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Spatial arrangement of the heart muscle fascicles and intramyocardial connective tissue in the Spanish fighting bull (*Bos taurus*)

D. SÁNCHEZ-QUINTANA[1], V. CLIMENT[1], V. GARCIA-MARTINEZ[1], M. ROJO[1] AND J. M. HURLÉ[2]

[1] *Department of Morphological Sciences, Faculty of Medicine, University of Extremadura, Badajoz, and* [2] *Department of Anatomy and Cell Biology, Faculty of Medicine, University of Cantabria, Santander, Spain*

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ABSTRACT

The spatial arrangement of the muscle fascicles and intramyocardial connective tissue was examined in the ventricles of the heart of the Spanish fighting bull (*Bos taurus*). In both ventricles, the muscle fascicles of the myocardium are arranged in 3 main directions, forming 3 muscle layers within the ventricular wall. The preferentially vertical arrangement of the muscle fascicles in the superficial and deep layers at the level of the fibrous aortic rings and the base of the semilunar valve leaflets suggests that these fascicles are actively involved in valvular dynamics. After controlled digestion of myocytes and elastic fibres with NaOH, a 3-dimensional arrangement of the scaffolding of connective tissue that supports the muscle fascicles and myocytes was observed. The arrangement and structure of this scaffolding may influence the order of contraction of muscle fascicles in different layers of the ventricle. In addition, differences were observed between the connective tissue scaffolding surrounding the myocytes of the 2 ventricles; these variations were correlated with the different biomechanical properties.

Key words: Cardiac muscle; collagen; heart valves.

INTRODUCTION

The beat-to-beat pumping ability of the mammalian heart is determined by at least 2 factors: (1) the spatial arrangement of muscle fibres in the ventricular walls (Streeter, 1979) and (2) the intramyocardial connective tissue (Weber, 1989). Evidence that the 1st of these factors influences the contractile efficiency of the heart comes mainly from morphological analyses based on gross dissection techniques (Robb, 1934; Thomas, 1957; Grant, 1965; Streeter, 1979; Anderson & Becker, 1980; Torrent-Guasp, 1980; Sánchez-Quintana & Hurlé, 1987), histological sections (Kashyap, 1975; Maron et al. 1981; Greenbaum et al. 1981; Fernandez-Terán & Hurlé, 1982) and computer-based methods of 3-dimensional reconstruction of serial sections (McLean & Prothero, 1991). These studies have shown that the muscle fibre bundles are precisely oriented within the ventricular wall and that changes in shape and the force generated during systole depend on the relative orientation of and interaction between these bundles.

Despite the many studies published on this subject, there is at present no general agreement as to the spatial arrangement of the ventricular muscle fibres, particularly with regard to the form of insertion into the fibrous skeleton, or as to the existence or otherwise of an independent muscular system for each ventricle (Anderson & Becker, 1980; Torrent-Guasp, 1980; Macias-Rodriguez et al. 1986; Sánchez-Quintana et al. 1990). Morphological evidence supports the involvement of the myocardium in the dynamics of the atrioventricular and semilunar valves.

The intramyocardial connective tissue, the 2nd factor involved in the heart's pumping ability, is now recognised as an important element responsible for the biomechanical properties of the heart (Weber, 1989). This tissue shows several levels of organisation, termed epimysium, perimysium and endomysium, which form a reticular extracellular network that

Correspondence to Dr D. Sánchez-Quintana, Departamento de Ciencias Morfológicas, Facultad de Medicina, Avda Elvas sn, Universidad de Extremadura, E-06071 Badajoz, Spain.

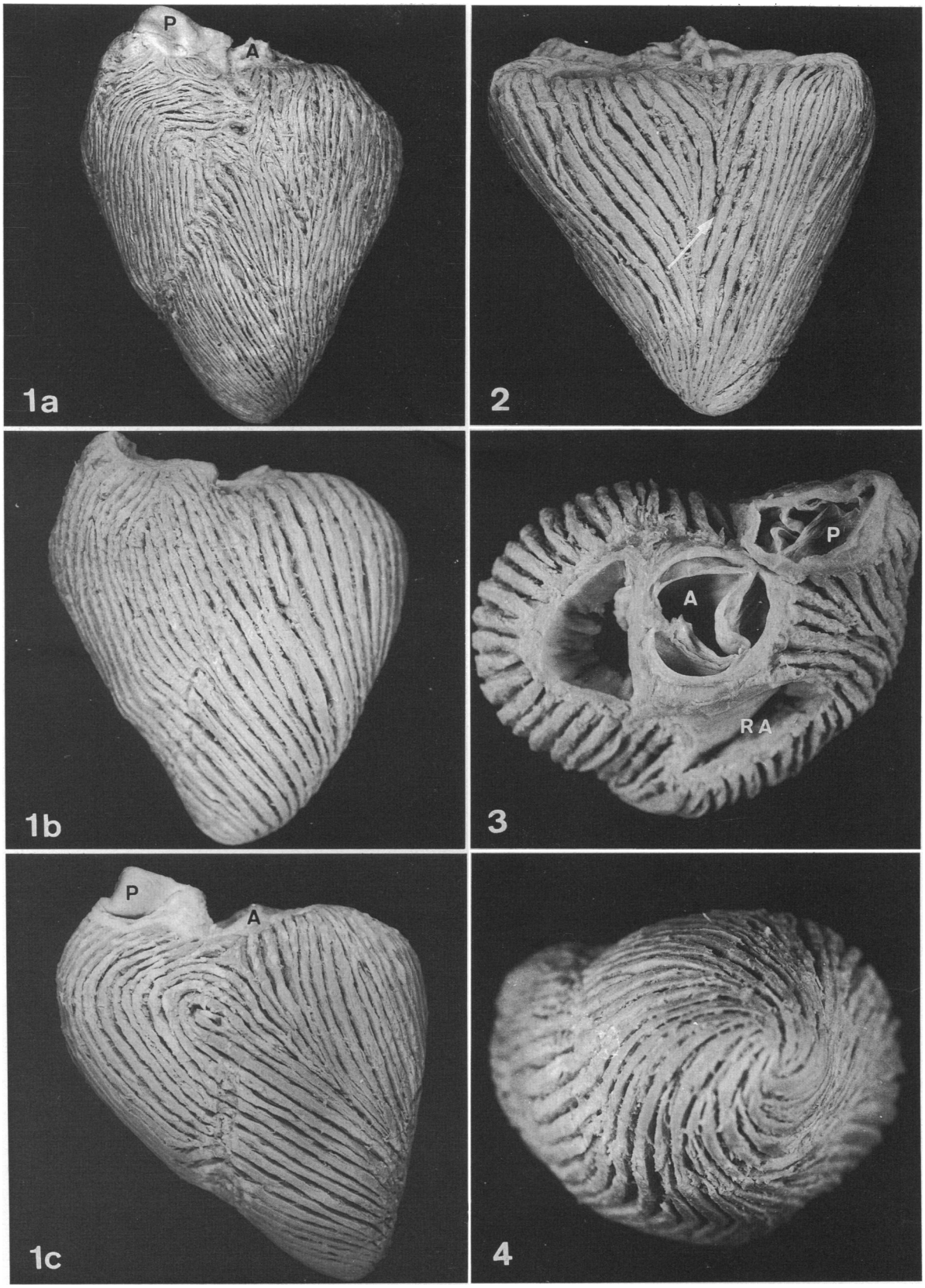

Fig. 1. (*a*) Anterior view of the heart showing the arrangement of the superficial layer of myocardial fascicles. (*b*) and (*c*) Variations in the pattern of muscle fascicle arrangement in the superficial layer. P, pulmonary artery; A, aorta.

Fig. 2. Dorsal view of the heart showing the arrangement of the superficial layer of myocardial fascicles. Arrow, dorsal interventricular sulcus.

interconnects myocytes, muscle bundles and blood vessels (Robison et al. 1983). It has been suggested that the intramyocardial connective tissue prevents overstretching of the myocardial cells and confers viscoelastic properties to the heart (Weber, 1989). However, the precise function of the perimysium and endomysium remains to be clarified (Morita et al. 1991).

The present study was designed to establish a more precise correlation between the spatial arrangement of the muscle fibres and the intramyocardial connective tissue, and to provide new knowledge concerning the adaptability and functional dynamics of the heart. Such information should help to make attempts to correct cardiac dysfunction more effective. We used dissection techniques and histological studies by light and scanning electron microscopy (SEM) to analyse the architecture of the muscle fibres and connective tissue in 37 hearts from Spanish fighting bulls. This animal was chosen because its physiological and behavioural characteristics (aggressiveness) are typical of a running animal that requires a high rate of metabolism (Montero-Aguera, 1976). Our observations show that the muscle fascicles of the myocardium in both ventricles are arranged in 3 main directions giving rise to 3 muscle layers, superficial, middle and deep. In addition, the organisation of the fascicles at the level of the fibrous rings of the pulmonary artery, aorta and atrioventricular orifices suggests that the myocardium is actively involved in valvular dynamics. Moreover, our findings after controlled digestion of myocytes and elastic fibres with NaOH reveal the presence of a skeletal framework of connective tissue which supports the muscle fascicles and cardiac muscle fibres. The orientation of this skeleton in the ventricular wall is similar to that of the cellular myocardial framework. We noted structural differences in the connective tissue skeleton surrounding the myocytes in either ventricle and correlated these variations with differences in the biomechanical properties of the 2 ventricles.

MATERIAL AND METHODS

We used a total of 37 hearts of Spanish fighting bull from different breeders. The animals weighed 510–580 kg; mean age at the time of death was 4 y. The whole heart was removed and washed several times in physiological saline solution. The organs weighed 2.2–2.4 kg. Tissues were studied by dissection, light microscopy and SEM.

Dissection technique

For dissection we used 26 hearts previously fixed in 10% formaldehyde for approximately 3 months and stored in 70% ethanol. Myocardial tissues were dissected with watchmakers' forceps and scissors, starting from the epicardial or endocardial surfaces (Fernandez-Teran & Hurlé, 1982; Sánchez-Quintana & Hurlé, 1987). After fixation, 5 hearts were boiled in water and acetic acid according to Pettigrew's method (1864).

Light microscopy

Six hearts were fixed in 10% formaldehyde for light microscopic examination. Several blocks of tissue from the walls of the right and left ventricles, interventricular septum, papillary muscles, and atrioventricular and arterial rings were dissected from formaldehyde-fixed hearts. The samples were then dehydrated in ethanol, embedded in paraffin and cut into sections 10–12 μm thick. Deparaffinised sections were then stained by the silver methenamine method according to Jones (1951), and the picrosirius red method as described by Dolber & Spach (1987).

Scanning electron microscopy

Five hearts were used for SEM observation. Small tissue blocks containing myocardium from the right and left ventricles, interventricular septum, papillary muscles and atrioventricular rings were fixed in Karnovsky's fixative at 4 °C for 10 h. To expose the architecture of the connective tissue sheaths surrounding working myocardial cells, the blocks were subjected to chemical digestion in 2 N NaOH at room temperature as described by Morita et al. (1991). The blocks were then dehydrated in a series of acetones of increasing concentration, dried by the critical point method, sputter-coated with gold, and observed in a JEOL T-100 scanning electron microscope.

Fig. 3. Basal view of the heart showing the arrangement of the superficial layer. Note the insertion of the muscle fascicles in the fibrous skeleton. A, Aorta; P, pulmonary artery; RA, right atrioventricular orifice.

Fig. 4. Apical view of the heart showing the arrangement of the superficial layer in the vortex cordis. Here the fascicles of the superficial layer invaginate, passing into the deep (subendocardial) layer.

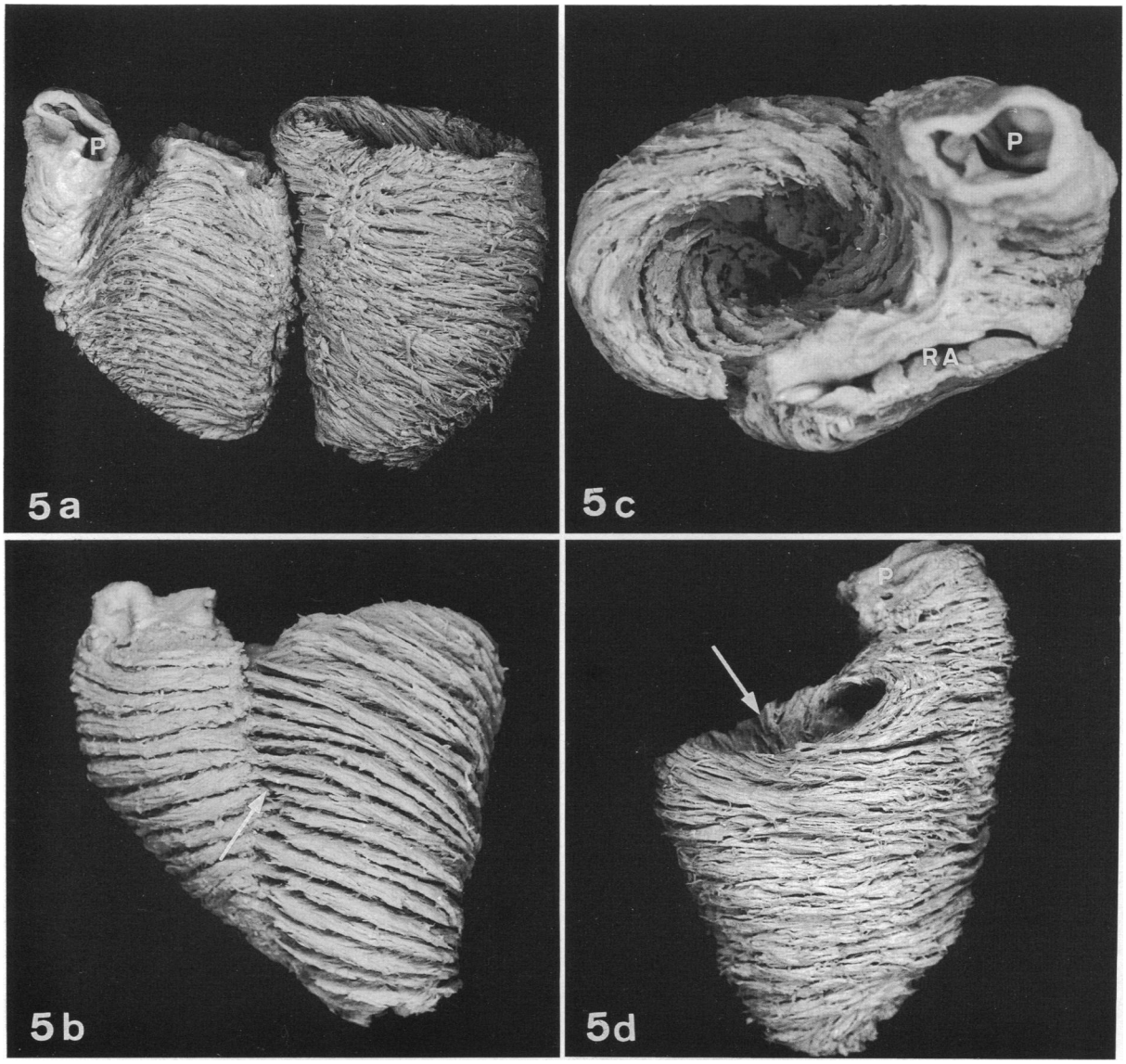

Fig. 5. (*a*) Septal view of the isolated ventricles, showing the arrangement of the middle layer. Note the preferential horizontal arrangement of muscle fascicles and the presence of a well defined middle layer of fibres in the right ventricle. P, pulmonary artery. (*b*) Anterior view of the heart showing the middle layer. Arrow, anterior interventricular sulcus. (*c*) Basal view of the heart showing the middle layer in the left ventricle. Note the circular arrangement of the muscle fascicles around the atrioventricular and aortic orifices; the fascicles then invaginate towards the inside of the ventricular cavity. P, pulmonary artery; RA, right atrioventricular orifice. (*d*) Right lateral view of an isolated right ventricle showing the middle layer. Note the circular arrangement at the level of the pulmonary artery and atrioventricular orifice, where the fascicles invaginate (arrow). P, pulmonary artery.

RESULTS

Gross anatomical features

To simplify the topographical terminology and anatomical descriptions, we will base our results on the 'free-standing' heart, oriented such that the longitudinal axis, which is diagonal in situ, is vertical. This obviates reference to the organ's anatomical relations and neighbouring structures. The heart is shaped like an inverted cone with a more or less acute apex, and is flattened through the anteroposterior plane. The anterior and especially the posterior interventricular sulci are shallow and reflect the marked difference in the size of the 2 ventricles, the left being the larger (Figs 1–4).

Myocardial fibre architecture

Dissection showed that the myocardial fibres of the ventricle are grouped into irregular muscle fascicles. We use the term 'muscle fascicle' in a macroscopic sense to refer to a group of muscle cells large enough to be seen by the naked eye through the ventricular mass. Our observations in dissections and histological sections showed that the muscle fascicles in both ventricles are arranged in 3 main directions, giving

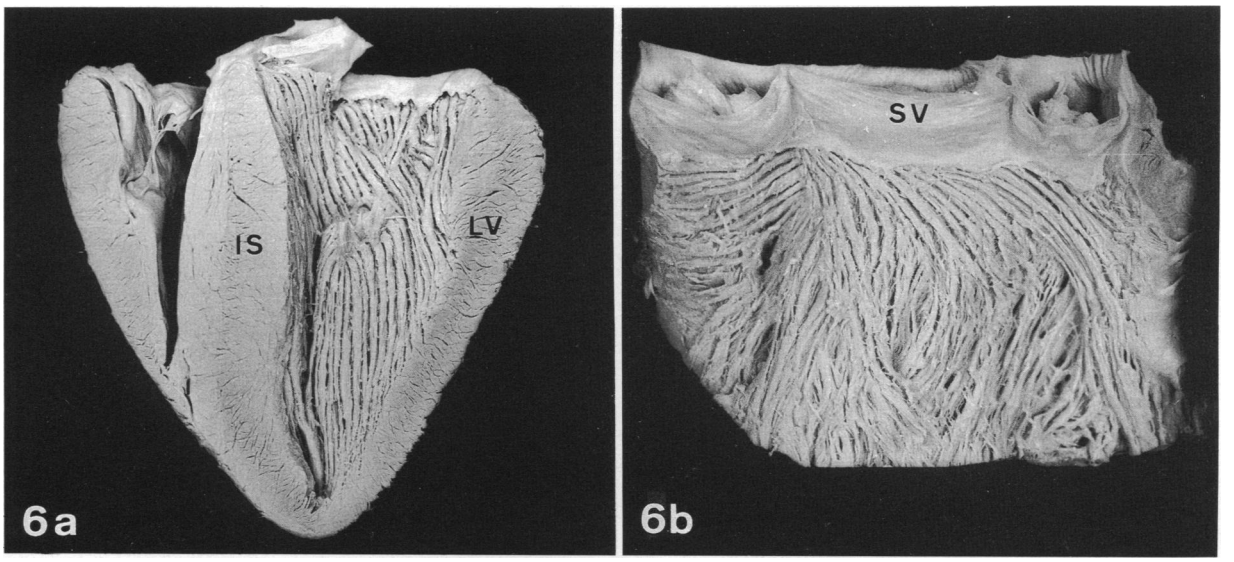

Fig. 6. (*a*) Frontal section of a heart through the aortic orifice. Note the vertical direction of muscle fascicles at the level of the deep layer of the left ventricle from the apex to the base of the ventricle. LV, left ventricle; IS, interventricular septum. (*b*) Longitudinal section of the pulmonary root showing the arrangement of the deep layer at the level of the base of the valvular leaflets. SV, semilunar valve leaflet.

rise to 3 muscle layers, superficial, middle and deep. However, we emphasise that there are no planes of connective tissue that allowed us to separate and distinguish between the 3 layers; instead, the distinction between one layer and the next is made on the basis of a gradual change in direction of the muscle fascicles. In fact, we found that some fibres changed from one muscle layer to another in the course of their trajectory around the ventricles.

Superficial layer

The superficial layer is characterised by fibres that are shared by the 2 ventricles. This layer originates in the base of the ventricles, partly in the fibrocartilaginous rings surrounding the 4 great orifices (aortic, pulmonary, right and left atrioventricular orifices) and partly in the fibrous structures connecting these orifices (Figs 1–3). After leaving their origin at the ventricular base, the muscle fascicles descend towards the apex of the heart (Figs 1–4). On the anterior surface, of 50% of the hearts dissected, the muscle fascicles are arranged vertically. The exception is the upper portion of the right ventricle, together with the origin of the pulmonary artery, where the fibres lie obliquely from left to right, and in the inferior portion of the left ventricle near the apex, where they run obliquely from right to left (Fig. 1*a*). Changes in the direction to the muscle fibres are frequent on the anterior surface: in 7 of 26 hearts (27%), the fibres ran obliquely downwards and leftwards (Fig. 1*b*), the trajectory being more vertical in the right than in the left ventricle. In 6 hearts (23%), characterised by their large transverse diameter at the base (22 cm, in comparison with 18 cm in the rest of the heart dissected), the fibres ran more obliquely (Fig. 1*c*). On the posterior surface, muscle fascicles originate in the posterior part of the atrioventricular orifices and right trigona fibrosa cordis, then run obliquely towards the apex. These fibres lie from left to right on the posterior surface of the left ventricle, and from right to left on the posterior surface of the right ventricle, forming the posterior interventricular sulcus between the ventricles (Fig. 2). At the apex, the fibres invaginate in a spiral pattern and subsequently give rise to the deep or subendocardial layer (Fig. 4).

Middle layer

Dissection revealed a middle layer of fibres forming proper sets in each ventricle (Fig. 5*a*), although this layer is more prominent in the left ventricle. The most noteworthy feature of the middle layer is that its origin and end in the muscle fascicles are not clearly distinguishable. In the left ventricle, the fascicles run obliquely around the chamber, forming a muscular cylinder open at its base and apex (Fig. 5*b*, *c*). At the base, the muscle fascicles form an elliptical ring encircling, but not inserted into, the mitral and aortic orifices (Fig. 5*c*). These fascicles invaginate posteriorly, forming a spiral pattern within the ventricular chamber, and are continuous with the muscle fascicles that invaginate at the apex. In the right ventricle the muscle fibres are arranged horizontally (Fig. 5*a*, *b*, *d*), but on reaching the tricuspid ring and the apex, they are not inserted, but invaginate towards the interior of

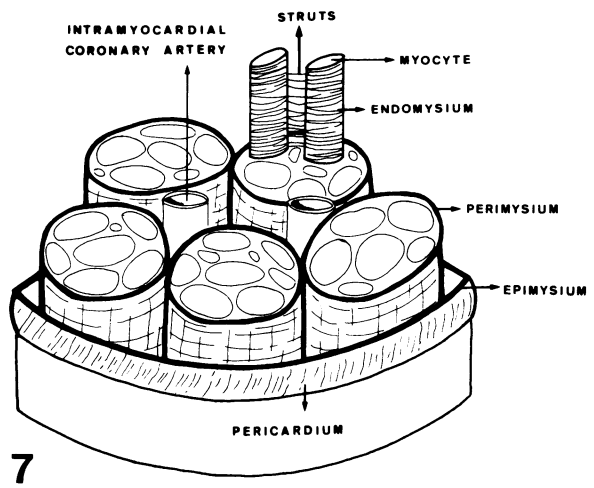

Fig. 7. Schematic representation of the connective tissue in a fragment of the wall of myocardium in the Spanish fighting bull. Note the arrangement of the epimysium, perimysium and endomysium.

the chamber (Fig. 5*d*). The muscle fibres surround the anterior and posterior portions of the pulmonary outflow tract in a sphincter-like manner, but are not inserted and do not invaginate through the fibrous ring (Fig. 5*a*, *c*, *d*).

Deep layer

The deep layer is composed of muscle fascicles that lie mainly vertically in both ventricles (Fig. 6*a*). In the left ventricle, the fascicles of the deep layer arise from the apex, and originate from the invaginated fascicles of the superficial layer. After they invaginate, the muscle fascicles are incorporated into the papillary muscles of the left ventricle, and either are inserted into the chordae tendineae, or ascend vertically along the subendocardial surface until they reach the perimeter of the left atrioventricular orifice and the base of the valvular leaflets of the aortic orifice (Fig. 6*a*). In the right ventricle, the origin of the muscle fascicles of the deep layer is more complex. Some of the fascicles arise from the superficial layer, at the site of its invagination at the apex; however, most deep layer fascicles originate from muscle fascicles of the middle layer that invaginate at the anterior and posterior atrioventricular sulcus (Fig. 5*a*, *d*). Some of these fascicles are continuous with the papillary muscles of the right ventricle; others ascend the subendocardial surface along a mainly vertical course, and are inserted in the perimeter of the right atrioventricular orifice, or in the base of semilunar valve leaflets of the pulmonary artery, where they come to lie obliquely (Fig. 6*b*).

Connective tissue architecture

We were able to visualise the 3-dimensional architecture and arrangement of the connective tissue in different regions of the myocardium of the left and right ventricles by using chemical digestion followed by SEM observation of fractured samples of myocardium. Controlled digestion with NaOH removes cellular elements such as myocytes, elastic fibres and ground substance, leaving fibrillar components (collagen and reticulin fibres) intact. This digestion procedure has been used by Shimada et al. (1986) and Morita et al. (1991) to expose the connective tissue sheaths surrounding Purkinje fibres in the sheep heart.

In the ventricular myocardium of the Spanish fighting bull, the intramyocardial connective tissue

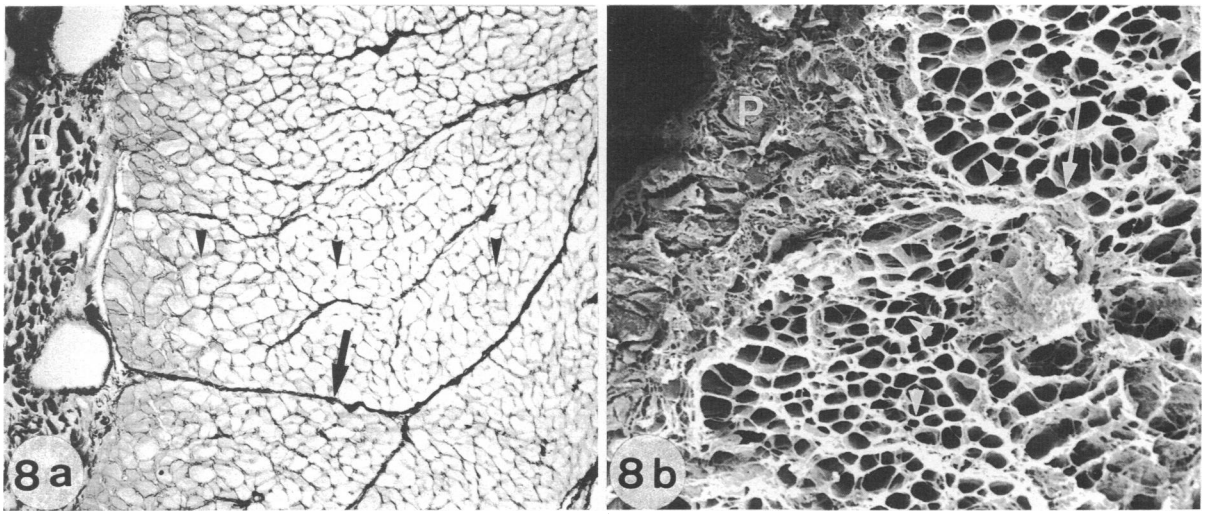

Fig. 8. Transverse section of the right ventricle, (*a*) with silver staining and (*b*) SEM micrograph. Note the arrangement of the connective tissue. Arrowheads, endomysium; arrow, perimysium; P, pericardium. (*a*) ×85; (*b*) ×150.

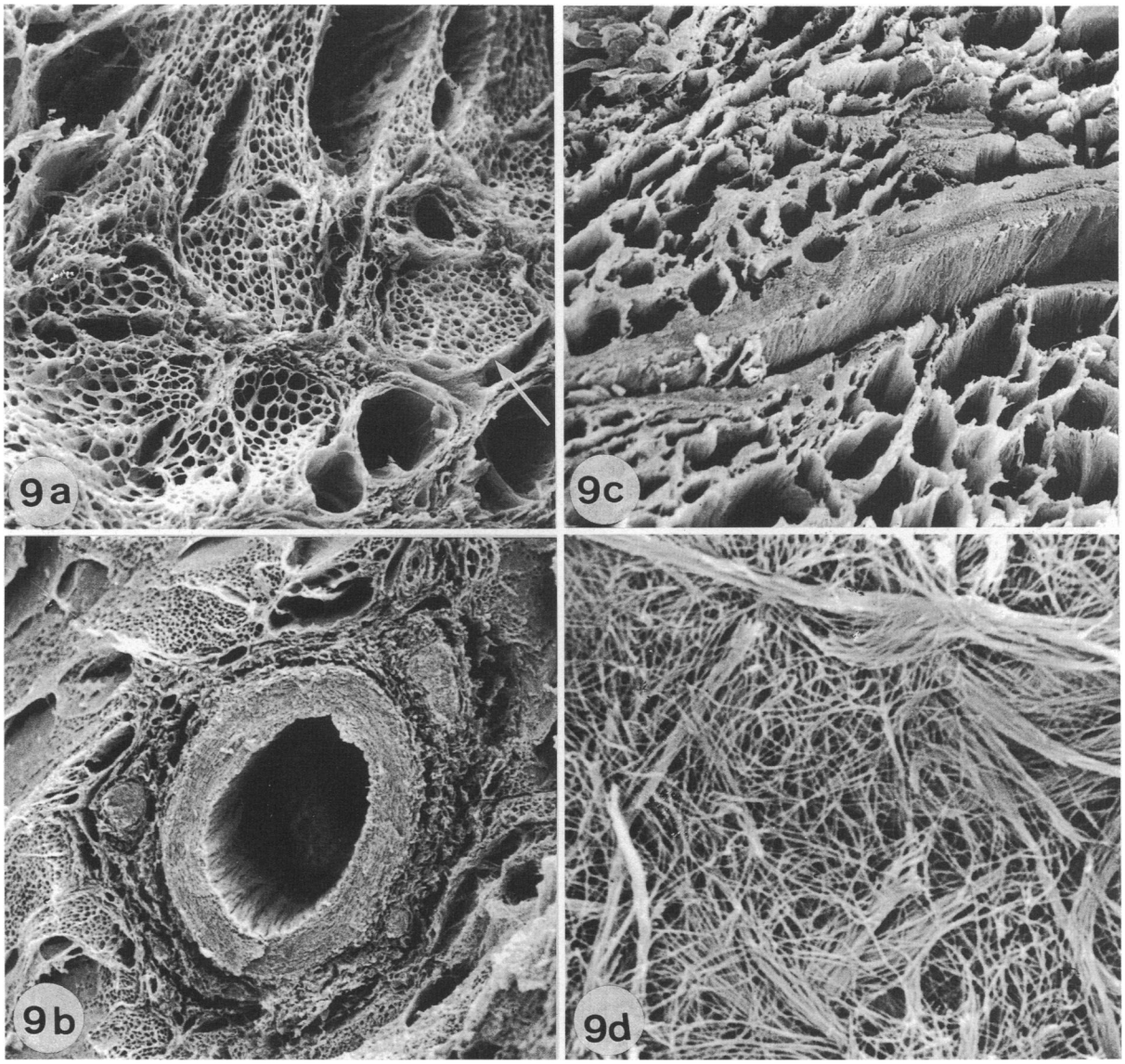

Fig. 9. SEM micrographs of the left ventricle of the Spanish fighting bull. (*a*) Note the perimysium (arrow) surrounding the muscle fascicles and (*b*) blood vessel. (*c*) Structurally, the perimysium is composed of thick sheaths that separate muscle fascicles from each other; (*d*) these sheaths are formed of densely meshed reticular fibrils. (*a*) ×160; (*b*) ×65; (*c*) ×590; (*d*) ×2600.

constitutes a well-defined architectural network that envelops the muscle fascicles and myocytes or cardiac muscle fibres. We distinguished 3 components on the basis of their distribution and organisation within the ventricular walls: epimysium, perimysium and endomysium. As shown in Figure 7, the epimysium comprises a sheath of connective tissue enveloping the myocardium and separating it from the epicardium and endocardium. Fibrillar tracts arise from this sheet and enter the thickness of the myocardium to form the perimysium (Fig. 8*a*, *b*). The second component forms a skeletal framework of connective tissue that supports the muscle fascicles (Fig. 9*a*) and blood vessels (Fig. 9*b*). Like the muscle fascicles, the perimysium is organised in 3 main directions within the ventricular walls. In structural terms the perimysium is composed of thick sheaths (Fig. 9*c*) formed of densely meshed reticular and collagen fibrils running in various directions (Fig. 9*d*) and forming connective tissue septae between the muscle fascicles.

The perimysium in turn gives rise to the 3rd component, the endomysium, which constitutes a skeletal framework enveloping each cardiac myofibre (Fig. 10*a*, *c*). This framework differs in arrangement and organisation in the left and right ventricles. In the latter, it is composed of a loose network of reticular and collagen fibrils of predominantly circular arrangement covering the surface of the myocyte (Fig. 10*a*). Each framework is connected to the neighbouring myocyte by thin closely arranged tracts that

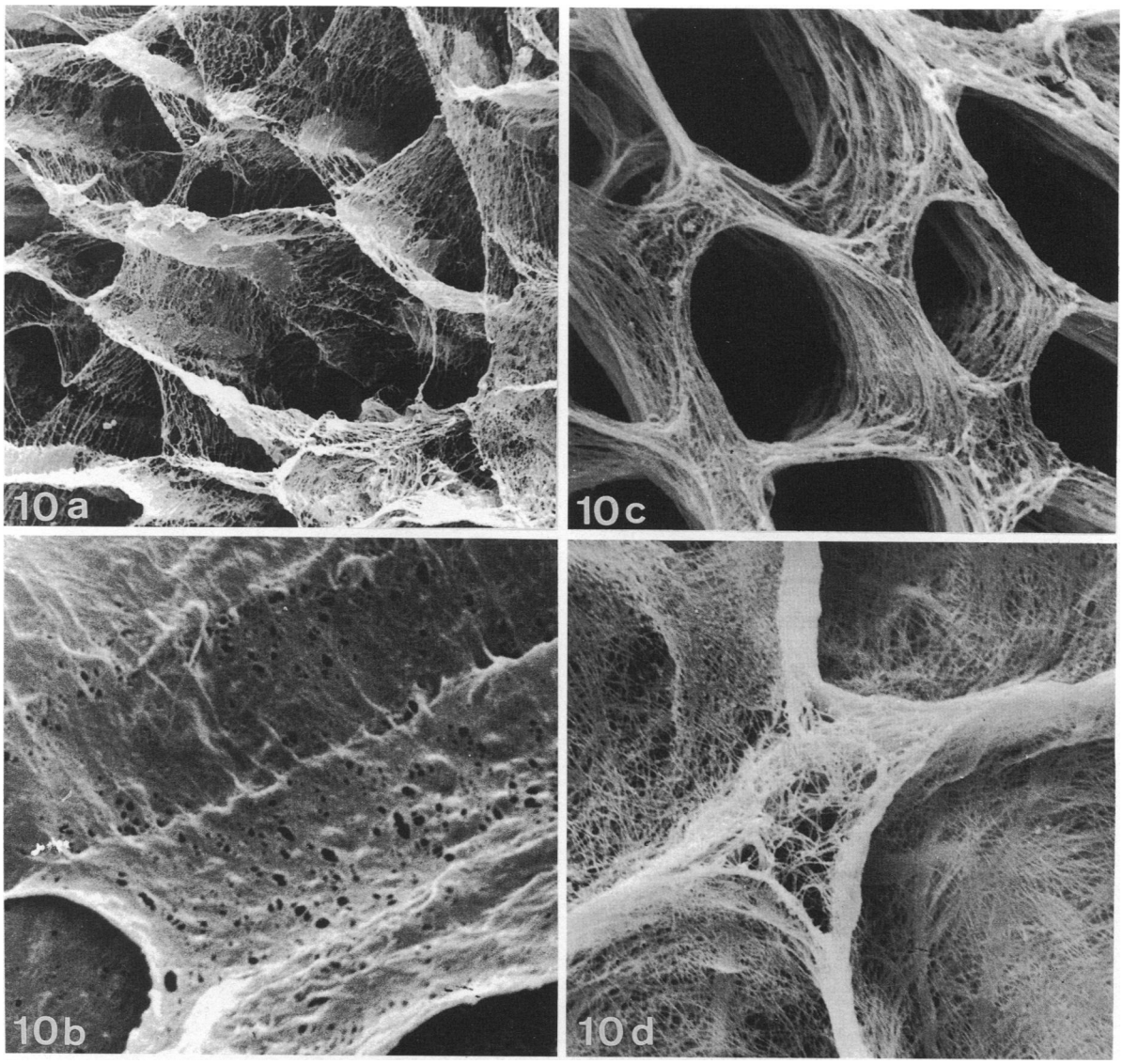

Fig. 10. SEM micrographs of the heart of the Spanish fighting bull showing endomysium surrounding the myocytes of (*a*) the right ventricle and (*c*) the left ventricle. The struts are arranged as transverse bridges in the right ventricle (*b*); in the left ventricle the struts lie in various directions (*d*). (*a*) ×1300; (*b*) ×1950; (*c*) ×1250; (*d*) ×2000.

form transverse bridges (struts) between myocytes (Fig. 10*b*). In the left ventricle, the framework is denser, and vertical fibrillar tracts are intermingled with circular tracts (Fig. 10*c*, *d*). Each framework is connected by thin transverse tracts and are more complex in arrangement, comprising fibrillar tracts that crisscross in different directions (Fig. 10*d*).

DISCUSSION

Most observations published to date on the architecture of muscle fibres in the ventricular wall were obtained by gross dissection (Pettigrew, 1864; Mall, 1911; Robb & Robb, 1942; Torrent-Guasp, 1980); other studies have been based on findings in histological sections (Kashyap, 1975; Greenbaum et al. 1981; Maron et al. 1981), and some have combined both procedures (Fernandez-Terán & Hurlé, 1982). However, different investigators have provided varied interpretations as to muscle fascicle patterns, even when computer-based methods of 3-dimensional reconstruction of serial semithin sections were used (McLean & Prothero, 1991). Despite these findings, new ideas are necessary to provide a precise morphological underpinning for, and better understanding of, cardiac biomechanics. In the present report, we used gross dissection and histological sections to elucidate the spatial orientation (architecture) of the muscle fascicles in the ventricles of the Spanish fighting bull heart, and also used histological sections and NaOH digestion followed by SEM examination to

describe the intramyocardial connective tissue architecture. In addition, we have attempted to correlate our observations of connective tissue architecture with the arrangement of the muscle fascicles.

Most authors agree that it is inappropriate to speak of the superimposition of anatomically different planes in the ventricular wall, as did Pettigrew (1864), who distinguished 7 layers in the yearling calf heart, and Mall (1911), who studied pig hearts. Instead, the muscle fascicles are said to show preferential spatial orientations within the ventricular wall (Streeter, 1979), without fibrous cleavage sheets of intramyocardial connective tissue between the superficial, middle and deep layers (Greenbaum et al. 1981; Fernandez-Terán & Hurlé, 1982). We observed a superficial layer encompassing both ventricles, in which the fascicles were mostly oriented in a vertical direction, and were inserted at the level of the fibrous skeleton of the heart (mainly at the trigones). The fascicles coursed toward the apex, where they invaginated to give rise to the deep or subendocardial layer. The fascicles in this vertically oriented layer were inserted into the chordae tendineae and in atrioventricular and arterial fibrous rings.

In many specimens we observed variations in the pattern of muscle fascicle arrangement in the superficial layer. The relatively high incidence of these variations suggests that they are unlikely to reflect pathology in the Spanish fighting bull, as all animals were of the same age and of similar size and body weight when killed. The variations we noted in the ventricular myoarchitecture may be compatible with normal heart functioning, as has been reported in the fish heart (Sánchez-Quintana & Hurlé, 1987).

The preferentially vertical arrangement of the muscle fascicles in the superficial and deep layers at the level of the fibrous arterial rings and the base of the semilunar valve leaflets suggests that the muscle fibres are actively involved in the dynamics of these valves. When the superficial fascicles contract, they may exert tension on the fibrous rings, a mechanism that may account for the presystolic valvular opening described by Thubrikar et al. (1979) and Deck et al. (1988) in the aortic valve. The deep fascicles may increase the tension on the fibrous core of the leaflets, thus helping to keep the semilunar valve leaflets from flattening against the sinus wall during ventricular ejection and permitting the formation of vortices in the ventricular cavities, an essential process for normal valvular function (Bellhouse & Bellhouse, 1968; Peacock, 1990).

In the heart of the Spanish fighting bull, a separate middle layer of muscle fascicles was seen in each ventricle. Anatomical dissection and histological sections failed to reveal insertions into the fibrous pulmonary artery or atrioventricular rings. Instead, the fascicles of the middle layer were arranged in a circular pattern around these orifices, and then invaginated towards the interior of the ventricular cavity. The exception to this pattern occurred at the level of the pulmonary artery ring, where the muscle fascicles did not invaginate and were arranged in a sphincter-like manner along the pulmonary artery outflow tract. These observations contrast with the results of dissections in other mammals such as the ox (Robb & Robb, 1942; Anderson & Becker, 1980; Torrent-Guasp, 1980; Greenbaum et al. 1981), in which no discrete middle layer was distinguishable in the right ventricle. However, in man a middle layer proper was found in a malformed heart with right ventricular hypertrophy (Sánchez-Quintana et al. 1990), an observation that raises the possibility that the variable presence or absence of the middle layer in mammals reflects an adaptation of the developing ventricles to the functional demands of the heart. Moreover, the circular arrangement of the muscle fascicles middle layer at the level of the aorticomitral orifice provides morphological support for the finding that the diameter of the aortic orifice is greatest at the start of systole, and decreases as this phase progresses (Thubrikar et al. 1980). Contraction of these muscles fascicles may be responsible for this decrease in diameter.

Previous studies of the developing postnatal and adult heart in mammals have used silver and picrosirius red staining (Robinson et al. 1983; Factor & Robinson, 1988; Sánchez-Quintana et al. 1991) to demonstrate the presence of different levels of organisation of the intramyocardial connective tissue. The epimysium is the connective tissue sheath that surrounds the entire muscle; the endomysium surrounds individual myocardial cells, and the perimysium connects the epimysium to the endomysium, and surrounds groups of muscle fascicles. With the techniques used in the present study, we observed the 3-dimensional architecture of the reticular and collagen sheaths that surround the myocardial cells, muscle fascicles and blood vessels in the Spanish fighting bull heart. Our findings showed that in both the left and right ventricles, the perimysium is composed of thick sheaths, whose reticular and collagen fibrils are woven into a compact felt-like texture. Like the muscle fascicles, these fibrils are arranged mainly in 3 directions within the ventricular wall. It is widely agreed that the contents of the connective tissue in the mammalian heart vary

according to species, region, age and pathological state (Borg & Caulfield, 1981; Caulfield, 1983; Robinson et al. 1983). These data suggest that the sheaths of the perimysium may modify the propagation of transverse impulses among muscle fascicles, as Dolber & Spach (1987) proposed in the ventricular papillary muscles of the dog and rabbit. As a consequence, the perimysium may influence the order of fascicle contraction in the different muscle planes. Alternatively, the thick sheaths may provide mechanical support for the intramyocardial coronary vessels, protecting them from external forces.

In contrast to the perimysium, the endomysium shows structural differences between the 2 ventricles. In the right ventricle, the myocardial cells were surrounded by a thin sheath consisting of looser networks of reticular and collagen fibrils, which were connected by thin lateral tracts (called struts by Borg & Caulfield, 1981). In the left ventricle, these networks are denser, and the struts are intermingled in a more complex pattern with myocytes. Although the role of the endomysium in myocardial function is not clear (Price, 1984; Weber, 1989), it is reasonable to assume that the different characteristic architectures of this structure reflect different functional aspects of the left and right ventricles and that a particular arrangement of the sheaths and struts can be induced in response to increasing pressure and volume of the left ventricle in comparison with the right. This hypothesis is supported by the absence of the endomysium in species with wholly trabeculated ventricles (Factor & Robinson, 1988).

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