

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 cm³ piece of myocardium (from segment B) was cut into smaller pieces of approximately 1 mm³ 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 NaKPO₄) was placed into a test tube to get a 2.5 % glutaraldehyde solution. These 1 mm³ 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— $\text{C}_8\text{H}_{14}\text{O}_4$), 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.

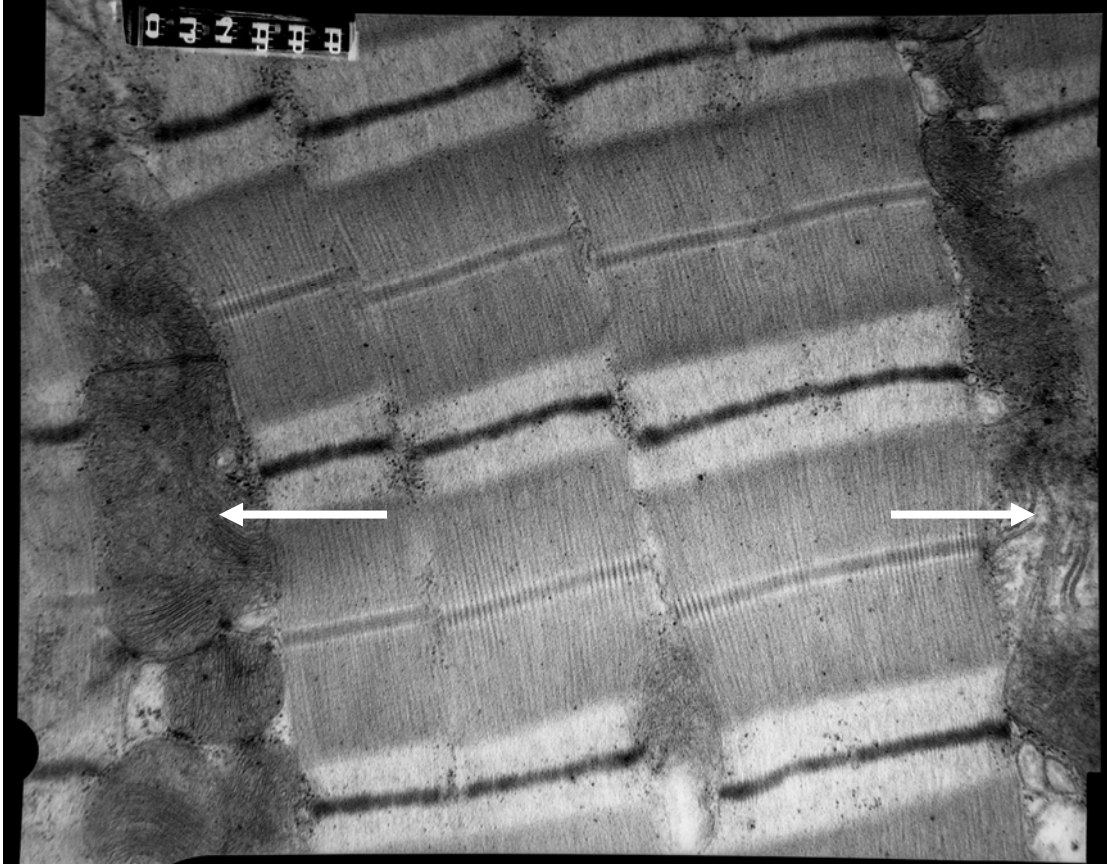


Figure 7.2. This is another section through the mid-region of the anterior wall of the left ventricle of a normal Dorper heart (x 45 000). Once again, note the organized arrangement of myofibrils, all in a parallel arrangement with a row of mitochondria (arrows) on the lateral aspects of these four bundles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

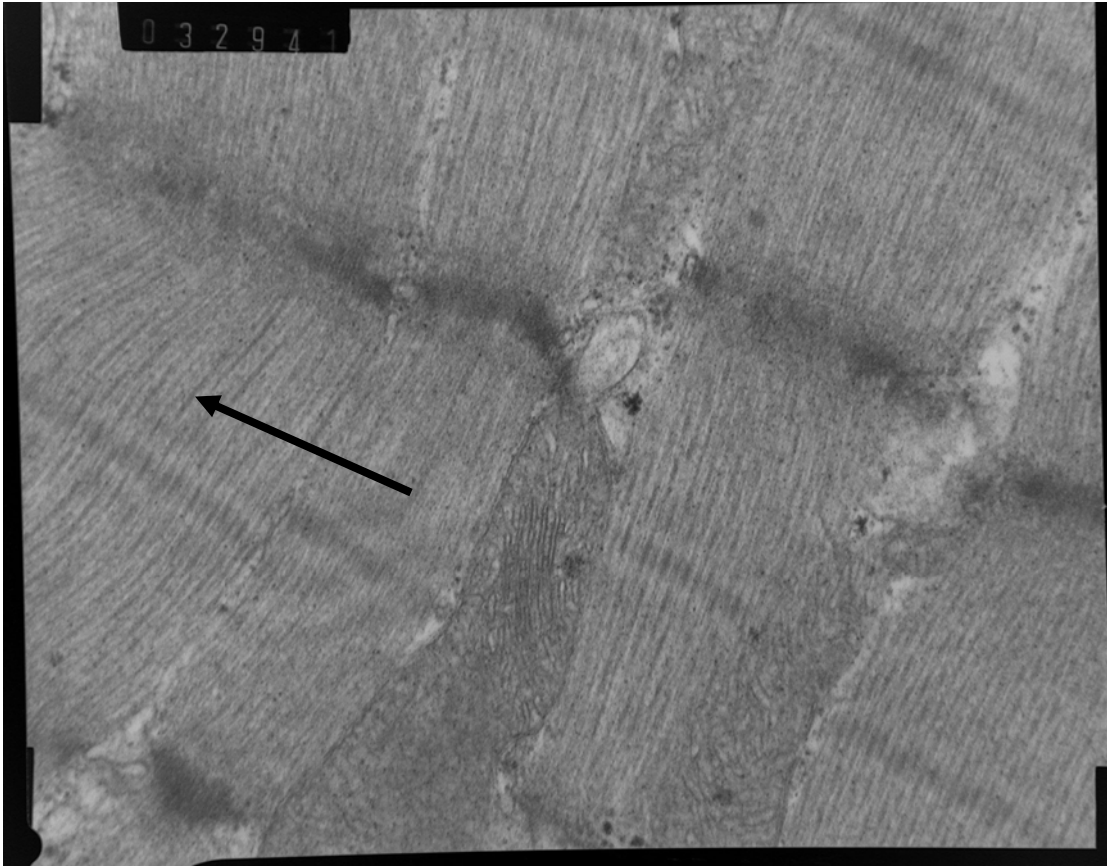


Figure 7.3. This is another section through the mid-region of the anterior left ventricular wall of the normal Dorper heart to once again demonstrate the organized, parallel arrangement of myofibrils (arrow) (x 50 000).

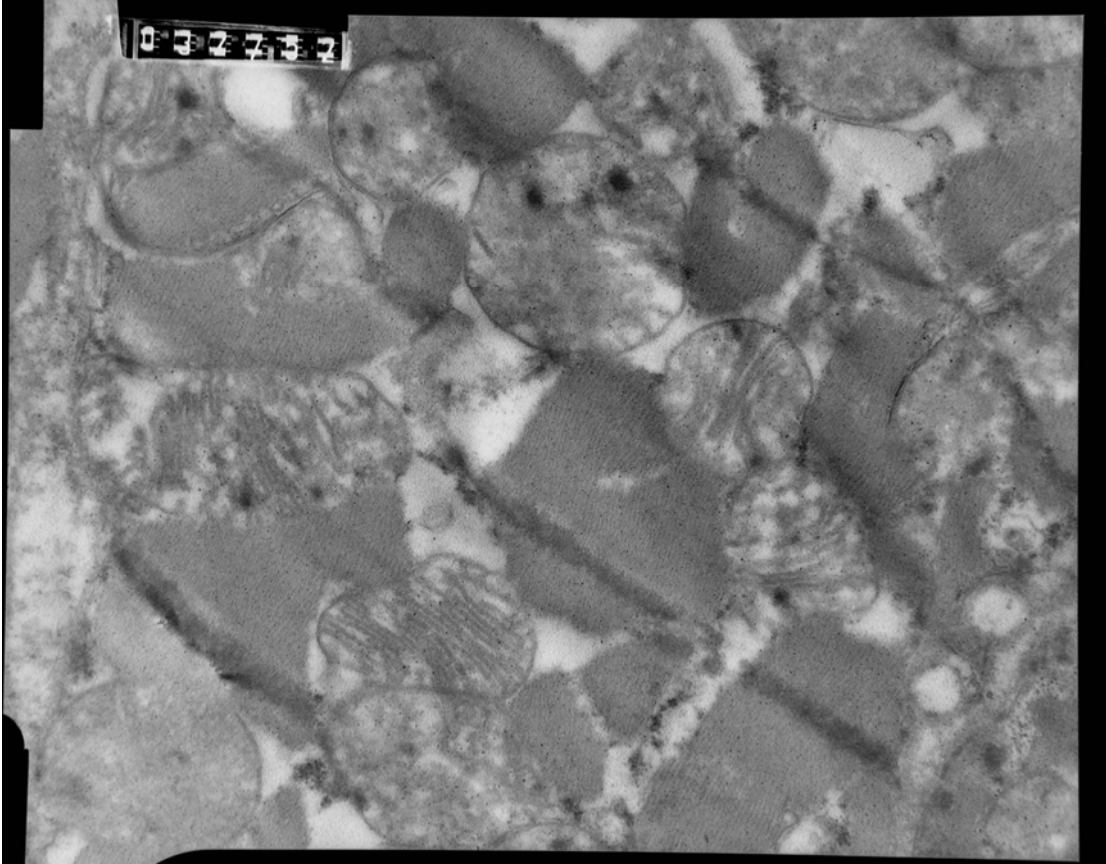


Figure 7.4. This section of the mid-region of the anterior left ventricular wall was taken from an experimental animal. Note the degeneration of myofibrils with the disorganized arrangement of mitochondria (x 50 000).

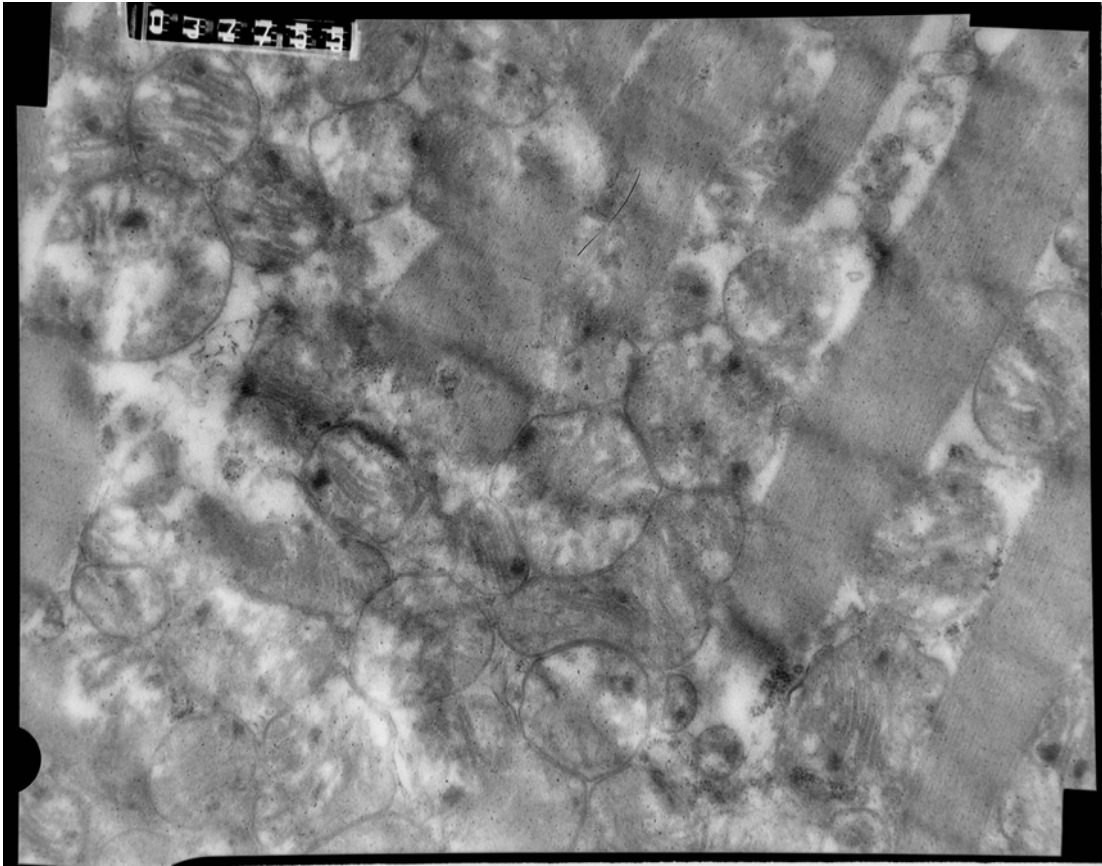


Figure 7.5. Section from the mid-region of the anterior left ventricular wall from another experimental animal. This picture also clearly shows degeneration of myofibrils, with a disorganized arrangement of mitochondria (x 50 000).

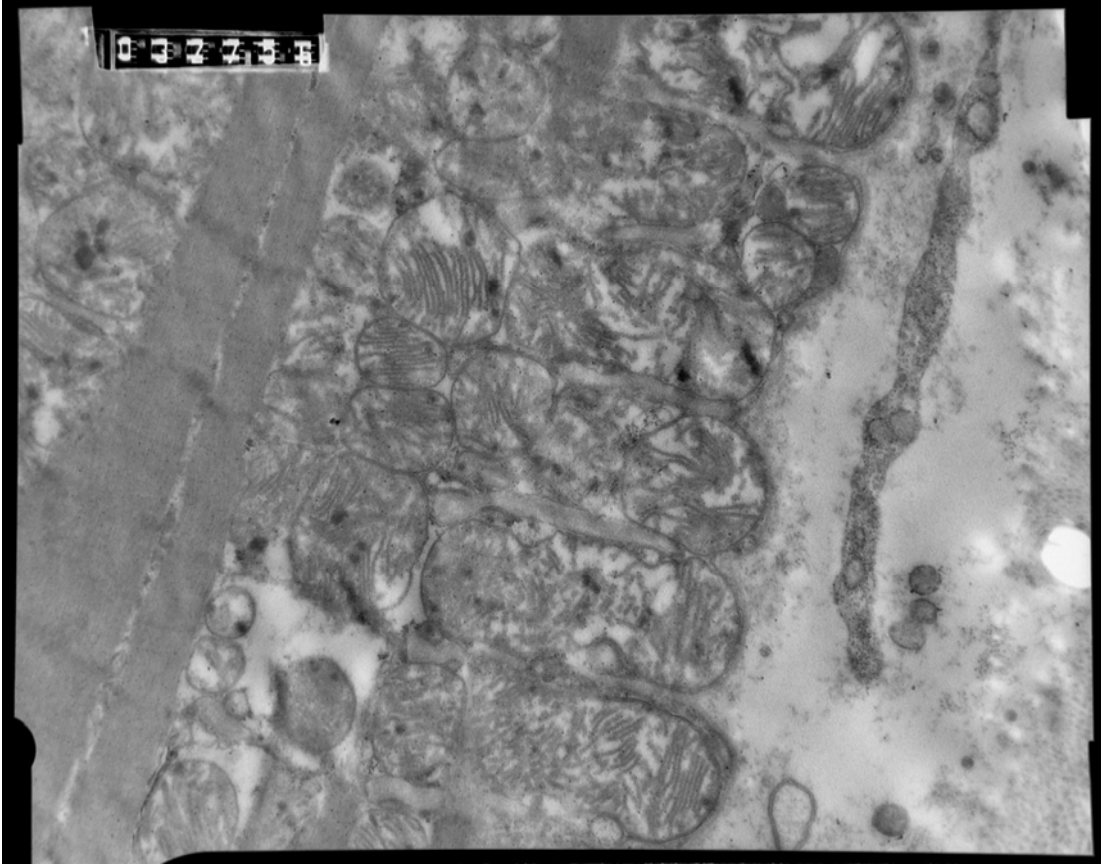


Figure 7.6. In this section, also from the mid-region of the anterior left ventricular wall from another experimental animal, almost no myofibrils are left (x 50 000).

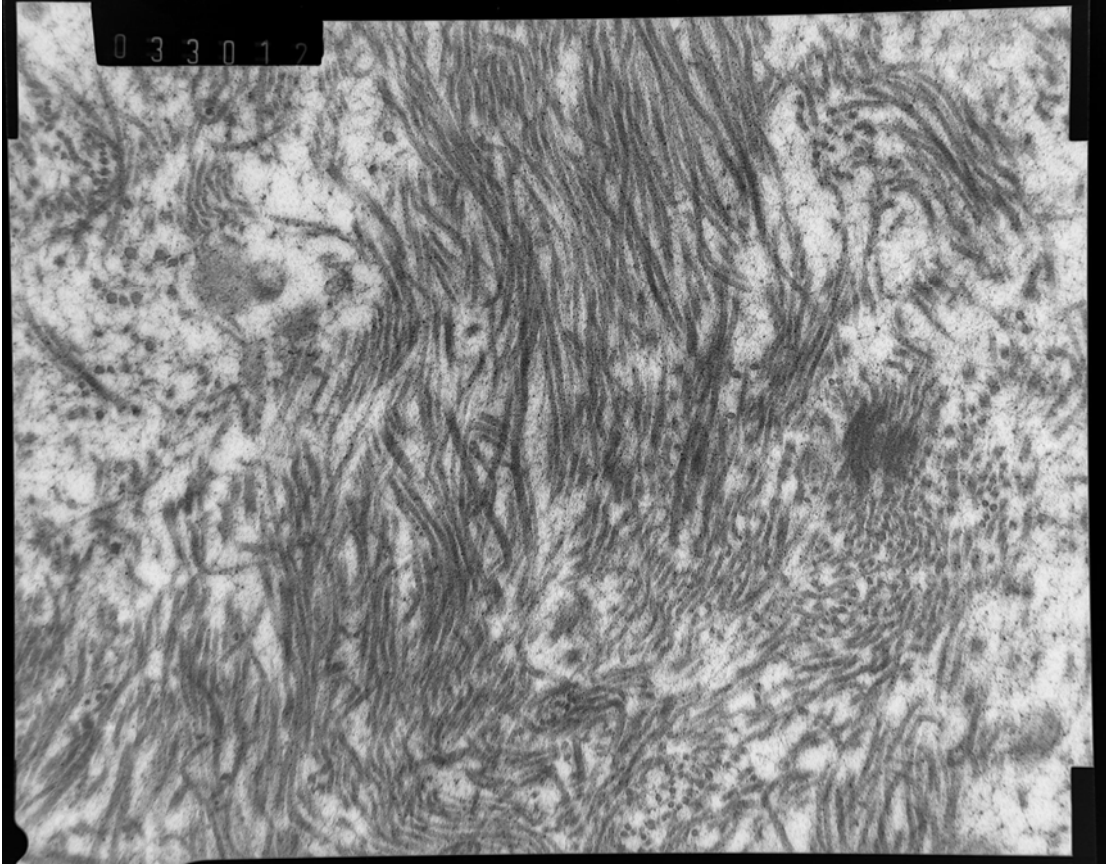


Figure 7.7. In this section, also from the mid-region of the anterior left ventricular wall from an experimental, no myofibrils or mitochondria are left. There is only fibrosis present (x 50 000).

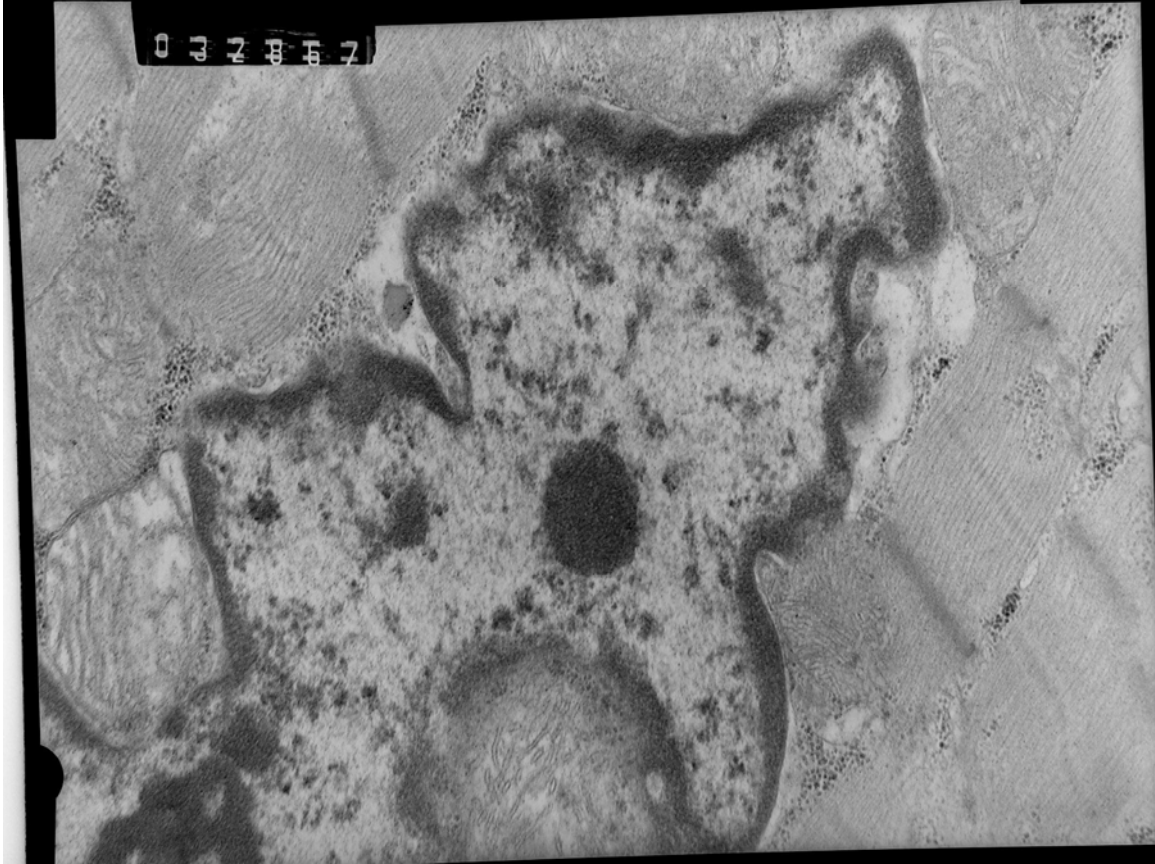


Figure 7.8. This section, from the mid-region of the anterior left ventricular wall from a normal Dorper heart, demonstrates the normal nucleus of the myocardium. Note the smooth border of the nuclear membrane (x 50 000).

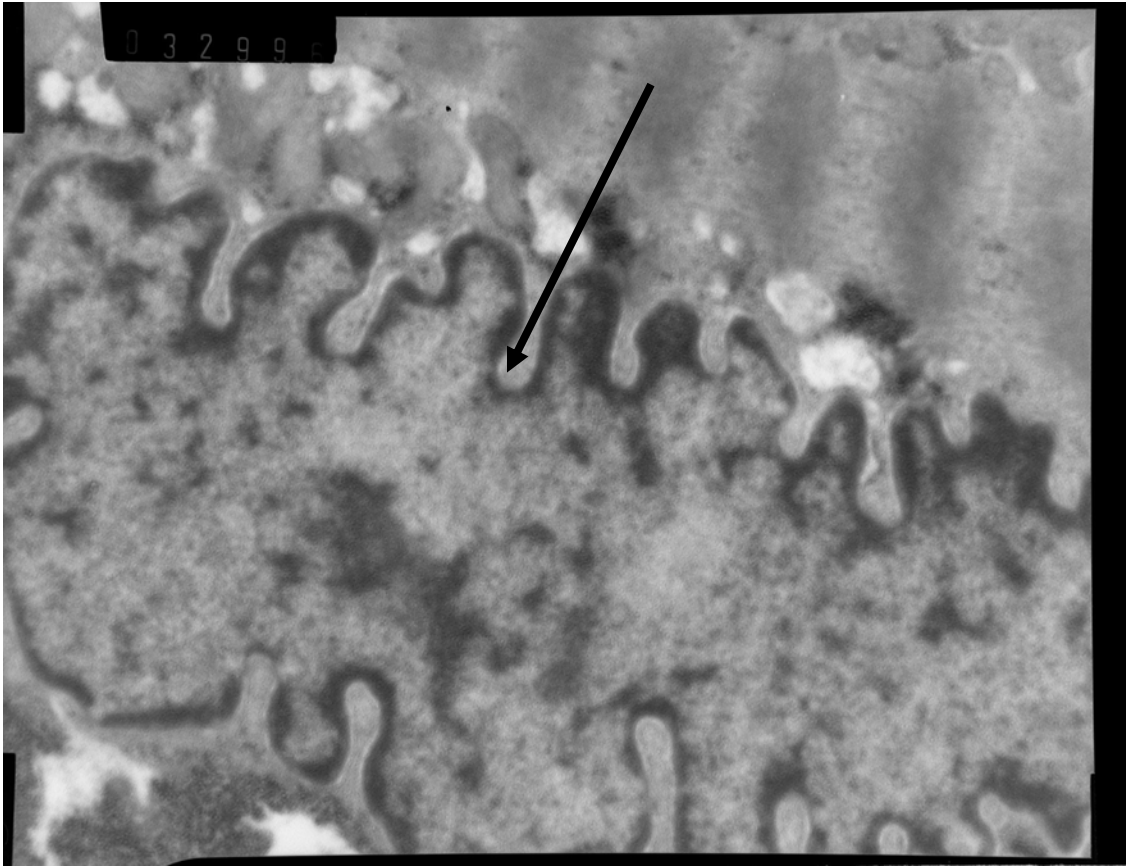


Figure 7.9. This section, from the mid-region of the anterior left ventricular wall, demonstrates a nucleus with numerous invaginations in the nuclear membrane (arrow) (x 50 000).

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
LBBS	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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