer. The heart was kept at 4°C during balloon filling.

The rupture threshold was defined as the LV pressure at which rupture occurred. The slope of the linear region of the LV filling curve (between 100 and 400 mm Hg) was used as an index of passive stiffness (Fig. 1). LV stress at rupture was calculated in LVs from shams and normals, assuming that the geometry of the LV was approximated by that of a thin-walled sphere (Mitsky, 1979) according to the following formula:

\[
\sigma_v = \frac{pR^2}{h} \left( \frac{2R_1 + h}{2R_1} \right)
\]

where \( \sigma_v \) is the wall stress in the meridional direction, \( R \) is the internal radius, \( p \) is the intraventricular pressure, and \( h \) is the LV wall thickness at rupture. At rupture, the trachea may be assumed to be relatively thin, that is \( h/R \ll 1 \), and thus, \( \sigma_v = pR/2h \) where \( R \) is the midwall radius.

The tensile strengths of LV muscle strips from normal rabbits (n = 6) and of excised scar strips from rabbits 7 days post-MI (n = 5) were directly measured.

Strips of muscle with initial unstressed dimensions 9.3 ± 1.2 mm long × 3.5 ± 0.4 mm thick × 3.0 ± 0.2 mm wide and strips of scar with initial dimensions 7.9 ± 1.1 mm long × 2.4 ± 0.3 mm thick × 3.6 ± 0.5 mm wide were excised in the meridional direction from the LV of rabbits immediately after sacrifice. Each excised strip was placed in oxygenated Krebs-Henseleit solution, and the width and thickness were measured at the base, midpoint, and apex with a Vernier caliper, and the average cross-sectional area (CSA) calculated. The strip then was clamped at the base and apex, mounted on an Instron tensile strength-testing apparatus, and initial length (l) was measured. Each specimen was stretched at a constant rate (50 mm/min) with continuous monitoring of passive tension until the point of rupture. The average cross-sectional area at rupture (CSAr) was calculated, assuming that cross-sectional area was inversely proportional to length, so that:

\[
CSA = CSA \times l/l_r
\]

where \( l_r \) is length at rupture. Tensile strength (TS) then was determined as \( TS = F/CSA \), where \( F \) is the force (g) at rupture.

**Biochemical Assays**

After the balloon-filling experiment was completed, the infarct area (determined by visual inspection of the endocardial surface) and a sample of distant noninfarcted LV were excised, placed on ice, and treated by the following methods.

All infarcts and post-MI scars were located in the lower anterior-lateral wall and involved the apex of the heart. Control tissue samples consisted of myocardial specimens taken from a distant normal-appearing location in the infarcted hearts. Additional controls consisted of low anterolateral wall specimens taken from normal rabbits and from rabbits subjected to sham coronary ligation.
Cardiac tissue was homogenized 3 × 12 seconds at 1500 rpm with a Tekmar model SDT Tissumizer homogenizer in 0.016 M potassium phosphate, 0.15 M sodium chloride, pH 7.7, using 3 ml of this buffer per gram of tissue wet weight. An aliquot of homogenate was removed and lyophilized for subsequent assays of hydroxyproline, DNA, and protein. The remaining homogenate was centrifuged at 15,000 g for 20 minutes. The supernatant (phosphate extract) was saved for assays of prolyl hydroxylase and lysyl oxidase activities and protein content. The pellet was resuspended in 4 M urea, 0.016 M potassium phosphate, 0.15 M sodium chloride, pH 7.7, using 2 ml of this buffer per gram of tissue wet weight, homogenized 3 × 9 seconds; after 35 minutes (to allow extraction of lysyl oxidase from the pellet by urea buffer), it was centrifuged again. The urea soluble supernatants obtained after centrifugation were dialyzed against 0.1 M sodium borate, 0.15 M sodium chloride, pH 8, overnight to remove the urea before assaying for lysyl oxidase activity.

Lysyl oxidase activity was quantified by the standard tritium release assay, using the insoluble chick aorta substrate (Pinnell and Martin, 1968). Two hundred thousand cpm of substrate were added to 0.1 M sodium borate, 0.15 M sodium chloride buffer, pH 8, and 0.25 ml of phosphate extract or dialyzed urea extract bringing the total volume to 0.75 ml. Assays were incubated at 45°C for 3 hours and stopped with the addition of 50 μl of 100% trichloroacetic acid. All lysyl oxidase activities were reported as the sum of activities from phosphate extract and dialyzed urea extract and were fully inhibited by 10^−4 M β-amino-propionitrile, a specific blocking agent (Pinnell and Martin, 1968).

Prolyl hydroxylase activity was quantified in the phosphate extract by the tritium release assay using collagen labeled with L-(4-3H(N))-proline prepared from embryonic chick calvaria (Hutton et al., 1966).

Hydroxyproline content was determined in lyophilized tissue which was solubilized in 0.1 N sodium hydroxide and then hydrolyzed in 6 M hydrochloric acid. Hydroxyproline, thus liberated, was measured colorimetrically with chloramine-T and Ehrlich's B reagent (Bergman and Lokey, 1969).

Determinations of protein were done using the Folin phenol reagent (Lowry et al., 1951).

DNA concentration was determined by the method described by Burton (1967), using calf thymus DNA as standard.

Statistical analyses were carried out on the Boston University IBM 370/158 time-shared computer, using analysis of variance (ANOVA) in a factorial design (Winer, 1962) and one-way ANOVA (Afifi and Azen, 1972), followed by Neuman-Keuls testing (Sokal and Rohlf, 1969) for infarct surface area, and infarct and LV weights, and by multiple comparisons testing with a control correcting for unequal size of control and treatment groups (Dunnett, 1964) for prolyl hydroxylase and lysyl oxidase activities, hydroxyproline content, DNA content, protein content, rupture threshold, and passive stiffness determinations.

To determine whether there was a significant difference in the site of ventricular rupture on days 1–4 vs. 6–8, we
Prolyl hydroxylase activity (Fig. 2) in the sham-operated controls did not vary throughout the 8 days post-surgery and was not statistically different from that of normals plotted at day 0 in Figure 2 or that of the noninfarcted LV region of ligated hearts. For statistical analysis, controls consisted of tissue taken from the apical anterolateral region in the combined group of sham-operated hearts from days 1-8 post-sham ligation (n = 18); the values from normal hearts (n = 4) and from the noninfarcted region of the ligated hearts were excluded. Prolyl hydroxylase activity in the infarct region expressed on the basis of total phosphate buffer extractable protein (Fig. 2, lower panel) increased significantly by 48 hours post-MI (control: 3970 ± 431; infarct: 9224 ± 643 cpm/mg extractable protein, P < 0.01), and was nearly maximal at 3 days post-MI (14,518 ± 2030 cpm/mg, P < 0.01), when it was three and a half times greater than that of the control group. This profile of the time course of prolyl hydroxylase activity was essentially the same regardless of whether enzyme activity was expressed per mg total phosphate buffer extractable protein (Fig. 2, lower panel), per mg tissue protein, per mg dry weight, or per µg DNA (Fig. 2, middle panel). In addition, total prolyl hydroxylase activity (Fig. 2, upper panel), computed per LV (cpm/LV), was elevated by 48 hours post-MI (P < 0.05 vs. sham-operated controls), maximal at 3 days post-MI (P < 0.01 vs. controls), and remained significantly elevated for the remainder of the 8-day post-MI period. There was no significant variation in LV dry weight (DW) throughout the 8 days post-thoracotomy (Fig. 3, upper panel). Tissue total protein in the infarct region (Fig. 3, lower panel) did not differ significantly compared with controls. Lower panel: post-MI prolyl hydroxylase activity per milligram of phosphate-extractable protein. Experimental protocol and groups are the same as in middle panel. employed the Fisher's exact probability test (Sokal and Rohlf, 1981a). Simple linear regression analysis (Sokal and Rohlf, 1981b) was used to correlate collagen content and passive stiffness. Results were expressed as the mean ± 1 SE.

Results

A total of 52 animals was subjected to coronary ligation and 26 to sham ligation; 5 ligated and 3 shams died at surgery; 5 ligated and 3 shams died after surgery but before the date of intended sacrifice; 5 ligated animals were excluded due to lack of visible infarctions; one ligated and one sham were excluded because of technical errors in assays; and one sham was excluded because of adhesions. The results of the remaining 36 ligated animals and 18 shams are reported. Gross examination of ligated LVs at the time of sacrifice revealed transmural Mls which were well defined even at one day post-MI.

Biochemical Studies

Prolyl hydroxylase activity (Fig. 2) in the sham-operated controls did not vary throughout the 8 days post-surgery and was not statistically different from that of normals plotted at day 0 in Figure 2 or that of the noninfarcted LV region of ligated hearts. For statistical analysis, controls consisted of tissue taken from the apical anterolateral region in the combined group of sham-operated hearts from days 1-8 post-sham ligation (n = 18); the values from normal hearts (n = 4) and from the noninfarcted region of the ligated hearts were excluded. Prolyl hydroxylase activity in the infarct region expressed on the basis of total phosphate buffer extractable protein (Fig. 2, lower panel) increased significantly by 48 hours post-MI (control: 3970 ± 431; infarct: 9224 ± 643 cpm/mg extractable protein, P < 0.01), and was nearly maximal at 3 days post-MI (14,518 ± 2030 cpm/mg, P < 0.01), when it was three and a half times greater than that of the control group. This profile of the time course of prolyl hydroxylase activity was essentially the same regardless of whether enzyme activity was expressed per mg total phosphate buffer extractable protein (Fig. 2, lower panel), per mg tissue protein, per mg dry weight, or per µg DNA (Fig. 2, middle panel). In addition, total prolyl hydroxylase activity (Fig. 2, upper panel), computed per LV (cpm/LV), was elevated by 48 hours post-MI (P < 0.05 vs. sham-operated controls), maximal at 3 days post-MI (P < 0.01 vs. controls), and remained significantly elevated for the remainder of the 8-day post-MI period. There was no significant variation in LV dry weight (DW) throughout the 8 days post-thoracotomy (Fig. 3, upper panel). Tissue total protein in the infarct region (Fig. 3, lower panel) did not differ significantly compared with controls. Lower panel: post-MI prolyl hydroxylase activity per milligram of phosphate-extractable protein. Experimental protocol and groups are the same as in middle panel.
### FIGURE 3.

**Upper panel:** LV weights post-MI. Total LV wet and dry weights were determined in hearts from rabbits subjected to coronary ligation (●) and from normals (n = 2) and sham-operated controls (n = 18) (○). Each point from ligated animals represents the mean ± se of the values obtained from five to six hearts. Each point from shams represents the mean ± se of the values obtained from three hearts.

**Middle panel:** DNA content post-MI. DNA content was determined in the infarct region (●) and in a noninfarcted region (▲) of LV of hearts from rabbits subjected to coronary ligation and killed at intervals post-MI. Each point represents the mean ± se of the values obtained from five to six hearts. In addition, the DNA content of normals (n = 2) and sham-operated animals was determined in low anterior-lateral LV wall specimens (○), each point representing mean ± se of the values obtained from three hearts. **P < 0.01 compared with combined group of shams, excluding normals.**

**Lower panel:** infarct region protein content post-MI. Infarct region protein content was determined in hearts from rabbits subjected to coronary ligation (●) and from sham-operated controls (○). Total collagen (hydroxyproline × 7.46) was also determined in the infarct region of hearts subjected to coronary ligation (●) and in low anterior-lateral LV wall specimens from shams (○). Non-collagen protein in infarct region of hearts subjected to coronary ligation (▲) and in low anterior-lateral LV wall specimens of shams (○) was calculated as the difference between total protein and collagen. Each point from ligated animals represents the mean ± se of the values obtained from five to six hearts. Each point from shams represents the mean ± se of the values obtained from three hearts. **P < 0.05 compared with combined group of shams, excluding normals.**

### FIGURE 4.

**Upper panel:** total LV lysyl oxidase activity post-MI. Total LV lysyl oxidase activity was determined in hearts from rabbits subjected to coronary ligation (●), from normals (n = 2), and from sham-operated controls (n = 18) (○). Each point from ligated animals represents the mean ± se of the values obtained from five to six hearts. Each point from shams represents the mean ± se of the values obtained from three hearts. *P < 0.05 compared with combined group of shams, excluding normals.**

**Middle panel:** post-MI lysyl oxidase activity per milligram of tissue DNA. Lysyl oxidase activity was determined in the infarct region (●) and in a noninfarcted LV region (▲) of hearts from rabbits subjected to coronary ligation and killed at intervals post-MI. Each point represents the mean ± se of the values obtained from five to six hearts. In addition, the lysyl oxidase activity of normals (n = 2) and sham-operated animals was determined in low anterolateral LV wall specimens (○) with each point representing mean ± se of the values obtained from three sham-operated hearts. **P < 0.01, *P < 0.05 compared with combined group of shams (n = 18, as defined in legend to Fig. 2, middle panel).**

**Lower panel:** post-MI lysyl oxidase activity per milligram of extractable protein. Experimental protocol and groups are the same as in middle panel. *P < 0.05, **P < 0.01 compared with combined group of shams (n = 18, as defined in legend to Fig. 2, middle panel).
between ligated and controls (n = 18, as defined in legend to Fig. 2), except for day 1 (day 1: 0.54 ± 0.04; controls: 0.46 ± 0.01 mg/mg DW, P < 0.05). DNA content (Fig. 3, middle panel) was higher in the infarct region of ligated hearts than controls (n = 18, as defined in legend to Fig. 2) at days 1, 3, and 4 post-MI (day 1: 1.9 ± 0.2; day 3: 2.1 ± 0.1; day 4: 1.9 ± 0.1 vs. controls: 1.4 ± 0.1 µg/g DW, P < 0.01) and did not differ on the remaining days. No significant differences were noted in extractable protein between infarct areas of ligated vs. controls.

Lysyl oxidase activity (Fig. 4) in the sham-operated controls did not vary with time after surgery and was not statistically different from that of normals (n = 2) or from that of the noninfarcted LV region of ligated hearts. Lysyl oxidase activity in the infarct region increased significantly relative to the controls (n = 18, as defined in legend to Fig. 2) at 72 hours post-MI (control: 29.6 ± 4.8; infarct: 74.7 ± 18.8 cpm/mg extractable protein, P < 0.01) and peaked at 4–6 days post-MI (121.5 ± 7.3 cpm/mg, P < 0.01).

This profile of time course of lysyl oxidase activity was essentially the same whether expressed on the basis of total extractable (urea and phosphate buffer) protein (Fig. 4, lower panel), tissue protein, DW, or DNA (Fig. 4, middle panel), with increases noted at 72 hours post-MI (P < 0.05 vs. controls) and peak at 4–6 days post-MI (P < 0.01 vs. controls). The pattern of elevation of total LV lysyl oxidase activity (Fig. 4, upper panel) was similar to that noted above, but reached a level of statistical significance only at 6 days post-MI (P < 0.05 vs. controls).

Hydroxyproline content (Fig. 5) in the sham-operated controls also did not vary with time after surgery and was not statistically different from that of normals (n = 2) or the noninfarcted LV region of ligated hearts. Hydroxyproline content in the infarct region (Fig. 5, lower panel) increased significantly at 72 hours post-MI compared with controls (n = 18) as defined in legend to Figure 2 (control: 2.8 ± 0.2; infarct: 5.3 ± 0.5 mg/g DW, P < 0.05) and appeared to continue to increase at 8 days post-MI (14.5 ± 1.7 mg/g DW, P < 0.01). Hydroxyproline content of the total LV (Fig. 5, upper panel) also increased with time post-MI, and was increased compared with controls (n = 18, as defined in legend to Fig. 2) by 4 days post-MI (control: 2.5 ± 0.2; infarct: 3.9 ± 0.4 mg/g DW, P < 0.05).

When hydroxyproline was expressed on the basis of DNA (Fig. 5, middle panel) and tissue protein, similar patterns of increases in content were noted, with the exception that statistical significance was first attained at 4 rather than 3 days post-MI.

**Mechanical Studies**

Rupture threshold in the intact LV (Fig. 6) was several-fold greater than physiological LV systolic pressure and did not differ significantly between sham-operated controls and animals subjected to coronary ligation. However, the site of ventricular rupture threshold in the intact LV (Fig. 6) was several-fold greater than physiological LV systolic pressure and did not differ significantly between sham-operated controls and animals subjected to coronary ligation. However, the site of ventricular

**FIGURE 5.** Upper panel: total LV hydroxyproline content post-MI. Total LV hydroxyproline content was determined in hearts from rabbits subjected to coronary ligation (O), from normals (n = 2), and from sham-operated controls (n = 18) (O). Each point from ligated animals represents the mean ± se of the values obtained from five to six hearts. Each point from shams represents the mean ± se of the values obtained from three hearts. *P < 0.05, **P < 0.01 compared with combined groups of shams, excluding normals. Middle panel: post-MI infarct region hydroxyproline content per milligram of DNA. Hydroxyproline content was determined in the infarct region (•) and in a noninfarcted LV region (△) of hearts from rabbits subjected to coronary ligation and killed at intervals post-MI. Each point from ligated animals represents the mean ± se of the values obtained from six hearts. Each point from shams represents the mean ± se of the values obtained from three hearts. *P < 0.05, **P < 0.01 compared with combined group of shams (n = 18, as defined in legend to Fig 2, middle panel). Lower panel: post-MI infarct region hydroxyproline content per gram dry weight. Experimental protocol and groups are the same as in middle panel.
rupture was related to the interval between infarction and the time the ventricle was subjected to progressive balloon filling (Table 1). The sites of LV rupture before marked increases in total LV hydroxyproline content occurred (days 1–4) were compared with those at days 6 and 8. Rupture through the infarcted area (center or border of infarct) occurred in 59% of ligated animals at days 1 to 4 post-MI and in only 18% at days 6 and 8 post-MI (P = 0.03).

Mean passive stiffness (Fig. 6) of the entire group of infarcted LVs averaged over 8 days post-MI (79 ± 4 mm Hg/100 μl) differed significantly from the combined group of all shams (61 ± 5 mm Hg/100 μl, P < 0.005).

Passive stiffness increased significantly at days 4 (94 ± 6 mm Hg/100 μl, P < 0.05) and 6 post-MI (110 ± 7 mm Hg/100 μl (P < 0.01 vs. the combined group of all shams). Stiffness correlated with hydroxyproline content over the 8 days post-MI (r = 0.599, P < 0.001, Fig. 7).

LV wall stress at the moment of rupture, calculated from balloon inflation experiments in 18 sham-operated animals and two normals, was 30 ± 2 g/mm². Tensile strength of the isolated normal LV muscle strips from six hearts was 25 ± 4 g/mm² and in isolated post-MI scar strips from five ligated rabbits at 7 days post-MI it was 59 ± 7 g/mm².

**TABLE 1**

<table>
<thead>
<tr>
<th>Site of Rupture with Progressive LV Balloon Filling</th>
<th>Infarct center or border*</th>
<th>Distant from infarct†</th>
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<tr>
<td>Days post-MI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–4</td>
<td>13/22 (59%)</td>
<td>9/22 (41%)</td>
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<tr>
<td>6–8</td>
<td>2/11 (18%)</td>
<td>9/11 (82%)</td>
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</tbody>
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* Number of hearts rupturing at the infarct center or border divided by total number of hearts studied. The numbers in parentheses indicate percent of hearts tested which ruptured at infarct center or border.

† Number of hearts rupturing through an area distant from infarct divided by total number of hearts studied. The numbers in parentheses indicate percent of hearts tested which ruptured distant from the infarct.

**FIGURE 6.** Upper panel: LV rupture threshold post-MI. LV rupture threshold was determined as depicted in Figure 1 in LV-RV preparations of hearts from rabbits subjected to coronary ligation (●) and killed at intervals post-MI. Each point represents the mean ± SE of values obtained from three to six individual hearts. In addition, LV rupture threshold was determined in normals (n = 4) and in animals subjected to sham coronary ligation (○) with each point representing the mean ± SE of values obtained from two to four hearts. Lower panel: infarct resistance to stretch post-MI. LV passive stiffness was determined in the same hearts described in the upper panel. *P < 0.05. **P < 0.01 compared with combined group of sham-operated hearts averaged over days 1–8 post-thoracotomy, excluding normals.

**FIGURE 7.** Relationship between post-MI total LV hydroxyproline content and LV passive stiffness. Total LV hydroxyproline content and LV passive stiffness were determined in hearts of rabbits subjected to coronary ligation (●) and in normals (n = 2) and those subjected to sham operation (n = 15) (○); each point represents the values obtained from an individual animal for hydroxyproline and passive stiffness (r = 0.599, P < 0.01).
Discussion

The present investigation was designed to study and correlate biochemical and mechanical parameters of infarct healing and repair in a well-defined model of myocardial infarction produced by coronary ligation in the rabbit. The results demonstrated a rapid increase in activities of enzymes which lead to collagen synthesis. Prolyl hydroxylase, an intracellular enzyme involved in post-translational modification of the polypeptide precursor of collagen, is dependent upon ferrous iron, ascorbic acid, α-ketoglutarate, and molecular oxygen for its activity (Hutton et al., 1967); its activity is increased by lactate (Comstock and Udenfriend, 1970). Increases in prolyl hydroxylase activity represent an early step in the collagen synthetic pathway. In the present study, prolyl hydroxylase activity rose significantly by 2 days and was nearly maximally elevated by 3 days post-MI. Lysyl oxidase is an extracellular copper- and oxygen-dependent enzyme involved in collagen cross-linking (Siegel, 1975), a step which imparts mechanical strength to collagen (Eyre, 1980). By 3 days post-MI, lysyl oxidase activity increased significantly reaching a peak at 4–6 days post-MI. Hydroxyproline content (a measure of collagen content) increased significantly at 3–4 days and appeared to be continuing to increase at 8 days post-MI. Thus, there appears to be an ordered progression of peaks of maximal changes in enzyme activities beginning with an increase in prolyl hydroxylase, an intracellular enzyme, and lysyl oxidase, an extracellular enzyme, leading to the formation of the end-product, collagen. These changes in enzyme activities and collagen content could be solely the result of new synthesis of these enzymes and of collagen; however, the present protocol cannot discriminate between stimulation of synthesis and inhibition of degradation of these macromolecules or some combination of these two processes.

The problem of a valid reference base for expressing the activities of prolyl hydroxylase and lysyl oxidase and the content of hydroxyproline was considered. Losses of non-collagen protein could have resulted in spurious elevations of these parameters when expressed with respect to dry weight or to total protein. Several reference bases were employed to confirm the real nature of the increases described. The extractable protein content from the infarcted areas of ligated animals did not differ from those of sham-operated controls. Enzyme activity increases expressed with respect to soluble protein, therefore, did not result from failure to extract the soluble protein from the infarcted regions. Tissue total protein, also used as a reference base, remained essentially constant throughout the 8-day period post-thoracotomy in both the ligated and the control group (a significant difference occurred only at day 1 (Fig. 3, lower panel). A progressive decrease in non-collagen protein was noted in the infarcted region of the ligated LVs as collagen content (hydroxyproline X 7.46) increased (Fig. 3, lower panel). We expressed enzyme activities and hydroxyproline content with respect to total protein and found patterns essentially identical to those described with respect to extractable protein. In view of the progressive decrease in non-collagen protein, this parameter was not used as a reference base, as it led to a calculation of greater increases in enzyme activity and hydroxyproline content. As these enzymes were most likely produced by fibroblasts, rather than by muscle tissue, total protein appears to be a better reference base than non-collagen protein. DNA content, which is directly related to cellularity and should not be influenced markedly by selective loss of non-collagenous tissue, was also used as a reference base. In the first 4 days post-MI, DNA content was generally found to be greater in the infarct region of the ligated hearts, compared with similar zones of the sham-operated animals (Fig. 3, middle panel). Nevertheless, the patterns of increases in enzyme activities were identical to those expressed with respect to protein. To further confirm that the increases in enzyme activity were not due to selective loss of non-collagenous tissue, we computed total enzyme activities and total hydroxyproline content in each LV. Although we did not expect this calculation to have much sensitivity due to the localized nature of the increases, we found that prolyl hydroxylase activity per total LV (Fig. 2, upper panel) had a similar profile with significant increases at 2 days, peak at 3 days, and continued elevations throughout the remaining days post-MI. Lysyl oxidase activity per total LV (Fig. 4, upper panel) was noted to increase significantly at 6 days post-MI with a qualitative pattern being similar to that noted when expressed in terms of protein or DNA. The lack of statistical significance at other time periods was thought to have been related to the localized nature of the increases in lysyl oxidase activity and the variability in lysyl oxidase activity in the non-infarcted LV regions. We also calculated total LV hydroxyproline (Fig. 5, upper panel) and demonstrated that an absolute increase occurred, reflecting new collagen synthesis.

We attempted to make some correlations between the post-MI changes in hydroxyproline content and the mechanical properties of the healing myocardium. A striking feature of the excised, infarcted ventricle was its strength and resistance to rupture. Even in the early post-MI period, the excised LV did not rupture until intra-LV pressure exceeded 500 mm Hg (Fig. 6). Our test of rupture threshold consisted of slow continuous filling of the intra-LV balloon until LV rupture occurred (Fig. 1). It is possible that the relatively slow, nonpulsatile increases in wall stress that were induced by this technique allowed greater stresses to be achieved before rupture took place than might have occurred had we imposed a pulsatile form of wall stress.
However, isolated strips of noninfarcted myocardium ruptured at similar stress values. Furthermore, isolated strips of post-MI scar, excised at 1 week post-MI, required two times as much stress as noninfarcted myocardium for rupture to occur, indicating that the infarct region was not the weakest part of the ventricle at this time.

We considered whether the results of the mechanical studies in small rabbit hearts could be extrapolated to much larger human hearts. The law of Laplace indicates that LV wall stress is directly related to intraventricular pressure and ventricular radii, and inversely proportional to LV wall thickness. Although the LV radii of these rabbit hearts were less than 1 cm, the wall thicknesses were correspondingly low. Thickness of the excised LV free wall strips used in this study averaged 3.5 ± 0.4 mm, a value similar to that measured with ultrasonic crystals in isolated beating rabbit hearts (Vogel et al., 1982). The ratio of LV mass to chamber volume and, thence, of wall thickness to radius has been shown to be comparable to rats, dogs, and humans in both normal and pressure-overloaded states (Ford, 1976). Thus, the relationship between the chamber pressure and wall stress required to rupture the myocardium should be similar in small and large hearts. LV wall stress at the rupture threshold in shams and normals calculated from the balloon inflation experiments was in good agreement with that calculated from the rupture of excised LV muscle strips. This suggests that the high rupture pressures we observed in normal noninfarcted hearts were not due to geometrical considerations such as the small radius of the rabbit heart or some interaction between the latex balloon and the wall of the heart. The high tensile strength of normal cardiac muscle and newly developing scar suggests that the occurrence of rupture requires a pathological process which decreases the tensile strength of the healing infarct or surrounding myocardium.

Although a recent MI and subsequent healing did not influence absolute rupture threshold as measured by our methodology, they did influence the site of ventricular rupture (Table 1). In the early post-MI period, the infarct region was usually the weakest portion of the ventricular wall; after 6–8 days, when new collagen synthesis had become evident (Figure 5), the infarct region appeared stronger than the noninfarcted myocardium. This apparent increase in mechanical strength of the infarct region could, in part, have been related to progressive contraction of the infarct at 4–8 days post-MI, so that the infarct region could have represented a smaller proportion of LV surface area in this later period. Accordingly, we examined the epicardial surface areas of the infarcts over the 8 days post-MI, but found no significant differences, although a tendency toward smaller infarct areas in the later period was noted (Fig. 8, lower panel).

The mean passive stiffness of the post-MI excised LV was increased significantly, compared with that of shams and normals. Passive stiffness in the infarcted LV rose significantly during the period of active collagen deposition (Fig. 6) and was directly related to hydroxyproline content (Fig. 7). Our measurement of passive stiffness reflected the contributions of edema, myocardial fibers, pre-MI collagenous fibrous elements, and infarct size, in addition to post-MI newly synthesized collagen. The lack of progressive increase in passive stiffness at 8 days post-MI despite continued increase in hydroxyproline content may have been related to contraction of the scar.
which occurred by 8 days post-MI as evidenced by the decrease in average weight of scar ($P < 0.05$ vs. days 2 and 4 post-MI, Fig. 8, middle panel), the progressive decrease in scar area (Fig. 8, lower panel) and the decrease in the fraction of LV mass contributed by the infarct zone by day 6 ($P < 0.05$, Fig. 8, upper panel). In the rabbit myocardium, contracture occurs after 15 minutes of ischemia at 37°C and is delayed at lower temperatures; this process can increase passive stiffness (Apstein and Ogilby, 1980). Our balloon-filling curves, however, were performed at a low temperature within 10 minutes of removal of the heart from the thorax before the onset of myocardial contracture. Thus, contracture was an unlikely factor influencing passive stiffness. We measured the stiffness of the excised LV at nonphysiological pressures, where the filling curves become linear and facilitated comparisons between experiments. Pressure volume data from the physiological range of pressures could not be measured accurately due to the scale required to record the high rupture pressures. Our intent was simply to demonstrate that the newly synthesized collagen had an influence on the mechanical properties of the infarcted LV, as shown in Figure 7.

Our results raise a number of questions and speculations regarding post-MI healing and repair. First, at least in this experimental model, the resulting infarcted ventricle was surprisingly resistant to rupture (Fig. 6) before new collagen synthesis could be detected (Fig. 5) suggesting that the collagenous network of the normal, pre-MI myocardium was an important protector against post-MI rupture. Studies by several investigators suggest that a pre-existing collagen network can provide mechanical stability even in the immediate post-MI period. Rabbit cardiac muscle hydroxyproline content is several times greater than that of rabbit skeletal muscle (Gay and Johnson, 1967). Dense collagen networks in noninfarcted rabbit and human hearts have been demonstrated by scanning electron microscopy (Caulfield and Borg, 1979). When noninfarcted rabbit hearts were incubated with collagenase, the hearts became more distensible as their hydroxyproline content diminished (O’Brien and Moore, 1966). Thus, the pre-existing collagenous skeleton of the normal myocardium may play an important role in preventing post-MI distortion and rupture.

The results of the present study expand previous studies of infarct healing. In autoradiographic studies of myocardial infarction induced by coronary ligation in rats, a proliferation of DNA-synthesizing connective tissue cells at the infarction border was demonstrated by 12 hours after ligation, and maximum proliferation of connective tissue cells was reached on the 2nd day post-MI (Kranz et al., 1971; Kranz, 1975). Studies of $[^{14}C]$glycine incorporation into subcellular fractions of cardiac protein and nucleic acids following coronary ligation in dogs demonstrated that the healing process started as early as 24 hours after infarction and reached a maximum on the 4th day post-MI (Gudbjarnason et al., 1964).

In studies of collagen metabolism in rats subjected to isoproterenol-induced myocardial necrosis, prolyl hydroxylase activity was elevated by day 2, as was total myocardial hydroxyproline beginning on day 4 (Judd and Wexler, 1975). Prolyl hydroxylase activity remained high through day 4 and then gradually declined, whereas hydroxyproline remained elevated throughout the 18-day study period.

Studies of the mechanical properties of infarcted myocardium in dogs indicated that passive LV compliance increased 1 hour after infarction (Forrester et al., 1972). In studies of a later phase of the healing process, ventricular compliance decreased by 3–5 days post-MI (Hood et al., 1970), and ventricular function improved over the first week after infarction (Kumar et al., 1970). Hood postulated that this time-dependent improvement of function was due to stiffening of the infarct region, which prevented systolic bulging and ventricular inefficiency. Healing of infarcted myocardium is unique in that the repair processes occur in infarcted tissue subjected to repetitive dynamic stresses imposed by cardiac contraction with each systole. Indeed, it might be expected that ventricular rupture would occur commonly in transmural infarction, but the overall incidence in man is only 1.5–8% of MIs (Rasmussen et al., 1979); nonetheless, this fatal complication accounts for 4–24% of MI deaths (Bates et al., 1977).

Coronary ligation and infarction initiate a complex set of metabolic reactions eventually leading to collagen synthesis and scar formation. Our investigations do not address the questions of whether the collagen synthetic reactions occur at a maximal rate, or whether the overall rate could be increased with the addition of selected co-factors and thus could lead to the ultimate deposition of more collagen or to the more rapid or stronger healing of the infarcted zone. These questions are of considerable clinical importance; their resolution has the potential to decrease the rupture- and aneurysm-related mortality rate.

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