Fibrinolytic Activator, Glutamic Oxaloacetic Transaminase and Potassium in Myocardial Tissue at Various Stages of Human Myocardial Infarction

The Institute of Pathology, Frederiksberg Hospital, Copenhagen, the Biological Institute of the Carlsberg Foundation, Copenhagen, and the Clinical Laboratory, Kommunehospitalet, Copenhagen, Denmark

Simon Fischer, Ole K. Albrechtsen and Nils U. Bang

An evaluation of the macroscopic and microscopic criteria of tissue necrosis and healing constitutes the classical method for the approximate determination of the age of the human myocardial infarct (13). In recent years it has been demonstrated that histochemical assays of tissue homogenates for intracellular enzymes and potassium may yield important additional information regarding time factors in the evolution of myocardial tissue necrosis (8, 14, 16).

Animal experiments with temporary occlusion of the coronary arteries have given fairly accurate information about early and late histological and histochemical changes in myocardial infarction (4, 5, 17).

The purpose of this paper is to present comparative studies of histological and biochemical changes in the human myocardial infarct at several stages of development.

In a study of the human myocardial infarct based on autopsy material certain complicating factors present themselves. Variations in mechanism and completeness of the occlusion and in the development of collateral circulation will give significant differences between patients with clinical symptoms of the same duration. Furthermore electrocardiographic studies and studies of the serum activities of myocardial enzymes released by myocardial infarction indicate that the occlusion and infarction in some instances occur without any time relationship to the onset of clinical symptoms. The varying time intervals between death and autopsy also make a comparison of histochemical changes from patient to patient difficult (11).

*) Aided by grants from Miss P. A. Brandt's Fund to the first author and from the Josiah Macy Jr. Foundation, New York, to Dr. Tage Astrup of the Biological Institute of the Carlsberg Foundation, Copenhagen.

In order to correlate the histological and biochemical changes in the human, myocardial infarct samples were examined from various zones in the same infarct in which the central zone represents fully developed tissue necrosis with a gradual transition through the ischemic areas in the periphery to normal myocardium. In a study of this kind the errors arising from differences in post mortem loss (e. g. diffusion) of intracellular enzymes and potassium from infarct to infarct would be eliminated to a certain extent. Potassium ion concentration and enzyme activities of normal compared with infarcted myocardium in the same heart should give more accurate information concerning the histochemical changes in the human infarct than would variations between different hearts. Estimation of concentrations of potassium ion, glutamic oxaloacetic transaminase and fibrinolytic activity (estimated as plasminogen activator) were chosen because determinations of these were felt to illustrate three important parameters of the infarct pathogenesis. Loss of intracellular potassium presumably takes place in the early phases of ischemia (7) and indicates loss of muscular function but not necessarily irreversible damage. Diminution of the intracellular GO transaminase activity seems to indicate loss of viability of the affected segment of the myocardium (14). Measurements of the activity of the plasminogen activator in the tissue was felt to be of interest in the evaluation of the pathogenesis of the hemorrhages observed in myocardial tissue necrosis. According to the studies of Astrup and co-workers the tissue activator activates a precursor in blood (pro-fibrinolysin or plasminogen) to the protease (fibrinolysin or plasmin) (2) which in turn may interfere with normal hemostatic control.

Materials and Methods

Seven human myocardial infarcts were studied. They varied in age from approximately two hours to 1 year. In one case rupture of the infarct was encountered (Case 6). Samples of tissue were taken from 12 different fixed points in one horizontal plane for each infarct. In some instances samples were taken in all three dimensions. The weight of each sample was approximately 1500 milligrams and was sub-divided as follows:

1 500 mg for histological examination (Hematoxylin-Eosin stain and van Gieson-Hansen stain).

2. 600 mg for potassium determination by flame photometry as described by Wallace (18). The normal potassium values according to Rodeck (15) for the left ventricle myocardium in wet tissue homogenates range between 300 and 400 mg%.

3. 200 mg for glutamic oxaloacetic transaminase determination. The GO-T assays were done spectrophotometrically as described by Henley and Pollard (16). By this method the number of mMol pyruvic acid formed after one hour's incubation of the tissue-substrate mixture is determined. 1 mMol pyruvic acid per g wet tissue per hour corresponds approximately to 20 spectrophotometrical units determined by the Karmen method (9). The range of activities for normal human myocardium samples was 150—350 mMol pyruvic acid, which is approximately one fifth of the values observed in tissue homogenates from dogs examined immediately after death.

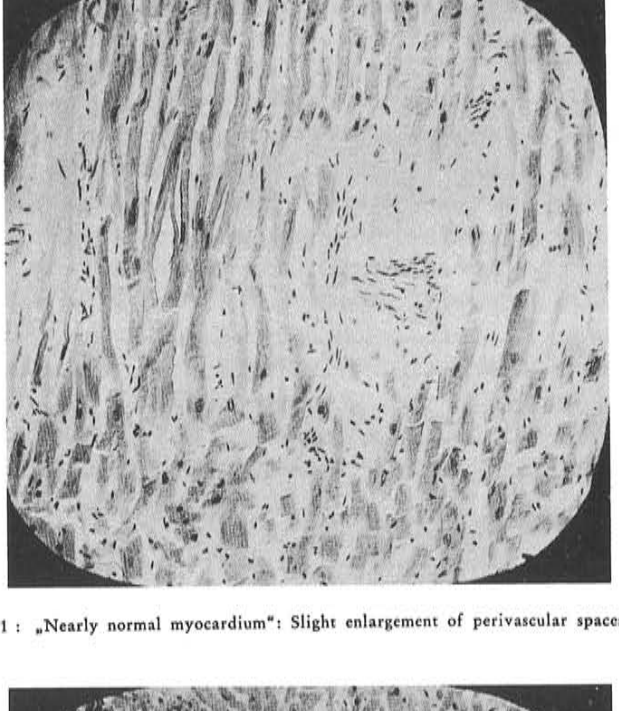

Fig. 1: „Nearly normal myocardium": Slight enlargement of perivascular spaces. 75 ×

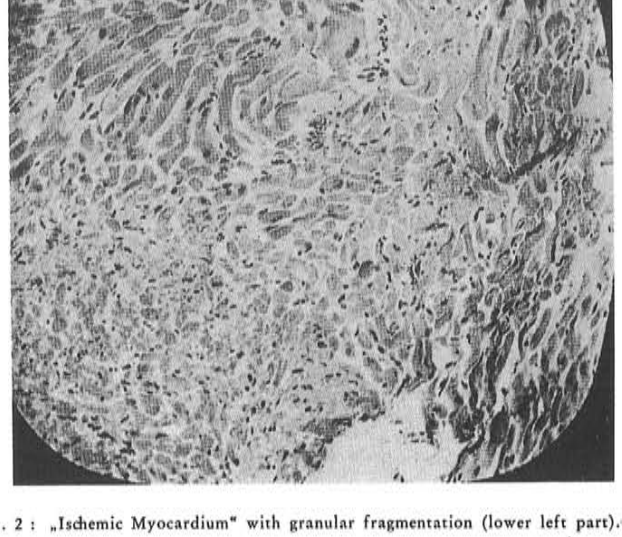

Fig. 2: „Ischemic Myocardium" with granular fragmentation (lower left part). 75 ×

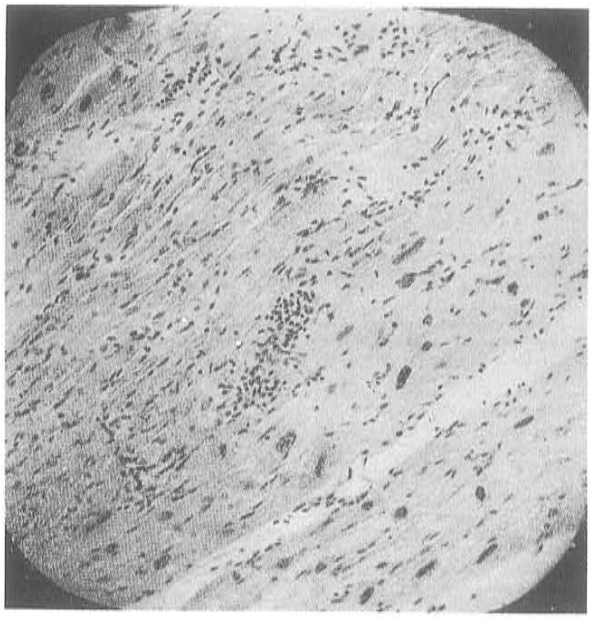

Fig. 3: „Necrosis +" about 50% (central part) 75 ×

Fig. 4: „Necrosis ++" about 100%. 75 ×

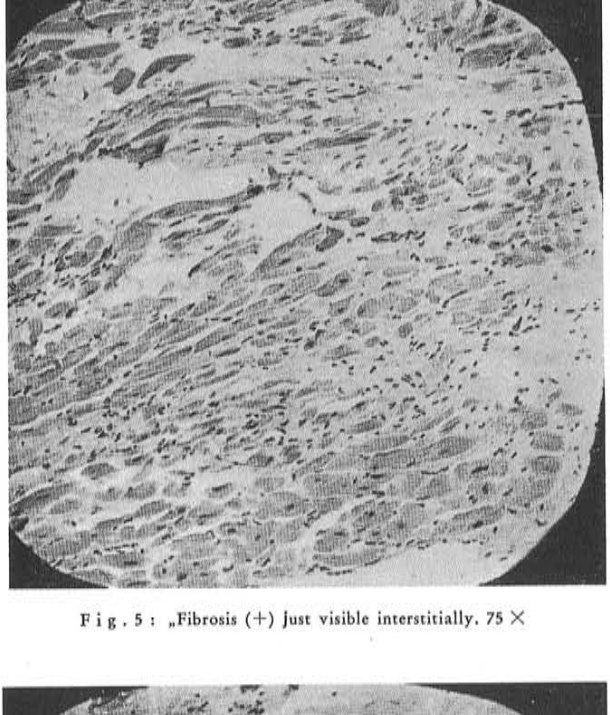

Fig. 5: „Fibrosis (+) just visible interstitially. 75 ×

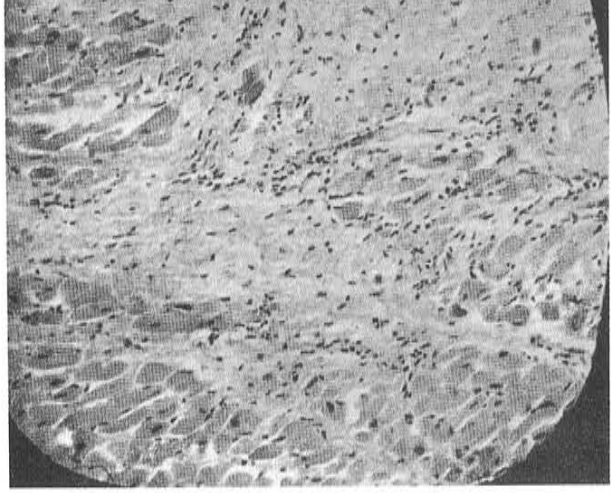

Fig. 6: „Fibrosis +" about 50%. 75 ×

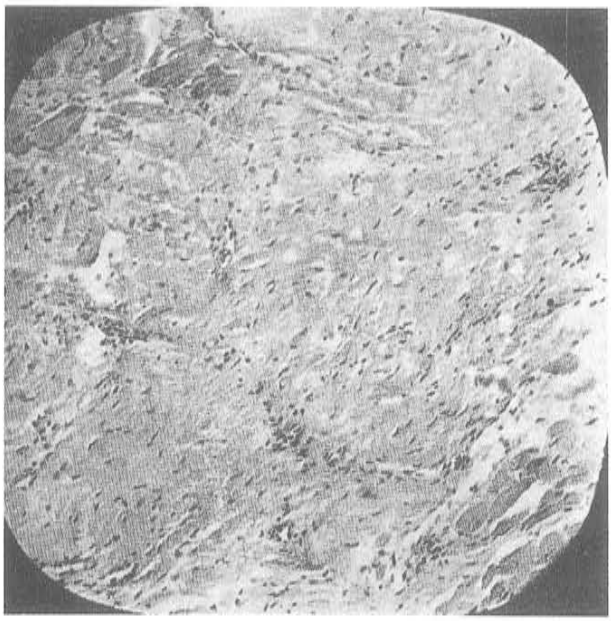

Fig. 7: „Fibrosis ++" about 100%. 75 ×

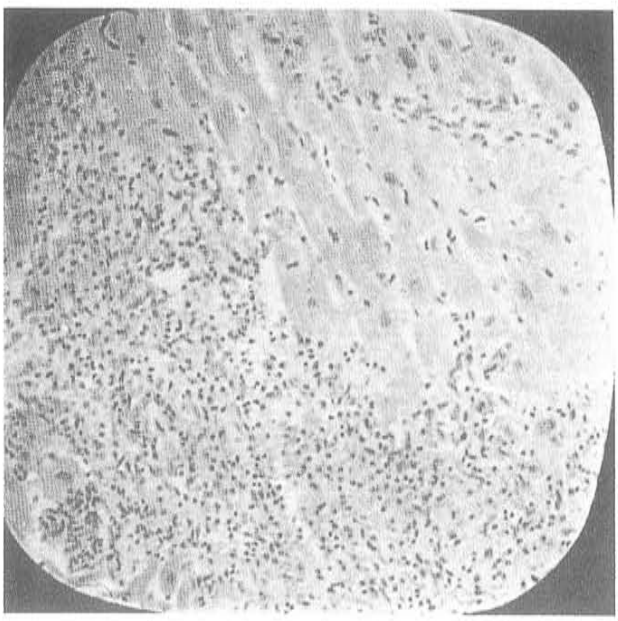

Fig. 8: Granulation tissue. 75 ×

4. 200 mg for fibrinolytic activity. The activator assays were performed as described by Astrup and Albrechtsen (3). The concentration of plasminogen activator (in units of a standard preparation per g fresh tissue) varies considerably from one individual to another in all human tissues examined. In an autopsy series the average tissue activator concentration in myocardium was 82 units as determined by the technique used in the present study (Albrechtsen [1]).

The samples for enzyme and potassium assays were thoroughly minced and homogenized in a Potter-Elvehjem grinder.

The tissue specimens were evaluated histologically according to the following criteria:

"Nearly normal myocardium": Normal tissue except for slight perivascular fibrosis (Fig. 1).

"Ischemic myocardium": Tissue with slight focal changes of granular fragmentation, increased eosinophilia, edema, vascular leucocytosis but without leucocytic infiltration of the tissues (Fig. 2).

"Necrosis'+": $< 50\%$ necrosis of the myocardium per field of vision at a 75 × magnification (Fig. 3).

"Necrosis++": 50—100% necrosis per field of vision at a 75 × magnification (Fig. 4).

"Fibrosis (+)": Slight fibrosis in the perivascular spaces and between the muscle fibers (Fig. 5).

"Fibrosis +": $< 50\%$ of the muscle fibers replaced by scar tissue per field of vision at a 75 × magnification (Fig. 6).

"Fibrosis ++": 50—100% of the muscle fibers replaced by scar tissue per field of vision at a 75 × magnification (Fig. 7).

"Granulation tissue": $> 50\%$ young fibrous tissue per field of vision at a 75 × magnification (Fig. 8).

Post mortem examination was done 12 hours after death on corpses which had been refrigerated for 6 hours. The samples of myocardium were kept frozen until the enzyme and potassium assays were done. The determinations were never done later than 72 hours after sampling of the tissues.

Results

Table 1 shows the results of the assays of GO transaminase, plasminogen activator, and potassium and gives the average values for 2 or more tissue samples of comparable histological appearance in each case. This table correlates the enzyme activities and potassium concentrations with the histological changes graded as indicated above. The average values of enzyme activities and potassium concentrations are given for all 7 infarcts and the percentage of GO transaminase, plasminogen activator and potassium found in tissue samples of varying histologic appearance are calculated in relation to the values for normal myocardium. Passing from normal to maximally necrotic myocardium a marked and significant decrease in the tissue GO transaminase was found in all instances. A less but equally significant decrease in GO transaminase activity was found in tissue samples from ischemic myocardium with only focal and very early, if any, necrotic changes. The average GO transaminase activity in the ischemic parts were 69% of normal as compared to 40% of normal for areas with maximum necrosis.

Table 1: Variations of GO-T Activities, *Plasminogen Activator* and Potassium Ion Concentration (—) in Tissue Homogenates from Various Zones of Myocardial Infarcts

Case	Age of Infarct	Normal Myocard.	Nearly Normal Myocard.	Ischemia	Necrosis +	Necrosis ++	Hemorrhage	Granulation Tissue	Fibrosis (+)	Fibrosis +	Fibrosis ++
1.	2 hrs. old	3100 *90* (223)	2750 *73* —	1750 — (246)	— — —	— — —	— — —	— — —	2950 *79* —	— — —	— — —
2.	Some hrs. old	900 *80* —	1250 *149* (202)	1250 *287* (174)	— — —	— — —	— — —	550 *159* —	3200 *172* (174)	1500 *154* (202)	— — —
3.	1 week old	2700 *195* (204)	1600 *172* —	2350 *178* (162)	1850 *171* —	1300 *127* (128)	— — —	1650 *172* (163)	1650 — (163)	4300 *172* —	— — —
4.	2 weeks old	2300 *108* (304)	2200 *74* (275)	— — —	1700 *79* (285)	600 *6* (273)	1150 *27* (281)	1350 *25* (281)	1700 *46* (238)	— — —	— — —
5.	3 weeks old	3100 *106* (244)	2750 *127* (218)	1450 *79* (211)	1350 *85* (169)	1400 *74* —	1350 *123* (169)	1400 *74* —	3050 — (218)	1700 *99* (211)	1200 *117* (212)
6.	6 weeks old	3450 *89* —	2500 *30* —	2250 *18* —	— — —	1000 *8* (164)	1500 *15* (164)	1800 *16* (164)	— — —	1200 *8* —	1200 319 (140)
7.	About 1 year	— — —	3450 382 (235)	— — —	— — —	— — —	— — —	— — —	— — —	3300 747 (235)	700 1412 (209)
	Average	2600 *111* (244)	2350 *144* (232)	1800 *140* (198)	1650 *111* (227)	1050 *54* (188)	1350 *55* (205)	1350 *89* (203)	2500 *99* (198)	2400 *236* (216)	1050 *616* (187)
	Percentage of normal values	100% *100%* (100%)	90% *129%* (95%)	69% *125%* (81%)	63% *100%* (93%)	40% *49%* (77%)	52% *49%* (84%)	52% *80%* (83%)	96% *90%* (81%)	92% *212%* (89%)	40% *555%* (76%)

The concentration of the plasminogen activator is seen to be significantly decreased only in areas with marked tissue necrosis whereas normal or slightly increased values are found in the ischemic areas. A surprising finding, based on a few observations, was an increase of plasminogen activator when fibrosis was present.

The results of the potassium determinations demonstrate significantly decreased values for both normal and infarcted areas. The post mortem loss of potassium from all cells undoubtedly has decreased the difference between the values for normal and diseased myocardium. For this reason the concentration gradients between normal, ischemic, and necrotic myocardium become rather small.

Table 2 illustrates an attempt to correlate the GO transaminase activities with the anatomical distribution of the lesion. GO transaminase estimations confirm the histological impression that necrosis more often affects the endocardial than pericardial parts of the myocardium and similarly that areas at the base are more frequently and more heavily affected than apical areas. In three cases (no. 1, 4, 5) GO transaminase is slightly increased at the border of the infarct as compared to normal tissue probably expressing the loss of GO transaminase from the center towards the periphery by diffusion.

Table 2: The Anatomical Distribution of GO-T in the Myocardial Infarcts

Case	Normal Myocard.	At the Demarcation	At Max. Necrosis	Endocard. Part.	Pericard. Part.	Apical Part.	Basal Part.
1.	3100	3400	1750	3000	2550	2650	3100
2.	900	1800	550	—	—	—	—
3.	2700	1800	850	1750	2850	3100	950
4.	2300	1750	300	1000	550	2800	800
5.	3100	2200	850	1250	1700	2500	1500
6.	3450	3300	650	1200	2500	3350	—
7.	3450	2850	700	—	—	—	—
Average	2600	2500	800	1650	2000	2850	1600
Percentage	100%	99%	30%	63%	77%	109%	61%

Discussion

The present series though limited in material demonstrates a correlation between the severity of histological changes and loss of intracellular GO transaminase in the human myocardial infarct. In fully developed necrotic areas the GO transaminase activity was 40% of that observed in normal myocardium with a gradual increase in activity through the peripheral areas towards normal myocardium. The degree of fibrosis and the amount of fibrous tissue per unit weight of myocardium influenced the GO transaminase activities. This would be expected since connective tissue is known to have little or no GO transaminase activity. An interesting observation is that a marked reduction of the GO transaminase activity was also found in ischemic areas where no clear histological signs of tissue necrosis were demonstrable. Decrease in GO transaminase activity to about 50% of the values for the normal myocardium was found in case 1 with ischemic changes not more than 2 hours old according to the clinical history. Only very slight focal changes of early necrosis were found by histological examination (figure 1). This seems to indicate that loss of intracellular enzymes has taken place at a time when little or no histological evidence of cellular death is present.

The present data raise the question; do moderately increased serum GO transaminase levels in the absence of unequivocal electrocardiographic evidence of myocardial infarction always indicate the presence of myocardial cell necrosis or does prolonged myocardial ischemia without irreversible cellular death give rise to increase serum GO transaminase levels? A release of GO transaminase from ischemic myocardium may, in part, explain the occurrence of "false-positive" transaminase tests not infrequently reported in recent literature (10).

The results of the potassium determinations, although of limited value for reasons mentioned above, demonstrate some loss of potassium ion in the peripheral ischemic areas. An equilibrium between extracellular and intra-cellular potassium will presumably occur as the necrosis becomes more pronounced in the central areas of the infarct. The concentration of potassium in tissue possibly will be of the same order of magnitude as that in serum.

The plasminogen activator in the tissue was found to be markedly decreased in areas with fully developed necrosis whereas ischemia areas did not show a decrease. No significant differences were observed for the tissue activator in necrotic areas with and without hemorrhage. In one infarct (case 6), in which rupture occurred on the ninth day after the onset of symptoms, only minimal amounts of activator was found. The lowest tissue activator concentration was found in necrotic areas of infarcts not older than one week. A few observations seemed to indicate that the plasminogen activator increased somewhat with increasing fibrosis and organization of the infarct and in firmly fibrosed areas the tissue activator concentration was 5 to 6 times that observed in normal myocardium (cases 6 and 7). The finding of low plasminogen activator concentration in the hemorrhagic, soft, necrotic areas, and high concentration in firm scar tissue indicates that local variations of plasminogen activator are not an essential factor in the pathogenesis of rupture (12).

Summary

1. The concentrations of GO transaminase, potassium and plasminogen activator have been estimated in tissue homogenates from different zones of 7 human myocardial infarcts of varying age.

2. Significantly decreased values for GO transaminase, plasminogen activator and potassium were found in areas with fully developed necrosis.

3. Ischemic areas showed a less marked but significant decrease in GO transaminase and potassium concentrations, whereas the plasminogen activator was normal or slightly increased.

4. Fibrosed infarcts showed increased concentrations of plasminogen activator.

Résumé

1. La concentration de transaminase GO, de potasse et de l'activateur du plasminogène a été déterminée dans les homogénats tissulaires de différentes zones de 7 infarctus de myocarde d'âge différents.

2. Une diminution interprétable des valeurs de transaminase GO, de l'activateur du plasminogène et du potasse est notée dans les zones de nécrose pleinement développée.

3. Les zones d'ischémie ont une diminution moins marquée mais toujours interprétable de la transaminase GO et du potasse; la concentration de l'activateur du plasminogène est normal ou légèrement augmentée.

4. Les infarctus fibrosés ont une concentration augmentée de l'activateur du plasminogène.

Zusammenfassung

1. In Gewebehomogenaten verschiedener Zonen von 7 menschlichen Myokardinfarkten verschiedenen Alters wurden die Konzentrationen von Kalium, Plasminogen-Aktivator und GO-Transaminase bestimmt.

2. Signifikant verminderte Werte von GO-Transaminase, Plasminogen-Aktivator und Kalium wurden im Bereich der vollständig entwickelten Nekrose gefunden.

3. Ischämische Gebiete zeigten eine weniger deutliche, aber doch signifikante Verminderung der GO-Transaminase und des Kaliums, während der Plasminogen-Aktivator normal oder leicht vermehrt war.

4. Eine erhöhte Konzentration des Plasminogen-Aktivators fand sich in fibrös umgewandelten Infarktgebieten.

References

(1) Albrechtsen, O. K.: The fibrinolytic activity of human tissues. Brit. J. Haematol. **3**: 284 (1957).
(2) Astrup, T.: Biological significance of fibrinolysis. Lancet **271**: 565 (1956).
(3) Astrup, T., Albrechtsen, O. K.: Estimation of the plasminogen activator and the trypsin inhibitor in animal and human tissues. Scand. J. clin. Lab. Invest. **9**: 3 (1957).
(4) Bing, R. J., Castellanos, A., Gradel, E., Lupton, C., Siegel, A.: Experimental myocardial infarction. Amer. J. med. Sci. **232**: 533 (1956).
(5) Blumgart, H. L., Gilligan, D. R., Schlesinger, M. J.: Experimental studies on the effect of temporary occlusion of coronary arteries. Amer. Heart J. **22**: 374 (1941).
(6) Henley, K. S., Pollard, H. M.: A new method for the determination of glutamic-oxaloacetic transaminase and glutamic pyruvic transaminase in plasma. J. Lab. clin. Med. **46**: 785 (1955).

(7) Jennings, R. B., Crout, J. R., Smetters, G. W.: Studies on distribution and localization of potassium in early myocardial ischemic injury. Arch. Path. (Chicago) 63: 586 (1957).
(8) Jennings, R. B., Kaltenbach, J. P., Smetters, G. W.: Enzymatic changes in acute myocardial ischemic injury. Arch. Path. (Chicago) 64: 10 (1957).
(9) Karmen, A.: A note on the spectrophotometric assay of glutamic oxaloacetic transaminase activity in human serum. J. clin. Invest. 34: 131 (1955).
(10) Kattus, A. A., Watanabe, R., Semenson, C.: Diagnostic and prognostic significance of serum transaminase levels in coronary occlusive disease. Circulation (N. Y.) 15: 502 (1957).
(11) Kent, S. P.: Effect of post mortem autolysis on certain histochemical reactions. Arch. Path. (Chicago) 64: 17 (1957).
(12) Lunseth, J. H., Ruwalt, M.: Pathogenesis of cardiac rupture due to myocardial infarction. Dis. Chest. 30: 499 (1956).
(13) Mallory, G. K., White, P. D., Salcedo, S. J.: The speed of healing of myocardial infarction. Amer. Heart J. 18: 647 (1939).
(14) Nydick, I., Wroblewski, F., La Due, J. S.: Evidence for increased serum glutamic oxaloacetic transaminase. Circulation (N. Y.) 12: 161 (1955).
(15) Rodeck, H.: Der Kalium-Inhalt im kranken Muskel. Schweiz. med. Wschr. 83: 1137 (1953).
(16) Siegel, A., Bing, R. J.: Plasma enzyme activity in myocardial infarction in dog and man. Proc. Soc. exp. Biol. (N. Y.) 91: 604 (1956).
(17) Tennant, R., Grayzel, D. M., Sutherland, F. A., Stringer, S. W.: Studies on experimental coronary occlusion. Chemical and anatomical changes in the myocardium after coronary ligation. Amer. Heart J. 12: 168 (1936).
(18) Wallace, W. M.: The application of the internal standard flame photometer to the analysis of biological material. J. Lab. clin. Med. 37: 621 (1951).