Expression, G. Stein and L. Kleinsmith, Eds. (Academic Press, New York, 1975), pp. 153–186; G. M. Pikler, R. A. Webster, T. C Spelsberg, *Biochem. J.* **156**, 399 (1976); R. A. Webster, G. M. Pikler, T. C. Spelsberg, *ibid.*, p. 409; T. C. Spelsberg, *ibid.*, p. 391.
11. W. T. Schrader and B. W. O'Malley, *J. Biol. Chem.* **247**, 51 (1972); W. T. Schrader, D. O. Toft, B. W. O'Malley, *ibid.*, p. 2401.
12. T. C. Spelsberg, A. W. Steggles, F. Chytil, B. W. O'Malley, *ibid.*, p. 1369.
13. We thank Mrs. P. Midthun, Mrs. B. Gosse, and Mr. B. Syverson for excellent technical assistance, and the National Institutes of Health (grant HD 9140-B) and the Mayo Foundation for financial support.

6 July 1976

Hyaluronidase-Induced Reductions in Myocardial Infarct Size

Abstract. *The size of myocardial infarctions following coronary artery occlusion in the rat was determined directly by measurement of creatine phosphokinase activity in homogenized whole left ventricles and by planimetric measurement of the area of the infarctions in histologic sections of serial slices of the left ventricles. Hyaluronidase was shown to produce significant reductions in expected infarct size both 48 hours and 3 weeks after occlusion without impairing fibrosis during the healing phase. Thus, the amount of myocardial necrosis that follows a coronary artery occlusion has been shown directly to be amenable to reduction with a pharmacological intervention.*

Acute myocardial infarction is the condition that occurs when interruption of blood flow in a coronary artery results in the necrosis of the myocardium supplied by that artery. In hospitalized patients with actue myocardial infarction the two most frequent causes of death are disturbances of heart rhythm and mechanical failure of the left ventricle. The mortality caused by the former has been reduced by the application of monitoring techniques and the use of antiarrhythmic drugs. Since mechanical failure of the left ventricle depends on the quantity of viable, contractile myocardium, reduction in the infarcted (nonviable) tissues should also improve prognosis. Indirect evidence now exists that various interventions modify the expected size of the myocardial infarct following experimental occlusion of a coronary artery (*1*). Based on the application of indirect criteria, hyaluronidase is thought to decrease the size of an infarction (*2*). This enzyme is known to depolymerize the mucopolysaccharides that are abundant in the interstitium of the myocardium. Consequently, it has been postulated that hyaluronidase may reduce infarct size by three mechanisms—(i) improved transport of nutrients to the ischemic myocardium, (ii) enhanced "washout" of noxious metabolic waste products, and (iii) increased collateral blood flow to the ischemic area (*3*), probably secondary to a decrease in myocardial edema (*4*). However, direct evidence that hyaluronidase (or other interventions) actually reduce infarct size is lacking. The goal of this investigation was to describe two independent methods that permit the direct measurement of the size of the myocardial infarct after experimental coronary artery occlusion in the rat and to determine whether these methods can be used to demonstrate directly the effects of interventions that may decrease infarct size.

A standard size infarct was produced in the left ventricles of albino Charles River male rats (weight, 250 to 300 g) by occluding the left coronary artery 2 to 3 mm from its origin, as described (*5*). In order to assess the effect of an intervention on expected infarct size, three groups of rats were studied: sham-operated (S-rats), rats with an occlusion but no intervention (O-rats), and rats in which an intervention was added to the occlusion (OI-rats). The intervention consisted of the intravenous administration of 1500 units of hyaluronidase (Alidase, Searle) per kilogram of body weight 5 minutes and 24 hours after coronary artery occlusion. The S-rats were not given the interventions, since we had previously found that in S-rats this dose of hyaluronidase does not affect the mean creatine phosphokinase (CPK) activity of the left ventricle when determined 48 hours later. Animals were killed either 48 hours after occlusion, that is, when the necrotic process was at its peak, or 21 days after occlusion, when the process of repair was complete. Just prior to death, and with the heart again exposed, 1 ml per kilogram of body weight of 10 percent carbon black (particle size, 300 Å) was injected intravenously. If the anterior surface of the left ventricle was not discolored by the carbon, a complete coronary artery occlusion was deemed to be present. The heart was then excised and either homogenized for enzymatic study or placed in 10 percent phosphate-buffered formalin for histologic processing and examination.

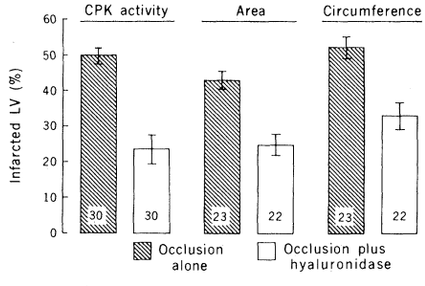

Fig. 1. Percentage of the left ventricle that was infarcted 48 hours after occlusion of the main left coronary artery in the untreated (O) rats (hatched columns) and hyaluronidase-treated rats (OI) (nonhatched columns). The extent of the infarcts was assessed by left ventricular (*LV*) creatine phosphokinase activity (*CPK*) and by two histologic methods based on the analysis of serial slices of the ventricle, that is, the infarcted area and the circumference overlying it. Bars indicate ± 1 S.E.M. The numbers in the columns are the numbers of rats studied in each group.

For the enzymatic studies, CPK activity of the homogenized whole left ventricle (free wall plus interventricular septum) was measured as described (*6*). In order to permit calculation of "enzymatic" infarct size, the minimum CPK activity in the infarcted tissue 48 hours after occlusion was also determined and was found to be 2.6 international units per milligram of protein. For histologic studies the left ventricles were sectioned into four slices (2 to 2.5 mm thick) from the apex to the base of the heart in a plane parallel to the atrioventricular groove. Paraffin-embedded sections (5 μm in thickness) were prepared from each slice, stained with hematoxylin and eosin, projected onto a screen, and planimetered to determine the cross-sectional area of the left ventricle and of the infarcted myocardium (*7*). The fraction of the left ventricle that was infarcted was calculated as a mean of this value in each of the four slices, then expressed as a percentage. Both the thickness of the interventricular septum and the ratio of the thickness of the infarcted portion of the left ventricle to that of the septum (representing normal left ventricular wall thickness) were also measured. The former would be expected to increase if compensatory hypertrophy developed in the remaining viable myocardium, and the latter is a useful index of the degree of thinning of the infarcted part of the free wall of the left ventricle. When the calculation of infarct size was based on the planimetered area, the thinning of the in-

farcted area, which was noted 3 weeks after occlusion, resulted in underestimation of the fraction of the left ventricle that had become infarcted. Therefore, the fraction of the circumference of the left ventricle that was infarcted was determined and was taken to be a more accurate reflection of the actual fraction of the left ventricle involved. There was little difference between the fraction of the endocardial and epicardial surfaces that were infarcted; the endocardial measurement was used because the infarct was more clearly defined there. When the animals were killed 48 hours after coronary artery ligation, CPK activity in the 23 S-rats was 12.7 ± 0.3 I.U. per milligram of protein (mean + 1 standard error) and in 30 O-rats 7.6 ± 0.2 I.U. per milligram of protein ($P < .0005$). Taking into account the residual CPK activity in the infarcted tissue, this represented a 49.7 ± 2.2 percent infarction of the left ventricle. In 30 OI-rats CPK activity was 10.4 ± 0.4 I.U. per milligram of protein. This represented a 23.6 ± 4.1 percent infarction of the left ventricle. The difference in infarct size between the O-rats and the OI-rats was highly significant ($P < .0005$) (Fig. 1).

When infarct size was assessed histologically 48 hours after occlusion, the 23 O-rats had 43.0 ± 2.4 percent (assessed by area) and 52.1 ± 2.8 percent (assessed by circumference) of their left ventricle infarcted. The corresponding values in 22 OI-rats were 24.9 ± 3.5 percent (by area) and 33.1 ± 3.7 percent (by circumference). The difference in infarct size between the O-rats and the OI-rats was highly significant ($P < .0005$) (Fig. 1).

Because of the extreme thinning of the infarcted area 21 days after occlusion, infarct size in rats killed at that time is more reliably calculated by the circumference method than by the area method. Infarct size 21 days after occlusion (Fig. 2) in ten O-rats assessed by circumference, was 38.0 ± 5.4 percent and in 17 OI-rats it was 26.3 ± 2.7 percent ($P < .025$). The hyaluronidase-induced preservation of jeopardized ischemic myocardium was also reflected in the reduced thinning of the left ventricular wall, as expressed by the ratio of the thickness of the infarct to that of the septal wall. The ratio of the thickness of the free wall to that of the septum in normal rats is 1.01 ± 0.01. At 21 days after the occlusion the ratio was 0.44 ± 0.05 in ten O-rats and was significantly higher in the 17 OI-rats, that is, 0.66 ± 0.06 ($P < .01$). The thickness of the interventricular septum in the three groups at 21 days (0.20 ± 0.04 mm for the S-rats and 0.20 ± 0.03 mm for both the O-rats and the OI-rats) showed no evidence of compensatory hypertrophy in the remaining viable myocardium.

These studies demonstrate that: (i) The rat model can be used to quantify directly the extent of experimental myocardial infarction by two independent methods—total left ventricular CPK activity and quantitative histology. (ii) The model can be used to demonstrate and to quantify intervention-induced changes in expected infarct size. (iii) Hyaluronidase, an agent thought, on the basis of indirect evidence, to be capable of reducing expected infarct size (*2*), has been shown directly to do so, and this effect has been quantitated. (iv) The beneficial effects of hyaluronidase are apparent not only at 48 hours but also at 21 days after occlusion when healing is complete, and this drug does not appear to interfere with normal scar formation. Caution should be used before applying these results from rats with normal coronary arteries to man with myocardial infarction and diffuse coronary artery disease.

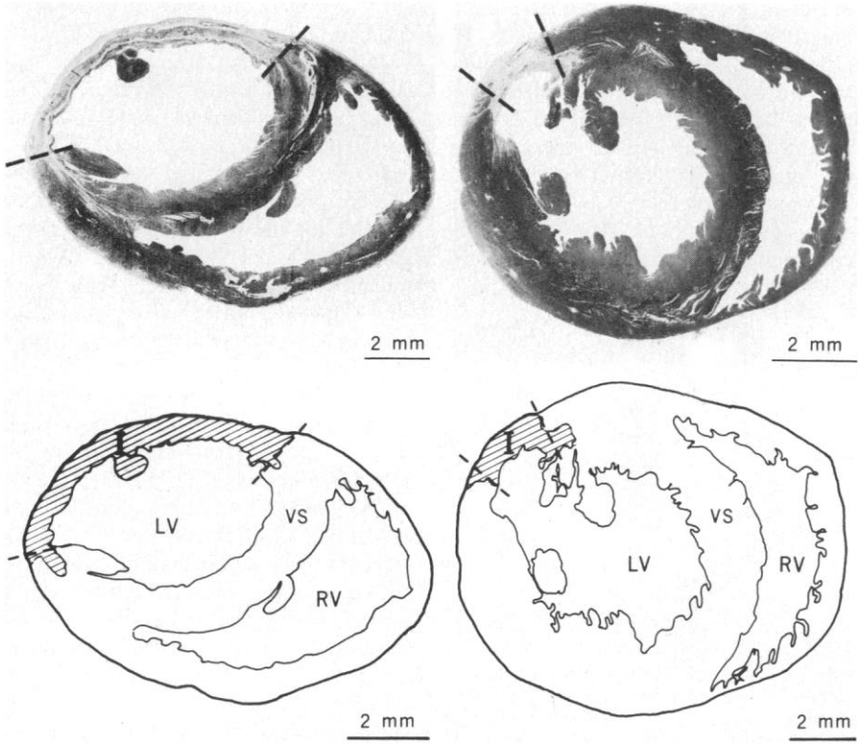

Fig. 2. Histologic sections (upper panels) and diagrams (lower panels) of transverse slices of hearts of rats killed 21 days after occlusion of the left main coronary artery without (left side) and with (right side) hyaluronidase treatment; *LV*, left ventricle; *RV*, right ventricle; *VS*, ventricular septum; and *I*, infarcted myocardium. The borders of the infarctions are shown by the interrupted lines and their areas by the shaded portions of the diagrams. Note that in the rat with an occlusion alone the infarction involved 54.1 percent of the endocardial circumference of the left ventricle, whereas in the hyaluronidase-treated rat it involved only 20.1 percent.

DEREK MACLEAN
MICHAEL C. FISHBEIN
PETER R. MAROKO
EUGENE BRAUNWALD
Departments of Medicine and *Pathology, Harvard Medical School,* and *Peter Bent Brigham Hospital, Boston, Massachusetts 02115*

References and Notes

1. P. R. Maroko, J. K. Kjekshus, B. E. Sobel, T. Watanabe, J. W. Covell, J. Ross, Jr., E. Braunwald, *Circulation* **43**, 67 (1971); P. R. Maroko and E. Braunwald, *Ann. Intern. Med.* **79**, 720 (1973).
2. P. R. Maroko, P. Libby, C. M. Bloor, B. E. Sobel, E. Braunwald, *Circulation* **46**, 430 (1972); L. D. Hillis, J. Askenazi, E. Braunwald, P. Radvany, J. E. Muller, M. C. Fishbein, P. R. Maroko, *Circulation*, in press; P. R. Maroko, D. M. Davidson, P. Libby, A. D. Hagan, E. Braunwald, *Ann. Intern. Med.* **82**, 516 (1975).
3. J. Askenazi, L. D. Hillis, P. E. Diaz, M. A. Davis, E. Braunwald, P. R. Maroko, *Am. J. Cardiol.* **37**, 118 (abstr.) (1976).
4. J. Martins de Oliveira and M. N. Levy, *Am. Heart J.* **60**, 106 (1960).
5. T. N. P. Johns and B. J. Olson, *Ann. Surg.* **140**, 675 (1954); H. Selye, E. Bajusz, S. Grassos, P. Mendell, *Angiology* **11**, 398 (1960); A. Deloche, F. Fontalira, J. N. Fabiani, G. Pennecot, A. Carpentier, C. Dubost, *Ann. Chir. Thorac. Cardio-Vasc.* **11**, 89 (1972).
6. J. K. Kjekshus and B. E. Sobel, *Circ. Res.* **27**, 403 (1970).
7. P. J. Boor, E. S. Reynolds, M. C. Fishbein, *Am. J. Pathol.* **82**, 26a (1976).
8. D.M. was in receipt of a British-American research fellowship (75-310) awarded by the British Heart Foundation and the American Heart Association. The support of the Wellcome and McCunn Trusts is acknowledged. The skilled technical assistance of Mr. J. Gannon, Ms. C. Morgan, and Ms. S. Hale is gratefully acknowledged.

7 July 1976