THE FOLLOWING (WALKING) ABILITY OF THE NEONATAL LAMB

by

KWAME OPPONG-ANANE, B.Sc.Agric. (Hons) Ghana; M.Sc Aberdeen

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Department of Animal Sciences
Waite Agricultural Research Institute
The University of Adelaide

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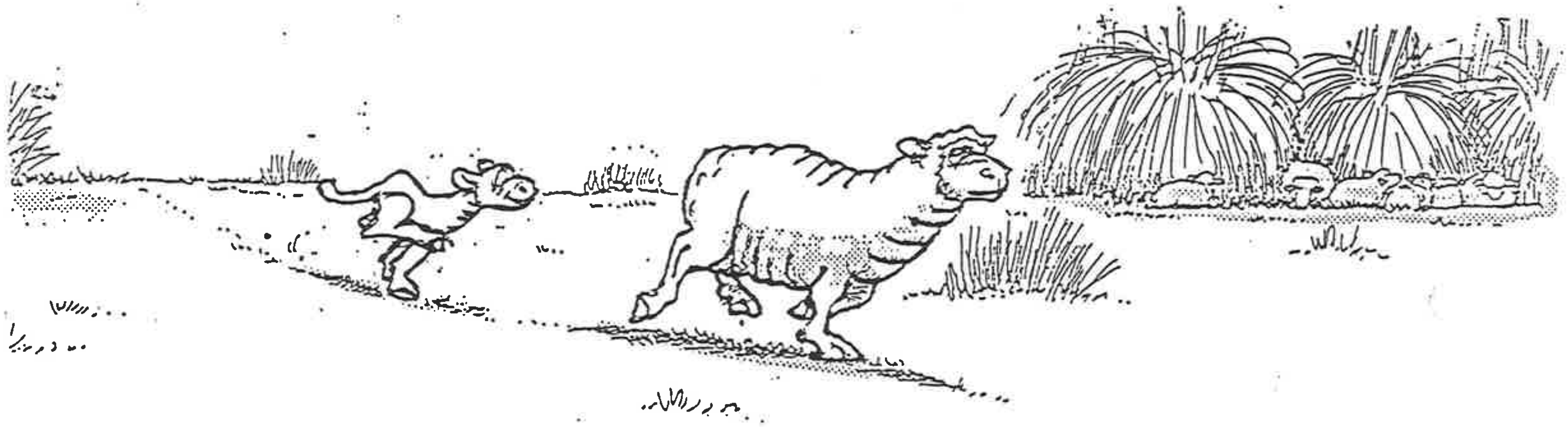
THE FOLLOWING (WALKING) ABILITY OF THE NEONATAL LAMB

A STUDY OF THE BEHAVIOURAL, METABOLIC AND PHYSIOLOGICAL RESPONSES OF THE NEONATAL LAMB TO WALKING



ABSTRACT

The majority of early lamb losses occur within the first 3 days of birth. Several factors such as the nutritional status of the ewe, maternal and lamb behaviour, adverse climatic condition and starvation contribute to the loss of lambs. The lamb's chances of obtaining adequate nourishment and surviving this critical period would be enhanced if it stays with the mother as she moves away from the birth site in her quest for food and water. The investigations presented in this thesis involve 18-h to 5-day old South Australian Merino lambs. In a few cases twin-born lambs were available for comparative studies. It examines the relationship between the strength of ewe-lamb bond and the lamb's ability to follow the mother, the energy availability and usage in the lamb, particularly for locomotion, and the metabolic and cardiorespiratory responses to locomotion.

Behavioural responses in lambs and ewes when separated 7 m away at 18 h post-partuum ranged from intense restlessness to no observable response. Approximately 58% of the lambs and 77% of the ewes exhibited the scaled maximal response to separation. The behavioural concern of ewe-lamb couples was significantly correlated ($r = 0.35$, $p < 0.02$). When released at a distance of 5 m from their own mother and an alien ewe 91% of the lambs were able to identify their mothers in a two-choice situation within 15 s. Similarly, 88% of the ewes were able to identify their lambs from a distance of 7 m. Mutual recognition tended to be higher in ewes and single lambs than in ewes with twins.

The behavioural interactions between 2-day old lambs and their mothers during long distance walking (1 or 2 km) in a narrow laneway showed that lambs with superior discriminatory ability of ewes followed their mothers better than those with lower recognition abilities. Ewes often succeeded in inducing a fair following response in lambs by emitting low-pitched bleats, performing naso-nasal contacts and allowing access to the udder for a few seconds.

Energy expenditure were determined by an isotopic dilution technique, and the distance moved by lambs, in simulated semi-arid conditions during the first 5 days of birth, measured with a pedometer. The mean daily distance moved by the lambs (2.3 km) was similar to the predicted value for the ewes. The proportion of the total daily energy demand of 1,300 kJ in the lambs which was devoted to locomotion amounted to approximately 1.4%.

Short and long periods of moderate treadmill exercise (3 x 5 min and 2 x 15 min work bouts at 1.0 m/s) and laneway following (2 x 0.5 km and 2 x 1.0 km) produced a greater than 2-fold increase in oxygen consumption as well as slight but significant elevations in blood lactate, pyruvate and free fatty acids, as well as in body temperature, respiratory and heart frequencies. The blood level of growth hormones was significantly elevated by exercise. Levels of most of the parameters returned to resting values within the 30-minute recovery period.

A linear relationship was observed between mass specific oxygen consumption and the speed of locomotion in lambs ($r = 0.90$, $p<0.001$). The energetic cost of locomotion in the neonates decreased with increasing speed of running and approached a minimum value. This minimum value, the incremental cost of locomotion, was identical to the slope of the curve relating oxygen consumption to the speed of locomotion (0.37 ml/kg/s). Body temperature, heart and respiratory frequencies, and stride length increased linearly as a function of locomotion. The lambs, like other mammals, responded to increased speed by changing both stride frequency and length. However stride frequency increased in a curvilinear fashion with increasing speed.

Maximal oxygen consumption (VO_2max) was determined in "untrained" and "trained" 5-day old lambs by treadmill exercise at a 7 or 16% incline respectively. "Trained" lambs had significantly higher levels of VO_2max (1.57 ml/kg/s) than "untrained" lambs (1.39 ml/kg/s). Similarly, values of aerobic scope were 6.6 and 5.6 for trained and untrained lambs respectively ($p < 0.05$). Maximal oxygen consumption measurements in the same individuals were highly repeatable ($r = 0.97$, $p < 0.001$).

The findings of this study suggest that the capability of lambs to seek and recognise their mothers within the first day of life is quite well developed. The results.highlight the well developed locomotory and discriminatory mechanisms that enable the lamb to successfully follow the mother, sometimes over long distances, during the neonatal stage. It shows that locomotory energy usage per se is not a limiting factor to survival provided the neonate is well fed. The results suggest that moderate running activities in neonatal lambs, like in other animals, are met primarily by energy produced aerobically. Above all, it points to the importance of mutual recognition in this critical period of the lamb's life.

DECLARATION

This thesis contains no material that has beenaccepted for the award of of any other degree or diploma in any University. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where the due reference is made in the text of the thesis.

I consent to this thesis being made available for photocopying and loan if accepted for the award of the degree

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PREFACE

Aspects of the work presented in this thesis have been reported elsewhere:

Abstracts

Oppong-Anane, K., Baudinette, R.V., Sabine, J.R., and Laube, S. (1989) Effect of mother-young recognition on following activities in neonatal lambs. Proc. Aust. Soc. Reprod. Biol. **13:** 57

Oppong-Anane, K., Baudinette, R.V., Sabine, J.R., and Laube, S. (1989) Metabolic responses and changes in blood growth hormone concentration during exercise in neonatal lambs. Proc.Nut.Soc. Aust..**14:** 110

Oppong-Anane, K., Baudinette, R.V., Sabine, J.R., and Laube, S. (1990) Following behaviour in single and twin born lambs. Proc Aust. Soc. Reprod. Biol. **14:** 127

Paper

Oppong-Anane,K.,Sabine,J.R.,Baudinette,R.V.and Laube, S. (1990) Metabolic and physiological response to sub-maximal intermittent exercise in neonatal lambs.Proc.Aust.Soc.Anim. Prod.**18:** 324,

GENERAL INTRODUCTION

The loss of lambs soon after birth constitutes a major source of reproductive wastage to the sheep industry (Hight and Jury, 1970; Haughey, 1973; Dennis, 1974; McCutcheon, 1981; Alexander, 1974, 1984; Slee and Stott, 1986). Although the impact of this loss varies greatly between breeds, flocks, localities, seasons and husbandry practices, a greater proportion of early lamb mortality, approximately 80%, occurs during the first 3 days of birth (Hight and Jury, 1970; Dalton et al, 1980).

The newborn lamb has a relatively small amount of body reserves compared with many other species (see Lindsay, 1986). These reserves, which amount to about 4000 kJ in an average sized lamb, are however sufficient to meet the energy requirements in the unsuckled lamb for less than a day in the cold, or about 3 days in a warm environment (Alexander, 1985). The neonatal lamb therefore needs to have access to the ewe's milk soon after birth if it is to survive the neonatal period.

Several factors including climatic conditions, nutritional status of the ewe, maternal and lamb behaviour and starvation are known to contribute to early lamb mortality (Hight and Jury, 1970; Haughey, 1983; Woolliams et al., 1983; McCutcheon et al., 1981; Alexander, 1960). Disease however plays a negligible role in lamb losses, and it takes very severe under-nutrition before a ewe has no milk for her lamb (Lindsay, 1986). In order to obtain adequate nourishment the neonate must follow the ewe as the latter moves away from the birth site in search of food and water. The following ability of the lamb assumes greater importance in maintaining mother-young contact in situations where food is scarce and watering points are far apart. Such situations occur in the semi-arid pastoral regions of Australia or under some traditional farming systems in tropical Africa where sheep are often left to fend for themselves far from the settlements. Following behaviour in the lamb possibly plays a major role in maintaining ewe-lamb contact (Winfield and Kilgour, 1976), particularly when lambs have to follow their mothers over long distances.

Several studies have suggested the existence of mutual recognition between the ewe and her lamb (Arnold et al., 1975; Shillito and Alexander, 1975; Alexander, 1977; Poindron and Schmidt, 1985). Nevertheless, most studies tend to place more emphasis on the role played by the ewe in the development and maintenance of the bond in view of the general observation by several workers, notably Smith et al. (1966) and Poindron and Le Neidre (1980) that the ewe was capable of exhibiting discriminatory behaviour between her own and alien lambs after a brief contact ranging from 30 to 120 min. All the major senses of recognition appear to be involved in the ewe's discriminating behaviour towards the neonate. Visual and auditory cues serve to bring dam and lamb together (Lindsay and Fletcher, 1968; Alexander, 1977; 1988; Shillito Walser, 1978), whereas the ultimate acceptance or rejection of the lamb by the ewe tends to depend on olfaction (Smith, 1965; Morgan et al., 1975; Alexander and Stevens, 1981).

The lamb's ability to discriminate between its own dam and other ewes was thought not to be well developed during the first week of life (Arnold et al., 1975). However, a later study by Shillito and Alexander (1975) showed that a high proportion of lambs were able to recognize a ewe within the first day of birth. Involvement of auditory and visual cues in this recognition has been suggested (Arnold, 1975; Shillito, 1975; Alexander, 1977), but these senses are poorly developed during the first week of life in the lamb in comparison with olfactory cues (Shillito, 1975; Alexander, 1977).

Lambs do not normally wander far from their birth sites nor run voluntarily at high speed during the first few days after birth. They often follow their mothers at walking pace, interspersed with submaximal running as the ewes move away from the birth sites in search of food and water. The neonates sometimes undergo short spells of exhaustive running in response to the mothers' bleats if they are separated or at the approach of predators. Some studies have been undertaken to evaluate substrate utilization and physiological changes in farm animals during work (see e.g. Faraci et al., 1984; Kuhlmann et al., 1985). However despite the numerous studies undertaken on causes of early lamb mortality and strategies for enhancing lamb survival, energy utilization and the physiological responses to locomotion in the neonatal lamb have not been studied. Similarly there are very few studies dealing with the following behaviour of the lamb (see Sguires, 1970; Winfield and Kilgour; 1976).

The investigations presented in this thesis involve 18-h to 5-day old single-born South Australian Merino lambs. In a few cases twin-born lambs were available for comparative studies. The project was designed to obtain relevant information in the following general areas:

(1) the relationship between the strength of ewe-lamb bond and the lamb's ability to follow the mother.

(2) the energy availability and usage in the lamb, particularly for locomotion.

(3) the metabolic and cardiorespiratory responses to locomotion.

The investigations were designed to provide answers to these specific questions:

(1) Is the lamb capable of establishing discriminatory behaviour for its mother within the first day of birth?

(2) Is the neonate capable of following and keeping in close proximity to the mother over long distances travelled? Is the lamb's ability to follow the mother influenced by its own discriminatory ability of the ewes, as well as the dam's ability to recognize it ?

(3) What is the daily energy requirement and how much of this is devoted to following the mother in her daily movement activities during the first 5 days of life? Secondly, how far does a lamb cover daily during the first 5 days of life under simulated semi-arid pastoral conditions?

(4) What are the effects of both short and long periods of running on substrate utilization and cardiorespiratory responses in the lamb?

(5) What are the metabolic and cardiorespiratory responses to the range of locomotory speeds likely to be employed by the neonate in the field?

(6) How much aerobic capacity is a 5-day old lamb capable of during exhaustive running such as may be required in escaping from mammalian predators?

The study is therefore a blend of behaviour and physiology; the combination of factors which is the basis of all animal adaptation. By investigating aspects of the critical early period in the life of the lamb the project aims to gain an insight into the behavioural, biochemical and physiological mechanisms involved in following behaviour of the neonatal lamb. The information provided by these investigations will be employed in a future study to determine if any of the factors involved in the following ability of the lamb has a significant influence on early lamb mortality.

CHAPTER 1. LITERATURE REVIEW

1.1. EARLY LAMB MORTALITY

1.1.1. INTRODUCTION

Early lamb mortality is a major source of reproductive wastage to the sheep industry (see Hight and Jury, 1970; Haughey, 1973; Dennis, 1974; McCutcheon et al., 1981; Alexander, 1984; Slee and Stott, 1986), but its impact varies greatly between breeds, flocks, localities, seasons and husbandry practices. Based on an average mortality of 20%, it is estimated that losses from birth to marking (when lambs are on the average four weeks old) amount to about 11 million lambs in Australia annually (McGuirk, 1982). In the extensive pastoral conditions of Australia, where lambing paddocks are often large and supervision is minimal or absent, early lamb mortality could, however, reach as high as 70% on some properties (Alexander, 1985). Early lamb mortality is economically significant even under more intensive systems of sheep farming, such as in the United Kingdom, where it accounts for as much as 4 million lambs annually before weaning (Weiner et al., 1983).

Lamb mortality does not only result in a loss for the purpose for which the lambs are intended, such as meat and/or wool, but also an economic cost in that the ewes produce less wool and require more feed than if they had not been pregnant. Furthermore, ram and management costs per unit of lamb produced increase (Hight and Jury, 1970).

Perinatal lamb mortality, defined as lamb losses during or within the first 7 days of birth, may approach 90% of all rearing losses (Dalton et al., 1980; Haughey, 1983), and in exceptional cases, may account for more than half the lambs born (Haughey, 1983). The majority of these failures, approximately 80%, occurs within the first three days of birth, the neonatal period (Hight and Jury, 1970; Dalton et al., 1980), with about a third of the deaths occurring at birth, and a similar proportion within 24 h of live birth (McMillan, 1983). The neonatal period is therefore critical in the reproductive mass and recruitment patterns of sheep populations.

The wide variation in early lamb mortality rates reported in the literature for various sheep production systems, and in particular for localities of similar environmental conditions, may sometimes arise as a result of different techniques applied in estimating the losses. This makes comparison of data on the magnitude of deaths difficult. Lamb mortality may be assessed directly from carcass collection, or indirectly by classifying ewes individually at marking on body condition, fleece quality and presence of parturient stains indicative of recent birth (see Alexander, 1984). The complete recovery of dead lambs for a direct estimation of rates of mortality is tedious and difficult to achieve. On the other hand, the figure obtained from the indirect method reflects minimal loss since it takes no account of cross mothering or the death of one individual from a multiple birth (Haughey, 1983).

Two broad approaches to assessing the causes of lamb deaths have been employed. The first method, using an autopsy, has been used in large scale surveys in which dead lambs are collected and examined for evidence of the causes of death. This also provides a basis for classifying lambs according to the stage at which death occurred relative to birth, i.e. ante-parturient, parturient or post-parturient (Haughey, 1983). The second approach depends on direct observation of behaviour at lambing, together with measurements of temperature and weight changes in lambs (see Alexander, 1985). This method is time consuming and demands a large number of observers and a reliable radio communication system to ensure continuous and comprehensive observations. Haughey (1983) indicated that the autopsy method, supported by appropriate microbiological and histopathological examination, is the simplest and most reliable method of determining causes of death, but an adequate sample (at least 50 carcasses) randomly collected to represent mortality throughout lambing, needs to be examined in order to arrive at a realistic assessment of the relative contributions that various entities make to total mortality. Again this is a labour intensive and expensive procedure.

1.1.2. CAUSES OF PERINATAL LAMB MORTALITY

Perinatal lamb mortality has a complex aetiology involving the actions and interactions of several factors (Hight and Jury, 1970; Haughey, 1983). Primary causes of lamb mortality, even in intensively shepherded flocks, are often poorly defined (Hight and Jury, 1970). Most researchers agree on the catalogue of factors that contribute to lamb mortality, though there is still controversy about their relative importance. Such causes have generally been classified as major or minor, but causes that are minor on a national scale can be catastrophic to the individual producer (see Alexander, 1984; 1985). Other than the two main prenatal factors influencing lamb survival, namely nutrition of the ewe and weather conditions during pregnancy, problems with parturition, starvation-exposure syndrome, arberrant maternal behaviour and predation are mostly considered as the major causes of perinatal mortality.

1.1.2.1. PROBLEMS WITH PARTURITION

The factors contributing to problems with parturition in sheep have not been fully determined (Alexander, 1985). It has been acknowledged, however, that a great variety of physical factors associated with the lamb malpresentation, feto-pelvic disproportion resulting from small maternal pelvic size or fetal oversize can contribute (Thompson, 1974; George, 1975; 1976; Haughey 1983; Woolliams et al., 1983). Failure of the cervix to dilate, primary or secondary inertia due to lack of uterine tone or exhaustion of the dam (Woolliams et al., 1983), or any of these factors combined may lead to delayed or difficult birth (dystocia). Injury to the central nervous system due to hypoxia may occur during difficult birth, frequently resulting in the death of the lamb during or within a few hours of birth (Haughey, 1983).

(1) Stillbirths

Stillbirth is defined as the expulsion of a fully developed but dead lamb which if born alive, might normally be expected to survive outside the uterus. Lambs in this category are characterised by the presence of some positive signs of viability during parturition, such as oedema of the head. Stillbirth often result from anoxia during a delayed birth (Woolliams et al., 1983). Though the study of Woolliams et al. (1983), showed no significant difference in the incidence of still births between singles and twin born lambs, relatively more triplets were either stillborn or died as consequence of delayed birth. This has been been attributed in part at least to intra-uterine competition among the fetuses (Hinch et al., 1986).

(2) Dystocia

Dystocia may be classified as to whether it arose from a disproportion in the relative dimension of the fetus to those of the maternal birth canal, (presentation usually being normal in the upright cranial presentation), or from malpresentation of the fetus or fetuses (Woolliams et al., 1983). Dystocia has been found to be responsible for between 15 to 20% of all lamb deaths (Dennis, 1965; Hight and Jury, 1970; George 1976; Dalton et al., 1980), while the incidence in individual flocks may vary between breeds, parity and age of ewes, season of lambing and stocking rate,.as well as ewes differing in pelvic inlet size.

(*i*) Parity and age of ewes

The incidence of dystocia in a flock tends to decrease with parity and age of ewes. George (1976) observed significantly higher numbers of dystocia case in nulliparious Dorset Horn ewes having both single and multiple births than in their multiparous counterparts. The two-year-old ewes in the flock exhibited significantly higher incidence of dystocia than any other age group. Smith (1977), on the other hand, found no ewe-age effect on dystocia in purebred lambs. Whereas the combined year-ewe effect significantly affected dystocia in crossbred lambs. However in both populations, multiple births increased dystocia in yearling and ewes four years and older, and reduced dystocia in two-and three-year old ewes. The increase in dystocia normally found in yearling or first parity ewes may be attributed to their smaller size (Smith,1977) which puts them at a disadvantage when faced with delivering large lambs (Woolliams et al., 1983), whereas the increased dystocia in older ewes may be related to age *per se* (Smith, 1977). In the case of malpresentation as the main cause of dystocia, Woolliams and colleagues (1983) found that ewes over the fourth parity were usually the worst.

(*ii*) Breed

The studies of George (1975; 1976) and Smith (1977) showed a significant influence of breed on the frequency of dystocia in purebred lambs. The level of dystocia in purebred populations observed by Smith (1977) ranged from 12% in coarse wool to 22% for Corriedale, and the level of dystocia in Finn crossbred lambs was less than half (6%) the incidence in Rainbouillet crossbred lambs. The renowned easy lambing in Merinos is indicated by a low dystocia incidence of a mere 4% as opposed to 36% of lamb deaths in Dorset Horn ewes lambing at the same time under similar conditions. The high incidence of dystocia observed in the Merinos was comparable with that reported for the Romney Marsh by Quinlivan et al. (1966). The reason for breed differences in incidence of dystocia may result from difference in the width of the pelvic inlet or the diameter of the pelvic passage (Groomers et al., 1985).

(*iii*) Season and stocking rate

Effects of season and stocking rate on frequency of dystocia is not well documented. However the work of George (1975; 1976) showed a significant effect of both parameters in Merino and Dorset Horn ewes studied in the same locality. Single bearing Dorset Horn ewes experienced significantly higher incidence of dystocia in winter and spring than in summer, whereas multiple bearing ewes showed no seasonal variation in dystocia frequency. Merinos on the other hand exhibited a high incidence of dystocia only in spring lambings.

The higher incidence of dystocia shown in spring lambings in the Dorset Horns and Merinos, as well as in winter in the Dorset Horns, may be due to differences in pastoral conditions during the last four to six weeks of gestation. In spring the rapid growth of pasture and improved nutrition may result in a rapid increase in fetal size in late pregnancy and a subsequent large lamb at birth. This is much less likely to happen in winter when pastures are mature. The high incidence of dystocia in spring lambing could also be due to the fact that the ewes were weaker, having been pregnant in the harsh winter months, whereas the winter and summer lambing groups were in stronger condition at lambing having been pregnant during autumn and spring respectively, where in general there was adequate pastures (George, 1975).

Effect of stocking rate on incidence of dystocia is conflicting. No significant difference between the population of ewes exhibiting dystocia was observed at three different stocking rates of eight, 12, and 16 ewes per hectare, either within season or for all seasons combined in the Dorset horn ewes (George, 1976). In case of the Merino ewes studied concurrently, the lowest incidence of dystocia observed at the lowest stocking rate (eight ewes per hectare), was probably due to the ewes being stronger than those at higher stocking rates as a result of bigger land available to the ewes for grazing. The mean body weight of ewes stocked at eight per hectare was slightly greater than the others, yet the mean birth weight of the lambs were almost identical (George, 1975). This could indicate that the ewes in the lowest stocking rate, being better fed prenatally, were capable of enduring the birth process with less difficulty than those at higher stocking rates which might have had a stressful pregnancy and lambing.

(/V) Birth weight and type of birth

That a dystocia as a major cause of death in large and single born lambs is strongly supported by the literature (Hight and Jury, 1970; George, 1976; Smith, 1977; Dalton et al., 1980; McMillan ,1983; Woolliams et al., 1983). However its importance in the death of multiple born lambs, particularly at low birth weights cannot be underrated. The proportion of both single and multiple born lambs dying from dystocia increases curvilianeary with birth weight (George, 1976; Dalton et al., 1980; Mc Millan, 1983). At low birth weights the risk of dystocia in multiple births is slightly higher than single births and is probably caused by weak lambs and ewes, the ewes having poor uterine contractions causing birth to be slow (Dalton et al., 1980). Birth weights have a minimal influence on dystocia at levels near the mean value(George, 1975; Smith, 1977). The importance of dystocia at higher birth weights, specially in single born lambs, suggests feto-pelvic disproportion as the main contributing factor.

(V) Pelvic inlet

The size of pelvic inlet has been implicated in dystocia in sheep in a number of studies (Quilivan, 1971; Fogarty and Thompson, 1974; Woolliams et al., 1983; Grommers et al., 1985). Ewes experiencing dystocia tend to have significantly smaller pelvis than those that have normal births (Quilivan, 971; Fogarty and Thompson, 1974). Dorset Horn ewes observed by Fogarty and Thompson (1974) were heavier than the Border Leicester ewes, however lamb birth weights were similar in both breeds. The larger pelvic area (estimated as the product of the conjugate and transverse diameters in the pelvis) due to a large conjugate diameter in the Border Leicester ewes, might have rendered the breed less prone to dystocia than the Dorset Horn ewes. The study of Naaktgeboren et al. (cited by McSporran and Fielden, 1979) in which dystocic Texel sheep were found to have approximately 10% greater conjugate and transverse diameters and a 40% higher lamb birth weight in comparison with the eutocic Heath ewes, supports the observation by Fogarty and Thompson (1974) that pelvic size does not necessarily correlate strongly with size of lamb at birth. The relevance of pelvic size to the incidence of dystocia was confirmed through radiographic studies on Romney ewes of different lambing performance by McSporran and Fielden (1979) who concluded that incompatibility in size between a ewe's pelvis and her lamb was largely responsible for the need of repeated assistance at birth in the dystocic group. They indicated that selection for large pelvic inlet by using external measurements as a means of reducing dystocia in a flock was not practical since it ignores the importance of birth weights. Selective breeding for freedom from dystocia in both ewes and rams appears to be a more effective way of overcoming the problem of difficult birth.

1.1.2.2. BIRTH INJURY TO THE CENTRAL NERVOUS SYSTEM

Birth injury to the central nervous system (CNS) has been indicated as the most important single cause of perinatal lamb mortality by Haughey (1983). He considered CNS injury as causing both neonatal and parturient deaths, with a combined prevalence of between 56 to 86% of all prenatal deaths. In lambs dying during or within a few hours after birth, apparently as a result of dystocia, the incidence of CNS injury was 100% This unexpectedly high importance of birth injury has been disputed by other workers (Alexander et al., 1980; Duff et al., 1982). A study by Duff and co workers (1982) showed birth injury in only a third of starved and exposed Romney lambs with an overall mortality of 16%. They concluded that birth injury to the CNS was only a minor cause in starvation implicated deaths because the injury observed was slight and the proportion of injured lambs that had milked prior to death was similar to that of uninjured lambs. It is however acknowledged that birth injury plays a significant role in deaths attributed to starvation-mismothering-exposure syndrome since moderate injury may prejudice lamb survival primarily by impairing locomotory and suckling drive (see McCutcheon et al., 1981; Haughey, 1983). This resulted in starvation and secondary hypothermia because they were unable to replenish with milk fetal energy reserves depleted by thermogenesis in the cold.

1.1.2.3. STARVATION-EXPOSURE SYNDROME

Post mortem examination in many lambs is seen to exhibit signs correlated with both starvation and exposure. Consequently these deaths are frequently combined into a single classification, which may include deaths considered to have been due to simple starvation, simple exposure and to combined effects of both. The starvation-exposure syndrome may account for up to half of all neonatal deaths (see McCutcheon et al., 1981).

(1) Starvation

Starvation occurs frequently in lambs of all types of birth, the incidence is however more common in multiple born lambs. It accounted for as much as 42% of all classified deaths as opposed to 15% mortalities in single born lambs in a New Zealand study of straightbred Romney and Border Leicester x Romney ewes (Hight and Jury 1970).

The newborn lambs depends on maternal milk supply for survival since their own energy reserves at birth amount to a mere 4000 kJ. The latter comprise of 60 to 70% in the form of lipid, about 15% in the form of glycogen in liver and muscles and the remainder from protein catabolism (see Alexander, 1974). Since the metabolic rate of lambs ranges from about 72 kJ/h at thermoneutrality to about 360 kJ/h at maximum thermogenic effort, and about 100 kJ/h at the maximal rate sustainable in the cold, the energy available to an average sized unsuckled lamb from the break down of body reserves will be sufficient to permit survival for about 3 days in a warm environment. Survival time may however be remarkedly reduced in cold environment to about 10 h or less (see Alexander, 1985).

Reasons for the failure of the lamb to obtain adequate nourishment include failure of the ewe to bond with her lamb, "accidental" separation after bonding, competition with litter mates, birth problems that affect the behaviour of ewe and lamb (see Alexander, 1984) and any impairment or loss of normal mammary function (Hayman et al., 1955). The decline in teat seeking activity when the lamb does not suck successfully would also be expected to reduce the chances of the lamb ever sucking successfully (Alexander and Williams, 1966). The chances of such a lamb surviving will be negligible.

The effect of depleting energy reserves on the behaviour of the lamb is not well documented. However Alexander (1962) observed that newborn lambs subjected to starvation, were apparently asleep for much of the time after initial teat seeking, or they tended to stand with back hunched uttering feeble cries. Muscular strength and activity in the lambs diminished progressively and finally a stage was reached where they were unable to stand and exhibited symptoms of coma, rolling eye movements, paddling leg movements and excessive salivation. The symptoms were more pronounced in the cold.

(2) The effect of high temperature on lambs

Factors influencing lamb mortality in hot environments such as the tropics or during summer in sub-temperate regions have received very little attention, mainly because most of the sheep breeding populations are found in the temperate and sub-temperate regions of the world where lambing usually occurs during cold weather. Deleterious effects of high ambient temperature on perinatal lamb survival have however been shown in a few studies, notably those of Morgan et al. (1972) and Stephenson and Suter (1984); neither of which however was undertaken in the tropics.

In a comparative study of lamb survival in winter (maximal daily temperature of 21°C to 28°C) and summer (maximum daily temperature exceeding 37°C) lambings, Morgan et al. (1972) observed no deaths in winter born lambs nor in summer born lambs held in the shade during the first day of life. By contrast 55% of summer-born lambs held in unshaded pen died, and lamb losses in this group to six weeks of age showed a 5-fold increase over winter-born lambs held in an unshaded pen. The surviving summer born lambs exhibited improved heat tolerance indicated by declining body temperature, heart and respiratory rates. It was not clear however whether the improvement in heat tolerance was due to improved efficiency of hoemeothermic mechanisms, such as respiratory evaporative heat loss, or to increasing protection from solar radiation by growing fleece. Similar evidence of a direct effect of high ambient temperatures on lamb survival was observed in the work of Stephenson and Suter (1984). In this study, lambs born and exposed to shadeless conditions during immediate post-natal life at 36°C had a lower survival rate than those born in moderate temperatures (28°C), though the difference (84 vs 65%) was not significant. The strong shade-seeking behaviour by ewes with lambs during the heat of the day in summer in the study of Morgan et al. (1972), was no doubt beneficial to the lambs' survival. These workers argued however, that because shade seeking was equally prevalent in pregnant ewes, the behaviour was probably a response to the ewes' own discomfort rather than a response aimed solely at protection of lambs.

Since lambs born in hot environments are severely stressed by heat, often accentuated by the lack of shade (Smith, 1961; Morgan et al., 1972), newborn lambs required to follow their mothers for any distance, whilst exposed to high ambient temperatures, are at a particularly high risk of rapid exhaustion and prostration. This could lead to mismothering and subsequent death from starvation, predation and heat failure (Alexander et al., 1982; Moule, 1954; Smith, 1961). The early post-natal death of lambs in hot environments also suggests hyperthemia and dehydration as causes of death (Haughey, 1983), with small lambs being more susceptible (Alexander, 1974).

(3) The effect of low temperature on lambs

The lamb is normally delivered in a physiologically advanced state from a warm uterine environment (about 39.5°C) to an external environment which could be lower than 0°C in the temperate region (Vermorel and Vernet ,1985). In order to survive the immediate post natal period in such an environment, the lamb has to maintain its body temperature by rapidly increasing its heat-producing capacity at least three times basal level (Dawes and Mott, 1959; Vermorel and Vernet, 1985). It is suggested that an increase of this magnitude is necessary since the lamb's oxygen uptake per unit weight in utero is similar to that of its mother, and since its surface area per unit weight is 3 times that of its mother (Dawes and Mott, 1959). The increased heat production may be achieved by muscular shivering and by non-shivering thermogenesis in brown adipose tissue, an important source of energy storage and heat generation in the newborn lamb (Alexander, 1979). When the lamb's "maximum sustainable" metabolic rate in the cold is exceeded by the rate at which heat is lost to the environment, deep body temperature falls resulting in hypothermia, a situation often encountered in lambs that have been separated from their mothers over a considerable period in a cold climate. The newborn's ability to withstand cold exposure may be an important factor in establishing a secure maternal bond with adequate suckling (Slee and Springbett, 1986), since ewes are known to loose interest in hypothermic lambs.

Deaths attributed to hypothermia may be classified as either primary or secondary. Primary hypothermia may be due to maximum heat production or "summit metabolism" being reached so that heat loss caused by severe climatic conditions such as cold, wet windy weather, exceeds the physiological capacity for heat production even in the presence of adequate energy reserves (Alexander, 1962 ; Andrew and Mercer, 1986; Haughey, 1983). Secondary hypothermia on the other hand, is due to exhaustion of energy reserves through sustained sub-maximal drain on the reserves by relatively mild climatic conditions not adverse enough to comply with the requirements for occurrence of primarily lethal hypothermia (Alexander, 1962 ; Haughey, 1983; Andrew and Mercer, 1986). In summation over time, the cost of this thermoregulatory heat production may exceed the sum of body energy reserves at birth, plus any exogenous supplies received in milk (Andrews and Mercer, 1986). However adequate colostrum intake enhances heat production capacity in the newborn lamb up to nearly 20% of summit metabolism as a consequence of increased supply of carbohydrate substrates to the heat-producing tissues. Colostrum intake soon after birth would therefore be of immediate survival benefit to the newborn lamb (Earles and Small, 1981).

Small lambs are particularly susceptible to cold exposure because they have a large surface area relative to body mass from which to lose body heat (Alexander, 1974,1985; Slee, 1978; Haughey, 1983). For example, the ratio is about 0.12 m^2/kg in a 1 kg lamb but only 0.07 m^2/kg in a 4 kg lamb. Thus small lambs must expend more energy per unit of body weight than larger lambs to produce heat under cold conditions. In addition they tend to have shorter and less dense coats than larger lambs, due largely to failure of secondary follicles to mature and produce wool. The thermal insulation of the coat of small lambs is therefore low, thus per unit of surface area, small lambs will tend to exchange heat more rapidly into the environment that large lambs (Alexander, 1974). Within breeds, small lambs would also tend to posses smaller energy reserves (mainly fat deposits), less efficient skin insulation in the form of subcutaneous fat (Alexander, 1974; Slee, 1978) and lower thyroid hormone levels (Vermorel and Vernet, 1985).

There may be variation between breeds, not only in lamb viability itself, but also in the relative importance of different components affecting both viability and susceptibility to cold exposure (Slee, 1981). Field observations by Sykes et al. (1976) showed that the Scottish Blackface newborn lamb was less susceptible to cold exposure than Tasmanian Merinos, indicated by the greater ability of the Blackface to maintain normal rectal temperature in conditions that caused the Merino to become hypothermic.

The importance of the birthcoat in cold exposure is also evident from the study of Slee (1978), where breed differences in cold resistance were paralleled by differences in birthcoat length. The two long-coated breed types exhibited about a 6-fold increase in cold resistance over the short-coated types. Moreover, within a breed, the long-coated Welsh lambs were over 4 times as cold resistant as the short-coated type. The relationship between birth coat type and lamb survival however is not a consistent finding in Australia (Mullane, 1966; Obst and Evans, 1970).

1.1.2.4. ABERRANT MATERNAL BEHAVIOUR

Aberrant maternal behaviour in sheep is generally considered a major contributory factor to deaths in newborn lambs (Alexander, 1960; Obst and Evans, 1970; Shelley, 1970; Arnold and Morgan, 1975; Stevens et al., 1982), particularly in flocks of sheep lambing for the first time (Alexander and Peterson, 1961). Any deviation from the normal pattern of maternal behaviour such as pre-lambing maternal interest in other lambs, leading to temporal or permanent adoption, delayed or lack of grooming of lambs, delay in first suckling or refusal to suckle, adoption and swapping of lambs after birth (Alexander, 1960) may leave the ewe without the necessary epistemic response to ensure survival of her offspring. Ewes that have not as yet lambed may exhibit maternal interest, such as being attracted to the placental fluids and newly-born lambs of the other ewes. This behaviour may lead to adoption or 'stealing' of alien lambs (Alexander, 1960), however most ewes loose interest in alien lambs prior to the birth of their own lamb (Alexander, 1960; Shelley, 1970; Arnold and Morgan, 1975) or during labour, and thereafter give their attention only to their own lambs (Shelley, 1970). The alien lambs which are left without maternal care may die, usually as a result of starvation and/or exposure.

Alexander (1960) observed occurrence of pre-lambing maternal interest as early as two weeks prior to lambing in 2% of the ewes in a flock. However Arnold and Morgan (1975) observed pre-lambing maternal interest only within 8 h of lambing with the majority of ewes doing so only within 2 h of lambing. Incidence of pre-lambing maternal interest among ewes, though generally low, varies quite considerably. Shelley (1970) and Alexander (1960) reported incidence between 6 and 15% among Merino ewes. High maternal interest however was displayed by 21% of a mixed flock of sheep studied by Arnold and Morgan (1975), and by 25 and 32% in two groups of Coriedale x Dorset Horn ewes studied by Winfield (1970). The interest is less prevalent in young than in older ewes (Shelley, 1970).

The incidence of lamb deaths resulting from pre-lambing maternal interest is also variable as, for instance, it accounted for only 3% loses in Merino lambs (Shelley, 1970), but 10% losses in a winter lambing flock studied by Arnold and Morgan (1975). Lamb losses attributed to pre-lambing maternal interest could be quite high in some isolated cases, such as the 20 and 34% losses observed in two groups of sheep by Winfield (1970). The incidence is however unlikely to be a major source of lamb mortality generally for a number of reasons. Firstly, the ewe usually loses interest in the alien lamb before the birth of her own lamb, thus allowing the mother of the alien lamb to resume normal care. Secondly, the mother of the lamb rarely deserts it or is forced away. Thirdly, if the interest continues into labour, the ewe usually becomes diverted and allows the lamb to be led away by its own mother. Finally, in the event of interest continuing throughout labour, the ewe normally rejects the alien once her own lamb is born. There may also be an adoption of a deserted lamb by a ewe that has lost her own lamb at birth (Alexander, 1960; Winfield, 1970; Arnold and Morgan 1975), which could be beneficial to the survival of the lamb where it does not result in a subsequent desertion (Arnold and Morgan 1975).

Ewes that lamb at about the same time and physically close together, as in high stocking intensities, facilitate contact between poorly mothered lambs and alien ewes interested in adopting lambs. However lambs reared by alien ewes do not grow as fast as those reared by their own dams, possibly due to some delay in being adopted by alien dams or, as in the case of some separated single born lambs, being reared as twin lambs (Winfield 1970). Desertion may take the form of a complete lack of interest in the lamb after birth, with the ewe walking away from the site of birth, or there may be temporal desertion for a few minutes to a few hours. Desertion is more common with twin than single births, especially among Merinos. For example, if twins are born several metres apart, one may be neglected and become lost or may be repelled as an alien if contact is remade after the critical receptive period has passed, as imprinting of one lamb does not guarantee acceptance of others in the same litter. However twin losses are more likely to occur where the ewe moves away from one site to another or is frightened away by the presence of man or another animal. One lamb is likely to be left behind, since some ewes, particularly Merinos, appear to take several days to recognise that they have more than one lamb (see Alexander et al., 1982). Incidence of desertion of lambs by ewes may be minor, as observed in the study of Arnold and Morgan (1975) where only 4% desertion were observed, leading to just 2% lamb mortality. However it is often considered an important aberrant maternal factor particularly in primaparious ewes where in one study it was found to be responsible for between 17 to 22% lamb deaths (Shelley 1970; Winfield, 1970).

Aberrant ewe behaviour may also influence a lamb's behaviour and its subsequent survival, by denying or restricting the its access to the udder or by delaying the time taken for it to have its first drink (Alexander, 1960; Arnold and Morgan, 1975). Such behaviour is more of a problem among primaparious ewes (Alexander, 1968). Though Arnold and Morgan (1975) found no ewe in the lambing flocks they studied denying her offspring access to the udder during the first few hours after birth, a few of the ewes failed to stand still while suckling attempts were being made. However the time from birth to first suckling was not influenced by the failure of the ewe to stand still. Such a ewe moved so as to remain facing her lamb as it commenced to seek the udder, thereby preventing it from suckling. The behaviour was usually temporary, but in 5% of the ewes persisted until the lambs ceased their nursing attempts (Alexander 1960).

1.1.2.5. PREDATION

Predation has long been considered by producers in certain localities as a major cause of loss in lambs, but there is little published evidence as to its importance to the sheep industry in general. Perinatal losses of 14 to 50% in Merino (Moule, 1954; Smith, 1964) and 36% in Border Leicester (Smith, 1964) lambs resulting from predation have been reported for some properties in Central Queensland. Predation was also found to be a problem on some individual farms in Western Australia (Davies, 1964). It is however the view of some workers that predators are often blamed for more deaths than they cause (Dennis, 1969; 1974) by farmers seeking to explain their losses. The farmers claims are based on the presence of potential predators on a farm or large proportions of carcasses showing mutilations (Dennis, 1969). This view is supported by a number of studies which showed lethal predation as a minor cause of lamb mortality, accounting for only 2 to 5% of lamb losses (Mc Farlane, 1964; Dennis, 1969; 1974).

Predators vary in importance in various sheep producing regions and localities. Lamb losses arising from predation in Australia are mainly attributed to dingo (*Canis familiaris dingo*), domestic dog (*Canis f. familiaris*), fox (*Vulpes vulpes*), feral pig (*Sus scrofa*) , crow (*Curvies spp.*), and wedge-tailed eagle (*Aguila audax*) (Rowley, 1970; Ferguson, 1982). In some parts of Tasmania, the Tasmania devil (*Sarcophilus harrisii*) may be a nuisance (Rowley, 1970). Although of minor occurrence, attack by ants appears to be a constant cause of lamb mortality in South Western Queensland (Smith, 1964). The magnitude of losses arising directly from predation however is difficult to assess, since some lambs are already dead when they are mutilated and others would have died even if they had not been attacked and mutilated (Smith, 1964; Watson, 1972). Furthermore, lambs may be completely devoured or removed by predators and thus not included in observed loss (Watson, 1972).

In view of the uncertainty of the accuracy of assessing losses due to predation, mortalities can only be attributed to primary predation when the carcass shows extensive and severe antimortem trauma and no other gross lesions (see Haughey, 1983), where the predator was seen to attack the lamb, where there was clear evidence of the lamb having been alive at the time of attack (e.g., haemorrhage from wounds), or the lamb still living when seen after an attack (Smith, 1964). Where predation is not the primary cause of death, damage is mostly inflicted after death or at the point of death. Most lethal effects of predation occur within two hours of birth, particularly during a prolonged parturition, or immediately after, or towards the end of the first week of life. Most of the damages observed on lambs in the Central Queensland studies were inflicted by dingoes and feral pigs. Foxes and ravens however were responsible for damages in Western Australia, where many carcasses had evidence of attack by both predators. Raven attacks commonly followed those from foxes attack but the reverse apparently did not occur. Foxes are generally selective in their approach and commonly attacked lambs from below by grasping the sternum and fracturing the ribs. In contrast, domestic dogs attack from above,grasping the lamb behind the withers, spreadeagling the lamb and crushing the chest (Dennis, 1969).

Although crow attacks on healthy lambs are rare compared to the other major predators in Australia, they are sometimes considered the most important cause of lamb mortality in some flocks. Lambs are usually attacked soon after birth and while still poorly coordinated, and the birds characteristically peck at the eyes, tongues, ears, umbilicus and perineum (Smith, 1964). The main feeding by the birds, however, occurs when the afterbirths have been shed. The birds will fight and squabble for this delicacy. Another favourite is the early faeces of the lamb which contain high proportions of fat and protein; these early faeces are probably a very nutritious food supply. Some of the pecking around the breech area of the lamb is likely to be an attempt to obtain faeces sticking to the hair coat, rather than a direct attack. Crows are normally driven off by the ewe or discouraged by the vigorous antics of a healthy pecked lamb. Even in a few cases where lambs do not resist their probing and pecking, the birds soon gave up the attack. Pecking has been observed more often on Border Leicester than on Merino lambs, probably due to the more sensitive skin of the Border Leicester breed. Even so, the injuries to the lambs appeared fairly minor (Anon., 1974). These observations, however, suggest that crows are mere scavengers, being more interested in the afterbirth and faeces of the lamb than inflicting injuries that could result in the death of lambs.

1.2. EWE AND LAMB BEHAVIOUR AT LAMBING

1.2.1. INTRODUCTION

The precocity of the newborn lamb enables it to stand and move away from the birth site within a few hours of birth. This could easily lead to separation and subsequent death, particularly from starvation, if the lamb is not sought by the dam or completely adopted by another ewe. It is therefore essential that a discriminative relationship or bond is established between the ewe and her lamb soon after birth, to ensure that they stay in close physical proximity to each other during the first few days after birth. This behaviour depends on the development of a rapid and exclusive mutual recognition between the mother and offspring which enables the ewe thereafter to actively reject all alien young (Collias 1956). The early attachment of ewe and lamb is a complex process in which apparently innate and acquired responses are involved (Smith, 1965) and normally commences a few hours, or in a few cases, a day or two prior to lambing.

1.2.2. PRE-PARTURIENT MATERNAL BEHAVIOUR OF THE EWE

Close to parturation the behaviour of the female changes, then and even more so after parturition. For the offspring the behaviour of the mother is critical for its learning and adjustment to the environment into which it is born. The mother, by her response to the separate acts of the young, may shape its behaviour (Arnold and Dudzinski, 1978). Most ewes cease grazing and withdraw from the flock an hour or so prior to lambing (Smith, 1965; Kilgour, 1972; Alexander, et al., 1982). There appear to be breed differences in the ewes' desire to seek isolation prior to lambing. This behaviour is exhibited to a greater degree by feral sheep and sheep that have reverted to semi-range conditions, which may move out of the flock groups for up to three to four days before their lambs are due (Kilgour, 1972). Whereas Romneys actively isolate themselves from the rest of the flock prior to lambing (Welch and Kilgour, 1970) parturition in the Merino usually begins while the ewe is with the flock. The parturient ewe and her newborn lamb are left behind while the flock graze on (Alexander et al., 1982). Unless anything untoward happens, such as a serious outside disturbance or having to lamb on very steep slopes, or a protracted birth process, the lamb will be delivered and bonding between ewe and lamb will take place at the birth site (Kilgour, 1985). Differences in stock management, such as paddock size and stocking density may account for much of the variation seen between breeds (Arnold and Dudzinski, 1978)

There would be obvious survival advantages in a ewe selecting a birth site that provided protection from weather and safety from misadventure (Alexander, 1988). There is, however, no indication that either wild or domestic sheep appear to have any innate wisdom or foresight in the selection of birth sites which will provide the maximum opportunity for the lamb survival (see Alexander, 1988; Knight et al., 1889). Many of the birth sites observed in a New Zealand study were exposed to the weather, were too small for both the ewe and lamb(s), or were situated so that lambs would easily slip into swamps or water courses. Choice of birth sites appear to be influenced by the comfort of the ewe rather than by any safety consideration for the lamb (Knight et al., 1889). Isolation-seeking behaviour by the pregnant ewe is not always a definite and distinct movement, but more in the nature of a gradual trend in movement. The distance moved by pre-parturient ewes from the main flock depends upon the shape of the enclosure. A slope or depression which could accentuate separation from the flock is frequently favoured, and so too is proximity to a hedge (Smith,1965). Mountain sheep, on the other hand, are said to be attracted by security of high cliffs (Pitzman; Geist, cited by Alexander, 1988).

Most ewes are strongly attracted to their own amniotic fluids or to fluids of other parturient ewes. The site of lambing appears therefore to be determined fortuitously by where the placental fluids are first spilled, and the ewes normally remain at the site of spillage unless disturbed, licking and pawing the ground (Alexander et al., 1982; Smith, 1965). This restriction of movement enhances the probability of the ewe finding lambs where they dropped (Smith, 1965). The attraction of the fluids to other parturient ewes,however,may in some cases result in undue interference in births.

The ewe's desire to seek isolation prior to lambing may be advantageous in facilitating the establishment of an exclusive satisfactory relationship between the ewe and her newborn lamb (Kilgour, 1972; Alexander et al., 1982). The process of bond development between the mother and young in seclusion progresses without any interruption from other ewes which might otherwise have shown interest in the lamb (Welch and Kilgour, 1970), with a possible consequence of lamb poaching. Isolation may provide the ewe a better chance of protecting the newborn lamb from predation. Under domestic conditions however the pre-parturient ewe's desire for isolation may sometimes lead her to a very exposed place which may be detrimental to the well-being and survival of her offspring (Kilgour, 1972), and may also make the ewe more susceptible to predator attack than if she is with the flock (Arnold and Dudzinski, 1978).

1.2.3. POST-PARTURIENT MATERNAL CARE AND BOND FORMATION

1.2.3.1. ONSET AND DEVELOPMENT OF MATERNAL CARE

Isolation seeking and interest in newborn lambs exhibited by some ewes prior to lambing suggest that the birth of their own lamb stimulates a pre-existing responsiveness. The appearance of the lamb not only allows manifestation of maternal behaviour in the dam, but also starts to influence the development of that behaviour (Poindron et al., 1979), which is crucial to the survival of the offspring. Immediately after birth there is a period during which the newborn must learn to find a teat and suck successfully if it is to survive. The mother must also learn to recognise her offspring otherwise it could be easily separated or lost. If ewes are not able to distinguish their own newly born lambs from those of other ewes, there could be considerable confusion over "ownership" of lambs, particularly where several ewes bearing twins give birth simultaneously (Lynch and Alexander, 1979).

Goats may recognise their kids soon after birth by 'labelling' them, which appears to be based upon cues passed from mother to her kid directly through licking and indirectly through the dam's milk. Licking may transfer rumen microfauna to the kid's body surface, while milk uptake and subsequent defecation may influence mouth, body and anal odours respectively (Gubernick et al., 1979; Gubernick, 1981). Although no such assertion has been made for the sheep, a study of maternal acceptance of naturally suckled lambs and artificially-fed lambs showed that ewes are able to distinguish between lambs of the two treatment groups, and it seems likely that ewe-fed lambs have been labelled in some way through close association with other dams (Alexander et al., 1989). It has been suggested however in a study by Alexander (1978) that the origin of the specific odour by which ewes recognise their lambs in the early post-partum period derives from the lamb and not from maternal labelling via milk or saliva. Smith (1965) suggested that licking and low pitch bleating in the ewe are instinctive and could provide a basis for latter attachment.

Insufficiency of contact between ewe and her lamb after birth, resulting from factors such as disturbance from management practices, approach of a predator, or interference of other ewes, may lead to failure of the development of an exclusive attachment resulting in subsequent rejection of the lamb when contact is remade later. The immediate post-partum period, during which ewes are still ready to accept newborn from whom they are separated at birth, has been called the sensitive period. The duration of this sensitive period in the attachment of the ewe and offspring is not clearly defined, though thought to be brief (approximately 12 h), varies considerably between individual ewes. Collias (1956) observed that separation of lambs or kids from their mothers shortly after birth for 2 h or more is likely to lead to rejection of the offspring by the mother. Such rejection is shown by persistent withdrawal from the nursing attempts of the young or by active butting. In contrast separation of shorter periods of 5 to 45 min resulted in complete acceptance by the mothers. On the other hand, Smith et al. (1966) observed that when lambs were separated from their mothers 5 min to 8 h after birth, all but one were readily accepted, and concluded that only a brief period of contact between ewe and offspring was required for the establishment of a discriminative and enduring attachment.

Poindron et al. (1979) observed a significant difference between ewes in maternal receptiveness to the length of separation of lambs. They found two out of eight Merino ewes to exhibit maternal care after 24 h of separation, though a decline in maternal care was most marked during the first 12 h after birth. These observations on the duration of the sensitive period are not conclusive in view of the fact that only a small number of ewes (ranging from 2 to 8) were used in each study. Furthermore experimental conditions for the studies varied widely. It may be inferred from these investigations that the appropriate stimulatory conditions for the development of discriminative maternal behaviour in sheep may be effective for only a brief period shortly after parturition. The effective period may however be considerably prolonged by enforced contact between dam and young, such as by preventing the behavioural response the dam normally exercises to avoid contact with alien young (Hersher et al., 1983).

1.2.3.2. PHYSIOLOGICAL REGULATION OF MATERNAL CARE

In sheep the onset of maternal behaviour is closely associated with parturition, which indicates that some physiological changes occurring at parturition may be involved in eliciting maternal responsiveness. This manifestation of maternal behaviour appears to be under endocrine regulation (see Le Neidre et al., 1979) since responsiveness in the ewe can be induced under the influence of rapidly declining progesterone and rapidly increasing oestrogen levels. Any decline in maternal responsiveness in the immediate post-partum period could also be delayed by administering oestradiol benzoate, a parturition inducing hormonal compound, to the ewe, resulting in high plasma concentrations of oestradiol and prolactin (Poindron et al., 1979). Vaginal-cervical stimulation (Keverne et al., 1983; Basiouni and Gonyou, 1988) and the presence of amniotic fluids (Basiouni and Gonyou, 1988) are also known to be associated with the development of maternal behaviour in the ewe.

Mechanical stimulation of the genital tract immediately prior to the introduction of alien lambs improved the maternal responsiveness of post-parturient ewes, indicated by intense licking and emission of low-pitched bleats toward newborn lambs (Keverne et al. 1983). By contrast, in the study of Basiouni and Gonyou (1988), cervical stimulation failed to affect the ewes' behavioural measures significantly, but did improve final acceptance of lambs when compared to controls . These different findings may be due to the different techniques used in the two studies. Basiouni and Gonyou (1988) used the fingertips to exert moderate pressure to expand the cervix of at least 48 h post partum ewes throughout the stimulus period, whereas Kervene et al. (1983) used either a vibrator to stimulate the vagina, or inserted a rubber bladder into the uterus. The bladder was inflated and maintained in place for 5 min before being expelled from recently delivered ewes, or ewes previously treated vaginally with progesterone impregnated sponges followed by subcutaneous injection of B-oestradiol. The maternal responsiveness of the treated ewes in terms of less aggression, intensive licking, emission of low pitched bleats and acceptance of suckling was much lower in the work of Basiouni and Gonyou (1988), as their ewes were presumably no longer in a susceptible hormonal state at the time of the trial.

In contrast to the above findings Poindron and Le Neidre (1980), working with Merino ewes, showed that passage of the lamb down the birth canal is not essential for multiparous ewes to become maternal. Their results suggest that, even in the absence of vaginal stimulation, the normal endogenous hormonal preparation of the ewe, together with previous experience, is sufficient for ewes to become maternal. Stimulation of the birth canal however may be necessary for the manifestation of normal maternal behaviour in inexperienced, primaparious ewes.

It has been suggested that the main difference between endocrine manipulation and cervix-vaginal stimulation is that hormones induce a response several hours or days after the end of treatment whereas the mechanical stimulation of the genital tract elicits an immediate acceptance of the lamb. This vaginal stimulation appears to have a major role in onset of maternal behaviour in second born lambs which may in some cases be dropped long after the ewes have established a selective behaviour pattern with the first-born lambs (Nowak, 1989).

1.2.3.3. INFLUENCE OF THE EWE ON EARLY ACTIVITIES IN NEONATAL LAMBS

A few minutes after its expulsion at birth the lamb lifts and shakes its head, bleats, and moves its head spasmodically. It then begins to "right" itself by turning into a sternally recumbent position, then intermittently, and at first without much effort, to push up on its hind legs. Later it gets up on its extended hind legs and "knees" (metacarpal bone) and then, at first unsteadily, it rises onto all four feet by extending its forelegs (Vince et al., 1985). After standing, the lamb attempts to find and suck the udder. Exploratory activities towards the dam typically take the form of "nosing" with the head fully extended on the same level as the trunk. For these activities the neonate may be assisted or frustrated by the strategic position of the dam. A ewe with a good maternal behaviour generally orients with its head towards the newborn and stands quietly while udder-seeking attempts are being initiated (Owens et al., 1985; Lynch and Alexander, 1983), while young and inexperienced ewes tend to rotate away from teat-seeking attempts (Lynch and Alexander,1983). In the first position, termed "parallel inverse", the ewe grooms and sniffs the anal area of the lamb. Most ewes arch their backs and crouch slightly when the lamb approaches to suck the udder, thereby lowering the udder closer to the lamb (Owens et al., 1985).

Stephens and Linzell (1974) showed in a study of newborn kids born both to normal mothers, and to does with 1 or 2 mammary glands transplanted to the neck, that the initial approach of kids appeared to be orientated primarily towards the mobile rounded form of the dam's ventral surfaces, with random yet directional movement. The udder was apparently identified by its tactile and thermal characteristics as sucking was attempted when the mouth contacted a smooth hairless surface, whether this was a teat located in either a normal or abnormal position, or even a surgically exteriorised skin-covered vein. Similar behaviour is expected in the newborn lamb. These cues however are unlikely to be the only sensory factors involved in teat seeking, since there is evidence that teat searching behaviour is possibly facilitated by olfactory cues from the inguinal wax which is secreted by a gland at the base of the udder (Vince and Ward, 1984)

Alexander and Williams (1964), working with Merino ewes, found that maternal assistance by grooming and directional orientation facilitated the progress of the lamb toward successful sucking. However neither grooming nor orientation alone facilitated the sucking drive. Maternal assistance also resulted in a significantly earlier increase in body mass in artificially or naturally groomed lambs. On the other hand Klopfer and Klopfer (1977) and Vince et al. (1985) showed that tactile stimulation had an inhibitory effect on the activity of neonatal kids and lambs respectively. Effects of touch vary with site stimulated; massage of the hind quarters elicits leg extension, whereas the same type of stimulation applied to the top of the head results in a forward and downward movement of the head with fewer leg movements, thus having a quietening effect (Vince et al., 1985).

1.2.3.4. THE INFLUENCE OF THE CHARACTERISTICS OF THE NEONATE ON MATERNAL CARE

The post-partum period, during which the maternal responsiveness to the lamb is under hormonal control, is limited to only a few hours. During this period the experience of the ewe by contact with the neonate stimulates the maintenance of maternal responses beyond the initial sensitive period (see Poindron et al., 1979; Poindron and Le Neidre, 1980). There are reasons to believe that the shift in the regulation of maternal behaviour from a hormonal to a non-hormonal basis may have evolved among mammals as a means of speeding reproduction. It serves to free the endocrine system to initiate a second reproductive cycle while completing the behavioural and lactational phases of the first cycle (Rosenblatt et al., cited by Rosenblatt, 1980).

Though the extent of the attractiveness of the newborn lamb to its dam has not been fully clarified, there is little doubt that the presence of amniotic fluids on the lamb is a major factor influencing the manifestation of post-parturient maternal behaviour (see Poindron and Le Neidre, 1980; Alexander et al., 1989; Vince et al., 1985; Lynch and Alexander, 1985; Smith, 1965). There is evidence to suggest that amniotic fluid from different births may vary decisively and that a ewe can be induced to follow cloth soaked in the amniotic fluid of her lamb (Collias, 1956; Smith, 1965). The same cloth presented to other ewes invariably induced a rapid sniffing, but not following (Smith, 1965). Though amniotic fluid repels non-pregnant ewes, the interest exhibited by pre-parturient ewes in the fluids and wet newborn lambs of other ewes confirms the probable role of amniotic fluids in maternal responsiveness.

The age of the lamb has been shown to influence the responsiveness of the ewe. Experiments in which the lambs of multiparous Prealpes-du-Sud and Merinos were exchanged with either an alien newborn lamb less than 18 min old and never licked by its own dam, or a 12 to 24 h old alien lamb, showed that the newborn lamb was significantly more acceptable. These results were consistent with those of a similar study by Poindron (1980) with Merino sheep in Western Australia where the exchange of 12 to 24 h old lamb with an alien newborn lamb 10 min later confirmed the fact that some characteristics of the lamb at birth influenced the initiation of maternal behaviour. The reduced maternal acceptance of both alien newborn lambs and 12 to 24 h old lambs after the initial 30 min or more of mother-young contact was also in agreement with the finding of Smith et al. (1966), who observed that a period of licking and nuzzling of approximately 20 to 30 min appears to establish a basis for discriminative behaviour in parturient ewes. In the study of Poindron (1980) and Smith et al., (1966) selective bonding with their lambs was established in all ewes within 30 min after delivery.

The behaviour of the newborn lamb could also be involved in its attractiveness to the dam. Since the newborn lamb is much less active than older neonates, its lower motor activity could influence acceptance by the mother. Further evidence of the lamb's behavioural influence on the dam's responsiveness is shown by the fact that ewes tend to butt alien lambs away, mainly when they try to stand up or approach but rarely when they lie down or stand still, suggesting that the relative immobility of younger lambs favours their acceptance (Poindron et al., 1980).

1.3. MOTHER-YOUNG RECOGNITION

1.3.1. INTRODUCTION

Within a few hours of its birth the lamb loses some features of attractiveness to the dam that were formerly important factors influencing its acceptance at delivery. Similarly, maternal responsiveness fades rapidly after parturition. The lamb provides cues essential for the maintenance of maternal behaviour and needs to be able to identify and stay in close proximity with its mother to enhance its chances of surviving the perinatal period. There is therefore a need for the development of a mechanism for the continuity of discriminative attachment between ewe and her lamb.

The roles of the special senses vitally involved in the development and maintenance of the infant-mother bond have been described in studies with many animal species. Despite several early reports indicating the involvement of sensory cues in the mutual recognition in sheep beyond the immediate post-partum period (see Morgan et al., 1975), it is only during the last 2 decades that the role played by the major senses of recognition, olfactory, visual, and auditory, has been studied in detail.

1.3.2. RECOGNITION OF THE LAMB BY THE MOTHER

The ability of the ewe to discriminate between her own and alien lambs is generally well developed within a few hours of parturition (Morgan et al., 1975; Shillito and Alexander 1977b). There are two distinct factors in the dam's discrimination between lambs; one is the recognition which can be made at some distance, whereby the mother goes directly to her own offspring, and the other is the recognition and final acceptance of the offspring when the dam is in close contact with the lamb (Morgan et al., 1975; Arnold and Dudzinski, 1978). All the major senses for recognition appear to play a role in the ewes' recognition of lambs, with visual and auditory cues possibly serving first to bring dam and lamb together. Ewes are known, however, to use olfactory signals as the most specific and decisive information in either accepting or rejecting suckling advances in lambs (see Smith, 1965; Morgan et al.,1975; Alexander and Stevens 1981; Alexander, 1988).

Olfactory cues for specific recognition could emanate from several regions of the lamb's body (Alexander, 1978), though ewes seem to prefer to spend more time investigating the anal region foı identification of lambs (Smith, 1965; Shillito and Alexander, 1975; Collias 1976; Alexander, 1978). The importance of olfactory cues in the recognition of the lamb by its mother has been demonstrated in experiments involving the deprivation of this cue in the ewe by surgical removal of olfactory bulbs (Baldwin and Shillito, 1974), by destruction of the olfactory epithelium with a solution of zinc sulphate (Poindron, 1976), or by scrubbing the lamb in detergent solution to suppress the olfactory cues (Alexander and Stevens, 1981). Suppressing the senses of smell prior to parturition in the studies of Baldwin and Shillito (1974) led to the inability of ewes to distinguish their own from alien lambs at close quarters, almost all the ewes allowed indiscriminate suckling. This might have influenced Morgan and co-workers (1975) to conclude from their study that without the use of olfactory cues the ewe will invariably accept an alien lamb irrespective of size. This assertion was not entirely in agreement with the results of an earlier study by Lindsay and Fletcher (1968). In the work of Morgan et al. (1975), the senses were impaired for periods of up to 3 months prior to lambing, and as such the ewes' sensory perceptions were certainly abnormal during the sensitive period of attachment to lambs. The deprivation of the olfactory senses may have well led to compensatory reliance of the other senses (Alexander and Shillito, 1977a).

Muting auditory cues by anesthetizing the vocal chords, and blackening various regions of the lamb's body to suppress visual cues provided evidence on the role of both auditory and visual cues in ewes recognition ability of lambs (Alexander, 1977; Alexander and Shillito 1977b). Suppressing the senses either separately or together slowed the movement of a ewe towards her lamb, indicating the dampen of the cues that elicit maternal attraction. Interference with visual cues only however introduced an element of uncertainty in the form of hesitation or dodging as the ewe approached her lamb; a behavioural response indicating possible recognition of an alien (Shillito and Alexander, 1980).

The high incidence of uncertain behaviour observed by Alexander (1977) and Alexander and Shillito (1977b) when the eye area or the crown area of the lamb was blackened indicated that visual cues that assist the ewe to distinguish her own lamb from aliens at a distance come largely from the head region. Colouring the front legs, tail, or rump together with the tail, had little effect on the ewe's recognition ability. The greater interest shown by ewes in mute lambs in comparison with those that have had movement cues impaired by anaesthetising indicates a complementary effect of lamb activity on visual cues (Alexander and Shillito, 1977a).

The bleating of lambs appears to be a call for attention, but whether their vocalisations are used in individual recognition by ewes or merely serve to orient the ewe towards the general direction of the lamb has been a matter of dispute. The general observation of Lindsay and Fletcher (1968) that a lamb's bleating acts as a non-specific signal serving to draw the attention of the ewe during separation from its young is in agreement with the findings of Alexander (1977). There appears, however, to be specificity of auditory cues in the lamb's call (Shillito Walser, 1978; Alexander and Shillito, 1980). Further evidence of this specificity was shown in studies involving Mesham and Clun Forest ewes by Smith (1965), and in Merinos by Poindron and Carrick (1976), in which ewes were able to recognise recorded bleats of their own lambs.

Though both the senses of hearing and of sight have been shown to be used in the identification of the lamb, visual stimuli are more important in assisting the ewe to approach the lamb and stay nearby. Lambs' bleats normally serve to attract attention and help the ewes to orient to the lambs' position (Shillito Walser, 1978).

1.3.3. RECOGNITION OF THE MOTHER BY THE LAMB

Newborn goats have the procedural and locomotion ability quickly to discriminate and achieve close proximity to their mothers within 48 h of birth. This represents a considerable achievement for so young an animal, for it involves the ability to distinguish identifying characteristics of the dam to match sensory information (Licketer and Herman, 1984). Shillito and Alexander (1975) observed that a high proportion of lambs were able to recognise a ewe as an alien even on the first day of life, with recognition ability improving with age. The earlier development of the lamb's ability to recognise a ewe as an alien is probably influenced by the fact that the lamb, as a "follower" species, spends more time with its mother during the neonatal period than the young of a goat, a 'hider' species, which is left behind while the dam moves away in search of food. It has been proposed however that the young of a "follower" species spend less time in close physical proximity to their dams as they mature, whereas the young of a "hider" species gradually come to spend more time in close proximity to their dams as they mature (Lent ; cited by Licketer, 1985). By contrast, studies by Arnold et al. (1975) indicated that recognition ability in lambs was not well developed within the first week of life. The discrepancy in these results has been suggested by Alexander (1977) to be due to variation between breeds, in that Merino lambs in the study of Arnold and associates (1975) would be slower in recognising their mothers than lambs of a number of English breeds. Since crossbred lambs are more active than purebred lambs (Stevens and Alexander, 1984), it is likely that crossbreds would be able to recognise their mothers earlier. Poindron and Schmidt (1985) found that 95% of four-week old lambs reared outside were able to recognise their mothers, compared to 72% of those reared indoors, suggesting that environmental conditions can significantly influence the lamb's ability to recognise its dams at a distance.

The role of olfaction in recognition of the dam is not well defined. Whereas olfactory cues are believed to assist a newborn lamb in the eventual location of the teats of the dam (Smith, 1965; Vince and Ward, 1984), it was suggested by Arnold et al. (1975) that olfactory cues could have very little effect on a lamb's overall ability to recognise its dam. It has been observed in several lambs that on reaching the udder region of an alien but apparently co-operative ewe, they rapidly withdraw, as if the udder region holds cues for recognition, such as scent and / or texture (Anon; cited by Shillito and Alexander, 1977). The importance of the udder region in olfactory examination by the lamb is indicated by the fact that a lamb, on reaching a ewe, shows no sign of nosing the ewe except in the udder region. Using newly born Clun Forest 'suckled' lambs, aged between 3 and 21 h old, and "unsuckled" lambs taken at birth with minimum olfactory experience of the dam, Vince and Ward (1984) observed that lambs could significantly discriminate between the inguinal wax of their mothers and that from alien ewes. They postulated that the wax probably contains individual characteristic substances which play a part in recognition of the dam, and hence in maintaining the bond between a ewe and her lamb. They felt that the fact that even unsuckled lambs could discriminate the smell of their own dam's from alien ewe's inguinal wax and amniotic fluids suggests that the ewe provides olfactory stimuli reflecting pre-natal experience which can facilitate teat-seeking activity.

The involvement of both auditory and visual cues in the recognition of ewes by lambs have been demonstrated in a number of studies (Arnold et al. 1975; Shillito, 1975; Alexander, 1977). These cues by contrast with olfactory ones, are however, poorly developed in the first week of life (Shillito, 1975; Alexander, 1977). Moreover, evidence for the relative importance of the 2 senses is conflicting. By depriving lambs of visual or auditory cues, Arnold et al. (1975) and Alexander (1977) demonstrated that the importance of visual cues relative to auditory cues increases with age, indicating some degree of learning in recognising ewes. The importance of visual cues in distance recognition of ewes by lambs was observed by Shillito (1975). She found that lambs of Clun forest, Finish, Jacob, Dalesbred and Soay breeds of sheep could identify their mothers from two alien ewes at a distance of 16 m, and would run to them. On the other hand, about 30%of the lambs failed to identify their mothers when the ewes were not visible. She suggested that the Jacob lambs rely more on sight than sound in recognition in comparison with Dalesbred lambs, since Jacob ewes vary more in individual appearance.

Lambs tend to respond to maternal calls by bleating, although the extent of this varies with breeds. Using recorded bleats, Shillito Walser and Walters (1987) demonstrated that week old lambs respond to the bleats of their mothers more frequently than those of alien ewes. Furthermore, most lambs do not search for their mothers during separation if the ewes are not bleating (Shillito, 1975).

1.4. SHEEP MOVEMENT ACTIVITIES

1.4.1. WALKING BEHAVIOUR IN ADULT SHEEP

Literature dealing specifically with routine daily movement activity in sheep is very scanty, mainly because of the difficulty in recording exactly where the sheep have travelled. Walking distance and frequency of drinking are very often closely associated when food is in short supply. In small paddocks, however, with abundant green feed and water, the distance walked by animals is small and involves mainly movement whilst grazing. Under extensive conditions, with natural water freely available, the animals will range further for feed and need not travel far for water, but when only limited watering points and dry feed are available walking to and from water accounts for most of the distance travelled (Arnold and Dudzinski, 1978).

Louw (cited by Creswell, 1960) used a checkerboard technique in studying walking behaviour in sheep, in which he divided a pasture into 8 ha squares by means of stakes. With hourly observation by means of binoculars he traced the movement of sheep breeds in relation to the stakes. It was concluded from 4 breeds and crosses studied that Blackhead Persian and Dorset x Persian were more active than Merino and Karakul breeds. All the sheep walked more in summer than in winter, possibly on account of the longer days in summer. Using a similar technique in an acre of lowland pasture over a period of 12 consecutive months, Tribe (cited by Creswell,1960) observed that sheep walked on the average 4.19 km daily; with about 80% of the walking distance covered during daylight hours (7.00 to 19.00 h)

The above studies on assessment of the distances walked by sheep were based on the observed positions of the ewe at specific intervals relative to that of previous sightings. The distance obtained therefore reflects the shortest distances that could have been undertaken during each two consecutive observation periods, but not the actual distances travelled. Creswell (1960) used a device consisting of a light-weight harness which was attached to two metal shafts on a wheeled frame to measure distance travelled by sheep in different terrains in New Zealand. An automatic distance recorder similar to that used on bicycles, recorded the number of wheel turns. He observed that, in general, Cheviot and Romney sheep walked about 12.9 and 8.2 km per week respectively on hill farms. Longer distances were undertaken on flat lowlands, 15.8 and 13.0 km per week by Cheviot and Romney sheep respectively, indicating that the shorter-legged Cheviots were more active. A rise in the observed activity of pregnant ewes, however, contradicts the shepherd's traditional belief that ewes in lamb spend more time lying down as parturition approaches. However lactation did not result in an increase in the distance covered.

Studies by Squires and associates (1970; 1971; 1972; 1974) in the semi-arid regions of Australia produced more precise estimations of daily movement distances covered by sheep. In these investigations, a large, treeless paddock covering 1,400 ha with watering points about 1.5 km apart was used. Further, an attempt was made to simulate the field situation in which sheep graze progressively further and further out from water as the season progresses. Accordingly a long and narrow (6 m) laneway, bare of vegetation, shadeless and flat, in which feed containing 15% sodium chloride was provided at points that were separated from the water supply by distances varying from zero to 5.6 km, was set up. An automatic unit incorporating both mechanical and photographic recorder was also used in the laneway. In a comparative study of walking behaviour in sheep of contrasting breed and physiological status, it was found that Border Leicester and Merino sheep both walked similar distances, maintaining 2 journeys daily to water until the distance separating food from water reached 4 km When the food was separated 4.8 km from the water source however, Border Leicesters maintained the twice daily drinking frequency and walked between 17.6 and 17.8 km per day, whereas Merinos reduced their drinking frequency to once a day and walking distance was 9.8 to 13.6 km per day (Squires and Wilson, 1971).

The mean daily distance travelled by a flock of 1,000 Merino sheep as a whole in the large paddock, determined by reference to established routes traced from air photos, as well as by fixed positions obtained during the course of observations, exceeded 7 km per day.

On its way to water the flock was led by a few sheep, usually in a single file walking along well defined paths, with the rest of the flock widely scattered, thus resulting in a rectangular-shaped configuration. On the return trip there was a tendency to reverse the configuration so that the flock moved on a broad front with a long tail of stragglers (Squires, 1974). It was concluded from another study (Squires, 1970), involving twenty Merino wethers carefully matched for weight and height, that Merino sheep must drink more than once daily if water intake is to exceed six litres. Though the observation by Squires and Wilson (1971) shows that it is possible for sheep to regularly walk 12 km or more per day, it must be realised that this consumes a large proportion of their potential grazing time, resulting in reduced feed intake despite the increased energy lost associated with walking (Squires, 1970). Sheep undertaking such a long journey over a prolonged period of time will undoubtedly loose body condition, concomitant with deterioration in their general welfare.

The speed of walking in the sheep observed by Squires et al. (1972) was mostly within the range of 1.5 to 2.9 km per hour (0.42 to 0.81 m/s). Walking speed on route to water was greater than the speed on the return journey and there was a general increase in speed as the sheep approached water (Squires et al., 1972). The mean values of 2.48 and 2.19 km per hour, representing speeds of sheep to and from water respectively in the first study of the series (Squires, 1969), showed a consistent and significant difference ($P < 0.01$).

1.4.2 F0LLOWING BEHAVIOUR IN LAMBS

In infant ungulates the following response occurs initially as a generalized response, by which the infant attempts to maintain its proximity to objects of an appropriate size range which are moving away or tangential to it (Winfield and Kilgour, 1976). When a ewe move away from the birth site, several hours after birth, the newborn lamb is required to follow and the ewe will return to the lamb if it does not walk after her. This following behaviour is particularly strong in sheep, and it is abnormal for ewes to move out of auditory range of their lambs during the first day or two of life (Alexander 1980). Nevertheless, a lamb could be easily separated from its mother at this stage as a result of flock movement associated with management practices, disturbance by man, other ewes or predators, and especially if the attachment between the two is not well developed.

The following response in lambs is especially important for survival of twins. As the ewe moves, perhaps to go to water or as the result of disturbance, only one twin may follow; this occurs especially if one lamb, usually the second-born, is less vigorous than the other. In the early post-partum phase some Merino ewes appear "satisfied" by the presence of one lamb and make no attempt to return to the deserted twin. Failure to ensure that both lambs 'follow' leads to widespread separation of the twins and the ewe may have difficulty in keeping track of, and suckling, both lambs (see Alexander, 1980).

A study involving lactating ewes with lambs at foot, either confined to a laneway which simulates field condition of a semi-arid region or to a small yard, showed that at no time did ewes and lambs operate independently in the former's quest for food and water. Furthermore, day old lambs were seen to follow their mothers even in moderate heat (27°C) over the 2.2 km separating food and water supplies (Squires, 1970). Following ability is particularly important in situations where food and water are sparse and newborn lambs have to follow over long distances in their mothers' search of food and water. Lamb losses prior to weaning can be very high (40 to 50%) under these conditions which are typical of the extensive and semi-arid region of Australia that support about 30% of the nation's sheep population (see Squires, 1970).

1.5. ENERGY INTAKE AND EXPENDITURE IN THE FIELD

1.5.1. FIELD METABOLIC RATES IN ANIMALS

1.5.1.1 INTRODUCTION

Indirect calorimetry has been widely used to measure metabolic rates of animals. In virtually all instances, however, the laboratory environment to which an animal is subjected during the period of study bears little or no resemblance to the natural environment. The application of the closed circuit system of indirect calorimetry depends simply upon physical gas laws. Into the typical metabolism (respiratory) chamber, usually dehumidified and carbon dioxide (CO_2) free, for instance, is placed an animal that is usually post absorptive. As the animal consumes O_2, the CO_2 and water produced are absorbed and thus any change in volume (after appropriate temperature and pressure conditions) is due to the O_2 consumed. In some instances, O_2 concentrations in the metabolism chamber may reach very low levels and affect the metabolism of the animal being studied. For prolonged experiments oxygen is added to maintain a specified pressure (Mullen, 1971; see Grodzinski and Wunder, 1975).

In the open circuit (flow) system of indirect calorimetry, the animal is placed in a relatively small volume respirometer (see Grodzinski and Wunder, 1975) or made to wear a face mask with tubing. Ambient air is drawn at a known rate of flow through the chamber or mask and the concentration of O_2 in the inflowing and outflowing gases are determined (see Fedak et al, 1981; Glesson and Baldwin, 1981). Both the closed and open systems of indirect calorimetry are restrictive in their use, and obviously can not be used in the estimation of metabolic rate in free-living animals.

Several methods have been devised for measuring field metabolic rates in animals. Such methods include radio isotope excretion rates, biotelemetry of heart rate and doubly-labelled water (DLW) techniques. The first two techniques are not completely satisfactory for many reasons but have been used with limited success. The most promising method, the

DLW technique, however, is quite expensive, complicated and normally gives only a long term average for integrated metabolic rates (see Grodzinski and Wunder, 1975). The shorter the attempted measurement period, the greater the sensitivity of the DLW technique to errors, and the less certain are some assumptions made in the data analysis (Stein et al., 1987). The costs of employing this technique in the determination of field metabolic rates have been reduced in recent years by the use of highly sensitive mass spectrometers which require minimal enrichment of body water (see Blaxter, 1989).

5.1.1.2 DOUBLY-LABELLED WATER TECHNIQUE

The rationale for obtaining a measurement of the rate of CO_2 production and thus energy expenditure using the DLW technique is based on the observation by Lifson et al. (1949) that when water labelled with oxygen-18 (^{18}O) was administered to animals, the O_2 in respiratory CO_2 and that in body water were in isotopic equilibrium with one another. Based on the above finding, Lifson et al (1955) demonstrated in a subsequent study using mice that the turnover rate of O_2 in the body water was greater than that of hydrogen. This is chiefly due to the fact that if the body water is labelled with deuterium (^2H) and ^{18}O as heavy water, after equilibration the ^2H is removed from the body almost entirely via water, whereas the ^{18}O is removed from the body by the same routes as the ^2H but also by respiratory CO_2. The difference between the turnover rates of ^2H and ^{18}O isotopes could therefore be measured and employed to calculate CO_2 production. Based on a set of assumptions listed below, an equation was derived to calculate CO_2 output and thus an estimate of energy output. This equation was subsequently modified by Lifson and McClintock (1966) as follows:

$$V_{CO_2} = \frac{N}{2.08} (K_O - K_H) - 0.015\, K_H \times N;$$

where V_{CO_2} is the rate of carbon dioxide production, the denominator 2.08 is the product of a fractionating factor ($^2H^{18}O$ liquid -- $C^{16}O^{18}$ gas = 1.04) and a stoichiometric factor (i.e.

CO_2. has an oxygen equivalent of 2 molecules), N is the estimate of total body water (TBW) rates, K_O and K_H are elimination rate constants for ^{18}O and ^{2}H respectively, and the remaining term ($- 0.015\ K_H \times N$) accounts for the fractionation effects of evaporative water loss on the assumption that this avenue involves half the total water loss (Lifson and McClintock, 1966).

A summary of the assumptions made for deriving the equation for rates of water turnover and CO_2 production as listed by Lifson and McClintock (1966) are:

(1) Total body water volume remains constant as does the weight and composition of body solids.

(2) All rates of intake and output remain constant through time.

(3) The volume of distribution of labelled water is equal to the TBW.

(4) Water is the only form in which hydrogen of body water is lost from the body, and water plus CO_2 are the only form of in which O_2 from the body water is lost.

(5) The specific activity of the hydrogen of water lost from the body is equal to that of the body water, and the specific activity of the O_2 of water lost from the body is equal to that of the body water.

(6) No water or CO_2 either isotopic or non-isotopic, enters the body with inspired air or through the skin surfaces.

Lifson and McClintock (1966) discussed the effects of inaccuracies and violations in these assumptions and showed that they did not introduce serious error in the calculated CO_2 output. However Nagy (1980), after extensive discussion of these basic assumptions in relation to field data, suggested modifications to the equation for calculating CO_2 production rates from isotope turnover in animals whose body water volumes remains constant, changes linearly, or changes exponentially through time. He stated that the use of an inappropriate equation produces errors of more than 5% only when body water volume decreases by more than 50% or increases by more than 100% during a measurement period.

The DLW dilution method is an ideal non-invasive technique for measuring energy expenditure in free-living animals over protracted periods. It cannot be used, however, to measure energy expenditure over a single day, or to assess energy expenditure in shorter periods such as during sleep (Jequier and Schutz, 1988). A major problem in the use of the technique is the recapture of the animals in order to determine the final concentration of ^2H and ^{18}O in the body water (see Blaxter, 1989). The method involves several steps:

(1) the measurement of ^2H and ^{18}O disappearance over 2 or 3 biological half lifes in body fluids and measurements of the ^2H_2O and $H_2{}^{18}O$ distribution spaces,

(2) the transformation of these values into water turnover, and water plus CO_2 from which CO_2 production is calculated by difference (Jequier and Schutz, 1988).

In order to obtain a good estimate of CO_2 production, and therefore energy expenditure by the DLW method, there is a need for obtaining an accurate estimate of TBW. Numerous indirect methods for the determination of TBW have been used, each of these has been based on the dilution principle. Though water labelled with ^2H and ^{18}O are the best and most frequently used tracers, tritium (^3H) has also been used with success (Conward et al., 1982; Donnelly and Freer, 1974; Searle, 1970). The main advantage of ^3H is that it is easily assayed by scintillation counting. However, the large dilution in the body requires that relatively large dose of 100 uCi or more of tritiated water be administrated in order to obtain adequate precision. Because of the radiation hazard, the ^3H cannot be readily used in environments where the use of radioactive nucleotide is restricted. Duterium and ^3H dilution volume is greater than the water space because of exchange with the labile hydrogen of protein and of the body constituents, and as such overestimates the TBW by between 1 and 5%. Oxygen-18 as a tracer for body water measurements has the advantages of both ^2H and ^3H without being itself radioactive. Furthermore, unlike ^2H it is non-toxic. Oxygen-18 dilution volume is also smaller than ^3H and ^2H dilution volumes, probably as a result of less exchange with non-aqueous body constituents, and should therefore be a more accurate measure of TBW (Schoeller et al., 1980).

The DLW method measures CO_2 production and not O_2 intake and thus is also a form of indirect calorimetry. The calculation of O_2 intake and energy expenditure from CO_2 production requires a knowledge of the metabolic fuel because the heat released per liter of CO_2 produced differs by 30% between carbohydrate and lipid utilization. The respiratory quotient (RQ) could be determined through continuous monitoring of respiratory gas exchange, but the use of such a method would defeat the non restrictive character of the DLW method (Schoeller, 1988). Since the value of the RQ is similar to the food quotient, it becomes more convenient predicting the value from dietary intake (Blaxter, 1989). The RQ in the short term can vary considerably, for example between 0.75 and 0.95 in the fasting and postprandial states of an animal respectively (Conward, 1988). The error in calculating energy expenditure from food quotient is, however, less than 3% in most situations (Black et al , cited by Schoeller, 1988).

5.2. MILK AND ENERGY INTAKE

The lamb depends solely on its energy reserves for survival during the first few hours after birth, it thereafter relies on the energy derived from the mother's milk for growth and daily activities for the first three weeks or so until it is able to utilize solid food. An accurate estimate of the milk intake is necessary in the determination of the energy available to the neonate.

The traditional methods of estimating milk intake in animals involve test-weighing the young before and after suckling or hand or machine milking of the dam after oxytocin administration . The test-weighing method requires that access to the dam is controlled so that suckling occurs only at designated times (see Oftendal, 1984), and the increase in the body mass of the young upon suckling is assumed to be representative of the amount of milk consumed over this period. This method is however of questionable value for species that suckle frequently. Restricting access to the dam could, for example, lead to a reduction in the normal level of suckling stimulation (Conward et al., 1982). Similarly, estimates of milk output derived from hand or machine milking offer no garantee of producing physiologically relevant results (Conward et al., 1982; Dove, 1988).

The isotopic dilution method of estimating milk intake in the young ruminant proposed by Macfarlane et al (1969) overcomes some of the problems associated with the traditional methods. This technique is based on the principle that all water consumed by the young is entirely from the milk. The water turnover as estimated from the rate of decline of an introduced isotope, usually tritium (^{3}H) or deuterium (^{2}H), over a period of time will then be a measure of milk intake. This method offers several advantages. Firstly, milk intake is estimated in free-living suckling young between, rather than during, periods of disturbance. Secondly, the method may give better estimates in multiple offspring by allowing sibling rivalry to have its effect on suckling behaviour (Dove, 1988). Finally, the method allows monitoring of body composition changes in the young, since body-water content is estimated as part of the method (Searle, 1974; Searle and Griffiths, 1976; Dove, 1988).

The estimated milk intake from the body water turnover method may differ by a small percentage from that of the actual milk intake. Equations have been derived however, to improve the accuracy of the turnover technique in estimating milk intake by taking into consideration the water liberated by oxidation of the organic constituents of the body (Donnelly and Freer, 1974; Dove and Freer, 1974; Pettigrew et al., 1987; Dove, 1988).

Estimated milk intake in lambs from water turnover measurements within the first 12 days from birth range from about 1 to 1.5 l per day (Wright and Wolf 1976; Dove and Freer, 1979; Conward et al 1982: Dove, 1988). Analysis of the ewe's milk yielded a composition of 78.6 to 84.0% water, 4.1 to 7.3% protein, 4.6 to 9.1% fat, and 4.0 to 5.0% carbohydrate (see Dove and Freer, 1974; Conward et al., 1982; Oftendal, 1984), with gross energy content of approximately 4.8 kJ per g liquid milk, or 26.6 kJ per g milk dry matter (Dove and Freer, 1974).

1.6. SUBSTRATE AVAILABILITY AND UTILIZATION FOR EXERCISE

1.6.1. INTRODUCTION

During exercise, the energy contained with chemical bounds is transformed into mechanical work by active muscles. The main sources of energy for such an activity are carbohydrate and fat stored in the body. The main carbohydrate stores of the body are glycogen and glucose, while fat is found mainly in the form of adipose tissue. Tissue protein is not normally used as a source of energy during exercise. It may however become essential to meet the blood sugar requirements of carbohydate-metabolising tissues where reserves of carbohydrates are exhausted, particularly during starvation (Shepherd, 1982).

The immediately available substrates for energy production are however those stored in muscle tissue itself, such as adenosine triphosphate (ATP) and creatine phosphate (CP) compounds, collectively known as phosphagen, and glycogen (Karlsson, 1971; Shepherd, 1982; Hultman and Sjoholm, 1983). Endogenous hexose and free glucose may also be available for immediate utilization. The intracellular contents of these substances are however low, and therefore only a few bounds of energy supply (Hultman and Sjoholm, 1983).

Various phosphagens act as carriers of energy within the cell to the point where the biologically meaningful reaction takes place. Phosphagen stores in the resting muscle are very limited and can only provide energy briefly during intense muscular contraction (Karlsson, 1971; Shepherd, 1982). The basis for replenishment of the high-energy phosphates, and in particular ATP, the dominant fraction of phosphagen and the primary source of energy for metabolic processes in the body are 2-fold. Firstly the breakdown of glycogen (via pyruvate) and fatty acids to carbon dioxide and water within the mitochondria

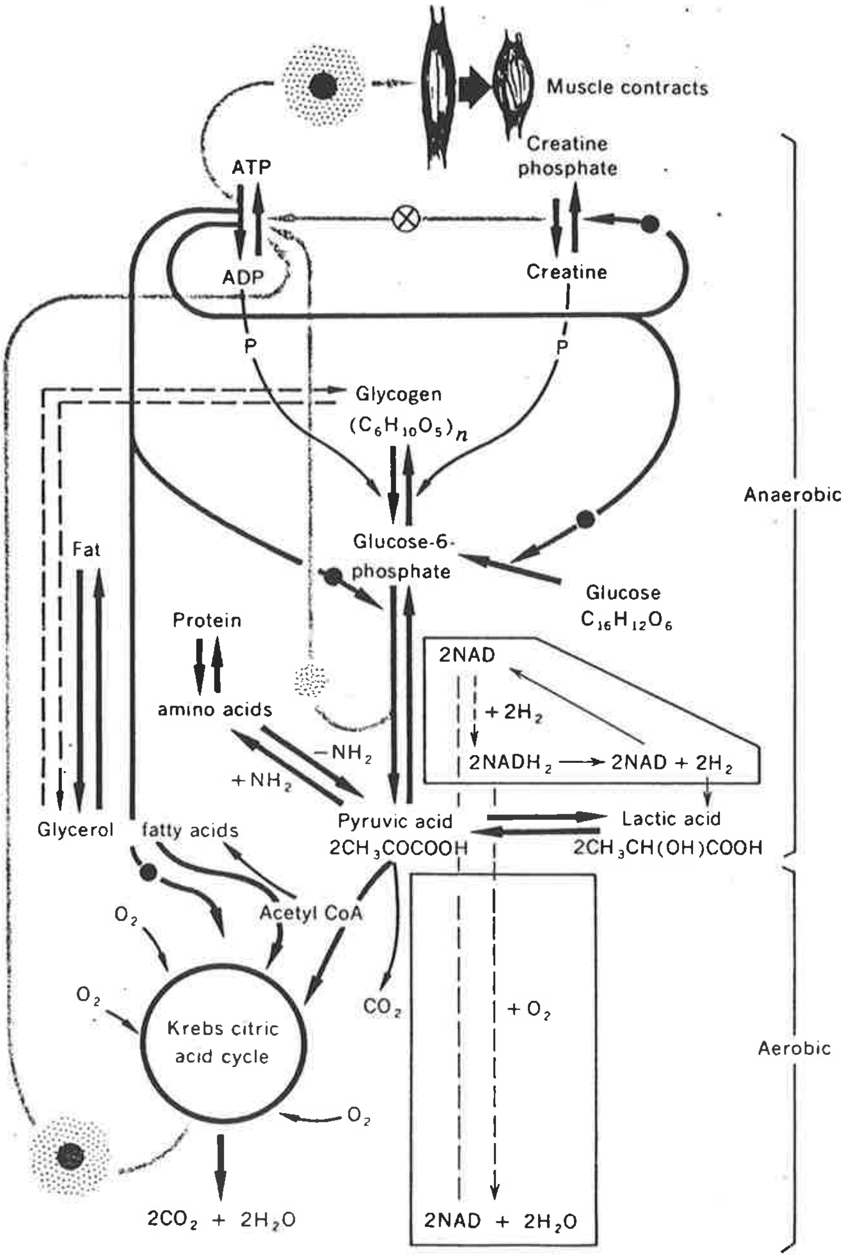

Figure 1.1 Simplified schematic diagram showing energy liberation and transfer in the living cell. (From Astrand and Rodahl, 1970.)

in the presence of oxygen (aerobic condition), and the breakdown of glycogen and/or glucose to pyruvic and lactic acids within the cytoplasm in the absence of oxygen (anaerobic condition) (see Figure 1.1). At the start of muscular activity, the store of oxygen available to the muscle cells in the form of oxymyoglobin and in the capillaries as oxyhaemoglobin is however extremely small. Delays in activation of mitochondrial respiration by phosphagens will further reduce the likely importance of this during the first increased energy demand (Hultman and Sjoholm, 1983).

6.2. CARBOHYDRATE

The final products of carbohydrate digestion in the alimentary tract, glucose, fructose and galactose are interconvertible, and as they pass through the liver they are converted almost entirely to glucose which then becomes available as an energy substrate by transportation in the bloodstream to muscles and other tissues. The glucose molecule is broken down into two molecules of pyruvic acid by glycolysis. The glycogen stored in the muscle may be broken down by the process of glycogenolysis which is dependant on the enzyme phosphorylase. At rest, cellular uptake of glucose is normally sufficient to support glycogen synthesis and glycolysis. During maximal exercise, glycogenolysis is probably sufficient to support rapid glycolysis, however, the depletion of intramuscular and liver glycogen can result in decreased capacity of substrate for glycolysis during prolonged exercise especially above anaerobic threshold. By limiting glucose uptake by other tissues, the fall in insulin level may indirectly serve to enhance uptake by active muscles during prolonged exercise. However, if blood glucose concentration falls during prolonged exercise, muscle glycolysis will be impaired for lack of initial substrate or fuel. Moreover, it is very likely that the ability to glycolyse limits the ability to utilize fat. The reversal of glycolysis, gluconeogenesis, is not possible without energy input and the intervention of specific enzymes (see Brooks and Faley,1984)

6.2.1. LACTATE AND PYRUVATE PRODUCTION AND UTILIZATION

Sufficient molecular oxygen for cellular respiration enables the formed pyruvate, as well as the extramitochondrial NADH, to be utilized as fuels for the subsequent mitochondrial processes. A relative lack of molecular oxygen diverts the pathway so that lactate becomes an end product. This enables the oxidation of extramitochondrial NADH and avoids the arrest of glycolysis or glycogenolysis. Lactic acid can however be formed in the presence of molecular oxygen due to an overstimulation of the glycogenolytic pathway with subsequent pyruvate formation and some reduction to lactate. The appearance of lactate in the contracting muscle can therefore be considered a normal feature of supposedly "steady state" condition and is related to the extent of stimulation of muscle metabolism (see Karlsson and Jacobs, 1982).

During exercise of moderate intensities of approximately 40 to 60% VO_2 max, production of lactate in the stimulated muscles results from the contribution of anaerobic processes to the energy output increases. The lactate produced diffuses into the blood stream and can be traced in the venous blood draining the muscle and eventually in the arterial blood if the amount of lactate produced is high enough (Karlsson, 1971; Senay et al, 1980, see Astrand and Rodahl, 1986). The translocation process for lactate is not simple diffusion, and does not immediately equilibrate the lactate concentration in the muscle cell with the lactate concentration in the blood. However, the highest blood lactate concentration reflects muscle lactate concentration fairly well after brief, exhaustive dynamic work, and is therefore useful as an indicator of maximal exercise in similar experimental situations (Karlsson, 1971). As the exercise proceeds, the blood lactate level falls again to the resting level and the exercise can continue for a long time. During strenuous and more exhaustive exercise, the oxygen demand of the active skeletal muscle is higher than can be met by the circulatory system. Hence the muscles continue to produce excessive amounts of lactate resulting in increased concentration in the blood during the exercise period (Karlsson, 1971; Lai and Lien, 1983; see Astrand and Rodahl, 1986). The blood lactate concentration may however reach a plateau when the rate of dispersal elsewhere in the body equals the rate of production (Lai and Lien, 1983).

During prolonged exercise, lactate and pyruvate concentrations in the blood vary only slightly from the rest value, and there is a tendency for both metabolites to increase slightly in the first few minutes and then to decrease slightly or remain constant (Karlsson et al, 1968). In contrast, Miniare and Forichon (1973) observed that the blood lactate concentration tended to decrease in the early period in mongrel dogs subject to prolonged exercise, while there was a tendency for the level to increase slightly in the final periods. The lack of hyperlactacidemia normally seen in animals subjected to prolonged physical exercise may be ascribed to a low lactate production, due to either an exhaustion of carbohydrate stores or to the restriction of lactate production at the onset of work, probably related to oxygen supply since running induces an increase in the rate of glycolysis which persits throughout exercise. The lack of lactate accumulation, in spite of an increased production, would require an existence of an equal increase in removal (Minaire and Forichon, 1973; Lai and Lien, 1983).

At the beginning of exercise, lactate and pyruvate levels in the blood increase at a rate related to the work intensity. The pyruvate evolution is, however, less systematic and may be independent of the lactate levels. Sometimes its level is only moderate in comparison with the lactate level, and sometimes it shows two peak values during exercise. (de Coster et al, 1969; Harris et al, 1968). The increase in blood pyruvate concentration with exercise does not often reflect a corresponding increase in muscle pyruvate concentration since the blood concentration at both rest and during a brief maximal exercise is higher than that in the muscle (Karlsson, 1971). Different evolution of lactate and pyruvate in both blood and the active muscle can be observed after exercise. Lactate concentration at the end of exercise is much higher in the active muscle than in the blood, but after 5 min of recovery the concentration in the two compartments runs parallel. When exercise is of high intensity and exhausting, blood lactate concentration still increases progressively 4 to 10 min after the muscular work is completed. (de Coster et al., 1969; see di Prampero, 1981). However, a clear decrease in lactate can be seen within the first minute after work when exercise is not exhaustive. Although there is no simple curve for the elimination of lactate from blood, it seems to have a half time of 15 min if the subject was resting during recovery independent of the peak concentration (see di Prampero, 1981). The time required for complete normalization however depends on the lactate level which was reached at the end of exercise. When blood lactate concentration is very high, it can sometimes take as much as 40 to 50 min before normalization is achieved (de Coster et al., 1969). Blood lactate concentration 60 min after exhaustive exercise in human subjects was 2-fold above the resting value (Lai and Lien, 1983).

There are many hypotheses concerning the fate of lactate after exercise. The two major mechanisms for lactate decrease are conversion into glycogen and oxidation into carbon dioxide and water. According to the classical concept, about 20% of the lactate produced during exercise is reoxidized to pyruvate and then released as carbon dioxide and water, and the remaining lactate is taken up by the liver and forms glucose, which can be reconverted into glycogen or delivered in the blood to be utilized by the muscle in its glycogenesis (see Astrand and Rodahl, 1986). It has however been shown that hepatic glycogenesis seems not to be fully brought into play in lactate elimination and that only a small proportion of the total lactate eliminated is removed by the liver (Hermassen et al., 1973: Miniaire and Forichon, 1973). The amount of lactate removed by excretion is probably less than 5% of the total amount removed. Another source of lactate removal is oxidation by the kidneys, and lactate may also be considered as fuel for the gut and myocardial metabolisms . However, due to the relatively low weight of these organs, this utilization accounts for just a small part of the lactate removal; estimated at about 10% at the most. Thus the major fraction of the lactate which is removed from the body must be eliminated in other organs. The working skeletal muscle, due to its large mass, is likely to be a major contributor to lactate oxidation (Hermassen et al, 1973; Miniaire and Forichon, 1973). Lactate may therefore not only be an end product of anaerobic glycolysis, but can also act as a substrate for energy release during exercise in skeletal muscle (Hermassen et al., 1973).

The disappearance of lactate from muscle after maximal exercise is rapid, while the disappearance from blood is slower. Only a small fraction (about 10%) of this lactate can be accounted for by an efflux from the muscle into the circulation during the recovery period, consequently more than 90% must be metabolized within the muscle itself. Because lactate is a metabolic 'dead-end', its most probable fate in skeletal muscle is conversion back to pyruvate. From pyruvate, there are at least two possible fates, which are oxidation to carbon dioxide and water, and conversion to alanine. Lactate (via pyruvate) is converted to alanine by the glutamate pyruvate transaminase reaction. However, the alanine cycle appears to be of a minor quantitative significance in accounting for the disappearance of lactate from skeletal muscle. This means that 75% must be metabolized by some other route (or routes) within the muscle, with the conversion to glycogen being a likely possibility (Hermansen and Vaage, 1977).

Lactate removal rates denote a balance between the removal and production of lactate; its disappearance from the blood after strenuous exercise occurs more rapidly during a period of light aerobic work which normally does not produce any lactate, rather than during a resting period (Hermassen et al., 1973; Belcastro and Boner, 1975; Hermansen and Vaage, 1977; Weber et al., 1987). This assertion is well illustrated by the study of Hermansen et al. (1973) in four trained human subjects. When the subjects rested in the recovery period, blood lactate concentration stayed at about the same level for some minutes, but from then on, a steady decrease was observed with a half-time of 25 min. However when the subjects performed exercise at 65% of their VO_2max during recovery, the blood lactate concentration was found to fall earlier and decreased faster with a half-time of 11 min. Thus the rate of lactate removal was more than twice as high during exercise than rest recovery. In another experiment, blood lactate concentration peaked at 3 min after maximal exercise before declining at 4 to 5 min while exercise continued at 70% of VO_2max. Peak value was however observed at 5 min after exercise at workload demanding 40% of VO_2max, suggesting a potential for a greater impact of higher intensity exercise on lactate removal during early recovery. At 30 and 40 min of the recovery period, 40% of VO_2max was significantly lower than recovery without exercise, whereas 70% of VO_2max was not. This suggests that low-intensity exercise may be more effective late in recovery than higher intensity exercise (Stanford et al., 1981). In general, lactate removal should occur most effectively during moderate exercise recovery intensities, since this provides for conditions where a minimal amount of lactate is produced and skeletal muscle blood flow is increased to transport the metabolite to the liver and muscle where removal occurs (Belcastro and Boner, 1975).

6.2.2. GLUCOSE PRODUCTION AND UTILIZATION

The concentration of blood glucose during exercise depends on the balance of the movement of glucose into and out of the blood stream (Harris et al, 1968; Pearson and Archibald, 1989), which is influenced by the duration and intensity of the work undertaken (Wahren et al., 1973; Alhborg et al., 1974). Observations in human subjects indicate that during exercise of mild intensity there is little or no change in blood glucose level. There is, however, a tendency for the concentration of glucose to fall slightly during the initial stages of exercise (about the first 10 min) after which there is a progressive rise. This is generally followed by a slight and progressive increase in the glucose concentration during the recovery period (Harris et al, 1968; Keppler et al, 1969; Ahlborg et al., 1974). The well known decrease in blood glucose level seen only during exercise of longer duration is illustrated by the observation of Alhborg et al. (1974) that arterial concentration of blood glucose at the end of 4 h exercise reduced by 30% as a response to exercise at 30% of VO_2 max. in human subjects.

Glucose uptake by working muscles increases significantly above the basal level during work, depending on the intensity and duration of the exercise. During prolonged submaximal exercise the glucose uptake increases for up to about 3 h and not until a further hour of exercise, when the blood glucose concentration had decreased by a third, was a small decline in glucose uptake noticeable (Wahren et al., 1973; Ahlborg et al., 1974). Decrease in the blood glucose concentration observed may be attributed to the failure of the hepatic glucose output to keep up with the augmented utilization by the working muscles (Ahlborg et al., 1974). The diminished utilization of glucose by inactive muscle during exercise makes available more glucose to the active muscles (Harris et al., 1974).

Draught oxen subjected to two work periods of 30 min each separated by a 15 min pause, and followed by a 15 min recovery period showed variable glucose concentrations before and after work. During work, glucose levels tended to decrease initially, at least during the first 10 min, but by 30 min of work, concentrations of the metabolite had started to rise again. The initial decrease at the start of each working session suggested a time lag between the increased demand for glucose in muscle and the start of exercise and increased mobilization from reserves. By 30 min of recovery this had been rectified and circulating levels of glucose had increased (Pearson and Archibald, 1989). This trend of blood glucose production contradicts the situation where there may be a transient net release of glucose during the first few minutes of exercise.

As exercise continues, blood glucose assumes an increasingly important role as a substrate for muscle oxidation, indicated by the net uptake. This increased glucose uptake is associated with a rapid and marked rise in splanchnic glucose production, related to the work load performed. Although glucose production from the hepatic glycogen dominates glucose output, particularly in the early phases of exercise, contribution from hepatic gluconeogenesis as evaluated from substrate balance in the study rose progressively as exercise continued. Thus as glycogen stores are depleted by prolonged exercise, glycogenic processes are accelerated (Wahren et al., 1971; Wahren et al., 1973). The fact that the high rate of glucose uptake is maintained while the blood insulin level falls during exercise underscores the hypothesis that glucose uptake by exercising muscles is not dependant on the ability to secret increased amounts of insulin (Wahren et al., 1973; Ahlborg et al., 1974).

Rapid recovery of blood glucose concentration back to resting values after cessation of exercise was shown in rats subjected to exhaustive exercise. The recovery of blood glucose concentration to control values occurred simultaneously with the removal of lactate from the blood stream; blood glucose showed an apparent decrease in concentration after 15 min of recovery. This trend in evolution of blood glucose after exercise was maintained throughout the observation period of 24 h (Brooks et al., 1973) and suggests a net uptake of glucose and presumably a relatively lower glucose release.

6.3. LIPID

It is well established that lipids are major sources of energy for muscular activity. The metabolism of lipids during exercise represents a highly integrated process that usually begins in the adipose tissue and is completed in the skeletal muscle mitochondria. Long chain fatty acids, transported as free fatty acids (FFA), form the major portion of this substrate (Pruett, 1970; Pethick et al., 1984).

As energy sources, circulating lipids complement glucose, but the relative importance of these metabolites is influenced by both nutritional and physiological status and is species dependant. In starvation, for example, plasma FFA and ketone bodies assume increasing importance relative to glucose. The ketone bodies, 3-hydroxybutyrate and acetoacetate, together with acetate are the other lipid sources of substrate for herbivores. These nutrients are derived either from the diet, or directly or indirectly from adipose tissue mobilized when nutrients absorbed from the alimentary tract fail to meet the energy requirements of the tissue (Pethick et al, 1984). Ketone bodies are usually considered fuels only during fasting or altered metabolic status such as diabetes, but they are more important in fed ruminants where they may account for up to 15% of the energy consumed by the skeletal muscles. Though about half the energy needs of ruminants are derived from ketone bodies and acetate, in contrast, these metabolites probably account for no more than 10% of energy utilized in human skeletal muscles. Their importance as fuel for skeletal muscle is further reduced in non-ruminant species fed a diet high in digestible carbohydrates (Pethick et al, 1984). The increasing importance of FFA as a fuel during exercise is not at the expense of other circulating metabolites utilized by muscle since the glucose uptake by muscle increased considerably over the resting state. The glucose consumed is however likely to be completely oxidized since only during the initial stages of exercise was there a tendency towards lactate and pyruvate release (Pethick et al, 1987).

6.3.1. FREE FATTY ACIDS

Free fatty acids originate from adipose tissue and circulatory lipoprotein, and are transported in plasma as an albumin complex. Although they account for only approximately 5% of the total lipids in the blood, they are the major form of lipid immediately available for utilization by cells. Their release from and incorporation into adipose tissue is a dynamic equilibrium influenced by a combination of humoral, nutritional and neural factors. Though they exist transiently in the circulation, the velocity of FFA turnover is such that if all FFA transported in the plasma were oxidized, it could provide essentially all the energy needs of the body (Van Duyne, 1966). The energy required for fatty acid synthesis is generated by the pentose cycle activity, and by the decarboxilation of malate via citrate cleavage pathway. Glucose is, however, not a significant precursor of FFA in ruminant tissues. This has been thought to stem from the virtual absence of an active citrate cleavage pathway essential for the translocation of acetyl Co A derived from pyruvate within the mitochondria to sites of fatty acid synthesis in cystosol (Pethick et al., 1984).

The liver is a major site for the uptake and metabolism of plasma FFA and is known to take about 25% of all FFA entering the blood stream in conscious fed sheep and post-absorbtive dogs. The gut tissue took up a further 8% of the total supply of FFA, so that the total splanchnic uptake accounted for about one third of FFA supply. Hepatic uptake of FFA does not appear to be regulated by intrahepatic metabolism, but rather by the plasma concentration of FFA in both non ruminant and ruminant species. The rate of FFA supply not only determines hepatic uptake, but also influences FFA metabolism in the liver, although the latter is modulated by the metabolic status of the animal (Pethick et al., 1984).

At rest, glucose is potentially the largest contributor to the energy requirements of skeletal muscle, while FFA makes only a small contribution. During exercise, the contribution made by FFA increased such that in the latter half of exercise it was the major energy source of skeletal muscle when sheep were subjected to submaximal exercise for 2 h. The contribution made by glucose was relatively constant throughout the exercise period in the sheep, however, exercise prompted a sustained shift towards fat mobilization, and the entry rate of FFA could have accounted for nearly 65% of the oxidative metabolisms if all the fat was oxidized. The actual contribution to the oxidative metabolism in the whole animal was however much lower at 20%, but this value represents an average contribution throughout the entire exercise period and still emphasizes the important role played by FFA in maintaining the fuel homeostasis during exercise (Pethick et al., 1987)

The direction of the change in plasma FFA during exercise is dependant on the relative severity of the work load. In addition, whether or not most of the mobilization of FFA occurs during or after work appears to depend on the proportion of energy which is derived anaerobically, as evidenced by the increase in blood lactate concentration. The point at which the plasma FFA levels cease to increase as the subject exercises to exhaustion lies between 70 to 80% of the VO2 max. It is also at this point that the mobilization of FFA after exercise becomes most pronounced and long lasting. Up to this work load, exercise activates agents which increase plasma FFA, thus increasing substrate availability as the exercise is prolonged. As exercise becomes more severe in relation to the aerobic capacity, so that a larger proportion of the energy is derived anaerobically, the mobilization of FFA is depressed for as long as the blood lactate remains high. This does not necessarily mean that FFA turnover and oxidation are completely suppressed. Whether the depression of FFA mobilization is directly regulated by the increased lactate concentration, or whether it is due to some other stimulus arising from anaerobic metabolism, is not clear. The FFA mobilization mechanism activated during exercise was apparently not affected by changes in plasma insulin concentration which stemmed from exercise. Plasma insulin concentrations were invariably reduced at all levels of exercise, whereas plasma FFA level either rose or fell depending on the severity of the work load (Pruett, 1970).

Several adaptations occur in skeletal muscle during exercise. The increased arterial concentration of FFA prompted by exercise, in addition to an increased rate of blood flow, resulted in a substantial increase with time in the utilization of FFA by skeletal muscle in humans. The blood flow remained constant between 15 and 240 min of exercise, and this agrees with work in humans where blood flow increases rapidly at the onset of exercise and then is maintained at a constant rate throughout the exercise period (Ahlborg et al., 1974).

6.4. PROTEIN

Proteins are recognized to be, in many aspects, the most remarkable chemical substance within the body, playing 2 distinct and separate roles as structural materials and as catalysts or enzymes that operate at the molecular level (Poortmans, 1973). It is generally believed that tissue proteins do not contribute as a source of energy for muscular activity (Poortmans, 1969;1973). However, protein metabolism plays a far more significant role in exercise energy production than had been previously indicated (see Cerny, 1973), particularly when carbohydrate sources of energy are exhausted or exercise is prolonged. In such a situation, protein degradation may become sufficient, providing as much as 10 to 80% of the required energy (see Lemon et al, 1973). The degradation is also essential to meet the blood sugar requirement of carbohydrate-metabolizing tissues such as the brain since lipids cannot be converted into glucose (Shepherd, 1982).

Protein catabolism occurs by a series of steps to yield keto acids which are then converted into substances that enter the Krebs cycle and hence proceed to complete degradation and energy formation (see de Vriers, 1980). Although it has been suggested that amino acids could be utilized as a source of carbon for energy production, studies in protein metabolism however reveal that amino acids are presumably not important as energy supply during exercise (Poortmans, 1969; see Cerny,1973). It seems more likely that protein metabolism occurs to provide an amino acid source for pyruvate removal and/or glucogenesis and controlling the total levels of Krebs cycle intermediates (see Cerny, 1973).

In general, the behavior of plasma protein during strenuous exercise resembles that of the corresponding hematocrits. Since there is usually a positive and significant relationship between exercise intensity and water movement from the vascular volume, plasma protein would be expected to increase accordingly. However, the increase in plasma protein during strenuous exercise is greater than seen in hematocrits (Poortmans, 1969; 1973; Senay et al., 1980). The increase in protein may therefore not be a direct influence of the corresponding hematocrits, the type of exercise may also influence the level of plasma protein attained during exercise. In a comparative study by Senay et al.(1980), no trend could be discerned during treadmill exercise and the plasma protein concentration seemed to be generally maintained, whereas there appeared to be a linear and significant relationship between increase in plasma protein concentration and cycling intensity.

1.7. ENDOCRINE RESPONSE DURING EXERCISE

Several endocrines are known to respond to exercise, however growth hormone is one of the most commonly measured endocrines during exercise. It originates from the adenohypophysis or anterior pituitary and circulates in the plasma in an unbound form. Its release is regulated by a specific hypothalamic hormone, the GH-releasing factor (Frohman, 1972: Martin, 1973).

A wide variety of physiological and pharmacological stimuli will promote growth hormone release. Some of these factors include muscular work, stress, heat exposure, hypoglycemia, arginine, vasopresin, propranolol, and derodopa. (see Metivier, 1973; Cronin et al, 1981). In addition, the growth hormone response to some of these factors appears to be additive. There is evidence of the existence of a growth hormone inhibiting factor (GIF) a tetradecapeptide (Effendic et al., 1978). The hormone is known to be associated with impairment of glycolysis, including the inhibition of glucose transport and promotion of growth in body tissues (Erikson et al., 1971) as well as increased mobilization of FFA for energy (Raben and Hollenberg, 1959) .

Growth hormone concentration in plasma may be influenced to varying degrees in response to muscular activity. For a group of individuals with fairly similar work capacity, increase in plasma growth hormone levels during submaximal work appears to be a function of the work intensity. However at very intense work loads, the increase in growth hormone may be less than that found at lesser work intensities. Growth hormone release is somewhat delayed at least 5 to 10 min from the onset of activity. Physical fitness of the subject is also important in the response to growth hormone to exercise. In general, growth hormone level increase is lower during moderate work loads in trained subjects (see Sutton et al., 1969).

Increased blood lactate levels resulting from stress associated with muscular activity has been suggested to influence growth hormone release (Sutton et al., 1969). Behavioural stress has also been demonstrated to cause alterations in plasma growth hormone concentrations in several species of animals. However, lambs subjected to acute behavioural stress from being retained in a dark cubicle for 10 min indicated that neither acute nor repeated behavioural stress appears to influence the secretion of growth hormone in lambs despite the fact that significantly increased amounts of corticosteroids occurred. Although secreation of growth hormone in the sheep does not appear to be influenced by behavioural stress, it may still be responsive to other stimulii, especially metabolic conditions (Cronin et al., 1981).

1.8. THE METABOLIC AND PHYSIOLOGICAL RESPONSES TO SPEED OF LOCOMOTION IN NEONATAL LAMBS

1.8.1. RESTING METABOLIC RATE

Since the early work of Kleiber (1932) there has been a rich literature concerning basal or standard metabolic rates in animals. Such determinations have shown that standard rates scale with body mass to the 0.75 power, although some controversy still exists as to the validity of this exponent for young animals (see reviews by McNab, 1980: Donhoffer, 1986)). In the present study it was decided to measure resting metabolic rate since this presents a more realistic state than the post-absorbtive condition in the defined basal level. Measurements of resting metabolic rates vary greatly, depending on the techniques used and the state of the animal. Variations may result from differences in nutritional status as observed by Slee et al (1990) in a comparative study involving lambs that had suckled for several hours and those prevented from sucking for about four hours where fasting significantly depressed resting metabolism. Resting metabolic rates of 0.11 to 0.16 ml/s/kg have been reported for neonatal lambs in a water bath at thermoneutrality (Robinson and Young 1988; Young et al , 1988; Slee et al., 1990), while a higher value of 0.32 ml/s/kg was obtained for lambs exposed to thermoneutral conditions in a respiratory chamber by Alexander et al. (1983).

The slightly lower resting metabolic rates recorded for lambs in a water bath, in comparison to those achieved with the more traditional methods involving the animal standing in an air environment at thermoneutrality, may be attributed in part to differences in procedure. The support provided by the buoyancy of the water may lead to a more relaxed state of the lambs since the skeletal muscles do not have to support the animals against gravitational force (Young et al., 1988).

1.8.2. METABOLIC RATE DURING EXERCISE

A linear relationship between metabolic rate and the speed of locomotion is seen in numerous species of quadrupeds (Fedak and Seeherman, 1979; Taylor et al., 1982), and in bipeds such as the ostrich (Fedak and Seeherman, 1979). However Hoyt and Taylor (1981) showed that metabolic rate increased curvilinearly with the speed of walking and trotting over a range of 1 to 6 m/s in three small horses weighing 110 - 120 kg. Sufficiently high speeds could however not be obtained to evaluate whether metabolic rate also increased curvilinearly or not during galloping. Earlier work of Margaria (cited by Hoyt and Taylor, 1981), showed a similar curvilinear relationship between metabolic rate and speed of walking in humans. The other exception to the linear relationship observed in a number of animals between metabolic rate and speed is in bipadal macropods. The independence of metabolism at speeds greater than 2.0 m/s observed in the tammar wallaby (*Macropus eugenii*) could not be attributed to maximal levels of oxygen consumption being reached, since blood lactate levels in the animals were low, suggesting that the energy for locomotion was provided mainly by aerobic metabolism (Baudinette et al., 1987).

The value of the intercept with the y axis of the regression line relating metabolic rate to the speed of locomotion; i.e., the extrapolated zero running speed, has been compared with the observed resting metabolism in several animals and human subjects within a wide range of speeds. The differences range from 0.05 to 0.39 ml/s/kg in 2.6 kg dog to 21 g white mouse in the study of Taylor et al. (1970). The discrepancy in the two values was 0.28 ml/s/kg in tammar wallabies studied by Baudinette and co-workers (1987). Resting on a treadmill or sitting passively on a bicycle ergometer yields a resting metabolic rate close to the y intercept, suggesting that the differences observed represent a postural cost of locomotion (see Schmidt-Nielsen, 1977). Estimates of the increased energy cost of standing over lying ranging from 5.9 to 11.7 W/kg per day for cattle and 7.1 to 11.7 W/kg per day for sheep were observed in a number of studies. Training however, reduced the difference between standing and lying metabolism (see Commonwealth Agricultural Bureau, 1990). A higher discrepancy between the two values of 13.5 W/kg was obtained for cattle of mean mass 273 kg (Vercoe, 1973).

The energetic cost of locomotion, obtained by dividing metabolic rate by speed of locomotion, decreases with increasing speed and approaches a minimum value for each individual. These asymptotic values are identical to the slope of the curves representing the relationship between energy consumption and speed, and are a constant for each animal (Taylor et al., 1970). This relationship was observed in horses within the range of trotting speed by Hoyt and Taylor (1981). However, as in the case of the curves relating metabolic rates to galloping speeds, the authors were unable to show if increases in the cost of locomotion occur at high galloping speeds. It was previously thought that the cost of locomotion in bipeds and quadrupeds differed and that this variation would be greater among large animals. However, comparison of the energy cost of locomotion for a wide variety of animal species showed no consistent difference between the cost for bipeds and quadrupeds of similar sizes, but suggested a difference between apparently "clumsy" and "graceful" animals (Fedak et al., 1981). The end results of this analysis for about 70 species is that the energy cost of locomotion varies in a regular fashion as a function of body mass. This allows the energy consumption of a running mammal to be predicted from the speed of locomotion and body mass.

The cost of locomotion has a convenient numerical value for animals and an intuitive meaning when it is expressed as mass specific oxygen consumption per unit distance. It is however a dimensionless number which is related to the efficiency of locomotion. Since the cost of locomotion is inversely proportional to speed, it would vary from infinity when the animal as at rest to some minimum value when speed is finite (Tucker, 1970).

1.8.3. PHYSIOLOGICAL RESPONSES

1.8.3.1. Heart frequency

Heart frequency increases linearly with the speed of locomotion in eutherian mammals (Wagner et al., 1977; Gleeson and Baldwin, 1981; Thomas and Fregin, 1981; and Faraci et al., 1984), and in marsupial mammals and man (Baudinette, 1978). Interestingly the linear relationship is not as well defined in birds (Butler and Woakes, 1980). The linear increase in heart frequency as a function of speed of locomotion over the range of speeds at which animals would run is clearly illustrated by the study of Faraci et al. (1984) involving domestic pigs subjected to progressive exercise from 1.0 to 1.8 m/s at a 3° incline. Heart frequency increased progressively with the increasing running speed and returned to the resting level within 15 min post-exercise in all but the highest running speed where normalization occurred at 30 min after exercise. There are however exceptions to the linearity between heart frequency and exercise intensity, perhaps more frequently among untrained subjects. Emotional factors, nervous behaviour and apprehension may also affect the heart frequency both at rest and during submaximal exercise (Hays, 1976; Baudinette et al., 1978).

Since a transitory overshot of heart frequency may occur at the commencement of running or when the running speed is suddenly changed, the duration of the exercise should be long enough to ensure achievement of steady state heart frequency (Donald and Ferguson, 1966). In the above-mentioned study of Faraci et al. (1984), steady state heart frequencies were achieved within 2 min of exercise at each speed level. However a longer duration of about 5 min at a fixed exercise intensity produces acceptable steady heart frequencies in most cases (Donald and Ferguson, 1966; Thomas and Fregin, 1981). On the other hand, steady state heart frequency has been assumed to have been reached when there is less than 5% variation in the observed frequency during the test (Baudinette, 1978).

Heart frequency may respond differently to exercise at a given VO_2, depending on whether the exercise is isometric or not and the type of limb involved (see Astrand and Rodahl 1986). However the significant increase in both metabolic and heart rates as a function of the speed of locomotion often seen in animals during submaximal work suggests the existence of a linear relationship between the 2 parameters. Such a linear relationship between metabolic and heart rates has been reported for several mammalian species including man (Astrand et al., 1973; Donald and Ferguson, 1966; Wagner at al., 1977; Brooks and White, 1978; Gleeson and Baldwin, 1981; Faraci et al., 1984). Although reproducible relationships between heart rate and VO_2 from test to test were observed in dogs by Donald and Ferguson (1966), they however cautioned the effectiveness of heart frequency as an indicator of work intensity where fear and excitement, resulting from unfamiliarity of the animal to the exercise procedure, are involved. The effect of excitement seems to be very pronounced in birds during flight (Butler et al., 1977; Butler and Woakes, 1980).

1.8.3.2. Body temperature

During muscular activity animals increase the rate at which they produce energy and therefore heat. The amount of energy utilized and the corresponding heat produced during exercise depends on a number of factors such as the type, severity and duration of the work (Warncke et al, 1988). During exercise involving mainly the leg muscles as in locomotion in eutherian mammals, the leg veins carry the warm blood draining the active muscles into the pelvic cavity, allowing rectal temperature to be taken as indicative of deep body temperature (see Buskirk, 1977).

The inability of regulatory mechanisms to totally dissipate the metabolic heat produced to the environment may force the cardiovascular system to its regulatory limit (Warncke et al, 1988: Burskirk, 1977). However much of the heat generated during exercise is temporarily stored and therefore reflected as a rise in body temperature. Physiological mechanisms for temperature regulation are such that in many species most of the heat produced during exercise can be dissipated to allow the animal to maintain constant body temperature (See Buskirk 1977, Taylor 1974). In other species heat is stored and may reflect a limit on the distance an animal can run. The strategy is a potential water-saving adaptation but can only be useful in large animals.

1.8.3.3 Respiratory frequency

The coupling of breathing and stride frequency in a 1:1 ratio has been reported for a number of animals including jack rabbits, dogs and horses (Bramble and Carrier, 1983) and the tammar wallaby (Baudinette et al, 1987). Clear entrainment between respiratory and stepping frequencies was observed by Iscoe (1981) in a third of cats walking on a treadmill between speeds of 0.31 to 1.67 m/s, while coupling in the remaining animals was either very weak or absent. Humans, which alone among modern eutherians utilize only a bipedal gait, differ from quadrupeds by employing several phase-locked patterns of respiratory and stride frequencies (4:1, 3:1, 2:1, 1:1, 5:2 and 3:2), with a 2:1 coupling ratio being the most prominent over a wide range of speeds and stride frequencies. However there is a change to a 4:1 ratio at lower running speeds. In both quadrupeds and humans, shift in coupling ratios may occur quickly and smoothly over just a few strides (Bechbache and Duffin, 1977). It has been suggested that the coupling of breaths and stride frequency may indicate the existence of neural circuitry linking the two variables, and changes in the ratio appear to be triggered by involuntary stimuli (Bramble and Carrier, 1983) .

1.8.3.4 Stride frequency and length

Increases in the frequency and length of stride are the only variables which contribute to an increase in an animals' running speed (Heglund et al, 1974; Taylor, 1977; Baudinette et al, 1987). A simple linear relationship between stride frequency and speed has been shown in a number of animal species ranging from a 9 g mouse to 680 kg horses. There are however exceptions; the frequency in horses remained constant as the speed increased during a gallop, indicating that all the increase in speed was achieved by increasing stride length (Heglund et al., 1974). Hopping frequency has also been shown to be independent of speed up to approximately 4 m/s in the tammar wallaby (Baudinette et al., 1987). Stride frequencies are higher in small animals than larger animals (Heglund et al., 1974). Smaller animals, in view of their shorter stride lengths in comparison with larger animals, would therefore be expected to run at higher stride frequencies to achieve the same running speed as larger animals.

1.8.3. MAXIMAL METABOLIC RATE

Animals, including man, exhibit linear relationships between the intensity of work performed and steady state metabolic rate until maximal oxygen consumption (VO_2max) is reached (Mitchell and Blomqvist, 1971; Shepherd and Gollnick, 1976; Bedford et al., 1979; Seeherman et al., 1981). Further increase in work intensity leads to an increased rate of metabolic energy utilization by the muscles without a corresponding increase in VO_2. The additional energy required is provided by anaerobic glycolysis which results in the production of excessive amounts of lactate in the active muscles. The increased concentration of lactate can be seen in the venous blood draining the muscles (Seeherman et al., 1981). The VO_2max of animals, including man, is considered to be the best single measure of aerobic capacity (Mitchell and Blomqvist, 1971; Shepherd and Gollnick, 1976; Bedford et al., 1979; Seeherman et al., 1981)

The most commonly used technique for eliciting maximal metabolism in normal lambs and calves involves the use of cold air with or without wind (Alexander, 1962 ; Alexander and Bell, 1975), and more recently, water progressively cooled from thermoneutrality to about 18^oC (Earles and Small 1985; Slee et al,1987). The cold induced summit metabolic techniques gave values of 0.77 to 1.04 ml/s/kg (15.5 to 20.9 w/kg) in the studies without impairing the subsequent health of the animals (Alexander, 1962 ; Alexander and Bell, 1971). Maximal oxygen consumption values as a factor of body mass have been determined from treadmill studies in a wide range of species by Seeherman et al. (1981)

In a comparative study of VO_2max Seeherman et al (1981) found that significantly lower VO_2max levels were elicited by cold exposure than treadmill exercise techniques in pygmy mice *(Baiomys taylori)*, chipmunks *(Tamias striatus* and white rats *(Rattus norvegicus)*. The study suggested that the cold exposure technique is limited to small animals. The treadmill procedure provides a clear experimental end point for VO_2max, once achievement of actual VO_2max could be confirmed, by ensuring that VO_2 remains constant with increasing exercise intensity and that the resulting additional energy is being supplied by anaerobic glycolysis. This stage is indicated by a significantly higher rate of increase in blood lactate (Seeherman et al , 1981).

Training tends to elevate VO_2max in animals and human subjects (see Mitchell and Blomqvist, 1971). This effect was demonstrated in rats by exercising one group for 10 days while the other was untrained. Maximal rates of oxygen consumption were 0.98 vs 1.24 ml/s/kg in male and 1.35 vs 1.50 in female untrained and trained rats respectively. The main objective of training is to get the animals accustomed to the treadmill (Bedford et al., 1979). However training of animals in the use of treadmill is normally conducted over two weeks, and may therefore lead to improvement in physical fitness in the animals. The greater mitochondrial density of their muscles would demand a greater amount of oxygen than their untrained counterparts (Seeherman et al., 1981). Mitchell and Blomqvist (1971) demonstrated that a decrease in physical activity in man caused a prompt fall in VO_2max, with bed rest resulting in a 20 to 25% drop below control values. He asserted that any increase in habitual physical activity of moderately heavy intensity involving large muscle groups will be expected to produce some change in VO_2max.

A range of 3.7 to 6.5-fold increase in VO_2 by cold exposure over pre-exercise values (aerobic scope) has been reported for lambs (Alexander, 1973; Young et al , 1988; Robinson and Young, 1988; Slee et al., 1990). The upper value of aerobic scope in the lambs fell within the six to eight fold increase in metabolic rates seen in rats, mice and hampsters (Pasquis et al, 1970; Shepherd and Gollnick, 1976), but is lower than the 11 to

15 that occurred in man (Shepherd and Gollnick, 1976; Mitchell and Blomqvist, 1971). The race thoroughbred horse is capable of extremely high aerobic scope up 50 during exercise (v.Engelhardt, 1977)

When an animal walks uphill, work has to be done to move the body vertically against gravity as well as horizontally. The higher the slope, the greater the work done. Higher levels but non significant differences in VO_2 max determined at different treadmill grades have been reported by Hermansen and Saltin (1969) and Kasch et al. (1976). The increase in VO_2max with a corresponding increase in treadmill slope is clearly illustrated by the study of Ciccone et al. (1982), where exercise at a speed of 2.7 m/s at treadmill grades of 0, 6 and 8% had increasing values of 0.36, 0.45 and 0.48 ml/s/kg, respectively.

A significantly high repeatability of the VO_2max level was obtained for repeated tests at horizontal and inclined treadmill running in human subjects (Kasch et al., 1976). Similar identical results were also obtained in treadmill and step test procedures involving the same subjects, which gave a significantly high correlation of 0.95.

CHAPTER 2. GENERAL MATERIALS AND METHODS

2.1. EXPERIMENTAL SITES

Ewe-lamb recognition and lamb following-bility tests were conducted at the Field Experiments Station,o Glenthorne", O'Hallaran Hill, located approximately 20 km. south of Adelaide (Lat. 34° 58' S., Long. 138° 38'E.). Treadmill exercise tests were conducted in a temperature controlled room at the Davies Laboratory, Waite Agricultural Research Institute, University of Adelaide, located approximately 7 km south east of Adelaide. Daily movement distance tests and energy intake and expenditure assessment (by doubly labelled water technique) were conducted at the Bolivar Experimental Station of the Waite Agricultural Research Institute, located approximately 30 km. north of Adelaide. All the experimental areas are in close proximity and experience a similar Mediterranean type of climate.

2.2. ANIMALS

The stock used in the investigations consisted of South Australian Merino sheep and their neonatal lambs. With the exception of the tests of ewe-lamb recognition (Chapter 3) and following-ability (Chapter 4), only lambs born and reared as singles were used in the experiments. Lambs for recognition and laneway following tests were dropped in a small paddock (0.5 ha.) adjacent to the test pens and laneway at the Glenthorne field site. Lambs for measurement of daily movement distance and energy intake and expenditure tests were born in the test paddocks at Bolivar, and those for the treadmill exercise tests were born in a 2-ha paddock at the Waite Agricultural Research Institute. All ewes were from the same breeding stock and there were no significant difference between the weight of lambs born at the different experimental sites.

2.3. MANAGEMENT OF FLOCK

2.3.1. MATING MANAGEMENT

Oestrus was synchronised by the intravaginal application of polyurethane sponges impregnated with progesterone (Repromap, 60 mg, Upjohn) for 14 days in each group of 20 to 50 ewes during the months of August and November 1987, March and September 1988, and January and May 1989. On withdrawal of the sponges 2 rams of proven fertility, fitted with harness and crayons, were introduced into each group. Ewes that were not raddled within 3 weeks of joining were removed from the flock.

2.3.2. MANAGEMENT DURING GESTATION

Ewes were kept on pasture and had unlimited watering facilities provided throughout gestation. During summer and autumn months, when pasture conditions were generally poor, supplementary hay was provided. Depending on the pasture availability and quality during the last eight weeks of gestation, ewes received an average of 200 g of oats/pea mixture (7:3) per head per day fed each other day, plus hay *ad libitum* . The ewes were transferred to a small paddock close to the test pens at Glenthorne, and to a paddock 100 m from the temperature controlled room at the Waite Agricultural Research Institute a week prior to the birth of the first lambs.

2.3.3. MANAGENT DURING LAMBING

So as to obtain an approximate time of birth of each lamb, the flock was either kept under continuous observation during peak lambing periods from 7.00 am to 7.00 pm daily, or inspected at 2-hourly intervals during slack lambing periods . Only lambs that were assessed from the wetness of the coat to have been born within an hour prior to inspection were used in the study.

At about 3 h post-partum each lamb was quietly removed from the ewe, given an identifying ear-tag, weighed, checked for sex and had a number corresponding to that of the mother sprayed on its right side. The lamb was then left with the dam until approximately 17 or 47 h post-partum, when it was removed in preparation for recognition or laneway and tredmill tests. On completion of these tests, usually after about an hour, the ewe and her lamb were returned to the flock.

2.4. CLIMATIC MEASUREMENTS

Ambient temperature during field tests was measured with a minimum-maximum mercury thermometer in the shade near the testing pen. Climatic measurements of mean minimum and maximum temperatures, wind speed at 2 m above ground and barometric pressure over the lambing and experimental periods were taken at the meteological recording centres at the Glenthorne Experimental Station, and the Waite Agricultural Research Institute (see Table 2.1).

Table 2.1 Mean climatic conditions in the field during lambing and experimental periods.

Study period	Temperature (°C)	Wind speed* (m/s)	Air pressure (mb)
Chapter 3	8.4 -14.2	1.7	1016.8
Chapter 4	13.1 -22.3	1.4	1019.3
Chapter 5	16 3 - 26.4	1.5	1018.4
Chapter 6			
Study 1 and 2	13.1 - 22.3	1.5	1019.3
Study 3 and 4	9.0 - 15.6	1.3	1002.1
Chapter 7			
Study 1	9.0 - 15.6	1.3	1022.1
Study 2	8.4 - 14.2	1.7	1016.8
Study 3	9.0 - 15.6	1.5	1019.3

*Wind speed was measured at 2 m above ground.

CHAPTER 3. MOTHER-OFFSPRING CONCERN AT SEPARATION AND RECOGNITION FROM A DISTANCE, AT EIGHTEEN HOURS POST-PARTUM

3.1. INTRODUCTION

Most lambs are relatively active during the first few days of life, and are generally able to follow and remain in close proximity to their mothers during the ewes' daily movement in search of food and water. Neonatal lambs often appear to undergo considerable stress in seeking their mothers during separation in open fields in the presence of other parturient ewes. The ability of a lamb to identify the dam in such a situation may be compromised. There are 2 behavioural patterns which indicate that a ewe and her lamb have identified each other. The first is the event of suckling; if a ewe permits a lamb to suck, it indicates that she has recognised the lamb as her own, or has failed to identify it as an alien. Secondly, when lambs are given the choice of ewes, they will run to their dams and stay very near them, and when ewes are given the choice of lambs they will approach their own lambs and stay near. This behaviour of ewes may be modified a little in the field, because lambs normally run to their ewes more frequently than ewes move to their lambs. If a ewe is searching for her lamb however, she will move very close to any lamb to investigate it (Shillito Walser and Alexander, 1980).

The existence of ewe-lamb mutual recognition in sheep has been shown by several workers (Arnold et al., 1975; Shillito and Alexander, 1975; Alexander, 1977; Poindron and Schmidt, 1985). However, most of these studies were undertaken beyond the immediate neonatal period, i.e. when the ewe and her lamb have had an adequate chance to develop discriminating attachment to each other and the most critical period of the lambs' vulnerability is over. Several studies have been undertaken on the neonates' ability to discriminate between ewes from a distance (Arnold et al., 1975; Shillito and Alexander, 1975; Poindron and Schimdt, 1985; Nowak et al., 1987). The influence of the ewe's discriminating response on her lamb's ability to identify her from other parturient ewes has however not been studied in detail. The present study was designed to assess the effects of body mass, sex and type of birth, on the ability of 18-h old lambs to identify their own mothers from another ewe of similar physiological status. The extent of the discriminating response of the ewe for her own lamb, and its influence on the ability of the lamb to recognize her were also studied. The results of the lambs' discriminating abilities will be employed in a subsequent study (see Chapter 4) to assess their relationship to following responses.

Preliminary investigation (not described here) aimed at developing a set of criteria for classifying the lamb recognition response, only 6 out of 10 eighteen-h old lambs could identify their mothers from an alien ewe in a 2-choice test when released from a distance of 8 metres. In contrast 8 out of 10 lambs made a correct choice of ewes from a distance of 5 metres within 3 min. It was also observed in this study that the behavioural concerns of the ewe and lamb to separation tended to correlate with the lamb's ability to discriminate between its dam and alien ewes. On the basis of this, a procedure (mostly adapted from unpublished work of Dr.George Alexander, CSIRO Division of Animal production, Australia) was used for investigations into the following:

(i) the behavioural responses of a ewe and her lamb to separation

(ii) the ability of a lamb to discriminate between its own mother and an alien ewe in a 2-choice test from from distance of 5 m

(iii) the ability of a ewe to discriminate between her own lamb and an alien lamb in a 2-choice test from a distance of 7 m

(iv) the comparative ability of single and twin-born lambs to discriminate between their mothers and alien ewes in a 2-choice test from a distance of 7 m

3.2. MATERIALS AND METHODS

3.2 1. Apparatus

In the design of the behavioural tests, the characteristics of the neonatal lamb in particular, had to be taken into consideration. A testing pen and the experimental procedures that seemed to provide the neonate optimum and unbiased chance of identifying its mother were adopted only after preliminary testing of various alternatives. The testing pen was designed to meet the following criteria (see Nowak, 1989):

(i) a choice of one additional ewe and her lamb of a similar age to the test lamb

(ii) both own and alien ewes to be close enough to see and be seen by the lambs at the start of a test

(iii) ewes placed in adjacent pens so as to allow unbiased comparative identification by the lamb

(iv) the size of the testing pen not too large to discourage lambs from seeking ewes when released

(v) test lambs to be released at a point neither too close nor too far to allow unbiased and optimum recognition of ewes

The testing pen (Figure 3.1) was located on sparse grass about 50 m from the flock lambing area. The tests were conducted in a funnel shaped enclosure (approximately 3 x 7 x 0.7 x 7 m) of 1 m high metal hurdles Two adjacent pens (1.5 x 1 m each) for holding ewes were located at one end of the enclosure, while 7 m away were sited 2 smaller pens (0.7 x 0.5 m) for the lambs. The partitions in adjacent pens were covered to prevent animals from seeing each other, and the 3 outer sides of the lamb pens were covered to prevent the lambs from seeing the observer.

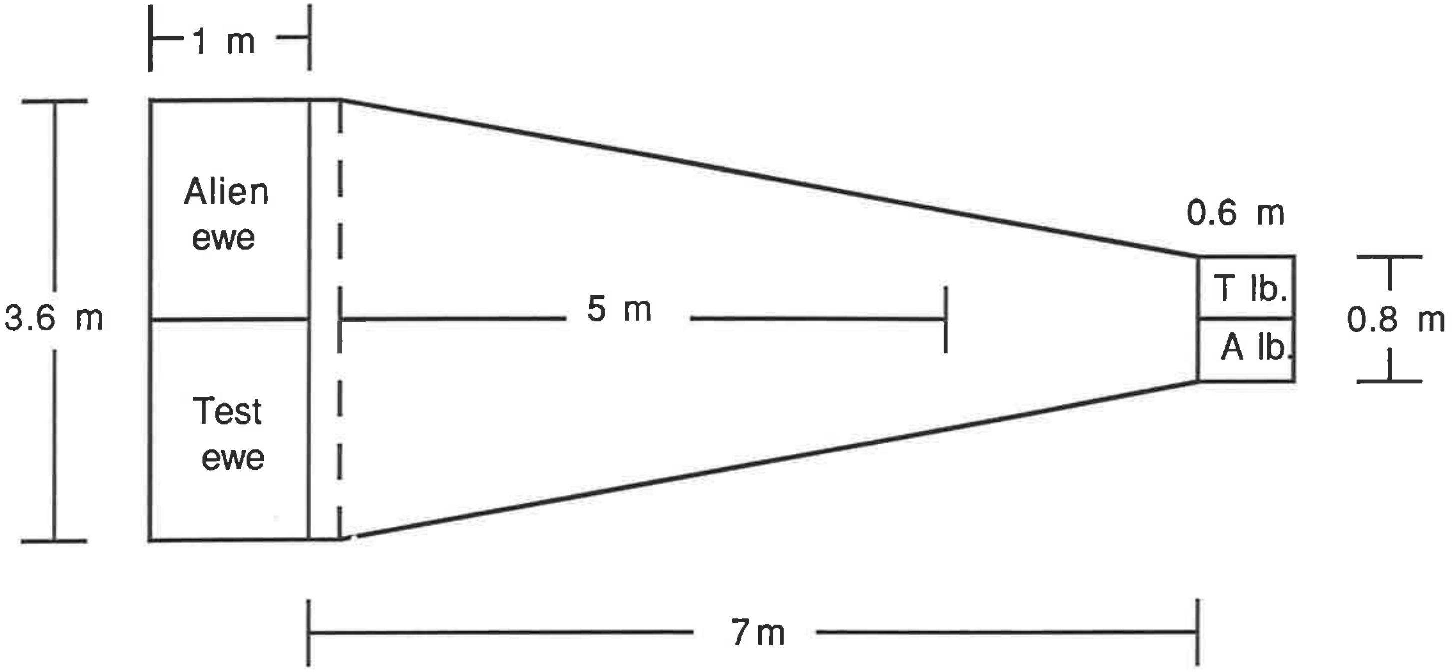

Figure 3.1 Testing pen used for ewe-lamb concern response to separation and two-choice recognition tests. T lb and A lb denote holding pens for test lamb and alien lamb respectively.

3.2.2. Animals

Lambs used in these investigations were chosen randomly from 2 groups of 97 and 36 South Australian Merino lambs born and reared as singles and twins respectively. A total of 43 single-born lambs dropped in either winter or autumn and their mothers were used in Studies 1, 2 and 3, and Study 4 involved a flock of 14 single- and 20 twin-born lambs dropped in autumn and their mothers.

3.2.3 Climatic conditions

The climatic conditions during the lambing period were generally cold an windy (see Table 2.1). The investigations were however carried out in fine climatic conditions. Tests were discontinued during rains and very windy weather.

3.2.4.Experimental conditions

3.2.4.1. Ewe-lamb behavioural concern at separation

At approximately 17 h post-partum the ewe and her lamb to be tested, as well as another parturient ewe (alien ewe) and her lamb (alien lamb) of a similar age to the test lamb, were held together in a holding yard (30 x 20 m) for about 30 min. Each ewe and her lamb were then confined to the ewe holding compartment of the testing pen for about 10 min during which period the lamb's acceptance by the mother, suggesting correct ewe-lamb pair, was confirmed. At about 18 h post-partum, the test and alien lambs were removed from their mothers and placed in adjacent cages 7 m away, in a standing position, facing alien and test ewes respectively. The concern behaviour or response of 43 ewes and their lambs to separation were observed and over a test period of 3 min. The ranking score for the response of the lamb and ewe are shown by Table 3.1.

Table 3.1 Ewe and lamb behavioural concern response.

Score	Lamb concern response	Ewe concern response
5	Very active, continuous high-pitch bleats, agitated	Restlessness, continuous high-pitch bleats
4	Continuous high-pitch bleats	Continuous high-pitch bleats, search
3	Active but a few bleats	Some bleat, little search
2	Inactive, a few bleats	Few bleats, no search
1	Down, inactive	No response

3.2.4.2. Ability of lambs to recognise their mothers from a distance of five metres

The lamb to be tested was gently removed from the pen, placed on the ground 5 m away facing the ewes and gently propelled towards the ewe by the assistant, who then withdrew. The ewe chosen by the lamb and the time taken to make a choice (i.e. the ewe it first approached within 25 cm, the range of proximal recognition indicated by Poindron and Schmidt, 1985) were recorded. Where the first choice was an alien the time taken to reach the lamb's own dam, if it occurred, was recorded. The tests lasted for a maximum of 3 min. Each lamb was tested only once to avoid possible bias resulting from previous experience in ewe selection. The test ewe and her lamb were used as 'aliens' in subsequent investigations where necessary. The ability of 43 single-born lambs to discriminate between their dams and an alien ewes were assessed as in Table 3.2.

3.2.4.2. Ability of ewes to recognise their lambs from a distance of seven metres

After each lamb recognition test, the lamb was placed with its dam in a pen for 3 min. After the lamb was seen to have been accepted by the mother, it was taken to a holding pen 7 m away and placed in standing position facing the ewes. The ewe to be tested was then released through a hatch and her ability to discriminate between her own and an alien lamb was assessed as in Table 3.2.

3.2.4.5 Comparative ability of single- and twin- born lambs to recognise their mothers

Similar procedures to those in Studies 1 and 2 were used to assess the concern of single- and twin-born lambs and their mothers at separation, and the ability of the lambs to discriminate between their dams and alien ewes. The twins were, however, tested separately with a minimum interval of 2 h.

Table 3.2 Ranking of ewe and lamb discriminative ability.

Score	Lamb behaviour	Ewe behaviour
5	Went directly to mother and stayed	Went directly to own lamb and stayed
4	Reached own mother after hesitsting between the two ewes	Reached own lamb after hesitsting between the two lambs
3	Reached alien ewe first but went to own mother later and stayed	Reached alien lamb first but went to own lamb and stayed
2	Went to alien ewe and stayed	Went to alien ewe and stayed
1	Did not reach either of the ewe	Did not reach either of the lambs

3.2.5. Statistical analysis

Values are presented as means with standard errors for quantitative data, and as average ranks for ranked variables such as separation concern and recognition responses. One way analysis of variance was used to determine the effect of body mass, sex, recognition class and type of birth on the time taken by a lamb to identifying a ewe and the speed of approach. This was followed by Student's t-test to evaluate the difference between groups when an effect was significant. Similarly, a Kruskal-Wallis one way analysis of variance was used to determine the effects on non-parametric variables such as lamb recognition responses and behavioural concern during separation. When an effect was significant, a Mann-Whitney test of 2-samples was used to analyse the difference (Zar, 1984).

The degree of the relationship between quantitative measurements was examined by a Simple Regression Analysis. The relationships between non-parametric variables, or between parametric and non parametric variables, were determined by Kendall's rank correlation tests. A Chi-squared distribution test was used to determine the randomness of a choice of ewe, following separation, by lambs according to body mass class, sex type, and the ewes' discriminating ability of lambs.

3.3. RESULTS

3.3.1. Ewe-lamb behavioural response at separation

During the 3 min separation of lambs at a distance of 7 m away from their mothers, all lambs and ewes, both test and aliens, exhibited varying degrees of concern. Behavioural response ranged from restlessness and high-pitch bleating, to relative inactivity with a few bleats (Figure 3.2). Fifty eight per cent of the test lambs (25/43), exhibited a high degree of agitation at separation, and emitted frequent high-pitched bleats throughout the observation period (Score 5). Eight of these lambs attempted to get out of the pen and in the process, hit their heads intermittently against the wire mesh at the front of the pen. Twenty six per cent

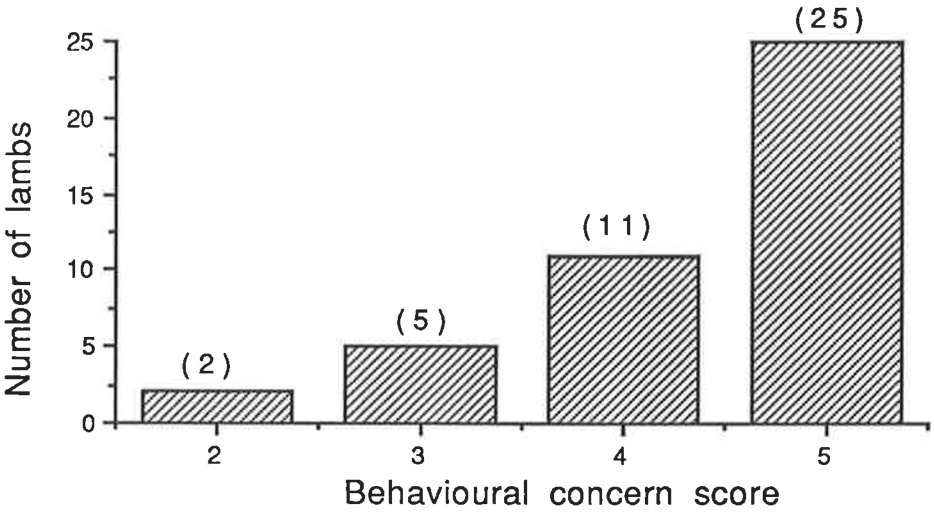

Figure 3.2 Behavioural concern shown by lambs to being separated from their mothers at a distance of seven metres.

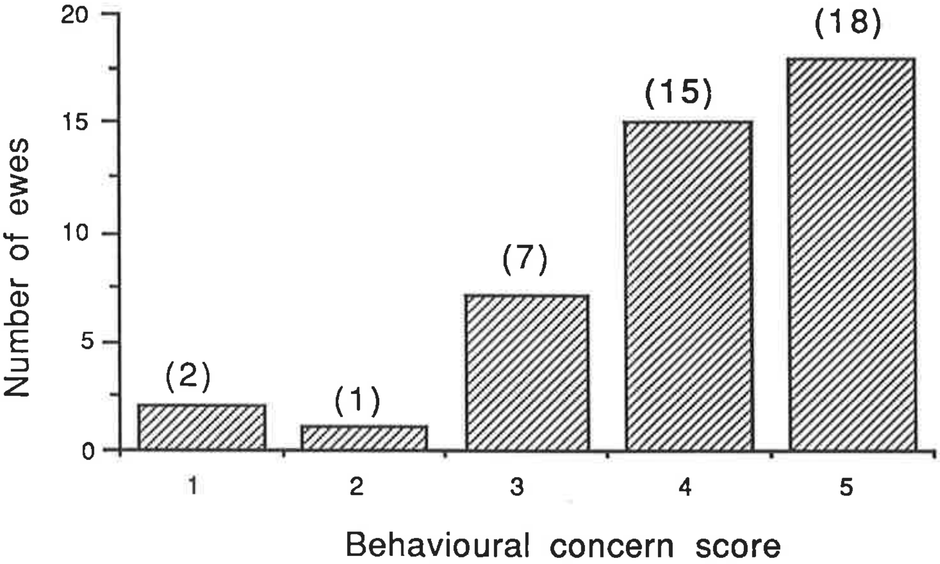

Figure 3.3 Behavioural concern shown by ewes to being separated from their lambs at a distance of seven metres.

of the lambs (11/43), comprising 8 males and 3 females (Score 4) exhibited similar behavioural concern to those in Score 5, though none of them was agitated. One of these lambs ceased bleating during the observation period for about 15 s, and resumed at a lower pitch. Twelve per cent of the lambs falling in Score 3 (5/43), comprising 2 males and 3 females, stood up facing the ewes, but did not seem to be concerned about being separated from their mothers. The rest of the lambs (Score 2), a female and a male, layed down throughout the observation period, and appeared unconcerned by the separation from their mothers. Attempts made to induce the lambs to stand up by prodding them with a stick failed. Neither sex type nor body mass of the lambs had any effect on the number of lambs exhibiting maximal response to separation.

The response of the test ewes to separation from their lambs ranged from extreme restlessness to complete lack of concern (Figure 3.3). Though the alien ewes' behavioural responses to separation were not recorded, they appeared to be as much concerned as the test ewes. Seventy seven per cent of the test ewes (33/43) exhibited a high degree of concern at separation, involving continuous high-pitched bleats, cycling and or pacing with the neck stretched most of the time as if searching for a lamb. Eighteen of these ewes exhibited extreme concern at the separation (Score 5), including 2 that attempted to jump out of the pen; the rest were ranked as Score 4. Sixteen per cent of the ewes falling in Score 3 (7/43) bleated intermittently at a high-pitch, but made little attempt at searching for their lambs. One ewe (Score 2) laid down in the pen throughout the observation period and made only a few bleats, mainly in response to the lambs call. The 2 ewes in Score 1 showed no concern whatsoever at the separation, despite the fact that their lambs were restless and bleated continuously. In general the lambs seemed to bleat more frequently in a response to the mothers' call, and ceased, or drastically reduced bleating, when the mothers were not calling. The behavioural concern at separation between the lambs and their mothers correlated significantly ($r = 0.35$; $P < 0.02$).

3.3.2. Ability of lambs to recognise their mothers at a distance of five metres

3.3.2.1 Qualitative behavioural observations

Eighty eight per cent of the test lambs (38/43) ceased bleating when being removed from their pens at the end of the separation tests. However, all the lambs rendered high-pitch bleats when being held on the ground by an assistant, at a distance of 5 m from the mother and an alien ewe, prior to and on being released. Eighty four per cent of the mothers (36/43) and most of the alien ewes rendered high-pitched bleats at the time of lamb release. Intermittent low-pitch bleats occurred in almost all the ewes as well as in 63% of the lambs (27/43) as they approached the ewes. The frequency of bleating in both ewes and lambs reduced considerably when contact between mother and offspring was achieved, or during the lambs' hesitation in choice of ewe.

On making contact with their mothers, most of the lambs attempted to reach them by placing their heads through the metal hurdles; at this time the ewes undertook olfactory examination of the protruding portion of the lambs' body. Following this, ewe and lamb nuzzled each other for a few seconds interspersed with occasional low pitch-bleating emanating mainly from the ewes. While the ewes were in apparent complacency after contact had been achieved, most lambs remained restless, actively nuzzled the ewes with their tails waggling and attempted to reach their mothers through the metal hurdles.

Where lambs first approached an alien ewe, they often hesitated in getting much closer, while the ewes tended to undertake olfactory examination of the lambs. Some lambs apparently realised that they had made a wrong choice of ewe, and continued to search for their mothers. On the other hand, a few of them seemed to accept an alien ewe, and made no attempt to go to their mothers, despite the latter's' agitation and bleating calls. Most alien ewes, on making contact with test lambs, became passive to their approaches. However 1 alien ewe exhibited an aggressive behaviour towards a lamb by continuously butting the lamb's head against the metal bars.

3.3.2.2 Quantitative observations

(1) Classification of dam recognition ability by lamb

Table 3.3 shows the distribution of tests according to sex, body mass and the lambs' ability to recognise their mothers. A similar proportion of male and female lambs were able to identify their mothers in the 2-choice test. All the lambs in the lower body mass class selected their mothers, whereas 1 and 2 lambs in the medium and high body mass classes respectively could not reach their mothers during the observation period. Distribution of time taken by lambs to identify their mothers from alien ewes in a 2-choice situation at a distance of 5 m is illustrated by Figure 3.4. Eighty eight percent of the lambs tested (38/43) that were able to recognise their mothers, irrespective of their first choice of ewe, did so within 3 to 60 s of being released (mean = 14.8 ± 2.0 s). Seventy four per cent of the lambs (25/43) falling into the Recognition Classes 5 and 4 showed a clear preference for their mothers, and reached them within 3 to 34 s (mean = 8.7 ± 1.4 s). However 30% of the lambs (14/43) in Class 3 went to an alien ewe first, within 6.0 ± 0.9 s, stayed for a short period, ranging from 5 to 54 s, before reaching their own mothers at 25.6 ± 4.2 s from being released (Table 3.4). Seven percent of the lambs ranked as Class 2 (3/43) went directly to the alien ewes and stayed, while 1 lamb (Class 1) did not make any attempt to seek her mother and consequently reached neither ewe within the observation period. Lambs in recognition Class 5 reached their mothers in a significantly shorter time than both those in Recognition Classes 4 ($p < 0.002$) and 3 ($p < 0.001$). Lambs in Recognition Class 4 also reached their mothers earlier than those in Class 3 ($p < 0.02$). There was, however, no significant difference in the time taken by lambs to alien ewe prior to reaching their own mothers (Class 3), and those that went directly to the alien ewe and stayed (Class 2). The overall ability of the lambs to recognise their mothers from alien ewes correlated significantly with both dam ($r = 0.59$, $p < 0.001$) and lamb ($r = 0.33$, $p < 0.03$) behavioural concerns to

Table 3.3. The number of tests done according sex, body mass and lambs' ability to recognise their mothers in a two-choice test.

Group of lambs	Class					Total
	1	2	3	4	5	
Sex						
Male	0	2	4	10	6	22
Female	1	1	10	2	7	21
Body mass						
< 4.8	0	0	7	5	6	18
4.8 - 5.7	1	0	4	3	4	12
> 5.7	0	3	3	4	3	13
Recognition class	1	3	14	12	13	43

Table 3.4 Relationship between the ability of 18-hour old single-born lambs to recognise their mothers and time taken to select a ewe in a two-choice test from a distance of five metres.

Recognition class	Time to recognise dam (s)	First choice of ewe	
		Dam (s)	Alien (s)
2	-	-	5.3 ± 0.9^{a} (3)
3	25.6 ± 4.2^{a} (14)	-	6.0 ± 0.9^{b} (14)
4	12.8 ± 2.3^{b} (12)	12.8 ± 2.3^{a} (12)	-
5	4.8 ± 0.3^{c} (13)	4.8 ± 0.3^{b} (13)	-

Values in the same row with different letters are significantly different; $p < 0.002$. Parantheses depict number of lambs in a class. Class 1 lamb did not reach a ewe during observation period.

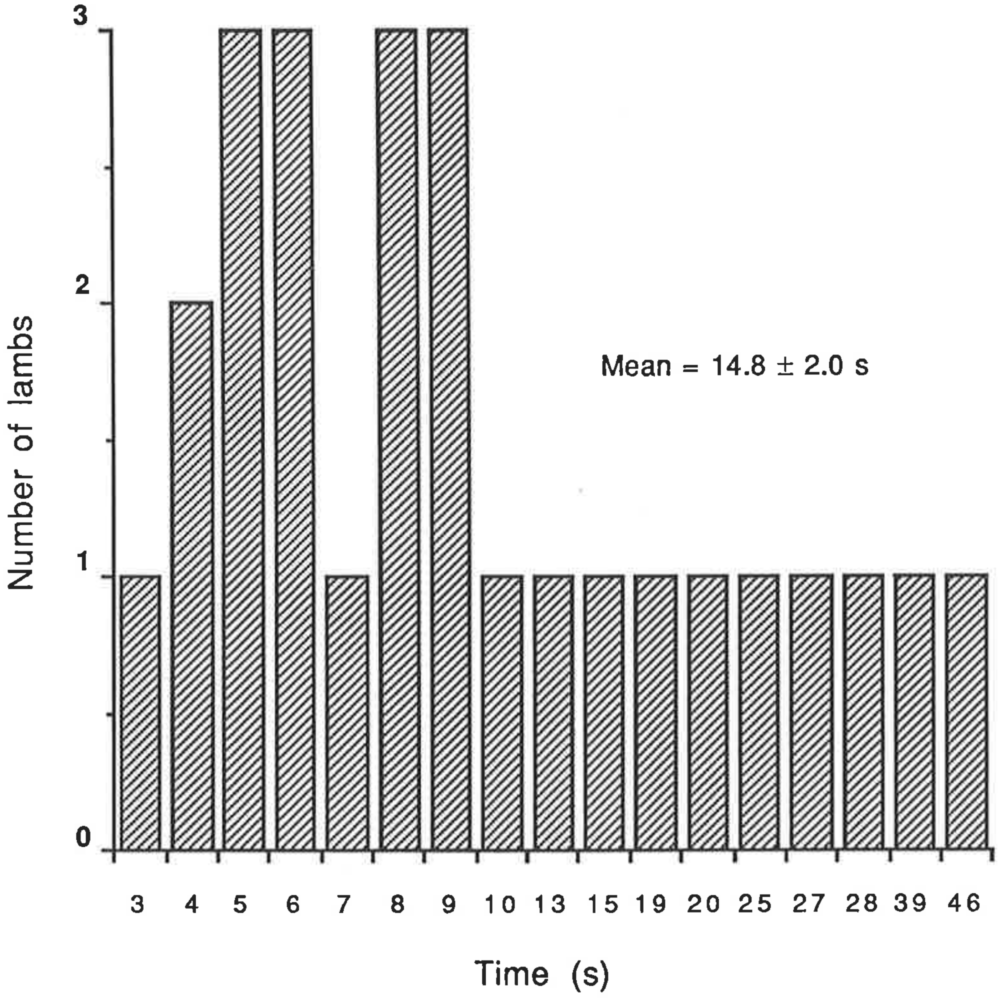

Figure 3.4 Time taken by 18-hour old single-born lambs to recognise their mothers in a two-choise test from a distance of five meters

the separation. The time taken by lambs to reach their mothers, irrespective of first choice of ewe, correlated significantly with the separation concern of their mothers ($r = 0.40$, $p < 0.01$) but not with their own concern at separation.

(2) Sex of lamb

The 91% of male lambs (20/22) that recognised their mothers tended to reach them in a shorter time than the 86% of females (18/21) that recognised their mothers (13.6 ± 2.4 s vs.16.1 ± 3.9 s). The difference in time was, however, not significant (Table 3.5). Male lambs tended to exhibit superior recognition of their mothers in comparison to the females, i.e., in terms of first choice of ewe (72 vs. 42%) (Figure 3.5). The females, nevertheless, took a slightly, but not significant, shorter time to achieve recognition (7.9 ± 2 .2 vs. 10.4 ± 2.0 s).

(3) Body mass of lambs

Table 3.6 shows the relationship between body mass class and time taken by a lamb to identify its mother in a 2-choice test. The lambs in Low (17/18) and Medium (11/12) body-mass classes that recognised their mothers did so within a slightly, though non significantly shorter period of time from being released than those in High body-mass class (13.7 vs 19.4 s). Sixty one percent of lambs in the Low body-mass class made a first choice of heir own mothers on release as compared to 58% and 54% of lambs in Medium and High body-mass classes respectively; this difference was not significant. The 39 percent of lambs in the Low body-mass group (7/18) that reached an alien ewe first did so at a significantly shorter time than those in Medium (4.3 vs. 8.5 s, $P < 0.06$) and High body-mass (4.3 vs. 6.0 s; $P < 0.01$) groups.

Table 3.5 Relationship between sex type and time taken by 18-hour old single-born lambs to select a ewe in a two-choice test from a distance of five metres.

Sex of lamb	Time to recognise dam (s)	First choice of ewe	
		Dam (s)	Alien (s)
Male	13.6 ± 2.4 (20)	10.4 ± 2.0 (16)	4.5 ±0.2 (6)
Female	16.1 ±3.9 (19)	7.9 ± 2.2 (9)	6.6 ± 1.0 (11)

Parenthesis depicts number of lambs in a class.

Table 3.6 Relationship between body mass and time taken by 18-h old single-born lambs to select a ewe in a two-choice test from a distance of five metres.

Body mass class	Time to recognise dam (s)	First choice of ewe	
		Dam (s)	Alien (s)
Low	13.1 ± 2.8 (18)	6.7 ± 0.7 (11)	4.3 ± 0.2 (7)^a
Medium	13.1 ± 3.4 (11)	10.9 ± 4.1 (7)	8.5 ± 2.6 (4)^b
High	19.4 ± 6.1 (10)	9.7 ± 2.7 (7)	6.0 ± 0.6 (6)^b

Values in the same row with different letters are significantly different; $p < 0.002$. Parantheses depict number of lambs in a class.

3.3.3. The ability of ewes to recognise their lambs from a distance of seven metres

The 3 ewes falling into Recognition Class 1 did not reach either of the lambs during the 3-min observation period after being released at a distance of 7 m , despite the fact that 1 ewe had her lamb bleating intermittently at high-pitch. The lambs of the other 2 ewes were inactive and did not bleat. Eleven ewes went directly to alien lambs, but 9 of them, after a few seconds of hesitation, went to their own lambs. A total of 29 ewes went to their lambs as first choice, though 14 of them hesitated between the alien and their own lambs.

Table 3.7 shows the number of lambs making a choice of a ewe according to the mother's discriminating ability of lambs, and Figure 3.5 shows the percentage initial choice of ewe by lambs after separation, according to the mother's ability to recognise their own lambs . Two out of 3 lambs belonging to ewes of Recognition Class 1 selected their own mothers as first choice, whereas neither of lambs born to ewes in Recognition Class 2 made a right choice of ewe. One of the 9 lambs belonging to ewes of Recognition Class 3 did not approach any of the ewes, and half of the remaining lambs made a right choice of ewe. Although a high proportion of lambs from ewes of Recognition Classes 5 (12/15) and 4 (9/14) made right choice of ewe, the dam's ability to recognise her lamb did not significantly influence the young's choice of ewe after separation.

3.3.4. Comparative ability of single- and twin-born lambs to recognise their mothers

Table 3.8 compares the ability of single- and twin-born lambs to identify their mothers in a 2-choice test. Single-born lambs (body mass 4.45 ± 0.21 kg) tended to perform better than twin-born lambs (body mass 3.42 ± 0.13 kg) in their ability to go directly to the dam after separation (86 vs. 40%). One single- and 2 twin-born lambs did not reach either ewe during the 3-min observation period, and subsequent observation indicated that these lambs were not attracted to any of the ewes. The single-born lambs that reached their ewes did so

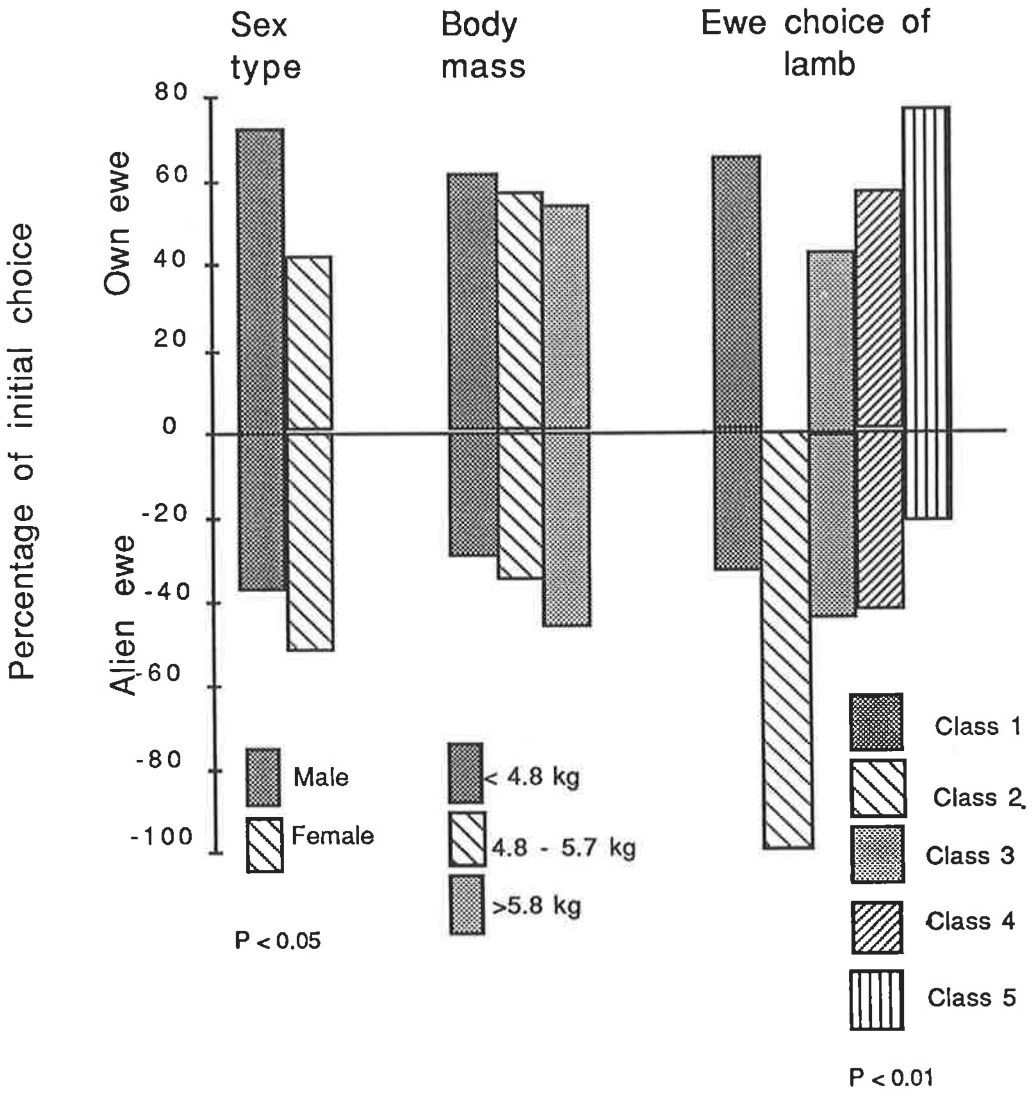

Figure 3.5 Ability of 18-hour old single-born lambs to select a ewe at a distance of five metres in a two-choice situation.

Table 3.7. Relationship between the ability of ewes to recognise their lambs at 18 hours post-partum and the number of single-born lambs selecting a ewe in a two-choice test.

Ewe rocog class	Number	Ewe selection by lamb		
		Dam	Alien	None
5	15	12	3	0
4	14	8	6	0
3	9	4	4	1
2	2	0	2	0
1	3	2	1	0

Table 3.8 Relationship between type of birth and time taken by 18-hour old single-born lambs to select a ewe in a two-choice test from a distance of five metres.

Type of birth	Time to recognise dam (s)	First choice of ewe	
		Dam (s)	Alien (s)
Single	18.2 ± 2.4 (12)	10.6 ± 2.2	7.5 ± 2.5 (4)
Twin	16.3 ± 2.6 (16)	12 7 ± 3.2	13.9 ± 2.9 (9)

Parentheses depict number of lambs in a class.

within of 18 s of being released. This did not differ significantly from the slightly shorter time of 16 s taken by the twins to reach their mothers.

Whereas no significant difference was observed in the single- and twin-born lambs' concern at being separated from their mothers prior to the recognition ability test, the twins tended to bleat slightly more frequently. Their mothers also emitted significantly more bleats than those of single-born lambs (average rank = 18.2 vs. 11.9; $P < 0.04$).

3.3.5. Ewe-lamb mutual recognition

Results of Studies 2 and 3 summarised in Table 3.9 indicate that 79% (34/43) of single-born lambs and their dams exhibited mutual recognition after separation. Among the mother-young couples not exhibiting mutual recognition, 5 lambs recognised their mothers, while 2 of their dams made a wrong choice of lamb with the rest (3) choosing neither lamb. In the remaining 4 couples, all the ewes chose correctly, whereas 3 lambs made wrong choices, and 1 did not go to any of the lams. Though a ewe or lamb might not succeed in selecting the partner, there was no occasion where both the dam and her offspring made wrong choices of partners.

The proportion of mother-young couples (Study 4) in which both animals exhibited mutual recognition was higher in single-born lambs and their mothers referred to as "single-couples" than twin-born lambs and their mothers referred to as "twin-couples" (10/13 vs. 14/20). In both "single-" and "twin-couple" groups, 2 lambs each made a correct choice of ewes, while their dams chose alien lambs. One lamb choosing an alien ewe was recognised by the mother. Also only 1 ewe-offspring couple from each birth type could not recognise each other.

Table 3.9 Ewe and lambs making correct choice of partners.

Lamb birth-type	Ewe-lamb units	Partners making correct choice			
		mutual	lamb alone	Ewe alone	None
Experiment 2					
Single	43	34	5	4	0
Experiment 4					
Single	14	10	2	1	1
Twin	20	14	2	3	1

3.4.DISCUSSION

3.4.1 Ewe-lamb behavioural concern at separation and dam recognition ability of single born-lambs

The large proportion of single-born lambs that exhibited high behavioural concern at being separated from their mothers, and were able to successfully discriminate between their dams and an alien ewe after separation in the present study, supports the contention that the mechanisms involved in the lambs' ability to recognise a ewe as an alien is reasonably well developed in most lambs during the first day of life (Shillito and Alexander 1977). This represents a considerable achievement for one so young, for it suggests an ability to distinguish identifying characteristics of the mother very soon after parturition, and a subsequent encounter to match sensory input with this stored information (Lickliter and Heron,1984). The high behavioural responses to separation shown by both ewes and lambs in this study may, however, not be a true representative of the concern behaviour to separation in field situations. The prevention of the ewe and her lamb from seeking each other by confinement might have led to greater restlessness in both animals. Nevertheless, ewe-lamb pairs exhibiting high mutual concern at separation in this study also tended to exhibit good recognition ability of the partner, and are therefore more likely to be active and succeed in their search for a separated partner in a field situation among a flock of parturient ewes and their lambs.

Single-born lambs in the present study took a relatively shorter time to search and choose their dams from alien ewes than Merino lambs of the same age but separated by 6 m from their ewes (15 s vs. 52 s.) observed by Arnold et al. (1975). Assessment of the comparative discriminating ability of the lambs in the 2 investigations is however difficult, since lambs in the study of Arnold et al. (1975) were separated from their mothers by an additional metre distance. They were also subjected to a choice of 3 ewes and as such, simultaneous calls from the ewes were likely to have compounded the difficulty of the dams' bleats serving as specific location signals. It is therefore not surprising that the proportion of lambs recognising their dams and going to them was relatively low, approximately 35%, compared to 91% in the present study. There was however, a progressive improvement in the discriminating ability of the lambs to 8 days of age in the study of Arnold et al. (1975), after which the proportion that could recognise their dams reached a similar value to that observed in the present study. The small proportion of lambs that went to alien ewes and stayed or made no choice of a ewe in the the 2 studies suggests that a small proportion of the lambs may probably have to rely more on their dams to re-initiate attachment, or on alien ewes for adoption if they are to survive in the field. It must be remembered however that in a field situation the ewe would be contributing more to achieving attachment after separation than is possible in these studies, and that in practice a few, if any, lambs would remain separated from their dams for long (Arnold et al., 1975).

The lack of significant difference in the time taken by a lamb to reach either a dam or an alien ewe in this study may be partially attributed to the fact that the capability for distant recognition might not have been well developed in some of the lambs so as to enable them to efficiently achieve recognition of a ewe as an alien from the distance of 5 m. Such a lamb would therefore respond at first sight to any ewe, since there are indications that the use of visual cues in recognition may not be efficient at more than a metre longer than the separation distance employed in this study (Shillito Walser et al., 1985). Field studies indicated that lambs bleated and scanned the field following separation, and often ran towards any ewe which stopped grazing and lifted her head. This initial orientation was checked by the lambs when they were 8 to 10 m away from the ewes. At this distance some of the lambs were close enough to have been able to commence relying on visual cues in conjunction with auditory cues to discriminate between the ewes. However, both senses are thought to be poorly developed in the neonate (Shillito, 1975; Alexander, 1977).

The high-pitched bleats emitted by almost all the ewes, both dam and alien, on release of a lamb and during the initial ewe seeking activities of the lamb in this study is consistent with the observations by Nowak et al. (1987) of Merino and Merino x Border Leceister crossbred sheep. In the present trial, since both test and alien ewes bleated simultaneously, it was not always possible to determine the source of maternal bleats. Neither could it be ascertained whether the dam's bleats served as specific cues to enhance the lamb's ability to make a definite identification, or were mere location signals. However in almost all cases, the louder and more frequent bleats emitted by the dams in comparison with those of the alien ewes during the initial approach of lambs is consistent with the observations of Lickliter and Heron (1984) involving goats. Some of the ewes having their lambs attracted by an alien ewe emitted intermittent high-pitched bleats with occasional rumbling sounds. Such a behaviour may indicate doubtful recognition or the ewe's inability to recognise their lambs, since high-pitched bleats are emitted only when the 2 are separated but not when they are at close proximity. Emitting of low-pitched bleats by the ewes as their lambs got closer might have provided cues to aid the lambs' discriminating behaviour as they made a choice of ewe (Nowak et al., 1987). The alien ewes, on the other hand, often continued with the high-pitched bleats which might have further helped in the lambs' discriminating action against them. These behavioural patterns were more apparent when a lamb hesitated in making a choice of an ewe. There appears also to be specificity of auditory cues in lamb vocalization, since dams that had decreased their concern at the separation resumed bleating, and often became agitated, as a response to the continuous high-pitched bleats from their own lambs, rather than alien lambs. The positive correlation between the frequency and pitch of bleats emitted by the ewes and their lambs during separation supports the contention that lambs tend to respond to maternal calls by bleating (Shillito Walser and Elisson, 1984).

On first making contact after separation, some of the ewes did not seem to effectively accept or reject lambs until olfactory examinations have been undertaken. This is not surprising in view of the importance of olfactory cues to the ewe in either accepting or rejecting a lamb at close quarters (Smith, 1965; Morgan et al., 1975; Alexander and Stevens, 1981). Although the ewes prefered investigating the anal region in comparison with other parts of the body for identification of lambs after separation in the field, the head, which was the only part of the lamb's body accessible for identification in the present study, seemed to have provided adequate olfactory cues for effective discriminating response. This supports the observations by Alexander (1978) and Shillito Walser (1980) that Merino ewes could use both the tail region and head of the lamb for olfactory identification, provided that the lamb was near to the ewe.

Though female lambs tended to recognise their dams earlier after separation than their male counterparts, the results, nevertheless, suggest that sex of a lamb does not influence its ability to discriminate between its own dam and an alien ewe. Nowak (1989) found that 18- and 24-h old female twin-born lambs performed better than the males in their recognising ability, and that 12-h old female lambs also spent more time with their mothers in a 5-min test of recognition. No obvious explanation could be attributed to the sex differences observed. However, the author suggested that the sex related differences might be emphasised only in situations where severe competition occurs.

3.4.2. Comparative ability of single- and twin born lambs to recognise their mothers

The insignificant difference in recognition response between single- and twin-born lambs, and the slightly shorter time taken by the twins to recognise their mothers, may be an indication of equally well developed discriminating ability of ewes in the 2 birth types, despite the lower maturity of the lamb at time of birth. This is in contrast to the finding of Nowak (1989) where the difference was significant and in favour of single-born lambs at 18 and 24-h after birth; nevertheless, his study did not show any difference in the recognition ability of 12-h old single- and twin-born lambs in terms of time spent with their mothers. The shorter gestation length in the twin-bearing ewes, and the disturbance in the process of bond formation by the presence of a sibling, were suggested by Nowak (1989) as possible explanation for the higher incidence of separation normally observed in twins. He suggested that twins need at least one day more to reach the level of maturity that is attained in singles at the time of birth in view of their shorter gestation length.

The percentage of ewe-lamb couples exhibiting mutual recognition in the present study (71 to 88%) was similar to that obtained in a flock of Merino d'Arles and Merino x

Romanov sheep kept outdoors, but higher than the 43% mutual recognition exhibited by those kept indoors in the study of Poindron and Schmidt (1985). The significant difference seen in ewe-lamb couples under the 2 experimental conditions suggests that distance recognition can be influenced by environmental conditions. Among the many factors thought to have possibly influenced the lower mutual response in the sheep kept indoors was that the animals, although able to recognise each other by visual and acoustic cues, may not be accustomed to discriminating at a distance and would therefore be inefficient in expressing a choice in the test presented to them. Alternatively, olfactory discrimination may be sufficient to ensure mother-young cohesion, and this may lead to the non-establishment of visual and auditory recognition (Poindron and Schmidt, 1985).

The higher, but non-significant difference in mutual recognition observed in the "single-couples", in comparison to the "twin-couples", may be due to the fact that the ewe spends more time during the immediate post-partum period with a single offspring than it could possibly achieve with twins, where sibling rivalry for attention could lead to disruption in mother-young attachment. In the present study, as well as that of Poindron and Schmidt (1985), the proportion of couples not exhibiting mutual recognition had more lambs selecting their dams in a 2-choice test than it was for the reverse. The mothering ability for twins in a flock varies widely during the neonatal period but improves with experience (Alexander et al., 1984). The ewes in this trial being multiparous were thus experienced in lamb care. This might have contributed significantly to the high recognition response of lamb by the dam and the mutual recognition observed in the "twin couples".

The test systems used in these investigations have the disadvantage that the responses of the dam and young are likely to be affected by the responses of the partner (Alexander,1977). However, the high behavioural response to separation exhibited by the single-born lambs, as well as the high success rate in the ability of both single- and twin-born lambs to recognise their dams following separation, suggests that the capability of lambs to seek and recognise their dams within the first day of life is quite well developed in the Merino lamb. The results also indicate that the widely held view that the mother plays the major role in the mutual identification during the first few days of the lamb's life, may not be correct. There is no doubt that the newborn lamb plays a much greater role in the maintenance of proximity and mother-offspring mutual attachment than that normally attributed to it, especially in multiple-born lambs. They therefore often have a "say" as to their survival during the neonatal period.

CHAPTER 4. FOLLOWING-BEHAVIOUR IN NEONATAL LAMBS

4.1. INTRODUCTION

Ewes and their lambs tend to stay closely together and the lambs do not wander far from the birth sites on their own during the neonatal period. However, since lambs tend to follow their mothers as they graze or move to water, the ewes need to restrict their own activities so as to remain near their lambs to facilitate frequent suckling episodes. This behaviour of the sheep contrasts with that of the "hider" species, such as cattle and goats, in which the young initially remains concealed between infrequent suckling episodes, while the mothers are free to move over long distances in search of food and water (Stevens et al., 1982).

The ability of a lamb to follow its mother in her quest for food and water, sometimes over long distances such as in the semi-arid pastoral regions of Australia, or under some traditional village farming systems in Africa and Asia where sheep are often left to fend for themselves on uncultivated lands far from the settlements, would undoubtedly be a necessity to obtaining nourishment and protection from the ewe and is therefore crucial to the survival of the lamb. Loss of energy resulting from locomotion is not a limiting factor to the ability of a suckled lamb to follow its mother over long distances since only about 1.4% of the total energy expenditure may be devoted to locomotion in such a situation (see Chapter 5.2). Following-behaviour possibly plays a major role in maintaining ewe-lamb contact (Winfield and Kilgour, 1976) particularly when lambs have to follow their mother over long distances.

There are a few studies dealing specifically with the following ability or behaviour of the ruminant neonate (Sguires, 1970; Winfield and Kilgour; 1976; Kishimoto, 1989) Of these the study of Winfield and Kilgour (1976) is the only detailed experimental investigation on the following-behaviour of neonatal lambs. However, the work presents observations on the reactions of lambs to a moving surrogate ewe in an environment that does not reflect the field situation. The present investigations examine the behavioural interactions between 48 h-old lambs and their dams during walking over long distances in a laneway. The effects of the ability of the lambs to discriminate between their own mothers and alien ewes (as determined in Chapter 3), type of birth, sex, and body mass of lambs on their following-responses are assessed

4.2. MATERIALS AND METHODS

4.2.1. Animals

A total of 30 healthy single-born 2-day old lambs weighing 5.14 ± 0.17 kg (range 3.5 to 6.8 kg) dropped in born in August, 1989 were used in Study 1. Study 2 involved 13 single- (4.9 ± 0.3 kg) and 17 twin-born (3.6 ± 0.2 kg) lambs dropped in April, 1989. Both groups of lambs were born and the experiments undertaken at the Waite Agricultural Research Institute Field Experiments Station, Glenthorne, Adelaide. The lambs were born outside and had access to their mothers prior to and during the experiment. Each lamb was used at approximately 48 h of age and only once.

4.2.2. Experimental procedure

(1) Ability of single-born lambs to follow their mothers over one kilometer distance (Study 1)

Prior to each testing session, the selected lambs (approximately 47-h old) and their dams were held in a small yard (15 x 10 m), shaded by the canopy of a tree. The lambs were weighed and then rested, together with their mothers, in the yard for a minimum of 30 min. The lamb to be tested was removed and held for about 10 min by an assistant sitting 2 m from the yard during which time resting state physiological measurements (rectal temperature, heart and respiratory frequencies) were measured and a blood sample taken for other studies being carried out simultaneously. The lamb and its mother were then held for 5 min in a pen (4 x 3 m) leading to a laneway (described below) to ensure that the lamb was accepted by the mother. The gate to the laneway was then opened and the lamb allowed to follow the mother at its own walking pace. An observer followed the animals at about 5 m behind and recorded the time taken by the lamb to cover each 0.25 km distance and the mean

Table 4.1. Ranking of following-responses in 24-hour old lambs.

Class	Ease of following	Frequency and pitch of bleating	Position of lamb
5	Very Good	Continuous high-pitched bleats	Ahead of ewe
4	Good	High-pitched bleats with few pauses	At the shoulder of ewe
3	Fair	Intermittent high-pitched bleats	At the hind leg
2	Poor	Few low-pitched bleats	Behind the ewe
1	Very Poor	No bleats	Does not follow

Ease of following is a subjective assessment of how well the lamb follows, frequency of bleating is a record of the pitch and frequency of bleats emitted by lamb, and position of lamb is a record of the futhermost sustained position of the lamb relative to the ewe.

distance between the ewe and her lamb during walking. The following-responses of the lambs (adapted from unpublished work of Dr. Alexander, CSIRO Division of Animal Production, Australia), ease of walking, following-position and bleating frequency and pitch, were subjectively assessed (see Table 4.1).

(2) Comparative ability of single- and twin-born lambs to follow their mothers over two kilometer distance (Study 2)

Fourteen single-born and 19 twin-born lambs were subjected to following tests similar to Study 1, but in this case, following was over 2 "laps" of 1.0 km distance. Twin-born lambs were tested one at a time to prevent sibling interaction during walking and the ewe's divided attention to the lambs. Testing of siblings were separated by a minimum of 2 h interval. The time to cover each 0.5 km distance and following-responses of the lambs completing the total distance were recorded according to the subjective assessment in Study 1.

4.2.3. Apparatus

Ewes and their lambs normally walk in various directions, often changing directions and at times retracing their steps. In order to obtain a good estimate of the actual distance covered during the following test, a laneway was designed to meet the following criteria:

1) Sufficiently long to minimize any artifact at the end of a return journey as ewes tend to run faster as they approach a known territory; in this case the starting point of the test.

2) Sufficiently narrow to restrict lateral movement and discourage ewes from turning back during walking.

3) Bare of vegetation in order to prevent grazing by ewes during walking which would otherwise interfere with lamb following ability.

The laneway consisted of a long and narrow path (2 m x 0.5 km) of gentle undulating terrain, shadeless and almost bare of vegetation as a result of herbicide application. The laneway was fenced 1 m high with woven wire, and the mid-point (0.25 km) marked. A pen (3 m x 2 m) was erected at the entrance to the laneway for holding the mother and her lamb prior to the test.

4.2.4 Statistical analysis

Values are presented as means with standard errors for quantitative data, such as speed and the time taken by the lamb to follow the mother, and as average ranks for subjective variables, such as the pitch and frequency of bleats, ease of following , and the following position of the lamb in the laneway. One way analysis of variance was used to determine the effect of body mass, sex, ewe recognition ability by lamb and type of birth on the time taken by the lamb and the speed to follow the mother in the laneway. This was followed by a Student t-test to evaluate the difference between the groups when an effect was significant. Similarly, a Kruskal-Wallis one way analysis of variance was used to determine the effects on the non-parametric variables. When an effect was significant, a Mann-Whitney comparison of 2-sample test was used to analyze the difference.

The degree of the relationship between quantitative measurements, such as speed of following and sustained distance between mother and lamb, were examined by simple regression analysis. The relationships among non-parametric variables, such as ease of following and bleating frequency, or parametric and non-parametric variables such as the speed and ease of following , were determined by Kendall's rank correlation tests.

4.3. RESULTS

4.3.1. Ability of single-born lambs to follow their mothers over one kilometer distance (Study 1)

(1) General following-responses

Two female lambs of body mass 4.2 kg and 4.8 kg refused to follow their mothers when released into the laneway, and 1 male lamb (body mass of 6.5 kg) walked for only about 100 m, stopped and refused to follow any longer. One of the female lambs whose sucking attempts were thwarted by the mother attempted to obtain milk from other ewes prior to and after the test, indicating poor development of mother-young attachment in the pair. The other female lamb however seemed to be active and had shown a good recognition ability of its mother in a previous study; its refusal to follow the ewe could therefore not be explained.. The male lamb which was delivered through difficult birth exhibited poor vigour at birth. Furthermore, its gait while following the mother seemed to be poorly coordinated. This might have resulted from a birth injury during the prolonged birth process. These 3 lambs were taken out of the study.

Immediately the gate between the holding pen and the laneway was opened, most of the ewes moved away at a fast pace for about 20 m, followed by their lambs. Thereafter if a lamb was not following within a body length (about 1 m) of the ewe, the mother often slowed down or stopped entirely to enable the lamb to catch up before the two moved off again.

Table 4.2 shows the number of single-born lambs tested according to their following-responses. Plates 1 and 2 show lambs following their mothers at the hind-leg and at 5 m behind respectively. The lambs followed their mothers in the laneway at a mean distance of 1.4 ± 0.3 m, with a range of 1.0 to 5.0 m, except 1 lamb which followed consistently at about 10 m behind the ewe. The mother of the lamb made no attempt to wait for it, but rather galloped off whenever the lamb was about to catch up with her. The furthermost sustained positions achieved by the majority of the lambs relative to the ewes were at the shoulder (36%, 11/30) and the hind-leg (40%, 12/30) of the ewes. Only 1 lamb occasionally maintained position ahead of the ewe. The time taken by the lambs to follow their dams were 2.94 ± 0.09 min at 0.25 km, 5.70 ± 0.17 min at 0.50 km and 9.01 ± 0.24 min at 0.75 km. The time for covering the entire distance was 12.17 ± 0.39 min at a speed of 1.41± 0.04 m/s (range = 0.99 to 1.95 m/s) . However, less than half the lambs studied were able to complete the following distance within the group average time (Table 4.3).

The narrowness of the laneway succeeded in restricting lateral movement of most of the ewes and lambs. Two ewes frequently attempted to turn back, and were prevented from

Plate1. A lamb following its mother by the hind leg (Following-position Score 3) in a long and narrow laneway.

Plate 2. A lamb following its mother at distance of five metres away in a long and narrow laneway (Following-position Score 2).

Table 4.2. The number of 48-hour old single-born lambs tested according to their their following-behaviour during a one kilometer walk.

Following behavior	Rank				
	1	2	3	4	5
Ease of following	2	6	6	8	8
Following position	0	7	11	12	8
Bleating frequency	0	23	4	12	2

Table 4.3 The time taken by single-born lambs to follow their mothers in minutes over a distance of one kilometer in relation to their discriminative ability of ewes.

Parameter	Recognition class				
	2	3	4	5	Total
Number of lambs following	3	10	8	9	30
Mean time to follow	13.16	12.73	12.10	11.28	12.17
Percentage of lambs above mean time	100	50	63	33	53

doing so by the observer, and 3 lambs completed the following distance by walking mostly in a zigzag manner. The ease of following was fair to very good (Classes 3 to 5) in 73% of the lambs (22/30), poor (Class 2) in 20% of the lambs (6/30), and very poor (Class 1) in 2 lambs. Despite the difficulty in following exhibited by 2 lambs, they nevertheless completed the following distance at a shorter time than a quarter of the lambs. The mother of one of these bleated frequently at a high pitch to induce her lamb to follow, and she had to turn back on 3 occasions, staying with the lamb for up to 30 s before setting off again together.

When separated by a few metres from each other during walking, the ewe and her lamb often emitted low pitched-bleats. There were no or minimal low-pitched bleats emitted by either of them when lambs were following at close proximity to their mothers. Bleating was at a high-pitch when the lambs were following far behind. This behaviour was clearly exhibited by a ewe which had her lamb following occasionally at a distance of about 10 m. On the whole, lambs bleated more frequently than their mothers during walking.

Seventy seven per cent of the lambs (25/30) urinated during walking with 63% (19/30) urinating more than 3 times during the 1 km distance. The frequency of defecation during walking was quite low in the lambs (27%, 8/30), however all the ewes defecated, with 90% (27/30) doing so at least twice during the trial. The ewes kept on walking while urinating or defecating, whereas the lambs always stopped following while urinating, which on the average took about 15 s, and resumed walking only on the completion of urination. They however continued following their mothers while defecating. A few sucking attempts were made by the lambs during the walk.

(2) Mother recognition ability by lambs and following-responses

The lambs' ability to follow their mothers in the laneway, in terms of ease of following, improved significantly with their increasing ability to discriminate between their own mothers and alien ewes ($r = 0.65$, $p < 0.001$). Lambs in Recognition Classes 5 and 4 followed their mothers better than those in Classes 3 ($p < 0.003$), and 2 ($p < 0.02$) (Table 4.4). There was, however, no significant difference in the ease of following between lambs in Classes 5 and 4, and Classes 3 and 2.

Table 4.4. Relationship between the ability of 18-hour old single-born lambs to recognise their mothers and their responses during a one kilometer walk at 48 hours of age.

Recogition class	No..of Lambs	Ease of following (av. rank)	Following position (av. rank)	Bleating frequency (av. rank)	Following distance (m)	Following speed (m/s)
2	3	7.2^{a}	13.8	28.2^{a}	1.8 ± 0.4	1.27 ± 0.01
3	10	11.0^{a}	13.8	15.5^{b}	2.3 ± 0.9	1.35 ± 0.08
4	8	18.6^{ab}	16.5	14.2^{b}	1.3 ± 0.2	1.39 ± 0.06
5	9	20.6^{b}	17.1	12.5^{b}	1.1 ± 0.1	1.54 ± 0.10

Values in the same column with different letters are significantly different at 5% level.

Table 4.5 Relationship between body mass and following-responses in single-born lambs during one kilometre walk at 48 hours after birth.

Body mass (kg)	Number of lambs tested	Ease of following (av rank)	Following position (av, rank)	Bleating frequency (av. rank)	Following distance (m)	Following speed (m/s)
<4.8	11	19.0^{a}	13.7^{a}	13.7	1.9 ± 0.1	1.41 ± 0.09
4.8 - 5.7	9	15.8^{ab}	12.6^{a}	15.8	1.2 ± 0.2	1.44 ± 0.05
>5.7	10	11.9^{b}	20.2^{b}	17.2	1.2 ± 0.2	1.38 ± 0.08

Values in the same row with different letters are different at 5 per cent level.

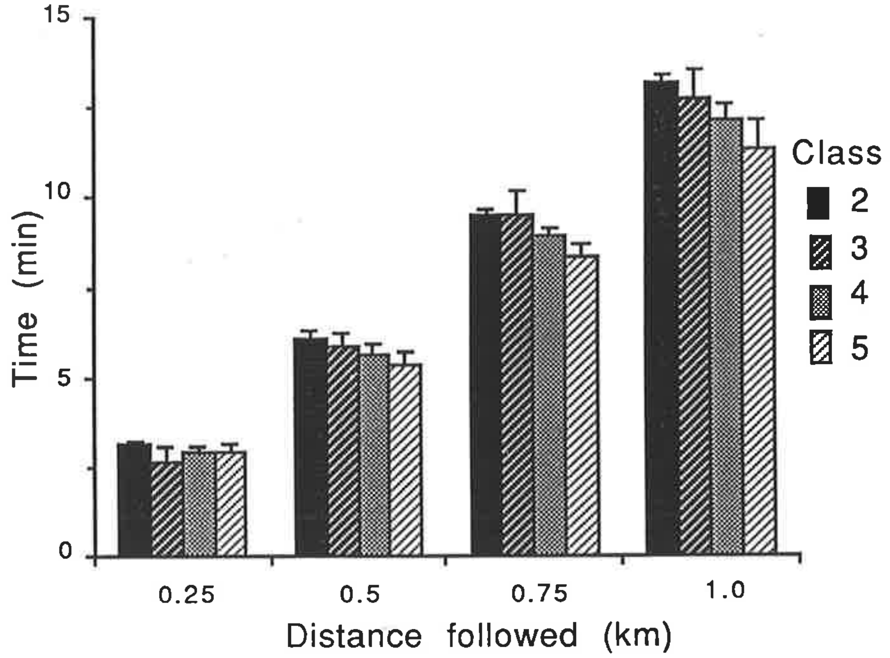

Figure 4.1 Relationship between the ability of 48-hour old lambs to recognise their mothers and and time taken to follow them over a distance of one kilometer.

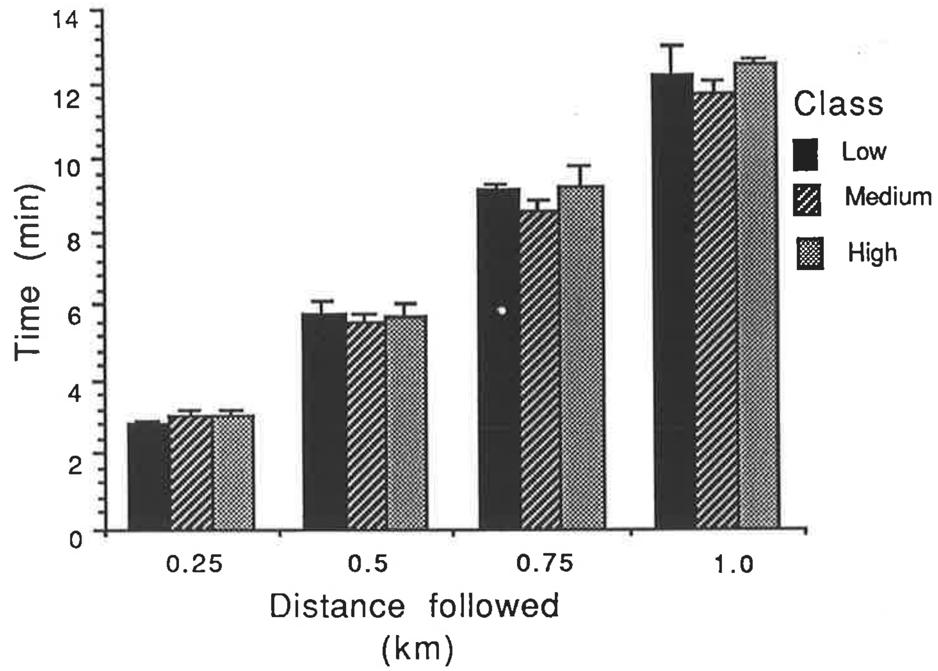

Figure 4.2 Relationship between body mass of 48-hour old lambs and time taken to follow their mothers over a distance of one kilometer.

The walking positions of the lambs relative to their mothers did not follow any particular trend. The only lamb which occasionally walked ahead of the ewe was in lamb Recognition Class 3. However lambs in Recognition Classes 3 and 2 tended to follow further behind their mothers than the Classes 5 and 4 lambs. Eighty nine per cent of Class 5 lambs (8/9) followed the ewes at either the shoulder or hind leg, compared to 75% (6/8)in Class 4 , 70% (7/10) in Class 3, and 67% (2/3) in Class 2.

The time taken by the lambs to cover the 1 km following distance tended to decrease, due to an increase in the speed of walking, with their increasing recognition ability of mother ($r = 0.36$; $p < 0.05$), though the differences were not significant (Figure 4.1, Table 4.8). Fifty three percent of the lambs (16/30) took more than the flock average time of 12.17 ± 0.03 min to follow their mothers over the 1 km distance. With the exception of Recognition Class 5 which had 33% of the lambs (3/9) covering the distance at a greater time than the flock average, all the other recognition classes had more than half their lambs taking more than the flock average to complete the walking.

(3) Lamb body mass and following-responses

The relationship between the body mass of lamb and the following-response is shown by Table 4.5. The body mass of lambs did not have any influence on their following-activities, and neither was there any difference in the responses between the 3 different groups of body mass, Low (below 4.8 kg), Medium (4.8 to 5.7 kg) and High (above 5.7 kg). However, lambs in the Low mass class tended to follow their mothers better than the High mass lambs ($p < 0.06$), and achieved a more distant sustained following-position than those in the Medium body mass class. The furthermost sustained position of the lambs relative to the ewes during walking in the High mass group was also slightly higher than the Medium and Low mass groups ($p < 0.08$).

Lambs in the High mass class tended to follow, on the average, 0.1 to 0.8 m closer to their mothers than those in the Medium and Low body mass classes respectively, though the differences between them were not significant. There was no difference in the frequency and pitch of bleats emitted during walking among lambs in the 3 body mass classes. Lambs in High and Medium body mass classes tended, however, to emit more high-pitched bleats than those in the Low body mass class. There was no significant difference between the time taken by the lambs to cover each 0.25 km distance among the body mass classes (Figure 4.2). Nevertheless, lambs in the Medium mass class tended to cover the 0.75 and 1.0 km distances at a shorter time than those in the other two classes

(4) Sex of lamb and following responses

The sex of the lamb had no significant effect on the following-activities during the 1 km walking distance (Table 4.6). The ease of following in the male lambs was however slightly better than the females, with 33% of males (5/15) falling into the maximum following ability class, compared to 20% (3/15) female lambs. The females, on the other hand, tended to take a slightly shorter time to cover the following distance at each 0.25 km than the males, though the differences were not significant (Figure 4.3). Both the minimum (0.99 m/s) and maximum (1.95 m/s) speeds of following were achieved by female lambs.

The average ranks for the two genders of lambs with respect to the furthermost sustained position relative to the ewes, and their frequency and pitch of bleats during walking did not differ. However, the males tended to follow closer to their mothers than the females, and a female lamb consistently emitted very high-pitched bleats throughout the walking.

4.3.2 Comparative ability of single- and twin-born lambs to follow their mothers over a two-kilometer distance (Study 2)

A twin-born lamb which exhibited a poor discriminating ability of ewes in a previous study refused to follow the mother when released in the laneway. One each of single- and twin-born lambs followed their mothers for 60 and 50 m respectively, stopped and refused to follow any further. These lambs were taken out of the study.

Single-born lambs tended to follow their mothers in the laneway slightly better than the twin-born lambs (Table 4.7). This was indicated by a large proportion of single-born lambs , 62% (8/13), assessed as being either very good or good in their ease of following. A

Table 4.6. Relationship between type of sex and following-responses in single-born lambs during one kilometre walk at 48 hour after birth.

Sex of lamb	No. of Lambs tested	Ease of following (av. rank)	Following position (av. rank)	Bleating frequency (av. rank)	Following distance (m)	Following speed (m/s)
Male	15	16.4	15.2	15.4	1.5 ± 0.1	1.37 ± 0.06
Female	15	14.6	15.8	15.6	1.7 ±0.6	1.47 ± 0.07

Table 4.7 Relationship between type of birth and following responses during two kilometre walk at 48-hour after birth.

Type of birth	Number of lambs tested	Ease of following (av. rank)	Following position (av. rank)	Bleating frequency (av. rank)	Following distance (m)	Following speed (m/s)
Single	13	16.5	18.3	13.5	1.4 ± 0.1^{a}	1.14 ± 0.04^{a}
Twin	17	14.8	13.3	17.0	3.2 ± 0.4^{b}	1.05 ± 0.04^{b}

Values in the same column with different letters are significantly differet at 5 per cent level.

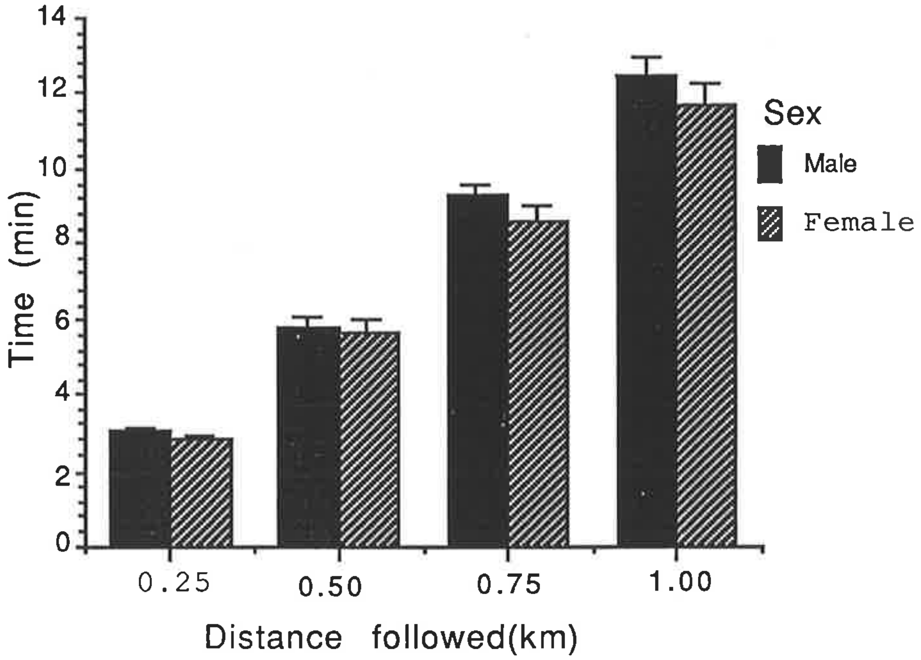

Figure 4.3 Relationship between sex of 48-hour old single-born lamb and time taken to follow their mothers over a distance of one kilometer.

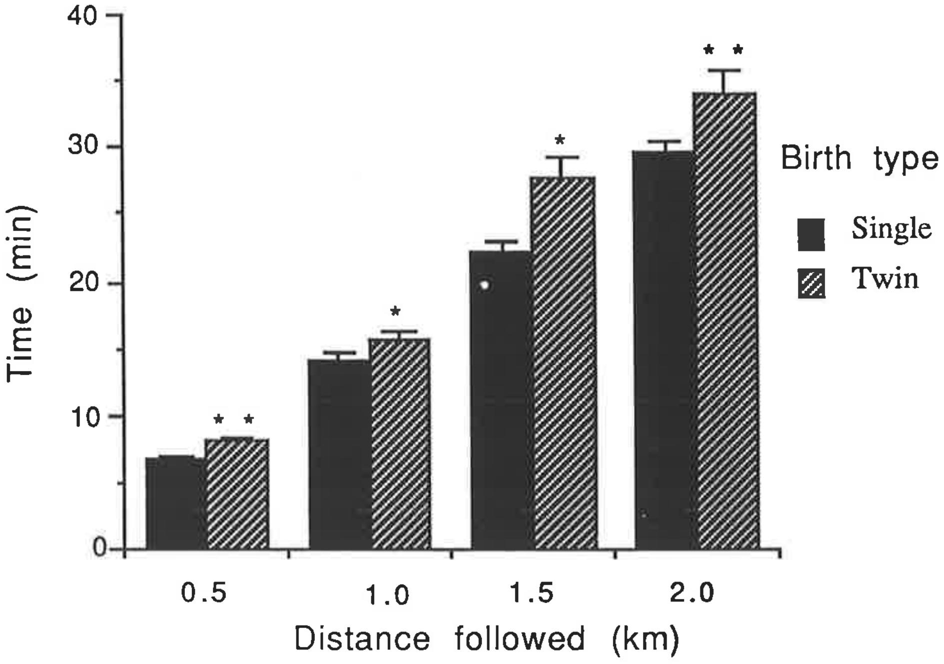

Figure 4.4 Relationship between type of birth and time taken by 48-hour old lamb to follow their mothers over a distance of two kilometers. Values are significantly different at 5 per cent *, and 1 per cent levels. **

lower proportion (53% (9/17) of twin-born lambs fell in the same category. All the single-born lambs kept in close proximity to their mothers during walking by following within 2 m while only 41% of the twins (7/17) did so Eighteen percent of the twin-born lambs (3/17) followed within 3 m, 29% (5/17) within 4 m, 12% (2/17) within 5 m, and 6% each (1/17) within 6 and 7 m from their mothers.

On the whole, single-born lambs tended to achieve "higher" sustained positions relative to their dams during walking than twin-born lambs, and 2 single-born lambs consistently walked ahead of their mothers. The twins tended to bleat more frequently and at a higher pitch than the singles while following , and one of them which consistently followed the mother at 10 m away emitted continuous high-pitched bleats throughout the 2-km distance.

There were considerable variations in the time taken by lambs to follow their mothers over each 0.5 km distance within both birth types (Figure 4.4). Single-born lambs completed the 2 km walk at a higher speed (1.14 ± 0.04 vs. 1.05 ± 0.04 m/s, $p < 0.05$) and covered each 0.5 km distance in a slightly shorter time than twin-born lambs. The time taken to complete the following distance ranged from 23.24 to 35.75 min and 26.33 to 50.05 min in single- and twin-born lambs respectively.

4.3.3 Inter-correlation among lamb discriminating ability of ewes and their following-responses

Table 4.8 depicts correlation matrices of various behavioural patterns observed in single-born lambs during following and their previously assessed discriminating ability of ewes. The frequency of lamb bleats correlated negatively, but significantly with their ease of following ($r = - 0.45$, $p < 0.016$) and their ability to discriminate between their own mothers and alien ewes ($r = - 0.58$, $p < 0.002$).

Table 4.8. Intercorrelation among ewe rocognition ability by the lamb and following-responses in neonatal lambs.

Variable	Recog. ability	Speed of following	Ease of following	Following distance	Following position	Bleating freq.
Recog. ability	1.00 (1.000)	0.36 (0.052)	0.57 (0.002)	-0.29 (0.116)	0.18 (0.338)	-0.54 (0.004)
Speed of following		1.00 (1.000)	0.30 (0.108)	-0.39 (0.034)	0.29 (0.123)	-0.33 (0.077)
Ease of following			1.00 (1.000)	-0.24 (0.193)	0.02 (0.898)	-0.51 (0.006)
Following diatance				1.00 (1.000)	-0.60 (0.001)	0.36 (0.051)
Following position					1.00 (1.000)	-0.32 (0.084)
Bleating frequency						1.00 (1.000)

For statistical significance, a correlation coeficient of 0.35 or larger is required at 5 per cent level.

4.4 DISCUSSION

The large proportion of the ewes that moved away at a gallop for a distance of about 20 m immediately the gate to the laneway was opened, slowed down within the first minute for their lambs to catch up with them. The galloping or "escaping behaviour" observed in the ewes is a normal response to fright or stress which in this case resulted from the confinement of ewe and her lamb prior to the test, as well as the previous experience of confinement during the recognition tests. The fact that most of the ewes slowed down for their lambs to catch up before moving again, and the lambs, readiness to follow the ewes despite being briefly abandoned, suggests an establishment of mutual mother-young attachment in most of the ewe-lamb pairs studied. In the open field situation most ewes would run away leaving their lambs to follow at the approach of a predator or when disturbed by man or other animals, and would only actively seek their offspring when the immediate danger was over. This behaviour could result in ewe-lamb separation, it is therefore necessary that continuation of mother-young attachment over time relies on the ability of one or both partners to be able to locate the other following periods of separation (Arnold et al, 1975).

Recognition or attachment of a lamb to the mother may be important for the young to voluntarily follow the mother. In the present investigation, most of the lambs followed within 5 m of their mothers and were therefore more likely to have maintained their attachment to the ewes by auditory and possibly visual cues, despite the assertion that these two senses are not well developed in the neonatal lamb (Shillito, 1975; Alexander, 1977; Shillito Walser and Alexander 1980). In the case of the lamb which exhibited a sustained following distance of 20 m, there would have been a higher probability of it being separated in an open field situation, since the mother showed no desire to move in concert with it.

The involvement of auditory cues in inducing the lamb to follow was clearly demonstrated when lambs following ewes beyond 10 m often rendered an immediate bleating response to the mothers' call and galloped to them. On the other hand, bleating by both ewe and lamb was nil or minimal and at a low pitch when the neonate was following well and close to the mother, indicating that auditory cues, as an aid to following, may be necessary only at a distance. Presumably, olfactory cues and sight were involved in proximal following. It was, however, very rare to see a ewe and her lamb sniffing each other during the test. On the other hand, the restriction imposed on the lateral movements of the animals by the narrow laneway might have cut down on the need to rely on senses of recognition as an aid to the lambs' following ability. Nevertheless, the high-pitched bleats of the ewes when the lambs were not following might well have concurrently served as location and stress signals. Auditory cues may assume greater importance in the field situation, where lambs following ewes at a distances would be more prone to separation. However, the lambs' ability to gallop and keep up with the ewes if sustained, despite the repeated unconcerned behaviour in some of the ewes in this study, would help them maintain proximal attachment to their mothers in the field.

Following-response in the lambs, in terms of ease of following, suggests that slightly more than half the lambs studied were capable of covering the 1 km distance with minimal stress, though the speed of walking, 0.99 to 1.95, m/s was generally higher than what is normally observed in the field. Most of the lambs occasionally had to undergo short spells of exhaustive running in response to the mothers' call following brief separation, particularly at the beginning of the walk. Such near maximal running speed could be achieved by healthy neonatal lambs in the field during the approach of predators such as dingoes (see Chapter 7). The general physical condition of the lambs at the end of the test suggests that they could have followed their mothers over longer periods without any detrimental effects. It is therefore not surprising that in the subsequent study, 93% (13/14) and 89% (17/19) of single- and twin-born lambs respectively, completed the 2-km following distance, with only a few of them exhibiting varying degrees of fatigue including considerable panting.

The general relationship between the lambs' ability to recognize their mothers in the 2-choice test (see Chapter 3) and their following-activities, suggests that lambs with superior discriminating ability of ewes stand a better chance of successfully following their mothers over long distances without being separated. The negative relationship existing between lambs' ability to recognize their mothers and the frequency of their high-pitched bleats further demonstrates the positive influence of the degree of a lamb's attachment to the mothers on its following-responses, since high-pitched bleats in the neonates suggest non-recognition of a ewe or a poor following. Most of the lambs exhibiting good recognition response of ewes also tended to "belong" to ewes of equally good recognition response for lambs. Where mutual attachment existed in a ewe-lamb pair, but the lamb did not exhibit good following-response, the ewe quite often induced a fair following-response in the lamb by adjusting her own pace of movement to suit the young, emitting intermittent low-pitched bleats when in close proximity, sniffing the lamb and/or performing naso-natal contacts.

Normally the ewes did not encourage sucking attempts when walking. However, when lambs were not exhibiting good following-response, the dams waited for them to catch up, and thereafter allowed the lambs access to the udder for a few seconds before setting off again. This practice also seemed to induce a "fair" following-response in most of the lambs who initially were poor followers. After successive sucking bursts, the lambs tended to follow their mothers well for a period of time before stopping to attempt another sucking. These intermittent pauses in following by the lambs may be aimed at seeking attention and nourishment from their mothers, or may be due to fatigue which necessitated the brief rest periods.

The furthermost sustained following-position of the lamb relative to the ewe did not seem to relate to the other following-activities in the lamb, with the exception of the following-distance between ewe and lamb. Most of the lambs maintained a sustained following-position at the shoulder or hind-leg, while a few of them occasionally explored the immediate environ resulting at times in a brief separation. This behaviour is similar to that often observed in neonatal lambs where lambs wandering independent of their dams occasionally separated (N. Edwards, personal communication). In the present study, the few of the lambs that tended to assume sustained following-positions ahead of the ewes, did not exhibit mutual recognition with their mothers in a previous test. This behaviour of the lambs may expose them to danger such as slipping on slopes, falling into ditches or to predator attack, and could therefore not indicate a superior following-position. The knowledge of the environment acquired by the neonates in such a short life, if any, would not be adequate to ensure them with intelligent directional decisions.

The shorter time taken by the medium mass lambs to cover the 0.5, 0.75 and 1.0 km distances and their better physical condition at the end of the exercise, compared to lambs in the extreme body mass classes, suggests an overall superior following-response in lambs neither too large nor too small. The poorer following-response in the small sized lambs may, however, be attributed to their lower energy reserves (Alexander, 1984; Slee, 1978). In cold and windy weather small sized lambs would be expected to expend more energy per unit of body mass than larger lambs to produce heat because of their large surface area relative to body mass (Haughey, 1983; Alexander,1984, 1986; Slee,1978). Problems at parturition and subsequent effects on vigour (see McCutcheon et al., 1981) could have affected the high mass group's overall following-response.

The usually better performance in exercise observed in males of many species of mammals may be attributed to their higher vigour and greater muscle mass, suggesting greater oxygen carrying capacity. However, this study suggests that there is little difference in body mass between the two sexes of neonatal lambs, resulting in a non-significant effect on the following-response.

The following-response, in terms of ease of walking, bleating frequency and the proportion of lambs following at the shoulder and hind leg of the ewe, was stronger in single-born lambs then twin-born lambs. Furthermore, the significantly lower sustained distance between mother and lamb and the following speed in the singles confirms their superiority over the twins. This superior performance might have been influenced by several factors, the neonates' following-response is in no doubt related to their degree of attachment to their mothers, and their ability to discriminate between their own and alien ewes. In the present study, the higher recognition response of the mother exhibited by the single-born lambs might have influenced their following-response. The shorter gestation length in twin-born lambs suggests that they are less mature at time of birth than single-born lambs, hence their delay or lower ability to discriminate between ewes (Nowak,1989). The twins, being small sized and "younger", might have found the exercise more strenuous than the single-born lambs. This supports the finding of Winfield and Kilgour (1976) that younger lambs quickly become fatigued and showed weak following-response while attempting to keep up with a moving surrogate ewe. Furthermore, twins differ from singles because each lamb follows not only its mother, but the mother and another lamb (Nowak, 1989). Since the twins had to follow the mother one at a time in the present study, the absence of the sibling might also have contributed to their poorer following-performance.

No attempt was made to determine the role of the various senses of recognition that may be involved in the following-response in lambs. To enhance our understanding of the following-behaviour in the early life of the lamb, there is a need for a systematic study into the relative importance of the various senses of recognition during following. Nonetheless, the unique role played by auditory cues in following as seen in the present study is unequivocal. When following the mother over long distances, such as pertains in the semi-arid pastoral regions of Australia, there is an occasional demand for the lamb to run at near maximal speed either to escape from a predator or to catch up with the mother during separation. In such a situation, it would be easier, as suggested by Nowak (1989), if recognition of the lamb by its mother relies on visual, auditory, or behavioural rather than olfactory characteristics.

5.1 DETERMINATION OF ENERGY INTAKE AND EXPENDITURE IN NEONATAL LAMBS BY LABELLED WATER TECHNIQUE

5.1.1 INTRODUCTION

Lifson et al (1955) observed that the oxygen of respiratory carbon dioxide is in isotopic equilibrium with the oxygen in the body water due to the activity of carbonic anhydrase. The difference in the turnover rates of tritium (^3H) or deuterium (^2H) and oxygen-18 (^{18}O) following the labelling of the body water with these isotopes led to the derivation of an equation by Lifson and McClintock (1966) to determine the rate of water flux and CO_2 in the animal. The estimated CO_2 produced was then used to determine energy metabolism by an appropriate respiratory quotient.

The largely non-invasive nature of the doubly-labelled water technique (DLW) using either $^3H_2{}^{18}O$ or $^2H_2{}^{18}O$ renders it ideal for the estimation of energy metabolism in free living animals. A problem in the use of this technique in the field involves the recapture of the animal in order to obtain body water samples for the determination of the final concentration of the introduced isotopes. Subjects must also refrain from drinking between administration of the labelled water and collection of the first sample (Forbes-Ewan et al., 1989). Although the DLW technique has been widely used on small animals including birds, insects and rodents, its application on large mammals, particularly in human subjects, was delayed by the high cost of the ^{18}O component in the dosage. Costs have been reduced in recent years by the use of highly sensitive mass spectrometers which requires minimal enrichment of body water (see Blaxter, 1989). This has enabled the DLW technique to be used extensively in larger animals and validated in humans from several laboratories.

The labelled water technique has been used in the estimation of water flux, and thus milk intake, in suckling lambs, based on the principle that if milk is the sole source of ingested water, water turnover as estimated from the exponential decline in the concentration of the introduced isotope in the body fluids of the young will be a measure of milk intake

(Macfarlane et al. 1969; 1967; Dove and Freer, 1974; Wright and Wolf, 1976; Conward et al., 1982; Dove, 1988). The method has, however, never been used for the estimation of energy expenditure in lambs. The purpose of the present study was to estimate field metabolic rate (FMR) in lambs during the first 5 days of life by DLW method, and to to use the value in a separate study to determine the proportion of the total energy that is devoted to locomotion in lambs.

5.1.2 MATERIALS AND METHODS

5.1.2.1 ANIMALS

Six multiparous ewes were selected at random 2 weeks prior to lambing, and allowed to graze a long but narrow paddock (0.1 x 2.5 km) of sparse vegetation, simulating conditions in the semi-arid pastoral regions of Australia at the Bolivar Experimental Station of the Waite Agricultural Research Institute.

Lambing was spread over 3 days in late summer, and subsequent observations showed that the ewes grazed as a group with their lambs following. A watering facility was available at one end of the paddock prior to lambing, however, no food supplements were provided. Hay was provided at the onset of lambing at the other end of the paddock. Three lambs were randomly selected at birth for energy expenditure studies.

5.1.2.2 LABELLED-WATER

The stock solution of DLW was prepared by adding 1 ml of 98.7 atom per cent ^2H_2O, and commercially packaged 2 ml of 96.1 atom per cent $H_2{}^{18}O$ (Amersham, Berkshire, U.K.) to 24 ml reverse osmosis water (ROH). Isotopes were administered in a pre-weighed dose providing approximately 20 mg ^2H_2O and 40 mg $H_2{}^{18}O$ per kg of body mass (body mass 4.0 ± 0.1 kg).

5.1.2.3 EXPERIMENTAL PROCEDURE

The experimental protocol of the DLW technique is shown by Table 5.1. At approximately 4 h post-partum, each lamb was captured and separated from the mother, weighed and confined to a small cage of wire mesh (0.5 x 0.5 m) for a period of 1 h. Three ml of blood were collected by venipuncture into a heparinised tube for a baseline isotopic level determination, after which an intra-muscular injection of approximately 2 ml DLW was administered to the lamb. Care was taken when withdrawing the needle to ensure that none of the injectate was lost via the puncture. The syringe and needle were weighed to the nearest 1 mg before and after the injection to determine the actual dose given. A second blood sample (3 ml) was taken after an equilibration period of 2 h (see Dove, 1988), after which the lamb was removed from the cage, and reunited with the mother. Where necessary, lamb acceptance by the ewe was facilitated by confining the couple for a brief period of about 15 min.

On the 3rd and 5th days after birth (approximately 72 and 120 h post-partum) the lamb was recaptured, separated from the mother for a period of 1 h, after which 3 ml of blood was collected. The blood samples were stored in tightly capped heparinised tubes at -20^{o}C until extraction of water.

5.1.2.4 WATER EXTRACTION

Water was extracted from the blood samples by azeotropic distillation (See Cuberthson et al, 1989) using a Dean and Starke apparatus consisting of a laboratory heating mantle (Electrothermal type EME 6 0250/CE), a 100 ml flask, a straight condenser, and a receiving cylinder of 5 ml capacity. A stopcock attached to the lower end of the cylinder permitted drainage of the heavier water layer on completion of distillation. A set of 6 heaters, receiving traps, and condensers installed in a fume cupboard was used. The azeotropic distillation technique is based on the principle that for most mixtures of liquids, the boiling

Figure 5.1 Schedule of procedures for the doubly-labelled water technique.

Day and time after birth	Experimental protocol
Day 0	Prevent sucking for 1 hour
4 hour	Take 1st blood sample
	Inject 1st DLW
	Prevent sucking for 2 hours
	Take 2nd blood sample
Day 3	Prevent sucking for 1 hour
76 h	Take 3rd blood sample
Day 5	Prevent sucking for 1 hour
124 h	Take 4th blood sample

Table 5.2. Levels of ^{2}H and ^{18}O enrichment in blood samples.

Lamb	^{2}H ‰				^{18}O ‰			
No	Pre-dose	Post-dose	Day 3	Day 5	Pre-dose	Post-dose	Day 3	Day 5
1	2.3	128.2	60.2	34.8	-7.27	15.66	4.50	-2.35
2	-17.0	150.5	68.5	24.2	-5.32	25.90	5.39	1.09
3	-15.5	162.3	64.8	38.2	-5.10	19.49	4.14	0.94
Mean	-10.1	147.0	64.5	32.4	-5.90	20.35	4.68	-0.11
SE	6.2	10.0	2.4	4.2	0.69	2.99	0.37	1.12

Values expressed as ‰ deviation from Vienna Standard Mean Ocean Water.

point of the mixture is below that of the pure liquids, and the vapour produced is also a mixture. When the vapour is cooled, the condensate separates into 2 layers if the liquid is immiscible and of different densities. The lighter liquid, in this case kerosene, is refluxed, but all of the other liquid, water, is removed from the material being treated. Any solids in either liquid remains behind.

A mixture of whole blood from the heparinised tube and 40 ml of Jet Al aviation fuel (kerosene, boiling point in the range of 160 to 240°C) was placed in a round-bottom flask which was then attached to the distillation apparatus. The mixture was vigorously boiled in a heating mantle until all the aqueous fraction had passed into the receiving cylinder, indicated by the absence of water droplets in the organic layer above the aqueous fraction. The aqueous layer was then carefully transferred to a stoppered glass vial (McCartney bottle) while ensuring that only a minimal amount of solvent was transferred. A small amount of shredded paraffin wax (1 g) was added to the extract and the tightly capped bottle was heated to 60°C to melt the wax. The wax melted and absorbed any traces of contaminating solvent in the extract as the sample cooled. The vials containing the sample were tightly capped and stored at room temperature until isotopic analysis. The solidified wax on top of the extract helped to prevent evaporation of the water during storage and handling. The apparatus was rinsed between distillations with acetone and allowed to dry before being re-used.

5.1.2 5 ANALYSIS OF ISOTOPIC ENRICHMENT OF ^{2}H AND ^{18}O IN BLOOD SAMPLES

Isotopic enrichment in the pre- and post-dose samples was measured by a stable isotopic gas ratio technique by means of a mass spectrometer (Micromass 602D, V.G. Isogas Ltd.) at the CSIRO Division of Water Resources Laboratory, Adelaide, South Australia.

(1) Deuterium (^2H) analysis

An aliquot of aqueous extract (25 ul) was introduced into the mass spectrometer through the inlet valve, and vaporized with a portable fan-forced heater. The level of ^2H in the extract was determined relative to an internal laboratory standard (ROA) which was calibrated against the internationally accepted standard, Vienna-Standard Mean Ocean Water-Standard Light Arctic Precipitate (V-SMOW-SLAP), following the reduction of the extract in a circulating system with uranium heated to 800°C.

(2) Oxygen-18 analysis

Samples were prepared for the determination of ^{18}O according to method of the Epstein and Mayeda (1953). An aliquot of 2 ml of the aqueous sample was injected into the bottom of an equilibration flask which was then connected to an evacuation system. Air was removed from the flask (by vacuum) and CO_2 with a containing a known isotopic composition of ^{18}O was added to restore the pressure to approximately 0.75 atm. The flask was then placed in a water bath at 30°C and shaken overnight (16 h). This time exceeds the known equilibriation time of 8 h for sample and CO_2.

An aliquot of CO_2 was cryogenically purified the next day using a -94°C ethanol slush trap to solidify water, and a -196°C liquid nitrogen trap to isolate the CO_2 (Schoeller et al, 1980). The flask was then attached to the mass spectrometer and an aliquot of CO_2 introduced for the determination of ^{18}O enrichment in the sample.

The isotopic abundance in the stock solution and V-SMOW-SLAP were determined to permit calculation of absolute values of ^2H and ^{18}O in the samples. Results were presented in parts per thousand (%o) units deviations to V-SMOW-SLAP and normalised according to the procedures defined in Gonfiantine (1984).

5.1.2.6 CALCULATIONS

(1) Total body water

Isotopic dilution space (IDS, kg) was determined according to Equation 3 of Wong et al (1986). For ^2H:

$$\text{IDS.} = \frac{d}{MW} \times \frac{APE}{100} \times 18.02 \times \frac{1}{R_{std} \times EOB} - \text{dose}; \tag{1}$$

where d is the amount of ^2H_2O in grams in the dose, MW is the molecular weight of the ^2H_2O, (19.99 g/mol), APE is the atom per cent of ^2H enrichment in the ^2H_2O (97.8‰), 18.02 is the molecular weight of the isotopic water in g/mol, R_{std} is the isotopic ratio of ^2H in the V-SMOW-SLAP standard, which has a value of 155.75×10^{-6} (Gonfiantini, 1984), and EOB refers to the enrichment of ^2H isotope in the sample over baseline value per mil. Isotopic dilution space for ^{18}O is determined in a similar manner. In this case, d is the amount of $H_2{}^{18}O$ in grams in the dose, MW is the molecular weight of the $H_2{}^{18}O$ (20.0 g/mol), APE is the atom percentage of ^{18}O enrichment of the $H_2{}^{18}O$ (96.1‰), Rstd is 2005.2 x 10-6, the ratio of ^{18}O in the V-SMOW-SLAP standard (Gonfiantini, 1984), and EOB refers to the enrichment of ^{18}O isotope in the sample over baseline value per mil.

Values of initial total body water (TBW) were determined as the means of the ^2H dilution space divided by 1.04, and the ^{18}O dilution space divided by 1.01, since ^2H and ^{18}O dilution overestimates the TBW, after correction for the volume of the dose injected (Schoeller et al., 1984).

(2) Milk consumed and metabolizable energy intake

Milk intake was estimated as 80 g milk DM/d consumed per kg $Mb^{0.75}$, where Mb is the body mass of lamb (see Agricultural Research Council, 1980). Metabolizable energy intake (MEI) from the estimated milk consumed was calculated as:

$$\text{MEI} = \text{MI} \times \text{GE} \times 0.94; \tag{2}$$

where MEI is in kJ, MI is milk intake in ml/d, GE, gross energy of milk, is 4.77 kJ/g (Dove and Freer, 1979), and the constant 0.94 represents the metabolic content of 1 ml of milk (H. Dove, personal communication).

(3) Energy expenditure

Carbon dioxide production (V_{CO_2}) was determined from Equation 4 of Schoeller et al.(1986):

$$V_{CO_2} = \frac{TBW}{2.078}(K_O - K_H) - 0.015\,K_H \times TBW; \qquad (3)$$

where TBW is the total body water determined from the mean of ^{2}H and ^{18}O dilution spaces, K_O and K_H are the fractional elimination constants for ^{18}O and ^{2}H, respectively, and the factor 0.015 assumes that half the total water loss from the animal's body leaves as evaporated water (see Lifson and McClintock, 1966).

The fractional elimination constant of ^{18}O was determined from Eq. 11 of Tatner, (1990).

$$K_O = \frac{\ln(C_0 - Bs) - \ln(C_1 - Bs)}{t} \qquad (4)$$

where C_0 is the initial enrichment of ^{18}O after equilibration with body water, C_1 is the final enrichment of ^{18}O, Bs is the background ^{18}O abundance in the animal, and t is the time in days between sampling the initial and final isotope enrichments. K_H is calculated in a similar manner.

Conversion of the estimate for the V_{CO_2} into values for energy expenditure assumed that the respiratory quotient (RQ) in the lambs during the experiment was equivalent to 0.82, the RQ attained by 24 h-old lambs studied by Alexander (1962).

Basal metabolic rate (BMR) in the lambs was estimated using Kleiber's (1961) formula (cited by Garland, 1983):

$$BMR = 293\ Mb^{0.75} \quad (5)$$

where BMR is in kJ/km and Mb is body mass of the animal in kg.

For comparison with the the calculated value, field metabolic rate (FMR) in the lambs was predicted by the regression equation for eutherian herbivores (Nagy, 1987):

$$\log y = 0.774 + 0.727 \log x, \quad (6)$$

where y is in kJ/d, and x is body mass in grams. Generalised equations were used to predict the metabolizable energy requirements for maintenance (see Australian Agricultural Council, 1990):

$$ME_m\ (MJ/d) = (K.S.M.\ (0.26\ Mb^{0.75} \exp(-0.03A)) + 0.09MEI)/k_m\ ; \quad (7)$$

where K is 1, S is 1, M is 1 , Mb is body mass in kg (4.7 kg), A is age in years (0.0068), k_m is net efficiency of use of ME for maintenance (about 0.84 for milk) and MEI is 6.72. Total expected energy expenditure was calculated as the sum of ME_m and "activity allowance". The "activity allowance" or cost of locomotion in the lambs was estimated as the product of the incremental cost of locomotion and the daily distance travelled (0.0179 MJ/d, see Chapter 4.2).

5.1.2.7. STATISTICAL ANALYSIS

Means are presented with the standard error. The difference and the degree of relationship between TBW determined by ^{18}O and ^{2}H dilutions was examined by Student's t-test and simple regression analysis respectively. The increase in body mass of lambs and the difference between K_O and K_H over the study period were also evaluated by Student's t-test.

5.1.3. RESULTS

Table 5.2 shows the time course of isotopic dilutions in the body water of the lambs during the first 5 days of life. The mean isotopic abundance of ^{2}H at 2 h post-treatment, 3 and 5 days after birth were 157.1, 74.6 and 42.5‰ excess over the pre-treatment value. The corresponding mean isotopic concentrations over the pre-treatment level for ^{18}O were 26.25, 10.58, and 5.79‰ at equilibration, Day 3 and Day 5 of life, respectively. The ^{2}H and ^{18}O enrichment in the lambs fell by 73 and 78% respectively during the present experimental period.

Physical characteristics of the lambs and TBW estimated by ^{2}H and ^{18}O dilutions are shown by Table 5.3. The mean TBW estimated by ^{18}O dilution tended to be higher than that estimated by ^{2}H dilution. The mean ratio of ^{18}O/^{2}H TBW was not significantly different from one. Body mass increased significantly by 1.3 fold ($p < 0.01$) during the experimental period. On average, the lambs added 270 g of body mass per day, of which approximately 66 g was dry matter. The predicted daily milk intake of 1500 ml provides a metabolizable energy intake of 6730 kJ/d .

Fractional elimination constants for ^{18}O and ^{2}H during the 5-day experimental period are shown in Table 5.4. K_O was on the average 0.0372 ± 0.0005 higher than K_H. The difference was, however, not significant. Estimated daily CO_2 production, O_2 consumption and FMR during the 5-day neonatal period are shown in Table 5.5. Mass specific O_2 consumption and energy expenditure were 14.08 ± 0.65 l/kg/day and 282.9 ± 13.2 kJ /kg/day respectively. Energy expenditure of 325 kJ/kg/day was predicted for the lambs.

Table 5.3 Lamb physical characteristics.

Lamb number (sex)	Body mass (kg) Day 0	Body mass (kg) Day 5	Total body water (kg) ^2H	Total body water (kg) ^{18}O	Total body water (kg) Mean	TBW/Mass	TBW $^{18}O/^2H$
1 (M)	4.5	5.6	3.35	2.79	3.07	0.68	0.83
2 (M)	3.6	5.2	2.50	2.02	2.26	0.63	0.81
3 (F)	3.8	5.2	2.38	2.60	2.49	0.66	1.09
Mean	4.0	5.4	2.74	2.47	2.61	0.66	0.91

Table 5.4 Fractional elimination rates from body water of excess ^{18}O and ^2H during the first five days of life

Lamb number	K_O / day	K_H / day	$(K_O - K_H)$ / day
1	0.3078	0.2709	0.0369
2	0.3167	0.2802	0.0365
3	0.2795	0.2413	0.0382
Mean	0.3013	0.2641	0.0372
SE	0.0112	0.0117	0.0005

K_O and K_H are elimination rate of excess ^{18}O and ^2H, respectively

5.1.4. DISCUSSION

5.1.4.1. ISOTOPIC ENRICHMENT OF BODY WATER

The introduced stable isotopes, ^2H and ^{18}O, are relatively common in the environment and small differences in the background level due to natural fluctuations would have a disproportionate effect in the final excess concentration and thus reduce the accuracy of the DLW method. This necessitated that the natural abundance of the isotopes in the lambs' body water was taken into account in the determination of their fractional turnover rates. The fall in ^2H and ^{18}O enrichment by 73 and 78% respectively during the present experimental period is similar to that seen during a 12-day experimental period in a subject with initial ^2H enrichment of 350‰ and ^{18}O enrichment of 110‰ in body water (Barrie and Coward, 1985). Using human neonates Schoeller (1983) suggested that the optimal metabolic period of ^2H and ^{18}O measurements is between 3 and 10 days; a range encompassing those in the present study. The dose of $^2H_2{}^{18}O$ administered to the lambs produced acceptable isotopic enrichment above the natural abundance levels by the end of the 5-day experimental period. However, extending the trial beyond 5 days might have resulted in the enrichment of isotopes in the final example, particularly ^{18}O, dropping so low as to become indistinguishable from the background values. The experimental period could be prolonged by increasing the dose of the labelled water, however larger doses would raise the isotope cost with only a minimal improvement in precision (see Schoeller, 1988).

5.1.4.2 TOTAL BODY WATER

The slightly higher estimates of the mean TBW by ^2H dilution compared to ^{18}O is consistent with the values obtained in a number of studies. The difference is, however, often less than 7% (Gales, 1989; Schoeller et al 1980, Lifson et al, 1955). The lower value of ^{18}O dilution space would make it a more accurate estimate of TBW. The mean value of ^2H and ^{18}O dilution spaces was used in estimating the TBW.

Table 5.5. Carbon dioxide production, oxygen consumption and energy expenditure in lambs during the first five days of life.

Lamb	Body mass*	CO_2 production	Oxygen consumption		Energy expenditure	
number	kg	l / day	l / day	l /kg / day	kJ/day	kJ/kg/day
1	5.1	56.46	68.84	13.50	1383.7	271.3
2	4.4	48.16	58.76	13.35	1180.5	268.3
3	4.6	58.02	70.76	15.38	1422.3	309.2
Mean	4.7	54.21	66.11	13.41	1328.8	282.9
±SE	0.4	3.06	3.73	1.16	75.0	13.2

* Mean of initial and final body masses; RQ = 0.82 (Alexander,1963).

The range in fresh body mass accounted for by TBW (65 to 68%) is consistent with the estimates for suckling lambs studied by Searle (1974), Wright and Wolff (1976) and Dove (1988). The 270 g mass gain in the lambs in the present study indicates an increase in body water pool of 178 ml/d, which is similar to that obtained by Dove and Freer (1974), assuming 66% of the body gain was water.

The value of milk intake predicted for the lambs, 1.5 l/d, is similar to that estimated for week old lambs by Dove and Freer (1979) and Conward et al. (1982). The predicted value was however higher than the range of reported values of milk intake in lambs by Searle and Griffith (1976) and Wright and Wolf (1976).

5.1.4.3 ENERGY INTAKE AND EXPENDITURE

The accuracy of the DLW technique for measuring energy metabolism in captive animals and human subjects have been validated by comparing simultaneous DLW measurements with CO_2 production or MEI measurements. The results obtained by the tracer technique and the other methods were in good agreement, with an average discrepancy of less than 4% (Lifson et al., 1955; Mullen, 1970; Schoeller et al., 1986; Nagy, 1987; Gales, 1989).

The mass specific field metabolic rate of 282.9 kJ/kg/d estimated for the lambs is comparable to the energy expenditure in premature infants (Wong et al., 1987) and 4-week to 4-month old exclusively breast-fed infants (Freymond et al., 1986; Lucas, 1987). The FMR obtained by DLW method in the present study may change slightly due to changes in RQ resulting form the rapid and large changes in milk composition which occur during early post-partum period. The calculated FMR in the lambs was 88% of that predicted using the generalised equations of Australian Agricultural Council (1990). This represents about a third of the predicted MEI, since MEI is converted to net energy for maintenance, in milk fed lambs, with an efficiency of about 0.84 (see Graham et al., 1976). Field metabolic rate, like

BMR, is strongly inversely correlated with body mass in eutherian mammals. This is clearly shown in data from a large number of free-living mammals summarized by Nagy (1987), illustrated by metabolic rates of 4,338 kJ/kg/d in a 13.3 g rodent (*Clethrionomys rutitis*) and 458 kJ/kg/d in a 84 kg sea lion (*Zalophus californius.*).

The BMR value predicted for the lambs in the present study was 965 kJ/d (see Garland,1983). Basal metabolic rate was not measured, due to the fact that such a determination would have disrupted the DLW regime. In fact it would be very difficult to determine when lambs are post-absorptive, and they may never reach the state of being completely inactive and post-absorptive in the field. The ratio of the FMR to the predicted BMR in the lambs was 1.4, a value which is slightly below that of human infants (Wong et al., 1987). For all mammalian species, the ratio of FMR to BMR is less than 7, with most values falling between 1.5 to 5 (Peterson et al., 1990). The ratio decreases with increasing body mass in marsupials, whereas it increases with increasing body mass in the eutherians studied to date (Nagy, 1987).

The metabolic rate in the present study may be assumed to be minimal for the sort of physical activities undertaken by the lambs, since the experimental period was in mid-summer, when the climatic conditions were mostly within the thermoneutral range for the animals. Lambs born in a mild winter would, however, require about 2,400 kJ for daily metabolism, representing the metabolic rate sustainable in the cold (see Alexander, 1985); a daily expenditure of about 40% of the predicted MEI in the lambs studied.

5.2. MOVEMENT DISTANCE AND ECOLOGICAL COST OF TRANSPORT IN NEONATAL LAMBS

5.2 1. INTRODUCTION

A few experimental investigations deal with the following behaviour of lambs (see Section 1.3), however no detailed study of the daily travelling distance in the neonatal lamb has been undertaken. None of the methods used so far provides an accurate measure of actual distance travelled during the routine activity of free-living animals. Furnival et al. (1982) used a pedometer to measure distance covered by 5 sheep grazing a 90 x 40 m paddock. They compared the distance recorded with the pedometer to the path of each sheep charted and measured with an opisometer, and concluded that the pedometer was not suited to study movement of grazing animals. The use of pedometers by human joggers in recent years has been widespread, and this has led to improvements in the design and effectiveness of the instrument. In the absence of an alternative method for estimating distance moved by a free-living animal, especially at night, it may be necessary to assess the effectiveness of newer pedometers for measuring the daily distance travelled by lambs. The availability of a treadmill makes verification of the technique possible.

A newborn lamb depends solely on its energy reserves for survival during the first few hours after birth. It thereafter increasingly depends on the energy obtained from milk, amounting to about 1 to 1.5 litres per day during the first week of life (Wright and Wolf, 1976; Dove and Freer, 1979; Conward et al., 1982), with a gross energy value of 4.77 kJ/g liquid milk (Dove and Freer, 1979). A large proportion of the energy obtained from the digestion and metabolism of milk may be used by the lamb for thermogenesis, especially under adverse cold climatic conditions. Since the intensity of motor activities in the neonates seem to be generally low, a greater proportion of the energy intake would be expected to go into growth rather than locomotion . Where a neonate does have to follow the dam over a long distance in her search for food and water, a substantial proportion of the milk energy would be expected to go into the cost of locomotion, resulting in a reduction in the energy available for growth and other activities. However, the work of Altmann (1987) suggests that cost of locomotion is a minor component of the animal's daily energy expenditure.

There have been two attempts to relate the energetic demands of walking to the overall daily energy requirements of an animal. The first of these was by Garland (1983) in which he defined a parameter, "the ecological cost of transport", as the ratio of locomotory costs to the total daily energy requirement. By using allometric equations for distance moved, energy required for locomotion and field metabolic rates, he was able to show that mammals spend a low proportion (usually less than 6%) of their daily energy budget on movement. Furthermore, larger animals were predicted to spend a greater proportion of their daily energy expenditure on locomotion than small animals. In a similar analysis, Altmann (1987) argued that the cost of locomotion should be divided by the non-locomotory daily energy expenditure in order to avoid the problem that animals that walk further would thereby have a greater value in the denominator. As it turns out, the formulae produce similar results, certainly within the limits of the allometric analysis later applied to them.

In the current study, a pedometer was employed for measuring the daily distance travelled by lambs in a simulated semi-arid condition during the first 5 days of life. Values obtained for daily travelling distance and incremental cost of locomotion determined from a separate study were then used to estimate the relative amounts of field metabolic rate (FMR) and non-locomotory energy (NLE) that were devoted to locomotion in the lambs.

5.2.2. MATERIALS AND METHODS

5.2.2.1. ANIMALS

Six single-born lambs with body mass of 3.8 ± 0.3 kg at birth were used for assessing the daily distance walked in a simulated semi-arid condition at Bolivar Experimental Station. These lambs were born during the same period and the trial carried out in the same long and narrow paddock as in Study 5.1.

5.2.2.2. EQUIPMENT

The distance measuring device (Micronta, Jog-mate™) illustrated by Plate 3 is a compact, easy to read electronic pedometer that displays the distance covered by a subject from 0 to 999 km. It has dimensions of 5.5 x 4.3 x 2.6 cm, weighs approximately 27 g and resembles a pocket watch. It can be used within a temperature range of 0^{O} to 40^{O}C. The manufacturer's instructions indicate that prior to being used, the pedometer is to be set to the appropriate mode (walk or jog) and stride length. It is then clipped over the belt of human subjects, halfway between the middle front of the body and the side. The unit might not work properly when not worn correctly, or when one skips or shuffles, climbs a steep hill, or takes short, quick steps down a hill.

After preliminary trials to select a position on the lamb's body for the attachment of the pedometer, a spot on the upper fore-leg, just below the head of the humerus, was considered the most appropriate position (see Plate 4). Two pedometers were used in the trials.

5.2.2.3. EXPERIMENTAL PROCEDURE

At approximately 6 h post-partum a pedometer set to walk mode and at 30 cm stride length (arrived at from the mean distance between foot prints on a concrete floor of a fore-leg of a 3-day old lamb) was fixed on to the shaved area of the fore-leg of the lamb with a glue. It was then covered with a water-proof tape, after which the lamb was taken to the mother, released and its acceptance by the ewe observed.

The lamb was recaptured after approximately 24 h (Day 1), and the pedometer reading taken. The pedometer was reset, and re-attached to the lamb. In some cases, the lamb was confined for 1 h after which blood sample was taken for a concurrent study, and then returned to the mother. The procedure was repeated at 24 h intervals up to 5 days after birth.

Plate 3 A distance measuring device, Micronta Jog-mate™ pedometer.


MICRONTA
Walk
Stride
Start
Jog

Plate 4. A pedometer attached to a spot on the upper leg of a lamb, just below the head of the humerous.

5.2.2.4. VALIDATION OF PEDOMETER READINGS

Prior to measuring the distance travelled, the accuracy of the pedometers was checked by the use of a motor-driven treadmill. After a 10-min familiarisation run on the treadmill by a 3- and a 5-day old lamb, the pedometers were reset at walk mode and 30 cm stride length and attached to the lambs. The animals were then subjected to treadmill exercise at approximately 0.48 m/s for 120 to 420 s. The distances covered by the lambs were calculated from the equation:

$$D = \frac{S \times T}{1000} \quad ; \qquad (1)$$

where D is the distance covered in km, S is the speed of the treadmill in m/s, and T is the duration of the exercise in s. The regression equation relating pedometer reading to distance walked was used to convert field readings of the pedometers to actual distance travelled by lambs during the experimental period. The relationship was:

$$y = .0.001 + 0.447x \qquad (r = 0.85) \; ; \qquad (2)$$

where y is the pedometer reading and x is the daily movement distance covered by lambs, both values are in km. Since the lambs tended to be with their mothers most of the time their respective movement distances are not be expected to differ significantly. For comparison with other mammals, the DMD of the ewes were predicted from the allometric equation for herbivorous mammals (Garland, 1983):

$$DMD = 0.875 \; Mb^{0.22 \pm 0.08} , \qquad (3)$$

where DMD is the mean daily movement distance covered by the ewes, and Mb is the mean body mass in kg.

5.2.2.5. CALCULATION OF ENERGY DEVOTED TO LOCOMOTION

The energy devoted to locomotion as a proportion of the total daily energy demand, the ecological cost of transport (ECT), may be estimated as follows. The distance an animal moves per day is multiplied by the incremental cost of locomotion (ICL), to yield the daily transport cost. This latter quantity is then divided by FMR to yield the parameter ECT:

$$\text{ECT (\% FMR)} = \frac{\text{100 x DMD (km/day) x (ICL kJ/km)}}{\text{FMR (kJ/day)}} \qquad \text{(Garland, 1983)} \qquad (4)$$

Using the alternate measure of the ecological cost of transport according to Altmann (1987), the relative amount of non-locomotory energy per day (NLE) that is devoted to locomotion, expressed as a percentage, (ECT (% NLE)), is calculated using the formula :

$$\text{ECT (\% NLE)} = \frac{\text{100 x (ICL x DMD)}}{\text{FMR - (ICL x DMD)}} \qquad (5)$$

The incremental cost of locomotion, also referred to as the minimum or net cost of locomotion, is defined as the slope of the relationship between metabolic power input and speed, and is expressed in units of energy used to transport a unit mass a given distance. Its value is independent of speed (Taylor et al., 1970). The value of the ICL employed in the present study, 7.44 kJ/km, was calculated from the slope of the curve for the relationship between metabolic rate and running speed in neonatal lambs subjected to 70 treadmill exercises in a separate trial (see Chapter 7).

The ecological cost of transport devoted to locomotion in both ewes and lambs were predicted from the allometric equation for herbivorous mammals as:

$$\text{ECT (\% FMR)} = 1.17\ \text{Mb}^{0.21} \qquad \text{(Garland, 1983);} \qquad (6)$$

and alternately as;

$$\text{ECT (\%NLE)} = 1.17\ \text{Mb}^{0.194} \qquad \text{(Altmann, 1987);} \qquad (7)$$

where Mb is the body mass of the animal in kg.

5.2.2.6. STATISTICAL ANALYSIS

Values are presented as means with their standard errors. One way analysis of variance was used to determine if differences existed between the mean daily distance travelled by the lambs. Where differences were significant, Student's t-test was used to compare distances moved in each day.

5.2.3. RESULTS

Table 5.6 shows the distance moved each day in individual lambs in the simulated semi-arid conditions which ranged from 1.6 to 3.2 km (mean = 2.4 ± 0.1 km), and the total distance covered over the first 5 days ranged from 11.0 to 13.7 km (mean = 11.9 ± 0.4 km). The daily distances travelled by the lambs were similar to the predicted values of 1.5 to 2.8 km (mean = 2.1 km) for their mothers. The mean daily walking distances within the first 3 days of life were not statistically different from each other. However, the mean distances travelled during Days 1 and 2 differed significantly from those for Days 4 ($p < 0.01$) and 5 ($p < 0.001$). The mean distance travelled during Days 3 and 4 were lower than that of Day 5 ($p < 0.01$ and $p = 0.01$, respectively).

Calculated and predicted values of ECT (% FMR and % NLE) in are shown in Table 5.7. Whereas the calculated values of ECT in the ideal lambing situation were significantly lower than the predicted values, the values of both measures of cost of locomotion in the lambs were similar to the predicted values.

Table 5.6. Distance moved by lambs during the first five days of life under simulated semi-arid conditions.

Lamb number	Lamb movement distance (km)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Total
1	2.1	2.2	2.1	2.3	2.9	11.6
2	2.0	2.0	2.2	2.6	3.0	11.9
3	2.2	2.2	2.1	2.5	2.9	11.9
4	2.3	2.1	3.2	2.90	3.2	13.7
5	1.9	1.6	2.3	2.2	3.1	11.0
6	1.7	1.9	2.3	2.9	2.9	11.6
Mean	2.0^{a}	2.0^{a}	2.4^{ac}	2.6^{bc}	3.0^{bd}	11.9
± SE	0.1	0.1	0.2	0.1	0.1	0.4

Column values with different superscripts differ at 1% significance level.

Table 5.7 Ecological cost of transport in neonatal lambs under simulated semi-arid conditions.

Lmab number	Body mass (kg)	Distance moved (km/d)	FMR (kJ/d)	Ecological cost of transport (%FMR)		Ecological cost of transport (%NLC)	
				Calcul.	Predic.	Calcul.	Predic.
1	5.1	2.3	1384	1.30	1.65	1.31	1.60
2	4.4	2.2	1181	1.45	1.60	1.47	1.56
3	4.6	2.7	1422	1.48	1.61	1.50	1.57
Mean	4.7	2.4	1329	1.41	1.62	1.43	1.58

5.2.4. DISCUSSION

5.2.4.1. DAILY TRAVELLING DISTANCE

Many studies have shown that even within species, movement distances are influenced by numerous factors, such as age, sex, resource density and distribution, habitat, weather, and season (Garland, 1983). Furthermore, almost all the methods used for estimating daily travelling distances systematically underestimate the true values; a point made more strongly by Altmann (1987). In the present study, the significantly high correlation observed between the distance recorded with the pedometers and the actual distance travelled by lambs during treadmill exercise ($r = 0.97$, $p < 0.001$) validated the suitability of the pedometer for estimating the distance travelled by lambs on a level terrain and at a steady speed, a situation that may not necessary apply to the present study. However, the estimated distances between flock locations at 4-hourly sightings over a period of 24 h suggest that the corrected pedometer readings represent fair estimates of the actual distances travelled by the lambs and their mothers.

Where forage is abundant and watering facilities are readily available, ewes and their lambs do not move far away from the lambing sites during the first week post-partum. The daily distance travelled by lambs during the neonatal period in such an environment would be significantly lower than that observed in the present study. The daily distances travelled by the lambs in the simulated semi-arid condition are similar to the 2.2 km undertaken by day-old lambs following their mothers in a study by Squires (1970), where the laneway used also simulated semi-arid conditions, and the value of 2.1 km predicted for the ewes, which initiated the movement of the couple, from the allometric equation derived by Garland (1983) for adult herbivorous mammals.

Walking is one of the activities that consume the most time and energy of sheep on rangeland, and can involve up to 40% of daylight hours for sheep travelling 12 to 16 km per day, a common phenomenon for flocks in inland Australia (see Squires, 1981). Since lambs do not operate independently from their mothers in their quest for food and water, they may sometimes follow the ewes over far longer distances than observed in the present study in situations where food and water supplies are very scarce. Such lambs could, nevertheless, survive and grow to satisfactory weaning weights even under a regime involving frequent long walks between food and water, however, the growth rates would be significantly lower in those which walk further (Squires, 1970).

Almost all the lambs subjected to 1 km of following, and at least half of those subjected to 2 km of following in a previous study (see Chapter 4), were in good condition and showed no signs of fatigue at the end of the exercises. This suggests that most lambs would be capable of covering far longer distances during the neonatal period than those recorded in the present study, without any significant ill effects.

5.2.4.2. ECOLOGICAL COST OF LOCOMOTION

The ecological cost of locomotion and the relative amount of non-locomotory energy devoted to locomotion in the lambs (1.41% and 1.43%) in the present study were only slightly lower than the predicted values, despite the fact that the lambs covered twice as much as the predicted daily travelling distance for adult mammals of the same body mass (Garland, 1983). This means that lambs are using proportionally less energy for locomotion than an adult mammal of equivalent body mass. The estimated values of ECT in the preliminary trial where the lamb walked on the average half a kilometer per day was about a quarter of the predicted values. The allometric equations for predicting the ECT might have been influenced by the non-inclusion of the energy associated with changing speed and direction in the allometric equations used (Altmann, 1987). Since daily travelling distance is by far the most variable component in the formulae for estimating the values of ECT, any inaccuracies in the measurements are therefore liable to affect the calculated values. As in the present study, daily movement generally has little consequence to the overall energy usage in mammals (Altmann, 1987; Baudinette, 1989). For example, locomotion accounts for a mere

0.5% of the total energy expenditure in a 10 g shrew, and even the elephant, a very large mammal, spends only 6% of its energy on locomotion (Altmann, 1987).

5.2.5. CONCLUSION

The daily distances travelled by lambs in the present study were likely to have been influenced by the location of the watering facility and feed supplement relative to the size and shape of the paddock and availability of food. Many paddocks in the semi-arid pastoral areas of Australia are so large that a minimum distance of 3 km might be expected between the water point and the corner of the paddock (see Squires 1981). Available grazing material closer to watering points would be expected to be impoverished towards the end of the dry season or summer, ewes will therefore have to graze further from water, and over longer distances in their search for adequate sustenance. This would subject the lambs to higher probability of loss through separation, adoption and subsequent rejection by alien ewes as well as to predator attack. The fraction of the metabolic energy that will be spent on locomotion by lambs following ewes in such a situation, though still low relative to FMR, could be significantly greater than in the present study. Using the Altmann equation for transport cost, there is a direct relationship between distance walked and the percentage contribution of energy to the exercise and daily cost.

CHAPTER 6. METABOLIC AND ENDOCRINE CHANGES ASSOCIATED WITH SUBMAXIMAL EXERCISES IN LAMBS

6.1. INTRODUCTION

During exercise, chemically bound energy stores of the body in the form of carbohydrate, fat, protein are transformed to mechanical work via ATP via active muscles. Several adaptations occur in the skeletal muscle during exercise, such as increased blood flow which enhances the transportation of substrates to the active muscles. The proportions of the major substrates utilized during muscular activity are influenced by the intensity and duration of work. However, the immediately available substrates for energy production are the phosphagens, hexose, free glucose and glycogen (see Astrand and Rodahl,1970; Karsson,1971; Shepherd, 1982; Hultman and Sjoholm, 1983).

As energy sources, circulating lipids complement carbohydrate sources but the relative importance of these metabolites are influenced by the nutritional and physiological status and is species dependant (Pethick et al., 1984). At rest, glucose is potentially the largest contributor to the energy requirements of the skeletal muscle, while free fatty acids (FFA) play only a minor role (Pethick et al., 1987; Pruett et al., 1987). Whereas cellular uptake of glucose is normally sufficient to support glycogen synthesis and glycolysis at rest, carbohydrate sources of energy may be limited during maximal exercise. Prolonged submaximal exercise prompts a sustained shift towards fat mobilization (Pethick et al., 1987), and free fatty acid (FFA) availability is increased up to the anaerobic threshold of the individual as exercise is prolonged. However, utilization of this metabolite for energy is depressed as exercise becomes more severe,and demands that a greater portion of the energy is derived anaerobically (Pruett, 1970); this stage is often indicated by very high blood lactate concentration. Tissue protein is not important as an energy source during muscular activity of short duration. However, it assumes a greater importance when carbohydrate sources are exhausted or if exercise is prolonged. Protein degradation in such cases may be essential to meet the blood glucose requirement of the carbohydrate-metabolising tissues, such as the central nervous system (see de Vries, 1980). Growth hormone level during submaximal exercise appears to related to the work intensity. However its release is often delayed for about 5 to 10 min from the beginning of exercise (Sutton et al., 1969).

Lambs do not travel much or run voluntarily at high speed during the first few days after birth. Nevertheless lambs often follow their mothers at walking pace interspersed with submaximal running as the ewes move away from the birth sites in search of food and water. The distance covered by the neonates may exceed 2 km per day in situations of scarce food and water (Squires, 1970). A few studies on the changes in the concentration of blood metabolites during work in farm animals has been undertaken (Farraci et al,. 1984; Pethick et al., 1984; 1987; Kuhlmann et al., 1988; Pearson and Archibald, 1989). There is also some information available on metabolic effects to cold exposure in lambs (Alexander et al., 1972; Eales and Small, 1985; Slee et al., 1990), but no such information is available for neonatal lambs during locomotion.

The aims of the present investigations were to measure the effects of short and prolonged periods of moderate exercise on changes in some blood-borne metabolites and growth hormone in 2-day old lambs in a field situation, and under a wide range of ambient temperature conditions as may occur during lambing periods in different climatic regions. These investigations were repeated in a controlled environment and at a fixed speed by the use of a treadmill in a climatic chamber in an attempt to minimize probable environmental effects. Measurement of blood metabolites was extended to 30 min after exercise so as to assess recovery response in the lamb to exercise stress such as may arise from walking and/or running in the field. The behaviour of the lambs during and after exercise was observed for possible detrimental effects that might be associated with the exercise protocols.The information provided by these studies may serve as a guide to understanding energy metabolism in neonatal lambs during locomotion.

6.2. MATERIALS AND METHODS

6.2.1. TREADMILL EXERCISES

6.2.1.1. Animals

Twelve single born lambs from well nourished ewes were used in Study 1. The lambs were born in spring 1989, and weighed 5.8 ± 0.2 kg at the commencement of the study. Study 2 involved 20 single born lambs, dropped in mid autumn and weighed 5.0 ± 0.2 kg during the trial. The ewes were provided with a supplement of 200 g oats/pea mixture (7:3) per head each other day in addition to hay *ad libitum* during the last 7 weeks of gestation. All lambs were born and kept in a 2-ha paddock up to approximately 1 h prior to the study.

6.2.1.2. Experimental procedure

(1) Animal preparation

At approximately 47 h post partum, each lamb to be tested was confined with its mother to a small cage (1.3 x 1.0 m) in a temperature controlled room at 25°C for a minimum period of 30 min. The lamb was then fitted with a lightweight plastic mask connected to an open flow calorimetry system and attached behind the ears by means of a velcro clasp. It was then held on the lap of an assistant for about 15 min during which time pre-exercise resting state blood sample was taken. Physiological measurements of oxygen consumption, rectal temperature, respiratory and heart rates were taken simultaneously for a separate study (see Ch 7 for details of methodology).

(2) Exercise protocol

Study 1.

Immediately after the pre-exercise measurements, the lamb was placed on a motor-driven treadmill set horizontally, with the dam held in a cage facing the lamb. The lamb was familiarized with the treadmill by walking or running at a low speed (0.3 to 0.5 m/s).for 1

Plate 5. A treadmill consisting of a firm running surface of heavy canvas driven by a 0.5 horsepower electric motor.

min. The treadmill was then run at 1.0 m/s, a speed well within the physical capabilities of neonatal lambs as observed in a previous study (see Chapter 4), and known to elicit between 50% to 60% of the maximal aerobic capacity of the lambs (see Chapter 7). The lamb was subjected to 3 five-min exercise bouts separated by 2 three-min rest pauses, and followed by 30 min of resting recovery period during which the lamb was again held on the lap of an assistant (Figure 6.1). Blood samples were drawn from the jugular vein following physiological measurements at the end of each exercise bout, and at 5, 15 and 30 min after cessation of the exercise. The lamb and dam were then held in a small paddock (40 x 50 m) and observed at specific intervals over a period of 24 h before being allowed to rejoin the flock.

The treadmill consisted of a firm 1.5 x 0.6 m running surface of heavy canvas driven by a 0.5 horse power electric motor (Pope A.C. Motor) (see Plate 5). The sides of the apparatus were covered with plywood, 0.5 m high, and erected 4 cm above the belt to prevent the lambs from catching their feet and tails between the belt and the wall. A panel of 2 x 2 cm wire mesh served as a barrier at the front of the treadmill but allowed ewe and lamb to see each other during exercise. An assistant sitting at the rear of the treadmill ensured that no lamb was harmed if it lost its footing. In general the lambs ran well in response to the sight and sound of the dam.

Study 2.

The preparation of lambs for the second exercise test was similar to that of Study 1. After the brief familiarization period on the treadmill, the lamb to be tested was subjected to 2 fifteen min exercise bouts at a speed of 1.0 m/s, separated by 3-min rest pause (Figure 6.2). Blood sampling and physiological measurements were taken at the end of each exercise bout, 15 and 30 min after the end of exercise. The lamb and mother were held together during the recovery period in a cage (1.5 x 1.0 m) which allowed suckling and minimal movement.

Figure 6.1 A schematic illustration of the experimental procedure in Study 1. Fifteen min intermittent exercise (3 x 5 min exercise bouts interspersed with 2 x 3 min rest paused) followed by 30 min recovery period. The dots indicate blood sampling times.

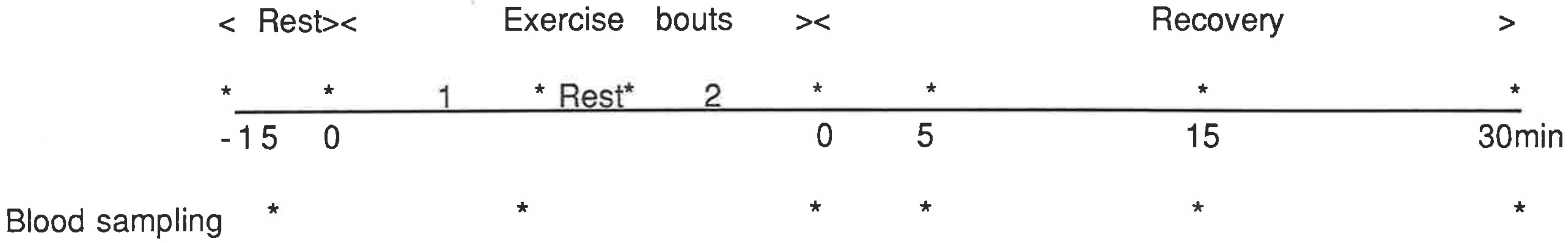

Figure 6.2 A schematic illustration of the experimental procedure in Study 3. Thirty min interval exercise (2 x 15 min exercise bouts interspersed with 3 min rest pause) followed by 30 min recovery period. The dots indicate blood sampling times.

6.2.2. LANEWAY EXERCISE

6.2.2..1 Animals

A total of 17 single-born lambs weighing 4.7 ± 0.4 kg, and 13 others weighing 4.3 ± 0.3 kg were used in Studies 3 and 4 respectively, which took place in late summer 1989 at Waite Agricultural Research Institute Field Experiments Station, Glenthorne, Adelaide. The lambs were born and held outdoors prior to during and after the studies. Each lamb was used only once when 2 days of age.

The exercises were undertaken in good climatic conditions with ambient temperature ranging from 12° to 26°C in Study 3, and from 15° to 33°C in Study 4. Light rain and windy weather, however, occurred in a few cases after the trial had commenced.

6.2.2.2. Experimental procedure

(1) Study 3

Preparation of animals for the laneway exercises is similar to that in Chapter 4. Briefly, at approximately 47 h post-partum the lamb to be tested was confined to a pen for 10 min after which a blood sample (3 ml) was taken following physiological measurements for a concurrent study while the lamb was held on the lap of an assistant.

Figure 6.3 illustrates the experimental protocol employed for the study. The ewe was released into a long and narrow laneway (2.0 m x 0.5 km) (see Chapter 4) and her lamb allowed to follow over 2 laps of the 0.5 km separated by a rest pause of 3 min. Further blood samples were taken at the end of each lap and at 5, 15, and 30 min after discontinuation of the exercise. The lamb was held on the lap of an assistant throughout the post-exercise recovery period after which the lamb and the ewe were confined to a pen (8 x 12 m) with 3 other ewe-lamb couples for a minimum of 1 h before allowed to rejoin the rest of the flock.

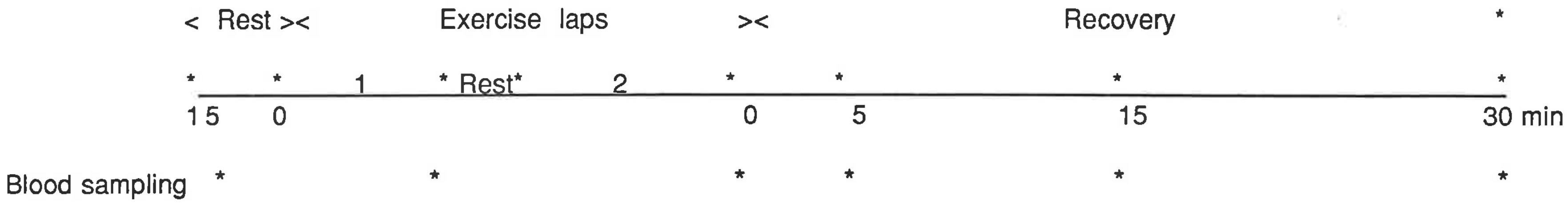

Figure 6.3 A schematic illustration of the experimental procedure in Study 3. One kilometer interval exercise (2 x 0.5 km exercise laps interspersed with 3 min rest pause) followed by 30 min recovery period. The dots indicate blood sampling times.

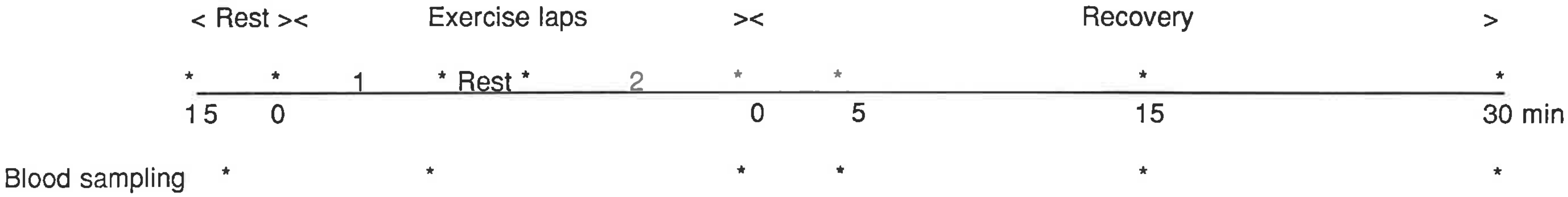

Figure 6.4 A schematic illustration of the experimental procedure in Study 3. Two-kilometer interval exercise (2 x1.0 km exercise bouts interspersed with 3 min rest pause) followed by 30 min recovery period. The dots indicate blood sampling times.

(2) Study 4

The preparation and treatment of the lambs used in this study was similar to that of Study 3 except that the test lamb was made to follow its mother over 4 laps of 0.5 km distance with rest pause of 3 min between the second and third laps (Figure 6.4). Blood samples were taken at rest, after 1.0 km and 2.0 km distances, and at 15 and 30 min during the post-exercise recovery period. The lamb being tested was held with her mother in a small pen (3 x 5 m) during the rest pause, and in a bigger pen (8 x 12 m) during the recovery period. Access to the ewe's udder was not prevented during exercise and recovery periods.

6.2.3. Blood sampling and handling

Blood samples (3 ml) were drawn via jugular venipuncture into 5 ml syringes and emptied into chilled tubes containing ethylenediaminetetraacetic acid (EDTA) which were immediately placed on ice. One mil of the blood was deproteinised by adding an equal volume of 8% (V/V) perchloric acid. The mixture was centrifuged at approximately 3000 g for 10 min at 4 °C and an aliquot of clear supernatant was removed and stored at -20°C for later determination of blood glucose, L-lactate and pyruvate concentrations. A second centrifugation of the supernatant was sometimes necessary to obtain a clear, protein-free solution. Plasma was prepared by centrifuging the rest of the blood at 3000 g for 5 to 10 min at 4 °C to sediment blood cells. The plasma was removed and stored at -20°C for later assay for total protein, FFA and growth hormone concentrations. Free fatty acid determination was undertaken within a week of blood collection, and the other metabolites were assayed over a period of 1 to 4 weeks.

6.2.4. Chemical analysis

The concentration of glucose in blood was measured by the glucose oxidase method of Bergmeyer and Burnt (1974b). An adaptation of the lactate dehydrogenase techniques of Gutmann and Wahlefeld (1974) and Bergmeyer and Bernt (1974a) were used for the estimation of L-lactate and pyruvate concentrations in blood respectively. An enzymatic calorimetric assay was used for the determination of FFA concentration in plasma (Test-Combination; Boehringer Mannheim Biochemical), and plasma total protein concentration was determined by a technique employing Commassie brilliant blue G250 (Sedmark and Grossberg, 1977). The measurement of plasma concentration of growth hormone was carried out using a radioimmunoassay for ovine growth hormone according to the method of Gabreath (1983).

6.2.5. Statistical analysis

Values of the parameters determined were expressed as means and standard errors of the means. Regression analysis was used to examine variations within the parameters at a sampling time that may be attributed to body mass and sex of the lambs, speed of following and ambient temperature The difference between mean values of parameters at rest, during exercise and post-exercise recovery were examined by one-way analysis of variance. Where significant difference was detected, a Student t-test for paired data was used to evaluate the significance of differences between mean values within a parameter determined at rest and at various sampling times during exercise and recovery. The Pearson product-moment correlation was used to test for significance of the relationships among the evolution of the blood-borne metabolites.

6.3. RESULTS

6.3.1. TREADMILL EXERCISES

6.3.1.1. Animal behaviour

The lambs fell into an apparent relaxed state within 3 to 5 min of being held on the lap of an assistant for the pre-exercise rest measurements; a far more standardized state than that seen if the animal was left standing alone. This state was usually preceded by mild struggling and bleating. During the treadmill familiarization and exercise test, the ewe often stood up in the cage with her head held high and close to the treadmill, and bleated intermittently. With the exception of 2 lambs in Study 1 and 3 lambs in Study 2 that refused to walk or did not perform satisfactorily during the familiarization trial, the rest of the lambs achieved steady state running by the end of the training period, by which time the bleating response of the lambs to their mothers' call had ceased or reduced considerably. Lambs not running well during the test were often encouraged by the sight and bleating calls of the dams. Most of the lambs urinated or defecated occasionally during exercise, thereby disrupting an otherwise uniform step frequency. Normal running posture was often regained within a few seconds of the elimination process.

Lambs in Study 1 remained still on the lap of the assistant throughout the recovery period, emitting occasional low-pitched bleats. They commenced suckling immediately on being returned to their mothers after the total separation period of about 1 h. Lambs in Study 2 resumed suckling within 5 min of the post-exercise recovery period, while being actively groomed by their mothers. Recovery from the stress of exercise in both studies occurred within 15 to 30 min after cessation of the exercise. On the whole, exercise did not seem to affect the behavior of the lambs and no detrimental affects resulting from the experimental procedure were subsequently observed within the 24 h post-exercise period.

6.3.1.2. Changes in concentration of blood-borne metabolites.

(1) Study 1 (three 5 min intermittent exercise).

Sex and body mass of lambs

Although there were considerable individual variations in the absolute concentrations in blood metabolites during exercise and recovery periods, the 2 variables, sex and body mass, did not seem to have significant effects on the variation within blood parameters observed at various sampling times.

Lactate

Changes in blood-borne metabolites are shown by Table 6.1 and Figure 6.5. Prior to the intermittent exercise, concentration of lactate in venous blood ranged from 0.84 to 2.02 mM, with a mean of 1.17 ± 0.10 mM. Blood lactate concentration increased significantly over the pre-exercise value during exercise and reached a maximum value of 2.01 ± 0.16 mM (range = 1.48 to 3.32 mM) during the last exercise bout, indicating a mean increase of 0.84 mM ($p < 0.001$) above the resting level. Lactate concentration after 5 min of the recovery period was not different from the exercise values; it subsequently decreased gradually to 1.19 ± 0.16 mM (range = 0.91 to 2.11 mM) at 30 min after cessation of exercise. The lactate concentration at 15 min of recovery, 1.43 ± 0.11 mM, though statistically similar to the pre-exercise level, represents a decrease of 69% of the excess lactate concentration achieved during exercise over the pre-exercise value, and a decrease of approximately 29% below exercise level.

The trend of blood lactate changes in most lambs was similar, with 7 of the lambs showing peak values in the third exercise bout while peak values were observed in 1 lamb during the first, and 2 others in the second exercise bouts (Figure 6.6). With the exception of 2 lambs which had lactate concentrations increasing into the first 5 min of recovery, lactate levels in the lambs decreased progressively throughout the recovery period.

Pyruvate

Blood pyruvate concentrations in the lambs at the end of the first exercise bout did not differ significantly from the mean pre-exercise value of 0.098 ± 0.006 mM (range = 0.064 to 0.110 mM). The pyruvate concentration however, increased progressively during the subsequent bouts, reaching 0.103 ± 0.005 mM (range = 0.080 to.137 mM) at the second bout and 0.115 ± 0.007 mM (range = 0.075 to 0.158 mM) at the end of the last bout. These represent increases of 0.017 mM ($p < 0.01$) at the second and 0.025 mM ($p < 0.001$) at the third exercise bouts over the pre-exercise level. Blood pyruvate levels 5 min after cessation

Table 6.1 Metabolic effects of three x 5 minutes of intermittent exercise and thirty minutes recovery period.

Sampling times	Lactate mM	Pyruvate mM	Glucose mM	Protein µg/l	Free fatty acids mM
Pre-exercise rest	1.17 ± 0.10	0.086 ± 0.004	5.08 ± 0.16	78.9 ± 4.9	0.345 ± 0.005
Exerc. bout					
1	1.69 ± 0.16**	0.098 ± 0.006	5.14 ±0.14	77.51 ±3.27	0.356 ± 0.007
2	1.83 ± 0.15**	0.103 ± 0.005*	4.92 ± 0.10	79.1 ± 1.9	0.376 ± 0.000**
3	2.01 ± 0.16***	0.115 ± 0.007**	4.94 ± 0.10	81.7 ± 4.3	0.400 ± 0.005**
Recovery min					
5	1.91 ± 0.17	0.114 ± 0.012*	4.92 ± 0.13	77.0 ± 3.0	0.370 ± 0.007**
15	1.43 ± 0.11	0.092 ± 0.006	5.12 ± 0.16	82.5 ± 2.8	0.348 ± 0.000
30	1.19 ± 0.10	0.086 ± 0.006	5.33 ± 0.16	82.2 ± 4.5	0.333 ± 0.005

Significantly diferrent from resting values at 5*, 1** and 0.1*** respectively.

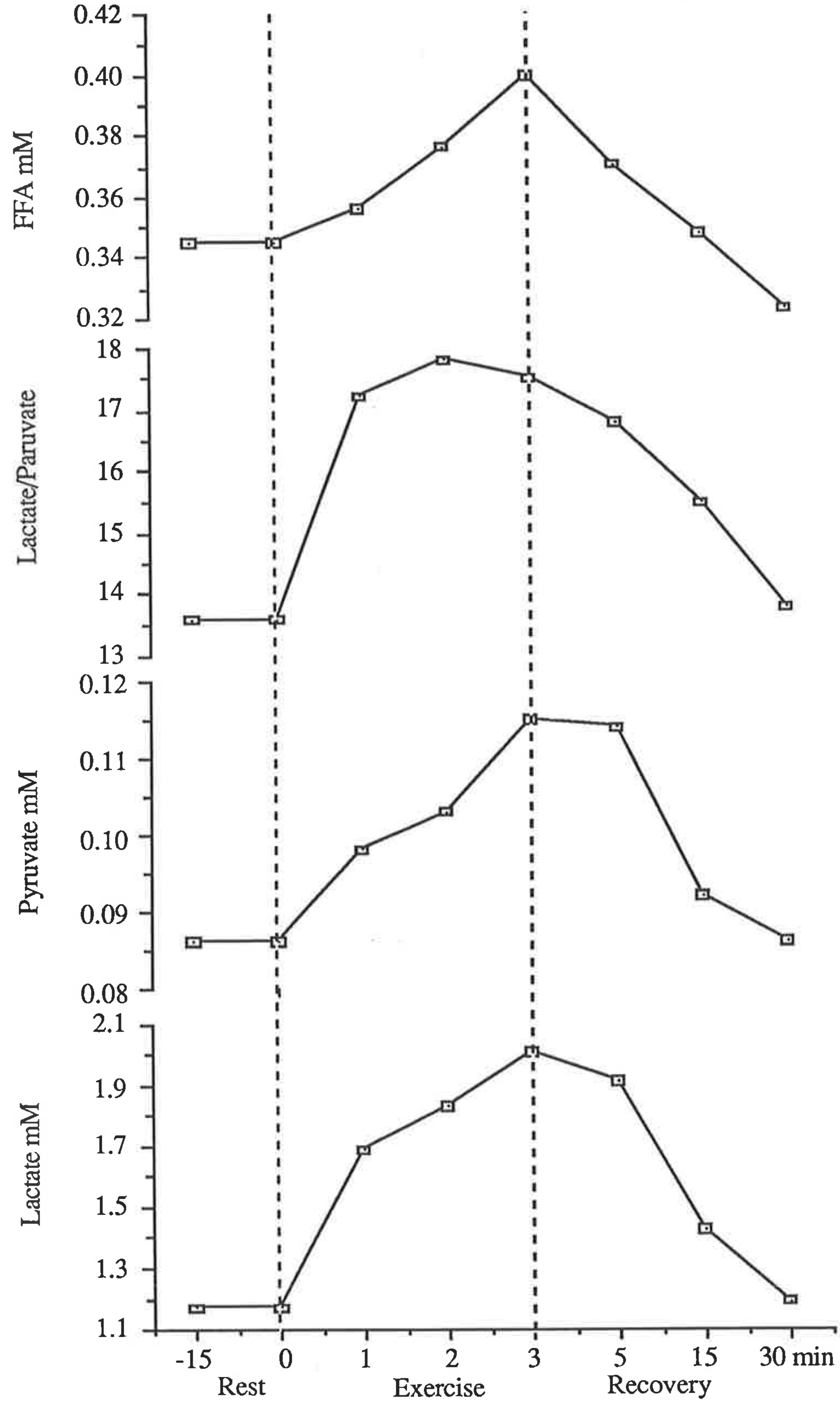

Figure 6.5 Evolution of lactate, pyruvate,lactate-pyruate ratio and FFA during15 min (3 x 5 min) intermittent exercise.

of exercise did not change from the level reached at the end of the intermittent exercise. The decline in blood concentration thereafter was rapid and reached the pre-exercise level after 15 min of recovery.

Lactate:pyruvate ratio

The lactate:pyruvate ratio increased significantly over the pre-exercise value of 13.6 to 17.2 by the end of the first bout of exercise, and reached a peak of 17.8 in the second bout. The ratio thereafter decreased gradually to the pre-exercise value by 30 min post-exercise recovery. The slight changes observed in the ratio during the subsequent exercise bouts indicated a tendency towards a plateau.

Glucose

Blood glucose concentrations during the intermittent exercise and subsequent recovery period did not differ significantly from that of the pre-exercise value of 5.08 ± 0.16 mM (range = 4.28 to 5.85 mM). Glucose concentration however tended to increase slightly to 5.14 ± 0.14 mM (range = 4.57 to 5.95 mM) during the first exercise bout before decreasing in subsequent bouts. There was a tendency towards a gradual increase in concentration after exercise to 5.33 ± 0.16 mM (range = 4.43 to 6.14 mM) by the end of the recovery period; a value significantly different from that seen at second and third exercise bouts ($p < 0.05$).

Protein

Total plasma protein concentration during the intermittent exercise and recovery periods did not follow any particular trend, nor did any significant changes occur from the pre-exercise value of 68.0 to 102.0 μg/l, with a mean of 78.9 ± 4.9 μg/l.

Free fatty acids

Plasma FFA concentration continuously increased during the intermittent exercise from the pre-exercise level of 0.345 mM (range = 0.311 to 0.365 mM) to a peak of 0.400 ± 0.005 mM (range = 0.369 to 0.427 mM) during the last exercise bout ($p < 0.001$). The FFA

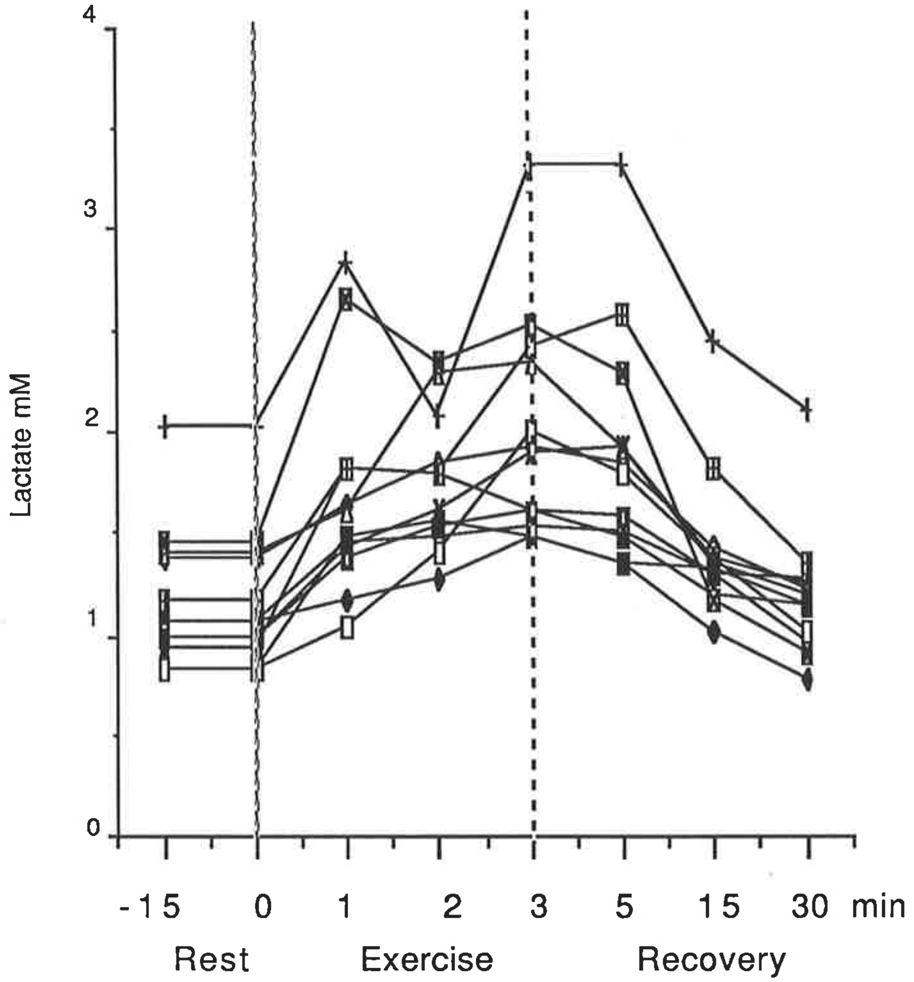

Figure 6.6 Blood lactate effects of 3 x 5 minutes treadmill intermittent exercise.

concentration 5 min after exercise 0.370 ± 0.007 mM (range = 0.397 to 0.390 mM) was higher than the pre-exercise value ($p < 0.01$). Decrease in plasma FFA concentration to the initial resting level occurred after 15 min of recovery.

Relationship between the changes in blood-borne metabolite levels

Table 6.2 depicts the association between the changes in blood-borne metabolite levels during exercise and recovery. A significantly high correlation was obtained between changes in the mean concentrations of lactate during the intermittent exercise and recovery and that of pyruvate ($r = 0.96$, $p < 0.001$) and plasma FFA ($r = 0.91$, $p < 0.01$). Blood pyruvate concentrations were also positively correlated with plasma FFA ($r = 0.87$, $p = 0.01$). Blood glucose concentrations during the test were negatively correlated with changes in the concentrations of lactate ($r = - 0.80$, $p < 0.05$), pyruvate ($r = - 0.81$, $p < 0.05$) and FFA ($r = - 0.84$, $p < 0.05$). Plasma total protein, on the other hand, was not associated with the changes in any of the other metabolites.

(2) Study 2 (two 15 min interval exercise)

Sex and body mass of lambs

Sex and body mass of lambs did not contribute to the variation in pre-exercise levels of blood-borne metabolites, nor that during exercise bouts and recovery period.

Blood lactate

Changes in the concentration in blood-borne metabolites studied during the 2 x 15 min exercise are illustrated in Table 6.3 and Figure 6.7. Blood lactate concentration in the lambs at rest, 1.92 ± 0.11 mM with a range of 1.31 to 2.74 mM, rose to 3.55 ± 0.25 mM (range = 2.25 to 4.59 mM) at the end of the second exercise bout ($p < 0.001$). It then decreased progressively to the resting value by 30 min. By 15 min of the recovery period, lactate concentration had decreased to 2.50 ± 0.18 mM (range = 1.77 to 3.77 mM) from the value at the end of work. This represents a decrease of 64% from the increase in lactate achieved

Table 6.2. Relationship among blood-borne metabolites determined at rest and different time points during three x 5 minutes exercise and recovery periods.

Parameter	Lactate	Pyruvate	FFA	Glucose	Protein
Lactate	1.00 (0.000)	0.96 (0.001)	0.91 (0.004)	-0.80 (0.033)	-0.32 (0.480)
Pyruvate		1.00 (0.000)	0.89 (0.012)	-0.81 (0.026)	-0.37 (0.408)
FFA			1.00 (0.000)	-0.84 (0.019)	-0.12 (0.792)
Glucose				1.00 (0.000)	0.42 (0.346)
Protein					1.00 (0.000)

First and second lines depict correlation coefficient and significance level respectively. For statistical significance a correlation of 0.553 or larger is required at 5% level.

over the pre-exercise value at the end of exercise; a decrease of 30% below the exercise level. Figure 6.8 depicts blood lactate evolution during exercise in individual lambs. Although 75% of the lambs exhibited peak lactate values by the end of the second exercise bout, there was no significant difference in the mean levels between the exercise bouts.

Pyruvate

Blood pyruvate concentration fell within the range of 0.075 to 0.125 mM, with a mean of 0.103 ± 0.004 mM, prior to the exercise test. By the end of the intermittent exercise, pyruvate concentration had increased progressively to a peak of 0.173 ± 0.013 mM (range = 0.107 to 0.243 mM), representing an increase of 0.070 mM over the resting value ($p < 0.001$). A significant decrease in the concentration occurred by 15 min after cessation of exercise ($p < 0.05$), however the value of 0.136 ± 0.012 mM (range = 0.84 to 2.07 mM) reached was slightly higher than the pre-exercise value ($p < 0.05$). The concentration at the end of the 30 min recovery period, 0.113 ± 0.004 mM (range = 0. 86 to 0.145 mM), did not differ from the resting value. Nevertheless, 8 of the lambs tended to exhibit slightly higher levels of the substrate in blood than their pre-exercise values.

Lactate:pyruvate ratio

The lactate:pyruvate ratio increased from a pre-exercise value of 18.6 to 21.3 by the end of the first 15 min of exercise. The ratio declined gradually to 30.5 at completion of exercise, and returned to pre-exercise levels at 15 min post-recovery.

Free fatty acids, glucose and total protein

The sampled concentrations of plasma FFA, total protein and blood glucose during exercise and recovery periods did not differ significantly from the pre-exercise levels of 0.406 ± 0.034 mM (range = 0.364 to 0.481 mM) for FFA, 5.63 ± 0.22 mM (range = 4.76 to 7.31 mM) for glucose, and 85.0 ± 7.6 µg/l (range = 69.8 to 118.3 µg/l) for total protein. However, the concentrations of glucose and total protein tended to be slightly lower during exercise than at rest and at the end of the recovery period. Plasma FFA showed a tendency

Table 6.3. Metabolic effects of two x 15 minutes exercise and thirty minutes recovery period

Sampling times	Lactate mM	Pyruvate mM	Glucose mM	Protein mM	FFA mM
Pre-exercise rest	1.92 ±0.11	0.103 ±0.004	5.63 ±0.22	85.0 ±7.6	0.406 ±0.034
Exercise bout					
1	3.56*** ±0.19	0.167** ±0.011	5.48 ±0.27	75.2 ±3.9	0.434 ±0.022
2	3.55** ±0.25	0.173*** ±0.013	5.12 ±0.28	79.4 ±5.5	0.429 ±0.037
Recov. (min)					
15	2.50** ±0.18	0.136* ±0.012	5.25 ±0.30	85.5 ±7.3	4.11 ±0.020
30	2.04 ±0.15	0.113 ±0.004	5.60 ±0.24	96.3 ±11.2	403 ±0.020

Significantly diferrent from resting values at *5 *, **1 ** and 0.1 *** levels.

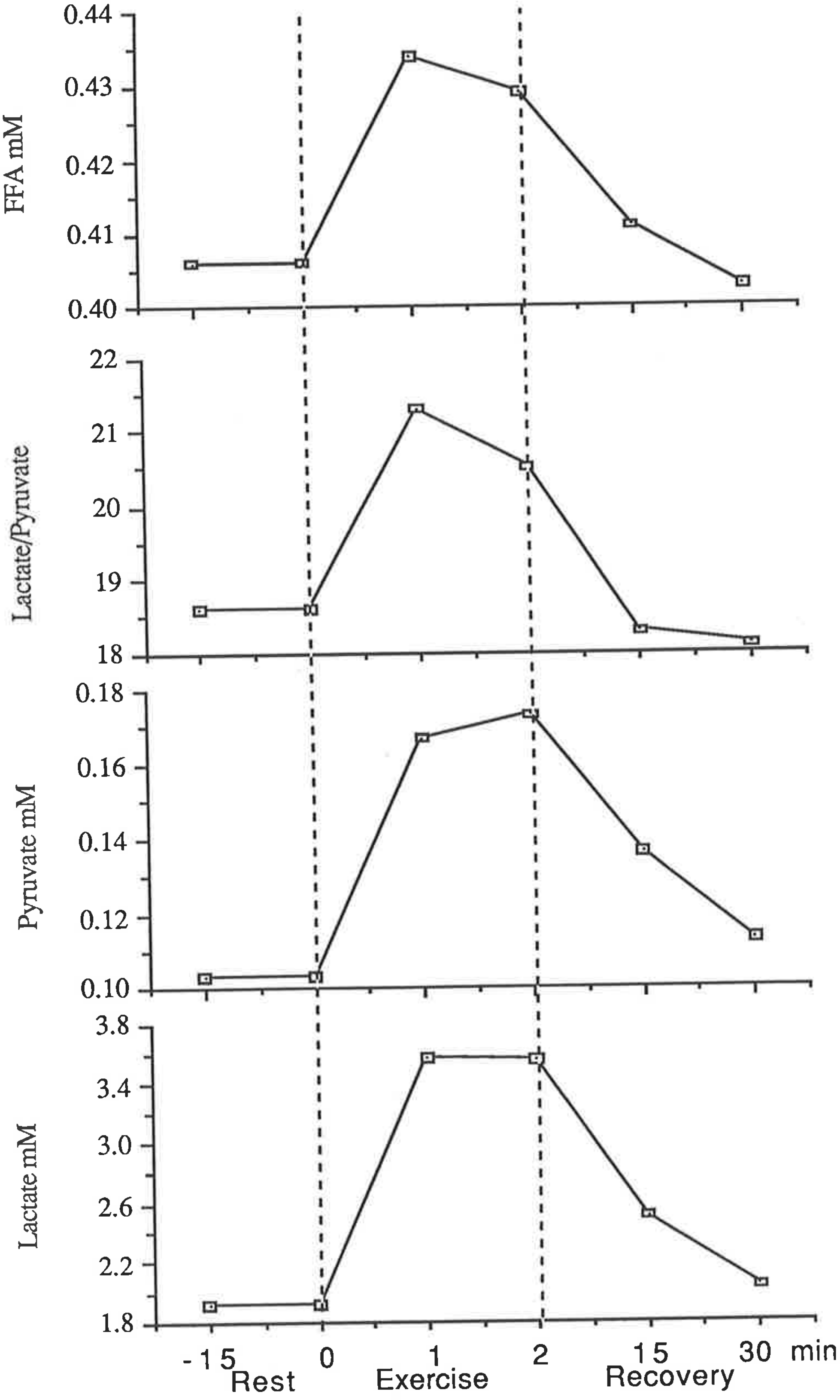

Figure 6.7 Evolution of blood lactate, pyruvate, lactate/pyru ratio during 2 x15 minutes treadmil interval exercise.

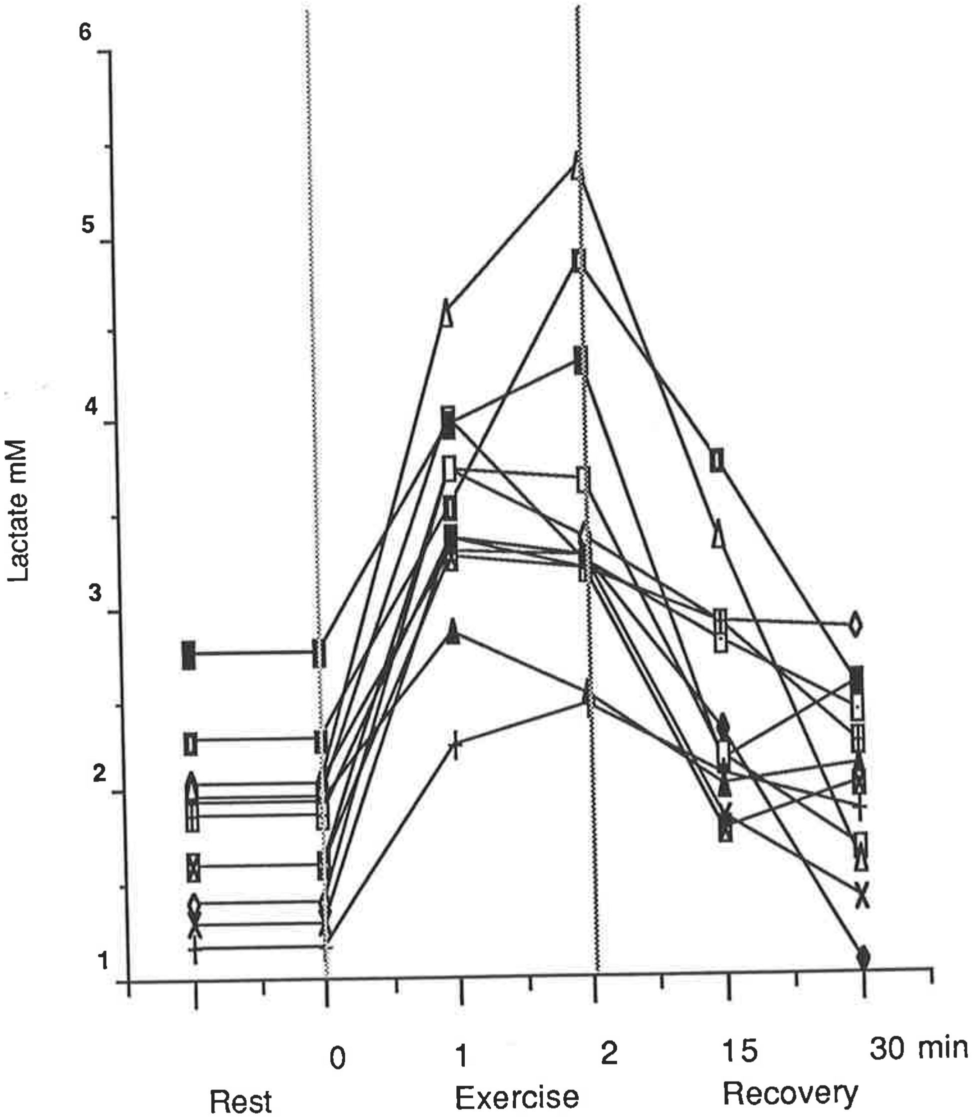

Figure 6.8 Blood lactate effects of 2 x15 minutes interval exercise

towards a rise in concentration during exercise and, thereafter, a gradual decrease to resting levels.

Relationship between the changes in blood-borne metabolite levels

Correlation matrices of the various blood-borne metabolites studied are shown by Table 6.4. Changes in the blood lactate levels during exercise and recovery was highly and positively correlated with that of pyruvate ($r = 0.99$, $p < 0.001$) and FFA ($r = 0.95$, $p < 0.01$). Changes in pyruvate concentration were also related to that of FFA ($r = 0.95$, $p < 0.05$). Changes in plasma FFA and total protein concentrations during exercise and recovery were not significant, nevertheless,changes at sampled times in both metabolites were negatively related to that of total protein ($r = -0.98$, $p < 0.043$). Blood glucose concentrations, on the other hand, did not correlate with any other metabolite.

(3) Comparative study of "intermittent" and "continuous" treadmill exercise

Comparison is made between the changes in blood-borne metabolites levels from pre-exercise values to the end of the 3 last exercise bouts in Study 1, referred to as "intermittent exercise", and to the end of the first exercise bout in Study 2, referred to as "continuous exercise". The intensity and duration (15 min) in the 2 types of exercise were similar. There were no differences in the pre-exercise venous concentrations of glucose, total protein and FFA in the 2 types of exercise. The absolute blood lactate and pyruvate concentrations by the end of the "continuous exercise" was higher than that of the "intermittent exercise", indicating an increase of 1.65 mM and 0.84 mM respectively ($p < 0.001$). A similar trend was seen in pyruvate concentrations where the increase during "intermittent exercise" was lower (0.012 vs 0.064, $p < 0.01$). The changes seen in plasma FFA by the end of the "intermittent exercise" was also lower than that of the continuous exercise ($p < 0.01$). The changes in blood glucose and plasma total protein over pre-exercise levels by the end of exercise in the 2 studies were not significantly different.

Table 6.4. Relationship among blood-borne metabolites determined at rest and different time points during two x 15 minutes of exercise and in the recovery periods.

Parameter	Lactate	Pyruvate	FFA	Glucose	Protein
Lactate	1.00 (0.000)	0.99 (0.001)	0.98 (0.003)	-0.64 (0.250)	-0.82 (0.092)
Pyruvate		1.00 (0.000)	0.95 (0.015)	-0.73 (0.165)	-0.77 (0.131)
FFA			1.00 (0.000)	-0.15 (0.378)	-0.98 (0.043)
Glucose				1.00 (0.000)	0.43 (0.474)
Protein					1.00 (0.000)

First and second lines depict correlation coeficient and significance level respectively. For statistical significance a correlation of 0.553 or larger is required at 5% level.

6.3.2. LANEWAY EXERCISES

6.3.2.1. Animal Behaviour

The general behaviour of the lambs during the laneway exercises and recovery periods is similar to that given in Chapter 4. All the lambs completing the exercises seemed to cope quite well. The lamb following at the lowest speed in Study 4 seemed to be tired and panted during the last half of the following distance and for 15 min after the exercise had stopped. Lambs in Study 3 exhibited vigorous sucking drives for about a minute soon after joining their mothers after the 30 min recovery period. This was followed by intermittent sucking during the next 10 min. The lambs spent most of the remaining recovery period resting near their mothers. Sucking after exercise was less vigorous in the Study 4 lambs; 6 of them did not commence sucking during the first 5 min after exercise.

6.3.3.2. Changes in concentration of blood-borne metabolites

(1) Study 3 (two 0.5 km interval exercise)

Lamb characteristics and environmental factors

Concentrations of blood metabolites in the lambs subjected to 2 x 0.5 km laneway exercise are shown by Table 6.6 and Figure 6.9. Despite the large variation seen in levels of all the blood metabolites studied prior to and during exercise, as well as the post-exercise recovery period, body mass and sex of lambs did not not account for any of the variations at sampling times, with the exception of the variations seen in plasma growth hormone at 15 min of recovery where sex had an effect (F = 7.65 on 1,15 df; $p < 0.05$).

The wide variation in ambient temperature during the trials (12°C to 26°C) and the speed of following (91.24 to 1.95 m/s) did not seem to influence the variations in the concentration of blood metabolites prior to exercise, during exercise or post-exercise recovery. However, higher ambient temperature tended to increase total protein levels at the 0.5 km distance.

Table 6.5 Metabolic effects of two x 0.5 kilometer interval laneway exercise and thirty minutes recovery.

Sampling times	Lactate mM	Pyruvate mM	Glucose mM	Protein mM	FFA mM	GH ng/l
Pre-exercise rest	1.37 ±0.17	0.087 ±0.006	4.90 ±0.12	98.4 ±4.8	0.364 ±0.017	8.1 ±0.8
Distance (km)						
0.5	3.22*** ±0.35	0.152** ±0.019	4.82 ±0.12	107.1 ±7.9	0.443 ±0.18	33.2*** ±5.32
1.0	3.42*** ±0.33	0.170*** ±0.018	4.60 ±0.14	100.1 ±5.6	0.506*** ±0.076	39.3*** ±5.3
Recovery min						
5	3.09*** ±0.36	0.167*** ±0.021	4.72 ±0.10	90.4 ±6.4	0.495** ±0.034	41.3*** ±5.7
15	2.36*** ±0.26	0.166** ±0.026	4.74 ±0.12	98.9 ±5.1	0.399 ±0.019	37.8*** ±6.3
30	1.82 ±0.21	0.113 ±0.004	4.99 ±0.12	101.1 ±5.0	0.397 ±0.016	31.8*** ±5.08

Significantly diferrent from resting values at *5 and *0.1% levels.

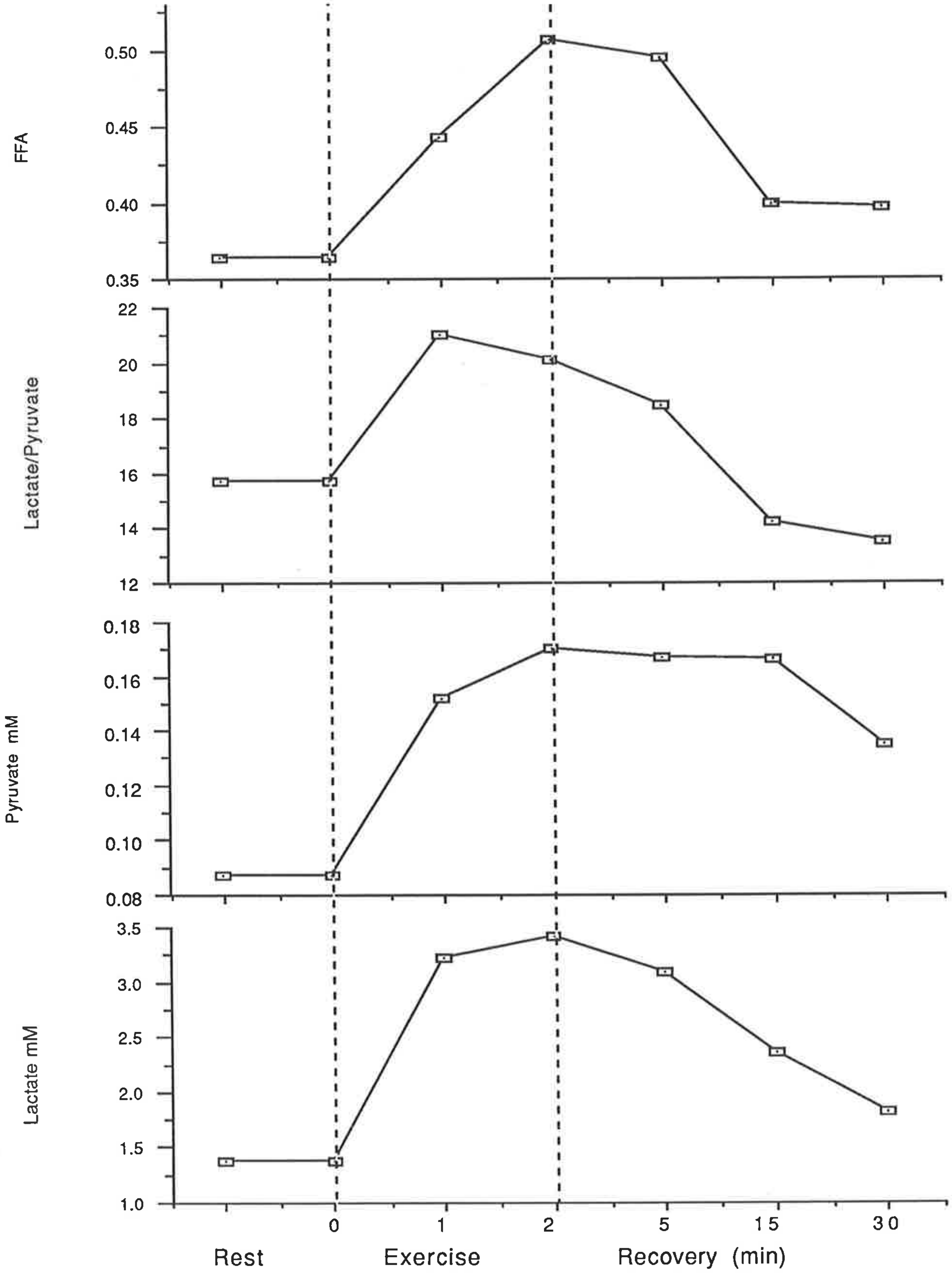

Figure 6.9. Metabolic effects of 2 x 0.5 kilometer laneway exercise.

Blood lactate

Concentration in blood lactate prior to exercise 1.37 ± 0.7 mM (range = 0.55 ± 2.92 mM) rose progressively to 3.22 ± 0.35 mM (range = 0.98 to 6.29 mM) at 0.50 km, and to 3.42 ± 0.33 mM (range = 1.30 to 5.74 mM) at 1.0 km following distance. The levels observed at the end of exercise bouts were not significantly different. Eight of the lambs exhibited peak lactate values at the end of the work, while blood lactate concentrations in the rest of the lambs reached peak values at 5 min after exercise (Figure 6.10). The mean lactate value of 3.09 ± 0.36 mM (range = 1.05 to 6.59 mM) after 5 min of exercise was significantly lower than the value at the end of exercise ($p < 0.05$). Clearance of blood lactate after exercise was gradual and reached pre-exercise values by 30 min recovery. The lactate concentrations of 2.36 ± 0.26 mM (range = 0.82 to 5.15 mM) seen 15 min after exercise, represented a decrease of 31% from the exercise level.

Pyruvate

Blood pyruvate concentration in the lambs prior to exercise ranged from 0.076 to 0.139, with a mean of 0.087 ± 0.006 mM. The laneway exercise elicited a progressive rise in pyruvate concentration to 0.152 ± 0.019 mM (range = 0.086 to 0.376) at 0.5 km ($p < 0.01$), and to 0.170 ± 0.018 mM (range = 0.094 to 0.384 mM) at 1.0 km distance ($p < 0.001$). The concentration of pyruvate remained at a plateau during the first 15 min after exercise, before reducing 21% from the post exercise value to 0.113 ± 0.014 mM (range = 0.051 to 0.327 mM) at 30 min recovery; a value which is 1.6-fold increase over the pre-exercise level.

Lactate:pyruvate ratio

The lactate:pyruvate ratio increased significantly from pre-exercise value of 15.7 to 21.0 by the end of the first 0.5 km distance. The ratio decreased to 20.1 at completion of the 1 km distance followed by a rapid decline to 18.5 by 5 min after exercise. The lactate:pyruvate

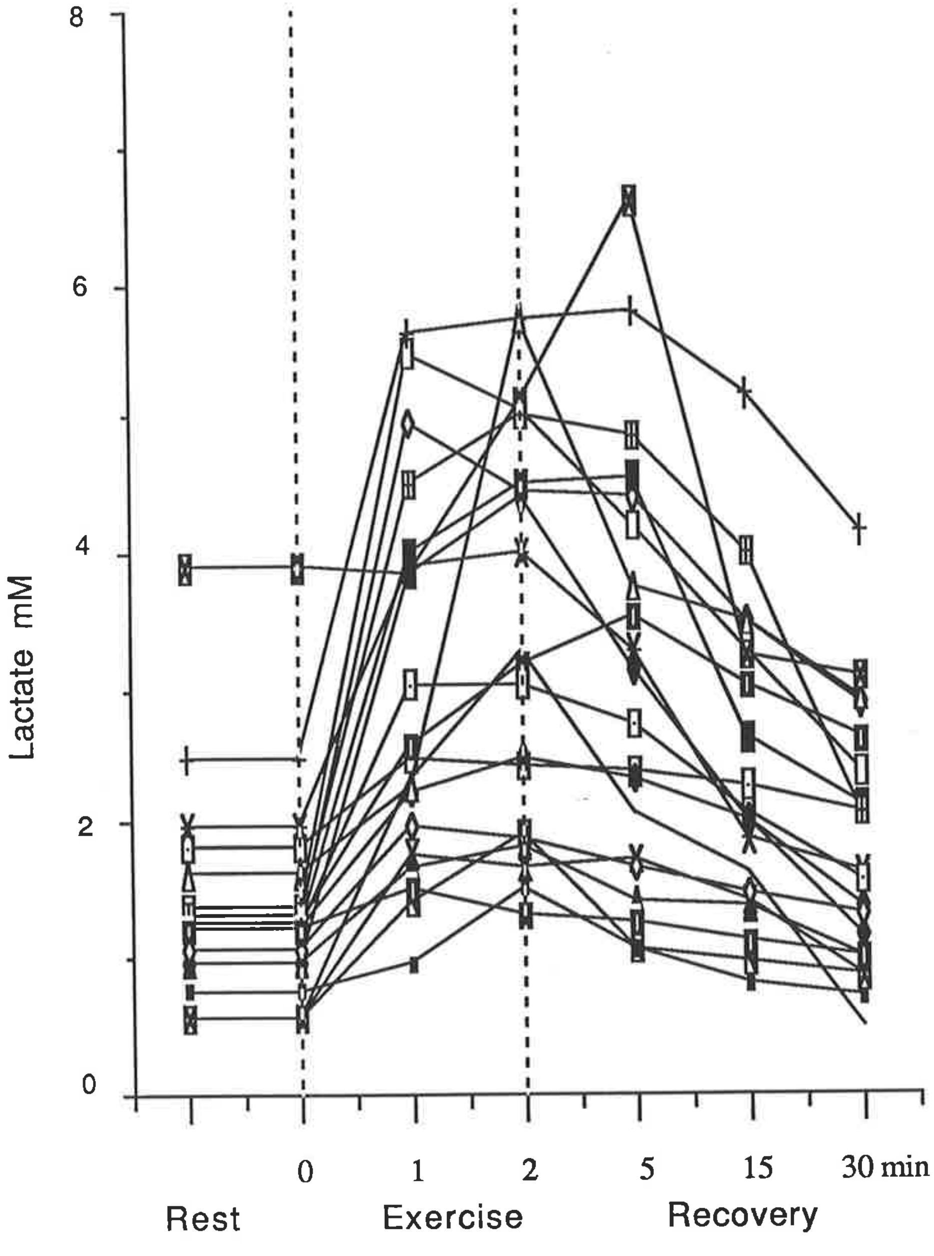

Figure 6.10 Lactate effects of 2 x 0.5 kilometer interval laneway exercise.

ratio at 15 and 30 min after exercise, 4.2 and 13.5, respectively, were lower than the pre-exercise value.

Glucose and total protein

Blood glucose and plasma total protein concentrations prior to exercise ranged from 3.91 to 5.57 mM (mean 4.90 ± 0.12 mM) and 77.1 to 148.0 µg/l (mean 98.4 ± 4.8 µg/l, respectively). Changes in the levels of these metabolites during the exercise and recovery periods did not differ from the resting values. However, total protein concentration at the end of the first lap of 0.5 km tended to be slightly higher than at other sampling points.

Free fatty acids

The concentration of plasma FFA prior to exercise ranged from 0.257 to 0.477 mM, with a mean of 0.364 ± 0.022 mM. A progressive increase from the resting value to 0.443 ± 0.018 mM, with a range of 0.310 to 0.501 mM, at the end of 0.5 km distance ($p < 0.001$), and then to 0.506 ± 0.024 mM (range = 0.313 to 0.512 mM) at 1.0 km distance ($p < 0.001$), occurred. Free fatty acids level reduced to 0.495 ± 0.031 mM (range = 0.372 to 0.688 mM; $p < 0.05$) at 5 min after exercise, a value which was significantly higher than the pre-exercise value ($p < 0.001$). The concentration thereafter reduced rapidly to pre-exercise values by 30 min after exercise.

Growth hormone

Plasma growth hormone concentration in the lambs prior to exercise was in the range of 2.8 to 13.1 mg/l, with a mean of 8.1 ± 0.8 ng/ml. Concentration of growth hormone rose rapidly from the resting value throughout the exercise period reaching 33.2 ± 1.3 at 0.5 ng/ml, and 39.3 5.3 mg/ml at the end of work. The increase extended to a peak of 41.3 ± 5.7 ng/ml at 5 min of the recovery period ($p < 0.001$), after which a slight reduction occurred. The mean level reached at 30 min of recovery was still significantly higher than the resting value ($p < 0.001$). One lamb consistently exhibited exceptionally high growth hormone levels at rest, during exercise and recovery (27.5 to 111.0 ng/ml)

Relationship between the changes in blood-borne metabolite levels

Table 6.7 illustrates the correlation matrices of the blood-borne metabolites studied. Blood lactate concentrations during exercise and recovery were highly and positively correlated with those of pyruvate ($r = 0.833$, $p < 0.040$) and FFA ($r = 0.907$, $p < 0.013$). Changes in growth hormone levels during exercise and recovery was highly correlated to that of pyruvate ($r = 0.982$, $p < 0.001$). Although the trend of changes in lactate and FFA concentrations tended to be similar to that of growth hormone, the correlations between them were not significant ($r = 0.776$, $p < 0.070$, and $r = 0.765$ $p< 0.076$, respectively). Blood glucose and plasma total protein levels during the test did not correlate with that of any other metabolite.

(2) Study 4

Lamb characteristics and environmental factors

Blood borne metabolite concentrations during the 2 x 1.0 km laneway exercise are shown by Table 6.8 and Figure 6.11. None of the variables, body mass and sex of lamb, speed of following and ambient temperature during the trial affected lamb to lamb variation in pre-exercise values of the blood metabolites. Speed of following did not exert any influence on the variation in the levels of the metabolites during the exercise nor recovery. Sex of lamb however contributed to the variation seen in the plasma total protein levels at 30 min after exercise ($F = 5.48$ on 1, 12 df; $p < 0.05$).

Lactate

Blood lactate level in lambs prior to the laneway exercise was 2.20 ± 0.14 mM, with a range of 1.27 to 3.21 mM. The concentration of the metabolite increased progressively to 6.30 ± 0.75 mM (range = 2.23 to 10.7 mM) at the end of the first kilometer ($p < 0\ .001$), and to 7.55 ± 0.91 mM (range = 2.69 to 10.14 mM) at the completion of exercise ($p < 0\ .001$), representing 2.9- and 3.4-fold increases over the resting value respectively. The

Table 6.6 Relationship among blood-borne metabolites determined at rest and different time points during two x 0.5 km interval laneway exercise and recovery periods.

Parameter	Lactate	Pyruvate	Glucose	Protein	FFA	GH
Lactate	1.00 (0.000)	0.83 (0.040)	-0.78 (0.068)	0.04 (0.937)	0.91 (0.013)	0.78 (0.067)
Pyruvate		1.00 (0.000)	-0.70 (0.118)	-0.11 (0.833)	0.75 (0.087)	0.98 (0.001)
Glucose			1.00 (0.000)	0.26 (0.614)	-0.77 (0.076)	-0.59 (0.223)
Protein				1.00 (0.000)	-0.26 (0.613)	-0.15 (0.779)
FFA					1.00 (0.000)	0.73 (0.098)
GH						1.00 (0.000)

First and second lines depict correlation coeficient and significance level, respectively. For statistical significance a correlation of 0.553 or larger is required at 5% level.

highest lactate concentration observed in the lambs (14.14 mM) occurred at the completion of exercise in the lightest lamb (3.2 kg) which also covered the 2.0 km distance at the lowest observed speed of 0.93 m/s. Concentration of lactate in blood decreased by 36% to 4.84 ± 0.68 mM at 30 min, and by 54 % to 3.44 ± 0.46 mM at 60 min of post-exercise recovery, but these levels were still significantly highly than the pre-exercise value ($p < 0.001$). Figure 6.11 depicts the evolution of blood lactate concentration in the lambs during exercise and recovery. Peak lactate levels were seen in 8 lambs at the completion of the exercise, while 1 lamb had its peak value at the end of the first exercise bout. Peak lactate levels achieved in 3 others at the end of the first bout did not differ from that achieved at the end of the exercise protocol.

Pyruvate

The pyruvate concentration in the blood ranged from 0.049 to 0.312 mM, with a mean of 0.214 ± 0.030 mM. There was a tendency towards an increase in the mean pyruvate concentration at the end of the first kilometer of exercise, however the value of 0.307 ± 0.47 mM (range = 0.122 to 0.642 mM) obtained was not significantly different from that of the pre-exercise level. The level of the metabolite at the completion of work, 0.354 ± 0.050 mM (range = 0.127 to 0.631 mM), showed a slight increase over the resting value ($p < 0.05$). The concentration thereafter reduced to resting values by 15 min of recovery, with that at 30 min of recovery being slightly lower. The difference in lactate levels between the 2 exercise periods were not statistically significant.

Lactate:pyruvate ratio

The lactate:pyruvate ratio increased 2-fold from a low pre-exercise value of 10.3 to 20.5 at the end of the first kilometer of following distance. The ratio increase further to 21.3 by the end of the second exercise period. The lactate:pyruvate ratio at both 15 (9.5) and 30 min (17.0) post-exercise were significantly higher than that obtained prior to the exercise.

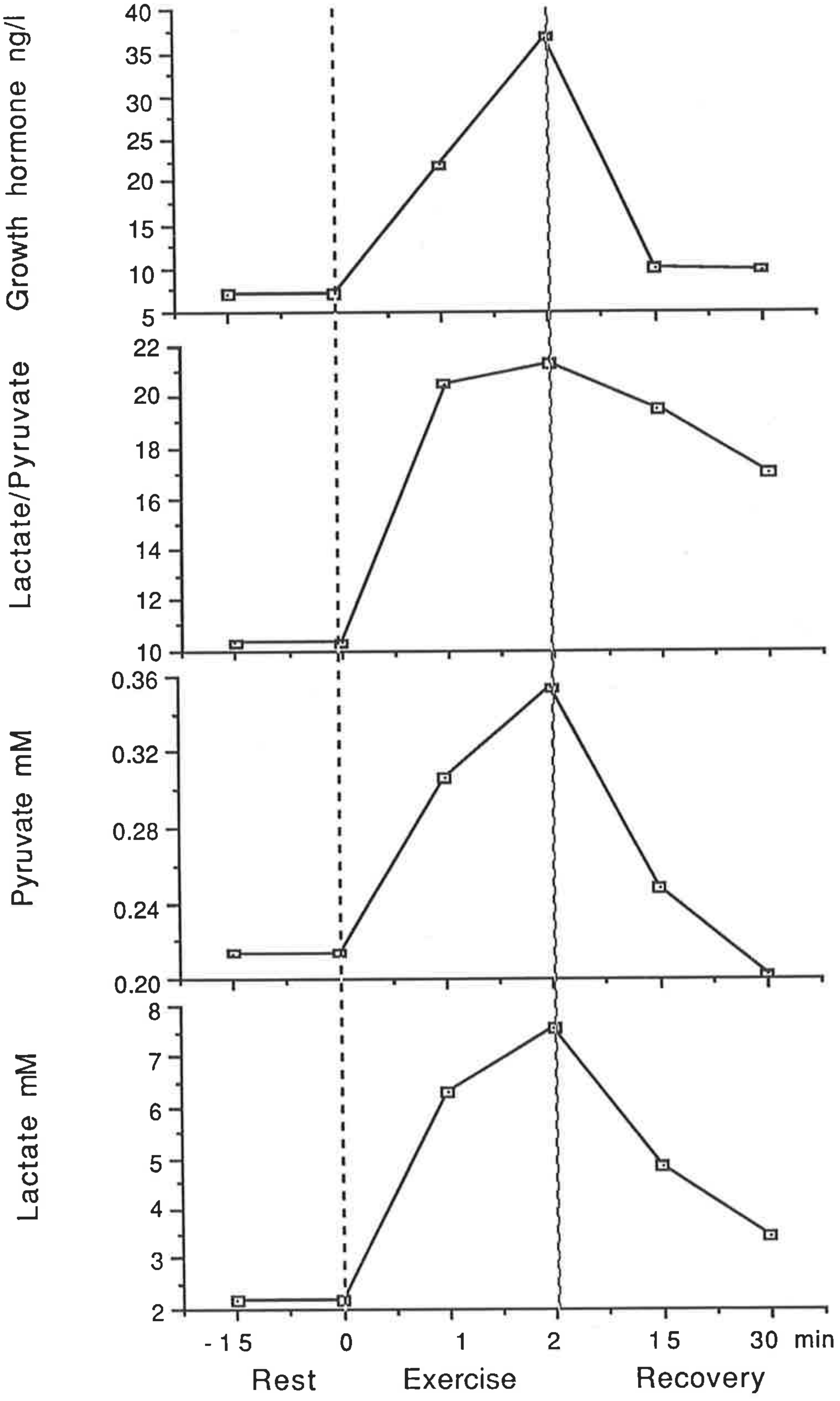

Figure 6.11 Evolution of lactate, pyruvate,lactate/pyruvate ratio and growth hormone during 2 x 1.0 kilometer interval laneway exercise.

Table 6.7 Metabolic effects of two x 1.0 kilometer interval laneway exercise and thirty minutes recovery.

Sampling times	Lactate mM	Pyruvate mM	Glucose mM	Protein mM	GH
Pre-exercise rest	2.20	0.214	5.73	81.3	7.1
(km)	±0.14	±0.030	±0.30	±5.5	±1.1
Exercise bout					
1.0	6.30***	0.307	6.54	94.3	22.0***
	±0.75	±0.047	±0.35	±5.0	±2.4
2.0	7.55***	0.354*	5.85	86.1	36.8***
	±0.91	±0.050	±0.39	±3.1	±5.5
Recovery (min)					
30	4.84	0.248	5.80	81.7	10.0
	±0.68	±0.036	±0.37	±3.4	±1.1
60	3.44***	0.202	6.39	82.3	9.7
	±0.46	±0.023	±0.33	±4.6	±2.0

Glucose and total protein

Concentration of blood glucose and plasma total protein during exercise and recovery did not change significantly from the pre-exercise value of 5.73 ± 0.30 mM (range = 4.15 to 7.64 mM) and 81.3 ± 5.5 μg/l (range = 42.2 to 118 3 μg/l), respectively. There was however a tendency for the concentrations of these 2 metabolites to rise above resting values by the end of the first kilometer distance, thereafter falling progressively to 30 min after exercise.

Growth hormone

Growth hormone concentration ranged from 1.9 to 16.0 ng/ml (7.1 to 1.1 ng/l) prior to the following in lambs. The level increase progressively to 22.0 ± 2.4 ng/ml, with a range of 12.3 to 44.0 ng/ml, at the end of 1.0 km of exercise ($p < 0.001$), and to 36.8 ± 5.5 ng/ml, with a range of 14.1 to 95.0 ng/ml, at the completion of the exercise ($p < 0$ 001). Growth hormone concentration had returned to pre-exercise values by 15 min of the recovery period.

Relationship between the changes in blood-borne metabolite levels

The correlation matrices in the changes in blood borne metabolite levels during the 2.0 km following distance are shown by Table 6 9 Changes in blood lactate concentrations during the exercise and recovery periods exhibited significant correlation with that of pyruvate ($r = 0.952$, $p < 0.013$) and growth hormone ($r = 0.913$, $p < 0.030$). Changes in the levels of blood pyruvate during the test also correlated significantly with that of growth hormone. Changes in glucose and total protein concentrations did not correlate with that of any other metabolites.

(3) Comparative study of "interval" and "continuous" laneway exercise

The effects of 2 x 0.5 km "interval "exercise in Study 3 and the first bout over 1.0 km in Study 4, referred to as "continuous" exercise, on blood metabolite levels are compared .

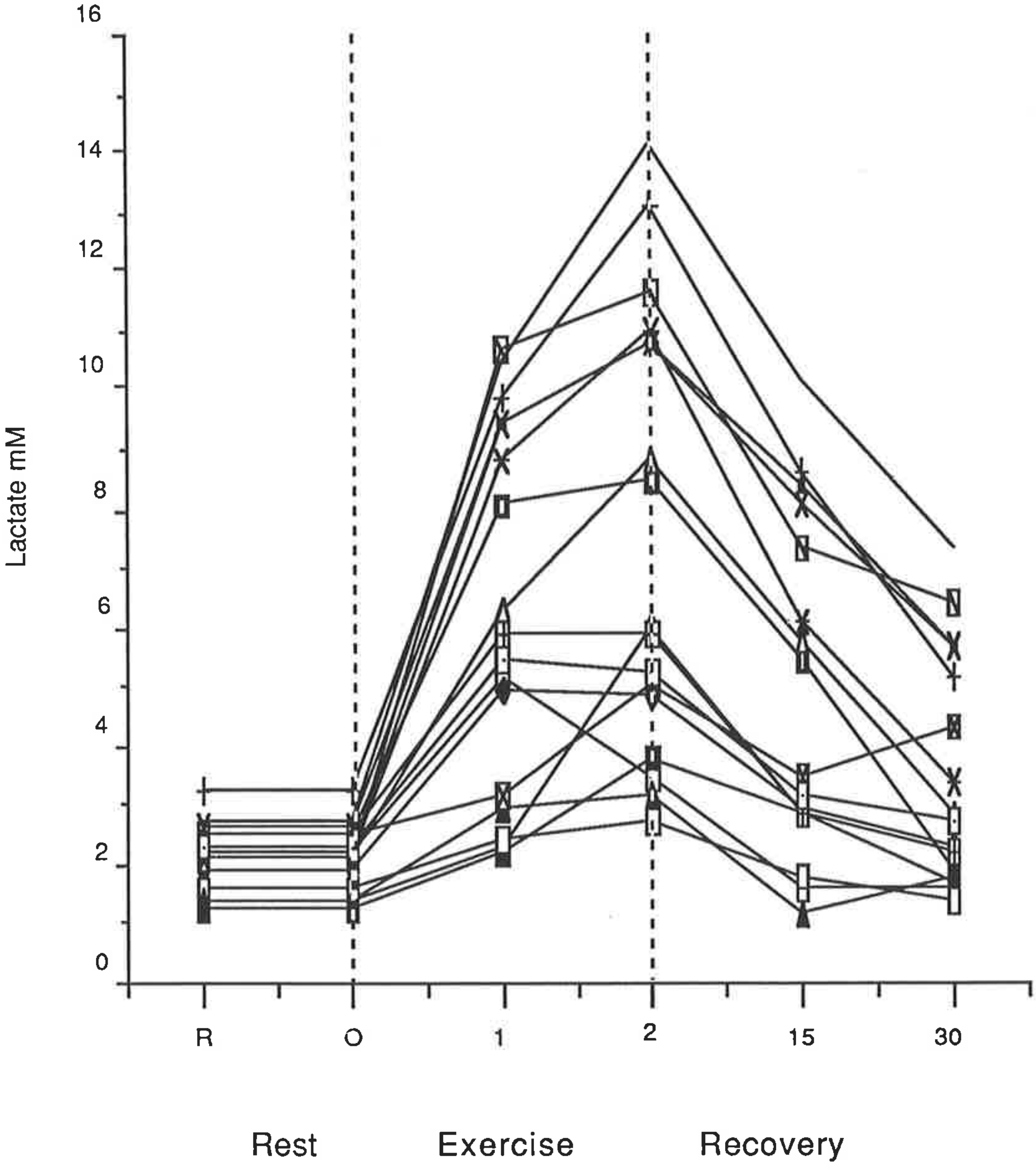

Figure 6.12 Blood lactate effects of 2 X 1.0 km laneway exercise

Although lambs used in the "interval" exercise tended to be slightly heavier than those in the 'continuous' exercise (5.3 ± 0.2 kg vs.4.8 ± 0.2 kg), the difference was however not significant. Although the "continuous" exercise was undertaken in a slightly higher range of ambient temperature than the "interval" exercises (15 to 33°C vs 12 to 26°C), the mean ambient temperature did not differ significantly (19.8 ± 1.1°C vs 17.01 ±1.2°C). Lambs involved in the "interval" exercise completed the 1.0 km distance in a shorter time than those in the "continuous" exercise.

With the exception of plasma growth hormone, the pre-exercise levels of blood-born metabolites in the "interval" and "continuous" exercises did not differ. The levels of lactate, pyruvate, and growth hormone at the end of both types of exercise were significantly elevated over resting values. Whereas the elevation in growth hormone levels at the end of the 2 types of exercises were similar, "continuous" exercise elicited significantly higher increases in blood lactate and pyruvate concentrations than interval exercise ($p < 0.01$).

6.4 DISCUSSION

Other than a previous publication emanating from the present study (Oppong-Anane et al., 1990) there are no published data on the changes in the concentration of blood-borne metabolites during exercise in the neonatal lamb. Nevertheless, studies undertaken to evaluate substrate utilization in mature farm animals indicate that considerable changes occur in the concentration of these metabolites during work (Faraci, 1984; Kuhlmann et al., 1985; Pearson and Archibald, 1989) and that levels of circulating blood metabolites during exercise depend on the balance between the rate of production and uptake of substrates, particularly by the active muscle which is in turn influenced by duration and intensity of the exercise. There are, however, difficulties in comparing the changes in blood metabolite levels during muscular activity in the present study with that reported for mature animals and human subjects, since energy metabolism in the exercising neonate may differ from that of the adult. Furthermore, most of the available data are from individuals that are trained to use exercising

Table 6.8 Relationship among blood-borne metabolites determined at rest and different time points during 2 x 1 km exercise and recovery periods.

Parameter	Lactate	Pyruvate	Glucose	Protein	GH
Lactate	1.00 (0.000)	0.95 (0.013)	0.17 (0.787)	0.64 (0.243)	0.91 (0.030)
Pyruvate		1.00 (0.000)	0.02 (0.975)	0.64 (0.242)	0.96 (0.011)
Glucose			1.00 (0.000)	0.66 (0.223)	0.05 (0.937)
Protein				1.00 (0.000)	0.54 (0.344)
Growth hormone					1.00 (0.000)

First and second lines depict correlation coeficient and significance level, respectively. For statistical significance a correlation of 0.553 or larger is required at 5% level.

equipment or are at least used to some form of exercise to a greater degree than can be achieved within 2 days of the lamb's life, and are therefore likely to have developed varying degrees of physiological adaptation to exercise. Other factors such as differences in sampling techniques, site of sampling (vein or artery) and moment of sampling in relation to time of activity may influence the values obtained for metabolite concentrations (de Coster et al, 1969). Even where the above factors are common, difference in analytical techniques used for determining the concentration of a blood-borne metabolite may be reflected in different values.

6.4.1. Lactate

The mean resting blood lactate concentration in the treadmill exercises, 1.37 ± 0.17 mM and 1.9 ± 0.11 mM for Studies 1 and 2 respectively, as well as the laneway exercises, 1.37 ± 0.70 mM for Study 3 and 2.20 ± 0.14 mM for Study 4, were considerably lower than the value of 3.8 ± 0.6 mM reported for dogs, but similar to that of pigs (Hastings et al., 1982). The resting values in the present study are similar or slightly higher than the range of 0.75 to 1.59 mM normally observed in human subjects at rest (Green et al, 1963; Keppler et al., 1969; Hermassen and Vaage, 1977; Lai and Lien, 1983). The significant elevation of blood lactate concentration over the pre-exercise values seen in the exercise bouts is consistent with that observed during moderate to heavy workloads (ie., 50 to 85% of the aerobic capacity). The increases in lactate concentration over pre-exercise values of 0.84 mM, 1.63 mM, 2.05 mM and 5.35 mM at the conclusion of exercise in Studies 1, 2, 3 and 4 respectively, were higher than that of Hereford calves subjected to treadmill exercise at speed of 1.0 m/s in the study of Kuhlmann et al. (1985) but lower than when the calves ran at speed of 1.4 to 2.2 m/s. The higher elevation of lactate observed during the laneway exercises compared to that of the treadmill exercises may be influenced by a number of factors including the higher speed of following and the relatively lower ambient temperature coupled with moderately windy conditions some of these lambs had to endure in the field.

The slight increase in blood lactate concentration of less than 0.30 mM observed in a few of the lambs over resting values at the end of the first 5 min of exercise in Studies 1 and 3 may be attributed to either individual variations in lactate response to exercise or relatively lower metabolic rate elicited by exercise in some of these lambs seen in a separate study (see Chapter 7.3). The rather low increase in lactate in these lambs during exercise was not unusual since, at muscular activity of moderate intensity of about 40 to 60% aerobic capacity, the lactate produced by the stimulated muscles, resulting from the contribution of anaerobic processes to the energy output and thus the lactate diffusing into the blood, usually correlates with the work intensity (Karlsson, 1971; Senay et al., 1980). Some of the lambs also had relatively higher metabolic rates and consequently greater lactate concentrations in blood. These lambs could have exceeded the average work load of 60% of their individual aerobic capacities which is considered the point at which lactate levels begins to increase rapidly (Karlsson 1971; Senay et al., 1980). Several studies have shown that blood lactate concentration remains almost unchanged during submaximal exercise at similar work loads as in the present studies. In view of the single-sampling of blood in each work bout, the level of lactate achieved at the end of the exercise bouts may not necessarily be an indication of the work load, but rather a reflection of many factors including the rate of lactate production in the muscles, muscle fibre type, the rate of release from these muscles, the rate of removal by the liver, the heart, the non active muscles and perhaps part of the working muscles and the volume of distribution. Other factors being equal, the lactate concentration is mainly an indication of the rate of production, but this is not always so (Cumming et al., 1980). The blood lactate levels observed in the present investigations may not necessarily represent peak values reached during the exercise period since the blood lactate may fall as exercise proceeds during moderate work intensities (Lai and Lien, 1983; Karlsson, 1971). Nevertheless, a lactate level may equal the peak value if a plateau was maintained to the end of an exercise bout as a result of equal rates of production and dispersal elsewhere in the body (Karlsson, 1968).

Lactate is a small and easily diffusible molecule, and the transportation of lactate from the muscle cells where it is produced to the blood and other tissues for removal is a fairly rapid process (see Gollnick and Hermassen, 1973). An immediate decrease may therefore occur in blood lactate concentration after cessation of submaximal exercise. The extension of blood lactate and pyruvate increases into the recovery period in some of the lambs with peak values at 5 min after exercise, may be attributed to the significantly higher metabolic rates exhibited during exercise in these lambs. This could result in high post-exercise metabolism, which in the case of the laneway exercises, may be influenced by the increased speed of following some of the lambs had to endure in their attempts to catch up with their mothers as they approached the holding pen. The increase in lactate levels in these lambs during the immediate post-exercise recovery is in agreement with the assertion of de Coster et al. (1969) that, when exercise is of high intensity and exhausting, blood lactate concentration still increases 4 or 5 min after the muscular work is completed. Increase in blood lactate during the initial part of the recovery period may also occur when the exercise is not sustained long enough to reach equilibrium for lactate flux between the muscle and blood (Kirbs, 1971).

The decrease of 29 to 36% in lactate concentration below exercise values observed at 15 min of recovery in the present studies suggests a good balance between the production and removal of lactic acid from the blood stream (Belcastro and Bonen, 1975). When work is not exhaustive a clear decrease in the lactate level could be seen within 0.5 min after exercise, and the time required for normalization depends on the level reached at the end of exercise. When lactates are very high it can take as much as 40 to 50 min before normalization is achieved (de Coster et al., 1975). Normalization occurred within 30 min after exercise in the present studies, with the exception of the laneway exercise of 2 km distance, confirming the submaximal nature of the work. Removal rates of lactate from the blood during recovery has been shown to be enhanced during periods of light intensity work of about 30 to 40% of the aerobic capacity of the individual rather than during a comparable rest period, since this range of work intensity provides the conditions where a minimal amount of lactic acid is produced and skeletal muscle blood flow is increased to transport lactic acid to removal sites

(Belcastro and Bonen, 1975). The similar rate of lactic acid disappearance in the lambs, particularly during the first 15 min after exercise, suggests that the recovery procedures used, the lamb held on the lap or with its mother in a small pen, did not affect the removal rates. This may be due to the fact that other than the initial burst of sucking when lambs were with their mothers, not much muscular activity occurred and that the lambs spent most of the recovery period lying down.

During the intermittent (Study 1) and the 2 laneway exercise protocols, blood lactate level continued to increase throughout the work bouts suggesting that the respiratory and cardiovascular adaptations were inadequate, presumably caused by the lowering of the oxygen demand and blood flow during the rest pauses. This prevented a balance being achieved between the removal and production of lactate. On the other hand, lactate concentrations were similar in the 2 bouts of exercise in Study 2 indicating probable attainment of complete cardiorespiratory mechanism during the exercise. This explanation may however be treated with caution since blood sampling was done only once in each exercise bout. The effect of "intermittent" and "continuous" exercise on lactate production is not very clear. The lower elevation in blood lactate levels in the "intermittent" compared to "continuous" exercises particularly in the laneway studies where the exercise intensities in some of the lambs were likely to have reached the anaerobic threshold is consistent with the observation of Essen et al. (1977) that increase in blood lactate may be slightly lower in intermittent exercise than continuous exercise when subjects perform at almost identical power output and oxygen consumption.

6.4.2. Pyruvate

The evolution of pyruvate during exercise and recovery was quite similar to that of lactate in both treadmill and laneway exercises. This trend was consistent with the observation of de Coster et al. (1969) and Keppler et al. (1969) in humans. Blood pyruvate changes during exercise are however less marked than those in lactate. The pyruvate concentration usually shows a transient increase during the first few minutes after discontinuation of exercise, then follows by a more sustained fall. The level is sometimes only moderate in comparison with lactate level, sometimes it shows 2 peak values while lactate shows only 1 peak. In other cases, pyruvate and lactate levels are similar; pyruvate may increase with the intensity of exercise while the lactate decreases during exercise. Blood lactate concentrations are always definitely higher (de Coster et al, 1968).

Blood pyruvate levels increased by 16% over the resting value by the end of the intermittent exercise in Study 1 while a significantly greater increase, ranging from 65 to 95%, was observed by the end of the other types of exercises. A decrease of 20 to 30% in blood pyruvate concentration below immediate post-exercise values was seen in Studies 1, 2 and 4 at 15 min of recovery. There was however no decrease in the case of the 2 x 0.5 km laneway exercise. The disappearance rate of pyruvate was however lower than lactate in all the studies.

6.4.3. Lactate:Pyruvate ratio

The relative degree of glycolytic metabolism reflected in the lactate:pyruvate peak ratio during exercise in the present studies, ranging from 17.2 to 21.3, is significantly lower than the peak values of 40 to 50 reported by Kuel et al. (1968) and Keppler et al. (1969) for normal human subjects and those suffering from respiratory diseases. The present ratio was however higher than the peak value of 12 for normal human subjects in the study of Harris et al.(1968).

The very significant increase in lactate:pyruvate ratio at the end of the first bout of exercise in the present work is consistant to that obtained in other studies. Results in Study 1 and that of Harris et al. (1968) indicated that peak lactate:pyruvate ratio may be reached by the 5th min of exercise. After the initial significant increase of lacate/pyruvate ratio observed during exercise, the ratio could increase continuously with time, reach a plateau, or decrease after the initial increase. The reason for the changes in lactate:pyruvate ratios during exercise are not clear, neither could any correlation be made with respiratory or a number of blood metabolites recorded and measured simultaneously in human subjects (de Coster et al., 1969). These different evolutions of lactate:pyruvate ratio in the present studies may be attributed to the non parallel, albeit quite similar, changes with time observed in lactate and pyruvate levels.

6.4.4. Glucose

Despite the non significant effect of exercise on blood glucose concentrations seen in the studies, there was a tendency for the levels of the metabolite to fall during exercise in Studies 2 and 3, whereas the other 2 types of exercise showed slight increases in glucose concentrations over the pre-exercise values during the first work bout. Since the levels of circulating glucose, like that of other metabolites, presumably depends on the balance of the movements of substrate into and out of the blood stream (Harris et al., 1968; Pearson and Archibald, 1989), the tendency for a decrease in glucose concentration during exercise observed in 2 of the present studies, though not significant, is in agreement with the trend observed in a number of investigations. It may be attributed to the temporal failure of the hepatic glucose output to keep up with the augmented utilization by the working muscles (Harris et al., 1968; Ahlborg et al., 1974).

The observed tendency for the glucose levels to initially decrease, at least during the first 10 min, followed by a progressive rise during exercise (Harris et al., 1968; Keppler et al., 1969; Ahlborg et al., 1974; Pearson and Archibald, 1989) may be due to the time lag between the increased demand for glucose in muscle and glucose production, particularly, from the hepatic glycogen. The tendency for blood glucose to increase by 15 min after the treadmill exercises is consistent with the observation in rats by Brooks et al. (1973). The rapid recovery of blood glucose in the rats, as well as in the present studies, occurred simultaneously with the removal of lactate from the blood stream. The well known clearance in blood glucose, is however, only seen during exercise of longer duration where blood glucose assumes an increasingly important role as a substrate for muscle oxidation (Keppler et al., 1969; Wahren et al., 1973).

6.4.5. Total protein

The plasma protein concentrations were not affected by the exercise in any of the studies, indicating that the duration of the work performed was probably neither long enough nor the intensity high enough to demand the use of protein sources of energy. Nevertheless, muscle protein could have been utilized without affecting the plasma protein levels. A positive and significant relationship has been observed between work intensity, water movement from the vascular volume and total protein in treadmill exercise (Senay et al., 1989). The lack of any changes in plasma total protein concentration during exercise from the resting values in the present studies therefore suggests that fluid shifts from the vascular compartment were unlikely to have occurred and would not have been responsible for the significant changes seen in some of the other blood-borne metabolites.

6.4.6. Free fatty acids

Changes in the FFA concentration during exercise are thought to indicate only an imbalance between the rate of release of FFA from the adipose tissue and the rate of removal by the working muscle. However FFA uptake is often related to the concentration in plasma (Hangenfeldt and Wahren, 1971).

Blood FFA concentration in lambs is generally high during the first day of life, having been elevated from considerably low values at birth (Van Dyune et al., 1960; Alexander and Mills, 1968; Alexander et al., 1972). The low plasma FFA concentrations seen in the lambs in the present studies may be due to the fact that the level of FFA in lambs decreases markedly during the second day of life, before gradually increasing with age to reach maximum values at about 45 days post-partum (Leat, 1967). Although the increases in FFA concentrations during exercise in Studies 2 and 4 were not significant, the progressive and significant increase seen in the other 2 experiments is consistent with the general observation of a marked rise during moderate intermittent exercise (Kuel et al, 1974), and suggests an increase in FFA utilization by the lambs. During exercise of moderate intensity, there is usually an initial fall in FFA level at the onset of work, after which concentration tends to increase progressively (Essen et al., 1977; Pearson and Archibald, 1989). The increase in concentration may be extended into the recovery period, as illustrated in a study of Pearson and Archibald (1989) involving draft oxen where the most rapid increase was seen 5 min after work stopped. Thereafter, FFA concentration decreased, reaching resting values 45 min after discontinuation of work. The lowering of blood FFA during exercise is valid only for continuously performed work and probably also within a given limits of exertion intensity.

The relationship between FFA, lactate and pyruvate production in the present studies is consistent with the observation that FFA levels in blood tends to correlate positively with lactate and pyruvate during exercise of moderate intensities up to 70 to 80% of the aerobic capacity . In human subjects moderate exercise activates agents which increase plasma FFA, thus increasing substrate availability as exercise is prolonged. As exercise becomes more severe in relation to the subject's aerobic capacity, thus demanding derivation of a larger proportion of the energy anaerobically, the mobilization of FFA is depressed for as long as the blood lactate remains high. This does not necessarily mean that FFA turnover and oxidation are completely suppressed (Pruett, 1970)

6.4.7 Growth hormone

The mean circulating levels of plasma growth hormone prior to exercise, 7.1 ± 1.1 ng/ml (range = 1.9 to 16.0 ng/ml) in Study 3, and 8.1 ± 0.8 (range = 2.8 to 13.1 ng/ml) in Study 4 lambs in the present investigations fell within the range of the mean concentration in 2 to 10, and 5 to 9 day-old lambs (Basset and Alexander, 1971; Cronin et al., 1981). Levels as high as 80 to 120 ng/ml were however observed in some 12 hour old Merino and crossbred lambs studied by Cronin et al. (1981). Circulatory growth hormone levels of 2 to 8 ng/ml during fasting, increasing to 15 ng/ml after 20 h of refeeding, have been reported for Southdown hoggets (Van Maanem et al., 1987).

A wide variation of physiological and pharmacological stimuli are known to elicitate increased levels of circulation growth hormone in animals (see Metivier 1973; Terjung 1979; Meyer and Knobel, 1967). On the other hand, rodents subjected to stress showed decreased levels of growth hormone concentrations (Muller et al., 1967). The secretion of growth hormone in the sheep does not appear to be significantly influenced by behavioural stress (Cronin et al., 1981), nor cold exposure. Nevertheless, concentration of growth hormone remained low, declining slightly during severe cold exposure, and then increased during recovery (Basset and Alexander, 1971). Exercise however, did elicited significant increases in circulating growth hormone levels in lambs in the present studies ($p < 0\ 001$), with 10% of the lambs exhibiting peak levels of 106 to 114 ng/ml.

Notwithstanding the significantly high increase in circulating growth hormone levels achieved during the laneway exercises, the metabolic effects of both treadmill and laneway work, especially the small but significant changes in blood lactate and pyruvate concentrations confirm that the work imposed on the lambs during the exercise protocols was at a submaximal level. This suggests that most running activities in neonatal lambs are undertaken by utilizing primarily aerobic pathways, with only minor contribution from anaerobic pathways of metabolism.

7.METABOLIC AND PHYSIOLOGICAL RESPONSES TO LOCOMOTION IN LAMBS.

7.1 INTRODUCTION

In quadruped animals running at a natural gait on a treadmill, metabolic rate measured as steady state oxygen consumption (VO_2) increases linearly with speed of locomotion until maximal oxygen consumption (VO_2max) is reached (Mitchell and Blomqvist, 1971; Shepherd and Gollnick, 1976; Bedford et al., 1979; Taylor et al., 1982; Baudinette, 1989). A similar relationship has been observed in some bipeds such as the ostrich over a wide range of speed (Fedak and Seeherman, 1979) and in the tammar wallaby, a hopping marsupial, at low speeds (Baudinette, 1978). Further increase in work intensity leads to an increased rate of metabolic energy utilization by the muscles without an increase in VO_2. Additional energy used is provided by anaerobic glycolysis which results in the production of excessive amounts of lactate in the active muscles and can be seen in the venous blood draining the muscles (Karlsson, 1971, Seeherman et al., 1981).

The energy cost of moving a unit distance for an animal declines to a minimum value with increasing speed (Taylor et al., 1970; 1977). This asymptotic minimum value is identical to the slope of the curve describing the relationship between energy consumption and speed, and is constant and a characteristic of each animal (Taylor et al., 1970; Taylor, 1977). It has been shown that energy cost of locomotion varies as function of body mass; this allows the energy consumption of a running mammal to be predicted if the speed of locomotion and body mass are known (Taylor and Raab, 1970; Taylor et al.,1970; 1982). Despite the significant data base on the energy demands of locomotion there are no published results on neonatal mammals. It follows that using the allometric equations derived for adult mammals to predict the metabolic cost of locomotion in lambs is unwise, and measurements were conducted as part of the present study.

Increases in the speed of locomotion are met by increases in stride frequency and length Taylor, 1977; Heglund et al., 1974; Baudinette, 1987). Stride frequency has been shown to initially increase linearly with speed of locomotion but then plateau (Heglund et al., 1974). Despite this independence of stride frequency from the speed of locomotion seen in some species, stride and respiratory frequencies tend to be phase locked at a 1:1 ratio in many animals (Bramble and Carrier, 1983; Baudinette et al., 1987). Again, all these studies have been conducted on adult animals.

A linear increase in heart frequency as a function of speed of locomotion has been observed in man and various species of animals (Donald and Ferguson, 1966; Wagner et al., 1977; Thomas and Fregin, 1981). Whereas heart frequency may be used as an indicator of exercise intensity, its effectiveness could be affected by emotional factors, particularly in untrained animals (Donald and Ferguson, 1966; Hays, 1976; Baudinette et al., 1978).

Maximal oxygen consumption, the greatest amount of oxygen an animal can take in during physical work, is considered to be the most useful single measure of aerobic capacity and cardiovascular function in the individual (see Mitchell and Blomqvist, 1971). The highly reproducible nature of VO_2max seen under similar experimental conditions makes it an important characteristic of each individual. However, there is scarcely any information on the aerobic capacity of domestic ruminants, and in particular the neonate. The most commonly used technique for eliciting maximal metabolic rates in young lambs involves the use of cold air with or without wind (Alexander, 1962; 1979). In recent years, water progressively cooled from thermoneutrality to about 18^oC has increasingly been used to induce summit metabolism in lambs without any detrimental effects (Slee et al., 1980; Earles and Small, 1981; Robinson and Young, 1988; Young et al., 1988). Peak VO_2 induced by cold exposure techniques often differs from that resulting from running since the voluntary activity of skeletal muscles in the former is insignificant, and may therefore not be a true representative of the aerobic capacity of the individual. For VO_2 to be acceptable as representing maximal aerobic capacity, the work done must involve large muscle groups and lasts for more than 2 min (Mitchell and Blomqvist, 1971). In the present study, VO_2max was derived from treadmill exercise.

The neonatal lamb normally follows the ewe at a slow walking pace interspersed with rest pauses. It sometimes undergoes short spells of exhaustive running in response to the mother's call during separation. The neonate's ability to run at near maximal speeds may be advantageous in escaping from mammalian predators, such as foxes and dingoes, known to be selective in their predation and which normally attack only weak lambs that are unable to escape with their mothers. Such lambs may have low aerobic capacity and are therefore not capable of sustaining high running speeds for the period required to escape.

There is no information available on cardiorespiratory and stride responses to exercise, nor VO_2max obtained in response to maximal exercise in very young animals. The observations in adult animals may not be directly applicable to the young in view of probable limitations in the efficiency of their cardiorespiratory and muscle systems. Nevertheless, the precocity of the lamb provides it with considerable locomotory capabilities to enable following the dams within the first day of birth. The first part of the present study investigates in 2 to 3-day old lambs the relationship between energy consumption, body temperature, heart, respiratory and stride frequencies, and the speed of locomotion. Possible entrainment of cardiorespiratory frequencies with stride frequency is also assessed. The second part looks at the responses of VO_2 and other physiological effects of treadmill and laneway interval exercises. The final part of the study determines the levels of VO_2max elicited by exercise in lambs and the corresponding blood and physiological parameters .

7.2 MATERIALS AND METHODS

7.2.1 ANIMALS AND EXPERIMENTAL PROCEDURES

7.2.1.1 METABOLIC AND PHYSIOLOGICAL RESPONSES TO CHANGES IN SPEED OF LOCOMOTION (STUDY 1)

(1) Animals

Twenty six 2 to 3-day old single-born lambs weighing 5.11 ± 0.10 kg (range = 3.59 to 7.30 kg) at the time of testing were used in the investigations. The lambs were born in June, 1988 in a 2.5 ha paddock at the Waite Agricultural Research Institute, and held with their mothers outdoors prior to and after each test. Water was provided *ad libitum* and lambs had access to their mother throughout the neonatal period with the exception of the time of exercise protocol.

(1) Experimental procedures

(A) Animal preparation

On the second day after birth each lamb to be tested and its mother were removed from the paddock and confined to a small cage in a temperature controlled room for a minimum of 30 min. The lamb was weighed to the nearest 100 g, fitted with a lightweight plastic mask attached behind the ears by means of a velcro clasp and then held gently on the lap of an assistant for approximately 15 min during which resting state oxygen consumption (VO_2) was measured. During this time pre-exercise physiological values of rectal temperature, respiratory and heart frequencies were measured in 11 of the lambs. Tests were performed at approximately 25 ^{o}C ambient, relative humidity of 40 to 60% and a normal room oxygen percentage between 20.89 to 20.93 vol per cent.

(B) Exercise protocol

The lamb was placed in a treadmill for 2 min while the mother was held in a cage facing the lamb. It was then run at a slow speed of about 0.2 m/s for 30 s, the speed was gradually raised to the required level and the lamb made to run at the selected speed for 5 min. Steady state stride frequency was counted twice over a period of 30 s in the fourth and fifth min, and oxygen consumption was measured continuously throughout the exercise. Where required, rectal temperature, respiratory and heart rates were measured within 1 min of cessation of exercise. A total of 16 lambs were tested once, 12 lambs were tested twice and 8 and 4 lambs tested 3 and 4 times respectively. Where a lamb was tested more than once, the next run was started only when all measured variables had returned to pre-exercise levels; in any case, not less than 2 h elapsed between each 2 tests. Work intensity of exercise, in terms of the percentage of maximal oxygen consumption (% VO_2max), was calculated from VO_2max determined in a separate study (see Section 7.2).

7.2.1.2 CARDIORESPIRATORY AND METABOLIC RESPONSES TO INTERVAL EXERCISE IN LAMBS (STUDY 2)

(A) TREADMILL EXERCISE

(1) Animals:

The study involved 14 two-day old single born lambs with body mass ranging from 4.8 to 6.7 kg (mean = 5.8 ± 0.1 kg). The lambs were born in August 1988 in a 2.5 ha paddock at the Waite Agricultural Research Institute. The neonates and their mothers were held outdoors prior to the test.

(2) Experimental procedure

After initial preparation (see section 7.2.1.1), the lambs were subjected to 3 five-min treadmill exercise bouts at 1.0 m/s, separated by 2 three-min rest pauses, followed by a 30 min recovery period. Steady state oxygen consumption and measurements of rectal temperature, respiratory and heart frequencies were taken simultaneously at rest, the end of the exercise bouts, and at 5, 15 and 30 min after exercise.

(B) LANEWAY EXERCISE

(1) Animals

A sample of 20 lambs was selected at random from the same parent flock and comprised 10 animals each born in June and August, 1989 at the Glenthorne Experimental Station of the Waite Agricultural Research Institute. Within each of the 2 groups the selection was random. The body mass of the lambs at commencement of the study was 5.3 ± 0.2 kg (range = 4.0 to 6.8 kg. The lambs were born and kept with their mother in a 1-ha paddock prior to the investigations. Water, mineral salt and hay were provided ad libitum. A grain mix supplement was also provided every other day.

(2) Experimental procedure

At approximately 47 h post partum, the lamb to be tested and its mother were removed from the flock and held in a small pen 50 m away from a long and narrow laneway (2 m x 0.5 km) (see Section 4.2.3) and left undisturbed for 30 min. The lamb was then held on the lap of an assistant for 10 min and pre-exercise rectal temperature, respiratory and heart frequency were measured. The ewe and her lamb were held in a cage adjacent to the laneway for 5 min after which the gate was opened and the ewe escaped into the laneway followed by the lamb. The animals walked 2 laps of 0.5 km each, separated by a rest pause of 3 min. An observer followed between 5 to 10 m behind. Physiological measurements were taken at the end of the exercise bouts and at 5, 15 and 30 min recovery while the lamb rested on the lap of an assistant. Additional physiological measurements for the August born lambs were taken at 10, 20 and 25 min after exercise.

7.2.1.3 MAXIMAL OXYGEN CONSUMPTION (STUDY 3)

(1) Animals

A total of 18 healthy 5-day old single-born lambs were used in the VO_{2max} investigation. The lambs were born and raised in a 2.5 ha paddock at the Waite Agricultural Research Institute in June, 1988. They were matched by sex and body mass into 2 groups of 9 for treadmill exercise tests at slopes of 7% (Group 1; body mass 6.07 ± 0.17 kg) and 16% (Group 2; body mass 6.02 ± 0.24 kg). Six lambs selected at random, 2 from Group 1 and the rest from Group 2, were used to evaluate the repeatability of VO_2max.

(2) Experimental procedure

(A) Animal preparation

Preparation of the lambs for the VO_2max tests was similar to that reported for lambs subjected to a range of locomotion speeds (see Section 7.2.1.1 for details). Physiological measurements were followed by blood sampling by jugular venipuncture. All tests were performed at a constant room temperature at 25 ^{o}C.

(B) Training protocol

Group 2 lambs were trained to use the treadmill prior to being tested, according to the protocol shown in Table 7.1. On the second day after birth, the treadmill speed was set at 0.2 m/s and each lamb in the Group encouraged to walk for 3 min prior to being exercised for 5 min at 0.5 m/s. The training was continued the next day by running the lamb twice for 5 min at 0.5 and at 1.0 m/s, separated by a rest pause of 3 min. The final training session

Table 7.1 Description of the treadmill training procedure.

Age of lamb (days)	Treadmill inclination (%)	Speed (m/s)	Duration (min)
2	0	0	3
2	0	0.2	3
2	0	0.5	5
3	0	0.5	5
3	0	1.0	5
4	7	0.5	5
4	7	1.0	5
4	7	1.5	5

Table 7.2 Description of the maximal oxygen consumption testing procedure.

Stage of test	Speed (m/s)	Duration (min)
1	0	0.5
2	0.5	1.0
3	1.0	1.0
4	1.5	1.0
5	maximal	4.5 - 8* 5 -15**

*Untrained lambs; **trained lambs.

was held on the fourth day. It involved 3 exercise bouts of 5 min each at 0.5, 1.0, and 1.5 m/s up a 7% grade, separated by 3 min rest pauses. During the rest periods the lamb continued to wear a face mask connected to the open flow calorimetry system. Three lambs that were not able to complete the training protocol were taken out of the study.

(c) Exercise protocol

Study 3a - Maximal oxygen consumption tests at seven per cent grade

Table 7.2 shows the exercise protocol used to determine VO_2max in the group that had no familiarisation with the motor-driven treadmill. After the completion of the pre-exercise measurements, the lamb was placed for 30 s on a stationary treadmill set at a slope of 7%. It was then subjected to a minute of exercise at 0.5 m/s. The speed was twice increased by 0.5 m/s for a duration of a minute each after which the speed, was gradually increased until the lamb reached its maximal running speed. At this stage the deflections on the chart recorder showing the level of VO_2 no longer increased with increasing speed indicating that VO_2max had been reached. The speed was held constant for the remainder of the test period which ended when the lamb could no longer maintain its normal position and tended to fall back. The maximum VO_2 reached prior to the onset of fatigue was taken as the VO_2max of the lamb. The safety of each lamb during the trial was assured by an assistant who sat at the rear of the treadmill and helped lambs which slipped to regain normal posture. Each lamb was promptly removed from the treadmill at the completion of the exercise regime or when it could no longer cope with the exercise.

Study 3b -Maximal oxygen consumption tests at 16 per cent grade

Lambs that had successfully completed the treadmill familiarisation protocol and were considered to be trained were subjected to a similar exercise protocol as in Study 3a with the treadmill inclined at a 16% grade.

Study 3c - Repeated test of maximal oxygen consumption

Steady state maximal oxygen consumption was determined twice at the same slope in 6 lambs selected at random. The tests were separated by a minimum interval of 4 h to ensure that blood lactate concentration and other physiological parameters had returned to resting levels before the second test.

7.2.2 MEASUREMENT TECHNOLOGY

(1) Rectal temperature

Rectal temperature, as an indicator of body temperature, was measured to 0.1°C using a thermocouple with an electronic digital display. The temperature probe was inserted 3.5 cm into the rectum and secured in place by attachment to the hair coat of the tail with a clip. The thermocouple was calibrated to 0.1°C against a mercury thermometer traceable to a national standard.

(2) Respiratory and heart frequencies

Heart frequency was measured by inserting 3 fine platignum electrodes under the skin of the lamb and held in place with adhesive tape. Heart frequency were determined at the end of the test since muscle potentials during running tended to swamp the cardiac potentials. Respiratory frequency was taken from a fine copper-constantan thermocouple taped across the right nostril of the lamb. Potential changes from the ECG leads and the thermocouple were displayed on a dual channel chart recorder (Duograph, Gilson Medical Electronics, see Figure 7.1). Sample time for both recordings were at least 30 s in duration.

During laneway tests, respiratory frequency was obtained by counting flank movements over a period of 1 min of uninterrupted breathing with a hand-held counter. However the frequency of rapid breathing, particularly after exercise, was obtained by

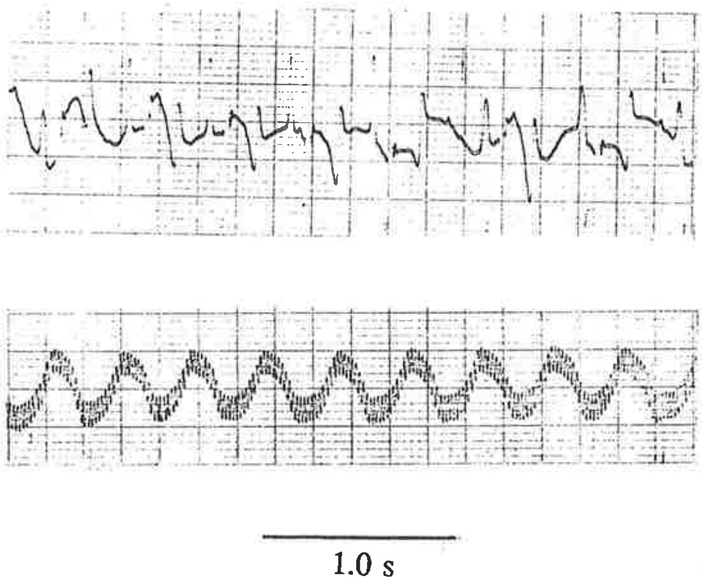

Figure 7.1 Electrocardiogram and respiratory patterns displayed on a dual channel chart recorder during exercise at 1.0 m/s. A positive upward gradient of respiratory pattern indicates inspiration and a negative pattern indicates inspiration. Speed of paper was 25 mm/s.

placing the receiver of an electronic stethoscope (Dif-stet^{TM}, Intech systems Corp) on the wall of the neck over the thorax.. The breaths were recorded with a micro-cassette recorder (National model No R N 2190, Matsusha Electric Industrial Co. Ltd, Japan, see Plate 6). Heart frequency was similarly obtained by the use of the stethoscope and recorder.

(3) Stride frequency

Stride frequency was manually counted twice over a period of 1 min with a hand held counter during steady state locomotion . In the case of treadmill interval exercise, stride frequency was counted during the second exercise bout.

(4) Oxygen consumption

Rates of oxygen uptake were measured using an open-flow system drawing respiratory gas from a loosely fitting latex mask worn by the lamb (see Plate 7). The mass of the mask and associated tubing was 0.18 kg. Flow in the system was monitored using a 300 ml/l rotameter (Brooks, Maryland). The difference in oxygen concentration of sub samples of the gas entering and leaving the mask was measured with an oxygen analyser (Applied Electrochemistry Model S 3A/I) and recorded on Rikadenki chart recorder (see Figure 7.2). The flow system and analyser were calibrated by bleeding a known amount of nitrogen gas into the mask through a calibrated rotameter and the deflections of the analyser determined (see Fedak et al, 1981). In determining Vo_2, steady state levels were assumed if less than 5% variation occurred in the recording over the duration of the tests. Oxygen uptake was calculated according to the equation 11c of Fedak et al (1981) and expressed as ml/s or ml/kg/s corrected to Standard Temperature and Pressure (273^oK, 1013mb). An energy equivalent of 20.1 kJ per litre of oxygen consumed was used for the derivation of metabolic heat production. The work load undertaken by the lambs was calculated as: work load in kg.m = mass of lamb in kg x treadmill speed in m/s x per cent grade.

Plate 6. An electronic stethescope (Dif-stet TM, Intech Syetems Corp.) connected to a micro-casset recorder (National Model No. RN 2190).

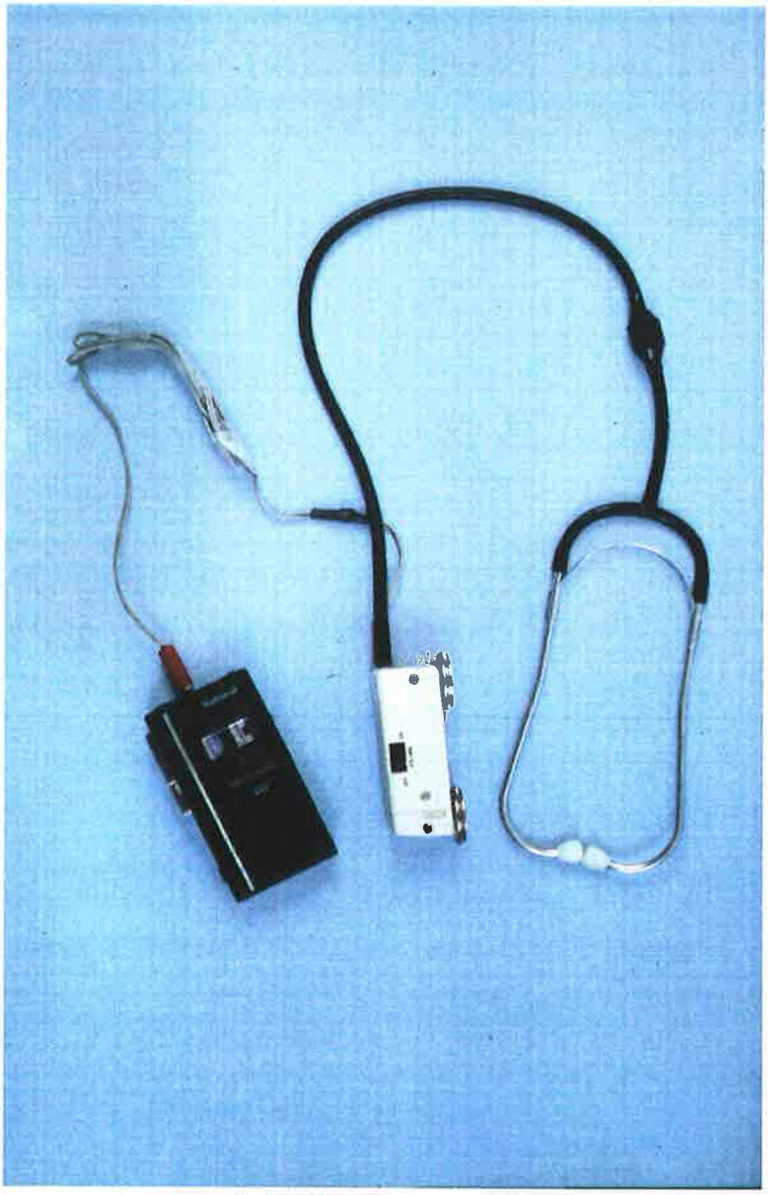

Plate 7. A lamb wearing loosely fitting latex mask connected to an open-flow system for the determination of oxygen consumption rates.

(5) Blood sampling

Blood samples were collected for lactate determination from the jugular vein of animals at rest and at the end of VO_2max determination. Details can be found in Section 6.2.3.

7.2.3 STATISTICAL ANALYSIS

Regression analysis was used to determine the relationship between the speed of locomotion and stride frequency, oxygen and energy consumption, energy cost of locomotion, stride length, rectal temperature, and heart and respiratory frequencies. Similarly the relationships between stride frequency and the physiological parameters studied were evaluated by regression analysis. Inter-correlation between the speed of locomotion and other parameters was determined by a partial correlation test, and Pearson moment-product correlation analysis was employed in the determination of the relationship in the changes between oxygen, physiological and stride parameters in 3 lambs at rest and at 3 running speeds.

Differences between pre-exercise and exercise values of blood lactate, VO_2, stride characteristics and physiological parameters in Studies 2 and 3, as well as the differences between similar parameters in the 2 groups in Study 3b, were evaluated by a Student t -test for paired data. Similar assessments between the end of interval exercise and recovery values were made. Least squares linear regression constants were computed to describe relationships between the speed of locomotion and stride frequency and the other parameters studied. The association between the VO_2max values achieved during the repeated tests, and the relationship between the changes in VO_2 and other physiological parameters in the interval exercises during exercise and recovery were assessed by Pearson moment - product correlation analysis. Values are presented as means with standard errors.

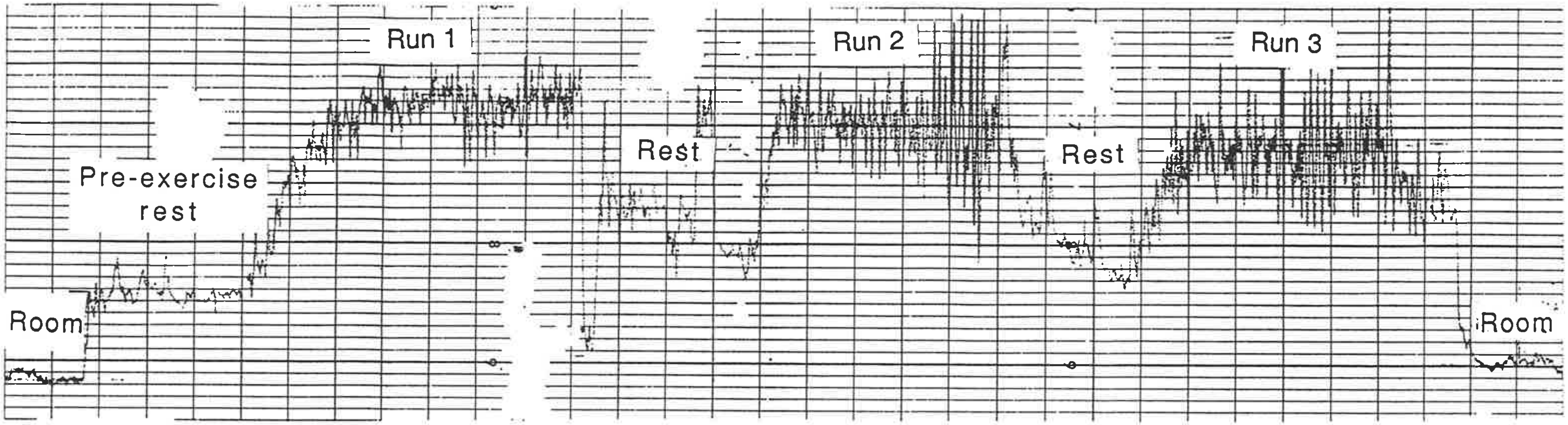

Figure 7.2 Chart recorder deflectons of the open-flow system representing oxygen concentration in the temperature controlled room and in gas leaving the mask at rest and during inttermittent exrcise.

7.3 RESULTS

7.3.1 METABOLIC AND PHYSIOLOGICAL RESPONSES TO CHANGES IN SPEED OF LOCOMOTION

All the lambs providing data for this study walked, trotted or galloped at a steady running speed for at least 3 out of the 5 min of exercise at the selected speed. It was however difficult maintaining consistent walking speed and stride frequency in 6 of the lambs tested at speeds below 0.5 m/s and they were withdrawn from the study. A lamb would occasionally run to the front of the treadmill as a response to the mother's bleats and, in the process of attempting to reach the mother, continuously hit its head against the wire mesh at the front of the treadmill. This tended to disrupt the steady state VO_2 and stride frequency attained. A few of the lambs also stopped running and were encouraged to move by gently pushing and slightly raising the caudal portion of the body. Those persistently interrupting steady state running were removed from the study.

7.3.1 1 Speed of locomotion and changes in gait

Table 7.3 depicts the frequency of treadmill speed used in the study. The speed of locomotion ranged from 0.15 to 2.34 m/s. A total of 45 tests were carried out at speeds below 1.0 m/s, 22 at 1.0 to 2.0 m/s and 3 above 2.0 m/s. The lambs changed gait from a walk to a trot to a gallop as the speed increased. They tended to walk at speeds below 0.50 m/s and changed gait to a trot above this speed. Galloping tended to commence at speeds approximating 2.0 m/s. Subjective assessment suggested that the lambs seemed to be more comfortable and readily achieved steady state running at speed ranging from 0.8 to 1.2 m/s.

7.3.1.2 Resting state oxygen consumption

Distribution of VO_2 and mass specific oxygen consumption rate (VO_2/Mb) are depicted in Figures 7.3a and b respectively. Resting state VO_2 was attained by most of the lambs within 5 to 10 min of being held on the lap of an assistant. Oxygen consumption levels ranged from 0.94 to 2.73 ml/s (mean = 1.52 ± 0.32 ml/s). The lower and upper quartiles

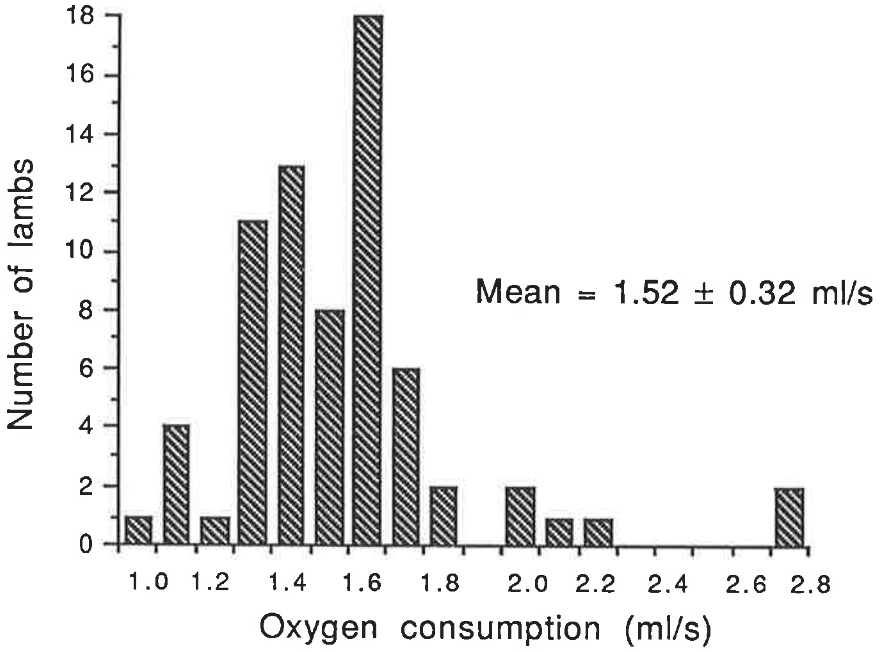

Figure 7.3a Distribution of steady state oxygen consumption at rest in lambs.

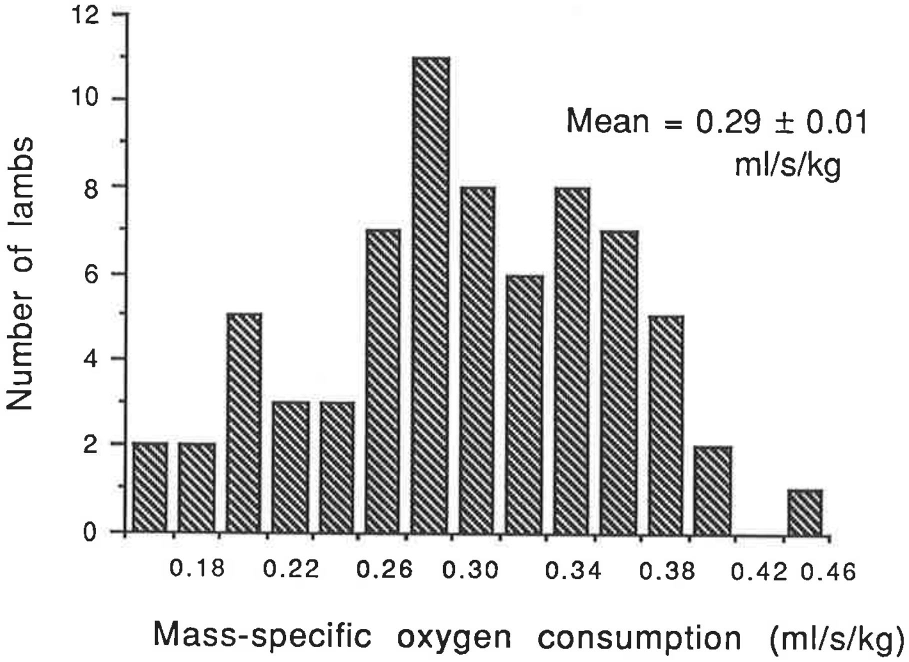

Figure 7.3b Distribution of mass-specific steady state oxygen consumption at rest in lambs.

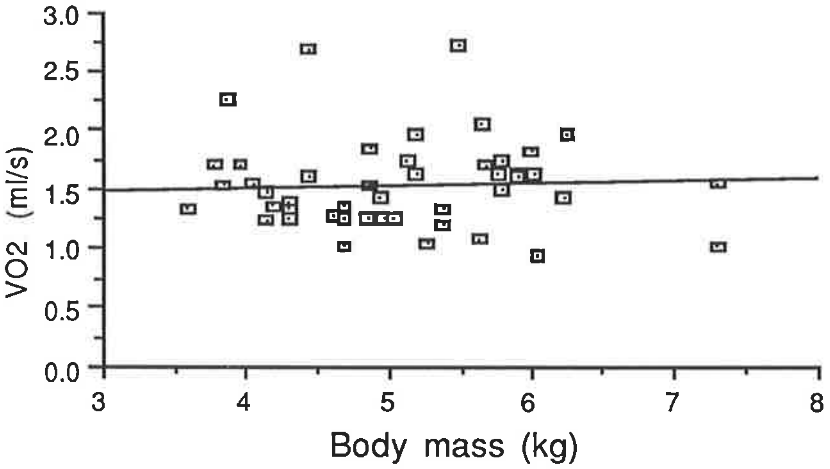

Figure 7.4a Steady state oxygen consumption at rest as a function of body mass.

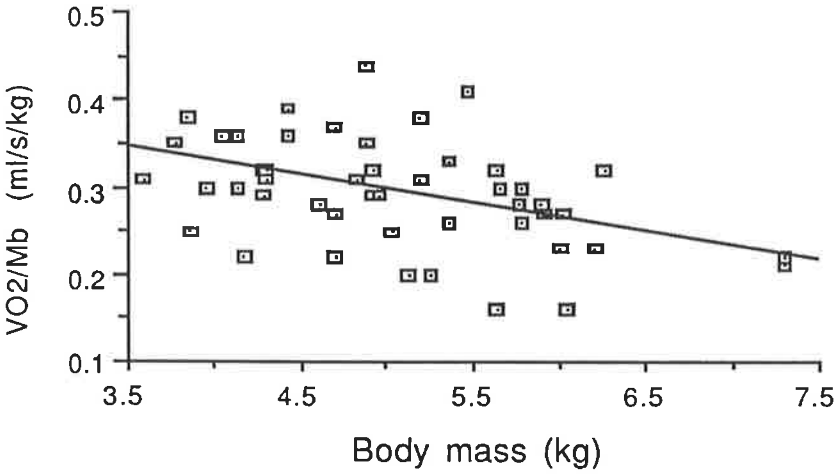

Figure 7.4b Steady state mass specific oxygen consumption as a function of body mass in lambs.

were 1.34 and 1.63 ml/s, respectively. The VO_2/Mb levels ranged from 0.16 to 0.44 ml/s/kg (mean = 0.29 ± 0.01 ml/s/kg). The lower and upper quartiles were 0.27 and 0.32 ml/s/kg, respectively.

Regression analysis indicated that VO_2 was independent of body mass (Figure 4.a), whereas VO_2/Mb decreased linearly with increasing body mass (Figure 4.2b). The relationship between VO_2/Mb and body mass is represented by the equation :

$$\log y = \log 0.46 - 0.03. \log x (r = -0.50, p < 0.001); \quad (1)$$

in which y is VO_2/Mb (ml/s/kg) and x is body mass in kg.

7.3.1.3 Oxygen and energy consumption as a function of speed of locomotion

The relationships between VO_2, VO_2/Mb and energy consumption (Emetab/Mb in J/s/kg) is shown by Table 7.4 and Figure 7.5. These variables increase linearly as a function of speed of locomotion in lambs. The relation between VO_2 (range = 1.49 to 6.09 ml/s) and speed (x, in units of m/s) is described by the regression equation:

$$y = 1.79 + 1.95 x \quad (r = 0.84, p < 0.001) ; \quad (2)$$

where y is VO_2 in ml/s.

Mass specific rates of oxygen consumption (range = 0.38 to 1.26 ml/s/kg) showed a remarkably high correlation with speed of locomotion; the relation being described by the linear equation:

$$y = 0.36 + 0.37 x \quad (r = 0.90, p < 0.001) ; \quad (3)$$

where y is VO_2/Mb in ml/s/kg.

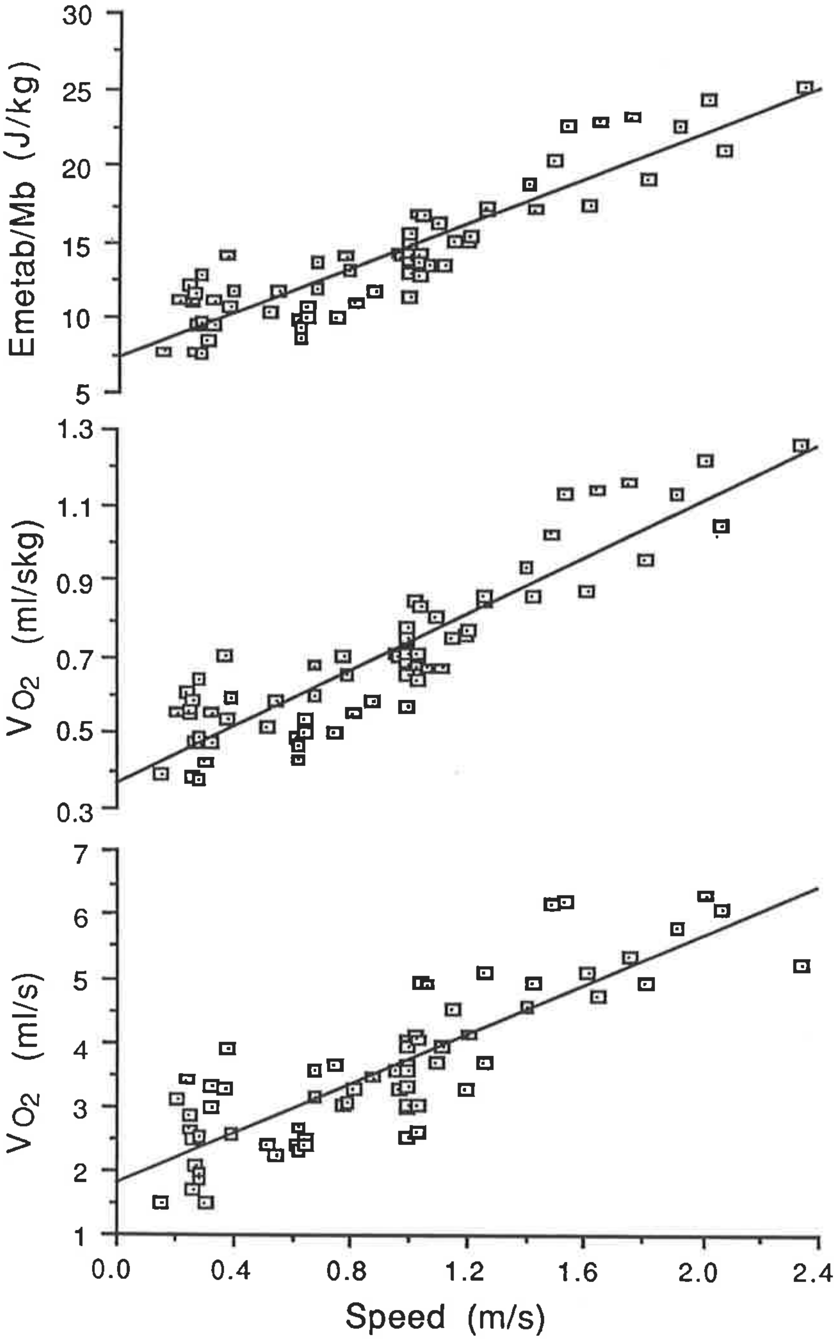

Figure 7.5 Steady state oxygen and energy consumption as a function of speed of locomotion in lambs.

Table 7.3 Frequency tabulation of treadmill speed (m/s) employed in the study.

Class	Lower limit	Upper limit	Midpoint	Frequency
1	0.00	0.50	0.25	17
2	0.50	1.00	0.75	28
3	1.00	1.50	1.25	16
4	1.50	2.00	1.75	6
5	2.00	2.50	2.25	3

Range = 0.15 to 2.34 m/s.

Table 7.4 Regression equations relating steady state oxygen and energy consumption, stride and physiological parameters to the speed of locomotion in lambs.

Parameter	Intercept	Slope	Correlation
VO_2 (ml/s)	1.79	1.95	0.84***
VO_2/Mb (ml/s/kg)	0.36	0.37	0.90***
Emetab/Mb (kJ/kg)	7.42	7.44	0.90***
Percent VO_2 max	26.42	24.00	0.80***
Stride frequency (H_z)	0.61	0.60	0.98***
Stride length (m)	0.28	0.25	0.94***
Rectal temp. (^oC)	39.97	0.32	0.40*
Respiratory freq. (H_z)	1.47	1.39	0.64***
Heart freq. (H_z)	2.44	1.57	0.73***

The equations are presented in the form of y = a + bx, except stride frequency where $y = a.x^b$. Letter y represents the parameter, a is the intercept, b is the slope, and x is the speed in m/s. *Significance at 5% and ***significance at 0.1% levels.

Conversion to energy units yielded a relationship between Emetab/Mb (range = 7.64 to 25.33 J/s/kg) of

$$y = 7.42 + 7.44\,x \qquad (r = 0.90,\ p < 0.001)\ ; \qquad (4)$$

in which y is Emetab/Mb in J/s/kg.

The steady state oxygen consumption as a percentage of VO_2mass (range =31 to 87%) in 26 tests increased linearly as a function of speed of locomotion. The regression line relating the 2 variables is given by the equation:

$$y = 26.42 + 24.00\,x \qquad (r = 0.80,\ p < 0.001)\ ; \qquad (5)$$

where y is percentage of VO_2mass. Standard errors of 3.6 and 3.7 were obtained for the intercept and slope respectively.

7.3.1.4 Stride frequency and length as a function of speed of locomotion

Table 7.4 and Figure 7.6 illustrate the relationship between stride variables and speed of locomotion in lambs. Stride frequency in the lambs during steady state locomotion ranged from 0.53 to 2.93 Hz and was highly dependant on speed. The curvilinear relationship between these variables showed a highly significant correlation;

$$\log y = \log 0.77 + 0.19 \log x \qquad (r= 0.97,\ p < 0.001)\ ; \qquad (6)$$

where y is stride frequency in Hz. The curvilinear nature of the relationship between the stride frequency and speed of locomotion is confirmed by the the linear relationship between the 2 variables plotted on logarithmic coordinates (Figure 7.7).

Stride length (range = 0.28 to 0.91 m) was also highly correlated with the speed of locomotion, but in a linear fashion described by the equation:

$$y = 0.28 + 0.25\,x \qquad (r = 0.94,\ p < 0.001)\ ; \qquad (7)$$

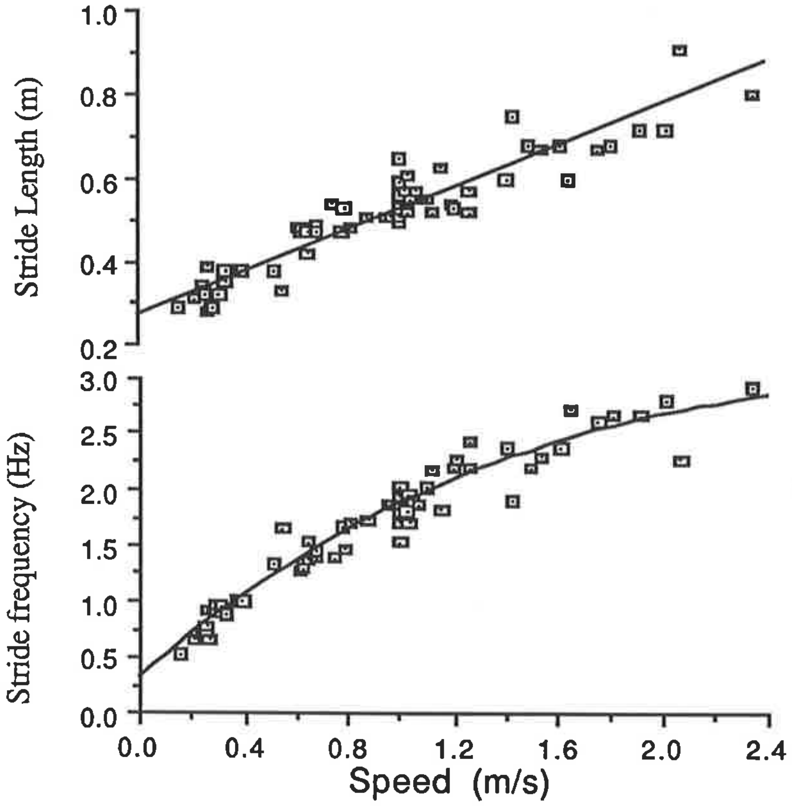

Figure 7.6 Stride frequency and length as a function of speed in lambs.

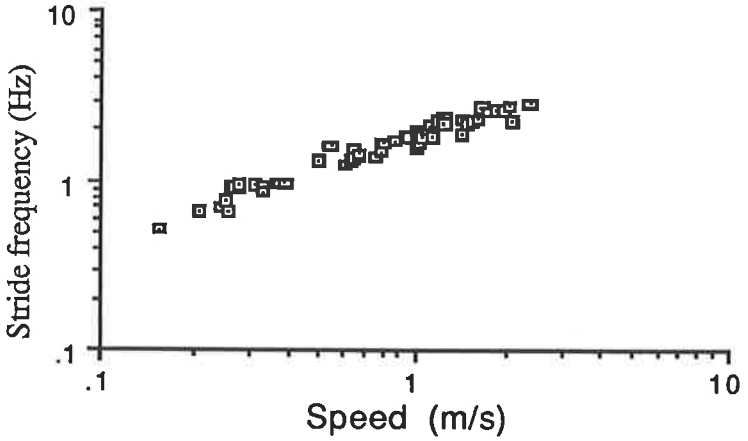

Figure7.7 Steady state stride frequency as a function of speed of locomotion in lambs plotted on logarithmic coordinates.

where y is stride length in metres. In both equations x refers to the speed of locomotion in m/s.

7.3.1.5 Rectal temperature, respiratory and heart frequencies as a function of speed of locomotion

Measurements of rectal temperature, respiratory and heart frequencies plotted as a function of speed of locomotion in lambs are shown by Table 7.4 and Figure 7.8. Rectal temperatures in the lambs (range = 39.5 to 41.0 °C) tended to be higher at higher running speeds. The relationship between the 2 variables;

$$y = 39.97 + 0.32\ x \qquad (r = 0.40,\ p < 0.05)\ ; \qquad (8)$$

in which y is the rectal temperature in °C. The slope is significant.

Respiratory and heart frequencies exhibited progressive increments of approximately 1.4 and 1.6 Hz to each increase of 1 m/s in speed of locomotion (x) respectively. The relationship between respiratory frequency (range = 1.4 to 4.2 Hz) and speed is described by the equation:

$$y = 1.47 + 1.39\ x \qquad (r = 0.64,\ p < 0.001)\ ; \qquad (9)$$

where y is the respiratory frequency measured as Hz. The slope of the relationship is significantly different from zero.

Heart frequency increased significantly as a function of speed of locomotion in lambs. The regression-derived line depicting the relationship between heart frequency (range= 2.4 to 6.2 Hz) and speed of locomotion (x) is described by the equation:

$$y = 2.44 + 1.57\ x \qquad (r = 0.73,\ p < 0.001)\ ; \qquad (10)$$

where y is the beating of the heart in Hz.

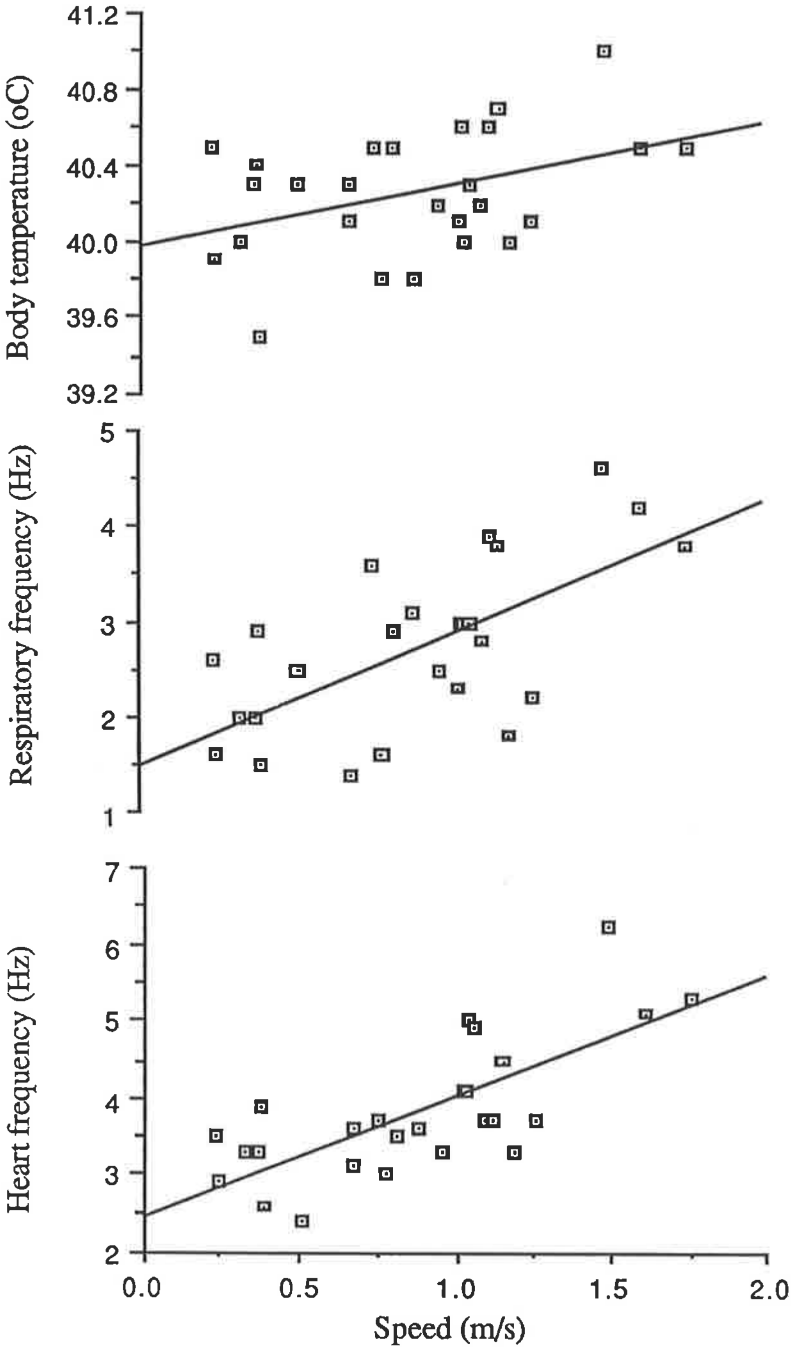

Figure 7.8 Rectal temperature, respiratory and heart frequencies as a function of speed of locomotion.

7.3.1.6 Rectal temperature, respiratory and heart frequencies as a function of stride frequency in running lambs.

Table 7.5 and Figure 7.9 show the relationships between stride frequency and rectal temperature, respiratory and heart frequencies. The regression line relating rectal temperature to stride frequency is described by the equation:

$$y = 39.94 \pm 0.19\ x \qquad (r = 0.30, \text{ not significant})\ ; \qquad (11)$$

where y is the rectal temperature in °C. The slope relating the variables shows that increases in rectal temperature in the lambs during locomotion tended to be independent of the stride frequency.

Respiratory frequency in the lambs increased linearly as a function of stride frequency, the relationship between the variables is given by the regression equation

$$y = 1.14 + 0.94\ x \quad (r= 0.54,\ p < 0.01)\ ; \qquad (12)$$

where y is the respiratory frequency in Hz. The slope of the regression line indicates that respiratory and stride frequencies approach a 1:1 coupling ratio over the range of speed employed in the test. Heart frequency also increased significantly with changes in stride frequency with a similar ratio. The linear relationship between the 2 variables is described by the regression equation:

$$y = 2.12 + 1.03\ x \qquad (r =0.60,\ p < 0.01)\ ; \qquad (13)$$

where y is the heart frequency in Hz. In all the equations x is the stride frequency per s.

7.3.1.7 Oxygen and energy cost of locomotion

The allometric equations relating VO_2, VO_2/Mb and the energy cost per unit distance moved (m) to the speed of locomotion, are shown in Table 7.6 . Figure 7.10 depicts the curves relating various measures of the efficiency of locomotion to the speed of locomotion.

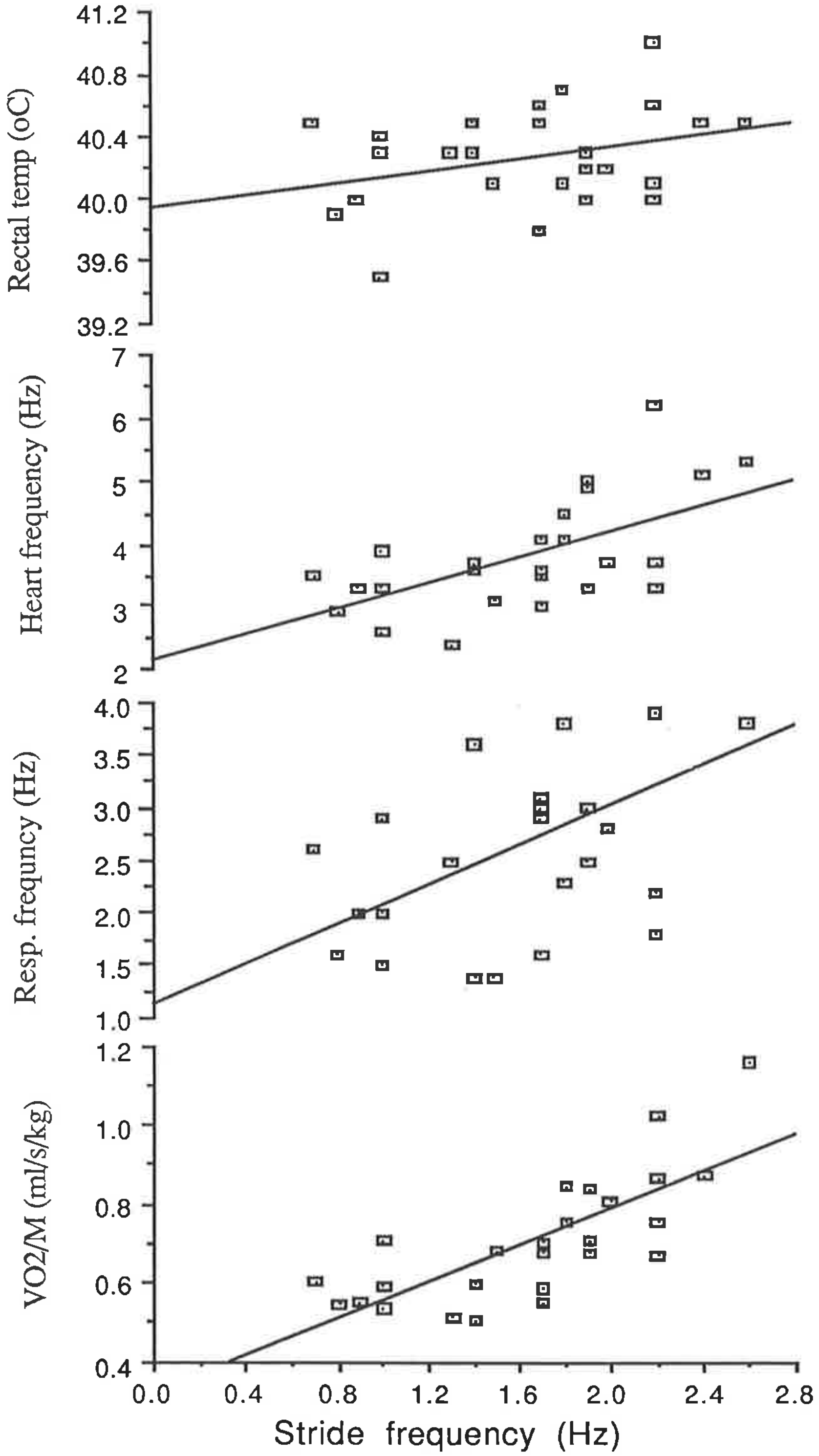

Figure 7.9 Mass specific oxygen consumption, rectal temperature,respiratory and heart frequencies as a function of stride frequency in running lambs.

Table 7.5 Regression equations relating rectal temperature, mass specific oxygen consumption, respiratory and heart frequencies to stride frequency in running lambs.

Parameter	Intercept	Slope	Correlation
Rectal temperature (^oC)	39.94	0.19	0.09
Respiratory freq. (Hz)	1.14	0.94	0.54
Heart freqency (Hz)	2.12	1.03	0.06
VO_2/Mb (ml/s/kg)	0.32	0.23	0.57

The equations are presented in the form of $y = a + bx$.

Table 7.6 Allometric equations relating the energetic cost of locomotion to speed in lambs.

Parameter	Intercept	Slope	Correlation
VO_2 (ml/m)	-0.30	-0.65	-0.93***
VO_2/Mb (ml/kg/m)	1.31	-0.60	-0.85***
VO_2/stride (ml/kg/stride)	-0.91	-0.25	-0.67***

Note: the intercept is equal to log a. The equations are presented in the form of $\log y = \log + b \log x$, where y is the intercept, b is the slope of the curve and x is the speed in m/s. ***Significant at 0.1% level.

The apparent costs of locomotion declined with increasing speed and approached minimum values towards the highest speed reached.

The relationship between the total oxygen consumed by the lambs to move a meter of distance and the speed of locomotion is expressed as:

$$\log y = -\log 0.298 - 0.65 \log x \qquad (r = -0.93,\ p < 0.001)\ ; \qquad (14)$$

where y is VO_2 in ml/m The standard error of the intercept and slope were 0.21 and 0.30, respectively. Mass-specific oxygen consumption per metre move was also highly correlated with the speed of locomotion in a curvilinear model. The equation representing the relationship between is expressed as:

$$\log y = \log 1.31 - 0.60 \log x \qquad (r = -0.85,\ p < 0.001)\ ; \qquad (15)$$

where y is ml O_2/kg/m. The values of 0.03 and 0.05 represent the standard error of the intercept and slope respectively. The mass specific oxygen cost per stride and the speed of locomotion are related by the allometric equation:

$$\log y = -\log 0.90 - 0.25 \log x \qquad (r = -0.67,\ p < 0.001)\ ; \qquad (16)$$

where y is ml O_2/kg/step.

7.3.1.8 Metabolic, physiological and stride parameters at rest and at three levels of speed of locomotion

A summary of steady state VO_2/Mb, the energy cost of locomotion, physiological and stride parameters measured in 3 lambs at rest and at 3 speeds of locomotion are shown in Table 7.7. Steady state VO_2/Mb, stride frequency and length were highly correlated with the speed of locomotion ($r = 0.98$ to 0 95, $p < 0.001$). Mass-specific oxygen costs during locomotion and respiratory frequency also increased significantly as a function of speed ($r = 0.78$ and 0.85, respectively, $p < 0.001$). Changes in heart frequency tended to increase as a function of speed ($r = 0.73$,$p < 0.$), however changes in oxygen cost per meter and per

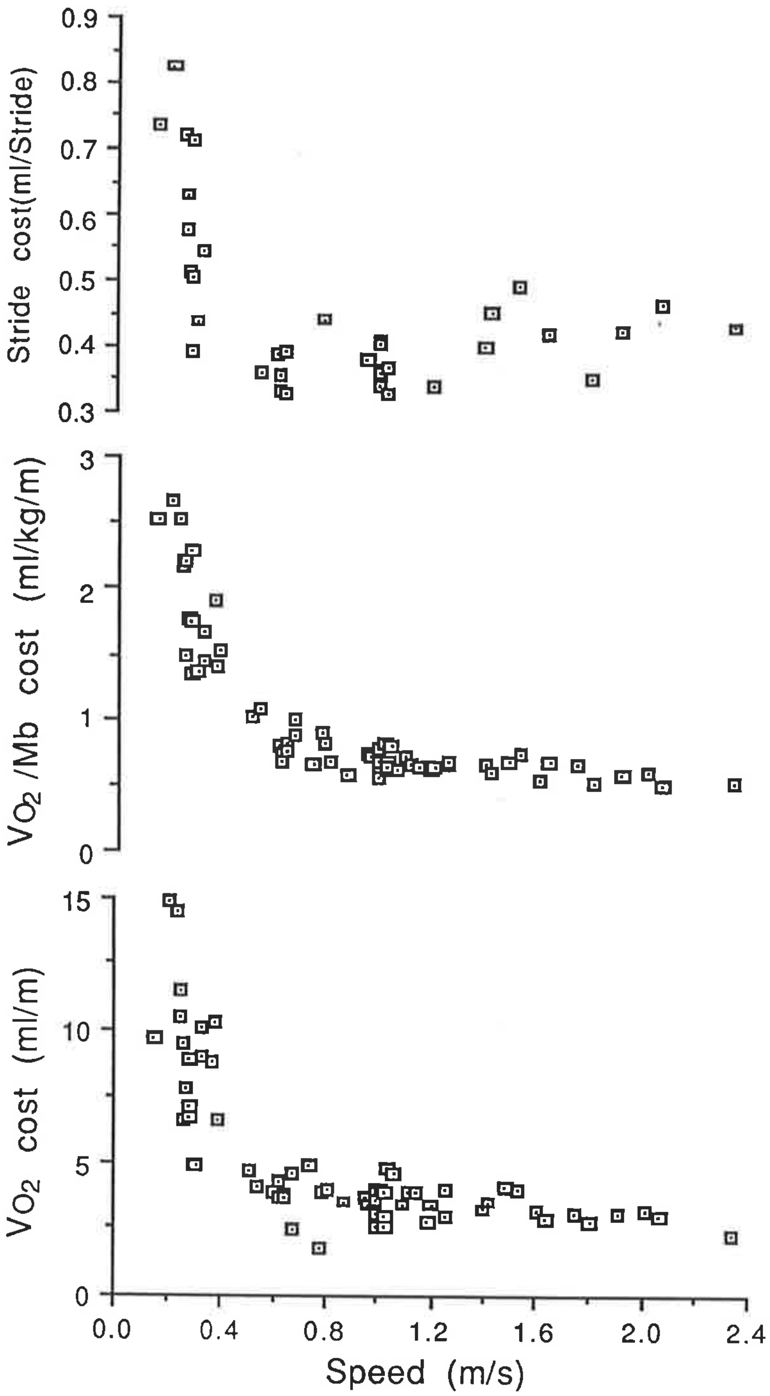

Figure 7.10 Three measures of the efficiency of locomotion in running lambs, Energy demand per distance moved, mass specific oxygen demand per distance and oxygen demand per stride are all plotted against speed.

Table 7.7 Mass specific oxygen consumption and cost per meter and per stride, physiological and stride parameters at rest, and at three speeds of locomotion in lambs.

Speed (m/s)	VO_2/Mb (ml/s/kg)	VO_2 cost per meter (ml/kg/m)	VO_2/Mb cost per stride (ml/kg/step)	Rectal temperature (°C)	Respiratory frequency (breaths/s)	Heart frequency (beats/s)	Stride frequncy (stride/s)	Stride length (m)
Rest	0.27 ± 0.0	NA	NA	39.7 ± 0.2	1.5 ± 0.7	3.2 ± 0.6	NA	NA
0.72	0.61 ± 0.04	0.85 ± 0.16	0.40 ± 0.07	40.3 ± 0.1	1.9 ± 0.5	3.3 ± 0.4	1.5 ± 0.1	0.48 ± 0.01
1.12	0.74 ± 0.04	0.68 ± 0.02	0.38 ± 0.06	40.5 ± 0.2	3.4 ± 0.3	3.4 ± 0.3	2.0 ± 0.1	0.57 ± 0.04
1.62	1.00 ± 0.08	0.63 ± 0.04	0.42 ± 0.05	40.7 ± 0.2	4.2 ± 0.2	4.6 ± 0.4	2.4 ± 0.1	0.68 ± 0.03

N =3, NA = values not applicable since the lambs were at zero speed of locomotion.

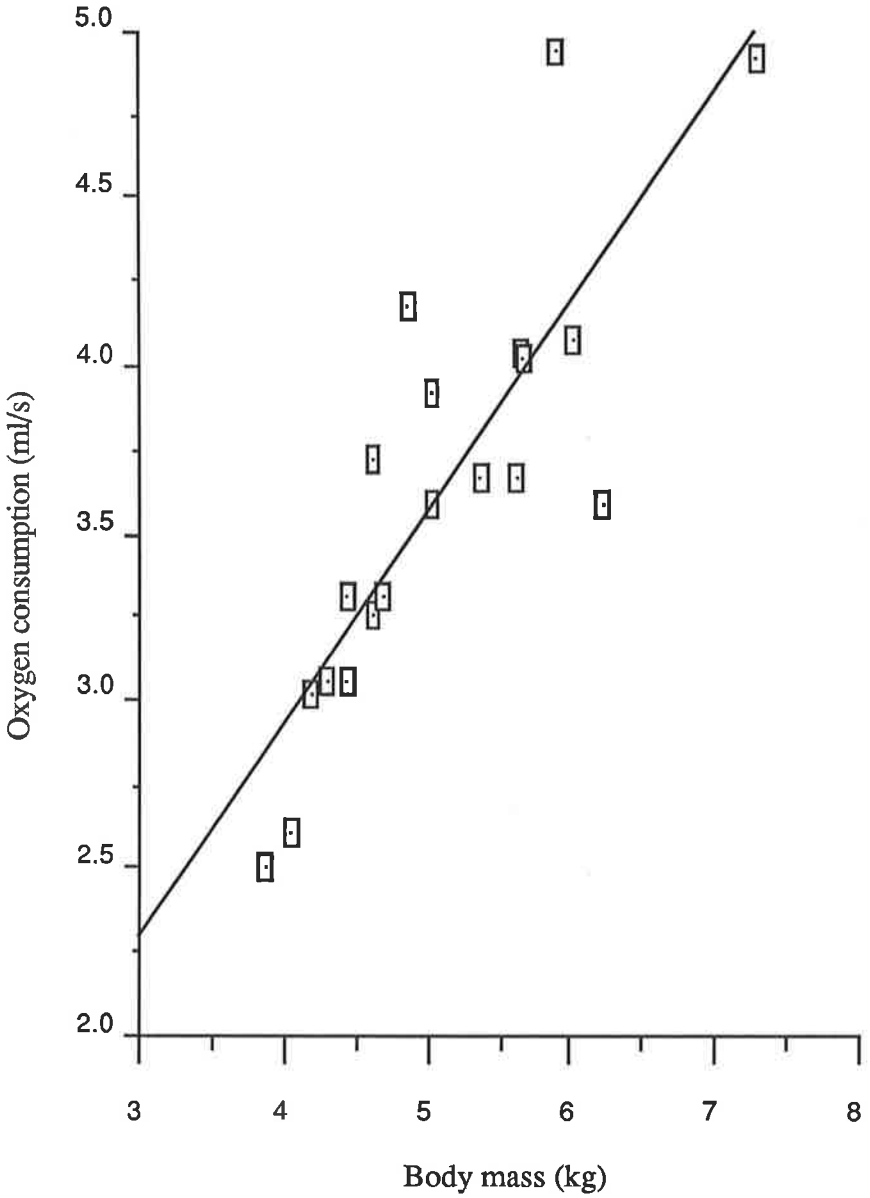

Figure 7.11 Steady state oxygen consumption as a function of body mass in lambs running at a speed of one metre per second.

stride, and rectal temperatures were independent of speed of locomotion in the lambs. There was little variation among lambs, either at rest or any of the speeds of locomotion, in the parameters studied with the exception of VO_2/Mb per meter at 0.72 m/s (0.85 ± 0.16 ml/kg/m). This indicates a marked increase in the efficiency of locomotion from lower to higher speeds of running.

Figure 7.11 shows that VO_2 increases linearly as a function of body mass when 19 lambs ran at a speed of approximately 1 m/s. The relationship between the 2 variables is described by the regression equation:

$$y = 0.39 + 0.63\ x \qquad (r = 0.84,\ p < 0.01)\ ; \tag{17}$$

where y is VO_2 ml/s in and x is body mass in kg.

7.3.1.9 Relationship between the speed of locomotion, oxygen consumption, energy consumption, gait characteristics and physiological parameters in running lambs.

Table 7.8 examines inter-correlations between the speed of locomotion and the parameters studied during a total of 26 runs. The assessment was evaluated by a partial correlation test. Speed was significantly related to changes in VO_2/Mb, stride frequency and length. Entire body VO_2 was significantly correlated with VO_2/Mb, and respiratory frequency. Mass-specific VO_2 showed negative relationship with stride frequency and length. Stride frequency and length were negatively correlated with each other, but both variables were positively correlated with speed. Rectal temperature exhibited a significant relationship with only respiratory frequency, while the latter correlated with VO_2 but exhibited negative relationship with VO_2/Mb. In contrast heart frequency was independent of all other variables.

Table 7.8 Intercorrelation values (r) between speed and other measured varables during exercise in lambs at varying speeds of locomotion.

Variables	Speed	VO_2	VO_2/Mb	Stride freq.	Stride length	Rectal temp.	Resp. freq.	Heart freq.
Speed	-1.000	0.036	0.663	0.906	0.754	-0.090	0.407	-0.093
VO_2		-1.000	0.497	-0.169	0.340	0.032	0.498	-0.013
VO_2/Mb			-1.000	-0.463	-0.547	0.100	-0.586	0.240
Stride frequency				-1.000	-0.458	0.001	-0.264	0.178
Stride length					-1.000	0.148	-0.320	-0.105
Rectal temp.						-1.000	0.522	-0.148
Respiratory freq.							-1.000	0.276
Heart frequency								-1.000

For statistical significance, a correlation coefficient of 0.444 or larger is required at the 5% level.

7.3.2 CARDIORESPIRATORY AND METABOLIC RESPONSES TO INTERVAL EXERCISE IN LAMBS

7.3.2.1 TREADMILL EXERCISE

Fourteen out of the 17 lambs subjected to the intermittent treadmill exercise successfully completed the protocol without any immediate or subsequent ill effects (see Section 6.3.1.1 for details of animal behaviour during the exercise).

Mean values for $V0_2$ and the measured physiological parameters prior to, during, and after exercise in the neonates are shown in Table 7.9. Graphical presentation of changes in the parameters studied are depicted by Figure 7.12. Oxygen consumption data during exercise recovery however covered only 2 lambs. Exercise significantly increased levels of all the physiological parameters, with peak values of respiratory frequency and rectal temperature occurring during the last exercise bout, whereas VO_2 and cardiac responses showed highest values during the first and second exercise bouts respectively.

(1) Rectal temperature

Rectal temperatures in the lambs prior to exercise ranged from 39.3 to 40.2 °C, with a mean of 39.6 ± 0.1 °C. Exercise elicited statistically significant elevations in rectal temperature of 1.0 °C over pre-exercise values during the exercise bout, to a peak of 40.6 ± 0.1 °C (range = 40.0 to 41.3 °C) at the end of the intermittent work ($p < 0.001$). Rectal temperature remained elevated during the first 15 min after exercise and had not returned to pre-exercise levels by the end of the 30 min recovery period. Whereas the rectal temperature 5 min after exercise did not differ significantly from that measured at the end of exercise, the temperature levels reached at 15 and 30 min after work, 40.1 ± 0.1°C and 39.9 ± 0.1 °C, respectively, denote significant reductions ($p < 0.01$).

(2) Respiratory frequency

Pre-exercise levels of respiratory frequency among the lambs ranged from a low 48 to a maximum 148 breaths per min (mean = 83 ± 7 b/min). Breathing frequency increased progressively and significantly to 209 ± 15 breaths per min (range 138 to 300 b/m) by the end of the intermittent exercise (Table 7.9). The increase extended into the first 5 min after exercise before decreasing to a mean of 183 ± 12 at 15 min of the recovery period. Respiratory frequency at the end of the recovery period, 154 ± 10 b/min (range = 100 to 225 b/min), represents an approximate 2-fold increase over the resting value ($p < 0.001$). Nevertheless, this value represents a considerable reduction of 26% from the peak level reached.

(3) Heart Frequency

Intermittent exercise in lambs tended to cause slight but significant increases in heart frequency from a mean pre-exercise level of 191 ± 9 beats/min (range = 148 to 270 beats/min) ($p < 0.05$). The peak value reached at the end of the second exercise represents a mere 16% rise over the resting value. There was an immediate and rapid fall to the resting value 5 min after cessation of exercise, followed by a further slight but insignificant reduction to 189 ± 9 (range = 132 to 248 beats/min) by the end of the recovery period.

Oxygen consumption rate increased 2.4-fold over the pre-exercise value of 0.323 ± 0.020 ml/kg/s (range = 0.231 to 0.477 ml/s/kg) during the first exercise bout and remained significantly elevated during the subsequent bouts ($p < 0.001$). Oxygen consumption had decreased significantly by the fifth min after exercise to 0.448 ± 0.015 ml/s/kg ($p < 0.05$). Further decrease to the resting level occured by 15 min after exercise. Intermittent exercise on average elicited 0.389 ± 0.029 ml O_2/kg/stride (range = 0.230 to 0.574), and the lambs ran at an overall stride frequency of 1.8 ± 0.1 per s (range = 1.4 to 2.2 strides/s), and at a stride length of 0.57 ± 0.02 m (range = 0.46 to 0.70 m).

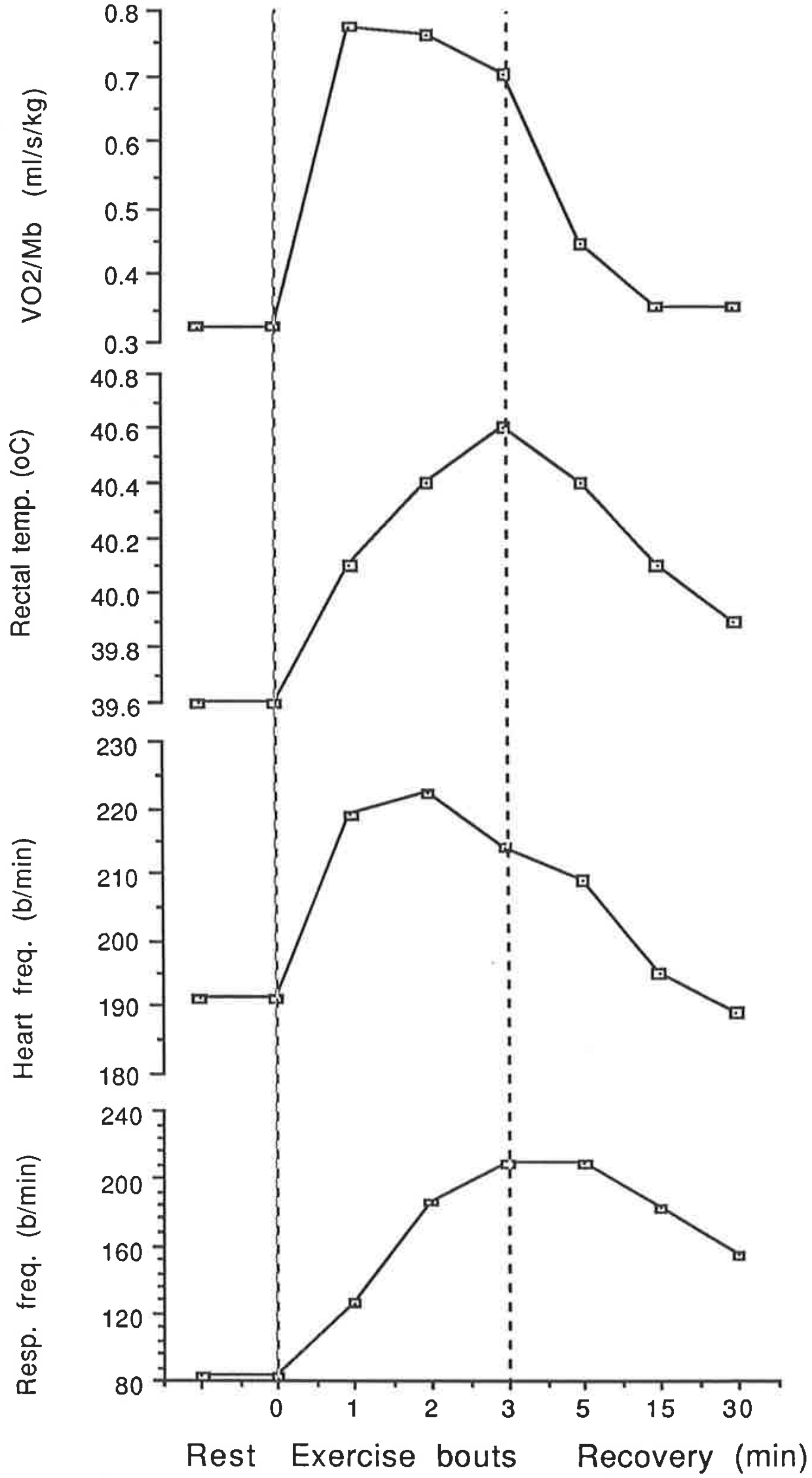

Figure 7.12 Physiological effects of 3 x 5 minutes intermittent exercise. Mean values from 14 lambs are shown.

Table 7.9 Physiological effects of three x 5 minutes of intermittent exercise in lambs.

Time of measurement	Respiratory frequency (breaths/min)	Heart frequency (beats/min)	Rectal temperature (oC)	Oxygen consumption (ml/s/kg)
Pre-exercise rest	83 ± 7	191 ± 9	39 6 ± 0.1	0.323 ± 0.020
Exercise bouts				
1	126 ± 10***	219 ± 11*	40.1 ± 0.1***	0.775 ± 0.057***
2	186 ± 12***	222 ± 10*	40 4 ± 0.1***	0.762 ± 0.043***
3	209 ± 15***	214 ± 7*	40.6 ± 0.1***	0.701 ± 0.043***
Recovery (min)				
5	209 ± 15***	209 ± 8	40.4 ± 0.1***	0.448 ± 0.015*
15	183 ± 12***	195 ± 8	40.1 ± 0.1***	0.352 ± 0.014
30	154 ± 10***	189 ± 9	39.9 ± 0.1*	0.350 ± 0.016

Significantly different from resting values at *5%,**1% and ***0.1% levels.

(4) Correlations between parameters during intermittent exercise.

Changes in respiratory frequency and rectal temperature during exercise and recovery correlated significantly ($r = 0.89$, $p < 0.01$). Changes in rectal temperature during exercise tended to correlate positively with that of heart frequency, but the relationship was not significant ($r = 0.74$, $p < 0.06$). Changes in heart frequency and oxygen consumption were highly correlated ($r = 0.95$, $p < 0.001$).

7.3.2.2 LANEWAY EXERCISE

The results depicted in Table 7.10 and Figure 7.13. are based on 20 out of the 23 lambs completing the 1 km distance. Data for 10, 20 and 25 min after exercise were however, obtained from only 10 lambs, 5 from each lambing group. The neonates followed their mothers at speeds ranging from 1.24 to 1.95 m/s (mean = 1.48 ± 0.04 m/s); the ambient temperature during the study ranged from 12°C in winter to 26°C in spring.

(1) Body temperature

Rectal temperature in the lambs was progressively and significantly elevated from 39.5 ± 0.1 °C (range = 38.4 to 41.0 °C) prior to exercise to a peak of 40.5 ± 0.1 °C (range = 38.9 to 41.8 °C) at the end of the prescribed distance ($p < 0.001$). The temperature remained steady during the first 5 min after exercise before reducing to the pre-exercise level within 10 min of the recovery period ($p < 0.01$). A further decrease of 0.2 °C by 20 min of the recovery period to 39.8 ± 0.1 °C (range = 39.5 to 40.4 °C), represented a significant decrease from the end of exercise value ($p < 0.001$).

(2) Respiratory frequency

Respiratory frequency in the lambs increased progressively during walking by 2.3 fold over the pre-exercise level of 114 ± 16 b/min (range = 45 to 291) to 257 ± 22 b/min (range 68 to 420 b/min) at the end of test period ($p < 0.001$). The rate thereafter decreased

Table 7.10 Physiological effects of two laneway walk each of half a kilometer following in lambs.

Point of measurement	Respiratory frequency (breaths/min)	Heart frequency (beats/min)	Rectal temperature (^{o}C)
Pre-following rest	114 ± 16	181 ± 7	39.5 ± 0.1
Following distance (km)			
0.5	198 ± 21**	235 ± 6***	40.1 ± 0.1**
1.0	257 ± 22***	240 ± 6***	40 5 ± 0.1***
Recovery (min)			
5	240 ± 19***	221 ± 6***	40.4 ± 0.1***
10	193 ± 22**	210 ± 6**	40.0 ± 0.1*
15	182 ± 16**	204 ± 4**	40.0 ± 0.1
20	143 ± 24	202 ± 7	39 8 ± 0.1
25	133 ± 24	197 ± 9	39 6 ± 0.2
30	30 ± 18	183 ± 8	39 8 ± 0.1

Significantly different from resting values at *5, **1, and ***0.1 levels.

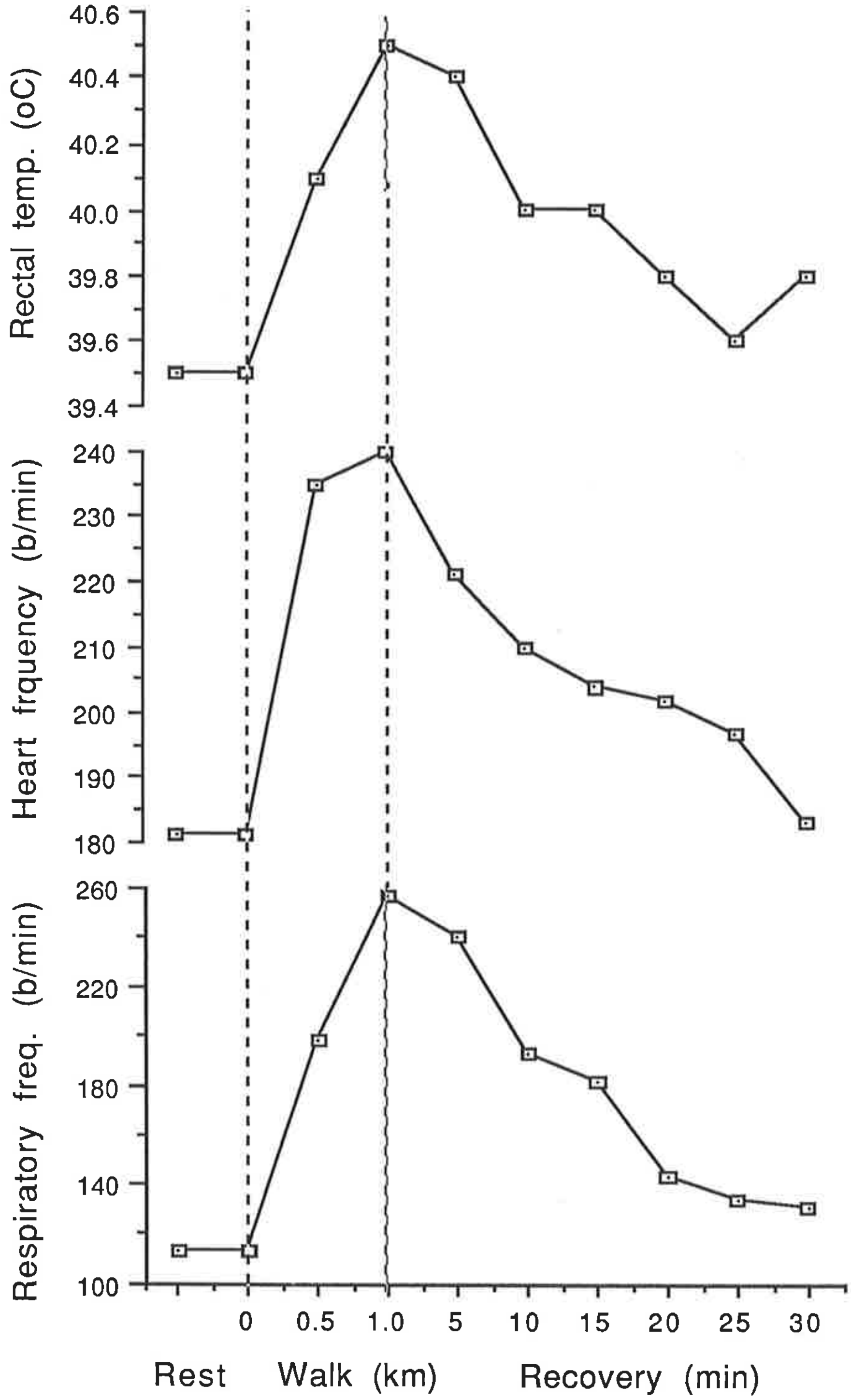

Figure 7.13 Physiological effects of a laneway walk of 2 x 0.5 kilometre in lambs while following their mothers.

gradually to pre-exercise levels within the 20 min recovery period. The respiratory frequency 5 min after exercise was not significantly different from the exercise value.

(3) Heart frequency

Heart frequency increased 1.3-fold above the pre-exercise level of 181 ± 7 (range = 150 to 228) to 240 ± 6 beats/min (range = 198 to 300 b/min) by the end of the test ($p < 0 .001$). It had returned to the resting level by 20 min after exercise. The mean value reached at this stage of recovery, 202 ± 7 beats/min (range = 168 to 240 b/min) represents a 15% decrease from the peak value at the end of exercise.

(4) The relationship among the physiological parameters measured during the laneway following exercise

Correlation analysis showed a significant relationship between changes in rectal temperature in the lambs and those of respiratory and heart frequencies, $r = 0.98$ ($p < 0.001$) and $r = 0.86$ ($p < 0.01$), respectively. Changes in respiratory frequency during exercise and recovery correlated significantly with that of heart frequency ($r = 0.90$, $p<0.01$).

7.3.3 MAXIMAL OXYGEN CONSUMPTION

Tables 7.11 and 7.12 show the pre- and post-exercise values of blood lactate concentrations, VO_2max, VO_2/Mbmax, stride and physiological parameters in lambs subjected to maximal work on 7% and 16% grades respectively. All the variables had increased significantly above pre-exercise values by the end of the VO_2max tests ($p < 0.01$ to 0.001), with rectal temperature and blood lactate values at the 7% slope producing the minimum and maximum differences respectively. The rate of VO_2/Mb in the lambs prior to maximal exercise at the 2 slopes did not differ statistically. Neither was there any significant difference in the pre-exercise levels for rectal temperature, heart rate and respiratory frequencies between the 2 groups.

Table 7.11 Blood lactate levels and physiological measurements before and at the end of the maximal exercise test at a 7% positive grade.

Parameter	Pre-exercise	End of exercise
Rectal temperature (°C)	39.4 ± 0.2	40.5 ± 0.3 *
Respiratory rate (breaths/min)	62 ± 10	195 ± 29 **
Heart rate (beats/min)	157 ± 7	222 ± 17**
Whole body V_{O_2} (ml/s)	1.50 ± 0.09	8.45 ± 0.57 **
Mass specific V_{O_2} (ml/s/kg)	0.25 ± 0.01	1.39 ± 0.07 **
Blood lactate (mM)	1.38 ± 0.12	4.17 ± 0.56 ***

Significant difference between columns at *5%, **1% and ***0.1% levels.

Table 7.12 Blood lactate levels and physiological measurements before and at the end of maximal exercise on a 16% positive grade.

Parameter	Pre-exercise	End of exercise
Rectal temperature (°C)	39.7 ± 0.1	41.2 ± 0.1 **
Respiratory rate (breaths/min)	85 ± 14	356 ± 32 **
Heart rate (beats/min)	156 ± 11	274 ± 6 **
Whole body V_{O_2} (mls)	1.43 ± 0.08	9.53 ± 0.62 **
Mass specific V_{O_2} (ml/s/kg)	0.24 ± 0.01	1.57 ± 0.05 **
Blood lactate (mM)	1.91 ± 0.07	5.17 ± 0.41 **

Significant difference between columns at *1% and **0.1% levels.

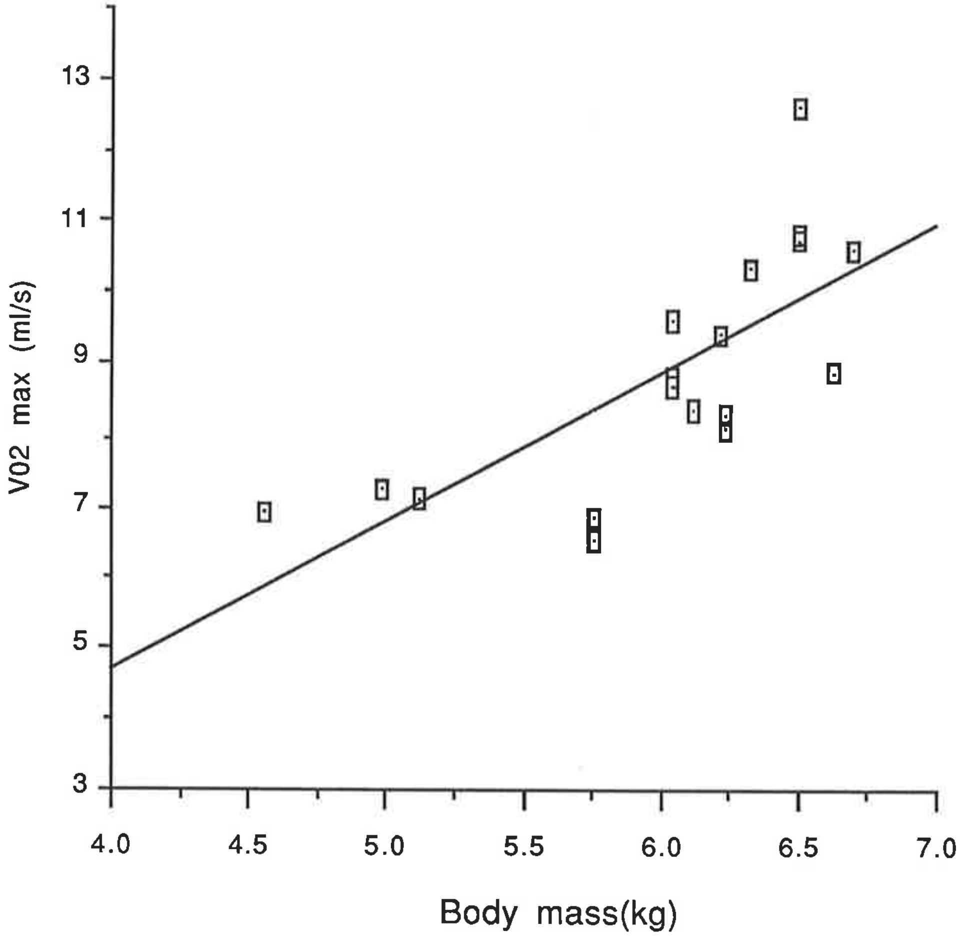

Figure 7.14 Maximal oxygen consumption as a function of body mass in lambs running at seven and sixteen per cent upward grade.

Figure 7.14 depicts steady state VO_2max elicited by both treadmill protocols as a function of body mass. The relationship between body mass and VO_2max is described by the regression equation:

$$y = - 3.69 + 2.09\ x \qquad (r = 0.73, p < 0.001)\ ; \qquad (18$$

where y is VO_2max (ml/s/kg) and x is the body mass in kg.

Comparative results of the variables measured at the end of exercise in the 2 exercise protocols are shown in Table 7.13. Maximal exercise at the 16% slope produced significantly higher values in almost all the parameters measured. Rectal temperature, heart and respiratory frequencies at the higher grade were significantly higher than those achieved at the 7% grade. The blood lactate level at the end of exercise tended to be higher in the trained than untrained lambs; the difference was however not significant. Nevertheless, the untrained lambs reached VO_2max in a significantly shorter time (5.39 ± 0.38 vs.9.44 ± 1.04 min) and tended to have a non-significantly higher rate of lactate production than the trained lambs (0.56 ± 0.09 vs. 0.39 ± 0.05 mM/min).

No statistical difference was found in entire body VO_2max between the lambs in Studies 1 and 2 (8.54 ± 0.57 vs. 9.53 ± 0.62 ml/s). Nevertheless, the lambs running at the higher slope tended to have higher values in both cases. Figures 7.15a and 15b show $VO_2/Mbmax$ at 7% and 16% treadmill grades. Steady state $VO_2/Mbmax$ achieved at the 16% slope was significantly higher than that of the lambs running at 7% grade (1.57 ± 0.05 vs. 1.39 ± 0.07 ml/s/kg, $p < 0.01$); the aerobic scopes also differed significantly (5.6 ± 0.3 vs. 6.6 ± 0.3, $p < 0.05$).

The $VO_2/Mbmax$ in the lambs tested twice under a similar exercise protocol (Table 7.14) showed a remarkably high correlation coefficient of 0.973 ($p<0.001$) and a negligible difference between the mean values for the initial test and repeat values (1.52 ± 0.02 vs. 1.49 ± 0.11 ml/s/kg). The aerobic scope, determined from $VO_2/Mbmax$ divided by

Table 7.13 Comparison of variables measured during maximal exercise test in untrained and trained lambs.

Parameter	Untrained (7% grade)	Trained (16% grade)	t-ratio
Body mass (kg)	6.07 ± 0.17	6.02 ± 0.24	-0.16
Test duration (min)	5.39 ± 0.38	9.44 ± 1.04	3.66**
Running speed (m/s)	1.96 ± 0.10	1.81 ± 0.08	-1.19
Rectal temp. (°C)	40.5 ± 0.3	41.2 ± 0.1	2.33
Respiratory freq. (breaths/min)	195 ± 29	356 ± 32	3.71**
Heart freq. (beats/m)	222 ± 17	274 ± 6	2.89**
Whole body VO_2 (ml/s)	8.45 ± 0.57	9.53 ± 0.62	1.70
Mass specific VO_2 (ml/s/kg)	1.39 ± 0.07	1.57 ± 0.05	3.10**
Heat production (kJ/kg)	27.3 ± 1.4	31.1 ± 0.9	3.26**
Work load (kg.m/s)	100 ± 4	75 ± 13	5.55***
Aerobic scope (VO_2max/VO_2rest)	5.6 ± 0.3	6.6 ± 0.3	2.78*
Blood lactate (mM)	4.17 ± 0.56	5.17 ± 0.41	-1.45
Lactate increase (mM/min)	0.56 ± 0.09	0.36 ± 0.05	1.47

Significance difference between columns at 5%*, 1%** and 0.1%*** levels

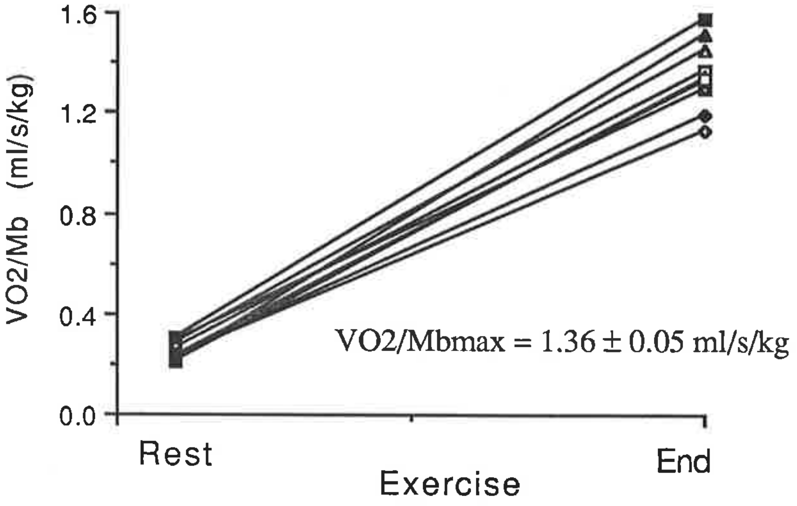

Figure 7.15a Mass-specific oxygen consumption at rest and end of 7% inclined treadmill exercise.

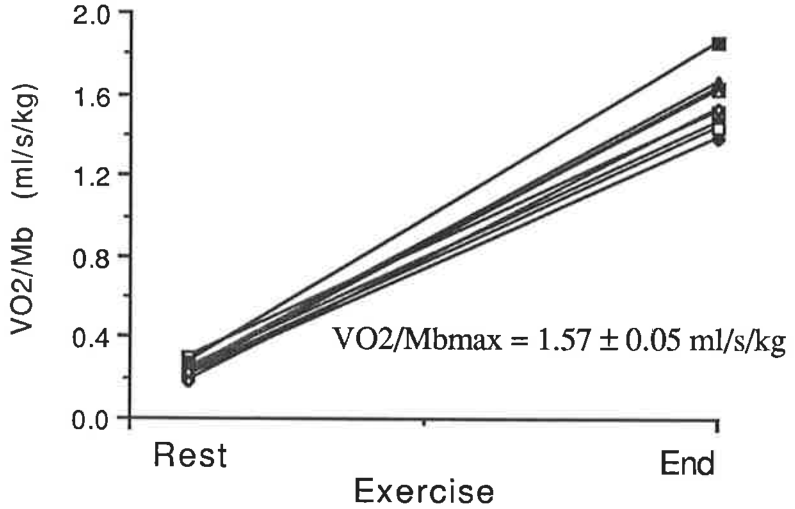

Figure 7.15b Mass-specific oxygen consumption at rest and end of 16% inclined treadmill exercise.

Table 7.14 Repeated tests of mass-specific maximal oxygen consumption at fixed treadmill grades.

Lamb (n = 6)	Slope (%)	Test VO2 max. ml/s/kg	Retest VO2 max. ml/s/kg
1	7	1.18	1.13
2	7	1.30	1.33
3	16	1.64	1.52
4	16	1.46	1.44
5	16	1.66	1.63
6	16	1.86	1.93
Mean		1.52 ± 0.10*	1.49 ± 0.11*

* Not significant at 5% level.

VO_2/Mbrest, exhibited by the lambs during the first and repeat tests were also similar (5.8 ± 0.6 vs. 6.1 ± 0.6) and highly correlated ($r = 0.89$, $p < 0.018$).

7.4 DISCUSSION

7.4.1 Resting metabolic and physiological values

Reported resting and maximal metabolic responses for animals of similar body mass vary considerably. Part of the variation is due to interspecific differences but the different techniques employed in the determination may also result in varying metabolic responses. Furthermore, it is difficult to obtain resting data while the lamb is under some sort of stress associated with the pre-treatment preparation for treadmill exercise and cold exposure tests. Indeed a "stress-free" lamb in a laboratory situation may be a difficult concept. However resting the lambs for 30 min prior to measurements in the present study, and taking the minimal sustained VO_2 over a period of 15 min, was an attempt to minimize the stress influencing the values. The metabolic rates obtained in the present study prior to the varying speed test (0.29 ± 0.01 ml/s/kg) and interval exercise test (0.32 ± 0.02 ml/s/kg) were higher than the previously reported values of 0.11 to 0.14 ml/s/kg for neonatal lambs and calves measured in a water bath (Robinson and Young, 1988; Young et al., 1988), as well as the 0.16 ml/s/kg obtained for Welsh Mountain crossbred lambs within an hour of birth (Slee et al., 1990). Mass-specific pre-exercise values were considerably higher than that of 0.06 to 0.1 ml/s/kg recorded for Yucatan miniature swine (Ciccone et al., 1982). The present values were similar to that obtained for 18 to 52 h old lambs tested in a water bath at 38.5 ^{o}C by Slee et al. (1990), but slightly lower than the 0.32 ml/s/kg reported by Alexander et al. (1983) for neonatal lambs exposed to thermoneutral conditions in a respiratory chamber.

The pre-exercise values of VO_2 in the present study, although generally higher in comparison to those for lambs tested in water, are nevertheless, lower than those achieved with more traditional methods involving the animal standing in an air environment at thermoneutrality (Alexander et al., 1983). The differences may be attributed to the support provided by the lap of the assistant in the present study, thus reducing significantly the voluntary muscular activities in the lambs. In the case of the water bath tests, the buoyancy effect of the water was likely to have contributed to the more relaxed state of the lambs as the skeletal muscles did not have to support the animal against gravitational force (Young et al., 1988). Variations in the resting metabolism that may result from differences in nutritional status is illustrated by the work of Slee et al. (1990) in a comparative study involving lambs that had sucked for several hours and those prevented from sucking for about 4 h where fasting significantly depressed resting metabolism. It should be stressed that the present measurements are those of "resting" animals and do not meet the requirement of basal or standard metabolic rate determinations.

7.4.2 Oxygen consumption as a function of speed of locomotion

The linear relationship between metabolic rate and the speed of locomotion (r = 0.84 to 0.90) is consistent with the findings involving other species of quadrupeds (Fedak and Seeherman, 1979. The slope of the regression equations relating the various measures of metabolic rates to the speed of locomotion in the present study indicate that on the average, increases of about 2.0 ml/s, 0.4 ml/s/kg and 7.4 J/s/kg in Vo_2, Vo_2/Mb and Emetab/Mb, respectively occurred for each increase of 1.0 m/s in speed. The slope relating the Vo_2/Mb and speed (0.37) is similar to that recorded for a 2.6 kg dog by Taylor et al., 1970) and a tammar wallaby of similar body mass as the present lambs running at speeds below 1.6 m/s (Baudinette et al , 1987). It is similar to the value of 0.32 predicted for a 5.1 kg adult animal by the equation of Taylor et al. (1982). This is the first time that a neonatal animal has been compared to the allometric equation.

The y-intercept of the regression line relating Vo_2/Mb to the speed of locomotion in the lambs, the extrapolated zero running speed, had a value of 0.36 ml/s/kg and was higher than the observed resting metabolism of 0.30 ± 0.01 ml/s/kg. The discrepancy in these values is consistent with that found in other species of animals and human subjects. The differences range from 0.05 to 0.39 ml/s/kg in a 2.6 kg dog to a 21 g white mouse in the study of Taylor et al. (1970), and 0.28 ml/s/kg in tammar wallaby (see Baudinette et al., 1987). Resting on a treadmill or sitting passively on a bicycle ergometer yields a resting metabolic rate close to the y intercept, suggesting that the differences observed represent a postural cost of locomotion (see Schmidt-Nielsen, 1977). It has been estimated as 13.5 W/kg between standing and lying in 273 kg cattle (Vercoe, 1973).

The non-significant difference in VO_2 during the 3 intermittent treadmill exercise bouts confirms the similarity of work intensity; a level which was about 60% of the aerobic capacity of the lambs. The 2-fold increase in VO_2 over resting levels achieved by the lambs was however lower than the 5.6 fold increase in dogs subjected to exercise at 50% VO_2max (Wagner et al., 1977) and the 4.6 fold increase in pigs at 30 % VO_2max during 40 min continuous exercise.

7.4.3 Energy cost of locomotion

The curvilinear relationship between a measure of efficiency of locomotion, energy use per mass per distance, showed a decrease with increasing speed and approached a minimum value towards the higher end of the speed. This pattern reflects that seen in other mammals (see Taylor et al., 1970). The lambs could not maintain position at speeds greater than 2.34 m/s, but at these speeds the value of the cost of transport appeared to be minimal.

7.4.4 Stride frequency and length as a function of of speed of locomotion

Increases in the frequency and length of stride are the only variables which actively contribute to an increase in an animal's running speed (see Heglund et al., 1974; Taylor, 1977; Baudinette et al. 1987). In the current study the lambs initially responded to increased treadmill speed by changing both stride frequency and length. Whereas the relationship between stride length and speed was linear, frequency changed in a curvilinear way with speed, indicating a proportionally lesser contribution to speed increase.

A simple linear relationship between stride length and speed has been shown in a number of animals species ranging from a 9 g mouse to 500 kg horses. However a plateau effect of stride frequency is common in many species; the animal's locomotory system acting as an oscillator. The most pronounced example occurs in the hopping kangaroo (see Heglund et al,. 1974; Baudinette et al., 1987).

7.4.5 Heart frequency effects of locomotion

The positive linear relationship seen between heart frequency and the speed of locomotion in lambs in the present study is consistent with that seen in other mammals (Wagner et al., 1977; Thomas and Fregin, 1981; and Faraci et al., 1984; Baudinette, 1978). Heart frequency was, however, independent of the 3 discrete speeds of locomotion at which the lambs ran (Table 7.5). In such an early stage of life, coupled with the unfamiliarity of the exercise protocol, fright and excitement may affect the heart rate achieved, particularly in the pre-exercise period and at lower speeds of locomotion (see 1969; Hays, 1976; Baudinette et al., 1978).

Since a transitory overshot of heart frequency may occur at the commencement of running, or when the running speed is suddenly changed, a prolonged duration of exercise should ensure a steady state heart frequency (Donald and Ferguson, 1966). In the study of Faraci et al. (1984) involving domestic pigs subjected to progressive exercise from 1.0 to 1.8 m/s , steady state heart frequencies were achieved within 2 min of exercise at each speed level. However in the present study, heart rate was sometimes still increasing 3 min into the exercise period in the few cases where attempts were made to measure it during locomotion. Since the exercise duration on the average exceeded 5 min, heart rate at the end of exercise should represent steady frequencies reached in the lambs (see Donald and Ferguson, 1966; Thomas and Fregin, 1981).

A linear relationship between heart rate and VO_2 has been reported for several mammalian species including man (Astrand et al., 1973; Donald and Ferguson, 1966;

Wagner at al., 1977; Brooks and White, 1978; Faraci et al., 1984). Although both metabolic and heart rates increased as a function of speed of locomotion in the present study, neither simple linear nor partial correlation tests showed significant relationship between the two. Donald and Ferguson (1966) cautioned the effectiveness of heart frequency as an indicator of work intensity where fear and excitement, resulting from unfamiliarity of the animal to the exercise procedure are involved.

The increase in heart frequency over resting values during laneway exercise in the present study was double the rise of 16% seen in the treadmill exercise. Both percentage increases are however lower than the increase of 133% observed in human subjects during mild intermittent exercise at 50% VO_2max, but similar to values observed at 25% VO_2max (Edwards et al., 1973). Whereas in lambs heart rate returned to the resting value by 5 min after the treadmill exercise, normalisation did not occur in the laneway exercise until 15 min after work. The reduction in heart frequency after exercise is similar to that seen in pigs subjected to 1.0 m/s at 3% incline, where a 2-fold increase over resting level was achieved by 5 min of exercise (Faraci et al., 1984).

7.4.6 Body temperature effects of locomotion

Body temperature, measured as rectal temperature, increased only slightly as a function of speed of locomotion in the present study, suggesting an efficiency of temperature regulatory mechanisms to dissipated most of the heat produced to the environment, thus allowing the lambs to maintain near constant body temperature and avoid overheating and hyperthermia (See Buskirk 1977; Astrand and Rodahl, 1986). Nevertheless, some metabolic heat was stored resulting in a slight rise in body temperature.

The significant elevation in rectal temperature elicited in the lamb by both the treadmill and laneway exercises (1.0^{o}C) in the present study was higher than the 0.3°C rise reported for calves subjected to exhaustive exercise (Kulhmann et al., 1985). If we assume a tissue specific heat of 3.47 kJ/(Kg ^{o}C), a 1.0^{o}C rise in body temperature at the most stressful test in the series of experiment, running up a 16^{o} slope for 9.4 min (Table 7.13) only results in the storage of 16% of the total heat produced. In a study of heat dissipation strategies, Taylor and Rowntree (1973) found that dogs and primates adopted the pattern seen in lambs and increased physical heat exchange during exercise. Cheetah and gazelle on the other hand, stored up to 80% of the total heat production and consequently were limited by body temperature in the distance they could run. The latter category has been termed "sprinter" as opposed to the "staying" capabilities of animals which dump heat. It would appear that lambs are in the latter category and the distance they can run is not limited by rises in body temperature. Panting in particular serves an important means of heat loss in lambs and was always present at the conclusion of treadmill and laneway tests.

7.4.7 Respiratory frequency as a function of stride frequency

The regression equation describing the relationship between respiratory and stride frequencies in the present study showed significantly high correlation (0.940), suggesting that the 2 variables tended to be phase locked in a 1:1 ratio. This coupling of breath per stride is consistent with that reported for jack rabbits, dogs and horses (Bramble and Carrier, 1983) and tammar wallaby (Baudinette et al., 1987). It has never been examined previously in neonate animals in which high growth and metabolic rate may have been expected to compromise the relationship. Iscoe (1981) observed clear entrainment between respiratory and stepping frequencies in a third of cats walking on a treadmill between speeds of 0.31 to 1.67 m/s, while coupling in the remainder was either very weak or absent.

Higher incidence in coupling of respiratory rhythm and stride frequencies in human subjects has been observed during running than walking (80 vs 53 %). Humans, which alone among modern eutherians utilize a bipedal gait, differ from quadrupeds by employing several phase-locked patterns of respiratory and stride frequencies (4:1, 3:1, 2:1, 1:1, 5:2 and 3:2). They tend to use the predominant coupling ratio of 2:1 over a wide range of speeds and stride frequencies, changing to 4:1 ratio at lower running speeds. In both quadrupeds and humans, a shift in coupling ratios may occur quickly and smoothly over just a few strides Bechbache and Duffin (1977). Bramble and Carrier (1983) suggested that coupling of respiratory and stride frequencies indicates the existence of neural circuitry linking the 2 variables, and changes in the ratio appear to be triggered by involuntary stimulii.

7.4.8 Maximal metabolic rates

The most commonly used technique for eliciting maximal metabolism in lambs and calves involves the use of cold air with or without wind (Alexander, 1962; Alexander and Bell, 1975), and more recently, water progressively cooled from thermoneutrality to about 18 °C (Slee et al., 1980; 1987; 1990; Earles and Small, 1985; Robinson and Young, 1988; Young et al., 1988). Other workers have used helium-oxygen mixtures in order to increase thermal conductance but these techniques have not been attempted on domestic animals, probably because of their relatively large size. These cold induced summit metabolic techniques give repeatable results without impairing the subsequent health of the animals. The VO_2max values obtained from those techniques may often differ from that resulting from treadmill exercises since the voluntary activities of the skeletal muscles in the former are insignificant, despite some limited amount of struggling that occurs in the water bath.

The VO_2max for the neonatal lambs determined as summit metabolism, 0.77 to 1.04 ml/s/kg (15.47 to 20.90 W/kg) in the studies of Alexander (1962), Alexander and Bell (1971) and Young et al. (1988) are significantly lower than the mean values of 1.39 ± 0.07 and 1.5 ± 0.05 ml/s/kg elicited by treadmill exercise in the present study. These values are however, significantly lower than the 2.6 and 2.8 ml/s/kg peak metabolic rate in Welsh mountain lambs tested within 1 to 52 h after birth by the fast cooling of a water bath (Slee et al., 1990). We have no information as to whether the Welsh animals have a higher capacity for VO_2max or if the difference is technique based.

In a comparative study of VO_2max, Seeherman et al. (1981) found that significantly lower VO_2max was elicited by cold exposure than treadmill exercise techniques ($p < 0.001$) in pygmy mice, chipmunks and white rats. They indicated that the cold exposure technique is limited to small animals in which conductance can be maximized by cold. The treadmill procedure provides a clearer experimental end point for VO_2max and explained the difference between the present data and summit metabolism (Alexander, 1973; Young et al , 1988; Robinson and Young, 1988; Slee et al., 1990).

In comparing the results from the present study with the survey of anaerobic capacity and VO_2max in mammals by Seeherman et al. (1981), the major difference is the slow rate of lactate production and the end concentration of lactate. In most mammals, end concentration of around 20 mM lactate are common at VO_2max. In lambs the level is about one quarter of this. Similarly the maximum rate of lactate production in animals about the same size of lambs is about 6 to 8 mM/min. In lambs it is about 10% of this rate of production. The conclusion is that lambs do not have the anaerobic capacity that adult mammals possess. Whether this is a common pattern in neonatal mammals would be an interesting question.

7.4.9 Aerobic scope of metabolic rates

An aerobic scope represented by the 5.6 and 6.6- fold increase in Vo_2 over the pre-exercise value by the end of the exercise protocols at 7% and 16 % slope, respectively, was similar to that of the lambs studied by Young et al. (1988), but higher than that obtained by Robinson and Young (1988), Alexander (1973) and Slee et al. (1990) by cold exposure.

In a comprehensive study of maximum aerobic capacity in mammals, Taylor et al. (1980) showed that the maximum rate of oxygen consumption is nearly a constant multiple of 10 times the resting metabolic rate and scales with body mass to the 0.75 power. A significant finding of this study was the wide range of maximal levels for animals of similar body mass, with domestic animals tending to the lower end of the range. The values for lambs suggest that these animals are at the lower extreme. Again the problem with any further generalisation is that the allometric equations in which we have such comparisons are derived from adult animals and their extension to neonates may be unwarranted.

7.4.10 Comparison of metabolic and physiological responses to seven and sixteen per cent inclined exercise protocols

Comparison of the results in the 2 treadmill exercise protocols is difficult in view of the compounding effect of training and differing slopes. However, the generally different physiological responses to the 2 protocols is consistent with that reported in the literature for trained and untrained subjects, or where aerobic capacity is determined at different treadmill grades. The slightly higher but non significant difference in VO_2 max at 16 % compared to that at 7 % (9.53 ± 0.60 vs 8.45 ± 0.57 ml/s) is in agreement with the findings in humans (Hermansen and Saltin, 1969; Kasch et al., 1976) . Nevertheless, the present exercise protocols produced significant differences in VO_2max per unit body mass, while those of Kasch et al. (1976) were not significant. The increase in VO_2max with corresponding increase in treadmill slope is clearly illustrated by the study of Ciccone et al. (1982) in which exercise at a speed of 2.7 m/s at treadmill grades of 0, 6 and 8% had increasing values of 0.36, 0.45 and 0.48 ml/s/kg respectively.

Numerous studies have shown that training tends to elevate VO_2max in animals and human subjects. Bedford et al. (1979) demonstrate this effect in rats by exercising one group for 10 days while the other was untrained. Maximal rates of oxygen consumption were 0.98 vs 1.24 ml/s/kg in male and 1.35 vs 1.50 in female untrained and trained rats respectively. The main objective of training is to get the animals accustomed to the treadmill. However, training of animals in the use of treadmill is normally over 2 weeks, a time which may lead to improvement in physical fitness in the animals. The greater mitochondrial density of their muscles would demand greater amounts of oxygen than their untrained counterparts (Seeherman et al., 1981). The short duration of the training period in the present study was unlikely to have had any significant effect on the mitochondrial densities in the lambs. Mitchell and Blomqvist (1971) demonstrated that a decrease in physical activity in man caused a prompt fall in VO_2max, with bed rest resulting in 20 to 25% drop below control values. He asserted that any increase in habitual physical activity of moderately heavy intensity involving large muscle groups will be expected to produce some change in VO_2max.

7.4.11 Repeatability of maximal oxygen consumption

The significantly high repeatability of the $VO_2/Mbmax$ in the lambs tested twice is consistent with the results of repeated tests at horizontal and inclined treadmill running in human subjects (Kasch et al., 1976). Similar identical results were also obtained in treadmill and step test procedures involving the same subjects, which gave a significantly high correlation of 0.95. The greatest difference in VO_2max between the same individual in the present study (0.11 ml/s/kg), although representing only 7% of the mean aerobic capacity of the lamb, was 2.8 times the reproducibility limits of 0.04 ml/s/kg (2.4 ml/min/kg) suggested by Taylor and co-workers (see Kasch et al, 1966).

7.5 CONCLUSION

In comparing metabolic responses of lambs to exercise with other animals we are restricted to allometric equations derived from adults, and little is known about the performance of neonates. However in the rate that they increment power to increase speed, and their dependance on stride length rather than frequency, lambs are similar to other species. In their aerobic scope they fall below the factor of 10 seen in many other mammals, but are grouped with other domestic species. One peculiarity is their low level of lactate production when all other signs suggest they have reached a maximal aerobic level. The finding should be further investigated in other neonatal mammals. In summary there is no indication in metabolic performance during locomotion to suggest a limit in following ability of neonatal lambs in the field situation.

8. GENERAL DISCUSSION

8.1 BEHAVIOURAL STUDIES

The ewe's ability to discriminate between her own and alien lamb(s) is generally well developed during the early post-partum period (Smith et al., 1966; Morgan et al., 1975). This early recognition ability of the parturient ewe was latter confirmed in a study by Poindron and Le Neidre (1980) in which ewes successfully recognised their offspring from alien lambs after a brief period of contact during the first 2 h following birth. The above findings led to the concept that the ewe plays the major role in the development of the mother-young bond, and that the lamb does not contribute much to the establishment of the bond. Nowak (1990). has recently criticised the conclusion and believes that the earlier studies underestimated the learning ability of the newborn lamb.

The proportion of lambs in the present study that exhibited high behavioural concern to being separated from their mothers at a distance of 7 m (25/43), and were able to discriminate between their own mothers and alien ewes in a 2-choice situation (38/43) in respective of the initial ewe approached, supports the contention that a high proportion of lambs are able to recognise a ewe as alien on the first day of life. This work supports the idea that a significant contribution is made by the new-born lamb to the establishment of mother-young mutual recognition (Nowak et al.,1987; Nowak, 1990). The lack of significance difference between the time taken in making an initial choice of a dam or an alien ewe (4.8 ± 0.3 s vs. 6.0 ± 0.9 s), and the number of lambs making the respective choices (13 vs. 14) in the present study suggests that distance recognition may not have been developed well enough in about a third of the lambs to enable them to successfully recognise their mothers from a distance of 5 m. In a field situation such lambs may respond at first sight to any ewes emitting high-pitched bleats before exhibiting discriminating behaviour when closer. This finding tends to support the contention that visual cues may not be effectively used at a distance by the new born lamb (Shillito Walser et al., 1980). Furthermore, the tendency of the lambs to respond to auditory cues from both their own mothers and alien ewes during separation and at the release of the lambs for the 2-choice test supports the suggestion that the ewe's bleats serve only as location signals at a distance. Nevertheless, involvement of auditory cues in enhancing the lamb's ability to recognise a ewe as a partner was clearly shown by the low-pitched, rumbling sounds from the mother when reached by the lamb.

Most of the lambs in the present study were able to follow and keep close to their mothers in the laneway, despite the fact that the speed of following was generally higher than what is normally observed in a field situation. The lamb's desire to follow and catch up with its mother when left behind, and the ewe's willingness to wait for her lamb, indicates establishment of mutual attachment in most ewe-lamb pairs. This, coupled with the proximity of ewe-lamb pair observed during the periodic sightings in the simulated semi-arid conditions, suggests that the neonate has the ability to follow and keep in close contact with the mother while she moves away in search of food and water over long distances as pertains in the inland pastoral regions of Australia.

The general relationship between the lambs' ability to recognise their mothers in the experimental 2-choice situation and their following response suggests that lambs with superior discriminating ability of ewes stand a better chance of successfully following their mothers over long distances without being separated. This will lead to a higher probability of these lambs obtaining adequate nourishment and protection, thus enhancing their chances of surviving the neonatal period. The negative relationship existing between a lamb's discriminating ability of ewes and the frequency of high-pitched bleats it emitted further demonstrates the positive influence of the degree of a lamb's attachment to the mother on its following response, since high-pitched bleats from the lamb during the present study tended to suggest a non recognition of a ewe as the mother or a poor performance in following.

Neither sex nor body mass of the lambs exerted significant influence on either the lamb's ability to recognise a ewe as alien or its ability to follow its mother. Nevertheless, female as well as medium sized lambs, weighing 4.7 to 5.8 kg at 18 h of age, tended to follow their mothers better, particularly in terms of the time taken to complete the 1.0 km distance. The non-significant effect of the sex of lamb on following-response may be attributed to the similar body mass in both sex types.

The restriction imposed on the lateral movements of the animals by the narrow laneway might have reduced the need to rely on senses of recognition as an aid to the following-ability. The involvement of auditory cues in inducing the lamb to follow was however clearly demonstrated when lambs following ewes at a distance of about 10 m often rendered immediate bleating response to the mothers' call and galloped to them. On the other hand, bleating by both ewe and lamb was nil or minimal, and at a low pitch, when the neonate was following well and close to the dam, indicating that auditory cues, as an aid to following, may be necessary only at a distance. Presumably, olfactory cues and sight were involved in proximal following. It was, however, very rare to see a ewe and her lamb sniffing each other during the test. Nevertheless, the high-pitched bleats of the ewes when the lambs were not following well might have served concurrently as location and stress signals. Auditory cues may assume greater importance in the field situation, where lambs following ewes at a distances would be more prone to separation. However, the lambs' ability to gallop and keep up with the ewes, if sustained, despite the repeated unconcerned behaviour in some of the ewes in this study, would enhance the maintenance of proximal attachment to their mothers in the field situation.

The shorter gestation time in twin-born lambs suggests that they are less mature at the time of birth than single-born lambs, hence their delay or lower ability to discriminate between ewes. This finding is consistent with that of Nowak (1989). Winfield and Kilgour (1976) found that younger lambs quickly become fatigued and showed weak following responses while attempting to keep up with a moving surrogate ewe. Furthermore, twins differ from singles because each lamb follows not only its mother, but a sibling as well (Nowak, 1989). Since the twins had to follow their mothers one at a time in the present study, the absence of the sibling might also have contributed to their weaker performance.

The stronger following response observed in the single-born lambs compared to twin-born lambs is most likely influenced by the superior discriminating ability of ewes.

8.2 PHYSIOLOGICAL STUDIES

The energy expenditure of the lambs under simulated semi-arid conditions estimated by the doubly-labelled water (DLW) technique in the present study, 1,320 kJ/d, was comparable to that of human infants of similar body mass (Freymond et al., 1986; Lucas, 1987; Wong et al., 1987). This is a surprising finding in view of the precocity of the newborn lambs and their higher muscular activity as a consequence of the considerable daily movement distance. The energy expenditure in the lambs determined by this method is also similar to the predicted value of 1 370 kJ/d from The Australian Agricultural Council (1990). In the interior pastoral regions of Australia a higher energy demand would be necessary to meet the increased thermogenesis sustainable in the cold winter nights of the lambing season (B.C.Jeffries, personal communication).

Despite the high correlation seen between the pedometer reading and the distance walked on the treadmill, there is a need for caution in employing a pedometer in the determination of distance travelled by animals in undulating or hilly terrain, or in situations where the animal undergoes frequent charges in speed of locomotion. Any of these factors influence the stride length and consequently the pedometer reading. In the present study, the level terrain and the periodic sightings of flock location in the paddock simulating semi-arid conditions suggest that the 2.3 km daily movement distance estimated for the lambs is representative of the true distance covered. This value approximates the 2.1 km daily movement distance predicted for ewes, the primary determinants of the neonates' distance travelled.

In nature, many animals travel at speeds which elicit close to their maximum rate of oxygen consumption. This has led to the general assumption that the energetic cost of locomotion in animals is high, and hence of considerable energetic and selective importance in view of the fact that VO_2 max is generally about 10-fold greater than the resting metabolism in mammals (see Garland, 1983). In the present study, the lambs were capable of treadmill speeds from 0.15 to 2.43 m/s, the range likely to be employed by the lamb in a field situation. Walking was "clumsy" towards the lower limit of the range, but the lambs tended to be more "comfortable" at speeds beyond 0.8 m/s. This probably is within the preferred speed range of the animal. The energy cost of locomotion as a proportion of the total energy demand, 1.4 %, is similar to predicted values (Garland, 1983; Altmann, 1987) and amounts to approximately half the predicted value for the ewes. The cost of locomotion in the neonates confirms the assertion that daily movement generally has little consequence for the overall energy usage in mammals (Altmann, 1987; et al., 1989).

The levels of blood metabolites during exercise at any time depend on the balance between the rate of production and uptake of substances from the blood particularly by the active muscles. This is in turn influenced by the intensity and duration of the exercise. In the present study, blood sampling was undertaken once prior to exercise, at the end of each exercise bout and at specific intervals during the 30 min recovery period. The changes observed in the concentration of the blood metabolites, particularly lactate, in conjunction with the rate of oxygen consumption provided some useful information on the energy metabolism in the lambs during exercise.

The slight but significant increase in blood lactate and pyruvate observed, both during treadmill exercise and laneway walking, is consistent with that observed in Hereford calves subjected to moderate treadmill exercise by Kuhlmann et al., (1985). The rather low increase in blood lactate concentration elicited by the exercise in most of the lambs was not unusual since the exercise was only 60% the aerobic capacity. The few lambs that showed high lactate concentrations in the blood, particularly during the laneway exercise, tended to follow their mothers at a higher speed than the group average. The slight increase in blood lactate concentration during exercise, coupled with a decrease of about a third of the exercise level of the metabolite 15 min after exercise, suggests a good balance between production and removal of lactic acid from the blood stream of the neonates. A similar effect might have occurred during the determination of maximal oxygen consumption in the lambs, resulting in a less than expected rise in blood lactate by the end of the exercise (see Seeherman et al., 1981). Alternatively the neonatal animals may have a low anaerobic capacity for exercise.

The effect of interval exercise and prolonged exercise in the present study is not clear. Nevertheless, blood lactate elevation during the intermittent exercise was lower than when work continued for 15 min, despite the fact that the work intensity and total duration in both exercise types were similar. This finding is similar to that of Essen et al. (1977). The decreased oxygen demand during the rest pauses in the intermittent exercise might have been sufficient to reduce the oxygen debt and thus limit the measured lactate levels in blood. The metabolic and physiological effects of both the treadmill and laneway exercises, especially the small changes in heart frequency, blood lactate and pyruvate concentrations, confirm that the work imposed on the lambs during the exercises was at a submaximal level, and suggests that most running activities in neonatal lambs are undertaken by primarily utilizing aerobic pathways, with only a minor contribution from anaerobic pathways of metabolism. There appeared to be no metabolic or physiologic limits apparent in following-behaviour.

The linear relationship between metabolic rate and speed of locomotion observed in the lambs is consistent with that seen in other quadrupedal species (Mitchell and Blomqvist , 1981; Shepherd and Gollnick, 1976; Bedford et al., 1979; Taylor et al., 1982; Baudinette, 1989). The lambs initially responded to increased treadmill speed by changing both stride frequency and length. Whereas the general relationship between stride length and speed was linear, frequency however changed in a curvilinear way with speed, indicating a proportionally lesser contribution to speed increase. This may result in a decreased cost of locomotion as speed increases, a probable advantage in escaping from mammalian predators. The reduction in the cost of locomotion with increasing speed is confirmed by the relationship describing the energy use per mass per distance moved which decreased with increasing speed and approached a minimum value towards the highest speed (see Taylor et al., 1970). An important finding from the treadmill study is that the allometric equations of Taylor's group predict the cost of locomotion in lambs. This is the first time that neonatal animals have been compared to the model.

Treadmill exercise seemed to be a more reliable technique for eliciting maximal oxygen consumption in the neonatal lambs than cold-induced techniques, either by cold air (Alexander, 1962; Alexander and Bell, 1975) or water progressively cooled from thermoneutrality (Slee et al.,1980; 1987; 1990). The cold-induced summit metabolism is usually lower than VO_2max resulting from treadmill exercise since the later involves maximal skeletal activities and consequently greater oxygen demand. The different VO_2max values from the 2 techniques was confirmed by Seeherman et al. (1981) in a study with mice, chipmunks and rats. Furthermore, the treadmill procedure provided a clear experimental end point for VO_2max since further increase in work intensity leads to increased lactate production without any increase in VO_2.

There are difficulties in comparing various measurements of metabolic scope due to the difference in assessing resting metabolic levels. In the present study the difficulty in obtaining resting VO_2 data while the lamb was under some sort of stress associated with the pre-treatment preparation might have led to lower values of aerobic scope of 5.5 and 6.6 achieved by the "untrained" and "trained" lambs in the present study. However, the aerobic capacities of the 2 groups of lambs, 1.39 and 1.5 ml/s/kg, were within the range of values determined for many mammals by Seeherman et al. (1981). The speed of running and the time taken by lambs to achieve VO_2max suggest that healthy 5-day old lambs would be able to undertake sustained, near-maximal speed long enough to at least provide a chance of avoiding a predator. Again, there is no real comparable data on VO_2 max levels from treadmill studies available for other neonatal mammals.

8.3 CONCLUSION

These investigations suggest that the ability of the lamb to follow its mother during the neonatal period may have significant influence on early lamb mortality, especially where the ewe has to travel over long distances in her quest for food and water.

During following, the lamb's ability to recognize the mother enhances its chances of maintaining close proximity with the ewe, and in the process obtain nourishment and protection. Other than in a few situations as when the lamb has to undergo exhaustive running while escaping from predators, running activities in the lamb are undertaken at submaximal levels, with energy being provided primarily by aerobic pathways. The lamb is however capable of sustained near-maximal running speed for about 5 to 15 min, a period long enough to enable a healthy lamb escape with the flock form an attack. Despite the fact that the neonatal lamb sometimes follows the mother over long distances, the cost of locomotion as a proportion of the total energy demand in the lamb is low. In the balance between the influence of physiological capacity and ewe-lamb behaviour, it would appear that the latter is the more important factor in determining a cause for early lamb mortality.

8. GENERAL DISCUSSION

8.1 BEHAVIOURAL STUDIES

The ewe's ability to discriminate between her own and alien lamb(s) is generally well developed during the early post-partum period (Smith et al., 1966; Morgan et al., 1975). This early recognition ability of the parturient ewe was latter confirmed in a study by Poindron and Le Neidre (1980) in which ewes successfully recognised their offspring from alien lambs after a brief period of contact during the first 2 h following birth. The above findings led to the concept that the ewe plays the major role in the development of the mother-young bond, and that the lamb does not contribute much to the establishment of the bond. Nowak (1990). has recently criticised the conclusion and believes that the earlier studies underestimated the learning ability of the newborn lamb.

The proportion of lambs in the present study that exhibited high behavioural concern to being separated from their mothers at a distance of 7 m (25/43), and were able to discriminate between their own mothers and alien ewes in a 2-choice situation (38/43) in respective of the initial ewe approached, supports the contention that a high proportion of lambs are able to recognise a ewe as alien on the first day of life. This work supports the idea that a significant contribution is made by the new-born lamb to the establishment of mother-young mutual recognition (Nowak et al.,1987; Nowak, 1990). The lack of significance difference between the time taken in making an initial choice of a dam or an alien ewe (4.8 ± 0.3 s vs. 6.0 ± 0.9 s), and the number of lambs making the respective choices (13 vs. 14) in the present study suggests that distance recognition may not have been developed well enough in about a third of the lambs to enable them to successfully recognise their mothers from a distance of 5 m. In a field situation such lambs may respond at first sight to any ewes emitting high-pitched bleats before exhibiting discriminating behaviour when closer. This finding tends to support the contention that visual cues may not be effectively used at a distance by the new born lamb (Shillito Walser et al., 1980). Furthermore, the tendency of the lambs to respond to auditory cues from both their own mothers and alien ewes during separation and at the release of the lambs for the 2-choice test supports the suggestion that the ewe's bleats serve only as location signals at a distance. Nevertheless, involvement of auditory cues in enhancing the lamb's ability to recognise a ewe as a partner was clearly shown by the low-pitched, rumbling sounds from the mother when reached by the lamb.

Most of the lambs in the present study were able to follow and keep close to their mothers in the laneway, despite the fact that the speed of following was generally higher than what is normally observed in a field situation. The lamb's desire to follow and catch up with its mother when left behind, and the ewe's willingness to wait for her lamb, indicates establishment of mutual attachment in most ewe-lamb pairs. This, coupled with the proximity of ewe-lamb pair observed during the periodic sightings in the simulated semi-arid conditions, suggests that the neonate has the ability to follow and keep in close contact with the mother while she moves away in search of food and water over long distances as pertains in the inland pastoral regions of Australia.

The general relationship between the lambs' ability to recognise their mothers in the experimental 2-choice situation and their following response suggests that lambs with superior discriminating ability of ewes stand a better chance of successfully following their mothers over long distances without being separated. This will lead to a higher probability of these lambs obtaining adequate nourishment and protection, thus enhancing their chances of surviving the neonatal period. The negative relationship existing between a lamb's discriminating ability of ewes and the frequency of high-pitched bleats it emitted further demonstrates the positive influence of the degree of a lamb's attachment to the mother on its following response, since high-pitched bleats from the lamb during the present study tended to suggest a non recognition of a ewe as the mother or a poor performance in following.

Neither sex nor body mass of the lambs exerted significant influence on either the lamb's ability to recognise a ewe as alien or its ability to follow its mother. Nevertheless, female as well as medium sized lambs, weighing 4.7 to 5.8 kg at 18 h of age, tended to follow their mothers better, particularly in terms of the time taken to complete the 1.0 km distance. The non-significant effect of the sex of lamb on following-response may be attributed to the similar body mass in both sex types.

The restriction imposed on the lateral movements of the animals by the narrow laneway might have reduced the need to rely on senses of recognition as an aid to the following-ability. The involvement of auditory cues in inducing the lamb to follow was however clearly demonstrated when lambs following ewes at a distance of about 10 m often rendered immediate bleating response to the mothers' call and galloped to them. On the other hand, bleating by both ewe and lamb was nil or minimal, and at a low pitch, when the neonate was following well and close to the dam, indicating that auditory cues, as an aid to following, may be necessary only at a distance. Presumably, olfactory cues and sight were involved in proximal following. It was, however, very rare to see a ewe and her lamb sniffing each other during the test. Nevertheless, the high-pitched bleats of the ewes when the lambs were not following well might have served concurrently as location and stress signals. Auditory cues may assume greater importance in the field situation, where lambs following ewes at a distances would be more prone to separation. However, the lambs' ability to gallop and keep up with the ewes, if sustained, despite the repeated unconcerned behaviour in some of the ewes in this study, would enhance the maintenance of proximal attachment to their mothers in the field situation.

The shorter gestation time in twin-born lambs suggests that they are less mature at the time of birth than single-born lambs, hence their delay or lower ability to discriminate between ewes. This finding is consistent with that of Nowak (1989). Winfield and Kilgour (1976) found that younger lambs quickly become fatigued and showed weak following responses while attempting to keep up with a moving surrogate ewe. Furthermore, twins differ from singles because each lamb follows not only its mother, but a sibling as well (Nowak, 1989). Since the twins had to follow their mothers one at a time in the present study, the absence of the sibling might also have contributed to their weaker performance.

The stronger following response observed in the single-born lambs compared to twin-born lambs is most likely influenced by the superior discriminating ability of ewes.

8.2 PHYSIOLOGICAL STUDIES

The energy expenditure of the lambs under simulated semi-arid conditions estimated by the doubly-labelled water (DLW) technique in the present study, 1,320 kJ/d, was comparable to that of human infants of similar body mass (Freymond et al., 1986; Lucas, 1987; Wong et al., 1987). This is a surprising finding in view of the precocity of the newborn lambs and their higher muscular activity as a consequence of the considerable daily movement distance. The energy expenditure in the lambs determined by this method is also similar to the predicted value of 1 370 kJ/d from The Australian Agricultural Council (1990). In the interior pastoral regions of Australia a higher energy demand would be necessary to meet the increased thermogenesis sustainable in the cold winter nights of the lambing season (B.C.Jeffries, personal communication).

Despite the high correlation seen between the pedometer reading and the distance walked on the treadmill, there is a need for caution in employing a pedometer in the determination of distance travelled by animals in undulating or hilly terrain, or in situations where the animal undergoes frequent charges in speed of locomotion. Any of these factors influence the stride length and consequently the pedometer reading. In the present study, the level terrain and the periodic sightings of flock location in the paddock simulating semi-arid conditions suggest that the 2.3 km daily movement distance estimated for the lambs is representative of the true distance covered. This value approximates the 2.1 km daily movement distance predicted for ewes, the primary determinants of the neonates' distance travelled.

In nature, many animals travel at speeds which elicit close to their maximum rate of oxygen consumption. This has led to the general assumption that the energetic cost of locomotion in animals is high, and hence of considerable energetic and selective importance in view of the fact that VO_2 max is generally about 10-fold greater than the resting metabolism in mammals (see Garland, 1983). In the present study, the lambs were capable of treadmill speeds from 0.15 to 2.43 m/s, the range likely to be employed by the lamb in a field situation. Walking was "clumsy" towards the lower limit of the range, but the lambs tended to be more "comfortable" at speeds beyond 0.8 m/s. This probably is within the preferred speed range of the animal. The energy cost of locomotion as a proportion of the total energy demand, 1.4 %, is similar to predicted values (Garland, 1983; Altmann, 1987) and amounts to approximately half the predicted value for the ewes. The cost of locomotion in the neonates confirms the assertion that daily movement generally has little consequence for the overall energy usage in mammals (Altmann, 1987; et al., 1989).

The levels of blood metabolites during exercise at any time depend on the balance between the rate of production and uptake of substances from the blood particularly by the active muscles. This is in turn influenced by the intensity and duration of the exercise. In the present study, blood sampling was undertaken once prior to exercise, at the end of each exercise bout and at specific intervals during the 30 min recovery period. The changes observed in the concentration of the blood metabolites, particularly lactate, in conjunction with the rate of oxygen consumption provided some useful information on the energy metabolism in the lambs during exercise.

The slight but significant increase in blood lactate and pyruvate observed, both during treadmill exercise and laneway walking, is consistent with that observed in Hereford calves subjected to moderate treadmill exercise by Kuhlmann et al., (1985). The rather low increase in blood lactate concentration elicited by the exercise in most of the lambs was not unusual since the exercise was only 60% the aerobic capacity. The few lambs that showed high lactate concentrations in the blood, particularly during the laneway exercise, tended to follow their mothers at a higher speed than the group average. The slight increase in blood lactate concentration during exercise, coupled with a decrease of about a third of the exercise level of the metabolite 15 min after exercise, suggests a good balance between production and removal of lactic acid from the blood stream of the neonates. A similar effect might have occurred during the determination of maximal oxygen consumption in the lambs, resulting in a less than expected rise in blood lactate by the end of the exercise (see Seeherman et al., 1981). Alternatively the neonatal animals may have a low anaerobic capacity for exercise.

The effect of interval exercise and prolonged exercise in the present study is not clear. Nevertheless, blood lactate elevation during the intermittent exercise was lower than when work continued for 15 min, despite the fact that the work intensity and total duration in both exercise types were similar. This finding is similar to that of Essen et al. (1977). The decreased oxygen demand during the rest pauses in the intermittent exercise might have been sufficient to reduce the oxygen debt and thus limit the measured lactate levels in blood. The metabolic and physiological effects of both the treadmill and laneway exercises, especially the small changes in heart frequency, blood lactate and pyruvate concentrations, confirm that the work imposed on the lambs during the exercises was at a submaximal level, and suggests that most running activities in neonatal lambs are undertaken by primarily utilizing aerobic pathways, with only a minor contribution from anaerobic pathways of metabolism. There appeared to be no metabolic or physiologic limits apparent in following-behaviour.

The linear relationship between metabolic rate and speed of locomotion observed in the lambs is consistent with that seen in other quadrupedal species (Mitchell and Blomqvist , 1981; Shepherd and Gollnick, 1976; Bedford et al., 1979; Taylor et al., 1982; Baudinette, 1989). The lambs initially responded to increased treadmill speed by changing both stride frequency and length. Whereas the general relationship between stride length and speed was linear, frequency however changed in a curvilinear way with speed, indicating a proportionally lesser contribution to speed increase. This may result in a decreased cost of locomotion as speed increases, a probable advantage in escaping from mammalian predators. The reduction in the cost of locomotion with increasing speed is confirmed by the relationship describing the energy use per mass per distance moved which decreased with increasing speed and approached a minimum value towards the highest speed (see Taylor et al., 1970). An important finding from the treadmill study is that the allometric equations of Taylor's group predict the cost of locomotion in lambs. This is the first time that neonatal animals have been compared to the model.

Treadmill exercise seemed to be a more reliable technique for eliciting maximal oxygen consumption in the neonatal lambs than cold-induced techniques, either by cold air (Alexander, 1962; Alexander and Bell, 1975) or water progressively cooled from thermoneutrality (Slee et al.,1980; 1987; 1990). The cold-induced summit metabolism is usually lower than VO_2max resulting from treadmill exercise since the later involves maximal skeletal activities and consequently greater oxygen demand. The different VO_2max values from the 2 techniques was confirmed by Seeherman et al. (1981) in a study with mice, chipmunks and rats. Furthermore, the treadmill procedure provided a clear experimental end point for VO_2max since further increase in work intensity leads to increased lactate production without any increase in VO_2.

There are difficulties in comparing various measurements of metabolic scope due to the difference in assessing resting metabolic levels. In the present study the difficulty in obtaining resting VO_2 data while the lamb was under some sort of stress associated with thc pre-treatment preparation might have led to lower values of aerobic scope of 5.5 and 6.6 achieved by the "untrained" and "trained" lambs in the present study. However, the aerobic capacities of the 2 groups of lambs, 1.39 and 1.5 ml/s/kg, were within the range of values determined for many mammals by Seeherman et al. (1981). The speed of running and the time taken by lambs to achieve VO_2max suggest that healthy 5-day old lambs would be able to undertake sustained, near-maximal speed long enough to at least provide a chance of avoiding a predator. Again, there is no real comparable data on VO_2 max levels from treadmill studies available for other neonatal mammals.

8.3 CONCLUSION

These investigations suggest that the ability of the lamb to follow its mother during the neonatal period may have significant influence on early lamb mortality, especially where the ewe has to travel over long distances in her quest for food and water.

During following, the lamb's ability to recognize the mother enhances its chances of maintaining close proximity with the ewe, and in the process obtain nourishment and protection. Other than in a few situations as when the lamb has to undergo exhaustive running while escaping from predators, running activities in the lamb are undertaken at submaximal levels, with energy being provided primarily by aerobic pathways. The lamb is however capable of sustained near-maximal running speed for about 5 to 15 min, a period long enough to enable a healthy lamb escape with the flock form an attack. Despite the fact that the neonatal lamb sometimes follows the mother over long distances, the cost of locomotion as a proportion of the total energy demand in the lamb is low. In the balance between the influence of physiological capacity and ewe-lamb behaviour, it would appear that the latter is the more important factor in determining a cause for early lamb mortality.

8. REFERENCES

Agricultural Research Council. 1980. The nutrient requirements of ruminant livestock. Slough. Commonwealth Agricultural Bureaux.

Ahlborg, G., P. Felig, L. Hagenfeldt, R. Hendler and J. Wahren. 1974. Substrate Turnover during Prolonged Exercise in Man Splanchnic and leg metabolism of glucose, free fatty acids and amino acids. J. Clin. Invest. **53**: 1080-1090.

Alexander, G. 1960. Maternal behaviour in the Merino ewe. Proc. Aust. Soc. Anim. Prod. **3**: 105-114.

Alexander, G., 1961. Temperature regulation in the new-born lamb. 3 Effect of environmental temperature on metabolic rate, body temperatures, and respiratory quotient. Aust. J. Agric Res. **12**: 1132-1174.

Alexander, G.. 1962a. Temperature regulation in the new-born lamb. 5. Summit metabolism. Aust. J. Agric Res. **13** :100-121.

Alexander, G. 1962b. Energy metabolism in the starved new-born lamb. Aust. J. Agric. Res., **13**: 144-164.

Alexander, G. 1964. Lamb survival: Physiological considerations. Proc. Aust. Soc. Anim. Prod.,5:113-121.

Alexander, G. 1974. Birth weight of lambs: influences and consequences. In: *Size at birth* (Eliot, K. and Knight, J. eds.). Associated Scientific Publishers.

Alexander, G. 1977. Role of auditory and visual cues in mutual recognition between ewes and lambs in merino sheep. Appl. Anim. Ethol., 3:65-81.

Alexander, G. 1978. Odour, and the recognition of Merino lambs by Merino ewes. Appl. Anim. Ethol., **4**: 153-158.

Alexander, G. 1979. Cold Thermogenesis. In: *International Review. of Physiology Environmental Physiology III.* (Robertshaw, D. ed.). Univ. Park Press, Baltimore.

Alexander, G. 1980. Husbandry practices in relation to maternal-offspring behaviour. In: *Behavior in relation to reproduction management and welfare of farm animals* (M. Wolzick Tomaszewska. ed.).

Alexander, G. 1984. Constraints to lamb survival. In: *Reproduction in sheep* (Lindsay, D.R. and Pearce, D.T. eds). Australian Academy of Science.

Alexander, G. 1985. Physiological and behavioural factors affecting lamb survival under pastoral conditions. In: *Agriculture: factors affecting the survival of newborn lambs* (Alexander, G., Barber, J.D. and Slee, J. eds.). Commission of the European Communities.

Alexander, G. and A. W. Bell. 1975. Quantity and Calculated Oxygen Consumption during Summit Metabolism of Brown Adipose Tissue in New-Born Lambs. Biol. Neonat. **26**: 214-220.

Alexander, G., A. W. Bell, and J.R.S. Hales. 1972. The Effect of Cold Exposure on the Plasma Levels of glucose Lactate, free Fatty Acids and Glycerol and on the Blood Gas and acid-Base Status in Young Lambs. Biol. Neonat. **20**: 9-21.

Alexander, G., R. Kilgour, D. Stevens and L.R. Bradley. 1984. The effect of experience on twin-care in New Zealand Romney Sheep. Appl. Anim. Behav. Sci. **12**: 363-372.

Alexander, G. and S.C. Mills. 1968. Free Fatty Acids and Glucose in the Plasma of Newly Born Lambs: Effects of Environmental Temperature. Biol. Neonat. **13**: 53-61.

Alexander, G.and J.E. Peterson. 1961. Neonatal mortality in lambs. Intensive observations during lambing in a flock of maiden Merino ewes. Aust. Vet. J. 37: 371-381.

Alexander, G. and E.E. Shillito. 1977a. Importance of visual clues from various body regions in maternal recognition of the young in merino sheep (Ovis aries). Appl. Anim. Ethol. **3**: 137-143.

Alexander, G. and E.E. Shillito. 1977. Maternal responses in merino ewes to artificially coloured lambs. Appl. Anim. Ethol. **4**: 141-152.

Alexander, G., J.P. Signoret, and E.S.E. Hafez. 1982. Sexual, maternal and neonatal behavior. In: *Reproduction in farm Anim*als. 4th edition. (E.S.E. Hafez ed.) Lea and Febiger Philadelphia.

Alexander, G., D. Stevens. 1981. Recognition of washed lambs by Merino ewes. Appl. Anim. Ethol. **7**: 77-86.

Alexander, G., D. Stevens, and L. R. Bradley. 1988. Maternal behaviour in ewes following caesarian section. Appl. Anim. Behav. Sci. **19**: 273-277.

Alexander, G., D. Stevens, and L. R. Bradley. 1989. Maternal acceptance of alien lambs in ewes treated and untreated with oestrogen at birth. Aust. J. of Exp. Agric. **29**:173-178.

Alexander, G., D. Stevens, R. Kilgour, and H. de Langen. 1983. Separation of ewes from twin lambs: Incidence in several sheep breeds. Appl. Anim. Ethol. **10**: 301-317.

Alexander, G., D. Williams. 1964. Maternal facilitation of sucking drive in newborn lambs. Science **146**: 665-666.

Alexander, G., D. Williams. 1966. Teat-seeking activity in lambs during the first hours of life. Anim. Behav. **14**: 166-176.

Altmann, S.A. 1987. The impact of locomotor energetics on mammalian foraging. J. Zool. Lond. **211**: 215-225.

Andrews, J.F. and J.B. .Mercer. 1985. Thermoregulation in the newborn lamb: the first 36 hours. In: In: *Agriculture: factors affecting the survival of newborn lambs* (Alexander, G., Barber, J.D. and Slee, J. eds.). Commission of the European Communities.

Anonymous. 1974. Crows and lambs. Rural research in CSIRO. 14-18.

Arnold, G.W., C.A. P. Boundy, P.D. Morgan, and G.Bartle. 1975. The roles of sight and hearing in the lamb in the location and discrimination between ewes. Appl. Anim. Ethol. **1**: 167-176.

Arnold, G. W. and A. Grassia. 1985. Spatial relationships between ewes and lambs. Appl. Anim. Behav. Sci. **14**: 253-261.

Arnold, G.W. and P.D. Morgan. 1975. Behavior of the ewe and lamb at lambing and its relationship to lamb mortality. Appl. Anim. Ethol. **2**: 25-46.

Astrand, I., P.O. Astrand, I. Hallback and A. Kilbom. 1973. Reduction in maximal oxygen uptake with age. J. Appl. Physiol. **35**: 649-654.

Astrand, P. O., E. H. Christensen. 1964. Aerobic work capacity. In: *Oxygen in Animal Organisms* (F. Dickens and E. Neil eds.). Pergamon Press. Oxford.

Astrand, P. O., K. Rdahl. 1970. Test book of work physiology: Physiological basis of exercise. McGraw-Hill Book Company New York. Second edition.

Astrand, P. O., K. Rdahl. 1986. Test book of work physiology: Physiological basis of exercise. McGraw-Hill Book Company New York. Third edition.

Australian Agricultural Council.. 1990. Feeding standards for Australian livestock: Ruminant Sub-committee. CSIRO Australia.

Baldwin, B A., E.E. Shillito. 1974. The effects of ablation of the olfactory bulbs on partuition and maternal behavior in soay sheep. Anim. Behav. **22**: 220-223.

Barrie, A., W. A. Coward. 1985. A rapid analytical technique for the determination of energy expenditure by the doubly labelled water method. Biomed. Mass Spectrom.**12**: 535-541.

Basiouni, G. F., H. W. Gonyou. 1988. Use of birth fluids and cervical stimulation in lamb fostering. J. Anim. Sci. **66**: 872-879.

Baudinette, R.V. 1978. Scaling of heart rate during locomotion in mammals. J. Comp Physiol. **127**: 337-342.

Baudinette, R.V. 1989. The Biomechanics and energetics of locomotion in Macropodoidea. In: *Kangaroos Wallabies and rat Kangaroos* (Grigg,G. Jarman, P. and Hume, I. eds.). Surrey Beauty and Sons Pty. Ltd., New South Wales

Baudinette, R.V., B. J. Gannon, W.B. Runciman, S. Wells, and J.B. Love. 1987. Do cardirespiratory frequencies show entrainment with hopping in the tammar wallaby.. J. exp. Biol. **129**: 251-263.

Bechbache, R. R. and J. Duffin. 1977. The entrainment of breathing frequency by exercise rythm. J. Physiol. **272**: 553-561.

Bedford, T. G., C.M. Tipton, N.C. Wilson, R.A. Oppliger, and C.V. Gisolfi. 1979. Maximum oxygen consumption of rats and its changes with various experimental procedures. J. Appl. Physiol. **47**:1278-1283.

Belcastro, A. N., A. Bonen. 1975. Lactic acid removal rates during controlled and uncontrolled recovery exercise. J. Appl. Physiol. **39**: 932-.

Bermeyer, H.U. and Bernt, E. 1974. D-Glucose determination with glucoes oxidase and peroxide. In *Methods of Enzymatic analysis. Second edition. vol 3.* (Bermeyer, H.U. and Bernt, E. eds.).Academic Press. Inc. New York.

Blaxter, K. 1989. Energy metabolism in animals and man. Cambridge University Press Cambridge.

Bramble, D.M. and D.R. Carrier. 1983. Running and breathing in mammals. Science. **219**: 251-256.

Brockman, R.P., 1987. Effect of exercise on net hepatic uptake of lactate, pyruvate, alanine and glycerol in sheep. Can. J. Physiol. and Pharmacol. **65**: 2065-2070.

Brooks, G. A., K. E. Brauner and G. Cassens. 1973. Glycogen synthesis and metabolism of lactic acid after exercise. Am. J. Physiol. **224**: 1162-1166.

Brooks, G.A., T.D. Fahey. 1984. Glycogenolysis, glycolysis and gluconeogenesis: the cellular degradation and synthesis of sugar and carbohydrate. In: *Exercise physiology Human bioenergetics and its applications.* John Wiley and sons. New York.

Brooks,G.A. and T.P. White. 1978. Determination of metabolic and heart rate responses of rats to treadmill exercise. J. Appl. Physiol. Respirat. Environ. Exercise Physiol. 45: 1009-1015,.

Buskirk, E. R., 1977. Temperature regulation with exercise. In: Exercise and Sports Sciences Review Vol. 5 (Hutton, R.S. ed.). Journal Publishing Affiliates. Santa Barbara. California:-.

Butler, P.J., N. H. West, and D. R. Jones. 1977. Respiratory and cardiovascular responses of the pigeon to sustained level flight in a wind-tunnel. J. exp. Biol. 82 :71-7.

Butler, P.J., A. J. Woakes. 1980. Heart rate, respiratory frequency and wing beat frequency of free flying barnacle geese . J. exp. Biol. **85**: 213-226.

Cerny, F. 1973. Protein metabolism during two hour ergometer exercise. In: Int. Symp. Biochem :232-237.

Christensen, E. H., R. Hedman and B. Saltin. 1960. Intermittent and continuous running. Acta Physiol. Scand. **50**: 269-286.

Ciccone, C. D., C.S. Lakas, and E.J. Zambraski. 1982. Oxygen consumption in treadmill exercised Yucatan miniature swine. Med. Sci. Sports and Exercise. **14**: 467-470.

Clapperton, J.L., 1964. The energy metabolism of sheep walking on the level and on gradients. Brit. J. Nutr. **18:** 47-54.

Collias, N.E., 1956. The analysis of socialization in sheep and goats. Ecology **37**: 228-239.

Coward, W.A.,. 1988. The doubly-labelled-water (2H2 18O) method: principles and practice. Proc. Br. Nutr. Soc. **47**: 209-218.

Coward, W.A., T.J. Cole, H. Gerber, S.B. Roberts and I. Fleet. 1982. Water Turnover and the Measurement of Milk Intake. Pflugers Arch. **393**: 344-347.

Cresswell, E., 1960. Ranging behaviour studies with Romney Marsh and Cheviot sheep in New Zealand. Anim. Behav. **8**: 32-38.

Cronin, M.T., B.J. Siegel and G.P. Moberg. 1981. Effect of behavioural stress on plasma levels of growth hormone in sheep. Physiol. Behav. **265**: 887-890.

Cumming, G. R., L. Hastman, J. McCort and S. McCullough. 1980. High Serum Lactates do Occur in Young Children after Maximal Work. Int. J. Sports Medicine. **1**: 66-69.

Cuthbertson, A. Z., J. C. Dighton, and B. D. Siebert. 1989. A modified distillation technique for the recovery of tritium from blood. Appl. Radiat. Isot. **40**: 545-546.

Dalton, D.C., T.W. Knight and D.L. Johnson. 1980. Lamb survival in sheep breeds in New Zealand hill country. N.Z. J. Agric. Res., **23**: 167-176.

Dawes, G.S.and J.C. Mott. 1959. The increase in oxygen consumption of the lamb after birth. J. Physiol. **146**: 295-315.

Daws, G.T.and V. R. Squires. 1974. Observations on the effects of temperature and distance to water on the behaviour of Merino and Border Leicester sheep. Agric. Sci. Camb. **82**: 383-390.

De Coster, A., H. Denolin, R. Messin, S. Degre, and P. Vandermoten. 1969. Role of the Metabolites in the Acid-Base Balance During Exercise. Biochemistry of Exercise Medicine and Sport. **3**: 15-34.

Dennis, S. M., 1969. Predators and perinatal mortality of lambs in Western Australia. Aust. Vet. J. **45**: 6-9.

Dennis, S. M. 1974. Perinatal lamb mortality in Western Australia 1. General procedures and results. Aust. Vet. J. **50**: 443-.

De Vries, H.A. 1980. Basic Physiology underlying the study of physiology of exercise. In: *Physiology of exercise* (de Vries, H.A. ed.). W.C. Brown Co. Publ. Dubugue. Iowa.

Di Pampero, P.E., U. Boutellier and P. Pietsch. 1983. Oxygen deficit and stores at onset of muscular exercise in humans. J. Appl. Physiol. **51:** 46-153.

Donald, D. E., D. Ferguson. 1966. Response of heart rate, oxygen consumption, and arterial blood pressure to graded exercise in dogs.. P.S.E.B.M. **121**: 626-630.

Donhoffer, S. 1986. Body size and metabolic rate: exponent and co-efficient of the allometric equation. The role of units.. J. Theor. Biol. **119:** 125-131.

Dove, H. 1988. Estimation of the intake of milk by lambs from the turnover of deuterium- or tritium-labelled water. Br. J. .Nutr. **60**: 375-387

Dove, H. M. and Freer. 1974. The accuracy of tririated water turnover rate as an estimate of milk intake in lambs. Aust. J. Agric. Res. **25**: 825-834.

Dove, H., M. Freer. 1979. The accuracy of tritated water turnover rate as an estimate of milk intake in lambs. Aust. J. Agric. Res. **30:** 725-739.

Duff, X.J., S.N. McCutcheon and M.F. McDonald. 1982. Central nervous system injury as a determinant of lamb mortality. Proc. N.Z. Soc. Anim. Prod. **42**: 15-17.

Eales, F.A.and J. Small. 1981. Effects of colostrum on summit metabolic rate in Scottish Blackface lambs at five hours old. Res.Vet. Sci. **30**: 266-269.

Eales, F.A. and J. Small. 1985. Determinants of heat production capacity in newborn lambs and their effects on survival: substrates fro heat production. In: *Agriculture: factors affecting the survival of newborn lambs* (Alexander, G., Barber, J.D. and Slee, J. eds.). Commission of the European Communities.

Eales, F. A., J. Small. 1985. Effects of acute hypoxia on heat production capacity in newborn lambs. Res. Vet. Sci. **39**: 212-215.

Edwards, R.H.T. 1983. Biochemical Bases of Fatigue in Exercise Performance: Catastrophe Theory of Muscular Fatigue. In: *Int. Series Sport Science Vol 13 (Biochemistry of Exercise)* (Kruttgen H.G Vogel J.N. and Portmans J. eds.) Human Kinetics Publ. Inc.

Edwards, R. H.T., L.G. Ekelund, R.C. Harris, C.M. Hesser, E. Hultman, A. Melcher and O. Wigertz. 1973. Cardiorespiratory and metabolic costs of continuous and intermittent exercise in man. J. Physiol. **234**: 481-497.

Effendic, S., T.Hokfelt and R. Luft. 1978. Somatostatin. Advances in Metab. Disord. **9:** 367-424.

Engelhardt, W.v.. 1977. Cardiovascular effects of exercise and training in horses.. Adv. Vet Sci. **21**:173-205.

Epstein, S. and T. Mayeda. 1953. Variation of oxygen- eighteen content of water from natural sources. Geochem. VCosmocheim Acta. **4**: 213-224.

Erickson, B. O., B. Pearson, and J.I. Thorell. 1971. The effects of repeated prolonged exercise on plasma growth hormone, insulin, glucose, free fatty acids, glycerol, lactate, and hydrobutyric acid in 13 year old boys and adults.. Acta. Pediat Scand. Suppl 217: 142-146.

Faraci, F. M., Olsen S.C. and H.H Erickson. 1984. Effect of exercise on oxygen consumption heart rate and the electrocardiogram of pigs.: Med. Sci. Sports Exerc. **16**: 406-410.

Fedak, M. A., L. Rome and H.J. Seeherman. 1982. One-step N2-dilution technique for calibrating open-ciucuit VO2 measuring systems.. J. Appl. Physiol: Respirat. Environ. Exercise Physiol. **51**:772-776.

Fedak, M.A. H.J. Seeherman. 1979. Reappraisal of energetics of locomotion shows identical cost in bipeds and quadrupeds including ostrich and horse. Nature. **282**: 713-716.

Fogarty, N.M., J.M. Thompson. 1974. Relationship between pelvic dimensions, other body measurements and dystocia in dorset horn ewes. Aust. Vet. J. **50**: 502-506.

Forbes-Ewan, C.H., B.L.L. Morressey, G.C. Gregg and D.R. Waters. 1989. Use of doubly labelled water technique in soldiers training for jungle warfare. J Appl. Physiol. **67**: 14-18.

Freymond K., Y. Schutz, J. Decombaz, J. L. Micheli, and E. Jequier. 1986. Energy balance, Physical Activity, and Thermogenic Effect of Feeding in Premature Infants. Pediat. Res. **20**: 638-645.

Frohman, L.A.. 1972. Clinical nuropharmacology of hypothalamic releasing factors. New Engl. J. Medic. **228:** 1384-1393.

Furnival, E. P., S. A. Philp and J. L. Corbett. 1982. Measurements with a pedometer of distances walked by sheep. Proc.Aust. Soc. Anim. Prod. **14:** 654.

Gabraith, D.F. 1983. Radioimmunoassay. Technical bulletin 2. New England Nuclear.

Gales, R. 1989. Validation of the use of tritiated water and doubly labeled water for estimating food, energy, and water intake in little penguins, Eudyptula minor. Physiol. Zool. **62:** 147-169.

Garland, T. 1983. Scaling the ecological cost of transport to body mass in terrestrial mammals.. Am. Nat. **121** : 571-587.

George, J.M. 1975. The incidence of dystocia in fine -wool Merino ewes. Aust. Vet. J. **51**: 262-265.

George, J.M. 1976. The incidence of dystocia in dorset horn ewes. Aust. Vet. J. **52**: 519-523.

Gollnick, P.D. and Hermansen, L. 1973. Biochemical Adaptations to Exercise: Anaerobic Metabolism In: Exercise and Sports Sciences Reviews Vol. 1 (Wilmore J. W. (ed.) Academic Press N.Y. and London.

Gonfinantini, R.. 1984. Advisory group meeting on stable isotope reference samples for geochemical and hydrological investigations. IAEA:Vienna, 19-21 September.1983.

Graham, N.M., J.L. Black, G.J. Faichney and G.W. Arnold 1976. Stimulation of growth and production in sheep-model 1: A computer programme to estimate energy and nitrogen utilization, body composition and empty live weight change, dat by day for sheep of any ag.. Agricultural Systems. **1**: 113-138.

Grodzinski, W.and B. A. Wunder. 1975. Ecological energetics of small mammals.. In: *Small mammals: their productivity and population dynamics*. (Golley, F.B., Petrusewicz, K. and Ryszkowiki, L. eds.) Camb. Univ. Press

Grommers, F.J., L. Elving and P. Van Eldik. 1985. Parturition difficulties in sheep. Anim. Reprod. Sci. **9**: 365-374.

Gubernick, D. J. 1980. Mechanisms of maternal labelling in goats. Anim. Behav. **29**: 305-306.

Gubernick, D. J., K. Corbeau Jones and P.H. Klopfer. 1978. Maternal "imprinting" in goats. Anim. Behav. **27**: 314-315.

Gutmann, I. and Wahkfeld, A.G. 1974. L-Lactate determination with lactate dehydrogenase and NAD. *In Methods of Enzymatic analysis. Second edition. Vol 3*. (Bermeyer, H.U. and Bernt, E. eds.).Academic Press. Inc. New York.

Hailey, A. 1988. How far do animals move? Routine movements in a tortoise. Can. J. Zool. **67**: 208-215.

Harris, P., M. Bateman, T.J. Bayley, K.W. Donald, J. Gloster and T. Whitehead 1968. Observations on the course of the metabolic events accompanying mild exercise. Q. J. exp Physiol. **53**: 43-64.

Haughey, K.G. 1973. Vascular abnormalities in the central nervous system associated with perinatal lamb mortality. Aust. Vet. J. **49**: 9-15.

Haughey, K. G. 1983. New insights into rearing failure and perinatal mortality. Postgraduate course in Vet. Sci. **67**: 135-147.

Hayes,J. P. 1989. Field and maximal metabolic rates of deer mice (Peromyscus maniculatus) at low and high altitudes. Physiol. Zool. **62**: 32-744.

Hayman, R. H., H. N. Turner and E. Turton. 1955. Observations on survival and growth to weaning of lambs from ewes with defective udders. Aust. J. Agric. Res. **6**: 446-455.

Hays, F.L. 1976. Alp and valley cattle. Exercise in cold, hot and high environments.. Pflugers Arch.,**362**: 185-192.

Heglund, N. C.and C. R. Taylor. 1988. Speed, stride frequency and energy cost per stride: How do they change with body size and gait?. J. exp. Biol. **138**: 301-318.

Heglund, N.C. , C.R. Taylor,and T.A. McMahon. 1974. Scaling stride frquency and gait to animal size: mice to horses. Science. **186**: 1112-1113.

Hermansen, L., S. Maehlum, D. R. Pruett, O. Vaage, H. Waldum, and T. Wessel-Aas. 1973. Lactate Removal at Rest and During Exercise. In: *Proc. 2nd Int. Symp. Biochem of Exercise* (Howard, H. and Poortmans, J.R. eds.).Magglingen.

Hermansen, L.and B. Saltin. 1969. Oxygen uptake during maximal treadmill and bicycle exercise. J.Appl. Physiol. **26**: 31-37.

Hermansen, L.and O. Vaage. 1977. Lactate disappearance and glycogen synthesis in human muscle after maximal exercise. Am. J. Physiol. **233**: E422-E429.

Herscher, L. and J.B. Richmond and A. U. Moore. 1963. Modifiabiltiy of the critical period for the development of maternal behaviour in sheep and goats. Behaviour. **20**: 311-320.

Hight, G.K. and K.E. .Jury. 1970. Hill country sheep production: 2. Lamb mortality and birth weights in romney and Border Leicester x Romney flocks. N.Z.J. Agric. Res. **13:**735:752

Hoyt, D.F.and C.R. Taylor. 1981. Gait and the energetics of locomotion in horses. Nature.292: 239-240.

Hultman, E. and H. Sjoholm. 1983. Substrate Availability. Int. Series Sport Sci. **13**: 63-75.

Hutson, G. D., D. K. Hitchcock. 1978. The movement of sheep around corners. Appl. Anim. Ethol. **4:** 349-355.

Iscoe, S. 1981. Respiratory and stepping frequencies in conscious exercising cats. J. Appl. Physiol. **51**: 835-839.

Jarrett, I.G., O.H. Filsell and F. J. Ballard. 1976. Utilization of Oxidizable Substrates by the Sheep Hind Limb: Effects of Starvation and Exercise. Metabolism. **25**: 523-531.

Jequier, E., Y. Schutz. 1988. Classical respirometry and the doubly-labelled-water method appropriate applications of the individual or combined techniques. Proc. Nutr. Soc. **47**: 19-225.

Karlsson, J. 1971. Lactate and phoshagen concentrations in working muscle of man. Acta Physiol. Scand. Supplement, **358**: 7-72.

Karlsson, J., B Diamant, B. Saltin. 1968. Lactate dehydrogenase activity in muscle after prolonged severe exercise in man J. Appl. Physiol.25: 88-91.

Kasch, F.W., W.H. Phillips, W.D. Ross, J.E.L. Carter and J.L. Boyer. 1966. A comparison of maximal oxygen uptake by treadmill and step-test procedures.. J. Appl. Physiol. **21**: 1387-1388.

Kasch, F. W., J. P. Wallace, R. R. Huhn, L. A. Krogh and P. M. Hurl. 1976. Maxiaml oxygen consumption during horizontal and inclined treadmill running. J. Appl. Physiol. **40**: 982-983.

Keppler, D., J. Keul and E. Doll. 1969. The Influence of the Form of Exercise on the Arterial Concentrations of Glucose, Lactate Pyruvate and Free Fatty Acids. Bioch..Exerc. Med. and Sport . **3**: 132-136.

Keul, J.and E. Doll. 1969. The Influence of Exercise and Hypoxia on the Substrate Uptake of Human Heart and Human Skeletal Muscles. Bioch. Exerc. Med. and Sport. **3**: 41-46.

Keul, J., G. Haralambie, and G. Trittin. 1974. Intermittent exercise: arterial lipid substrates and arteriovenous differences. J. Appl. Physiol. 36: 159-161.

Keverne, E.B., F. Levy, P. Poindron and D. Lindsay. 1983. Vaginal stimulation: an important determinant of maternal bonding in sheep. Science. **219**: 81-83.

Kilgour, R., 1972. Behaviour of sheep at lambing. N.Z. J. Agric. **125**: 24-27.

Kishimoto R. 1989. Early mother and kid behavior of a typical "follower" Japanese serow Capricornis crispus. Mammalia. **53:** 165-176.

Kleiber, M. 1932. Body size and metabolism. Hilgardia **6**: 315-353.

Klopfer, P.H.and M.S. Klopfer. 1977. Compensatory responses of goat mothers to their impaired young. Anim. Behav. **25**: 2 86-291.

Knight, T. W, G. K. Hight and G. W. Winn. 1979. The influence of sires on lamb survival. Proc. N.Z. Soc. Anim. Prod. **39**: 87-93.

Knight, T.W., W.H. McMillan, R. Kilgour, H.U.P. Hockey, and D.R.H. Hall. 1989. Effect of slope of lambing site on lambs slipping and lamb mortality. N.Z. J. Agric. Res. **32:** 199-206.

Kuhlmann, W.D., D. S. Hodgson and M.R. Fedde. 1985. Respiratory, cardiovascular and metabolic adjustments to exercise in the Hereford calf. J. Appl Physiol. **58**: 1273-1280.

Lai, J. S.and I.N. Lien. 1983. Maximal blood lactic acid concentration and its recovery course after exhaustive graded treadmill exercise in young men. Int. Series Sport Sci. **13**: 411-414.

Leat, W.M.F. 1967. Plasma lipids of newborn and adult ruminants and of lambs from birth to weaning. J. agric. Sci. Camb. **69**: 241-246.

Lemon, P.W.R. and D.G. Dolny and B.A. Sherman. 1983. Effect of intense prolonged running on protein catabolism. Int. Symp. Sport. Sci. 13: 367-372.

Le Neinder, P.P. Poindron and C., Delouis. 1979. Hormonal induction of maternal behavior in non-pregnant ewes. Physiol. Behav. **22**: 731-734.

Lickliter, R.E. 1984. Mother-infant spatial relationships in domestic goats. Appl. Anim. Behav. Sci. **13**: 93-100.

Lickliter, R. E.and J. R. Heron. 1984. Recognition of mother by newborn goats. Appl. Anim. Behav. Sci. **12**: 187-192.

Lifson, N., G.B. Gordon and R. McClintock. 1955. Measurement of total carbon dioxide production by means of D^2O^{18}. J. Appl. Physiol. **7**: 704-710.

Lifson, N., C.B. Gordon, M.B. Uischer and A. O. Nier. 1949. The fate of utilized molecular oxygen and the source of heavy oxygen of respiratory carbon dioxide studied with the aid of heavy oxygen. J. Biol.Chem. **180**: 803-811.

Lifson, N and R McClintock 1966. Theory of use of the turnover rates of body water for measuring energy and material balance J. Theoret. Biol. **12**: 46-74.

Mitchell, J. H., G. Blomqvist.. 1971. Maximal Oxygen Uptake. N. Engl. J. Med. **284:** 1018-1022.

Morgan, P.D., G.W. Arnold and C.A.P. Boundy. 1972. The bahavior of ewes and their new born lambs in a hot climate. Proc. Aust. Soc. Anim. Prod. **9:** 371-375.

Moule, G.R. 1954. Observations on mortality amongst lambs in Queensland. Aust. Vet. J. **153**: 153-171.

Mullaney, P.D. 1966. The relation of birth coat and lamb survival. Aust. J. Expt. Anim. Husb. **6:** 4-87.

Mullen, R. K. 1971. Energy metabolism and body water turnover rates of two species of free-living kangaroo rats, Dipodomys merriami and Dipodomys microps. Comp. Biochem. Physiol. **39A**: 379-390.

Muller, E.E., T. Saita, A. Arimura and A. V. Scnally. 1967. Hypoglyceamia, stress and growth hormone release: blockage of growth hormone release by drugs acting on the central nervous system. Endocrinology. **80**: 109-117.

Nagy, K. A.. 1980. CO2 production in animals: of potential errors in the doubly labeled water method. Am. J. Physiol. **238**: R466-R473.

Nagy, K. A. 1987. Field metabolic rate and food requ irement scaling in mammals and birds. Ecological Monographs. **57**: 111-128.

Nagy, K.A. 1988. Doubly labelled water studies of vertebrate physiological ecology. Ecological Studies. **68**: 270-287.

Nowak, R. 1989. Early recognition of the mother by the newborn lamb: effect of breed and litter size. Ph.D. Thesis. University of Western Australia.

Nowak, R. 1990. Mother and sibling discrimination at a distance by three to seven day old lambs. Developmental Psychobiology. **23**: 285-295.

Nowak, R., P. Poindron, P. Le Neindre and I. G. Putu. 1987. Ability of 12 hour old merino and crossbred lambs to recognise mothers. Appl. Anim. Behav. Sci. **17**: 263-271.

Nowak, R., P. Poindron and I. G. Putu. 1989. Development of mother discrimination by single and multiple newborn lambs. Developmental Pscycobiology. **22**: 833-845.

Obst, J, M. and J. V. Evans. 1970. Genotype-environment interactions in lamb mortality with particular reference to birth coat and haemoglobin type. Proc. Aust. Soc. Anim. Prod. **8:** 149-153.

Oftedal, O.T. 1984. Milk composition, milk yield and energy output at peak lactation: a comparative review. Symp. Zool. Soc. Lond. **51**: 33-85.

Oppong-Anane, K., R.V. Baudinette, S. Laube and J.R. Sabine. 1989. Metabolic and physiological responses to submaximal intermittent exercise in neonatal lambs. Proc. Aust. Soc. Anim. Prod. **18**: 324-327.

Owens, J.L., B.M. Bindon, T.N. Edey and L.R. Piper. 1985. A note on the effects of dexamethasone-induced partuition on ewe behaviour and lamb survival in prolific booroola ewes. Anim. Prod.**41**: 417-420.

Passoneau, J.V. and O.H. Lowry. Pyruvate fluorimetric assay. 1974. In Methods of Enzymatic analysis. Second edition. Vol 3. (Bermeyer, H.U. and Bernt, E. eds.).Academic Press. Inc. New York.

Pasquis, P., A. Lacaisse, and P. Dejours. 1970. Maximal oxygen uptake in four species of small mammals. Resp. Physiol. **9:** 298-309.

Pearson, R.A., R.F. Archibald. 1989. Biochemical and haematological changes associated with short periods of work in draught oxen. Anim. Prod. **48**: 375-384.

Peterson, C.C., K.A. Nagy, and J. Diamond. 1990. Sustained metabolic slope. Proc. Natl. Acad. Sci., **87**: 2324-2328.

Pethick, D.W., A. W. Bell and E. F. Annison. 1984. Fats as energy sources in animal tissues. In: *Fats in Animal Nutrition.* (W. Seman ed.).

Pethick, D.W., N. Harman and J.K. Chong. 1987. Non-esterified Long-chain Fatty Acid Metabolism in Fed Sheep at Rest During Exercise. Aust. J. Biol. Sci. **40** :221-234.

Pethick, D.W., D.B. Lindsay, P.J. Barker and A.J. Northrop. 1983. The metabolism of circulating non-esterified fatty acids by the whole animal, hind-limb muscle and uterus of pregnant ewes. Br. J. Nutr. **49:** 29-143.

Pettigrew, J. E., S. G. Cornelius, R. L. Moser, and A. F. Sower. 1987. A refinement and evaluation of the isotope dilution method for estimating milk intake by piglets. Livestock Production Science. **16**: 163-174.

Poindron, P. M.and Carrick. 1976. Hearing recognition of the lamb by its mother. Anim. Behav. **24**: 600-602.

Poindron, P.and P.Le Neidre. 1980. Endocrine and sensory regulation of the maternal behaviour in the ewe. In: Adv. Stud. Behav. 11: 75-119.

Poindron, P., P.Le Neindre, I. Raksanyi, G. Trillat and P. Orgeur. 1980. Importance of the characteristics of the young in the manifestation and establishment of maternal behavior in sheep. Reprod. Nutr. Develop. **20**: 817-826.

Poindron, P., G.B. Martin and R.D. Hooley. 1979. Effects of lambing induction on the sensitive period for the establishment of maternal behavior in sheep. Physiol. Behav. **23**: 1081-1087.

Poindron, P.and P. Schmidt. 1985. Distance recognition in ewes and lambs kept permanently indoors at pasture. Appl. Anim. Behav. Sci. **13**: 267-273.

Poortmans, J. R. 1969. Influence of physical exercise on proteins in biological fluids. Bioch. Exerc. Med. Sport . 3: 312-327.

Poortmans, J. R. 1973. Effects of long lasting physical exercise and training on protein metabolism. In *Int. Synp. Biochem. Exerc.*

Pruett, E.D.R. 1970. Free fatty acid mobilization during and after prolonged severe muscular work in men. J. Appl. Physiol. 29: 809-815.

Quinlivan, T.D. 1971. Dystocia in sheep:preliminary observations on within- and between breed differences in various skelatal measurements. N.Z.Vet. J. **19** 75-77.

Raben, M.S.and C.H. Hollenberg. 1959. Effects of growth hormone on plasma fatty acids. J. Clin. Invest. **38:** 484-498.

Robinson, J. B.and B. A. Young. 1988. Recovery of neonatal lambs from hypothermia with thermal assistance. Can. J. Anim. Sci., **68**: 183-190.

Rosenblatt, J.S. 1980. Hormonal and nonhormonal regulation of maternal behavior: A theoretical study. Reprod. Nutr. Develop. **2**: 791-800.

Rowley, I. 1970. Lamb predation in Australia: incidence, predisposing conditions, and the identificatiuon of wounds. C.S.I.R.O. Wildl. Res. **15**: 79-123.

Schmidt-Nielsen, K. 1972. Locomotion: energy cost of swimming, flying and running. Science, **177**: 222-227.

Schmidt-Nielsen, K. 1977. 1. Problems of scaling: Locomotion and physiological correlates. In: *Scale effects in animal locomotion* (T.J. Pedley ed.) Academic Press London.

Schoeller, D.A. 1983. Energy expenditure from doubly labeled water; some fundamental considerations in humans. Am. J. Clin. Nutr. **38**: 999-1005.

Schoeller, D.A. 1988. Measurement of energy expenditure in free living humans by using doubly labelled water. Am. J. Clin. Nutr. **118:** 1278-1289.

Schoeller, D.A., E. Van Santen, D.W. Peterson, W. Dietz, J. Jaspan and P.D. Klein. 1980. Total body water measurement in humans with ^{18}O and ^{2}H labelled water. Am. J. Clin. Nutr., **33**: 2686-2693.

Seale, J., C. Miles and C.E. Bodwell. 1989. Sensitivity of methods for calculating energy expenditure by ues of doubly labeled water. J. Appl. Physiol. **66**: 644-653.

Searle, T.W., D.A. Griffiths. 1976. The body composition of growing sheep during milk feeding and the effect on composition of weaning at various body weights. J. agric. Sci. Camb. **86:** 83-493.

Sedmark, J.J. and Grossberg, E. 1977. Total protein determination. Annal. Biochem. **77**: 544

Seeherman, H.J., C.R. Taylor, G.M.O. Maloiy and R.B. Armstrong. 1981. Design of the mammalian respiratory system.. II measuring maximum aerobic capacity. Resp. Physiol. **44**: 11-23.

Senay, L.C., G. Rogers, and P. Jooste. 1980. Changes in blood plasma during progressive treadmill and cycle exercise. J. Appl. Physiol. 49: 59-65.

Shelley, L 1970. Interrelations between the duration of partuition, post natal behaviour of lambs and the incidence of neonatal mortality. Proc. Aust. Soc. Anim Prod **8:** 348-352.

Shepherd, R.E. and P.D. Gollnick. 1976. Oxygen uptake of rats at different work intensities. Pflugers Arch. **362**: 219-222.

Shillito, E. 1975. A comparison between the role of vision and hearing in lambs finding their own dams. Appl. Anim. Ethol. **1**: 369-377.

Shillito, E. and G. Alexander. 1977. Mutual recognition amongst ewes and lambs of four breeds. Appl. Anim. Ethol. **1:** 151-165.

Shillito Walser, E. and G. Alexander. 1980. Mutual recognition between ewes and lambs. Reprod. Nutr. Develop. **20**: 807-816.

Shillito Walser, E.E. 1978. A comparison of the role of vision and hearing in ewes finding their own lambs. Appl. Anim. Ethol. **4:** 71-79.

Shillito Walser, E.E. 1980. Maternal recognition and breed identity in lambs living in a mixed flock of Jacob, Clun Forrest and Dalesbred sheep. Appl. Anim. Ethol. **6**: 221-231.

Shillito Walser, E., E. Walters, P. Hague and T. Williams. 1985. Responses of lambs to model ewes. Behav. **95:** 110-120.

Slee, J. 1978. The effects of breed, birthcoat and body weight on the cold resistance of newborn lambs.. Anim. Prod. **27**: 43-49.

Slee, J. 1981. A review of genetic aspects of survival and resistance to cold in newborn lambs.. Liv. Prod. Sci. **8**: 419-429.

Slee, J., R.G. Griffiths and D.E. Samson. 1980. Hypothermia in new born lambs induced by experimental immersion in a water bath and by natural exposure outdoors. Research in Veterinary Science **28**: 275-280.

Slee, J., J.P. Simpson and J.A.. Woolliams. 1987. Metabolic rate responses to cold and to exogenous noradrenaline in newborn Scottish blackface lambs genetically selected for high or low resistance to cold. Anim. Prod. **45**: 69-74.

Slee, J., S.P. Simpson, A.W. Stott, J.C. Williams, and D. E. Samson. 1990. An improved water-bath test to study effects of age and previous sucking on metabolic rate and resistance to cold in newborn lambs. Amin. Prod. **50**: 319-331.

Slee, J.,S.P. Simpson and S.B. Wilson. 987. Comparative methods for inducing and measuring non-shivering thermogenesis in newborn lambs. Anim. Prod. **45**: 61-67.

Slee, J. and A. Springbett. 1986. Early post natal behaviour in lambs of ten breeds. Appl. Anim. Behav. **15**: 229-240.

Slee, J. and A.W. Stott. 1986. Genetic selection for cold resistance in Scottish Blackface lambs.. Animal Prod. **43:** 397-404.

Smith, F.V. 1965. Instinct and learning in the attachment of lamb and ewe. Anim. Behav. **13**: 84-86.

Smith, F.V., C. Van-Toller and T. Boyes. 1966. The "critical period" in attachment of lambs and ewes. Anim. Behav. **14**: 120-125.

Smith, G.M. 1977. Factors affecting birth weight, dystocia, and prewaening survival in sheep. J. Anim. Sci. **44**: 745-753.

Squires, V. R. 1970. Growth of lambs in a semi-arid region as influenced by distance walked to water. Proc. Aust. Soc. Anim. Prod. **8**: 219-225.

Squires, V.R. 1974. Grazing distribution and activity patterns of merino sheep on a saltbush community in south east Australia. Appl. Anim. Ethol. **1**: 17-30.

Squires, V.R. 1981. Livestock management in the arid zone. Iketa Press. Melbourne.

Squires, V.R., G.T. Daws and R.A. Bawden. 1969. An automatic recording unit for photographic studies of behavior of domestic animals. J. Biol. Photo. Assoc. **37**: 188-198.

Squires, V.R. and A.D. Wilson. 1971. Distance between food and water supply and its effect on drinking frequency, and food and water intake of Meroino and Border Leicester sheep. Aust. J. Agric. **22**: 283-290.

Squires, V.R., A. D. Wilson and G.T. Daws. 1972. Comparisons of the walking activity of some australian sheep. Proc. Aust. Soc. Anim. Prod. **9**: 376-380.

Stamford, B.A., A. Weltman, R. Moffatt and S. Sady. 1981. Exercise recovery above and below anaerobic threshold following maximal work.. J. Appl. Physiol. Respirat. Environ. Exercise Physiol. **51**: 840-844.

Stein, T.P., R.W. Hoyt, R.G. Settle, M. O'Toole and W.D.B. Hiller. 1987. Determination of energy expenditure during heavy exercise, normal daily activity, and sleep using the doubly-labelled-water ($^2H_2{}^{18}O$). Am. J. Clin. Nutr. **45**: 534-539.

Stephenson, R.G.A. and G.R. Suter. 1984. Reduction of the effects of heat stress on lamb birth weight and survival by provision of shade. In: *Reproductioon in sheep*. (Lindsay, D.R. and Pearce, D.T.eds). Australia Academic of Science.

Stevens, D., G. Alexander, and J.J. Lynch. 1982. Lamb mortality due to inadequate care of twins by merino ewes. Appl. Anim. Ethol. **8**: 243-252.

Stevens, D., G. Alexander, B. Mottershead,and J.J. Lynch. 1984. Role of the lamb in post-partum separation of ewes from twin lambs. Proc. Aust. Soc. Anim. Prod., **15**: 751-752.

Stevens, D.B., J.L. Linzell. 1974. The development of sucking behaviour in the newborn goat. Anim. Behav. **22**: 628-633.

Stott, A.W., J. Slee. 1987. The effects of litter size, sex, age, body weight, dam age and genetic selection for cold resistance on the physiological responses to cold exposure of Scottish Blackface lambs in a progressively cooled water bath. Anim. Prod. **45**: 477-491.

Sutton, J. R., J.D. Young, L. Lazarus, J.B. Hickie and J. Maksvytis. 1969. The hormonal response to physical exercise. Aust.. Ann. Med. **18:** 84-90.

Taylor, C.R. 1970. Dehydration and heat: effects on temperature regulation of East African ungulates. Am. J. Physiol., **219**: 1136-1139.

Taylor, C.R. 1977. The energetics of terrestrial locomotion and body size in vertebrates. In: *Scale effects in animal locomotion* (T.J. Pedley ed.). Academic Press. London.

Taylor, C.R., M.C. Heglund and G.M.O. Molory. 1982. Energetics and mechanism of terrestrial locomotion. 1. metabolic energy consumption as a function of speed and body size in birds and mammals.. J. exp. Biol. **97**: 1-21.

Taylor, C.R., M.C. Heglund, T.A. McMahon and T.R. Looney. 1980. Energetic cost of generating muscular force during running. A comparison of large and small animals. J. exp. Biol. 86: 9-18.

Taylor, C., R., G.M.O. Maloiy, E. R. Weihel, V. A. Longman, J.M.Z. Kama, H.J. Seeherman, and M.C. Heglund. 1980. Design of the mammalian respiratory system: 3. Resp.Physiol. **44**: 25-37.

Taylor, C.R., V.J. Ruwntree. 1972. Temperature regulation and heat balance in running cheetahs: a strategy for sprinters?. Am. J. Physiol. **224:** 848-851.

Taylor, C R., K. Schmidt-Nielson and J.L. Raab. 1970. Scaling of energetic cost of running to body size in mammals.. Am.J. Physiol.. **219**: 1104-1107.

Taylor, C.R., A. Shkolnik, R. Dmi'el and D. Baharav. 1974. Running in cheetahs, gazelles, and goats: energy cost and limb configuration. Am. J.Physiol.. **227**: 848-850.

Terjung, R. 1979. Endocrine response to exercise.In: *Exercise and Sports Rev*. (Hottoky, R.S and Miller, D. eds) Am. Coll. Sport Medicine.

Thomas, D. P. G.F. Fregin. 1981. Cardiovascular and metabolic responses to treadmill exercise in the horse. J. Appl. Physiol. Respirat. Environ. Exercise Physiol. **50**: 864-868.

Tucker, V.A., 1970. Energetic cost of locomotion in animals. Comp. Biochem. Physiol **34**: 841-846.

Van Duyne, Ch. M. 1965/66. Free Fatty Acid Metabolism during Perinatal Life. Biol. Neonat. **9**: 15-123.

Van Duyne, C. M., H.R. Parker, R.J. Havel and L.W. Holm. 1960. Free fatty acid metabolism in fetal and newborn sheep. Am. J. Physiol. **199**: 987-990.

Van Duyne, C., H. Parker, L. W. Holm, T. Hirai and J. Gallager. 1963. Metabolism of free fatty acids during perinatal life of lambs. Am. J. Obst. and Gynec. **91**: 277-285.

Van Maanen, M.C., S.N. Mc Cutcheon, and R.W. Purchas. 1989.Plasma metabolite and hormone concentrations in southdown ram hoggets from lines divergently selected on the basis of backfat thickness. N.Z. J. Agric. Res. **32**: 219-226.

Vercoe, J.E. 1973. The energy cost of standing and lying in adult cattle. Br. J. Nutr. **30**: 207-210.

Vermorel, M.and J. Vernet. 1985. Major factors affecting the thermogenesis and cold resistance of newborn lambs. In: *Agriculture: factors affecting the survival of newborn lambs* (Alexander, G., Barber, J.D. and Slee, J. eds.). Commission of the European Communities.

Vince, M.A.and T.M. Ward. 1984. The responsiveness of newly born clun forest lambs to odour sources in the ewe. Behavior. **89**: 117-127.

Vince, M.A.,T.M. Ward and M. Reader. 1984. Tactile stimulation and teat-seeking behavior in newly born lambs. Anim. Behav. **32**: 1179-1184.

Wagner, J.A., S.M. Harvath and T.E. Dahms. 1977. Cardiovascular respiratory and metabolic adjustments to exercise in dogs. J. Appl. Physiol. Respirat. Environ. Exercise Physiol. **42**: 403-407.

Wahren, J., G. Ahlborg, P. Felig, and L. Jorfeldt.. 1971. Glucose Metabolism during exercise in man. In: *Muscle metabolism during exercise*. (Pernow B. and Saltin B. eds.). Adv. Expl. Med. Plenum Press. New York.

Wahren, J., Ph. .. Felig, L. Hagenfeldt, R. Hendler, and G. Ahlborg. 1973. Splanchnic and Leg Metabolism of Glucose, Free Fatty Acids and Amino Acids during Prolonged Exercise in Man. In: *Proc. 2nd Int. Symp. Biochem. Exercise.*

Warncke, G.,J. Bandholtz and P. Schultze-Motel. 1988. Metabolic cost and body temperatures during grade running in quail.. Comp. Biochem. Physiol. **89A**: 93-96.

Weber, J.M., W.S. Parkhouse, G.P. Dobson, J.C. Harman, D.H. Snow and P.W. Hochachka. 1987. Lactate kinetics in exercising Thoroughbred horses: regulation of turnover rate in plasma. Am. J. Physiol. **253**: R896-R903.

Wiener, G., C.Woolliams and N.S.W. MacLeod. 1983. The effects of breed, breeding system and other factors on lamb mortality 1 : Causes of death and effects on the incidence of losses. J. Agric Sci. **100**: 539-551.

Winfield, C.G.,. 1970. The effect of stocking intensity at lambing on lambing survival and ewe and lamb behaviour. Proc. Aust. Soc. Ani. Prod. **8**: 348-352.

Winfield, C.G. and R. Kilgour. 1976. A study of the following behaviour in young lambs. J. Anim. Ethol. **2**: 235-243.

Wong,W.W.,W.J. Cochran,W.J. Klish, E O'Brien Smith, L.S. Lee, P.D.Klein. 1988. In vivo isotope-fractionation factors and the measurement of deuterium- and oxygen-18-dilkution spaces from plasma urine:saliva-respiratory water vapour. Am. J. Clin. Nutr. **47**: 1-6.

Woolliams, C., G. Wiener and N.S.W. MacLeod. 1983a The effects of breed, breeding system and other factors on lamb mortality. 2: Factors influencing the incidence of delayed birth, Dystocia, congenital defects and miscellaneous causes of early death. J. Agric. Sci., **100**: 553-561.

Wright, D.E. and Wolth, J E. 1976. Proc. N.Z. soc.Anim. Prod. **36**: 99-102.

Young, B. A., V.A. Walker and W.T. Whitmore. 1988. Procedure for measuring resting and summit metabolism in sheep and calves.. Can. J. Animal Sci. **68**: 1 73-182.

Zar, J.H. 1984. Biostatical analysis. Second edition. Hall Inc. London.