Contents

Chapter 1

Introduction

Electrocardiography of the normal T wave:

On a twelve lead electrocardiogram (ECG) a normal T wave is the result of repolarization of ventricular muscle fibers from an active to a resting transmembrane voltage [1]. The voltage changes of the repolarization process traverse the same range as those of the depolarization process, except in the reverse direction, and therefore the T wave may be viewed as the final result of a reversed depolarization process [1]. On the normal human ECG the net areas of the QRS complex and the T wave in any lead are neither characteristically equal, nor opposite in direction [2]. This means that the sequence of ventricular depolarization and repolarization are not in the same direction, but opposite, resulting in an average mean electrical axis of the QRS complex of +60° and for that of the T wave only about 10-12° to the left of this [2].

Three electrocardiographic features of the T wave are noted. The direction (or polarity), the shape (or contour) and the height (or amplitude) [3]. On the normal human ECG the direction (or polarity) of the T wave is as follow [3]: upright in leads I, II and V3-V6; inverted in lead aVR and variable in leads III, aVL, aVF and V1-V2. The shape (or contour) of the normal T wave is normally slightly rounded and asymmetrical [3]. The height (or amplitude) of the normal

T wave does not exceed 5 mm in any standard lead and 10 mm in any precordial lead [3].

The Tp wave, formerly called the Ta wave, represents the repolarization process in the atria and this wave is opposite in direction (polarity) to the P wave [3]. The repolarization process in the atria differs from that in the ventricles. In the ventricles repolarization proceeds in a direction opposite to that of depolarization, yielding a T wave with the same polarity as that of the QRS complex [3]. However, in the atria repolarization proceeds in the same direction as that of depolarization, yielding a Tp wave with a polarity opposite to that of the P wave [3].

T waves can undergo changes in their polarity, amplitude and/or contour [4]. For many years T wave changes were classified according to the Wilson formulation as either primary or secondary in nature and furthermore, it was firmly believed that this classification could explain all possible T wave abnormalities [5]. According to the Wilson formulation secondary T wave changes is the result of changes in the preceding QRS complex, and these QRS complex changes are the result of an altered sequence of ventricular activation [5, 6, 7]. Secondary T wave changes are thus the result of an altered sequence of ventricular activation and are not related to changes/ pathology of ventricular muscle [5, 6, 7]. Primary T wave changes are not associated with changes in the preceding QRS complex and are caused by changes/ pathology of the

ventricular muscle [2] . However, all T wave abnormalities are indicative of a disturbance in ventricular repolarization [8].

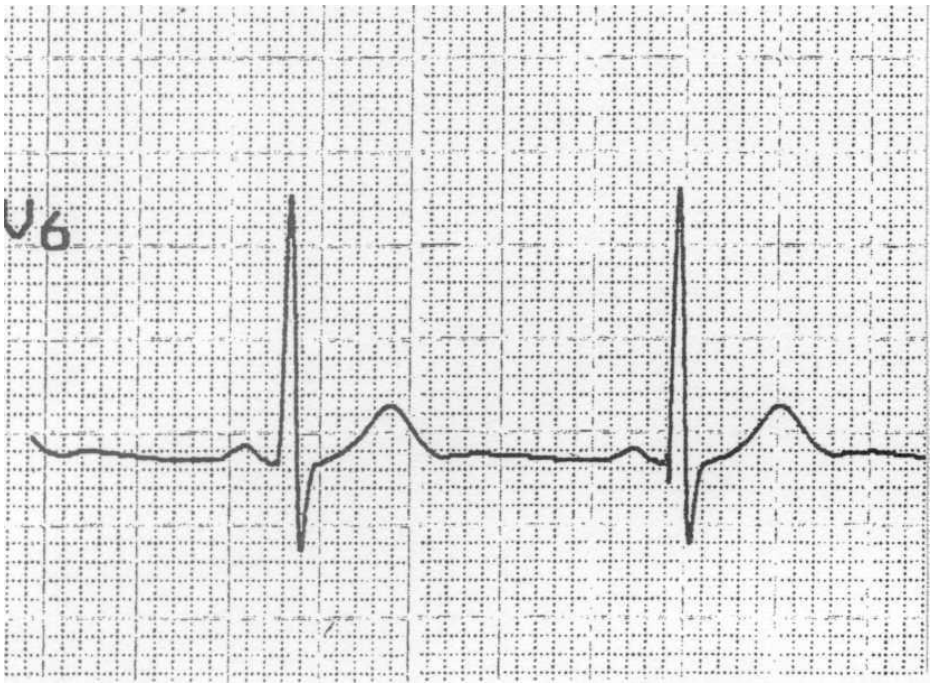

Figure 1. This is an example of normal T waves, taken from lead V6 of a 12-leaded electrocardiogram. Note the positive polarity of the T waves with amplitudes of 0.35 mV. Note the morphology of the T waves: the ascending limbs are asymmetric relative to the descending limbs (The ECG tracing is the property of the author).

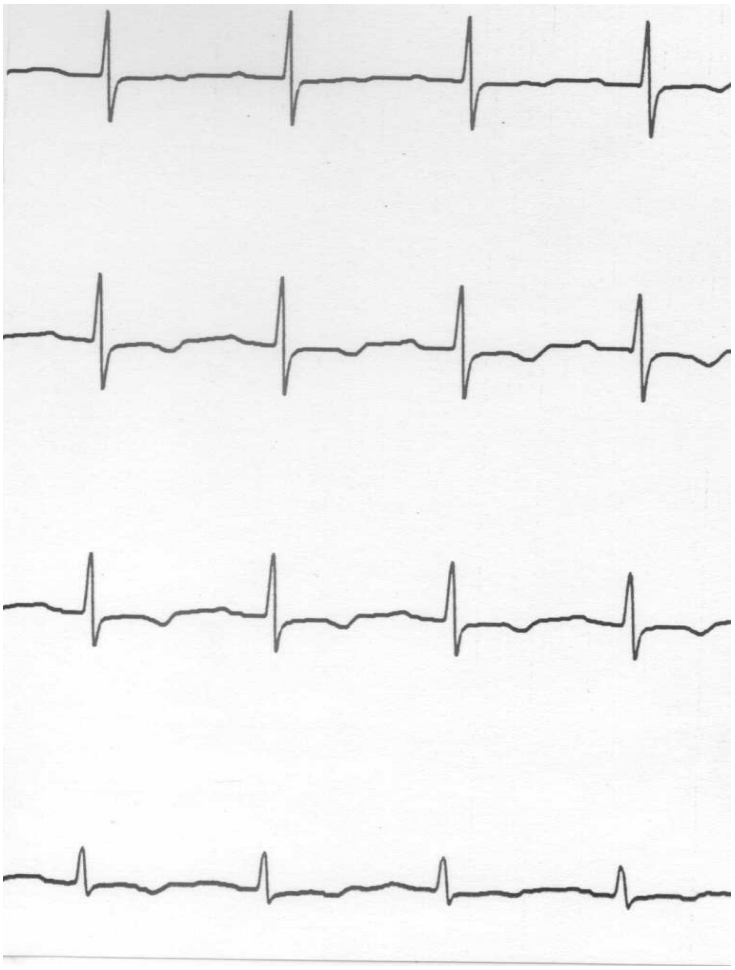

Figure 2. This is an example of primary T wave abnormalities. Note the normal preceding QRS complexes, which are followed by inverted T waves (The ECG tracing is the property of the author).

Primary T wave abnormalities can be caused by one of two mechanisms [8]. Firstly, by a uniform and secondly by a nonuniform alteration of the shape and/or duration of ventricular action potentials [8]. Clinically, primary T wave abnormalities are divided into a functional and an organic group [8, 9]. However, this division is based on a clinical evaluation of the subject and not on any electrocardiographic characteristics [8]. Functional T wave

abnormalities can be distinguished from the organic group by the administration of oral potassium salts, which has been shown to correct the former [9].

The first category of primary T wave abnormalities, those due to uniform alteration of the shape and/or duration of ventricular action potentials, can be recognized electrocardiographically by alterations (more specifically depression) of the ST segment[8]. Causes include drugs, such as cardiac glycosides, quinidine, procainamide, disopyramide, amiodarone and phenothiazines, and electrolyte disturbances, such as hyper- and hypokalemia and hypocalcemia [8]. The second category of primary T wave abnormalities, those due to nonuniform alterations of the shape and/or duration of ventricular action potentials, include T wave abnormalities due to post-ischaemic changes, pericarditis, acute cor pulmonale, pulmonary embolism, myocardial tumors, myocarditis and other primary myocardial diseases [8]. Characteristically these abnormalities do not affect the course or duration of the ST segment [8].

Secondary T wave abnormalities can be found during any condition of an altered sequence of ventricular activation (depolarization) [8]. These conditions include ventricular hypertrophy, bundle branch block, ventricular pre-excitation and during periods of ventricular pacing or ventricular ectopy [8]. In patients with primary myocardial diseases T wave abnormalities are usually secondary to alterations in the duration, amplitude or axis of the preceding

QRS complex which are due to ventricular hypertrophy, but sometimes primary T wave abnormalities can precede these QRS complex alterations [8]. Finally, T wave abnormalities can also be the result of various combinations of these primary and secondary mechanisms [8].

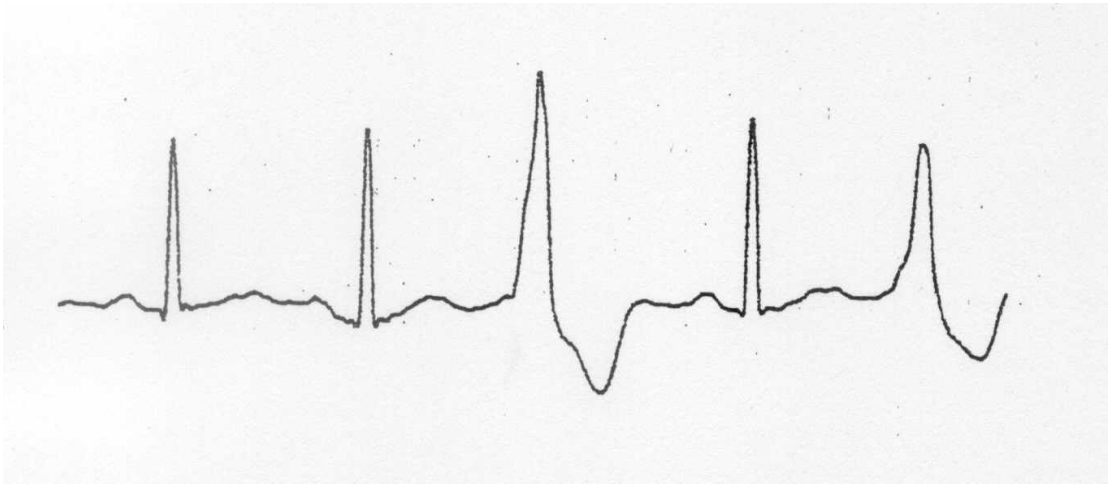

Figure 3. This is an example of a secondary T wave change.The third and fifth beats in the tracing are premature ventricular complexes (PVC's). Note the abnormal QRS complexes of the PVC's, which are followed by inverted T waves—classic secondary T wave changes (The ECG tracing is the property of the author).

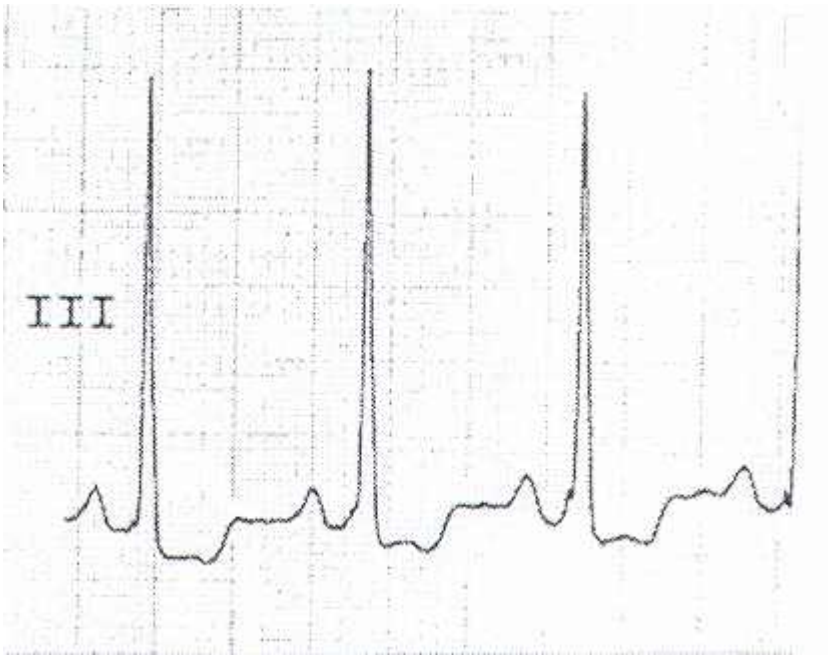

Figure 4. This is an example of ventricular preexcitation (Wolff-Parkinson-White syndrome). Note the delta waves in every QRS complex, which is followed by secondary ST depression and secondary T wave changes (The ECG tracing is the property of the author).

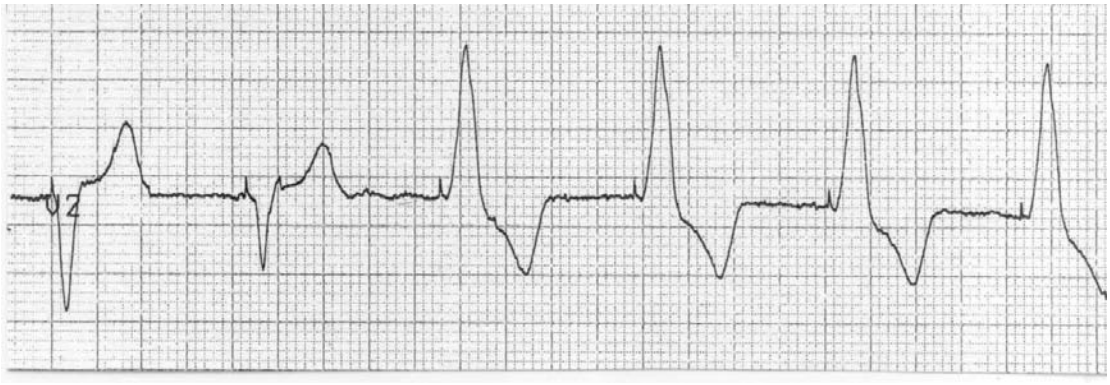

Figure 5. An example of ventricular pacing. Note the pacemaker spikes in front of every QRS complex. Ventricular pacing causes abnormal QRS complexes, which are followed by secondary T wave changes (The ECG tracing is the property of the author).

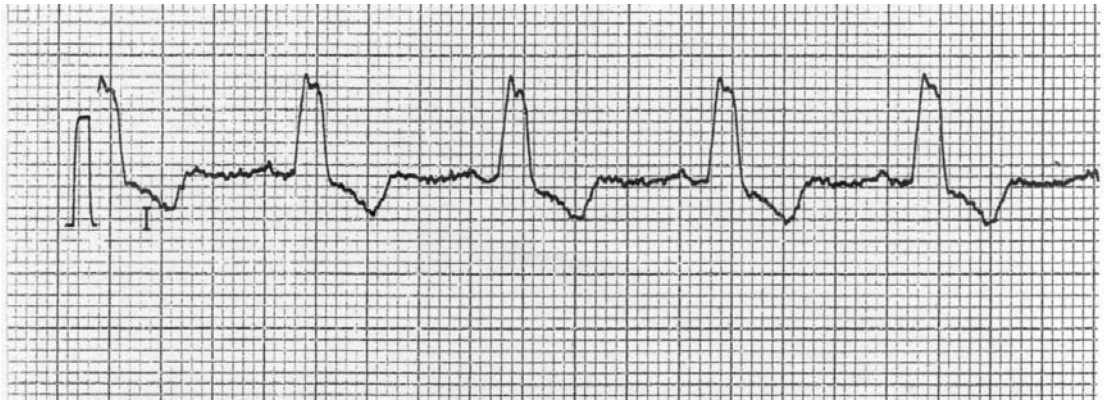

Figure 6 An example of a left bundle branch block, another cause of secondary T wave changes. Note the abnormal and broad QRS complexes, which are followed by inverted T waves—classic secondary T wave changes (The ECG tracing is the property of the author).

However, during the years following Wilson's formulation it became quite clear that not all T wave abnormalities could be satisfactorily classified as either primary or secondary in nature [5].

1. Conditions associated with T-wave changes that cannot be explained by Wilson's formulation:

Since Wilson's formulation in 1943 it has been observed that several conditions can be associated with T wave changes that appear to be primary, but without any manifestations of myocardial pathology [5, 6]. Therefore, there emerged a third category of T wave changes, known as "pseudoprimary" T wave changes [6].

1.1 Wolff-Parkinson-White syndrome

Wolff, Parkinson and White were the first to describe an electrocardiographic syndrome of ventricular preexcitation [10]. In these hearts bypass tracts, which are remnants of embryonic conduction tissue, cause activation to pass directly from the atria to the ventricles without an appreciable degree of delay—bypassing the atrioventricular nodal system, hence the term "preexcitation" [10]. These bypass tracts can be present at several sites around the atrioventricular valve rings and can be single or multiple [10]. Ventricular preexcitation may be permanent or episodic, with constant or intermittent conduction through the bypass tract(s) respectively. During preexcitation ventricular activation spreads through the ventricles by myocardial conduction from the preexcited area until it encounters the normal activation wavefront, which results from atrioventricular nodal

conduction and spread via the specialized conduction system [10]. This results in a fusion beat, which can be seen as a delta wave on the electrocardiogram[10]. Two types of Wolff-Parkinson-White (WPW) syndrome are recognized: type A with left ventricular pre-excitation and type B with right ventricular preexcitation[10].

Two categories of T wave abnormalities can be present in WPW syndrome. Firstly, during periods of ventricular preexcitation there is an altered sequence of ventricular activation and classic secondary T wave abnormalities can be seen. However, in most patients ventricular preexcitation is not permanent. Ventricular preexcitation can remit spontaneously [11] or can be cured by radiofrequency catheter ablation of the accessory pathway(s) [12]. The second category of T wave abnormality in WPW syndrome is seen during periods of normal sinus rhythm, when there is no ventricular preexcitation, for variable periods after which they gradually disappear [11]. These T wave abnormalities can also be seen after permanent cure of preexcitation by radiofrequency catheter ablation of the bypass tract(s) [12, 13, 14]. Furthermore, these T wave changes in the ablation group do not correlate with any markers of tissue injury [12] and are thus not primary T wave changes. They are seen during periods with a normal ventricular activation sequence and are thus also not secondary T wave changes. They are regarded as so-called “pseudoprimary” T wave changes [6].

Currently, these T wave changes are regarded to be the consequence of "cardiac memory" [12, 13, 14]. The concept of cardiac memory will be discussed in detail later on (page 21). These pseudoprimary T wave changes, or cardiac memory T waves, can also be seen after right ventricular pacing, periods of intermittent left bundle branch block, paroxysmal tachycardia and premature ventricular complexes [12].

1.2 T Wave abnormalities following periods of intermittent left bundle branch block

Left bundle branch block (LBBB) can be the result of conduction delay (or block) of the left bundle branch of the specialized conduction system and can be the result of disease in the main left bundle branch or fibers of the bundle of His (predivisional) or in each of the two fascicles, or in any of several sites in the intraventricular conduction system (postdivisional) [15, 16]. LBBB produces a prolonged QRS duration, marked distortion of the QRS complex and ST-T wave abnormalities [15, 16]. Commonly accepted diagnostic criteria for LBBB are as follow [15]: QRS duration > 120 msec; broad, notched R waves in the lateral precordial leads (V_5, V_6) and usually also leads I and aVL; small or absent initial r waves in the right precordial leads (V_1, V_2) followed by deep S waves and absent septal q waves in left-sided leads.

Normally the interventricular septum is activated from left to right and this results in an initial r wave in the right precordial leads (V_1, V_2) and a q wave in leads I and aVL, as well as the left precordial leads (V_5, V_6) [16]. During periods of LBBB the septum is activated from right to left and the result is the disappearance of initial r waves in leads V_1 and V_2 and the disappearance of q waves in leads V_5 and V_6 [16]. The ventricular activation front then proceeds from the left side of the interventricular septum to the anterior wall of the left ventricle, then to the inferior wall and from there to the postero-lateral free wall [16]. This altered sequence of ventricular activation during LBBB produces two electrocardiographic abnormalities [15, 16]. The first is monophasic QRS complexes: QS in lead V_1 and R in leads I, aVL and V_6 [16]. The second is secondary repolarization abnormalities [15, 16]. In most cases the ST segment and T wave are discordant with the QRS complex—the ST segment is depressed and the T wave is inverted in leads with positive QRS waves (leads I, aVL, V_5 and V_6), while there is elevated ST segments and positive T waves in leads with negative QRS complexes (V_1 and V_2) [15]. According to Wilson's formulation these T wave changes are secondary, as there is an altered sequence of ventricular activation [5, 6, 7].

Rosenbaum *et al* were the first to describe symmetrical T wave inversions during periods of normal conduction in 26 of 35 patients with intermittent left bundle branch block [17]. Denes *et al* confirmed

this finding and documented a high prevalence (83%) of deep, symmetrical, precordial T wave inversions during normal conduction in patients with intermittent left bundle branch block [17]. In that same year (1978), Engel *et al* also described a series of patients with T wave inversion during normal conduction after a period of LBBB [18]. These T wave abnormalities occurred during normal conduction and are thus not secondary T wave changes. Most of these patients had no organic heart disease and therefore, these T wave changes are also not primary. Therefore, these are another example of "pseudoprimary" T wave changes and Denes *et al* became the first authors to refer to the concept that the heart muscle can "remember" periods of abnormal ventricular activation, causing abnormal repolarization to persist beyond the period of abnormal ventricular activation[17].

1.3 T wave abnormalities following periods of paroxysmal tachycardia

Inversion of T waves on the electrocardiogram may persist for long periods after an episode (or episodes) of paroxysmal tachycardia in the absence of organic heart disease[19]. This phenomenon will occur in about 20% of patients with episodes of paroxysmal tachycardia of both ventricular and supraventricular origin, and is totally unrelated to the

age of the patient or the presence or absence of organic heart disease [8, 19, 20, 21, 22, 23].

Once again, these T wave abnormalities are present during normal conduction and are therefore not secondary. They can occur in the absence of organic heart disease and are thus also not primary. Therefore, the post-tachycardia syndrome, consisting of T wave inversion after an episode (or episodes) of supra- or ventricular tachycardia is another example where the heart muscle "remembers" periods of altered ventricular activation [5].

1.4 T wave abnormalities following periods of ventricular pacing

In 1958 artificial cardiac stimulation by way of implantation of a permanent pacing system was introduced as a treatment for patients with complete heart block [24, 25]. Since that time both the indications for cardiac pacing, as well as the complexity of pacing-system design have expanded [25]. In transvenous ventricular pacing systems the lead tip is positioned in the right ventricle and during ventricular stimulation the ECG will demonstrate a left bundle branch block pattern, as ventricular activation is now initiated in the right ventricle [24].

During right ventricular pacing the ECG will demonstrate a left bundle branch block pattern and therefore secondary T wave abnormalities will be observed during the period of pacing [15, 16]. Once again, these T wave changes are secondary, because there is an alteration of the normal sequence of ventricular activation—Wilson's formulation [5, 6, 7]. However, it is well known that T wave inversions can occur in the unpaced ECG subsequent to ventricular pacing and that these T wave abnormalities can persist for variable periods once ventricular pacing is terminated [5, 26, 27]. Therefore, ventricular pacing is another example of a situation where the heart muscle "remembers" periods of altered ventricular activation[5].

1.5 T wave abnormalities following premature ventricular complexes

Premature ventricular complexes (PVCs), also known as ventricular extrasystoles, are the result of premature depolarization of the myocardium below the bifurcation of the bundle of His (distal to the atrioventricular junction) [28]. PVCs may have their origin from either of the two ventricles, the interventricular septum or the fascicular system [28, 29]. Electrocardiographically they are recognized by the premature occurrence of a QRS complex that is bizarre in shape with a duration exceeding that of the dominant QRS complex (typically greater than

120 ms) with a large T wave, opposite in direction to that of the major deflection of the QRS complex [30]. However, PVCs may also have narrow QRS complexes if they originate from a point equidistant from each ventricle in the interventricular septum or high in the fascicular system itself [29].

PVCs lead to an alteration in the sequence of ventricular activation and therefore, classic secondary T wave abnormalities are seen in a PVC [31]. However, PVCs also lead to changes in both the amplitude and polarity of the T waves of sinus beats following PVCs [32, 33, 34, 35, 36, 37]. This phenomenon was first described by White in 1915 [32]. These are T wave abnormalities of normal sinus beats after the PVC and are therefore not secondary T wave abnormalities. These post-extrasystolic T wave changes can follow one of two patterns: a subepicardial or a subendocardial pattern [35]. The majority (more than two thirds) of post-extrasystolic T wave changes follow a subepicardial pattern, where there is a decrease in the amplitude or even total inversion of the T wave of sinus beats following the PVC [35]. The remaining third follow a subendocardial pattern, where there is ST-segment depression with less pronounced inversion, or even an increase in amplitude of the T wave of post-extrasystolic sinus beats [35].

These post-extrasystolic T wave changes can persist for variable periods and is another example where the heart muscle "remembers" periods of altered ventricular activation [5, 32, 33, 34, 35, 36, 37].

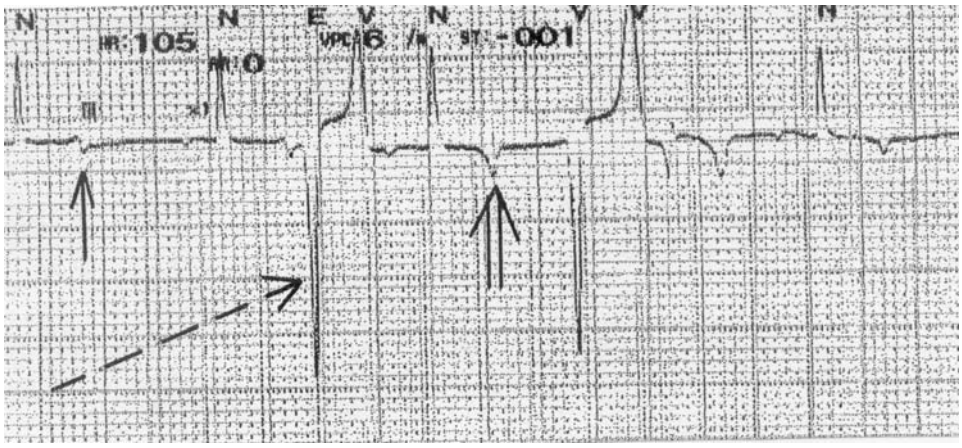

Figure 7. An example of cardiac memory. The third and fifth beats are PVC's. Note the bifid T wave (arrow) before the PVC (broken arrow). The T wave of the first normal beat after the PVC (double arrow) is inverted. In this instance the T wave of the first normal beat after the PVC "remembers" the direction of the abnormal QRS complex, therefore the term "cardiac memory" (The ECG tracing is the property of the author).

2. Cardiac Memory:

Memory is a property of several biological systems, such as the brain, the gastrointestinal tract and the immune system [6, 38]. It is now quite clear that the heart also remembers and this memory is seen electrocardiographically in the T wave [6]. As stated previously, for many years it has been assumed that Wilson's formulation, classifying all T wave changes as either primary or secondary in nature, covers all

possible T wave abnormalities occurring on the human electrocardiogram [5]. Secondary T wave abnormalities are the result of an altered sequence of ventricular activation and are thus dependent on the preceding QRS complex [6]. As discussed previously these are the T wave changes seen during periods of ventricular pacing, left bundle branch block, ventricular pre-excitation and the T wave abnormalities of premature ventricular complexes. Primary T wave abnormalities are independent of the QRS complex and are the result of alterations in ventricular ion channels and/or myocardial pathology [5, 6].

In the years after Wilson published his formulation on T wave changes a third category of T wave change became evident. These are T wave changes seen after periods of altered ventricular activation when normal sinus rhythm has returned. Rosenbaum *et al* coined the term pseudoprimary for these T wave changes [5]. However, these "pseudoprimary" T wave changes actually has characteristics of both secondary (they are dependent on a previous period of altered ventricular activation) and primary T wave changes (they are the result of changes in the ion-channel determinants of repolarisation, as will be discussed shortly) [6]. As already discussed, these T wave changes are seen during normal sinus rhythm after preceding periods of ventricular pacing, ventricular preexcitation, left bundle branch block, paroxysmal tachycardia and premature ventricular complexes.

Rosenbaum *et al* were the first to coin the term “cardiac memory” to refer to the phenomenon where T waves during normally conducted beats seem to “remember” the QRS complex of the previous abnormally conducted beats [5]. Cardiac memory can be characterised in the following way: after a period (or periods) of abnormal ventricular activation, the T wave during subsequent normal sinus rhythm retains the vector of the previously abnormal QRS complex (es) [5, 6, 39, 40, 41, 42]. Furthermore, with recurrent periods of altered ventricular activation these T wave changes may increase in magnitude—referred to as accumulation [5, 6]. When cardiac memory is noted on the ECG, the direction of the T wave(s) is similar to the direction of the QRS complex(es) noted during the period(s) of abnormal ventricular activation and these cardiac memory T waves may be observed after either short or long periods of abnormal ventricular activation and are thus referred to as short- and long term cardiac memory respectively [40]. However, the time period required to separate short- from long term cardiac memory has not been defined at present [40].

The T wave reflects transmural and apico-basal gradients for ventricular repolarisation and certain changes in ventricular ion currents has been described in cardiac memory [43]. T waves are the result of a balance between inward and outward ion currents in individual ventricular myocytes [6]. During the action potential inward current is carried by sodium ions during phase 0, the action potential

upstroke and during the phase 2 plateau [6]. Calcium ions also contribute towards the inward current during the plateau [6]. Outward, repolarising currents are carried by potassium [6]. An initial- and three types of delayed rectifier potassium currents contribute to ventricular repolarisation [6]. The initial, transient, outward (I_{to}) potassium current contributes to the notch during phase 0 of the action potential [6]. Three types of delayed rectifier potassium currents contribute to phases 2 and 3 of the action potential: I_{ks} (slow), I_{kr} (rapid) and I_{kur} (ultra-rapid) [6]. There is also an inward rectifying potassium current (I_{ki}) that contributes to phase 3 [6].

Regional differences in these ionic currents, the resulting action potentials and the temporal sequence of ventricular activation are all factors that affect expression of the T wave, as they create voltage gradients apico-basally, as well as transmurally in the ventricular myocardium [6]. For example, the action potential duration is longer midmyocardial than that in the endocardium or epicardium [6].

Cardiac memory is associated with decreases in I_{to} density and mRNA for Kv4.3 (this encodes the α-subunit of the I_{to} channel protein) [43]. Furthermore, I_{to} kinetics are also altered, such that recovery from inactivation prolongs 20-fold and as a result there is an altered transmural repolarisation gradient [43]. Further evidence for the role of I_{to} in inducing cardiac memory is that it's absence prevents memory

from occurring [43]. This was shown by administering the I_{to} blocking agent, 4-aminopyridine to intact dogs and isolated tissue, after which cardiac memory could no longer be induced [43]. Cardiac memory is also associated with ultrastructural changes in the myocardium: there is a reduction in the density of the gap junctional protein connexin 43 (Cx43) and there is also changes in the distribution of Cx43—from being concentrated at the longitudinal poles of myocytes to a more uniform distribution across the lateral cell margins [43, 44]. These changes are not uniform as they are greater epicardially than endocardially [44]. Together, these molecular and ultrastructural changes contribute to an altered transmural repolarisation gradient with resultant memory T waves.

Periods of altered ventricular activation alters the stress/strain relationship in the myocardium and it is well known that an altered stress/strain relationship increases the release of angiotensin II [43]. Angiotensin II is a known mitogenic molecule and is closely linked to myocardial remodeling, hypertrophy and fibrosis in a variety of cardiac disorders [45, 46, 47, 48]. Therefore, a very important, but as of yet an unanswered question is: might cardiac memory be an initial, electrocardiographic sign of structural myocardial disease to come?

There is no data available on cardiac memory T waves as an electrocardiographic warning for myocardial disease to come. However, current available data suggest that there is a very definite

risk for structural myocardial disease in patients exposed to prolonged and/or repetitive episodes of altered ventricular activation. As these episodes are a cause of cardiac memory T waves, it is a possibility that these memory T waves may serve as a warning to the practising clinician.

The available data on the risk for structural myocardial disease in patients exposed to prolonged and/or repetitive episodes of altered ventricular activation will now be reviewed.

3. Conditions associated with cardiac memory T waves that may cause myocardial disease:

As there is no data available on the possibility that cardiac memory T waves may be an electrocardiographic warning of pending myocardial disease, the available data on the five conditions associated with cardiac memory T waves and all the possible myocardial diseases associated with these five conditions: ventricular preexcitation (Wolff-Parkinson-White syndrome), left bundle branch block, paroxysmal tachycardia, ventricular pacing and frequent premature ventricular contractions will be reviewed.

3.1 Myocardial diseases associated with ventricular preexcitation

Wolff-Parkinson-White syndrome has been associated with various cardiac disorders, such as hypertrophic cardiomyopathy [49, 50], dilated cardiomyopathy [51, 52, 53], histiocytoid cardiomyopathy [54] and peripartum cardiomyopathy [55]. Interestingly, even myocarditis—both atrial and myocardial—has been associated with Wolff-Parkinson-White syndrome [56, 57]. In 1995 Lopez *et al* [56]described a case report of a patient with lymphocytic myocarditis who also has Wolff-Parkinson-White syndrome and in 2001 Basso *et al* [57] in a post-mortem study, demonstrated the presence of focal, active, atrial myocarditis in 50% of a series of Wolff-Parkinson-White patients who died suddenly.

All of the above interesting observations raise the question: “can ventricular pre-excitation lead to myocardial disease?”. If this proves to be the case, can cardiac memory T waves serve as a warning to the clinician performing electrocardiography? To date this question has not been answered.

3.2 Myocardial disorders associated with left bundle branch block

Among patients with established heart disease, especially acute myocardial infarction, the presence of complete bundle branch block (both left and right) is associated with an increase in mortality [58]. What about new and permanent versus old or transient bundle branch block in acute myocardial infarction? Two studies in the thrombolytic era have shown that the development of new and permanent bundle branch block (both left and right) during acute myocardial infarction is an independent predictor of an increase in mortality, whereas an old or transient bundle branch block is associated with only a slight increase in mortality [58, 59, 60].

But what is the situation in the general population? Fahy *et al* [58] found a prevalence for bundle branch block of 0.3% which increased to 1.6% in persons older than 64 years. The Reykjavic study [58] found a prevalence for left bundle branch block of 0.4% in middle aged men, while the Tecumseh study [58] reported a prevalence of bundle branch block of 2.4% in men older than 50 years. In all of these studies an increase in mortality was only observed in patients with established coronary artery disease [58].

In 1998 Eriksson *et al* [58, 61] reported that the prevalence of complete bundle branch block (both left and right) increases from 1% to 17% in

men between 50 and 80 years of age and that there is no relation to coronary artery disease or mortality. However, those who subsequently developed bundle branch block were more likely to develop congestive heart failure than men without bundle branch block [58, 61].

In 2001 Hesse *et al* [62] published a paper, in effect linking data from population studies with those in acute myocardial infarction. They evaluated 7073 patients with either known or suspected coronary artery disease who were referred for nuclear exercise testing. After a mean follow-up of 6 years both left and right bundle branch block were associated with an increase in mortality, even after adjusting for demographic, clinical, exercise and nuclear scintigraphic parameters [58, 62].

However, based on these epidemiological studies it is unclear if left bundle branch block can cause heart disease in healthy individuals. Therefore, let's look at the functional effects of left bundle branch block. Left bundle branch block has profound hemodynamic effects [63]: it leads to asynchronous myocardial activation which may trigger ventricular remodeling, it impairs both systolic and diastolic function and it causes mitral regurgitation which can also trigger ventricular remodeling [63].

In patients with dilated cardiomyopathy and left bundle branch block it is not always clear whether left bundle branch block is the cause or the

consequence of left ventricular dilatation: a classic chicken-egg dilemma [63].

Therefore, there is another gap in the literature: it is unclear whether left bundle branch block can be a cause of myocardial disease and if so, whether cardiac memory T waves can serve as an electrocardiographic warning in that subgroup of patients with intermittent left bundle branch block.

3.3 Myocardial disorders associated with repeated episodes of paroxysmal tachycardia

Exposure of the mammalian heart to repeated episodes of paroxysmal tachycardia can lead to cardiomyopathy, a condition termed tachycardia-induced cardiomyopathy, also known as arrhythmia-induced cardiomyopathy [64, 65, 66, 67, 68, 69]. Tachycardia-induced cardiomyopathy is a partially or totally reversible left ventricular dysfunction after normalisation of the tachycardia [65]. There are two forms [65]: a pure form that occur in apparently normal hearts and the more common form in which there is underlying cardiac disease associated with the tachycardia.

Episodes of paroxysmal tachycardia are a known cause of cardiac memory T waves [8, 19, 20, 21, 22, 23]. Disappointingly, there is no literature examining whether cardiac memory T waves can serve as a warning for impending tachycardia-induced cardiomyopathy.

3.4 Myocardial disorders associated with ventricular pacing

In 1990 Karpawich *et al* [70]examined the hemodynamic, electrophysiologic and histologic complications of right ventricular apical pacing in 12 beagle puppies. They found significant elevations of right atrial and pulmonary artery pressures, as well as alterations in sinus node function and prolongation of ventricular refractory periods in the paced group. Furthermore, they found significant histological alterations in the paced hearts which consisted of myofibrillar disarray, dystrophic calcifications, prominent subendocardial Purkinje cells and various mitochondrial alterations [70]. They concluded that chronic right ventricular apical pacing leads to various adverse cellular changes, which are associated with hemodynamic and electrophysiological deterioration. Right ventricular pacing has also been demonstrated to be associated with hemodynamic deterioration in human adults [70]. In 1999 Karpawich *et al* [71] also performed myocardial biopsies in young, human patients (median age 16 years) who were being paced due to congenital atrioventricular block. They identified the same histological

alterations: myofiber size variation, fibrosis, fat deposition, sclerosis and mitochondrial changes [71].

It has been known for a long time that right ventricular apical pacing alters the sequence of ventricular activation profoundly and results in major interventricular dyssynchrony, delaying left ventricular activation from 30 to 60 msec in normal hearts and up to 180 msec in diseased hearts [72]. In 2001 Tantengco *et al* [73] published a paper, proving that chronic right ventricular apical pacing in young patients is a cause of left ventricular systolic and diastolic dysfunction.

Thus, it is clear that altering the normal sequence of ventricular activation by right ventricular apical pacing is a cause of left ventricular dysfunction and structural alterations. However, once again there is no data on the possibility that cardiac memory T waves, seen during interspersed periods of normal sinus rhythm may be a warning for these structural sequelae.

3.5 Myocardial disorders associated with premature ventricular complexes

According to current knowledge all textbooks on cardiovascular diseases have consensus that in the absence of underlying heart disease, premature ventricular complexes have little significance and treatment is not indicated [28, 29, 30, 31]. However, it is also known that the

presence of premature ventricular complexes (PVC's) in apparently healthy, middle-aged men is associated with an increased incidence of coronary heart disease and a greater risk of subsequent death [30, 74, 75, 76, 77, 78].

The discrepancy is clear: "PVC's occur in many healthy individuals and in the absence of heart disease, there is little or no increased risk [31]. But, in apparently healthy middle-aged men PVC's are associated with an increased incidence of coronary heart disease. Where does this leave the practising clinician ?

Chronic PVC's increase both the total and sudden death rate in patients with chronic heart disease, such as ischemic heart disease, hypertensive heart disease and all of the cardiomyopathies, especially in patients with a reduced ejection fraction [31].

Once again we are faced with a chicken-egg dilemma. PVC's are a very common occurrence in both normal and cardiomyopathic hearts [28, 29, 30, 31]. But can they be a cause of heart disease? Only one publication in the literature addresses this question [81]: Redfearn *et al* [81] presented a case where persistent PVC's from the right ventricular outflow tract resulted in left ventricular dilatation and systolic dysfunction. After successful ablation of the ectopic focus the cardiomyopathy resolved [81]. This case raises the possibility that frequent PVC's may be a cause of

left ventricular dysfunction. Once again, there is no data available on the possibility that cardiac memory T waves may serve as a warning for cardiomyopathy that may be caused by PVC's.

In summary, the available data on the five conditions able to induce cardiac memory T waves (ventricular preexcitation, left bundle branch block, paroxysmal tachycardia, ventricular pacing and premature ventricular contractions) and the evidence linking them to structural myocardial disease was presented. Of the five conditions PVC's are the most common and furthermore, the evidence linking PVC's as a possible cause of cardiomyopathy is the weakest.

PVC's originating from the right ventricle initiate depolarisation from the right ventricle, inducing ventricular asynchrony. They share this feature with right ventricular outflow tract tachycardia and right ventricular pacing. The major difference between ventricular tachycardia, ventricular pacing and PVC's are basically only the frequency of depolarisations. Ventricular tachycardia is defined as more than 3 PVC's with a rate more than 100/min [82] and ventricular pacing is usually set at a physiologic rate of about 70/min.

As discussed before, both ventricular tachycardia and ventricular pacing are known causes of left ventricular structural changes. Therefore, it is very plausible that PVC's, originating from the right

ventricle, can be a cause of left ventricular structural changes. And indeed, a case report was found in the literature where the authors believed that persistent PVC's from the right ventricular outflow tract caused left ventricular dilatation and systolic dysfunction [81]. Once again, there is no data that cardiac memory T waves can serve as an electrocardiographic indicator of myocardial dysfunction arising in such a scenario.

Hypothesis

Cardiac memory T waves can serve as an electrocardiographic surrogate for structural myocardial alteration in the hearts of Dorper sheep.

Null Hypothesis

Cardiac memory T waves can not serve as an electrocardiographic surrogate for structural myocardial alteration in the hearts of Dorper sheep.

Research needed in order to prove this hypothesis

Dorper wethers, aged between 10 and 12 months, were chosen for this project. When asked why Dorper wethers I refer the reader to the statement made by Albert Szentgyörgyi: "Life is a similar process in cabbages and kings; I choose to work on cabbages because they are cheaper and easier to come by." [43] But on a serious note, sheep are a well known animal model for the study of cardiac dysrhythmias and the ovine anatomy makes access to the heart via the internal jugular vein with a catheter, using the Seldinger technique, relatively easy.

In this proposed ovine model, the following will need to be achieved:

- Establish a method to produce consistent and reliable 12-lead, surface electrocardiograms.
- Establish a method to induce PVC's, originating from the right ventricle.
- Document whether these PVC's are in fact able to induce cardiac memory T waves, as cardiac memory has never before been described in the ovine heart.
- Document the normal histological appearance of the ovine myocardium.

- Afterwards, it will be determined whether any structural changes can be seen in the ovine hearts subjected to persistent right ventricular PVC's and if so, if any correlation can be found with cardiac memory T waves.

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Chapter 2

The normal ovine electrocardiogram: A 12-leaded approach

As a non-invasive laboratory procedure for evaluating the heart, electrocardiography is without equal. The technique is simple and reproducible and the record lends itself to repetitive and serial studies.

Sheep are popular among electrophysiologists for the study of various primary and secondary electrophysiological perturbations [1, 2, 3, 4, 5]. However, a search of the Medline and Premedline databases, between 1966 and 2002, did not reveal any literature describing a practical method of obtaining a 12-lead electrocardiogram (ECG) with a six channel electrocardiograph in sheep. Using the standard limb and unipolar leads with a one channel electrocardiograph Pretorius *et al* could not reproducibly demonstrate a constant electrocardiographic tracing [6]. However, by moving Einthoven's triangle from the frontal to the sagittal plane, by the moving the standard limb and unipolar electrodes, Schultz *et al* were able to demonstrate a constant electrocardiographic tracing [7]. This study, however was done with a one channel electrocardiograph and constancy was only demonstrated in the standard limb and unipolar electrodes.

A practical and reliable method for obtaining a 12-lead electrocardiographic tracing in sheep is essential for two reasons. First, an ovine model can be utilized in various cardiovascular experimental protocols and second, the hearts of sheep are affected in several plant poisoning syndromes in South Africa with obvious economic importance to farmers [6, 7] and if it can be shown that a reliable electrocardiographic tracing can be utilized to detect such poisoning syndromes it will have importance to such farmers.

In order to be able to utilize an ovine model in proving or rejecting the hypothesis it is therefore essential that a practical, reliable and reproducible method of performing a 12-lead ECG with a six channel electrocardiograph is obtained. The purpose of this study therefore was to develop such a method of obtaining electrocardiographic tracings with a six channel electrocardiograph.

Materials and Methods

This study was performed with the approval of the ethics committee of the University of Pretoria's Biomedical Research Centre.

15 clinically normal Dorper wethers, all between the ages of 10 and 12 months, and weighing between 35 and 40 kg were used in this study. They were fed on lucerne hay *ad libitum*, received 300g/day of pelleted concentrate (10 MJ ME/kg DM with 14% crude protein) and had free access to water at all times.

The sheep were sedated and placed in the right lateral decubitus position (Figure 2.2). There were two groups: In the first group (n=8), the sheep were sedated with ketamine hydrochloride (Brevinaze) at a dose of 100 mg IM once only and in the second group (n=7) all the sheep were sedated with midazolam (Dormicum) at a dose of 30 mg IM once only. After an interval of approximately 10 minutes in every case there were no spontaneous movements and the sheep were placed in the right lateral decubitus position. This method of sedating the animal made it possible for one person to perform electrocardiography without any help and it also eliminated all limb movements which will cause disturbances on the electrocardiographic tracing. The reason for having two groups regarding anaesthetic agent was to evaluate the possibility that the choice of anaesthetic agent will have a major modifying effect on the ECG tracing.

In an attempt to reduce the ECG variations encountered in normal animals, Einthoven's triangle was moved from the frontal to the sagittal plane as previously described by Schultz *et al* [7] by moving the standard and unipolar limb electrodes as follow:

(a) The right arm lead was moved from the right fore limb to the head between the ears (aVR).
(b) The left arm lead was moved from the left fore limb to the dorsum of the sacrum (aVL).

(c) The left leg lead was moved from the left hind limb to the sternal angle (aVF).

(d) the earth electrode was placed on the right hind leg, just above the hock.

The six precordial electrodes were placed as follow:

(a) V1: Placed 7 cm to the right of the sternal angle.

(b) V2: Placed 7 cm to the left of the sternal angle.

(c) V3: Placed 4.5 cm below and 1 cm to the left of V2.

(d) V4: Placed 4.5 cm below and 1 cm to the left of V3.

(e) V5: Placed 4.5 cm below and 1 cm to the left of V4.

(f) V6: Placed 4.5 cm below and 1 cm to the left of V5.

This specific positioning of the six chest leads was arbitrary as we wanted to demonstrate reproducibility and constancy in the morphology of the electrocardiographic tracings between all 15 sheep and in the right lateral decubitus position this specific positioning of the leads is easily obtained.

The electrodes used in all 12 leads were Meditrace 200, disposable ECG, conductive, adhesive electrodes. The skin areas where ECG electrodes were placed were shaven and the electrodes secured to the skin with Super Glue (Bostik). A 12-lead electrocardiogram was then performed in every case with a

Schiller AT-2 plus 6-channel electrocardiograph. The paper speed was set at 25 mm/s and calibrated at 1 mm height = 0.1 mV.

Results

The electrocardiographic tracings of all 15 sheep demonstrated the same constant configuration of the precordial leads (V1 – V6).

During normal sinus rhythm an rS pattern was constantly and repetitively shown in all 6 the precordial leads. Importantly, the polarity of the T wave was also constantly positive in all 6 of the precordial leads. Table I describes the characteristics of the ECG components.

Table 1 Characteristics of the normal ovine ECG

	Ketamine (n=8)			Midazolam (n=7)		
	Mean	**Min**	**Max**	**Mean**	**Min**	**Max**
Heart rate (beats/min)	171.00	163.00	181.00	151.00	130.00	185.00
P-wave duration (msec)	64.00	50.00	74.00	52.00	47.00	56.00
P-wave amplitude (mV)	0.09	0.04	0.16	0.07	0.06	0.09
PR interval (msec)	80.00	74.00	90.00	85.00	70.00	96.00
QRS duration (msec)	44.00	42.00	48.00	51.00	38.00	60.00
QT interval (msec)	220.00	208.00	228.00	258.00	212.00	288.00
QRS axis (°)	-151.00	-138.00	-165.00	-147.00	-93.00	-168.00
T-wave axis (°)	43.00	22.00	58.00	40.00	23.00	59.00
Sokolow index (SV1 + RV5 in mV)	0.42	0.29	0.61	0.54	0.31	0.77

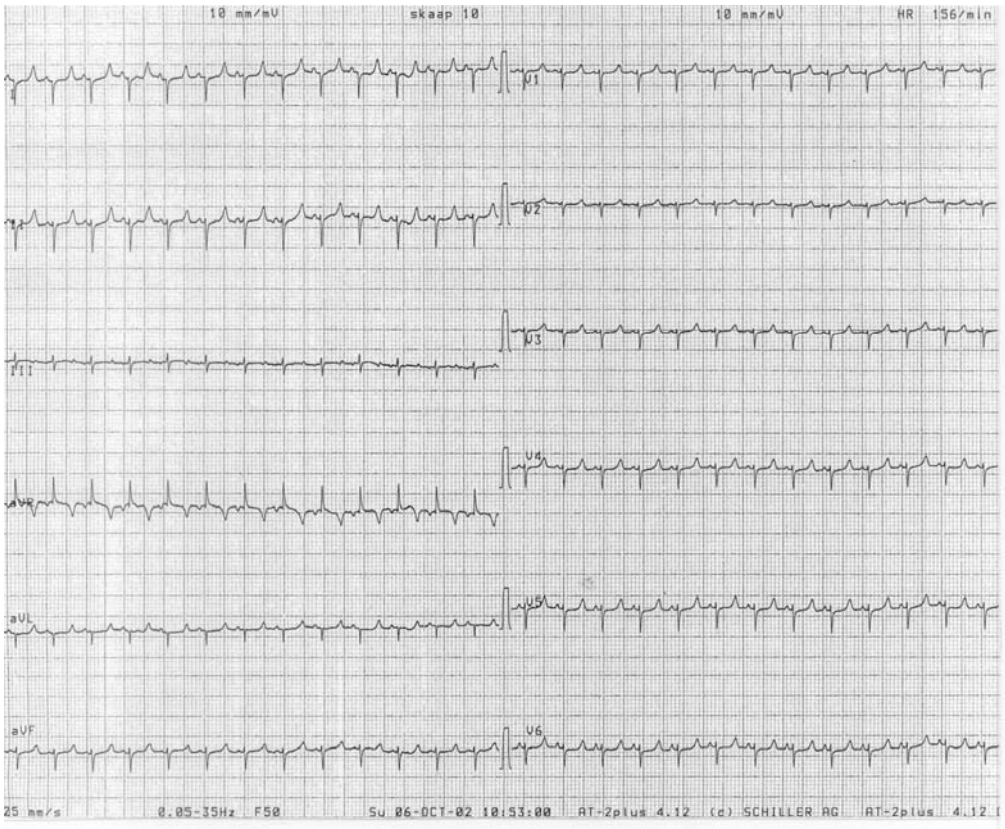

Figure 2.1. The normal ovine 12-lead electrocardiogram.

Discussion

Schultz *et al* [7] demonstrated a technique by which reproducible 6-lead electrocardiograms (utilizing the 3 standard limb-: aVR, aVL, aVF and unipolar leads: I, II, III) can be recorded in normal sheep. In this study their method of placing the limb and unipolar leads was used, but a new method to obtain a reproducible 12-lead electrocardiogram, including the six precordial electrodes with a 6-channel electrocardiograph was developed. Of interest is the positive polarity of the tracing obtained with lead aVR. This was also seen in the study by Schultz *et al* [7] and furthermore the rS pattern of the six precordial leads was consistent. Note that this is most probably due to the fact that lead V6 is not facing the left ventricle. However, the purpose of this study was to find a practical way of placing the six precordial leads that will lead to a consistent tracing among different sheep and in sheep in the right lateral decubitus position this is the most lateral placement possible, without the sheep overlying the electrocardiographic leads.

The main purpose of this experiment was twofold. Firstly, to determine whether our positioning of the 6 precordial electrodes in a 12-lead ovine electrocardiogram yields reproducible tracing between different sheep and secondly, to note the polarity of the T waves in the six chest leads, as later on we will be looking at post-extrasystolic T wave changes.

There were 2 groups of sheep with regard to choice of anaesthetic agent—the first group were sedated with ketamine and the second with midazolam. This

was done in order to determine whether there would be any possible differences in the 6 precordial leads with regard to the QRS pattern and/or QRS-, P-, and T wave polarity. The 6 precordial tracings were identical in all 15 sheep: rS pattern of the QRS complex with positive P and T waves.

This method of obtaining a 12-lead, 6-channel ECG in sheep is proposed as valid, easy and reproducible. Furthermore, the method of sedation makes it possible for one person to perform electrocardiography in sheep, without any concern that this will influence the tracing.

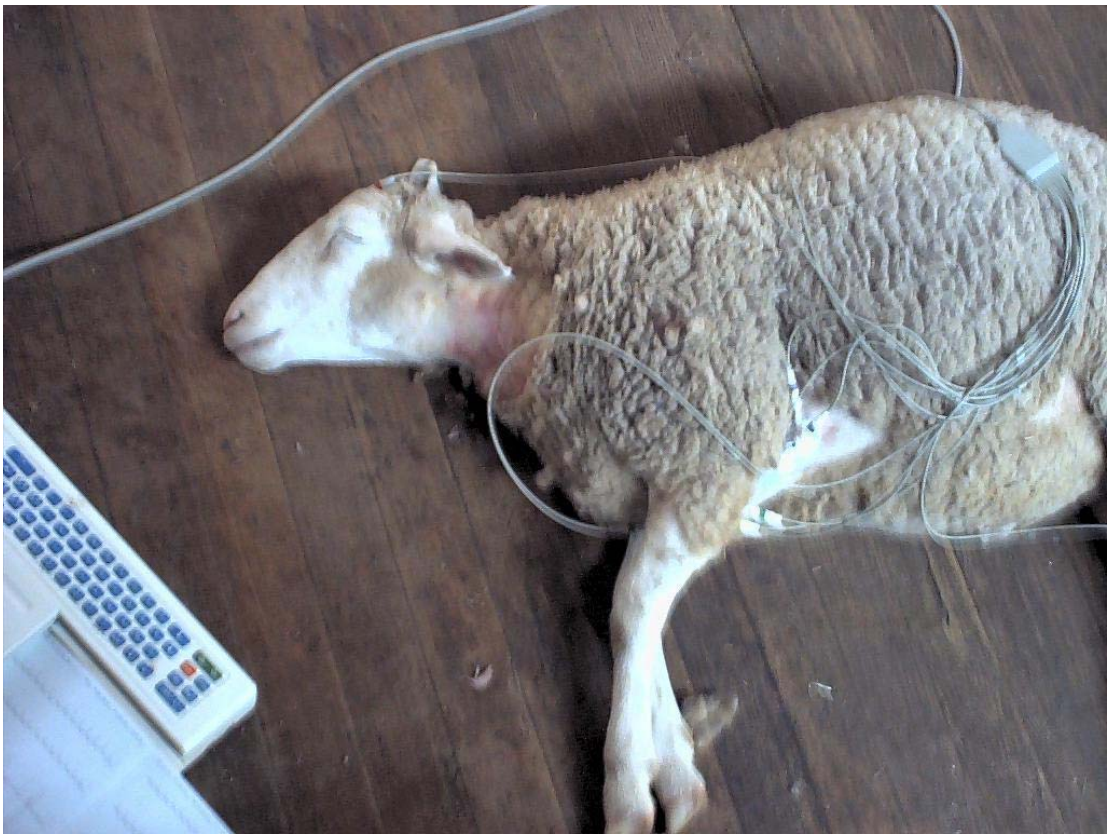

Figure 2.2. Dorper wether in right lateral decubitus position

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Chapter 3

The morphology of premature ventricular complexes in the dorper sheep heart

A premature ventricular complex (PVC) is the expression of an impulse that arises prematurely in an ectopic ventricular focus and can originate in the specialized conduction tissue distal to the bifurcation of His or in the ventricular myocardium itself [1, 2]. On the 12-lead, surface electrocardiogram PVC's are recognized by the premature occurrence of a QRS complex that is abnormal in shape and with a duration that exceeds that of the dominant QRS complex. The T wave is opposite in direction to the major deflection of the QRS complex [2]. However, narrow PVC's can occur and have been explained as originating at a point equidistant from each ventricle in the ventricular septum or by arising high in the fascicular system [3]. Currently three mechanisms of PVC generation are recognized: enhanced automaticity, triggered activity and reentry [4].

In the human heart it has been shown that certain characteristics of a PVC can reflect either the presence or absence of myocardial disease [1, 5, 6]. Normal PVC's (those not complicated by myocardial disease) has the following characteristics [1, 5]: 1) The QRS amplitude is large and exceeds 20 mm or

higher. 2) The QRS complex does not exceed 120 msec in duration. 3) The QRS deflection has a smooth contour with no notching. 4) The ST segment and T wave are opposite in direction to the QRS deflection. 5) The ST segment does not display any isoelectric period. 6) The ST segment blends imperceptibly with the proximal limb of the T wave so that the two cannot be separated. 7) The T wave has asymmetrical limbs.

It has been shown that primary myocardial disease can alter these features of "uncomplicated" PVC's and any one or more of the following changes can occur [1, 5], thus yielding a "complicated" PVC:

1) The QRS complex is diminished in amplitude.
2) The QRS complex widens and exceeds 120 msec.
3) There is marked notching and irregularity of the QRS complex.
4) The ST segment displays an isoelectric period.
5) The T waves tend to be sharply pointed and symmetrical.
6) The T wave has the same direction as that of the QRS complex.

For the purpose of this study it is necessary to document the morphological features of PVC's in the normal Dorper sheep heart as this has not been described in the literature before. This is important for two reasons. First, it is essential that PVC's can be accurately identified on the 12-lead surface electrocardiogram, as they will be used later on to induce cardiac memory T waves, and secondly we want to evaluate the possibility that these

morphological features may change if individual sheep are exposed to prolonged periods of PVC`s.

Materials and Methods

The first 5 sheep that were used in chapter 2 were taken out of this study: they were slaughtered and their hearts were subjected to histological examination so that they can serve as normal histological controls (see chapter 4). The remaining 10 sheep that were used in chapter 2 were used in this study. These 10 sheep were designated sheep 1 to 10.

These 10 Dorper wethers were sedated and electrocardiography was performed as described in chapter 2. Right ventricular PVC's were induced in all 10 sheep. The guidewire of an Arrow central venous pressure catheter set (spring-wire guide with diameter 0.81 mm and length 60 cm, straight soft tip on one end and J tip on the other end.) were advanced into the right ventricle via the left internal jugular vein by the Seldinger technique. The guidewire was introduced with the soft tip in front with the sheep lying sedated in the right lateral decubitus position. The position of the guidewire was confirmed with an X-ray and PVC's were then induced by mechanical movement of the guidewire. This method of inducing PVC's was chosen because of the observation that so-called "tip extrasystoles" are often seen during the implantation of ventricular pacemakers in humans [7].

These 10 Dorper wethers were then exposed for variable periods to PVC's (see table 3.1).

Table 3.1. PVC load

Sheep number	Days subjected to PVC's	Number of PVC's counted
1	60 minutes	55
2	15 days	221
3	1 day	80
4	36 days	575
5	9 days	150
6	28 days	371
7	16 days	1887
8	13 days	210
9	53 days	902
10	34 days	908

*Electrocardiography was performed once daily. Ten serial electrocardiographic tracings were printed over a period of 30 minutes every morning.

Results

In these 10 wethers a total of 5359 PVC's were actually counted and documented on a 12-lead surface electrocardiogram. The morphological characteristics of PVC's on the first and last day of PVC exposure were analyzed separately in every wether in order to detect any possible changes in PVC morphology (see tables 2-6).

Table 3.2. PVC exposure on the first and last day of study

Sheep Number	PVC's on 1st day	PVC's on last day
1	55	55
2	14	16
3	80	80
4	16	18
5	17	21
6	13	14
7	118	135
8	16	19
9	17	14
10	27	31
Total:	373	403

Table 3.3. PVC QRS duration on the first and last day of study

Sheep number	Mean QRS duration on 1st day	Mean QRS duration on last day	Difference*
1	0.038	0.06	0.022
2	0.040	0.07	0.030
3	0.042	0.08	0.038
4	0.040	0.06	0.020
5	0.038	0.08	0.042
6	0.040	0.06	0.020
7	0.038	0.07	0.032
8	0.04	0.07	0.030
9	0.038	0.06	0.022
10	0.038	0.08	0.042

*p = 0.000001 (paired t-test)

Mean QRS duration of PVC's on first day= 0.0392 (95% CI: 0.038-0.040).

Mean QRS duration of PVC's on last day= 0.069 (95% CI: 0.06-0.08).

In this study three changes were noted in PVC morphology between the first and last day of PVC exposure. First, the QRS duration of the PVC's increased (see table 3.4), secondly, notching appeared in the QRS complexes of the PVC's (see table 5), and lastly, the isoelectric ST-segments of the PVC's disappeared (see table 6).

Table with graph 3.4. Increase in PVC QRS duration during chronic PVC exposure

Sheep number	Number of PVC's >0.06s on 1st day of study	Number of PVC's >0.06s on last day of study	Difference*
1	20	20	0
2	1	15	14
3	8	48	40
4	4	16	12
5	4	18	14
6	2	10	8
7	32	130	98
8	3	18	15
9	3	13	10
10	3	30	27

*p = 0.013 (paired t-test).

O.R= 13.93 (Odds ratio that the QRS duration of PVC's will increase from < 0.06 s on the first day of PVC exposure to > 0.06 s on the last day of PVC exposure).

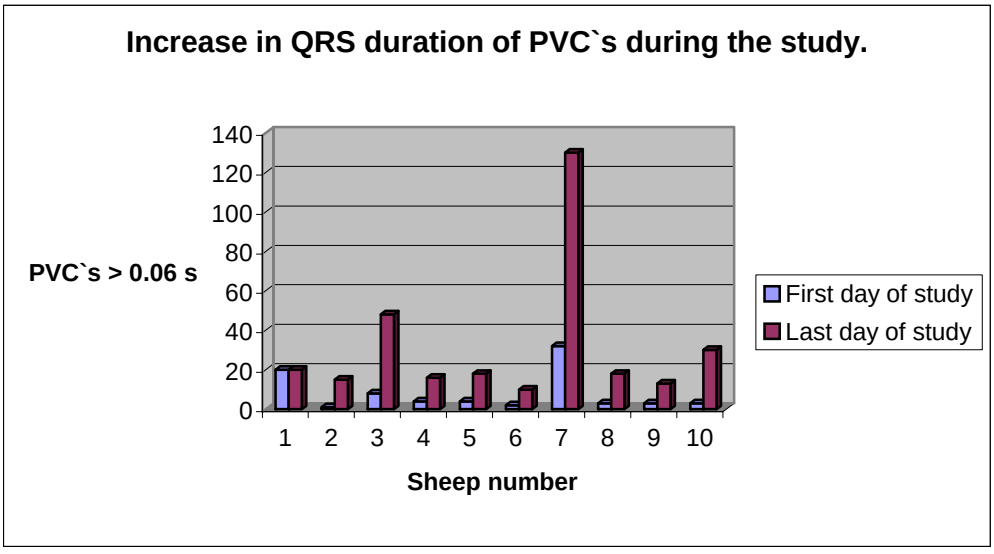


Table with graph 3.5. PVC's with notching of QRS complexes

Sheep number	Number of PVC's with notching on 1st day	Number of PVC's with notching on last day	Difference*
1	2	2	0
2	0	9	9
3	3	3	0
4	1	10	9
5	1	11	10
6	1	7	6
7	9	81	72
8	2	9	7
9	1	8	7
10	3	22	19

*p = 0.034 (paired t-test).

O.R = 11.17 (Odds ratio that PVC's will have notching of their QRS complexes on the last day of the study).

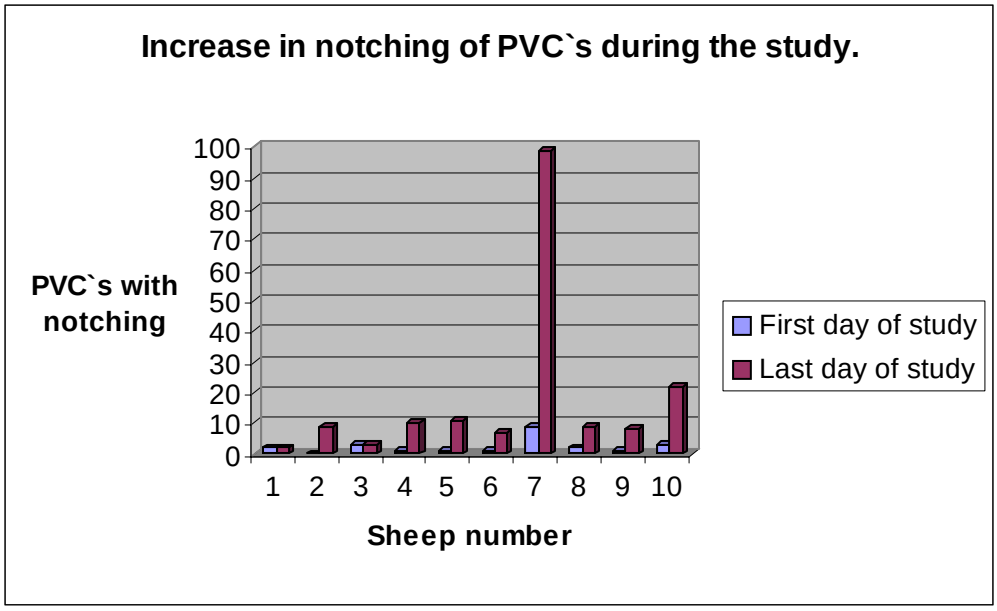
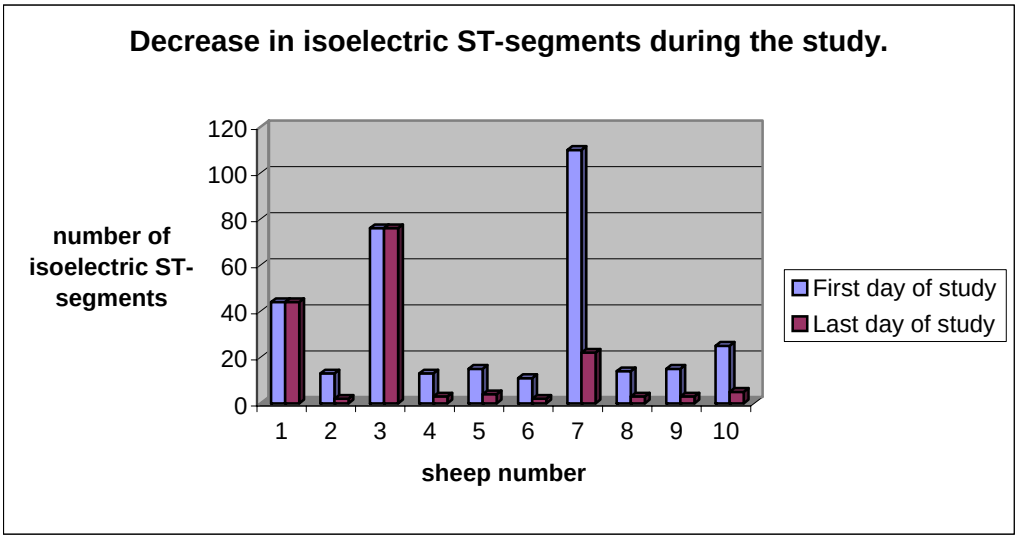


Table with graph 3.6. Number of PVC's with isoelectric ST-segments

Sheep number	Number of PVC's with isoelectric ST-segments on first day	Number of PVC's with isoelectric ST-segments on last day	Difference*
1	44	44	0
2	13	2	11
3	76	76	0
4	13	3	10
5	15	4	11
6	11	2	9
7	110	22	88
8	14	3	11
9	15	3	12
10	25	5	20

*p = 0.031 (paired t-test).

O.R = 12.86 (Odds ratio that the isoelectric ST-segment of the PVC's will disappear at the end of the study).



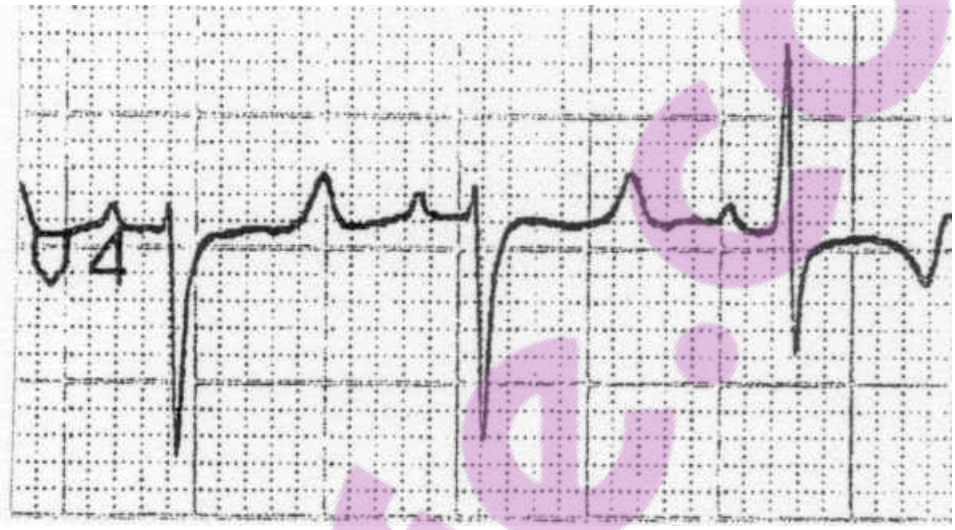


Figure 3.1. An example of an uncomplicated PVC occurring in the normal ovine heart during the first day of being subjected to PVC's. The third beat in this tracing from lead V4 is a PVC. Note the narrow QRS complex, with a duration of 40 msec and the isoelectric ST-segment. No notching of the QRS complex is present.

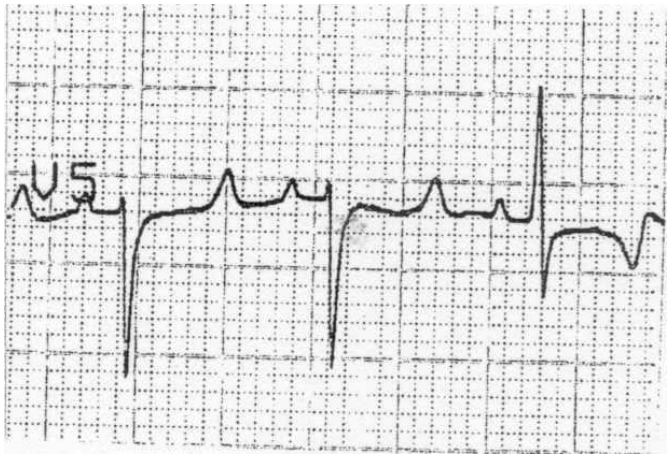


Figure 3.2. Another example of an uncomplicated PVC in the ovine heart, this time seen in lead V5. The third beat in the tracing is a PVC. Note once again the narrow QRS complex with no notching and the isoelectric ST-segment.

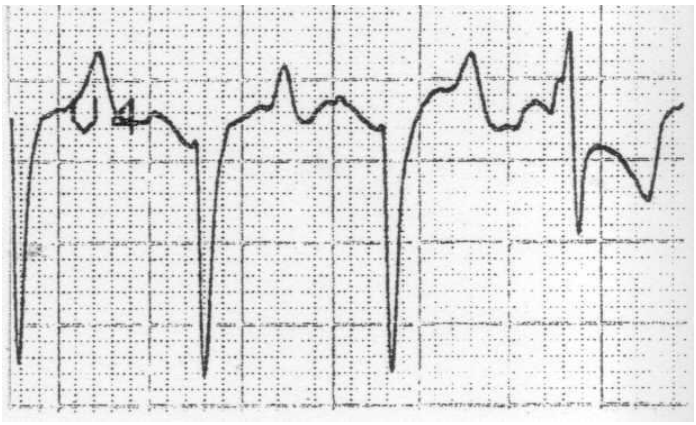


Figure 3.3. This tracing was taken from lead V4 from the same sheep as in figure 3.2, but this time after 14 days of exposure to PVC's. Now we see changes, possibly indicative of myocardial pathology. The third beat is a PVC. Note the notching of the QRS complex, the QRS complex is much broader than in figure 3.2, with a duration of 80 msec. Note also the prominent loss of the isoelectric ST-segment.

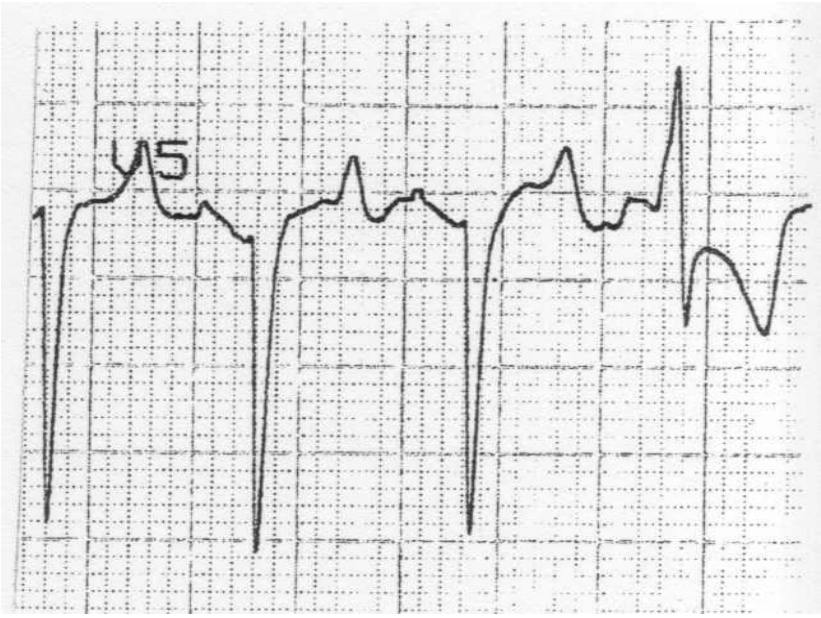


Figure 3.4. Tracing from lead V5 from the same sheep as in figure 3.3, also after 14 days of exposure to PVC's. Note once again the broader QRS complex with notching and loss of the isoelectric ST-segment, features suggestive of a complicated PVC.

Discussion

In all 10 wethers (with a total of 5359 PVC's counted) the following was seen. At the beginning of the study (during the first day) the PVC's had the following characteristics (see figures 3.1 and 3.2):

1) The QRS duration did not exceed 60 msec.
2) There was no notching of the QRS complexes.
3) The ST-segments were isoelectric.

During the course of the study, certain changes were noted in the PVC's (see figures 3.3 and 3.4):

1) The QRS duration prolonged to > 60 msec.
2) Notching of the QRS complexes appeared.
3) The isoelectric ST-segments disappeared.

A very clear and consistent change was thus seen in the morphology of ovine PVC`s in all the animals exposed to prolonged periods of PVC`s. We started the study with 10 normal sheep. The question that has arisen now is whether these 10 heart are still normal, as the morphological characteristics of "complicated" PVC`s has appeared. This question will be answered in the following chapter when these hearts will be examined histologically.

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Chapter 4

Structural myocardial alterations

The first 5 sheep that were used in chapter 2 to study the normal ovine electrocardiogram were used to study the normal histological appearance of the Dorper sheep heart.

These sheep were slaughtered and the hearts removed.

Left ventricular dissection

The musculature of each left ventricle (LV) was dissected into three regions: Two transverse incisions were made, one at the level of the base and the other at the level of the apex of the posteromedial papillary muscle (see figure 4.1). This divides the LV into three regions: base, mid-region and apex. Each of these three regions were then dissected into four parts: anterior, posterior, septal and lateral. In this way every LV was dissected into 12 pieces, representing the musculature of the entire LV, which were subsequently all subjected to histological examination.

These 12 segments were numbered as follow:

A = anterior part of base

B = anterior part of mid-region

C = anterior part of apex

D = septal part of base

E = septal part of mid-region

F = septal part of apex

G = lateral part of base

H = lateral part of mid-region

I = lateral part of apex

J = posterior part of base

K = posterior part of mid-region

L = posterior part of apex

Histological evaluation

Tissue blocks from these 12 sites were fixed in 10 % buffered formalin and paraffin-embedded sections for light microscopy were prepared using routine histological procedures. They were stained with hematoxylin and eosin (HE). All the sections were then histologically examined.

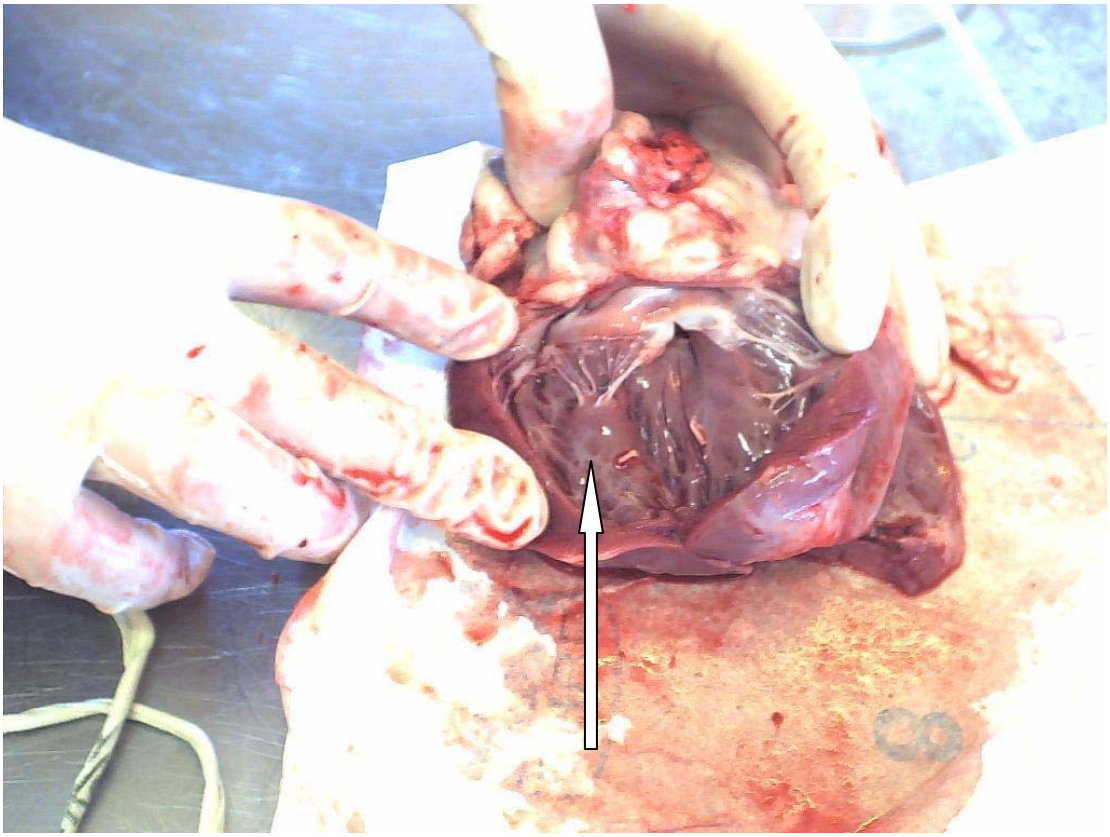

Figure 4.1. The posteromedial papillary muscle of the Dorper sheep heart (arrow).

Myocardial histological appearance of the normal Dorper sheep heart.

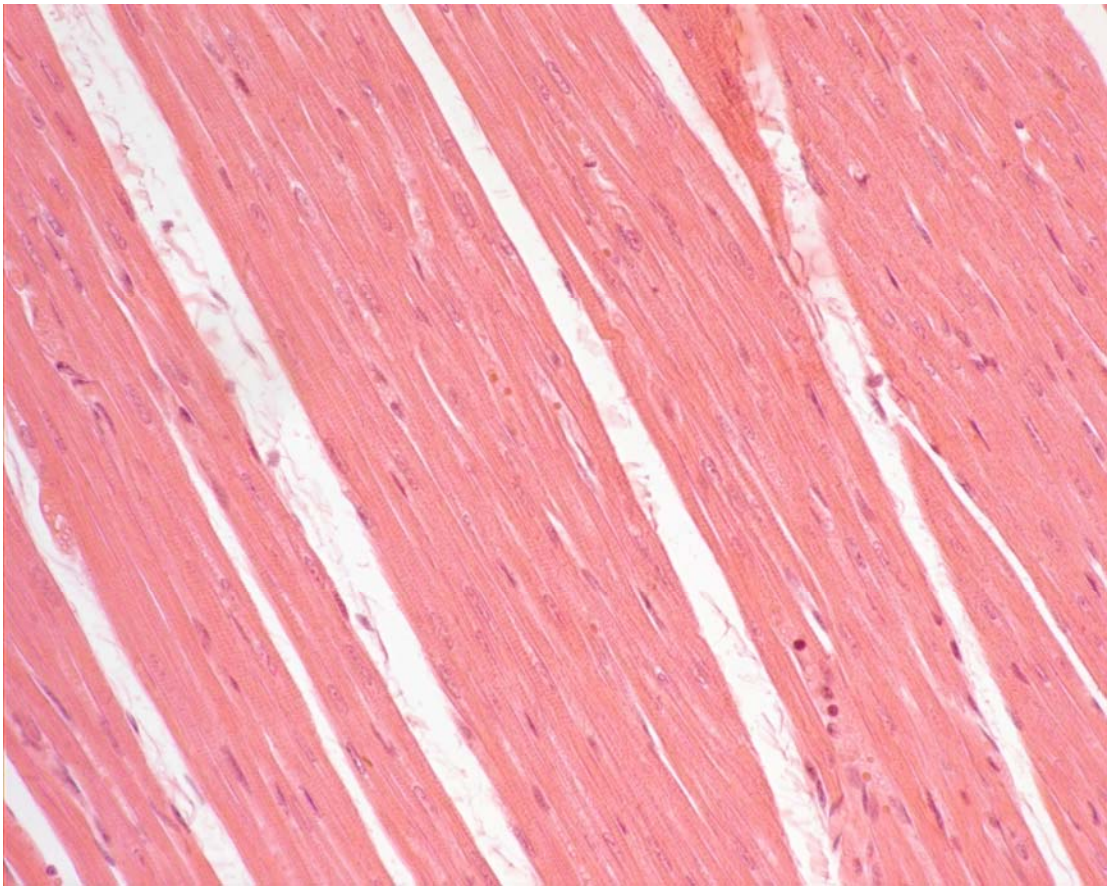

Figure 4.2. Longitudinal section (x 100) from sheep 1.

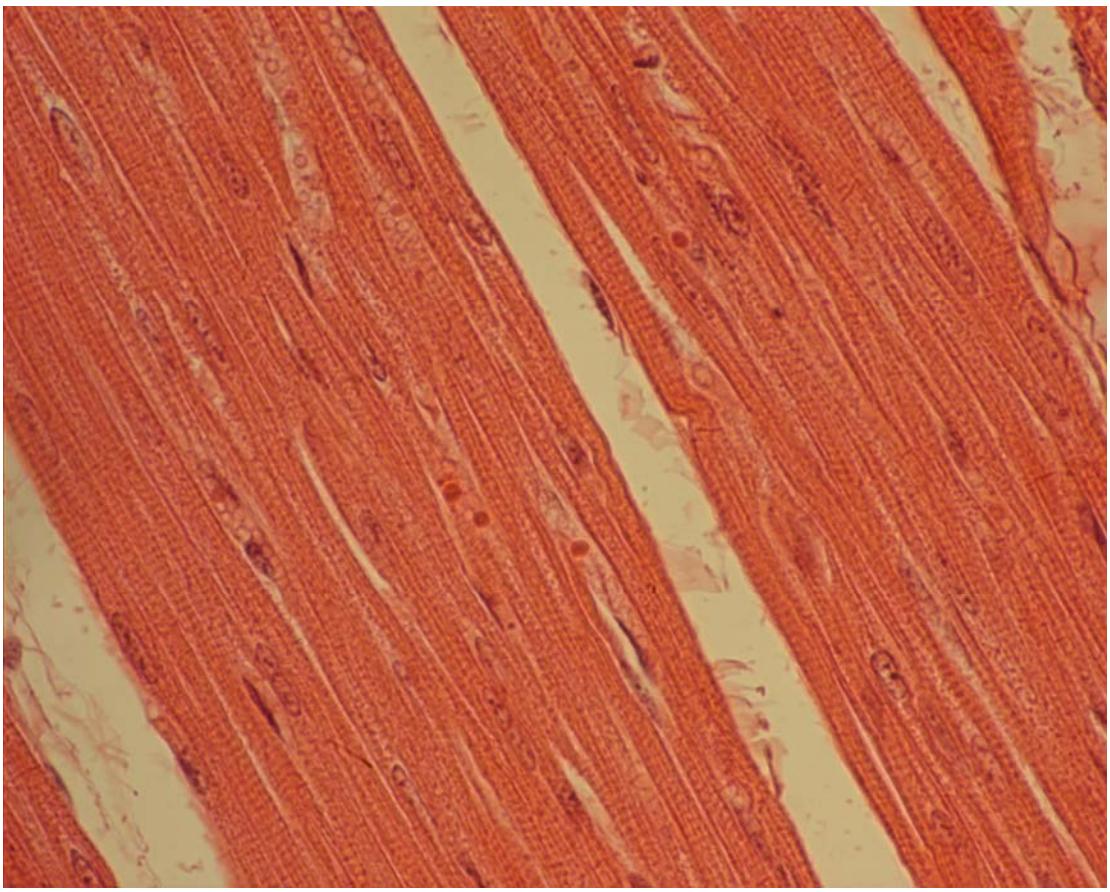

Figure 4.3. Longitudinal section (x 200) from sheep 2.

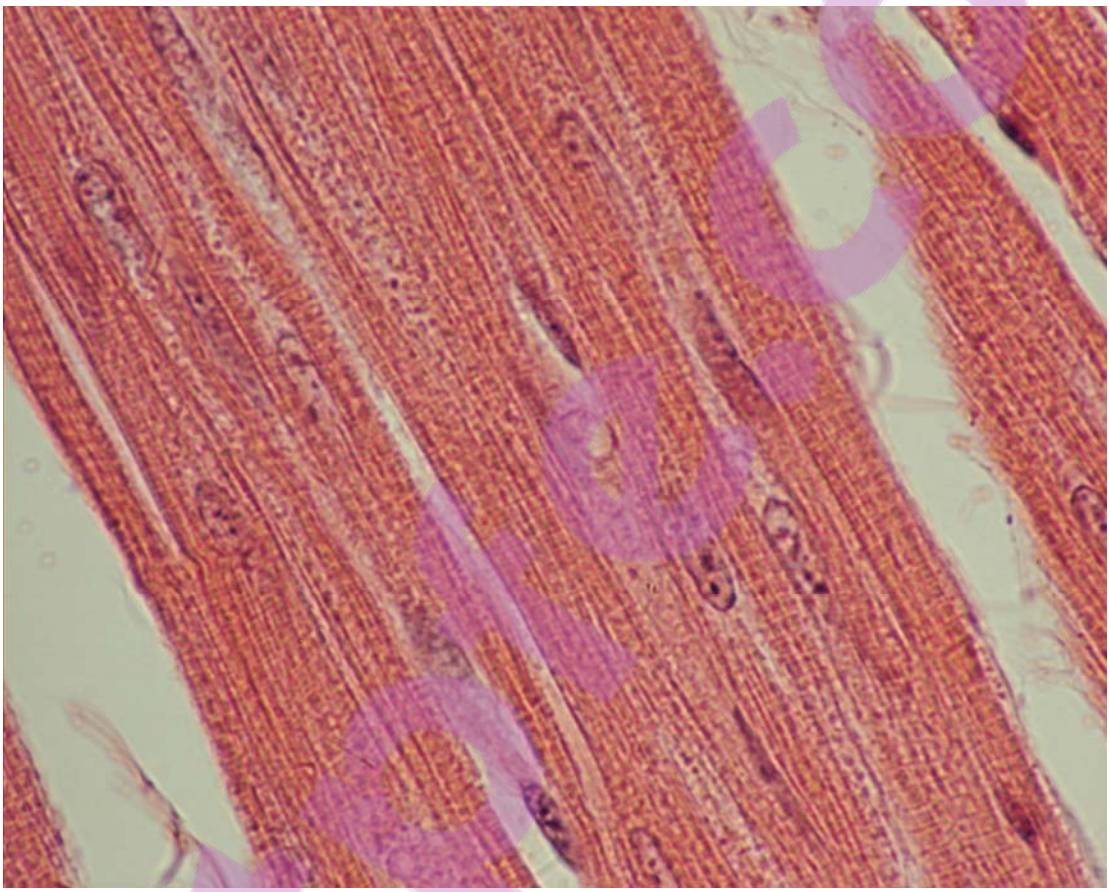

Figure 4.4. Longitudinal section (x 400) from sheep 3.

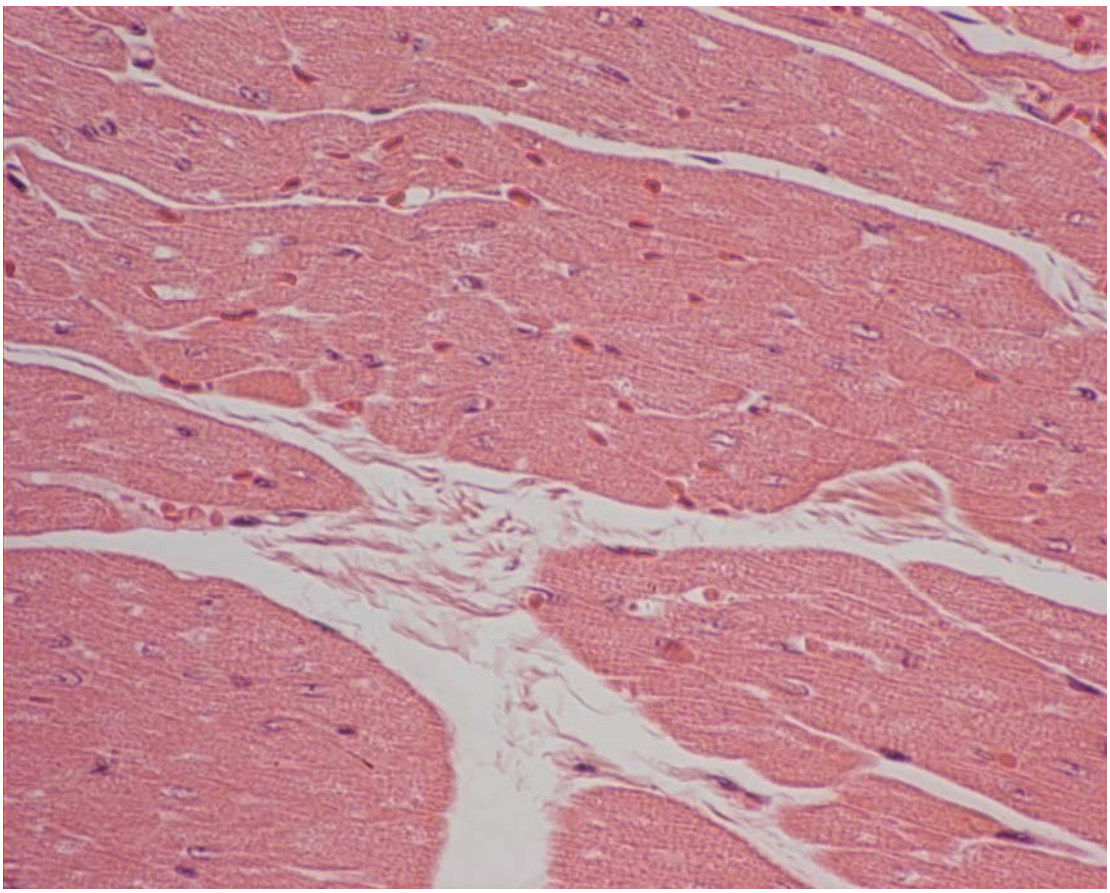

Figure 4.5. Transverse section (x 200) from sheep 4.

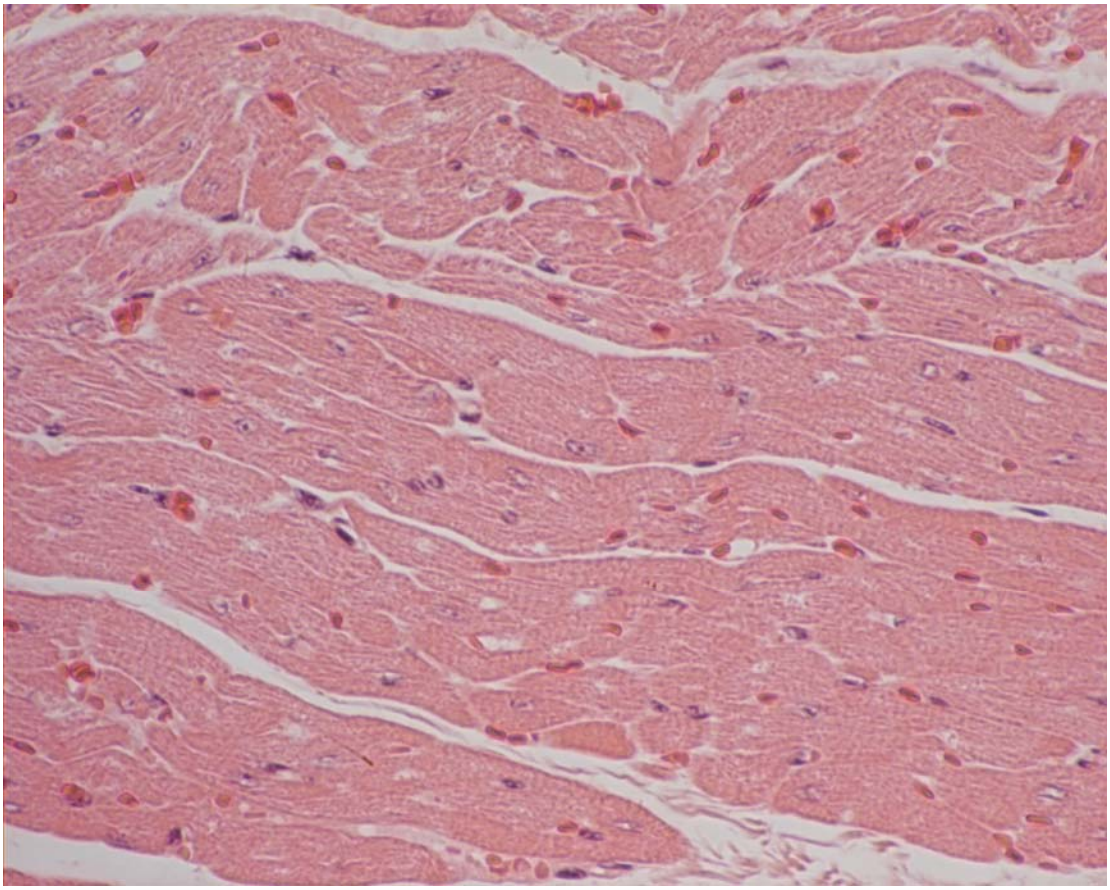

Figure 4.6. Transverse section (x 200) from sheep 5.

All 12 sections from the left ventricles of all 5 normal wethers had the same normal histological appearance (see figures 4.2 to 4.6).

6 of the 10 wethers that were exposed to prolonged periods of PVC`s were subsequently slaughtered and their hearts were also subjected to histological examination in order to determine if any histological differences exist between the two groups. This was done because of the peculiar finding that the morphology of PVC`s differed between the first and last day of study, findings consistent with possible myocardial pathology, as discussed in chaper 3. Six of these 10 wethers were chosen at random for histological evaluation, the reason for excluding 4 wethers were because of financial constraints. The 6 chosen wethers were: sheep number 2, 4, 6, 7, 9 and 10.

When compared to the 5 histological control animals (see figures 4.2 to 4.6) histological changes occurred in all 6 experimental animals. These changes consisted of both myocardial cellular and interstitial abnormalities (see figures 4.7 to 4.12). According to the Dallas criteria [1, 2, 3, 4] these observed myocardial cellular and interstitial changes are indicative of myocarditis.

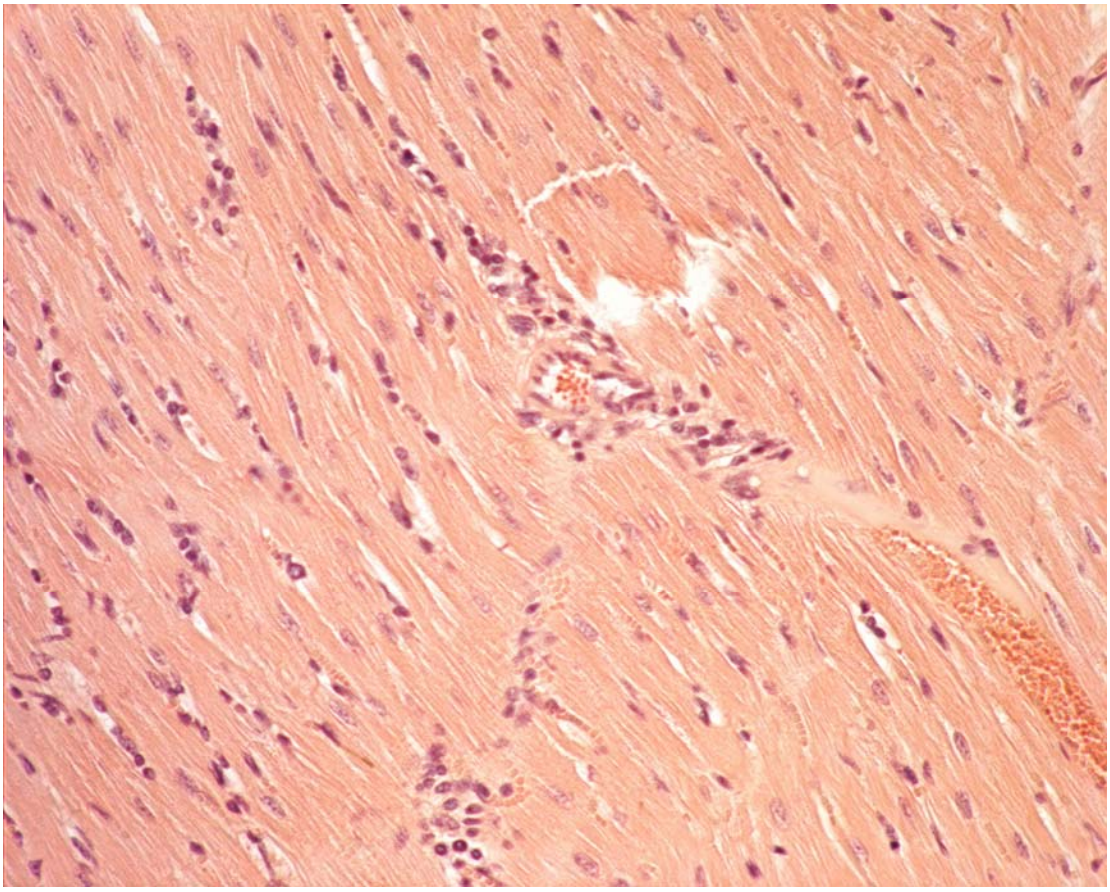

Figure 4.7. Longitudinal section (x 100) from sheep 2. Note the infiltration of the left ventricular interstitium by a mixed inflammatory cell infiltrate, a feature of myocarditis.

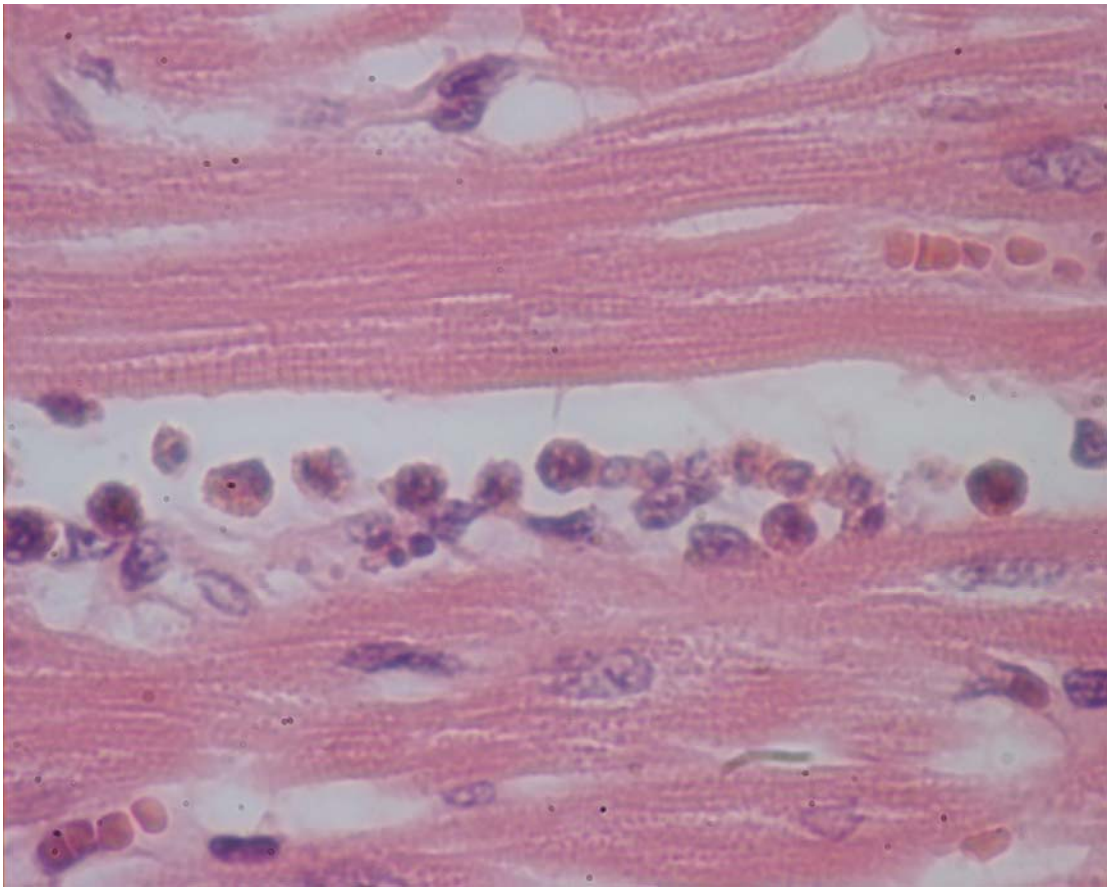

Figure 4.8. Longitudinal section (x 400) from sheep 4. Note the interstitial infiltration by inflammatory cells with myocytolysis.

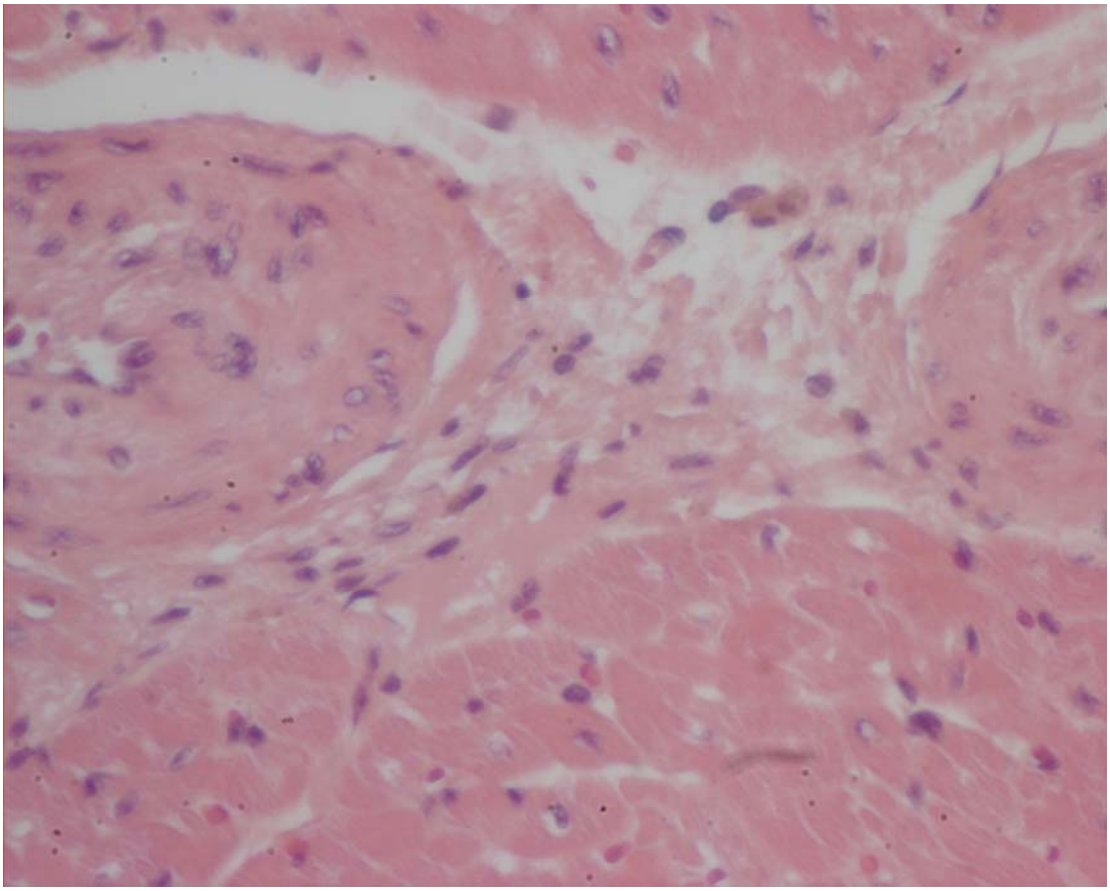

Figure 4.9. Transverse section (x 200) from sheep 6. Once again with an interstitial infiltration of inflammatory cells.

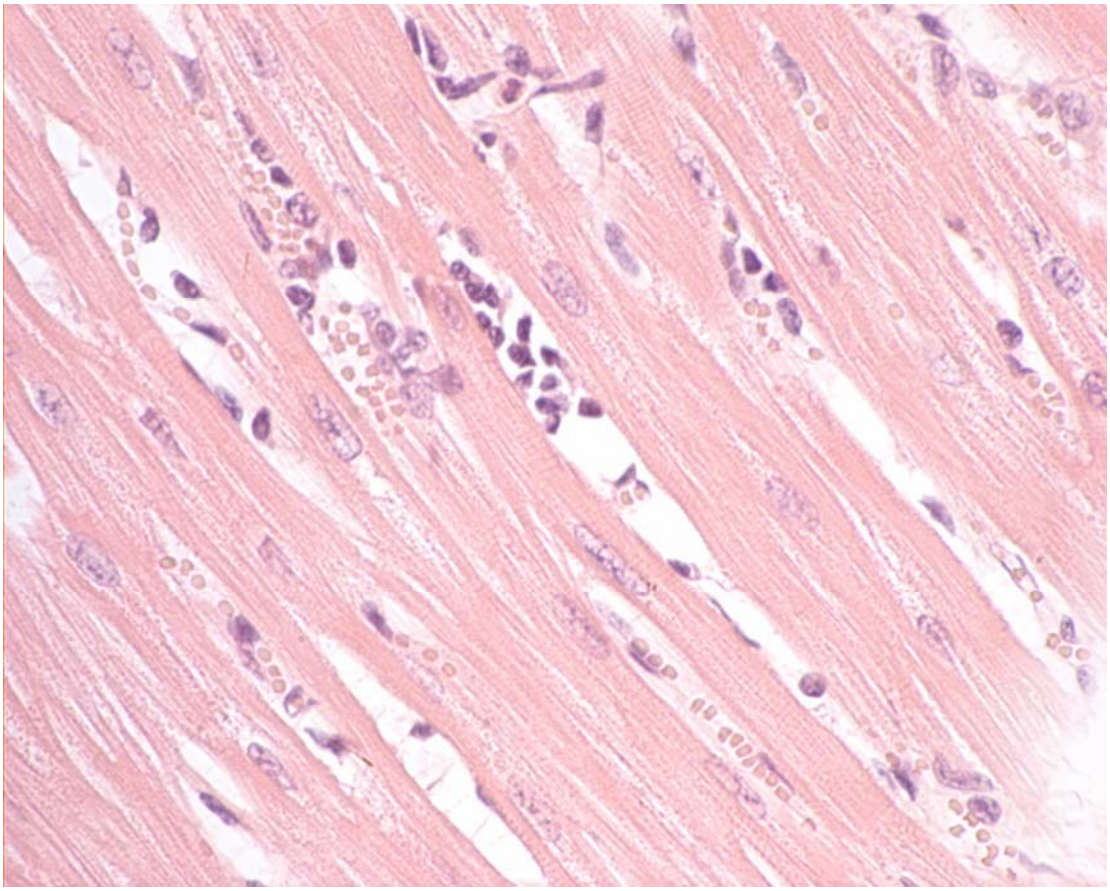

Figure 4.10. Longitudinal section (x 200) from sheep 7. Interstitial infiltration of inflammatory cells.

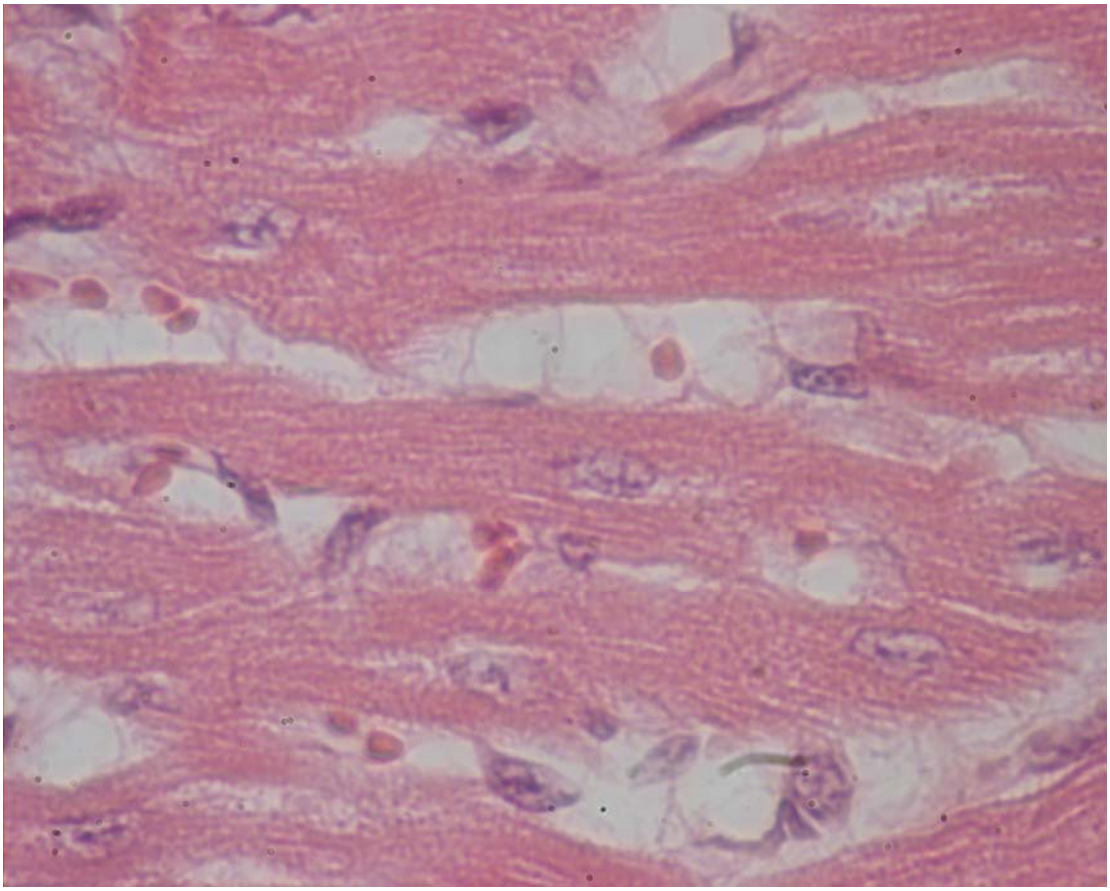

Figure 4.11. Longitudinal section (x 400) from sheep 9, demonstrating myocytolysis.

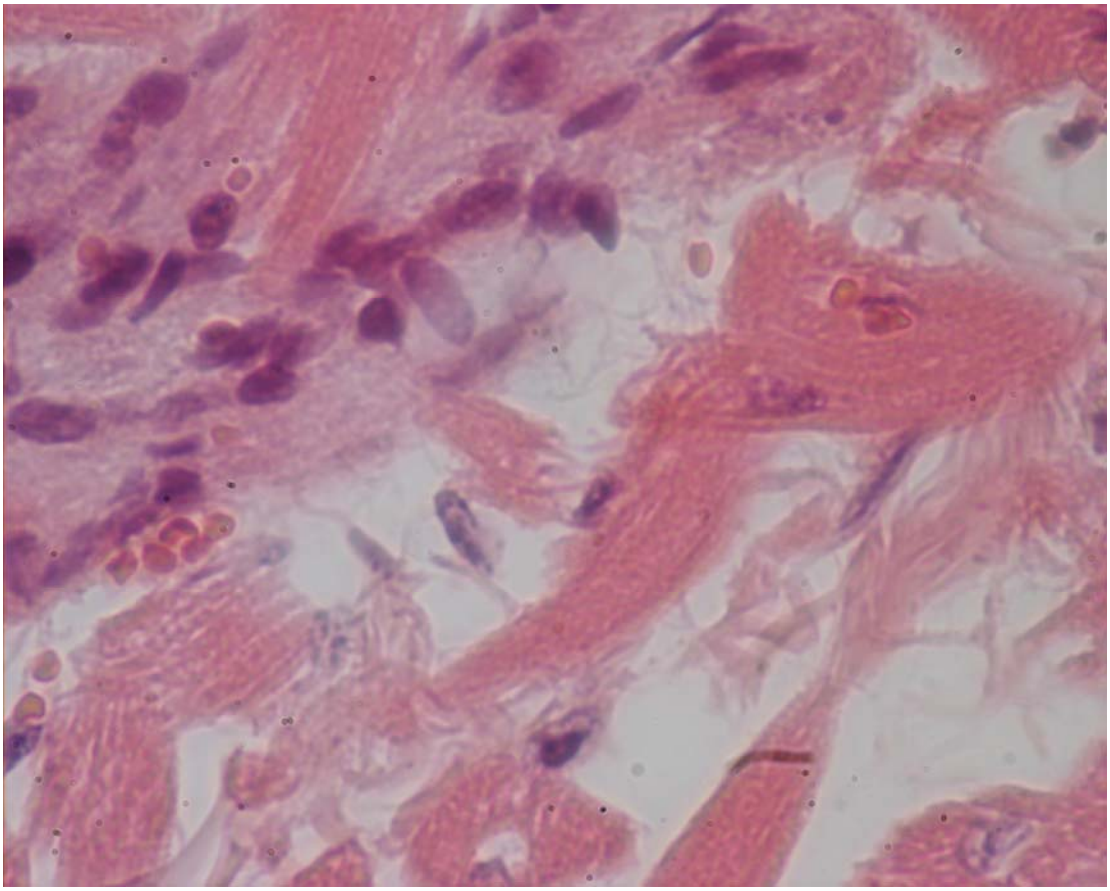

Figure 4.12. Longitudinal section (x 400) from sheep 10 demonstrating an interstitial inflammatory cell infiltrate and myocytolysis.

It has thus been shown clearly that in Dorper sheep exposed to prolonged periods of PVC`s, induced by a guidewire situated in the right ventricle, certain morphological changes appeared in these PVC`s, which are indicative of myocardial pathology. As discussed in chapter 3, these changes consist of a prolongation of the QRS complex of PVC`s, the appearance of notching of PVC`s and the disappearance of the ST segment of PVC`s. Every wether served as it`s own control—-at the beginning of the study when normal wethers entered the study, the PVC`s had different characteristics than at the end of the study when myocardial pathology was present. This association does not at any stage take the cause of myocardial pathology into account: we are looking at electrocardiographic surrogates of myocardial pathology and thus far, three morphological changes of PVC`s have been found as valid surrogates. The possible causes of myocardial pathology in these sheep will be discussed in chapter 6. Now, we will look if any characteristics of cardiac memory T waves can serve as an electrocardiographic surrogate for myocardial pathology.

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Chapter 5

Cardiac memory T wave frequency in the normal and diseased Dorper sheep heart.

Memory is a property common to a diverse range of tissues, such as the brain, the gastrointestinal tract and the immune system [1, 2], but is it possible for the heart to remember ? Indeed, this appears to be the case—cardiac memory has been demonstrated in the heart of the human, dog, cat and rabbit [3, 4, 5, 6].

Cardiac memory is an electrocardiographic phenomenon seen in the T wave, when T waves of normally conducted beats seem to "remember" the polarity of the QRS complexes of previous abnormally conducted beats [1, 3]. Only one event is remembered by the heart and that is a period (or periods) of altered ventricular activation [1, 3, 4, 6]. A variety of clinical scenarios are able to cause abnormal ventricular activation and these include: ventricular pacing, left bundle branch block, ventricular preexcitation and premature ventricular complexes [3, 4, 7, 8, 9, 10].

Rosenbaum and Blanco [3] , in their original description of cardiac memory, noted a specific sequence in cardiac memory. Periods of abnormal ventricular activation (leading to an altered sequence of ventricular depolarization) may induce a change in the T wave, which will be noted after return to a normal

sequence of ventricular activation. The T wave will retain the vector of the previous abnormal QRS complex—the polarity or direction of this T wave will be the same as that of the abnormal QRS complex(es).

Cardiac memory has never before been documented in the ovine heart. The objective of this study was therefore to examine the possibility that cardiac memory can be induced and documented in the hearts of normal Dorper wethers.

Materials and methods

The 10 clinically normal Dorper wethers that were used in chapter 3 were used in this study.

These 10 wethers were exposed to right ventricular PVC's for variable periods, as described in chapter 3 (table 5.1). The objective was to determine whether right ventricular PVC's are able to induce cardiac memory T waves. The second objective was to see if there is any difference in the frequency of cardiac memory T waves at the beginning and end of the study period.

Table 5.1. PVC load

Sheep number:	Number of PVC's counted:
1	55
2	221
3	80
4	575
5	150
6	371
7	1887
8	210
9	902
10	908

Results

A total of 5359 PVC's were counted and documented on a 12-lead surface electrocardiogram. In order to detect if there is any difference between the early and late occurrence of cardiac memory T waves the first and last 10 % of PVC's were evaluated in every wether. The T wave of the first normal beat after every PVC were evaluated in order to determine whether these T waves retained the vector of the previous PVC QRS complex (table 5.2). Only lead III of the 12-lead, surface electrocardiogram were used to assess for the presence

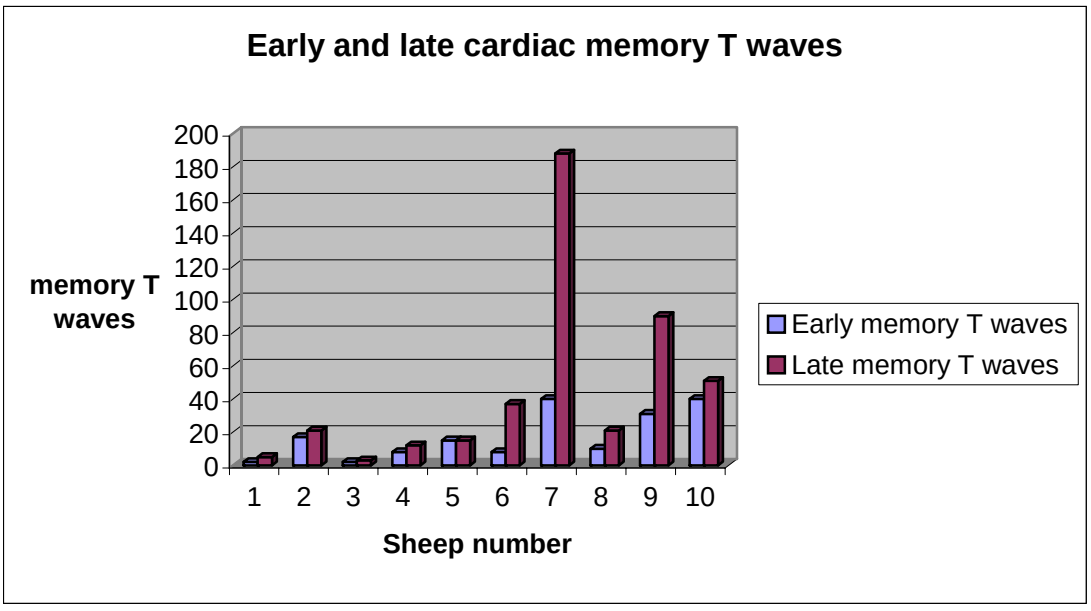
of cardiac memory T waves as a pilot study showed that this is the lead with the highest yield for cardiac memory T waves [11].

Table and graph 5.2. Early and late cardiac memory T waves

Sheep number:	Number of early memory T waves:	Number of late memory T waves:	Difference*:
1	2	5	3
2	17	21	4
3	2	3	1
4	8	12	4
5	15	15	0
6	8	37	29
7	40	188	148
8	10	21	11
9	31	90	59
10	40	51	11

* $p = 0.049$ (paired t-test).

OR = 10.38 (Odds ratio that an ovine heart that does not demonstrate cardiac memory T waves during the first 10 % of PVC's will do so during the last 10 % of PVC's.)



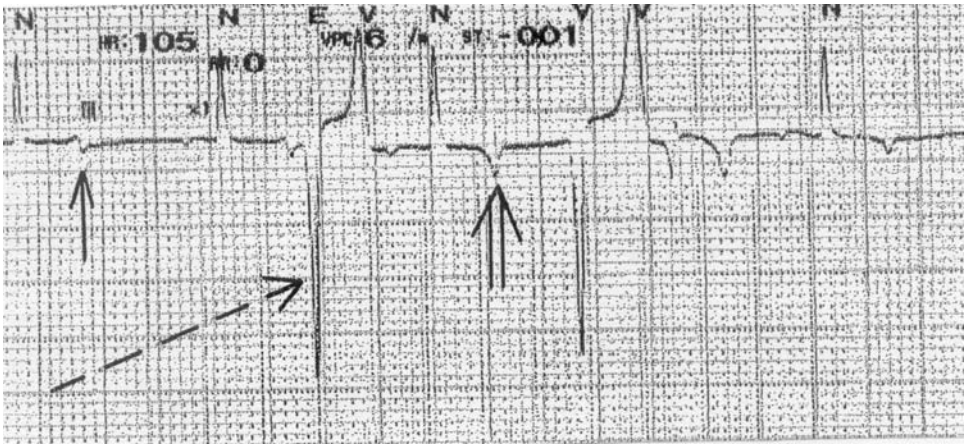


Figure 5.1. An example of cardiac memory T waves. The third and fifth beats in this tracing are PVC's (broken arrow). Note the bifid T waves before the first PVC (arrow). Note the inverted T waves after the PVC's (double arrow)—the T wave retains the vector of the QRS complex of the PVC—thus the term "cardiac memory".

Discussion

This is the first report of cardiac memory in sheep [11]. Cardiac memory T waves may appear after either short or long periods of altered ventricular activation [1, 4]. However, there is no consensus yet in the literature on the time period required to separate short- from long-term cardiac memory [4]. Rosenbaum and Blanco [3] in the first cardiac memory experiments needed 15 minutes of right ventricular pacing to demonstrate memory T waves in the human heart. Goyal and Syed [12] were able to induce cardiac memory after only 1 minute of right ventricular pacing in humans. This study demonstrates 2 concepts: First, the ovine heart is able to manifest cardiac memory T waves, and secondly the higher the load of altered ventricular activation (PVC's were used in this study) the more likely the manifestation of cardiac memory, as demonstrated by an odds ratio (OR) of 10.38 (the OR=10.38 that the amount of cardiac memory T waves will increase during the last 10% of PVC's as compared to during the first 10% of PVC's).

Currently, it is not known whether cardiac memory T waves can serve as an electrocardiographic warning for future myocardial pathology. In this study, it was shown that the true value of using cardiac memory T waves as an electrocardiographic surrogate for structural myocardial alteration in the Dorper sheep heart does not lie in an instantaneous electrocardiographic assessment, but in electrocardiographic follow-up in order to determine if there is an increase in the frequency of cardiac memory T waves. As shown in

this study an increase of at least 42 % in the frequency of cardiac memory T waves, following PVC`s is indicative of underlying structural myocardial changes in the Dorper sheep heart.

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Chapter 6

Summary

In this study we evaluated the validity of well-known human electrocardiographic markers of myocardial pathology in Dorper sheep. These markers are all properties of PVC`s, namely the duration of the QRS complex of PVC`s, the presence of notching of the QRS complex of PVC`s and change in the ST segment of PVC`s. It was shown that these three electrocardiographic phenomena correlate with myocardial pathology in the hearts of Dorper sheep. We also described a new electrocardiographic indicator of myocardial pathology in the hearts of Dorper sheep, namely an increase in the frequency of cardiac memory T waves, induced by PVC`s, as a new electrocardiographic surrogate for myocardial pathology. This study was possible, because we knew from a pilot study that our specific method of inducing right ventricular PVC`s is known to induce structural alterations in the myocardium of Dorper sheep. The guidewire was situated in the right ventricle and we examined the histological appearance of only the left ventricle, in order to exclude any possible changes caused by the wire itself. Although this study was not designed to answer the question of whether PVC`s can be a cause of, rather than a consequence of, structural myocardial disease, it is an important method, because in this way every wether serves as it`s own histological control for electrocardiographic changes. We started with normal Dorper wethers, induced right ventricular PVC`s and these PVC`s had certain characteristics, as described in chapter 3. We know what the normal histological appearance of Dorper wethers are and the electrocardiographic

appearance of PVC`s in the normal heart. At the end of the study certain changes appeared in the PVC`s, namely the QRS duration increased, notching appeared and the ST segment disappeared. Furthermore, at this stage the histological appearance of the left ventricle resembled that of myocarditis. At the end of the study (abnormal myocardial histology) we also noted an increase of 42 % in the incidence of cardiac memory T waves following PVC`s, when compared to the beginning of the study (normal myocardial histology).

What might the reason be for the abnormal left ventricular histology ? As this study was not designed to answer that question this is open to debate. It might be the anaesthetic, the wire itself or the PVC`s. As already discussed we induced right ventricular PVC`s and afterwards we examined the left ventricles, therefore these histological alterations cannot be a direct consequence of the guidewire itself.

It is suggested that it will be worthwhile to explore the possibility that PVC`s may be a cause of myocardial disease and that it is not always a consequence of established myocardial disease.

Addendum

A qualitative assessment of the electron-microscopic appearance of the normal and experimental dorper heart

During electronmicroscopical analysis only the anterior part of the mid-region of the left ventricle (segment B) was assessed in a normal Dorper wether and compared with the six experimental animals used in chapter 5.

The specimens were subjected to three phases of preparation, before microscopy was performed. This consisted of fixation, dehydration and embedding, as described in various textbooks on electron microscopy [1, 2, 3, 4] as used by various authors in the literature [5, 6, 7, 8, 9]. The first phase consisted of fixation:

A 1 cm^3 piece of myocardium (from segment B) was cut into smaller pieces of approximately 1 mm^3 in a petri dish, filled with 2.5 % glutaraldehyde in a phosphate buffer. This was made up as follow: 1 ml of 25 % glutaraldehyde (Cidex), 4 ml of distilled water and 5 ml of buffer (1.5 M $NaKPO_4$) was placed into a test tube to get a 2.5 % glutaraldehyde solution. These 1 mm^3 pieces of myocardium were then left in this solution for one and a half hour to complete primary fixation. After one and a half hour the specimens were washed three

times with phosphate buffer at 10 minute intervals, in order to remove all the glutaraldehyde. The specimens were then placed for one and a half hour in osmiumtetroxide (1 % $AgOsO_4$) to complete secondary fixation. Afterwards the specimens were again washed three times, at 10 minute intervals, with phosphate buffer.

The second phase consisted of dehydration:

The specimens were placed at 10 minute intervals in 30 %, 50 %, 70 %, 90 % and 100 % ethanol. Afterwards all the specimens were left overnight in 100 % ethanol in order to complete the dehydration process.

The third phase consisted of embedding:

Quetol resin was used and was prepared as follow: 1.94 g of Quetol (Ethylene glycol Diglycidyl ether—$C_8H_{14}O_4$), 2.23 g of NMA (Nadic Methyl Anhydride), 0.83 g of DDSA (Dodecenyl Succinic Anhydride) and 0.10 g of RD2 were mixed and rotated for 5 minutes. Then 0.05 g of S1 (DMAE: 2-dimethylamino-ethanol) was added to the mixture and this yielded a 5 g mixture of Quetol resin.

The specimens were then placed in a mixture, consisting of 50 % resin and 50 % of 100 % ethanol, for 1 hour. The specimens were then placed in 100 % resin for 6 hours.

The specimens were then placed in a fresh mixture of resin and placed in the oven at 65°C for 36 hours in order to allow polymerization to occur.

The specimens were then cut into 100 nm ultrathin sections, by way of a standardized ultramicrotomic technique, placed on copper grids and stained for 15 minutes with uranylacetate and then for a further 5 minutes with leadcitrate.

The specimens were then subjected to transmission electronmicroscopy, using a Philips transmission electron microscope.

In order to prevent any possible post-mortem, autolytic changes influencing the morphological analysis, all specimens were subjected to primary fixation within five minutes of death of each experimental animal.

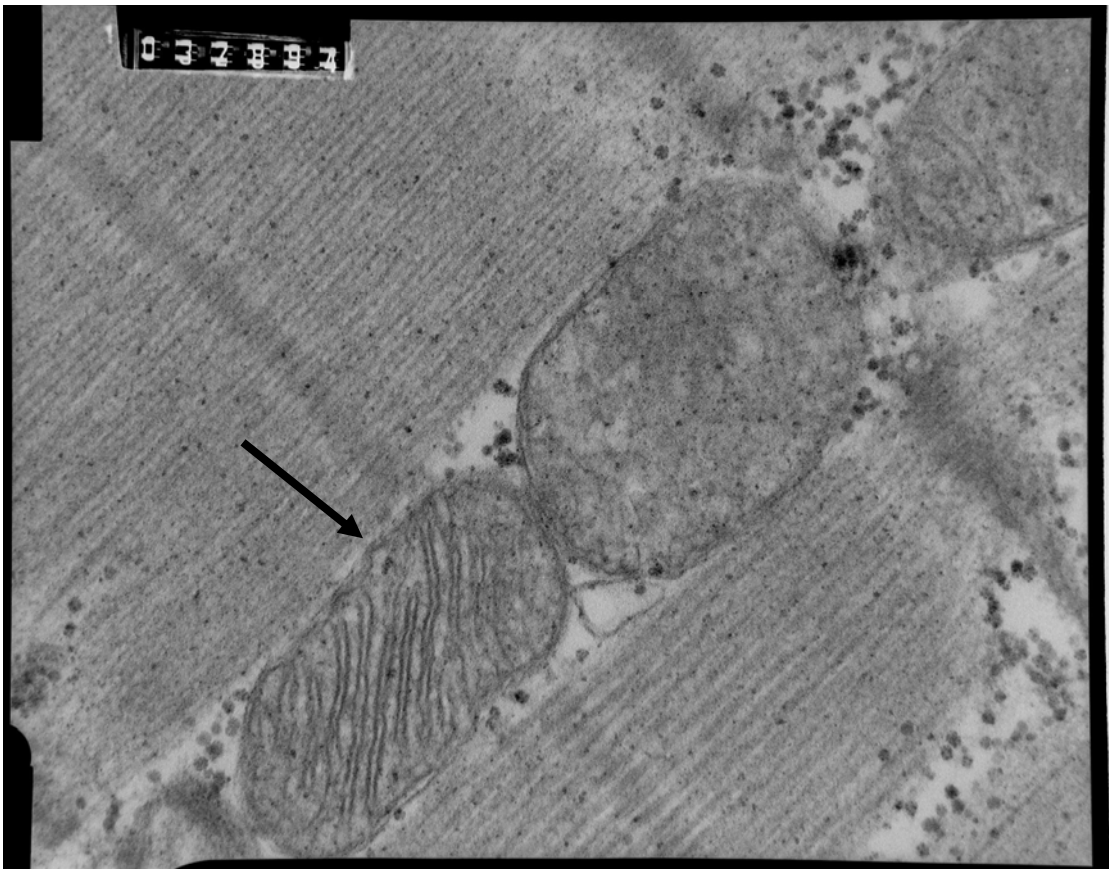

Figure 7.1. A section through the mid-region of the anterior wall of the left ventricle of a normal Dorper wether (x 55 000). Note the organized, parallel arrangement of myofibrils and the row of mitochondria (arrow) between two adjacent bundles of myofibrils.

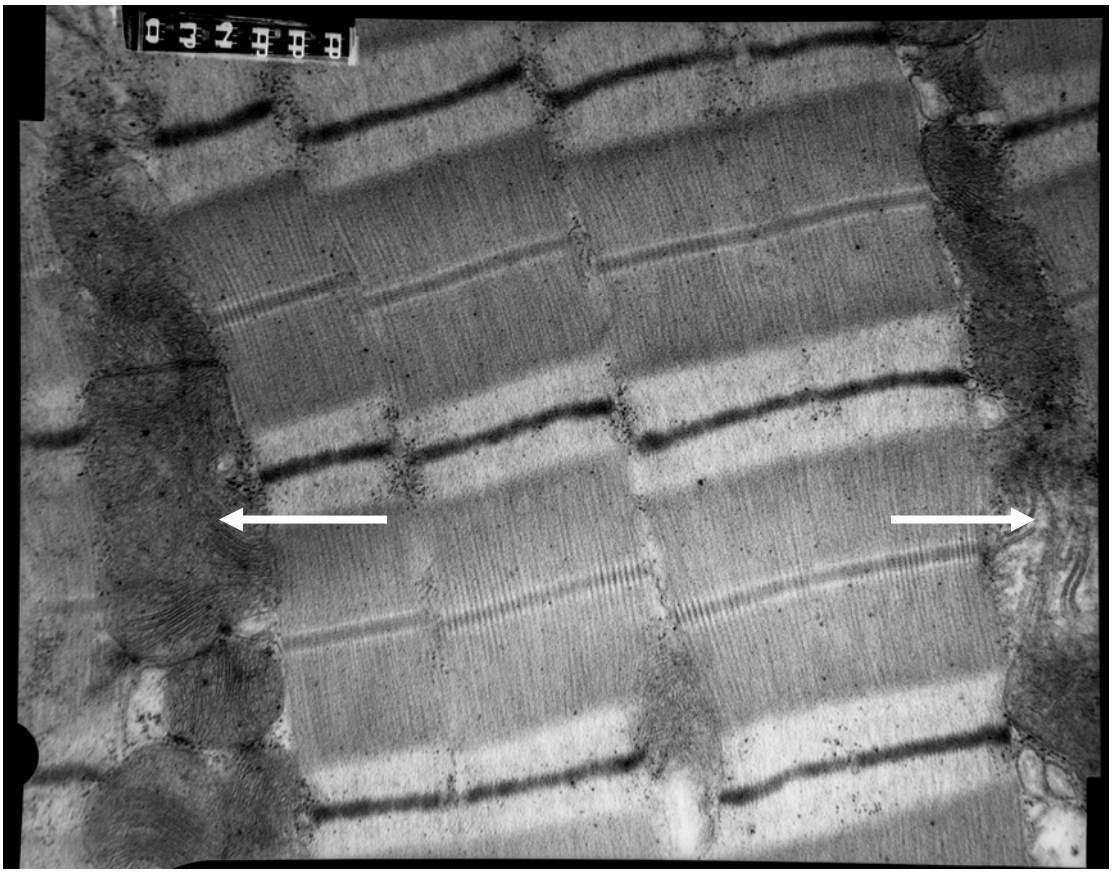

Figure 7.2. This is another section through the mid-region of the anterior wall of the left ventricle of a normal Dorper heart (x 45 000). Once again, note the organized arrangement of myofibrils, all in a parallel arrangement with a row of mitochondria (arrows) on the lateral aspects of these four bundles of myofibrils.

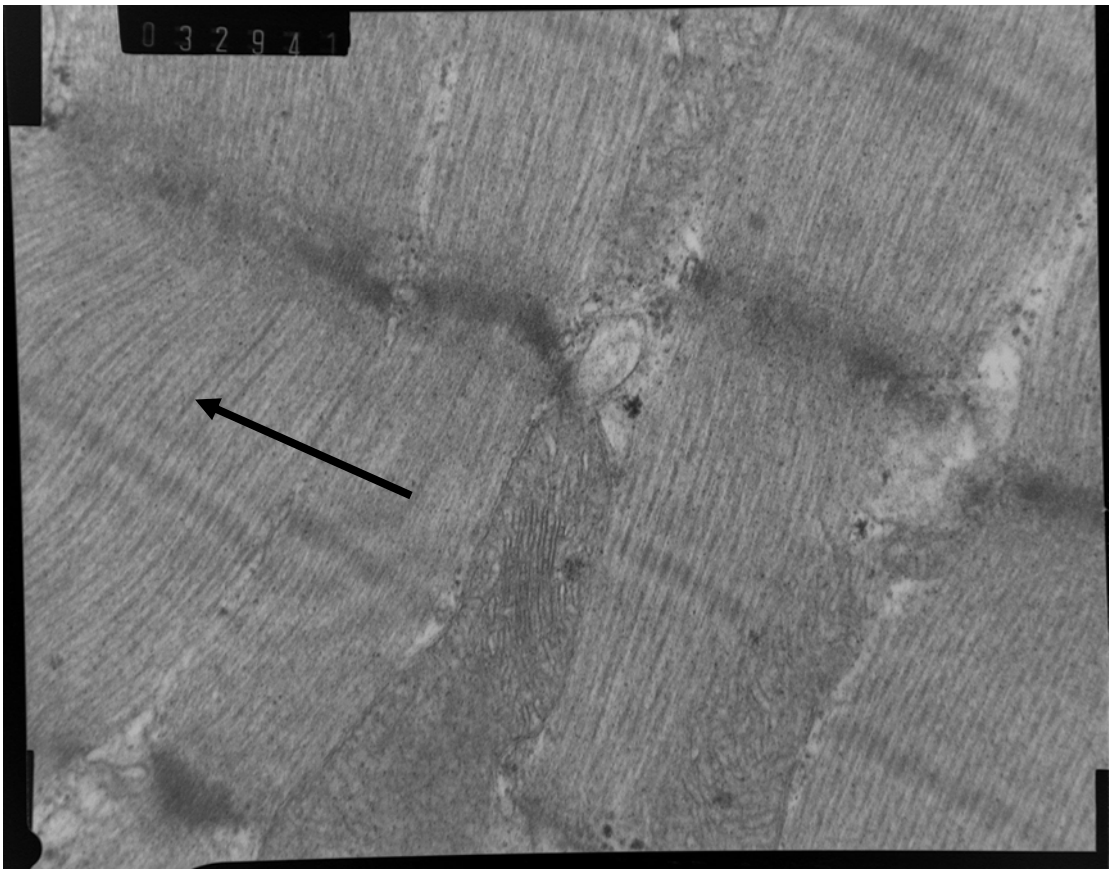

Figure 7.3. This is another section through the mid-region of the anterior left ventricular wall of the normal Dorper heart to once again demonstrate the organized, parallel arrangement of myofibrils (arrow) (x 50 000).

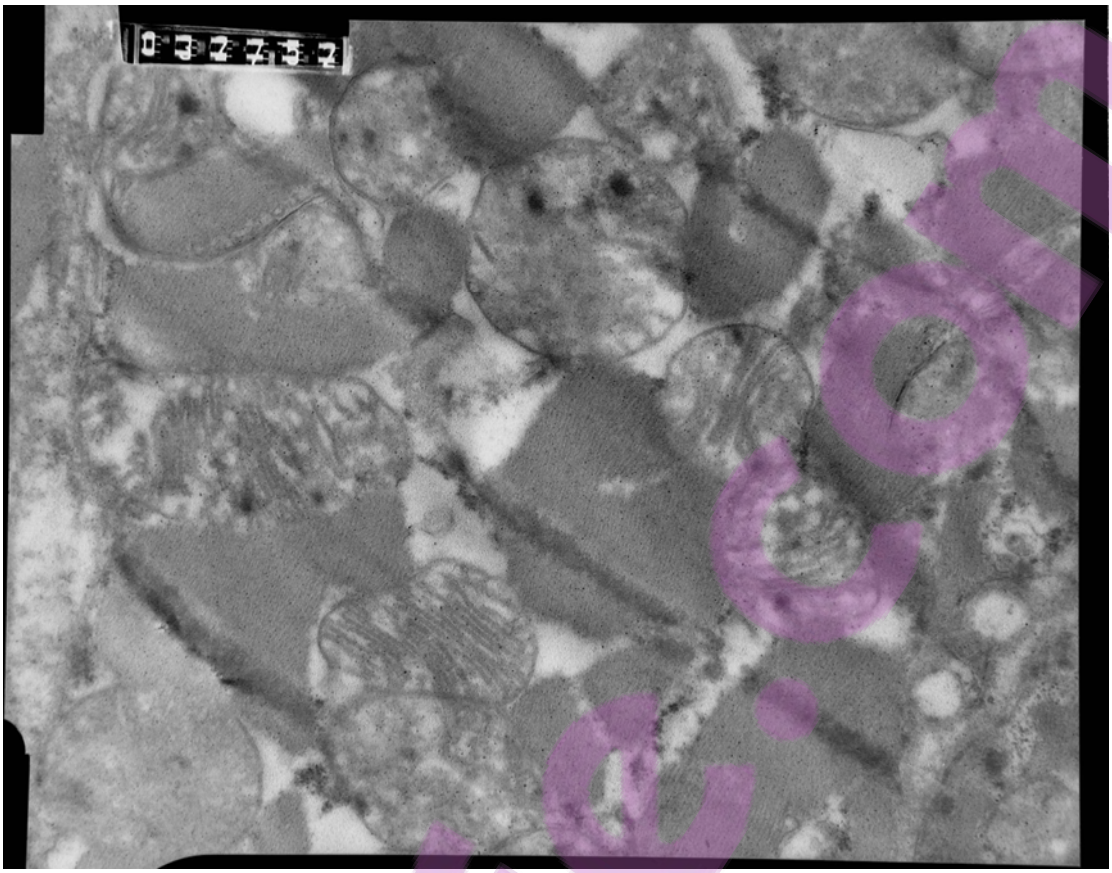

Figure 7.4. This section of the mid-region of the anterior left ventricular wall was taken from an experimental animal. Note the degeneration of myofibrils with the disorganized arrangement of mitochondria (x 50 000).

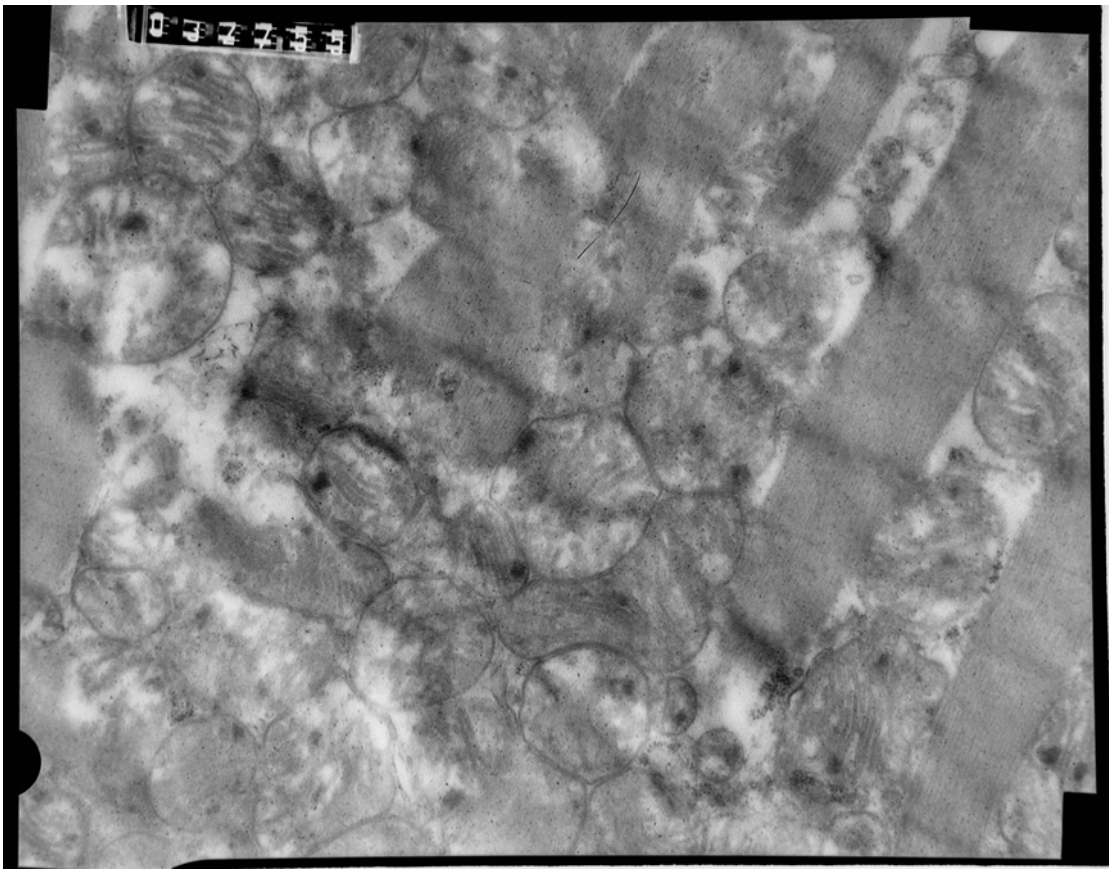

Figure 7.5. Section from the mid-region of the anterior left ventricular wall from another experimental animal. This picture also clearly shows degeneration of myofibrils, with a disorganized arrangement of mitochondria (x 50 000).

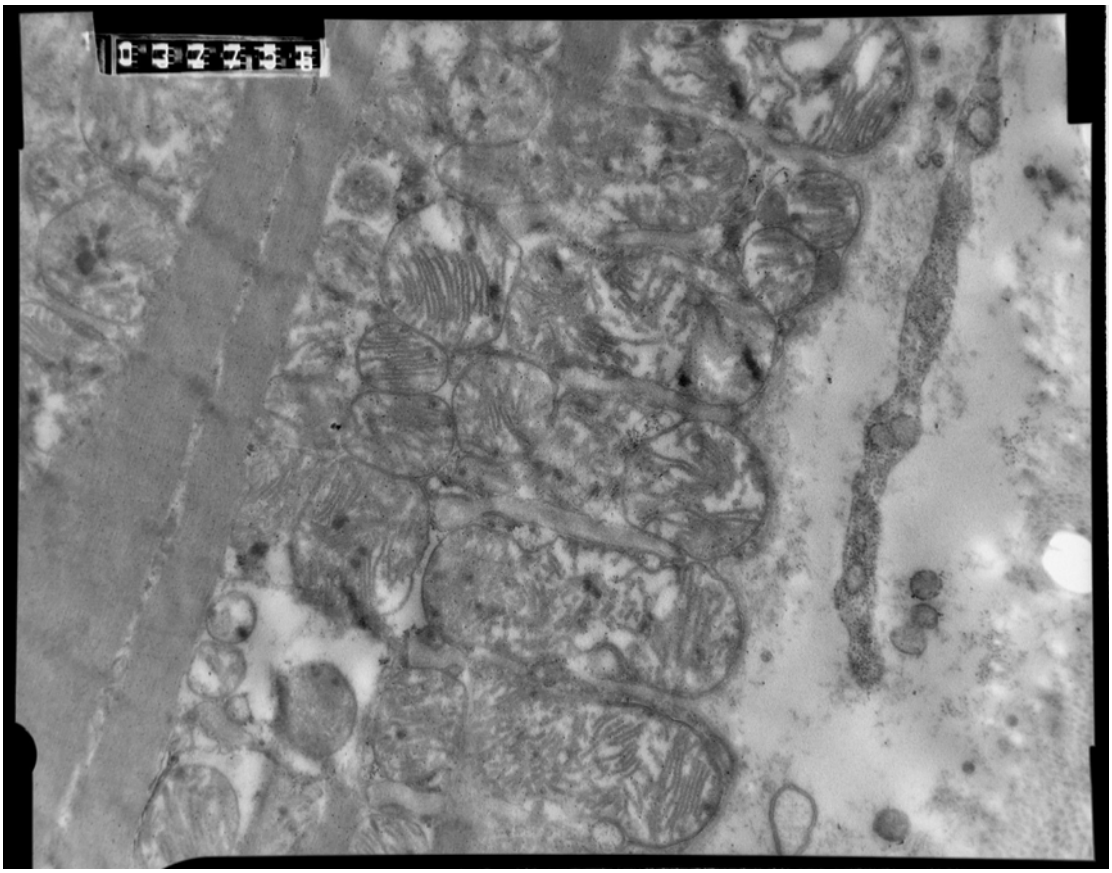

Figure 7.6. In this section, also from the mid-region of the anterior left ventricular wall from another experimental animal, almost no myofibrils are left (x 50 000).

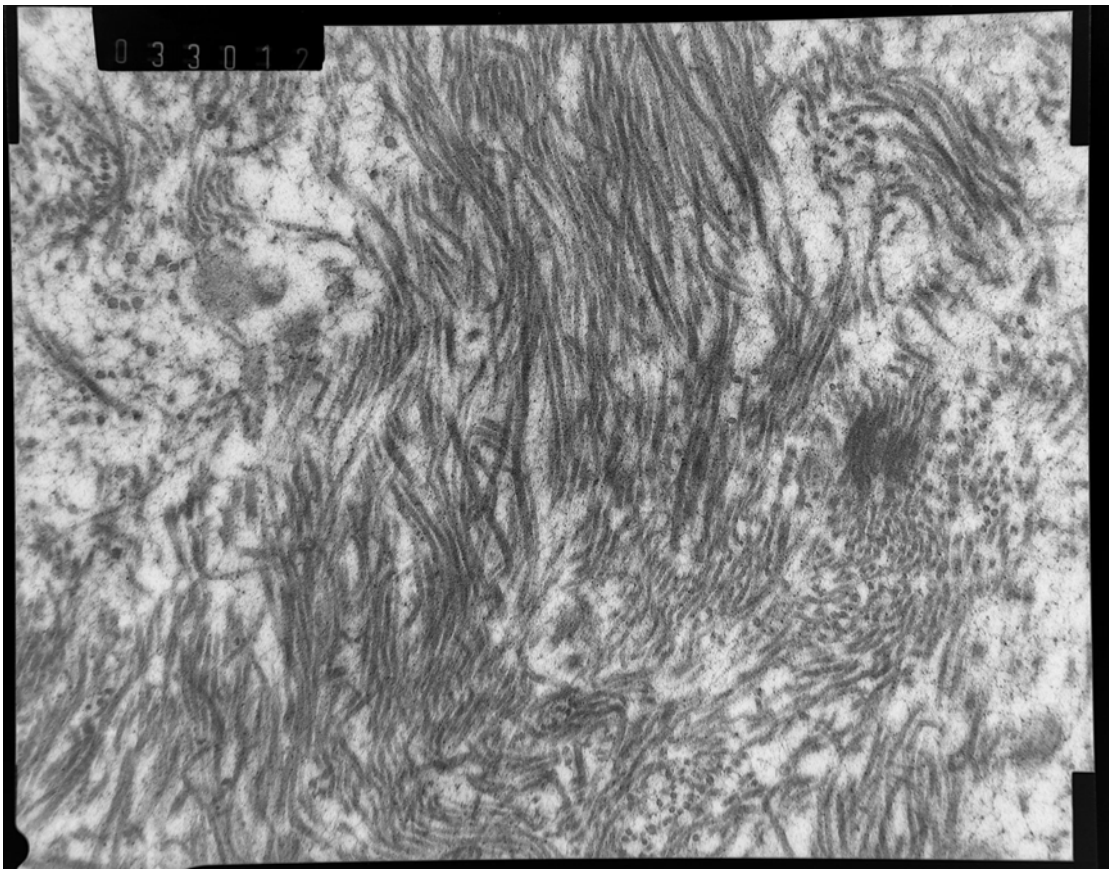

Figure 7.7. In this section, also from the mid-region of the anterior left ventricular wall from an experimental, no myofibrils or mitochondria are left. There is only fibrosis present (x 50 000).

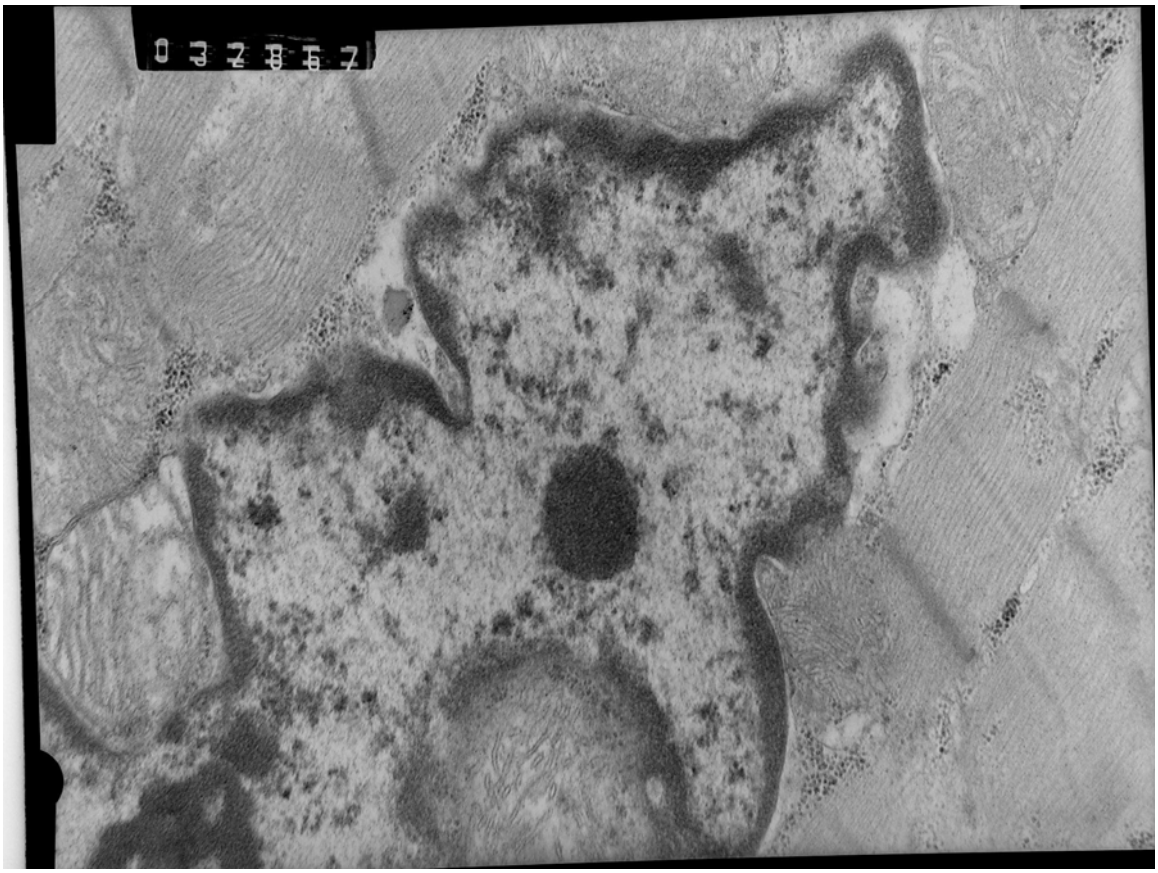

Figure 7.8. This section, from the mid-region of the anterior left ventricular wall from a normal Dorper heart, demonstrates the normal nucleus of the myocardium. Note the smooth border of the nuclear membrane (x 50 000).

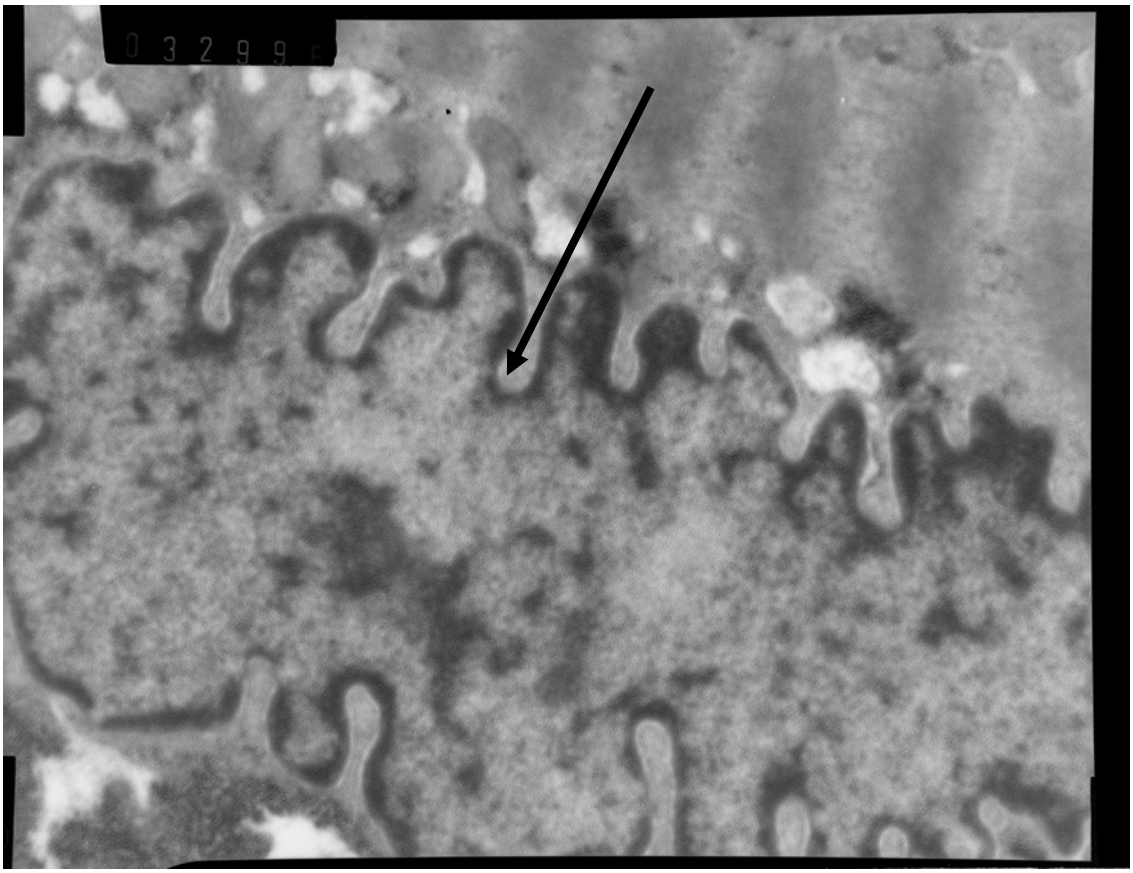

Figure 7.9. This section, from the mid-region of the anterior left ventricular wall, demonstrates a nucleus with nu-merous invaginations in the nuclear membrane (arrow) (x 50 000).

This was a purely qualitative assessment and therefore, the changes were not quantified. However, the following changes were consistently seen in all the experimental animals. Firstly, there was degeneration of myofibrils, a condition known as myocytolysis. Secondly, areas appeared where the myofibrils lost their normal, organized arrangement, a condition known as

myofibrillar disarray [10] [11]. Lastly, peculiar invaginations appeared in the nuclear membrane in all of the experimental animals.

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ABBREVIATIONS

DM	Dry mass
dP/dt	Rate of left ventricular pressure increase during left ventricular systole
ECG	Electrocardiogram
LA	Left atrium
LBBB	Left bundle branch block
LV	Left ventricle
OR	Odds ratio
PVC	Premature ventricular complex
SCS	Specialized conduction system
WPW	Wolff-Parkinson-White

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