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Myocardial Infarction without Coronary Occlusion

A morphologic study in sheep

G. Helmius[1], B. Gerdin[3] and B. Nyström[2]

From the Departments of Diagnostic Radiology[1] and Neurosurgery[2], University Hospital and Institute of Forensic Medicine[3], Uppsala University, Uppsala, Sweden

ABSTRACT

The macroscopic, histologic and enzyme-histochemical characteristics of the myocardial lesion obtained after heating of a thermosonde in a branch of the left coronary artery in sheep is reported. In 13 sheep such myocardial lesions were produced distal to the location of the thermosonde. Alterations were observed in accordance with generally accepted morphologic criteria for myocardial infarction. The coronary artery branch in which the thermosonde was located showed erythrocyte and platelet aggregates immediately after the heating episode, which disappeared within a few min, as demonstrated by coronary arteriography. Injection of radiolabelled microspheres into the coronary circulation after induction of the myocardial lesion, cryosectioning of the heart and autoradiography revealed a lack of blood flow in the damaged myocardial region. We consider this new method a suitable tool for further studies on the complex pathology involved in the development of myocardial infarction.

INTRODUCTION

Much effort has been invested in attempts to clarify the true nature of myocardial infarction, and several animal models have been developed as tools for experimental studies. Most models are based on a mechanically achieved coronary occlusion. The argument that these models reflect events that correspond to the clinical situation has implied acceptance that coronary occlusion is a necessary prerequisite for the development of an ischaemic myocardial lesion. This assumption has been questioned by several authors in recent years (2,18,56,60,68).

This paper describes the morphologic appearance of the myocardial lesion caused by heating of a thermosonde introduced into a major branch of the left coronary artery in sheep (28). This model, as evaluated by left ventricular angiography, results in an altered left ventricular function, similar to that

found in connection with myocardial infarction, although no mechanical occlusion is observed at coronary arteriography (29). It might therefore serve as a complementary tool in further studies on the pathogenesis of myocardial infarction.

MATERIAL AND METHODS

Twelve rams and one ewe of a fine-wool breed, less than 36 months old and with an average weight of 45 kg (range 31-66 kg), were used. They were anaesthetized with chloral hydrate and thiomebumal sodium as described in a previous publication (28). Ventilation was controlled in a partial rebreathing system and anaesthesia was maintained by 75% nitrous oxide in oxygen with the addition of small amounts of halothane.

Experimental procedure

The different stages of the experimental procedures are described in detail elsewhere (28). Of the 13 sheep, six were used in the autoradiographic investigations and the other seven for histology. In two of the latter animals thoracotomy was performed before the induction of the experimental myocardial lesion, and in the other five after this induction. Four of these five sheep were killed 6 h after, and one 7 h after the experimental infarction procedure.

Catheterization was performed as described elsewhere (28). After coronary arteriography and left ventricular angiography (29), the coronary catheter was left in the orifice of the left coronary artery and a thermosonde with an NTC-type thermistor at the tip (Siemens, K 19) was introduced through the catheter into one of the major branches of the left coronary artery. The thermistor functioned as a heater as well as a thermometer.

Myocardial damage was produced by intermittent heating of the thermistor (28). After a total heating period of 38-1120 s (average 202 s) the heating was stopped and the thermosonde withdrawn.

In two sheep biopsies about 1.5 x 3 mm in size were taken at different time intervals from the macroscopically damaged myocardium as well as from supposedly undamaged myocardium, with the aid of a conchotome. The material so obtained was used in histochemical studies.

In the two sheep subjected to thoracotomy before induction of the myocardial lesion, the development of the macroscopic appearance of the myocardial damage could be recorded during the intracoronary heating, on a 16 mm colour motion film (Eastman Kodak Company, Rochester, New York, USA).

Autoradiographic investigation

In 6 animals radioactively labelled microspheres were injected selectively

into the left coronary artery. In one animal this was performed before, and in five animals at different time intervals (2.5 h, 3.5 h, 7.5 h, 7 days, 13 days) after the induction of the myocardial lesion. For this purpose carbonized microspheres labelled with ^{141}Ce and with a diameter of 15 $\pm$ 5 µm (3M Company, Saint Paul, Minnesota, USA) delivered in dextran and mixed with either Isopaque Coronar^{R}, 370 mg I/ml, or Urografin^{R}, 290 mg I/ml, were used. The approximate number of particles injected varied between 1.1 - 5.7 x 10^{6} and the amount of radioactivity injected varied from 1 to 9 MBq. This amount was shown to create good autoradiographies from sections 100 µm thick.

Immediately after death the hearts were excised, examined macroscopically and photographed. After removing the left and right atrium and the right ventricle, with the exception of the interventricular septum, the hearts were sectioned transversely into two or three pieces and mounted on a microtome stage with a metal frame (40 x 120 mm) with the apex uppermost. The hearts were embedded in carboxymethyl cellulose and rapidly frozen by immersion on the microtome stage in hexane and solid CO_2 (-75°C) for 10 min. The metal frame was then disconnected and the stage with the firmly fixed heart was mounted in a cryostat at a temperature of -20°C. Transverse sections of the heart tissue (20 and 100 µm) were cut parallel to the microtome stage every mm, beginning at the left ventricular apex. Sections for autoradiography were picked up onto 810 Scotch brand adhesive tape (3M Company) as described by Ullberg (71) and Ullberg & Appelgren (72). The sections were mounted on wooden frames and after storage in dry air at -20°C for 2 days the sections were placed in contact with Industrex C x-ray film (Eastman Kodak Company) for exposure. The sections and the film were sandwiched in ordinary x-ray cassettes in which the intensifying screens had been replaced by cardboard, and were kept at -20°C for 2 months. After exposure, the sections and x-ray films were carefully separated. The films were processed in a Pakorol XR processing machine (Pako Co, Minnesota, USA) with developer G 137 (Agfa Gevaert) at 24°C. The developing time was 105 s.

Light microscopy

Seven sheep were used for these studies. Immediately after death the hearts were excised, opened by an incision through the left ventricle and fixed in excessive amounts of 4% buffered formaldehyde (Histofix, Bethlehem Trading). Several pieces were taken from the damaged myocardial region, i.e. a visibly recognized region distal to the site of the thermosonde, as well as from the interventricular septum, from the posterior wall including the posterior papillary muscle, and from an undamaged region in the upper part of the anterior wall. The pericoronary tissue, including the coronary artery at the level of

the thermosonde, was dissected and serially sectioned transversely to the coronary vessel. Sections were stained with Mayer's acid haematoxylin-eosin (38), Mallory's phosphotungstic acid haematoxylin (40) with the precautions suggested by Voigt (73), the picro-Mallory stain of Carstairs (9) and the van Gieson stain of Weigert (38) and with nuclear fast red (38).

Cryotome sections from biopsies (see below) were stained with PAS as described by Mowry & Millican (49) for evaluation of the amount of tissue glycogen.

Enzyme histochemistry

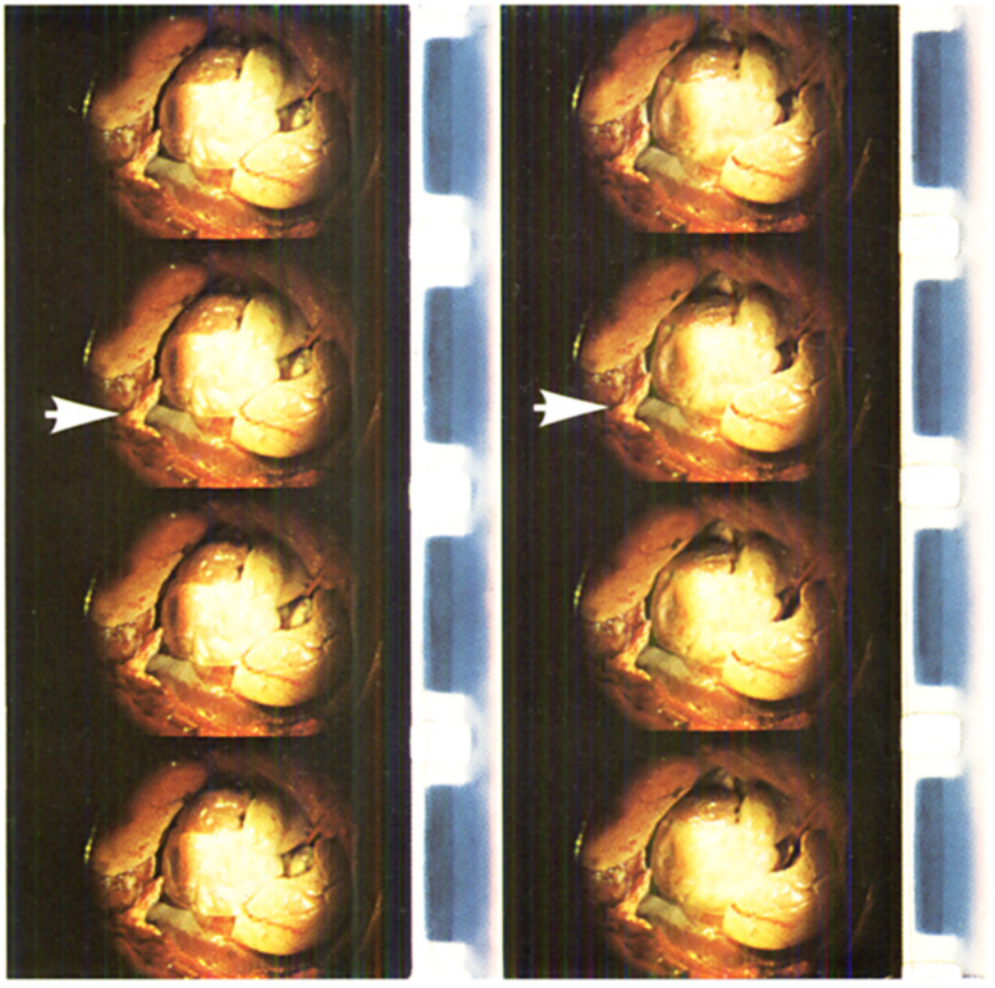
In two sheep, biopsies taken at various times after the heating were immediately frozen in liquid nitrogen and kept at -70°C until sectioned. Sections 16 µm thick were cut on a cryotome and studied for the presence of phosphorylase according to the method of Takeuchi & Kuriaki (70) with the modification of Eränkö & Palkama (19), myofibrillar ATPase (55), succinic dehydrogenase (SDH) (51) and NADH-dependent dehydrogenase activity - so called NADH-tetrazolium reductase (NADH-tr) (62); the two latter with some modifications by Nyström (52).

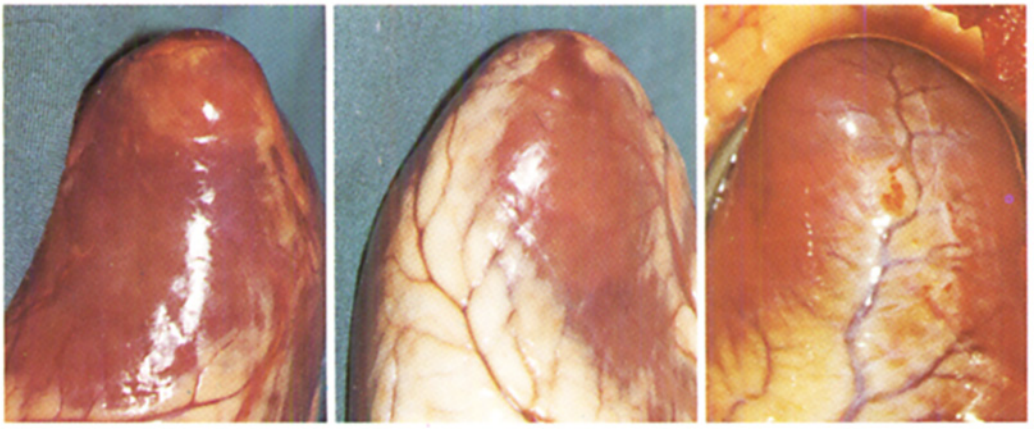
RESULTS

Macroscopic appearance

Immediately after the heating episode an area of the myocardium with its proximal margin, usually more than 10 mm distal to the location of the thermosonde, became cyanotic (Fig. 1 top left). It lost its contractile properties and, as observed on 16 mm film, was slightly depressed compared with the surrounding musculature and completely adynamic. On palpation in vivo, reduced tone was found in the cyanotic area. The appearance during one typical systole and one diastole about 1 min after heating is seen in Fig. 1 bottom. This corresponds to the presence of a hypokinetic region as visualized at left ventricular angiography (29). A few h later the cyanotic area became paler and after about 8 h a hyperaemic border with small haemorrhages in the margin was seen. In hearts investigated up to 13 and 15 days after heating, the previously cyanotic area remained soft and became more pale yellow than the surrounding undamaged muscle.

Fig.1. Macroscopic appearance of the myocardial lesion in four different animals at various time points after the heating procedure. Top left: after 45 min. Top middle: after 7.5 h. Top right: after 50 h. The motion picture frames show the myocardial lesion *in vivo* immediately after the heating. Maximal systolic contraction (left) and diastolic dilatation (right) are indicated by arrows.





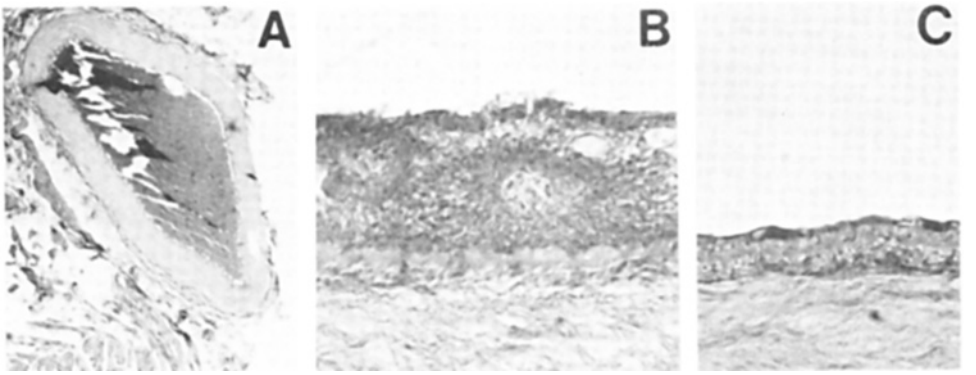


Fig. 2 A. A coronary artery occluded by a massive aggregate of mainly red blood cells but also platelets and some white cells. Picro-Mallory, 23 x.

B. Platelet aggregates are covering an area of a coronary arterial wall in a sheep killed 6 h after intracoronary heating. Picro-Mallory, 920 x.

C. Normal intima of a coronary artery with intact endothelial cells in the same animal as in B. Picro-Mallory, 920 x.

Microscopic findings

Immediately after the heating procedure, platelet and erythrocyte aggregates occluded the coronary artery in the region where the thermosonde was located (Fig. 2 A). In sections from animals killed after 6 h no coronary occlusions were seen. At that time, however, minor platelet aggregates adhered to presumably damaged endothelium (Fig. 2 B). In the intramural arterial branches and in the microcirculation no fibrin- or platelet-containing aggregations were observed. In one section a minor fibrin-containing thrombus occluded the lumen of an intramural vein.

The first light microscopic sign of a myocardial lesion was seen immediately after the heating procedure in the form of discrete areas in which the individual fibres exhibited contraction bands characteristic of myofibrillar degeneration (Fig. 3 A). At this time a capillary stasis was also observed. Six h later the alterations were more profound. In the transition zone to healthy myocardium, myofibrillar degeneration was evident, with contraction bands as well as many relaxed wavy fibres (Fig. 3 B). In the central part of the affected region myocardial damage was observed in the form of altered staining properties. Muscle cells were also swollen and nuclei exhibited both pyknotic and karyorrhectic (Fig. 3 D) changes. Although identifiable, the cross-striations were obscured by a diffuse granularity. The fibres were swollen with varying widths, and the distance between the individual fibres was sometimes increased, indicating interstitial oedema. In a few sections the cellular phase of an inflammatory reaction was identified in the form of margination of granulocytes in capillaries and also as perivascular granulocyte aggregates (Fig. 3 C).

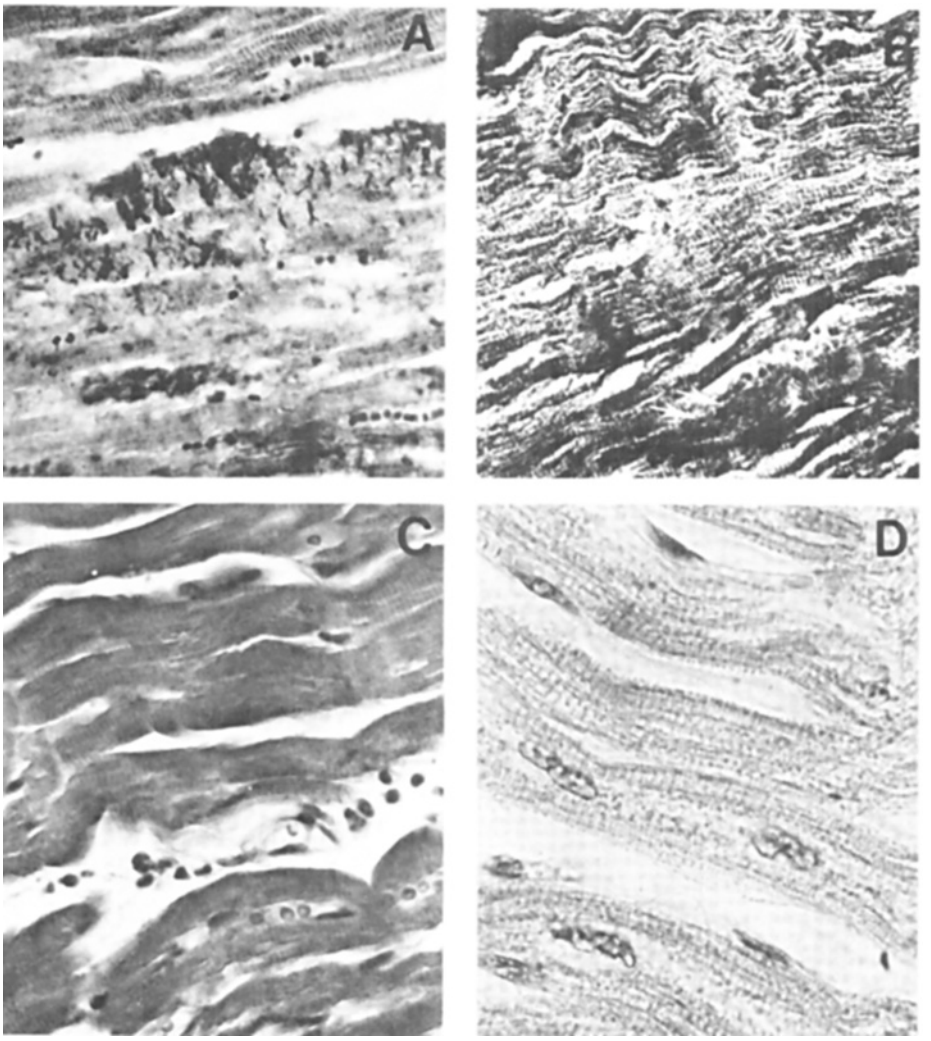


Fig. 3 A. Myofibrillar degeneration with characteristic contraction bands and capillary stasis in a sheep dying immediately after the heating procedure. PTAH, 370 x.

B. Wavy fibres in the borderline area against healthy myocardium in the upper half of the figure. In the lower part myofibrillar degeneration (arrows) and capillary stasis are seen. PTAH, 370 x.

C. Early perivascular inflammatory reaction in a sheep killed 6 h after the heating. Haematoxylin and eosin, 920 x.

D. Nuclei in various stages of degeneration in a sheep killed 6 h after the heating. Nuclear fast red, 720 x.

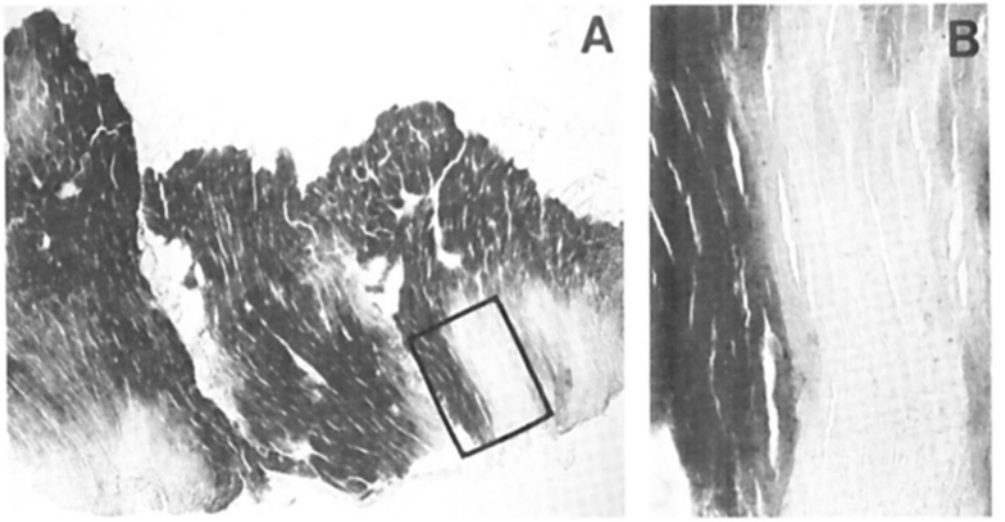


Fig. 4. Sections of a biopsy taken from damaged myocardium 45 min after induction of the lesion. Staining for phosphorylase. In A at low magnification (60 x) a patchy loss of staining is seen. The marked area is shown in a higher magnification (230 x) in B.

Enzyme histochemistry

In the first biopsies taken 45-60 min after the heating procedure, a patchy decrease in myocardial phosphorylase activity was seen (Fig. 4). At this time a likewise patchy decrease in the amount of muscle glycogen was identified as pale zones in PAS-stained sections. Sections stained for ATPase, SDH and NADH-tr exhibited no change in enzyme distribution or activity at any of the times following the injury, i.e. up to 6 h.

Autoradiography

Examination of the autoradiographs from the sheep in which the radioactively labelled microspheres were injected before induction of myocardial damage revealed a homogeneous blood supply to the entire left ventricular myocardial tissue. This is in accordance with the finding at arteriography in a previous study (29) that the left coronary artery (into which the microspheres were injected) is completely responsible for the blood supply of the left ventricle.

In the five animals in which microspheres were injected at various time intervals after heating, there was a virtual absence of radioactivity in a region corresponding to the area where the infarction was observed either at gross examination, examination of the cryotome sections, or left ventricular

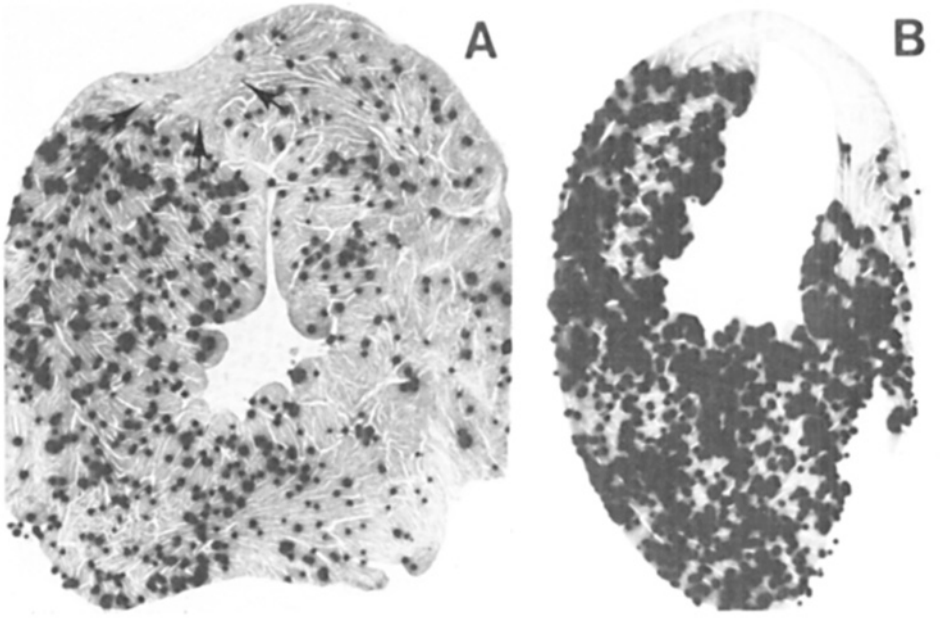


Fig. 5 A. Autoradiography of a heart from an animal injected with radiolabelled microspheres 13 days after the intracoronary heating procedure. Note the visibly infarcted area, marked by arrows, which is devoid of radioactivity.

B. Autoradiography of the only heart with arteriographically verified coronary arterial obstruction. The thin-walled infarcted area is devoid of radioactivity.

angiography (Fig. 5 A), or at two or all of these examinations. In one of these sheep a larger transmural area was devoid of radioactivity, in good accordance with the macroscopic and left ventricular angiographic location of the myocardial lesion (Fig. 5 B). Here the infarcted area was very thin and distended, with a tendency to aneurysmatic formation. This was the only case (29), i.e. the only one out of 11, in which coronary arteriography performed after the heating procedure revealed an occlusion of the vessel lumen.

In four of the experiments no side-effects of the microsphere injections were noted ($1.6 - 3.8 \times 10^6$ injected). In two cases the experiments were ended after injection of 5.6 and 5.7×10^6 microspheres respectively, which in both cases gave rise to ventricular fibrillation.

DISCUSSION

The methods used in this investigation to study the myocardial lesion observed by the naked eye revealed that the lesion is very similar to that seen in myocardial ischaemia and infarction. The histologic observations of contraction bands, altered staining properties, swollen muscle cells and nuclear changes have all been generally accepted as indicating an early ischaemic

lesion (1,3,5,67,69).

Coagulative myocytolysis or myofibrillar degeneration (3) with hypercontraction of muscle fibres and the presence of coarse transverse contraction bands due to coagulation of hypercontracted sarcomeres has been related to the effect of high local concentrations of catecholamines (33,43,44,45,74) and considered to represent an extreme exhaustive effect on the myocyte. The appearance of such lesions immediately after the heating procedure is in accordance with the general forensic experience that this type of lesion can develop in conditions associated with high adrenergic tone even if death ensues very rapidly (10,17, 65). The changes might also reflect effects of local arrhythmias, as suggested by Rajs et al. (57).

Histochemistry is nowadays a frequently employed tool in studies of myocardial ischaemia (34,46,61,67). Within the affected area there was a decrease in glycogen 45-60 min after heating. When myocardial infarction is induced by ligation of the coronary artery, this glycogen loss seems even more rapid and complete (67,69). In our model we found a clear loss of phosphorylase activity only 45 min after the heating procedure. After coronary artery ligation phosphorylase activity has been found to decrease in the rat within 2 h (14) and in the dog within 4 h (39). The latter authors also found a patchy loss of myofibrillar ATPase after 4 h in the dog. In our experimental model no change in this enzyme activity was found even 6 h after the heating. Neither was any change in SDH nor NADH-tr activity observed. Although no consistent change in activity of these two enzymes has been reported following coronary artery ligation, most authors describe a decrease (13,14,36,64,75). This has also been found in myocardial infarction in man (20).

The apparent decrease in phosphorylase activity following injury might be a mere reflection of muscular fatigue, as found in skeletal muscle (35) and as is known to be associated with a decrease in the active form of the enzyme (11,12). Since there is no loss of total phosphorylase activity during fatigue, the histochemical phosphorylase method of Takeuchi & Kuriaki (70) will probably reflect the level of active phosphorylase, as pointed out by Kugelberg & Edström (35).

Another explanation for the decrease in phosphorylase activity together with unchanged activities of ATPase, SDH and NADH-tr might be that the phosphorylase is primarily located in the cytoplasm, and might therefore leak to the extracellular space through a defective cell membrane after damage to the cell more easily than the latter three enzymes, which are mainly linked to mitochondria or actin filaments.

No other changes in enzyme activity were observed in our experimental model. It must be emphasized, however, that the material was obtained with a conchotome and consisted of a very small piece of subepicardial muscle. As, at least

after coronary ligation, the myocardial infarction is first seen subendocardially and then spreads progressively towards the epicardium (59,63), it is possible that subendocardial tissue samples might have exhibited more profound alterations. Labelled microspheres have been used previously in studies on myocardial perfusion during ischaemia (6,15,31,58). Budd et al (7) used macroaggregated albumin instead of carbonized beads, together with the Ullberg technique, to visualize the size of an experimental myocardial infarction. They found, as we have done, that zones of tissue damage in infarcted myocardium can be localized and measured with this technique. This possibility is due to the fact that the damaged myocardium lacks local blood flow. Such impairment of local tissue perfusion might be a consequence of the infarction process and does not necessarily require the occurrence of a mechanical occlusion of a major vessel, discernible on coronary arteriography.

The connecting events between the heating and the myocardial lesion have not been revealed by our studies. The amount of energy liberated, 700-5700 mJ (28), is several ten-potencies lower than can be expected to be necessary to produce thermal damage.

Transient arterial spasm is a possible explanation for the observed effect. Such spasm has been supposed to be responsible for the transient myocardial dysfunction seen in Prinzmetal's angina (8,16,27,42,53) and also for certain cases of acute myocardial infarction (21,23,24,30,54,66).

There are at least two different ways in which spasm could influence the myocardial function. One is by a primary decrease in blood flow due to a reduction of the transsectional area, of such pronounced degree as to cause an ischaemic myocardial lesion. Another possibility is that arterial spasm leads to endothelial damage at the site of the most contracted arterial segment, followed by platelet aggregation and microembolism of such aggregates to the myocardium (22).

Embolization of platelet aggregates has been shown to cause permanent damage to the myocardium in swine (32) and has also been found to occur distal to developing thrombi in coronary arteries in dogs (47,48,50) and in the coronary macro- and microcirculation in sudden deaths due to coronary disease (25,26). In baboons, Leinberger et al. (37) observed local aggregation of platelets in the potentially salvageable marginal zone early after coronary occlusion. They supposed that such aggregation was responsible for the extent of infarct evolution. These results support the findings in the present investigation and it seems plausible that platelet aggregation is one probable pathogenetic factor in the ischaemic damage. One possibility is that local haemolysis occurs in the vicinity of the warm tip of the thermosonde, which could facilitate platelet aggregation,as described by Born (4). The platelet aggregation

is obviously a reversible event, as no occlusion was seen 6 h after the heating procedure in the microscopic sections or earlier at coronary arteriography.

The purpose of this study was not to reveal in detail the pathogenetic pattern of the myocardial lesion obtained after heating by the thermosonde. This cannot be done without thorough studies of the myocardial metabolism and of the rheological alterations occurring on induction of the cardiac lesion.

The present investigation shows,however, that the myocardial lesion created is accompanied by morphologic alterations that can be described as myocardial infarction. In spite of our lack of knowledge of the precise mechanisms involved, we consider this method a suitable tool for further studies on the complex pathology involved in the development of myocardial infarction.

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Address for reprints:
Gunnar Helmius, M.D.
Dept for Diagnostic Radiology
University Hospital
S-750 14 Uppsala Sweden