CHAPTER ONE

GENERAL INTRODUCTION

In veterinary practice, anaesthetics and anaesthetic adjuncts are used in order to perform both surgically invasive and non-invasive manipulations of varying degrees of magnitude. This often happens in environments where temperature variations are difficult to control and this is even more so, under field conditions. Seasonal and diurnal changes in temperature create extremes of environmental temperature variations. Encounters with restless animals in a variety of environmental conditions may require that these animals be sedated for varying periods of time. This calls for an agent which should ideally be easy and safe to administer, one that gives good and excitement free sedation without prolonging the recovery period, one with analgesic action thus making supplementation with local anaesthetics unnecessary, one that is not toxic or irritant to tissues and not toxic to organs even after repeated doses, one that does not com pregnant animals and which can be given in supplemental doses without deleterious effects (Fessl, 1970). It is therefore important for the clinician to understand the effects of environmental temperature variations on the body temperatures of animals and the resultant effects of these changes on

the pharmacokinetics of some anaesthetics and anaesthetic adjuncts in order to facilitate prevention and management of any anaesthetic complications that may arise.

If an animal has to resort to abnormal behaviour or undergo extreme adjustments in its behaviour or physiology in order to cope with adverse aspects of its environment, then it can be said to be in a state of stress (Fraser *et al.*, 1975; Friend, 1980), and intense heat or cold are recognized as such agents that may cause stress in animals (Guyton, 1992). In the presence of a stressful situation, physiological and behavioural adjustments are made to in attempt to eliminate that event from being a stressor (Moberg, 1976; Friend, 1980). It is not how pleasant or unpleasant the stressor is that counts but, its intensity (Seyle, 1973).

During anaesthesia and surgery, several factors including, abolition of behavioural responses, attenuated hypothalamic function, reduced metabolic rate, reduced effector responses and abnormally large thermal stresses combine to interfere with normal thermoregulation (Imrie and Hall, 1990). Thus, deviations of internal temperatures from normothermia that elicit vigorous regulatory responses in unanaesthetized animals, may elicit diminished or no responses in animals anaesthetized for surgery. General anaesthetic agents, with

the exception of ketamine, impair thermoregulation, presumably by attenuation of hypothalamic function. General anaesthetics increase warm response thresholds (active vasodilation and sweating) and decrease cold response thresholds (vasoconstriction, shivering and non-shivering thermogenesis). They increase the threshold range from approximately 0.2° C to 4° C. The effect of this is a widened range of core temperatures over which no thermoregulatory responses occur (Imrie and Hall, 1990).

Other agents used during general anaesthesia also have a major effect on the general heat balance. Neuromuscular blocking drugs abolish shivering thus making paralysed anaesthetized patients cool more rapidly than unparalysed ones during surgery. However, this may relate more to the type of surgery being performed than to the neuromuscular blocking (Goldberg and Roe, 1966; Imrie and Hall, 1990), as patients undergoing open body cavity surgeries will incur greater heat losses when compared to those undergoing operations which do not involve open body cavities (Morris and Wilkey, 1970). Agents which produce vasodilation redistribute heat to the periphery thus increasing heat loss to the environment while opioids, barbiturates, phenothiazines and butyrophenones have both central and peripheral actions which tend to decrease body temperature (Imrie and Hall, 1990).

The duration of action of anaesthetics and other drugs used during anaesthesia at different body temperatures is not well understood. In man, reported studies indicate that perioperative hypothermia markedly decreases drug metabolism. The duration of action of the muscle relaxant vecuronium is more than doubled by a 2° C reduction in body core temperature (Heier *et al.*, 1991). This prolongation of the duration of action is the result of altered pharmacokinetics (Sessler, 1994). Studies on other drugs indicate that the effect of the muscle relaxant atracurium is less dependent on body core temperature. A 3° C decrease in body core temperature increases its duration of action by only 60%. Under the same conditions of body core hypothermia (3° C), plasma concentrations of propofol, which was being infused constantly, were 30% higher than normal. However, the effect of mild hypothermia on the metabolism and pharmacodynamics of most other drugs have yet to be reported. The results for muscle relaxants and propofol indicate that the effects of body temperature on the duration of drug action are likely to be substantial (Sessler, 1994).

Xylazine (Rompun®) was first synthesized in 1962 and given the code name Bay Va 1470. Chemically, it is 2[2,6-dimethylphenylamino]-4H-5,6-dihydro-1,3-thiazine. Pharmacologically, xylazine is classified as an analgesic as well as a sedative and skeletal muscle relaxant (Booth, 1988). Xylazine has been used extensively in various animal species because of its potent sedative, analgesic

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and myorelaxant properties (Clarke and Hall, 1969). It may not possess all the attributes of an ideal sedative but it seems to possess certain properties that earned it its place in veterinary practice for may years. The development of a number of xylazine antagonists that can be used to reverse its adverse effects, has improved its safety in veterinary practice (McDonnell et al., 1993).

The veterinary anaesthetist is called upon to deal with a number of species of animals that exhibit great variation in size and temperament as well as in anatomical and physiological development. Apart from differing response of each species to the various anaesthetic agents, there is often marked variation in response between breeds within each particular species (Hall and Clarke, 1983).

The sedative and anaesthetic effects of xylazine hydrochloride not only show considerable variation from species to species but, the variation is also exhibited among individual animals of the same species (Neophytou, 1982; Raptopoulos and Weaver, 1984). Studies carried out in heifers injected with xylazine hydrochloride and exposed to heat stress and thermoneutral environmental temperature conditions (Fayed et al., 1989) showed that there are significant differences in the response of some physiological variables to different environmental temperature conditions. In these studies in heifers, significant differences were noted in serum insulin and glucose concentrations, body

temperature, pulse rate, respiration rate, duration of salivation and recovery from the sedative effects of xylazine under the different environmental temperature conditions (Fayed *et al.*, 1989). In other studies carried out with xylazine in cats exposed to different environmental temperature conditions (Ponder and Clark, 1980), it was shown that thermoregulatory function was affected with the body temperature decreasing in the cold and increasing in the hot environments without any thermoregulatory mechanism intervening to counter these changes.

From the available literature, there is evidence that:

[i]   the duration of action of some anaesthetics and anaesthetic adjuncts is influenced by body core temperature in man and may be variable in animals acclimatised to different environmental conditions.

[ii]   anaesthetics and anaesthetic adjuncts induced biochemical and physiologic changes that vary with different body temperatures in animals.

[iii]   some clinical manifestations with some drugs are difficult to relate to the plasma kinetics of these drugs.

From the literature available, there is inadequate explantion for the understanding of the effects of thermostress on the metabolism and pharmacodynamics of most anaesthetics and anaesthetic adjuncts in animals. It

is essential for this to be understood to explain the clinical manifestations seen

in animals as a result of the use of these drugs.

OBJECTIVES

I.   To investigate the influence of environmental conditions on some

biochemical, physiologic and clinical effects of xylazine hydrochloride in

goats.

II.   To investigate the effects of different environmental conditions on the

pharmacokinetics of xylazine hydrochloride in goats.

CHAPTER TWO

LITERATURE REVIEW.

2.1 INTRODUCTION.

2.1.1 STRESS

An animal may be considered to be in a state of stress if abnormal behaviour or extreme adjustments in its behaviour or physiology are necessary in order to cope with adverse aspects of its environment (Fraser *et al.,* 1975; Friend, 1980). In the presence of a stressful situation, physiological and behavioural adjustments come into play and eliminate that event from being a stressor (Moberg, 1976; Friend, 1980). It has been established that it is always immaterial whether the stressor is pleasant or unpleasant. All that counts is its intensity (Selye, 1973). To cope with the stress, the hypothalamic-pituitary-adrenal cortex axis is vital (Seyle, 1973).

Intense heat or cold is recognized as an agent that causes stress in animals, leading to increased cortisol release by the adrenal gland (Guyton, 1992). Approximately 48 hours after initiation of stress, detectable hyperplasia and hypertrophy of the

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adrenal glands occurs (Selye, 1936; Friend *et al.*, 1977). Increased glucocorticoid secretion then continues until either the stress is removed, adaptation occurs, or adrenal exhaustion occurs and the animal dies (Selye, 1973).

Glucocorticoid surge can be measured by obtaining blood samples at close intervals after the onset of stress (Smith *et al.*, 1972). However, plasma concentrations of glucocorticoids as a measure of stress can be erroneous since the amount in blood is a product of secretion and clearance. It is important to know the turnover rate of glucocorticoids as an indicator to what is actually occurring (Friend, 1980). Christison and Johnson (1972), showed that under conditions of prolonged heat exposure, adjustments are made in the turnover rate of glucocorticoids. One indicator of stress which has been widely used as a measure of stress exceeding 48 hours is the change in the weight of the adrenal glands (Christian, 1961; Selye, 1973). However, this requires euthanising the animal. Since glucocorticoids are known to be gluconeogenic, a measure of changes in plasma glucose concentration has been found to relate well to the occurrence of stress (Pearson and Mellor, 1976).

In the determination of whether an animal is stressed or not, it is suggested that an integrated approach employing both behavioural and physiological measures is necessary since behavioural methods alone are too subjective and thus highly erroneous (Friend, 1980).

2.1.2 PHYSIOLOGY OF TEMPERATURE REGULATION.

The need for body temperature regulation (thermoregulation) arises because biological reactions in the body are enzyme catalysed and the rate of the reactions increase with a rise temperature. However, this increase reaches a maximum beyond which protein denaturation occurs and enzyme activity either decreases or stops. This is because many biological reactions have a $10°$ C temperature coefficient (Q10) of 2, that is, their rate of reaction doubles for a $10°$ C temperature increase (Rodwell, 1979). In mammals, protein denaturation begins at $-1°$ C causing physical disruption of cells (Vick, 1984). In man, consciousness is lost at a body temperature of about $30°$ C and death usually occurs at temperatures of less than $25°$ C as a result of ventricular fibrillation. The benefits of maintaining the internal body temperature at a value close to the optimum for enzymatic activity therefore cannot be overemphasized. These include a constant high rate of metabolic activity, rapid nerve conduction and muscular contraction, decreased viscosity of blood and, perhaps most important of all, freedom from the constraints of environmental conditions (Imrie and Hall, 1990).

2.1.3 HOMEOTHERMY.

This is a pattern of temperature regulation in which the cyclic variation in core temperature either nychthermally or seasonally, is maintained within arbitrary limits of ±2° C despite much larger variation in ambient temperature (Hensel, 1981).

2.1.4 THERMAL BALANCE.

Homeothermy is achieved by balancing heat gains with heat losses. Within a range of ambient temperatures, this may be achieved without metabolic expenditure by control of peripheral vasculature. Beyond the limits of this thermoneutral zone (approximately 20-35° C in resting human adults), energy must be expended in order to maintain body thermal balance.

Heat gains can be achieved through: basal metabolism (the major source is muscular exercise), feeding, storage, growth, shivering and non-shivering thermogenesis. Shivering may produce a four to six-fold increase in heat production (Iampietro *et al.*, 1960). Severe exercise can produce up to 20-fold increase in metabolic rate.

Heat losses from the body are through the points of contact with the environment, namely the skin and the respiratory tract. Losses through convection, conduction

and radiation account for 75% of basal heat production at rest while losses through insensible perspiration and the respiratory tract account for the remaining 25%. In man, sweating is the main thermoregulatory mechanism responsible for heat loss (Imrie and Hall, 1990).

2.1.5 TEMPERATURE RECEPTORS.

There is growing evidence that temperature receptors exist in many body tissues. They may be classified into central - which sense the body core temperature and, peripheral - which sense the temperature of the body shell and the environment. Although neurones in the pre-optic and hypothalamic (includes both anterior and posterior hypothalamus) nuclei appear to be essential for activation of normal responses to external and internal thermal stresses (Hammel, 1968; Satinof, 1978), more recently, it has become apparent that many body tissues contain temperature receptors, including the spinal cord, the midbrain, the lower brainstem, abdominal organs and skeletal muscle. Thus the concept of the hypothalamus as the sole sensor of core temperature has given way to one of multiple thermal inputs with the hypothalamus contributing probably as little as 20% of the total (Simon et al., 1986).

2.1.6 HEAT BALANCE DURING ANAESTHESIA.

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During anaesthesia and surgery, the factors which combine to interfere with normal thermoregulation include: abolition of behavioural responses, attenuated hypothalamic function, reduced metabolic rate, reduced effector responses and abnormally large thermal stresses (Imrie and Hall, 1990). Thus, deviations of internal body temperatures from normothermia that elicit vigorous regulatory responses in unanaesthetized animals, elicit diminished or no responses in anaesthetized animals. Although loss of consciousness during general anaesthesia abolishes thermal sensation, it is generally held that the sensitivity of the thermoreceptors is not impaired (Hammel, 1988). Thermoregulatory responses are still possible during light general anaesthesia as evidenced by shivering and vasoconstriction in anaesthetized patients (Imrie and Hall, 1990). General anaesthetic agents, with the exception of ketamine, impair thermoregulation, presumably by attenuation of hypothalamic function. General anaesthetics increase warm response thresholds (for active vasodilation and sweating) and decrease cold response thresholds (vasoconstriction, shivering and non-shivering thermogenesis). They increase the threshold range from approximately 0.2° C to 4° C. The effect of this is a widened range of body core temperatures over which no thermoregulatory responses occur (Imrie and Hall, 1990).

The pattern of body temperature change during general anaesthesia consists of three

phases: an initial large decrease in core temperature on induction of anaesthesia and for the first hour, a slow linear reduction in core temperature and finally, core temperature stabilises and remains virtually unchanged. This results in an inadvertent intra-operative hypothermia (Sessler, 1994). The reasons for the rapid decline in body core temperature following induction of anaesthesia can be attributed to an anaesthetic induced decrease in metabolic heat production by about 20% and an anaesthetic induced vasodilation which allows redistribution of heat from the core to the peripheral tissues (Goldberg and Roe, 1966; Holdcroft *et al.*, 1979; Sessler, 1994). This markedly decreases body core temperature but hardly changes the mean body temperature and heat content of the body (Sessler, 1994). Vasodilation following induction of general anaesthesia slightly increases cutaneous heat loss (Sessler, 1994) and therefore, it contributes minimally to the rapid decline in body core temperature in the first one hour following induction of general anaesthesia.

The phase of slow and linear decline in body temperature after the redistribution of body heat is due to heat loss exceeding heat production (Goldberg and Roe, 1966; Holdcroft *et al.*, 1979; Hynson and Sessler, 1992).

In the third phase where the temperature remains virtually unchanged, it is suggested that this is possibly due to a thermal steady state when heat production

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is equal to heat loss. But, this is only possible where patients remain relatively warm (Sessler *et al.*, 1987). Another possible explanation is that the resultant body core hypothermia triggers thermoregulatory vasoconstriction which reduces heat loss to the environment when body core temperature is between 33 to 35° C (Belani *et al.*, 1993).

Even though thermoregulatory vasoconstriction decreases cutaneous heat loss (Sessler *et al.*, 1992), it cannot produce a thermal steady state. A thermal steady state is not achieved because of the confinement of metabolic heat to the core compartment where most of it is produced and therefore the peripheral tissues temperature starts to decline as the supply of heat from the core is discontinued. This in effect re-establishes the core to periphery thermal gradient and this does not represent a thermal steady state (Sessler, 1994).

Other agents used during general anaesthesia also have a major effect on the general heat balance. Neuromuscular blocking drugs abolish shivering thus making paralysed anaesthetized patients cool more rapidly than unparalysed ones during surgery. However, this may relate more to the type of surgery being performed than to the neuromuscular blocking (Goldberg and Roe, 1966; Imrie and Hall, 1990). Agents which produce vasodilation redistribute heat to the periphery thus increasing heat loss to the environment while opioids, barbiturates, phenothiazines

and butyrophenones have both central and peripheral actions tending to decrease body temperature (Imrie and Hall, 1990).

2.1.7 TEMPERATURE MONITORING.

During general anaesthesia, body core temperature monitoring is important because it can help quantify hyperthermia and hypothermia. Inadvertent hypothermia is the most common perioperative thermal disturbance, when core temperature may decrease by 0.5 to 1.5° C within the first 30 minutes (Sessler, 1994).

A rise in body core temperature is not the first sign of acute malignant hyperthermia as other non-specific signs of the syndrome e.g tachycardia and hypercarbia may precede it but, it can help to confirm the diagnosis (Sessler, 1994). Intraoperative hyperthermia can also be as a result of infectious fevers, mismatched transfusions, blood in the fourth ventricle and excessive warming (Sessler, 1994).

2.1.8 TEMPERATURE MONITORING SITES.

Body temperatures measured at each site have different physiologic and practical significance (Sessler, 1994). Since the body core compartment tissues are highly

perfused and have a uniform temperature, their temperature is the most accurate measure of body temperature. This temperature can be measured in the pulmonary artery, distal one third of the oesophagus, the tympanic membrane and the nasopharynx. Skin surface temperatures are considerably less than core temperatures and the difference between the two may vary unpredictably. For this reason, measurements of skin temperature should not be substituted for valid measures of core temperature (Sessler, 1994). Both rectal and urinary bladder temperatures can be considered to be intermediate between the core and skin surface temperature in terms of their relative representation of body temperature.

2.1.9 THERMOMETERS

Since mercury in glass thermometers are cumbersome and slow, electronic thermometers which include thermistors and thermocouples are very useful and accurate for body temperature measurements. Also useful and accurate are infra-red tympanic membrane thermometers and "deep tissue" thermometers based on actively reducing cutaneous heat flux to zero (Sessler, 1994).

2.2   XYLAZINE HYDROCHLORIDE.

2.2.1 CLASSIFICATION.

Xylazine (Rompun®) was first synthesized in 1962 and given the code name Bay Va 1470. Chemically, it is 2[2,6-dimethylphenylamino]-4H-5,6-dihydro-1,3-thiazine. Pharmacologically, xylazine is classified as an analgesic as well as a sedative and skeletal muscle relaxant (Booth, 1988). Xylazine has been used extensively in various animal species because of its potent sedative, analgesic and myorelaxant properties (Clarke and Hall, 1969).

2.2.2 PHARMACOLOGIC PROPERTIES.

Xylazine is a potent α_2- adrenergic agonist. It acts upon the central nervous system by activation or stimulation of α-adrenoreceptors e.g the α_2- adrenergic receptors. Because xylazine has also α_1-adrenergic effects, it causes peripheral in addition to central actions upon these adrenergic receptor subtypes. Within the central nervous system, activation of α_2-adrenoceptors induces both analgesia and sedation. These result from the binding of xylazine to the α_2-adrenoceptors in the presynaptic membrane resulting in activation of membrane-associated G-proteins and this leads to activation of potassium channels in the postsynaptic neuron, causing the cell to lose potassium and become hyperpolarized. This action makes the cell unresponsive to excitatory input (Thurmon et al., 1996). Xylazine also produces muscle relaxation by inhibition of intraneural transmission of impulses at the central level of the

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central nervous system (Booth, 1988; Thurmon *et al.*, 1996).

There is evidence that α_2-agonists and μ-opioid agonists can produce the same pharmacodynamic events (Thurmon *et al.*, 1996). This is because the adrenergic and opioid receptors although different, may be found in the same location of the brain and, even on the same neuron. Furthermore, these receptor types are coupled to the same signal transducer and the signal transduction mechanism is linked to the same effector mechanism (Thurmon *et al.*, 1996).

2.2.3 ABSORPTION

Following intramuscular injection, absorption of xylazine is rapid, with a half-life of 2.8-5.4 minutes. It is incompletely absorbed, its bioavailability ranging from 52 to 90 % in the dog, 17-73 % in sheep and 40-48 % in the horse (Garcia-Villar *et al.*, 1981).

2.2.4 METABOLISM AND ELIMINATION.

Experiments conducted in rats and cattle have demonstrated that in rats, only 8 % of intact or unchanged drug appears in urine while in cattle, less than 1 % of the unchanged drug is eliminated in urine 2 hours after its administration (Duhm *et al.*,

1969; Garcia-Villar *et al.*, 1981). Xylazine undergoes rapid metabolism yielding about 20 metabolites in rats. In cattle, peak excretion of metabolites in urine occurs between 2-4 hours following administration of xylazine. This suggests that xylazine is extensively metabolised. One metabolite rapidly formed in cattle is 1,amino-2-6-dimethybenzene (ADB) which appears in urine within 4 hours of the injection of a therapeutic dose of xylazine. ADB probably forms from oxidative or hydrolytic breakdown of the thiazine ring (Putter and Sagner, 1973).

2.2.5 PHARMACOKINETICS

After a rapid distribution with a half-life of between 1.2 and 6 minutes, the apparent volume of distribution for xylazine is between 1.9 and 2.7 l/kg in the dog, horse, sheep and cow. The peak drug concentration in plasma is reached after 12 to 14 minutes in horse, cattle, sheep and dog following intramuscular administration. The half-life of elimination of xylazine after intravenous administration of a single dose is 49.5 minutes in horse, 36.5 minutes in cattle, 23 minutes in sheep and 30 minutes in the dog. No data is available in other species (Garcia-Villar *et al.*, 1981).

In cattle, the plasma kinetics of xylazine are difficult to relate to some of the sustained clinical effects it causes. The short half-life of xylazine in cattle (36.5 minutes) contrasts with the duration of polyuria which lasts for up to 5 hours

(Thurmon *et al.*, 1978); hyperthermia lasting for up to 18 hours (Young, 1979); hyperglycaemia for 24 hours (Eichner *et al.*, 1979); hypothermia lasting for up to 24 hours and prostration after a high dose lasting for 36 hours (Clarke and Hall, 1969) and/or appearance of diarrhoea 12-24 hours after injection (Hopkins, 1972). Plasma half-life or elimination half-life cannot be related to these peculiar biochemical and physiological effects. It is obvious that much more needs to be elucidated about the mechanism(s) of action of xylazine (Booth, 1988).

2.2.6 DETERMINATION OF XYLAZINE HYDROCHLORIDE IN BIOLOGICAL MATERIALS.

Three properties of xylazine make it possible for it to be determined quantitatively in biological materials and these are; it is extractable from water and biological fluids at weak alkaline pH, its alkaline character which makes it be readily transferable from an organic solvent into acid aqueous solutions and, it possesses an UV absorption spectrum with a maximum at 240 nm (Putter and Sagner, 1973).

Several authors have reported techniques for determination of xylazine in biological materials. High-performance liquid chromatography (HPLC) has been used to detect xylazine in sheep plasma (Alvinerie and Toutain, 1981), the urine of Greyhounds (Moore and Oliver, 1989), horse plasma (McDonell *et al.*, 1993), and

plasma of horse, cattle, sheep and dog (Garcia-Villar *et al.*, 1981). Other methods include spectrophotometry and thin-layer chromatography (Putter and Sagner, 1973), used to detect residues of xylazine in meat, milk, and urine and gas chromatography, used in the determination of xylazine in serum and tissues of various animals (Rogstad and Yndestad, 1981). More recently, Muge *et al.* (1995) have described a radioreceptor assay technique for determination of xylazine and medetomidine in sheep plasma. However, the high performance liquid chromatography (HPLC) technique is the most widely used owing to its simplicity, rapidity, high selectivity and range of sensitivity (McDonnell *et al.*, 1993). Because xylazine is administered in small doses, sensitive methods are required for its detection in biological materials (Putter and Sagner, 1973).

Xylazine, a strongly basic substance has previously been separated by reverse-phase HPLC on octadecylsilica (Akbari *et al.*, 1988; Moore and Oliver, 1989). This sometimes requires the addition of organic amines to mask residual silanol groups on the silica surface, with resultant elevation in mobile phase pH and marked electrostatic interaction between basic compounds and residual silanol groups, which in turn affect efficient separation. Efficient separation, however, can be achieved by addition of an ion pairing reagent which allows for operation at a relatively low pH where xylazine is ionized (McDonnell *et al.*, 1993).

Several extraction methods and mobile phases have been used in determination of xylazine concentration in biological fluids using the HPLC technique (Alvinerie and Toutain, 1981; Akbari *et al.*, 1988; Psomas and Fletouris, 1992; McDonnell *et al.*, 1993). During extraction, partitioning into ethyl acetate with prior pH adjustments with boric acid gave better recoveries compared to partitioning into chloroform after buffering of plasma at pH 11 with boric acid or extracting into ethyl ether (McDonnell *et al.*, 1993). The various extraction methods, mobile phases and operating HPLC conditions applied in the determination of xylazine concentration in blood in various animal species have demonstrated differences in the magnitudes of recovery from and limits of detection of xylazine in biological fluids (Garcia-Villar *et al.*, 1981; Alvinerie and Toutain, 1981; Akbari *et al.*, 1988; Psomas and Fletouris, 1992; McDonnell *et al.*, 1993). It has been suggested that increasing the volume of the sample used can lower the limit of detection of the method employed (Alvinerie and Toutain, 1981; Akbari *et al.*, 1988).

2.3 BEHAVIOURAL AND CLINICAL EFFECTS OF XYLAZINE IN ANIMALS.

2.3.1 INTRODUCTION.

Xylazine is a widely used sedative in biological research and veterinary medicine

(Goldfine and Arieff, 1979; Hsu and Hummel, 1981). Pharmacologically classified as an analgesic as well as a sedative and skeletal muscle relaxant (Booth, 1988), it has been used in various animal species because of its potent sedative, analgesic and myorelaxant properties (Clarke and Hall, 1969). Its use in both domestic and wild animal species either alone or in combination with other tranquillizers and anaesthetic agents has been relatively safe (Knight, 1980). Because xylazine strongly potentiates the effects of all tranquilizers, sedatives, cataleptics, dissociative anaesthetics, and anaesthetic agents, care should be exercised when it is used in combination with drugs in these groups (Knight, 1980). Xylazine induces a number of physiological and pharmacological changes in the species in which its use has been studied (Knight, 1980).

2.3.2 SEDATION

The ensitivity to xylazine between animal species differs (Thurmon *et al.*, 1996). Goats are much more sensitive to xylazine than sheep and doses of 0.05 mg/kg may result in profound sedation for 12 or more hours (Dehghani *et al.*, 1991). Signs of sedation following the intramuscular administration of 0.5 mg/kg xylazine in goats have been seen as early as 3 minutes of the administration of the drug. These signs include lowering of the head and neck, partial drooping of the upper eyelid, protrusion of the nictitating membrane and tongue, muscular incoordination and

staggering gait (Saleh, 1993). Similar effects of xylazine in horses have been reported (Clarke and Hall, 1969). The onset and duration of action of xylazine when given in goats via the intramuscular route has been shown to be quite variable. Dehghani *et al.* (1991) reported the onset of action as 22.5 ± 6.23 minutes and a duration of effect of 96.25 ± 37.04 minutes in goats injected with 0.2 mg/kg xylazine hydrochloride. Keller and Bauman (1978) reported that goats attained sternal recumbency in 5 to 15 minutes with the duration of sedation being 20 to 90 minutes and recovery occurred in 60 to 240 minutes. In other studies, Mohammed and Yelwa (1993) injected goats with 0.05 mg/kg xylazine hydrochloride intramuscularly and reported the onset of action as 7.0 ± 3.11 minutes post-xylazine injection with recumbency resulting in 18.0 ± 11.8 minutes and taking 78.8 ± 26.47 minutes to full recovery. The same authors reported crying and neck flexing as effects of xylazine hydrochloride administration.

While comparing three different dosages of 0.15, 0.20, and 0.25 mg/kg of xylazine injected intramuscularly in goats, Prajapathi *et al.* (1994) were able to show that the duration of sedation and recovery times were dose-dependent, being significantly longer with the higher dosages as compared to the lower dosages. When given as an intravenous infusion over a wide dose range, it was shown that xylazine-induced sedation was dose-dependent and that the onset of sedation was 2 to 8 minutes with full recovery varying from 1 to 3 hours. At the highest dose, the goats were unable

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to stand for 30 to 60 minutes.

In sheep, 1.1 mg/kg of xylazine injected intramuscularly produced sedation lasting for 60 to 90 minutes (O'Hair *et al.*, 1986). In cats, Dehghani *et al.* (1991) reported that the onset of action of xylazine hydrochloride given at 2.2 mg/kg body weight intramuscularly was 22.5 ± 6.23 minutes with a duration of action of 96.25 ± 37.04 minutes and recovery was in 441.66 ± 32.0 minutes. In horses and cattle, intramuscular administration of xylazine resulted in sedation in 10-15 minutes post-drug administration (Clarke and Hall, 1969).

While evaluating the effects of xylazine in heifers under thermoneutral (18° C ; 42% humidity) or heat-stress (33° C ; 63% humidity) conditions, Fayed *et al.* (1989) reported that heifers in the heat-stress conditions took significantly longer time (107 ± 20 minutes) to stand compared with those in the thermoneutral conditions (41 ± 6 minutes). It was suggested that animals in the heat-stress environment were more sensitive to xylazine than those in the thermoneutral environmental conditions. This might be due to hormonal or neural changes that develop in heat-stressed animals whereby, heat-stressed animals have decreased metabolic rate resulting from decreased thyroid hormones which might alter xylazine kinetic properties (Magdub *et al.*, 1982).

2.3.3 SALIVATION

Intravenous injection of xylazine hydrochloride induces profuse salivation in ruminants (Booth, 1988). Salivation following administration of xylazine hydrochloride has been reported in goats (Neophytou, 1982; Kokkonen and Eriksson, 1987; Mohammed and Yelwa, 1993; Saleh, 1993) and cattle (Fessl, 1970; Raptopoulos and Weaver, 1984; Fayed *et al.*, 1989). Following intramuscular administration of xylazine, Mohammed and Yelwa (1993) and Saleh (1993) reported of profuse watery salivation starting at 14 ± 5.7 minutes and 5 to 7 minutes post-xylazine injection, respectively. Following intravenous infusions of various doses of xylazine hydrochloride in goats, Kokkonen and Eriksson (1987) reported of salivation starting to drip a few minutes after infusion of the higher doses of xylazine and this continued for 30 to 60 minutes. This dripping of saliva after xylazine was attributed to the inability of the goats to swallow saliva during sedation (Kokkonen and Eriksson, 1987). While evaluating the effect of xylazine in heifers under thermoneutral and heat-stress conditions, Fayed *et al.* (1989) noted that it took longer (80 ± 18 minutes) for salivation to subside in heifers placed under heat-stress conditions ($33°$ C ; 63% humidity) than in those under thermoneutral conditions ($18°$ C ; 42% humidity) where the duration was 56 ± 10 minutes. It was

suggested that animals in the heat-stress environment were more sensitive to xylazine than those in the thermoneutral environmental conditions. This might be due to hormonal or neural changes that develop in heat-stressed animals whereby, heat-stressed animals have a decreased metabolic rate resulting from decreased thyroid hormones which might alter xylazine kinetic properties (Magdub et al., 1982).

2.3.4 ANALGESIA

Xylazine has been shown to provide good analgesia in goats (Dehghani et al., 1991; Saleh, 1993; Aithal et al., 1996), sheep (O'Hair et al., 1986), and cattle (Fayed et al., 1989). Its analgesic activity is due to its action on the autonomic and central nervous system (CNS) (Knight, 1980). There are reports in the literature, however, that it has only moderate analgesic action in the region of the distal extremities (Fessl, 1970; Knight, 1980; Mogoa, 1990). Injection of 0.5 mg/kg of xylazine, intramuscularly in goats caused skin insensibility to pin pricks and loss of the interdigital reflex (Saleh, 1993). In sheep, O'Hair et al. (1986) reported that the onset of analgesia was from 5 to 20 minutes and this lasted for 20 to 35 minutes following intramuscular administration at a dosage of 1.1 mg/kg body weight. Xylazine also has a potent anaesthetic effect when injected locally (Knight, 1980). In cattle, Fayed et al. (1989) noticed that analgesia was significantly prolonged in

the animals that were exposed to heat-stress conditions (33° C ; 63% humidity) than in those under thermoneutral conditions (18° C ; 42% humidity). It was suggested that animals in the heat-stress environment were more sensitive to xylazine than those in the thermoneutral environmental conditions. This might be due to hormonal or neural changes that develop in heat-stressed animals whereby, heat-stressed animals have decreased metabolic rate resulting from decreased thyroid hormones which might alter xylazine pharmacokinetic properties (Magdub et al., 1982).

2.3.5 URINATION

Urination following the administration of xylazine hydrochloride is a common occurrence in goats (Neophytou, 1982; Mohammed and Yelwa, 1993; Aithal et al., 1996). Polyuria in goats injected with xylazine via the intramuscular route has been observed starting 47.8 ± 11.8 minutes post-xylazine injection (Mohammed and Yelwa, 1993). Urination has also been reported in some goats after epidural xylazine (Aithal et al., 1996). In cattle, Thurmon et al. (1978) reported of increased urine output lasting several hours, accompanied by the presence of glucose in the urine and increased urine pH following administration of xylazine hydrochloride.

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Not only does the quantity of urine increase but, micturition frequency also increases following administration of xylazine in cattle (Raptopoulos and Weaver, 1984). Polyuria which is observed in animals following administration of xylazine hydrochloride is thought to be due to prolonged hyperglycaemia which has been shown to persist for up to 150 minutes post-xylazine injection in goats (Mohammed and Yelwa, 1993).

In cattle, polyuria has been reported to last for up to 5 hours (Thurmon *et al.*, 1978). The increased urine output attributed to xylazine could be due to its effects on one or more of the animal's water-conserving mechanisms such as anti-diuretic hormone (ADH) formation or release, as a result of its action on the distal tubules, or due to increased osmotic attraction of water into the renal tubules by failure to reabsorb glucose (Thurmon *et al.*, 1978).

2.4　EFFECTS OF XYLAZINE ON HAEMATOLOGY AND CARDIOPULMONARY FUNCTION.

Xylazine hydrochloride when injected intravenously in animals induces hypotension, bradycardia, heart block, respiratory depression and in ruminants, marked salivation (Booth, 1988). The cardiopulmonary and haematological effects of xylazine hydrochloride have been investigated in several animal species,

including goats (Kumar and Thurmon, 1979; Kokkonen and Eriksson, 1987; Mohammed and Yelwa, 1993; Prajapathi *et al.*, 1994; Saleh, 1993; Dehghani *et al.*, 1991; Bafi-Yeboa and Huvos, 1980; Aithal *et al.*, 1996), cattle (Campbell *et al.*, 1979; DeMoor and Desmet, 1971; Aouad *et al.*, 1981), sheep (Aziz and Carlyle, 1978; Celly *et al.*, 1997), cats (Dehghani *et al.*, 1991), horses (Clarke and Hall, 1969; Garner *et al.*, 1971), dogs (Klide *et al.*, 1975) and donkeys (Mogoa, 1990).

Studies conducted in horses (Clarke and Hall, 1969), dogs (Klide *et al.*, 1975), and sheep (Aziz and Carlyle, 1978) with intravenously injected xylazine showed that partial cardiac blockade with decreased conductivity and bradycardia do occur. In ponies, intravenous xylazine has been shown to produce sino-atrial (SA) block, transient atrio-ventricular (AV) block and bradycardia within one minute of its injection (Garner *et al.*, 1971).

Xylazine hydrochloride, given intravenously or intramuscularly caused significant reduction in heart rate in goats (Kumar and Thurmon, 1979; Kokkonen and Eriksson, 1987; Mohammed and Yelwa, 1993; Dehghani *et al.*, 1991), calves (Campbell *et al.*, 1979; Young, 1979; Aouad *et al.*, 1981), sheep (Aziz and Carlyle, 1978; Celly *et al.*, 1997) and horses (Clarke and Hall, 1969; Garner *et al.*, 1971). Even when given epidurally to goats, significant reduction in heart rate has been reported (Aithal *et al.*, 1996). Similar effects of epidural injection of xylazine

hydrochloride have been reported in cattle (Skarda and Muir, 1992).

The reduction in heart rate in goats has been reported to be dose-dependent (Kokkonen and Eriksson, 1987). The xylazine-induced reduction in heart rate following intramuscular administration of the drug has been shown to commence at 15 to 30 minutes post drug administration and the heart rate remains depressed for several hours, returning to baseline values sometimes after 24 hours (Dehghani et al., 1991; Mohammed and Yelwa, 1993; Prajapathi et al., 1994). Accompanying this bradycardia, occasional incidences of missed heart beat (Aziz and Carlyle., 1978; Bafi-Yeboa and Huvos, 1980) and irregular heart beats (Aithal et al., 1996) have been encountered. In calves, a decrease of up to 37% of baseline heart rate value, 15 minutes following xylazine injection has been recorded (Aouad et al., 1981). In the same species, decrease in cardiac output and stroke volume have been shown to accompany reduction in heart rate (Campbell et al., 1979). In sheep, progressive bradycardia for up to 60 minutes and marked decrease in cardiac output to 37% of the control output following intravenous xylazine administration have been reported (Aziz and Carlyle, 1978). Decrease in cardiac output following intravenous administration of xylazine has also been reported in ponies (Garner et al., 1971).

It has been suggested that xylazine hydrochloride causes decrease in heart rate via

central and peripheral suppression of the sympathetic trunk since pre-administration with atropine negated the change in heart rate in goats (Kumar and Thurmon, 1979). Decrease in heart rate by xylazine has also been attributed to withdrawal of the sympathetic tone, to the increase in parasympathetic tone, and to the direct depressive actions of xylazine on cardiac pacemaker and conduction tissue (Schmitt et al., 1970; Antonaccio et al., 1973; Klide et al., 1975; Aziz and Carlyle, 1978; Knight, 1980). Another possible cause of this also could be in response to transient hypertension that xylazine hydrochloride induces (Clark et al., 1982).

Intravenous infusion of different doses of xylazine causes dose-dependent decrease in arterial blood pressure in goats with maximal decrease of about 10 mm Hg (Kokkonen and Eriksson, 1987). Decrease in mean arterial blood pressure following administration of xylazine has also been reported in calves (Campbell et al., 1979; Aouad et al., 1981) and sheep (Celly et al., 1997). Although Kumar and Thurmon (1979) reported neither xylazine nor the combination of atropine and xylazine significantly altered the arterial blood pressure in the goat, it has been suggested that the small responses in blood pressure despite the high doses they used was due to the intramuscular route of administration. Greater responses in blood pressure were achieved when the intravenous route was used despite the smaller doses injected (Kokkonen and Eriksson, 1987). Initial increase followed by a decrease in mean arterial blood pressure has been reported in sheep (Aziz and Carlyle, 1978;

Celly *et al.*, 1997), calves (Aouad *et al.*, 1981), dogs (Schmitt *et al.*, 1970), cattle (Clarke and Hall , 1969) and horses (Garner *et al.*, 1971; Muir *et al.*, 1977). In most species, the cardiovascular effects of intravenous xylazine include hypotension with bradycardia which apparently are mediated by central α_2-adrenoreceptors. After administration of higher doses of xylazine, a brief initial vasopressor effect may be evident (Kerr *et al.*, 1972; Klide *et al.*, 1975). Increase in total peripheral resistance following xylazine administration has been reported in calves (Campbell *et al.*, 1979) and sheep (Aziz and Carlyle, 1978). This increase in total peripheral resistance has been attributed to direct α-adrenoreceptor stimulation of peripheral vessels, causing peripheral vasoconstriction (Greene and Thurmon, 1988).

In goats, xylazine has been shown to cause significant reductions in respiratory rate at maximal sedation period (Kumar and Thurmon, 1979; Dehghani *et al.*, 1991). Reduction in respiratory rate has been reported in the same species following xylazine administration (Mohammed and Yelwa, 1993; Prajapathi *et al.*, 1994; Aithal *et al.*, 1996). Similar trends in changes in respiration rate due to xylazine have also been reported in other species (Fessl, 1970; Kerr *et al.*, 1972; McCashin and Gabel, 1975; Aziz and Carlyle, 1978; Young, 1979; Aouad *et al.*, 1981; White *et al*, 1987). Incidences of xylazine causing bradypnoea, alternating with tachypnoea, forced breathing and periodic apnoea in goats and other species are not

uncommon (Aziz and Carlyle, 1978; Bafi-Yeboa and Huvos, 1980; Kokkonen and Eriksson, 1987; Saleh, 1993). The reduction in respiration rate has been shown to remain below baseline values for several hours following xylazine administration (Dehghani *et al.*, 1991; Mohammed and Yelwa, 1993; Saleh, 1993; Prajapathi *et al.*, 1994). Despite the above, xylazine has been reported to have caused increase in mean respiratory rate to 28% above baseline values in sheep (O'Hair *et al.*, 1986). Aithal *et al.* (1996) and Skarda and Muir (1992) have reported a reduced respiratory rate and irregularity in the pattern of respiration following epidural injection of xylazine in both goats and cattle respectively. Apart from causing a reduction in respiratory rate, concurrent decrease in tidal volume in goats (Mohammed and Yelwa, 1993) and sheep (Aziz and Carlyle, 1978) has been reported. These effects of xylazine hydrochloride on pulmonary function have been attributed to its central α_2-adrenoreceptor mediated activity which results in respiratory depression (Prajapathi *et al.*, 1994; Aithal *et al.*, 1996).

In goats (Kumar and Thurmon, 1979), xylazine causes a decrease in total number of erythrocytes, leukocytes, haemoglobin concentration and haematocrit at maximal depth of analgesia. Dehghani *et al.* (1991) also reported of significant reduction in the packed cell volume, haemoglobin concentration, white blood cell count and red blood cell count during deep sedation following administration of xylazine to caprine and feline. In cattle, DeMoor and Desmet (1971) reported that xylazine

caused a significant decline in packed cell volume and they attributed this to xylazine's lytic effect, an effect also found with the phenothiazine derivative, propionylpromazine. The decrease in red blood cell count, haematocrit and haemoglobin concentration following administration of xylazine might have resulted from pooling of blood in the spleen as has been reported with other tranquillisers in goats (Monzally *et al.*, 1972). Total protein, albumin and globulin levels were not altered by xylazine hydrochloride in goats (Kumar and Thurmon, 1979).

2.5 EFFECTS OF XYLAZINE ON ARTERIAL BLOOD GAS TENSIONS AND ACID-BASE BALANCE.

During anaesthesia, it is important that arterial pH should not deviate greatly from normal and that the arterial oxygen supply should be adequate (DeMoor and Desmet, 1971). Severe hypoxaemia following intravenous administration of xylazine hydrochloride at both sedative and non-sedative doses has been reported in various animal species (DeMoor and Desmet, 1971; Doherty *et al.*, 1986; Nolan *et al.*, 1986; Celly *et al.*, 1997).

In goats, Kumar and Thurmon (1979) reported a significant reduction in arterial pH and oxygen tension and a rise in carbon dioxide tension, 15 minutes after xylazine

was administered intramuscularly. These values returned to near pre-administration level after 2 hours. Decrease in pH and oxygen tension and increase in carbon dioxide tension in arterial blood attributed to the effect of xylazine has also been reported in goats (Kumar et al., 1976). Kumar and Thurmon (1979) while reporting on the significant changes in pH, oxygen and carbon dioxide tensions due to xylazine, however, noted that standard bicarbonate and base excess remained within normal range during the entire period of observation. Similar observations on changes in base excess (Saleh, 1993) and actual bicarbonate and buffer base (DeMoor and Desmet, 1971), have been made in goats and cattle respectively.

Although Saleh (1993) evaluated the effect of xylazine hydrochloride on the venous blood gas tensions and acid-base balance in goats, the trend of changes in the variables evaluated was similar to that seen in arterial blood in goats by Kumar and Thurmon, (1979). Saleh (1993) reported of a marked drop in pH values within 30 minutes post-xylazine administration with a concomitant drop in mean values of bicarbonate [HCO_3^-] and total carbon dioxide (TCO_2), then a gradual increase in pH values. The gradual increase in pH values as above was also accompanied by increase in the [HCO_3^-]and TCO_2 values. Venous carbon dioxide tension increased reaching a peak at 90 minutes post-xylazine injection.

In sheep, O'Hair et al. (1986) reported that xylazine hydrochloride caused mean

arterial oxygen tension (PaO_2) to drop to 58% of baseline values after 10 minutes and returned to 85% of baseline values after 70 minutes post-xylazine injection. The same authors reported that by 60 minutes post-xylazine injection, mean arterial carbon dioxide tension ($PaCO_2$) had increased progressively to a peak 30% above baseline. While comparing the hypoxaemic effects of equipotent sedative doses of α-2 adrenoreceptor agonists xylazine, romifidine, detomidine, and medetomidine in sheep, Celly et al. (1997) reported that all the α-2 agonists significantly decreased PaO_2 levels without significant change in $PaCO_2$. Maximum decrease in PaO_2 was seen by 10 minutes post-administration of the drugs and the PaO_2 values were still significantly lower at 60 minutes post-administration. Similar findings have been reported in the same species (Aziz and Carlyle, 1978). In the similar (Celly et al., 1997), the authors reported that the lowest PaO_2 values for xylazine were 32.6 mmHg. No significant changes were seen in arterial blood pH and base excess values. In calves, Aouad et al. (1981) observed that pH and PaO_2 decreased while $PaCO_2$ increased within 15 minutes of xylazine injection.

In cattle, DeMoor and Desmet (1971) reported that xylazine caused a decrease in pH from injection to 15 minutes post-injection which then started rising to be significantly higher after 45 to 120 minute period than at zero time. Arterial oxygen

tension was significantly reduced after 15, 30, 45, 60 , and 90 minutes compared with the initial values while arterial carbon dioxide tension values were significantly higher after 15 minutes than after 120 minutes. The same authors also observed that pH was significantly higher in animals in dorsal than in lateral position from 15-120 minutes, that the arterial carbon dioxide was significantly higher in animals in lateral position compared with the dorsal position after 120 minutes and, buffer base was significantly higher in animals in dorsal position as compared with those in lateral position after 0, 30, 45, and 60 minutes. During the studies, DeMoor and Desmet (1971) observed that pH, standard bicarbonate and base excess tend to increase after xylazine administration but the increase was not significant throughout. Moderate increase in arterial carbon dioxide tension following intravenous or intramuscular xylazine administration in horses has also been reported (Clarke and Hall, 1969).

The hypoxaemia and carbon dioxide retention seen in goats and other species following xylazine administration may be partly due to hypoventilation during sedation due to depression of the respiratory centre and due to recumbency, since lateral recumbency during surgery period probably causes some mismatching of ventilation and perfusion of lung tissue. This would contribute to the low O_2 tension and high CO_2 tension (Kumar *et al.*, 1976; Kumar and Thurmon, 1979). It has been shown that dorsal and lateral recumbency do contribute to acid-base disturbances

in cattle injected with xylazine (DeMoor and Desmet, 1971), even though differences in the size between cattle and goats may be overwhelming.

The clinical significance of a fall in arterial oxygen tension and / or packed cell volume is that, although healthy animals can tolerate these changes, animals with acute or chronic anaemia or with disorders having an unfavourable effect on tissue oxygenation including reduced blood supply to the heart and respiratory disease, may be in danger as such changes may have a fatal outcome. Such animals would require adequate ventilation in order to avoid hypoxia (DeMoor and Desmet, 1971; Kumar *et al.*, 1976).

2.6   EFFECTS OF XYLAZINE ON PLASMA GLUCOSE AND INSULIN.

Xylazine induces a number of physiological and pharmacological changes in the species in which its use has been studied (Knight, 1980). Among these changes, hyperglycaemia and hypoinsulinaemia have been demonstrated in several animal species including cattle (Symonds, 1976; Symonds and Mallinson, 1978; Eichner *et al.,* 1979; Hsu and Hummel, 1981; Fayed *et al.*, 1989), cats (Feldberg and Symonds, 1980), dogs (Goldfine and Arieff, 1979; Benson *et al.*, 1984), sheep (Brockman, 1981; Muggaberg and Brockman, 1982) and horses (Thurmon *et al.,* 1982; Greene *et al.*, 1987). Xylazine's hyperglycaemic effect has been reported in

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goats (Kumar and Thurmon, 1979; Dilipkumar *et al.*, 1997).

The mechanism by which xylazine induces its hyperglycaemic and hypoinsulinaemic effects has been investigated in various animal species. Earlier studies in cattle (Symonds, 1976; Symonds and Mallinson, 1978; and Eichner *et al.*, 1979) and cats (Feldberg and Symonds, 1980) suggested that the state of hyperglycaemia was due to increased hepatic glucose production and this was directly related to a decrease in insulin concentration as a result of decreased pancreatic secretion of insulin. Although the mechanism was not fully understood, xylazine's hyperglycaemic and hypoinsulinaemic effects have been associated with a direct hepatic glycogenolytic or gluconeogenic effect, inhibited or reduced insulin synthesis or inhibited insulin secretion (Symonds, 1976; Symonds and Mallinson, 1978; Eichner *et al.*, 1979). Hsu and Hummel (1981) in studies conducted in cattle reported that the mechanism of hyperglycaemia appeared to be α-adrenergically mediated.

Studies carried out in cats (Feldberg and Symonds, 1980) were able to show that xylazine-induced hyperglycaemia was not a central effect, nor was it due to adrenaline release from the adrenals or due to xylazine's direct action on the liver but, due to a fall in plasma insulin produced by an action of xylazine on the pancreas, inhibiting insulin secretion. However, this does not affect glucagon

secretion in cats but, increases the glucagon/insulin ratio. Increase in glucagon/insulin ratio, by stimulating glucose production in the liver has also been suggested as a probable reason for xylazine induced hyperglycaemia (Feldberg and Symonds, 1980). In sheep, Brockman (1981) reported of increased glucagon concentrations immediately after xylazine administration and this was attributed to sympathetic stimulation by xylazine and therefore, possibly accounting for the hepatic events leading to hyperglycaemia. Further studies in cattle (Hsu and Hummel, 1981) showed that xylazine produced a dose dependent hyperglycaemia and hypoinsulinaemia. By using the α_2-adrenergic blocking agent yohimbine, α_1 and α_2-adrenergic blocking agent phentolamine, and α_1-adrenergic blocking agents prazosin and phenoxybenzamine, Hsu and Hummel (1981) were able to demonstrate that xylazine-induced hyperglycaemia and hypoinsulinaemia are mediated predominantly by α_2-adrenergic receptors, possibly in β-cells of the pancreatic islets which inhibit the release of insulin. Alpha$_2$-adrenergic receptors are present in the membrane of the pancreatic beta cells (Exton, 1982) and apparently inhibit the release of insulin as xylazine is an α_2-adrenergic agonist. Xylazine may also directly stimulate hepatic glucose production through its weak α_1-agonistic effect (Hsu and Hummel, 1981).

In dogs (Benson *et al.*, 1984) and horses (Thurmon *et al.*, 1982), the trend in

glucose concentration was found to be directly opposite in direction to that of insulin, indicating that the hyperglycaemia induced by xylazine in these species would appear to be caused by decreased insulin secretion as previously reported in other species (Symonds, 1976; Thurmon *et al.*, 1978; Eichner *et al.*, 1979; Feldberg and Symonds, 1980).

While evaluating the effect of xylazine in heifers under thermoneutral and heat stress conditions, Fayed *et al.* (1989) reported of xylazine-induced hypoinsulinaemia associated with hyperglycaemia under both environmental temperature conditions. The hypoinsulinaemia and the hyperglycaemia persisted for 2 hours and 6 hours respectively, following the administration of xylazine. The hypoinsulinaemic effect of xylazine was more pronounced in heifers in the heat stress environment (33° C; 63% humidity) whereas the magnitude of hyperglycaemia was more pronounced in the animals placed in the thermoneutral (18° C; 42% humidity) environmental temperature conditions than in those under heat stress environmental conditions. However, the reasons for the differences in the magnitudes of hypoinsulinaemia and hyperglycaemia at different environmental temperatures are not clear.

2.7   XYLAZINE HYDROCHLORIDE AND BODY TEMPERATURE.

Ambient temperature is an important factor affecting thermal responsiveness of animals to systemically injected drugs (Buckley *et al.*, 1969; Avery, 1972; Tsoucaris-Kupfer and Schmitt, 1972; Ponder and Clark, 1980; Livingston *et al.*, 1984; Fayed *et al.*, 1989). Agents that depress thermoregulation (e.g anaesthetics and some anaesthetic adjuncts) allow the body temperature to drift passively upwards in a hot environment and downwards in cold. These changes in body temperature are not opposed by effector mechanisms to prevent or lessen variation in temperature (Clark, 1979).

The effect of xylazine hydrochloride on body temperature in various species varies. In goats, xylazine has been shown to decrease (Mohammed and Yelwa, 1993; Prajapathi *et al.*, 1994; Saleh, 1993), increase (Aithal *et al.*, 1996; Dehghani *et al.*, 1991) or, have no effect on body temperature (Kumar and Thurmon, 1979). s in body temperature caused by xylazine are known to take up to 24 hours before they return to baseline values (Prajapathi *et al.*, 1994). In cats, Dehghani *et al.* (1991) reported significant reduction in body temperature attributed to xylazine as did Tsoucaris-Kupfer and Schmitt, (1972) and Livingston *et al.* (1984) in rats. In cattle, increase in body temperature following xylazine administration has been reported (Fessl, 1970; Hopkins, 1972; Young, 1979). Increases of 1.6 to 1.9° C, peaking 4-5 hours following xylazine administration to cattle kept in an environmental

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temperature range of 25-28° C, with the temperature returning to pre-injection values after 18 hours have been reported (Young, 1979).

Fayed *et al.* (1989) carried out studies to evaluate the effect of xylazine in heifers under thermoneutral (18° C; 42% humidity) or heat- stress (33° C; 63% humidity) conditions. After 35 days of conditioning the heifers to the respective environments, intravenously administered xylazine hydrochloride induced marked hyperthermia in the heat-stressed heifers that lasted 6 hours whereas the drug had no significant effect on body temperature in heifers in the thermoneutral environmental conditions. The hyperthermic effect of xylazine in the heat-stress environment was attributed to inhibition of the thermoregulatory centre by xylazine and also, partly due to suppression of the respiration rate in the heat-stressed group, thus, the heifers could not get rid of the excess heat through the respiratory system.

When different dosages of xylazine were injected into cats subcutaneously or intravenously at three different environmental temperatures of 4, 22, and 32° C (Ponder and Clark, 1980), xylazine consistently caused a dose- related decrease in body temperature in the 4 and 22° C environments which was maximal 3-4 hours after injection and the hypothermia lasted for at least 12 hours. In the 32° C environment, xylazine induced hyperthermia in the cats. Further, the xylazine-

induced hypothermia developed more rapidly in cats in the 4° C environment than in the 22° C environment and the hypothermic response developed somewhat sooner after intravenous than subcutaneous administration of xylazine. The maximum decrease in body temperature was attained much sooner in the 4° C environment as this created a greater temperature gradient between the body and the environment than at 32° C. The authors also noted that neither the hypothermia nor the hyperthermia was opposed by compensatory thermoregulatory effector activities e.g shivering to oppose fall in body temperature or increased respiration rate to oppose increase in body temperature. Ponder and Clark (1980) have suggested that animals given xylazine should not be exposed to extreme heat or cold for several hours to avoid the development of hyperthermia or hypothermia. Because full recovery to the baseline body temperature values in the cats required 12 hours or more, cats which appear to have fully recovered from the effects of xylazine may still be unable to maintain normal body temperature, especially if exposed to extremes of environmental temperature.

Studies carried out in rats (Tsoucaris-Kupfer and Schmitt, 1972; Livingston *et al.*, 1984) using xylazine and clonidine, another α-sympathomimetic agent, in environmental temperature range of 20 to 24° C showed that both agents produced a dose-dependent hypothermia in the rats. Using an α_2-antagonist yohimbine, the

hypothermic effect of xylazine was antagonised, whereas that of clonidine was potentiated (Livingston *et al.*, 1984). Whether xylazine and clonidine were injected intraperitoneally (Livingston *et al.*, 1984) or into the cerebral ventricles or hypothalamus (Tsoucaris-Kupfer and Schmitt, 1972), the hypothermia induced by the two drugs in the rats was similar. This suggested that the hypothermic action of α-sympathomimetic agents seems to be due to a dual effect, central and peripheral and the receptors involved in this effect seem to have some properties common with the classical adrenergic receptors (Tsoucaris-Kupfer and Schmitt, 1972). They also suggested that possible causes of hypothermia by α-sympathomimetic drugs could be through cutaneous vasodilation or a decrease in oxygen consumption at cellular level.

Avery (1972) evaluated the thermoregulatory effects of intrahypothalamic injections of noradrenaline and carbamylcholine chloride (carbachol, a direct acting parasympathomimetic drug) in rats at environmental temperatures of 5, 24, and 35°C. In these studies, noradrenaline and carbachol gave rise to hyperthermia at 5° C while at 24° C, noradrenaline significantly lowered body temperature. At 35°C, noradrenaline defended against the normal hyperthermia observed in this environment.

Acclimation of animals to a new environment is generally known to involve participation of the endocrine and metabolic systems (Yousef *et al.*, 1967). Adaptation to a new environment has three steps namely; initial shock reaction (lasting seconds to minutes), stabilised state (minutes to hours), and the compensation stage or acclimation which spans over a period of days to weeks (Prosser, 1958). It has been shown that acute exposure to heat and cold lead to changes in thyroid function (Johnson *et al.*, 1958; Yousef *et al.*, 1967). Acute exposure of cows from a temperature of 18° C to a hot environment of a temperature of 38° C did not have significant effects on rectal temperature of the cows in the initial 36 hours of exposure but significant changes in both rectal temperature and thyroid activity only occurred after 60 hours of exposure (Yousef *et al*, 1967). Exposure of the same cows from 18° C to 1° C environment did not significantly affect rectal temperature. These observations suggest that metabolic rate and thyroid activity are not involved in the early (first 2 days) stage of adjustment to a hot temperature environment (Yousef *et al*, 1967).

CHAPTER THREE

CLINICAL, CARDIOPULMONARY AND HAEMOCYTOLOGIC EFFECTS OF XYLAZINE HYDROCHLORIDE IN GOATS UNDER DIFFERENT ENVIRONMENTAL TEMPERATURE AND HUMIDITY CONDITIONS.

3.1   INTRODUCTION

Xylazine, pharmacologically classified as an analgesic as well as a sedative and skeletal muscle relaxant (Booth, 1988) has been widely used in biological research and veterinary medicine (Knight, 1980; Greene and Thurmon, 1988). The drug induces a number of physiological and pharmacological changes in the species in which its use has been studied (Knight, 1980). The cardiopulmonary and haematological effects of xylazine hydrochloride have been investigated in several animal species including goats (Kumar and Thurmon, 1979; Bafi-Yeboa and Huvos, 1980; Kokkonen and Eriksson, 1987; Mohammed and Yelwa, 1993; Saleh, 1993; Prajapathi *et al.*, 1994; Dehghani *et al.*, 1991; Aithal *et al.*, 1996), cattle (DeMoor and Desmet 1971; Campbell *et al.*, 1979; Aouad *et al.*, 1981), sheep (Aziz and Carlyle, 1978; Celly *et al.*, 1997), cats (Dehghani *et al.*, 1991),

horses (Clarke and Hall, 1969; Garner *et al.*, 1971), dogs (Klide *et al.*, 1975) and donkeys (Mogoa , 1990).

Xylazine hydrochloride, when injected intravenously in animals induces hypotension, bradycardia, heart block, respiratory depression and in ruminants, marked salivation (Booth, 1988). In horses (Clarke and Hall, 1969), dogs (Klide *et al.*, 1975), and sheep (Aziz and Carlyle, 1978), when injected intravenously, xylazine causes partial cardiac blockade with decreased conductivity, arrhythmia and bradycardia. In ponies, intravenous xylazine has been shown to produce sino-atrial (SA) block, transient atrio-ventricular (AV) block and bradycardia within one minute of its injection (Garner *et al.*, 1971).

A study in heifers (Fayed *et al.*, 1989) revealed that the recovery period, duration of salivation and sedation following xylazine administration was longer in animals acclimatised to hot, humid conditions than in animals under thermoneutral conditions. There is no report in the literature that describes the influence of different environmental temperature and humidity conditions on the clinical, cardiopulmonary and haemocytological effects of xylazine in goats. The purpose of this study was to investigate the clinical, cardiopulmonary and haemocytological effects of xylazine hydrochloride in goats under three different sets of environmental conditions.

3.2   MATERIALS AND METHODS.

3.2.1 EXPERIMENTAL ANIMALS

Six adult, indigenous breed, castrated male goats, weighing between 21.0 and 34.0 kg (mean = 28.2 ± 1.0,SEM), were purchased locally and transported to the University of Pretoria's Faculty of Veterinary Science at Onderstepoort. They were housed indoors in individual crates at the Faculty's Veterinary Academic Research Unit to acclimatise for 14 days. On arrival, they were dewormed (Panacur®- Hoechst, Ag-Vet, South Africa) and clinically examined to ascertain the status of their health. They were fed on a diet of lucerne, hay and water, which were provided *ad libitum*. Three weeks after arrival, the goats underwent surgery to subcutaneously relocate the carotid arteries for experimental monitoring and sampling purposes. The protocol for this study was approved by the Ethics and Research Committees of the Faculty of Veterinary Science, University of Pretoria.

3.2.2 SURGICAL RELOCATION OF THE CAROTID ARTERIES.

Food was withheld for 24 hours and water for 12 hours before the surgical operation. On the day of the surgical operation, the goats were weighed and the hair of the neck was clipped with an electrical clipper. Before induction of anaesthesia, the goats were premedicated with midazolam (Dormicum®, Roche), injected intramuscularly at a dose of 0.4 mg/kg body weight. After they became recumbent, they were restrained in lateral recumbency and a 18G catheter (Jelco®, Critikon) was percutaneously introduced into the median vein on the medial aspect of the carpus and secured with tape on to the skin. Anaesthesia was intravenously induced with ketamine hydrochloride (Anaket®, Centaur) at a dose of 4.0 mg/kg body weight and the trachea intubated with the aid of a laryngoscope. The right jugular furrow and adjacent areas were surgically prepared. The goats were transferred into theatre and connected to a circle circuit anaesthetic machine, and anaesthesia maintained with 2% halothane (Fluothane®, Zeneca) in oxygen during the surgical procedure. Fresh gas flow rate was set at 15 ml/kg/minute until surgical anaesthesia was reached and thereafter maintained at 5 ml/kg/minute during surgery. The surgical relocation of the carotid arteries was performed as described by Bone *et al.* (1962) in sheep with a slight modification. Bone *et al.* (1962) after freeing the carotid artery and moving it to the surface of the musculature, made a secondary skin incision parallel with the line of initial incision to form a skin flap connected to the body

at both ends. This skin flap was then wrapped around the carotid artery and sutured together at the edges to form a tube enclosing the carotid artery. In our case, after freeing the carotid artery and bringing it to the surface of the body, the initial incision in the cutaneous musculature was sutured with interrupted sutures of no. 0 chromic catgut, (Ethicon) sutures, leaving 1 cm unsutured at both the proximal and distal ends of the incision. The skin incision was closed with interrupted no. 3/0 nylon (Ethilon) sutures. After uneventful recovery from anaesthesia, the goats were postoperatively injected with procaine penicillin intramuscularly for 4 days. Vital body parameters and the healing process were monitored daily for the following 14 days. After the 14 days, the surgical wounds had healed and the nylon skin sutures were removed under midazolam sedation at a dose of 1 mg/kg body weight, injected intramuscularly. The healing of the surgical wounds was uneventful in all the goats and after removal of the skin sutures, the carotid arteries were easily palpable under the skin and the pulsations were easily visible.

3.2.3 EXPERIMENTAL PROCEDURE.

3.2.3.1   EXPERIMENTAL DESIGN

A single group, three phase repeated measures study design was used to study the effect of environmental temperature and humidity on the clinical, cardiopulmonary and haemocytologic effects of xylazine in six goats. The clinical, cardiopulmonary and haemocytologic effects of the drug were repeatedly examined in the goats during a low, medium and high temperature and humidity exposure treatment phases. A washout period of at least seven days was allowed between treatment phases.

3.2.3.2   TREATMENT PHASES.

The experiments in this study were conducted in temperature and humidity controlled environments. Phase one of the study was conducted in the medium temperature and humidity environment with room temperature set at $24 \pm 1°$ C and a relative humidity of $55 \pm 1\%$. Phase two of the study was conducted in the high temperature and humidity environment with room temperature set at $34 \pm 1°$ C and a relative humidity of $65 \pm 1\%$. Phase three of the study was conducted in the low temperature and humidity environment with the room temperature set at $14 \pm 1°$ C and a relative humidity of $33 \pm 1\%$. The temperature and humidity

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settings were done several hours before any experiment was started so as to produce uniform conditions in the rooms. Between experiments, the goats were housed and fed in individual crates, in housing premises devoid of temperature and humidity control.

Before any experiment was carried out, the goats had food withdrawn for 24 hours and water for 12 hours. On the day of the experiment, each goat was weighed and taken into a preparation room adjacent to the temperature and humidity controlled room. The average weight of goats during the experiments in the 14° C environment was 28.6 ± 1.8 kg, 26.9 ± 1.5 kg in the 24° C environment and 29.0 ± 1.9 kg in the 34° C environment.

In the preparation room, the goats were restrained in lateral recumbency on a surgical table with a waterproof foam mattress. The jugular furrows on both sides of the neck were thoroughly clipped and surgically prepared. A 18G intravenous catheter (Jelco®- Critikon) was percutaneously introduced into the left jugular vein, flushed with heparinised saline, capped and secured on to the skin with nylon No. 2/0 (Ethilon) sutures. Another 20G catheter (Medican® - Medical Specialities) was percutaneously introduced into the subcutaneously lying right carotid artery, flushed with heparinised saline, capped and secured on to the skin with nylon sutures. The animals were then transferred into the

temperature and humidity controlled rooms. A strain gauge blood pressure transducer attached to a multi- parameter physiological monitor (Propaq®104EL- Protocol Systems Inc., Oregon, USA) was connected to the carotid catheter via a non-compliant tubing and a three- way stop cock to monitor mean, systolic and diastolic blood pressure. The zero-point for the strain gauge blood pressure transducer was taken at the level of the sternum. The transducer was calibrated with a mercury sphygmomanometer. After a stabilisation period of 10 to 15 minutes, baseline readings were taken and then xylazine hydrochloride (Rompun®- Bayer Animal Health (Pty), Isando, South Africa) at 0.1 mg/kg body weight was injected intravenously through the jugular catheter.

Pulse rate was read off the multi-parameter physiological monitor (Propaq®104EL- Protocol Systems Inc., Oregon, USA) and the respiratory rate was determined using a capnograph or by counting thoracic excursions over a one minute period. The respiratory rate, heart rate, mean, systolic, and diastolic arterial blood pressures were recorded at 'time zero' (baseline) and at 5, 15, 30, 45, and 60 minutes post-xylazine injection.Venous blood for the determination of haemoglobin concentration, red cell count, white cell count, haematocrit, mean corpuscular haemoglobin concentration, mean corpuscular volume and total protein were drawn from the jugular vein into EDTA tubes at 'time zero'

and 15, 30, and 60 minutes post-xylazine injection, and these determinations made within 6 hours of the sample collection. All cell counts and blood cell parameters were determined using System 9000 Diff Model Automated Cell Counter (Serono Diagnostics Inc., PA 18103, USA). Total protein was determined using a RA-1000 Analyser (Technicon Instruments Corporation, New York, USA) based on the Biuret reaction of Weichselbaum and as described by Skeggs and Hochstrasser(1964). Other variables evaluated over the entire monitoring period were, sedation, analgesia, salivation, muscle relaxation and recovery time. Sedation was assessed by assessing the drooping of the upper eyelids and protrusion of the tongue. Analgesia was assessed by the response of the goats to needle pricks to the flank, thorax, coronary band and manual pinching of the interdigital skin. Muscle relaxation was judged by the muscle tone of the jaw and uppermost fore and hind limbs during passive flexing and extending.

3.2.4 STATISTICAL ANALYSIS.

The data in these studies was analysed on a personal computer equipped with statistical software (Sigma Stat- Jandel Scientific Software®- Jandel Corporation, San Rafael, CA). Results are given as mean and standard error of the mean. To test for significance of the effect of treatment over time as well as

for differences among treatments between groups, data collected over time were analysed using a two way repeated measures analysis of variance (ANOVA). When a significant effect of treament was observed, comparisons between treatments were performed using one way analysis of variance (ANOVA) for repeated measures followed by Bonferroni's t-test to examine for least significant difference. Significant changes with time within any group were also analysed using one way analysis of variance (ANOVA) for repeated measures followed by Bonferroni's t-test to examine deviation from control (baseline, 'time zero') values. Where the data was either not normally distributed or the equal variance test failed, the data was analysed using Friedman Repeated Measures Analysis of Variance (ANOVA) on Ranks followed by Dunnett's method to examine deviations from baseline or 'time zero' (control) values. $P < 0.05$ was considered significant.

3.3 RESULTS

3.3.1 CLINICAL AND BEHAVIOURAL EFFECTS.

Under the three environmental conditions, xylazine administration to the goats caused an initial excitement and restlessness characterised by bleating, groaning, grunting and limb movements which lasted for between 1 and 5 minutes.

Nystagmus was also evident over this period of time. This was followed by a period of calmness and the animals remained sedated for the next 25 to 30 minutes with occassional bleating and tail switching being seen in some of the goats. Under all three environmental conditions, signs of recovery as evidenced by leg movements and raising of the head occurred within 40 to 50 minutes following xylazine administration. The monitoring period under all the three environmental conditions was one hour. At the end of the one hour monitoring, the jugular vein and arterial indwelling catheters and the oesophageal thermometer probe were removed and the animals left undisturbed to rise on their own. The goats arose unaided after 73.83 ± 4.87, 70.50 ± 0.76, and 68.00 ± 1.03 minutes from the time of xylazine administration in the 24, 34, and 14° C environments, respectively. There was no significant difference in the time they took to stand under the three different environmental conditions. On standing, all the animals had stable gait and ate and drank water immediately without any difficulty.

Following intravenous administration of xylazine, dripping of saliva started at 6.33 ± 0.80, 5.65 ± 1.36, and 6.50 ± 1.23 minutes in the 24, 34, and 14° C environments, respectively. Although the amount of saliva was small at the beginning, this became copious in the following minutes and then subsided to very light amounts. The duration of salivation was 45.67 ± 2.01, 45.17 ± 4.59,

and 44.33 ± 3.06 minutes in the 24, 34, and 14° C environments, respectively. There were no significant differences in the time salivation started or the duration of salivation under the three different environmental conditions.

Following administration of xylazine to the goats, insensitivity of the abdominal and thoracic skin to pin pricks was present starting at 7.40 ± 1.50 minutes in the 24° C environment and the duration of analgesia was 40.00 ± 3.73 minutes. In the 34° C environment, analgesia of the thorax and abdomen was present at 6.17 ± 0.83 minutes and lasted for 43.83 ± 3.92 minutes, whereas the analgesia onset was at 5.50 ± 0.50 and the duration was 39.50 ± 2.15 minutes in the 14° C environment. In all cases, there was no analgesia from the coronary band, distally. The analgesia of the abdominal skin persisted relatively longer than that of the thoracic wall skin. The onset and duration of analgesia was not statistically different under the three different environmental conditions.

Following injection of xylazine hydrochloride to the goats under the three environmental conditions, urination of relatively large amounts of urine occurred after 21.00 ± 1.88 minutes and also after 69.60 ± 3.42 minutes.

Xylazine hydrochloride provided good muscle relaxation in all the goats under the three different environmental conditions and this lasted over the 25 to 30 minutes/period of sedation.

3.3.2 CARDIOPULMONARY AND HAEMOCYTOLOGICAL EFFECTS.

The trend in heart rate following intravenous injection of xylazine hydrochloride to the goats under the three different environmental conditions was similar (Figure 3.1). Within the first minute following injection of the drug, one-half of the goats exhibited bradycardia and heart block that gave way to tachycardia. Bradycardia alternated with tachycardia in most of the goats for the 5 minutes following administration of xylazine and this persisted for up to 10 minutes in some goats. Mean heart rates then started declining in all the three environments for the rest of the monitoring period. The heart rates had not returned to baseline values at the end of the monitoring period. The mean heart rate for the goats exposed to an environmental temperature of 24° C declined from 74.00 ± 6.05 beats/minute at 'time zero' to 63.00 ± 5.96 beats/minute at 30 minutes and declined further to 58.00 ± 4.20 beats/minute at 60 minutes post-xylazine injection, a decrease of 22%. However, these changes were not significant.

In the 34° C environment, mean heart rate increased from baseline value of 76.50 ± 3.91 beats/minute to 82.50 ± 11.93 at 5 minutes and then declined to 66.67 ± 4.40 beats/minute at 30 minutes and then further to 64.33 ± 7.17 beats/minute at 60 minutes, a decline of 16%. All these changes were not significant when compared to baseline values. The mean heart rates of goats in the 14° C environment declined from 85.00 ± 10.88 beats/minute at time zero to 72.83 ± 9.36 at 30 minutes and then further to 60.00 ± 3.14 beats/minute at 60 minutes, a decline of 29%. Mean heart rate at 60 minutes post-xylazine injection in this group was significantly ($P < 0.05$) lower that the baseline value. There was great individual variation in heart rate under the different environmental conditions. Environmental conditions did not have a significant effect on heart rate.
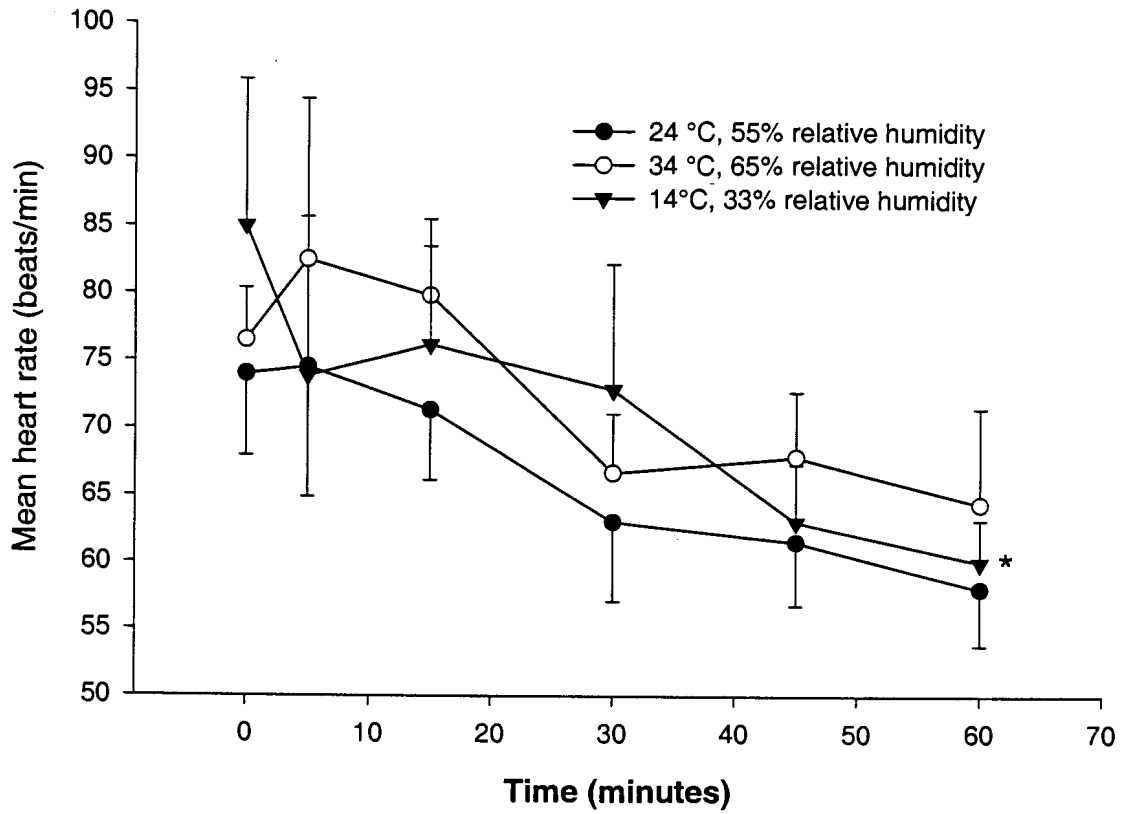
Figure 3.1 : Temporal changes in mean and standard error of mean of heart rate of goats following intravenous injection of 0.1 mg/kg xylazine under three different environmental conditions
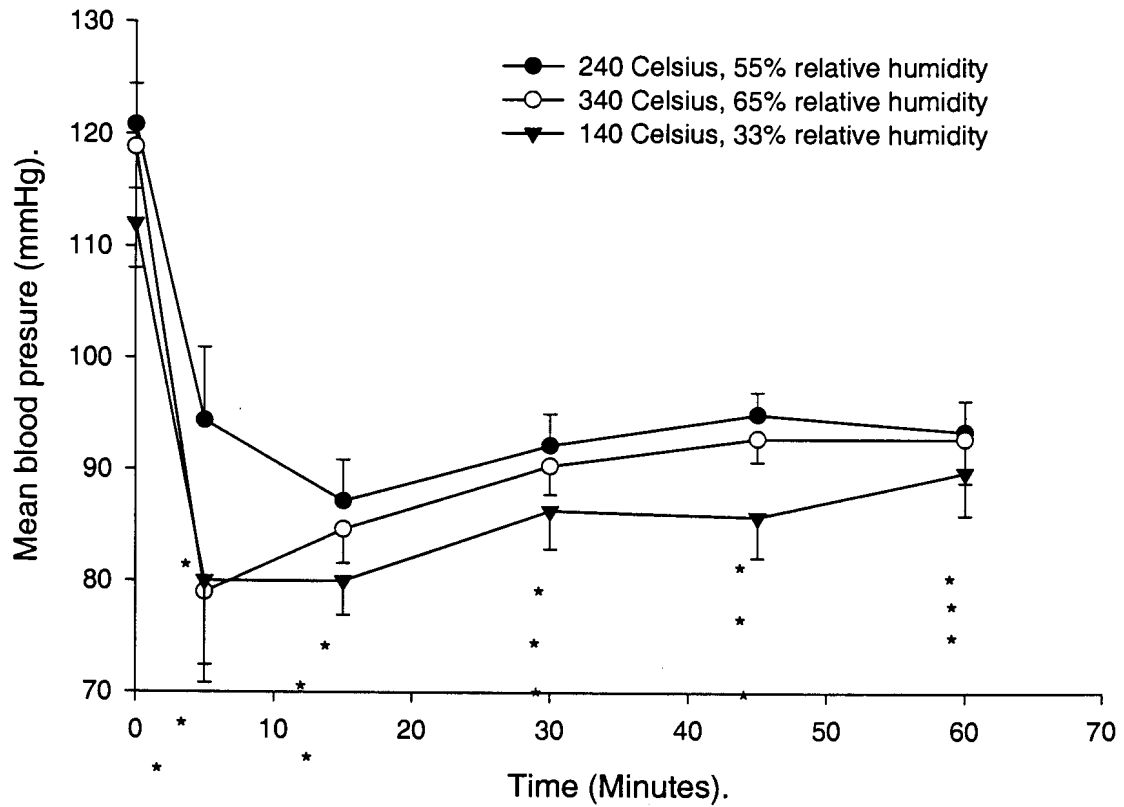*- Significantly (P<0.05) different from values at time 0.

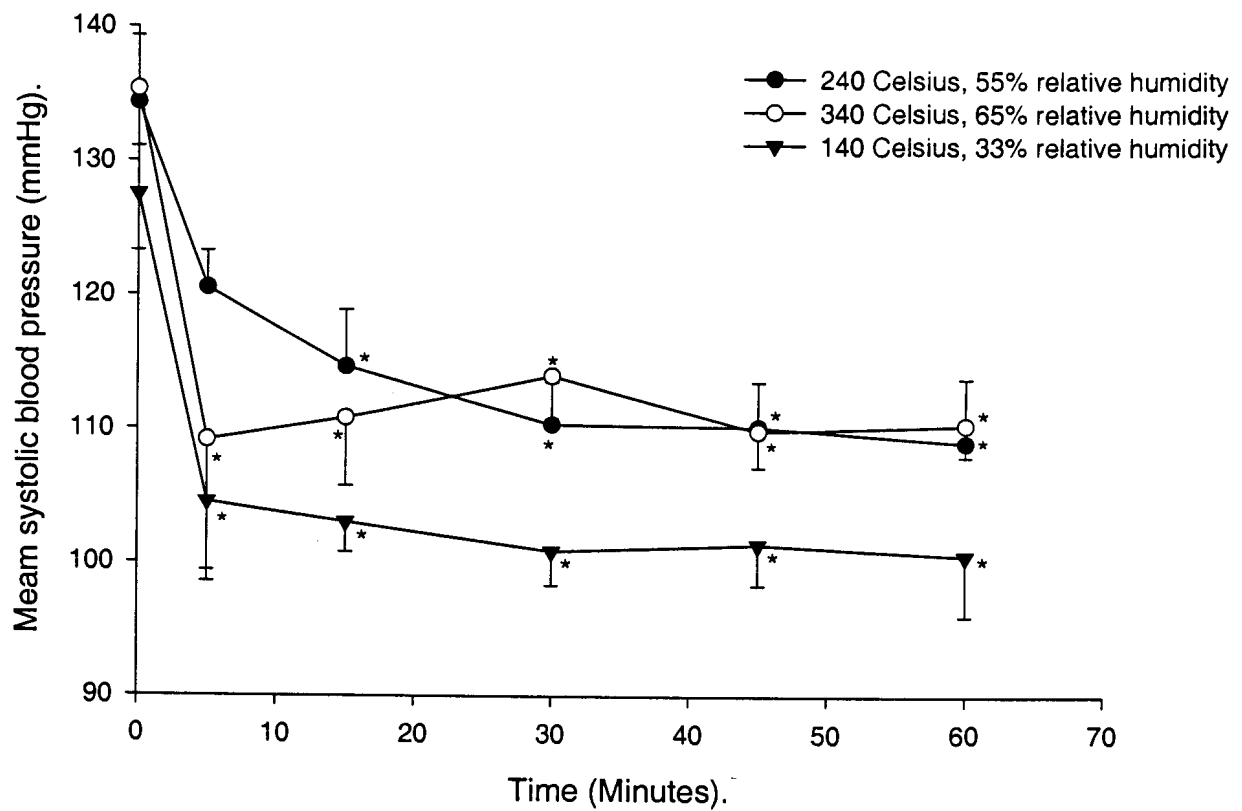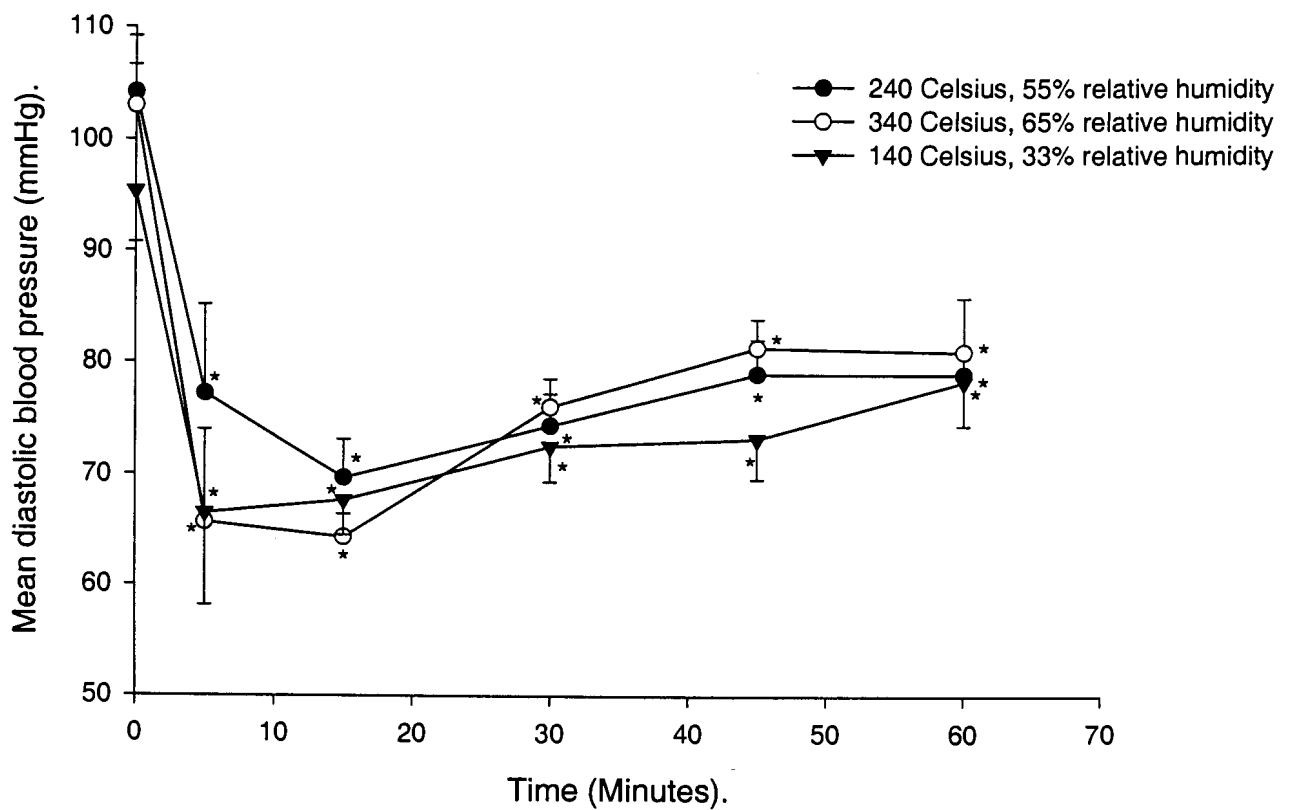Figure 3.2 : Temporal changes in mean and standard error of mean of mean blood pressure of goats following intravenous injection of 0.1 mg/kg xylazine in goats under three different environmental conditions
*- Significantly($P<0.05$) different from values at time 0.

Figure 3.3 : Temporal changes in mean and standard error of mean of systolic blood pressure in goats following intravenous injection of 0.1 mg/kg xylazine under three different environmental conditions
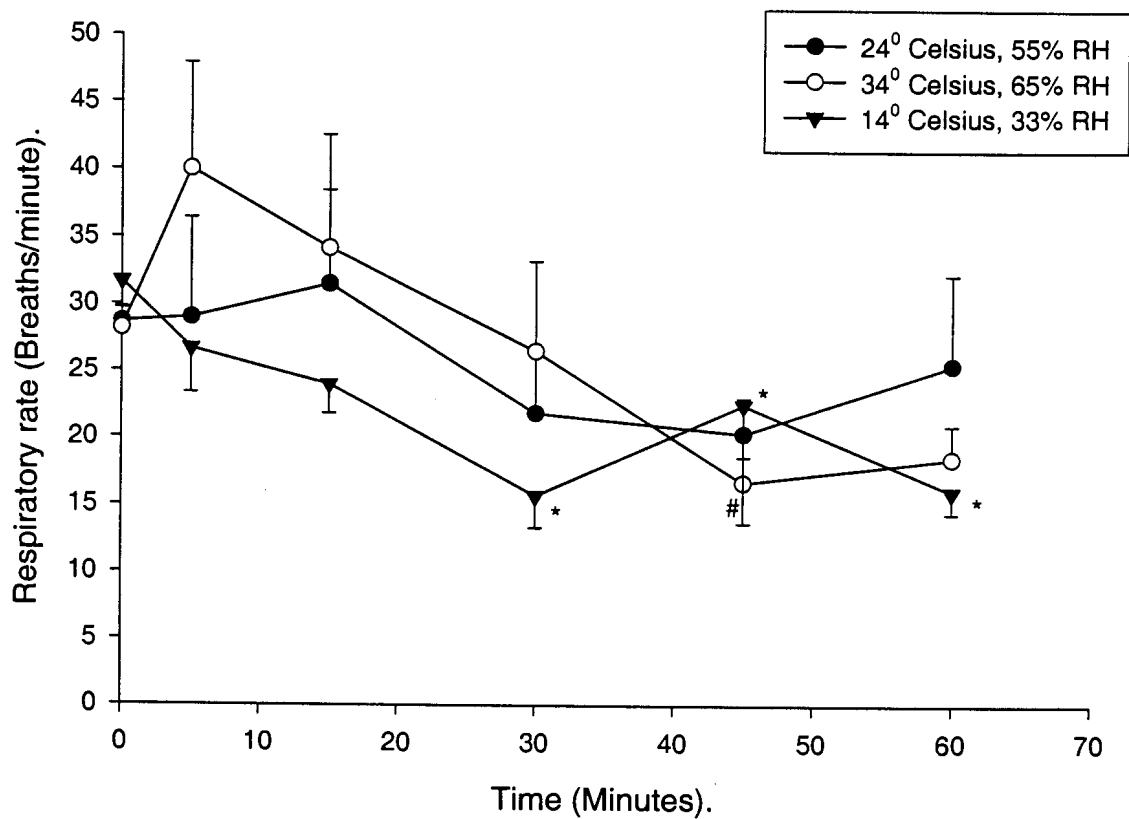*- Significantly (P<0.05) different from values at time 0.

Figure 3.4 : Temporal changes in mean and standard error of mean of diastolic blood pressure of goats following intravenous injection of 0.1 mg/ml xylazine under three different environmental conditions
*- Significantly (P<0.05) different from values at time 0.

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Figure 3.5 : Temporal changes in mean and standard error of mean
of respiratory rate of goats following intravenous injection of 0.1 mg/kg
xylazine under three different environmental conditions
*- Significantly (P<0.05) different from values at time 0.
- Significantly (P<0.05) different from values at 5 minutes in same group

Mean arterial blood pressure (MAP) changes following injection of xylazine hydrochloride were similar under the three environmental conditions (Figure 3.2). Within 1 to 2 minutes of the injection of the drug, all goats developed hypertension. In the 24° C environment, MAP rose from 120.83 ± 3.66 mmHg at 'time zero' to 156.83 ± 7.87 mmHg. In the 34 and 14° C environments, MAP rose from 118.83 ± 3.73 and 112.00 ± 3.96 mmHg at 'time zero' to 140.50 ± 1.65 and 142.00 ± 5.01 mmHg, respectively. The MAP then started to decrease, recording a maximum decrease at 15 minutes post-xylazine injection of 34 mmHg in the 24° C environment. In the 34 and 14° C environments, maximum decrease in MAP of 40 and 32 mmHg were recorded at 5 minutes post-xylazine injection, respectively. Following the maximal decline, MAP started increasing in all the three environments but these had not returned to baseline values at the end of the 60 minutes monitoring period. The MAP values at 5, 15, 30, 45, and 60 minutes post-xylazine injection were significantly ($P < 0.05$) lower than baseline values under all the three environmental conditions. Similar trends were observed for systolic and diastolic blood pressure (Figures 3.3 and 3.4) .There were no significant differences in arterial blood presssure attributable to the differences in temperature and humidity in the three environments.

Following administration of xylazine to the goats under the three environmental conditions, there were very dramatic changes in the respiratory pattern and rate

in all the goats (Figure 3.5). Within 1 to 5 minutes of drug administration, the goats exhibited apnoea that lasted for up to 1 minute in some goats. The apnoea alternated with irregular, laboured respiration while some goats showed open mouth breathing and gasping for air. There was also cyanosis of the oral mucous membranes. For the rest of the monitoring period, the respiration pattern alternated between rapid-shallow breathing to deep and slow respiration as judged by the thoraco-abdominal excursions. Regular respiration patterns were observed in most goats after 45 to 50 minutes post-xylazine administration.

The pattern of respiration rate varied under the different environmental conditions. In the 24° C environment, mean respiration rate increased from baseline values (28.67 ± 3.17 breaths/minute) to 31.50 ± 6.96 breaths/minute at 15 minutes and then decreased to 20.33 ± 2.69 breaths/minute at 45 minutes from when it started to increase again. However, these changes were not significant. In the 34° C environment, mean respiration rate rose from a baseline value of 28.17 ± 1.52 breaths/minute to 40.00 ± 7.93 breaths/minute at 5 minutes, then decreased to 16.67 ± 1.91 breaths/minute at 45 minutes and then started rising. Respiration rate was significantly ($P < 0.05$) lower at 45 minutes as compared to that at 5 minutes in this group.

In the 14° C environment, mean respiration rate declined from baseline value (31.67 ± 1.89 breaths/minute) steadily reaching a minimum at 30 minutes (15.67 ± 2.39 breaths/minute) only to rise at 45 minutes and then decrease to 16.00 ± 1.63 breaths/minute at 60 minutes. The mean respiration rate in this environment at 30, 45, and 60 minutes post-xylazine injection was significantly ($P < 0.05$) lower than the baseline value. Under all the three environmental conditions, the respiration rates remained lower than baseline values at the end of the 60 minutes monitoring period. Environmental conditions did not have significant effects on the respiration rate.

The results of the effects of xylazine on haemocytological variables under the three different environmental conditions are as shown on Table 3.1. Xylazine hydrochloride significantly ($P < 0.05$) reduced haemoglobin concentration and mean corpuscular volume (MCV) 15 minutes post-injection to the end of the 60 minutes monitoring period in the goats under all the three environmental conditions. A similar significant ($P < 0.05$) reduction in total protein over the same period of time occurred in goats in the 24 and 34° C environments, while there was no significant change in total protein in the 14° C environment. Significant ($P < 0.05$) reductions in haematocrit and red cell count occurred at 15, 30, and 60 minutes post-xylazine injection in all the three environmental conditions. Mean corpuscular haemoglobin concentration (MCHC) significantly

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(P < 0.05) increased at 30 and 60 minutes and 60 minutes post-xylazine injection in the 24 and 14° C environments, respectively, whereas, there was no significant change in the 34° C environment. Xylazine hydrochloride caused a significant (P < 0.05) reduction in the total white cell count at 30 mintes post-injection in the 24° C environment whereas this reduction was significant at 15, 30, and 60 minutes and at 30 and 60 minutes in the 34 and 14° C environments, respectively. The values of the above variables had not returned to the baseline values at the end of the 60 minutes monitoring period.

Xylazine caused haemolysis and this was seen in the plasma of all blood samples drawn within the first 5 minutes following its injection. Environmental conditions did not have significant effects on the variables evaluated above.

Table 3.1

Changes in haemocytologic variables (Mean±SEM) in goats following intravenous injection of 0.1 mg/kg xylazine hydrochloride under three different environmental conditions, (n=6).

Variable	Environmental Temperature (° C)	Time post-xylazine injection (minutes)			
		0	15	30	60
Hb (g/l)	14	106.33±2.89	94.77±3.08*	91.30±3.12*	93.85±3.45*
	24	103.98±2.61	90.33±7.77*	87.85±2.91*	89.37±3.05*
	34	104.17±4.79	88.23±1.48*	85.10±2.34*	88.67±3.38*
RCC $(x10^{12}/l)$	14	17.44±0.60	15.60±0.33*	14.92±0.46*	15.53±0.61*
	24	17.25±0.54	15.32±0.44*	14.59±0.39*	14.89±0.43*
	34	16.98±0.52	14.82±0.25*	14.11±0.31*	14.47±0.48*
TSP (g/dl)	14	61.18±1.75	59.35±1.70	59.20±1.68	59.40±1.20
	24	65.62±1.08	62.20±0.81*	61.30±0.93*	61.40±1.06*
	34	62.30±1.23	60.15±1.01*	59.38±0.74*	59.08±0.71*
HT (l/l)	14	0.320±0.013	0.280±0.011*	0.268±0.011*	0.272±0.011*
	24	0.317±0.011	0.257±0.013*	0.255±0.012*	0.257±0.013*
	34	0.312±0.007	0.262±0.005*	0.250±0.008*	0.257±0.012*
MCV (f/l)	14	17.23±0.19	16.88±0.20*	16.88±0.24*	16.97±0.20*
	24	16.92±0.17	16.65±0.15*	16.53±0.15*	16.48±0.15*
	34	17.12±0.22	16.78±0.19*	16.72±0.23*	16.73±0.23*
MCHC (g/dl cells)	14	33.27±0.62	33.82±0.32	34.33±0.50	34.77±0.64*
	24	32.83±0.60	33.33±0.96	34.83±0.70*	35.17±0.83*
	34	33.50±0.99	34.00±0.63	34.00±0.45	34.83±0.79
WCC $(x10^{9}/l)$	14	18.32±1.28	15.67±1.10	13.98±1.07*	14.08±1.62*
	24	18.77±2.41	16.73±2.18	15.40±1.90*	16.72±1.58
	34	16.93±0.91	13.88±1.05*	13.03±0.85*	13.15±1.26*

*- Significantly ($P < 0.05$) different from values at time 0 (baseline values). Hb- Haemoglobin concentration; RCC- Red blood cell count; TSP- Total serum protein; HT- Haematocrit; MCV- Mean corpuscular volume; MCHC- Mean corpuscular haemoglobin concentration; WCC- White cell count.

3.4   DISCUSSION.

3.4.1 CLINICAL AND BEHAVIOURAL EFFECTS

3.4.1.1   SEDATION

Goats are much more sensitive to xylazine than sheep and doses of 0.05 mg/kg may result in profound sedation for 12 or more hours (Dehghani *et al.*, 1991). The time it takes for signs of sedation to show, the duration of action and recovery time following administration of xylazine in goats varies and depends on among others the dose and the route of administration of the drug (Keller and Bauman, 1978; Dehghani *et al.*, 1991; Saleh, 1993; Mohammed and Yelwa, 1993; Prajapathi *et al.*, 1994).

In this study, xylazine administration to the goats initially caused restlessness characterised by bleating, groaning, grunting and limb movements which lasted for 1 to 5 minutes. This phenomenon has not been reported previously. This was followed by a period of calm and the animals remained sedated for 25 to 30 minutes. Over the same period of sedation, xylazine provided good muscle relaxation. Vocalisation and neck flexing following administration of xylazine in goats has been reported (Mohammed and Yelwa, 1993). Signs of sedation following administration of xylazine in goats include lowering of the head and

neck, partial drooping of the upper eyelid, protrusion of the nictitating membrane and tongue, muscular incoordination and staggering gait (Saleh, 1993). Protrusion of the tongue following administration of xylazine to steers has also been reported (Raptopoulos and Weaver, 1984). Although the goats in our study were restrained in lateral recumbency before the intravenous injection of xylazine, classical signs of sedation due to xylazine such as protrusion of the nictitating membrane and tongue and drooping of the upper eyelid were evident within 5 minutes of the administration of the drug. In this study, sedation occurred within the first 5 minutes of the administration of xylazine with the duration of sedation lasting for 25 to 30 minutes and recovery in 68 to 74 minutes, which is in agreement with previous findings (Keller and Bauman, 1978; Prajapathi *et al.*, 1994) in the same species. It has been shown (Fayed *et al.*, 1989) that heifers injected with xylazine and exposed to heat-stress conditions (33° C; 63% humidity) took significantly longer to stand from recumbency when compared to those exposed to thermoneutral (18° C; 42% humidity) environmental conditions, contrary to our findings in goats. Fayed *et al.* (1989) suggested that this could be due to increased sensitivity to xylazine by animals in the heat-stress environment.

3.4.1.2   SALIVATION

Intravenous injection of xylazine hydrochloride induces profuse salivation in ruminants (Booth, 1988). In this study, xylazine induced salivation, starting as light amounts and proceeding to copious amounts over time. Our findings agree with what has been reported in goats (Neophytou, 1982; Kokkonen and Eriksson, 1987; Mohammed and Yelwa, 1993; Saleh, 1993;), cattle (Raptopoulos and Weaver, 1984; Fayed *et al.*, 1989) and donkeys (Mogoa, 1990). Following intramuscular administration of xylazine in goats, profuse salivation has been shown to start at 14.4 ± 5.7 minutes (Mohammed and Yelwa, 1993) and in 5 to 7 minutes (Saleh, 1993) post-xylazine administration. Following intravenous infusions of various doses of xylazine in goats, Kokkonen and Eriksson (1987) reported of saliva starting to drip a few minutes after infusion of the higher doses of xylazine and this continued for 30 to 60 minutes. In this study, dripping of saliva in the three environmental conditions started as early as 5.67 ± 1.36 minutes and lasted for as long as 45.67 ± 2.10 minutes post-xylazine injection, which correlates well with previous findings (Kokkonen and Eriksson, 1987, Saleh, 1993) in the same species. The dripping of saliva after xylazine administration in goats has been attributed to the inability of the goats to swallow saliva during sedation (Kokkonen and Eriksson, 1987). In this study, there was no significant difference in the duration

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of salivation in the goats at the three different environmental conditions, which was contrary to the findings of Fayed *et al.* (1989) in heifers. Fayed *et al.* (1989) who evaluated the effect of xylazine in heifers under thermoneutral or heat-stress conditions reported that it took longer for salivation to subside in heifers under heat-stress conditions than in those exposed to thermoneutral conditions. The authors suggested that animals in the heat-stress environment were more sensitive to xylazine than those in the thermoneutral environmental conditions.

3.4.1.3   ANALGESIA

Xylazine hydrochloride provided good analgesia of abdominal and thoracic skin but no analgesia was present below the coronet. Xylazine has been reported to provide hardly any to moderate analgesic action in the region of the distal extremities (Fessl, 1970; Knight, 1980; Mogoa, 1990). As in this study, xylazine has been shown to provide good analgesia in goats (Dehghani *et al.*, 1991; Saleh, 1993; Aithal *et al.*, 1996), sheep (O'Hair *et al.*, 1986) and cattle (Fayed *et al.*, 1989), and this analgesic property is through its action on the autonomic and central nervous system (Knight, 1980). Even when it is injected locally, xylazine has been shown to have a potent local anaesthetic effect (Knight, 1980). The analgesia provided by xylazine is sometimes not adequate as some animals have been shown to react to surgical stimulation, even when supportive

local or regional analgesia was used in combination with intravenous xylazine (Raptopoulos and Weaver, 1984). Xylazine should be supplemented with some form of effective analgesia when a surgical procedure is to be performed (Raptopoulos and Weaver, 1984). The analgesia provided by xylazine in goats in this study lasted for up to 44 minutes, longer than has been reported in sheep (O'Hair *et al.,* 1986). Differences in environmental conditions in this study did not have significant effects on duration of analgesia as has been seen in cattle (Fayed *et al.,* 1989). The long duration of analgesia could be due to the greater sensitivity goats have to xylazine as compared to sheep (Dehghani *et al.,* 1991).

Longer recovery periods, duration of salivation and duration of analgesia in animals exposed to heat-stress conditions as compared to thermoneutral conditions has been attributed to increased sensitivity to xylazine in animals under heat-stress conditions (Fayed *et al.,* 1989). There is a possibility that hormonal or neural changes develop in heat-stressed animals whereby, heat-stressed animals have decreased metabolic rate resulting from decreased thyroid hormones which might alter xylazine kinetic properties (Magdub *et al.,* 1982). A possible explanation for the differences between our study and that of Fayed *et al.* (1989) apart from species differences could be in the study design. Whereas Fayed *et al.* (1989) had the heifers conditioned to their respective environmental conditions for 35 days prior to the study, the goats in our study

were acutely exposed to the three different environmental conditions without prior conditioning. It has been shown that thyroid activity following acute exposure of cattle to 1° C environment was significantly increased as from 36 hours following exposure whereas it was significantly depressed at 38° C, only after 60 hours following the acute exposure (Yousef *et al.*, 1967). In this study, the goats were exposed to the three study environmental conditions for only 1 hour, a duration of time not long enough to alter thyroid activity and subsequent changes in metabolic rate to alter xylazine kinetic properties, going by the findings of Yousef *et al.* (1967).

3.4.1.4 URINATION

Urination following administration of xylazine hydrochloride is a common occurrence in goats (Neophytou, 1982; Mohammed and Yelwa, 1993; Aithal *et al.*, 1996). In cattle, Thurmon *et al.* (1978) reported of increased urine output lasting for up to 5 hours, accompanied with the presence of glucose in the urine and increased urine pH following administration of xylazine hydrochloride. Not only does the quantity of urine increase but, the frequency of urination also increases following administration of xylazine in steers (Raptopoulos and Weaver, 1984). In our study, polyuria occurred after 21.00 ± 1.88 minutes and also after 69.60 ± 3.42 minutes. This is in agreement with previous reports in

goats (Mohammed and Yelwa, 1993; Aithal *et al.*, 1996). The polyuria observed in animals following administration of xylazine is attributed to the hyperglycaemia which has been shown to persist for up to 150 minutes post-xylazine injection in goats (Mohammed and Yelwa, 1993). Increased urine output following xylazine administration as has been seen in cattle could also be due to its effects on one or more of the animal's water-conserving mechanisms such as anti-diuretic hormone (ADH) formation or release, as a result of its actions on the distal tubules or, due to increased osmotic attraction of water into the renal tubules by failure to reabsorb glucose (Thurmon *et al.*, 1978). Polyuria seen in goats in our study coincided with the period of hyperglycaemia which started at 15 minutes post-xylazine injection, peaking at 30 minutes and was still present at the end of the 60 minutes monitoring period. It has been suggested that caution should be exercised when using xylazine in dehydrated or hypovolemic cattle to avoid further aggravation of these conditions. Furthermore, similar caution should be exercised when administering xylazine to cattle with urinary tract obstruction as this can lead to rupture of the urinary bladder as has been witnessed previously (Thurmon *et al.*, 1978).

3.4.2 CARDIOPULMONARY AND HAEMOCYTOLOGICAL EFFECTS.

A significant reduction in mean arterial blood pressure following administration of xylazine has been reported in goats and other species previously (Campbell *et al.*, 1979; Aouad *et al.*, 1981; Kokkonen and Eriksson, 1987; Celly *et al.*, 1997). A significant reduction in mean arterial blood pressure was observed under all three environmental conditions in the present study. Decrease in arterial blood pressure due to xylazine in goats is dose related (Kokkonen and Eriksson, 1987) and the magnitude of decrease in blood pressure depends on the route of administration of the drug. Following intramuscular administration, xylazine may not significantly alter blood pressure despite the higher doses used (Thurmon *et al.*, 1978) but greater responses in arterial blood pressure are achieved when the intravenous route is used despite the smaller doses injected (Kokkonen and Eriksson, 1987). Following the administration of xylazine hydrochloride, there was an initial increase in mean arterial blood pressure in all the three environmental conditions followed by a decrease. An initial increase in mean arterial blood pressure followed by a decrease due to xylazine has been shown to occur in sheep (Celly *et al.*, 1997) and calves (Aouad *et al.*, 1981). In most species, some of the reported cardiovascular effects of intravenous or intramuscular injection of xylazine including hypotension with bradycardia were present in the goats in our study. These effects are apparently mediated by

central α_2-adrenoreceptors. After higher doses, a brief initial vasopressor effect is not uncommon (Kerr *et al.*, 1972; Klide *et al.*, 1975). Increase in total peripheral resistance after xylazine administration has been reported in calves (Campbell *et al.*, 1979) and sheep (Aziz and Carlyle, 1978) and this has been attributed to its direct α-adrenoreceptor stimulation of peripheral vessels, causing peripheral vasoconstriction (Greene and Thurmon, 1988).

Xylazine hydrochloride did not cause significant changes in heart rate in the goats. Although the drug tended to reduce the mean heart rate towards the end of the study period and, even though the mean heart rates remained below baseline values at the end of the study, it was only in the 14° C environment that the mean heart rate was still significantly ($P < 0.05$) lower than baseline values. This could be attributed to the hypothermia the goats suffered in this environment as their mean oesophageal temperature was much lower (37.97 ± 0.24° C) as compared to the 24° C environment where the mean oesophageal temperature was 38.78 ± 0.20° C. There was great individual variation in heart rate under the different environmental conditions.

Xylazine hydrochloride administered intravenously or intramuscularly, causes a significant reduction in heart rate in goats (Kumar and Thurmon, 1979; Kokkonen and Eriksson, 1987; Dehghani *et al.*, 1991; Mohammed and Yelwa,

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1993; Saleh, 1993; Prajapathi *et al.*, 1994), cattle (Young, 1979; Campbell *et al.*, 1979), sheep (Aziz and Carlyle, 1978; Celly *et al.*, 1997) and horses (Clarke and Hall, 1969; Garner *et al.*, 1971). Similar effects are seen when the drug is administered epidurally in goats (Aithal *et al.*, 1996) and cattle (Skarda and Muir, 1992). The reduction in heart rate in goats has been shown to be dose dependent (Kokkonen and Eriksson, 1987) and the heart rate can remain depressed from a few hours to 24 hours (Dehghani *et al.*, 1991; Mohammed and Yelwa, 1993; Prajapathi *et al.*, 1994). In this study, there was great individual variation in heart rate under the different environmental conditions however, the differences in environmental conditions did not have significant effects on the mean heart rates of the goats. Xylazine also caused bradycardia and atrio-ventricular block followed by tachycardia. It is not uncommon to observe incidences of missed heart beat (Aziz and Carlyle, 1978; Bafi-Yeboa and Huvos, 1980) and irregular heart beats (Aithal *et al.*, 1996) accompanying bradycardia following xylazine administration in animals. In sheep, progressive bradycardia for up to 60 minutes and marked decrease in cardiac output to 37% of the control output following intravenous administration of xylazine have been reported (Aziz and Carlyle, 1978). In ponies (Garner *et al.*, 1971), intravenously administered xylazine is reported to have caused sino-atrial (SA) block and transient atrio-ventricular (AV) block within the first minute of its injection, and, these changes were accompanied by bradycardia and reduced

cardiac output. However, the authors were not certain whether the cause of the SA block was due to baroreceptor reflex and/or vagotonic effects. In calves, it has been shown that decreased cardiac output and stroke volume may also accompany bradycardia (Campbell *et al.,* 1979). It has been suggested that xylazine hydrochloride causes a decrease in heart rate via its central and peripheral suppression of the sympathetic trunk since pre-administration of the anticholinergic agent atropine before xylazine administration negated the change in heart rate in goats (Kumar and Thurmon, 1979). The ability of xylazine hydrochloride to decrease heart rate has been attributed to the withdrawal of the sympathetic tone, and to the direct depressive action on cardiac pacemaker and conduction tissue (Schmitt *et al.*, 1970; Antonaccio *et al.*, 1973; Klide *et al.*, 1975; Aziz and Carlyle, 1978; Knight, 1980). Another possible cause also could be in response to the transient hypertension that the drug induces (Clark *et al.*, 1982).

Xylazine hydrochloride significantly reduced respiration rate in goats in the 14° C environment starting at 30 minutes post-injection to the end of the study period. Xylazine has been shown to have similar effects on respiration at maximal sedation period in goats and other species (Fessl, 1970; Kerr *et al.*, 1972; McCashin and Gabel, 1975; Aziz and Carlyle, 1978; Kumar and Thurmon, 1979; White *et al.*, 1987; Dehghani *et al.*, 1991; Mohammed and

Yelwa, 1993; Aithal *et al.*, 1996) and the respiratory rate can remain below baseline values for hours following its administration (Saleh, 1993; Prajapathi *et al.*, 1994; Mohammed and Yelwa, 1993). Even when injected epidurally, xylazine can cause reduction in respiratory rate and irregularity in the pattern of respiration (Skarda and Muir, 1992; Aithal *et al.*, 1996). In our study, following xylazine injection, bradypnoea alternating with tachypnoea, forced breathing and periodic apnoea giving way to regular and deep respiration occurred. This has previously been reported in goats and other species (Aziz and Carlyle, 1978; Bafi-Yeboa and Huvos, 1980; Kokkonen and Eriksson, 1987; Saleh, 1993). Apart from causing a reduction in respiratory rate, a concurrent decrease in tidal volume in goats (Mohammed and Yelwa, 1993) and sheep (Aziz and Carlyle, 1978) has been reported. These effects of xylazine hydrochloride on pulmonary function have been attributed to its central α_2-adrenoreceptor mediated activity which results in respiratory depression (Prajapathi *et al.*, 1994; Aithal *et al.*, 1996).

Xylazine hydrochloride induced significant changes in haemoglobin concentration, red blood cell count, total white blood cell count, total protein, haematocrit, mean corpuscular volume, and mean corpuscular haemoglobin concentration in the goats in the three different environmental conditions. Decrease in total number of erythrocytes, leukocytes, haemoglobin

concentration (Kumar and Thurmon, 1979; Dehghani *et al.*, 1991) and haematocrit (DeMoor and Desmet, 1971; Kumar and Thurmon, 1979; Dehghani *et al.*, 1991), during the period of sedation following administration of xylazine have been reported in goats and other species and, this is consistent with our findings in the goats in this study. Contrary to the findings of Kumar and Thurmon (1979) in goats, xylazine hydochloride caused significant ($P < 0.05$) reduction in total proteins in the goats exposed to 24 and 34° C temperature environments in our study.

In this study, haemolysis was observed in the plasma of blood samples drawn in the first 5 minutes following injection of xylazine. Xylazine's lytic effect has been reported as have similar effects to the phenothiazine derivative propionylpromazine (DeMoor and Desmet, 1971). This lytic effect has been suggested to contributing to significant decline in packed cell volume (DeMoor and Desmet, 1971). Pooling of blood in the spleen as has been reported with other tranquilisers in goats (Monzally *et al.*, 1972) can also lead to decrease in red blood cell count, haematocrit and haemoglobin concentration following administration of xylazine hydrochloride. Engorgement of the spleen leads to reduced amount of the circulating blood components in the peripheral circulation (Schalm, 1965). The fall can also be attributed to haemodilution due to an influx of intestinal fluids due in part to the decreased heart rate and to the

low blood pressure (Drevemo and Karstad, 1974). The fall in total white cell count is probably be due to adrenocortical stimulation and the subsequent effect of glucocorticoids on circulating neutrophils and lymphocytes following xylazine injection. Increased adrenocortical activity causes depression of lymphocytic tissue and disappearance of lymphocytes from the peripheral blood (Schalm, 1965).

CHAPTER FOUR

EFFECTS OF XYLAZINE HYDROCHLORIDE ON ACID-BASE BALANCE AND ARTERIAL BLOOD GAS TENSIONS IN GOATS UNDER DIFFERENT ENVIRONMENTAL TEMPERATURE AND HUMIDITY CONDITIONS.

4.1 INTRODUCTION

Xylazine hydrochloride is widely used in various animal species because of its potent sedative, analgesic and myorelaxant properties (Clarke and Hall, 1969). Intravenous administration of xylazine hydrochloride at both sedative and non-sedative doses has been followed by severe hypoxaemia in various species (DeMoor and Desmet, 1971; Doherty *et al.*, 1986; Nolan *et al.*, 1986; Celly *et al.*, 1997). Apart from hypoxaemia, carbon dioxide retention and acid-base disturbances are also known to occur following its administration to animals (Clarke and Hall; 1969; DeMoor and Desmet, 1971; Kumar *et al.*, 1976; Kumar and Thurmon, 1979; Saleh, 1993; O'Hair *et al.*, 1986; Celly *et al.*, 1997).

Studies on the effects of xylazine hydrochloride on arterial blood gas tensions and

acid-base balance in goats under different environmental temperature and humidity conditions have not been reported. The purpose of this study was therefore to evaluate the effects of xylazine hydrochloride on arterial blood gas tensions and acid-base balance in goats under three different sets of environmental temperature and humidity conditions.

4.2 MATERIALS AND METHODS.

4.2.1 ANIMALS

Six adult, indigenous breed, castrated male goats, weighing between 21.0 and 34.0 kg (mean = 28.2 ± 1.0,SEM), were purchased locally and transported to the University of Pretoria's Faculty of Veterinary Science at Onderstepoort. They were housed indoors in individual crates at the Faculty's Veterinary Academic Research Unit to acclimatise for 14 days. On arrival, they were dewormed (Panacur[®]-Hoechst, Ag-Vet, South Africa) and routinely clinically examined to ascertain the status of their health. They were fed on a diet of lucerne, hay and water, which were provided *ad libitum*. Three weeks after arrival, the goats underwent surgery to subcutaneously relocate the carotid arteries for experimental monitoring and sampling purposes. The protocol for this study was approved by the Ethics and Research Committees of the Faculty of Veterinary Science, University of Pretoria.

4.2.2 SURGICAL RELOCATION OF THE CAROTID ARTERIES.

This was performed as described in Chapter Three (3.2.2).

4.2.3 EXPERIMENTAL PROCEDURE.

4.2.3.1 EXPERIMENTAL DESIGN

A single group, three phase repeated measures study design, was used to study the effect of environmental temperature and humidity on the acid-base balance and arterial gas tensions in six goats following intravenous injection of xylazine hydrochloride. The effects of the drug on acid-base balance and arterial gas tensions were repeatedly examined in the goats during a low, medium and high temperature and humidity exposure treatment phases. A resting period of at least seven days was allowed between treatment phases.

4.2.3.2 TREATMENT PHASES.

The experiments in this study were conducted in temperature and humidity controlled environments. Phase one of the study was conducted in the medium

temperature and humidity environment with room temperature set at 24 ± 1° C and a relative humidity of 55 ± 1%. Phase two of the study was conducted in the high temperature and humidity environment with room temperature set at 34 ± 1° C and a relative humidity of 65 ± 1%. Phase three of the study was conducted in the low temperature and humidity environment with the room temperature set at 14 ± 1° C and a relative humidity of 33 ± 1%. The temperature and humidity settings were done several hours before any experiment was started so as to have uniformity of conditions in the rooms. Between experiments, the goats were housed and fed in individual crates, in housing premises devoid of temperature and humidity control.

Before any experiment was carried out, the goats had food withdrawn for 24 hours and water for 12 hours. On the day of the experiment, each goat was weighed and taken into a preparation room adjacent to the temperature and humidity controlled room. The average weight of goats during the experiments in the 14° C environment was 28.6 ± 1.8 kg, 26.9 ± 1.5 kg in the 24° C environment and 29.0 ± 1.9 kg in the 34° C environment.

In the preparation room, the goats were restrained in lateral recumbency on a surgical table with a waterproof foam mattress. The jugular furrows on both sides of the neck were thoroughly clipped and surgically prepared. A 18G intravenous

catheter (Jelco®- Critikon) was per cutaneously introduced into the left jugular vein, flushed with heparinised saline, capped and secured on to the skin with No.2/0 nylon (Ethilon) sutures. Another 20G catheter (Medican® - Medical Specialities) was per cutaneously introduced into the subcutaneously lying right carotid artery, flushed with heparinised saline, capped and sutured to the skin with nylon sutures. The animals were then transferred into the temperature and humidity controlled rooms. An oesophageal thermometer probe was introduced through the right ventral nasal meatus so that its tip lay in the distal third of the oesophagus to monitor oesophageal temperature and this was connected to a multi- parameter physiological monitor (Propaq® 104EL- Protocol Systems Inc., Oregon, USA). After a stabilisation period of 10 to 15 minutes, baseline readings were taken and then xylazine hydrochloride (Rompun®- Bayer Animal Health (Pty), Isando, South Africa) at 0.1 mg/kg body weight was injected intravenously through the jugular catheter.

Arterial blood samples (1 ml) were drawn from the carotid artery into 2.5 ml syringes whose dead space contained 10 μl of heparin, any air expelled and the end of the needle stoppered. The syringes were placed in iced water and the samples analysed within 2 hours of collection using an autoanalyser (Radiometer ABL 300-Copenhagen, Denmark). The arterial blood samples taken at 'time zero' and 5, 15, 30, 45, and 60 minutes post-xylazine injection were analysed for pH, arterial partial

pressures of oxygen and carbon dioxide, oxygen saturation, bicarbonate ion concentration, actual bicarbonate excess, total carbon dioxide, standard base content and oxygen content. All the measurements were corrected for the oesophageal temperature of each animal at the time of sampling. Other variables evaluated over the entire monitoring period were, sedation, analgesia, salivation, muscle relaxation and recovery time. Sedation was assessed by observation of the drooping of the upper eyelids and protrusion of the tongue. Analgesia was assessed by the response of the goats to needle pricks to the flank, thorax, coronary band and manual pinching of the interdigital skin. Muscle relaxation was judged by the muscle tone of the jaw and uppermost fore and hind limbs during passive flexing and extending.

4.2.4 STATISTICAL ANALYSIS.

The data in these studies was analysed on a personal computer equipped with statistical software (Sigma Stat- Jandel Scientific Software®- Jandel Corporation, San Rafael, CA). Results are given as mean and standard error of the mean. To test for significance of the effect of treatment over time as well as for differences among treatments between groups, data collected over time were analysed using a two way repeated measures analysis of variance (ANOVA). When a significant effect of treament was observed, comparisons between treatments were performed using one

way analysis of variance (ANOVA) for repeated measures followed by Bonferroni's t-test to examine for least significant difference. Significant changes with time within any group were also analysed using one way analysis of variance (ANOVA) for repeated measures followed by Bonferroni's t-test to examine deviation from control (baseline, 'time zero') values. Where the data was either not normally distributed or the Equal variance test failed, the data was analysed using Friedman Repeated Measures Analysis of Variance (ANOVA) on Ranks followed by Dunnett's method to examine deviations from baseline or 'time zero' (control) values. $P < 0.05$ was considered significant.

4.3   RESULTS.

4.3.1 CLINICAL AND BEHAVIOURAL EFFECTS.

These were as reported and discussed in Chapter Three.

4.3.2 ARTERIAL BLOOD GAS TENSIONS AND ACID-BASE BALANCE.

The results of the arterial gas tension and acid-base values of the goats at three different environmental conditions are as shown in Table 4.1. Xylazine

hydrochloride caused a significant (P < 0.05) reduction in the mean pH values in the goats at 24, 34, and 14° C environments 5 minutes after its administration when compared to baseline ('time zero') values. In the 24° C environment, the pH was still significantly (P < 0.05) lower at 15 minutes post-xylazine injection and this started increasing to surpass the baseline value of 7.378 ± 0.010 and stand at 7.411 ± 0.009 at the end of the 60 minutes monitoring period. In the 34° C environment, pH was still significantly (P < 0.05) lower at 15 minutes as compared to baseline value and this started to increase as from 30 minutes and as at 60 minutes, the pH was 7.430 ± 0.012, significantly (P < 0.05) higher than baseline value of 7.395 ± 0.010. In the 14° C environment, after 5 minutes following injection of xylazine, the pH started to increase and this rose beyond the baseline value of 7.392 ± 0.014 to stand at 7.428 ± 0.013 at 60 minutes. However, these changes were not significant.

Table 4.1

Means (±SEM) of arterial blood gas and acid-base values in goats following intravenous injection of 0.1 mg/kg xylazine hydrochloride under three different environmental conditions, (n = 6).

Variable	Time (Minutes)	Environmental temperature (° C)		
		14	24	34
pH	0	7.392 ± 0.014	7.387 ± 0.010	7.395 ± 0.010
	5	7.336 ± 0.014*	7.331 ± 0.009*	7.351 ± 0.009*
	15	7.346 ± 0.014	7.331 ± 0.008*	7.358 ± 0.012*
	30	7.374 ± 0.012	7.376 ± 0.011	7.390 ± 0.012
	60	7.428 ± 0.013	7.411 ± 0.009	7.430 ± 0.012*
$PaCO_2$ (mmHg)	0	36.65 ± 1.61	36.72 ± 1.03	37.45 ± 1.10
	5	44.22 ± 1.95*	44.87 ± 0.94*	45.77 ± 1.11*
	15	44.32 ± 2.09*	47.08 ± 1.79*	45.37 ± 1.44*
	30	42.68 ± 1.86*	43.70 ± 2.47*	44.22 ± 0.94*
	60	38.98 ± 1.44	41.15 ± 1.10	39.25 ± 1.08
PaO_2 (mmHg)	0	80.53 ± 4.14	79.23 ± 3.84	75.35 ± 1.81
	5	30.17 ± 3.28*	29.77 ± 2.79*	29.78 ± 4.87*
	15	39.37 ± 3.48*	35.88 ± 3.72*	36.92 ± 4.77*
	30	43.60 ± 3.82*	43.22 ± 3.32*	42.47 ± 3.94*
	60	51.62 ± 3.54*	61.52 ± 6.31*	62.28 ± 7.23*
O_2 Saturation (%)	0	93.18 ± 1.02	92.93 ± 2.50	92.58 ± 0.82
	5	43.53 ± 6.31*	43.45 ± 5.57*	42.00 ± 8.81*
	15	62.28 ± 5.99*	56.10 ± 6.41*	56.57 ± 7.21*
	30	71.48 ± 5.19*	70.98 ± 4.04*	68.42 ± 5.03*
	60	84.15 ± 2.48	86.50 ± 3.25	85.48 ± 3.23
$[HCO_3$-] (mmol/l)	0	21.28 ± 0.74	20.68 ± 0.61	21.98 ± 0.29
	5	22.42 ± 0.77	22.50 ± 0.50*	24.08 ± 0.50*
	15	23.12 ± 0.69*	23.35 ± 0.70*	24.30 ± 0.63*
	30	24.00 ± 0.75*	24.50 ± 0.79*	25.67 ± 0.70*
	60	25.18 ± 0.96*	25.32 ± 1.05*	25.03 ± 0.78*
Total CO_2 (mmol/l)	0	22.30 ± 0.78	21.68 ± 0.63	23.00 ± 0.31
	5	23.62 ± 0.80*	23.47 ± 0.55	25.32 ± 0.52*
	15	24.35 ± 0.73*	24.63 ± 0.74*	25.55 ± 0.65*
	30	25.23 ± 0.79*	25.72 ± 0.85*	26.87 ± 0.70*
	60	26.30 ± 0.99*	26.47 ± 1.09*	26.10 ± 0.80*
ABE (mmol/l)	0	-2.15 ± 0.70	-2.85 ± 0.64	-1.42 ± 0.34
	5	-2.33 ± 0.72	-2.60 ± 0.45	-0.60 ± 0.55
	15	-1.63 ± 0.64	-1.82 ± 0.59	-0.33 ± 0.66
	30	-0.45 ± 0.68	0.07 ± 0.52*	1.47 ± 0.77*
	60	1.43 ± 0.89*	1.48 ± 1.01*	1.83 ± 0.82*

*- Significantly (P < 0.05) different from values at time 0 (baseline values).

pH-arterial hydrogen ion concentration; $PaCO_2$-arterial carbon dioxide tension; PaO_2-arterial oxygen tension; O_2-arterial oxygen saturation; $[HCO_3$]- arterial bicarbonate ion concentration; Total CO_2-total arterial carbon dioxide; ABE- arterial actual base excess.

There was a concomitant significant ($P < 0.05$) increase in mean arterial carbon dioxide tension ($PaCO_2$) in the goats starting at 5 minutes post-xylazine injection in the three environmental conditions and this remained significantly ($P < 0.05$) higher at 15 and 30 minutes post-xylazine injection as compared to baseline values. At the end of the 60 minutes monitoring period, these values still remained higher than baseline values.

Peak $PaCO_2$ attained were 47.08 ± 1.79 mmHg at 15 minutes for the 24° C environment, 45.77 ± 1.11 mmHg at 5 minutes in the 34° C environment and 44.32 ± 2.09 mmHg in the 14° C environment at 15 minutes. Baseline values for $PaCO_2$ were 36.72 ± 1.03, 37.45 ± 1.10, and 36.65 ± 1.61 mmHg in the 24, 34, and 14° C environments, respectively.

The bicarbonate ion concentration [HCO_3^-] was significantly ($P < 0.05$) increased in the goats starting at 5 minutes post-xylazine injection in the 24 and 34° C environments while this started after 15 minutes in the 14° C environment. At the end of the 60 minutes monitoring period, these values were still significantly higher than baseline values. Total arterial carbon dioxide content (TCO_2) increased in the same pattern, being significantly ($P < 0.05$) higher than baseline values, starting at

15 minutes post-xylazine injection in the 24° C environment and at 5 minutes in the 34 and 14° C environments. Total arterial carbon dioxide content remained significantly higher than baseline values at the end of the 60 minutes monitoring period. Actual base excess (ABE) following xylazine administration increased in all the three environments and these values were significantly ($P < 0.05$) higher at 30 and 60 minutes post-xylazine injection in the 24 and 34° C environments and at 60 minutes in the 14° C environment as compared to baseline values.

Xylazine had very dramatic effects on arterial blood oxygen tension (PaO_2). Under all three environmental conditions, xylazine caused significant ($P < 0.05$) decreases in PaO_2 starting at 5 minutes post-xylazine injection and these values remained lower than baseline values at 15, 30 and 60 minutes post-xylazine injection. Maximal reductions in PaO_2 were at 5 minutes post-xylazine injection in all the three environments. In the 24° C environment, PaO_2 values were reduced by 62% from 79.23 ± 3.84 to 29.77 ± 2.79 mmHg at 5 minutes. In the 34° C environment, these values were reduced from 75.35 ± 1.81 to 29.78 ± 4.87 mmHg over the same period, a reduction of 60%. In the 14° C environment, PaO_2 was reduced from 80.53 ± 4.14 mmHg at baseline to 30.17 ± 3.28 mmHg at 5 minutes, a reduction of

63%. Although PaO_2 started increasing after maximal reduction at 5 minutes post-xylazine injection, these values were still significantly (P < 0.05) lower at the end of the 60 minutes monitoring period. The effects of xylazine hydrochloride on oxygen saturation under the different environmental conditions were similar to that of PaO_2. Xylazine caused significant (P < 0.05) reductions in arterial oxygen saturation starting at 5 minutes after its administration in all the three environments and arterial oxygen saturation values remained significantly lower at 15 and 30 minutes post-xylazine injection. At the end of the 60 minutes monitoring period, the arterial oxygen saturation values were approaching baseline values. Although xylazine hydrochloride had significant effects on the pH, arterial oxygen and carbon dioxide tensions, oxygen saturation, arterial bicarbonate ion concentration, total arterial carbon dioxide content and actual base excess in the goats, the different environmental temperature and humidity conditions did not have significant effects on the values of these variables.

4.4 DISCUSSION.

4.4.1 ARTERIAL GAS TENSIONS AND ACID-BASE BALANCE.

Xylazine hydrochloride caused significant changes in pH, arterial oxygen tension

(PaO_2), arterial oxygen saturation, arterial carbon dioxide tension ($PaCO_2$), total

carbon dioxide (TCO_2), arterial bicarbonate ion concentration [HCO_3-] and actual

base excess (ABE) in all three different environmental conditions. Significant

reduction in arterial pH and oxygen tension and a rise in carbon dioxide tension in

this study correlates well with similar findings in goats (Kumar *et al.*, 1976; Kumar

and Thurmon, 1979) and cattle (DeMoor and Desmet, 1971; Aouad *et al.*, 1981;

Raptopoulos and Weaver, 1984). Even in venous blood, similar changes occurred

following administration of xylazine hydrochloride (Saleh, 1993). In this study,

these changes developed more rapidly, within 5 minutes of the administration of the

drug, than has been reported in the same species in other studies (DeMoor and

Desmet, 1971; Kumar *et al.*, 1976; Kumar and Thurmon, 1979; Aouad *et al.*, 1981),

in which these changes occurred 15 minutes after xylazine administration. This

difference may be explained by the fact that in our study, xylazine was injected

intravenously whereas in the other studies, it was injected intramuscularly, thus the

delay in development of these changes following intramuscular administration of

xylazine.

In this study, pH, $PaCO_2$, [HCO_3-], TCO_2, and actual base excess increased to

above baseline values at the end of the 60 minutes monitoring period. This indicated

that xylazine had a marked effect on the acid-base balance tending to shift the

balance towards acidosis in the early stages following its administration and then towards alkalosis in the later stages as seen at 60 minutes post-injection. Similar findings have been reported (Saleh, 1993) in goats. In sheep, following intramuscular injection of xylazine, a decrease in mean arterial oxygen tension to 58% of baseline values within 10 minutes occurred (O'Hair *et al.*, 1986). In our study, arterial oxygen tension decreased to between 37 and 40% of baseline values within 5 minutes of the administration of xylazine and these values had not returned to their baseline levels 60 minutes later. The same trend was seen with oxygen saturation. Since xylazine also caused significant reduction in blood haemoglobin concentration, this could also potentiate the effects of low haemoglobin oxygen saturation (Raekallio *et al.*, 1998). The difference in magnitude between these two studies could be attributed to the route of administration of xylazine. The greater changes seen in this study could be due to the intravenous route used to administer the xylazine, despite the smaller doses given. Our findings compare with the findings of Aziz and Carlyle (1978) and Celly *et al.* (1997) in sheep in which they reported of significant decrease in PaO_2 levels, with maximum decrease seen by 15 and 10 minutes respectively, following intravenous administration of xylazine. The PaO_2 levels remained significantly lower at 60 minutes post-drug administration. In their study, Celly *et al.* (1997) reported that the lowest PaO_2 level attained in sheep was 32.6 mmHg, which compares well with the 29.8-30.2 mmHg

observed in goats in the present study. In our study, differences in environmental conditions did not have significant effects on the variables evaluated as above.

Severe hypoxaemia following administration of xylazine at both sedative and non-sedative doses has been reported in various animal species (DeMoor and Desmet, 1971; Doherty *et al.*, 1986; Nolan *et al.*, 1986; Celly *et al.*, 1997). The hypoxaemia and carbon dioxide retention seen in this and other studies following xylazine administration may be partly due to hypoventilation during sedation due to depression of the respiratory centre and due to recumbency. The lateral recumbency in which these goats were placed could also have caused mismatching of ventilation and perfusion of lung tissue. These factors could have led to the low oxygen tension and high carbon dioxide retention (Kumar *et al.*, 1976; Kumar and Thurmon, 1979). It has been shown that dorsal and lateral recumbency contribute to acid-base disturbances in cattle injected with xylazine hydrochloride (DeMoor and Desmet, 1971) and this could have been the case in our study, even though there are differences in size between cattle and goats. In other studies, Raptopoulos and Weaver (1984) attributed the increased carbon dioxide retention in steers injected with xylazine to be partly due to upper respiratory obstruction which was usually more evident on inspiration. From recent studies carried out in sheep with α_2-adrenoreceptor agonists, Celly *et al.* (1997) suggested that increased transpulmonary pressure observed following administration of these drugs could

contribute to a decrease in PaO_2. Incidences of xylazine causing bradypnoea alternating with tachypnoea, forced breathing and periodic apnoea as seen in this study are not uncommon (Bafi-Yeboa and Huvos, 1980; Kokkonen and Eriksson, 1987; Saleh, 1993; Celly *et al.*, 1997). Apart from causing a reduction in respiratory rate, concurrent decrease in tidal volume in goats has been shown to occur (Mohammed and Yelwa, 1993) and all these factors could have contributed to the changes in arterial blood gases and acid-base balance in the goats in this study. Effects of xylazine hydrochloride on pulmonary function have been attributed to its α_2-adrenoreceptor mediated activity which results in respiratory depression (Prajapathi *et al.*, 1994; Aithal *et al.*, 1996).

A fall in arterial oxygen tension and/or packed cell volume (PCV) is clinically important in animals with acute or chronic anaemia or with disorders having an unfavourable effect on tissue oxygenation including reduced blood supply to the heart and in respiratory disease, in which case these changes may pose a danger and lead to fatal consequences. Such animals would require adequate ventilation in order to avoid hypoxia (De Moor and Desmet, 1971; Kumar *et al.*, 1976). During anaesthesia, care should be taken to avoid major deviations of arterial pH from normal and the adequacy of arterial oxygen supply should be ensured (DeMoor and Desmet, 1971).

CHAPTER FIVE

THE HYPERGLYCAEMIC AND HYPOINSULINAEMIC EFFECTS OF XYLAZINE HYDROCHLORIDE IN GOATS UNDER DIFFERENT ENVIRONMENTAL TEMPERATURE AND HUMIDITY CONDITIONS.

5.1 INTRODUCTION.

Xylazine hydrochloride has been widely used as a sedative in biological research and veterinary medicine (Goldfine and Arieff, 1979; Hsu and Hummel, 1981). It has been used in both domestic and wild animal species either alone or in combination with other tranquillizers and anaesthetic agents with relative safety (Knight, 1980).

Among the physiological and pharmacological changes it induces in animals, hyperglycaemia and hypoinsulinaemia have been demonstrated in several species including cattle (Symonds, 1976; Symonds and Mallinson, 1978; Eichner *et al.*, 1979; Hsu and Hummel, 1981; Fayed *et al.*, 1989), cats (Feldberg and Symonds, 1980), dogs (Goldfine and Arieff, 1979; Benson *et al.*, 1984), sheep (Brockman,

1981; Muggaberg and Brockman, 1982) and horses (Thurmon *et al.*, 1982; Greene *et al.*, 1987). Xylazine's hyperglycaemic effect has been reported in goats (Kumar and Thurmon, 1979; Dilipkumar *et al.*, 1997). The mechanism by which xylazine induces its hyperglycaemic and hypoinsulinaemic effects in cattle appeares to be α-adrenergically mediated (Hsu and Hummel, 1981).

Previous studies conducted in heifers (Fayed *et al.*, 1989) showed that the hyperglycaemic effects of xylazine were more pronounced in heifers kept in thermoneutral environmental conditions whereas its hypoinsulinaemic effects were more pronounced in heifers exposed to heat stress conditions. In the available literature, no studies appear to have been reported that quantify the hyperglycaemia and hypoinsulinaemia induced by xylazine in goats under different environmental conditions. The aim of this study was to evaluate the hyperglycaemia and hypoinsulinaemia induced by xylazine hydrochloride in goats under different sets of environmental temperature and humidity conditions.

5.2   MATERIALS AND METHODS.

5.2.1 ANIMALS

Six adult, indigenous breed, castrated male goats, weighing between 21.0 and 34.0

kg (mean = 28.2 ± 1.0,SEM), were purchased locally and transported to the University of Pretoria's Faculty of Veterinary Science at Onderstepoort. They were housed indoors in individual crates at the Faculty's Veterinary Academic Research Unit to acclimatise for 14 days. On arrival, they were dewormed (Panacur[®]-Hoechst, Ag-Vet, South Africa) and routinely clinically examined to ascertain the status of their health. They were fed on a diet of lucerne, hay and water, which were provided *ad libitum*. The protocol for this study was approved by the Ethics and Research Committees of the Faculty of Veterinary Science, University of Pretoria.

5.2.2 EXPERIMENTAL PROCEDURE.

5.2.2.1 EXPERIMENTAL DESIGN

A single group, three phase repeated measures study design was used to study the effect of environmental temperature and humidity on the hyperglycaemic and hypoinsulinaemic effects of xylazine in six goats.The effects of the drug on plasma glucose and insulin were repeatedly examined in the goats during a low, medium and high temperature and humidity exposure treatment phases. A washout period of at least seven days was allowed between treatment phases.

5.2.2.2   TREATMENT PHASES.

The experiments in this study were conducted in temperature and humidity controlled environments. Phase one of the study was conducted in the medium temperature and humidity environment with room temperature set at $24 \pm 1°$ C and a relative humidity of $55 \pm 1\%$. Phase two of the study was conducted in the high temperature and humidity environment with room temperature set at $34 \pm 1°$ C and a relative humidity of $65 \pm 1\%$. Phase three of the study was conducted in the low temperature and humidity environment with the room temperature set at $14 \pm 1°$ C and a relative humidity of $33 \pm 1\%$. The temperature and humidity settings were done several hours before any experiment was started so as to have uniformity of conditions in the rooms. Between experiments, the goats were housed and fed in individual crates, in housing premises devoid of temperature and humidity control.

Before any experiment was carried out, the goats had food withdrawn for 24 hours and water for 12 hours. On the day of the experiment, each goat was weighed and taken into a preparation room adjacent to the temperature and humidity controlled room. The average weight of goats during the experiments in the $14°$ C environment was 28.6 ± 1.8 kg, 26.9 ± 1.5 kg in the $24°$ C environment and 29.0 ± 1.9 kg in the $34°$ C environment.

In the preparation room, the goats were restrained in lateral recumbency on a surgical table with a waterproof foam mattress. The jugular furrow on the left side of the neck was thoroughly clipped and surgically prepared. A 18G intravenous catheter (Jelco®- Critikon) was per cutaneously introduced into the left jugular vein, flushed with heparinised saline, capped and secured on to the skin with nylon No.2/0 (Ethilon) sutures. The animals were then transferred into the temperature and humidity controlled rooms. After a stabilisation period of 10 to 15 minutes, a baseline blood sample was taken and then xylazine hydrochloride (Rompun®- Bayer Animal Health (Pty), Isando, South Africa) at 0.1 mg/kg body weight was injected intravenously through the jugular catheter.

Venous blood samples for plasma glucose and insulin determination were drawn at 'time zero' and 15, 30, and 60 minutes post-xylazine injection. For plasma glucose determination, samples were drawn into tubes containing sodium fluoride and potassium oxalate as preservatives and determined using a RA- 1000 Analyser (Technicon Instruments Corporation, New York, USA) based on the hexokinase procedure for glucose determination as described by Leon et al. (1977). For plasma insulin concentration determination, venous blood samples were drawn into heparinised vacutainer tubes. These samples were centrifuged, the plasma harvested and stored at -20° C until the analysis was done later using a $[^{125}I]$

radioimmunoassay procedure using a commercial kit (Coat-A-Count®- Diagnostic products Corporation, Los Angeles, CA, USA). Other variables evaluated over the entire monitoring period were, sedation, analgesia, salivation, muscle relaxation and recovery time. Sedation was assessed by observing for drooping of the upper eyelids and protrusion of the tongue. Analgesia was assessed by the response of the goats to needle pricks to the flank, thorax, coronary band and manual pinching of the interdigital skin. Muscle relaxation was judged by the muscle tone of the jaw and uppermost fore and hind limbs during passive flexing and extending.

5.2.2.3   RADIO IMMUNOASSAY PROCEDURE FOR THE DETERMINATION OF PLASMA INSULIN CONCENTRATION.

Plasma insulin concentrations were determined by a $[^{125}I]$ radio immunoassay procedure using a commercial kit (Coat-A-Count® - Diagnostic Products Corporation, Los Angeles, CA, USA). 200 µl of goat plasma was pipetted into antibody-coated polypropylene tubes (12x75 mm) followed by 1.0 ml of $[^{125}I]$-insulin tracer into every tube. The tubes were vortexed and then incubated in a water bath set at 37° C for 3 hours. Liquid was decanted from the tubes and, to determine the amount of radioactivity present, each tube was counted for one minute on an automatic gamma counter (Wizard 3"®-Model 1480-Wallac Oy,

Finland). Standards (0, 5, 15, 50, 100, 200, and 400 µU/ml) and controls were included with each set of unknowns. The concentrations of unknown samples were interpolated from the logit-log representation of the calculated percent bound versus the standards.

The percent bound was calculated for each standard using the equation:

$$Percent\ bound = \frac{Average\ counts}{Net\ maximum\ binding\ counts} \times 100$$

5.2.3 STATISTICAL ANALYSIS.

The data in these studies was analysed on a personal computer equipped with statistical software (Sigma Stat - Jandel Scientific Software®- Jandel Corporation, San Rafael, CA). Results are given as mean and standard error of the mean. To test for significance of the effect of treatment over time as well as for differences among treatments between groups, data collected over time were analysed using a two way repeated measures analysis of variance (ANOVA). When a significant effect of treament was observed, comparisons between treatments were performed using one way analysis of variance (ANOVA) for repeated measures followed by Bonferroni's t-test to examine for least significant difference. Significant changes with time within any group were also analysed using one way analysis of variance (ANOVA) for repeated measures followed by Bonferroni's t-test to examine

deviation from control (baseline, 'time zero') values. Where the data was either not normally distributed or the Equal variance test failed, the data was analysed using Friedman Repeated Measures Analysis of Variance (ANOVA) on Ranks followed by Dunnett's method to examine deviations from baseline or 'time zero' (control) values. $P < 0.05$ was considered significant.

5.3   RESULTS.

5.3.1 CLINICAL AND BEHAVIOURAL EFFECTS

These were as reported and discussed in Chapter Three.

5.3.2 EFFECTS OF XYLAZINE ON PLASMA GLUCOSE AND INSULIN

Mean plasma glucose concentrations increased from baseline values ('time zero' values) in all the three environmental conditions (Figure 5.1). This hyperglycaemia was evident at 15 minutes post-xylazine injection, reaching a maximum at 30 minutes post-xylazine injection in all three environmental conditions. Mean plasma glucose concentrations were significantly ($P < 0.05$) higher than baseline values from 15 minutes post-xylazine injection and remained so until the end of the 60

minutes monitoring period. The magnitude of hyperglycaemia was greatest in the

24° C environment, followed by the 34° C environment and lowest in the 14° C
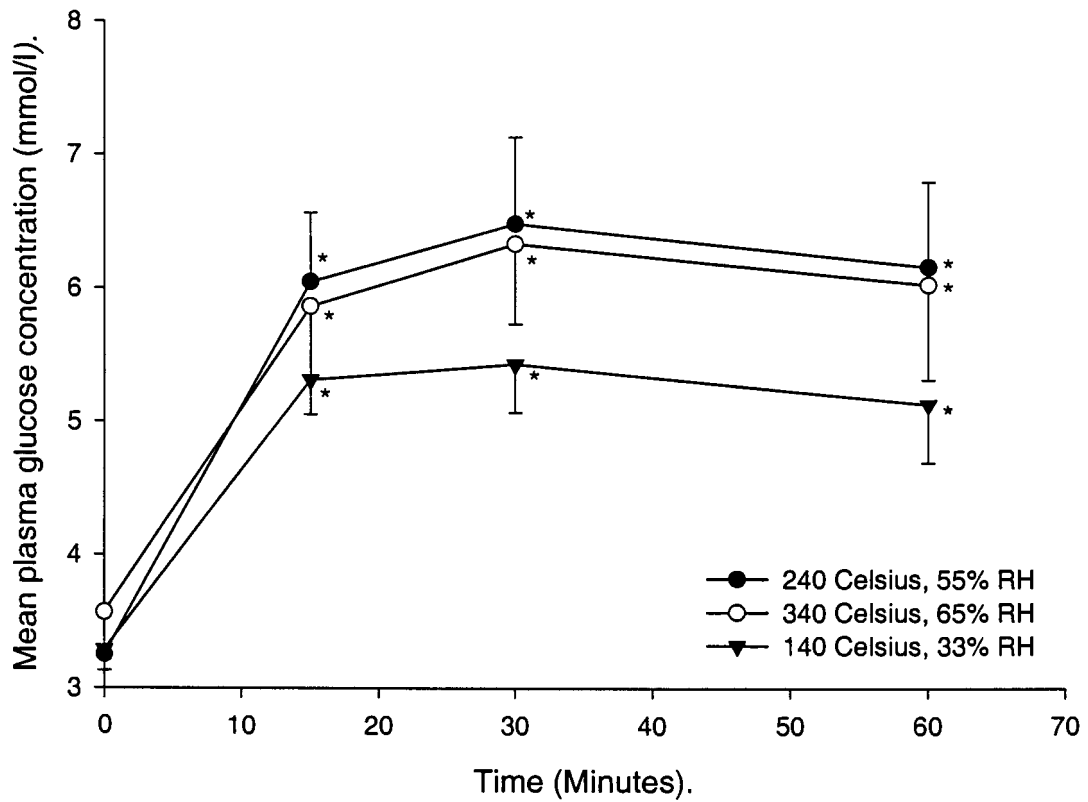
environment.

Figure 5.1: Temporal changes in mean and standard error of mean of plasma glucose concentration of goats following injection of 0.1 mg/kg xylazine under three different environmental conditions.

* Significantly different (p <0.05) from values at time 0.

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At 15 minutes post-xylazine injection, plasma glucose concentrations were 186 % (6.05 ± 0.52 mmol/l) of the baseline value (3.25 ± 0.06 mmol/l) in the 24° C, 164% (5.87 ± 0.53 mmol/l) of the baseline value (3.57 ± 0.35 mmol/l) in the 34° C environment and 162% (5.32 ± 0.26 mmol/l) of the baseline value (3.28 ± 0.15 mmol/l) in the 14° C environment. These concentrations increased to 199%, 178%, and 165% of baseline values in the 24, 34, and 14° C environments, respectively, at 30 minutes post-xylazine injection, when the xylazine-induced hyperglycaemia reached the peak. Although plasma glucose concentrations started to decline after 30 minutes, at 60 minutes post-xylazine injection, these concentrations were still higher than baseline values, the values being 188%, 169%, and 156% of baseline values in the 24, 34, and 14° C environments, respectively. Differences in the environmental conditions did not have significant effects on plasma glucose concentrations.

The trend in changes in plasma insulin concentrations was directly opposite to that of plasma glucose concentrations (Figure 5.2).
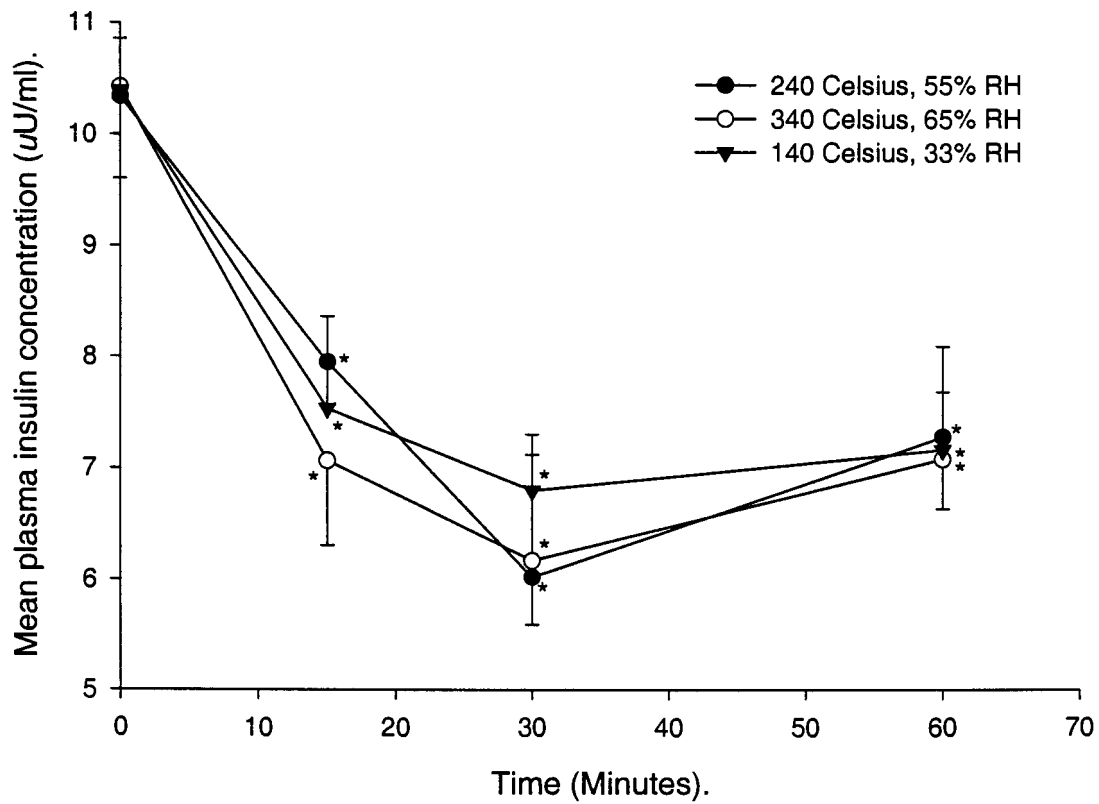
Figure 5.2: Temporal changes in mean and standard error of mean of plasma insulin concentration of goats following intravenous injection of 0.1 mg/kg xylazine under three different environmental conditions.

* Significantly different)p<0.05) from values at time 0.

Mean plasma insulin concentrations showed a decline at 15 minutes with maximum decline at 30 minutes post-xylazine injection in all the three environmental conditions. Plasma insulin concentrations were significantly ($P < 0.05$) lower than baseline values from 15 minutes post-xylazine injection to the end of the 60 minutes monitoring period. At 15 minutes post-xyalzine injection, mean plasma insulin concentrations decreased from 10.33 ± 0.68 µU/ml to 7.95 ± 0.41 µU/ml in 24° C environment; 10.42 ± 0.82 µU/ml to 7.07 ± 0.76 µU/ml in the 34° C environment and and 10.38 ± 0.47 µU/ml to 7.53 ± 0.41 µU/ml in the 14° C environment. The mean plasma insulin concentrations were 77%, 68%, and 73% lower than the baseline values at 24, 34, and 14° C environmental conditions, respectively. The mean plasma insulin concentrations continued to decrease in all three environmental conditions reaching the lowest level at 30 minutes post-xylazine injection. At this highest hypoinsulinaemic effect, plasma insulin concentrations were 58%, 59% and 65% of the baseline values at 24, 34, and 14° C environmental conditions, respectively. At this maximal hypoinsulinaemic effect of xylazine, the magnitude of hypoinsulinaemia was greatest in the 24° C environment followed by the 34 and 14° C environments, respectively. Although mean plasma insulin concentrations started to increase from 30 minutes onwards in all three environmental conditions, as at 60 minutes post-xylazine injection, these values were still lower than baseline values, being 70%, 68%, and 69% lower than

baseline values at 24, 34, and 14° C environments, respectively. Differences in environmental conditions did not have significant effects on the mean plasma insulin concentrations.

5.4   DISCUSSION.

Xylazine hydrochloride injected intravenously in goats exposed to the three environmental conditions induced significant hyperglycaemia associated with hypoinsulinaemia which persisted to the end of the study period. The findings in the present study agree with the observations in other studies carried out in cattle (Symonds, 1976; Symonds and Mallinson, 1978; Eichner *et al.*, 1979; Hsu and Hummel, 1981; Fayed *et al.*, 1989), cats (Feldberg and Symonds, 1980), dogs (Goldfine and Arieff, 1979; Benson *et al.*, 1984), sheep (Brockman, 1981; Muggaberg and Brockman, 1982) and horses (Thurmon *et al.*, 1982; Greene *et al.*, 1987). In our study, the trend in changes in plasma insulin concentrations was directly opposite to that of plasma glucose concentrations under all three different environmental conditions. This trend has been reported in other species (Eichner *et al.*, 1979; Goldfine and Arieff, 1979; Brockman, 1981; Thurmon *et al.*, 1982).

Rapid onset of hypoinsulinaemia and hyperglycaemia occurring within 15 minutes following intravenous administration of xylazine hydrochloride as seen in this study

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is similar to other studies carried out in cattle (Eichner *et al.*, 1979) and horses (Thurmon *et al.*, 1982). Maximum hyperglycaemic and hypoinsulinaemic effects of xylazine hydrochloride produced 30 minutes following its injection in goats in this study also correlated well with other findings in cattle (Symonds and Mallinson, 1978), horses (Thurmon *et al.*, 1982) and sheep (Brockman, 1981). Following maximal change of plasma glucose and insulin concentrations at 30 minutes post-xylazine injection, plasma glucose concentrations started to decrease while the plasma insulin concentrations started to increase but, these values had not returned to baseline levels at the end of the 60 minutes monitoring period. Although we did not monitor plasma glucose and insulin concentrations beyond 1 hour, xylazine-induced hyperglycaemia and hypoinsulinaemia has been known to persist for 3 to 6 hours post-xylazine injection (Brockman, 1981; Hsu and Hummel, 1981; Benson *et al.*, 1984; Fayed *et al.*, 1989) and even for 24 hours (Eichner *et al.*, 1979).

It has been shown that xylazine-induced hyperglycaemia and hypoinsulinaemia is dose-dependent (Hsu & Hummel., 1981) and that the time interval from xylazine injection to maximal plasma glucose concentration differs between species. Furthermore, xylazine pharmacokinetics correspond closely with clinical responses in horses and dogs but not in cattle (Garcia-Villar *et al.*, 1981), suggesting that there are differences in mechanisms between species. The magnitude of hyperglycaemia

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and hypoinsulinaemia due to xylazine hydrochloride will equally differ depending on whether the drug was injected via the intramuscular or intravenous routes as the time course of events is delayed following intramuscular as compared to the intravenous route (Benson *et al.*, 1984).

In our study, the hyperglycaemic and hypoinsulinaemic effects of xylazine were not significantly different in the three different environmental conditions. This is contrary to findings in heifers (Fayed *et al.*, 1989) in which the hypoinsulinaemic effect of xylazine was more pronounced in heifers exposed to heat stress conditions (33° C ; 63% humidity) whereas the magnitude of hyperglycaemia was more pronounced in heifers kept in a thermoneutral environment (18° C ; 42% humidity). This difference might have arisen from the fact that, in our study, the goats were acutely exposed to the three different environmental conditions whereas Fayed *et al.* (1989) had the heifers conditioned to the respective environmental conditions for 35 days prior to the studies. Possible hormonal or neural changes develop in heat stressed animals which lead to decreased metabolic rate resulting from decreased thyroid hormones, which might alter xylazine kinetic properties (Magdub *et al.*, 1982) and this might account for the differences.

The mechanism by which xylazine causes hypoinsulinaemia and hyperglycaemia

is not well understood but, it may be associated with increased gluconeogenesis, decrease in insulin synthesis, or inhibition of insulin release from the pancreas (Eichner *et al.*, 1979). Since the trend in glucose concentration has been found to be directly opposite in direction to that of insulin, this indicates that the hyperglycaemia induced by xylazine would appear to be caused by decreased insulin secretion as reported in other species (Symonds, 1976; Symonds and Mallinson, 1978; Thurmon *et al.*, 1978; Eichner *et al.*, 1979; Feldberg and Symonds, 1980). There is a strong possibility that xylazine-induced hyperglycaemia and hypoinsulinaemia involves stimulation of α_2- adrenergic receptors, possibly in β-cells of the pancreatic islets which inhibit the release of insulin (Hsu and Hummel, 1981). Alpha$_2$-adrenergic receptors are present in the membrane of pancreatic beta-cells (Exton, 1982) and apparently inhibit the release of insulin as xylazine is an α_2-adrenergic agonist. Xylazine may also directly stimulate hepatic glucose production through its weak α_1-agonist effect (Hsu and Hummel, 1981).

Although the importance of xylazine-induced hyperglycaemia remains speculative, some conditions associated with hyperglycamia (e.g. hyperosmolality exceeding 33.6 mmol/l) can cause central nervous system changes, including blindness. It is not uncommon to encounter blood glucose concentrations as high as 50.4 mmol/l in horses following intestinal resection (Thurmon *et al.*, 1982) and since xylazine

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is often used as a sedative in horses with acute abdominal pain, its hyperglycaemic effect may worsen an existing condition. It has also been suggested (Goldfine and Arieff, 1979) that xylazine should be avoided as a tranquiliser in experiments where either insulin secretion or plasma glucose are critical variables. Furthermore, the effects of xylazine must be taken into consideration when carrying out investigations involving carbohydrate metabolism or when working with tissues sensitive to insulin and perhaps other hormones like growth hormone, *in vitro* or *in vivo* (Eichner *et al.*, 1979; Thurmon *et al.*, 1982). Xylazine hydrochloride has positive attributes in this area as it can be useful in examining the role of α-adrenergic receptors in the regulation of insulin release and simulation of diabetes mellitus (Goldfine and Arieff, 1979; Hsu and Hummel, 1981).

CHAPTER SIX

THE EFFECT OF DIFFERENT AMBIENT TEMPERATURE AND HUMIDITY CONDITIONS ON THERMAL RESPONSES TO XYLAZINE IN GOATS.

6.1   INTRODUCTION.

Xylazine is a non-narcotic analgesic with sedative, muscle relaxant and complex autonomic activity (Booth, 1988). In view of these properties, it is used widely in both domestic and wild animals (Raptopoulos and Weaver, 1984).

The effect of xylazine hydrochloride on body temperature in various animal species varies. In goats, xylazine has been shown to decrease (Mohammed and Yelwa, 1993; Saleh, 1993; Prajapathi *et al.*, 1994), increase (Dehghani *et al.*, 1991; Aithal *et al.*, 1996) or, have no effect on body temperature (Kumar and Thurmon, 1979). Changes in body temperature caused by xylazine are known to take up to 24 hours before they return to baseline values (Prajapathi *et al.*, 1994).

Previous studies in cats show that animals given xylazine were unable to

thermoregulate, becoming hypothermic in cold environments and hyperthermic in hot environments (Ponder and Clark, 1980). Other studies reported that rats injected with xylazine under environmental temperatures of 20 to 24° C suffered from hypothermia (Tsoucaris-Kupfer and Schmitt, 1972; Livingston *et al.*, 1984), whereas cattle given the same drug in an environmental temperature of 33° C suffered from hyperthermia (Fayed *et al.*, 1989).

There is no report in the available literature on the effects of xylazine hydrochloride on body temperature in goats under different environmental conditions. The purpose of this study was therefore to evaluate the effects of xylazine hydrochloride on body temperature in goats under three different sets of environmental temperature and humidity conditions.

6.2 MATERIALS AND METHODS.

6.2.1 ANIMALS

Six adult, indigenous breed, castrated male goats, weighing between 21.0 and 34.0 kg (mean = 28.2 ± 1.0,SEM), were purchased locally and transported to the University of Pretoria's Faculty of Veterinary Science at Onderstepoort. They were

housed indoors in individual crates at the Faculty's Veterinary Academic Research Unit to acclimatise for 14 days. On arrival, they were dewormed (Panacur®-Hoechst, Ag-Vet, South Africa) and routinely clinically examined to ascertain the status of their health. They were fed on a diet of lucerne, hay and water, which were provided *ad libitum*. The protocol for this study was approved by the Ethics and Research Committees of the Faculty of Veterinary Science, University of Pretoria.

6.2.2 EXPERIMENTAL PROCEDURE.

6.2.2.1　EXPERIMENTAL DESIGN

A single group, three phase repeated measures study design was used to study the effect of environmental temperature and humidity on the thermal responses to xylazine in six goats. The effects of the drug on both rectal and oesophageal temperatures were repeatedly examined in the goats during a low, medium and high temperature and humidity exposure treatment phases. The effect of the three environmental conditions on oesophageal and rectal temperatures of the untreated goats was also examined. A washout period of at least seven days was allowed between treatment phases.

6.2.2.2   TREATMENT PHASES.

The experiments in this study were conducted in temperature and humidity controlled environments. Phase one of the study was conducted in the medium temperature and humidity environment with room temperature set at 24 ± 1° C and a relative humidity of 55 ± 1%. Phase two of the study was conducted in the high temperature and humidity environment with room temperature set at 34 ± 1° C and a relative humidity of 65 ± 1%. Phase three of the study was conducted in the low temperature and humidity environment with the room temperature set at 14 ± 1° C and a relative humidity of 33 ± 1%. The temperature and humidity settings were done several hours before any experiment was started so as to have uniformity of conditions in the rooms. Between experiments, the goats were housed and fed in individual crates, in housing premises devoid of temperature and humidity control.

Before any experiment was carried out, the goats had food withdrawn for 24 hours and water for 12 hours. On the day of the experiment, each goat was weighed and taken into a preparation room adjacent to the temperature and humidity controlled room. The average weight of goats during the experiments in the 14° C environment was 28.6 ± 1.8 kg, 26.9 ± 1.5 kg in the 24° C environment and 29.0 ± 1.9 kg in the 34° C environment.

In the preparation room, the goats were restrained in lateral recumbency on a surgical table with a waterproof foam mattress. The jugular furrow on the left side of the neck was thoroughly clipped and surgically prepared. A 18G intravenous catheter (Jelco®- Critikon) was percutaneously introduced into the left jugular vein, flushed with heparinised saline, capped and sutured to the skin with nylon No.2/0 (Ethilon) sutures. The animals were then transferred into the temperature and humidity controlled rooms. An oesophageal thermometer probe was introduced through the right ventral nasal meatus so that its tip lay in the distal third of the oesophagus to monitor oesophageal temperature and this was connected to a multi-parameter physiological monitor (Propaq®104EL- Protocol Systems Inc., Oregon, USA). After a stabilisation period of 10 to 15 minutes, baseline readings of oesophageal and rectal temperature were taken and then xylazine hydrochloride (Rompun®- Bayer Animal Health (Pty), Isando, South Africa) at 0.1 mg/kg body weight was injected intravenously through the jugular catheter. Rectal temperature was determined by using a clinical thermometer inserted at least 9 cm into the rectum and read 1 minute after its insertion. Other variables evaluated over the entire monitoring period were, sedation, analgesia, salivation, muscle relaxation and recovery time. Sedation was assessed by observing for drooping of the upper eyelids and protrusion of the tongue. Analgesia was assessed by the response of the goats to needle pricks to the flank, thorax, coronary band and manual pinching of

the interdigital skin. Muscle relaxation was judged by the muscle tone of the jaw and uppermost fore and hind limbs during passive flexing and extending.

6.2.3 STATISTICAL ANALYSIS.

The data in these studies was analysed on a personal computer equipped with statistical software (Sigma Stat- Jandel Scientific Software®- Jandel Corporation, San Rafael, CA). Results are given as mean and standard error of the mean. To test for significance of the effect of treatment over time as well as for differences among treatments between groups, data collected over time were analysed using a two way repeated measures analysis of variance (ANOVA). When a significant effect of treament was observed, comparisons between treatments were performed using one way analysis of variance (ANOVA) for repeated measures followed by Bonferroni's t-test to examine for least significant difference. Significant changes with time within any group were also analysed using one way analysis of variance (ANOVA) for repeated measures followed by Bonferroni's t-test to examine deviation from control (baseline, 'time zero') values. Where the data was either not normally distributed or the Equal variance test failed, the data was analysed using Friedman Repeated Measures Analysis of Variance (ANOVA) on Ranks followed by Dunnett's method to examine deviations from baseline or 'time zero' (control)

values. $P < 0.05$ was considered significant.

6.3 RESULTS.

6.3.1 CLINICAL AND BEHAVIOURAL EFFECTS

Clinical and behavioural effects of xylazine in the goats were as reported previously in Chapter Three.

6.3.2 EFFECTS OF XYLAZINE ON BODY TEMPERATURE

Xylazine hydrochloride caused an initial rise in oesophageal temperature in the goats in the 24° C environment 5 minutes following its injection whereas the rectal temperature declined over the same period (Figures 6.1 and 6.2). However, these changes were not statistically significant. After the initial 5 minutes following its injection, both rectal and oesophageal temperatures in the 24° C environment continued to decrease and these remained significantly (P < 0.05) lower than the mean temperature values at 'time zero' at 30, 45, and 60 minutes for oesophageal temperature and at 15, 30, 45, and 60 minutes for the rectal temperature. Maximal decrease in mean oesophageal temperature occurred at 30 minutes post-xylazine injection when mean temperature was 38.75 ± 0.13° C as compared to the baseline

('time zero') value of $39.15 \pm 0.22°$ C, a decrease of $0.4°$ C. The maximal decrease in mean rectal temperature for the $24°$ C environment occurred at 60 minutes post-xylazine injection when mean rectal temperature was $38.62 \pm 0.23°$ C compared to baseline value of $39.42 \pm 0.11°$ C, a decrease of $0.8°$ C. Both rectal and oesophageal temperatures were significantly ($P < 0.05$) lower than the mean baseline values at the end of the 60 minutes monitoring period. After 24 hours, rectal temperature although approaching baseline values still remained lower than the baseline values.

In the $34°$ C environment, xylazine caused an initial increase in both oesophageal and rectal temperatures in the goats in the first 5 minutes. However, the increase was not statistically significant. This increase was then followed by decline in both oesophageal and rectal temperatures reaching the lowest at 15 minutes and then a steady increase followed to record maximal readings at the end of the 60 minutes monitoring period. At 60 minutes post-xylazine injection, oesophageal ($39.75 \pm 0.14°$ C) and rectal ($39.65 \pm 0.16°$ C) temperatures were significantly ($P < 0.05$) higher than baseline values of $39.28 \pm 0.11°$ C and $39.33 \pm 0.13°$ C, respectively. At 60 minutes post-xylazine injection, mean oesophageal and rectal temperatures were 0.47 and $0.32°$ C above baseline values, respectively. After 24 hours, mean rectal temperature had declined even below the baseline values.

In the 14° C environment, xylazine hydrochloride caused a decline in both oesophageal and rectal temperatures of the goats starting at 5 minutes post-administration and this steady decline continued for the rest of the 60 minutes monitoring period. Both mean oesophageal and rectal temperatures were significantly ($P < 0.05$) lower than baseline values at 15, 30, 45, and 60 minutes post-xylazine injection. Maximal decline in mean oesophageal and rectal temperatures were recorded at 60 minutes post-xylazine administration when the oesophageal temperature of $37.97 \pm 0.24°$ C was $1.5°$ C below the mean baseline value of $39.47°$ C. The rectal temperature at 60 minutes post-xylazine administration was $38.05 \pm 0.19°$ C, $1.57°$ C below the baseline value of $39.62 \pm 0.11°$ C. Rectal temperature was still below baseline values after 24 hours.

One goat in this group exposed to the 14° C environment and which weighed 21.5 kg, the lowest in the group, shivered for more than 2 hours from the time it stood up. Over the 60 minutes monitoring period, its oesophageal temperature had declined by $2.2°$ C while the rectal temperature declined by $2.3°$ C.

Following the injection of xylazine hydrochloride, goats in the 14° C environment had significantly ($P < 0.05$) lower oesophageal and rectal temperatures as compared

to when they were exposed to 34° C temperature environment. Equally, the goats in the 24° C environment attained significantly (P < 0.05) lower oesophageal temperatures as compared to when they were exposed to the 34° C environment. When the goats were exposed to the three different environmental temperature and humidity conditions without sedation with xylazine hydrochloride, environmental conditions did not have a significant (P>0.05) effect on either oesophageal or rectal temperature.
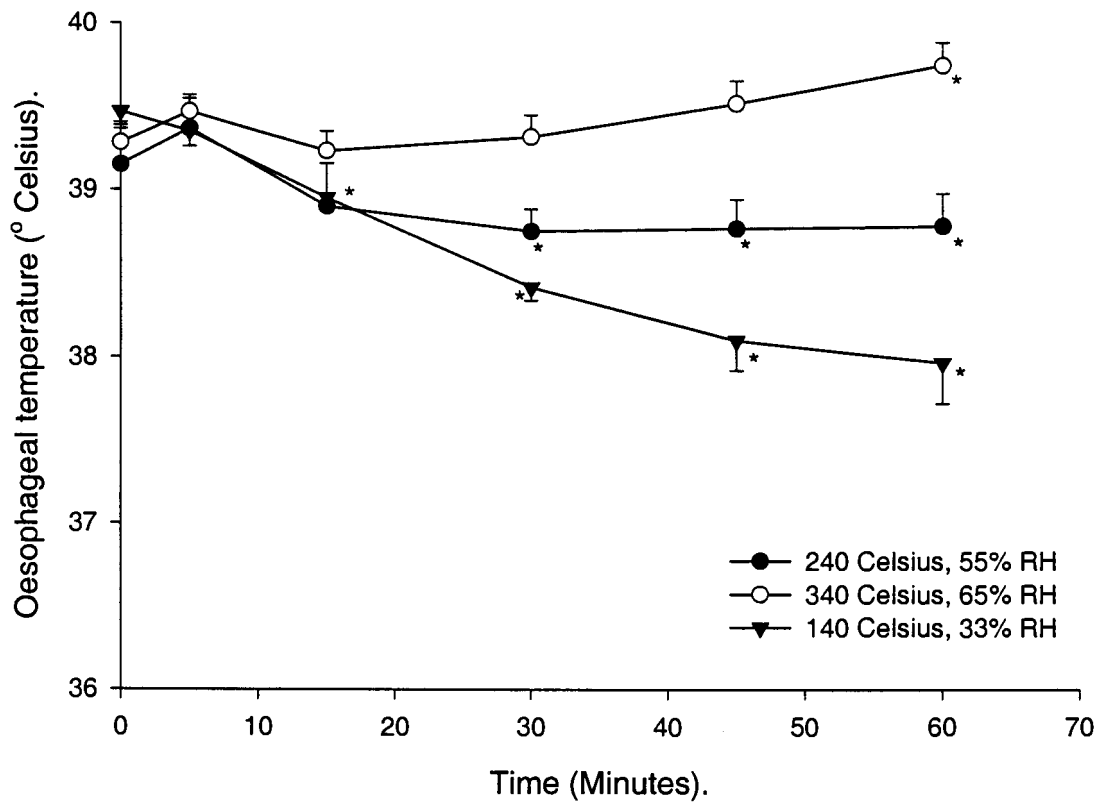
Figure 6.1: Temporal changes in mean and standard error of mean of
oesophageal temperature of goats following intravenous injection
of 0.1 mg/kg xylazine under three different environmental conditions.

* Significantly different (p<0.05) from values at time 0.
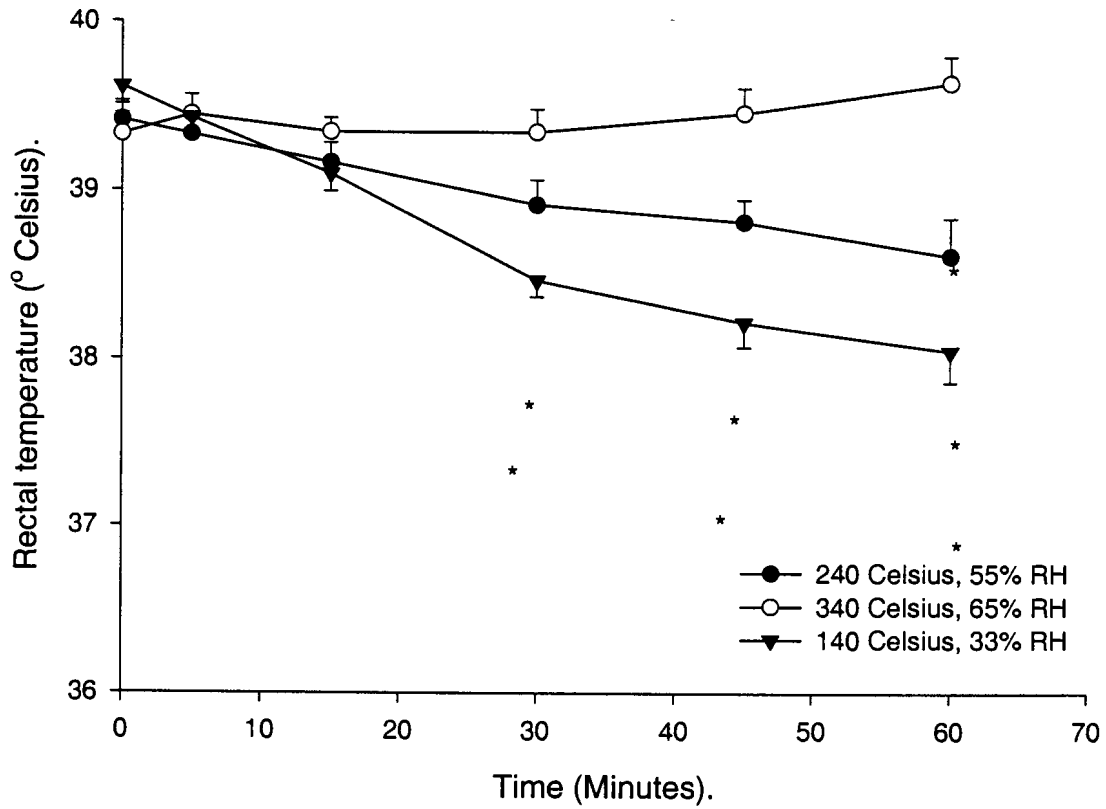
Figure 6.2: Temporal changes in mean and standard error of mean of rectal temperature of goats under three different environmental conditions.

* Significantly different (p<0.05) from values at time 0.

6.4   DISCUSSION.

Ambient temperature is an important element affecting thermal responsiveness of animals to systemically injected drugs (Buckley *et al.*, 1969; Avery, 1972; Tsoucaris-Kupfer and Schmitt, 1972; Ponder and Clark, 1980; Livingston *et al.*, 1984; Fayed *et al.*, 1989). Furthermore, body temperature responses to xylazine hydrochloride in various animals appear to differ markedly.

Injection of xylazine hydrochloride in goats in this study resulted in significant reduction in body temperature in the 14 and 24° C environments, starting at 15 minutes post-xylazine injection whereas in the 34° C environment, significant increase in body temperature occurred at 60 minutes post-xylazine injection. In goats, xylazine has been shown to decrease (Mohammed and Yelwa, 1993; Saleh, 1993; Prajapathi *et al.*, 1994), increase (Dehghani *et al.*, 1991; Aithal *et al.*, 1996) or, have no effect on body temperature (Kumar and Thurmon, 1979). In cats, (Ponder and Clark, 1980; Dehghani *et al.*, 1991) and rats (Tsoucaris-Kupfer and Schmitt, 1972; Livingston *et al.*, 1984), decrease in body temperature following administration of xylazine has been reported, while in cattle (Fessl, 1970; Hopkins, 1972; Young, 1979), xylazine caused an increase in body temperature.

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General anaesthetic agents with the exception of ketamine, impair thermoregulation, presumably by attenuation of hypothalamic function, giving rise to a widened range of core temperatures over which no thermoregulatory responses occur (Imrie and Hall, 1990). Vasodilators redistribute heat to the periphery, thus increasing heat loss to the environment while opioids, barbiturates, phenothiazines and butyrophenones have both central and peripheral actions tending to decrease body temperature (Imrie and Hall, 1990).

In this study, xylazine caused a decrease in body temperature in the 14 and 24° C environments while the body temperature increased in the 34° C environment. This is consistent with findings in cattle (Fayed *et al.*, 1989), cats (Ponder and Clark, 1980) and rats (Tsoucaris-Kupfer and Schmitt, 1972; Livingston *et al.*, 1984). In the studies carried out in cattle by Fayed *et al.* (1989), xylazine caused hyperthermia in the 33° C temperature environment while it did not have any effect on body temperature of the cattle in the 18° C environment. Cats injected with xylazine and exposed to environmental temperatures of 4 and 22° C (Ponder and Clark, 1980) and rats injected with the same drug and exposed to environmental temperature range of 20 to 24° C (Tsoucaris-Kupfer and Schmitt, 1972; Livingston *et al.*, 1984) suffered hypothermia. When the cats in the above study were exposed to environmental temperature of 32° C, they suffered hyperthermia (Ponder and Clark,

1980). In this study, the goats exposed to environmental temperatures of 14 and 24° C showed a decrease in mean oesophageal temperature of 1.5 and 0.37° C, respectively, 60 minutes following xylazine administration. Over the same period, rectal temperature in the 14 and 24° C environments decreased by 1.57 and 0.80° C, respectively. The goats in the 14° C environment attained lower body temperatures sooner compared to the 24° C environment. These effects are in agreement with findings in cats (Ponder and Clark, 1980) in which cats kept in a 4° C environment attained maximum decrease in body temperature earlier than those in the 22° C environment. This difference in the rate of development of hypothermia in cats was attributed to the greater temperature gradient between the body and the environment at 4° C as opposed to 22° C (Ponder and Clark, 1980), and this may explain the difference seen in decrease in body temperature in this study. Furthermore, the developments seen in cats would be expected as they were kept in an environment with a lower temperature (4° C) and because of their greater surface area to body mass ratio, owing to their smaller size as compared to goats in the present study. The possible causes of hypothermia by xylazine could be through cutaneous vasodilation or a decrease in oxygen consumption at cellular level (Tsoucaris-Kupfer and Schmitt, 1972). In the 34° C environment, mean oesophageal and rectal temperatures increased by 0.47 and 0.32° C, respectively over the 60 minute monitoring period. Hyperthermia that develops in hot

environments has been attributed to the inhibition of the thermoregulatory centre by xylazine and also, partly due to the suppression of the respiration rate in heat-stressed animals, thus making them unable to get rid of excess heat through the respiratory system (Fayed *et al.*, 1989). Agents that depress thermoregulation allow the body temperature to drift passively upwards in a hot environment and downwards in the cold. These changes in body temperature are not opposed by effector mechanisms to prevent or lessen variation in temperature (Clark, 1979). In contrast, environmental temperature did not have a significant effect on body temperature in the unsedated goats in the present study, indicating that the effector mechanisms responsible for countering variation in body temperature resulting from variations in environmental temperature were intact. It has been suggested (Ponder and Clark, 1980) that animals injected with xylazine should not be exposed to extreme heat or cold for several hours to avoid the development of hyperthermia or hypothermia. Because full recovery to the baseline body temperature values in cats (Ponder and Clark, 1980) have been shown to take up to 12 hours, some animals which may appear to have fully recovered from the effects of xylazine may still be unable to maintain normal body temperature, especially if exposed to extremes of environmental temperature. Hyperthermia induced by xylazine may be important when long sedation is needed under hot conditions, especially in black animals thus, provision of a shade may be advisable under hot conditions (Hopkins, 1972).

In this study, one goat which weighed 21.5 kg, the lowest in the group, shivered for more than 2 hours from the time it stood up from the 14° C environment. Over the 60 minutes monitoring period, its oesophageal temperature had decreased by 2.2° C (39.6 to 37.4° C) while the rectal temperature had decreased by 2.3° C (40.0 to 37.7° C).

Hypothermia, defined as the clinical state of subnormal body temperature (Smith, 1985) is as a result of heat loss exceeding heat production. Complications of perioperative hypothermia include post-operative shivering which unacceptably increases metabolic rates of patients, as it can increase oxygen consumption from 100 to 600%, impaired coagulation, prolonged drug action, increased chances of wound infection and negative post-operative nitrogen balance (Sessler, 1994). Apart from hypothermia sharply reducing basal metabolic rate, cardiovascular effects including lowered blood pressure, gradual decrease in heart rate and output, cardiac arrhythmias and conduction disturbances often ensue. Additional effects of hypothermia are disturbances in acid-base balance, left shift of the oxyhaemoglobin dissociation curve, internal fluid shifts from the extracellular compartment, and decreased peripheral glucose utilisation, causing hyperglycaemia (Zenoble and Hill, 1979).

Mild core hypothermia is common during surgery and anaesthesia due to factors like cold operating theatres, cold skin preparation lotions, cold irrigating fluids, cold intravenous fluids, dry anaesthetic gases and exposure of the body viscera in abdominal surgeries. Because these impose large thermal stresses on patients, normothermia should be maintained intraoperatively. This could be achieved through the use of warm operating theatres, adequate heating, cutaneous warming, humidification of inspired gases, warming of intravenous fluids and adequate cutaneous insulation (Imrie and Hall, 1990; Sessler, 1994). Hyperthermia may also occur during anesthesia and the cause should be sought and appropriate treatment given.

CHAPTER SEVEN

THE EFFECTS OF AMBIENT TEMPERATURE AND HUMIDITY ON THE PHARMACOKINETICS OF XYLAZINE IN GOATS.

7.1   INTRODUCTION.

Xylazine (Rompun®, Bay Va 1470) was first synthesized in 1962 and given the code name Bay Va 1470. Chemically, it is 2(2,6-dimethylphenylamino)-4H-5,6-dihydro-1,3-thiazine. Pharmacologically, xylazine is classified as a sedative, analgesic and a central acting skeletal muscle relaxant (Booth, 1988). It has been used extensively in various animal species because of its potent sedative, analgesic and myorelaxant properties (Clarke and Hall, 1969). The sedative effects of xylazine are due to its stimulation of the central nervous system presynaptic α_2-adrenergic receptors. Although this was originally considered an adverse effect, the development of a number of xylazine antagonists which can reverse its sedative effects have improved its safer use in veterinary practice (McDonnell et al., 1993).

Previous studies on the pharmacokinetics of xylazine hydrochloride in horse, cattle, sheep, and dog (Garcia-Villar *et al.*, 1981) following its intravenous injection showed that its half-time of distribution was very short, ranging from 1.2 to 5.9 minutes, with half-time of elimination varying between 23 and 50 minutes. The volume of distribution was large (1.9 - 2.7 *l*/kg). Studies carried out in rats (Duhm *et al.*, 1969) with radio-labelled xylazine injected intravenously showed that only 8% of intact or unchanged xylazine appeared in urine while 70% of the radio-activity was eliminated in urine with half-life of about 2 to 3 hours. The authors reported that xylazine was rapidly metabolised yielding about 20 metabolites. In cattle, Putter and Sagner (1973) reported that 1% of the unchanged drug was eliminated in urine 2 hours after its administration with an apparent half-life of 40 minutes. Peak excretion of metabolites occurred between 2 and 4 hours following its administration.

Knowledge of the pharmacokinetics of xylazine hydrochloride in different animal species is important in order to establish the relationship between the pharmacokinetics and the clinical effects it causes. It has been established that plasma kinetics of xylazine relate well to some its clinical effects in the horse and dog, whereas it is difficult to relate plasma kinetics to some of the sustained clinical effects that are observed in cattle (Garcia-Villar *et al.*, 1981).

From the available literature, there is no report on the pharmacokinetics of xylazine hydrochloride in goats. This study reports on the pharmacokinetics of xylazine hydrochloride in goats under three different sets of environmental conditions.

7.2 MATERIALS AND METHODS.

7.2.1 ANIMALS

Six adult, indigenous breed, castrated male goats, weighing between 21.0 and 34.0 kg (mean = 28.2 ± 1.0,SEM), were purchased locally and transported to the University of Pretoria's Faculty of Veterinary Science at Onderstepoort. They were housed indoors in individual crates within the Veterinary Academic Research Unit and acclimatised for 14 days. On arrival, they were dewormed (Panacur®- Hoechst, Ag-Vet, South Africa) and routinely clinically examined to ascertain the status of their health. They were fed on a diet of lucerne, hay and water, which were provided *ad libitum*. The protocol for this study was approved by the Ethics and Research Committees of the Faculty of Veterinary Science, University of Pretoria.

7.2.2 EXPERIMENTAL DESIGN

A single group, three phase, repeated measures study design was used to study the effect of environmental temperature and humidity on the pharmacokinetics of xylazine in six goats. The intravascular pharmacokinetics of xylazine were repeatedly examined in the goats during a low, medium and high temperature and humidity exposure. A washout period of at least seven days was allowed between treatment phases.

7.2.3 TREATMENT PHASES

The experiments in this study were conducted under temperature and humidity controlled environments. Phase one of the study was conducted under the medium temperature and humidity environment, with room temperature set at 24 ± 1° C and a relative humidity of 55 ± 1%. Phase two of the study was conducted under the high temperature and humidity environment, with room temperature set at 34 ± 1° C and a relative humidity of 65 ± 1%. Phase three of the study was conducted in the low temperature and humidity environment with the room temperature set at 14 ± 1° C and a relative humidity of 33 ± 1%. The temperature and humidity settings were done several hours before any experiment was started so as to have uniformity of conditions in the rooms.

Between experiments, the goats were housed and fed in individual crates, in housing premises devoid of temperature and humidity control.

Before any experiment was carried out, food was withdrawn from the animal(s) for 24 hours and water for 12 hours. On the day of the experiment, each goat was weighed and taken into a preparation room adjacent to the temperature and humidity controlled room. The average body weight of goats during the experiments in the low temperature environment was 28.6 ± 1.8 kg, 26.9 ± 1.5 kg in the medium environment, and 29.0 ± 1.9 kg in the high temperature environment.

In the preparation room, the goats were restrained in lateral recumbency on a surgical table with a waterproof foam mattress. The left jugular furrow was thoroughly clipped and surgically prepared. A 18G intravenous catheter (Jelco®- Critikon) was percutaneously introduced into the left jugular vein, flushed with heparinised saline, capped and secured to the skin with nylon No. 2/0 sutures (Ethilon). Following preparation, the animals were transferred to the temperature and humidity controlled room.

After a stabilisation period of 10 to 15 minutes, baseline blood samples were taken and then xylazine hydrochloride (Rompun®- Bayer Animal Health(Pty),

Isando, South Africa), at 0.1 mg/kg body weight, was injected intravenously through the jugular catheter. For determination of plasma concentrations of xylazine hydrochloride, venous blood samples were drawn into 10 ml heparinised vacutainer tubes immediately prior to treatment (0 minutes) and then at 1, 2, 4, 6, 8, 15, 30, and 60 minutes post-xylazine injection.The intravenous jugular catheter was flushed with heparinised saline after every sampling. The collected samples were centrifuged at 3500 rpm, the plasma harvested and stored at -20° C until analysis for xylazine content was performed.

7.2.4   MEASUREMENT OF XYLAZINE CONTENT IN PLASMA.

7.2.4.1   INTRODUCTION.

Although several techniques have been used for the determination of xylazine in biological materials (Alvinerie and Toutain, 1981; Moore and Oliver, 1989; Garcia-Villar *et al.*, 1981; Putter and Sagner, 1973; Muge *et al.*, 1995), the high performance liquid chromatography (HPLC) technique is the most widely used. This is because of its simplicity, rapidity, high selectivity, and range of sensitivity (McDonnell *et al.*, 1993). As xylazine is administered in relatively

small doses, sensitive methods are required for its detection in biological matrixes (Putter and Sagner, 1973).

In the determination of xylazine in equine plasma, using the high performance liquid chromatographic (HPLC) technique, McDonnell *et al.* (1993) separated the drug and the internal standard, pindolol, on a 5 μm cyanopropyl-modified column (250 x 4.6 mm i.d), using a buffer-acetonitrile mixture containing an ion-pairing reagent. Xylazine and the internal standard were isolated from plasma by liquid extraction into ethyl acetate. The method used was validated over a xylazine concentration range of 50 - 2000 ng/ml in plasma with a reproducibility of < 5% for both inter-day and intra-day replicate determinations. The method was linear over the concentration range studied, with no interferences from endogenous plasma components and a limit of detection of 20 ng/ml.

7.2.4.2 REAGENTS.

The following reagents were used in the HPLC procedure:

*   Analytical grade xylazine hydrochloride (Sigma Chemical Co., St Louis, MO, USA).

*   Methanol- HPLC grade (Merck NT. Laboratory Supplies, Midrand, S.A.).

*   Sodium hydrochloride- HPLC grade (Merck NT. Laboratory Supplies,

Midrand, S.A)

*   Boric acid- HPLC grade (Merck NT. Laboratory supplies, Midrand,

S.A.).

*   Nitrogen gas- Ultra pure grade (Afrox, S.A.).

*   Acetonitrile- HPLC grade (Merck NT. Laboratory Supplies, Midrand,

S.A.).

*   Phosphoric acid - HPLC grade (Saarchem, Krugersdorp, S.A.).

*   Ethyl acetate- HPLC grade (Merck NT. Laboratory Supplies, Midrand,

S.A.).

*   Sodium 1-Heptanesulphonic acid - HPLC grade (BDH Laboratory

Supplies,   Poole, England)

7.2.4.3   INSTRUMENTATION.

The HPLC system consisted of a Waters Model 600E single piston pump

(Waters, Milford, USA), which was used to deliver the mobile phase and the

eluent   stream   was   monitored   with   a   Waters   490E   programmable

multiwavelength ultraviolet absorbance detector (Waters, Milford, USA).

Samples were introduced via a Waters 712 WISP autosampler injector (Waters,

Milford, USA). Drug separation was done using a lichrospher 100 CN (5 µm),

250 x 4 mm i.d, column (E.Merck, Darmstadt, Germany). Quantification was based on peak area measurements using a 386 personal computer programmed with System Gold, version 5, supplied by Beckman Instruments, USA.

7.2.4.4   HPLC CONDITIONS.

The mobile phase consisted of acetonitrile: phosphoric acid (1% v/v), at a ratio of 70 : 30 v/v with 2 ml/l PIC-B7 (Paired Ion Chromatography - Sodium 1-Heptanesulphonic acid) ion-pairing reagent. The mobile phase was degassed by using an ultrasonic bath for 10 minutes and filtered under vacuum through a 0.45 µm filter using a solvent filtration system. Pump flow rate was set at 0.8 ml/minute. The system operated at ambient temperature. The autosampler injection volume was 50 µl. The detector was set at 231 nm UV wavelength and run time was 10 minutes.

7.2.4.5   STOCK SOLUTIONS, SECONDARY XYLAZINE SOLUTIONS
            AND REFERENCE SAMPLES.

A xylazine standard stock solution of 100 µg/ml was prepared in methanol. Secondary xylazine solutions were prepared from the standard stock solution by

dilution in methanol to achieve 50, 25, 10, 5, 1, and 0.5 µg/ml concentrations. These solutions were kept in the refrigerator at 4 °C.

Reference samples (n = 4) of goat plasma spiked with xylazine were prepared daily for validation of the analytical technique and for calibration curves during the analytical phase. Plasma, obtained from heparinised fresh blood samples, was spiked with xylazine to achieve concentrations 5, 10, 20, 50, 100, 250, and 500 ng/ml. These represented the lowest and highest concentrations required for pharmacological analysis. On the day before use, the reference samples were prepared by pipetting 20 µl of each secondary solution into 10 ml silinized glass tubes. The contents of each tube were evaporated to near dryness using a Turbo Vap at 50 °C, under a stream of nitrogen gas and then 2 ml of plasma added. The mixture was shaken in a vortexer for 10 minutes and stored in a refrigerator overnight for use the next day.

7.2.4.6   EXTRACTION PROCEDURE AND EFFICIENCY.

Aliquots (500µl) of plasma (reference samples and/or samples collected from goats) were pipetted into 15 ml silinized glass tubes. Boric acid (1 ml of 1%) was added to each sample and the solution vortexed for 1 minute. Ethyl acetate (5 ml) was then added to each sample, the solution again vortexed for 1 minute.

Thereafter, the samples were centrifuged at 3 500 r.p.m for 10 minutes. An aliquot (4 ml) of the organic layer was transferred to clean 10 ml silinized glass tubes and evaporated in a Turbo Vap LV evaporator (Zymark) at room temperature (20 - 25° C) under a nitrogen stream. The residue obtained was then dissolved in 500 μl of acetonitrile-water (75 : 25) and the mixture vortexed for 1 minute. A 50 μl aliquot of the dissolved residue of each sample was injected into the HPLC system using an autosampler.

Extraction efficiency was determined by comparison of measured drug concentration, with and without extraction of goat plasma containing a low, medium and high concentration of xylazine. With extraction, reference plasma samples (n = 6) containing 10, 50, and 100 ng/ml of xylazine were extracted as previously described and the aliquots collected for HPLC analysis. Without extraction, blank plasma samples (n = 6) from untreated goats were first processed according to the extraction procedure and thereafter, 100 μl of the xylazine standards containing 10, 50, and 100 ng/ml xylazine, then added, vortexed for 1 minute and the 50 μl aliquot for HPLC analysis collected. The extraction efficiency was determined as the percentage of the ratio of the concentration of xylazine with and without extraction.

7.2.4.7   CALIBRATION PROCEDURE.

Calibration curves were constructed by HPLC analysis from a series of reference standards (5, 10, 20, 50, 100, 250, and 500 ng/ml). Six (n = 6) replicates of reference standards for each xylazine concentration were used to construct the initial calibration curve. Linear regression analysis of the area under the curve of the xylazine chromatogram for each reference standard versus the xylazine spiked concentration in the sample, were performed. The response factor representing the slope (b) of the curve $y = bx + a$ and the y intercept (a) were used to calculate the xylazine concentration in plasma.

The description of the initial calibration curve was used to validate all subsequent calibration curves determined on each day of analysis. Daily calibration curves consisted of a single series of the reference samples. The initial calibration curves were accepted if the coefficient of correlation (r) was ≥ 0.95% and the coefficient of variation was < 10% between replicates. Daily calibration curves were accepted if the response factor was within ± 10% and the y intercept within ± 20% of the initial calibration curve.

7.2.4.8   LIMIT OF QUANTIFICATION AND LINEARITY.

Linearity and limit of quantification (LOC) were determined from the calibration graphs. Linearity was determined by the coefficient of determination of the least square regression analysis, whereas the limit of detection was defined as the lowest concentration that could be measured precisely and accurately by the analytical method.

7.2.4.9   ACCURACY, PRECISION AND REPRODUCIBILITY.

These were determined from the initial calibration curves and from *in vitro* quality control samples. Precision was accepted if the coefficient of variation (CV) of the mean concentrations of xylazine measured for each sample concentration was $\leq 10\%$. Accuracy was deemed satisfactory if the percentage of error of mean of concentration measured versus concentration spiked was less than 10%.

7.2.4.10   *IN VITRO* QUALITY CONTROL SAMPLES.

On each day of analysis, three replicates of 10, 50, and 100 ng/ml of the spiked

plasma (*in vitro* samples), representing a range of low, medium and high values

were analysed.

Intra-day and interday accuracy and precision were determined from the results

of the *in vitro* samples. Reproducibility of the method was established from the

intra-day and inter-day variation. Reproducibility was also determined on

duplicate analysis of at least 10% of plasma samples.

7.2.5 PHARMACOKINETIC ANALYSIS.

Both compartmental and non-compartmental models were used for the analysis

of pharmacokinetic data.   Non-linear compartmental analysis of the xylazine

plasma data was performed by means of WinNonLin Version 2.1 (Statistical

Consultants, Inc., New York, USA) computer programme using the Nelder-

Mead algorithm (Nelder and Mead, 1965). Initial pharmacokinetic parameter

estimates, used for the non-linear analysis, were derived automatically by initial

linear analysis performed by the programme. Akaike's information criterion

(Yamaoka *et al.*, 1978), based upon the mean values of the final estimates of the

associated pharmacokinetic parameters and lack of systematic deviations around the fitted disposition curve, was used to determine the number of exponential terms that best described the data.

Primary pharmacokinetic parameters in this study were derived from a two compartmental analysis with IV-push input, first order output using macroconstants as primary parameters (Model 8), yielding the microconstants (K10, K12, and K21), the partial exponents (α and β) and the coefficients (A and B). Secondary disposition parameters, including area under curve (AUC) of the xylazine plasma concentration versus time curves, distribution half-life (T½α), biological half-life (T½β), elimination half life (K10-HL), total body clearance (Cl = Dose/AUC) volume of central compartment (Vc), and apparent volume of distribution at steady state (Vss), were derived from the primary parameters. Total plasma concentration of xylazine at pseudoequilibrium (Cp^o) was calculated as the sum of the coefficients (A+B).

In addition, non-compartmental analysis of the plasma concentration versus time data of xylazine for intravascular input (Model 201) was also performed. The area under the plasma concentration versus time curve (AUC, zero-moment) and the first non-normalized moment (AUMC) were calculated according to the non-linear trapezoidal method from time zero to the last sample

time (Gibaldi and Perrier, 1982). Extrapolation of AUC to infinity (AUCinf) was performed using the slope of the terminal phase (β). The mean residence time (MRT, first moment) was derived from $AUMC_{inf}/AUC_{inf}$. The mean plasma concentration of (Cav) was calculated as AUC/Tlast. Total body clearance and V_{ss} were derived by similar formulae as compartmental analysis.

7.2.6 STATISTICAL ANALYSES.

The descriptive statistics (Mean ± SEM) were calculated for all pharmacokinetic parameters under the three different environmental temperature conditions. The means of the pharmacokinetic parameters between the different temperature conditions were statistically compared for differences using one way analysis of variance (ANOVA). All statistical procedures were performed on a personal computer using a statistical software programme (SigmaStat - Jandel Scientific Corporation, San Rafael, CA, USA) for Windows 95.

7.3   RESULTS.

7.3.1 HPLC VALIDATION RESULTS.

7.3.1.1   CHARACTERISTICS OF CALIBRATION CURVES AND LINEARITY.

The linearity of the calibration graph for xylazine in plasma from 5 ng/ml through 500 ng/ml is illustrated in Figure 7.1. Linearity, as measured by coefficient of determination (r^2) was > 99% for all other daily calibration graphs. A mean response factor of 11.64 was generated for calculation of xylazine content in plasma.

7.3.1.2   SPECIFICITY AND SENSITIVITY.

Xylazine was identified according to its retention time. No interfering peaks occurred for xylazine in blank plasma samples as well as in plasma samples collected before treatment. Good baseline separation of xylazine chromatographic peaks was achieved as illustrated in a representative chromatogram in Figure 7.2. The lowest limit of quantification for determination of xylazine in plasma was 5 ng/ml and the linear range of the

HPLC-assay was 5-500 ng/ml. Concentrations below the limit of quantification were recorded as non-quantifiable.

7.3.1.3   ACCURACY AND PRECISION.

The accuracy and precision of the HPLC analytical method is summarized in Table 7.1. An overall mean accuracy of 98.3 ± 7.1 % and a precision below 15% were achieved for xylazine analysis in plasma at concentrations between 5 and 250 ng/ml.

Table 7.1: Accuracy and precision of xylazine in plasma.

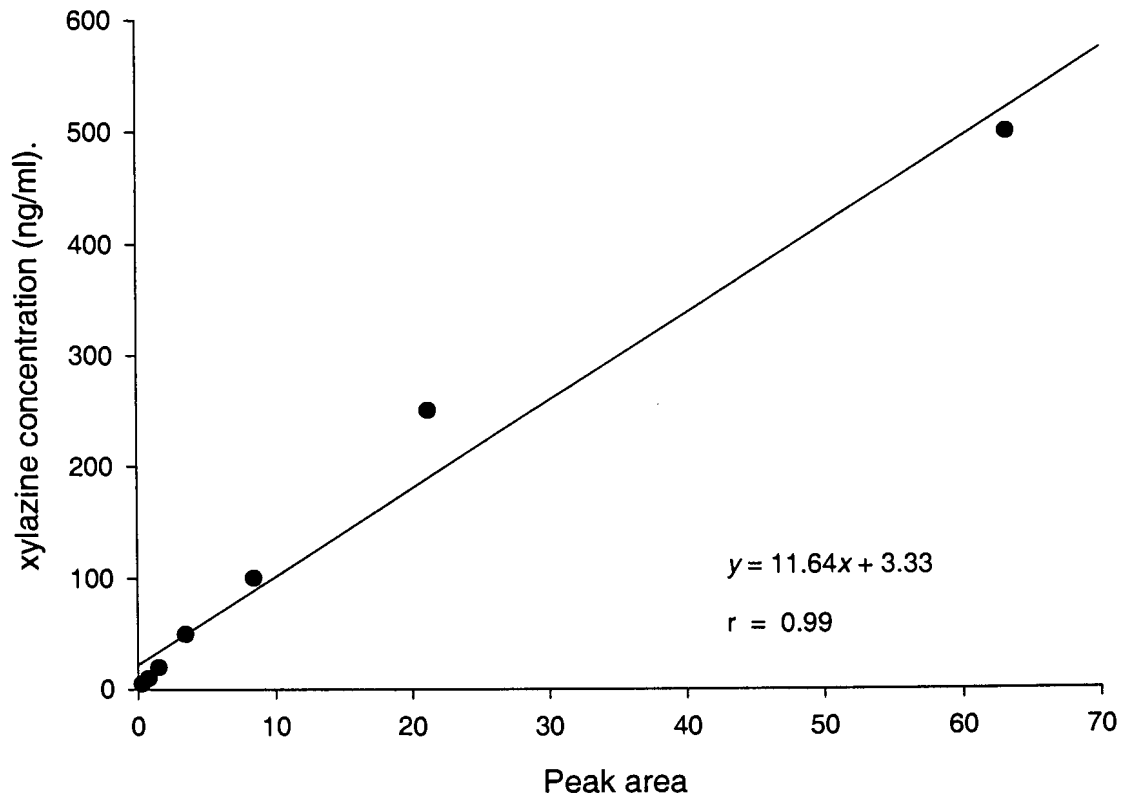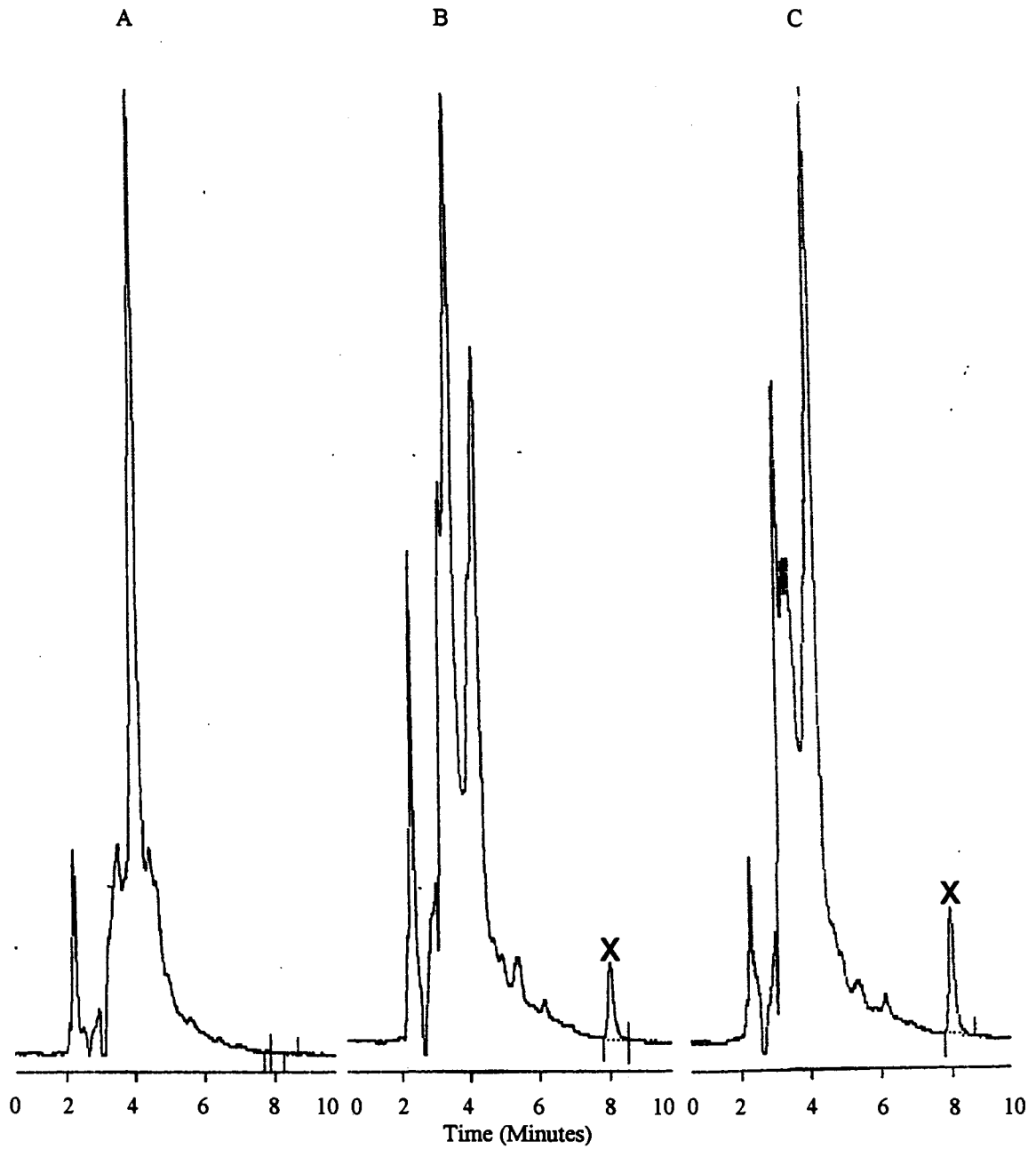| Spiked concentration (ng/ml)	Measured concentration (ng/ml)	Accuracy (%)	Precision (CV%)
5	4.4 ±1.8	87.7 ±36.5	9.0 ±3.5
10	10.7 ±1.6	107.2 ±16.0	7.7 ±2.7
20	19.8 ±1.7	98.8 ±8.7	9.7 ±0.8
50	46.5 ±7.4	93.0 ±14.8	13.5 ±6.4
100	103.3 ±4.6	103.3 ±4.6	10.3 ±2.3
250	250.4 ±1.3	100.2 ±0.5	8.5 ±0.7

$y = 11.64x + 3.33$

r = 0.99

Figure 7.1 : Calibration curve in plasma of the mean xylazine concentration and peak areas

Figure 7.2: Representative chromatograms of blank goat plasma extract (A), goat plasma spiked with 100 ng/ml xylazine (x) with extraction (B) and without extraction (C).

7.3.1.4   REPEATABILITY.

Highly repeatable results were obtained for xylazine from plasma and from *in vitro* control samples analysed each day and on different days of analysis. Repeat analyses (≤ 10% of samples) for xylazine were within 13% of the original measurement.

7.3.1.5   EXTRACTION EFFICIENCY.

The mean percentage recovery of xylazine from plasma spiked with xylazine after extraction was 79.09% for xylazine within the concentration range of 10 - 100 ng/ml while, the mean recovery from plasma spiked with xylazine before extraction was 98.1 % for the same xylazine concentration range, giving a mean extraction efficiency of 80.6 %. Mean percentage recovery of xylazine from plasma spiked with 100 ng/ml of xylazine was > 100%. Lower extraction efficiency was achieved at the lower concentrations (Table 7.2).

Table 7.2: Mean percentage recovery of xylazine from spiked plasma samples.

Spiked concentration (ng/ml)	Recovery (%)
Spiked before extraction	
10	68.6 ±9.4
50	65.8 ±10.2
100	102.9 ±2.6
Spiked after extraction	
10	101.9 ±4.4
50	91.8 ±14.8
100	100.6 ±7.2

7.3.2 INTRAVASCULAR DISPOSITION OF XYLAZINE.

The results of the individual pharmacokinetic analysis at the three different environmental temperature and humidity conditions for the non-compartmental and compartmental models are presented in Annex 1 & 2.

The plasma versus time profile data after IV-push for xylazine (Figures 7.3a and 7.3b) was best described by a two-compartmental open model with first-order rate constants. A coefficient of determination percentage (r^2) of > 99% for predicted versus actual plasma xylazine concentration was obtained in all cases.
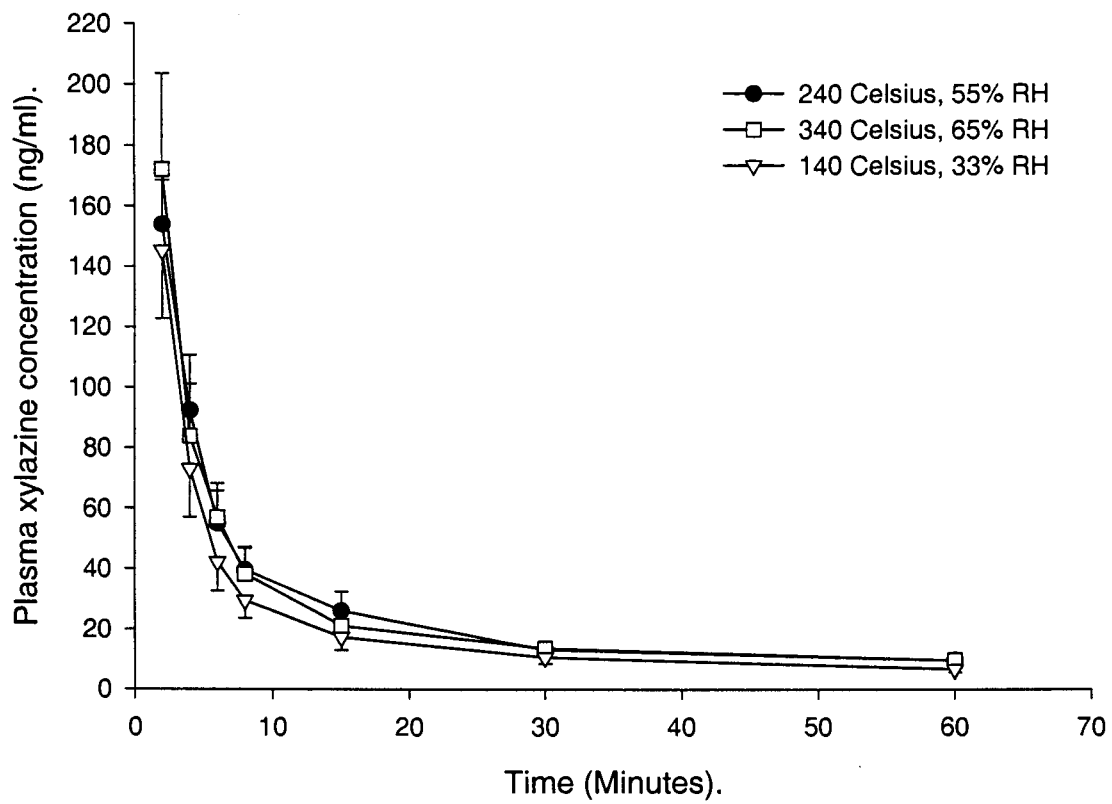
Figure 7.3a : Plasma xylazine concentration versus time in goats under three different environmental conditions.
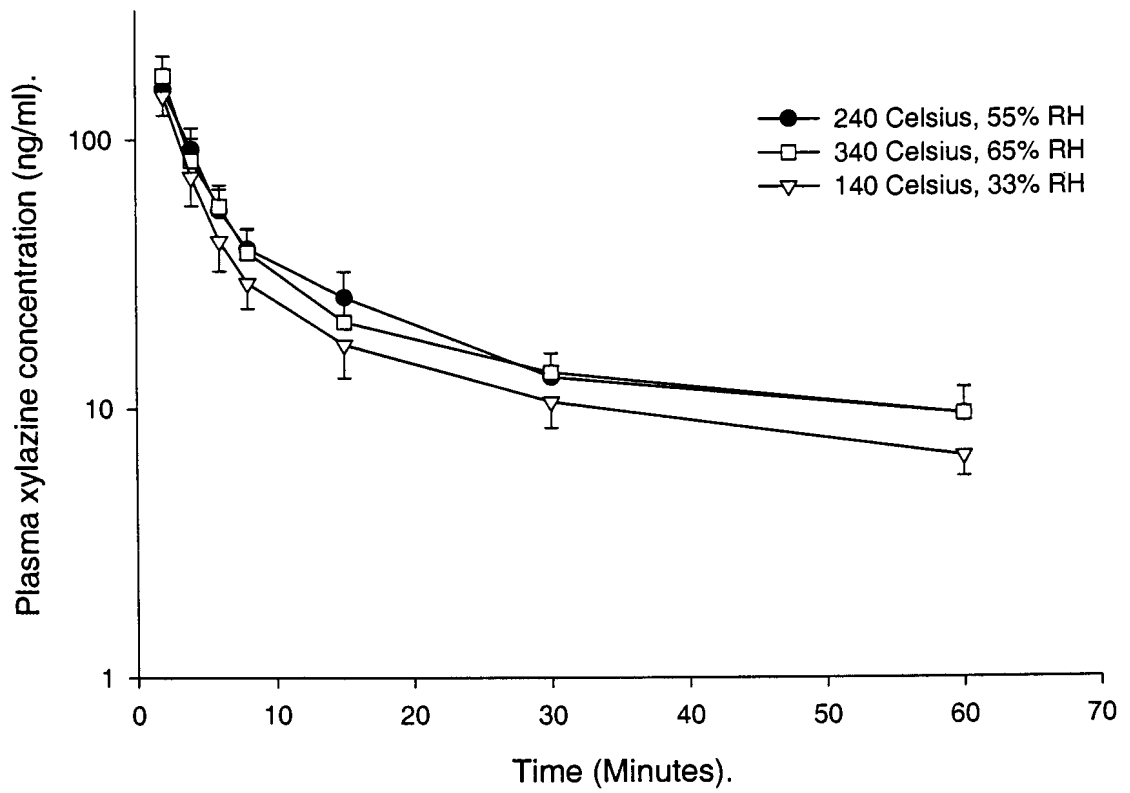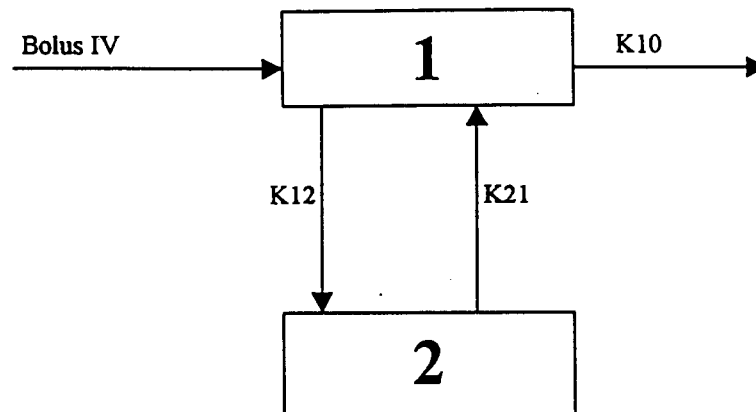
Figure 7.3b : Semi-logarithmic plot of plasma xylazine concentration versus time in goats under three different environmental conditions.

Figure 7.4: Two compartment open model with first order rate constants.

K12 (14 °C) 0.27 (±0.05)   K21 (14 °C) 0.09 (±0.04)   K10 (14 °C) 0.48 (±0.23)

 (24 °C) 0.17 (±0.05)   (24 °C) 0.09 (±0.01)   (24 °C) 0.22 (±0.11)

 (34 °C) 0.32 (±0.06)   (34 °C) 0.09 (±0.04)   (34 °C) 0.31 (±0.10)

The results of the pharmacokinetic parameters describing the plasma disposition of xylazine in goats after intravenous administration at three different environmental temperatures are presented in Table 7.3 and Fig. 7.4. Similar pharmacokinetic results of xylazine, not withstanding large individual variation, were obtained in the three different environmental temperature conditions. There were no significant $(P > 0.05)$ differences between the three different environmental temperature conditions for any pharmacokinetic parameter.

Table 7.3: Mean pharmacokinetic parameters describing the plasma disposition of xylazine in goats after intravenous administration under three different environmental conditions derived by both non-compartmental and compartmental pharmacokinetic analysis.

Pharmacokinetic variable	Mean ± SEM		
	14 °C	24 °C[*]	34 °C
Non-compartmental:			
AUClast (ng.min/ml)	1509 ±217	2022 ±204	1885 ±300
AUCinf (ng.min/ml)	1779 ±229	2347 ±243	2371 ±450
AUMCinf (ng.min^2/ml)	49016 ±5615	60197 ±13857	51974 ±7869
MRT (min)	27.1 ±2.3	25.3 ±3.6	32.0 ±5.6
T½lambda	29.9 ±3.5	24.6 ±3.4	32.0 ±4.6
Cav (ng/ml)	25.2 ±3.6	33.7 ±3.4	31.4 ±5.0
Cl (ml/kg/min)	55.5 ±7.0	44.7 ±4.4	54.5 ±7.4
Vss (l/kg)	1.5 ±0.30	0.80 ±0.11	1.62 ±0.44
Compartmental:			
AUC (ng.min/ml)	2195 ±245	2322 ±309	2105 ±350
AUMC (ng.min^2/ml)	77240 ±40202	53189 ±17578	69807 ±27426
A (ng/ml)	835.2 ±446.6	396.9 ±225.5	646.2 ±243.3
B (ng/ml)	37.7 ±12.9	550.0 ±6.5	54.0 ±27.8
Cp^0 (ng/ml)	872.8 ±458.4	451.9 ±226.1	700.2 ±266.7
α (min^{-1})	0.78 ±0.26	0.4387 ±0.15	0.67 ±0.16
β (min^{-1})	0.06 ±0.03	0.04 ±0.01	0.04 ±0.02
K10 (min^{-1})	0.48 ±0.23	0.22 ±0.11	0.31 ±0.10
K12 (min^{-1})	0.27 ±0.05	0.17 ±0.05	0.32 ±0.06
K21 (min^{-1})	0.09 ±0.03	0.09 ±.01	0.09 ±0.04
K10-HL (min)	3.8 ±1.49	6.6 ±2.16	4.0 ±1.3
T½α (min)	1.5 ±0.43	2.3 ±0.6	1.6 ±0.48
T½β (min)	31.0 ±10.0	20.0 ±3.2	31.0 ±7.2
Vc (min^{-1})	0.25 ±0.07	0.38 ±0.09	0.29 ±0.07
Cl (ml/kg/min)	48.4 ±5.8	46.4 ±6.2	54.4 ±10.3
MRT (min)	31.9 ±13.0	21.2 ±4.8	31.1 ±8.8
Vss (l/kg)	1.4 ±0.4	0.92 ±0.18	0.8 ±0.4

* n = 4 (See Tables 10.1 &10.2 in the Annexure)

There was a rapid decline in plasma xylazine concentration in all three environmental conditions within 10 minutes following its injection followed by a slower gradual decline over the rest of the 60 minutes monitoring period. Mean plasma xylazine concentrations in plasma of goats in the 14° C temperature environment were generally lower, although not significantly (p > 0.05) different, as compared to concentrations in the other two environments.

Under the three different environmental conditions, large individual differences were observed in Cp^o, rates of distribution, K10-HL, T $\frac{1}{2}\beta$, and Cl. In the 24 and 34° C temperature environments, results of AUClast, and β, were more consistent between the groups. T$\frac{1}{2}el$ results of goats were similar in the 14° C and 34° C environments.

7.4   DISCUSSION

7.4.1 HPLC ANALYTICAL METHOD

Many different analytical methods are available for analysis of xylazine in biological fluids but, the HPLC technique is the most widely used owing to its simplicity, high selectivity and range of sensitivity (McDonnell *et al.*, 1993).

The determination of the concentration of xylazine in goat plasma in the present study was based on the HPLC technique as described by McDonnell *et al.* (1993) in horses. However, in our study, no internal standard was used and the drug separation was done using a different column. As in the previous study in horses (McDonnell *et al.*, 1993), the chromatographic conditions selected for the present study in goats proved suitable, yielding sharp peaks with a retention time of 7.9 minutes as compared to the study in equines of 10.6 minutes. The method used was linear over the concentration range studied, very selective, yielding clean plasma extracts, free from endogenous interfering peaks, as was the case in horses (McDonnell *et al.*, 1993).

In this study, an overall mean accuracy of 98.3 ± 7.1 % and a precision (expressed as the mean coefficient of variation) below 15% were achieved in plasma concentrations 5 - 500 ng/ml. An extraction efficiency of 80.6 % was achieved. McDonnell *et al.* (1993), using the same technique in horses reported precision of < 5% and achieved overall mean recovery of xylazine from plasma of 89.2%, in the concentration range of 50 - 2000 ng/ml. Since the chromatographic conditions they used were similar to the ones in the present study, the differences in the results obtained in these two studies may originate from the different columns used and species differences. Furthermore, this study showed that lower extraction efficiencies are attained at lower xylazine

concentrations as compared to higher xylazine concentrations. The higher extraction efficiency achieved by McDonnell *et al.* (1993) in their studies in horses could be due to the higher xylazine concentration (50 - 2000ng/ml) range they used as compared to the concentration range (5 - 500 ng/ml) used in the present study. In our study, we used a lichrosper 100 CN (5 μm), 250 x 4 mm I.D column, whereas McDonnell *et al.* (1993) used a spherisorb column (250 x 4.6 mm I.D) packed with 5 μm cyanopropyl-modified silica.

The various extraction methods, mobile phases and operating HPLC conditions applied in the determination of xylazine concentration in blood in various animal species have demonstrated differences in the magnitudes of recovery and limits of detection of xylazine from plasma (Alvinerie and Toutain, 1981; Garcia-Villar *et al.*, 1981; Akbari *et al.*, 1988; Psomas and Fletouris, 1992; McDonnell *et al.*, 1993). During extraction, partitioning into ethyl acetate rather than into chloroform or ethyl ether gave better recoveries and efficient separation can be achieved by addition of an ion-pairing reagent which allows for operation at a relatively low pH where xylazine is ionized (McDonnell *et al.*, 1993). This was achieved in the present study in goats.

7.4.2 PHARMACOKINETIC RESULTS.

The pharmacokinetic results derived by both non-compartmental and compartmental pharmacokinetic analysis show similar trends within and between treatment groups. Non-compartmental analysis is easier to perform and assumes a more general mathmatical property such as linearity (Gillespie, 1991). In some cases the use of a specific compartmental model is necessary and may lead to error due to the use of invalid assumptions, inherent in the model. In the current study a standard two-compartmental analysis was used.

Intravenous injection of a single dose of xylazine in horse, cattle, sheep and dog (Garcia-Villar *et al.*, 1981), showed a very short T½α (1.2 - 5.9 minutes), a T½β varying between 23 to 50 minutes and a large volume of distribution (1.9 - 2.7 l/kg). In this study in goats, a similar T½α (1.47 - 2.31 minutes) and T½β (20 to 30 minutes) was observed, whereas the apparent volume of distribution at steady state in goats (0.92 ±0.18 *l*/kg) was smaller than that reported for sheep (2.74 *l*/kg) at the medium temperature and humidity conditions. This may be due to the leanness of goat body composition. Large apparent volumes of distribution suggest that xylazine diffuses extensively from the central compartment. The rapid elimination seen in our and other studies (Garcia-Villar *et al.*, 1981) is probably related more to rapid metabolism rather than to a rapid

renal excretion of the unchanged drug. Previous studies carried out in rats (Duhm *et al.*, 1969) and cattle (Putter and Sagner, 1973) demonstrated that in rats, only 8% of intact or unchanged xylazine appears in urine while in cattle, less than 1% of the unchanged drug is eliminated in urine, 2 hours after its administration. It was further noted that xylazine undergoes rapid metabolism yielding about 20 metabolites in rats (Duhm *et al.*, 1969). In cattle, peak excretion of metabolites in urine has been shown to occur between 2 and 4 hours following administration of xylazine (Putter and Sagner, 1973), suggesting that xylazine is rapidly metabolized.

Following intravenous injection of xylazine in rats, Duhm *et al.* (1969) reported that the drug was rapidly distributed to the central nervous system, kidneys, pancreas, liver and thyroid. In this study, the half-time of distribution was very short (2.3 ± 0.6 minutes), indicating rapid distribution to, among other organs, the central nervous system, which could have been responsible for the initial excitement and restlessness noted within 1 to 5 minutes of the administration of xylazine. As has been reported in the horse and dog (Garcia-Villar *et al.*, 1981), plasma kinetics of xylazine relate well to some of the clinical effects it causes in goats. In our study, sedation produced by xylazine lasted for 25 to 30 minutes and recovery occurred within 40 - 50 minutes from the time of injection of the

drug, which correlates well with the half-life of plasma xylazine concentration observed in the present study.

Maximal effects of xylazine on arterial blood pH and blood gases occurred within 5 minutes of its injection. Its rapid distribution probably led to depression of the respiratory centre with rapid changes in the arterial blood pH and blood gases. Maximal changes in values of plasma glucose and insulin, haemoglobin concentration, red cell count, total serum protein, haematocrit, mean corpuscular volume, and total white cell count occurred 30 minutes after xylazine injection. This relates well to the 20 to 30 minutes half-time of plasma xylazine concentration. In contrast, in cattle, plasma kinetics of xylazine are difficult to relate to some of the sustained clinical effects it causes. The short half-life of 36.5 minutes (Garcia-Villar *et al.*, 1981) is in contrast with hyperthermia which can last for up to 18 hour post-injection (Young, 1979); hyperglycaemia of up to 24 hours (Eichner *et al.*, 1979); a duration of polyuria of up to 5 hours (Thurmon *et al.*, 1978); prostration after a high dose lasting for 36 hours (Clarke and Hall, 1969); and/or appearance of diarrhoea 12 to 24 hours after its injection (Hopkins, 1972). Sensitivity of cattle to xylazine may be related to its metabolism that may result in long-acting metabolites or could be as a result of a species difference in the number of α- receptors (Greene and Thurmon, 1988). One such metabolite (1, amino-2-6-dimethybenzene) is known

to be formed rapidly, probably from oxidative or hydrolytic breakdown of the thiazine ring, and appears in the urine of cattle within 4 hours following intramuscular injection of xylazine (Putter and Sagner, 1973).

In the present study, none of the differences observed in any of the pharmacokinetic parameters evaluated were statistically (P > 0.05) different between the three different environmental temperature and humidity conditions. This may be partly due to the small population sample size in the present study. Another reason to explain the lack of significant differences could be the mode of exposure of the goats to these conditions. In the present study, the goats were acutely exposed to the three respective environmental conditions without prior acclimatisation and, for only one hour. Previous studies conducted in cattle injected with xylazine (Fayed *et al.*, 1989) following acclimatisation to heat-stress (33° C; 63% humidity) and thermoneutral (18° C; 42% humidity) environmental conditions for 35 days showed that the sensitivity of cattle to xylazine under the two environmental conditions were different. The authors reported that animals in the heat-stress environment were more sensitive to xylazine than those in the thermoneutral environmental conditions. This might be due to the hormonal or neural changes that develop in heat-stressed animals whereby, heat-stressed animals have decreased metabolic rate as a result of decreased thyroid hormones, which might alter xylazine pharmacokinetic

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properties (Magdub *et al.*, 1982). Furthermore, it has been shown that acute exposure to heat and cold lead to changes in thyroid function (Johnson *et al.*, 1958; Yousef *et al.*, 1967) but, significant changes in thyroid activity occurred only after 60 hours of exposure in hot environments (Yousef *et al.*, 1967).

From the present study, there is evidence that acute exposure of goats injected with xylazine hydrochloride to three different temperature and humidity controlled environments for one hour does not significantly alter xylazine kinetic properties. Previous studies in cattle (Fayed *et al.*, 1989) injected with xylazine following their acclimatisation to heat-stress and thermoneutral environmental conditions for 35 days showed that the sensitivities of the animals to xylazine under the two environmental conditions were different, possibly due to altered hormonal or neural changes that result in altered xylazine kinetics and thus differences in sensitivity to xylazine. There is a need to investigate further the pharmacokinetics of xylazine in goats and other animal species which have had prior acclimatisation to different environmental temperature and humidity conditions for varying time periods. A bigger sample size than used in the present study is suggested in order to highlight significant differences in xylazine pharmacokinetic parameters where they may exist.

CHAPTER EIGHT

GENERAL CONCLUSIONS

From these studies, the following conclusions were drawn:

Xylazine hydrochloride, given intravenously at 0.1 mg/kg body weight in goats exposed to the three different environmental temperature and humidity conditions provided similar sedation and muscle relaxation for 25 to 30 minutes with uneventful recovery. The drug induced profuse salivation, urination and provided analgesia of the thoracic and abdominal skin, but analgesia was absent distal to the coronary band. However, environmental conditions did not significantly ($P > 0.05$) influence the onset and duration of these effects.

Acute exposure to the three environmental conditions did not have significant ($P > 0.05$) effects on cardiopulmonary and haemocytological variables, acid-base balance, arterial blood gas tensions, plasma glucose and insulin concentrations.

Xylazine hydrochloride induced bradycardia in all goats and atrio-ventricular heart block in some goats. Reductions in heart rate of 16 to 29% of baseline values in the

three environmental conditions were recorded. Even though mean heart rates remained below baseline values at the end of the 60 minutes monitoring period, it was only in the 14° C environment that the rate was significantly (P < 0.05) lower than baseline values.

Xylazine hydrochloride induced initial hypertension in the goats in the three environments within 1 to 2 minutes of its injection, followed by significant (P < 0.05) decline in mean, systolic and diastolic blood pressures.

Administration of xylazine to the goats in the three environments was followed by apnoea, irregular, laboured respiration, open mouth breathing and cyanosis of the visible mucous membranes. Although mean respiration rates remained below baseline values throughout the study period, it was only in the 14° C environment these values were significantly (P < 0.05) below baseline values.

Xylazine hydrochloride caused significant (P < 0.05) reduction in haemoglobin concentration, haematocrit, red blood cell count, mean corpuscular volume, total protein concentration and total white blood cell count while it significantly (P < 0.05) increased mean corpuscular haemoglobin concentration. All the above variables had not returned to baseline values at the end of the 60 minutes

monitoring period.

In all three environmental conditions, xylazine induced severe hypoxaemia, significant reduction in arterial blood oxygen saturation, and carbon dioxide retention at 5 minutes post-injection. Significant ($P < 0.05$) reduction in arterial blood pH with concomitant significant ($P < 0.05$) increases in arterial blood carbon dioxide tension, arterial bicarbonate ion concentration, total arterial carbon dioxide and actual base excess occurred as early as 5 minutes post-drug injection. These variables had not returned to baseline values at the end of the monitoring period.

Xylazine hydrochloride induced significant ($P < 0.05$) hyperglycaemia and hypoinsulinaemia in all three environmental conditions starting 15 minutes post-drug injection to the end of the study period. Maximal hyperglycaemic and hypoinsulinaemic effects were achieved at 30 minutes post-drug injection.

The body temperature of goats not injected with xylazine was not significantly affected by the three environmental conditions. Environmental conditions had significant ($P < 0.05$) effects on the body temperature of xylazine-injected goats. Xylazine-injected goats attained significantly ($P < 0.05$) lower body temperatures in the 14 and 24° C environments while they attained higher body temperatures in the 34° C environment. Body temperature had not returned to baseline values at the

end of the monitoring period. No thermoregulatory effector mechanisms came into effect to counter the decrease or increase in body temperature over the 60 minutes monitoring period.

Similar pharmacokinetic results of xylazine, not withstanding large individual variation were obtained, with no significant (P > 0.05) differences between the three environmental conditions for all pharmacokinetic parameters evaluated. Xylazine had a very short half-time of distribution and a large volume of distribution.

Changes in values of plasma glucose and insulin concentration, haemocytological, arterial blood gases and acid-base balance variables and duration of sedation correlated well with the half-life of plasma concentration of xylazine observed in this study.