THE PERICARDIUM AND PERICARDIAL ADHESIONS IN RELATION TO REOPERATIVE CARDIAC SURGERY

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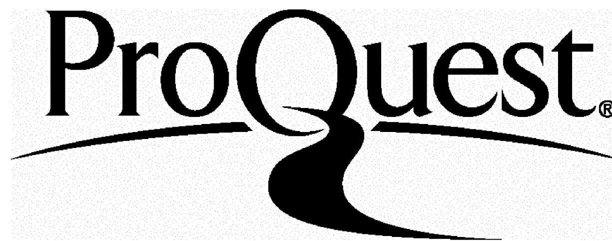

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GUIDE TO THESIS

The "guide to thesis" was composed so as to promptly direct the attention of the reader to the author's style and format within this work. By gaining this early attention it is hoped that less time and effort would be spent by the reader in attempting to appreciate the manuscript's direction of flow.

The abstract has given a summary of the subject and main conclusions of this thesis. The lists of: content, figures and tables are to simplify cross referencing. These lists are followed by the acknowledgements.

Chapter 1. The introduction has initially taken an overview of the evolution of cardiac surgery and highlighted some of the areas that may have hindered or encouraged the progress of the speciality. As some of the main developmental steps are reviewed, one eventually comes to the prevalent modern day phenomenon of reoperative cardiac surgery and the risk imposed upon it by the presence of pericardial adhesions. By reviewing the evolution in this way, it is hoped that the surgical problems encountered due to the presence of pericardial adhesions might be placed in the context of the many other evolutionary problems that cardiac surgery has encountered and overcome during its development.

The normal basic morphology and function of pericardium is discussed so as to prepare the reader to appreciate the abnormal situation following surgery. Similarly the fibrinolytic system, in relation to the pericardium, is discussed so as to remind the reader of certain background facts relating to the fibrinolytic system.

Chapter 2. Not only are adhesions encountered as a problem in reoperative cardiac surgery but also in reoperative surgery in general. Therefore part of the aim of this chapter is to concisely place reoperative adhesions in the context of reoperative surgery in general. In addition, it reviews the work that has been done with regard to overcoming the problems of pericardial adhesions in reoperative cardiac surgery. The volume of publications on the subject is perhaps a testament to the interest that the subject provokes

and to the magnitude of the problem that researchers perceive this aspect of cardiac surgery to be.

Chapters 3 & 4. An outline of the aim and design of the study into three phases is given in chapter 3. The study developed in phases as a response to fundamental questions about the pericardium and its response to surgery. Therefore, the separation and presentation of this study into phases seemed most appropriate to enable the reader to follow and appreciate the evolution of the work.

Chapter 4 contains the "Patients, Materials and Method" and indicates those aspects of the study the are common to each phase. Those parts of the study that are peculiar to one phase rather than another are also elaborated. In subsequent chapters when "Patients, Materials and Methods" is mentioned then chapter 4 will be briefly referred to.

Chapters 5, 6, 7 and 8. The formats of chapters 5, 6, and 7 are similar and follow the pattern of a:
1. Summary, 2. Introduction, 3. Patients, Material & Methods, 4. Results, and 5. Discussion.

It is anticipated that the summary will enable the reader to obtain a rapid overview of the salient points before reviewing the more elaborate detail. The introduction presents the background to the phase that it introduces and, where relevant, links its development from the preceding phase. The "Patients, Materials & Methods" will be a summarised version of chapter 4 and will refer back to the relevant parts of that chapter. The result and discussion sections present and briefly discuss the experimental data for each phase of the study and considers the clinical implication of these results.

The final chapter reviews the results of the work, considers its present clinical implications and discusses the possible future prospects with respect to the pericardium and pericardial substitution.

ABSTRACT

The presence of pericardial adhesions prolongs the operation time and increases the risk of serious damage to the heart and other major vascular structures during resternotomy. The reported incidence of such damage is 2-6%. Pericardial mesothelial cells have regenerative potential and exhibit fibrinolytic activity. The pericardial mesothelium therefore has the capacity to recover following cell loss or damage and has an actual or potential role in the break down of the fibrinous adhesions that serve as the initial scaffolding for the firm collagenous adhesions seen at reoperative surgery. These features of the pericardium may have been underestimated in much of the previous research into pericardial substitution. The studies in this thesis have demonstrated:

- That during cardiac surgery there is a significant reduction in the pericardial fibrinolytic activity in comparison to initial activity. The recovery in fibrinolytic activity that occurs towards the end of the surgical procedure and afterwards never reaches the preoperative magnitude.

- That during cardiac surgery, simultaneous with the changes in the fibrinolytic activity, increasing pericardial inflammation occurs with a concomitant increase in mesothelial damage. Permanent residual damage is apparent in the reoperative pericardium.

- That during the reduction in pericardial fibrinolytic activity the simultaneous plasma tPA activity rises to a peak suggesting that the fall in pericardial fibrinolytic activity is independent of the plasma tPA and haemodilution.

- That the cellular content of pericardial fluid is mainly mesothelial which may therefore contribute to mesothelial regeneration and recovery. The pericardial fluid, by virtue of its enzyme content, has the potential to take part in fibrinolytic activity.

- That the polyhydroxybutyrate pericardial patch does not confer any obvious short-term reoperative advantage in calves exposed to CPB surgery.

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CHAPTER 1

INTRODUCTION

INTRODUCTION

Pericardial Adhesions In Relation to the Evolution of Cardiac Surgery

Adhesions across the pericardial space are a typical sequel to cardiac surgery. Yet the problems that adhesions present to present day reoperative cardiac surgery could not have been anticipated by the pioneers of the specialty when they began to lay down its foundations. At present, despite the research that has gone into this field, there is no universally accepted method of overcoming the problems posed by adhesions at reoperation. Indeed, in recognising this fact it was Gabbay (Gabbay, 1990) who proposed "the need for intensive study of pericardial substitution after open heart surgery" as one means toward overcoming the problems that pericardial adhesions present. For those that would undertake such research, the problems presented by pericardial adhesions must be seen in the context of the many obstacles that have arisen, and been overcome, during the evolution of cardiac surgery. These obstacles have often been of a practical nature, such as anaesthesia, cardiopulmonary bypass circuits, myocardial preservation, and blood transfusion. Moreover certain early day discouraging attitudes, such as short-sighted and pessimistic prognostications of the speciality, have also served as obstacles to progress. If the problem presented by pericardial adhesion are to be addressed then such difficulties must be overcome with the appropriate research.

As will be shown in the following sections, the steps in the evolution of cardiac surgery have been a mixture of experimental endeavours and clinical application. Therefore, in order to view the problems of pericardial adhesions in context rather than as an isolated phenomenon, it is of pertinent interest to take a brief overview of the evolution of cardiac surgery and the pioneering spirits that have given this evolution impetus. Such an overview might enable the reader to more easily regard the problems of pericardial adhesion in a context that incorporates the evolution of the speciality.

An Overview of the Evolution of Cardiac Surgery

An example of the type of attitude and fear that have hampered the progress of the speciality is exemplified by the comment made by the renowned Theodore Billroth (Harken, 1989), in about 1894:

"Any man who would operate on the heart should lose the respect of his colleagues."

Later Sir Steven Paget, 1896, expressed the view that:

"Surgery of the heart has reached the limits set by Nature toward all surgery, no new method and no new technique will overcome the natural obstacles surrounding a wound of the heart."

Yet soon after these comments, in Sept. 9, 1896, Rehn of Frankfurt successfully sutured a stab wound of the right ventricle. Many such operations were to follow.

Early Operative Developments

Not long after the famous Paget prediction, 1896, and Rehn's stab wound success, Sir Lauder Brunton, in 1902, wrote about the mechanical nature and treatment for mitral stenosis (Brunton, 1902). Cutler, initially at Western Reserve University Medical School and later the Mosley Professor of Surgery at the Harvard Medical School and the Peter Bent Brigham Hospital in Boston, did experimental work, as did others, related to treating mitral stenosis surgically. He and Levine in 1923 reported a case operated on through a median sternotomy incision in which a special curved knife was inserted through the left ventricular apex to cut a stenotic mitral valve (Cutler and Levine, 1923). It was soon after this, in 1925, that Sir Henry Souttar, at the London Hospital, digitally opened a stenotic mitral valve through the left atrial appendage (Souttar, 1925). However, the referring Physicians, for their own reasons, appeared not to be impressed and did not make any more such referrals. They perhaps felt that the operation was unjustifiable and ahead of its time. Sir Henry was therefore unable to repeat the operation (Harken, 1989). Perhaps because of this attitude, the stimulus to the speciality that might

have been gained from the success of this early operation was not exploited. Instead, a number of rather compromise operations surfaced (Harken, 1989).

Cardiac surgery received further impetus in August 1938, when Robert E. Gross of the Boston Children's Hospital, performed successful ligation of a patent ductus arteriosus (PDA) in a 7 year old girl (Gross and Hubbard, 1939). He subsequently developed division rather than ligation of the PDA as the surgical method of choice. During World War II, Dwight Harken reported successful removal of a large number of metallic fragments from in and about the heart (Harken, 1946). This was encouraging in that it showed that the heart could be manipulated and operated on.

After the second World War, Harken (Harken et al, 1948) and Bailey (Bailey, 1949) in the United States and Brock (Baker et al, 1950) in London continued with a surgical approach to mitral stenosis. Their approach of opening the stenotic mitral valve through the left atrial appendage was similar. A number of new techniques were subsequently added to the operation of closed mitral valvotomy. One of these was the Tubb's transventricular dilator used with digital control by a finger inserted through the left atrial appendage (Austen and Wooler, 1960). The results of closed mitral valvotomy were satisfactory and sometimes very good such that its use continued beyond the 1960s.

Until the mid-1950s, with the exception of valvotomy for mitral stenosis, cardiac surgery consisted of operations for extracardiac lesions, such as patent ductus arteriosus, coarctations, Blalock-Taussig shunts, and pulmonary valvotomies. Aortic valve surgery lagged behind, the first report having reference to aortic valve surgery was by Smithy and Parker, on their study on aortic valvotomy (Smithy and Parker, 1947). In the early 1950s clinical attempts to resolve aortic stenosis were limited to closed methods, using either a dilator introduced transventricularly or a digital approach through a pursue-string hole sown into the aorta (Bailey et al, 1950). Their success was only modest. In 1951, Hufnagel, in Washington, D.C., developed a chambered ball valve that was to be inserted into the descending thoracic aorta in the hope of palliating aortic regurgitation (Hufnagel, 1951; Hufnagel and Harvey, 1953). It did not resolve the signs of aortic

regurgitation in the upper body and further progress here had to wait until the introduction of cardiopulmonary bypass (CPB).

The Introduction of Cardiopulmonary Bypass

Whilst the existing operative procedures were being refined, several groups and noted surgeons were enthusiastically pursuing the development of a mechanical method of temporarily replacing the function of the heart and lung. Gibbon, with his pioneering experimental work at the Massachusetts General Hospital in Boston in the late 1930s was a major contributor to this. Other workers included Crafoord and Senning in Stockholm, Cleland in London, Dennis and later Lillehei in Minnesota. However, it was Gibbon who is credited with the first successful clinical use of the CPB circuit, when he repaired an atrial septal defect in a young woman in 1953. Not long after that, perhaps because of subsequent disappointing clinical cases, Dr Gibbon discontinued his participation in open heart surgery. Nevertheless, the Gibbon machine was adopted and modified by the Mayo Clinic under the aegis of Dr John Kirklin. Other successes soon followed. In June 1954, Lillehei performed his first successful operation on a small child with cross-circulation. In July 1954 Crafoord removed a left atrial myxoma from a 40 year old woman using CPB, electrical fibrillation and hypothermia.

The experimental work done by Senning before this successful operation by Crafoord is of particular interest because it illustrates the type of unanticipated problems that can arise and how they were overcome. Having been invited onto the Crafoord research team, Senning noted that a number of those experiments that involved opening the heart were complicated by air emboli. To overcome this problem they elected to stop the heart's pumping activity by electrically induced ventricular fibrillation (Senning, 1952; Senning, 1955). Having arrested the heart for a time, the often oedematous heart had to be electrically paced after defibrillation until spontaneous activity resumed. Furthermore, to reduce body oxygen consumption, and therefore the need for the type of high CPB flow that would traumatise the blood constituent, Senning and his colleges combined CPB with hypothermia. A technical combination that they used as standard in many of their operative cases (Senning, 1989).

With the introduction of CPB an open approach to the problem of mitral stenosis was first made with CPB by Lillehei in 1957 (Lillehei et al, 1957) and independently by Merendino (Merendino and Bruce, 1957) in the same year. About this time it was becoming increasingly realised by many surgeons that some valves were irreparable and that a prosthetic valve would be required. Successful mitral valve replacement was first reported by Starr and Edwards, in 1961 (Starr and Edwards, 1961). Similarly a more effective approach to aortic valve disease in adults came with the introduction of CPB. In aortic valve surgery also, surgical intervention was initially limited to valvotomy and the removal of calcific deposits. The use of a prosthesis in the aortic position graduated through many stages, but it was not until the introduction of the ball valve prosthesis for aortic valve replacement by Harken (Harken et al, 1960) and Starr (Starr et al, 1963) in 1960 that such surgery became established.

Myocardial Revascularisation

Myocardial revascularisation took a relatively independent evolutionary path. Alexis Carral (Carral, 1902; Carrel, 1910), who was later awarded the Nobel Prize for medicine, presented a paper to the American Surgical Association in which he describes the first documented attempt at direct coronary surgery. He described the anastomoses of a graft of homologous carotid artery between the descending thoracic aorta and the left coronary artery of a dog. He stated, "unfortunately the operation was too slow. Three minutes after the interruption of the circulation fibrillary contraction appeared, but the anastomosis took five minutes. By cardiac massage the dog was kept alive, but it died two hours afterwards.

Indirect myocardial revascularisation was attempt by Claude Beck in the mid1930s. He noticed that cutting a band of adhesions compressing the heart resulted in brisk bleeding from each end of the transected adhesion. By scarification of the epicardium new vessels would grow into the heart, this combined with a pectoral muscle flap was to increase the chances of revacularisation. Many modification of this procedure were developed, by Beck (Starr, Edwards et al., 1963) and others. However, the operative mortality was high (38%), but the vast majority of surviving patients were said to have improved.

The next major development in myocardial revascularisation came when Vineberg, in Montreal 1951, reported the direct implantation of an internal mammary artery into the myocardium in order to create new coronary collaterals (Vineberg and Miller, 1951), a procedure that the Cleveland Clinic group showed years later brought new blood to the left ventricle (Effler et al, 1965). Howbeit the amount of blood volume delivered was too small and of limited distribution to be of any long-term good.

With a more direct approach to revascularisation, in 1954 Murray and associates reported experimental studies of the anastomosis of the internal mammary artery to a coronary artery (Murray et al, 1954). Further contributions to progress came shortly after this when Longmire and colleagues, at the University of California in Los Angeles, reported a series of patients in whom direct-vision coronary endarterectomy was carried out without the use of CPB (Longmire et al, 1958). Then Senning in 1961 (Senning, 1961), reported patch grafting of a stenotic coronary artery.

The Advent of Coronary Angiography

As cardiopulmonary bypass continued to evolve, a technique for selective coronary arteriography that was safe and provided adequate detail of the coronary artery anatomy was being perfected by the pioneering work of Mason Sones at the Cleveland Clinic, in the early 1960's (Sones and Shirey, 1962). Sones had been injecting a bolus, retrograde injection of dye across the aortic valve into the left ventricle and noticed that the dye had inadvertently passed directly down the right coronary artery. Visualisation was far superior than the hitherto procedure of root injection and the patient tolerated the procedure well. The continued development of the technique led to a rapid development and application of direct coronary surgery. There was further progress when Favaloro and Effler, in May 1967, at the Cleveland Clinic began performing reversed saphenous vein bypass grafting and by January 1971 had performed 741 such operation (Loop et al, 1979).

Myocardial Preservation

With the growth of general cardiac surgery in the late 1960s and early 1970s came the recognition of the need for improved myocardial preservation. Surgeons and cardiologists were seeing a disturbing number of patients return from theatre having developed a transmural myocardial infarction perioperatively and dying soon after surgery. In particular the studies of Taber (Taber et al, 1967), showed scattered small areas of myocardial necrosis, involving approximately 30% of the left ventricular myocardium, in a group of patients dying in the early postoperative period. Furthermore, Najafi and colleagues (Najafi et al, 1969), showed that acute diffuse subendocardial myocardial infarction was found frequently in patients dying early after valve replacement and suggested that this was related to the intraoperative myocardial protection. The cause of these infarctions became apparent after regional blood flow measurements were made. These studies showed that there is considerable myocardial oxygen demand during ventricular fibrillation, and that subendocardial perfusion in particularly is impaired due to the relative compression of subendocardial vessels especially if the heart becomes distended (Buckberg et al, 1977). However, hypothermia reduces the vigour of ventricular fibrillation, thereby limiting the compression of intramyocardial vessels.

As mentioned earlier, Senning and his colleagues introduced ventricular fibrillation and varying degrees of hypothermia, with or without topical cooling. Others would combine this with either aortic or local vessel occlusion (Bigelow et al, 1950; Shumway and Lower, 1959). This technique, with some variation from surgeon to surgeon, was used extensively until the early 1970s. However, with the problems cited above accompanying ventricular fibrillation, the introduction of intermittent aortic cross-clamp ischaemia in the mid-1970s markedly reduced the incidence of low output syndrome (Buckberg et al, 1975).

Clinical cardioplegia was introduced in 1955 by Melrose et al (Melrose et al, 1955) with the use of hypertonic potassium citrate blood to facilitate arrest and a 'quiet' operating field. It was reintroduced by Bretschneider (Bretschneider et al, 1975) in the 1960s and by Gay and Ebert (Gay and Ebert, 1973) in the 1970s, and is now used routinely by

most cardiac surgeons throughout the world to prevent ischaemic injury. Continued evolution of cardioprotective strategies has brought us, via the routes of crystalloid and blood cardioplegia, to a recent trend toward continuous warm blood cardioplegic infusion (Lichtenstein et al, 1991; Salemo et al, 1991).

The Increasing Reoperative Workload

At pace with the development of the specialty has come an increasing operative work load and with that an inevitable, but perhaps unanticipated, volume of reoperative surgery. The risk of reoperative cardiac surgery is significantly higher than in primary surgery (Foster et al, 1984; Lamas et al, 1986; Lytle et al, 1987; Salomon et al, 1990). In reoperative coronary surgery this increased risk is multifactorial (Cohn, 1993). First, a ubiquitous problem that affects all forms of reoperative surgery, the risk of re-entry sternotomy owing to the presence of adhesions, and the consequent potential for cardiac injury and catastrophic bleeding. Second, there is advancement of the native coronary disease so that there now may be more diffuse coronary disease causing an impedance to coronary arterial run-off, a major determinant of adequacy of myocardial revascularisation. Third, there may be the presence of partially open, atherosclerotic saphenous vein coronary bypass grafts; this constitutes a major risk of reoperative coronary artery bypass, and the operative mortality incidence is much higher in patients with patent vein grafts as opposed to those where all vein grafts were closed for equal degrees of left ventricular dysfunction (Grondin et al, 1984; Keon et al, 1982). Handling of the atherosclerotic vein grafts may cause micro- or macro-emboli, which lead to myocardial infarctions of varying size that may be fatal. Fourth, there may be varying degree of left ventricular failure as a result of the repeated ischaemic and/or infarctive episodes that have caused the patient to represent. Finally, there may be a shortage of conduit for revascularisation.

Similar risk factors exist in reoperative valve surgery including worsening cardiac failure giving rise to dilated left and right ventricular chambers. As in reoperative coronary surgery, the presence of pericardial adhesions and consequent adherence of the dilated heart to the sternum further increases the risk of structural damage to the heart during

resternotomy. It is the widespread problem of pericardial adhesions that will be addressed in this manuscript.

Reasons for Reoperation

The increasing volume of reoperative cardiac cases take up to 10-20% of the annual workload in some centres (Gabbay, 1990). Various reasons for repeat sternotomies exist, including recurrent angina due to occlusion of aortocoronary bypasses or progression of coronary artery disease, failure of valve substitutes, infection on cardiac prostheses, primary palliation in congenital heart disease, residual or recurrent defects, and replacement of "out -grown" conduits. With the increasing reoperative volume the technical difficulties and consequent morbidity (Mary et al, 1974) and occasional mortality (Dobell and Jain, 1984; Loop et al, 1981), imposed by a resternotomy in the presence of pericardial adhesions are being increasingly realised. Surgical complications, including severe blood loss, are also a greater in the reoperative population.

Difficulty of Primary closure of the Pericardium

Although primary closure of the pericardium affords some protection against adhesion formation (Cunningham et al, 1975; Nandi et al, 1976) its completion is not always practical and indeed can be hazardous because of the risk of graft kinking and cardiac tamponade (Engelman and Spencer, 1970). Indeed, recognising that after surgery the heart is often oedematous and larger than usual, subsequent closure of the native pericardium may induce such complications. It is the consideration of such potential problems that prompt surgeon's to preferentially leave the pericardium open.

Consequence of not closing the Pericardium

When the native pericardium has not been closed, the adhesions formed after the primary operation practically "glue" the heart to the sternum, consequently making the reoperation tedious and increasing the danger of rupture of the heart. In particular, the right ventricle and right atrium may become adherent to the sternum as may the aorta and innominate vein thereby placing all these structures at risk during reopening. Moreover, with a reported incidence of 2-6% (Loop, 1984) for catastrophic haemorrhage during

resternotomy, the problem is by no means trivial. Indeed, some surgeons will prophylactically put the patient on femorofemoral bypass as a precaution in case the heart is accidentally injured while opening the sternum.

Other untoward affects of Pericardial Adhesions

Apart from their unwelcome imposition on reoperative cardiac surgery, there is evidence implicating adhesions as a cause of ventricular dysfunction after cardiac surgery (Bailey et al, 1984) and as a factor promoting graft occlusion (Shapira et al, 1989; Urschel et al, 1976).

Assessment and Precautions Prior to Resternotomy

There are a number of publications that have dealt with the preoperative assessment and surgical considerations in patients undergoing resternotomy (Dobell and Jain, 1984; English and Milstein, 1978; Macmanus et al, 1974; Verkkala et al, 1990). The advice that they give is broadly similar but involves the need for much more care and attention to detail. It is generally agreed that the pericardium does confer some protection against pericardial adhesions, it should therefore be closed if a second operation is conceivable or even anticipated. However, as mentioned above, this is not always practical and perhaps may even be hazardous. In many instances surgeons will just interpose thymic tissue, pleura or pericardial fat pad between the heart and the sternum.

As part of the preoperative assessment of reoperative cases the previous operating notes are usually reviewed. However, it is not infrequently the case that the primary procedure has been done by another surgeon and, if pericardial closure was a feasible option at the time of primary surgery, there is no record as to whether the pericardium has been closed. In such cases it is perhaps better to assume that the pericardium has not been closed and therefore a thorough assessment of reoperative risk factors becomes more crucial. Accordingly, preoperative preparation ought to include assessment of the size of the heart. In particular the size of the right ventricle and right atrium which, if distended and adherent to the sternum, can be entered during resternotomy leading to rapid exsanguination. A lateral chest radiograph may show a retrosternal space and the situation

of the ascending aorta. Computer tomographic scanning may further delineate the extent of structural adhesions.

At reoperation the groin should be prepared and the CPB lines in readiness for immediate commencement of femorofemoral or femoral to 'pump sucker' bypass. A number of surgeons use a posterior paddle placed behind each patient so that defibrillation can be accomplished easily without extensive dissection of the heart. If the resternotomy is judged to entail a high risk for vascular damage, the femoral vessels should at least be exposed, or even cannulated. If haemorrhage is virtually certain than femorofemoral bypass should be instituted. This will empty the heart and perhaps cause it to fall away from the sternum. It is the practice of some surgeons, including myself, to leave the sternal wires in position but untwisted and open, using them as a posterior sternal limitation for the oscillating saw. Using the saw the outer cortex and medulla of the sternum are divided. The linea alba and xiphisternum are incised, and then the two edges of the wound are gently retracted upward and laterally so that the posterior cortex of the sternum can be opened under direct vision and with great caution such that if a vascular compartment were opened, the blood loss would be limited. Having divided the sternum, sharp dissection is then continued laterally into the immediate substernal plane and often out into both pleural spaces before a retractor is inserted. Premature placement of the retractors could be dangerous in that the presence of dense adhesions may provoke damage to the heart or great vessels either as the retractor is being placed or retracted. The right side of the heart and the aorta should be dissected first to allow cannulation and the institution of CPB during which time the remaining adhesions may be dissected with the heart decompressed.

Pericardial Morphology

Normal Pericardium - Macroscopically

Macroscopically the pericardium consists of an outer sac, called the fibrous pericardium, consisting of fibrous tissue, and an inner double-layered sac, the serous pericardium, which lines the fibrous sac and is invaginated by the heart which it in turn covers. The two opposed layers of serous membrane go to make up the visceral and parietal portions

of the pericardium. The visceral portion, or epicardium, not only covers the heart but also the great vessels, and from the latter is reflected to form the parietal layer that lines the fibrous pericardium. The visceral and parietal layers are separated by a fluid medium of up to 50 ml of pericardial fluid, which provides a complete cleavage between the heart and its surroundings, allowing it freedom of movement within the pericardium. Only a few cells are found in normal pericardial fluid, mainly serosal mesothelial cells and macrophages.

The fibrous pericardium is also attached to the posterior surface of the sternum by superior and inferior connective tissue ligament. By means of these ligaments it is securely anchored within the thoracic cavity and therefore maintains the heart in position within the chest.

The arteries of the pericardium are derived from the internal mammary and their musculophrenic branches, and from the descending thoracic aorta; its veins are tributaries of the azygos system. Its nerve supply is derived from the vagus and phrenic nerves, and sympathetic trunks.

Parietal Pericardium - Microscopically

Histologically, the parietal pericardium is composed of three distinct layers: (Ishihara et al, 1980; Leak et al, 1987) 1) the serosa, 2) the fibrosa, and 3) an outer layer of epipericardial connective tissue (figure 1). The serosa consists of a single layer of mesothelial cells and of a narrow submesothelial space (about 2 μ in width) that separate the mesothelial cells from the fibrosa, the underlying layer. The mesothelial cells are flat and polygonal in outline possessing numerous microvilli and have a mild bulge in the region of the nuclei. The fibrosa is made up of connective tissue that contains fibroblasts, macrophages, and a network of blood and lymphatic vessels. The cells and vascular network are separated by compactly arranged collagen fibrils and small, inconspicuous elastic fibers. The epipericardial connective tissue layer contains loosely arranged collagenous and elastic fibers, adipose tissue cells, nerve fibers, blood and lymphatic vessels, and connective tissue cells.

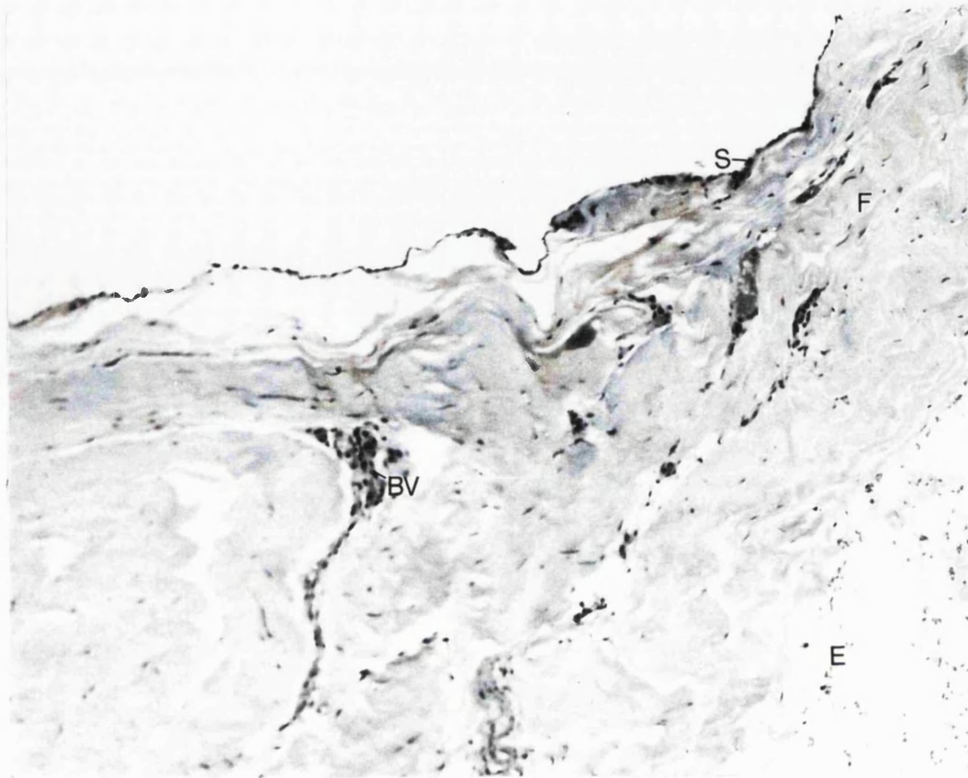


Figure 1. Light micrograph. The three layers are indicated by the following: S, serosal layer; F, fibrosal layer within which are blood vessels (BV); E, epipericardial connective tissue layer.

Visceral Pericardium - Microscopically

The epicardial surface of the heart is composed of a layer of loose connective tissue that is covered by a single layer of serosal mesothelial cells similar in structure to those of the parietal pericardial mesothelium. At the sites of attachment of the great vessels to the heart both these serosal surfaces are continuous with each other. Underlying the mesothelium is a layer of loose connective tissue that contains blood and lymphatic vessels and nerves.

Importance of Mesothelial Cells

The properties of mesothelial cells, such as the ability to produce prostacyclin (Carlin et al, 1983) and thrombomodulin (Boffa et al, 1987) have been discovered in recent years. In particular, mesothelial pericardial tissue has its own inherent fibrinolytic activity (Gervin et al, 1975; Porter et al, 1971) and is therefore, to a degree, able to remove fibrin molecules and prevent adhesion formation. One of the first to demonstrate a reduction in mesothelial (pleural, Pericardial, and peritoneal) fibrinolytic activity in response to trauma was Porter (Porter et al, 1969).

Pericardial Fluid

Little is known about the formation and removal of pericardial fluid. Similarly, the details of the lymphatic drainage of the pericardium are still disputed. Even the fundamental question of whether pericardial fluid is an ultrafilterate of arterial plasma as suggested by the work of Gibson and Segal (Gibson and Segal, 1978) or an overflow of myocardial interstitial fluid that oozes from the epicardium as suggested by Miller and colleges (Miller et al, 1971), is unsettled. This uncertainty surrounding the normal physiology of pericardial fluid will remain until individual studies are performed on normal pericardial fluid; pericardial transudate in cardiac failure; pericardial exudate in acute and chronic infection; pericardial fluid of metabolic diseases and other such variation of pericardial fluid. Such a study of pericardial fluid goes beyond the extent of this present work.

The composition of pericardial fluid was studied in a variety species by Gibson and Segal (Gibson and Segal, 1978). They found its electrolyte and protein content to be consistent with the expected composition of an ultrafilterate of blood and, as would be expected

from an ultrafilterate, the osmolarity of pericardial fluid was slightly lower than that of the plasma. Only the potassium was present in concentrations appreciably higher than those of plasma. They attributed this to the "lability of the intracellular potassium during contraction." Enzymes such as; creatine kinase, lactic dehydrogenase, and glutamic oxoacetic transferase are present in human pericardial fluid. The role of pericardial fluid in the prevention or minimisation of pericardial adhesions is to be discussed in this work.

Functions of the Pericardium

Limiting Mobility of the Heart

This double-layered membrane with its potential space filled with pericardial fluid lubricates the surface of the heart so that it can undergo motion without friction or damage to the epicardium (Holt, 1970). A proposed extension of this concept states that the pericardium with its enclosed liquid serves to equalise gravitational forces acting on the heart (Avasthey and Wood, 1974). The heart shifts with alteration of the subject's posture, but the pericardium still limits the extent of this mobility of the heart within the thorax. In patients with congenital partial absence of the pericardium the heart is not in the midline, and it moves excessively when the posture is changed (Morgan et al, 1971). This phenomenon is not observed after cardiac surgery or surgical removal of the pericardium because of the dense pericardial adhesions that fix the heart in the mediastinum.

Limiting Cardiac Dimensions and Preventing Acute Distension

A number of authors, Bernard (Bernard, 1898), Kuno (Kuno, 1916), and Gibbon and Churchill (Gibbon and Churchill, 1931), to name a few, have shared the view that the pericardium limits cardiac distension and prevents acute distension. The pericardium is also an important determinant of the relation between the diastolic pressure and the dimensions of the left and right ventricles. Holt et al measured intracardiac and intrapericardial pressures in dogs when they were normovolaemic, after hypervolaemia had been induced by transfusion and after hypovolaemia created by haemorrhage. They noted the anticipated increase in the diastolic pressures of the left and right ventricle and of right atrial pressure during acute hypervolaemia and observed that pericardial pressure

increased during hypervolaemia. When the ventricular diastolic pressure increased from 0 to 10 mmHg an equal and parallel increase in intrapericardial pressure occurred. As a result, the ventricular transmural diastolic pressure, the filling pressure of the ventricles, was not increased. After further transfusion, the ventricular diastolic pressure increased to more than 10 mmHg but there was little further increase in intrapericardial pressure, suggesting that the pericardium had reached the limit of its acute distensibility.

Role of the Pericardium In Right Heart Failure In Left Ventricular Disease

Oedema and increased systemic venous pressure has been observed in patients with severe left ventricular disease. Much of the right heart failure can be explained by elevation of the left ventricular diastolic pressure causing pulmonary hypertension and eventual failure of the right ventricle. In such cases right ventricular function may also be further impaired by bulging of the ventricular septum into the right ventricle (Bernheim syndrome) (East and Bain, 1949). Reversed Bernheim physiology (bulging of the ventricular septum into the left ventricle with resulting left ventricular dysfunction) has been postulated to explain evidence of left ventricular failure in patients with predominantly right ventricular disease.

It has been speculated that the pericardium may oppose the effects of left ventricular dilatation on right ventricular function (Shabetai, 1978) in a manner now described: dilatation of the left ventricle increases intrapericardial pressure, which thus limits right ventricular filling and thereby reduces forward flow into the lungs, perhaps preventing pulmonary oedema. The pericardium thus causes reciprocal shifts of the Frank-Starling curves of the two ventricles and so balances the outputs.

Role of the Pericardium In Haemodynamic interaction between the left and Right Ventricles

On a beat to beat basis, evidence exists for a linear relation between ventricular end-diastolic volume and the subsequent stroke volume. A major function of Starling's law in normal man is to maintain nearly equal time-average outputs from the right and left

ventricles to keep a proper volume equilibrium between the pulmonary and systemic circulations. The pericardium, by influencing the effects of diastolic pressure and dimensions of one ventricle on the opposite ventricle, may facilitate haemodynamic interaction between the ventricles to balance the right and left ventricular out-puts (Shabetai, 1978).

The Fibrinolytic System and The Pericardium

Fibrinolysis

Fibrinolysis is a basic regulatory mechanism that ensures that life threatening fibrin deposits in blood vessels are removed, and that clot lysis at the site of injury is not removed prematurely leading to haemorrhage. It also helps maintain control of fibrin deposition in other areas such as the pleural, peritoneal, and pericardial cavities and urinary system.

Historical Note

The earliest observations that can be directly connected with the fibrinolytic system are dated to the second half of the eighteenth century. Autopsies were documented where the blood was found to be entirely fluid (Morgagni, 1769). Hunter (Hunter, 1794) noted this phenomenon in cases of sudden death and reported the failure of such postmortem blood to clot when removed from the body. This phenomenon became well established by the twentieth century (Morawitz, 1906). As a result of his pioneering work in the study of blood coagulation, Nolf (Nolf, 1905) believed that blood clot lysis was due to the action of a proteolytic enzyme and later proposed the existence of a dynamic equilibrium, whereby the fibrin formed during coagulation was removed by an active lytic substance from the blood vessel wall (Nolf, 1908). In 1936, Yudine, working in Russia, turned this knowledge to practical use when he described transfusing blood from corpses who met with sudden or violent death (Yudine, 1936). Having turned to a fluid state the blood could be transported without the use of anticoagulant.

Further observation, initially thought to be unrelated to blood fluidity, concerned a proteolytic enzyme in plasma. Denys and Marbaix in 1889 noted the development of

fibrinolytic or proteolytic activity in blood treated with "inert" solvents such as chloroform. This work was extended by Delezene and Pozerski, 1903 who incubated dog serum with chloroform for several hours. When the chloroform was removed the serum possessed the ability to digest caesin or gelatin, a process that was inhibited by the addition of a trace of untreated serum. It was concluded that chloroform treatment removed an inhibitor to proteolytic enzymes normally present in excess, thereby leading to the appearance of the active enzyme. This active enzyme, with its lytic activity, was shown to be concentrated in the ammonium sulphate precipitated fraction of chloroform treated serum (Tagnon et al, 1943). Similarities were observed between this lytic enzyme and trypsin (Opie et al, 1911) therefore its inhibitor was understandably called "antitrypsin", in addition it was suggested that chloroform destroyed the inhibitor component of this lytic enzyme (Dale and Walpole, 1916). The trypsin inhibitor and the inhibitor of fibrinolytic and proteolytic activities of normal blood or serum were therefore thought to be the same substance.

Further research, involving "fibrinolysin" produced by Streptococcal bacteria, ultimately provided the link between previous reports and our current knowledge of the fibrinolytic system. Tillet, and Garner (Tillet and Garner, 1933) drew attention to the marked lysis of human plasma clots by filtrates of certain haemolytic Streptococci in culture. This filtrate was thought to contain an agent acting directly on fibrin. An important discovery followed in 1941, when Milstone (Milstone, 1941) working in the Department of Bacteriology at New York University demonstrated that fibrin formed from purified fibrinogen was not lysed by the Streptococcal "fibrinolysin". This pure fibrin was only lysed by the bacterial agent if a small amount of the acetic acid precipitated fraction (euglobulin) of plasma was present. Milstone concluded that the bacterial "fibrinolysin" and a "lytic factor" present in the euglobulin fraction of plasma reacted to produce a "lysin" that digested fibrin. Further studies by Kaplan (Kaplan, 1944) showed that Milstone's "lytic factor" was an enzyme precursor that could be changed into a fibrin-splitting proteolytic enzyme by chloroform treatment. Subsequent studies by Christensen showed that the enzyme produced by the action of chloroform and the Streptococcal filtrate on serum was identical (Christensen, 1945; Christensen, 1946). Therefore there

was a common final pathway activated in different ways (figure 2). Because of these findings, a change in terminology was proposed, the lytic enzyme being called plasmin, its precursor called plasminogen, the inhibitor removed by chloroform called antiplasmin (rather than antitrypsin), and the bacterial filtrate called Streptokinase (Christensen and MacLeod, 1945).

Although these findings demonstrated the mechanism by which Streptococcal fibrinolysis occurs, it did not elucidate how fibrinolytic activity was acquired in man and animals. Further experiments, mimicking the conditions of interaction occurring in tissue cultivation and in the organism, were undertaken to clarify this state of affairs (Permin, 1947; Permin, 1950a; Permin, 1950b). The basis of this method was the estimation of the lytic activity on a film of fibrin formed by clotting fibrinogen on a Petri dish (fibrin plate method). In this fibrin plate method (Astrup and Mullertz, 1952) bovine or human solutions containing plasminogen were poured into a Petri dish and clotted with thrombin to form a solid medium containing fibrin. After applying fragments of tissue, a quantitative estimate of activity was gained by measuring the area of lysis produced in a given time by measured amounts of the test specimen. If the fibrin layer was then heated (Lassen, 1952) for 30 to 45 minutes at 80 ^{0}C the plasminogen present in the plate was denatured without seriously interfering with the clot. If the test specimen was added to this preheated plate and no zone of lysis occurred this would indicate that the specimen acted by way of plasminogen as did streptokinase. This test enabled differentiation between plasminogen activating agents and proteolytic enzyme activity (which could cause lysis irrespective of the presence of plasminogen). Fibrinolytic activators have been extracted from a number of tissues such as thyroid, kidney, uterus, ovary, lymph glands, brain, heart and many others (Astrup and Sterndorff, 1952).

The fibrinolytic system, (Pâques, 1988) as it is currently understood, is a complex pathway simplified in figure 2, involving enzymes and their inhibitors that enable precise control of fibrin deposition and removal (Collen, 1980). A number of authors, such as Collen 1980 (Collen, 1980), and Pâques 1988 (Pâques, 1988), have recently reviewed

the fibrinolytic system in detail. Some of the individual molecules involved in the fibrinolytic system and their interactions are now briefly described.

Individual Components of the Fibrinolytic System

Plasminogen

Plasminogen is the precursor molecule of the serine protease plasmin. It is a single chain glycoprotein with a molecular weight of approximately 90 000 daltons. Its plasma concentration is around 120 mg/l. Plasminogen exists in two forms: the native molecule called "Glu-plasminogen" and a slightly degraded form called "Lys-plasminogen". These are converted to "Glu-plasmin" and "Lys-plasmin" by plasminogen activator cleavage. Plasmin then catalyses the conversion of "Gly-plasminogen" to "Lys-plasminogen" and "Gly-plasmin" to "Lys-plasmin".

Plasmin

Plasmin is a double chain molecule consisting of a heavy (or A) chain originating from the amino-terminal part of the plasminogen molecule, and light (or B) chain that is derived from the carboxyl-terminal part and contains the active site (Robbins et al, 1967). Plasmin is a relatively non-specific protease, being responsible not only for the degradation of

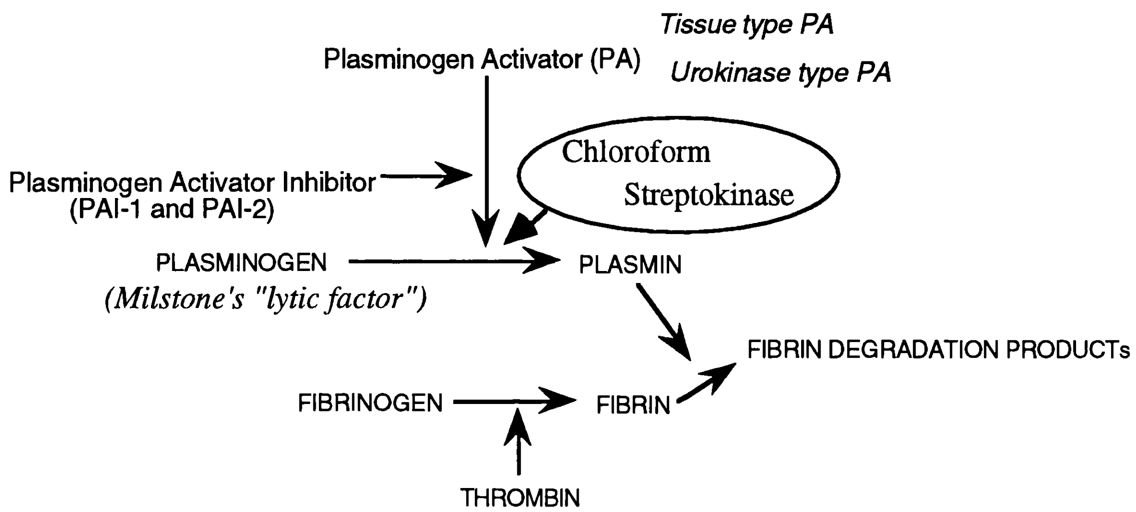


Figure 2

fibrin, but also for hydrolysing other proteins and peptides at lysyl and arginyl bonds (Castellino, 1984).

Plasminogen Activators and Plasminogen Activator Inhibitors

There are two principle plasminogen activators (PA), tissue type PA (tPA), which is present in plasma (Rijken et al, 1980) and a number of tissues and body fluids, and urokinase PA (uPA), which is present in urine (Williams, 1951) and some tissue mucosa (Larsson et al, 1984). The activity of these enzymes is mainly controlled by the action of plasminogen activator inhibitor-1 (PAI-1), synthesised by endothelial cells, granulosa cells, melanoma cells and is found in the alpha-granules of platelets and in plasma, and plasminogen activator inhibitor-2 (PAI-2). Plasma levels of tPA and PAI-1 are controlled by the rate of their synthesis, release and clearance from the circulation (Ranby and Brandstrom, 1988). Synthesis of PAI-1 and tPA are regulated and will respond to hormonal, pharmacological and neural stimuli (Hsueh et al, 1984).

When fibrin is formed, tPA and plasminogen are adsorbed to the clot in an ordered and sequential way. The local concentration of both tPA and plasminogen is thus greatly increased at the site of fibrin deposition. The binding of tPA and plasminogen to fibrin lead to conformational changes in both molecules that facilitates efficient plasminogen activation. However, in plasma minimal plasminogen activation by tPA does occur and alpha-2-antiplasmin, the most important physiological inhibitor of plasmin, rapidly binds to free plasmin thus preventing fibrinogen degradation. By these mechanisms the normal fibrinolytic process is both triggered by, and localised to, sites of fibrin formation (Collen, 1980).

Although the vascular endothelium is thought to be the site of continuous release of tPA into the circulation (Todd, 1958) (thereby helping to maintain haemostatic equilibrium), the occurrence of tPA and uPA activity in a variety of different tissue and cell types, including the pericardium, suggests that they play a role in a number of different biological processes. Furthermore, the different distribution of uPA and tPA suggests that the two types of activators have different functions. With current knowledge, it

seems most likely that all processes in which plasminogen activators play a role involve extracellular proteolysis (Saksela, 1985).

Streptokinase

Streptokinase is non-enzyme protein produced by the Lancefield C ß-haemolytic streptococci. As indicated in the "historical note", studies of this bacterial product proved crucial in the clarification of the fibrinolytic pathway. More recent research (Brodgen et al, 1973) has revealed that Streptokinase activates the fibrinolytic system indirectly, by forming a one to one complex with plasminogen that produces conformational change in the plasminogen molecule exposing an active site. This Streptokinase-plasminogen complex is then converted to Streptokinase-plasmin that enzymatically converts plasminogen to plasmin (Brodgen, Speight et al., 1973).

Alpha-2-antiplasmin

Human plasma exerts a strong inhibitory action on plasmin. Alpha-2-antiplasmin is the most important physiological inhibitor of plasmin, the reaction being one of the fastest protein interactions known (Wiman and Collen, 1978). Alpha-2-antiplasmin was first isolated and described in 1976 by Moroi and other groups of workers (Moroi and Aoki, 1976). Synthesis of this inhibitor occurs in the liver. It reacts with plasmin to form a one to one inactive complex stabilised by the formation of an ester bond between antiplasmin and the active site of plasmin (Holmes et al, 1987).

Alpha-2-Macroglobulin

This high molecular weight proteinase inhibitor has a wide specificity and inhibits plasmin, but at a much slower rate than alpha-2-antiplasmin (Ganrot, 1967). It may function as a reserve inhibitor of plasmin one the plasma alpha-2-antiplasmin is exhausted during thrombolytic therapy or acute disseminated intravascular coagulation (Chmielewska, 1988).

Pericardial tissue injury and subsequent inflammation result in an outpouring of a fibrin rich exudate (Roberts and Spray, 1977). This exudate together with the fibrin that is in

the spilled blood, can either undergo organisation by fibroblast infilteration and collagen deposition to form dense adhesions or, as a result of fibrinolysis, be resolved. This capacity that pericardial mesothelial cells have to cause resolution of fibrinous adhesions makes their potential role in the control of reoperative adhesions so crucial.

CHAPTER 2

REVIEW OF PREVIOUS WORK ON ADHESIONS

REVIEW OF PREVIOUS WORK ON ADHESIONS

Adhesions in General

The problems imposed upon reoperative surgery by the presence of adhesions are not unique to cardiac surgery. In general surgery adhesions are the commonest cause of intestinal obstruction. It was Becker (Becker, 1952) who noted that 90% of 412 cases of adhesive obstruction were postoperative. Since the early days of abdominal surgery, surgeons have observed that injury or inflammation within the peritoneal cavity produced an out pouring of fibrin that would cause adherence of adjacent structures. These fibrinous adhesions would either become absorbed completely or else become organised into fibrous adhesions. Ellis (Ellis, 1962) showed that it was not just injury alone that prompted adhesion formation, but injury accompanied by vascular damage. Buckman and his colleagues (Buckman et al, 1976) showed how denuded peritoneum, because of its fibrinolytic activity, could heal without permanent adhesions. However, this activity was lost if the peritoneum was rendered ischaemic. Moreover, such ischaemic tissue could actively inhibit fibrinolysis by normal tissues. This explains the phenomenon of the failure of intact peritoneum to liberate itself from fibrinous adhesions to ischaemic tissue. This work was also confirmed by the studies of Raftery in 1981 (Raftery, 1981).

Ischaemic tissue is not unique in stimulating an inflammatory vascular response in adjacent intra-abdominal structures. Many of the substances that may contaminate the peritoneal cavity at the time of laparotomy may be responsible for the formation of granulomas and intra-abdominal fibrous adhesions. These include fragments of gauze, lint and cotton wool, clumps of antibiotic powder, antiseptics and dusting powders that have been used for surgical gloves (Myllarniemi, 1967). Some of the early products, such as talc (magnesium silicate) and lycopodium , produced extensive granuloma formation. More recent replacement products such starch powder are still capable of provoking adhesion formation (Jagelman and Ellis, 1973).

Although the problems produced by the presence of adhesions are well documented, it is important to note that the process of adhesion formation is often beneficial and constitutes

part of normal healing. Indeed, in experimental models where intestinal anastomoses were performed, there was a high incidence of peritonitis when agents preventing adhesion formation were tested (Fabri et al, 1983). In cardiac surgery they may prevent excessive movement of the heart void of its normal pericardial encasement. Therefore it may be more appropriate to regard adhesions as an ally who's excesses must be restrained.

As in cardiac surgery, so likewise in general surgery great efforts have been made to overcome the problems of postoperative adhesions (Ellis, 1971). These efforts include:
1. The instillation of various fluids or distension with gas introduced into the peritoneal cavity to hold the damaged surfaces away from each other (Reissman et al, 1993).
2. Enhancement of peristalsis in an attempt to disrupt early fibrinous adhesions.
3. The use of enzymes such as trypsin to digest adhesions.
4. The covering of peritoneal surfaces with a variety of inert membranes, lubricants or with grafts of peritoneum.
5. The instillation of substances to inhibit deposition of fibrin, including steroids, anticoagulants and fibrinolytic agents.

The majority of these efforts have either been ineffectual or actually, in some studies, harmful in that they increase the incidence of adhesion. However, simple precautions to prevent unnecessary abdominal and pelvic adhesions from developing include, prevention of granuloma formation from foreign materials such as gauze and glove powder. Peritoneal defects should be left rather than pulled together under tension thereby producing an area of ischaemia. To avoid adhesions between bowel and the under-surface of the abdominal wall incision the omentum should be drawn down over the other abdominal organs before closing the laparatomy incision and should be carefully tacked around an anastomotic suture line (Ellis, 1971).

Many of the basic pathophysiological principals learnt from the work on peritoneum can be used as guidelines for the work on pericardium. In particular the peritoneum, like pericardium, has regenerative potential and fibrinolytic activity. In both, the responses to trauma and pathogenesis of adhesion formation are similar. However, there are

dissimilarities in the interplay of structures and organs, e.g. there is no intra-pericardial equivalent of the omentum, and in the microbiological milieu. Nevertheless, it is with this background knowledge of the peritoneal work that specific questions relating to the pericardium can be formulated and answered by research directed at the pericardium.

Methods Used to Overcome the Problem of Pericardial Adhesions

Attempts at overcoming the problems of adhesions have resulted in numerous pericardial substitutes (Gabbay et al, 1989) and a number of techniques for closing the pericardium (Milgalter et al, 1985; Zapolanski et al, 1990). Preliminary experiments with these pericardial substitutes have been carried out in laboratory animals using a thoracotomy or median sternotomy approach. Few studies have included exposure of the animals to cardiopulmonary bypass (CPB). Most of the tested materials have given good results in the animal studies (Heydorn et al, 1987; Rhodes et al, 1989), but the long term clinical outcome, has been disappointing (Eng et al, 1989; Gallo et al, 1985).

Dextran pericardial washout (Reikerås et al, 1987), and pharmacological agents such as corticosteriod have also been used to limit adhesion formation (Vander Salm et al, 1986). Cardiopulmonary bypass through a left thoracotomy approach (Gandjbakhch et al, 1989) is used by some in attempt to avoid substernal adhesions.

Pericardial Substitutes

The variety of biomaterials that have been used in pericardial substitution includes those both natural and synthetic in origin. The natural materials (pericardial xenografts) include glutaraldehyde processed pericardial tissue from bovine , porcine, and equine sources (Gallo, Artinano et al., 1985; Heydorn, Daniel et al., 1987). Some of the synthetic materials studied include Dacron polyester, silicone rubber, silicone coated polyester fabrics, polytetrafluoroethylene (PTFE), and silicone filled PTFE (Laks et al, 1981; Reder et al, 1983).

Biologically Derived Materials

Bovine Pericardium

Bovine pericardium undergoes fixation in glutaraldehyde thereby making the material easier to handle but also destroying viable cells. In a case report of a 47 year old man, Skinner and co-workers described a dense epicardial reaction to the processed bovine pericardium (Skinner et al, 1984). In 1989 Eng and colleagues (Eng, Ravichandran et al., 1989) reported 4 patients undergoing resternotomy 3 to 8 years after the implantation of glutaraldehyde-preserved bovine pericardium as a pericardial substitute. In all cases, the patch was frozen to the inner aspect of the sternum, increasing the difficulty of resternotomy. Histological examinations of the patches confirmed dense fibrous connective tissue, patchy calcification, and foreign body giant cell reaction. They concluded that Bovine pericardium appears to increase the difficulty of repeat sternotomy and recommended against its continued use. These clinical findings showed a marked difference to the animal studies (Gallo et al, 1978; Heydorn, Daniel et al., 1987; Mathisen et al, 1986) that suggested that, at least in the short-term, bovine pericardium prevented severe adhesion.

Frasca et al (Frasca et al, 1985) found that, within 3 weeks, mineral deposition occurred first in the cells of the glutaraldehyde-fixed bovine pericardium when this was implanted as myocardial windows in young sheep. In particular the nuclei, because of its large number of anionic groups that could bind calcium ions and thereby initiate mineral formation, were prone to calcification. This is contrary to calcium processes in viable cells where calcium deposition is associated with formation of calcium granules in mitochondria. In the same study Frasca et al noted that mineralisation of collagen occurred later, and was not seen without cellular calcification. It was therefore postulated that the presence of cells promotes the mineralisation of non viable biological materials. The normal healing process of inflammation and fibrosis occurs in all substitute materials, but because glutaraldehyde fixed bovine pericardium is dead tissue it is incapable of participating in local metabolic process such as the replacement of biodegradable substrate with newly formed collagen. Moreover, the cells appear to act as

foci of mineralisation this may explain the long-term observations with bovine pericardium where formation of cartilage and calcification is a frequent finding (Mathisen, Wu et al., 1986) making it impractical as a pericardial substitute.

Equine Pericardium

Equine pericardial substitutes have been used as a pericardial substitute by Gallo (Gallo, Artinano et al., 1985) and by Segesser et al (Segesser et al, 1987), mainly in children. In the work of Segesser et al, they implanted a glutaraldehyde fixed equine pericardial patch into 200 consecutive patients. Serving as controls were patients scheduled for reoperation in whom the pericardial sac had primarily been left open. Adhesions were scored 0-5. Of the pericardial patch group, 9 patients came for reoperation. The mean grade of adhesions was significantly lower (blunt - moderate) in the patch group than in those with an open pericardium (Severe - very severe). However, in some cases where adhesions did not appear epicardial thickening was noticeable. The authors considered equine pericardial patches to be suitable for closure of the pericardium in cases where primary closure is not feasible. However, the experience of Mathisen, using the same material and having found areas of significant multifocal calcific degeneration in one of his experimental groups, prompted him to be more guarded in his recommendation of the material.

Porcine Pericardium

In 1978, Gallo and colleagues (Gallo, Artinano et al., 1978) described the use of glutaraldehyde-fixed porcine pericardium as a pericardial substitute in dogs. There was a low incidence of adhesions to the cardiac surface and they went on to place this type of patch in eight patients. By 1985 they (Gallo, Artinano et al., 1985) had implanted 87 glutaraldehyde-preserved xenografts patches (34 porcine, 28 bovine, and 25 equine) and had reoperated on 13 patients. In those 13 patients, they found that the epicardial surface of the heart beneath the implant was thicker and more fibrous than the remaining epicardium to the extent that recognition of coronary vessels was impeded.

Human Amniotic Membrane

In a study comparing amniotic membrane (AM) and high porosity 0.2-mm PTFE, Muralidharan and colleagues (Muralidharan et al, 1991) concluded that, at 18 weeks after implantation, AM performed satisfactorily as a pericardial substitute and was superior to PTFE. They attributed much of its success to its lack of antigenicity (Goodfellow et al, 1976) and resultant minimal host immunoreaction preventing adhesions between the pericardium and the patch.

Nondegradable Synthetic Materials

Dacron was well established in the garment industry. When it became available to medical fabricators, besides its other applications, it became the most popular material for replacement of large arteries and closure of ventricular septal defects. Its more recent use as a pericardial substitute, although in modified forms, is yet to be established (Gabbay, Guindy et al., 1989; Mazuji and Lett, 1963). However, the experimental results of Youmans and associates (Youman et al, 1968) led to the clinical use of Dacron patch material coated with silicone rubber and silicone rubber (Laks, Hammond et al., 1981) itself as a pericardial substitute.

Teflon

Silicon-filled and high-porosity polytetrafluoroethylene (PTFE), when used as a pericardial substitute, reduced postoperative adhesions but produced a severe epicardial reaction in dogs (Meus et al, 1983). However, the type of PTFE used in these studies were of a different texture. Its structural characteristics and porosity favour cellular infilteration and tissue attachment.

There are a number of reports evaluating the efficacy of expanded PTFE. When it was used in patients the patch showed no cellular ingrowth (Minale et al, 1988). Furthermore, in 1988, Harada and colleagues, reported the clinical use of expanded PTFE in 61 children operated on for congenital heart disease, of which 23 underwent reoperation. Their conclusion was that the clinical use of expanded PTFE as a pericardial substitute is valuable in children with congenital heart disease who are expected to undergo

reoperation soon. In another report, which involved the implantation of expanded PTFE into 96 patients who had undergone repair of congenital heart defects, Amato (Amato et al, 1989) in essence concluded that: (1) the use of PTFE as a pericardial substitute was feasible; (2) at reoperation there were not significant adhesion to the chest was or epicardium, and coronary visualisation was not obscured; (3) the substitute material caused some difficulty in the echocardiographic examination of patient in the parasternal view only. Other studies have similarly recommended the use of PTFE (Revuelta et al, 1985; Sakamoto et al, 1978). Sakamoto and colleagues have extensive clinical experience with the material. Revuelta recommended that the thickness of the patch be no more than 0.1 mm, whereas thicker samples were noted to provoke a filmy adhesion when used in dogs.

The use of 0.1 mm expanded PTFE as an interim pericardial substitute in patients awaiting heart transplantation and supported with a ventricular assist device (VAD) has been assessed by Holman and colleagues (Holman et al, 1993). Aware of reports that the use of PTFE may obscure coronary visibility at reoperation, they felt that the patients need for a subsequent heart transplant did not make this possible complication significant. Their conclusion was that the PTFE protected the device cannulas during repeat sternotomy and expedited the dissection of the heart and great vessels. Reconstruction of the pericardium with expanded PTFE was therefore recommended at the time of VAD insertion.

The experience of Gabbay (Gabbay, 1990) and Bunton et al, (Bunton et al, 1990), with expanded PTFE are not as optimistic as these previous reports would suggest. When Gabbay and colleagues used expanded PTFE in animals not subjected to CPB the results were good, but when subjected to CPB the results showed an epicardial reaction with some difficulty in separating the heart from the epicardium. In a disturbing number of the animals studied Bunton noted a fibrous peel that obscured coronary anatomy and recommended against its use.

Hydrogel Composite Pericardial Patch

Work on the hydrogel composite patch has been published in two main studies (Allder et al, 1990; Blue et al, 1991). The patch is made of a hydrogel, poly 2-hydroxyethyl methacrylate (PHEMA) as an outer coat, reinforced with either ethylene tetrafluoroethylene (ETFE) or polyethylene terephthalate (PET). The preliminary study by Allder, 1990, assessed the mechanical properties of the material and found that its properties closely matched those of canine pericardium. The results of its use as an in vivo pericardial patch in greyhounds not subjected to CPB where mixed. The ETFE patches were implanted for 12 weeks and for 24 weeks (n = 6). The PET patches were implanted for 12 weeks. The ETFE composite patch caused an unacceptable epicardial reaction. The PET patch results were more encouraging in that the epicardium was largely unaffected. In view of its more acceptable short term effects, long term studies on PET were conducted where the material was implanted for 6, 9, and 12 months (Walker et al, 1992). They concluded that it was a material having potential as a pericardial substitute in that it greatly limited epicardial and pericardial adhesion formation, allowing direct access to the heart at reoperation. However, one disconcerting feature that they noticed was the tendency of the patch to calcify after 9 and 12 months in vivo.

Biodegradable Materials

The use of an absorbable graft as a transient scaffold for tissue regeneration is a concept that has been employed in vascular reconstruction. Carral and Guthrie were two pioneers at the beginning of this century who worked on vascular substitutes. Charles Guthrie concluded as early as in 1919 that "to restore and maintain function, an implanted segment need only temporarily restore mechanical continuity and serve as a scaffolding or bridge for the laying down of an ingrowth of tissue derived from the host" (Guthrie, 1919).

Bowald et al were one of the first to use a totally absorbable compound, polyglactin 910, as a patch graft in the aorta, in the atrial wall, and as a tubular graft in the aorta in pigs (Bowald et al, 1978; Bowald et al, 1979; Bowald et al, 1980; Bowald et al, 1981). The material was found to sustain endothelial regeneration (Audell et al, 1980) but was

sometimes lacking in the tensile strength necessary to withstand long-term systolic pressures (Bowald, Busch et al., 1980). Similar findings were true for other absorbable materials such as polyglycolic acid (PGA)(Greisler, 1982) and polyurethane/polylactic L-acid (Lommen et al, 1987).

The feasibility of using an absorbable material as a pericardial substitute has been investigated by workers such as Gabbay et al (Gabbay, Guindy et al., 1989) and Malm et al (Malm et al, 1992a). Gabbay and co-workers studied pericardial patches in animals that had or had not undergone CPB. In those animals subjected to CPB they found that the PGA mesh that they used was replaced in 3 to 4 months by a thin layer of autogenous collagen that did not adhere to the heart and allowed the lung and other surrounding tissue to be peeled away from the heart. The anatomical visibility of the heart and coronary vessels was well maintained. From their work they concluded that PGA may be an excellent material for preventing adhesions between the lung and chest or between the heart and the surrounding tissue. However, it lacked the necessary strength that would enable it to afford protection to the heart at the time of reoperation. In sheep not subjected to CPB Malm et al (Malm et al, 1992b) used polyhydroxybutyrate (PHB) as an absorbable pericardial substitute patch. They demonstrated regeneration of mesothelium-like cells onto the patch. The cells displayed many of the morphological, histochemical and ultrastructural characteristics of native mesothelium.

In a letter to the editor Fradin and colleagues (Fradin et al, 1993) presented some of the preliminary experimental results of a new resorbable biomaterial implanted into Yucatan pigs. The artificial connective matrix was left in vivo for 27 to 31 weeks postoperatively. No remnants of the biologic substitute could be found either grossly or at the microscopic examination. Instead there was a white neotissue layer, which histologically proved to made of young fibrosis with little inflammatory reaction. There was evidence of mesothelium-like cells. Though optimistic with the material they felt it necessary to assess the material in conditions mimicking cardiac operating conditions before clinical trial could be considered.

Modified Pericardial Closure

Pericardial Meshing

In the animal work of Milgalter et al (Milgalter, Uretzky et al., 1985), three groups of mongrel dogs were used. There were 6 animals in each group. In Group 1 (control) the pericardial flap was fashioned and then left exposed whilst the rest of the pericardium was abraded. The pericardium was closed by a continuous 5-0 polypropylene suture in all animals. The dogs were maintained on antibiotics for 72 hours postoperatively. In Group 2 the same procedure was followed except that the pericardial patch was meshed by multiple incisions parallel to the long axis of the flap. In Group 3 the same procedure again was completed except that the flap was replaced by a measured pericardial substitute, either silicone (three dogs) or porcine glutaraldehyde-preserved pericardium (three dogs). The animals were sacrificed at 8 weeks. Their results showed dense adhesions and severe epicardial reaction in Group 1 animals. The situation was worse in Group 3 animal, but in Group 2 the degree of adhesions and epicardial reaction was none to minimal and the meshed pericardium was regenerated by normal pericardium and mesothelial lining. However, this study did not employ CPB and therefore perhaps underestimated the additional insult to the pericardium attributable to the affects of CPB.

Trapezoidal Flap

This technique is described by Zapolanski et al (Zapolanski, Fishman et al., 1990), It basically involves the use of native pericardium that has been dissected into a trapezoid shape with its base remaining attached at the diaphragm. However, they had not yet had the opportunity to reoperate on any of the patients that they used this technique on.

A similar technique was advocated by Nugent and by Canver (Canver et al, 1993; Nugent et al, 1988). In this technique the pericardial flap has its base on the right side rather than the diaphragmatic side of the patient. In both these techniques of pericardial closure (pericardial meshing and trapezoidal flap), the basic aim was to reemploy the native pericardium to protect the heart at resternotomy.

Combined Mediastinal Fat Pad and Anterior Pericardium

This technique is described by Berry and colleagues (Berry et al, 1993), and involves the use of the mediastinal fat dissected free as a flap from the patients right and anterior pericardium incised eccentrically from the left. These tissues are then approximated at the end of the operation. The authors have used the technique in over 500 cases and of the 3 reoperative patients the results were favourable.

Other Methods

Dextran 70

Dextran 70 has been used as a dilutant of maize starch powder (found as a lubricant on some surgical gloves) thereby reducing adhesion formation in rats (Reikerås, Nordstrand et al., 1987). The same substance has also been used to similar effects in rabbits (Robinson et al, 1984).

Pharmacological Manipulation

In a study by Vander Salm et al (Vander Salm, Okike et al., 1986), they used three groups of 15 dogs: a control group, a methylprednisolone group, and an ibuprofen group. All groups had pericardial adhesions induced in the same manner. The pericardium was opened and left open for two hours. The epicardial surface of the heart was then sprinkled with cotton fibers and, after 50 ml of autologous blood was instilled into the pericardium, the pericardium was closed with nylon sutures. In the methylprednisolone group 500 mg of methylprednisolone sodium succinate was given intravenously at the time of surgery. For seven days thereafter, 0.3 mg/kg was given orally three times a day. In the ibuprofen group, having created the adhesions, ibuprofen (12.5 mg/kg) was given intravenously at the time of operation, followed by 12.5 mg/kg orally three times in one day and then 6 mg/kg orally three times a day for six days. The animals were sacrificed at about four weeks. The conclusions were that, in dogs, ibuprofen has modest effect in reducing pericardial adhesion, and that high doses of methylprednisolone nearly eliminate these adhesions. However, there was an episode of a superficial wound infection and three cases of sternal dehiscence. The control group

had three cases of sternal dehiscence but no wound infections. There was one such case in the ibuprofen group.

The use of such drugs and their potential effects in the clinical setting would provoke some ethical questions. The use of steroids could hinder the healing of postinfarct myocardium and provoke ulcer formation.

Left Thoracotomy

This technique (Gandjbakhch, Acar et al., 1989) is aimed at avoiding the substernal adhesions and keeping the mobilisation of the heart to a minimum. An anterolateral left thoracotomy through the fourth intercostal space is used. This provides a good exposure to the lateral aspect of the left ventricle and gives direct access to the circumflex coronary arterial system. The patient is placed in the right lateral decubitus position. The vein is harvested from either the right or left leg, and the left femoral vessels are exposed for cannulation.

With femorofemoral bypass instituted the main pulmonary artery can be cannulated to increase venous drainage. Exposure of the left anterior descending coronary artery and the right coronary arterial system is poor. Those who have used the approach consider it to be of value in a select group of patients where: (1) there is a need for isolated circumflex coronary bypass grafting in patients with pericardial adhesions due to previous coronary operation or mediastinal irradiation and (2) patent internal mammary artery or vein graft constructed on the left anterior descending coronary artery that might be injured during sternal reentry.

The Influence of Cardiopulmonary Bypass on Adhesion Formation

Factors that Embarrass Pericardial Mesothelium

As mentioned earlier, the early work by Porter et al (Porter, Ball et al., 1971) and Gervin et al (Gervin, Jacobs et al., 1975) demonstrated how the fibrinolytic activity of mesothelial cells, in particular pericardial mesothelial cells, decreases as the mesothelial cell layer is traumatised. During cardiac surgery such damage will occur as the heart and pericardial cavity are exposed to a number of traumas including handling, drying, cooling

and diathermy. However, the additional insult of CPB is a factor that needs to be considered. During cardiopulmonary bypass contact between blood and synthetic surfaces (oxygenator and tubing) and with the exposed subendothelium of the surgically cut vessels results in the activation of factor XII. This in turn leads to activation of closely related enzyme systems, including the kallikrein, complement, coagulation and fibrinolytic systems. These produce white cell activation and a whole body inflammatory response (Kirklin et al, 1983). This response to CPB may have a significant influence on the pericardium's ability to recover from its insult and regain its full fibrinolytic capacity.

The Importance of Using CPB as part of the Evaluation of a Pericardial Substitute

Gabbay and coworkers (Gabbay, Guindy et al., 1989) alluded to the importance of CPB as a factor influencing adhesion formation when they compared the degree of adhesion formation in animals that had undergone CPB and those that had not. By putting some of their animals on CPB they reproduced the severe adhesions observed in the clinical setting thereby exposing the pericardial patches to the type of environment that would be met clinically. At reoperation the pericardial patches in the non CPB animals showed little or no adhesions to the heart and lungs compared with what was observed in the CPB animals. Moreover, they noted more severe adhesions between the heart and native pericardium in those animals that had undergone CPB. From these observations they suggested that the explanation for the superior results with pericardial substitutes in animal studies compared with clinical findings are not species related but result from the actual operating conditions and damage to the heart and the pericardium incurred at the time of operation. More specifically, the experimental animal protocols have not included CPB techniques common to human experience, as the animal experiment was performed solely for the implantation of test pericardium. They therefore proposed that future experimental evaluation of potential pericardial substitutes incorporate the use of CPB.

Similar observations were made by Gallo and colleagues (Gallo et al, 1988) in their use of bovine and porcine as pericardial substitutes. In the experimental setting the results were encouraging. However, the clinical situation was rather different in that the

epicardial area facing the implant became very thick and more fibrous than the remaining epicardium thereby greatly obscuring the coronary visibility.

CHAPTER 3

AIM

&

THESIS DESIGN

Aim of Study

The Specific Aims of this Study were:-

1. To confirm the presence and determine the magnitude of fibrinolytic activity within the pericardium. To identify the principal fibrinolytic activators and inhibitors. To measure quantitative variation in the activity of these enzymes during CPB.
2. To document simultaneous changes in pericardial morphology with changes in the fibrinolytic activity.
3. To compare the morphological and fibrinolytic changes in primary and reoperative pericardium.
4. To relate the changes in the plasma tPA activity (IU/cm^2) with the changes in the pericardial plasminogen activating activity (PAA).
5. To assess the cytological and biochemical profile (in terms of its electrolyte content and fibrinolytic activity) of pericardial fluid and equate this with a potential role in minimising adhesion formation.
6. To compare the changes in the morphological and fibrinolytic features of pericardium in animal models (calves) that: 1) have been subjected to CPB, and 2) have not been subjected to CPB.
7. To investigate the ability of polyhydroxybutyrate (PHB) to act as a scaffold for regeneration of pericardial tissue in animals subjected to CPB surgery.
8. To evaluate the PHB patch in the pericardial position, with special reference to its affects on pericardial adhesion formation, the visibility of the coronary anatomy, and infectivity.

Thesis Design

Phase One: Clinical Study

At this phase of the study the changes in the pericardial fibrinolytic activity and morphology in response to CPB were observed and evaluated. Questions arising out of this phase would be addressed in phase two. Samples were taken at fixed times in relation to the time of initial pericardiotomy.

Phase Two: Clinical study

The changes in phase one were again reviewed. Patients undergoing primary elective CPB surgery and patients undergoing first time reoperative surgery were assessed. Samples were taken at times related to three events during surgery: (1) initial pericardiotomy (in the reoperative group this would be when the pericardium is first exposed), (2) commencement of CPB, (3) rewarming of patient to 37 ^{0}C. The plasma tPA activity was measured in order to relate its changes to those changes in primary pericardial PAA. Pericardial fluid from those patients undergoing a primary operation was assessed.

Phase Three: Animal Studies

This phase of the study was to evaluate PHB as a pericardial substitute and assess the reaction to the material in terms of: mesothelial cell regeneration, postoperative adhesion formation, coronary anatomy visibility, and infectivity. Morphological and fibrinolytic changes during CPB were noted to confirm whether these changes where similar to those noted in clinical studies.

CHAPTER 4

PATIENTS, MATERIALS
&
METHODS

Patients

Phase One Patients

Ten patients, undergoing primary elective open heart surgery, were studied. Eight patients had coronary artery bypass grafts, one patient had mitral valve replacement and one patient had aortic valve replacement. There were 7 males and 3 females. The median age was 62.5 years (range 42-74 years). Myocardial protection was maintained with cardioplegia (St Thomas' solution), moderate systemic hypothermia (28^0 C, nasopharyngeal) and topical hypothermia. The median cross-clamp time was 55.5 minutes (range 29-90 minutes). The median perfusion time was 99.5 minutes (range 49-124 minutes).

Phase Two Patients

Ten patients undergoing primary elective open heart surgery and ten undergoing first time reoperative open heart surgery were studied. Of those patients undergoing a primary elective operation, seven patients underwent coronary artery bypass grafting, two had mitral valve replacement and one patient had combined aortic valve replacement and coronary artery bypass. There were 9 males and 1 female. The median age was 61.5 years (range 50 - 72 years). In both the primary and reoperative groups, myocardial protection was maintained with blood cardioplegia, moderate systemic hypothermia (28^0 C, nasopharyngeal) and topical hypothermia. The median cross-clamp time was 47 minutes (range 24 - 66 minutes). The median perfusion time was 75 minutes (range 38 - 99 minutes).

The ten reoperative patients underwent surgery a median of 9 years (range 1 - 30 years) after their first operation; eight of them underwent coronary artery bypass, one had aortic valve replacement, and another had a mitral valve replacement. There were 7 males and 3 females. The median age was 57 years (range 40 - 70). The median cross-clamp time was 42 minutes (range 32 - 63 minutes). The median perfusion time was 66.5 minutes (range 52 - 103 minutes).

Exclusion Categories

The following patients were excluded from the study:-

(1) Patients requiring emergency procedures.

(2) Patients requiring drugs which would interfere with the assay measures e.g. aspirin within 5 days of the operation, steroids, and aprotinin.

(3) Patients with significant pathology in addition to the cardiac disease, e.g. diabetes, renal failure or hepatic failure.

(4) Patients who at primary pericardiotomy were noted to already have pericardial adhesions.

There was no significant difference in age, cross-clamp time and perfusion time in the phase two primary and reoperative groups.

Anaesthetic Technique

A standard anaesthetic regime was employed in all patients studied in both phase one and phase two patients, i.e.

Induction	Intravenous fentanyl and midazolam with muscle relaxant.
Maintenance prebypass	Enflurane, oxygen and air.
During bypass	Further bolus of fentanyl or midazolam as required
Post bypass	Propofol infusion and intravenous omnopon (2.5 - 5 mg) as required

All patients were intubated after induction of anaesthesia and maintained on intermittent positive pressure ventilation (IPPV) prebypass and postbypass for about 4 to 18 hours postoperatively. Thereafter, spontaneous ventilation was re-established and once the patient was rewarmed with satisfactory arterial blood gases, extubation was completed.

Cardiopulmonary Bypass Technique

The perfusion technique employed was a conventional one of moderate hypothermia with topical cooling, non-pulsatile flow using one venous cannula for coronary artery bypass and aortic valve surgery and two for mitral valve surgery, a hollow fibre membrane oxygenator, an arterial line filter (pore 40 μ), and ascending aorta arterial return.

The pump flow on bypass was calculated on the standard formula: Flow = 2.4 $L/m^2/min$. The perfusion pressure was maintained at a mean of 50 - 65 mm Hg. The prime solution was 2 L of Hartmann's.

Pericardial Biopsy Technique

Pericardial biopsies were taken using a disposable, 6 mm diameter biopsy punch (Stiefel Laboratories (UK) Ltd, Wooburn Green, UK). The samples were obtained from the non-diaphragmatic sides of the open pericardium, all within 3 cm of its cut edges. It was felt that this area, because of its relative exposure, would be more representative of pericardium that is subject to the maximum injury sustained during surgery (handling, instrumentation, dehydration, diathermy, abrasion, temperature variation, et cetera). Samples for electron microscopic (EM) studies were placed in 2% glutaraldehyde. Those for histological analysis were fixed in 10% buffered formalin.

Sample Timing for Phase One

Four (a paired sample for PAA assay, one for histological analysis, and one for EM study) pericardial samples were taken at times 0, 75, and 135 minutes from the time of opening the pericardium.

Sample Timing for Phase Two

Pericardial samples were taken at four time points during the operation. At 0 and 30 minutes from the time of pericardiotomy (before the commencement of CPB). At 30 - 50 minutes after the commence of CPB, and then finally 10 minutes after the patient had been rewarmed (for convenience these times are respectively designated, times A, B, C, and D). Equivalent reoperative samples were taken from those areas of the non

diaphragmatic pericardium that had little or no adhesions and that required at most only blunt dissection to liberate it from the heart's surface.

In 5 of the 10 primary patients and at each of the pericardial sampling times 5 ml of blood was collected into citrated tubes. Two millilitres of this was immediately acidified with 2 ml of acetate buffer solution (2 to 8 ^{o}C) kept on ice, until centrifuged within 15 minutes at 2000 - 5000 g for 20 minutes at 4 ^{o}C. Duplicated samples of 0.3 ml of the separated acetate treated plasma was aliquoted with 0.01 ml of hydrochloric acid 1 mol/l and rapidly frozen in liquid nitrogen before storage at -35 ^{0}C until analysed for tPA activity. The remaining 3 ml of citrated blood was also centrifuged at 2000 - 5000 g for 20 minutes at 4°C. Of the separated plasma, 1.5 ml was stored in plain tubes at -35°C until assayed for tPA and uPA antigen. All the acidified plasma was assayed within 4 weeks of storage. Samples were thawed rapidly at 37°C and the samples assayed according to the manufacturer's instructions (KABI Diagnostica COA-SET® tPA, KabiVitrum Ltd. KabiVitrum House Riverside Way Uxbridge, Middx, UB8 2YF, England). In brief, samples were incubated with plasminogen in the presence of fibrin fragments. The plasmin generated by any tPA in the sample cleaves a chromogenic substrate resulting in a coloured product. By the inclusion of standards, the photometric determination of tPA activity in plasma can be calculated.

In 15 patients (the 10 primary operative patients mentioned plus an additional 5 primary cases), 3 - 5 ml of pericardial fluid was collected with a syringe on immediately opening the pericardium. For the various assays, the 15 samples were further treated like the blood samples above. In 5 of the 15 samples at least 2 ml of pericardial fluid was sent to our biochemistry laboratory for a biochemical profile and 5 samples were cytologically reviewed.

Sample Timing for Phase Three

Samples were taken at times 0, 60, and 90 minutes from the time of pericardiotomy, and then again at reoperation 4 weeks later. For convenience these times were also designated

letters A, B, C, and D respectively. Only pericardial samples from groups A (CPB) and C (non-CPB) were compared.

Preparation of Pericardial Homogenates

All samples were assayed within 2 weeks. The pericardial biopsy specimens were thawed at room temperature and weighed. Each biopsy was washed with 0.5 ml rinsing solution (5 mmol/l sodium dihydrogen phosphate, 0.15 mol/l sodium chloride, pH 7.4) and placed into a small plastic test tube, on ice, containing 1 ml homogenising solution (2.5 mmol/l sodium dihydrogen phosphate, 0.075 mol/l sodium chloride, 0.25 % 'Triton X100' Sigma Chemical Co. Ltd., UK, pH 7.8). The tissues were then homogenised using an 'Ultra-Turrax' (Janke and Kunzel, Staufen, Germany) homogeniser for 30 S. The homogenates were centrifuged at 12 000 g for 20 min at 4^0 C. Aliquots of 0.25 ml of supernatant were stored at -20 to -35 ^{0}C until assay.

Assay For Plasminogen Activating activity (PAA)

PAA in the pericardial tissue extracts was assayed using a standard fibrin plate technique (Astrup and Mullertz, 1952). A layer of fibrin was produced in the base of a 5 cm diameter plastic Petri dishes (Sterlin Ltd., UK) by pouring in a solution containing 0.9 ml buffer (0.03 mol/l sodium acetate, 0.03 mol/l sodium barbitone, 0.02 mol/l hydrochloric acid, 1.2 mmol/l sodium chloride; pH 7.4), 3 ml of 1% human fibrinogen (KabiVitrum Ltd., Uxbridge, U.K), 0.3 ml of human plasminogen (KabiVitrum Ltd., 2.5 units/ml) and 0.2 ml bovine thrombin (Armour Pharmaceutical Co., USA, 20 units/ml). After the fibrin solution had set, a 6 mm diameter well was cut in the centre of the plate. The pericardial tissue homogenates were brought to room temperature and 20 μl of each specimen was placed in the central well of a fibrin plate. Standard solutions of human tissue plasminogen activator (Second International Standard, National Institute of Biological Standards and Controls Mill Hill, U.K) reconstituted in sterile water were placed on fibrin plates at the time of each batch of assays.

All plates were then incubated at 37^0 C for 24 hours and the diameter of the zone of lysis around the central well was measured directly by placing the plates on a measuring scale.

The maximum and minimum diameters were recorded and the mean value used. A standard plot of diameter of fibrin plate lysis against log t-PA was constructed for each assay and the line of best fit calculated using least squares regression analysis. The mean PAA level for the paired samples was calculated. To establish whether fibrinolysis was plasminogen mediated, homogenate supernatant was also applied to fibrin plates that had been previously heated to 80^0 C for 20 minutes (thereby destroying the plasminogen). Lysis did not occur in those plates so treated, confirming that fibrinolysis was mediated through plasminogen (Lassen, 1952). The PAA was expressed in International units per square centimetre of pericardium. The lower limit of sensitivity of the assay was 0.07 IU/cm^2 for the human data and 0.04 IU/cm^2 for the animal data.

Enzyme-Linked ImmunoSorbent Assays (ELISA)

These kits are complete enzyme-linked immunosorbent assay (ELISA) based kits for rapid antigen determination. Precoated microplate strips simplify application and provide flexibility in assay numbers. The principles behind the measurements are the same for each fibrinolytic component. However, that for tPA measurement will be briefly described here. The microplate strips coated with a monoclonal anti-tPA antibody are used to capture any tPA present in the sample. A second antibody, conjugated to an enzyme label, is added. After incubation and removal of unreacted conjugate, enzyme substrate is added. Incubation with enzyme substrate produces a blue colour that turns yellow when the reaction is stopped with sulphuric acid. The amount of colour produced in the wells, as measured chromogenically by a light meter, is proportional to the amount of tPA originally present in the sample. The same principle applies also to the other substrates assayed.

All the homogenates were assayed for the following fibrinolytic components using commercially available ELISA assays (Tintylse, Porton products, Maidenhead, U.K.): tissue type plasminogen activator (t-PA), urokinase plasminogen activator (u-PA), plasminogen activator inhibitor-1 (PAI-1), and plasminogen activator inhibitor-2 (PAI-2). The lower limits of detection were 0.75 ng/ml for t-PA, 0.1 ng/ml for u-PA, 2.5 ng/ml for PAI-1, and 6 ng/ml for PAI-2.

Histological Evaluation

Histological Evaluation for Phase One

Formalin fixed paraffin embedded sections of pericardial tissue were stained with haematoxylin and eosin and assessed by a histopathologist having no knowledge of the patient nor of the time sequence of biopsy. Using a consistent magnification to compare each feature, various features were assessed including vascular congestion and oedema (figure 3), margination (figure 4) and the presence, nature and severity of cellular infilteration of connective tissue.

In addition, mesothelial cell damage was assessed on morphological grounds, and the degree of mesothelial cell rounding (figure 5) and loss (figure 6) was recorded.

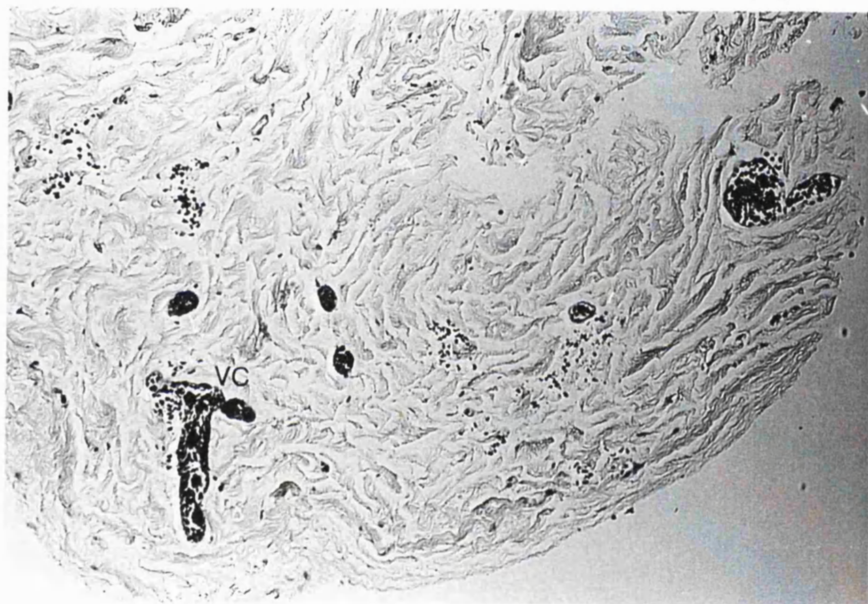


Figure 3. Histology of parietal pericardium. Section through the fibrosal and outer epipericardial layers showing vascular congestion (VC).

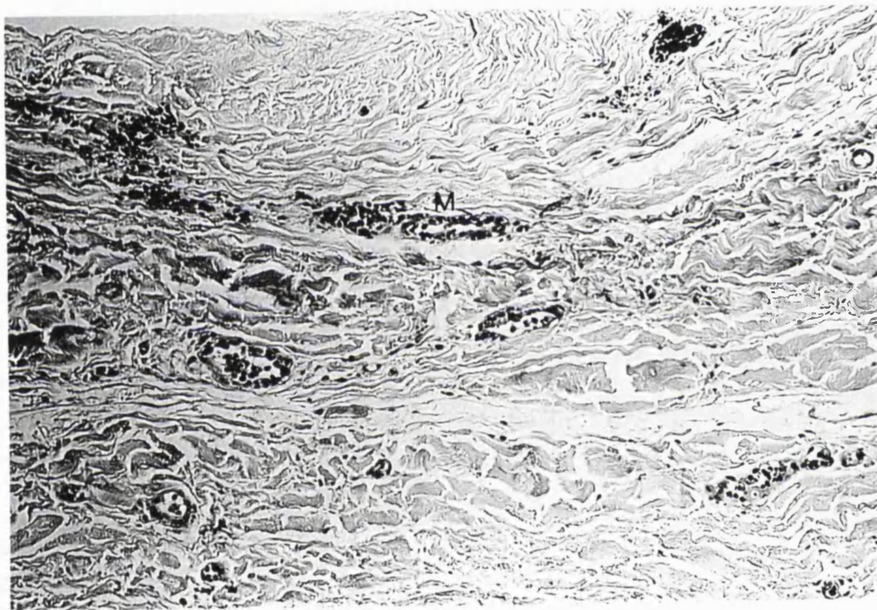


Figure 4. Histology of parietal pericardium. Section showing margination of neutrophils (M).

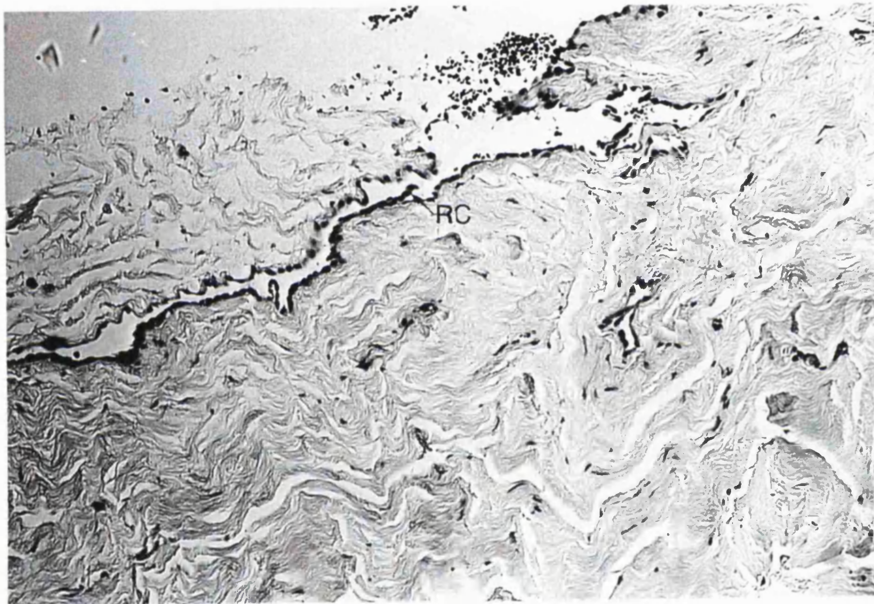


Figure 5. Histology of parietal pericardium. Section showing rounded mesothelial cells (RC) becoming detached from the fibrosal layer.

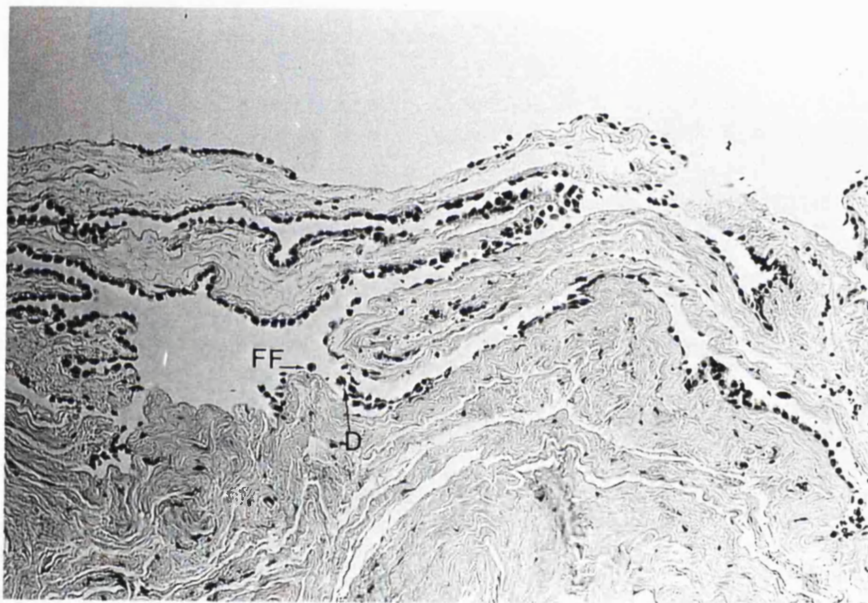


Figure 6. Histology of parietal pericardium. Section showing detached (D) and free-floating cells (FF).

Histological Evaluation for Phase Two and Three

The histological assessments here were as for phase-one samples. However, for statistical analysis, the severity of these individual features of inflammation was scored in the following manner: absent = 1, mild = 2, moderate = 3, severe = 4. The sum of the inflammatory features for each time period was totalled, and the resultant total inflammatory score for each time period was compared.

Mesothelial cell damage was assessed on morphological grounds, and the degree of mesothelial loss and cell rounding was scored for statistical analysis. The method used for scoring mesothelial damage was as follows: predominantly normal mesothelium = 1, predominantly rounded mesothelial covering = 2, mesothelium stripped from pericardial surface = 3, mesothelium stripped from pericardial surface with fibrin deposition = 4.

Transmission and Scanning Electron Microscopic Studies

Tissue samples, not exceeding 4 mm^3 in volume were fixed in 2% glutaraldehyde for 2 hours. Further preparation of samples continued according to whether they were for transmission or scanning electron microscopy.

Specimens for transmission electron microscopy after washing in phosphate buffer, were osmicated and dehydrated in acidified DMP before routine embedding in Taab resin. One micron sections were cut and stained with toludine blue for observation at light microscope level, followed by ultra thin sections of approximately 100nm, collected on nickel grids and stained with uranyl acetate and lead citrate, for observation on the Philips CM-10 electron microscope at 80KV.

Following fixation in 2% glutaraldehyde, tissue samples for scanning electron microscopy were washed in distilled water, then dehydrated in ethanol before immersion in liquid carbon dioxide in a critical point drier. After drying, specimens were mounted on stubs and coated with gold in a Polaron sputter coater before examination in a Hitachi S520 scanning electron microscope using an accelerating voltage of 20 kV.

Material and Methods Peculiar to the Animal Studies

Animals

Twenty calves, were divided into 3 groups of study. The test group (group A) consisted of 6 females, median weight 47.5 kg (range 39 to 60 kg) receiving a PHB pericardial patch. The first control group (group B) consisted of 9 female animals, median weight 46 kg (range 35-56 kg) in whom the pericardium was left well open. The second control group (group C) also had their pericardium left open and consisted of 5 female animals, median weight 45 kg (range 39.5 to 60 kg). The group C animals did not undergo CPB.

Anaesthesia and Operative Procedure

In the concomitant study the animals were randomised to undergo either functional denervation of the heart using cryo-ablation or to act as controls (non-denervations). In group A there were 3 of each type. In group B, there were 4 non-denervations and 5 denervations. In group C there were 2 non-denervations and 3 denervations. Both procedures (denervation and non-denervation) required that the heart and pericardium be handled and manipulated in a manner equivalent to that which occurs in clinical patients. The aim of this part of the study was to study the effects of functional denervation of the heart by cryoablation (Gordon et al, 1993) on post-traumatic coronary endothelial recovery following deliberate injury to the endothelial surface of the left anterior descending (LAD) artery. The type of operation performed under CPB was not directly relevant to this study. However, the use of CPB and surgical manipulation enabled us to imitate the clinical situation.

All animals were pre-medicated with ace promazine (ACP) 0.05 mg/kg before general anaesthesia was induced by inhalation using a mixture of oxygen and nitrous oxide with halothane 5%. Once anaesthesia was induced, endotracheal intubation was undertaken and oro-gastric tube passed. Anaesthesia was maintained using a concentration of 1-1.5% halothane, i.e. the minimum compatible with maintained anaesthesia. Antibiotic prophylaxis was given intravenously at induction and continued for five postoperative

days by way of intramuscular injection. The right side of the neck and thorax were then clipped.

The right chest was entered through a postero-lateral thoracotomy, at the bed of the fifth rib. The pericardium was incised parallel and anterior to phrenic nerve. Pericardial stay sutures were placed and a nylon tape positioned to encircle the ascending aorta. Following placement of purse strings and administration of heparin at a dosage of 300 units/kg, the ascending aorta was cannulated at its junction with the first arch vessel (left common carotid artery) and bicaval cannulation was undertaken through the right atrium (figure 7). Cardiopulmonary bypass was established at a flow rate of 2.4 $l/min/m^2$ whilst cooling from 38^0C to a core temperature of 35^0C. St. Thomas' crystalloid cardioplegia and topical cooling (with cold ringers solution) were employed for myocardial preservation. The cardioplegia was infused through an aortic cannula with a side arm for venting. The perfusion circuit included an arterial line filter and bubble oxygenator.

When the cardiac activity had ceased, the vent side arm was opened for venting. The heart was elevated on two large swabs and a size 15 scalpel blade used to make an arteriotomy in the mid-portion of the LAD. The arteriotomy was enlarged with a Pott's scissors. A 5F balloon catheter with an external inflated diameter of 5 mm was introduced into the LAD and inflated to a pressure of 7 atmospheres for 2 minutes, deflated, and re-inflated again for two minutes at the same pressure. The catheter was then removed and a 5F introducer containing a stainless steel wire with a rounded tip inserted into the LAD. The wire was advanced out of the tip of the introducer until the tip could be palpated and visualised against the LAD wall. The combined catheter-wire assembly was withdrawn ensuring that the tip of the wire rubbed along the artery wall. This was repeated three times. Re-warming was commenced whilst the arteriotomy was closed with a continuos 7/0 prolene suture.

For group A, the median cross-clamp time was 26 minutes (range 19 to 39 minutes, n = 6) and the median perfusion time was 58.5 minutes (range 45 to 120 minutes, n = 6). For group B, the median cross-clamp time was 33 minutes (range 17 to 41 minutes, n = 9) and the median perfusion time was 62 minutes (range 32 to 175 minutes, n = 9). The

variation in cross-clamp and perfusion time is attributed to the variable ease of the procedure and recovery of the heart following cardiopulmonary bypass. However, there was no significant difference between the two groups (A & B) in the cross-clamp time $p > 0.05$ or perfusion time $p > 0.05$. In the group C animals the median pericardial exposure time was 60 0 minutes (range 45 to 87 minutes, n = 5).

The groups A and B underwent the same procedure under cardiopulmonary bypass, without significant differences in the cross-clamp and cardiopulmonary bypass time. The assumption made is that the similar pericardial exposure of groups A and B would produce equivalent pericardial changes in them. Therefore these two groups were not compared for the extent of their mesothelial damage, pericardial inflammation and change in plasminogen activating activity. Moreover, our aim was to compare the CPB and non-CPB situation to assess the possible additional affects of CPB. Therefore, group B acted as a control to compare the extent and degree of adhesion formation, infectivity and preservation of the visibility of the coronary anatomy with that of the study group (group A) and the non-CPB control group C.

Following successful completion of the procedure the animal was rewarmed to 39^0C. Having come off bypass de-cannulation was completed, protamine administered, and haemostasis secured. In group A the pericardial defect (average size 8 x 12 cm) was closed by inserting a patch secured with a 5.0 Prolene running suture (figures 8 and 9). The chest was closed over a single 36F drain placed in the pericardial cavity through a small defect in the native pericardium. In group B and C the chest was closed in a like manner, but without the patch.

The animals were restudied at four weeks. Premedication, induction and maintenance of general anaesthesia, and monitoring was undertaken in an identical fashion to the initial procedure. A median sternotomy was used as the route of exposure at reoperation. The animals were sacrificed after this stage of the study.

Comments on Materials and Methods

The Use of Calves

Calves were used for the animal study in which CPB was necessary because they are known to tolerate CPB, run a low risk of postoperative infection, and develop postoperative pericardial adhesions within a relatively short time. Furthermore, the size of the calf is suitable for CPB in an experimental set-up.

Polyhydroxybutyrate Pericardial Patch

Polyhydroxybutyrate is a polymer of ß-hydroxybutyric acid. It is produced in nature by a wide range of microorganism that use it to store carbon and energy in the same way that humans accumulate fat. It was first isolated and characterised in 1926 at the Pasteur Institute in Paris. It remained an academic curiosity until W. R. Grace & Company in the USA produced small quantities for commercial evaluation in the late 1950's and early 1960's. Patents relating to the extraction and use of PHB were filed in the USA at about this time. It is manufactured in the UK by Imperial Chemical Industries (ICI) PLC, Billingham in the North East of England.

Polyhydroxybutyrate is produced by fermentation. Although a wide range of micro-organisms will make it, the organism used is Alcaligenes eutrohus a non-pathogenic organism that is safe to handle in large quantities. These micro-organisms are ubiquitous in the environment, where they can grow on a wide range of carbon substrates in both aerobic an anaerobic condition. Current production methods involve allowing the organism to grow on glucose.

The physical properties of the manufactured material vary with the amount of copolymer (hyrdoxyvalerate) it contains. Its biodegradable property has prompted various medical specialities to evaluate its potential use. In orthopaedics it has been assessed as an implant into the femur (Knowles et al, 1992). In cardiac surgery, as already mentioned, it has been used as a pericardial substitute (Malm, Bowald et al., 1992a) and intra-atrial patch.

The decision to use PHB as a trial pericardial patch was made considering its proven ability to sustain the regeneration of pericardial mesothelial cells onto its surface (Malm, Bowald et al., 1992b). However, the work that showed this was not done without CPB. Therefore, being aware that the encouraging results of experimental studies often do not extrapolate themselves into clinical practice, we chose to subject this promising substrate to the type of environment that is met with in clinical practice. This necessitated CPB surgery. The product was supplied by Astra Meditec, Mölndal, Sweden. The physical characteristics of the product supplied are summarised in table 1.

Table 1 Technical data on the PHB-patch.

Content of patch	Poly-ß-hydroxybutyrate (PHB)
Dimensions	10 x 15 cm
Fibre thickness	2 - 20 μm
Patch thickness	0.2 - 0.6 mm
Weight	12 - 20 mg/cm^2
Strength	> 5 N in a 10 mm wide patch

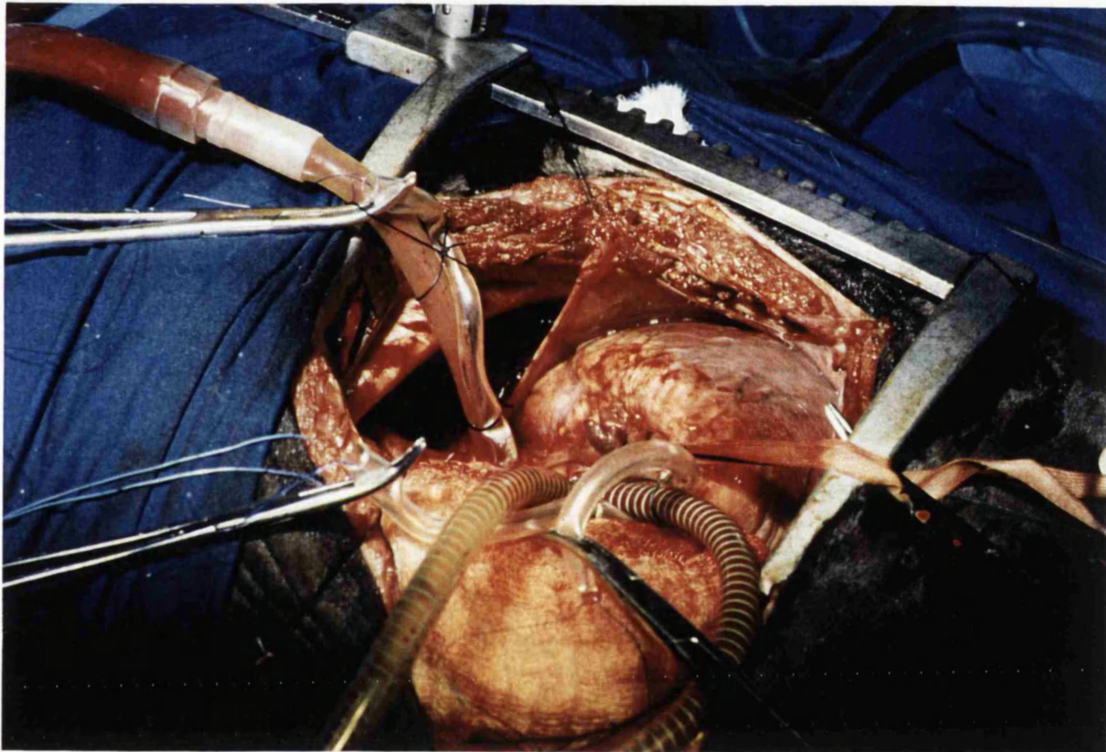


Figure 7. Part of cardiopulmonary bypass circuit with aortic cannula and bicaval cannulation.

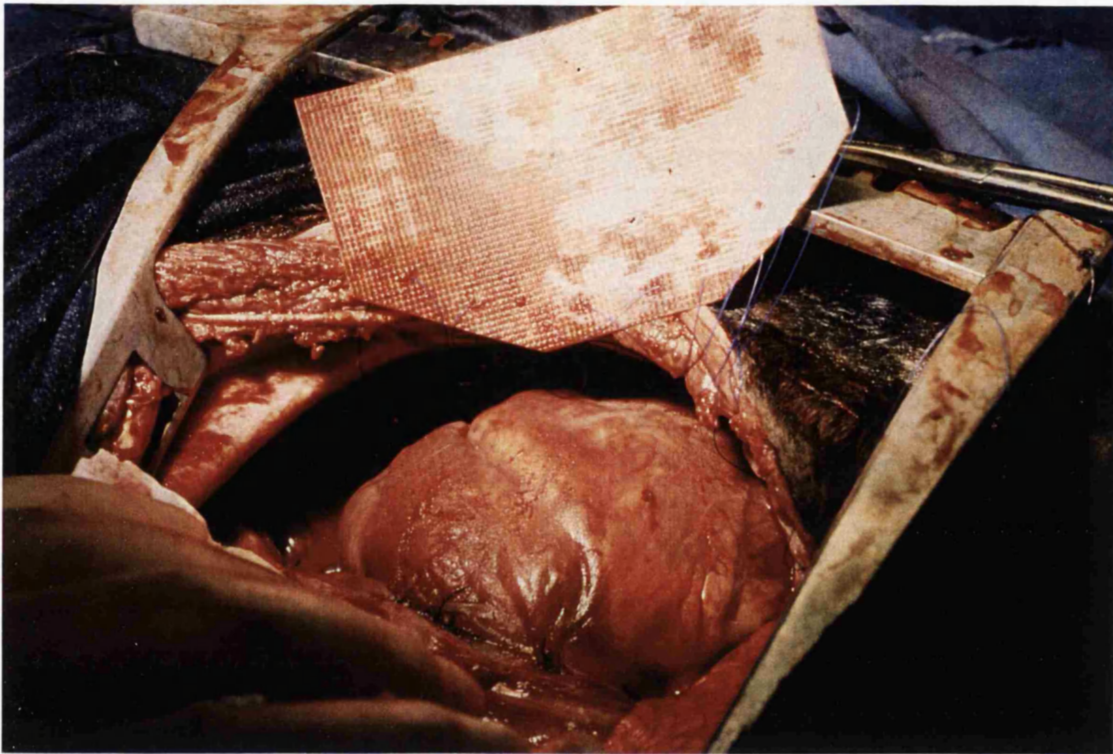


Figure 8. Pericardial substitute in the process of being sown into position.

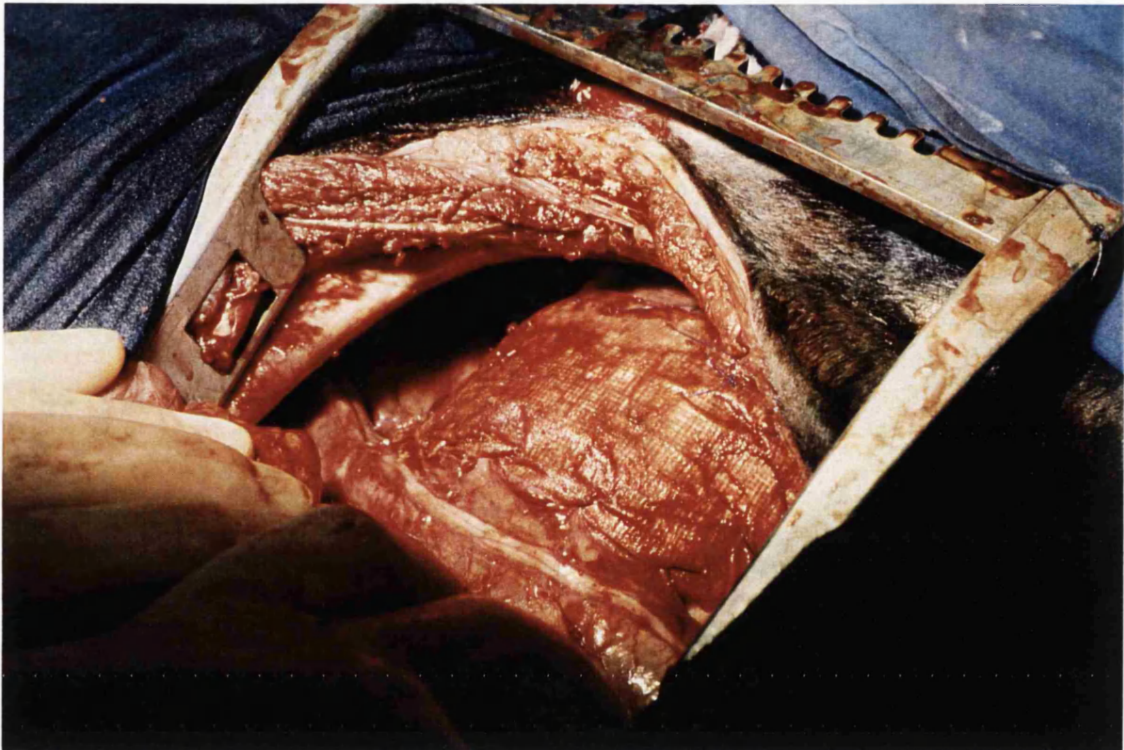


Figure 9. Pericardial substitute in position

Statistical Methods

Early histogram inspection of the raw data showed that its distribution was not of the normal (Gaussian) type. Therefore, assumptions could not be made that the data being analysed was suitable for parametric analysis. Accordingly, non-parametric measures of significance were used. The statistical difference between paired median values was analysed using Wilcoxon matched-pairs test, unpaired values were compared using the Mann-Whitney U tests.

Where the information obtained is of a categorical nature (e.g. none, mild, moderate, or severe) inefficient methods of analysis may be applied by the reduction of data to a two-point scale (e.g. none to mild, or moderate to severe). By doing so information may be sacrificed, moreover, this entails avoidable subjectivity in the choice of the cutting point that divides the two categories. With such data the chi-squared test should not be used for comparison of two groups for information would be wasted (Moses et al, 1984). In a number of areas some of our categorical data was given a numerical value or score corresponding with the severity of the category. These numerical values could then be analysed with the non-parametric tests mentioned above.

The Friedman's non-parametric two way analysis of variance was used to investigate whether there was any overall discernible reduction in the median PAA and t-PA levels with time. The Wilcoxon matched-pairs test was then used to look more closely at where the discernible reduction lay. The chi-squared test was used to analyse the significance of the histological changes and Spearman's rank correlation coefficient was used to detect the linear association between PAA and t-PA, and PAA or t-PA and the mean sample weight of the pericardial biopsies.

CHAPTER 5

CHANGES IN PERICARDIAL MORPHOLOGY AND FIBRINOLYTIC ACTIVITY DURING CARDIOPULMONARY BYPASS SURGERY.

(Phase One: Clinical Study)

Summary

The presence of pericardial adhesions at resternotomy not only increases the operation time, but also increases the risk of serious damage to the heart, great vessels, or extracardiac grafts. The reported incidence of damage is 2-6%. The fibrinolytic activity of pericardial tissue may be a crucial factor in determining the extent of adhesion formation following primary operation.

Ten patients undergoing cardiac surgery were studied to assess the plasminogen activating activity (PAA) of homogenates of pericardial tissue samples. Samples were taken at three times during the operation and the PAA was measured using a standard fibrin plate technique. Tissue-type plasminogen activator (t-PA), urokinase plasminogen activator (u-PA), plasminogen activator inhibitor-1 (PAI-1), and plasminogen activator inhibitor-2 (PAI-2) were also measured using enzyme linked immunosorbent assays (ELISA).

Compared with its initial levels (median 2.06 IU/cm^2, range 1.28-6.48 IU/cm^2), the PAA of pericardial biopsies was significantly reduced at 75 minutes (median 0.64 IU/cm^2, range 0.12-2.44 IU/cm^2, $p < 0.05$) with some recovery at 135 minutes (median 1.45 IU/cm^2, range 0.12-4.39 IU/cm^2, $p < 0.059$).

The major plasminogen activator present was t-PA. Compared with its initial levels (median 2.34 ng/ml, range 1.03-6.42 ng/ml) subsequent t-PA values were also significantly reduced at 75 minutes (median 0.83 ng/ml, range 0.75-5.13 ng/ml, $p < 0.01$) with some recovery at 135 minutes (median 1.24 ng/ml, range 0.75-6.67 ng/ml, $p = 0.093$). Low levels of u-PA were found in 5 of 10 patients. However, neither plasminogen activator inhibitor-1 nor plasminogen activator inhibitor-2 was detected.

Light microscopy showed both increasing pericardial mesothelial damage and increasing features of acute inflammatory changes with time. Compared with samples taken at initial pericardiotomy, those taken at 75, and 135 minutes demonstrated a significant progress in mesothelial cell damage ($p < 0.01$), together with increasing features of pericardial

inflammation ($p < 0.01$). Electron microscope studies confirmed and supplemented these findings.

This study shows that PAA is present in pericardial tissue and that t-PA is the major plasminogen activator. The observed inflammatory changes and concomitant damage to the pericardial mesothelium, and the significant reductions in pericardial t-PA and PAA seen during cardiac surgery may be important factors contributing to the early development of pericardial adhesions.

Introduction

Adhesions are a well-described sequel to primary surgery and are potentially a major problem if further reoperative intervention is required. In general surgery great efforts have been made to overcome the well documented problems produced by the presence of adhesions at reoperative surgery (Ellis, 1971). As discussed in chapter 2, many of the basic pathophysiological principals learnt from the work on peritoneum can be used as guidelines for the work on pericardium. This background knowledge of the peritoneal work may enable specific questions relating to the pericardium to be formulated and answered by research directed at the pericardium. However, it is important to remember that the process of adhesion formation is often beneficial and constitutes part of normal healing. Indeed, in experimental models where intestinal anastomoses were performed, there was a high incidence of peritonitis when agents preventing adhesion formation were tested (Fabri, Ellison et al., 1983). In cardiac surgery adhesions may prevent excessive movement of the heart that is void of its normal pericardial encasement. Therefore it may be more appropriate to regard adhesions as an ally who's excesses must be restrained. To appreciate these excesses in the context of cardiac surgery, a greater understanding of the basic morphological and biochemical changes that take place in the pericardium during and in response to cardiac surgery would seem pertinent. It is with this idea in mind that the initial phase of this study set out to establish the affects of CPB surgery on pericardial tissue, in particular on its fibrinolytic activity and morphology.

It is known that pericardial tissue injury and subsequent inflammation may cause an outpouring of fibrin rich exudate (Roberts and Spray, 1977). This fibrin, together with that in spilled pericardial blood, gives rise to fibrinous adhesions. This may then either undergo organisation by fibroblast infilteration and collagen deposition to form dense adhesions or, as a result of fibrinolysis, be resolved. Damage to pericardial tissue and a reduction in the fibrinolytic capacity of pericardial tissue, as reflected in the plasminogen activating activity (PAA), may be crucial in allowing these pericardial adhesions to form.

Summary of Patients, Materials and Methods for Phase One

Ten patients undergoing primary elective open heart surgery were studied. There were 7 males and 3 females. The median age was 62.5 years (range 42-74 years). Myocardial protection was maintained with cardioplegia (St Thomas' solution), moderate systemic hypothermia (28^0 C, nasopharyngeal) and topical hypothermia.

The pericardium was opened vertically and biopsies were taken using a disposable, 6 mm diameter biopsy punch. The samples were obtained from the non-diaphragmatic sides of the open pericardium, all within 3 cm of its cut edges. Samples were taken at 0, 75, and 135 minutes from the time of pericardiotomy. Samples for electron microscopic (EM) studies were placed in 2% glutaraldehyde. Those for histological analysis were fixed in 10% buffered formalin.

The following are elaborated in chapter 4:

1. Preparation of Pericardial Homogenates.
2. Assay For Plasminogen Activating Activity (PAA).
3. Enzyme-Linked ImmunoSorbent Assays (ELISA).
4. Histological Evaluation.
5. Transmission and Scanning Electron Microscopic Studies.

Results

Fibrinolytic Activity

Examination of the data using Friedman's non-parametric two way analysis of variance, suggested that there was an overall discernible reduction in the PAA ($p = 0.05$) and t-PA ($p= 0.004$) levels with time. This possibility was then investigated more closely, using Wilcoxon matched-pairs test, to see where the difference lies. The results are as shown below.

For each of the ten cases, the PAA of the pericardial biopsies was measured in all the homogenate samples. Compared with initial levels (median 2.06 IU/cm^2, range 1.28-6.48 IU/cm^2), the PAA of pericardial biopsies was significantly reduced at 75 minutes (median 0.64 IU/cm^2, range 0.12-2.44 IU/cm^2, $p < 0.05$) with some recovery at 135 minutes (median 1.45 IU/cm^2, range 0.12-4.39 IU/cm^2, $p < 0.059$). There was no significant difference between the PAA levels at 75 and 135 minutes.

The major plasminogen activator present was t-PA. Compared with its initial levels (median 2.34 ng/ml, range 1.03-6.42 ng/ml) subsequent t-PA values were also significantly reduced at 75 minutes (median 0.83 ng/ml, range 0.75-5.13 ng/ml, $p < 0.01$) and at 135 minutes (median 1.24 ng/ml, range 0.75-6.67 ng/ml, $p < 0.093$). Again, there was no significant difference between the t-PA levels at 75 and 135 minutes. Low levels of u-PA were detected in 5 of 10 patients (median 0.125 ng/ml, range 0.1-0.3 ng/ml, lower limit of detection was 0.1 ng/ml). However, neither PAI-1 nor PAI-2 was detected (lower limit of detection ≤ 2.5 ng/ml and ≤ 6 ng/ml respectively).

There was a significant positive correlation between the PAA and t-PA level (figure 10). Together with the fact that t-PA levels were approximately 10 times higher than u-PA levels, this suggests that t-PA rather than u-PA is responsible for the PAA exhibited by pericardium.

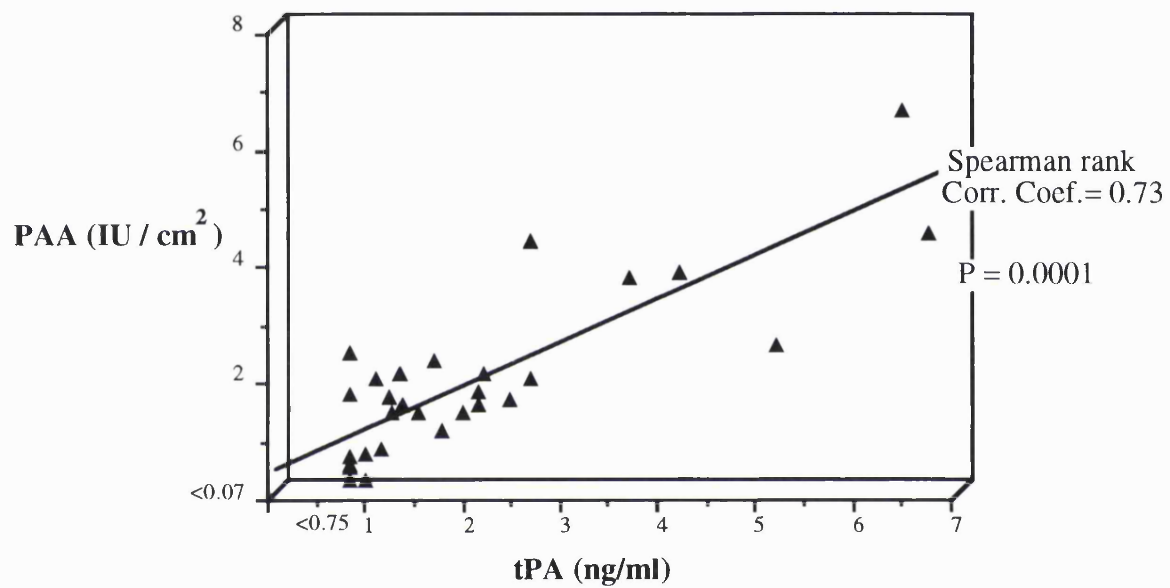


Figure 10. The plasminogen activating activity of all pericardial biopsies plotted against the corresponding tissue-type plasminogen activator (tPA) level.

Histology

Although a consistent magnification was used by the histopathologist to compare each feature, for clarity, those magnifications that best illustrated the changes discussed are presented. In early samples, three distinctive layers of parietal pericardium were noted (figure 11): the serosa (S), the fibrosa (F), and outer layer of epipericardial connective tissue (E). The serosa consisted mainly of a surface of flattened mesothelial cells and of a narrow submesothelial space that separated the mesothelial cells from the underlying fibrosa. The fibrosa was composed of connective tissue cells sparsely scattered about compactly arranged collagen fibers and small rather inconspicuous elastic fibers. There were also a few small blood vessels (BV). The connective tissue of the epipericardium was mainly loosely arranged collagen and elastic fibers interspersed with adipose cells. Small blood vessels and nerve fibers were also present with a scant population of connective tissue cells.

Later samples (figure 12) showed a layer of rounded mesothelial cells (RM) with focal cell loss and oedema of the submesothelial connective tissue. The latest samples (figure 13) showed a predominance of areas of denuded mesothelium exposing underlying fibrosa that was, in many areas, covered by a thin fibrinous exudate.

The histological findings of mesothelial damage and the extent of pericardial inflammation are graphically illustrated in figures 14 and 15 respectively. Although these show clear evidence of increasing mesothelial damage and progressive inflammatory changes with time, at zero time it is noticeable that there is already evidence of mild mesothelial damage in four of the ten samples and inflammatory changes in eight of the ten samples. This can be explained by an inconstant degree of injury to the closed pericardium during the variable period between initial sternotomy, intra-mediastinal manipulation (e.g. dissection and handling of fat on the anterior pericardium, dissection of internal mammary), and subsequent pericardiotomy.

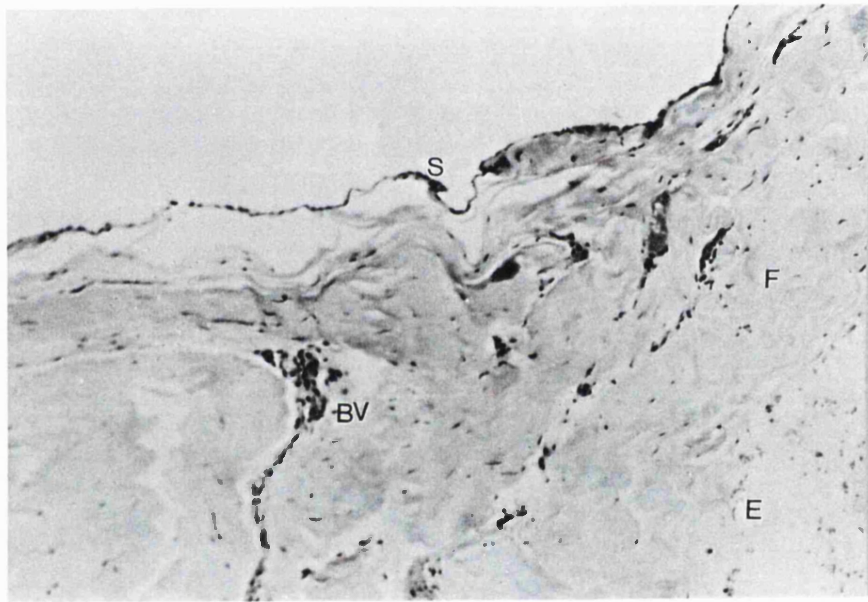


Figure 11. Light micrograph. The three layers are indicated by the following: S, serosal layer; F, fibrosal layer within which are blood vessels (BV); E, epipericardial connective tissue layer.

Figure 12. Light micrograph. The serosal layer of later pericardial sample showing rounded mesothelial cell (RM) with areas of focal cell loss.

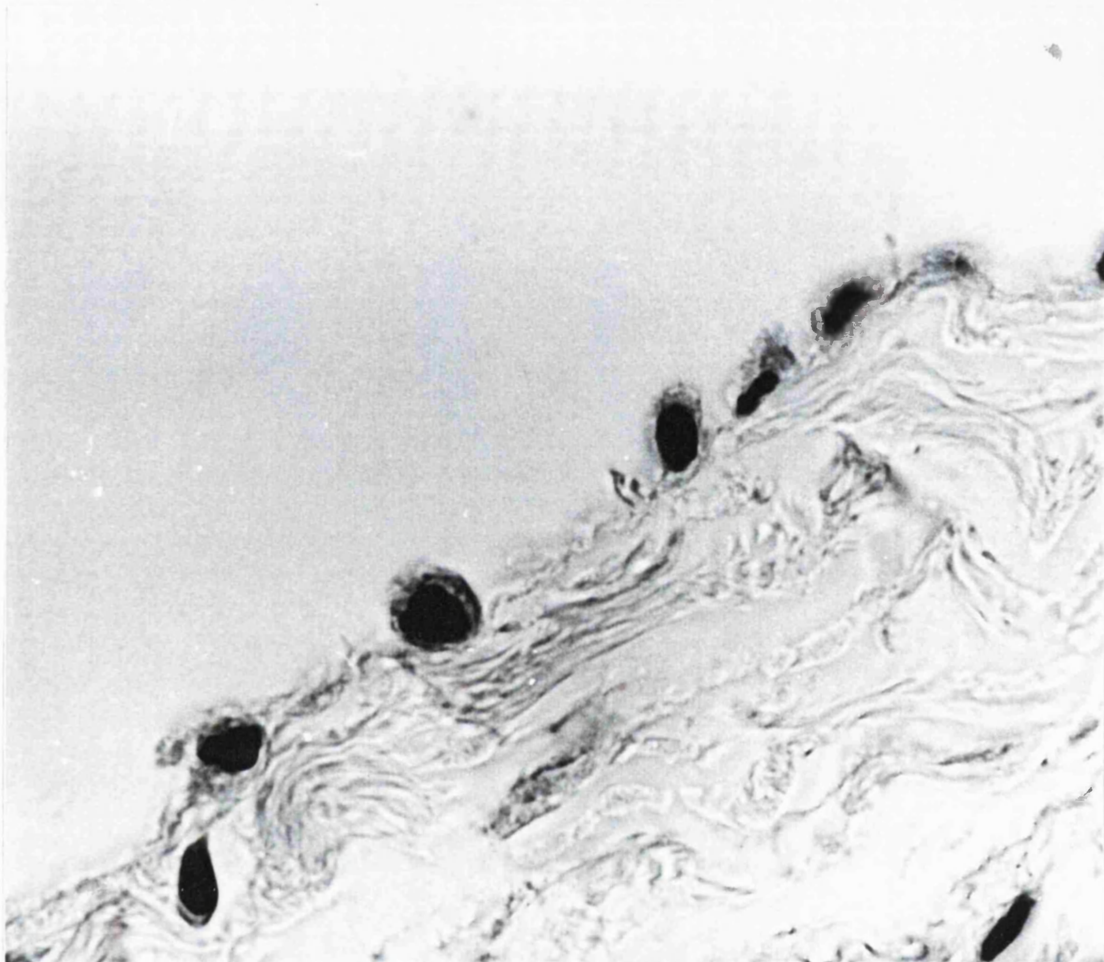
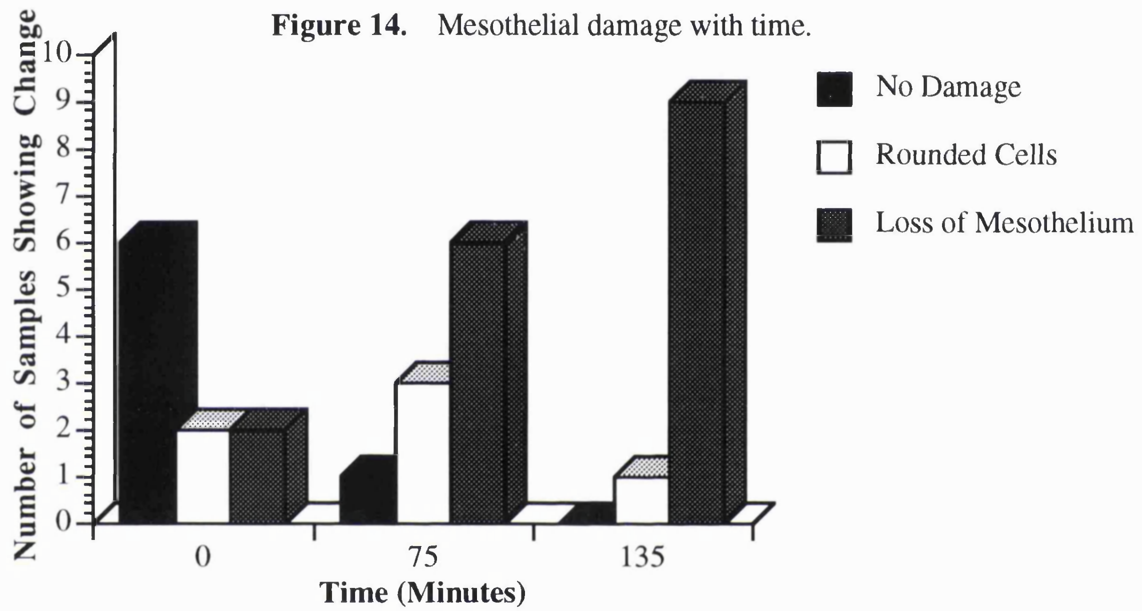


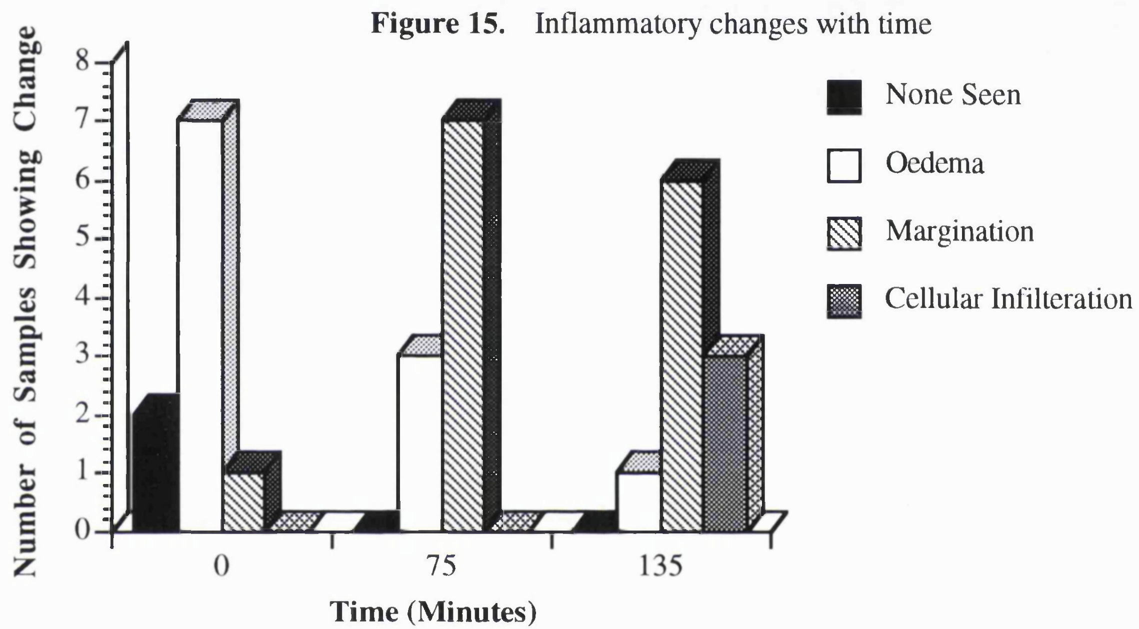
Figure 13. Light micrograph. Rounded mesothelial cells with areas of denuded pericardium covered by thin layers of fibrin.

Figure 14. Mesothelial damage with time.



Extent of mesothelial damage at times 75 and 135 minutes compared with that at zero time shows a significant, $p < 0.01$, preponderance of tissue with either rounded mesothelial cells or with mesothelial cell loss.

Figure 15. Inflammatory changes with time



Pericardial samples at times 75 and 135 minutes compared with those at 0 time show a significant, $p < 0.01$, preponderance of tissue with margination and frank inflammation.

Nevertheless, the extent of mesothelial damage at times 75 and 135 (figure 14) minutes compared with that at zero time show a significant ($p < 0.01$) preponderance of pericardial tissue with either rounded mesothelial cells or with mesothelial cell loss. Similarly, a significant ($p < 0.01$) preponderance of tissue with features of neutrophil margination and frank inflammation, rather than normal or congested tissue, was noted at times 75 and 135 minutes compared with that at 0 time.

Scanning Electron Microscopy

Examination of early pericardial samples at low magnification (Fig. 16), showed the serosal surface to be mostly covered by mesothelial cells (M). The surface of the "healthy" mesothelial cells contained numerous microvilli; these were one of the first cell structures to disappear during the operative period and were not at all apparent in later samples. Even at this stage there were rounded mesothelial cells (RM) and small areas of denuded pericardium exposing underlying fibrosa (F).

Later structural changes included a greater proportion of rounded mesothelial cells and cells detached or residually attached to the basal lamina and adjacent cells. In those areas (figure 17) where the mesothelial cells had become detached (D) and free floating, the serosal surface was covered by a relatively smooth layer of material corresponding to the submesothelial basal laminar (BL). At various locations (figure 18) this submesothelial layer was partially removed, exposing underlying bundles of collagen fibrils (CF) seen as discrete units. With the mesothelium and basal laminar completely removed (figure 19), the discrete collagen units (C) could be seen together with detached or free floating mesothelial cells (DM).

Transmission Electron Microscopy

Early pericardial samples (Fig. 20) showed the mesothelium most often to be a continuous single layer of 2 - 2.5 μm broad cells joined at the surface by tight junctions (T) and desmosomes, with highly convoluted intercellular channels (IC), sometimes clearly proceeding from beneath the junctions to the basement membrane.

Figure 16. Scanning electron micrograph. Serosal surface covered with mesothelial cells (M). The surfaces of the cells are covered with numerous microvilli (which appear as white specks). Some mesothelial cells (RM) were beginning to round off and loss their microvilli. At other sites the denuded pericardium was exposing the underlying fibrosa.

Figure 17. Scanning electron micrograph. This micrograph depicts the difference between the denuded submesothelial basal lamina (BL) on the left hand side and the surviving microvillous appearance of the mesothelium on the right. Mesothelial cells (D), demarcating the two areas, have become detached from the basal lamina (BL). Although some cells retain the microvillous covering, others are becoming rounded and have lost most of their microvilli.

Figure 18. Scanning electron micrograph. There is a complete loss of the mesothelial cell layer with exposure of the submesothelial basal laminar. At various locations the basal laminar is absent and exposes the collagen fibrils (CF) of the fibrosal layer.

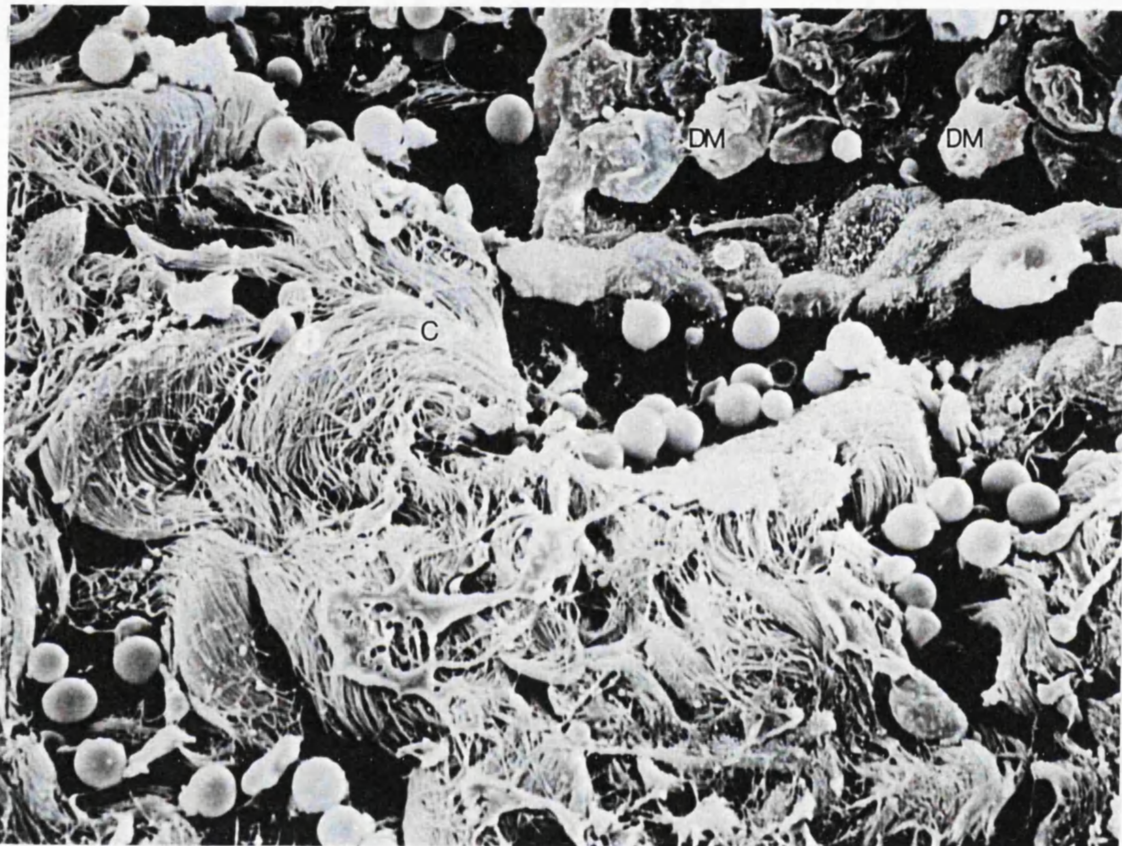


Figure 19. Scanning electron micrograph. In the later samples the mesothelial cells have almost all been shed to reveal the fibrosal collagen units (C). A few scattered free floating mesothelial cells (DM) are visible.

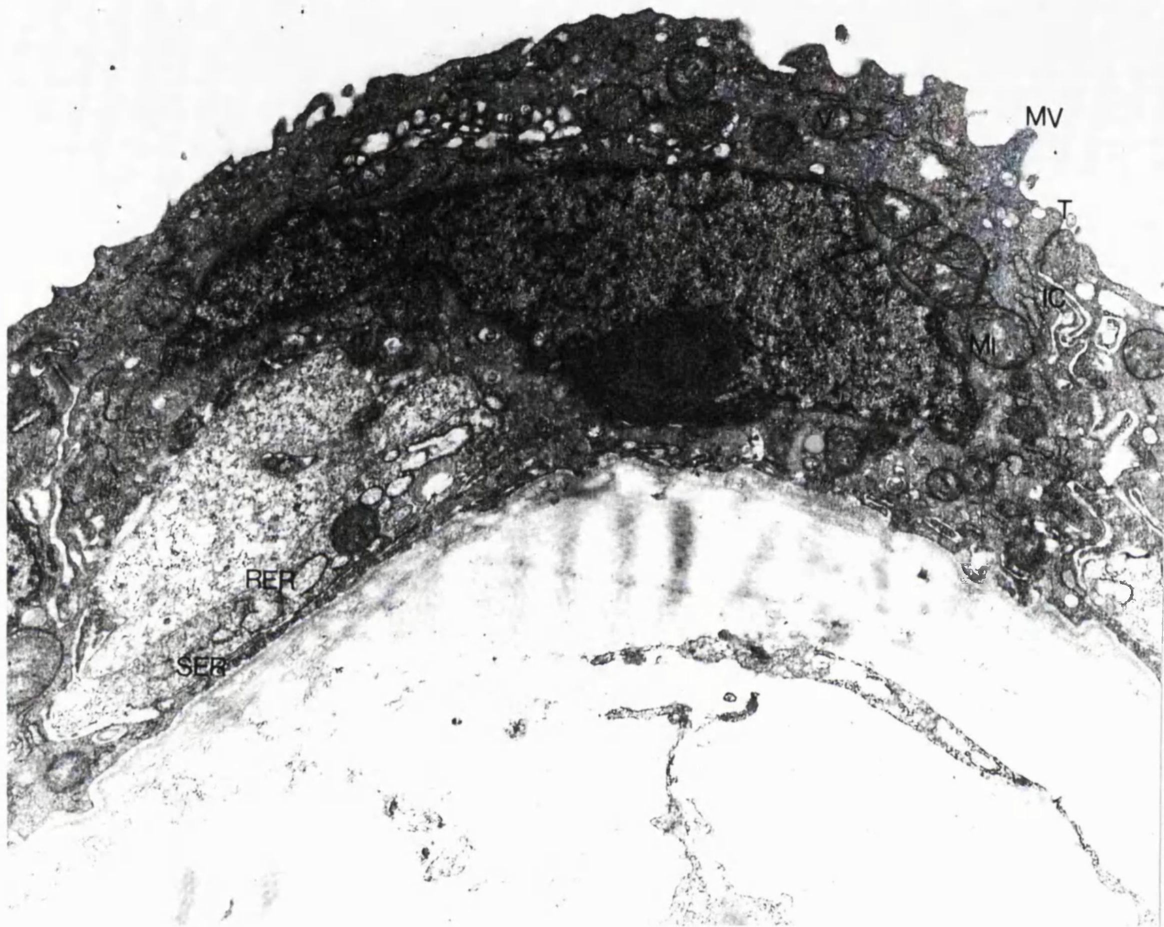


Figure 20. Transmission electron micrograph. Micrograph of an early pericardial sample showing three mesothelial cells (only one cell completely shown). They are joined at their surfaces by a tight junction (T) (right side) and desmosomes (left side). Convoluted intercellular channels (IC) run from beneath the junctions to the basal laminar. Mitochondria (Mi), microvilli (MV), vesicles (V), smooth and rough endoplasmic reticulum (SER, RER) were also present.

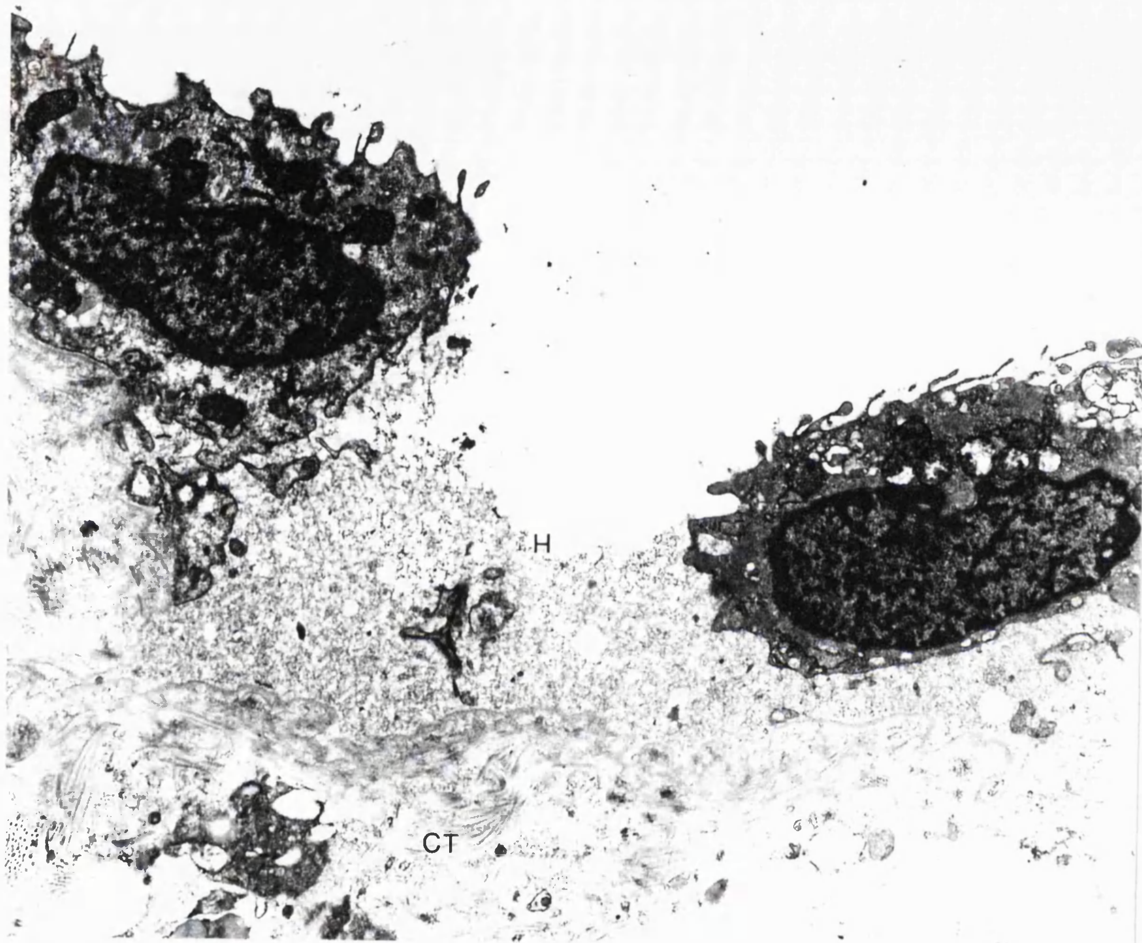


Figure 21. Transmission electron micrograph. This micrograph shows two mesothelial cells of a later pericardial sample. They have become separated and the gap between them is haemorrhagic (H). Beneath this haemorrhagic area lies connective tissue (CT).

Nuclei were slightly irregular in shape with prominent nucleoli. There were large mitochondria that were distinct from the occasional lamellar bodies present. Both rough and smooth endoplasmic reticulum (SER and RER) and Golgi apparatus was usual. The cytoplasm contained many vesicles (V). Microvilli (MV) were present on the surfaces of the cells, but these were never densely arranged.

In later categories sloughing of mesothelial layer became apparent revealing the submesothelial connective tissue (Fig. 21). Some areas showed heavy deposition of fibrin on the mesothelium. In addition, a few mesothelial cells showed hypoxic changes as suggested by swollen and distended organelles, particularly the endoplasmic reticulum. There were also inflammatory changes in blood vessels beneath the mesothelial layer with margination of neutrophils and total occlusion of the vessels.

Discussion

This study, in keeping with previous work by Porter (Porter, Ball et al., 1971) and again by Gervin (Gervin, Jacobs et al., 1975), has shown that mesothelial pericardial tissue expresses fibrinolytic activity. Pericardial tissue should therefore be capable of removing fibrin and hence, at least potentially, be able to prevent or minimise adhesion formation following cardiac surgery. The fibrinolytic activity is reflected by the PAA of pericardial tissue; this activity, as suggested by the positive correlation between PAA and t-PA, appears to be primarily due to the presence of t-PA rather than u-PA. In comparison with initial values, the PAA and the levels of t-PA were significantly lower at later stages of cardiac surgical procedures. The reduction in pericardial fibrinolytic activity, seen during cardiac surgery, suggests a reduced capacity of pericardial tissue to prevent adhesion formation. As appears to be the case in the peritoneum where the reduction in peritoneal PAA is strongly implicated as a causal factor in peritoneal adhesion formation (Gervin et al, 1973; Thompson et al, 1989; Whitaker and Papadimitriou, 1985), this reduced pericardial PAA may be a significant factor in the pathophysiology of pericardial adhesions.

Although the PAA and t-PA levels at times 75 minutes and 135 minutes were not statistically different, inspection of the data suggests that these values are on the increase at 135 minutes. Furthermore, the statistical difference between the PAA and t-PA levels at times 0 and 75 minutes are greater than at 0 and 135 minutes. All cases were rewarmed and off CPB by 135 minutes and so, therefore, the apparent recovery in fibrinolytic activity may have some relation to the affects of CPB and temperature. The extent of this recovery and the degree to which it is impaired by the mesothelial damage and the simultaneous inflammatory response has not been answered by this study.

The presence of PAI-1 and PAI-2 was not detected in the pericardial tissue within the duration of CPB, suggesting that they play no part in the observed early fall in PAA. However, in peritoneal studies (Vipond et al, 1990), normal peritoneum has, likewise, no detectable PAI-1 nor PAI-2 whereas inflamed peritoneum has reduced PAA and

detectable PAI-1 and PAI-2. It may be that, as in peritoneum, PAI-1 and PAI-2 appear in pericardial tissue at a later stage after the initial insult.

The lack of correlation between the PAA, or t-PA levels, and the mean sample weight of the pericardial biopsies (figure 22), provides indirect evidence for the site of production of PA. The use of a 6 mm diameter punch biopsy standardised the area of mesothelium sampled. Therefore the different weights of the biopsies thus for the most part reflect the different amounts of underlying tissues sampled. If sub-mesothelial tissues were the major site of plasminogen activity, a positive correlation between PAA and mean sample weight might be expected. The fact that several low weight samples exhibited high PAA values and vice versa supports the theory that the surface mesothelial layer is responsible for most of the pericardial fibrinolytic activity (Merlo et al, 1980). However, the precise identification of the exact cellular origin and tissue localisation of pericardial PAA requires further study using techniques such as in situ m-RNA hybridisation.

Previous studies have not only shown the presence of PAA in pericardium and other mesothelial structures (Gervin, Jacobs et al., 1975; Porter, Ball et al., 1971), but have also indicated that injury (Ryan et al, 1973) (e.g., physical trauma, drying, diathermy, and ischaemia) decreases this activity (Buckman, Woods et al., 1976; Raftery, 1979). It is possible that mesothelial damage during surgery, caused by drying or manipulation, may be responsible for the decrease in PAA and t-PA observed in this study. However, it is not yet clear whether the diminished PAA demonstrated in the pericardium is a causative factor in the process of formation of the dense adhesions seen at reoperation. Nevertheless, it has been shown by Dorr and others (Dorr et al, 1990; Menzies and Ellis, 1989) that, following mesothelial injury, enhancing PAA or fibrinolytic activity, in the form of topical t-PA, may prevent adhesion formation in the peritoneum.

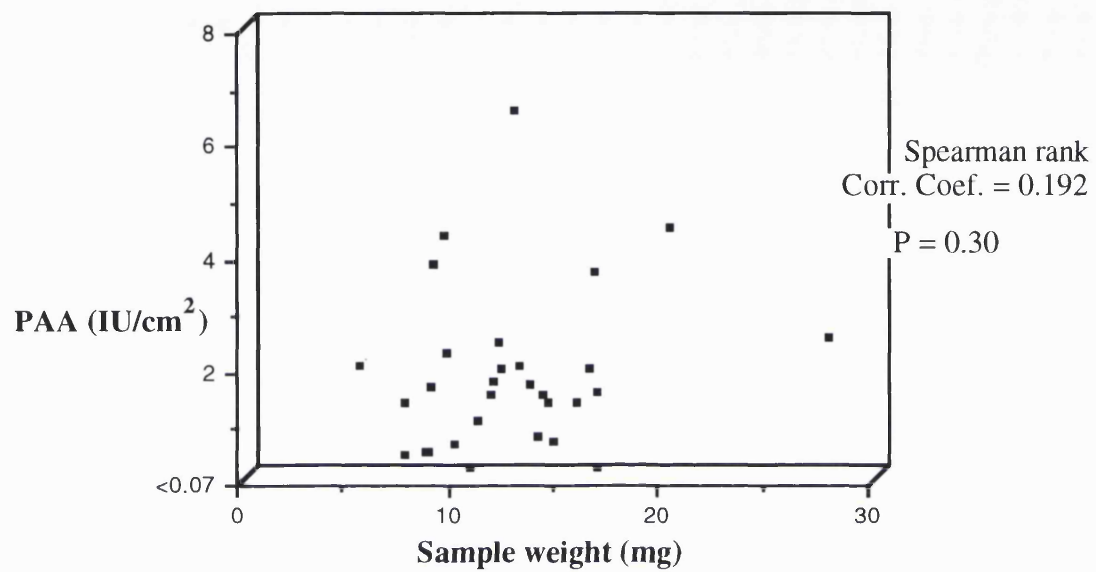


Figure 22
The plasminogen activating activity of all pericardial biopsies plotted against the corresponding mean sample weight.

The present study also confirms that there is an early inflammatory reaction in pericardial tissue in response to the insult of cardiac surgery. The response of mesothelium to injury has been described by previous investigators using bacterial toxins (Onderdonk et al, 1978), silica (Shade and Williamson, 1968), and various chemical and mechanical (Cliff et al, 1973) stimuli. In general this mesothelial cell response takes the form of the following progressive changes. Firstly there is a change in cell shape from a flat to a cuboidal one together with a total loss or reduction in the number of microvilli, then separation of adjacent cells, and finally shedding or detachment of cells from the basal lamina. The alteration in mesothelial cell shape is, as described by Majno et al (Majno et al, 1969), indicative of cellular contraction. Because these cells contain actin filaments (Tsilibary and Wissig, 1983) as well as a number of intermediate filaments scattered throughout the cytoplasm, it is assumed that much of the change in cell shape is due to redistribution of these filaments.

The mechanism of cellular detachment from the basal lamina is open to speculation. However, those cells that have become detached are sometimes found to be floating above the basal lamina but still anchored by their attachment to adjacent cells. With the loss of the mesothelial cells, large amounts of fibrin are deposited onto the exposed underlying connective tissue. The fibrinous adhesions that develop may undergo organisation by fibrosis. Evidence suggests that mesothelial cells themselves play a role in this tissue organisation by producing a chemoattractant for fibroblasts (Kuwahara et al, 1991).

The extent of tissue recovery following bypass surgery and the degree to which this recovery is hindered by the continued presence of noxious stimuli, such as cytotoxic products from inflammatory cells, remains unknown. Similarly, the degree of recovery of fibrinolytic activity and the extent to which the benefits of this recovery are offset by the pericardial response to insult is unknown. Nevertheless, as shown in work published by Malm et al (Malm, Bowald et al., 1992a) pericardial mesothelial regeneration does occur. In their work on sheep (Malm, Bowald et al., 1992b) they were able to show the regeneration of pericardial mesothelial cells onto a framework of biodegradable material.

However, this work did not use CPB, perhaps thereby underestimating the additional noxious affects of CPB on pericardium and therefore on its potential for recovery. As alluded to by Gabbay et al (Gabbay, Guindy et al., 1989) the regenerative potential may be further impaired by the additional insult of CPB as well as surgical manipulation. Considering human clinical experience requiring CPB, conducting animal experiments without administration of CPB may overlook the adverse affects of CPB and therefore perhaps make the results obtained less meaningful.

Though regeneration does occur, and with it some recovery of fibrinolytic activity with new mesothelial cells having an enhanced fibrinolytic activity (Raftery, 1979; Ryan, Grobety et al., 1973), the regenerative process may not be uniform. If areas of pericardium remain denuded these sites are more likely to develop adhesions owing to the localised deficiency in mesothelial fibrinolytic activity. It is generally agreed that this process of mesothelial recovery begins within 24 hours of pericardial insult and is completed by 8-10 days. However, there are a number of theories as to the sources of the new mesothelial cells, these include: 1) The replication and migration of mature mesothelial cells adjacent to the injured area (Watters and Buck, 1972), 2) Pre-existing free-floating serosal cells settle on the denuded surface, where they spread out, attach themselves to one another, and differentiate into mesothelial cells (Ryan, Grobety et al., 1973), 3) Migration of underlying connective tissue cells to the denuded surface, which then undergo metaplasia to become mesothelial cells (Raftery, 1973).

The extent to which the local mesothelial fibrinolytic activity is reduced by the haemodilution due to CPB and the underlying submesothelial inflammatory exudation and oedema is unknown. The haemodilution that occurs as a result of the institution of CPB is known to decrease the blood protein concentrations. Hence it could be argued that the observed reduction in PAA and t-PA are, in part, a reflection of this haemodilution. In addition, systemic and local hypothermia may play a role in bringing about a degree of decreased enzyme activity. However, it has been observed that a similar per-operative reduction in mesothelial PAA occurs in patients undergoing elective laparotomy (Scott-Coombes et al, 1992) in whom haemodilution and hypothermia do not play as major a

role. This suggests that local factors, such as extensive damage to mesothelial cells and submesothelial inflammation, rather than haemodilution are more important determinants of fibrinolytic activity. These local factors appear to provide a favourable environment for the accumulation of fibrin, which after organisation lead to fibrous adhesions.

This work has not researched the effects of warm heart surgery, without cooling and without cold topical hypothermic solutions, on the fibrinolytic properties of the pericardium. However, one can speculate that, because the enzyme activity is not suppressed by general and local hypothermia, the reduction in the PA activity may not be as great. However, since a reduction in the PA activity is not the only factor in the pathogenesis of adhesions the effects of warm heart surgery on adhesion formation are unknown.

In conclusion, this study documents some of the early ultrastructural and histological changes that occur within the pericardium during cardiac surgery. The effects of other factors, such as local infection, retained thrombus, pericardial effusions, and post-perfusion syndrome, on the formation of adhesions have not been addressed by this study but are factors that will need further investigation. The changes that we have observed appear to have a diminutive affect on the effective fibrinolytic activity of pericardium. One can hypothesis that any recovery in mesothelial cell fibrinolytic activity would appear to be offset by concomitant damage to the mesothelial layer. The consequent the loss of fibrinolytic activity in denuded areas would tend to favour the process of organisation of fibrinous adhesions. As a result these tentative fibrinous adhesions are eventually converted into the fibrous adhesion encountered at reoperative surgery. Moreover, in those areas where there is no pericardial covering, fibrous adhesions will occur owing to the unrestrained process of organisation. Therefore in tackling the problem of pericardial adhesions with a pericardial substitute, a substitute is required that will not only permit the regeneration of mesothelial cells, but will also delay organisation of fibrinous adhesions long enough for mesothelial recovery to occur. To achieve this delay, the feasibility of imposing upon the substitute material a localised and limited degree of fibrinolytic activity, as has been done to a limited degree in abdominal

work (Menzies and Ellis, 1989), needs to be tested. Nevertheless, at this time in the evolution of cardiac surgery the problems imposed upon reoperative surgery by the presence of pericardial adhesions are yet to be resolved. Further knowledge of the early extracellular and cellular events leading to adhesion formation may enable a rational manipulation of these processes. This may eventually lead to the emergence of a suitable pericardial substitute that will incorporate the regenerative and fibrinolytic capacity of normal pericardium.

CHAPTER 6

EVALUATION AND COMPARISON OF THE RESPONSE OF PRIMARY AND REOPERATIVE PERICARDIAL MORPHOLOGY AND FIBRINOLYTIC ACTIVITY TO CARDIOPULMONARY BYPASS SURGERY

(Phase Two: Clinical study)

Summary

In an attempt to appreciate the changes that favour adhesion formation the morphological and fibrinolytic changes that occur in human primary and reoperative pericardium were compared. Ten patients undergoing primary elective open heart surgery and ten undergoing first time reoperative open heart surgery were studied to assess these changes. Pericardial samples were taken at four time points during the operation. At 0 (time A), 30 (time B), minutes from the time of pericardiotomy (before the commencement of CPB), 30 - 50 minutes (time C) after the commence of CPB, and then finally 10 minutes (time D) after the patient had been rewarmed. The fibrinolytic activity, as measured by the plasminogen activating activity (PAA), in the pericardial samples of the ten primary cases was compared with that in 5 of the reoperative cases. For the primary group, the PAA after 30 minutes of exposure (median 6.65 IU/cm^2, range 3.85 - 11.89 IU/cm^2, $p = 0.14$, n=10) was not significantly reduced when compared to the initial activity (median 8.74 IU/cm^2, range 2.22 - 17.68 IU/cm2, n=10). After 30 - 50 minutes CPB the PAA was significantly reduced (median 3.93 IU/cm^2, range 1.5 - 13.24 IU/cm^2, $p = 0.028$, n=10) and, although there was some recovery of activity, still reduced after rewarming for 10 minutes (median 3.12 IU/cm^2, range 0.88 - 19.93 IU/cm^2, $p = 0.047$, n=10). The simultaneous plasma tPA activity showed a significant ($p < 0.05$) increase after 30 - 50 minutes bypass with a later decline. The changes in the reoperative pericardial PAA were similar to those observed in primary tissue. In addition, the degree of PAA in reoperative pericardium was consistently lower than that observed in primary tissue. This difference only reached significance at the sample times B ($p < 0.01$) and C ($p < 0.01$). The extent of primary pericardial mesothelial damage at times B, C, and D compared with that at time A showed a significant ($p < 0.01$ for times B, C, and D) preponderance of pericardial tissue with either rounded mesothelial cells, mesothelial cell loss, or mesothelial cell loss with fibrin deposition. Similarly there was a significant worsening of the degree of inflammation ($p < 0.05$) with a preponderance of samples from the later stages of the operation having a relatively higher inflammatory score. Compared with primary pericardium, the reoperative samples showed a significant

($p < 0.01$ for times A, B, and C) preponderance of damaged mesothelium at the earlier stages of the operation. However, as the extent of mesothelial damage in the primary group increased with time, this difference was no longer significant at time D. In addition, the reoperative fibrosal and epipericardial layers tended to contain more fibrous tissue than the equivalent primary pericardium. It appears that, following the initial bypass surgery, there is an inadequate recovery of the pericardial regeneration and fibrinolytic activity. With a better understanding of the changes that take place in the pericardium it may be possible to avert this tendency to adhesion formation by a pericardial substitute that will promote pericardial regeneration following CPB surgery.

Introduction

In phase one of this study we documented the morphological and fibrinolytic changes in the pericardium in response to trauma, injury, and cardiopulmonary bypass surgery. In essence these changes consisted of a significant reduction in the pericardial fibrinolytic activity and increasing pericardial inflammation and mesothelial damage. In the literature there are few descriptions of these early ultrastructural and biochemical changes that occur in the human pericardium in response to the insult of cardiac surgery but it is known that the pericardium reacts to acute injury by exuding fluid, fibrin, and/or cells (Roberts and Spray, 1977). Nevertheless, the precise mechanism responsible for adhesion formation and pericardial fibrosis are still to be fully appreciated. It is presumed that the changes that we have noted are clinically significant because they may be responsible for determining the subsequent extent of adhesion formation around the heart (Cliff, Grobety et al., 1973).

The events that transpire within the pericardium during the period between the primary operation and subsequent reoperation are unknown, but may possibly be surmised by observing primary and reoperative pericardium and their response to CPB surgery. Therefore, in phase two of this study we documented the fibrinolytic, cellular and extracellular events within human primary and reoperative pericardium during CPB surgery. In the primary group salient cytological and biochemical features of pericardial fluid were also inspected. Furthermore, to confirm whether or not the changes in the plasma tPA activity reflected the concomitant change in the pericardial PAA these two activities were measured and compared.

Summary of Patients, Materials and Methods for Phase Two

Ten patients undergoing primary elective open heart surgery and ten undergoing first time reoperative open heart surgery were studied. Of those patients undergoing primary surgery, there were 9 males and 1 female. The median age was 61.5 years (range 50 - 72 years). In both the primary and reoperative groups, myocardial protection was maintained with blood cardioplegia, moderate systemic hypothermia (28^0 C, nasopharyngeal) and topical hypothermia. The median cross-clamp time was 47 minutes (range 24 - 66 minutes). The median perfusion time was 75 minutes (range 38 - 99 minutes).

The ten reoperative patients underwent surgery a median of 9 years (range 1 - 30 years) after their first operation. There were 7 males and 3 females. The median age was 57 years (range 40 - 70). The median cross-clamp time was 42 minutes (range 32 - 63 minutes). The median perfusion time was 66.5 minutes (range 52 - 103 minutes).

For the primary pericardial samples the pericardium was opened vertically. Pericardial biopsies were taken using a disposable, 6 mm diameter biopsy punch. Samples were taken at four time points during the operation. At 0 and 30 minutes from the time of pericardiotomy (before the commencement of CPB). At 30 - 50 minutes after the commence of CPB, and then finally 10 minutes after the patient had been rewarmed (for convenience these times are designated, times A, B, C, and D). Samples for electron microscopic (EM) studies were placed in 2% glutaraldehyde. Those for histological analysis were fixed in 10% buffered formalin.

Equivalent reoperative samples were taken from those areas of the non diaphragmatic pericardium that had little or no adhesions and that required at most only blunt dissection to liberate it from the heart's surface.

The following are elaborated in chapter 4:

1. Collection and Preparation of Blood for tPA Activity and Assays.
2. Collection of Pericardial Fluid.
3. Preparation of Pericardial Homogenates.

4. Assay For Plasminogen Activating activity (PAA).

5. Enzyme-Linked ImmunoSorbent Assays (ELISA).

6. Histological Evaluation.

7. Transmission and Scanning Electron Microscopic Studies.

Results

The PAA in the pericardial samples of the ten primary cases was assessed and compared with that in 5 of the reoperative cases. In both the primary and reoperative groups when the pericardial PAA of later samples was compared with the initial activity at time zero, there was a significant reduction in the activity. For the primary group in particular, when the initial pericardial PAA (median 8.74 IU/cm^2, range 2.22 - 17.68 IU/cm^2, n=10) was compared with the activity after 30 minutes of exposure it was not significantly reduced (median 6.65 IU/cm^2, range 3.85 - 11.89 IU/cm^2, P = 0.14, n=10). However, after 30 - 50 minutes CPB the PAA was significantly reduced (median 3.93 IU/cm^2, range 1.5 - 13.24 IU/cm^2, p = 0.028, n=10) and, although there was some recovery of activity, still significantly reduced after rewarming for 10 minutes (median 3.12 IU/cm^2, range 0.88 - 19.93 IU/cm^2, p = 0.047, n=10). The simultaneous plasma tPA activity (figure 23) showed a significant ($p < 0.05$) increase after 30 - 50 minutes bypass with a later decline.

A similar picture was observed in the reoperative pericardium. Compared with the initial PAA (median 2.78 IU/cm^2, range 2.64 - 8.26 IU/cm^2, n=5), the activity was not significantly reduced after 30 minutes of exposure (median 2.78 IU/cm^2, range 1.47 - 4.98 IU/cm^2, p = 0.23, n=5). However, the reoperative pericardial PAA after 30 - 50 minutes CPB was significantly reduced (median 1.00 IU/cm^2, range 0.78 - 1.83 IU/cm^2, p = 0.043, n=5) as was the activity after 10 minutes rewarming (median 1.66 IU/cm^2, range 0.3 - 4.7 IU/cm^2, p = 0.043, n=5).

The PAA was consistently lower in reoperative pericardium than in primary (figure 24). This difference only reached significance at the sample times B ($p < 0.01$) and C ($p < 0.01$). At every stage of sampling (times A, B, C, and D) the amount of tPA present in primary and reoperative pericardium (table 2) was not significantly different ($p > 0.05$). This was also true for the uPA. In addition the amount of uPA detected (table 3) in pericardium was consistently lower than that of tPA. In spite of the significant change in PAA, neither tPA nor uPA showed a significant quantitative change during the operative

period. In neither primary or reoperative pericardium could PAI-1 or PAI-2 be detected within the duration of CPB.

Figure 23. Box Plot of the plasma tPA activity during surgery against time period of sample

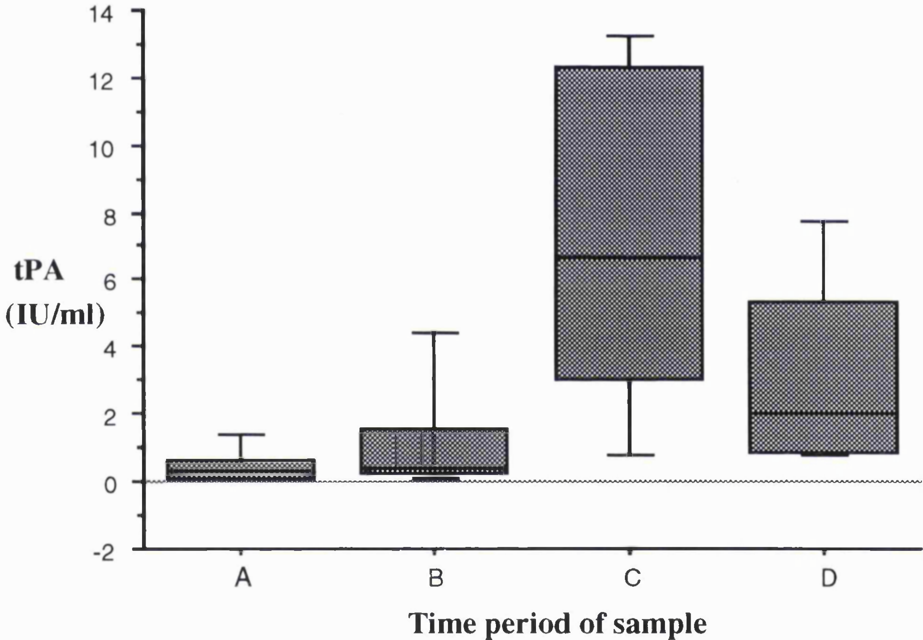


Figure 24. Box plot of primary and reoperative pericardial PAA during surgery against period of sample

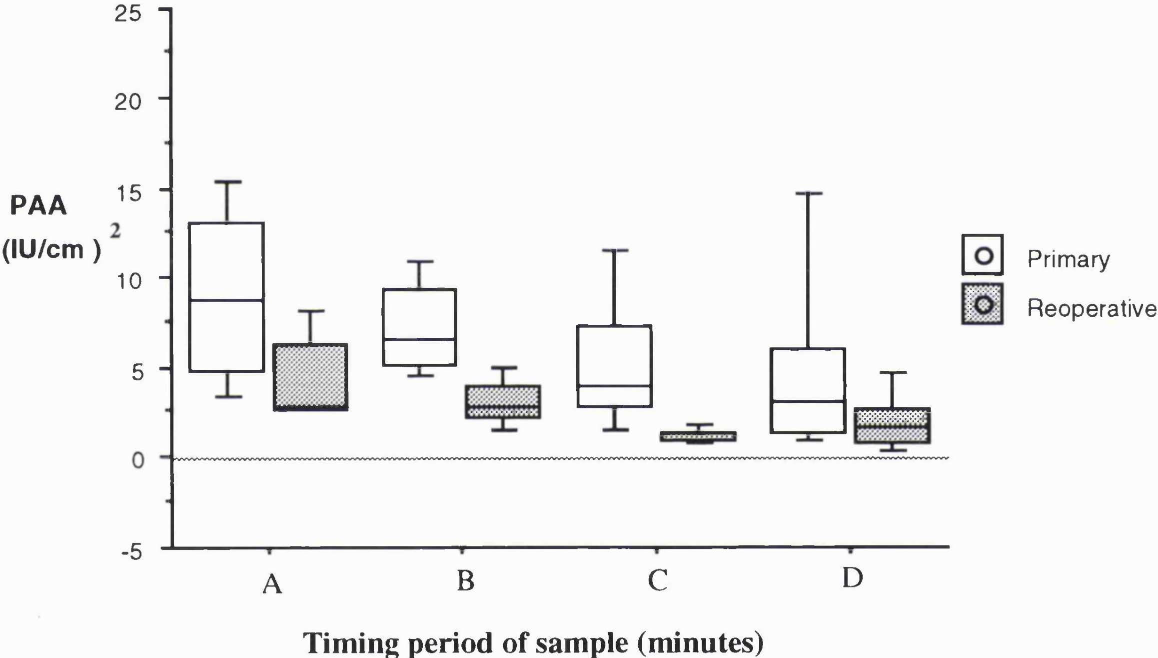


Table 2. Quantity of tPA (ng/ml) in primary and reoperative pericardium.

		Time period of sample			
Pericardium		A	B	C	D
Primary	Median	1.81	1.94	1.5	1.67
	Range	1.50 - 6.20	1.50 - 4.99	1.50 - 6.45	1.50 - 6.54
Reoperative	Median	2.54	2.20	1.91	2.30
	Range	1.75 - 3.10	1.88 - 2.70	1.74 - 2.96	1.50 - 3.32

Table 3. Quantity of uPA (ng/ml) in primary and reoperative pericardium.

		Time period of sample			
Pericardium		A	B	C	D
Primary	Median	0.62	0.46	0.52	0.49
	Range	0.17 - 1.12	0.22 - 1.42	0.21 - 1.92	0.25 - 1.95
Reoperative	Median	0.57	0.85	0.68	0.76
	Range	0.40 - 1.39	0.39 - 0.91	0.47 - 0.87	0.40 - 1.28

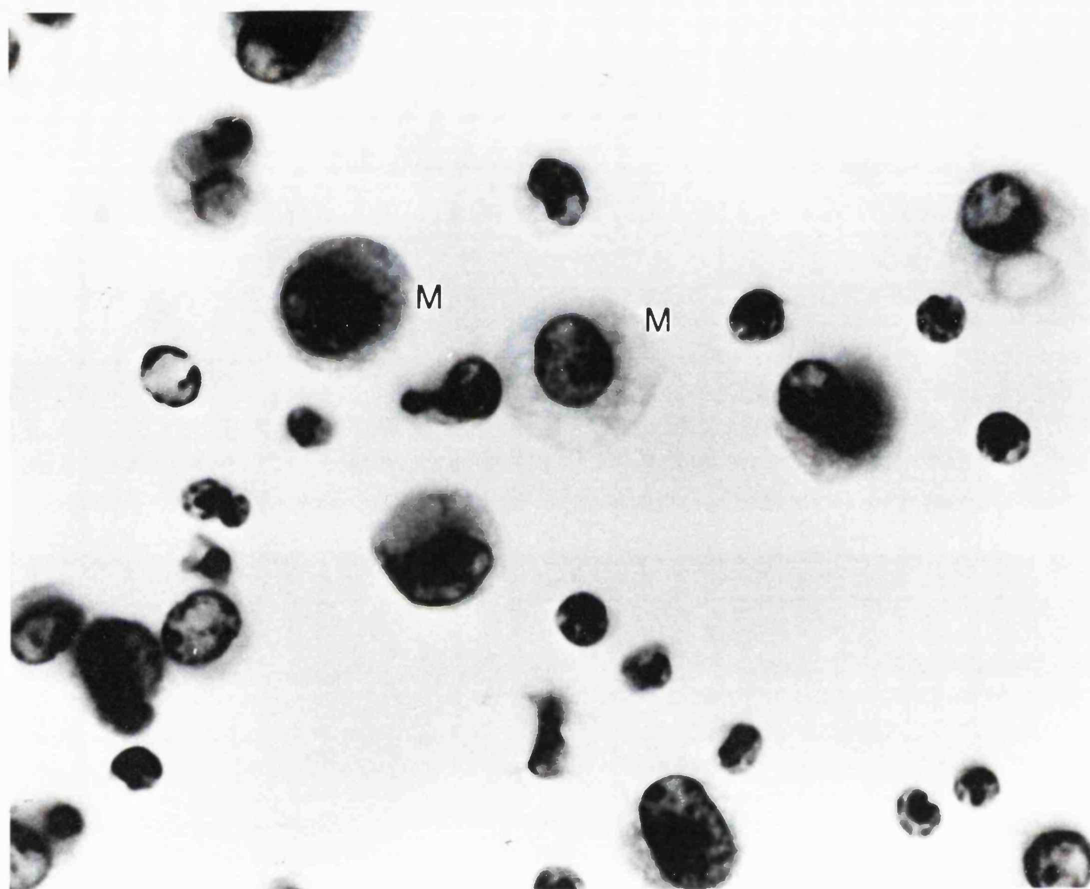
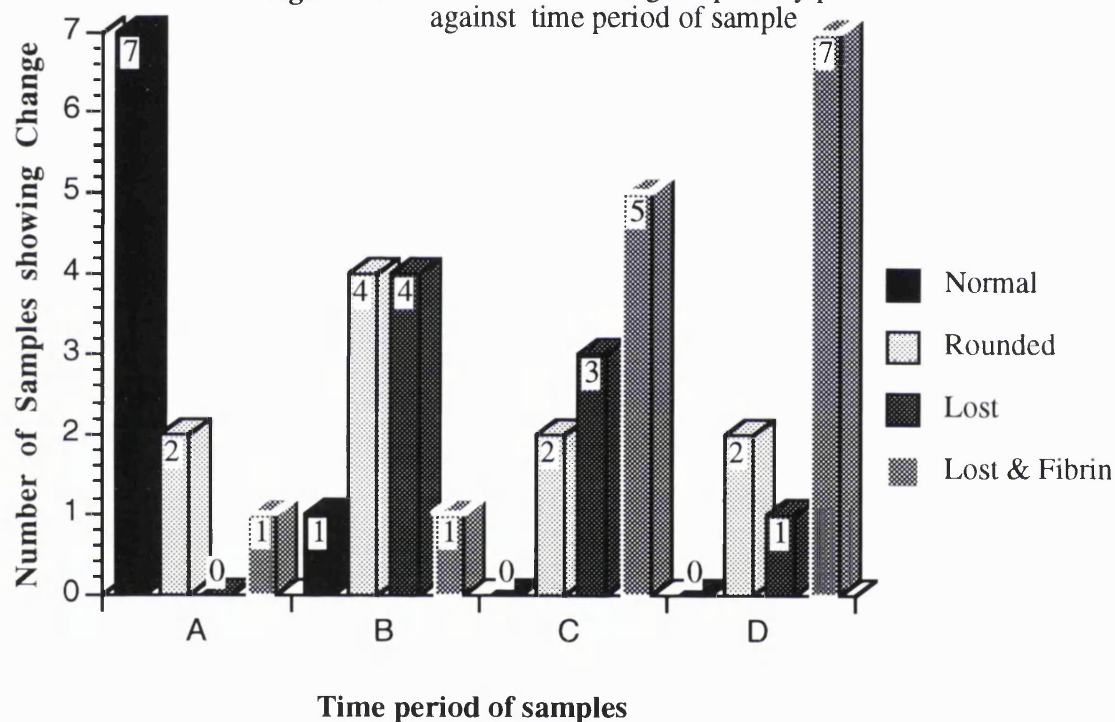


Figure 25. Cytological sample of pericardial fluid showing some of its cellular constituents. The principal cell type present (more than 75%) was the mesothelial cells (M).

Figure 26. Mesothelial damage in primary pericardium against time period of sample



Time period of samples

Figure 27. Mesothelial damage in reoperative pericardium against time period of sample

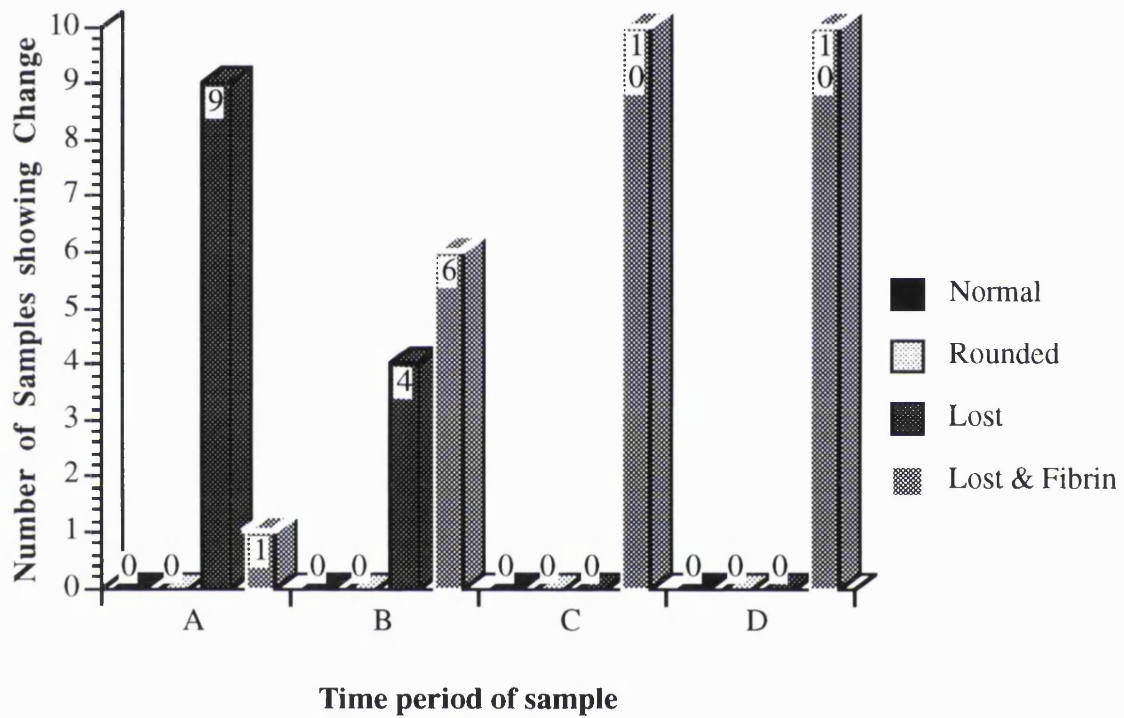


Table 4.	**Pericardial Fluid**		**Blood**	
	n = 5		n = 5	
	Median	Range	Median	Range
Sodium	136	(133 -138)	138.6	(137 -140)
Potassium	4.4	(3.9 - 4.9)	4.2	(3.6 - 5.1)
Bicarbonate	27.2	(25 - 29)	25	(23 - 28)
Urea	8.6	(5.3 - 53)	7.8	(4.8 - 10.2)
Creatinine	80	(61 - 148)	97	(62 -153)
Total Protein	24	(17 - 29)	75	(43 - 87)
Albumin	21	(14 - 25)	44	(27 - 51)
Calcium	1.63	(1.57 - 2.23)	2.4	(2.06 - 2/85)
Inorganic Phosphate	0.53	(0.28 - 0.9)	0.98	(0.81 - 1.34)
Total Bilirubin	6	(5 - 14)	12	(8 - 15)
Alkaline Phosphate	11	(6 - 16)	55	(34 - 107)
Aspartate Transaminase	24	(15 - 54)	42	(23 - 61)
Uric Acid	0.41	(0.3 - 0.5)	0.43	(0.27 - 0.63)

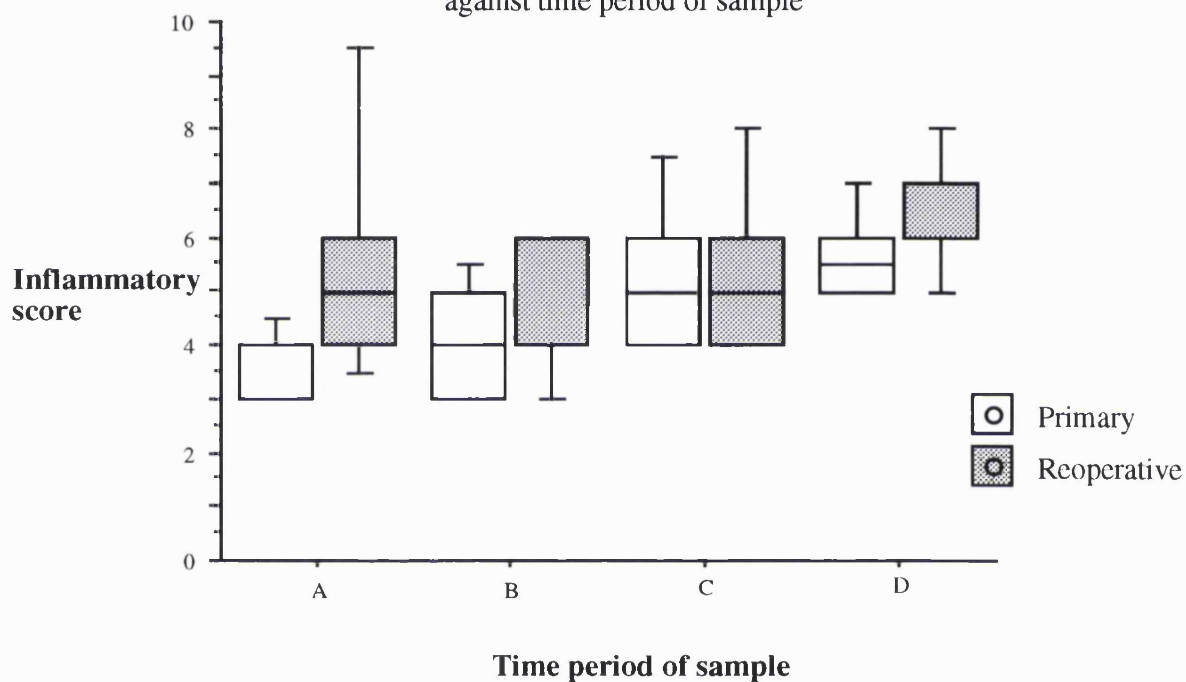
Five specimens of pericardial fluid taken at the time of coronary artery bypass surgery were examined in regards to their cytological and biochemical features. From the cytological point of view, each of the specimens showed similar features; the principal cell type present (more than 75%) was the mesothelial cell; also seen were a few macrophages, red blood cells, neutrophil granulocytes and an occasional small lymphocyte. The mesothelial cells in air dried giemsa stained preparations varied in diameter from 15 to 30 microns; the cells were separated and did not form aggregates or sheets. The cell surface was slightly blurred, due to the large number of long slender microvilli that cannot be resolved using the light microscope. Almost all the cells had moderate amounts of azurophilic cytoplasm which is rich in intermediate filaments. The nucleous was fairly large and some contained an inconspicuous nucleolus. The nuclear chromatin was finely dispersed throughout the nucleus. Small cytoplasmic vacuoles were present in some cases, often closely applied to the nuclear membrane forming a ring-like appearance (figure 25). The comparative biochemical profile of the pericardial fluid and the blood sampled at the same time is shown in table 4. Although quantities of tPA (median 2.5 ng/ml, range 1.19 - 8.49 ng/ml, n=15), uPA (median 0.92 ng/ml, range 0.56 - 1.81, n=15), and PAI-1 (median 4.87 ng/ml, range 2.5 - 15.03 ng/ml, n=15), were present in the pericardial fluid, only 3 of 15 samples had low levels of plasminogen activating activity (0.027, 0.01, and 0.36 IU/cm^2 respectively) in the other samples no such activity was detected. Only two of the pericardial fluid samples contained detectable quantities of PAI-2, (6.1 and 11.3 ng/ml respectively).

For each sampling period the histological findings of mesothelial damage and extent of pericardial inflammation in primary and reoperative pericardium are illustrated in figures 26 to 28. In some of the primary cases evidently at zero time there is already mild mesothelial damage and inflammatory changes in some of the ten samples. This can be explained by an inconstant degree of injury to the closed pericardium during the variable period between initial sternotomy, intramediastinal manipulation (e.g. dissection and handling of fat on the anterior pericardium, dissection of internal mammary), and subsequent pericardiotomy. The extent of mesothelial damage in the primary pericardium

at times B, C, and D (as represented by the damage score, figure 29) compared with that at time A shows a significant ($p < 0.01$ for times B, C and D) preponderance of pericardial tissue with either rounded mesothelial cells, mesothelial cell loss, or mesothelial cell loss with fibrin deposition. Similarly, for each of the inflammatory features, except oedema, there was a significant worsening of the degree of margination and infilteration. Therefore at time C and D there was a significant ($p < 0.05$) preponderance of primary pericardial samples with relatively high inflammatory scores.

Compared to the primary pericardial samples, the reoperative pericardium showed a significant ($p < 0.01$ for time A, B, and C) preponderance of damaged mesothelium at the earlier stages of the operation, time A, B, and C. However, as the extent of mesothelial damage in the primary group increased with time, this difference was no longer significant at time D. None of the early reoperative samples demonstrated a normal mesothelial surface, all had lost most of their mesothelial covering and had fibrin deposition on their surface. In addition, the reoperative fibrosal and epipericardial layers tended to contain more fibrous tissue than the equivalent primary pericardium.

Figure 28. Box plot of the inflammatory score against time period of sample



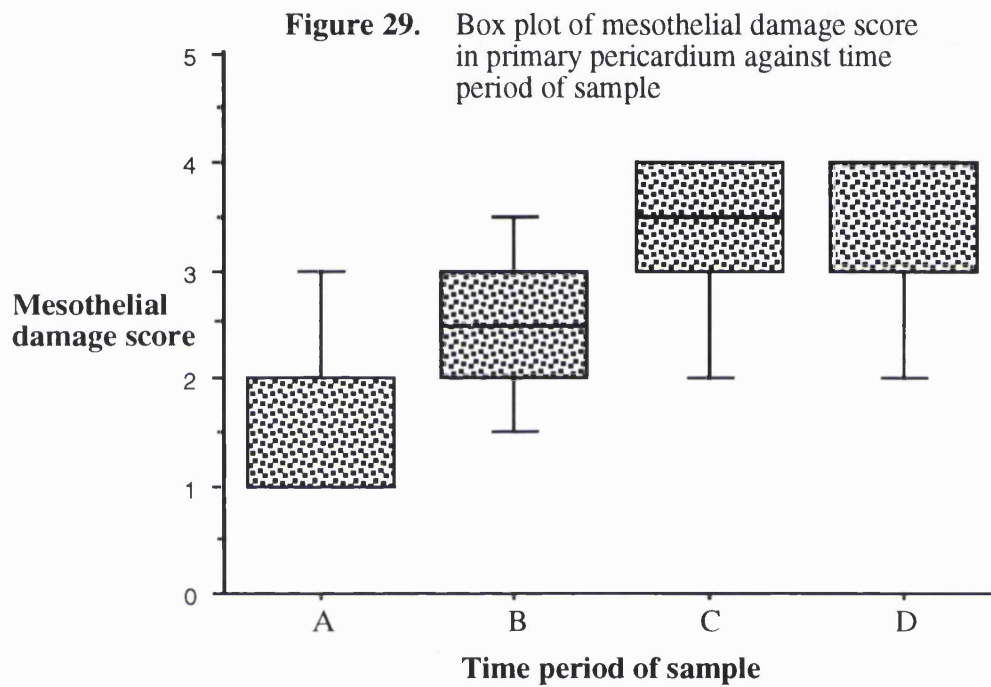


Figure 29. Box plot of mesothelial damage score in primary pericardium against time period of sample

Discussion

The pericardial mesothelium, because of its fibrinolytic activity, has the potential to remove the fibrinous adhesions that may serve as the initial scaffolding for the firm collagenous adhesions seen at reoperative surgery. We hypothesise therefore that pericardium has the actual or potential capacity to limit adhesion formation. However, because of surgical trauma and subsequent histological and ultrastructural derangement (Gervin, Jacobs et al., 1975; Porter, Ball et al., 1971) this activity is depressed thereby reducing its potential to reduce adhesion formation. The result is that early fibrinous adhesions become infiltrated by proliferating fibroblasts, followed by vascularization and cellular ingrowth. This resultant granulation tissue is subsequently mature into permanent adhesion formation (Holtz, 1984).

Our results have already confirmed that there is an increasing morphological destruction of the pericardium with concomitant loss of fibrinolytic activity (as measured by the PAA). This fall in pericardial PAA is not a reflection of plasma levels of PAA for, as the results show, the plasma tPA activity rises to a peak at the same time as the pericardial PAA falls to its trough. The manner and degree of pericardial recovery that occurs during the postoperative and subsequent period is not known but can be inferred by inspection and assessment of reoperative pericardium in terms of its histological and fibrinolytic activity.

In reoperative pericardium the histological features, as one might expect, start off in a worse state being practically void of normal mesothelial covering, possessing increased interstitial fibrosis and a relatively low PAA per unit of pericardial tissue. Bearing in mind the potential for mesothelial regeneration (Malm, Bowald et al., 1992b; Raftery, 1979) and looking at our apparent late recovery of PAA in primary pericardial tissue one might expect a normal or near normal PAA at reoperation. However, it appears that the processes that cause pericardial and mesothelial healing with recovery of PAA (the preferred outcome) compete with those leading to pericardial adhesions and fibrosis

(which cause the complications). The histological and biochemical outcome seen in reoperative pericardium are the result of these competitive actions.

As has been mentioned in the previous discussion, the process of mesothelial recovery begins within 24 hours of pericardial insult and is completed by 8-10 days (Whitaker and Papadimitriou, 1985). Whether these new mesothelial cells arise from the replication and migration of adjacent mesothelial cells, or the pre-existing free-floating serosal cells (which we found to constitute about 75% of the cells in pericardial fluid) or the migration of underlying connective tissue cells to the denuded surface is something yet to be confirmed. However, if fibrin and fibrinous adhesions are not removed by the fibrinolytic activity of the mesothelial cells (which is maximally present in normal pericardium but deficient in injured and reoperative pericardium) and the pericardial exudate is not absorbed, tissue organisation will begin and by 21 days, although further fibrosis will occur, adhesions are histologically established (Johnson and Whitting, 1962; Milligan and Raftery, 1974). In addition, it may be that pericardial fibrosis, which we have noted to be more extensive in reoperative pericardium, compromise the blood supply to the mesothelial surface thereby further interfering with its regenerative recovery and return of PAA.

Therefore, it appears that the competitive balance between regenerative processes and tissue organisation is tipped in favour of the latter. However, it may be possible to avert this tendency to organisation by incorporating a fibrinolytic agent into an absorbable pericardial substitute that will delay the organisational process long enough for mesothelial regeneration to be established. At every sampling stage, for both primary and reoperative pericardium, the plasminogen activator present in greatest quantities was tPA rather than uPA. The possibility is there for tPA to be incorporated into such an absorbable pericardial patch.

The role of pericardial fluid in the prevention of adhesions is open to speculation. Most of the fluid samples analysed showed no PAA, but because of its tPA and uPA content it has at least the potential to prevent adhesions forming. The PAA of pericardial fluid is apparently kept in check by the presence of PAI-1 and PAI-2. The mechanism whereby

the inhibitory affects of PAI-1 and PAI-2 are removed has not been demonstrated here. Nevertheless, the absence of pericardial fluid in the postoperative period (and therefore the absence of its potential PAA and lubricating affect) may be another factor that encourages adhesion formation.

An attempt has been made to elucidate the potential role of the pericardium and, to some extent, the pericardial fluid in the prevention of post surgical adhesions. With a better understanding of the changes that take place in the pericardium it may be possible to avert or at least minimise the tendency to adhesion formation with a pericardial substitute that will promote pericardial regeneration following CPB surgery.

CHAPTER 7

PERICARDIAL SUBSTITUTION AFTER CARDIOPULMONARY BYPASS SURGERY: A TRIAL OF AN ABSORBABLE PATCH

(Phase 3: Animal studies)

Summary

Primary closure of the pericardium affords some protection against adhesion formation and the consequent hazards of resternotomy, but its completion is not always practical therefore the pursuit of an ideal pericardial substitute has prompted much research. The pericardial mesothelium has fibrinolytic activity and regenerative potential. These features of the pericardium may have been underestimated in much of the previous research into pericardial substitution. Twenty calves undergoing surgery, were divided into 3 groups of study. The test group (group A), consisting of 6 females, median weight 47.5 kg (range 39 to 60 kg), received a poly-ß-hydroxybutyrate patch (PHB) following cardiopulmonary bypass (CPB). The first control group (group B) consisted of 9 female animals, median weight 46 kg (range 35-56 kg) in whom the pericardium was left open following CPB. The second control group (group C) also had their pericardium left open and consisted of 5 female animals, median weight 45 kg (range 39.5 to 60 kg). The group C animals did not undergo CPB. The surgery required that the heart and pericardium be handled and manipulated in a manner equivalent to that which occurs in clinical patients. The plasminogen activating activity (PAA) of homogenates of pericardial tissue samples were measured in 5 animals in group A, and 5 in group C. Samples were taken at times 0, 60, and 90 minutes from the time of pericardiotomy, and then again at reoperation 4 weeks later. In group A there was a significant reduction in the PAA during the operation. Compared with the activity at 0 time (median 0.088 IU/cm^2, range 0.055 to 0.214 IU/cm^2) there was a significant reduction in the PAA at 60 minutes (median 0.046 IU/cm^2, range 0.044 to 0.05 IU/cm^2, $p < 0.05$) and at 90 minutes (median 0.043 IU/cm^2, 0.044 to 0.048 IU/cm^2, $p < 0.05$). However, by the time of reoperation there was some recovery (median 0.061 IU/cm^2, range 0.045 to 0.102 IU/cm^2, $p > 0.05$). The reduction in the pericardial PAA of group C animals did not reach significance. For both the CPB and non-CPB samples the progress of mesothelial damage, compared with that at zero time, showed a significant preponderance of pericardial tissue with a high degree of mesothelial damage at times 60 minutes, 90 minutes and at reoperation ($p < 0.05$ at each time point). In both the CPB and non CPB animals the inflammatory features of the pericardium became more apparent in the later samples but more significantly in group C.

No significant difference in adhesion formation, and postoperative coronary anatomy visibility was found between any of the groups. At reoperation the patch material did not possess any regenerative mesothelium but there was pronounced macrophage activity within the patch material. There were no infective episodes in any of the animals studied. The patch material conferred no obvious short term reoperative advantage in terms of adhesion formation and coronary anatomy visibility. Despite these results, the combination of these results and that of other workers suggests that what is required is a device or agent that will delay the overwhelming tendency to adhesion formation long enough for mesothelial regeneration, and therefore fibrinolytic activity, to re-establish itself following CPB surgery.

Introduction

In phase one of this study an attempt was made to obtain a greater understanding of the basic morphological and biochemical changes that take place in the pericardium during and in response to cardiac surgery. In phase two the idea was to build upon the foundation of phase one by: 1. comparing the response of primary and reoperative pericardium, 2. examining the biochemical nature of pericardial fluid, 3. examining the change in the plasma PAA activity in conjunction with the changes in pericardial PAA. The information enabled the formation of a working hypothesis that might explain the changes that occur within the pericardium following primary surgery, elucidate the potential role of the pericardium, and to some extent the pericardial fluid, in the prevention of post surgical adhesions.

It appears that although pericardium has the inherent potential to minimise adhesion formation this potential is overwhelmed by a deficiency of pericardial covering and a milieu that would tend toward fibrosis and adhesions formation. With a better understanding of the changes that take place in the pericardium it may become possible to avert or at least minimise the tendency to adhesion formation with a pericardial substitute that incorporates the inherent features of the pericardium by promoting pericardial regeneration onto its structure.

The use of the fibrinolytic activity (Gervin, Jacobs et al., 1975; Porter, Ball et al., 1971) and regenerative potential of pericardium is illustrated in the study by Malm et al (Malm, Bowald et al., 1992a; Malm, Bowald et al., 1992b). They demonstrated the "prevention of postoperative pericardial adhesions by closure of the pericardium with absorbable polymer patches". This patch, made of polyhydroxybutyrate (PHB), supported the regeneration of pericardium and pericardial mesothelium in particular. However, the work did not include the use of cardiopulmonary bypass (CPB) which, causes additional insult to the pericardium (Gabbay, 1990; Nkere et al, 1993).

Although primary closure of the pericardium affords some protection against adhesion formation (Cunningham, Spencer et al., 1975; Nandi, Leung et al., 1976) its completion

is not always practical and indeed can be hazardous because of the risk of graft kinking and cardiac tamponade (Engelman and Spencer, 1970). Faced with the problem of an inadequate amount of pericardium to provide an unrestrictive closure, it is not surprising that numerous pericardial substitutes have arisen (Gabbay, Guindy et al., 1989) and a number of techniques for closing the pericardium (Milgalter, Uretzky et al., 1985; Zapolanski, Fishman et al., 1990). The results of these materials have been good in the animal studies (Heydorn, Daniel et al., 1987; Rhodes, Brandon et al., 1989) but the long term clinical outcome, has been disappointing (Eng, Ravichandran et al., 1989; Gallo, Artinano et al., 1985).

It is with this background that we have undertaken this phase of the study to produce an animal model of the clinical situation. The model would produce equivalent morphological and biochemical pericardial changes to those seen clinically. In this study all the calves were subjected to cardiac instrumentation and manipulations. In addition, some were subjected to CPB. Polyhydroxybutyrate (described in more detail in chapter 4, Patients, Materials & Methods) was used as an absorbable pericardial patch. The absorbable patch was implanted following bypass surgery, and its effects on adhesion formation, infectivity, preservation of the visibility of the coronary anatomy and pericardial regeneration were investigated.

Summary of Materials and Methods for Phase Three

Twenty calves, were divided into 3 groups of study. The test group (group A) consisted of 6 females, median weight 47.5 kg (range 39 to 60 kg) receiving a PHB pericardial patch. The first control group (group B) consisted of 9 female animals, median weight 46 kg (range 35-56 kg) in whom the pericardium was left well open. The second control group (group C) also had their pericardium left open and consisted of 5 female animals, median weight 45 kg (range 39.5 to 60 kg). The group C animals did not undergo CPB.

Group A and B underwent an operative procedure under cardiopulmonary bypass. For group A, the median cross-clamp time was 26 minutes (range 19 to 39 minutes, n = 6) and the median perfusion time was 58.5 minutes (range 45 to 120 minutes, n = 6). For group B, the median cross-clamp time was 33 minutes (range 17 to 41 minutes, n = 9) and the median perfusion time was 62 minutes (range 32 to 175 minutes, n = 9). The variation in cross-clamp and perfusion time is attributed to the variable ease of the procedure and recovery of the heart following cardiopulmonary bypass. However, there was no significant difference between the two groups (A & B) in the cross-clamp time, $p > 0.05$, or perfusion time, $p > 0.05$. In the group C animals the median pericardial exposure time was 60 minutes (range 45 to 87 minutes, n = 5).

Groups A and B underwent the same procedure under cardiopulmonary bypass, without significant differences in the cross-clamp and cardiopulmonary bypass time. The assumption made is that the similar pericardial exposure of groups A and B would produce equivalent pericardial changes in them. Therefore, groups A and B were not compared for the extent of their mesothelial damage, pericardial inflammation and change in plasminogen activating activity. Group B acted as a control to compare the extent and degree of adhesion formation, infectivity and preservation of the visibility of the coronary anatomy with that of the study group A and the non-CPB control group C.

Following successful completion of the procedure the animal was rewarmed to 39^0C. Having come off bypass de-cannulation was completed, protamine administered, and haemostasis secured. In group A the pericardial defect (average size 8 x 12 cm) was

closed by inserting a patch secured with a 5.0 Prolene running suture. The chest was closed over a single 36F drain placed in the pericardial cavity through a small defect in the native pericardium. In group B and C the chest was closed in a like manner, but without the patch.

Only groups A and C were sampled and compared for changes in for change in morphological and biochemical features. The pericardium was incised parallel and anterior to the phrenic nerve. Pericardial biopsies were taken using a disposable, 6 mm diameter biopsy punch. The samples were obtained from the non-diaphragmatic sides of the open pericardium, all within 5 cm of its cut edges. Samples were taken at times 0, 60, and 90 minutes from the time of pericardiotomy, and then again at reoperation 4 weeks later. For convenience these times were also designated letters A, B, C, and D respectively. Two samples that would be used for assays were then wrapped in aluminium foil and snap frozen before transfer to a storage freezer at -20^0 C. Samples for electron microscopic (EM) studies were placed in 2% glutaraldehyde. Those for histological analysis were fixed in 10% buffered formalin.

The following are elaborated in chapter 4:

1. Polyhydroxybutyrate Pericardial Patch.
2. Anaesthesia and Operative Procedure .
3. Pericardial biopsy technique.
4. Preparation of pericardial homogenates.
5. Assay For Plasminogen Activating Activity (PAA).
6. Macroscopic evaluation.
7. Histological evaluation.
8. Transmission and scanning electron microscopic studies.

Results

The PAA was measured and compared in 5 of the animals that had undergone CPB (group A) and in 5 that had not undergone CPB (group C). The reduction in the pericardial PAA in response to surgery was consistently greater in the CPB group of animals (figure 30). The resultant difference between the two groups only reached significance at 60 minutes ($p < 0.05$) and 90 minutes ($p < 0.05$). At reoperation there was no significant difference between the PAA of the two groups. In group A there was a significant reduction in the PAA during the operation. Compared with the activity at 0 time (median 0.088 IU/cm^2, range 0.055 to 0.214 IU/cm^2) there was a significant reduction in the PAA at 60 minutes (median 0.046 IU/cm^2, range 0.044 to 0.05 IU/cm^2, $p < 0.05$) and at 90 minutes (median 0.043 IU/cm^2, 0.044 to 0.048 IU/cm^2, $p < 0.05$). However, by the time of reoperation there was some recovery (median 0.061 IU/cm^2, range 0.045 to 0.102 IU/cm^2) such that the observed reduction in the PAA compared with the initial value was no longer significant $p > 0.05$.

In those animals not subjected to CPB (group C), the reduction in the initial PAA (median 0.119 IU/cm^2, range 0.051 to 0.252 IU/cm^2) did not reach significance at 60 minutes (median 0.065 IU/cm^2, range 0.054 to 0.153 IU/cm^2, $p > 0.05$) nor at 90 minutes (median 0.58 IU/cm^2, 0.051 to 0.126 IU/cm^2, $p > 0.05$). However, as in group A (the CPB group), at the time of reoperation there was some recovery (median 0.085 IU/cm^2, range 0.052 to 0.137 IU/cm^2). The reduction in the PAA remained insignificant in comparison with the initial value, $p > 0.05$.

The histological finding of increasing mesothelial damage with time is graphically illustrated in figures 31 and 32. These show clear evidence of increasing mesothelial damage in both the CPB(group A) and non CPB (group C) animals respectively. At zero time it is noticeable that there is already evidence of mesothelial damage in one of the CPB samples. This can be explained by an inconstant degree of injury to the closed pericardium during the variable period between initial thoracotomy, intra-thoracic manipulation, and subsequent pericardiotomy. In both these groups the samples showed a progress of mesothelial damage. Compared with that at zero time, there was a

significant preponderance of pericardial tissue with a high score for mesothelial damage (table 5) at times 60 minutes, 90 minutes and at reoperation ($p < 0.05$ at each time point). Nevertheless, for each sampling time there was no significant difference between the mesothelial damage scores of the two groups. Figures 33 and 34 shows the appearance of early, mid, and late mesothelial surface damage as seen by scanning electron microscopy. Figure 35 is a transmission electron micrograph that shows a mesothelial cell becoming detached from the underlying basal laminar and fibrosal connective tissue layer.

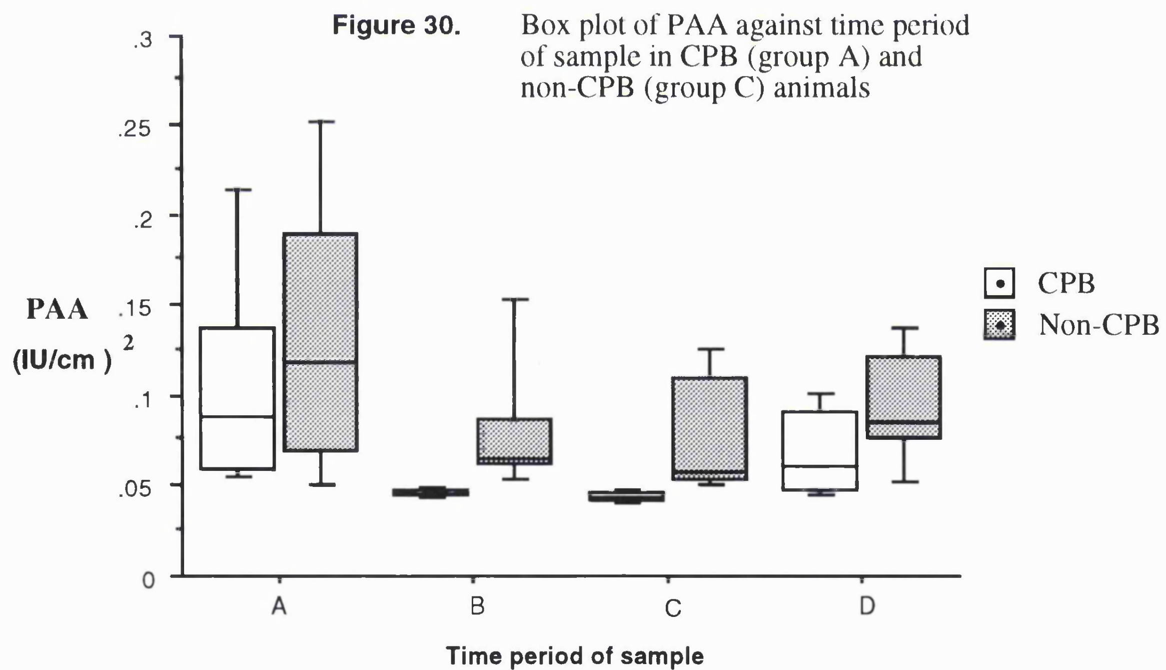


Figure 30. Box plot of PAA against time period of sample in CPB (group A) and non-CPB (group C) animals

Time period of sample: A = 0 minutes at pericardiotomy, B = 60 minutes after pericardiotomy, C = 90 minutes after pericardiotomy, D = Reoperation at 4 weeks.

Table 5. Showing mesothelial damage score for the CPB (group A) and non-CPB (group C) animals.

Number of samples for each sampling time with a particular mesothelial damage score

Score	Time 0		Time 60		Time 90		Reoperation	
	CPB	NoCPB	CPB	NoCPB	CPB	NoCPB	CPB	NoCPB
1	5	5	0	0	0	0	0	0
2	1	1	2	5	0	2	0	0
3	0	0	2	1	2	1	3	5
4	0	0	2	0	4	3	3	1
Total	6	6	6	6	6	6	6	6

Time period of sample: 0 minutes at pericardiotomy, 60 minutes after pericardiotomy, 90 minutes after pericardiotomy, Reoperation at 4 weeks.

Scoring of mesothelial damage was as follows: predominantly normal mesothelium = 1, predominantly rounded mesothelial covering = 2, mesothelium stripped from pericardial surface = 3, mesothelium stripped from pericardial surface with fibrin deposition = 4.

For each sampling time there was no significant difference between the mesothelial damage scores of the two groups, $p > 0.05$.

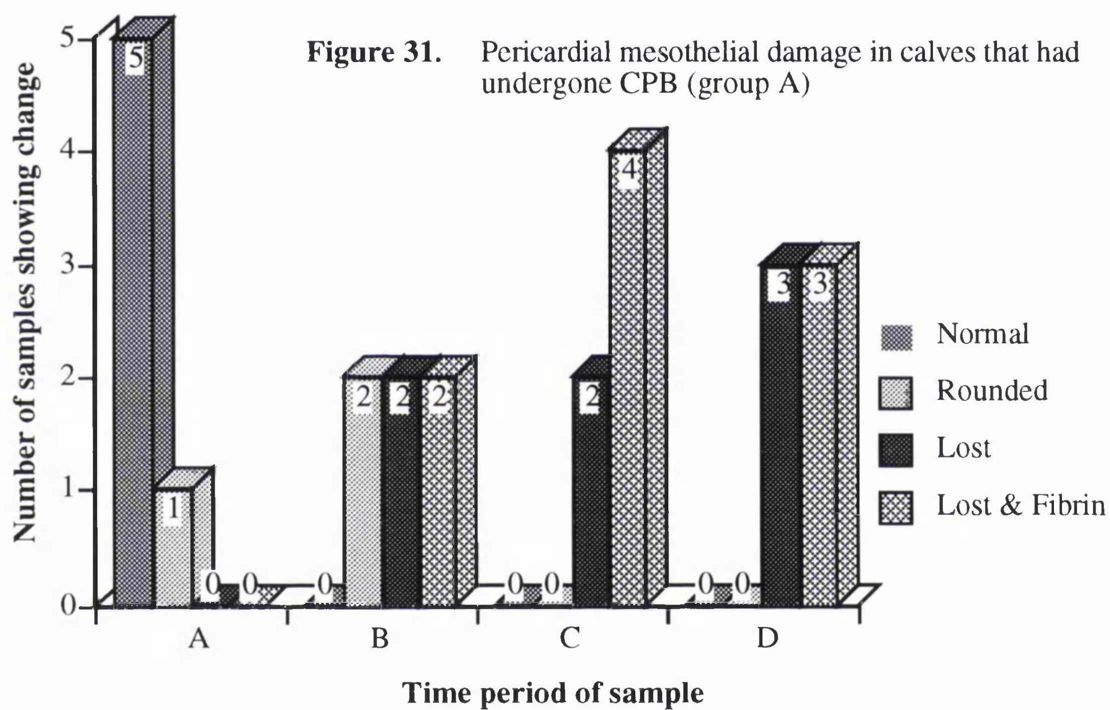
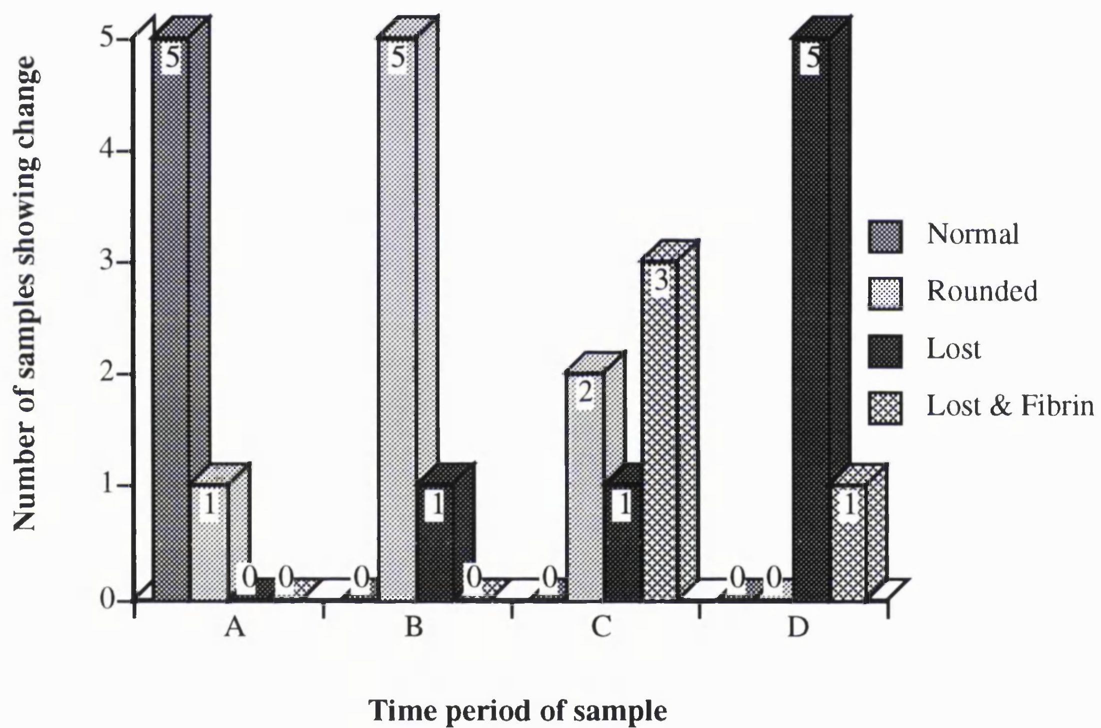


Figure 31. Pericardial mesothelial damage in calves that had undergone CPB (group A)

Time period of sample: A = 0 minutes at pericardiotomy, B = 60 minutes after pericardiotomy, C = 90 minutes after pericardiotomy, D = Reoperation at 4 weeks.

Figure 32. Pericardial mesothelial damage in calves that had not undergone CPB (group C)



Time period of sample: A = 0 minutes at pericardiotomy, B = 60 minutes after pericardiotomy, C = 90 minutes after pericardiotomy, D = Reoperation at 4 weeks.

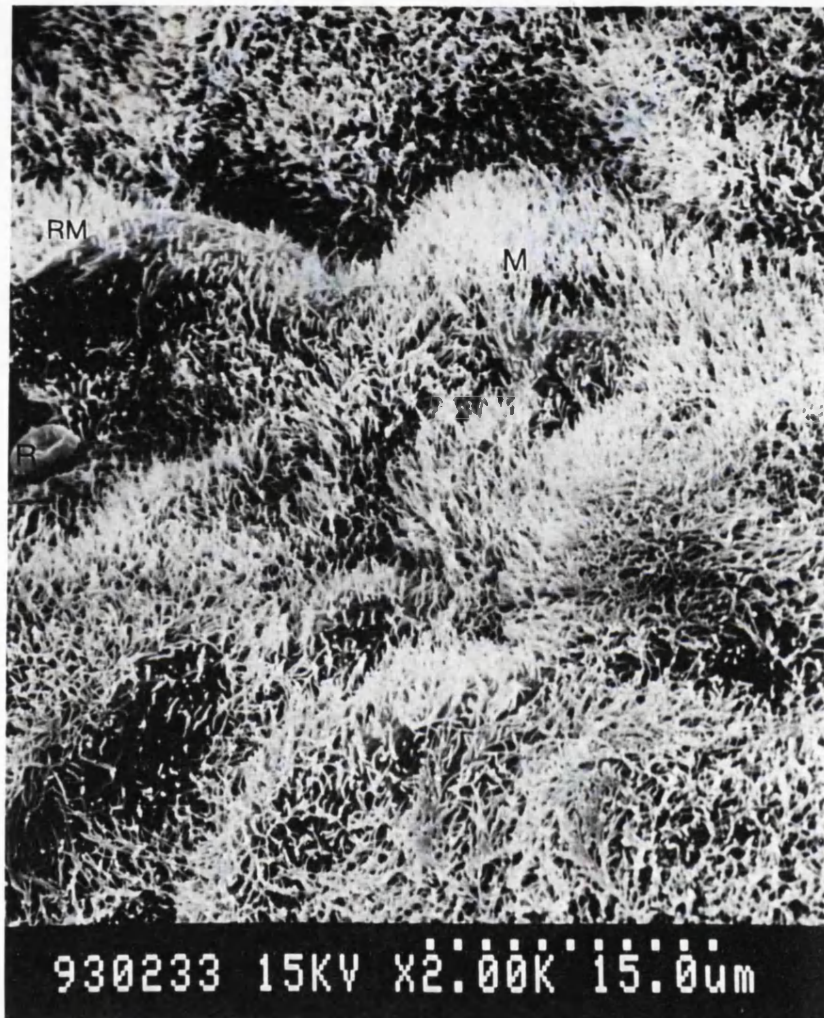


Figure 33. Scanning electron micrograph. Serosal surface covered with mesothelial cells (M). The surfaces of the cells are covered with numerous microvilli (which appear as white specks). Some mesothelial cells (RM) were beginning to round off and loss their microvilli. There is also a red blood cell (R) present on the surface of the mesothelial cells.

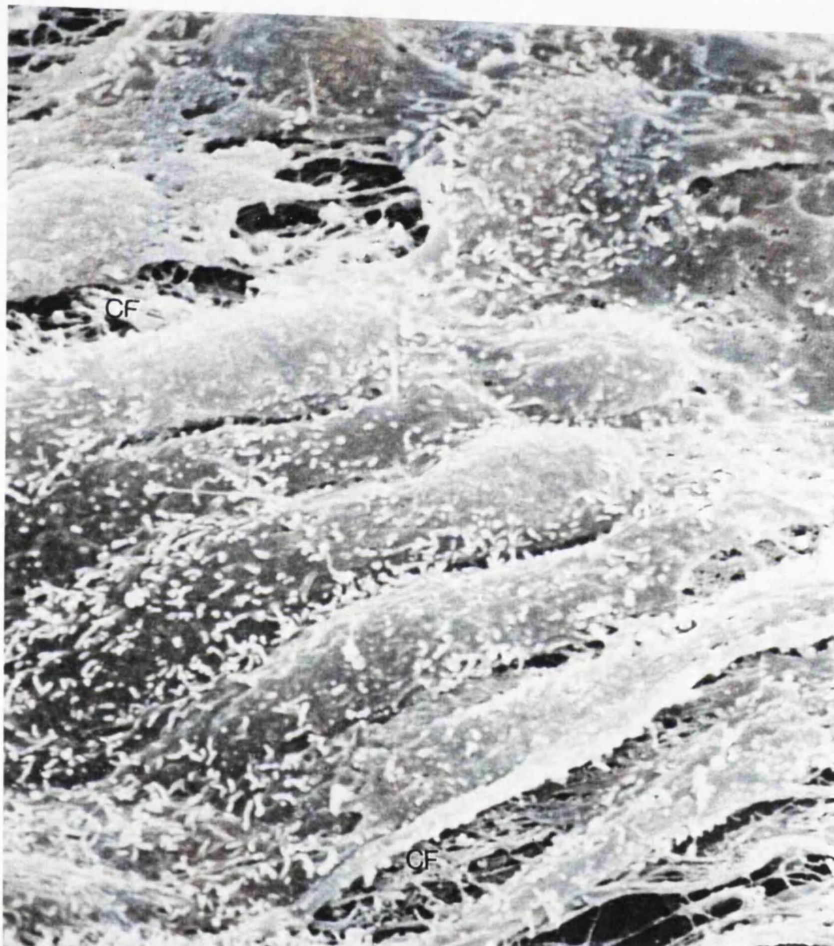


Figure 34. Scanning electron micrograph. This micrograph depicts the difference between a denuded area exposing the collagen fibrils (CF) of the fibrosal layer and the surviving microvillous appearance of the mesothelium on the upper right.

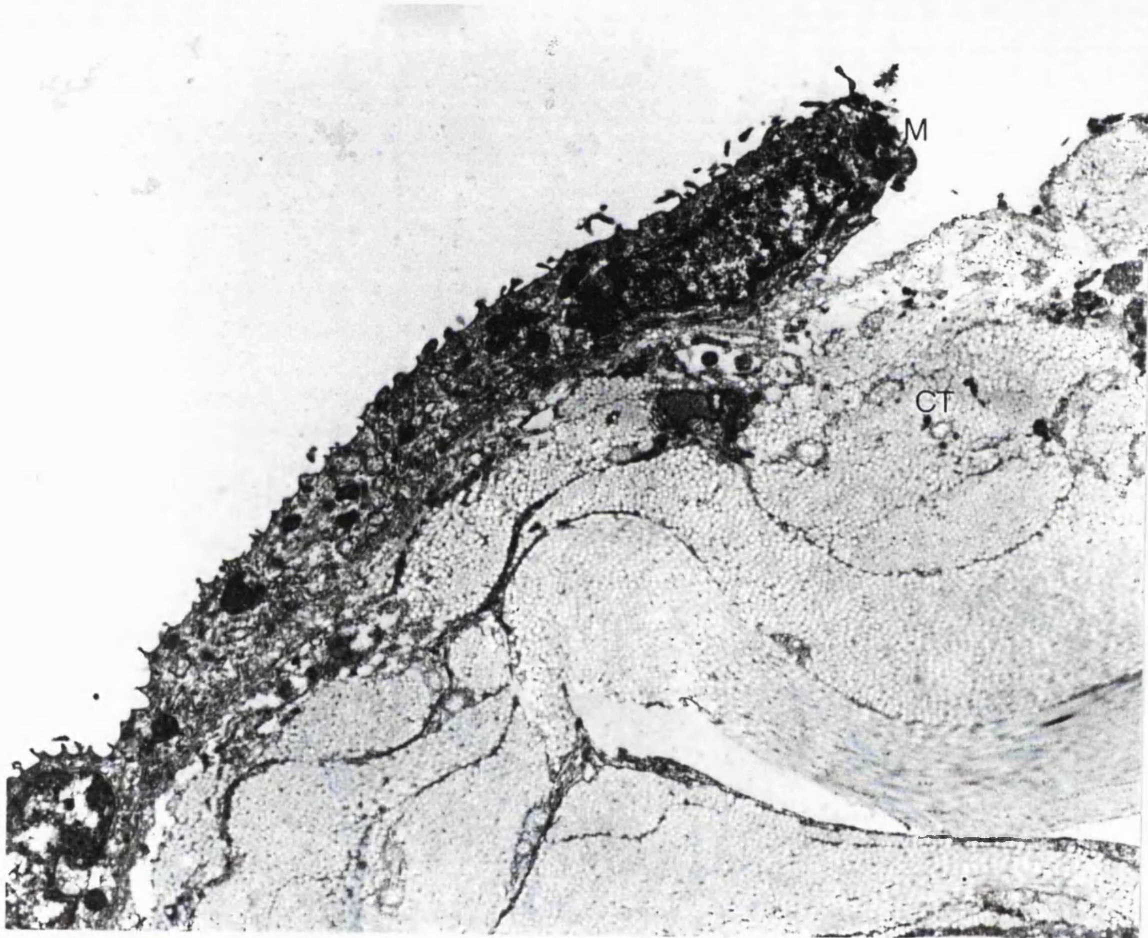


Figure 35. Transmission electron micrograph that shows a mesothelial cell (M) becoming detached from the underlying basal laminar and fibrosal connective tissue layer (CT).

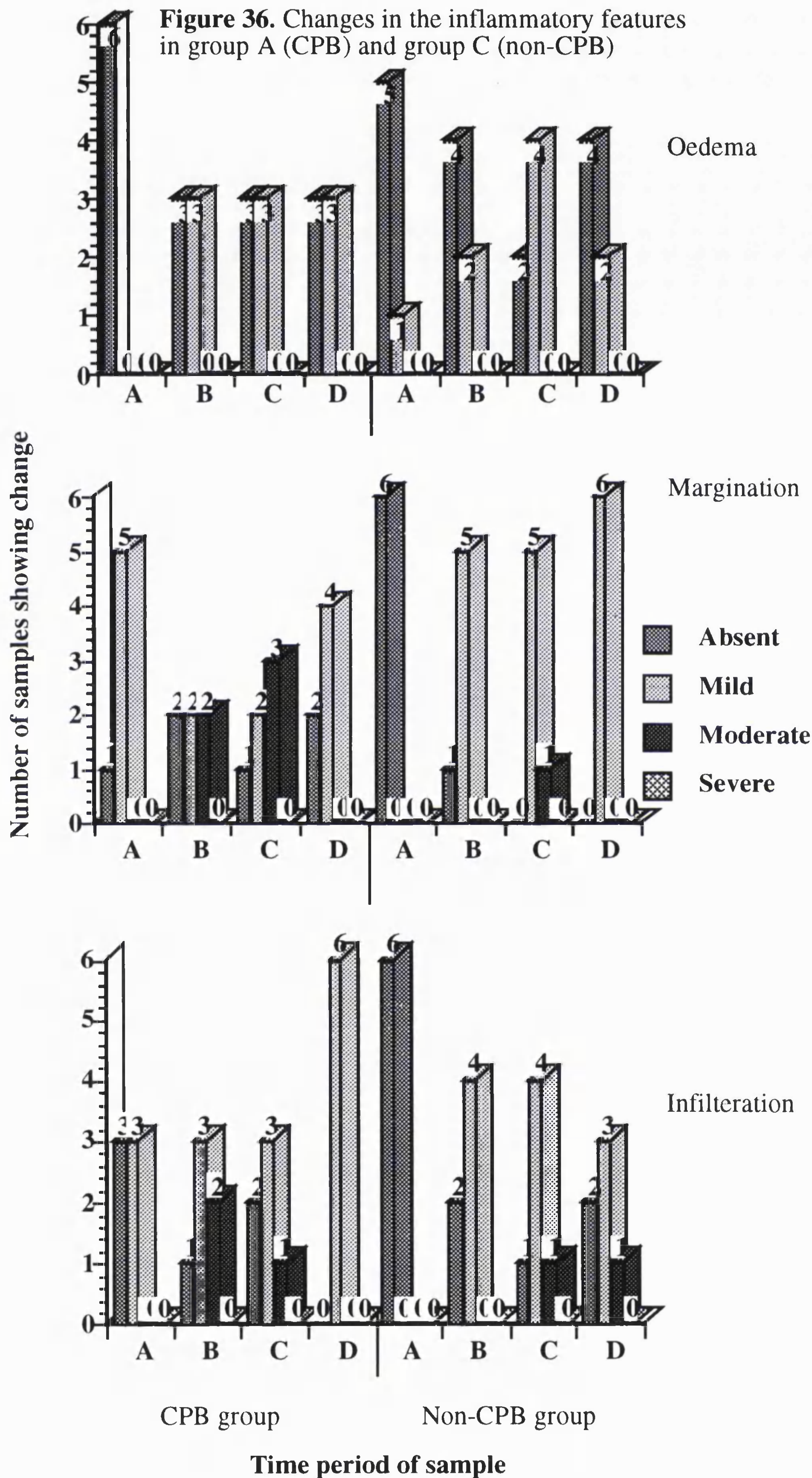


Figure 36. Changes in the inflammatory features in group A (CPB) and group C (non-CPB)

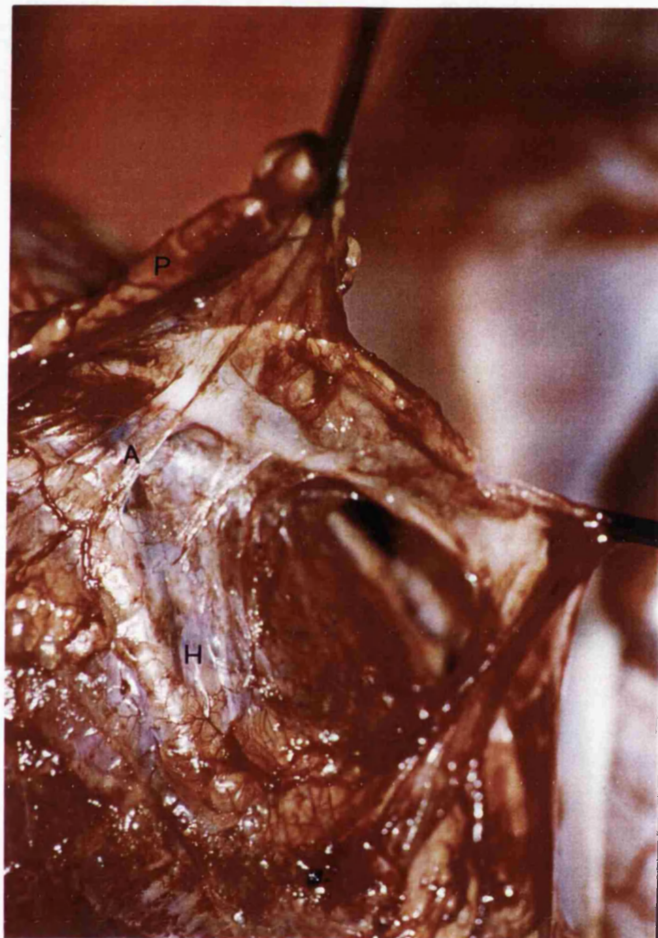


Figure 37. Postoperative pericardial adhesions (A) between the heart (H) and native pericardium (P) which is being held up with surgical instruments.

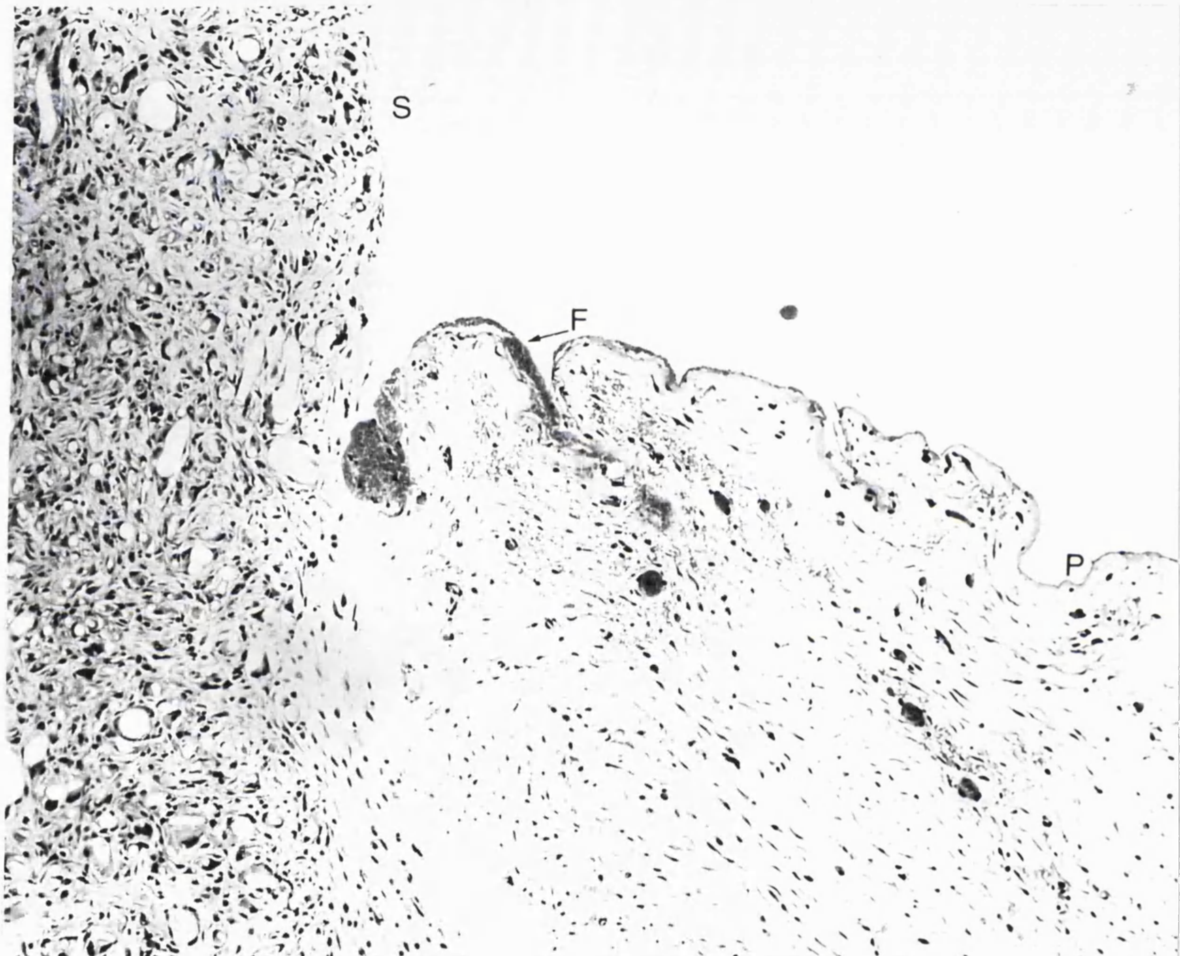


Figure 38. Reoperative specimen showing pericardial substitute (S) and denuded native pericardium (P) covered by a thin layer of fibrin (F). The substitute material shows a marked giant cell infilteration.

In both the CPB(group A) and non-CPB (group C) animals the inflammatory features (oedema, margination, and infilteration) of the pericardium became more apparent in the later samples (figure 36). In the CPB animals the changes in the score of the inflammatory features did not reach statistical significance. However, in the non-CPB animals there was a significant increase in the degree of margination at times 60 minutes ($p < 0.05$), 90 minutes ($p < 0.05$), and at reoperation ($p < 0.05$) compared with that seen at 0 time. Furthermore, the samples from the non-CPB animals showed a significant predominance of infilteration at 90 minutes.

The pericardial cavities of all those animals that had undergone CPB (both group A and B) were evaluated for adhesion formation between: the patch and heart, the patch and the chest wall, the heart and native pericardium, and the heart and the chest wall (in those group B animals without a patch, table 6). The visibility of the coronary anatomy in the areas of: the patch, the native pericardium, and the area of the exposed heart (in those animals without a patch, table 7) was also assessed and scored. A similar evaluation was made for those 5 group C animals that did not undergo CPB nor receive a patch. No significant difference in adhesion formation, and postoperative coronary anatomy visibility was found between any of the group comparisons.

Even after four weeks the macroscopic postoperative adhesions were quite advanced (figure 37). Histologically, the patch material was firmly adherent to the underlying heart and overlying pleura being effectively sandwiched between the two. The patch material did not possess any regenerative mesothelium. There was pronounced macrophage activity associated with the patch material (figure 38). The function of these macrophages appeared to be removal and degradation of the patch.

There were no infective episodes in any of the animals studied. In particular, in those receiving a patch there was no incidence of local or systemic infection.

Table 6. Showing the severity of adhesions and the resultant score for each group.

		Adjacent areas between which adhesions were assessed at reoperation and number of animals per group with a particular adhesion score								
Severity of adhesions	Adhesion score	H-P or H-CW			P-CW or H-CW			H-NP		
		Group			Group			Group		
		A	B	C	A	B	C	A	B	C
Nil	1	0	0	0	0	0	0	0	0	0
Mild	2	1	4	0	0	4	0	4	6	4
Moderate	3	3	4	3	6	4	3	2	3	1
Severe	4	2	1	2	0	1	2	0	0	0

Group A, n = 6, Group B, n = 9, Group C, n = 5.

Adhesion score: 1 = no adhesions, 2 = mild, could be dissected bluntly, 3 = moderate, a need for some blunt dissection, 4 = severe, unable to liberate without sharp dissection. H-P = Heart to Patch, H-CW = Heart to Chest Wall, P-CW = Patch to Chest Wall, H-NP = Heart to Native Pericardium.

Table 7. Showing the coronary anatomy visibility and the resultant score.

		Coronary anatomy visibility score in the area indicated and number of animals per group with a particular score					
		Area of patch/area of open pericardium			Beneath area of native pericardium		
Visibility	Visibility score	A	B	C	A	B	C
Clear	1	0	0	1	0	5	2
Blurred	2	0	4	1	6	3	3
Obscure	3	6	5	2	0	1	0
Covered	4	0	0	1	0	0	0

Group A, n = 6, Group B, n = 9, Group C, n = 5.

Coronary anatomy visibility score: 1 = clear, 2 = blurred, vessels can be visualised easily but, owing to inflammatory changes, not as clearly as the non operative heart, 3 = obscure, vessels only detected after careful inspection of the heart's surface, 4 = covered, vessels only visualised by removal of adhesions and/or adherent tissue.

There was no significant difference between the groups in the coronary anatomy visibility, $p > 0.05$.

Discussion

It was Gabbay who put forward criteria for the 'ideal' pericardial substitute (Gabbay, 1990). These criteria included: 1) non adherence to the heart and easy separation from it upon reoperation; 2) non adherence to the sternum upon reoperation, so that repeat sternotomy is technically no different from the original procedure; 3) capability of mechanical attributes, and maintenance of the barrier integrity of the native pericardial sac; 4) freedom form dimensional distortion or shrinkage upon prolonged implantation; 5) convenience and technical ease of handling; 6) immunologic inertia, so as not to provoke inflammatory host response; and 7) capability of acquiring fibrinolytic activity similar to native pericardial tissue. Such a prospective pericardial substitute will have to be able to overcome the early postoperative pericardial micro-environment that, as a result of the insult of CPB surgery, is so conducive to adhesion formation. The insult upon the pericardium during CPB is manifest in three main ways, 1) the time related increase in the mesothelial cell damage and loss, 2) the reduction in the mesothelial fibrinolytic activity (as measured by the PAA), 3) the generalised pericardial inflammatory response and the consequent transpericardial fibrosis (which may impair subsequent mesothelial recovery as a result of its diminished blood supply in the presence of fibrosis).

The various pericardial substitutes that have been used (Gabbay, Guindy et al., 1989) in an attempt to overcome the problems associated with adhesions, have not pandered to the fibrinolytic capacity of mesothelium which may prevent or at least minimise adhesion formation. This underestimation of the role of the pericardium, and the fact that few of the experimental trials have been conducted with cardiopulmonary bypass, which has an additional injurious affect on the pericardium (Gabbay, 1990), may explain why the various methods have not been shown to produce significant clinical benefit.

The results of this study give some understanding as to the affects of non-CPB and CPB experiments on the pericardium. The similarities between the two are: the time related significant increase in the mesothelial cell damage and loss, and the generalised pericardial inflammatory response. The more significant increase in inflammatory features noted in the non-CPB (group C) animals compared to the CPB (group A) animals may be

attributable to the hypothermia induced during CPB and by the topical cooling. The hypothermia in the CPB group would have had the affect of slowing down the inflammatory process . What is not known is the long term recovery of these factors and how much this recovery is comparatively hindered by the local and systemic affects of CPB. The dissimilarities are perhaps more important in that, although there is a reduction in the PAA of both non-CPB and CPB pericardium, only the CPB pericardium showed a significant reduction in PAA during the operative period. At reoperation both groups showed a recovery in the PAA such that the difference between them was not significant. What is not known is the time scale of this recovery and how much the available PAA was overwhelmed by the local and generalised factors favouring adhesion formation.

The outcome, as far as adhesion formation is concerned, is dependant upon the result of the competition between mesothelial regeneration and recovery of PAA (the preferred outcome), and organisation (with its attendant adhesion formation). Other factors such as local infection, retained thrombus, pericardial effusions, and post-perfusion syndrome, may have an additional affect on the competition between recovery and organisation. However, judging from those parameters that we have measured the main factor that might have a notable affect on pericardial recovery is the significant reduction in PAA seen in pericardium exposed to CPB.

Interestingly we noticed no significant difference in the degree of adhesion formation among the three groups of animals (CPB with patch, CPB without patch, and non-CPB without patch). The same was true for the visibility of the coronary anatomy. Indeed, the patch material conferred no obvious advantage in terms of adhesion formation and coronary anatomy visibility. This result contrast with that obtained by Malm et al (Malm, Bowald et al., 1992b). They showed regeneration of pericardial mesothelial cells onto a framework of the same biodegradable material, a significant reduction in postoperative pericardial adhesion formation, and improved coronary anatomy visibility. However, a number of important differences in the study method ought to be noted: 1) in this study the animals with a patch were subjected to CPB which was not the case in the study mentioned. However, the use of CPB would seem to be an appropriate methodology as it

is more comparable to the clinical situation. 2) in Malm's study the patch material was left in-situ for a longer period of time (2 - 30 months as opposed to our 4 weeks), 3) there is the possibility of species variation in that we conducted our experiments on calves whereas their work was done on sheep.

Such was the close adherence of the patch to the surrounding structures, in particular the underlying heart, that it was difficult to see quite how regenerative mesothelium would have migrated to cover its surface. In the study by Malm et al (Malm, Bowald et al., 1992a) even the earliest samples, i.e. those after two months, had the surface of the patch completely covered with mesothelium-like cells. In this study, at 4 weeks, one would have expected to see at least a few strips or islands of mesothelial regeneration. Yet even after careful inspection of the patch, in particular the edges that where continuous with the native pericardium, no regenerative mesothelial cells were found. These results suggest that the processes of adhesion formation have completely overtaken the regenerative processes to the extent that the adhesions themselves, by obliterating the pericardial space, impeded mesothelial regeneration.

Histologically the patch material was insulated and phagaocytosed by polynucleated macrophages. This picture was noted by Malm et al in their earlier samples of pericardial regeneration. In their later samples, the regenerative tissue was well vascularised and had fewer macrophages. The macrophages could be seen to have phagocytosed much of the patch material.

Chapter 8

Final Discussion, Future Possibilities and Conclusion

FINAL DISCUSSION

At this stage in the evolution of the speciality of cardiac surgery, the presence of pericardial adhesions continues to impose a considerable problem (with technical difficulties and subsequent morbidity and occasional mortality) in reoperative cardiac surgery. The recognition of this fact is evident from the many research endeavours that have gone into finding means to overcome the problem. Yet, no universally accepted method has arisen.

This work has confirmed the presence of fibrinolytic activity (as measured by the PAA) in human pericardial tissue, particularly in its mesothelium. This activity, although it has the actual or potential role of preventing or at least minimising post surgical adhesion formation, is compromised by trauma, injury, and in particular by CPB surgery. Concomitant destructive changes in the pericardial morphology compound the deleterious affect on the mesothelial fibrinolytic potential.

It may be suggested that the observed diminution in the pericardial PAA is a reflection of haemodilutional effects. However, this reservation does not take into consideration, the reduction in mesothelial PAA that occurs in other fields of surgery, for it has been observed that a similar per-operative reduction in peritoneal mesothelial PAA occurs in patients undergoing elective laparotomy (Scott-Coombes, Whawell et al., 1992) in whom haemodilution and hypothermia do not play as major a role. This suggests that local factors, such as extensive damage to mesothelial cells and submesothelial inflammation, rather than haemodilution are more important determinants of fibrinolytic activity. These local factors appear to provide a favourable environment for the accumulation of fibrin, which after organisation lead to fibrous adhesions. In addition, the concomitant plasma tPA activity, a measure of the effect of haemodilution, was observed to reach a peak at the same time the pericardial PAA reached its trough, therefore strongly suggesting that these changes are independent of each other.

Our work on primary pericardial tissue suggested that, towards the end of the operative procedure, some recovery of the PAA had begun. However, the work on reoperative

pericardium suggests that although some recovery does occur (in calf pericardium as well as human pericardium), it never returns to the preoperative magnitude. In addition, the pericardium tends to be thicker with an increased fibrous tissue content, and the mesothelium is deficient. It is likely that the reduced fibrinolytic activity seen in reoperative pericardial tissue is due to this mesothelial deficiency that occurs as a result of inadequate mesothelial recovery. This recovery being impeded by a number of unknown factors and the fibrous reaction in the pericardium compromising the blood supply to the mesothelium.

In those areas where there is a deficiency of pericardial covering, and therefore of fibrinolytic activity, a pericardial substitute would seem appropriate. The function of which would be to take over the role of the deficient pericardium and affords some protection against adhesion formation, resternotomy and subsequent dissection. A number of materials have been used for pericardial substitution, but few with the idea of encouraging pericardial tissue to regenerate onto it and exert its influence on adhesion formation. This idea of exploiting the inherent fibrinolytic and regenerative potential of pericardial tissue in order to overcome the problems associated with pericardial adhesions at resternotomy has received relatively little direct research attention. Much of the past research work appears to have underestimated at least two factors that we believe to be important. The first is the affects of CPB on pericardial recovery and adhesion formation. Much of the past work has been done without CPB. However, our previous discussions have alluded to the importance of CPB as well as surgical manipulation, and trauma as factors (Gabbay, Guindy et al., 1989) affecting the regenerative potential of mesothelial cells. Furthermore, this work suggests that the PAA of pericardial tissue is reduced more significantly in an environment that incorporates CPB in comparison to one that does not. Therefore, considering human clinical experience requiring CPB, it is appropriate to recreate these conditions in animal experiments by including CPB into the experimental protocol. The second is the neglect or underestimation of the fibrinolytic activity and regenerative capacity of pericardium, to prevent adhesion formation. This potential has been demonstrated by the work of Malm et al (Malm, Bowald et al., 1992b).

The place of pericardial fluid in the prevention of adhesions is unknown. We have found that its constitution is very similar to that of plasma. In addition it contains tPA, uPA, PAI-1, and PAI-2. The balance between these plasminogen activators, e.g. tPA and plasminogen activator inhibitors, e.g. PAI-1 is such that in most (12 of 15) samples that we examined, there was no fibrinolytic activity. In those 3 samples where activity was found, the magnitude of this activity was low. However, the presence of such enzymes suggests that pericardial fluid does play a role in the prevention of adhesions. Furthermore, because of its cellular content, being predominantly mesothelial cells (but also lymphocytes, macrophages, red blood cells, and neutrophils), it may contribute to the cellular turnover and regeneration of the mesothelium. This it could do by donating pre-existing free-floating mesothelial cell, which we found to constitute about 75% of the cells in pericardial fluid, to settle on denuded areas, where they spread out, attach themselves to one another, and differentiate into mono-layered mesothelial cells (Ryan, Grobety et al., 1973).

Future Possibilities

Although our result did not show the patch material to be of benefit in minimising adhesions, the combination of these results and that of Malm suggests that what is required is a device or agent that will delay the overwhelming early tendency to adhesion formation long enough for mesothelial regeneration to establish itself. Bioactive coatings (Hubbell, 1993) may have this desired influence by the incorporation of enzymatic agents such as thrombolytic agents, anticoagulant agents, and cell adhesion-promoting agents (e.g. fibronectin which promotes endothelial cell adhesion) that improve the biocompatibility of cardiovascular materials by promoting the rapid coverage of the biomaterial by cells, e.g. endothelial cells. Hence, it may be possible to impregnate the material with optimal concentrations of tPA thereby arming it with fibrinolytic potential for the period that it is crucial for mesothelial cells to colonise the patch without being impeded by the adhesions. Indeed, a system has been developed for the release of immobilised streptokinase within a poly(methacrylic acid-g-ethylene oxide) copolymer, a high degree of retention of enzymatic activity was observed (Drummond and Peppas, 1991). Immobilised streptokinase has been examined in vivo (Mercer et al, 1978), and

retention of fibrinolytic activity was observed for greater than 150 days. The kinetics of immobilised urokinase also has been examined in vitro (Senatore and Berneth, 1986). An alternative is heparin coating the material with the same aim in mind.

The results of this study and others suggests that a biological material such as PHB may acquire those features of an ideal pericardial substitute. Nevertheless further modification of this material, such as those suggested, is required to overcome the early tendency to adhesion formation and to facilitate mesothelial regeneration.

CONCLUSION

This study confirms the presence of fibrinolytic activity within primary pericardial tissue. During the period of CPB the magnitude of this activity, which is largely due to the presence of tPA, significantly diminishes. The presence of uPA in smaller quantities has also been confirmed but there was no evidence of PAI-1 or PAI-2 during the period of CPB.

Accompanying the changes in the fibrinolytic activity where morphological changes in the pericardium. The latter changes were characterised by increasing histological and ultrastructural damage of the mesothelium and connective tissue layers.

When these events in primary pericardial tissue were compared to those occurring in reoperative pericardial tissue equivalent changes were noted. There was also a reduction in the fibrinolytic activity of reoperative pericardial tissue. However, this activity was consistently lower than that observed in primary pericardial tissue. Furthermore, at the earlier stages of the operation the reoperative tissue showed a preponderance of damaged mesothelium and connective tissue with fibrosis.

The plasma tPA activity rose to a peak level during the same period that the pericardial fibrinolytic activity in primary and reoperative pericardium fell to its trough. This suggests that the plasma and pericardial fibrinolytic activity are unrelated during the period of CPB.

The role of pericardial fluid in the prevention of adhesions is rather speculative at the moment. We found that serosal cells formed 75% of the cells in pericardial fluid. These cells could form part of the regenerative pool of cells that enable the process of mesothelial regeneration to occur. Furthermore, the presence of tPA and uPA would confer plasminogen activating activity on pericardial fluid. However, it appears that this PAA is kept in check by the simultaneous presence of PAI-1 and PAI-2 in pericardial fluid. Therefore, one can speculate that the postoperative absence of this resource of serosal cells and the loss of potential PAA may be factors that further encourage adhesion formation.

The work on animal models reflected much of the morphological and fibrinolytic activity changes observed in the clinical setting. During the operative period there was increasing morphological destruction of the pericardium with a reduction in its fibrinolytic activity. Although the morphological changes were similar for animals subjected to CPB and those not subjected to CPB, the reduction in the fibrinolytic activity only reached significance in those animals subjected to CPB. This suggests that there is a contributory factor from the affects of CPB that further reduce the pericardial fibrinolytic activity.

For the period of time that we maintained the PHB patch in the pericardial position we found no pericardial mesothelial regeneration. The patch material conferred no obvious advantage in terms of adhesion formation and coronary anatomy visibility, and there was no adverse affects in terms of postoperative infectivity.

The presence of pericardial adhesions at resternotomy continues to increase the risk associated with resternotomy and dissection. Despite the experimental use of a number of pericardial substitute materials, there is no universally accepted material. However, in the context of pericardial substitution, the idea of using a material that will provide a scaffold for the regeneration of pericardial tissue is relatively new and warrants further research. Such research, if it is to be a fair representation of the clinical situation, must include those conditions that are common to cardiac surgery, i.e. pericardial and myocardial manipulation and trauma, and CPB.

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ADDENDUM

Publications to date Arising from this Work

Nkere UU, Whawell SA, Thompson EM, Thompson JN, Taylor KM. Changes in the pericardial morphology and fibrinolytic activity during cardiopulmonary bypass. J Thorac Cardiovasc Surg 1993; **106**: 339-345.

Nkere UU, Schofield JB, Whawell SA, Thompson JN, Sarraf CE, Taylor KM. Perioperative Histological and Ultrastructural changes in the Pericardium and Adhesions. Ann Thorac Surg 1994; **58**: 437-444.

Changes in pericardial fibrinolytic activity during cardiopulmonary bypass. Published in a Supplement (July 1992) to the International Journal of Fibrinolysis (Abstract). **Nkere UU**, Whawell SA, Thompson JN, Thompson EM, Taylor KM.

Nkere UU, Whawell SA, Sarraf CE, Schofield JB, O'Keefe PA. Pericardial Substitution After Cardiopulmonary Bypass Surgery: A Trial Of An Absorbable Patch. Ann Thorac Surg (In Print).

Nkere UU, Whawell SA, Sarraf CE, Schofield JB, Thompson JN, Taylor KM. The Pericardium and Pericardial Adhesions. Thorac Cardiovasc Surg (In Print).

Copies of the published papers are provided within the inside pocket of the back page.