GENERAL DISCUSSION AND CONCLUSIONS ON THE PATHOGENESIS OF GOUSIEKTE

6.1 INTRODUCTION

Gousiekte is a disease of ruminants characterised by heart failure four to six weeks after the ingestion of toxic rubiaceous plants. Signs of congestive heart failure are present in most field cases, including lung oedema, accumulation of fluid in body cavities, hydropericardium and generalised congestion, and various degrees of cardiac dilatation. Histopathological lesions characterised by myofibre hypertrophy, necrosis with replacement fibrosis and an associated round cell infiltration and myofibre atrophy are found most consistently in the left free ventricular wall with a predilection for the subendocardial region. In animals with an extended latent period, lesions extend to involve the interventricular septum and right free ventricular wall (Kellerman *et al*. 2005). "Atypical" lesions have also been reported and are characterised by myofibre degeneration or the absence of lesions (Hurter *et al*. 1972; Adelaar, Terblanche & Smit 1966).

Sheep that were examined in the present study after short latent periods (<35 days) were representative of "atypical" lesions including fibre hypertrophy and small, scattered foci of necrosis throughout the ventricular wall (transmural) with mononuclear cell infiltration and focal areas of fibrosis. A less common lesion was diffuse atrophy without replacement fibrosis.

Cases with longer latent periods (>35 days) were more representative of the "typical" lesions characterised by fibre hypertrophy, replacement fibrosis, myofibre atrophy and necrosis, mononuclear cell infiltration, endocardial thickening, and arterial medial hypertrophy and oedema. The necrotic foci were

either evenly scattered throughout the ventricular wall (transmural) or associated with the areas of subendocardial fibrosis.

In rats twice exposed to pavetamine lesions were characterised by multifocal, mild to moderate transmural necrosis with fibrosis, and atrophy of fibres in the subendocardial region.

Ultrastructural lesions included breakdown of thick (myosin) filaments, fragmentation of Z bands, and selective proliferation of mitochondria and sarcoplasmic reticulum in areas previously occupied by myofibrils. Advanced myocardial injury was characterised by complete loss of intercellular connections and necrosis of myocardial cells.

In both ruminants and rats, the following data was considered in an attempt to elucidate the pathogenesis of the myocardial lesions emanating from the current research, research conducted in collaboration with other scientists and information from the literature:

- characteristics of the toxic principle of pavetamine;
- anatomical pathological changes in sheep and rats exposed to either pavetamine, crude plant extracts, or plants associated with gousiekte;
- clinical pathological parameters to monitor the toxic effect of gousiekte on the myocardium during latency;
- cardiodynamic parameters in sheep and rats exposed to pavetamine, crude plant extracts or gousiekte-inducing plants to demonstrate initial heart failure;
- biochemical findings in isolated fragmented sarcoplasmic reticulum from sheep with gousiekte; and
- the influence of pavetamine on gene expression in the rat heart.

Based on the information emanating from this study and previous research the following deductions are made to explain the pathogenesis of the myocardial lesions.

1. Pavetamine has a prolonged effect on the myocardium owing to the inhibition of protein synthesis, and also influences the energy production system that affects the function of the myocytes. The structure of the myocytes is not affected during the early stages of the latent period but eventually myofibre hypertrophy, atrophy, degeneration and necrosis are seen.

2. Replacement fibrosis in the subendocardial region is a sequel to the effect of pavetamine on myofibres and the consequence of ischaemia owing to impaired myocardial perfusion of, particularly, the subendocardial region as a result of decreased myocardial contraction, increased diastolic pressure, tachycardia and myofibre hypertrophy.

3. Cardiac dilatation is a compensatory mechanism, a result of the myofibre damage inflicted by pavetamine and ischaemia (pathological dilatation).

4. Lesions in animals with gousiekte represent a final, common pathway of cellular damage rather than a manifestation of a specific type of heart disease. Animals may die during any stage in the development of the lesions. “Atypical” lesions represent a manifestation of the disease in a progression that terminates with dilated cardiomyopathy if the animal does not die during the early stages.

These hypotheses provide an explanation, for the first time, for the latent period between ingestion of the plant and the onset of illness in gousiekte. They also explain the wide range of lesions seen in experimental cases, and demonstrate that the “typical” lesions of gousiekte are not pathognomonic and that the absence of “typical” lesions does not rule out a diagnosis of gousiekte in situations where exposure to the causative plants and the clinical history support such a diagnosis.

6.2 EFFECT OF PAVETAMINE ON HEART MUSCLE

The term protein turnover is used to describe the continuous synthesis and degradation of muscle protein in the body, including the myocardium (Swick & Song 1974; Earl *et al.* 1978). In rats, dogs, fowls and mice it was found that the average protein turnover rate of cardiac muscle was more rapid than that of skeletal muscle. In the rat a ten-fold difference in turnover rate between the ventricular and *tensor fasciae latae* myofibres was detected using [^{3}H]leucine (Earl *et al*. 1978). Swick and Song (1974) reported a six-fold difference in turnover rate between myosin from cardiac muscle and skeletal muscle in the rat.

Pavetamine is an inhibitor of myocardial protein synthesis, and in rats exposed to pavetamine the inhibition was sustained for at least 48 hours, in contrast to the other organs that were either unaffected or recovered rapidly (Schultz *et al*. 2001). Compared to other organs, cardiac muscle is therefore particularly susceptible to any disturbance of protein synthesis, and Schultz *et al.* (2001) postulated that while myocardial protein is degraded during physiological turnover, pavetamine inhibits the synthesis of new myocardial protein. Depending on the half-life of the affected cardiac protein, a point will be reached where breakdown of myocardial contractile protein exceeds synthesis to the extent that heart failure will occur.

One of the most striking, consistent features of gousiekte is the latent period of about four to eight weeks (Theiler, Du Toit & Mitchell 1923), and any proposed mechanism to elucidate the pathogenesis will have to take this into consideration. The duration of the latent period varies significantly between animals, as demonstrated in chapter 3. Based on the available information from field cases and experimentally induced cases of gousiekte, including the current study, it appears that the duration of the latent period depends on, amongst other factors, individual variation within and between species, the dosage of pavetamine, and the route of exposure.

Rats exposed to pavetamine on day 0 and day 27, as outlined in chapter 5, showed a dramatic weight loss between five and ten days after the first exposure, then regained weight until about three days after the second exposure (figure 5.1 and table 5.2) and finally kept on losing weight until the trial was terminated. It is assumed that there is a correlation between weight gain and the effect of pavetamine on the myocardium. These findings suggest that animals can recover if exposed to a non-lethal dose of pavetamine, which must be taken into consideration in formulating the pathogenesis of the myocardial lesions. To elucidate this aspect of the pathogenesis, rats exposed to a single dose of 5 mg/kg pavetamine will have to be monitored over an extended period of time to establish if they eventually develop cardiac failure or recover from the initial damage reflected in the weight loss.

The residual effect of a single dose of pavetamine was investigated by Theiler, Du Toit and Mitchell (1923) who reported that during an outbreak of gousiekte 1 047 of 1 761 sheep died 4-6 weeks after being on gousiekte veld for less than 24 hours. Furthermore, it was confirmed that limited exposure to the plant could induce typical gousiekte lesions when a sheep died 37 days after eating 0,9 kg *Pachystigma pygmaeum* in a single dose (Fourie 1994).

Gousiekte therefore does not necessary result from protracted intake of pavetamine, which supports the hypothesis that in animals exposed to pavetamine, whether in a single dose or repeated doses, a stage is reached after which the lesions are irreversible and self-perpetuating owing to the progressive deleterious effect of pavetamine on the myocardium. The self-perpetuating nature of the lesions during the later stages of the disease is supported by the presence of chronic active myocardial lesions in animals long after exposure to pavetamine has ceased (Theiler, Du Toit & Mitchell 1923). This was also noted in the current study. It must be pointed out that most of the experimental cases of gousiekte reported in the literature were produced by exposing animals to repeated doses of pavetamine, as was done in this study.

A study of the early stages of the latent period is problematic owing to the absence of morphological myocardial lesions during the first approximately

three weeks after exposure to gousiekte-inducing plants (Fourie *et al*. 1989). Additional evidence of the timing of myocardial degeneration and necrosis during the development of gousiekte was obtained by measuring the activity of serum enzymes in affected animals. Myocardial damage in 20 sheep dosed with either *P. pygmaeum* or *F. homblei* and 15 field cases of the disease in sheep was confirmed by an increase in serum activity of aspartate trans-aminase (AST) and, to a lesser extent, lactate dehydrogenase (LD) during latency (Fourie *et al*. 1989). Fourie *et al.* measured the serum activity of AST and LD as a diagnostic aid in studying cardiac damage in sheep. Elevated levels of particularly AST from about 21 days onwards after commencement of feeding trials were found to be the most reliable clinical pathological parameter that could be used for the identification of affected animals during latency. In the experimental animals the enzyme activity followed a peculiar pattern in that no increases were found during the first two to three weeks of the latent period, followed by a peak in activity that could occur up to 30 days after dosing had ceased.

Considering the short clearance half-life of about 12 hours of AST (Latimer, Mahaffey & Prasse 2003) it appears that myocardial damage occurs approximately three weeks after exposure to pavetamine and is sustained long after dosing has stopped, which indicates self-perpetuating degeneration. Tachycardia, abnormal heart sounds on auscultation, increased CPFI values and/or arrhythmia were recorded terminally in these cases (Fourie *et al*. 1989).

According to Zak, Ratkitzis and Rabinowitz (1971) the half-life of the major cardiac myofibrillar proteins myosin, actin and tropomyosin in rats vary between 11 and 12 days. A possible explanation for the latent period in ruminants is that initially the protein inhibition and negative energy metabolism effect (bio-chemical lesion) of pavetamine are inadequate to cause heart failure and no morphological lesions are evident. Depending on the variables that affect the latent period as outlined, a critical period is eventually reached when sufficient proteins are depleted and not replaced resulting in heart failure and ischaemia that give rise to myofibre degeneration and necrosis. The situation is different in

rats under the experimental conditions used, where cardiac malfunction sets in too early for the inhibition of myocardial protein synthesis to explain it in the same way as in sheep. The possibility that rats are more susceptible to the energy metabolism effects of pavetamine should be considered.

The effect of pavetamine on myocardial proteins, and more particularly myosin, was demonstrated by Ellis, Schultz & Basson (2007) who showed that in rats exposed to pavetamine, immuno-labeling of myosin revealed an altered expression of myosin, whereas the expression of actin remained unaltered. In the rats down-regulation of the myosin light chain 2 gene resulted in impaired contractility of the heart, and expression of the beta isoform of cardiac proteins resulted in a slower contraction and saving of energy. This corroborates the findings of Snyman *et al*. (1982a) who demonstrated that the lesions in gousiekte are characterised by impaired energy utilisation in the myofibre contractile system with diminished sensitivity to activating calcium ions. This has serious consequences for the myocardium since it is almost totally dependant on aerobic respiration for energy (Snyman *et al*. 1982a). The effect of pavetamine on the myocardium is therefore not confined to the inhibition of protein synthesis but also negatively affects the energy metabolism of myofibres.

The most striking ultrastructural lesions in sheep in this study include a preferential loss of thick (myosin) filaments in degenerative myocytes, which results in a frayed appearance. This is in line with the findings of Schutte *et al.* (1984) and supports the findings of Schultz *et al*. (2001) and Ellis, Basson and Schultz (2007) that pavetamine inhibits myocardial protein synthesis. It also explains the decreased contractility of the myocardium in sheep (Van der Walt & Van Rooyen 1977; Van der Walt *et al*. 1981; Fourie *et al.* 1989) and in rats (Hay *et al.* 2001; Hay, Schultz & Schutte 2008). Owing to the limited extent of the lesions in rats it was not possible to identify which filaments were most affected.

In an attempt to explain the latent period in gousiekte, Fourie (1994) considered the notion that cardiac injury is caused by autoimmune processes. The possibility was considered that antibodies formed against plant protein may cross-react with heart muscle antigens, or that auto-antibodies may be formed against cardiac antigens released by damage to the heart caused by the toxin associated with gousiekte.

Neither a humoral, nor a cellular immune response was demonstrated against any of the prepared cardiac antigens in affected sheep (Fourie 1994). Schultheiss *et al.* (1986) reported that sera from human patients with dilated cardiomyopathy contained circulating auto-antibodies directed against the adenosine diphosphate/adenosine triphosphate (ADP/ATP) carrier of the inner mitochondrial membrane. Anti-myosin antibodies have been detected in the sera of humans following cardiac surgery (De Scheerder *et al.* 1985). The fact that animals can die of gousiekte without any anti-heart antibodies being present strongly suggests that gousiekte is not an autoimmune disease (Fourie 1994). The association of round cells, and particularly lymphocytes, with the myocardial lesions in gousiekte is therefore most likely due to release of myocardial antigens following necrosis of myofibres.

It is therefore hypothesised that:

- The action of pavetamine on the myocardium is initially purely functional and induces no morphological lesions.
- The lesions that are recognised develop over a period of time after a single or repeated exposure that is not immediately fatal, resulting mainly from the effect of pavetamine on protein synthesis and energy metabolism of myocytes and attempts by the heart to compensate for impaired function, and from myocardial ischaemia (*vide infra).*

6.3 MYOCARDIAL LESIONS

Hypertrophy of myocardial fibres in the subendocardial region was present in all the sheep exposed to *P. pygmaeum* in this study. This is the first study to show that myofibre hypertrophy is a constant lesion in sheep dosed with *P. pygmaeum* (see chapter 3). Subendocardial fibrosis and myofibre anisocytosis and necrosis of particularly the left free ventricular wall are a hallmark in animals with “typical” gousiekte lesions, and any mechanism proposed for the pathogenesis of the myocardial lesions would have to take this into account.

Although it is believed that myocardial myocytes are terminally differentiated, approximately 15 % to 20 % of myocytes in rats retain the capacity to undergo hyperplasia (Kajstura *et al.* 2000; Leri *et al.* 2000). The proportion of terminally differentiated myocytes and those that retain the capacity to replicate, changes with age, and there is no point during the life span of rats at which all myocytes are comparable in terms of age, size, shape and molecular properties. Myocytes respond to pathological insults by means of hypertrophy or hyperplasia, and these responses are influenced by cell volume, which in turn reflects age (Kajstura *et al.* 2000; Leri *et al.* 2000).

According to Dunlop and Malbert (2004), hypertrophy of myocardial fibres is an intrinsic compensatory mechanism to meet the body’s demand for increased cardiac output. Enlargement of individual fibres is due to an increase in the rate of protein synthesis and the addition of new sarcomeres. Furthermore, large (old) myocytes do not react to growth stimuli and are more prone to becoming necrotic. Small cells, on the other hand, are younger, can undergo hypertrophy and are less susceptible to necrosis (Kajstura *et al.* 2000; Leri et al. 2000). It is postulated that the morphophysiological differences of myofibres, i.e. age, size, shape and molecular properties, may explain the different responses of myofibres to pavetamine, which result in anisocytosis that is particularly prominent in the subendocardial region in sheep with long latent periods (refer to chapter 3).

In normal myocardial tissue of rats and humans there is a constant density of capillaries per unit area of tissue (Rabinowitz & Zak 1972). This is because in neonatal animals the development of capillaries occurs in proportion to the increase in the size of the cells. On the other hand, in older animals very little capillary proliferation occurs, and myofibre hypertrophy will therefore lead to an increased diffusion distance between myofibres and may eventually lead to ischaemia with replacement fibrosis (Rabinowitz & Zak 1972; Unverferth 1985).

Atrophy, varying from focal to diffuse and involving individual or small groups of fibres, was present in seven sheep (table 3.3). Diffuse atrophy occurred in one animal with a short latent period. Causes of atrophy include a decreased workload, loss of innervation, reduced blood supply, inadequate nutrition, loss of endocrine stimulation, ageing and decreased protein synthesis (Schultz *et al.* 2001; Kumar, Cotran & Robbins 2003). Irrespective of the cause, atrophy represents a reduction in structural components of the cell owing to an altered balance in the production and degradation of cellular proteins (Jubb, Kennedy & Palmer 1993).

The main ultrastructural changes in degenerative/atrophic fibres, as outlined in chapter 4, comprised degradation of myofibrils and proliferation of particularly mitochondria and sarcoplasmic reticulum, confirming that the effects of pavetamine on protein synthesis and energy metabolism are most probably the major reasons for myofibre atrophy in gousiekte. However, reduced perfusion of the myocardium following heart failure may be a contributing factor (*vide infra)*. The nature of the distribution of atrophy ranging from individual fibres to diffuse atrophy in animals with a short latent period is difficult to explain. In the current study the source of pavetamine was from a plant origin yet diffuse atrophy was noted in only one animal (see chapter 3), which confirms the wide individual variation in response to the same dosage rate of pavetamine.

Myocardial necrosis that was either distributed throughout the left ventricular wall (transmural) or associated with areas of fibrosis in the subendocardial region occurred in seven of the ten experimental sheep in the *Pachystigma* study (table 3.3) and all the sheep in the *Fadogia* study (chapter 4), and was

present in all four rats dosed twice with pavetamine (table 5.1). Based on the transmural distribution of the myocardial lesions in the sheep with a short latent period and a few with medium to long latent periods, as outlined in chapter 3, and the rats euthanased on day 42 (see chapter 5), there is no evidence that pavetamine selectively affects myocardial fibres in the subendocardial region.

As pointed out above, the half-lives of the major cardiac myofibrillar proteins myosin, actin and tropomyosin in rats vary between 11 and 12 days (Zak, Ratkitzis & Rabinowitz 1971). Myocardial protein is degraded during physiological turnover and if pavetamine inhibits the synthesis of new myocardial protein and energy metabolism, a stage would be reached where breakdown of myocardial contractile protein exceeds synthesis and results in myofibre atrophy and eventually necrosis.

Subendocardial fibrosis that varied in extent from multiple small foci to almost diffuse fibrosis was present in seven sheep in the *Pachystigma* study (table 3.3). Fibrosis is common in the left free ventricular wall and apex, and in animals with more advanced lesions fibrosis extends to the interventricular septum and occasionally the right free ventricular wall (Theiler, Du Toit & Mitchell 1923; Smit 1959; Newsholme & Coetzer 1984). It was deemed necessary to investigate possible causes of this lesion, since this may be central to the pathogenesis of the myocardial lesions.

A predilection for the development of subendocardial fibrosis may be attributed to an abnormal oxygen supply-to-demand ratio (ischaemia), increased wall stress and cellular hypertrophy (Baandrup *et al.* 1981; Unverferth 1985).

According to the law of LePlace, wall stress = radius x pressure/2 x wall thickness. Tissue pressure is greater in the subendocardial tissue than in the subepicardial tissue, which means that subendocardial tissue is more prone to injury (Unverferth 1985). This is illustrated in humans, where there is overwhelming evidence that subendocardial myofibres demonstrate greater potential for ischaemic injury than subepicardial myofibres (Unverferth 1985). In

dogs, subendocardial tissue has a 20 % greater oxygen consumption per unit weight than subepicardial tissue, indicating a higher metabolic activity of subendocardial myofibres (Weiss *et al.* 1978).

Various workers have studied transmural coronary blood flow (Rudolph & Heyman 1967). Transmural distribution of coronary blood flow depends on an increasing gradient of extravascular pressure from epicardium to endocardium (highest in the endocardial region) and on vascular resistance (decreasing from epicardium to endocardium). An increase in either extravascular pressure or vascular resistance will result in decreased coronary blood flow to the myocardium (Knieriem 1978).

Dilated cardiomyopathy is characterised by low cardiac output and low coronary blood flow (Weiss *et al.* 1976). This results in inadequate myocardial perfusion, most notably of subendocardial tissue, because of an increased left ventricular end diastolic pressure. The latter produces high extravascular pressure in the subendocardial region and this is readily transmitted to, principally, subendocardial coronary arteries during diastole inhibiting flow to this area (Breithardt, Kuhn & Knieriem 1978; Unverferth 1985; Dunlop & Malbert 2004).

Blood flow to the subendocardial region normally occurs during diastole, whereas subepicardial flow is maintained during systole and diastole (Rouleau, Boerboom & Surjadhana 1979). Tachycardia will therefore reduce blood flow to particularly the subendocardial tissue, thereby exacerbating ischaemia in the already oxygen-deprived subendocardial tissue. Tachycardia is a common clinical sign in animals with gousiekte, as was noted in this and other studies (Pretorius & Terblanche 1967), and an increase in end diastolic pressure (*vide supra*) is a feature of animals with gousiekte during the later stages of the disease (Pretorius *et al*. 1973).

A number of differences are apparent between the left and right ventricle under physiological and pathological conditions (Kvasnicka & Vokrouhlichky 1991). Owing to the high intramural pressure the coronary flow in the wall of the left ventricle occurs only during diastole whereas in the right ventricle it is limited

only if there is a significant intrathoracic pressure. According to Kvasnicka and Vokrouhlichky (1991) it is difficult to evaluate the differences in the response between the left and right ventricles to a long-term volume overload because of too many variables.

From a pathophysiological point of view, sheep with gousiekte exposed to pavetamine demonstrate several characteristics that increase the risk of myocardial ischaemia of, in particular, the left ventricular subendocardial tissue. The effect of ischaemia is exacerbated by the direct effects of pavetamine on protein synthesis and energy metabolism of myocytes that are almost totally dependant on aerobic respiration for energy (Snyman *et al*. 1982a). The increased lactate and nicotinamide adenine dinucleotide (NADH) levels that were found in myocytes probably constitute an attempt by the myocardium to compensate for the shortfall in energy by increasing anaerobic metabolism (Kellerman, Coetzer & Naudé 1988). Myocardial necrosis is therefore likely to have been caused by amongst other disturbances in the energy metabolism.

Considering the available information it would appear that the deviations in myocardial energy metabolism reflected in the biochemical lesions described here are most probably a secondary consequence to, or were exacerbated by, the myocardial ischaemia resulting from impaired ventricular contraction (*vide supra*). Based on ultrastructural observations in this study, myocardial cells with advanced injury revealed selective proliferation of certain organelles associated with energy production and protein synthesis viz. mitochondria and sarcoplasmic reticulum. This supports the findings of Snyman *et al*. (1982a) and Schultz *et al*. (2001) and serves as additional evidence of impaired energy and protein metabolism (Ghadially 1988).

Medial hypertrophy and oedema of myocardial arteries and arterioles was found in six of the experimental sheep in the *Pachystigma* study (table 3.3). Similar lesions have been reported in humans with subendocardial fibrosis and the increased vascular resistance associated with the lesion is considered to be an additional cause of reduced coronary blood flow that will exacerbate the already

impeded perfusion of the myocardium (Andrade & Teixeira 1973; Unverferth, 1985; Kumar, Cotran & Robbins 2003).

This is the first detailed study of the myocardial lesions in animals where both "typical" and "atypical" lesions occurred in a single group of sheep. The study has clearly shown the tremendous variation in lesions associated with gousiekte and that animals can succumb during any stage of the development of the lesions. Considering the different changes myofibres undergo during the development of lesions the following pathogenesis is postulated for the development of subendocardial fibrosis:

- The subendocardial fibrosis has a multifactorial origin and the most important contributing factors include: (1) the effect of pavetamine on myofibre protein synthesis and energy metabolism resulting in myofibre hypertrophy, degeneration and eventually necrosis; (2) ischaemia resulting from heart failure, (3) fibre hypertrophy and replacement fibrosis of necrotic fibres, exacerbating impeded perfusion of the tissue.
- From a pathophysiological point of view, compared to the rest of the myocardium, the left ventricular subendocardial tissue appears to be particularly exposed to ischaemia in animals with heart failure.
- During the later stages of the disease the lesions are irreversible and self-perpetuating.
- Contrary to what is currently believed by veterinarians, the myocardial changes in animals with gousiekte represent a final, common pathway of cellular injury rather than a manifestation of a specific type of heart disease.
- "Atypical" lesions represent a manifestation of the disease in a progression that terminates with dilated cardiomyopathy if the animal does not die during the early stages. In animals with "atypical" lesions, the time from exposure to pavetamine to death of the animal is too short for "typical" lesions to develop.

Myocardial lesions in the rats in the current study differed significantly from the "typical" gousiekte lesions and were more comparable with the "atypical" lesions. The absence of myofibre hypertrophy in the rats could be attributed to,

amongst others, the dosage rate, duration of the latent period, and a species variation, and needs further investigation.

Myofibre atrophy noted in the rats in this study has not been reported previously and supports the hypothesis of ischaemia as a cause of myofibre degeneration/necrosis reported in ruminants. The dose and route of pavetamine can have a significant effect on the latent period and extent of the myocardial lesions, as pointed out earlier in the chapter. The induction of “typical” lesions in rats with pavetamine is an aspect of the disease that needs further investigation because this could facilitate future gousiekte research.

6.4 EVIDENCE OF VENTRICULAR FAILURE

As outlined in chapter 3, macroscopical lesions associated with left heart failure, such as pulmonary oedema and hydropericardium, were present in most of the sheep dosed with *P. pygmaeum*, which confirms that gousiekte causes left-sided congestive heart failure (table 3.2). Features suggestive of biventricular congestive heart failure, such as generalised congestion and ascites, were less obvious. Even though the terms left and right heart failure are often used, it should be kept in mind that the heart is a closed system and that it is therefore not uncommon for left heart failure to induce right heart failure and *vice versa (*Jubb, Kennedy & Palmer 1993; Cunningham & Klein 2007). Biventricular congestive heart failure would therefore be expected to be more common in animals with a long latent period, as was the case in this study.

Ventricular failure appears to be the underlying cause of death in sheep with gousiekte. Tachycardia (table 3.1) and an increase in the cardiac pulmonary flow index (CPFI) are common cardiodynamic features of sheep with gousiekte, mainly during the later stages of the disease (from around 42 days after exposure) (Van der Walt & Van Rooyen 1977; Van der Walt *et al*. 1981; Fourie *et al.* 1989; Fourie 1994).

As outlined in chapter 2, an increase in CPFI is attributed to a decrease in both the stroke volume and the pumping efficiency (contractile force) of the left ventricle relative to the right ventricle, which results in an increase in the

ventricular filling pressure (left ventricular end diastolic pressure) and pulmonary blood volume. It can also be described as the ratio of the cardiopulmonary blood volume to stroke volume and this ratio is equivalent to the number of heart beats necessary to pump blood from the right side to the left side of the heart through the lungs (Pretorius *et al.* 1973; Van der Walt & Van Rooyen 1977; Van Rooyen *et al.* 1984; Fourie *et al.* 1989).

Pipedi (1999) and Hay, Schultz and Schutte (2008) also demonstrated left ventricular failure in rats (reduced systolic function). According to Pipedi (1999) there was a significant difference in the contractility index (dP/dt_{max}) between control rats and rats exposed subcutaneously to crude *P. harborii* extract. Contractility was used as a measure of cardiac function, and the reduced contractility was considered to be the main factor responsible for heart failure. The decline in the contractile force of the heart reduced left ventricular systolic pressure significantly and resulted in an increased left ventricular end diastolic pressure. These results coincide with the findings in rats treated with pavetamine, where reduced systolic function was shown (Hay, Schultz & Schutte 2008), and in sheep fed *P. pygmaeum* (Pretorius *et al.* 1973; Van der Walt & Van Rooyen 1977; Van Rooyen *et al*. 1984). However, according to Hay, Schultz and Schutte (2008), in the rats treated with pavetamine the diastolic component of the cardiodynamic function was not affected. This was attributed to the low concentration of pavetamine used in the study or else the latent period was too short for an increased left ventricular end diastolic pressure to develop.

The left ventricular systolic pressure is the maximum pressure attained by the left ventricle before blood is ejected into the systemic circulation that supplies all the tissues of the body except the lungs. The low left ventricular systolic pressure observed in rats exposed to *P. harborii* extract and in sheep exposed to gousiekte plants (*vide supra*) is indicative of reduced systolic function resulting in more blood being retained by the ventricle at the end of the systolic ejection, which results in an increased left ventricular end diastolic pressure. The blood retained within the left ventricle exerts back-pressure in the

pulmonary veins, increasing pulmonary capillary pressure and giving rise to pulmonary oedema (Cunningham & Klein 2007).

No signs of congestive heart failure were noted macro- or microscopically in the rats injected with pavetamine and euthanased on day 6 and day 42 respectively in this study, nor in rats exposed to *Pavetta harborii* extract (Pipedi 1999). Mild pulmonary oedema was noted in rats exposed to pavetamine intraperitoneally (Schultz *et al*. 2001). In both of the latter studies no light-microscopically discernable myocardial lesions were present in the experimental animals and only mild ultrastructural lesions were reported. Pavetamine significantly reduced the systolic cardiodynamic function of rats but did not affect the diastolic component (Hay, Schultz & Schutte 2008).

In rats exposed to *P. harborii* extract the latent period was three weeks (Pipedi 1999), and the rats that received pavetamine were euthanased four to sixteen days after exposure (Schultz *et al*. 2001). The rapid course of the disease in rats, compared to that in sheep that show signs of congestive heart failure, may be one of the reasons why the rats did not show lesions associated with congestive heart failure. Other reasons may be a variation in susceptibility between species and differences in the relative quantities of material administered.

Death in ruminants exposed to gousiekte-inducing plants under natural or experimental conditions without any premonitory signs, macro- or microscopical lesions, was reported by various researchers (Smit 1959; Adelaar, Terblanche & Smit 1966; Hurter *et al.* 1972). Animals can therefore succumb to gousiekte without signs of congestive heart failure. In these animals it therefore appears that the compensatory mechanisms were adequate to meet the body's perfusion demands up to the point of death.

During a natural outbreak of gousiekte in the Ventersdorp district of the North-West Province in 1988 a farmer lost 60 of 90 sheep on veld sparsely infested with *P. pygmaeum.* During a visit to the farm electrocardiograms (ECG) were recorded on four animals, all of which dropped dead within 100 m of the

recording site. All the animals had “typical” myocardial lesions even though the ECG recordings were normal, which shows that changes in electrical activity do not necessarily occur in gousiekte (L. Prozesky. R.A. Schultz & N. Fourie, unpublished data 1988). On the other hand, Pretorius *et al*. (1973) reported sino-atrial node (SA node) arrhythmias in 56 % of animals on average 11 days before death. They speculated that cardiac dilatation causes gallop rhythm, bundle branch block and an increase in P wave duration.

Considering the abovementioned data the following conclusions are drawn:

- Most animals that succumb to gousiekte do so as the result of left ventricular failure.
- In animals with advanced lesions clinical signs of left and right ventricular failure are present.
- Animals can die during any stage in the development of the lesions and changes in electrical activity do not necessarily occur in gousiekte.

6.5 COMPENSATORY MECHANISMS

Two morphological indicators of compensation were seen in sheep, namely cardiac dilatation and myofibre hypertrophy. The decline in the contractile force of the heart (*vide supra*) resulting in increased left ventricular end diastolic pressure, which is a sign of ventricular overloading, will initiate adaptive mechanisms in an attempt to meet the body’s demands for adequate perfusion.

The major intrinsic compensatory mechanism is the Frank Starling mechanism of increased preload to control ventricular performance, resulting in dilatation of the heart and a systemic response that includes increased heart rate, and increased release of catecholamines by the adrenergic cardiac nerves and the adrenal medulla. This results in intensified contractibility and the activation of the renin-angiotensin system and other neurohormonal mechanisms that maintain arterial blood pressure (Braunwald 1992; Jubb, Kennedy & Palmer 1993; Guyton & Hall 2000; Radostits *et al.* 2000; Dunlop & Malbert 2004; Cunningham & Klein 2007). When cardiac dilatation is the result of a

pathological condition, as is the case with gousiekte, it is characterised by impaired systolic function with a reduced ejection fraction and increased preload (volume overload) (Dec & Fuster 1994; Weekes *et al*. 1999).

Cardiac dilatation occurs over a period of time and can be difficult to evaluate macroscopically, since the measurements used are subjective and may be difficult to quantify, particularly during the initial stages of development (Jubb, Kennedy & Palmer 1993; Radostits *et al.* 2000; Kumar, Cotran & Robbins 2003).

In this study, based on the subjective criteria used for identifying dilated hearts, dilatation of particularly the left ventricle occurred in two of the animals with extended latent periods (51 days) (table 3.2). On the other hand, based on the microscopically detectable endocardial thickening (Jubb, Kennedy & Palmer 1993), it was concluded that varying degrees of cardiac dilatation (fig. 3.14) were present in seven of the experimental sheep (table 3.2). These results concur with those of Theiler, Du Toit and Mitchell (1923) who claimed that cardiac dilatation was present in the majority of animals that succumb to gousiekte.

Hypertrophy of myocardial fibres in the subendocardial region was present in all the sheep exposed to *P. pygmaeum* in this study *(vide supra)*. From the available information it is concluded that two morphological indicators of compensation were seen in sheep with gousiekte, viz. myofibre hypertrophy that was present in all the sheep exposed to *P. pygmaeum,* and cardiac dilatation that occurs mainly during the later stages of the disease (animals with medium to long latent periods).

6.6 CONCLUSIONS

This investigation has clearly shown that the study of the pathogenesis of myocardial lesions in gousiekte is problematic, owing to –

- the long latent period;
- the tremendous variation in the range and extent of the macroscopical and microscopical lesions in animals that succumb to the disease;
- the inability to quantify the toxicity of plants associated with the disease; and
- the unavailability of sufficient amounts of pavetamine to induce the disease in a sufficient number of ruminants to enable statistical analysis of the lesions.

The most important findings of this study are the following:

- During the early stages of the latent period (around 21 days) the action of pavetamine on the myocardium is purely functional and induces no morphological lesions.
- The transmural distribution of myocardial necrosis in rats exposed to pavetamine twice, sheep with a short latent period and even in some of the sheep with an intermediate latent period, suggests that pavetamine does not selectively affect myofibres in the subendocardial region *per se.*
- Lesions that are recognised develop over a period of time after single or repeated exposure to pavetamine that is not immediately fatal.
- Hypertrophy of subendocardial fibres is the first discernable light-microscopical lesion. Hypertrophy and cardiac dilatation are considered compensatory mechanisms of the heart in its attempt to meet the body's demand for increased cardiac output.
- Most animals that succumb to gousiekte do so as the result of left ventricular failure. Animals can die during any stage in the development of the lesions and changes in electrical activity do not necessary occur in gousiekte.
- In animals with advanced lesions pathological lesions of left and right ventricular failure are present.
- "Atypical" lesions represent a manifestation of the disease in a progression that terminates with dilated cardiomyopathy if the animal does not die during the early stages. In animals with "atypical" lesions,

the time from exposure to pavetamine to the death is too short for "typical" lesions to develop.

- There is a correlation with regard to distribution and type of lesion between the "typical" lesions associated with gousiekte and those reported in humans with certain forms of dilated cardiomyopathy. Contrary to what is currently believed, the myocardial changes in animals with gousiekte represent a final, common pathway of cellular injury rather than a manifestation of a specific type of heart disease.

6.7 PROPOSED FUTURE RESEARCH AREAS

1. Attempt to produce "typical" gousiekte lesions in rats to facilitate gousiekte research.

2. Study the proteins affected in myocytes by pavetamine in more detail.

3. Develop methods to identify and quantify the toxicity of plants associated with gousiekte for purposes of research and diagnostics.

4. Determine the cause of death in animals with little or no myocardial damage.

5. Elucidate the structure of pavetamine to enable further study of the pharmacological properties of the toxin.

6. Myocyte cell culture studies comparing pavetamine with other metabolic, ion channel and biosynthetic toxins to document changes in homeostasis, ion potential, energy production and calcium metabolism.