In fictional murder mysteries the person responsible for the crime is often the one least likely to be involved. In contrast, in real life, the culprit is usually the most obvious candidate right from the start. In this particular case, the myocardial collagen matrix is the suspect (and as I shall illustrate, a likely culprit) responsible for at least some of the complications that occur after myocardial infarction.

Collagen is at the same time the most obvious suspect and yet one that has been frequently overlooked. There are two reasons for this oversight: first, muscle is the major component of the heart and collagen makes up a small percentage of heart mass. Second, relatively specialised techniques are required to reveal the presence and organisation of the myocardial collagen matrix. For example, the most frequently used histological stain, haematoxylin and eosin, is poor in terms of differentiating collagen and therefore gives the false impression that there is hardly any collagen in the heart. Furthermore, a stain commonly used to detect collagen, trichrome, is in fact not specific for collagen and its use can lead to a substantial underestimation of collagen content [1]. Only with preparations such as scanning electron microscopy, examination of silver impregnated sections using light microscopy, and the examination of picrosirius red stained sections with polarised light can the presence and organisation of the collagen matrix be appreciated.

Why is collagen the obvious suspect? One reason becomes evident when the collagen matrix is visualised. Its organisation, for example connecting neighbouring myocytes, is consistent with a role in maintaining structural integrity. In addition, the strength and stiffness of type I collagen are comparable to steel and are several orders of magnitude greater than muscle. Thus the amount, organisation, and type of collagen are important factors in determining the mechanical properties of the heart.

While recent reviews have dealt with the general role that collagen plays in myocardial structure and function [2,3], my objective is to focus on the fate of collagen during and after myocardial ischaemia and in cicatrix formation after infarction, with an emphasis on recently published work.

1. Ischaemia related collagen injury

It has recently been appreciated that ischaemia can result in collagen damage; however, the exact time course, nature, and mechanism of this damage are not yet known. Collagen injury can even occur in the absence of muscle necrosis. For example, 12 five minute coronary artery occlusions each followed by 10 minutes of reperfusion do not kill myocytes but do cause disruption, breakage, and abnormal staining of collagen fibres [4,5]. Although such damage was initially thought to be the cause of postischaemic contractile dysfunction or myocardial stunning, the absence of collagen damage after induction of stunning with a single 15 minute coronary artery occlusion indicates that there is no causal relationship between collagen damage and stunning [5,6].

In cases of sustained ischaemia, it has been suggested that the onset of collagen damage is early and extensive. For example, Takahashi et al. [7] reported that 50% of the native myocardial collagen matrix was lost after only three hours of ischaemia in rats. Increased activity of collagenase and other enzymes was also found over the same period. However, it is surprising that such extensive loss of
collagen is not associated with an equally rapid compromise of the heart’s structural integrity. For example, pronounced infarct expansion does not occur until several days after infarction [8], and myocardial rupture has not been reported in rats. In fact, in contrast to the above biochemical study, no appreciable collagen loss was found by light microscopy even several days after myocardial infarction in rat hearts [8].

Light microscopy did, however, reveal a reduction in the birefringence of silver stained collagen fibres in the first four days after infarction. These changes in optical properties are consistent with degradation; however, because similar changes were not seen with other stains, we speculated that the degradation may involve injury to glycoproteins associated with collagen fibres. Such injury would adversely affect the mechanical properties of collagen and be responsible for changes in birefringence. Indeed, we found that the number of collagen fibres with reduced birefringence correlated with the degree of infarct expansion [8]. The proposed hypothesis was that collagen fibres weakened by such injury would not be able to withstand the haemodynamic forces to which the tissue was subjected and so expansion would occur. Although the time course and nature of collagen injury is unclear, there is agreement that injury does occur, probably by the activation of latent matrix metalloproteinases present in the myocardium [9].

The later arrival in the infarct of inflammatory cells which are also capable of releasing proteolytic enzymes is likely to enhance collagen injury. If the degradation is extensive enough and no collagen is left in a particular region (figure, panel B), cardiac rupture could occur. In this respect, the heart will only be as strong as its weakest link. Although studies have shown a substantial decrease or even an absence of collagen around rupture sites in humans [10], cardiac rupture occurs in only a small percentage of cases, which suggests that complete destruction of the collagen matrix, even on a regional basis, is a rare event. In fact, an important factor limiting the complete loss of the collagen is that several days after infarction the healing process begins and new collagen is produced.

2. Healing of the infarct

Time course: when is healing complete?

Most studies of myocardial ischaemia have focused on either muscle necrosis or the remodelling that occurs in non-infarcted tissue in the weeks after infarction. Infarct healing has received little attention because it is assumed to be a passive process. However, healing is dynamic in nature, and open to manipulation. Increases in steady-state mRNA levels for type I and III collagen have been found on the second day after infarction in rabbit hearts [11]. New collagen is produced by fibroblasts or fibroblast-like cells in the infarct [12] and, in rats, can be seen by light microscopy as early as three or four days after infarction [8]. The amount of collagen present increases with time [13]. It is well known that immature scar collagen lacks the strength and stiffness of mature collagen. Therefore in the early stages of healing the scar may still be vulnerable to stretch by distending forces. Exactly how long the scar takes to achieve the same level of maturity as normal collagen is unknown. We found that the brightness of scar collagen viewed with polarised light (a reflection of subcellular structure), measured six weeks after infarction, is approximately 25% less than that of pericardial collagen [1]. Even 15 weeks after infarction, scars from rabbit hearts were susceptible to irreversible strain when subjected to repetitive stretching in vitro [14], which suggests that the scar is still not sufficiently stiff to resist the forces to which it could be subjected. These findings are consistent with a prolonged period of potential vulnerability. On the other hand, collagen in scars may be different from that found in normal myocardium in terms of both collagen types and cross links. For example, the early stages of wound healing are associated with increased amounts of type III collagen. Typically, tissues with a high type III collagen content are neither as stiff nor as strong as tissues in which type I collagen predominates. Whether the ratio of type I to type III collagen in the scar returns to the same levels found in normal myocardium is unknown. In addition, the concentration of hydroxylysylpyridinoline cross links (thought to be important in determining tensile strength) in scar collagen has been found to be greater than that in non-infarcted tissue 13 weeks after coronary artery occlusion in rats [15], implying that scar collagen may be stronger than normal myocardial collagen.

Healing is not a homogeneous process. Several studies have demonstrated striking anisotropy within the scar. Light microscopic examination of scar tissue in dog hearts revealed a trilayered structure [16]. Within each layer, collagen fibres were aligned in approximately the same direction, but individual layers had different three dimensional orientations. These orientations appeared to correspond to the orientation of muscle that had previously occupied the space. Structural examination of transmural scars in canine hearts using acoustic techniques (analysis

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Fig. 1. (A) Myocardial scar tissue six weeks after infarction. Much of the necrotic muscle has been replaced by thick collagen fibres. A network of thin collagen fibres is present in areas where the healing process is not so advanced. (B) In contrast, there is an absence of collagen in the vicinity of a suspected dissection (marked by arrows) through the ventricular wall one week after infarction. Both micrographs show picrosirius red stained tissue viewed with circularly polarised light (bar = 50 μm).
of integrated backscatter) supported the concept of a trilayered structure of collagen fibres with different orientations in different layers [17]. Given such anisotropic structure, it is not surprising that Gupta et al. [8] found that myocardial scars, formed after permanent coronary artery occlusion in sheep, had anisotropic mechanical properties. Six weeks after infarction they found that scar tissue was less extensible in the circumferential than in the longitudinal direction. Furthermore, they found that mechanical anisotropy of the scar varied with time. Stiffness did not consistently increase, but rather increased during the first two weeks, and subsequently decreased at six weeks. Such findings are compatible with ongoing remodelling in the scar. Although structural changes in the scar as a function of time have not been rigorously examined, we noted differences in structure at different stages of healing (figure, panel A). In regions where cellular debris was present, we saw a lattice-like arrangement of thin collagen fibres [16]. In regions where debris had been largely removed, thicker fibres appeared to be woven through the lattice and finally the lattice was replaced by highly aligned parallel fibres.

It is also possible that other forms of scar remodelling may occur. Holmes et al. [19] monitored the position of gold beads implanted in pig myocardium after myocardial infarction, and observed progressive scar contraction during the first three weeks, primarily in the circumferential direction. Contraction of scar tissue is thought to be mediated by cells (probably phenotypically transformed fibroblasts) containing F actin and also α smooth muscle actin filaments. Indeed fibroblast-like cells with positive staining for α smooth muscle actin have been found in human myocardial scars as early as four days and as late as 17 years after infarction [20]. These findings further illustrate the dynamic nature of healing.

Other connective tissue matrix elements

In this review, I have taken the connective tissue matrix to be synonymous with collagen. However, this is a simplification, and other elements such as glycoproteins and integrins may also be important. For example, the glycoproteins fibronectin and osteopontin are expressed as early as one day after infarction and therefore precede collagen expression [11,21]. It appears likely that these components play a key role in the early healing process by mediating cell adhesion and guidance [21,22].

Role of pharmacological therapy after infarction

The question of when healing is complete is important because various pharmacological agents are being used to treat patients after myocardial infarction, often without any idea of their effect on healing. For example, an appreciation that drug therapy may interfere in healing occurred when some anti-inflammatory agents (that is, ibuprofen and methylprednisolone) were found to result in scar thinning in animal models. Such drugs were often used to treat pericardial inflammation and pain after infarction and so the experimental finding was of considerable interest. Although this subject was extensively studied in the 1980s, the mechanism of the adverse reaction was not determined and, fortunately, not all anti-inflammatory agents (for example, aspirin) caused scar thinning.

Recently, attention has shifted to the role of angiotensin converting enzyme (ACE) inhibitors in limiting ventricular remodelling, most frequently focusing on changes in non-infarcted tissue. For example, captopril has been shown to reduce or prevent the increase in fibrosis found in non-infarcted tissue after myocardial infarction. Although this is considered to be a beneficial effect, it is reasonable to suppose that if ACE inhibition affects fibrosis in non-infarcted tissue, there might also be an effect on scar colla-

gen. This concept is supported by the finding that collagen formation is associated with an increase in tissue ACE binding activity [12]. The apparent divergent clinical effects of captopril and enalapril on patient mortality, particularly when treatment was begun in the first few hours after infarction, has led to speculation that these drugs affect scar healing [23]. Although this possibility has not been examined directly, there is evidence that early treat-

ment with the ACE inhibitor quinapril retards healing in spontaneously hypertensive rats examined nine weeks after infarction [24].

Increased collagen in non-infarcted tissue could adversely influence electrical conduction by reducing cell to cell contact and so increasing the likelihood of abnormal conduction. Bélichard et al. found captopril and propranalol reduced the susceptibility of rat hearts to electrically stimulated arrhythmias four weeks after infarction [25]. This reduction in arrhythmias occurred even though the drugs had divergent effects on ventricular remodelling. Captopril reduced ventricular dilatation, septal hypertrophy, and fibrosis in non-infarcted tissue compared with untreated hearts - changes consistent with the reduced incidence of arrhythmias. In contrast, propranolol increased ventricular dilatation and the amount of fibrosis in non-infarcted tissue. Although increased fibrosis should increase susceptibility to arrhythmias, the authors speculated that it might have reduced the degree of tissue inhomogeneity.

Pharmacological treatment could also have indirect effects on collagen. For example, nitrate treatment for the first two to six weeks after myocardial infarction in dogs produced no difference in collagen content between treated and untreated scars, but reduced infarct thinning and ventricular dilatation [26]. Echocardiography revealed ongoing remodelling (progressive infarct thinning) in control hearts, consistent with the concept that newly produced collagen fibres are unable to resist the stresses to which they are subjected. Nitrates, because of their ability to reduce preload and afterload, would hence exert a positive effect on remodelling by not subjecting immature collagen to high wall stress.
3. Reperfusion after infarction

As stated earlier, it is possible, using multiple brief coronary artery occlusions, to produce collagen damage in the absence of muscle necrosis. In addition, the degree of damage produced is more severe than that seen after a similar period of sustained occlusion. These observations are consistent with the hypothesis that some component of reperfusion may be detrimental to collagen. Is it then possible that reperfusion after longer periods of coronary artery occlusion results in more collagen damage than permanent occlusion?

Many studies have shown that only transmural infarcts result in significant expansion of the infarcted tissue. Early reperfusion of an occluded artery is required to limit the amount of muscle necrosis and prevent the development of transmural infarction. As it is collagen fibres that provide tensile strength and stiffness to the myocardium, the lack of expansion in non-transmural infarcts is consistent with early reperfusion protecting both collagen and muscle from ischaemia related injury.

A more complex situation arises when reperfusion occurs too late to salvage any myocytes. The suggestion from clinical reports that late reperfusion reduces mortality has been supported by recent trials specifically designed to address this issue. For example, the LATE trial [27] examined the effect of reperfusion using recombinant human tissue plasminogen activator in patients treated between 6 and 24 hours after the onset of chest pain. There was a significant reduction in 35 day mortality in those patients reperfused within 12 hours, and a suggestion of a possible benefit in some patients treated even later. The incidence of cardiac rupture in the LATE trial was not increased by reperfusion; however, rupture tended to occur earlier in reperfused patients. Moreover, a meta-analysis of other thrombolytic trials indicated that there is an increase in the odds ratio of cardiac rupture if reperfusion occurs more than 17 hours after the onset of chest pain [28]. Thus, although there is evidence that late reperfusion (within 12 hours) is protective, there is also evidence that very late (> 17 h) reperfusion could be detrimental.

A suggested mechanism for the protection derived from late reperfusion is acceleration of healing. Although many papers have mentioned this possibility, there has been very little systematic investigation. Several studies found that late reperfusion in rats (as long as six hours after occlusion) resulted in a significant increase in the resorption of necrotic myocytes measured one or two weeks after infarction [29-31], a finding consistent with acceleration of healing. However, these studies did not examine collagen structure. Three weeks after infarction, one of these studies performed a qualitative assessment of scar tissue density and found no difference between reperfused and non-reperfused hearts [31]. This is consistent with a previous study done in rabbits in which similar collagen contents were found three weeks after infarction in reperfused and non-reperfused hearts [32]. However, reperfused scars did have a reduced density of collagen cross links, which correlated with a modest reduction in tensile strength in reperfused versus non-reperfused scars [30]. Thus whether reperfusion accelerates or interferes with healing is unclear.

4. Genetic manipulation of scar tissue

The effects of the pharmacological agents discussed earlier may ultimately be due to modulation of collagen gene expression. Appreciation of this point and the various transcriptional and posttranscriptional steps involved in positive and negative regulation of collagen gene expression may allow precise modification of healing. However, even though enhanced synthesis of proteins by treatment with bovine growth hormone was reported 30 years ago by Bing and colleagues [33], there have been few attempts to accelerate myocardial scar healing. We found that treatment with human growth hormone increased the collagen content of rat myocardial scars one week after permanent coronary artery occlusion, but did not limit the degree of ventricular expansion [34]. Another study reported that growth hormone treatment reduced the incidence of ventricular aneurysm formation after infarction in rat hearts [35]; however, significant differences in infarct size between treated and untreated groups probably confound the results. Enhanced scar healing may be of the greatest benefit in disease states with abnormal collagen metabolism. For example, diabetes is thought to exert several effects on wound healing, including inhibition of collagen synthesis and stimulated degradation of procol-
gen. Although evidence of impaired myocardial healing in diabetic patients is lacking, it may be responsible for the worse than expected outcome after infarction even when the usually higher incidence of risk factors is taken into account [36].

One form of genetic manipulation that is currently the focus of intense research is injection of a virus containing the myoD gene into an infarct to convert fibroblasts into muscle and restore contractile function [37]. This is an appealing idea; however, caution is warranted. Fibroblasts have a specific role in the infarcted myocardium, and any subversion of their function could be detrimental. For example, early fibroblast conversion could reduce collagen production and retard healing. Even successful production of muscle in the scar may not be beneficial. If the muscle were surrounded by collagen, it would probably atrophy. Conversely, islands of muscle within the scar might provide pathways for abnormal conduction and the generation of arrhythmias. Although such experiments are interesting, they provide another illustration of the potential for trouble when the importance of collagen in myocardial structure and function is overlooked.

5. Conclusion

Recent studies have shown that myocardial collagen is not an inert material, but is one that is vulnerable to ischaemia related injury. In addition, healing after myocardial infarction is a multifaceted process that is amenable, for better or worse, to manipulation. The table summarises the current evidence implicating collagen in an array of ischaemia related events. Although the evidence for collagen as a culprit in most of these events is compelling, the modus operandi is yet to be elucidated. Furthermore, the evidence comes almost exclusively from animal models of ischaemia and infarction. Although there is no reason to suppose that collagen's role is different in human hearts, there has been little direct study.

Apart from the previous neglect of the extracellular matrix in the heart, another factor limiting the available information is that a comprehensive analysis of the collagen matrix requires a multidisciplinary approach. Scar properties are determined not only by how much collagen is present, but also by what type, in what organisation, and by what amount and kind of cross links. In addition, the molecular signalling mechanisms responsible for collagen production or degradation must be considered, and for clinical study non-invasive methods of collagen analysis must be employed.

As we unravel the mysteries of the collagen matrix, the possibility of new treatment strategies for myocardial infarction should be revealed. For example, the reduction of collagen degradation and stimulation of healing have the potential to limit ventricular expansion and remodelling. Furthermore, these connective tissue changes occur over the course of weeks or months and so provide considerably more opportunity for intervention than attempts to limit muscle necrosis which must be initiated within hours of the onset of ischaemia.

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