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Research Article

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served myocardium was then assessed by two inde-
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serial histologic sections and creatine kinase ac-
tivity of the whole left ventricle. The amount of
normal myocardium preserved at 21 days postocclusion
was significantly increased, by $22.3\pm7.8\%$ ($P < 0.025$)
after the administration of hyaluronidase, by 25.3
$\pm5.8\%$ ($P < 0.005$) after cobra venom factor, by 14.5
$\pm6.9\%$ ($P < 0.05$) after hydrocortisone, by $20.8\pm8.2\%$
($P < 0.025$) after the single dose of MP, by $20.9\pm3.9\%$
($P < 0.001$) after the four doses of MP, and by 10.2

$\pm3.7\%$ ($P < 0.05$) as a result of pretreatment with
reserpine. The four doses of MP significantly thinned
the infarct—by $25.6\pm2.9\%$ ($P < 0.001$)—and although
ventricular rupture did not occur, the intervention
caused distension of the left ventricle as a result of
stretching of the infarcted tissue during scar formation.
Thus, myocardium acutely jeopardized by ischemia
can be preserved on a long-term basis.

INTRODUCTION

The prognosis after acute myocardial infarction de-
pends directly on the quantity of remaining viable,
normally functioning myocardium (1, 2). If myo-
cardial cell death after acute coronary occlusion could
be reduced, a greater quantity of viable myocardium
would remain and this might be expected to reduce
the incidence both of intractable cardiogenic shock
and of pulmonary edema; immediate mortality would
thereby be reduced and chronic heart failure would
be less likely.

Animal experiments have shown that salvage of
myocardium acutely jeopardized by ischemia may be
feasible. After coronary artery occlusion, the speed at
which acutely ischemic cells become irreversibly in-
jured is not uniform and depends on their location as
well as on a number of other factors (3–5). However,
the fate of these cells can be modified, at least in
the short term, by various therapeutic interventions,
as was shown indirectly by epicardial ST segment
(4, 5) and QRS (6) mapping, by the relationship be-
tween epicardial ST segment elevation and the creatine
kinase (CK)[1] content of the subjacent myocardium (4, 5),
and by serial changes in serum CK activity (7, 8). These
experiments involved the use of the indirect tech-
niques mentioned above and the examination of the
effects of interventions 24 h after the occlusion,

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[1] *Abbreviations used in this paper*: CK, creatine kinase;
CVF, cobra venom factor; H, hyaluronidase; HC, hydro-
cortisone; MP, methylprednisolone; R, reserpine.

when the process of infarction is still incomplete. The long-term effects of apparently favorable interventions on myocardial salvage have not been investigated.

The present study was designed to ultilze the model of experimental myocardial infarction after coronary artery occlusion in the rat, in order to measure directly and compare the effectiveness of different interventions in preserving acutely jeopardized myocardium at the time of peak necrosis and when healing is complete. Two independent techniques were used to measure the quantity of viable left ventricular myocardium: total CK activity and quantitative histology of the left ventricle. A preliminary report describing this method has been published (9).

To achieve these goals four interventions that have previously been shown by indirect techniques to limit myocardial necrosis, hyaluronidase (5, 6, 10–14), cobra venom factor (15), hydrocortisone (16), and methylprednisolone (17), and one other (reserpine) thought likely to be favorable because of its catecholamine-depleting action (18) were administered, and their effects on myocardial preservation compared.

METHODS

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, Mass.), weighing between 200 and 300 g, were lightly anesthetized with ether, the chest opened in the fifth intercostal space and, while the heart was briefly everted from the thoracic cavity, the main left coronary artery was ligated 1–2 mm from its origin with a 4-0 suture (9, 19–21). The heart was then repositioned, and the muscle and skin layers closed with a "purse-string" 0 suture. The operative mortality was 21%. With the exception of the reserpine-treated group, all treated rats were given the drug within 5 min after the occlusion—hyaluronidase (H; Alidase; Amersham/Searle Corp., Arlington Heights, Ill.), 1,500 NF U/kg of body weight i.v. (this dose was repeated 24 h later); cobra venom factor (CVF), 20 U/kg i.v.; hydrocortisone (HC; Solucortef; The Upjohn Company, Kalamazoo, Mich.), 50 mg/kg i.v.; methylprednisolone (MP; Solumedrol; Upjohn), 50 mg/kg i.v.—one group (MP × 1) received this dose once; a second group (MP × 4) received three further doses of 50 mg/kg i.m. 3, 6, and 24 h after the occlusion. The reserpine (R; Serpasil; CIBA/GEIGY Corporation, Summit, N. J.)-treated rats were given 0.5 mg/kg of body weight i.m. once on each of the 3 days before the occlusion. The effect of each drug was studied simultaneously with a separate group of control animals in which the coronary artery was ligated, but the drug not administered. These are termed C^1, C^2, C^3, C^4, C^5, and C^6, denoting the controls for the H-, CVF-, HC-, MP × 1-, MP × 4-, and R-treated rats, respectively. Sham-operated rats, subjected to the same procedure except that the suture placed around the coronary artery was not tied, were used during the enzymatic studies to establish whether the drug per se influenced CK activity and as a base line for defining CK activity in hearts not subjected to coronary artery occlusion. None of the drugs altered the myocardial CK activity 48 h postoperatively. All rats were given penicillin G benzathine suspension (Bicillin L-A; Wyeth Laboratories, Philadelphia, Pa.) 120,000 U i.m. 2 h after the coronary ligation.

In a preliminary study of 79 nontreated rats with coronary artery occlusion sacrificed at regular intervals between 1 and 21 days postocclusion, the necrotic process was found to be at its peak 48 h after the coronary occlusion, and healing was complete 3 wk after the occlusion. In this study, therefore, these two times, i.e. 48 h and 21 days after occlusion, were chosen for study; the rats were reanesthetized with ether and their hearts excised. The success of each coronary artery occlusion was tested by the injection of 1 ml/kg of 10% carbon black (particle size 300 Å), either i.v. just before sacrifice or into the left coronary artery after sacrifice. Those rats in which the entire left ventricular free wall became discolored with carbon were considered to have had nonoccluded coronaries and were considered to be sham-operated animals.

After the hearts were excised, the amount of myocardium preserved was measured directly, in the 48-h rats both by histology and left ventricular CK and in the 21-day rats usually only by histology. Left ventricular CK activity at 21 days after occlusion was studied only in the H-treated rats and their controls. For the histologic study, the hearts were fixed in 10% phosphate-buffered formalin for 24 h and the ventricles were then sectioned into four slices, each 2- to 2.5-mm thick, from apex to base and in a plane parallel to the atrioventricular groove. After dehydration and clearing, all four slices from each heart were embedded. 5-μm thick sections were then cut and mounted on 2 × 2-inch glass slides and stained either by hematoxylin-eosin in the rats sacrificed 48 h after occlusion or by Masson's trichrome method to demonstrate collagen in the rats sacrificed 21 days after occlusion.

To assess changes in the quantity of preserved myocardium at the time of peak necrosis (48 h) and after healing is complete (21 days), the histologic sections of all four slices from each occluded or sham-operated heart were projected onto a screen at a 10-fold magnification and the following measurements were made by planimetry (22, 23): In each heart obtained from rats sacrificed 48 h after coronary occlusion (a) the areas of normal and infarcted left ventricular myocardium, and (b) the lengths of the arcs of the endocardial circumference of the left ventricle underlying both the normal and the infarcted myocardium were determined. From these measurements the percentage, both by area and by circumference, of the left ventricle that remained normal was calculated. In the hearts obtained from rats sacrificed 21 days after coronary occlusion, the total area of preserved normal myocardium in the infarcted hearts was measured and expressed per kilogram of body weight; this amount of preserved myocardium was then expressed as a percentage of the mean of the total area of normal myocardium found in 21-day sham-operated rats. Infarct size, as in the 48-h rats, was calculated as a fraction of the left ventricular circumference (Fig. 1). The area percentages represent estimates of the fraction of the volume of the normal left ventricular myocardium that was preserved or infarcted, and the circumference percentages represent the fraction of the surface area of the normal left ventricle that was preserved or comprised the infarct. The variation in the histologic measurements of preserved myocardium and infarct size, when performed by the same individual on different days, was <5%. The mathematical justification for using two-dimensional tissue sections to make quantitative estimates of three-dimensional structures (stereology) is detailed elsewhere (23).

The quantity of salvaged myocardium was expressed as follows: (a) the preserved left ventricular CK activity, expressed as a percentage of the normal left ventricular CK activity; (b) the percentages of the left ventricular endocardial circumference underlying the preserved and the infarcted myocardium, (c) in rats sacrificed 48 h after
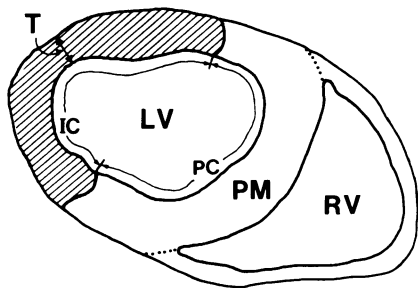
FIGURE 1 Diagram of transverse slice of a rat heart after occlusion of the left main coronary artery; LV, left ventricular cavity; RV, right ventricular cavity; infarcted myocardium is shaded; PM, left ventricular preserved myocardium which includes the interventricular septum (unshaded-dotted line separates LV from RV myocardium); T, site of measurement of the thinnest point of the infarcted tissue; IC, endocardial circumference underlying the infarcted myocardium; PC, endocardial circumference underlying the preserved myocardium.

coronary occlusion, the preserved and infarcted left ventricular areas were expressed as percentages of the total left ventricular area; (d) in rats sacrificed 21 days after coronary occlusion, the area of preserved left ventricular myocardium, corrected for rat weight, was expressed as a percentage of the normal left ventricular area in sham-operated rats also corrected for body weight.

In hearts destined for enzymatic analysis, the atria, right ventricle, and great vessels were dissected from the left ventricle, which was then homogenized to permit measurement of its CK activity by spectrophotometric assay (24). To permit calculation of the amount of preserved myocardium by the enzymatic method, the minimum CK activity in the center of the infarcted tissue was determined and found to average 2.6 ± 0.4 IU/mg protein. After allowing for this "residual" CK, the percentage of the normal left ventricular mass preserved was calculated, using each operating day's nontreated and treated sham-operated rats for reference. Thus, for example, with a mean left ventricular CK activity of 12.6 IU/mg of protein in nontreated sham-operated rats and of 7.3 IU/mg protein in a rat with an occluded coronary artery, 42.1% of the left ventricular CK activity in the rat with the occlusion has been lost. Infarct size, corrected for the CK activity of 2.6 IU/mg protein in the infarct center, thus equals $42.1 + (2.6 \times 42.1/12.6) = 42.1 + 8.7 = 50.8\%$ of the left ventricle. Thus, by this method of assessment, 100.0 − 50.8, i.e., 49.2% of the left ventricle has been spared.

In the CVF-treated rats, venous blood samples were taken at sacrifice from both the control and treated rats for measurement of serum complement by the CH_{50} inhibition method (25). Additional venous blood samples from CVF-treated sham-operated rats were taken from groups of three animals each at half-hourly intervals for 4 h after the administration of the CVF, as well as daily thereafter for 1 wk.

Purification of the CVF. Lyophilized *Naja naja* venom (Ross Allen's Reptile Institute, Inc., Silver Springs, Fla.) was reconstituted and chromatographed on DEAE-cellulose and Sephadex G-200 (25; Pharmacia Fine Chemicals, Piscataway, N. J.). After dissolving 1 g of venom in 65 ml sodium phosphate buffer, 0.03 M, pH 7.4, at 37°C, the sample was applied to a 2.5 × 40-cm column of equilibrated DEAE-cellulose. The nonbound proteins, mostly phospholipase, were washed through at 50 ml/h for 16–18 h. A linear salt gradient to 0.5 M NaCl was then begun, and the anti-

complementary peak was concentrated and applied to a Sephadex G-200 column in isotonic saline phosphate buffer, pH 7.4. The final peak was a single homogeneous band on polyacrylamide electrophoresis (26), and when assayed by the CH_{50} inhibition method (25) had a specific activity in the range of 4 μg protein/U. In purification of CVF, care was taken to avoid contact with unsiliconized glass.

In all series, Student's t test for group observations was used to compare the differences in the amount of left ventricular myocardium preserved and in the extent of the healed infarcts between each of the treated and control groups. The χ^2 test (short form) was used to compare the difference in the incidence of subendocardial infarction between the control occluded and all of the treated animals.

RESULTS

Hyaluronidase. CK activity of the homogenized left ventricles in 48-h sham-operated rats averaged 12.7 ± 0.3 (SEM) IU/mg of protein ($n = 23$). In the untreated occluded control rats (C^1), left ventricular CK activity was 7.6 ± 0.2 IU/mg of protein ($P < 0.001$). Taking the "residual" CK activity in the infarcted tissue into account, the C^1-rats were left with 50% of the normal left ventricular myocardium. In the rats given H after occlusion (H-rats), CK activity averaged 10.4 ± 0.4 IU/mg of protein, an activity corresponding to that of 76% of the normal left ventricular myocardium. The difference between the H-rats and the C^1-rats, i.e. 76 compared with 50%, indicates that H resulted in a 52% increase in the amount of myocardium preserved compared to that in the C^1-rats ($P < 0.001$; Table I). When the amount of myocardium preserved at 48 h after occlusion was assessed histologically, the H-rats had 32% ($P < 0.001$) more normal myocardium by area and 40% more by circumference ($P < 0.001$) than did the C^1-rats (Table I).

In the rats sacrificed 21 days after the occlusion, the amount of normal left ventricular myocardium preserved when assessed histologically by area was 22% ($P < 0.025$) greater in the H-treated rats than in the C^1-rats (Table II). Infarct size, measured by circumference, was reduced by an average of 36% ($P < 0.01$; Table III). These differences were less striking than in the 48-h groups, a finding confirmed by the CK method which showed that 22 H-rats had $16.6 \pm 6.5\%$ more preserved myocardium than did 17 C^1-rats ($P < 0.025$).

Cobra venom factor. CVF reduced the mean CH_{50} level from 188 ± 9 U ($\pm$SEM) to less than 50 U within 30 min of its administration, and this depression of serum complement persisted for 4–5 days. At 48 h after occlusion there was, by the CK method, a 22% ($P < 0.025$) increase in the amount of normal myocardium preserved in the CVF-rats compared with the C^2-rats (Table I). Assessed histologically, CVF also increased the amount of myocardium preserved by 10% ($P < 0.05$) when assessed by area and by 16%

TABLE I

Effect of Interventions on the Preservation of Left Ventricular Myocardium (Expressed as Percentage of the Whole Left Ventricle) by the CK, Area, and Circumference Methods, at 48 h after the Coronary Artery Occlusion

Intervention and method of assessment	Control groups (occlusion alone)		Experimental groups (occlusion plus intervention)		Increase of preserved left ventricular myocardium	P‡
	(% NM)*	n	(% NM)*	n		
					%	
Hyaluronidase						
	(C¹)		(H)			
CK	50.3±2.2§	30	76.4±4.1	30	52.0±8.1	<0.001
Area	57.0±2.5	23	75.1±3.2	22	31.8±5.6	<0.001
Circumference	47.9±2.8	23	66.9±3.8	22	39.7±7.9	<0.001
Cobra venom factor						
	(C²)		(CVF)			
CK	53.6±3.8	15	65.3±3.8	22	21.8±7.2	<0.025
Area	57.4±2.2	21	62.9±2.2	22	9.5±3.8	<0.05
Circumference	41.7±2.1	21	48.2±2.9	22	15.7±7.0	<0.05
Hydrocortisone						
	(C³)		(HC)			
CK	51.7±2.1	22	61.7±3.6	20	19.4±6.9	<0.005
Area	55.7±1.9	30	63.8±2.9	26	14.5±5.2	<0.01
Circumference	42.4±1.9	30	50.4±3.3	26	19.0±7.8	<0.025
Methylprednisolone × 1						
	(C⁴)		(MP × 1)			
CK	50.9±2.5	26	62.9±3.8	25	23.6±7.4	<0.005
Area	55.7±1.9	30	61.6±2.7	27	10.5±4.8	<0.05
Circumference	42.4±1.9	30	49.9±3.1	27	17.7±7.3	<0.025
Methylprednisolone × 4						
	(C⁵)		(MP × 4)			
CK	52.2±3.2	22	68.3±5.0	19	30.9±9.6	<0.005
Area	55.7±1.9	30	58.2±2.5	27	4.5±4.6	NS
Circumference	42.4±1.9	30	45.6±2.5	27	7.5±5.8	NS
Reserpine						
	(C⁶)		(R)			
CK	49.6±3.2	25	60.4±2.5	29	21.8±5.6	<0.005
Area	53.6±2.2	28	58.5±2.1	34	9.1±4.0	NS
Circumference	41.4±2.2	28	49.1±2.3	34	18.7±5.6	<0.01

* Normal myocardium preserved, expressed as a percentage of the myocardium in the whole left ventricle.

‡ P values calculated for the differences between control and experimental groups.

§ All values are presented as mean±1 SEM.

($P < 0.05$) when assessed by the circumference method (Fig. 2, Table I).

21 days postocclusion, the amount of normal left ventricular myocardium preserved, when assessed by area, was 25% ($P < 0.01$) greater in the CVF-rats than in the C²-rats (Table II). Infarct size, measured by circumference, was reduced by an average of 19% ($P < 0.02$; Table III).

Glucocorticoids. By the CK method the amount of normal myocardium preserved 48 h after the occlusion was increased by 19% ($P < 0.01$) in the HC-rats, by 24% ($p < 0.005$) in the MP × 1-rats, and by 31% (P

TABLE II

*Effect of Interventions on the Preservation of Left Ventricular Myocardium, Calculated by Expressing the Total
Area of Preserved Myocardium 21 Days after the Coronary Artery Occlusion as a Percentage
of the Total Area of Normal Myocardium in 21-Day Sham-Operated Rats*

Intervention	Control groups (occlusion alone)		Experimental groups (occlusion plus intervention)		Increase of preserved left ventricular myocardium	$P\ddagger$
	(% NM)*	n	(% NM)	n	%	
Hyaluronidase	(C^1) 51.6±3.7§	15	(H) 63.1±4.0	17	22.3±7.8	<0.025
Cobra venom factor	(C^2) 50.0±2.3	30	(CVF) 62.6±2.9	35	25.3±5.8	<0.01
Hydrocortisone	(C^3) 52.0±2.7	25	(HC) 59.5±2.5	16	14.5±6.9	<0.05
Methylprednisolone × 1	(C^4) 52.0±2.7	25	(MP × 1) 62.8±4.2	18	20.8±8.2	<0.025
Methylprednisolone × 4	(C^5) 54.8±1.7	49	(MP × 4) 66.3±2.1	50	20.9±3.9	<0.001
Reserpine	(C^6) 50.6±2.3	26	(R) 55.7±1.9	29	10.2±3.7	<0.05

* Normal myocardium preserved, expressed as a percentage of the myocardium in the whole of the left ventricle.
‡ P values, calculated for the differences between control and experimental groups.
§ All values are presented as mean±1 SEM.

< 0.005) in the MP × 4-rats (Table I). Histologically, the increases in the amount of preserved myocardium at 48 h, compared to that in concurrently studied non-treated controls (C^3-, C^4-, and C^5-rats), were, by area, 15% (P < 0.01) for the HC-rats, 11% (P < 0.05) for the MP × 1-rats, and 5% (NS) for the MP × 4-rats, and, by circumference, 19% (P < 0.025) for the HC-rats, 18% (P < 0.025) for the MP × 1-rats, and 8% (NS) for the MP × 4-rats (Table I).

In the rats sacrificed 21 days after the occlusion, the preserved left ventricular myocardium, assessed by area, was increased by 15% (P < 0.05) in the HC-rats (Fig. 3), by 21% (P < 0.025) in the MP × 1-rats, and by 21% (P < 0.001) in the MP × 4-rats (Table II). However, the fraction of the left ventricular circumference

comprising the healed infarct at 21 days was not significantly decreased in the glucocorticoid-treated rats compared to their respective controls (Table III). Actually, in the MP × 4-rats it was increased by 1% although this difference was not statistically significant. This discrepancy between the effect on area and circumference can be explained by stretching of the infarcted tissue during scar formation. Indeed, the infarct thickness was obviously reduced; at its thinnest point in any of the four sections, the transmural infarct averaged 0.63±0.03 mm in the C^5-rats and was significantly reduced in the MP × 4-rats in which it averaged 0.47±0.02 mm (P < 0.001).

Reserpine. When measured by the CK method 48 h postocclusion, pretreatment with R achieved a 22%

TABLE III

*Effects of Interventions on Infarct Size (Expressed as Percentage of Left Ventricular Circumference)
21 Days after the Coronary Artery Occlusion*

Intervention	Control groups (occlusion alone)		Experimental groups (occlusion plus intervention)		Decrease	P*
	Scar size (% LV)	n	Scar size (% LV)	n	%	
Hyaluronidase	(C^1) 41.4±3.7‡	15	(H) 26.3±2.8	17	36.4±6.8	<0.01
Cobra venom factor	(C^2) 43.4±2.2	30	(CVF) 35.3±2.5	35	18.7±5.9	<0.02
Hydrocortisone	(C^3) 42.9±2.3	25	(HC) 39.3±3.1	16	8.4±7.2	NS
Methylprednisolone × 1	(C^4) 42.9±2.3	25	(MP × 1) 37.9±2.7	18	11.5±6.4	NS
Methylprednisolone × 4	(C^5) 43.9±1.7	49	(MP × 4) 44.3±1.7	50	−0.8±3.9	NS
Reserpine	(C^6) 42.6±2.5	26	(R) 39.9±2.0	29	6.3±4.7	NS

* P values, calculated for the differences between control and experimental groups.
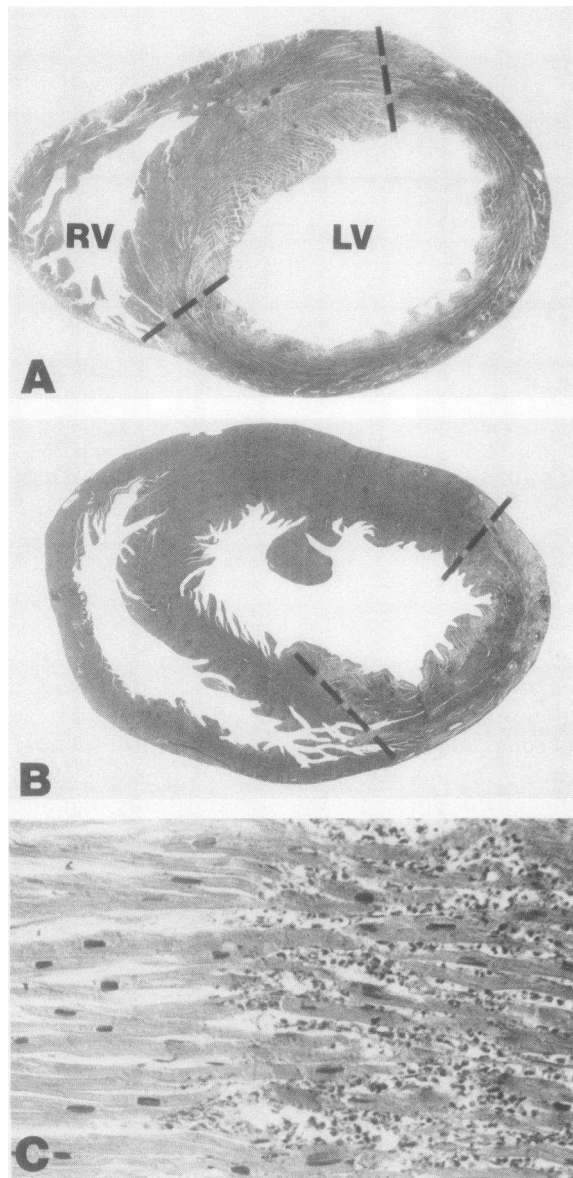‡ All values are presented as mean±1 SEM.

FIGURE 2 CVF-induced preservation of ischemic myo-cardium: (A) representative histologic section from untreated occluded rat at 48 h postocclusion; (B) section from rat treated with CVF 48 h postocclusion. The preserved left ventricular myocardium is to the left of the broken lines. Section B shows greater preservation of myocardium than section A (hematoxylin and eosin stain, ×7). (C) At higher magnifica-tion, the infarcted myocardium in a nontreated rat 48 h post-occlusion (to the right) is recognized by a marked inflamma-tory cell infiltrate and the darker staining of the necrotic myocardial fibers (hematoxylin and eosin stain, ×40). LV, left ventricular cavity; RV, right ventricular cavity.

FIGURE 3 Effects of HC on preservation of ischemic myo-cardium and the myocardial scar. The four histologic sec-tions from a representative untreated occluded rat at 21 days postocclusion are shown in A and those from a HC-treated rat in B. The preserved left ventricular myo-cardium, which lies to the left of the broken lines, is greater in the treated rat (B). The scar in B is not ab-normally thin (trichrome stain, ×3). (C) At higher magnifica-tion, the healed infarct in an untreated rat at 21 days post-occlusion (to the right) consists of dense collagen, and there is no residual inflammation (trichrome stain, ×40).

($P < 0.005$) increase in the amount of spared myo-cardium (Table I), whereas by the histologic methods the increase was 9% (NS) by area and 19% ($P < 0.01$) by circumference (Table I). In the rats sacrificed 21 days after the occlusion, the preserved myocardium in the R-pretreated rats as assessed by area was 10%
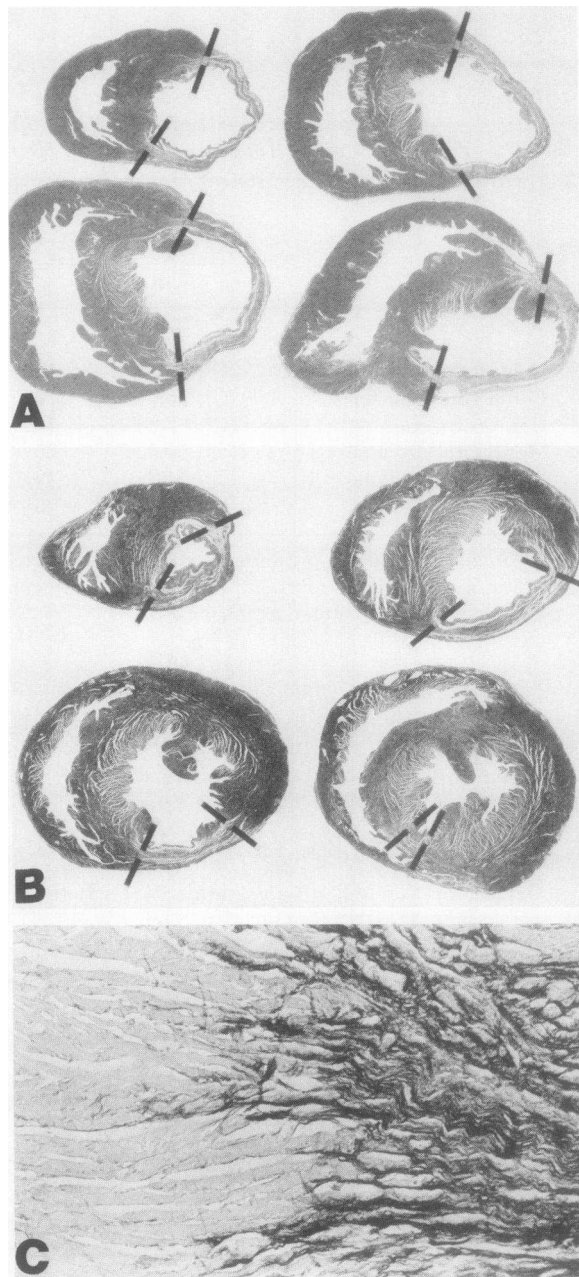
($P < 0.05$) greater than in the C^6-rats (Table II). Infarct size in the R-rats, as determined by circumference, was 6% (NS) less than in the C^6-rats (Table III).

All interventions. The occlusion of the left main

coronary artery in the rats usually resulted in transmural infarcts, only 3 of 171 nontreated occluded (C^1–C^6) rats having a subendocardial infarction. However, when the treated rats from all the groups were considered together, the number of subendocardial infarcts was greater (15 of 165) ($\chi^2 = 10.4$; $P < 0.01$).

DISCUSSION

The direct measurement of the viable myocardium remaining after coronary occlusion has demonstrated that acutely jeopardized ischemic myocardium, otherwise destined to undergo necrosis, can be salvaged by a number of interventions. This conclusion is based on the preservation of CK activity and on the quantitative histology of the left ventricle 48 h after coronary occlusion, i.e., at the peak of the necrotic process. The present investigation also shows that long-term salvage of acutely ischemic myocardium can be achieved by interventions administered during a brief period or even as a single dose. It may be postulated, therefore, that after coronary occlusion an intervention may need to ensure myocardial cell survival only until a collateral circulation that can maintain cellular viability over the long term has developed. The experimental model used also permits a quantitative comparison of the effect of different interventions on myocardial salvage.

It has previously been shown, by indirect techniques, that H can limit myocardial necrosis for 24 h when it is administered before or up to 6 h after coronary artery occlusion in the dog (10, 13). It also appears to be beneficial for at least 1 wk in patients with acute myocardial infarction when administered within 8 h of the onset of chest pain (12). The mechanism by which this agent protects the acutely ischemic heart is uncertain. It is known to hydrolyze interstitial glycoproteins and it may therefore improve the transport of nutrients to the ischemic myocardium and enhance the washout of its noxious metabolites. It has also been shown to increase collateral blood flow to the ischemic area (14), probably secondary to a decrease in myocardial edema.

Our initial observations on the effectiveness of H in limiting myocardial infarct size in this animal model (9) have been extended in the present study to include a large number of animals studied histologically 21 days after occlusion as well as two further groups studied by the CK activity method at this time. Also, in the present study the amount of myocardium salvaged at 48 h and 21 days after occlusion has been quantified. The beneficial results obtained with this drug during the first 48 h exceeded those produced by the other interventions tested in this study. By the CK method there was ≅50% more residual myocardium in the H-treated than in control-occluded rats at the peak of the necrotic process (Table I). After the infarct had healed, i.e., 3 wk after

occlusion, the amount of preserved myocardium in the treated rats both by histology and by CK was still significantly greater than in the control-occluded rats, although this difference was less than at 48 h. Thus, although much of the early benefit derived from H administration appears to persist (Table II), the loss of some of the early benefit may indicate that the drug does not protect the ischemic myocardium from all the factors that normally control its evolution to complete infarction.

CVF has also been shown by indirect techniques in the dog to limit myocardial necrosis 24 h after coronary occlusion (15), but it has not been applied clinically. This substance depletes the third component of complement, thereby reducing the generation of leukotactic factors; it may act by reducing the myocardial injury that results from the release of lysosomal enzymes from infiltrating polymorphonuclear leukocytes and by reducing the capillary permeability and the injury to cell membranes which follow activation of the complement system. The present study extends previous observations by showing that the ischemic myocardium salvaged shortly after coronary occlusion by a single dose of CVF remains viable for a prolonged period.

Considerable controversy has surrounded the possible effects of glucocorticoids on infarct size. Pharmacologic doses of HC have been shown to diminish myocardial necrosis in the dog for up to 24 h (16), whereas pharmacologic doses of MP have been shown to reduce infarct size in some studies (17), but fail to do so in others (27). The longer-term effects of these glucocorticoids in the dog are unknown. In man, nonpharmacologic doses of HC have not consistently influenced mortality after acute myocardial infarction (28, 29). With the use of CK curve analysis, pharmacologic doses of MP have been reported by Morrison et al. (30) to decrease infarct size, whereas deMello et al. (31) have reported an increase in infarct size and mortality. Multiple doses of MP administered to patients with acute myocardial infarction have also been reported possibly to increase the incidence of ventricular rupture (32). The mechanism by which glucocorticoids may benefit acutely ischemic myocardium remains conjectural. They may stabilize cell membranes, prevent or delay the release of lysosomal enzymes (33, 34), stabilize the phagocytic vacuoles in infiltrating inflammatory cells, thereby reducing their heterolytic activity (16), inhibit the generation of prostaglandins and thromboxanes (35), exert other anti-inflammatory actions, or increase collateral blood flow to the ischemic myocardium (36).

When administered during the first 24 h after a skin incision, glucocorticoids interfere with wound healing (37). Although the rat is particularly sensitive to the metabolic effects of steroids, it is no more sensitive to steroid-induced interference with healing than are other species (38). Furthermore, in the dog, pharmaco-

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logic doses of MP adversely affect the early healing phase of acute myocardial infarction (39), and in one patient receiving prolonged HC therapy after acute myocardial infarction, the development of a ventricular aneurysm was associated with delayed myocardial healing (40).

Multiple doses of MP (equivalent to a 20-fold greater glucocorticoid effect than that of the single dose of HC) resulted in thinning of the infarcts, either because of the high dose or its prolonged administration. At the time of sacrifice these abnormally thin infarcts had already developed into ventricular aneurysms (Fig. 4). In the MP × 4-treated rats, the distension of the infarct 3 wk after occlusion may give the false impression that myocardial necrosis has been more extensive than is actually the case, there simply being greater representation of the infarct as a percent of the left ventricular surface. This situation represents expansion of the infarct rather than extension or reinfarction on account of a true increase in necrosis (41).

Pretreatment with R has the theoretical attraction that the catecholamine-depleted ischemic myocardium

(18) may benefit not only from the inhibition of adrenergically mediated stimulation of myocardial oxygen consumption, as occurs with propranolol-induced beta blockade (4, 5, 42–45), but also by the prevention of additional myocardial damage by the local release of catecholamines from their stores in the cardiac adrenergic nerve endings as myocardial necrosis occurs. However, pretreatment with R resulted in only borderline long-term salvage of ischemic myocardium and was less than that achieved by the other interventions (Table II).

In conclusion, in this rat model of local myocardial ischemia after coronary artery occlusion, H, CVF, HC, MP × 1, MP × 4, and R all spared acutely ischemic myocardium for 21 days. Inasmuch as left ventricular hypertrophy cannot be detected until more than 6 wk postocclusion (46), the increased amount of myocardium present at 21 days after occlusion in the treated rats represents true salvage of jeopardized myocardium. Although the sparing of myocardium at 21 days was significant for all of the interventions studied, with H it was less than at 48 h whereas it was generally similar at these two times with the other interventions (Tables I and II, Fig. 5).

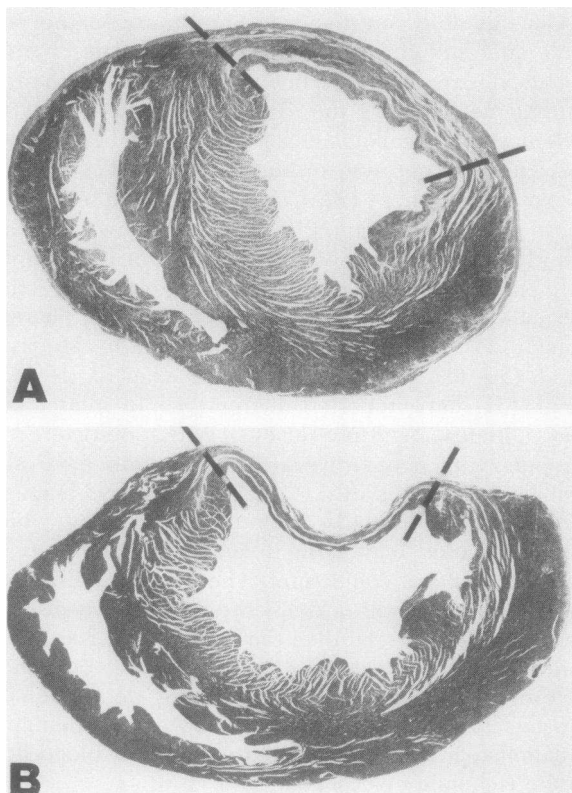Occlusion of the left main coronary artery in the rat 1–2 mm from its origin results in infarction of

FIGURE 4 Scar thinning resulting from administration of MP × 4. A representative section from an untreated occluded rat at 21 days postocclusion is shown in A and one from a MP × 4-treated rat in B. Note that for a scar of comparable size, that from the MP-treated rat is abnormally thin (trichrome stain, ×3).
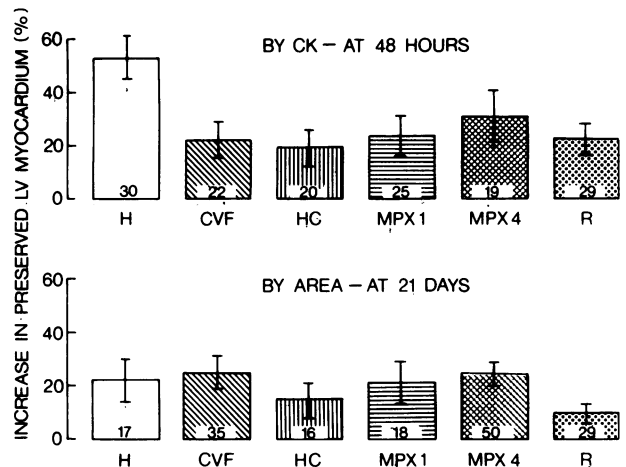

FIGURE 5 Comparative short- and long-term effectiveness of the six interventions on myocardial salvage. (Upper panel) Measurements based on CK activity made 48 h postocclusion; (lower panel) measurements based on histologic (area) analysis 21 days postocclusion. The benefit from each intervention is expressed as the mean percent increase in the amount of left ventricular myocardium preserved compared to that found in the nontreated occluded rats. Bars indicate ±1 SEM. The numbers in each column represent the number of treated rats in each group. H, hyaluronidase-treated rats; CVF, cobra venom factor-treated rats; HC, hydrocortisone-treated rats; MP × 1, rats treated with only a single dose of methylprednisolone; MP × 4, rats given four doses of methylprednisolone over the first 24 h after occlusion; R, rats pretreated with reserpine for 3 days before occlusion.

≅50% of the left ventricle. The rat has a relatively rich network of collateral vessels; this can readily be demonstrated by injecting carbon black into the ostium of the right coronary artery and noting its penetration into the portion of the left ventricle ordinarily perfused by the left coronary artery. Differences in the extent of collateralization may well influence the effectiveness of interventions designed to limit infarct size. The normal human and pig have relatively sparse collaterals, whereas the normal dog and the patient with diffuse coronary artery disease generally have a richer collateral network. The rat's collaterals appear to resemble the latter, but a more detailed analysis must be carried out in this species.

There are many obvious differences between this experimental model and the patient with acute myocardial infarction. The most important is the presence in most patients of diffuse atherosclerotic coronary artery disease, whereas in the rat only one coronary artery is occluded in a normal heart whose other coronary vessels are normal. Also, the oxygen consumption of the rat heart, expressed on a weight basis, greatly exceeds that of the human heart. Therefore, myocardial necrosis would be expected to occur much more rapidly in the rat than in man after coronary occlusion. Therefore, it is not clear what time intervals between occlusion and intervention would be comparable in these two species. However, we have noted in the dog that the effectiveness of H diminishes progressively as this interval lengthens; efficacy can still be demonstrated at 6 h but not at 9 h after coronary occlusion (13). In addition, the pathological evolution of the infarct in the rat is telescoped in comparison to that in man; 48 h after coronary occlusion the infarct in the rat resembles one which is 4-days old in the human; the 21-day-old infarct in the rat is comparable to a 42-day-old infarct in man (47). However, despite the aforementioned differences between the rat and the human, the fundamental tissue changes after coronary occlusion are essentially identical in all mammalian species. Even though there may be quantitative differences between the effects of interventions in various species, we have noted no qualitative differences between the responses of the rat and dog. The principal advantage of this model is that it allows a relatively precise direct assessment of the effects of various interventions on infarct size.

The results of this study lend support to the suggestion that timely therapeutic interventions after coronary occlusion in man may limit infarct size and thereby reduce the incidence of myocardial failure and its attendant mortality and morbidity. Of the various interventions tested in this study, the administration of H or CVF early after the occlusive event offers the greatest promise for further study. The results of the preliminary clinical studies with H have

been encouraging (11, 12), but the definitive role of this agent in clinical practice needs to be determined in a much larger number of patients. CVF has not so far been given to patients; its role in clinical practice is still to be determined. Before glucocorticoids can be recommended for further clinical trial, their long-term effects deserve further study so as to determine the optimal dose and frequency of administration that will achieve the maximum myocardial salvage without causing thinning and distension of the infarcted tissue during healing (48). It is possible that, as in the experimental animal, the administration of a single dose will reduce myocardial damage, but that multiple doses are required in order to interfere with the healing process. R might be useful when administered prophylactically, but in this study its beneficial effects were relatively small. An important, unresolved question is whether combinations of these interventions, and others, such as beta adrenergic blockers, which appear to act by fundamentally dissimilar mechanisms, will provide greater benefit than single interventions.

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