STUDIES OF CASTRATION AND TAILING IN YOUNG LAMBS; INFLUENCES OF DIFFERENT METHODS ON ACUTE DISTRESS RESPONSES.

A thesis in partial fulfilment of the requirements for the Degree of

MASTER OF SCIENCE

in
Physiology
at
Massey University.

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1991

ABSTRACT

An investigation of the acute effects of several methods (rubber rings, knife and docking iron) of castrating and tailing 4 to 5 week old lambs was performed. The investigation consisted of a study of certain anatomical aspects of the sites of castration and tailing, experimental validation of the behavioural responses as indices of distress, ranking of the various methods of castration and tailing according to the acute responses and testing of the effects of handling on lambs castrated plus tailed with the knife

The innervation of the external genitalia and the tail was described and the pattern of innervation was discussed with respect to castration and tailing.

The behaviour elicited by castration and tailing was dependent upon the method used. Following rubber ring application the behaviours exhibited were increased activity, increased recumbency of which a high proportion was lateral, and these behaviours were associated with elevated plasma cortisol concentrations and are therefore suggested to be indicative of distress.

After use of the knife or the docking iron abnormal standing/walking behaviour was associated with elevated plasma cortisol concentrations and therefore appeared to indicate distress. The behavioural and plasma cortisol responses continued beyond the 4 hour observational period of the first experiment so a second experiment was performed. It demonstrated that the response to castration plus tailing with the knife approached completion at 8 hours. Handling of lambs to effect blood sampling did not appear to significantly affect the response.

Ranking of the methods of castration plus tailing, castration only and tailing only showed that the use of the knife was apparently more distressing than any other procedure. Apparently less distressing than the use of the knife but similar to each other were castration plus tailing with the rings, castration only with the ring, short-scrotum plus tailing with the rings and castration with the ring plus tailing with the docking iron. Tailing only with the ring and tailing only with the docking iron were apparently as distressing as handling with jugular venipuncture, although it is

suggested that the quality of the distress apparently experienced would have been different.

Thus in order to minimise the acute effects on lambs, there is evidence that the best methods are as follows.

1. For castration plus tailing: castration plus tailing with the rubber rings, short-scrotum plus tailing with the rubber rings or castration with the rubber ring plus tailing with the docking iron.

2. For castration only: castration only with the rubber ring.

3. For tailing only: tailing only with the rubber ring or the docking iron.

However there are other factors to be considered in conjunction with the above recommendations, and these are discussed.

ACKNOWLEDGEMENTS

All those who have experienced research as a student will know that the completion of a research project is only possible with direction and help from others. I would like to thank all those who have helped me over the past two years.

Thanks is particularly directed to my chief supervisor Professor David Mellor for his input and support both practical and philosophical. Also to my other supervisor Dr Ted Kirk for all his help and perseverance. Dr Robert Holmes helped with aspects of the experimental design and I appreciate his guidance.

I would like to thank Neil Ward and Nick Broomfield for all their technical help, especially in setting up the docking trials and help with the computer functions. Many thanks to the team who helped with the castration and tailing experiment: Professor Mellor, Dr Holmes, Neil Ward, Nick Broomfield, Rae Ming Ong, Roy Sparksman, Irene Hall, Debbie Anthony, Linda Randle and the Massey Sheep and Beef Cattle Research Unit staff, especially Lynley Watt, Tim Harvey and Mike Hogan. Thanks to Dr Keith Lapwood and Jane Candy for help and direction with the RIA procedures.

Also I would like to thank the many people in the Physiology and Anatomy Department for their comments and suggestions throughout the project, particularly Dr Heather Simpson and Dr Craig Franklin. Finally, I would like to thank my flatmates for all their support.

CONTENTS

LIST OF TABLES:

LIST OF FIGURES:

PAGE Note that Fa indicates facing page; Fo indicates following page and the number pertains to the page of text.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 ANIMAL WELFARE:

The relationship between humans and animals has always been queried by society in order to give an ethical basis to our treatment of animals (Uvarov, 1985). Historically there has been an acceptance of the separateness of man and animal, and even with the scientific elucidation of man's place in the animal kingdom we still view animals as separate from us: man and beast. Religious and philosophical doctrine has enshrined in most societies the concept that humans are not only separate from animals but are also superior. The concept of superiority has been said to stem from the presence of an immortal soul and the ability to perceive good and evil, right and wrong (Uvarov, 1985; Gee, 1986). The perceived lack of these traits in animals has given credence to the assumption that there was no obligation to avoid causing animals discomfort or provide care for them over and above that which was necessary to keep them functioning. The sixteenth century Cartesian view of animals as merely mechanistic represents societies' acceptance of lack of care for and overwork of domestic animals and the use of animals for entertainment (bear and bull-baiting, cock fighting, racing etc.)(Gee, 1986). With the rise of science came the use of animals in experimentation. In the context of the time, the use of animals in experiments that today would be considered hideous and cruel, was accepted as proper, and animals were used almost as of right with scant regard for their welfare.

From the times of the early vivisectioners the conditions under which animals are used have undergone a steady improvement (Uvarov, 1985; Gee, 1986). The improvement in conditions mirrors an increasing tide of humanitarianism as evidenced by recognition of the rights of man and advances in health and hygiene (Gee, 1986). The early animal welfare lobbyists targeted as unacceptable gross abuses of animals used purely for entertainment, such as blood sports, animal fights and baiting (Uvarov, 1985). The first legislation passed to protect animal welfare was Martin's Act of 1822 which prohibited "cruel and improper treatment of cattle" - specifically bull baiting. In 1824 the Society for the Prevention of Cruelty to Animals (later to be royally patronised) was founded, and this evidences a groundswell of animal welfare concerns. However many still failed to recognise the need or desirability for changes in the treatment of animals and it was only in 1876 that the first effective legislation governing experimental use of animals was passed (Uvarov, 1985). The Cruelty to Animals Act of 1876 was, and with some recent EEC modifications, still is the basis for British and worldwide treatment of experimental animals. Importantly the Royal Commission investigating animal experimentation prior to the act being passed concluded that "it would be neither reasonable nor practicable absolutely to prevent experiments on animals" (Uvarov, 1985). This statement reflects the already strongly placed view that the use of animals was an integral and indispensable part of science. Indeed the spectre of the poor state of health and medical treatment for both people and animals in the absence of animal experimentation was being held up as justification for continuing animal experimentation.

Today there is an increasingly strong move towards minimising discomfort in any sphere of animal use and thus improving the well-being of animals (Kilgour, 1980; Blackshaw, 1986; Gee, 1986). Animal well-being "is a state or condition of physical and psychological harmony between the organism and its surroundings" (Hurnik, 1988). There has been some difficulty in defining the key word in the above statement; "harmony" can be interpreted in different ways by different groups of people. In the context of animal husbandry the well-being of sheep can be disrupted by disease and parasitism. An example is the occurrence of parasitism by blowfly larvae known as flystrike. In order to prevent such disruption to well-being the tail can be removed thus reducing the prevalence of infection.

However when the majority of people exist without contact with areas of animal use misunderstanding can occur over what are considered normal practices (Kilgour, 1980; Gee, 1986). For example, the urbanisation of modern societies has reduced the contact many people have with farming and the practices used to bring animal products to the consumer. This may lead to concern at the use of practices which are or appear to be distressing without adequate knowledge of the practices themselves. In order to defuse any potential cases of such confusion it is essential to fully define the reasons for the action and therefore justify it, and examine the effect such actions have on the animal involved. There is a need for proper assessment of the well-being of animals used by humans, and this assessment should be based on objective information rather than emotional responses (Moberg, 1985; Broom, 1988; Hurnik, 1988). To provide this information Molony (1985) suggests that "vigorous efforts are needed to observe and publish details of the behavioural and physiological changes which occur in as many species as possible and for a variety of noxious procedures".

1.2 AIMS:

This study examines certain animal husbandry procedures in detail. It sets out to delineate the acute effects certain routine animal husbandry procedures have on lambs, and comment on the comparative distressing nature of the procedures. The reasons for these procedures will be defined and are of sufficient moment to justify their use. In the interests of animal welfare a comparison of methods of carrying out the necessary manipulations (castration and tailing) will be made to assess the relative acute effects of these various manipulations in terms of the distress elicited. In terms of ensuring that animal well-being is protected "unnecessary" suffering must be examined (Hurnik, 1988). "Unnecessary" suffering includes situations in which the purpose necessitating the suffering are not suitably important, and secondly suffering that may be reduced or avoided by using alternatives to achieve the same purpose (Hurnik, 1988). This study sets out to provide information on the second of these situations.

Also, validation of behavioural indices of distress will be performed, via utilisation of known hormonal factors. Hormonal and behavioural responses to stressors are intimately related (Dantzer and Mormede, 1983). The relationship of the hormonal responses and behavioural responses have been previously examined in the castration and tailing situation (Shutt, Fell, Connell, Bell, 1988; Mellor and Murray, 1989a; Wood, Molony, Hodgson, Mellor, Fleetwood-Walker, 1991). These studies have shown that characteristic behaviours can be observed when animals (specifically castrated and tailed lambs) are assumed to experience pain. Zayan (1986) makes 5 recommendations for the use of behavioural indicators: (i) look for changes due to pain in a social context, (ii) use systematic observation of behaviours after surgical procedures, (iii) look for behavioural indicators in a comparative perspective, (iv) look for more than one behavioural indicator to increase reliability, (v) relate behavioural indicators to indicators of other kinds. The present study directly addresses three of these five recommendations. Systematic observation of behaviours following routine husbandry operations was used rather than creating artificial experimental pain. Also a number of behavioural activities were recorded and the relationship of these was examined, and finally the behavioural observations were related to hormonal responses in order to validate their use as indicators of distress.

The successful elucidation of behaviours as indicators of distress may provide those involved in animal husbandry (farmers, veterinarians and welfare groups) with detailed information for determining the presence or degree of distress in animals.

1.3 STRESS/DISTRESS:

Stress is a much used term that has proved difficult to define, and while many people use the term few understand its essence. In the physical sciences stress has a precise definition: it is a force applied to a body that causes a change (Ewbank, 1985). Thus in terms of a spring the force that stretches or compresses is the stress (Yousef, 1988). This firm definition has not been applied to the biological situation. In biological systems the term stress becomes confusing and definitions vague. For example, Selye (1976) states that "stress is not a nonspecific reaction", and also that "stress is not a specific reaction". From the literature it seems that the most widely agreed upon view of stress is not as the force causing change, but as the change itself (Selye, 1976; Ramsey, 1986; Yousef, 1988). In terms of a working definition the best seems to be one that incorporates two basic features: a disturbance or perceived threat to the homeostasis of the organism, and a response that counteracts the disturbance. Thus, stress is a state elicited by specific challenges to homeostasis, a state manifested by nonspecifically-induced changes, that are outside normal fluctuations, within a biological system.

Some researchers state that the negative connotation of the term "stress" is unhelpful in the concept of animal stress, but stress can be a positive adaptive process providing a protective mechanism for the integrity of the organism (Selye, 1976; Ewbank, 1985; Rushen, 1986; Yousef, 1988). In the case of the use of castration and tailing techniques on lambs, the situation is perhaps greatly different from situations to which the concept of stress has been commonly applied. We are dealing not with environmental stress in which there is a grey area as to whether the effects are adaptive or maladaptive, whether stress has positive or negative implications for the animal. Rather we are dealing with an acute noxious physical trauma which, via psychological and physiological mechanisms, causes activation of a general response. For the purposes of this study it has not been found helpful to use the term

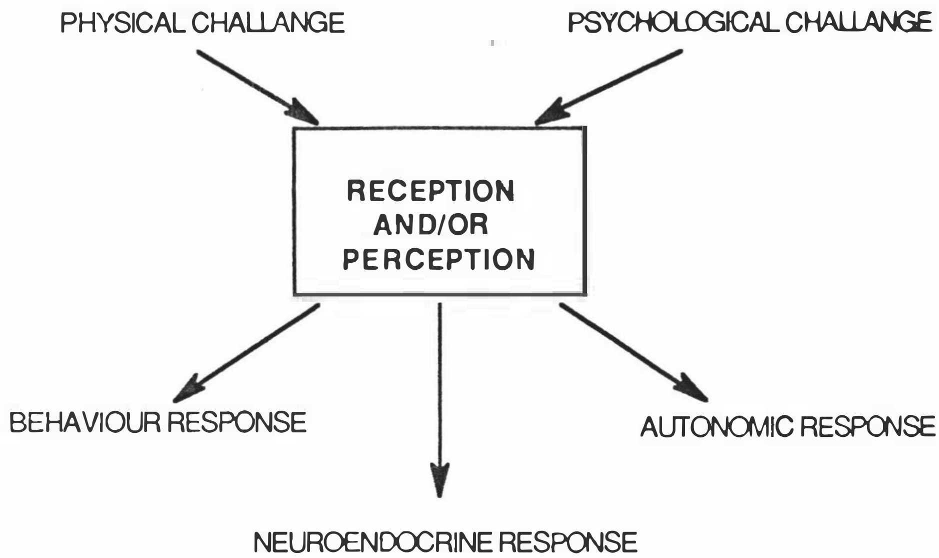


Fig. 1-1: Diagramatic illustration of an 'hourglass' view of stress.

stress, largely due to its vague nature. Rather the study is based upon the assumption that the procedures used (castration and tailing) are painful and will cause distress.

The views of Ewbank (1985) encompass a dual concept of stress: physiological stress and distress. Physiological stress is defined as adaptive mechanisms that aid the ability to cope with new, challenging events. The responses to coitus or voluntary exercise would fall into this category. Distress however involves damage to the animal as a result of the response to a stimulus. This means that distress occurs as a result of damage due to a response to a stimulus not as a result of damage due to the stimulus itself. Such a view of physiological stress and distress is valuable in dealing with the apparently confounding evidence used by Rushen (1986) which will be discussed below, but does not seem to adequately define distress in terms of situations of acute physical trauma. The term 'distress' in this study will be applied to animals that have a total conscious experience of the response to aversive or noxious stimulation. Thus distress involves an element of suffering due to a stimulus, but distress is considered to be a subset of stress and thus the physiology of the stress response as stated by various workers will be discussed.

1.4 MEASUREMENT OF STRESS/DISTRESS:

The work of Hans Selye has been used as a basis for much of the understanding of stress. His view can be termed an 'hourglass' concept of stress: that suggests that exposure to a wide variety of nonspecific stimuli can elicit physiological responses that include components that can be measured experimentally (Fig 1-1) .

In the case of harmful or potentially harmful stimuli the problem that arises is what factors actually represent the amount of distress, and whether these can be measured.

Selye's view of stress was based on observations that the responses to challenging stimuli always included a neuroendocrine activation involving the release of corticosteroids from the adrenal cortex. Since there were no cases of obvious stress that did not include adrenal gland activation it was believed that corticosteroid release could be used as proof of stress (Moberg, 1985), although

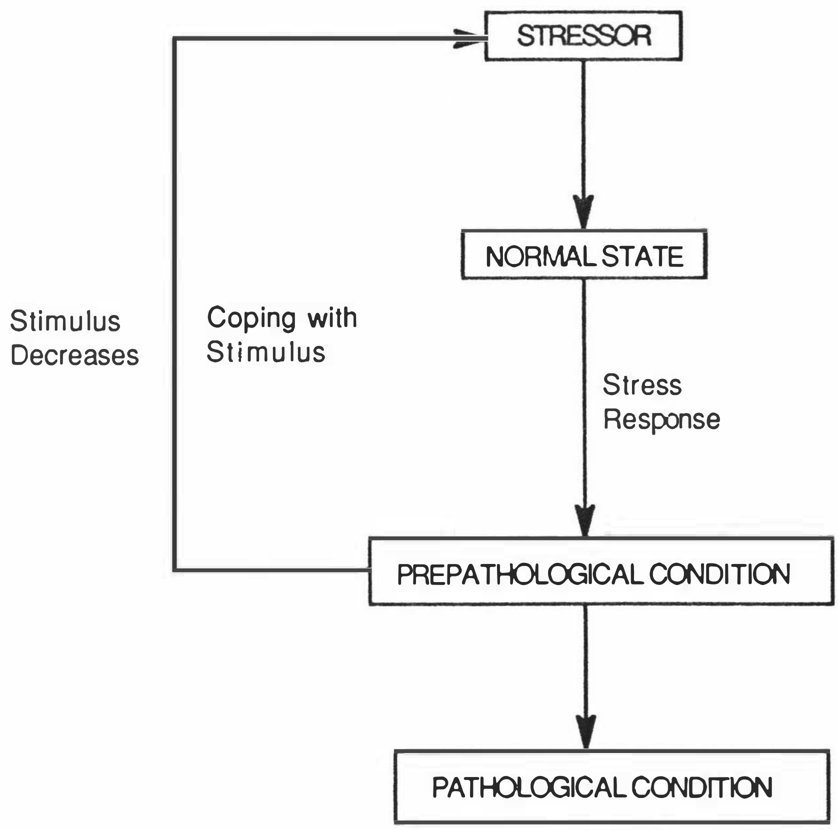


Fig 1-2: A model of the response to stress based on Moberg (1985)

Selye (1976) recognised that the release of corticosteroids was one component of a range of reactions that could be used to assess the stress response.

Moberg (1985) asserts that stress is best indicated by the onset of a prepathological condition, induced by a stressor, which may prevent the onset or decrease the effects of a pathological condition (Fig 1-2).

An animal in a normal condition, subjected to a stimulus that actually does or is perceived to threaten its homeostatic integrity, will show a stress response. A stressor can be an emotional disturbance such as fear or anxiety, or an actual or potential noxious stimulus. The stress response has been viewed as nonspecific: a wide range of dissimilar stimuli can evoke a relatively standard neuroendocrine response.

The response to a stressor involves a physiological activation mediated by the hypothalamic-pituitary-adrenal axis and by the autonomic nervous system. Also specific behaviour may be stimulated by a stressor; the behavioural response appears to include some nonspecific components, but the validity of these as indicators of stress has yet to be defined fully. The response to stressors may lead to physiological amelioration of the effects of the stimulus or behavioural escape from the stimulus and a resulting return to a normal state. Animals that lack the ability to respond to a stressor, such as after adrenalectomy, show an inability to survive even minor stress (Ramsey, 1982).

As a result of the physiological and behavioural responses that constitute a stress response, the organism will enter a state of increased vulnerability: the prepathological condition (Moberg, 1985). If the stimulus continues or the effects of the stimulus are prolonged then the stress condition may develop into a pathological condition. This can be seen most readily in the corticosteroids' immunosuppressive properties allowing opportunistic infections , but other examples include the development of peptic ulcers (Selye, 1976), excessive feather-pecking in poultry (Ewbank, 1985), stress-induced behavior leading to changes in nutrition (Solomon, Amkraut and Rubin, 1985). However, it is an adaptive function of the stress response that it confers physiological and behavioural amelioration of the effects of the stressor, thus allowing a return to a normal state. So while a stressed animal may be said to be in a vulnerable or prepathological condition it does not always follow that a pathological condition will develop. Thus, the stress response is a protective mechanism, adaptive to coping with challenge to the animal's homeostasis.

This view of stress, proposed by Moberg (1985), leads to his suggestion that the onset of a prepathological state be used as indicative of stress. If one accepts Moberg's model, the problem of how to measure the onset of a prepathological state arises. At what point can the immune system be said to be suppressed or inflammation reduced? When is an animal considered prone to disease or dysfunction?

Such recent concepts of stress reduce the importance of a reliance on adrenocortical activity as an index of stress (Dantzer and Mormede, 1983; Moberg, 1985; Veith-Flanigan and Sandman, 1985; Rushen, 1986; Yousef, 1988). The impetus for this trend was evidence that the relationship of components of the response to stressors may change with different types of stimulation (Dantzer and Mormede, 1983; Moberg, 1985; Rushen, 1986). Indeed Rushen (1986) states that it is a mistake to rely on measurement of plasma corticosteroid levels when animals are exposed to particular husbandry procedures in order to determine how distressing the procedure is for the animal.

Since the design of the following experiment involves the measurement of plasma cortisol as an index of distress as in the studies of Shutt et al (1987,1988), Mellor and Murray (1989a,b)and Wood et al (1991), Rushen's (1986) views must be explored fully.

The review by Rushen (1986) promulgates several reasons for the suspicion of plasma cortisol as an index of stress. First Rushen states that plasma corticosteroids do not give a sensitive enough assessment of the distressfulness of a given situation. The evidence for this statement comes from Freidman, Ader, Grota and Larson (1967) who showed that in rats increasing footshock gradations were not accompanied by progressively higher plasma cortisol corticosterone concentrations. However, while low amperage footshock (0.25, 0.5 and 1 mA) did not significantly elevate the plasma corticosterone concentration more than the controls, it should be noted that control animals were handled in the same manner as the experimental animals. Thus it may be that the distress elicited by handling, blood sampling and placing the rats in small cages was similar in magnitude to handling and subsequent low amperage footshock. Perhaps only at higher amperages footshock was a sufficiently noxious stimulus to distress the rats enough to be measurable as a higher plasma corticosterone concentration.

Another reason stated by Rushen (1986) and implied by other workers, against reliance on plasma corticosteroids as an index of distress is that corticosteroids can be elevated by events that are not considered unpleasant or distressing. The much used example is that of coitus but other examples include voluntary exercise in humans. Whether these examples cause a problem for people using cortisol as an index of stress really depends upon their view of stress. The views of Ewbank (1985) help in placing this evidence in perspective. The performance of coitus or of voluntary exercise could conceivably necessitate work of such intensity or duration that the normal physiology of the organism must respond to deal with the challenge and thus induce a stress response. On the other hand, and especially in the case of coitus, the accompanying psychological arousal may be sufficient to induce the release of corticosteroids without any abnormal physiological change. According to Ewbank (1985) if there is no suffering accompanying the general response, and in usual circumstances of coitus and voluntary exercise there would not be, then the organism is not distressed.

While there may be some inadequacy in using plasma cortisol as an index of distress in some situations, the castration and tailing situation is not one of them. Morton and Griffiths (1985) state in relation to assessing pain and distress that "physiological parameters (e.g. cortisol levels) may be of use, especially if 'normal' values have been evaluated". It may be that when the physical and psychological components of stress are separated (as Moberg, 1985; and Dantzer and Mormede, 1983 suggest is possible) the adrenal response may only be of limited use as an indicator. An example of the separation of physical and psychological components is cited from the work of Mason (1968), when monkeys starved of nutrients but fed flavoured, non-nutritive cellulose pellets to alleviate the discomfort of hunger, showed no rise in plasma cortisol concentration. However it is not clear whether the starvation was sufficient to create a stress response.

There is evidence that physical damage without conscious perception can elicit the classical adrenal component of the stress response. The evidence is that sheep and goats undergoing laporotomy while under general anaesthesia show a plasma cortisol elevation (Person and Mellor, 1975). It must be remembered that the stimuli that castration and tailing provide are without doubt potent physical and experiential challenges. Separation of the physiological and emotional components of the procedure is not possible within the bounds of routine docking procedures, so in terms of the distress of these husbandry procedures the evidence of Mason (1968) is not applicable.

1.5 PAIN:

Castration and tailing have been shown to cause distress (Shutt et al, 1988; Mellor and Murray, 1989a,b; Wood et al, 1991) which may stem from the intensity of pain elicited by those procedures. While the perception of pain in animals is unprovable, by analogy with our own anatomy, physiology and behaviour (Morton and Griffiths, 1985) and a bias towards humane treatment it seems reasonable to accept that animals do feel pain.

Pain can be defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Zayan, 1986). The sensation of pain depends upon a noxious stimulus that is potentially or actually damaging to tissues, that elicits nociceptor discharge. Nociception may be processed solely at the spinal reflex level and thus is not necessarily perceived as pain. If the stimulus is of sufficient intensity it may be processed at higher CNS levels and conscious perception of the stimulus as pain can occur. There have been discrete neural structures associated with noxious stimulation: superficial or 'fast' noxious stimulation is received by A ∂-fibres and transmitted in the neospinothalamic tract, and deep or 'slow' noxious stimulation is received by the C-fibres and transmitted in the paleospinothalamic tract. The perception of noxious stimulation as pain is not always associated with distress. For the perception of pain to be distressing there must be one or more of several factors in operation: (a) the pain is of sufficient intensity, (b) the pain may cause psychological arousal such as fear or anxiety if the cause or outcome of the pain is not known, (c) the pain may cause physical debility and/or psychological arousal if the animal experiences dysfunction as a result of the pain. For example lambs may be distressed by the pain resulting from application of a ring and from not being able to escape from the ring that is causing the pain. An important factor associated with the stimulation that castration and tailing provide is that the lambs will be distressed both by the physiological challenge (tissue trauma, nerve discharge and pathology of the treatments) and the emotional perception (pain, fear, arousal).

1.6 Stress and the Hypothalamic-Pituitary-Adrenal Axis:

The experience of distress in animals has been said to cause a response that includes activation of the hypothalamic-pituitary-adrenal axis. What factors mediate and regulate this axis and what are its effects?

The physiology of the stress response has been widely described in the literature. The following discussion is based on these texts: Selye (1976); Daughaday (1981); Liddle (1981); Ramsey (1982); Groer and Shekleton (1983); Kellerwood and Dallman (1984); Munck, Guyre and Holbrook (1984).

The hypothalamic-pituitary-adrenal axis refers to a neuroendocrine pathway that plays a central role in the stress response (Selye, 1976). The regulation of the pathway is initiated at the hypothalamus. Neural processing of aversive or noxious stimuli occurs at various levels of the nervous system, afferent signals from the special senses and somatic receptors will be perceived at higher centres. However the hypothalamus is central to the transfer of afferent input into the cognitive cortex and subsequent efferent signals. For example the effect of sensory information on the emotional condition is performed by the limbic system (which Papez described as the 'seat of emotions') and the hypothalamus is a major part of the limbic system. Thus, via the hypothalamus, emotional factors can affect the endocrine status of the animal. Stimulation of the hypothalamus can release neurosecretions originating in specific nerve cell regions which when carried in the portal venous system can control anterior pituitary activity. The hypothalamic neurosecretions include specific releasing factors for pituitary hormones. An example is adrenocorticotropin hormone (ACTH) which is released by corticotropic releasing factor (CRF). ACTH is a true hormone, released from the anterior pituitary, transported in the blood and acting at a target site to stimulate the secretion of corticosteroids from the adrenal gland which then have systemic actions. This then is the hypothalamic-pituitary-adrenal axis, it is mediated by CRF, ACTH and corticosteroids.

1.6.1 REGULATION:

There is negative feedback regulation of the hypothalamic-pituitary-adrenal axis in that corticosteroids inhibit the hypothalamic-pituitary component of the system. This inhibition operates by three mechanisms: (a) inhibition of the CRF-mediated ACTH release due to corticosteroid actions on the cell membrane of the anterior pituitary, (b) inhibition of CRF synthesis and release and (c) inhibition of ACTH synthesis by corticosteroid inhibition of the mRNA responsible for ACTH production. Thus the corticosteroid response to transient stimulation will itself not persist. However this does not indicate that the stress response cannot persist. In chronic or persisting stress the release of ACTH can occur regardless of the circulating corticosteroid levels. Furthermore it appears the corticosteroid level for inhibition of ACTH or CRF rises thus allowing a continuing stress response.

1.6.2 METABOLIC EFFECTS:

The release of cortisol from the adrenal cortex acts to elevate plasma levels of glucose, amino-acids and lipids. Plasma glucose levels are increased by cortisol-mediated stimulation of gluconeogenesis in the liver; this can be a 6 to 10 fold increase in the conversion of amino acids through keto-acids to glucose. Also cortisol inhibits the uptake of glucose from the blood into many cells, and by inhibition of hexokinase activity reduces oxidation of glucose already in the cells. Cortisol enhances plasma glucose levels by its synergistic action on other hormones such as adrenaline, glucagon and growth hormone (GH). The function of cortisol in its insulin antagonism has long been seen as providing glucose for tissue repair and muscle work in stressed animals. Munck et al (1984) propose that this property is secondary or coincidental, and that the primary function of corticosteroids is to prevent insulin from causing dangerous hypoglycaemia.

Cortisol mobilises body proteins by stimulating protein catabolism and suppressing cellular protein synthesis. This gives an increase in circulating amino acids which provide substrate for hepatic gluconeogenesis and an available resource for damaged cells. Similarly cortisol increases free fatty acid concentrations and cholesterol levels in the blood.

1.6.3 IMMUNOSUPRESSIVE EFFECTS:

Glucocorticoids block Fc-receptor augmenting factor (FRAF) which is a lymphokine that causes an increase in the number of Fc receptors on normal monocytes. Since the Fc receptors on monocytes and macrophages are important for specifically binding immunoglobins the inhibition of the increase in number of Fc receptors reduces the effectiveness of immune activity because recognition of antibody-tagged anitgens is not as efficient. Other lymphokines, immune interferon and T cell growth factor are known to stimulate granulocyte production and macrophage production and activation. Glucocorticoids have been shown to inhibit the production of such lymphokines from lymphocytes.

Natural killer cell (modified T lymphocytes) have the ability to lyse target tumour cells, and this forms part of the primary resistance to tumour growth. Evidence shows that glucocorticoids reduce the natural killer cell activity in peripheral blood.

1.6.4 ANTI-INFLAMMATORY EFFECTS:

Inflammation can be mediated by a raft of endogenous compounds: prostaglandins, leucotrienes, bradykinin, serotonin and histamine. These compounds cause an increase in the permeability of the vascular bed by causing a drawing apart of endothelial cells at their junctions. The action of histamine is immediate (over the first 2 to 3 minutes after stimulation) while the prostaglandin, leucotriene (products of arachidonic acid metabolism) and bradykinin participate in the delayed phase of inflammation. Glucocorticoids inhibit prostaglandin and leucotriene production by blocking the release of arachidonic acid from phospholipids. Similarly glucocorticoids block or reduce the molecular mechanisms of bradykinin, serotonin and histamine. Thus by decreasing chemical mediator levels, preventing vasodilation, capillary permeability and phagocytosis inflammation is reduced in the stressed animal.

1.6.5 THEORIES ON THE STRESS/DISTRESS RESPONSE:

There is an apparent paradox in the glucocorticoid immunosupression and anti-inflammatory actions. In the stressed animal the anti-inflammatory actions may be important for preventing damage to tissue due to hydrolytic effects and the steroid activity would re-establish membrane stability essential for the repair process (Ramsey, 1982). But the immunosupression associated with the stress response seems less beneficial. Munck et al (1984) hypothesise that immunosupression is vital in preventing the over-reaction of the immune system which would lead to auto-immune pathology. This is a unifying concept in the functional reason for glucocorticoid activity during the stress response: to prevent insulin from causing hypoglycaemia, to prevent auto-immune damage. So the stress-induced glucocorticoid elevation protects against the usual defense reactions to stress over-reacting and themselves becoming a challenge to the integrity of the organism (Munck et al, 1984).

1.7 OUTLINE OF THE PRESENT STUDY:

The thesis is divided into five chapters. Chapter One consists of a general introduction. Chapter Two embodies the investigation of the anatomy of the scrotum and the tail. The main castration and tailing trial is described in chapter Three, which investigates the behavioural and hormonal responses and also involves a comparison of methods of castration and tailing. Chapter Four embodies a follow up experiment that investigates the effects of handling on lambs that have been castrated and tailed. A synopsis of the main findings of the study is provided in chapter Five. As an aid to following the thesis there are chapter summaries provided at the start of each chapter listing the sections as they are presented.

CHAPTER TWO

ANATOMY

2.1 INTRODUCTION:

Tailing and castration procedures in sheep usually involve the application of various tools to the tail and scrotum of lambs to effect the removal of most of the tail and the removal or the inactivation (as in the short-scrotum procedure) of the testes. The process of amputating the tail and removing or inactivating the testes causes local tissue trauma and afferent nerve discharge from nociceptors. The noxious stimulation provided by the tailing and castration procedures is assumed to cause pain. Therefore, a study of the nerves that subserve the perception of pain due to castration and tailing have particular reference to animal welfare.

Somatic nerves that supply the periphery originate from the spinal cord at each spinal segment in the manner shown in Figure 2-1. The spinal cord receives sensory afferent fibres via the dorsal roots of the spinal nerves. The sensory nerve fibres in dorsal roots come from both dorsal and ventral branches of the spinal nerves. The spinal nerves tend to supply sensory and motor fibres to the region of the body corresponding to the segmental level at which they emerge from the spinal cord (Frandson, 1974). The appendages are supplied with sensory and motor fibres by braid-like arrangements known as plexuses which are formed by the intermingling of fibres of several successive regional spinal nerves (Frandson, 1974).

The integument of an animal can be divided up into sensory areas that are each supplied by one spinal nerve. Each area is known as a dermatome (Sinclair, 1981), and successive dermatomes overlap, in sheep as in other animals (Kirk, 1968). The area of the integument that is supplied by afferent fibres of a peripheral nerve is known as the cutaneous area (CA) of that nerve (Spurgeon and Kitchell, 1982; Kirk, Kitchell and Carr, 1987a): on the trunk CA can correspond to dermatomes; in the limbs, because the peripheral nerves in the limbs arise from plexuses, they do not.

The determination of the nerve supply to a cutaneous area can be made primarily in two ways: anatomical observation and neurophysiological mapping. By dissection of tissues the structural neural connections can be observed and the anatomical arrangement of the nerves defined. However, the functional cutaneous area supplied by nerves cannot be determined by anatomical observation because even with a dissecting microscope it is not possible to trace fine nerve fibres to their terminations. Neurophysiological mapping provides an opportunity to demonstrate the cutaneous area supplied by an intact nerve and to determine the degree of functional interconnection between spinal nerves.

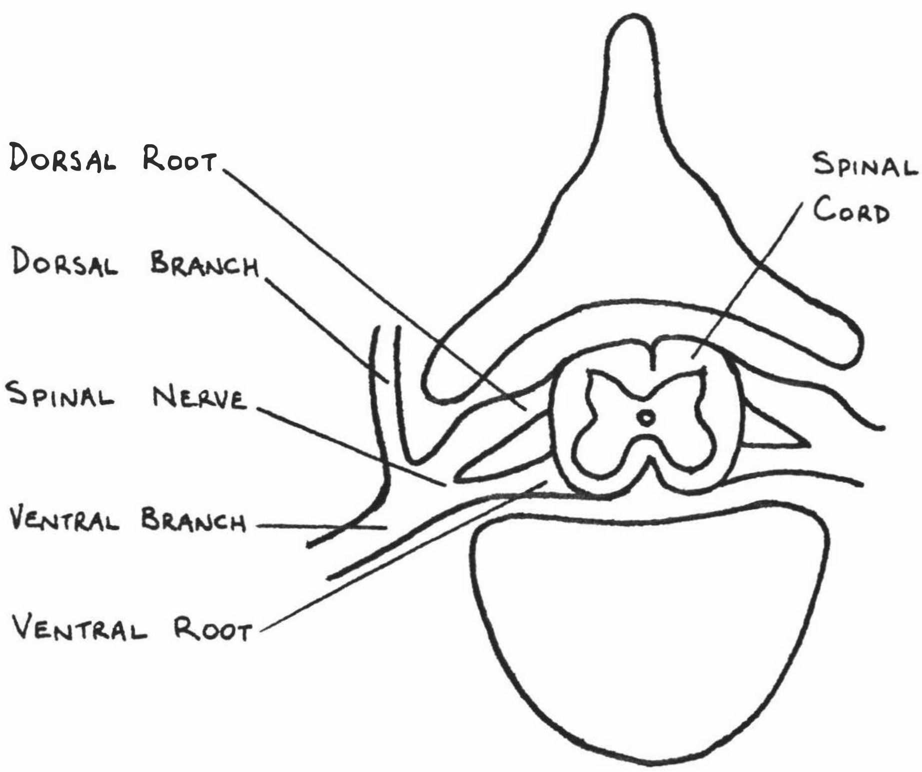


FIGURE 2·1 : DIAGRAM OF A SPINAL NERVE.

(ADAPTED FROM FRANDSON, 1974)

Some previous studies have used neurophysiological mapping techniques to determine the arrangements of cutaneous areas of nerves in monkeys and cats (Kuhn, 1953), bulls (Larson and Kitchell, 1958), dogs (Fletcher and Kitchell, 1966; Kitchell, Whalen, Bailey and Lohse, 1980; Spurgeon and Kitchell, 1982) and sheep (Larson and Kitchell, 1958; Ghoshal and Getty, 1971; Kirk et al, 1987a,b; 1988). It has been noted, however, that there is inherent variation in the extent of the CA from subject to subject (Fletcher and Kitchell, 1966; Kirk et al, 1987a,b; 1988).

The study of Larson and Kitchell (1958) showed that the external genitalia of bulls and rams receive their nerve supply via three routes: cranial, middle and caudal. The cranial route is via ventral branches of the spinal nerves from C8,T1,T2,T9-12 and L1,2; the middle route is via the genitofemoral nerve (also known as the inguinal or external spermatic nerve) which is made up of ventral branches of the spinal nerves from L2-4; and the caudal route made up of ventral branches of the sacral spinal nerves (Larson and Kitchell, 1958).

The ventral branches of T9-12 and L1,2 in the sheep are said to supply CA on the skin in the region of the protractor preputiae muscle, especially around the preputial orifice (Larson and Kitchell, 1958). In the dog the CA of the cranial iliohypogastric nerve that arises from the spinal nerve from L1 and of the caudal iliohypogastric nerve arising from the spinal nerve from L2 were found to occasionally extend onto the cranial prepuce (Spurgeon and Kitchell, 1982).

The genitofemoral nerve (GFN) is formed from a plexus originating from the spinal nerves from L1-4 in the sheep, and passes through the inguinal canal on the lateral surface of the cremaster muscle as two components that later anastomose and are designated the cranial and caudal branches of the GFN (Larson and Kitchell 1958). Both the cranial and caudal branches supply the cremaster muscle. The former is stated to terminate in the skin of the fold of the flank, while the latter has a cutaneous branch that reaches as far cranially as the preputial orifice and includes the lateral scrotal wall (Larson and Kitchell, 1958). In contrast Kirk et al (1987a) demonstrated that the CA of the genitofemoral nerve included only small areas of the caudal portion of the prepuce and the proximal portion of the base of the scrotum.

The sacral plexus is formed by branches of the spinal nerves from S2-4, and from this plexus three nerves arise which supply the CA of the external genitalia in the ram (Larson and Kitchell, 1958). The pudendal nerve arises from the plexus and terminates by dividing into the dorsal nerve of the penis (DNP) and the scrotal nerve (Kirk et al, 1987a). In the bull the pudendal nerve terminates by dividing into the DNP and the superficial perineal nerve (SPN)(Larson and Kitchell, 1958). In rams the CA of the DNP includes the prepuce as far caudally as the base of the scrotum (Kirk et al, 1987a). The CA of the scrotal nerve includes the caudal and cranial aspects of the scrotum and occasionally an ipsilateral area of the caudomedial aspect of the thigh (Kirk et al, 1987a).

The pudendal plexus also gives rise to three other cutaneous branches: the proximal cutaneous branch (PCB), the distal cutaneous branch (DCB) and the deep perineal branch (DPB). The anatomical arrangement varies (Kirk et al, 1987a; 1988) and although Larson and Kitchell (1958) state that excepting the deep perineal branch only one cutaneous branch is present in the ram, Kirk et al (1987a) describe both the proximal and distal cutaneous branches. The proximal cutaneous branch of the sacral plexus has a CA that includes the caudolateral aspect of the hindlimb without extending onto to the scrotum (Kirk et al, 1987a). The distal cutaneous branch of the sacral plexus has a CA that includes the perineum typically from the caudal midline laterally to overlap the CA of the PCB, and distally onto the caudal aspect of the scrotum (Kirk et al, 1987a). The terminal portion of the DCB of the sacral plexus that supplies the CA on the scrotum was identified and termed the caudal scrotal nerve by Larson and Kitchell (1958). The deep perineal branch of the sacral plexus has a CA that includes the skin immediately ventrolateral to the anus and occasionally may extend along the caudal midline of the perineum (Kirk et al, 1987a).

Thus nerves that provide cutaneous innervation to the external genitalia in the ram include the:

Ventral branches of spinal nerves from T9-12 and L1,2 (cranial and caudal iliohypogastric nerves).

Ventral branches of the spinal nerves from L1-4 (genitofemoral nerve)

Ventral branches of spinal nerves from S2-4 after forming a sacral plexus (dorsal nerve of the penis, scrotal nerve and the distal cutaneous branch of the sacral plexus).

The cutaneous innervation of the tail in the sheep has not been defined in the literature. Kirk (1968) and Kirk et al (1987b) have shown CA on the tail dock that originate from branches of the spinal nerves from S4, Ca1 and Ca2. However, due to the fact that sheep are usually tail docked as lambs the CA of the tail have not been more fully defined, and the present study was undertaken in part to study a greater length of the tail of the sheep.

MATERIALS AND METHODS

2.1 Preparation:

Nine entire adult rams, aged 2-5 years and weighing between 51.5kg and 66.5kg, and 10 entire hogget rams weighing between 26kg and 37kg were used in the study. Preparation of each animal was the same and was carried out the evening prior to the experiment being performed. The animals were weighed and the caudal regions (dorsal and lateral rump, perineum, scrotum, preputial skin and tail) were closely clipped. On the skin of the squarely standing animal the following reference points were marked using a black felt-tip pen: tuber coxae, greater trochanter of the femur, ischiatic tuberosity, lumbosacral junction and the midlines of the dorsal and caudal surfaces of the trunk. Measurement of the distances between these standard points enabled scaling factors to be calculated during the later transposition of mapped areas onto standard diagrams.

The ventral area of the neck was closely clipped and the right external jugular vein raised for intravenous injection of sodium pentobarbitone at a concentration of 60 mg/ml and a dose rate of 30 mg/kg bodyweight. The anaesthetic solution was administered as a bolus of two thirds the total calculated dose and then in 2 ml increments until a surgical plane of anaesthesia was induced, as determined by loss of jaw tone and the disappearance of the palpebral reflex. The trachea was then cannulated to prevent occlusion of the breathing passages due to regurgitation of ingesta, and to facilitate use of a respiratory pump (Palmer Co. Ltd., London) for brief periods when anaesthesia became too deep. The right common carotid artery was cannulated to enable blood pressure to be monitored via a physiological pressure transducer (Bell and Howell, model 4-327-I) and an amplifier (Neurolog Unit, NL 105) to a chart recorder. The right external jugular vein was cannulated to enable a drip of sodium pentobarbitone solution at a concentration of 20 mg/ml and a rate sufficient to maintain a surgical plane of anaesthesia throughout the experiment. Euthanasia was carried out at the end of the surgical procedures by overdosing the animal with sodium pentobarbitone solution while the animal was still deeply anaesthetised.

2.2 Surgical Exposure of Nerves:

Surgery involved a combination of three basic procedures. First, exposure of spinal nerve rootlets which was achieved by laminectomy at the level of the sacrum. Laminectomy was performed in 7 adult rams and 5 hogget rams. The removal of the dorsal process of the sacrum revealed the spinal cord with nerve rootlets in the spinal canal. Discrete nerve rootlets and nerve fascicles contained in the spinal cord were dissected away from the arachnoid mater to enable recordings to be made with the bipolar recording electrode. Care was taken to reduce the presence of cerebrospinal fluid (CSF), which interfered with recording, by suction or swabs. Paraffin was applied to the nerves from which recordings were made, with a brush, in order to limit other fluids from interfering in the recording and to prevent nerves from drying out. In order to allow free access to the spinal cord and accompanying nerve rootlets muscles attached to the spine at this level were removed and the crest of the ilium on the side from which recording were made was also removed if necessary.

The second surgical procedure was the isolation of the cutaneous branches from the sacral plexus, performed in rams (3 adult and 1 hogget). Removal of the gluteal muscles overlying the sacrotuberous ligament and subsequent incision through the ligament revealed the sacral plexus. Exposure of the nerves arising from the pudendal plexus, at points immediately distal to the plexus revealed cutaneous branches from which recordings were made. Recordings from the scrotal and preputial branches of the pudendal nerve were performed in 3 adult and 6 hogget rams.

The scrotal nerve and the dorsal nerve of the penis (DNP) were isolated by incision 2-3 cm lateral of the midline and midway between the anus and the neck of the scrotum on the perineal skin. Dissection around one side of the penis at this level revealed the scrotal nerve and DNP for recording.

The final surgical procedure involved dissection of the skin away from the subcutaneous faschia to expose cutaneous nerves. Dorsal Cutaneous Branches of the sacral spinal nerves were revealed by dissection parallel to the midline, began approximately 2 cm contralateral to the side from which recordings were made, at a level equal or caudal to the lumbosacral junction (performed in 4 adult and 3 hogget rams). Continuation of the dissection caudally along the lateral edge of the tail in 8 hogget rams exposed the main superficial nerve trunk which runs ventrally and slightly laterally to the coccygeal vein. Dissection midway between the midline and lateral edge of the ventral surface of the tail exposed the main nerve trunk that supplied the ventral cutaneous areas. Once the nerve trunks on the tail were found, surgical dissection was used to subdivide them in order to define the areas innervated by the trunks.

2.3 Neurophysiological Recording

(a) Nerves that were exposed by surgical means were placed on a bipolar recording electrode which was connected to a Neurolog system (Fig. 2.2)

(b) Mapping was performed to identify the cutaneous area (CA) that was supplied by the nerve isolated by surgery. Stimulation involved brushing the closely clipped hairs without moving the underlying structures.

(c) The nerve action potentials elicited by stimulation of the cutaneous area which they supply were heard as distinctive noise in the loudspeaker and seen as action potentials on the oscilloscope screen.

(d) The boundaries of the CA of the nerve that had been isolated and was on the recording electrode were thus defined and could be delineated on the skin with coloured paint.

(e) The isolated nerve was then marked for later identification by attaching a flagged ligature.

2.4 Confirmation of Identification of Nerve Branches:

An autopsy was carried out on each subject after it had been euthanased. Each flagged nerve or nerve rootlet was identified by

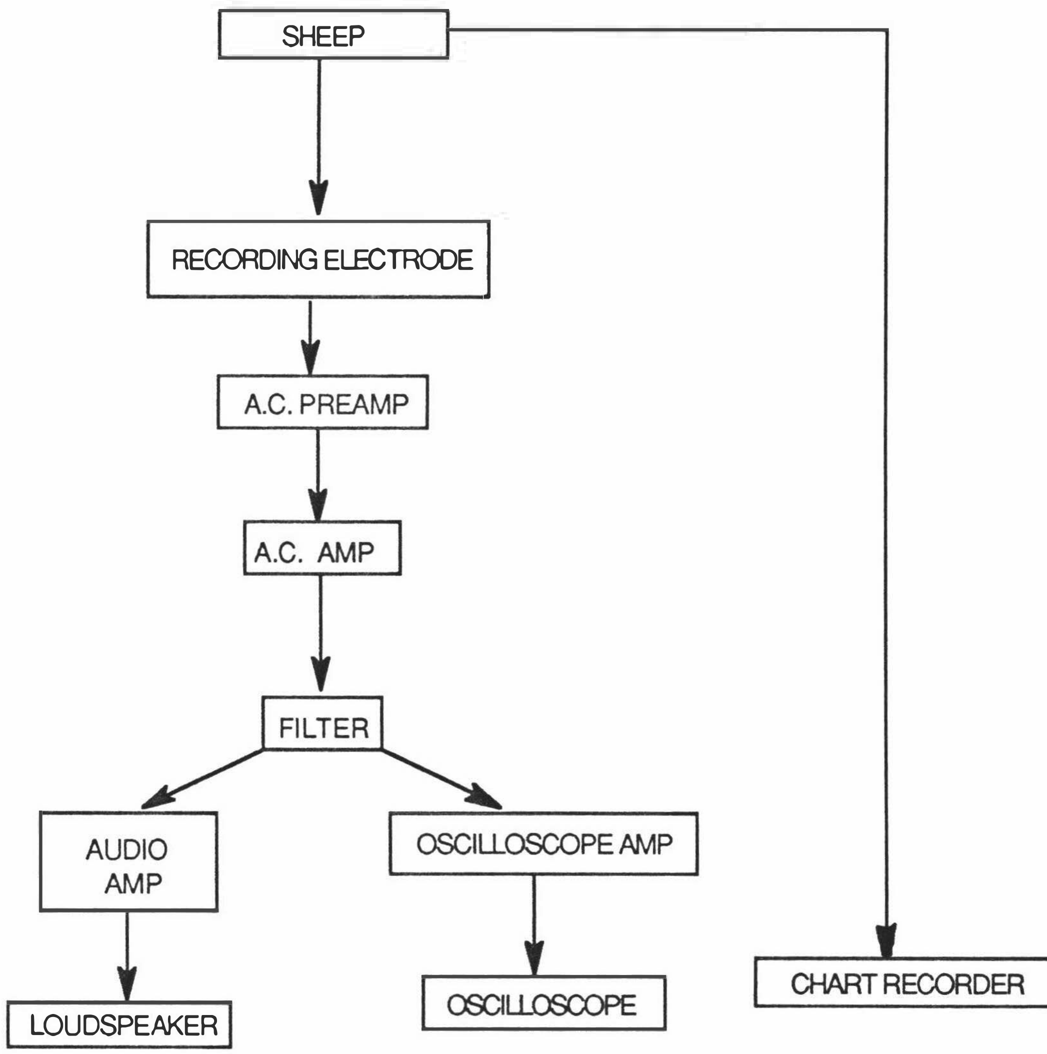


Fig. 2-2: Diagram of the Neurolog System used for neurophysiological mapping

dissection back to its point of entry into a vertebral foramen.

2.5 Preparation of Figures:

In order to map the fields found by neurophysiological methods accurately onto diagrams standard figurines were prepared and used. Measurements were taken from squarely standing sheep of landmarks and these measurements were used to transfer the dimensions of the experimental animals onto the prepared figurines. Thus by measuring distances such as that between the tuber coxae and the greater trochanter of the femur, between the greater trochanter of the femur and the ischiatic tuberosity, between the lumbosacral junction and the end of the tail the actual dimensions of the sheep could be scaled to the size of the figurines.

2.3 RESULTS

2.3.1 Innervation of the Perineum:

A. Anatomical Arrangements:

The sacral nerve plexus was identified on one side of each of 4 rams. It was formed by S2, S3, S4 and occasionally Ca1 spinal nerves, with several anatomical arrangements (Fig. 2-3a and b). The Proximal Cutaneous Branch and Distal Cutaneous Branch arose either singly (Fig. 2-3a) or as a common trunk from the plexus (Fig. 2-3b). In some cases the Proximal Cutaneous Branch (more commonly) and the Distal Cutaneous Branch (occasionally) had a direct nerve connection with S4.

In one ram a single branch of the S2 nerve entered the plexus (Fig. 2-3b); in over half the remaining three rams a second smaller branch located more dorsally was also present (Fig. 2-3a).

A caudal rectal nerve was isolated in 2 adult rams and the 3 hogget rams. It arose either with or without connection to the proximal and distal cutaneous branches.

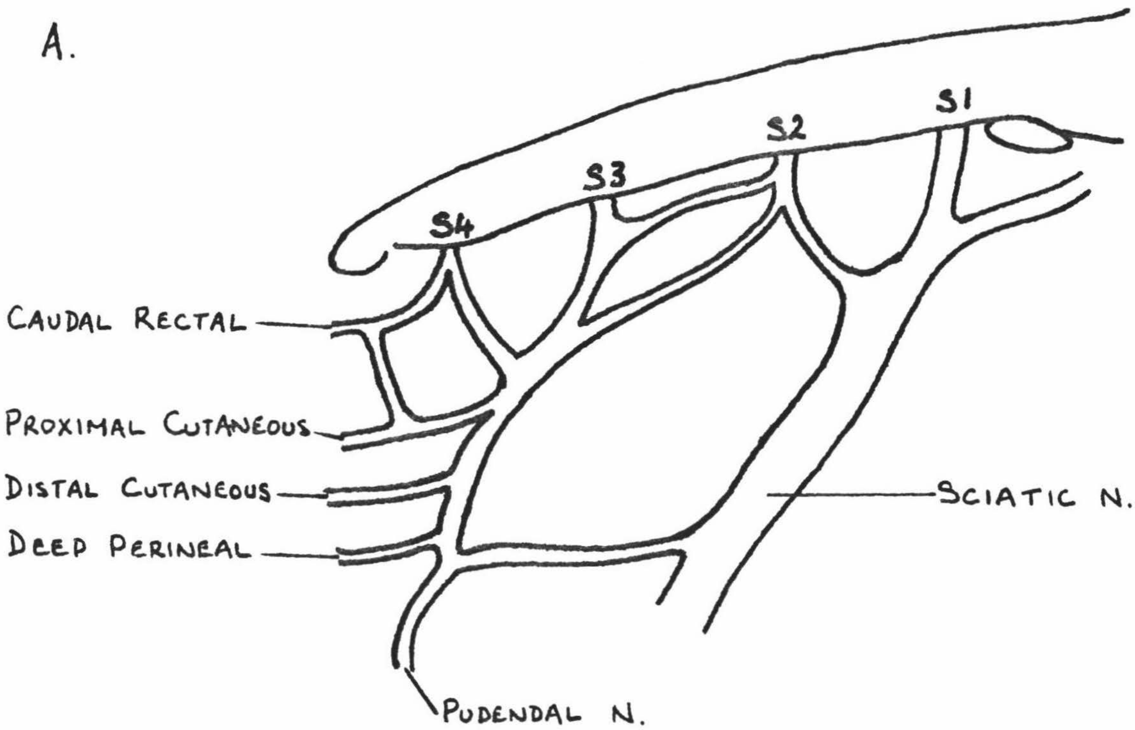
B. Cutaneous Areas (CA):

The CA of the Proximal Cutaneous Branch was mapped in 3 adult rams and 1 hogget rams, and in each case it included an area lateral and slightly ventral to the dock or tail (Fig. 2-4). It varied among the rams in its extent down the caudal aspect of the leg and its extent cranial to the ischiatic tuberosity on the dorsolateral aspect of the leg (Fig. 2-4). In no instance did the CA touch the perineal midline of the body.

The Distal Cutaneous Branch's CA was mapped in 2 of 3 adult rams and the hogget ram. The CA most commonly lay ventrolateral to the anus without involving the anal skin. It always covered an area that extended, from the ventral edge of the anal skin close to and abutting the midline, ventrally to a point short of the beginning of the neck of the scrotum (Fig. 2-5). The combined CA for all subjects extended ventrally from the level of the tail, to include an ipsilateral strip of the caudal aspect of the scrotum (Fig. 2-5).

The CA of the Deep Perineal Branch was mapped in 2 adult rams and 1 hogget ram. It always included the ventral anal skin and an area ventral to the anus (Fig. 2-6). A combination of all the

A.



B.

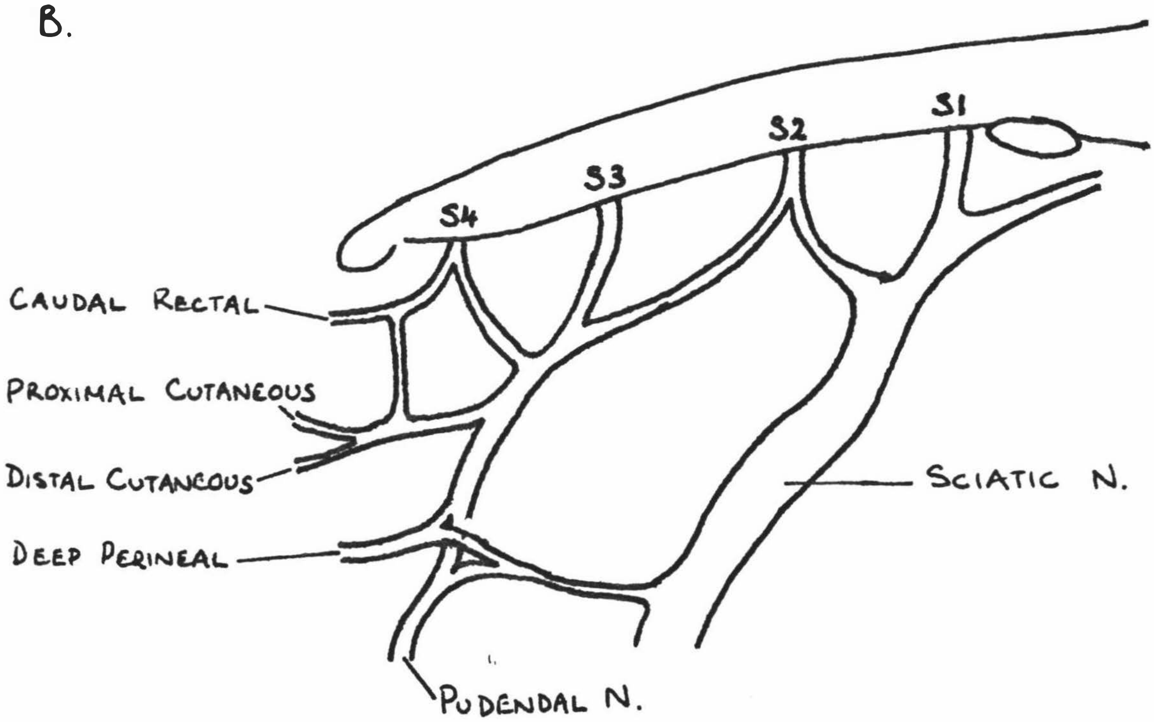


FIGURE 2·3 : ARRANGEMENTS OF THE SACRAL PLEXUS AND BRANCHES

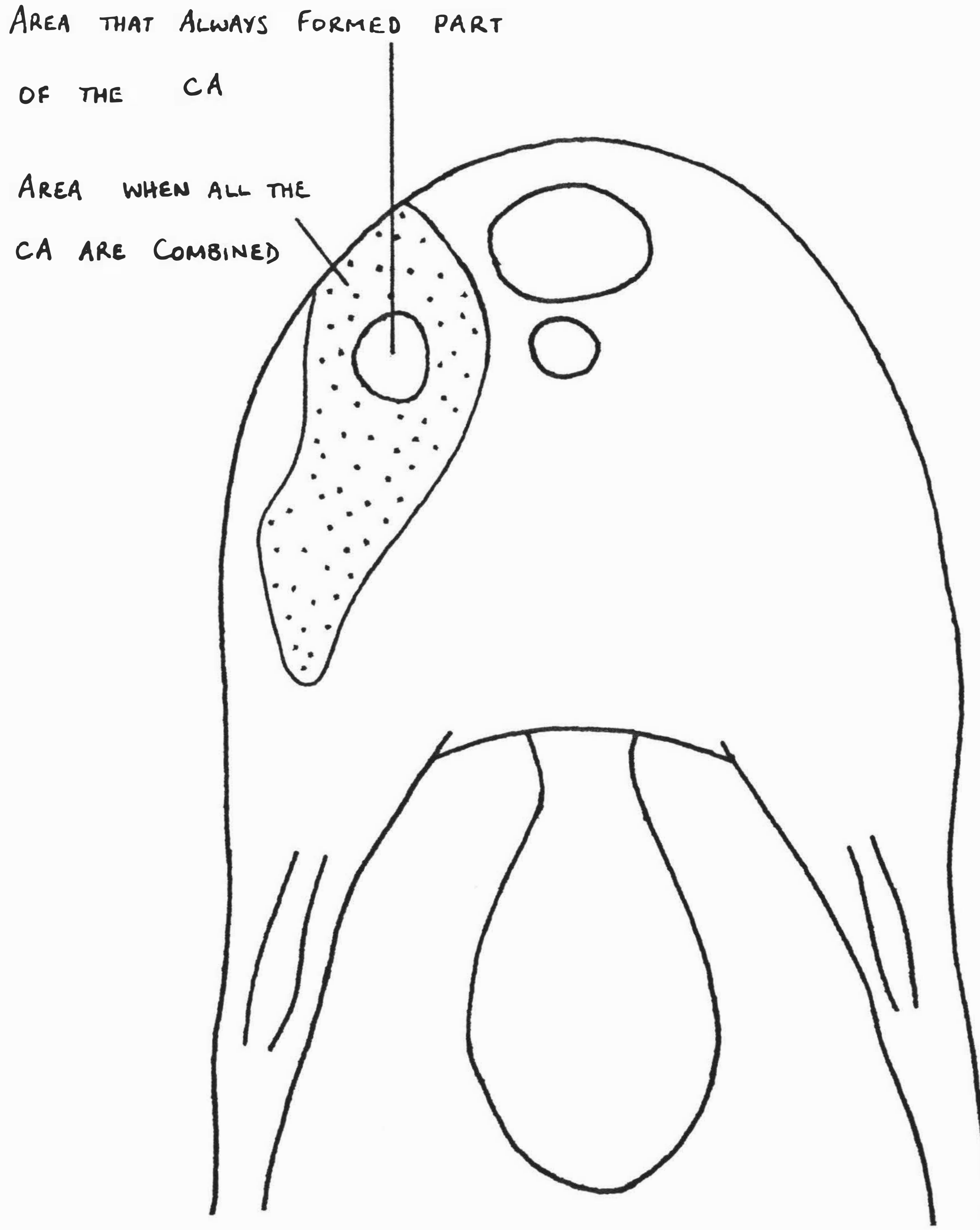


FIGURE 2·4: CUTANEOUS AREAS RECORDED FROM THE PROXIMAL CUTANEOUS BRANCH OF THE SACRAL PLEXUS (LEFT SIDE).

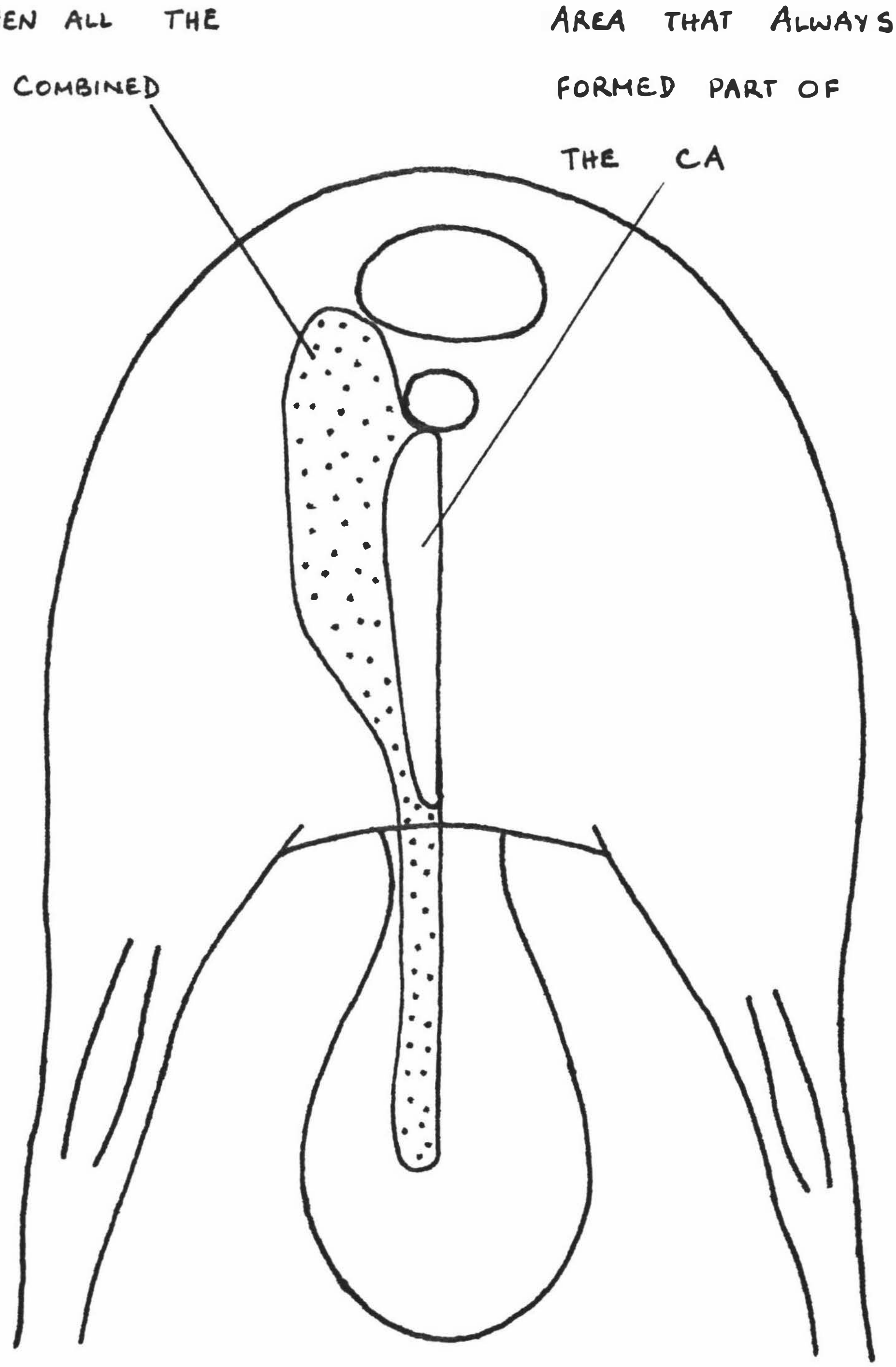


FIGURE 2.5 : CUTANEOUS AREAS RECORDED FROM THE DISTAL CUTANEOUS BRANCH OF THE SACRAL PLEXUS (LEFT SIDE).

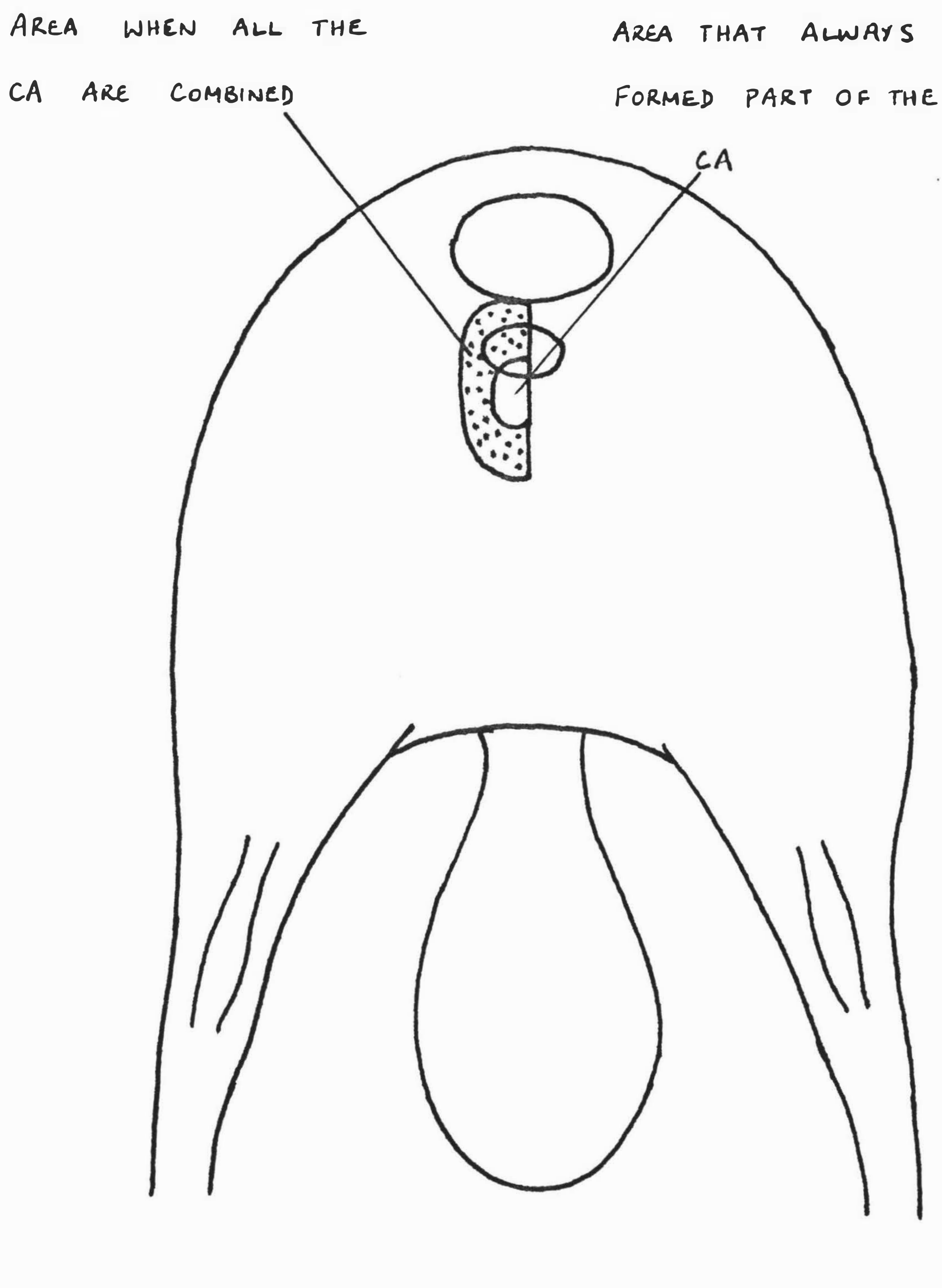


FIGURE 2·6 : CUTANEOUS AREAS RECORDED FROM THE DEEP PERINEAL BRANCH OF THE SACRAL PLEXUS (LEFT SIDE).

subjects' CA covered an area extending from the base of the tail to a point a quarter of the distance between the anus and the neck of the scrotum (Fig. 2-6).

2.3.2 Innervation of the External Genitalia

A. Anatomical Arrangements:

At a point distal to the point of connection between the pudendal plexus and the sciatic nerve the pudendal nerve became apparent as a discrete entity. Its path proceeded caudally and ventrally between the tail and the ischiatic tuberosity, arcing around the ischiatic arch to lie along the dorsal surface of the penis (Fig. 2-7a).

The terminal branches of the pudendal nerve are the Dorsal Nerve of the Penis (DNP) and the Scrotal Nerve. These two branches separated from each other at a point between the distal limit of the sacral plexus and the ischiatic arch (Fig. 2-7b). Such a pattern was found in 8 of 9 rams studied. In one hogget ram the branches were separate at a level proximal to the entry of the deep perineal branch into the sacral plexus. From the point of separation to a point just proximal to the sigmoid flexure of the penis the two branches were enclosed within a thin sheet of connective tissue and without dissection could not be observed as discrete nerves (Fig. 2-7b).

There was no apparent connection between the DNP or Scrotal Nerve from their point of separation until a point just proximal to the sigmoid flexure of the penis. At this point some variation of connection was observed. The most common arrangement (in 2 of 3 adult and all 6 hogget rams) was a single macroscopic connection between the Scrotal Nerve and the DNP (Fig. 2-7c). In one adult ram no connection was observed between the two branches. The DNP divided into 2 branches (3 in 1 adult ram) at a point proximal to the connection with the Scrotal Nerve. In all animals one branch could be identified as supplying the preputial CA (Fig. 2-8) while the one or two other branches appeared to supply deep penile structures. The most dorsal of the DNP branches did not appear to supply a cutaneous area. The other

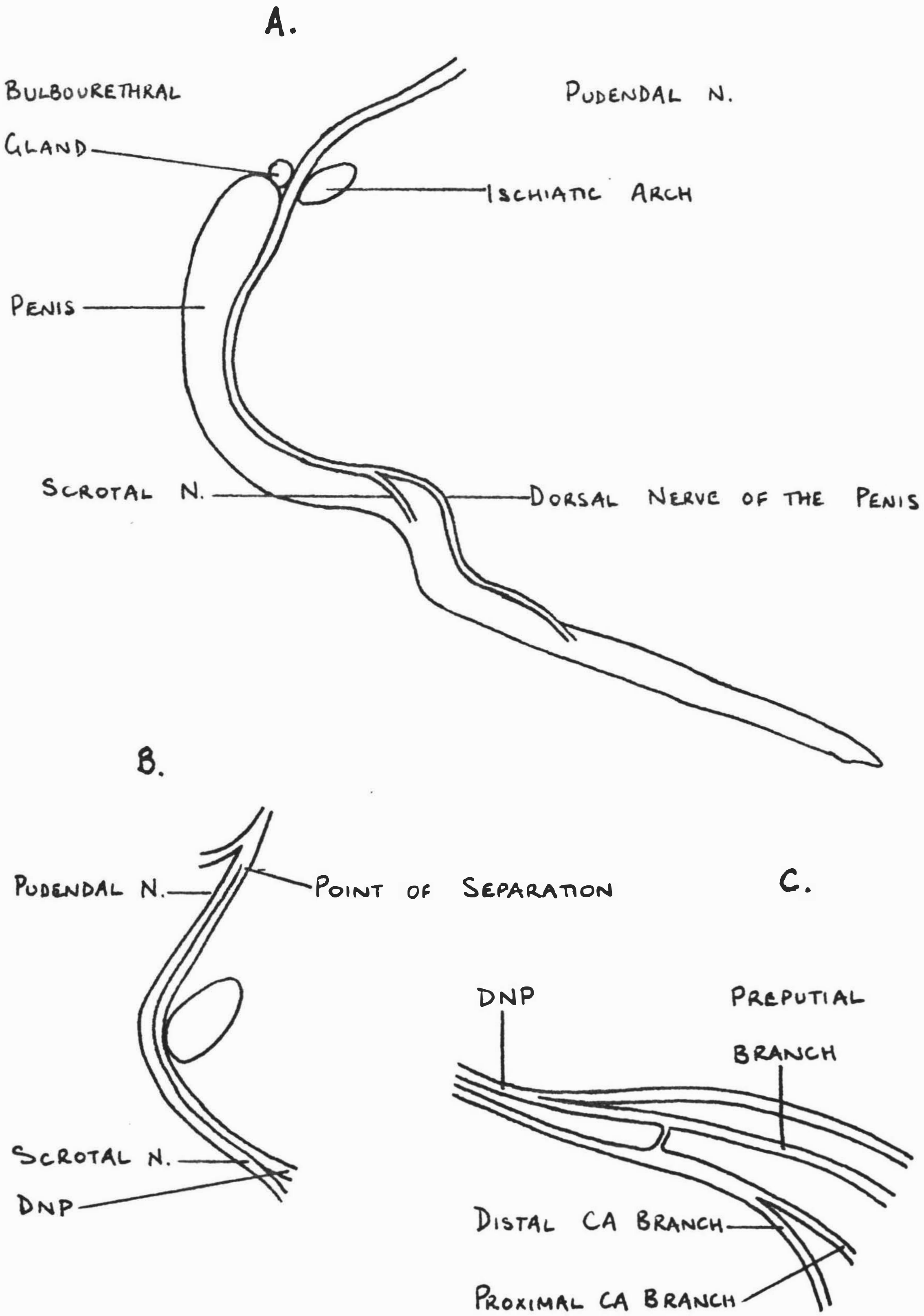


FIGURE 2·7: NERVES OF THE EXTERNAL GENITALIA ARISING FROM THE PUDENDAL NERVE.

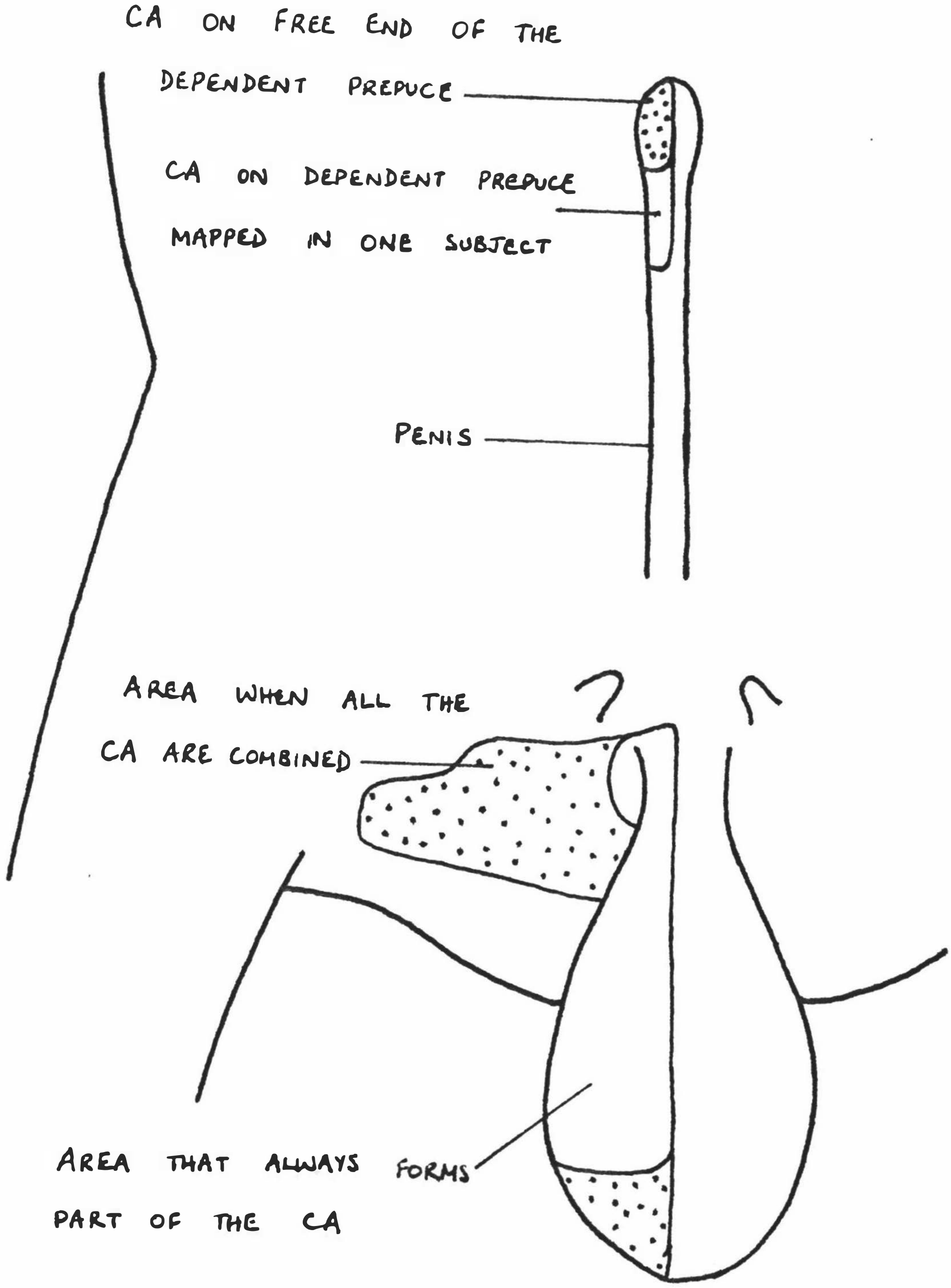


FIGURE 2.8 : CUTANEOUS AREAS RECORDED FROM THE DNP AND THE SCROTAL NERVE (LEFT SIDE).

VENTRAL ASPECT

branch, always located closer to the Scrotal Nerve supplied the preputial CA and had (in all but one adult ram) connection to the Scrotal Nerve (Fig. 2-7c).

B. Cutaneous Areas:

The CA supplied by the DNP were located ipsilaterally and in all but one hogget was restricted to the dependent free end of the prepuce (Fig. 2-8). In one hogget a CA on the prepuce proximal to the dependent free end was demonstrated. Attempts to identify this CA in other sheep failed, but stimulation of deep structures along the prepuce induced nerve discharge (Fig. 2-8).

The Scrotal Nerve was observed to bifurcate at a point distal to the connection between the scrotal nerve and the DNP. In one adult and one hogget ram the dorsal branch of the scrotal nerve supplied a CA that extended over the proximal area of the scrotum and the ventral branch of the same nerve supplied a CA extending over the distal area of the scrotum (Fig. 2-9). The CA supplied by the entire Scrotal Nerve (Fig. 2-8, 2-10) extended over the ipsilateral side of the scrotum from the abdomen caudal to the ipsilateral teat to a level near the distal end of the scrotum. Some difficulty was experienced mapping the CA on the distal end of the scrotum due to an inability to keep the scrotum absolutely still. In some animals the CA of the Scrotal Nerve extended laterally from the base of the scrotum onto an area of thigh (Fig. 2-8, 2-10)

2.3.3 INNERVATION OF THE TAIL

A. Cauda Equina of the Adult and Hogget Ram:

In the adult ram the cauda equina became apparent within the vertebral canal at the level of the fourth sacral vertebra (S4). In 4 of 7 rams the cauda equina began at the level of the S4/Ca1 intervertebral foramina(Fig. 2-11). In the other 3 rams was not apparent until the level of the first caudal vertebra.

The Cutaneous Areas (CA) supplied by individual dorsal spinal nerve rootlets were in many subjects not well defined. Cutaneous

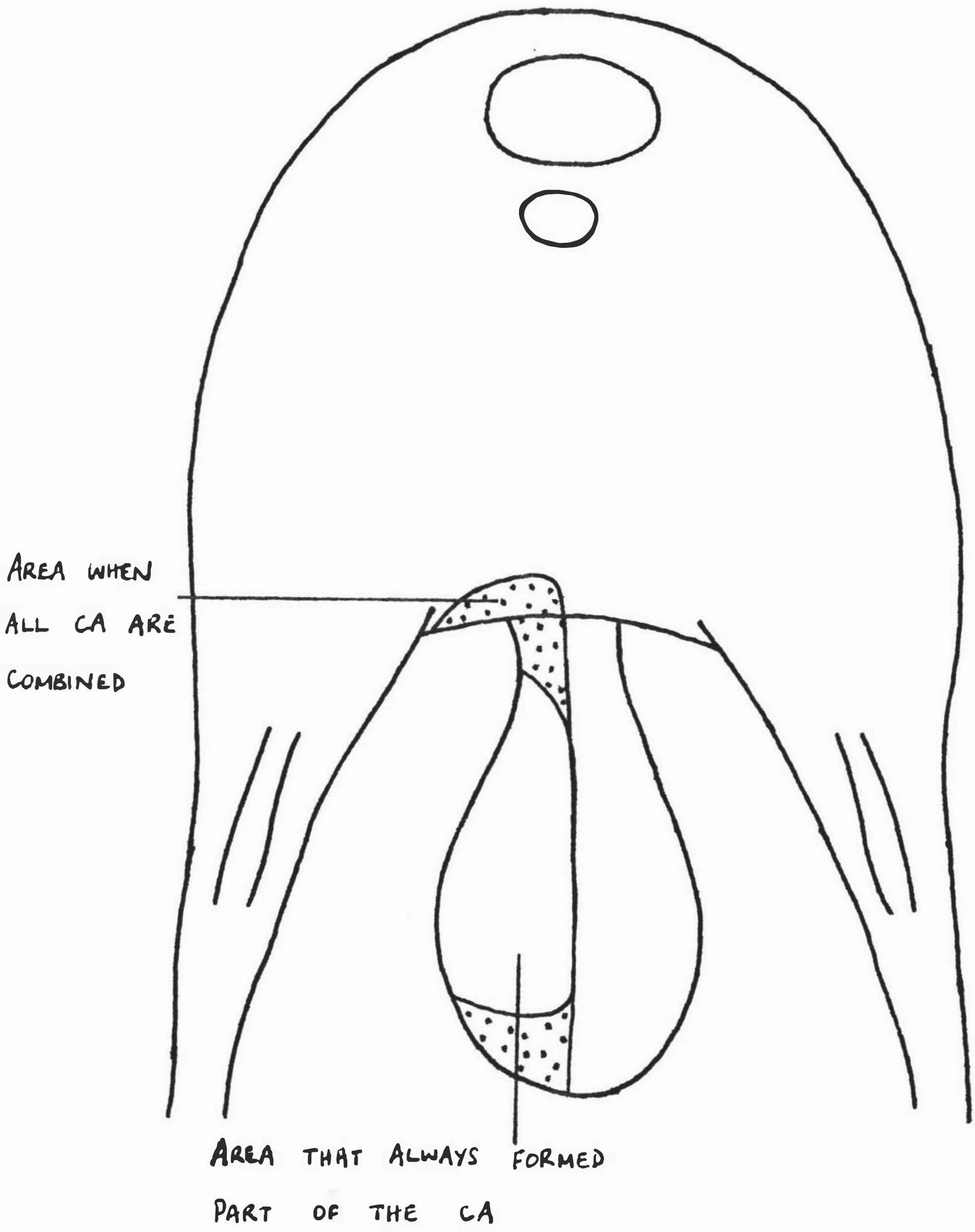


FIGURE 2.9 : CUTANEOUS AREAS RECORDED FROM THE SCROTAL NERVE (LEFT SIDE).

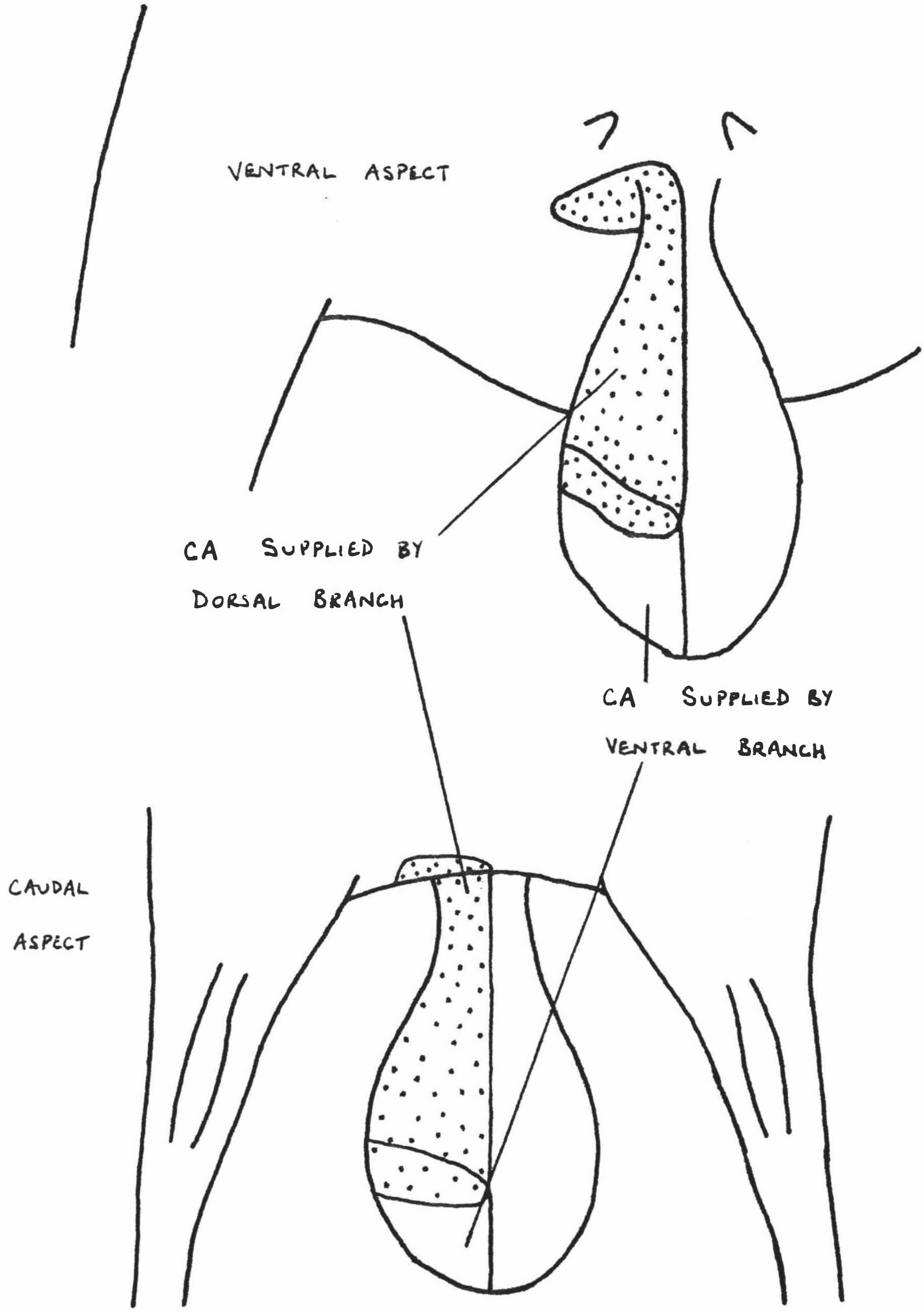


FIGURE 2·10: CUTANEOUS AREAS RECORDED FROM THE DORSAL AND VENTRAL BRANCHES OF THE SCROTAL NERVE.

areas (CA) recorded from nerve rootlets of the S2 spinal nerve were found on the lateral perineal skin (Fig. 2-12), extending from a point lateral to and at the level of the dock, ventrally on the perineal surface. Two CA were mapped (in 3 rams) that had the common feature of extending from the dorsal midline above the dock, coursing around the dock and terminating against the midline on the perineal surface (Fig. 2-12). These CA were recorded from rootlets of the S3 and S4 spinal nerves.

Cauda Equina of the Hogget Ram:

The cauda equina of the hogget rams at the at the level of Ca 1 (Fig. 2-13). In 1 of 5 hoggets examined the cauda equina did not begin until a point caudal to Ca 1. In both hogget and adult rams the cauda equina comprised 5 distinct nerve bundles each bundle being a composite of many discrete nerve fibres.

The CA of spinal nerve rootlets were difficult to define both in hoggets and adult rams. CA of S3 and S4 spinal nerve rootlets were mapped (Fig.2-14). Recording from caudal (Ca1, Ca2) spinal nerve rootlets showed CA on the tail that were ipsilateral and extended from the midline on the dorsal surface to the ventral border of the lateral surface (Fig. 2-14, 2-15). At the caudal limit of the CA supplied by Ca2 the field extended ipsilaterally from the dorsal midline to the ventral midline. Where this was the case the tail had lost the dorsoventral flattening that was characteristic of the proximal four tenths of the length of the tail.

Ventral tail CA from the recording of spinal nerve rootlets showed a similar pattern to the dorsal CA. However they did not extend as far cranially as the dorsal CA and showed more overlapping (Fig. 2-15). The most caudal ventral CA was recorded from the Ca2 spinal nerve rootlets (as with the dorsal CA) but it did not extend to the non-dorsoventrally flattened part of the tail. The ventral CA extended from the ventral midline laterally onto the dorsal surface but did not reach the dorsal midline (Fig. 2-15, 2-16).

B. Anatomical Arrangements

The DCB arrangement in the tail was seen as components in a

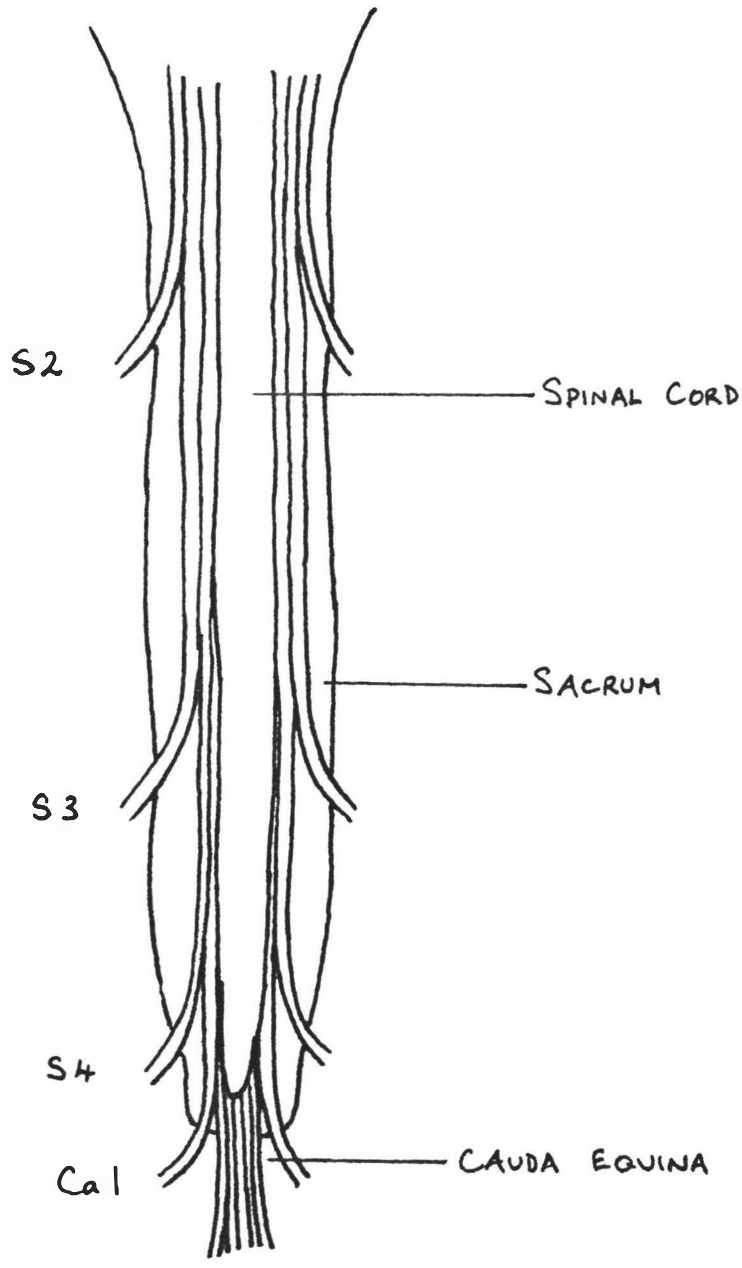


FIGURE 2·11 : DIAGRAM OF THE CAUDA EQUINA OF THE ADULT RAM.

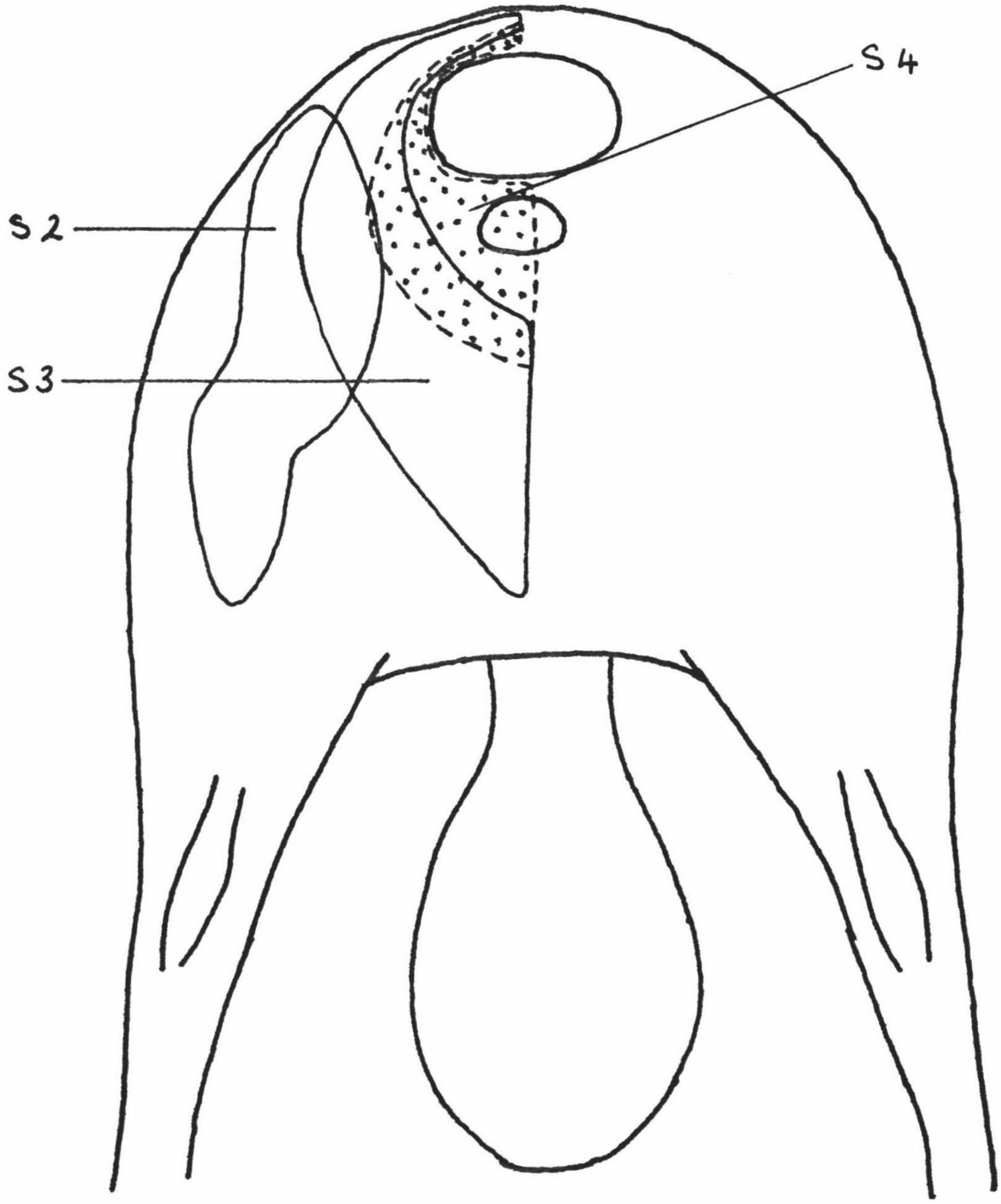


FIGURE 2.12: CUTANEOUS AREAS RECORDED FROM SPINAL ROOTLETS AT S2, S3 AND S4.

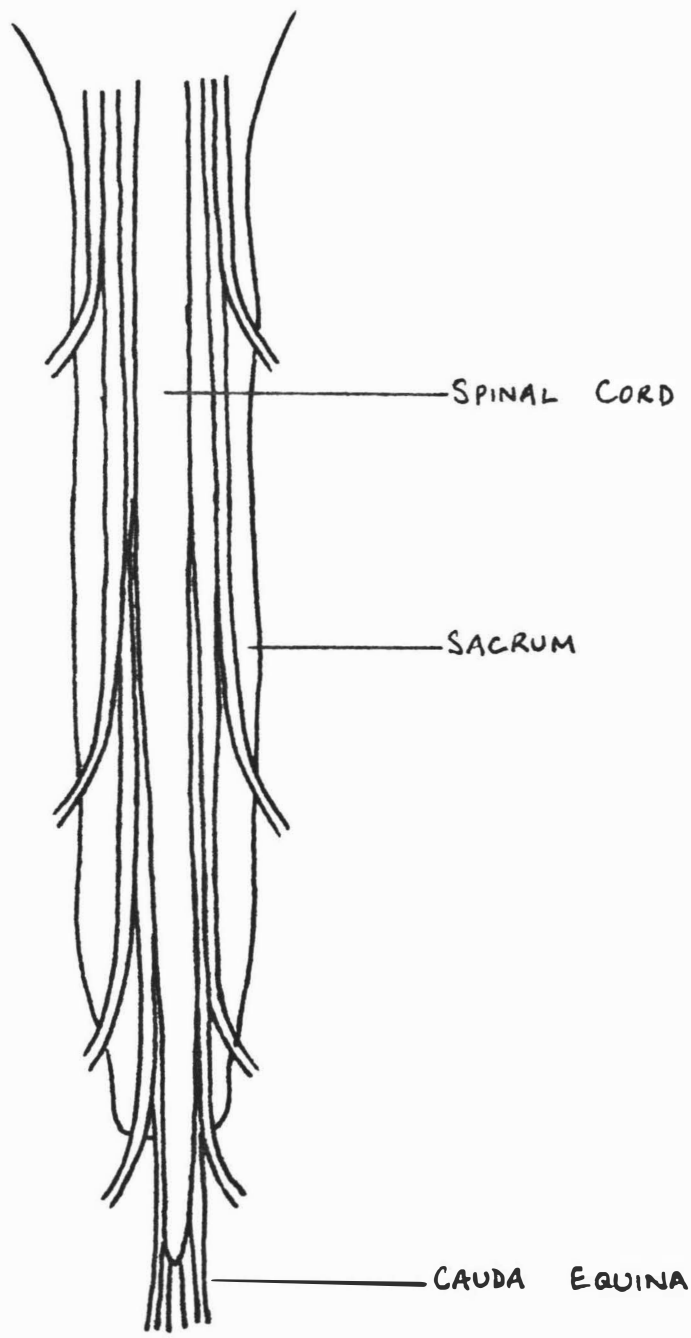


FIGURE 2.13 : DIAGRAM OF THE CAUDA EQUINA OF THE HOGGET RAM

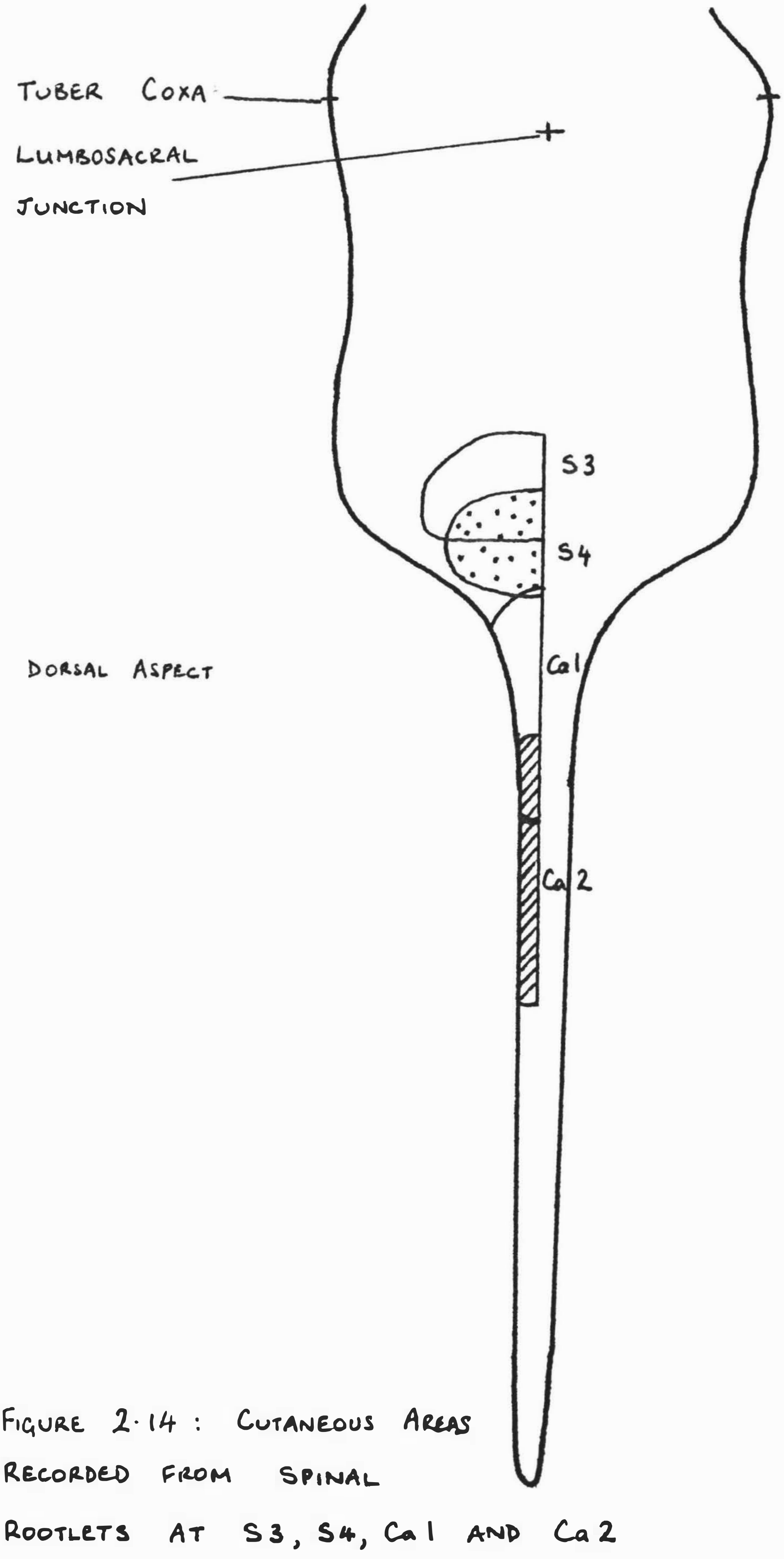


FIGURE 2·14 : CUTANEOUS AREAS RECORDED FROM SPINAL ROOTLETS AT S3, S4, Ca 1 AND Ca 2

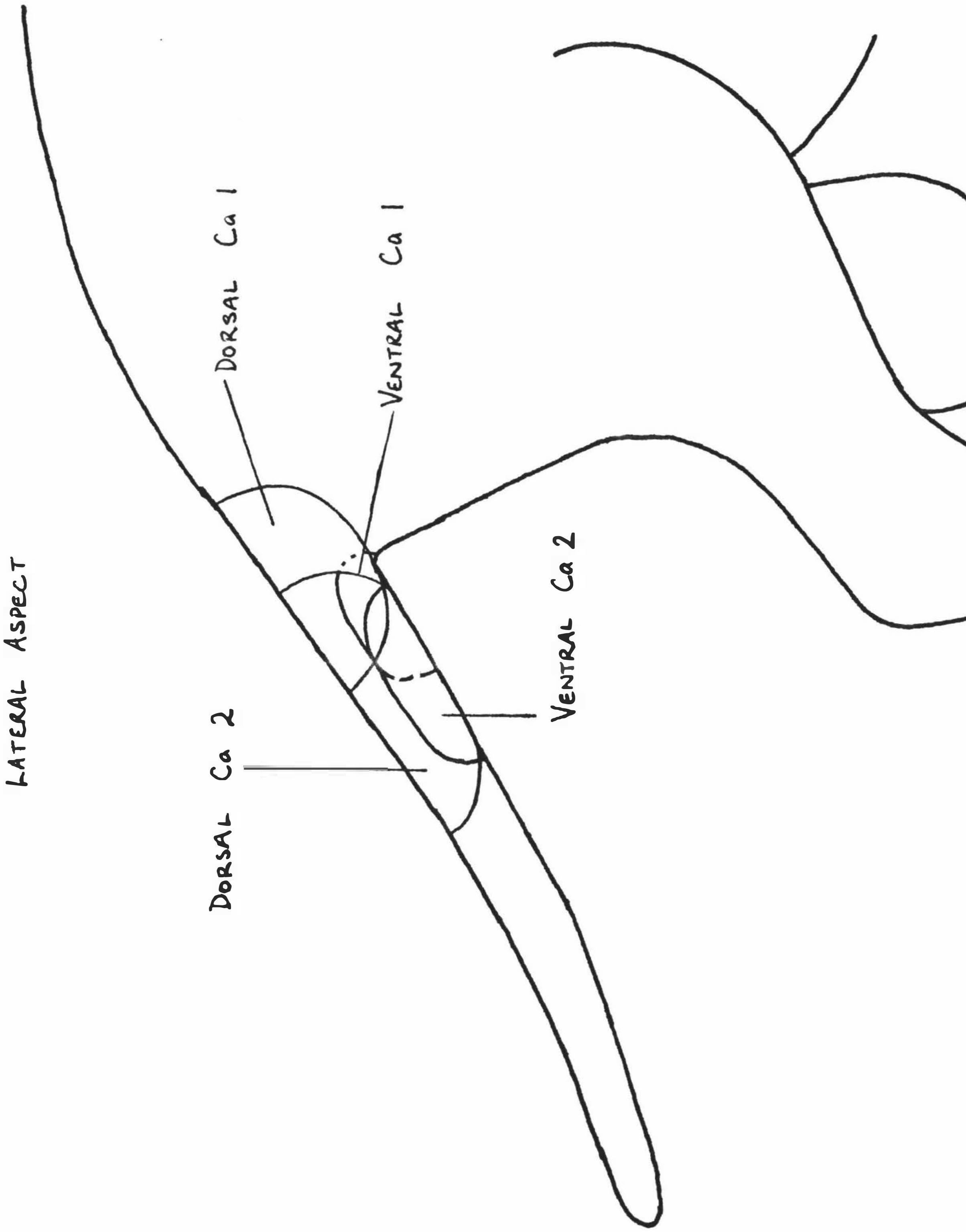


FIGURE 2·15: CUTANEOUS AREAS RECORDED FROM DORSAL AND VENTRAL SPINAL ROOTLETS AT Ca1 AND Ca2.

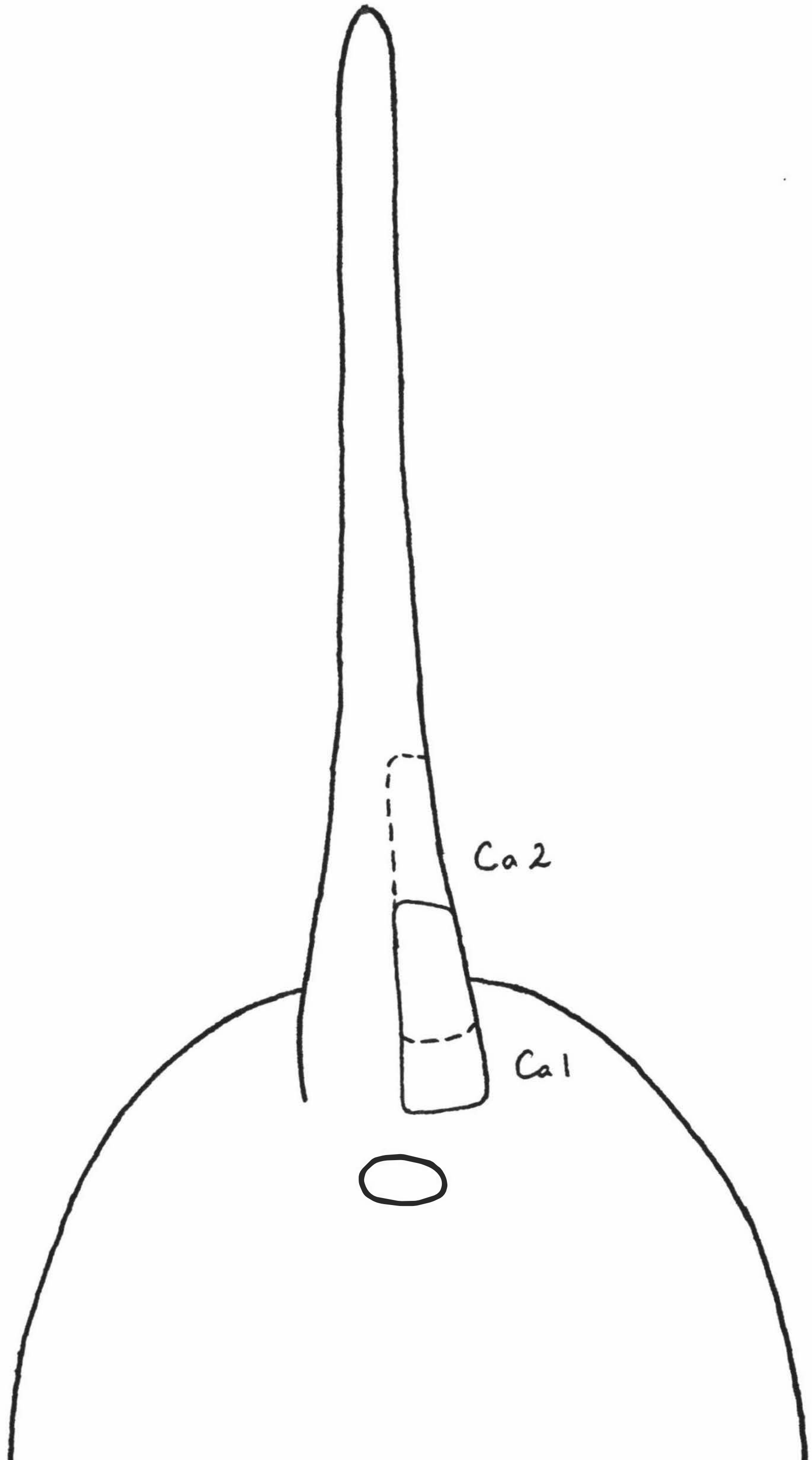


FIGURE 2.16: CUTANEOUS AREAS RECORDED FROM VENTRAL SPINAL ROOTLETS AT Ca 1 AND Ca 2.

number of hoggets. The dorsal cutaneous branches (DoCB) arose from a superficial nerve trunk that extended from the level of Ca1 (a connection to the Ca1 spinal nerve was observed in all but 2 hoggets) to the level of Ca10-Ca12 (Fig. 2-17). From Ca1 to Ca5-Ca6 the superficial nerve trunk had connections at each vertebral level with a deep nerve trunk coursing along the dorsolateral surface of the vertebral column. Distal to Ca6 the superficial nerve trunk had connection directly into each spinal cord segment (Fig 2.-17). The deep nerve trunk also extended from Ca1, with connection into the spinal cord from Ca1 to Ca5-Ca7. Distal to Ca5-Ca7 the deep nerve trunk became more superficial and had no connection into the spinal cord (Fig. 2-17). The deep nerve trunk was observed as far distally as Ca 13 in 2 hoggets and at least as far as Ca10 in 3 others. The superficial nerve trunk was located at least as far distally as Ca11, running lateral to the main caudal vein. While the deep nerve trunk was located dorsally and medially compared to the main caudal vein.

A ventral nerve trunk was also located, midway between the ventral midline and the lateral edge of the ventral surface. There was no ventral deep nerve trunk located although in 2 hoggets interconnection from Ca1, Ca2 and Ca3 was found against the vertebral surface.

C. Cutaneous Areas:

No satisfactory recordings could be made from any of the hoggets from approximately the distal 10 cm of the tail (Fig. 2-19). Recording from the dorsal superficial nerve trunk at each vertebral level demonstrated successive CA that extended from the dorsal midline, laterally to the lateral border of the dorsal surface (Fig. 2-20). In the areas distal to the dorsoventral flattening of the tail the dorsal CA extended from the dorsal midline to a point midway around the arc between the most lateral and the most ventral points of the circular tail. The CA on the dorsal surface became successively shorter at more distal points. The mapping of CA from the superficial nerve trunk was clearer at the proximal areas of the tail, as a result the superficial nerve trunk only supplied measurable CA to a level approximately 10 cm from the distal end of the tail

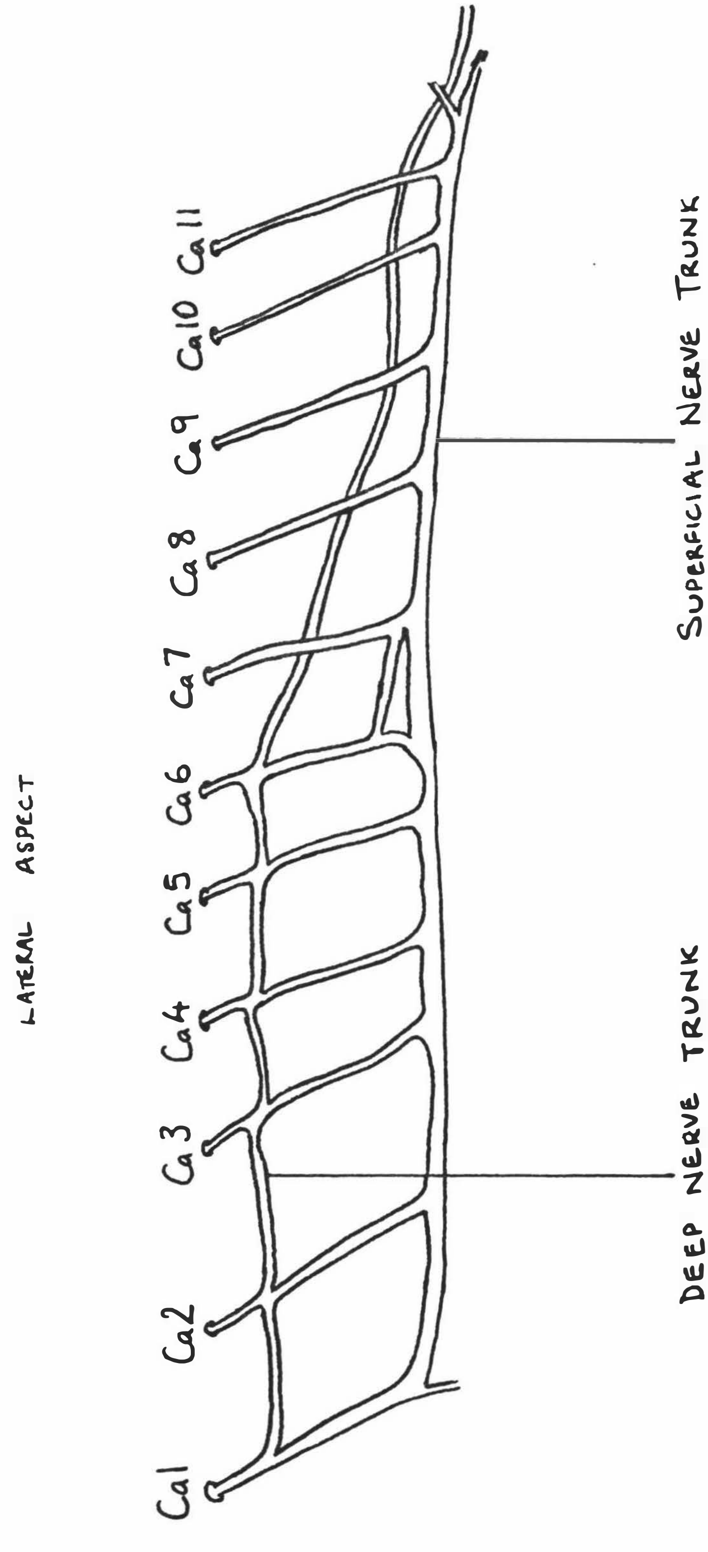


FIGURE 2·17 : ARRANGEMENT OF THE NERVE TRUNKS IN THE TAIL.

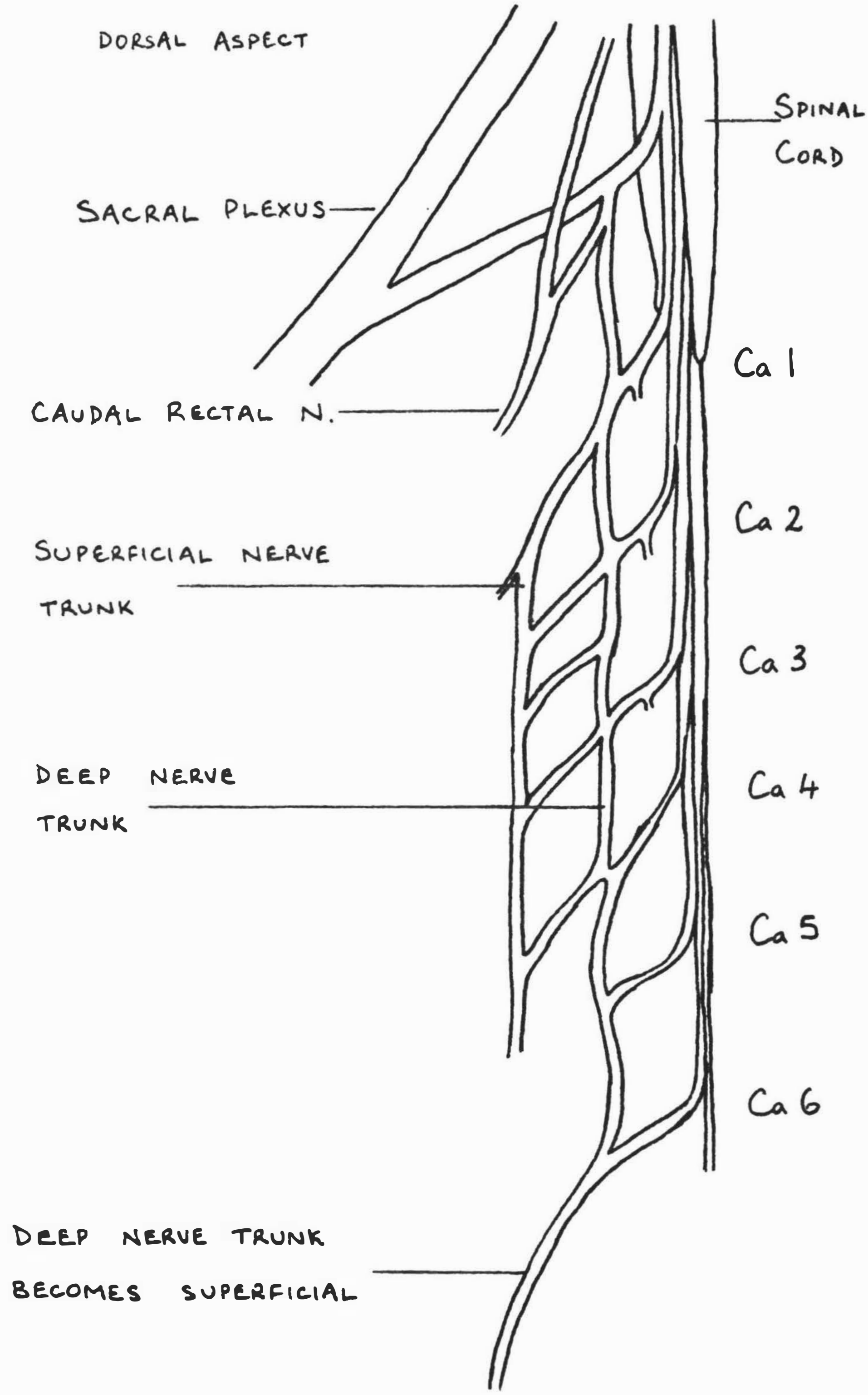


FIGURE 2.18 : ARRANGENTS OF SPINAL AND PERIPHERAL NERVES AT THE PROXIMAL END OF THE TAIL (LEFT SIDE).

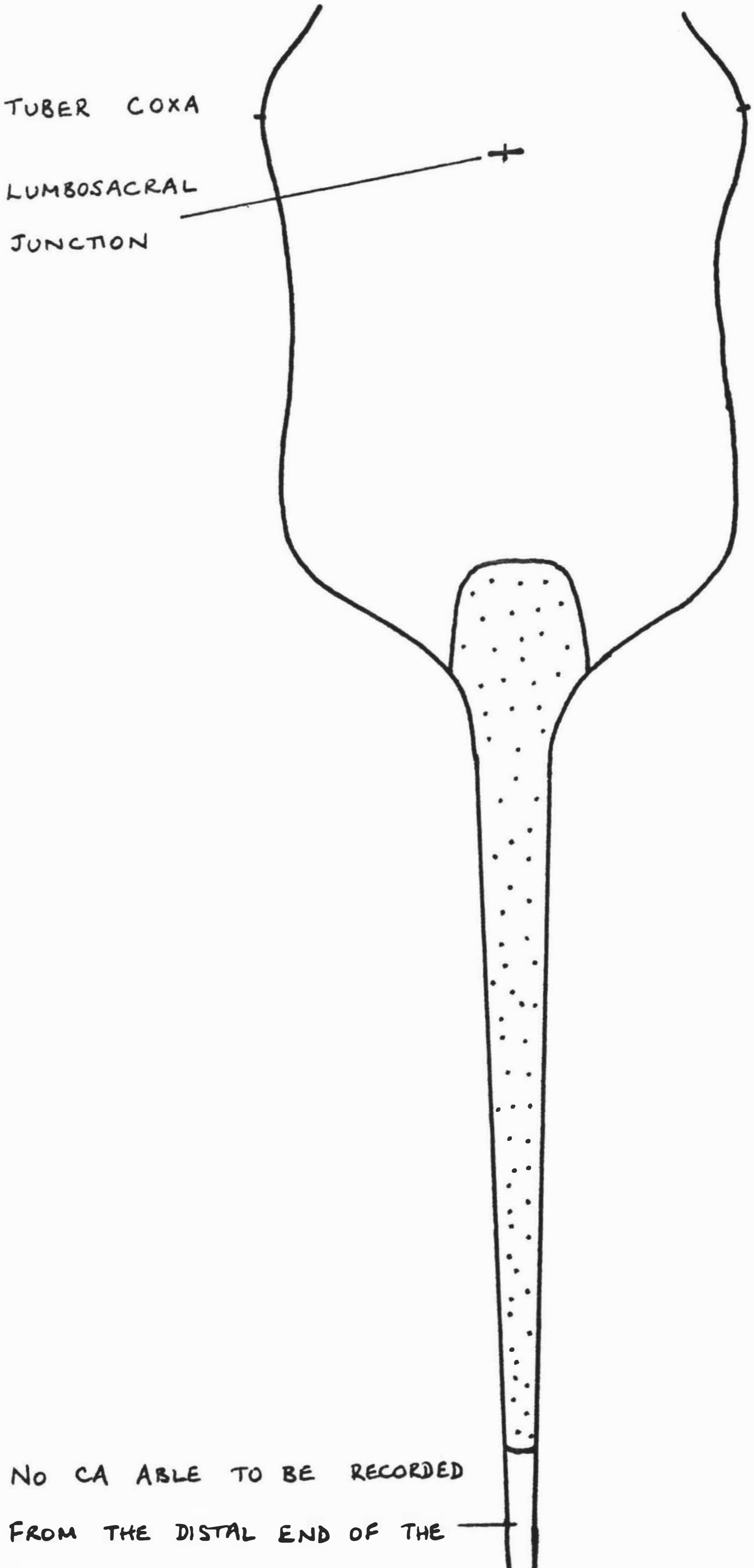


FIGURE 2·19 : MAXIMUM EXTENT OF THE CA RECORDED FROM THE TAIL.

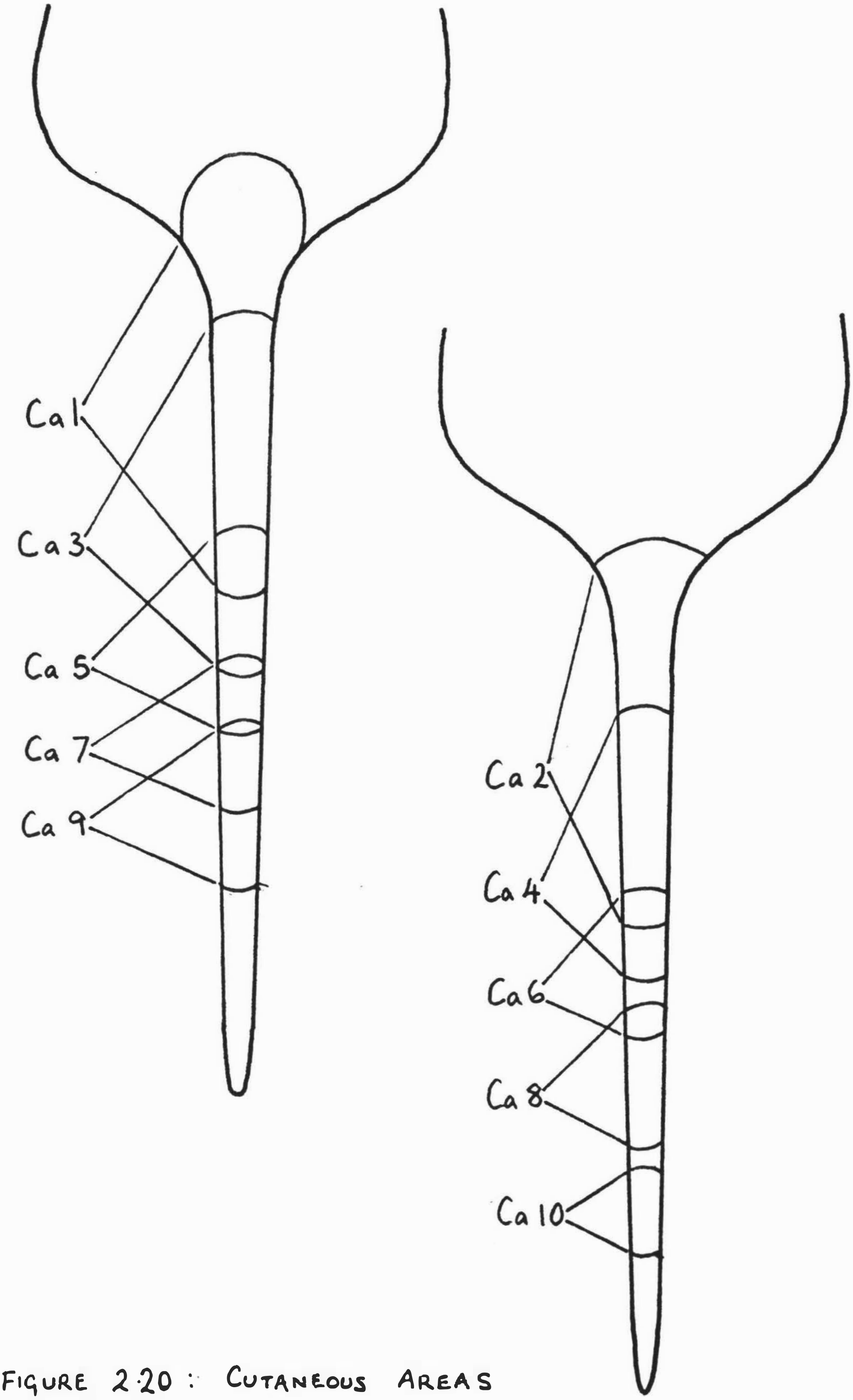


FIGURE 2.20: CUTANEOUS AREAS RECORDED FROM THE SUPERFICIAL NERVE TRUNK OF THE TAIL AT EACH VERTEBRAL LEVEL.

(Fig. 2-19).

The dorsal deep nerve trunk also supplied CA on the dorsal surface which were of a similar size and position to those supplied by the superficial nerve trunk. However recording from the deep nerve trunk was often obscured by deep sensory/motor stimuli which made mapping of the cutaneous areas (CA) difficult.

Recording from the ventral nerve trunk at each vertebral level demonstrated successive CA that extended from the ventral midline, laterally to overlap the ipsilateral dorsal CA. At levels distal to the dorsolateral flattening of the tail the ventral CA extended from the ventral CA extended from the ventral midline to a point midway around the arc between the most lateral and the most dorsal points of the circular tail. The CA on the ventral surface became successively shorter further caudally.

2.4 DISCUSSION

2.4.1 Innervation of the Perineum:

The sacral plexus arising from ventral branches of spinal nerves from S2-4 was found to vary in its configuration. The arrangements were similar to the arrangements described by Kirk et al (1987a). Additionally, the CA of the proximal cutaneous, distal cutaneous and deep perineal branches of the sacral plexus covered similar regions as those described by Kirk et al (1987a).

2.4.2 Innervation of the External Genitalia:

That the caudal aspect of the scrotum was innervated by the distal cutaneous branch of the sacral plexus supports the findings of Kirk et al (1987a) and this nerve is assumed to be homologous to the caudal scrotal nerve of Larson and Kitchell (1958).

The terminal branches of the pudendal nerve were identified as the dorsal nerve of the penis (DNP) and the scrotal nerve. The DNP and the scrotal nerve were found to anastomose at a point proximal to the sigmoid flexure of the penis and such connection has not been previously described in the literature. The scrotal nerve was generally found to innervate a CA on the ipsilateral scrotum and an area on the thigh lateral to the base of the scrotum which is in agreement with the observations of Kirk et al (1987a). However there was some indication that two branches of the scrotal nerve could supply a proximal and distal CA on the scrotum (Fig 7).

The DNP also was found to bifurcate and only one of these branches was cutaneous, it supplied a CA on the dependent free end of the prepuce. Only in one subject was the CA of this branch of the DNP found to extend caudally along the prepuce from the dependent free end. Kirk et al (1987a) described a preputial CA of the DNP extending along the length of the prepuce, but it appears in that study, as in the present study that such a CA was difficult to consistently demonstrate, perhaps due to variation between rams or due to mixture of proprioceptive nerve fibres with the tactile nerve fibres.

In terms of the nerves affected by the castration procedures, there will be differences that depend on the tool used. For example, the application of a tight rubber ring to the neck of the scrotum, as in the castration and short-scrotum procedures, can be assumed to involve stimulation of both the scrotal nerve and the distal cutaneous branch of the sacral plexus. Whereas use of the knife for castration can be assumed to cause stimulation of the scrotal nerve but will probably not involve the distal cutaneous branch of the sacral plexus since only the distal third of the scrotum is severed. However, in the cases of castration with the ring and the knife the nerves that descend through the inguinal ring will be important. The actual removal of the testes that is the final part of castration with the knife may be expected to involve the testicular nerve which will be stretched and broken by this action. Castration with the ring may be expected to not only stimulate the cutaneous nerves but also stimulate the deep sensory nerves such as the terminal branches of the genitofemoral nerve that course over the external cremaster muscle and the testicular nerve within the spermatic cord. These nerves along with the scrotal nerve and the distal cutaneous branch will subserve the pain caused by the hypoxia and then anoxia associated with reduction of blood flow to the scrotal and testicular tissues. The short-scrotum procedure, however, would not be expected to involve the testicular nerve because the testes and the spermatic cords are pushed up and not included under the ring. While this may suggest the short-scrotum procedure stimulates mainly the cutaneous nerves (distal cutaneous branch, scrotal and genitofemoral nerves) it should be remembered that the act of pushing the testes up against the abdominal wall may in itself involve noxious stimulation subserved by other nerves.

2.4.3 Innervation of the Tail:

Observations in this study confirmed previous reports Frandon, 1974) that in young adult sheep the spinal cord does not extend down into the tail as it is foreshortened in the lumbar and sacral regions. The distal portions of the spinal cord give off paired spinal nerves that course along the vertebral canal and supply areas caudal to the end of the spinal cord, this is termed the cauda equina (Frandon 1974). The cauda equina became apparent at a more cranial level in the adult ram (S4) than in the hogget ram (Ca1), although the same number of spinal nerves (5) make up the cauda equina.

Innervation of the proximal portion of the tail has been shown in the sheep to arise from spinal nerve roots from S3, S4, Ca1 and Ca2 (Kirk 1968). The present results also show that spinal nerves from S3, S4, Ca1 and Ca2 supply CA on the tail. The CA of the sacral spinal nerves of sheep do not extend as far caudally as those of the cat (Kuhn 1953). Also, the cat has S1 and S2 dermatomes that extend onto the tail (Kuhn 1953), but these were not demonstrated in the sheep. The dermatomes on the tail of the dog are supplied by spinal nerves from S3 and the five caudal segments (Fletcher and Kitchell 1966) which is relatively similar to the arrangement in the sheep.

The majority of the cutaneous innervation on the length of the tail had not previously been mapped. In the dog the tail dermatomes are described as "forming serially overlapping bands around the tail" (Fletcher and Kitchell 1966), and the cat has been shown to have a similar arrangement (Kuhn 1953).

Recording from the dorsal spinal nerves confirmed that the cutaneous innervation of the tail was arranged in serially overlapping bands which extended from the dorsal midline to the lateral edge of the ventral surface (Figs 12, 13). The ventral CA from spinal nerves also showed serially overlapping bands that extended from the ventral midline to a point midway between the lateral edge of the ventral surface and the dorsal midline (Figs 13, 14) This pattern seems similar to that described in the dog (Fletcher and Kitchell 1966) although recordings from spinal nerves supplying cutaneous areas distal to Ca2 could not be confirmed.

The arrangement of the peripheral has not been described in the sheep. The dorsal branches of the spinal nerves leaving the cauda equina at each segment form a dorsal superficial nerve trunk which has connections to a deep nerve trunk that courses along the vertebral surface until the level of Ca6 at which point it becomes superficial (Fig15 , 16). From the level of Ca6 the superficial nerve trunk has direct connection into the cauda equina. A ventral nerve trunk was also identified but the arrangement was different to that of the dorsal nerve trunk with no ventral deep nerve trunk observed, although an interconnection between the Ca1, 2 and 3 spinal nerves may represent the deep nerve trunk identified on the dorsal aspect.

Recording from the peripheral nerves was difficult due to problems in preventing proprioceptive discharge when stimulating the cutaneous areas. It appears that the distal CA on the tail were supplied by the nerve trunk that was deep proximal to Ca6, and that the mixing of cutaneous and proprioceptive fibres obscured mapping of the distal CA. However, the pattern of CA on the tail as mapped from the dorsal superficial nerve trunk at each segmental level demonstrated that they formed serially overlapping bands. The CA were longer more proximally and became progressively shorter at more distal levels, although this may be an artifact of the poor recording conditions experienced at distal points.

The performance of tailing occurs at the same place, at the level of the junction of the caudal fold and the tail, regardless of the method used. However, the effect of the different methods in terms of nerve stimulation may be different. Application of a rubber ring to the tail will impinge on the superficial nerve trunks and thus stimulate discharge from the nerve fibres in that nerve trunk. This means that fibres innervating areas distal to the ring will be stimulated as well as those at the level of the ring and thus cause greater a afferent barrage.

The pain associated with the reduction in blood flow will also be subserved by the superficial and deep nerve trunks. The pressure of the ring on the superficial nerve trunks and possibly on the deep nerve trunks may prevent the passage of impulses along these nerves after the initial discharge and thus reduce their input of afferent signals. This may occur increasingly with time as the tissues under the ring become compressed. However, the presence of spinal nerves of the cauda equina within the vertebral column will enable more prolonged peripheral afferent signals to occur. Thus the noxious stimulation provided by blood flow reduction may continue for a longer period than would otherwise be expected. The positioning of the ring assumes importance when this fact is considered because positioning of the ring at an intervertebral space may allow more rapid compression of the spinal nerves of the cauda equina and therefore reduce afferent signals in a shorter period. If the ring is positioned over a vertebra the bone will resist compression and the spinal nerves within will be protected and may remain active for longer.

In the cases of tailing with the knife or docking iron the protection afforded by the vertebrae will be negated as all the tissues including the spinal nerves of the cauda equina are severed. This suggests that not only will there be noxious input via the peripheral nerves but there will be an accompanying input of all the spinal nerves transected by the procedure.

CHAPTER THREE
CASTRATION AND TAILING TRIAL

3.1 Introduction

3.2 Materials and Methods

3.2.1 Experimental Animals

3.2.2 Experimental Procedures

3.2.3 Experimental Equipment

3.2.4 Blood Sampling

3.2.5 Behaviour

3.2.6 RIA

3.2.7 Presentation of Results

3.3 Results

3.3.1 Behavioural and Cortisol Responses

A. General Summary

B. Control Lambs

C. Rings

(i) Castration plus Tailing

(ii) Castration Only

(iii) Tailing Only

(iv) Short-scrotum plus Tailing

D. Knife

(i) Castration plus Tailing

(ii) Castration Only

(iii) Tailing

E. Docking Iron

(i) Tailing Only

(ii) Castration with the Ring plus Tailing with the Iron

F. Restlessness

3.3.2 Integrated Cortisol Responses

A. Control Lambs

B. Tailing

C. Castration

D. Castration plus Tailing

E. Effects of Different Tools

3.1 INTRODUCTION

Tailing of lambs is an important, routine animal husbandry procedure carried out to prevent build-up of faeces in the wool of the tail and breech, and thereby reduce the likelihood of infection or parasitism, primarily flystrike, a debilitating often fatal condition (Wohlt, Wright, Sirois, Kniffen and Lelkes, 1982; Charleston and Tenquist, 1985; Henderson, 1990). Tailing also aids mating efficiency and dressed lamb carcasses have a neater appearance and thus receive a higher market price (Wohlt et al, 1982). Castration of lambs is variously said to be a necessary (Kilgour, 1985) and an unnecessary (Gee, 1986) practice, but common reasons advanced to justify castration of ram lambs are to reduce unmanaged breeding, promote fleece growth and to increase ease of handling (Wohlt et al, 1982). In New Zealand tailing and castration are usually carried out at an age of 3-6 weeks.

There are a variety of methods for tailing and castrating lambs, including use of a knife, a docking iron and constricting rubber rings. Use of a knife is the most basic method, involving cutting through the tail to leave a stump and severing the tip of the scrotum to reveal the testes and pulling them out. The bleeding associated with use of the knife on the tail is undesirable and can be avoided by using a docking iron. A docking iron is a tool with a sharp edge that is heated (usually with a built in gas nozzle) and so sears the tissues as it cuts through them. The searing action of the iron cauterises the wound and therefore prevents bleeding. Further development of bloodless emasculation techniques produced tight rubber rings which, when applied to the tail or scrotum, constrict the tissues and occlude the blood vessels. Application of a rubber ring causes hypoxia and then anoxia in the tissues at the level of and distal to the ring, and as a result death of the tissues which then slough off. It takes between two and three weeks from application of a rubber ring for the sloughing off to occur.

Studies of tailing and castration methods have tended to concentrate on the effect on the lamb over a period of weeks after the procedure. Initially this involved investigating the effect different methods of tailing and castration had on lamb production and growth (Garner and Sanders, 1936; Barrowman, Boaz and Towers, 1953;1954). A comparison of the rubber ring and bloodless crushing (Burdizzo) techniques (Barrowman et al, 1953) of castrating lambs concentrated on live weight gain as the response parameter. No significant difference was found between the two methods in terms of time taken to reach slaughter weight. Similarly, an experimental evaluation of rubber ring application at different ages (Barrowman et al, 1954) was largely made on production factors. In both cases, however, written descriptions of the immediate discomfort elicited by the castration and tailing methods were provided, despite the absence of techniques to "accurately estimate the degree of discomfort or pain" (Barrowman et al, 1953;1954). Also clinical inspection of the sites of treatment was used to determine local wound extent and healing.

The effect of the various treatments on live weight gain has been central to past research, i.e. how did different castration techniques affect the productivity of the lambs, and therefore their economic viability. Thus, while production factors (time to reach slaughter weight, live weight gain) were of primary importance in investigating castration and tailing, welfare aspects were limited to descriptive passages because of a lack of quantitative evaluative techniques (Barrowman et al, 1953;1954). Matthews, Matthews and Ogden (1960) investigated several methods of castration and tailing in terms of retardation of weight gain after the procedures. No attempt was made to examine the relative distress or pain effects of the methods, indeed the authors determined that "no differences in castration and docking methods were found". Similarly, Garner and Sanders (1936) recorded no difference in growth rates between lambs castrated with the knife and lambs castrated with the Burdizzo. More recently Wohlt et al (1982) showed that tailing by either knife or rubber ring had no sustained effect on growth. It has thus been widely shown that the practice of castrating and/or tailing lambs does not adversely affect the lambs' ability to grow, nor do different methods have a sustained effect on production.

Changes in the emphasis of animal studies have shifted towards investigating the effects of castration and tailing on the physiological condition and health of the lamb. Having already ascertained the effects of the procedures on the animal's productivity, it is the welfare considerations that are becoming important. Examination of blood parameters and metabolites (haematocrit, haemoglobin level, glucose and protein concentration), as indices of health and evidence of physiological challenge, showed a short term disturbance due to tailing with the rubber ring (Wohlt et al, 1982). The disturbance to the lambs' health following tailing was not of sufficient severity that the advantages of tailing were negated (Wohlt et al, 1982). This emphasis on the effects of the animal husbandry procedures on the health of the lamb is part of an increasing awareness that the welfare aspects themselves deserve study.

Lambs show pronounced behavioural characteristics when rubber rings are applied. These have been variously described as: "often nosing the scrotal region", "passing through 5-10 minutes of great uneasiness", "the lamb would walk a few steps fall down, sometimes sideways sometimes backwards and would occasionally roll completely from one side to the other" (Barrowman et al, 1953;1954). In apposition to that type of behavioural response is that to the Burdizzo, which apparently caused "no obvious upset after the operation" (Barrowman et al, 1953;1954). Such disparity in behavioural response must be considered critically when the relative effects of castration and tailing methods are investigated.

With the development of reliable methods to measure components of the distress reaction came the evaluation of castration and tailing as stressors. The relative postoperative distress of routine surgical practices involves a hierarchy in intensity from tailing alone to castration plus tailing and the greatest distress due to castration plus tailing with mulesing (Shutt et al, 1987). The distress caused by use of rubber rings is also more intense in lambs castrated plus tailed than lambs tailed only (Mellor and Murray, 1989a). Even without documented evidence of the relative distress elicited by different methods of tailing and castration some apparently authoritative views have been documented. There is a view that if the knife is sharp and wielded by experienced operators the process of surgical castration will be relatively painless (Henderson, 1990), and thus have an advantage over other methods. Indeed the opinion that application of the rubber ring causes pain and discomfort for some hours while use of the knife induces a substantially shorter period of after pain (Henderson, 1990) has a seemingly wide acceptance. Other methods of castration and tailing such as the docking iron and short-scrotum procedure also have not been investigated.

The stress response of lambs was investigated in a comparison of rubber ring use and surgical cutting with a knife as methods of tailing and castration (Shutt et al, 1988). The results were interpreted as indicating that surgical techniques caused less distress than the application of rubber rings, and probably a shorter period of pain (sic). The primary evidence to support this appears to have been behavioural. The observation that ringed lambs were very active and their activity obviously indicated distress, while cut lambs were less active was used to suggest that ringed lambs were experiencing more distress. Re-interpretation of their plasma cortisol concentration results showed that surgically tailed and castrated lambs had a greater response than ringed lambs (Mellor and Holmes, 1988). Since cortisol was being used as an index of distress one would assume surgically tailed and castrated lambs experienced greater distress than ringed lambs. Endorphin levels, which were higher in surgically tailed and castrated lambs, were said to show that cut lambs had greater analgesia and therefore less distress. However that train of thought, if extended, would suggest that the more severe the noxious stimulus or challenge the more analgesia would be induced and thus the lower the distress experienced - a contradictory concept which is novel to say the least. Furthermore it remains to be demonstrated that endorphins have analgesic actions in 4 to 5 week old lambs because Wood et al, (1991) found that young lambs (less than 1 week of age) cannot significantly reduce pain and distress by endogenous opioid activity. Concern has been expressed (Mellor and Holmes, 1988) over the method and interpretation of Shutt et al's (1988) study. The relative stress effects of different methods of castrating and tailing lambs therefore remain to be established.

Indices of distress in lambs have usually involved measurement of hormonal factors associated with the response to a stressor; in particular cortisol. Mellor and Murray (1989a;b) have used castration and tailing as episodes to investigate the distress response in relation to postnatal maturation and to establish correlations between hormonal and behavioural responses in stress. It has been shown that there are characteristic behaviours exhibited after castration and tailing with rubber rings, and that these correlate with high cortisol concentrations in the blood; these are therefore described as indicative of marked distress. Tailing alone and castration alone elicit less dramatic behavioural responses which are associated with lower cortisol levels (Mellor and Murray, 1989a; D.J.Mellor, V.Molony and I.Robertson, Unpublished Data).

The present study was designed to address several of these issues. Trials were conducted to study, comparatively , the distress elicited by various techniques of castrating and tailing lambs. The major purposes were, first, to rank castration methods (surgery and rubber ring application), and tailing methods (surgery, rubber ring application and searing), and secondly to provide more quantitative information on the relationship between behavioural and hormonal responses in those distressed states. Regarding the second objective, the behavioural indices, used by Mellor and Murray (1989a) and refined by G.N.Wood and V.Molony (Personal communication), were further adapted and extended. One of the conclusions of Mellor and Murray (1989a) was that restlessness was a feature of the onset of distress and that restlessness was related to the distress experienced as indicated by the plasma cortisol concentrations. They (Mellor and Murray 1989a) recommended that restlessness be quantified and G.Wood and V.Molony (Personal Communication) described a restlessness score that consisted of quantifying the restlessness as the number of times the lambs stood up or lay down.

3.2 MATERIALS and METHODS

3.2.1 Experimental Animals:

One hundred and nineteen Romney lambs with an mean postpartum age of 33 days (range 28 to 37 d), and an mean weight of 13.7 kg (range 7.2 kg to 19.1 kg) were investigated during routine castration and tailing on the Massey Sheep and Beef Cattle Research Unit. The mob of ewes and lambs provided for the trial were mustered into the yards the day prior to each experimental day. Sufficient lambs and their mothers for each trial day were separated out and penned, the remainder were returned to the paddock. Experimental days were on alternate days over a two week period in September, with 24 lambs used each day.

On the day prior to each experimental day the lambs were weighed and the ventral surface of the neck was clipped to allow jugular venipuncture. The lambs were penned with their mothers overnight with water available in each pen. The pens (1.2 m x 0.9 m) were created using wire mesh gates so each sheep could see, smell and hear others (Fig. 3-5).

On the day of the experiment, lambs were separated from their mothers and placed in adjacent pens about one hour before the experimental period. Twin lambs were kept together and single lambs were put into a pen with one other with both mothers adjacent. Water buckets were removed.

No lambs showing clinical signs of ill-health were selected. One lamb was excluded during observation, due to gastrointestinal problems causing diarrhoea.

At the end of each experimental day each lamb was sprayed with an antiflystrike solution, Vetrazin (Ciba Pharmaceuticals, Auckland), any untailed lambs were tailed using the docking iron (the method favoured by the Massey Sheep and Beef Cattle Research Unit shepherds), and ram lambs were ear-marked. Ram lambs not castrated as part of the experiment were left entire.

3.2.2 Experimental Procedures:

The person performing the castration and tailing remained in one place and lambs were carried to the treatment position.

There were twelve treatment groups to which lambs were allocated according to sex during the experiment (Table 3-1); the castration and tailing procedures were carried out in the following manner:.

1. Castration plus tailing control: Lambs were handled as if they were being castrated plus tailed, but were left intact.

2. Castration plus tailing with rubber rings: Lambs had a tight rubber ring (Elastator Ltd, Blenheim) applied to the neck of the scrotum at a level clearing the teats, with both testes distal to the ring (Fig. 3-1). A rubber ring was applied to the tail at the point of junction of the caudal fold and the tail.

3. Castration plus tailing with a knife: Lambs had the distal one third of the scrotum cut off with a sharp knife to expose the testes in situ (Fig. 3-2). The testes were then removed by grasping each with serrated tongs and drawing them out (Fig. 3-3). The tail was then amputated with the knife at the level of the junction of the caudal fold and the tail. There was no treatment of the wounds.

4. Castration with rubber rings plus tailing with a docking iron: Lambs were castrated with a rubber ring and tailed by amputating the tail with an anvil-scissor docking iron (Te Pari Products Ltd.; Palmerston North) at the point of junction of the caudal fold and tail (Fig. 3-4).

5. Short-scrotum plus tailing with rubber ring: Lambs had a rubber ring applied to the scrotum at a level clearing the teats with both testes proximal to the ring. Thus the testes are pushed up against the abdominal wall and rendered infertile by the elevated temperature (cryptorchidism). The tail was docked with a rubber ring.

6. Castration with rubber ring: Lambs were castrated only with a rubber ring (as above).

7. Castration with a knife: Lambs were castrated only with the knife (as above) .

8. Tailing control: Lambs were handled as if they were being tailed, but were left intact.

9. Tailing with rubber ring: Lambs were tailed only with a rubber ring (as above).

10. Tailing with the knife: Lambs were tailed only with the

Fig. 3-1: Application of a rubber ring to the neck of the scrotum.

Fig 3-2: Severing the distal third of the scrotum with the knife to expose the testes.

Fig. 3-3: Withdrawing a testis with the serrated tongs.

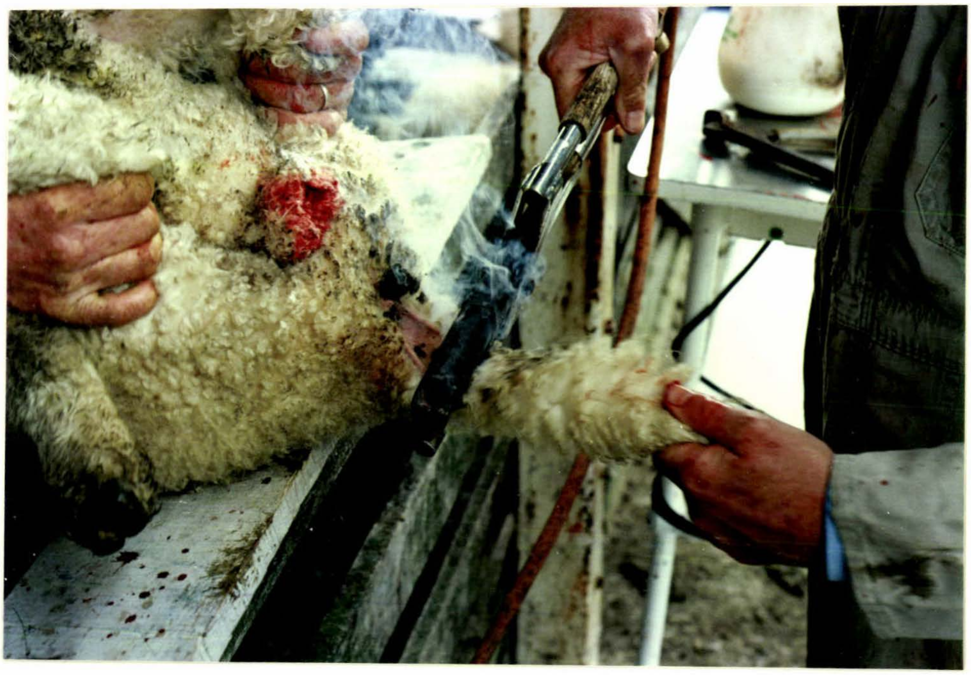


Fig. 3-4: Amputating the tail at the level of the junction of the caudal fold and tail using the docking iron.

knife (as above).

11. Tailing with the docking iron: Lambs were tailed only with the docking iron (as above).

12. ACTH injection: Lambs were injected into a jugular vein with 14 μg/kg body weight synthetic adrenocoticotropin (Synacthen; Ciba Pharmaceuticals, Auckland) to elicit a high cortisol response; this ACTH dose was the same as that used by Mellor and Murray (1989b).

Females were used in the tailing only groups (Groups 8 to 11) and 5 females/5 males were used in the ACTH injection group. Obviously, males were used in all groups subjected to castration (Groups 1 to 7).

All castration and tailing techniques used during the experiment were checked by Massey SBCRU staff.

TABLE 3-1 Details of treatment groups, mean ages, numbers used and sex of lambs used in the castration and tailing experiment.

GROUP	TREATMENT	MEAN AGE	Nº USED	SEX
1	CT CONTROL	33	10	male
2	CT RINGS	33	9	male
3	CT KNIFE	33	10	male
4	C RING T IRON	33	10	male
5	SS RING T RING	34	10	male
6	C RING	33	10	male
7	C KNIFE	33	10	male
8	T CONTROL	33	10	female
9	T RING	34	10	female
10	T KNIFE	34	10	female
11	T IRON	33	10	female
12	ACTH Inj.	30	10	male/female
		TOTAL:	119 (74 male, 45 female)	

CT=castration plus tailing, C=castration, T=tailing, SS=short-scrotum, ACTH Inj.=adrenocorticotropin injection. Mean age in days.

3.2.3 Experimental Equipment:

The treatment tools (Fig. 3-6) were prepared prior to their use, both the knife and the docking iron were cleaned of blood, faeces and, in the case of the docking iron, burnt wool. In accord with normal farm practice no antiseptic solution was used. The docking iron was ignited and heated for 15 minutes before use and the knife was sharpened. On the days between the experimental periods, the duckboards on which the sheep were penned, were cleaned with water and sprayed with an antiseptic solution (2% formalin).

3.2.4 Blood Sampling:

Time 0 was taken as the time of completion of each lamb's treatment. A blood sample was taken just prior to treatment and subsequent blood samples were taken at 15, 30, 60, 90, 120, 150, 180, 210 and 240 minutes after treatment. Blood sampling involved each lamb being picked up and blood taken by venipuncture of the jugular vein with a 5 ml heparinised vacutainer. This procedure usually took 12-15 seconds. Lambs were studied in units of eight and sampling of lambs in a unit was done serially so pretreatment sampling was completed for all lambs in a unit before procedures were performed on that unit. The blood samples collected in vacutainers were immediately centrifuged (1000 rpm for 15 minutes) and the plasma aspirated off into storage vials and frozen for later radioimmunoassay (RIA).

3.2.5 BEHAVIOUR.

The behaviour of each lamb was observed for a period of 4 hours from the time of treatment. The initial hour involved continuous recording of the restlessness of each lamb. Restlessness was defined by the number of times a lamb stood up or lay down (standing was designated by the sternum and the abdomen both being off the floor).

The behavioural activity, i.e. the position or activity assumed by the lamb, was recorded instantaneously on the minute for the first 60 minutes (60 observations) and on every fifth minute for

Fig. 3-5: Experimental site with pens created from wire mesh gates. Lambs are adjacent to their mothers.

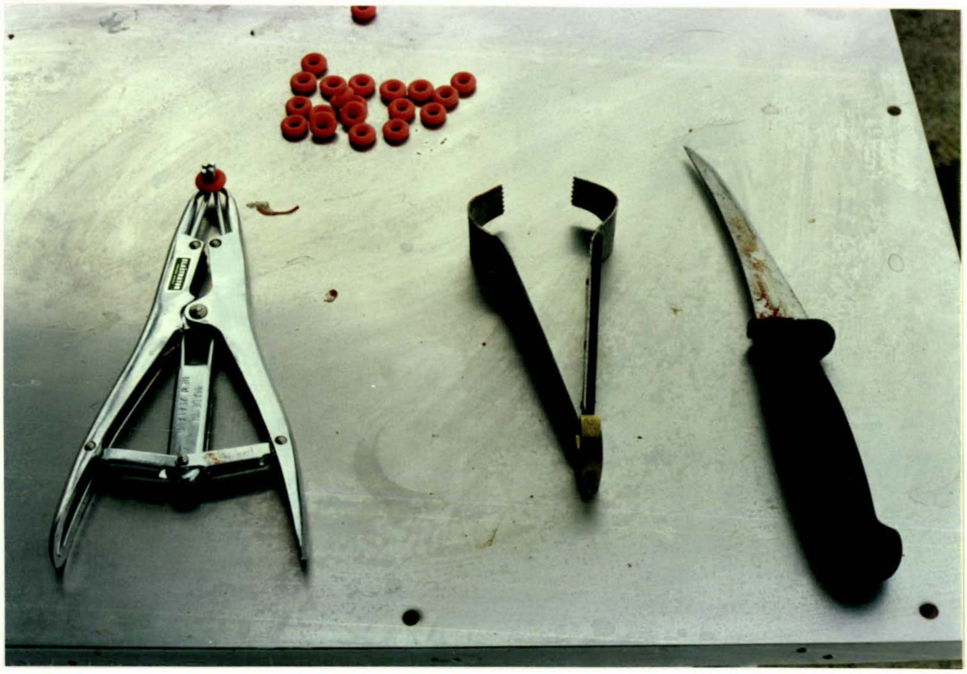


Fig. 3-6: Treatment tools; (from left to right) rubber ring applicator with rubber ring in place, serrated tongs, knife.

Fig. 3-7: Example of an abnormal standing posture.

Fig. 3-8: Example of ventral recumbency flexed posture.

the subsequent 180 minutes (36 observations). The recording medium was a checksheet (see Appendix A) with predetermined behavioural categories indicated as follows:

STANDING/WALKING

N : Normal a lamb standing (sternum and abdomen are off the ground) normally or walking normally.

Ab S : Abnormal standing a lamb standing in an abnormal position. May be indicated by a rounded, hunched stance or unusual head position (Fig. 3-7).

Ab N : Abnormal walking a lamb walking with some abnormality of gait. May be indicated by stiffness, swaying, stamping or ataxia of the legs.

VENTRAL RECUMBENCY

N : Normal a lamb lying on its sternum and abdomen with all 4 legs tucked in.

F : Flexed a lamb lying on its sternum and abdomen with one or both hindlegs out but flexed (bent at hock)(Fig. 3-8).

E : Extended a lamb lying on its sternum and abdomen with one or both hindlegs out and extended (hock joint straight).

LATERAL RECUMBENCY

U : Head Up a lamb lying on its side (with one or both forelegs and both hindlegs out), with head elevated off the floor.

D : Head down a lamb lying on its side (as above), with its head down on the floor (Fig. 3-9).

Fig. 3-9: Example of lateral recumbency head down posture.

Fig. 3-10: Example of lateral recumbency with rolling and kicking.

R : Rolling/Kicking a lamb lying on its side with all four legs out and rolling from one side to the other and/or kicking any of its legs (Fig. 3-10).

SLEEPING : The presence or absence of sleep as indicated by closed eyes and relaxed ventral recumbency, was noted.

FAST BREATHING: The presence of abnormally rapid ventilation was noted.

TREMOR :Any tremor or quivering of body, limbs or head was noted.

Comments on any of behaviours exhibited by the lambs but not covered specifically by the checksheet, or any factors that may have had an effect on the lambs being observed, were recorded. Any unnecessary interaction between the sheep and experimenters was avoided.

The behaviour of the lambs injected with exogenous ACTH was not observed and recorded because it has been shown previously that lambs given exogenous ACTH at the dose rate used did not exhibit any significant behavioural differences from control lambs (Mellor and Murray 1989a).

3.2.6 RADIOIMMUNOASSAY:

Assay of the concentration of cortisol in each plasma sample was performed using radioimmunoassay. The experimental samples were assayed with standard plasma samples to provide within and between assay coefficients of variation of 9.2% and 14.7% respectively; and a sensitivity of 7.3 nmol/l.

The assay involved competitive binding to an antibody of the sample plasma cortisol and radioactively labelled cortisol. After binding was complete the ratio of the plasma cortisol to the radioactively labelled cortisol was used to determine the concentration of cortisol in the plasma sample.

The Process: Extraction of the cortisol from the plasma sample was performed by serial mixture with solvents (dichloroethane and ethanol). Once the cortisol had been extracted it was combined with tritiated cortisol, cortisol antiserum and bovine gamma-Globulin (Serva, Heidelberg) to allow competitive binding overnight at 4°C. Samples were assayed in duplicate. The bound cortisol (cortisol bound to the antibody) was then separated from the free cortisol using polyethylene glycol 4000 (BDH Ltd., Poole) precipitation. The bound cortisol was then resuspended in water and mixed with scintillation fluid and the radioactivity was measured using a Beckman LS 8000 scintillation counter. The radioactivity of each tube was inversely proportional to the concentration of the plasma cortisol and hence the concentration could be determined by comparison with a series of standard cortisol solutions.

3.2.7 PRESENTATION OF RESULTS

Results will be considered in two sections; the first is a detailed description of the patterns of the plasma cortisol concentrations and the behavioural responses throughout the 4 hours of measurement, and the second involves a comparison of the castration and tailing techniques used by reference to the integrated cortisol responses to treatment (see below).

Behavioural and cortisol responses:

The cortisol responses are presented as the mean plasma cortisol concentrations (mean ± s.e.m.) for each group at each sample time. Comparison between the groups at a single time point is made on the incremental change from the pretreatment plasma cortisol concentrations, using the Student T test.

Behaviours were also presented as the mean ± standard error of the mean and compared between treatment groups using the Student T test at the same time periods.

Comparison of Tailing and castration techniques:

For comparison of the castration and tailing techniques the integrated cortisol response (Mellor and Murray 1989) will be used. The integrated cortisol response is the area under the plasma cortisol concentration curve above the pretreatment value. This gives an integrated value that combines the magnitude and duration of the response. For the purposes of this study the integrated cortisol response was calculated for every lamb and the results were analysed within each treatment group to find the mean and standard error of the mean. The mean integrated cortisol responses for each treatment group were then compared with other appropriate treatment groups using the Student 't' test.

Calculation of the Integrated Cortisol Response:

The area under the cortisol response curve was calculated using the formulae for finding the areas of the triangles and trapezia formed in each segment of the area under the cortisol response curve. The diagram below (Fig 3-11) does not represent any lamb's response but illustrates the situations faced in the calculation of the area under the response curve above the pretreatment level.

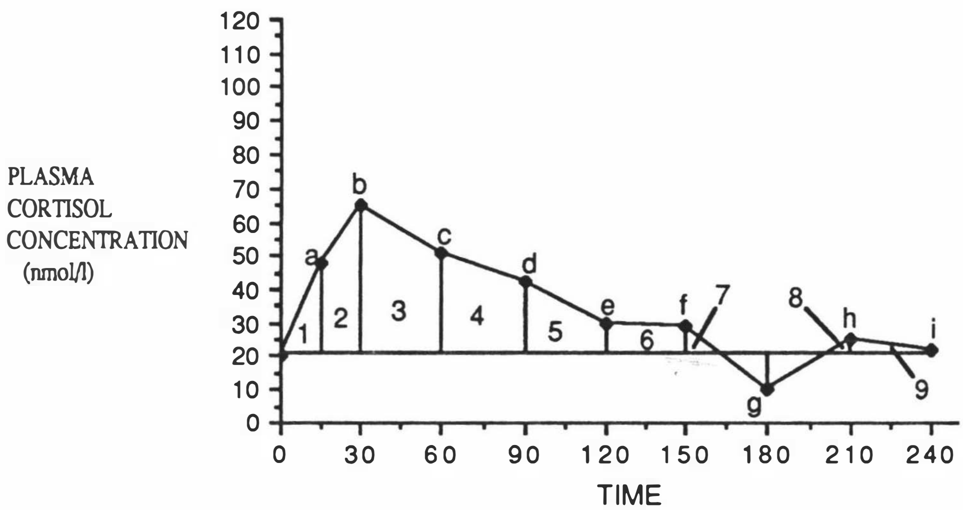


Figure 3-11: Graph of changes in plasma cortisol concentration to illustrate calculation of the integrated cortisol response.

The area under the above response line (Fig. 3-1) can be calculated by summing the individual areas labelled 1 through 9.

Thus:

$$A_{TOTAL} = A_1 + A_2 + A_3 + A_4 + A_5 + A_6 + A_7 + A_8 + A_9$$

By using the formulae for the area of a triangle:

$$\text{Area} = \frac{\text{base x height}}{2}$$

and a trapezium: $$\text{Area} = \text{base x} \frac{(\text{height}_1 + \text{height}_2)}{2}$$

Therefore:

$$A_1 = 1/2 \;.\; a \;.\; 15$$

$$A_2 = 15 \;.\; \frac{(a + b)}{2}$$

$$\Sigma(A_3{:}A_6) = 30 \;.\; \left(\frac{b}{2} + c + d + e + \frac{f}{2} \right)$$

$$A_9 = 30 \;.\; \frac{(h + i)}{2}$$

However for Area 7 and Area 8 the length along the x axis is not given, thus trigonometry is used to find the lengths. Note that of 119 lambs only 29 individuals had responses that needed trigonometry to calculate the area under the line.

Thus the situation can be demonstrated diagrammatically:

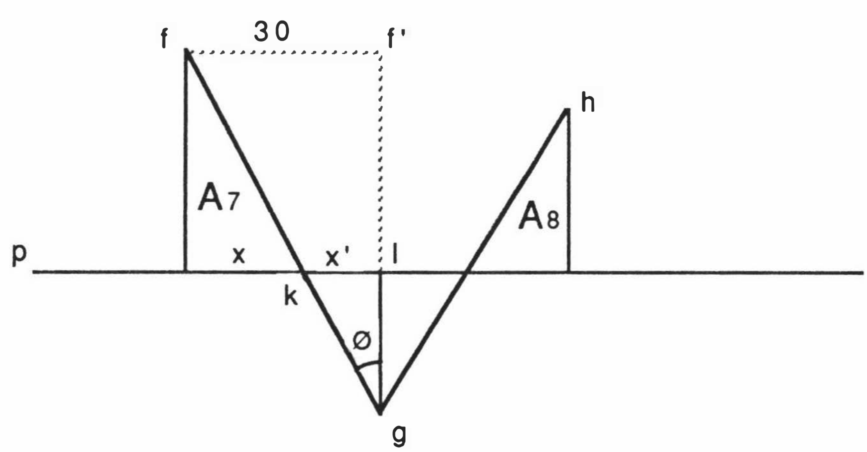


Figure 3-12: Diagram representing the trigonometry used to calculate parts of the area under the cortisol response curve

Where f, g and h are plasma cortisol concentrations and p is the level of the pretreatment plasma cortisol concentration. Therefore to find Area 7 the triangle f.f'.g can be used to calculate tan Ø , because:

$$\tan \varnothing = \frac{\text{opposite}}{\text{adjacent}}$$

Thus $\tan \varnothing = 30/(f' + g)$

The value for tan Ø can now be used in the triangle g.k.l to find the length of x' :

$$\tan \varnothing = \frac{\text{opposite}}{\text{adjacent}}$$

$$\Rightarrow \quad \tan \varnothing = \frac{x'}{g}$$

$$\Rightarrow \quad x' = \tan \varnothing \times g$$

Once x' has been found the length of x can be calculated because:

$$x + x' = 30$$

$$\Rightarrow \quad x = 30 - x'$$

Using the values calculated the area of Area 7 can now be found:

$$A7 = \frac{(f \, . \, x)}{2}$$

and similarly this process can be used to find Area 8.

3.3 RESULTS

Lambs that were found to be abnormally distressed prior to treatment were excluded from the results. The determination of abnormal distress for exclusion purposes was made on the basis of the pretreatment plasma cortisol concentrations: if these were greater than the mean 15 minute plasma cortisol concentration for the treatment group then the animals were excluded. This basis for exclusion was to ensure that animals suffering from an unrecognised source of abnormal distress did not influence the mean responses to each of the treatments used. Four lambs were excluded on the basis of abnormal prior distress and their plasma cortisol responses are provided in Appendix D.

3.3.1 Behavioural and Cortisol Responses:

The behaviours recorded on the checksheets were recorded in all the categories set out on the checksheets. However, it did not prove informative to consider each of the ventral and lateral subdivisions separately. Thus the behavioural responses were considered as standing/walking or recumbent and as the proportion of each that was abnormal. Previous work (Mellor and Murray, 1989a) has shown that lateral recumbency is an abnormal behaviour. Thus the adoption of a lateral position in recumbency was considered abnormal.

The behavioural responses observed as sleeping, fast breathing and tremor also did not provide any useful information. Sleeping was not observed in any lambs. The fast breathing behaviour was not considered informative because confusion arose over what constituted fast breathing and whether slow deep breathing was as important. Tremor was often masked by the movements associated with breathing.

A. General Summary:

In response to the experimental procedure all lambs had a transient rise in plasma cortisol concentration that varied in magnitude and duration depending upon the treatment. In this results section there is a detailed description of the responses to individual treatments in comparison to control lamb responses; between treatment comparisons will be made in the discussion of those results. The pattern of the cortisol response is taken here to indicate the pattern of the distress experienced by the lambs. A comparison of the plasma cortisol response and the behaviour therefore gives an indication of the usefulness of the behavioural parameters in determining the level of distress experienced. Thus these results will be presented within broad treatment sets, with a description of the plasma cortisol response followed by a description of the behaviour exhibited. All times given are time from the treatment. Lambs exposed to different treatments exhibited behaviours specific to the broad treatment categories which are summarised here:

Rubber Rings: Lambs treated with the application of rubber rings invariably showed high levels of restlessness. This restlessness, defined as the number of times lambs stood up or lay down, usually did not persist longer than 1 hour. Ringed lambs also exhibited a greater incidence of recumbent behaviour than did those receiving other treatments and of this recumbent behaviour a greater proportion was lateral recumbency than after other treatments. The occurrence of lateral recumbency was virtually confined to lambs that had rubber rings applied. Lambs that had rubber rings applied also showed reduced standing/walking behaviour in the first 45 minutes after treatment and the standing/walking behaviour in this period was mainly abnormal.

Knife: Lambs that were castrated and tailed with the knife characteristically showed a normal level of restlessness. The incidence of standing/walking behaviour was high and of this standing/walking behaviour much was abnormal. The level of abnormal standing/walking was effectively the only difference from control lambs, and it persisted across the whole observation time period (4 hours).

Docking Iron: Lambs that were tailed with the docking iron

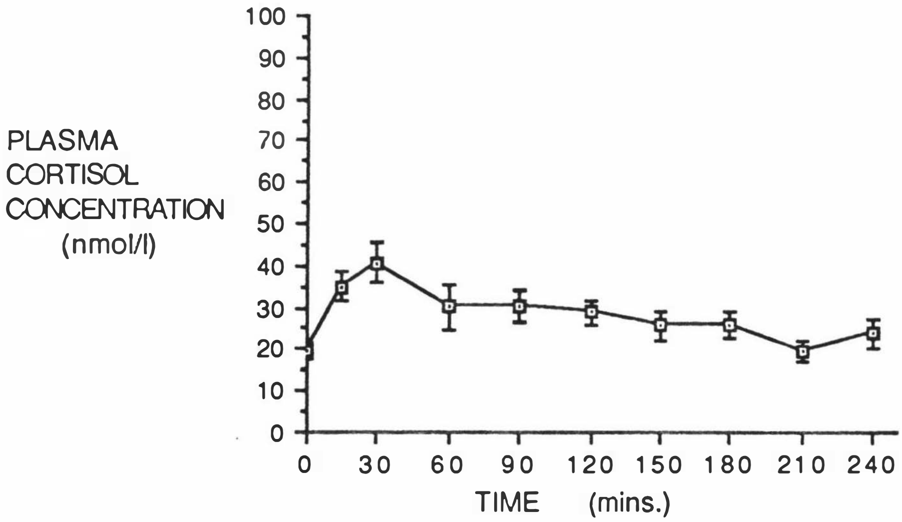


Fig 3-13 : Changes in Plasma Cortisol Concentrations (mean ± s.e.m.) in Control Lambs

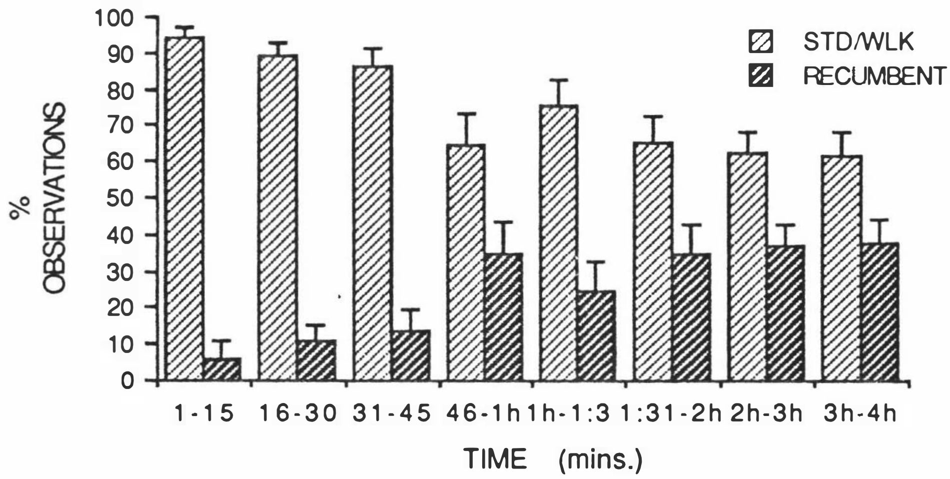


Fig 3-14 : Incidence (mean ± s.e.m.) of Standing/Walking and Recumbent Behaviour of Control Lambs

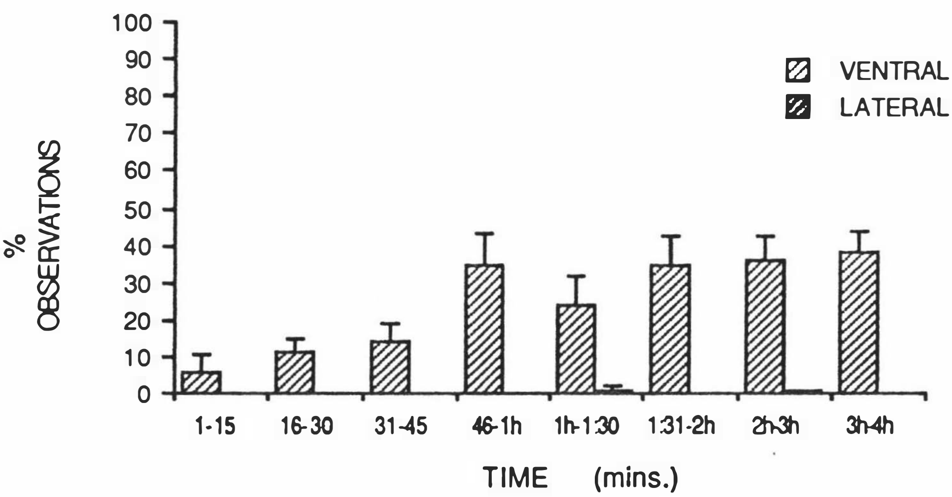


Fig 3-15 : Incidence (mean ± s.e.m.) of Ventral and Lateral Recumbent Behaviour of Control Lambs

typically showed a normal level of restlessness. The incidence of standing/walking behaviour was high and in the first 90 minutes a significant proportion was abnormal. The behaviour of these lambs was very similar to the control lambs in terms of both restlessness and the incidence of standing/walking behaviour. Only in the proportion of standing/walking behaviour that was abnormal during the first 90 minutes were these lambs different from control lambs.

B. Responses to Control Handling:

The responses of the two control groups were analysed separately to see if there was any difference between them. No difference was found so the two groups were pooled (n= 18) and the combined results used.

Lambs exposed to handling and blood sampling showed a mean cortisol response that peaked at 15 to 30 minutes with a plasma cortisol concentration of 35 to 41 nmol/l, and returned to the pretreatment levels by 210 minutes after treatment (Fig. 3-13).

Recumbent behaviour was not observed commonly in the first 45 minutes (8% to 13%), but from 46 minutes the incidence of recumbent behaviour increased to levels of 25% to 35% of observations (Fig. 3-14). Of that recumbent behaviour virtually none was lateral, it was almost entirely ventral recumbency (Fig. 3-15).

Standing/walking behaviour dominated control lamb activity throughout the 4 hour experimental period (Fig. 3-14), particularly in the first 45 minutes (>90 %). Thereafter standing/walking behaviour made up 65% to 77% of observations. While there was some abnormal standing/walking behaviour observed it only constituted 18% to 25% of standing/walking observations in the first 90 minutes. In the last 2.5 hours the proportion of standing/walking behaviour that was abnormal increased to a maximum of 30% (Fig. 3-16).

Control lambs showed very little restlessness (restlessness score = 3.1 ± 1; mean ± s.e.m.) in the first hour after treatment (Fig. 3-17).

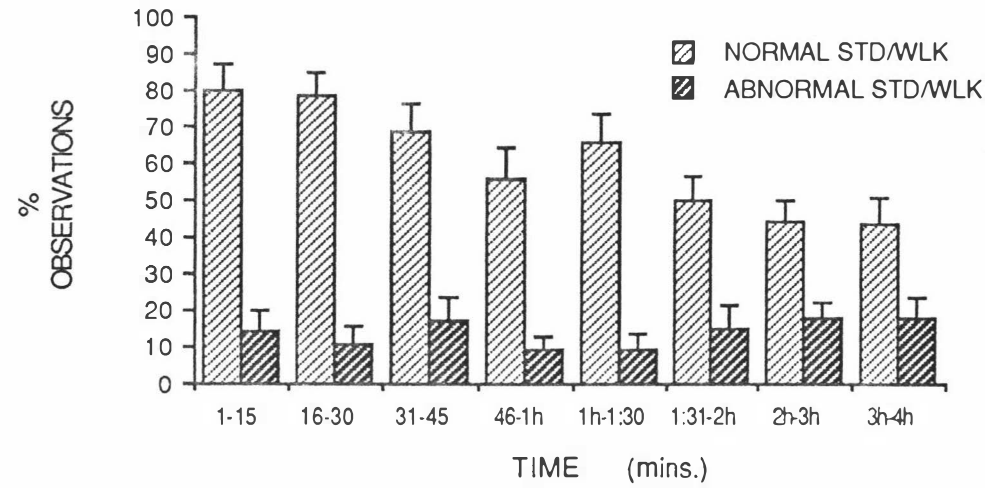


Fig 3-16 : Incidence (mean ± s.e.m.) of Normal and Abnormal Standing/Walking Behaviour of Control Lambs

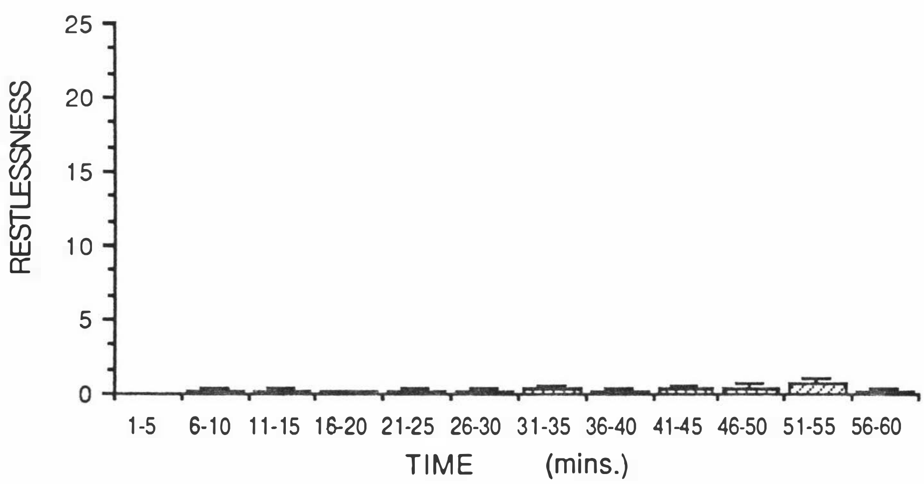


Fig 3-17 : Incidence (mean ± s.e.m.) of Restlessness of Control Lambs

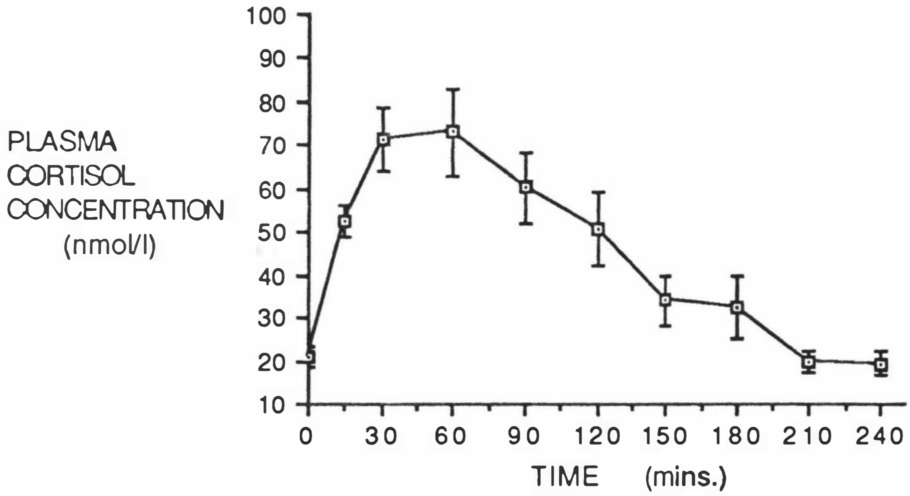


Fig 3-18 : Changes in Plasma Cortisol Concentrations (mean ± s.e.m.) in Lambs Castrated plus Tailed with the Rings

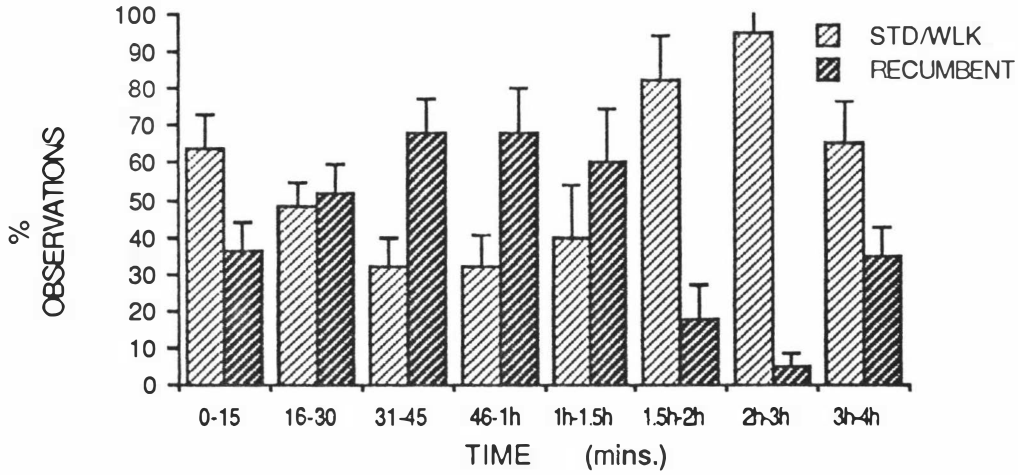


Fig 3-19 : Incidence(mean ± s.e.m.) of Standing/Walking and Recumbent Behaviour of Lambs Castrated plus Tailed with the Rings

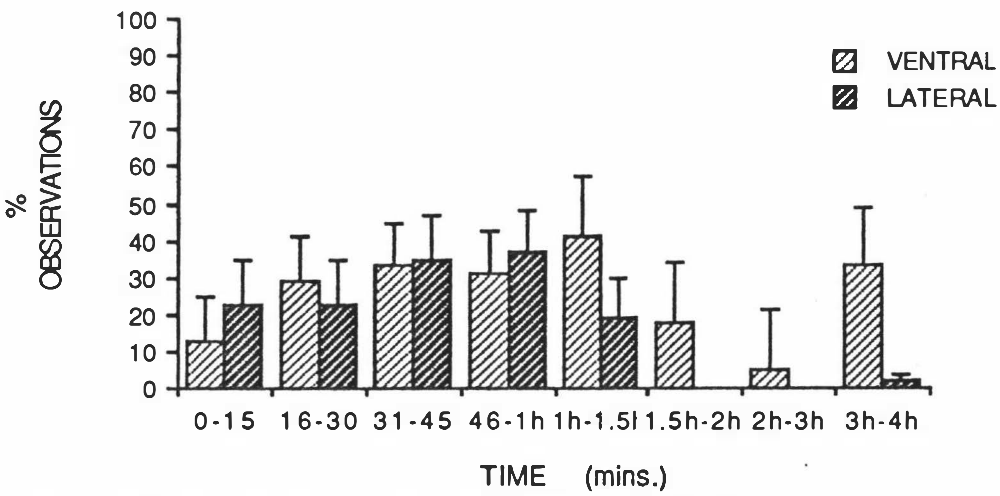


Fig 3-20 : Incidence (mean ± s.e.m.) of Ventral and Lateral Recumbent Behaviour of Lambs Castrated plus Tailed with the Rings

C. Responses to Rubber Rings:

(i) Castration plus Tailing: Lambs castrated plus tailed with the rings showed a cortisol response that peaked at 30 to 90 minutes with a mean plasma cortisol concentration of 60 to 73 nmol/l. The plasma cortisol concentration remained elevated above the pretreatment level until 210 minutes after treatment (Fig. 3-18).

The incidence of recumbent behaviour was significantly higher ($p < 0.01$) in castrated plus tailed lambs than control lambs over the first 1.5 hours, it ranged from 37% to 60% (Fig. 3-19). Of that recumbent behaviour a significant proportion ($p<0.01$)(32% to 65%) was lateral recumbency(Fig. 3-20). Between 1.5 hours and 4 hours, the level of recumbency was similar to control lambs, except in the hour from 2 to 3 hours when castrated and tailed lambs showed significantly less recumbency than control lambs. Of the recumbent behaviour in the period from 1.5 hours to 4 hours there was no lateral recumbency except for 5% in the last hour.

The incidence of standing/walking behaviour was significantly lower ($p<0.01$) in castrated and tailed lambs than control lambs over the first 1.5 hours; it ranged from 32% to 64% (Fig. 3-19). Of that standing/walking behaviour a high proportion (40% to 100%) was abnormal (Fig. 3-21). In comparison, control lambs exhibit below 20% of standing/walking behaviour that was abnormal. Between 1.5 and 4 hours, the level of standing/walking behaviour was similar to control lambs, except in the hour from 2 to 3 hours when castrated and tailed lambs showed significantly ($p<0.01$) more standing/walking behaviour than control lambs. In the period 1.5 to 4 hours there were no significant differences in the incidences of abnormal standing/walking behaviour, except between 1.5 and 2 hours when there was a higher proportion of abnormal standing/walking behaviour than in control lambs (Fig. 3-21).

Castrated and tailed lambs stood up and lay down very frequently (148 times ± 14; mean ± s.e.m.) in the first hour after treatment (Fig. 3-22). The restlessness was highest from 6 to 30 minutes and thereafter decreased gradually.

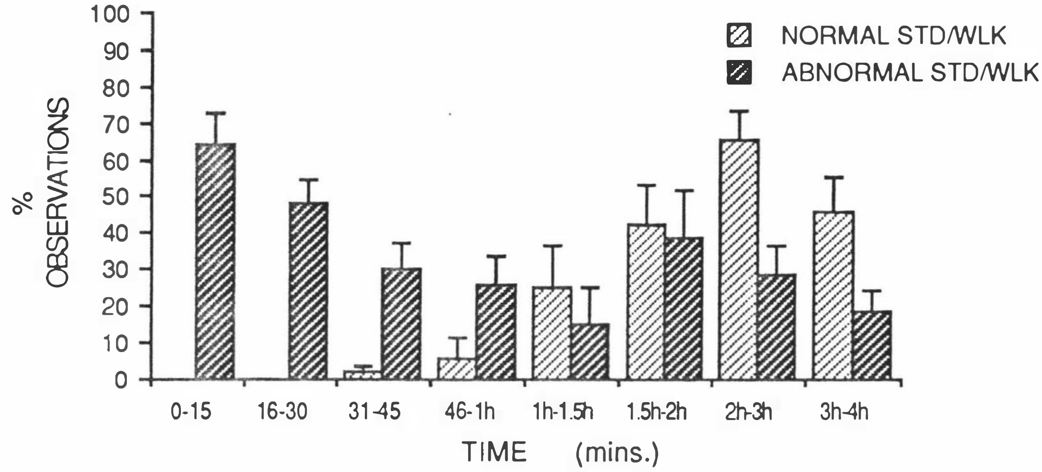


Fig 3-21 : Incidence (mean ± s.e.m.) of Normal and Abnormal Standing/Walking Behaviour of Lambs Castrated plus Tailed with the Rings

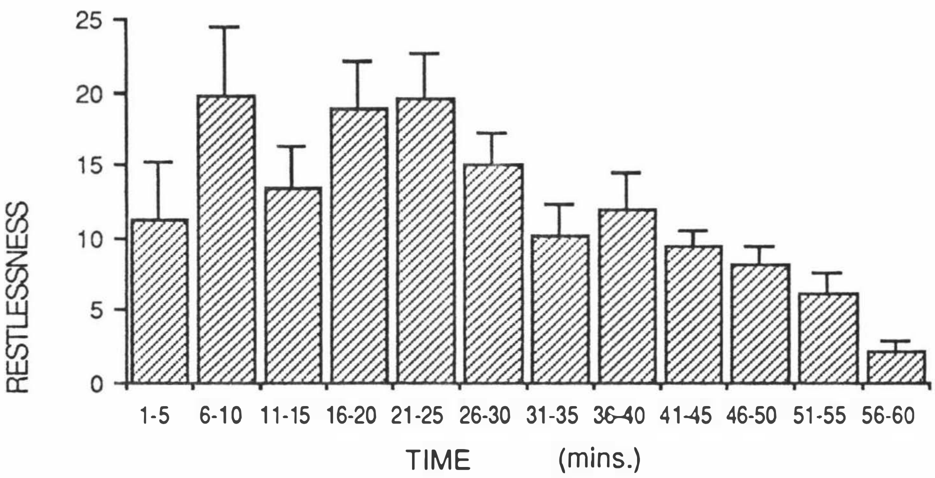


Fig 3-22 : Incidence (mean ± s.e.m.) of Restlessness of Lambs Castrated plus Tailed with the Rings

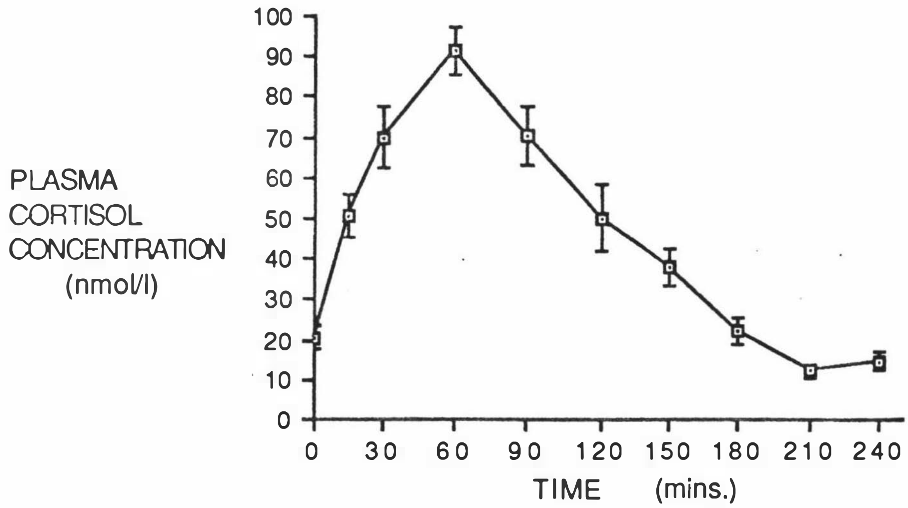


Fig 3-23: Changes in Plasma Cortisol Concentration (mean ± s.e.m.) in Lambs Castrated with the Ring

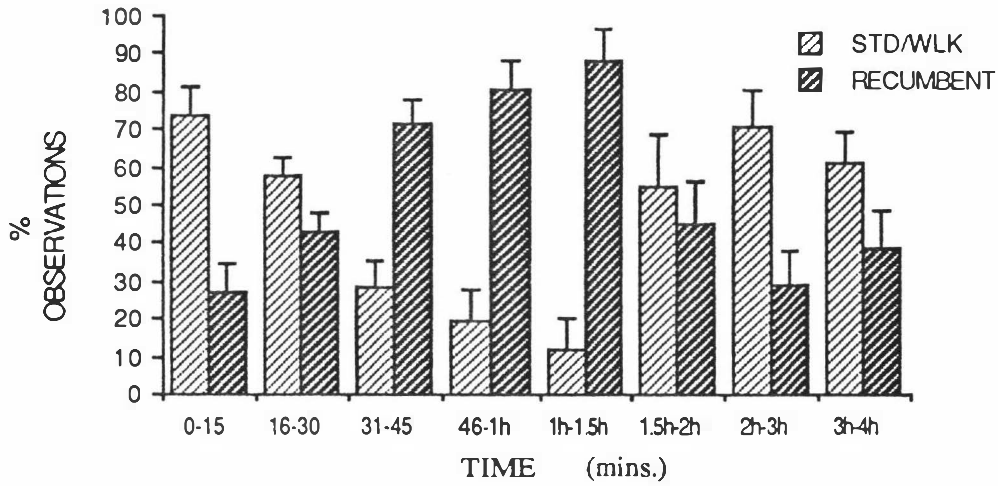


Fig 3-24: Incidence (mean ± s.e.m.) of Standing/Walking and Recumbent Behaviour of Lambs Castrated with the Ring

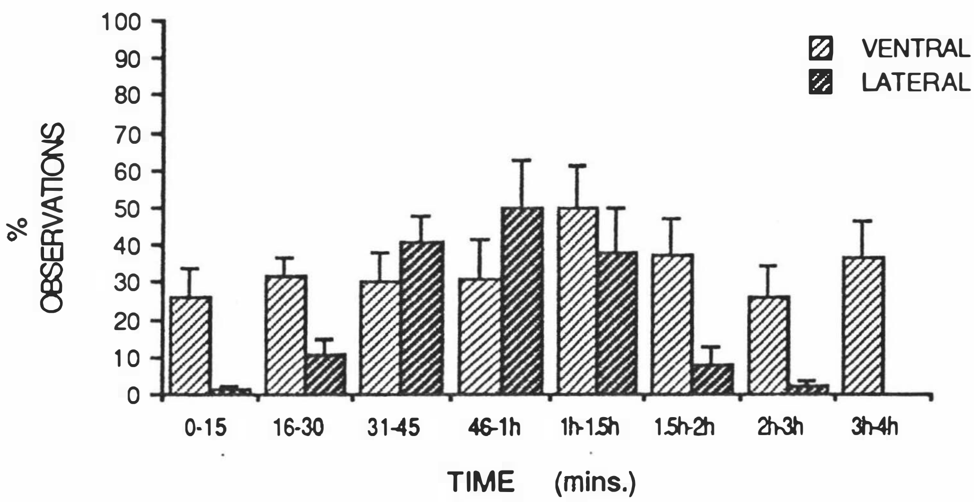


Fig 3-25: Incidence (mean ± s.e.m.) of Ventral and Lateral Recumbent Behaviour of Lambs Castrated with the Ring

C(ii) Castration Only: Lambs castrated alone with the ring showed a cortisol response that peaked at 60 minutes with a mean plasma cortisol concentration of 92 nmol/l. The plasma cortisol concentration remained elevated above the pretreatment level until 180 minutes after treatment (Fig. 3-23).

The incidence of recumbent behaviour was significantly higher ($p<0.01$) in lambs castrated with the ring than in control lambs over the first 1.5 hours; ranging from 28% to 88% (Fig. 3-24). Of the recumbent behaviour a significant proportion ($p<0.01$)(26% to 62%) was lateral recumbency in the period 16 minutes to 1.5 hours (Fig. 3-25). In comparison control lambs exhibit almost no lateral recumbency.

The incidence of standing/walking behaviour was significantly lower in castrated lambs than control lambs over the first 1.5 hours ($p<0.01$); ranging from 13% to 73% (decreasing progressively with time)(Fig. 3-24). Of that standing/walking behaviour a proportion (43% to 98%) was abnormal (Fig. 3-26). After 1.5 hours, while the incidence of standing/walking behaviour was similar to control lambs, the proportion that was abnormal was higher in the period 1.5 to 3 hours. In the last hour (3 to 4 hours after treatment) there was no significant difference in the standing/walking behaviour between lambs castrated with the ring and control lambs.

Lambs castrated with the ring showed a high level of restlessness, standing up or lying down 77 ± 9 times (mean ± s.e.m.) in the first hour (Fig. 3-27). The restlessness peaked between 16 and 40 minutes and then decreased.

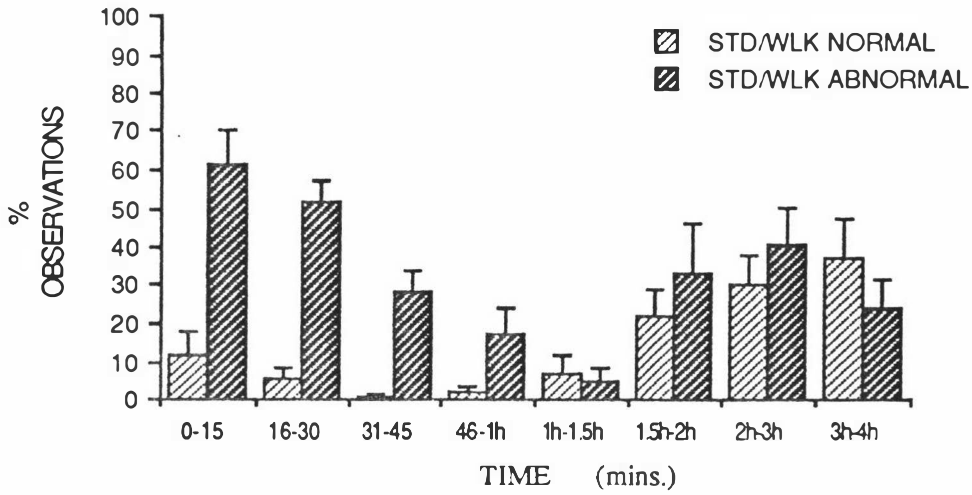


Fig 3-26: Incidence (mean ± s.e.m.) of Normal and Abnormal Standing/Walking Behaviour of Lambs Castrated with the Ring

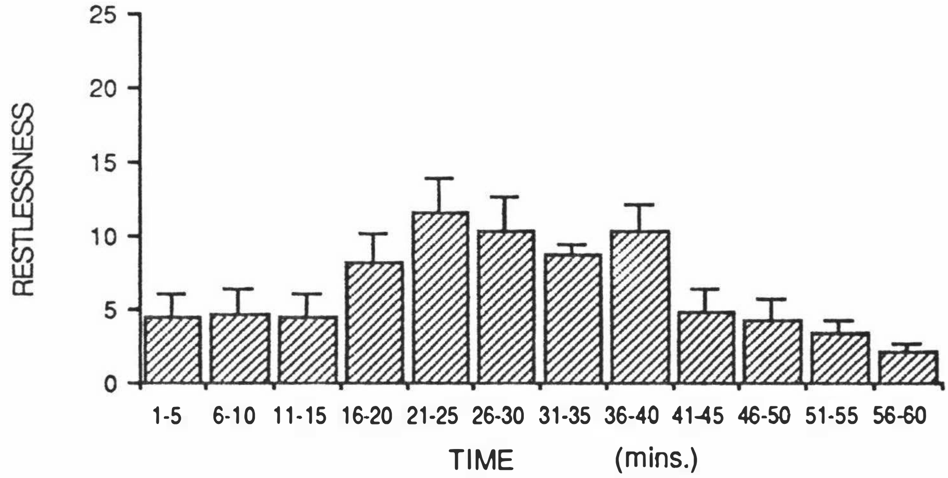


Fig 3-27: Incidence (mean ± s.e.m.) of Restlessness of Lambs Castrated with the Ring

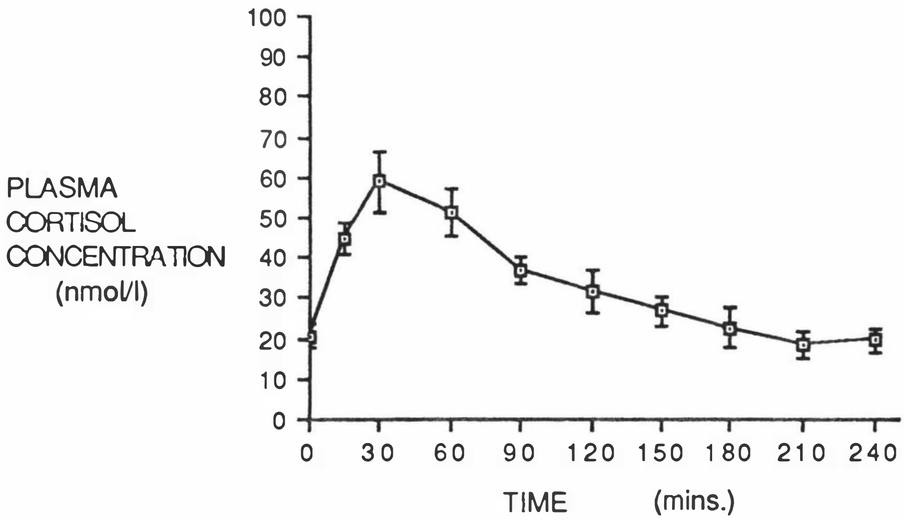


Fig 3-28: Changes in the Plasma Cortisol Concentration (mean ± s.e.m.) in lambs Tailed with the Ring

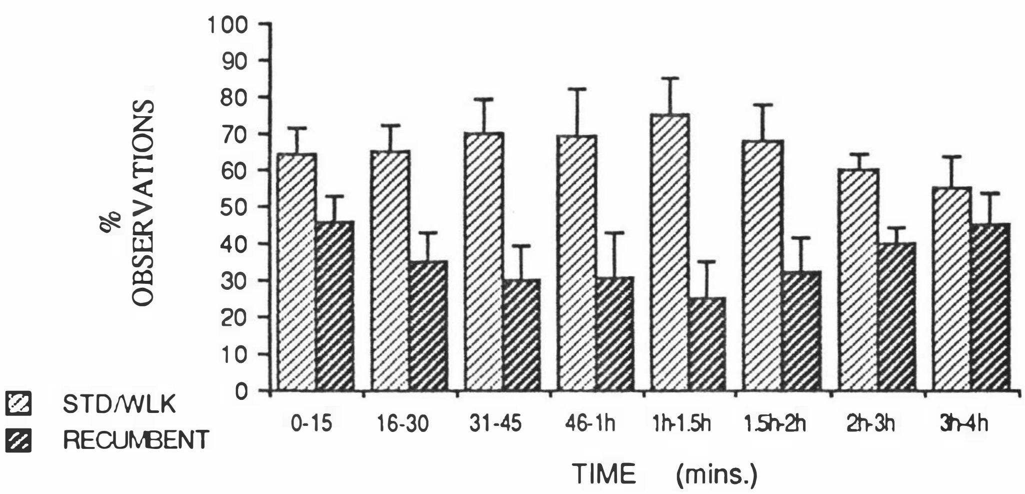


Fig 3-29: Incidence (mean ± s.e.m.) of Standing/Walking and Recumbent Behaviour of Lambs Tailed with the Ring

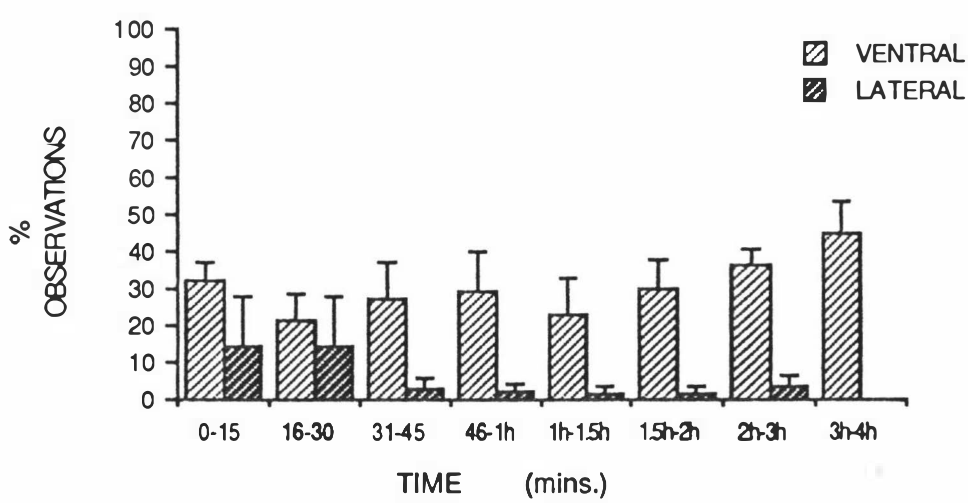


Fig 3-30: Incidence (mean ± s.e.m.) of Ventral and Lateral Recumbent Behaviour of Lambs Tailed with the Ring

C(iii) Tailing Only: Lambs tailed with the ring showed a cortisol response that peaked at 30 to 60 minutes after treatment with mean plasma cortisol concentrations of 51 to 59 nmol/l. The plasma cortisol concentration remained elevated above the pretreatment level until 180 minutes after treatment (Fig 3-28).

The incidence of recumbent behaviour was significantly higher ($p<0.01$) in lambs tailed with the ring than control lambs over the first 45 minutes; it ranged from 30% to 37% (Fig. 3-29). Of the recumbent behaviour a significant proportion ($p<0.05$)(40% to 54%) was lateral in the first 30 minutes (Fig. 3-30). After the first 45 minutes the incidence of recumbent behaviour was not significantly different from control lambs. The proportion of recumbent behaviour that was lateral after the first 30 minutes was also not different from control lambs.

The incidence of standing/walking behaviour was significantly lower ($p<0.01$) in lambs tailed with the ring than control lambs over the first 45 minutes, ranging from 64% to 70% (Fig 3-29). Of the standing/walking behaviour in that period a significant ($p<0.01$) proportion (96% to 100%) was abnormal, and this high proportion of abnormal standing/walking behaviour extended until 1.5 hours after treatment (Fig. 3-31). From 1.5 until 4 hours the proportion of standing/walking behaviour that was abnormal was similar to that in control lambs.

Lambs tailed with the ring showed a high level of restlessness, standing up or lying down 100 ± 20 times (mean ± s.e.m.) in the first hour after treatment (Fig. 3-32). The restlessness increased to a peak at 21 to 25 minutes and thereafter decreased rapidly.

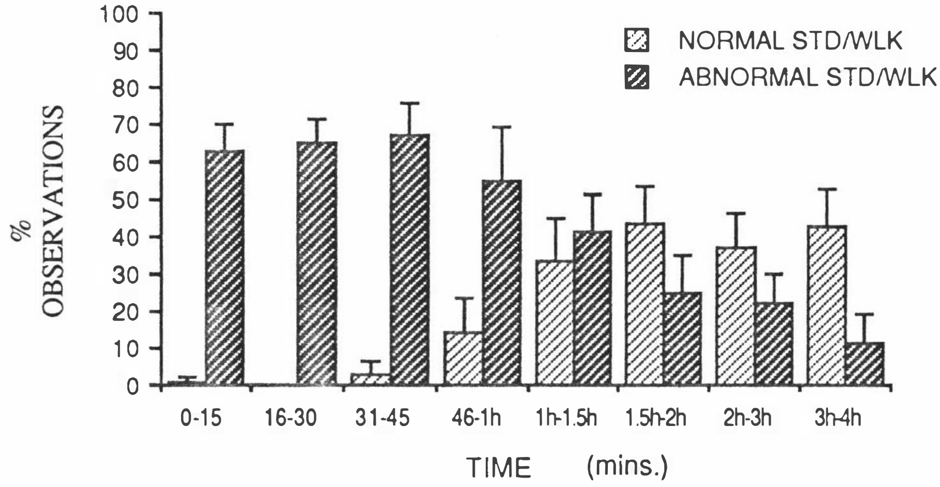


Fig 3-31: Incidence (mean ± s.e.m.) of Normal and Abnormal Standing/Walking Behaviour of Lambs Tailed with the Ring

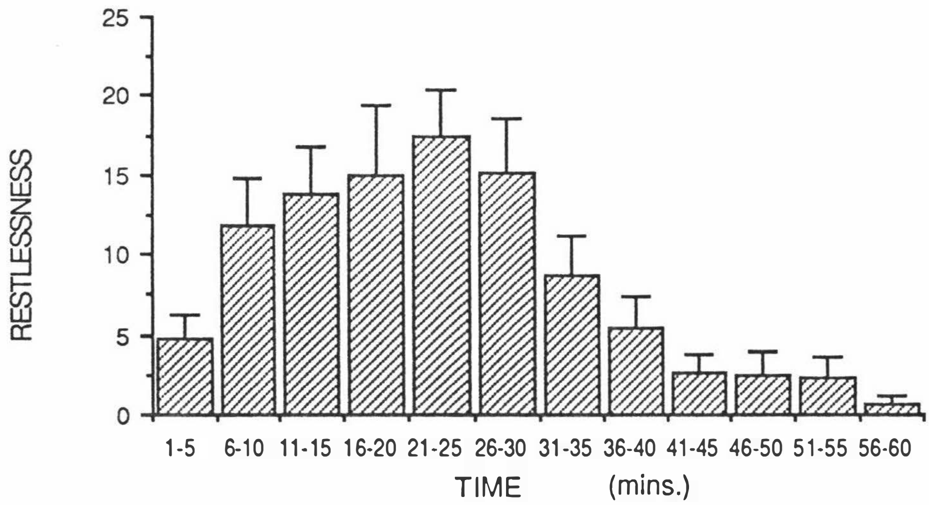


Fig 3-32: Incidence (mean ± s.e.m.) of Restlessness of Lambs Tailed with the Ring

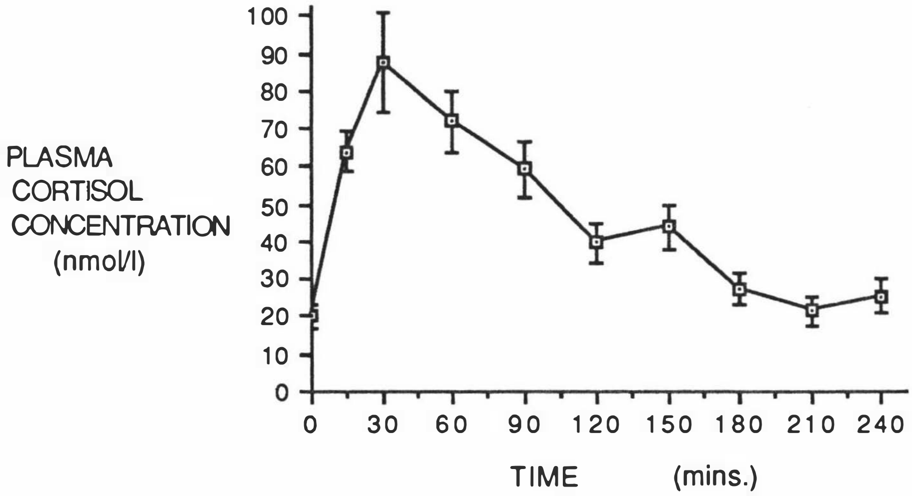


Fig 3-33: Changes in Plasma Cortisol Concentration (mean ± s.e.m.) in Lambs Short-Scrotum plus Tailed with the Rings

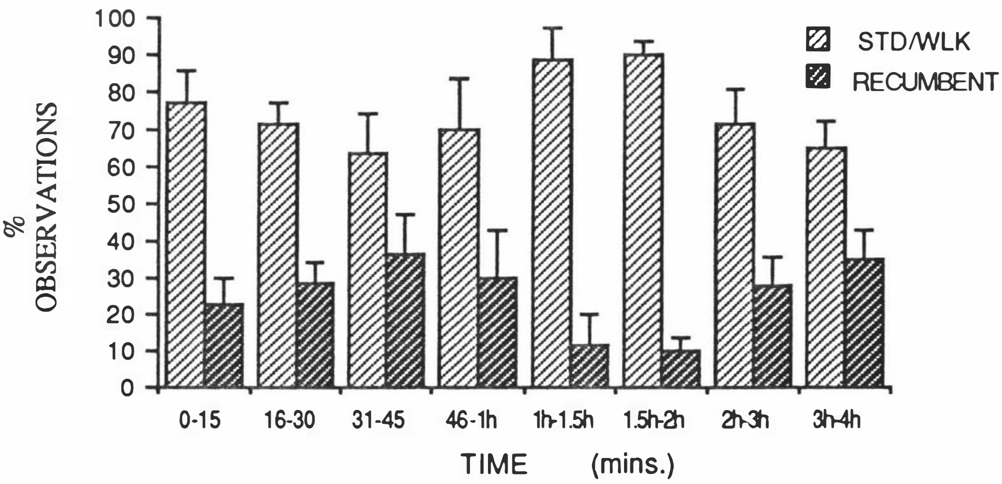


Fig 3-34: Incidence (mean ± s.e.m.) of Standing/Walking and Recumbent Behaviour of Lambs Short-scrotumed plus Tailed with the Rings

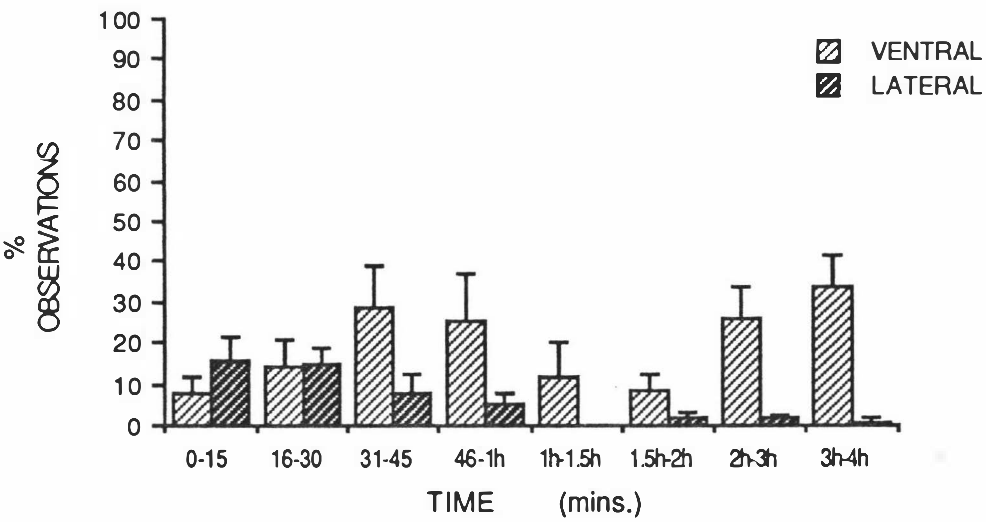


Fig 3-35: Incidence (mean ± s.e.m.) of Ventral and Lateral Recumbency of Lambs Short-Scrotumed plus Tailed with the Rings

C(iv) Short-Scrotum plus Tailing: Lambs that were short-scrotumed plus tailed showed a cortisol response that peaked at 30 to 60 minutes with mean plasma cortisol concentrations of 72 to 88 nmol/l. Plasma cortisol concentrations remained elevated above the pretreatment level until 180 minutes after treatment (Fig. 3-33).

The incidence of recumbent behaviour was significantly higher ($p<0.01$) in short-scrotumed plus tailed lambs than control lambs over the first 45 minutes, ranging from 23% to 37% (Fig. 3-34). In the periods 1 to 2 hours after treatment the incidence of recumbent behaviour was significantly lower ($p<0.01$) than for control lambs. Of the recumbent behaviour there was a significant proportion ($p<0.05$) that was lateral in the first 30 minutes (52% to 62%) (Fig. 3-35).

The incidence of standing/walking behaviour was significantly lower ($p<0.01$) than for control lambs over the first 45 minutes, ranging from 63% to 77%, and significantly higher ($p<0.01$) in the period 1.5 to 2 hours (90%)(Fig. 3-34). Of the standing/walking behaviour a significant ($p<0.01$) proportion (51% to 100%) was abnormal over the first three hours after treatment (Fig. 3-36). Between 3 and 4 hours, the incidence of standing/walking behaviour that was abnormal was similar to that of control lambs.

Lambs short-scrotumed plus tailed with the rings showed high restlessness, standing up and lying down 111 ± 18 times (mean ± s.e.m.) in the first hour. The restlessness peaked at 21-25 minutes and thereafter decreased gradually (Fig 3-37).

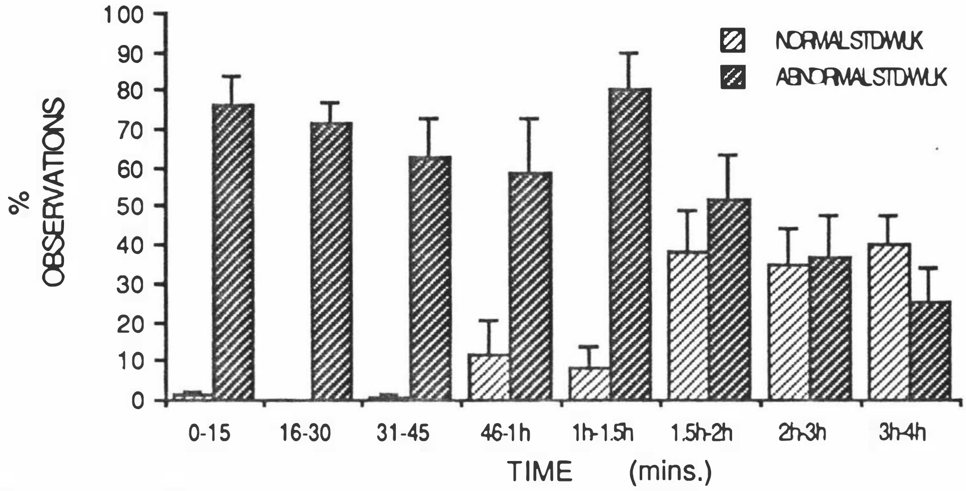


Fig 3-36: Incidence (mean ± s.e.m.) of Normal and Abnormal Standing/Walking Behaviour of Lambs Short-Scrotumed plus Tailed with the Ring

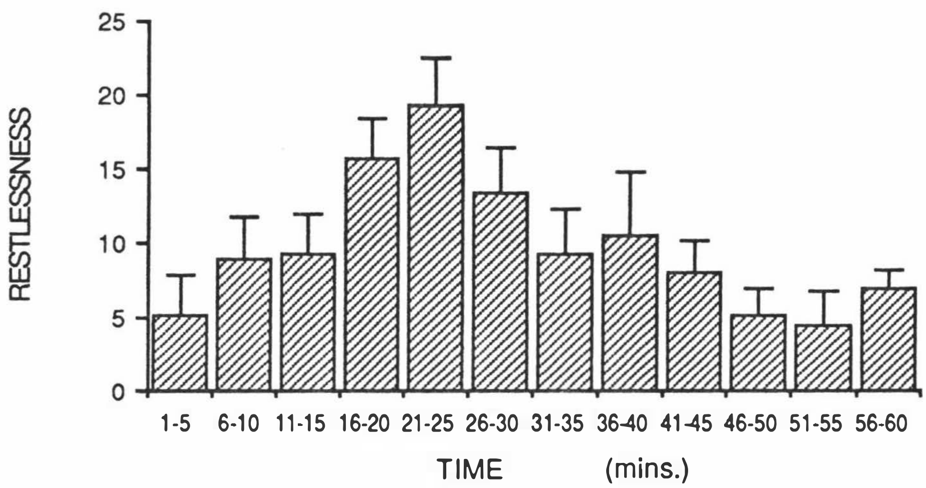


Fig 3-37: Incidence (mean ± s.e.m.) of Restlessness of Lambs Short-Scrotumed plus Tailed with the Rings

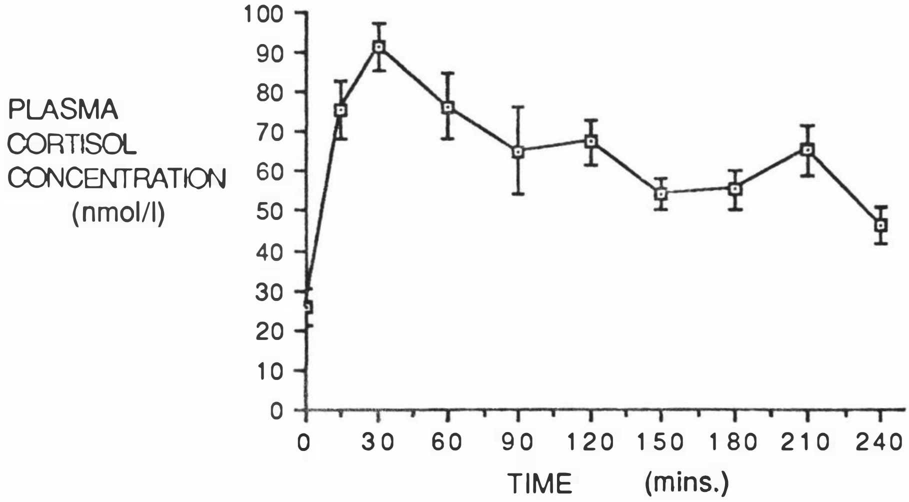


Fig 3-38: Changes in Plasma Cortisol Concentrations (mean ± s.e.m.) in Lambs Castrated plus Tailed with the Knife

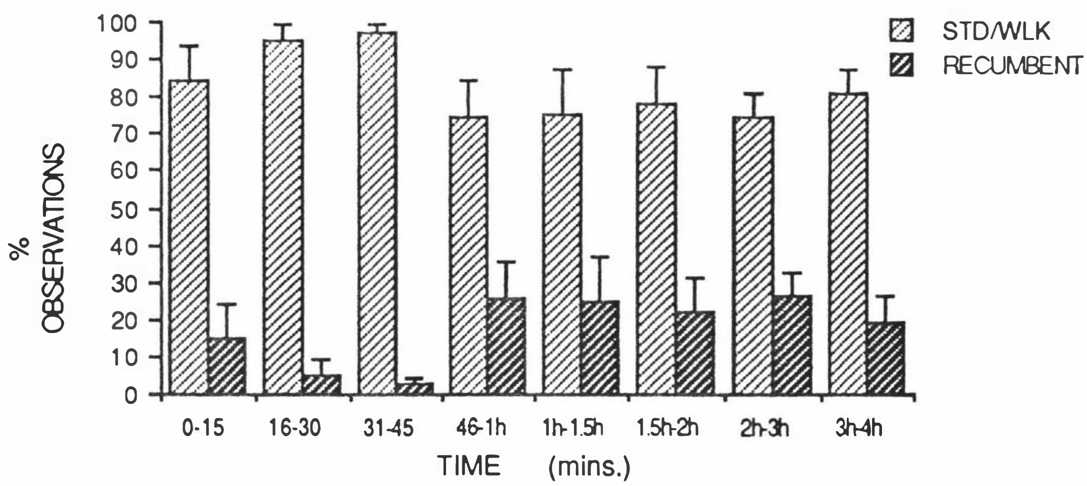


Fig 3-39 : Incidence (mean ± s.e.m.) of Standing/Walking and Recumbent Behaviour of Lambs Castrated plus Tailed with the Knife

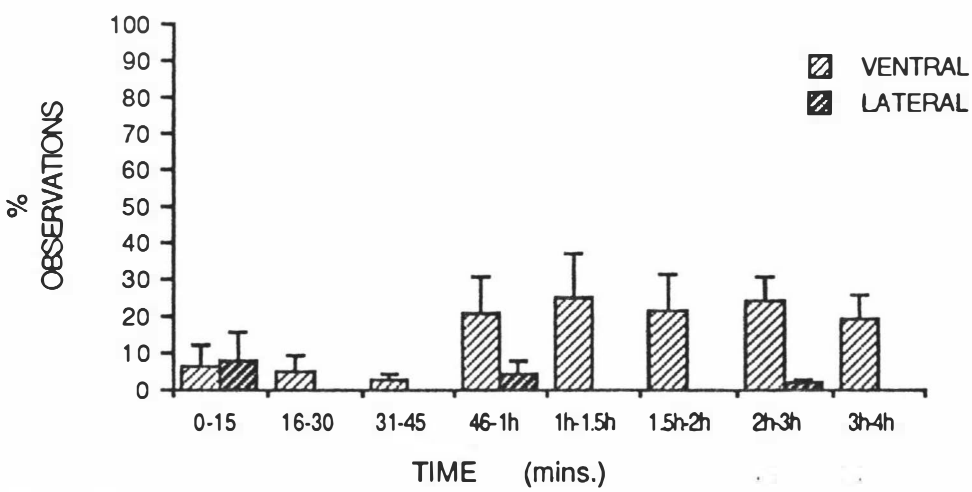


Fig 3-40: Incidence (mean ± s.e.m.) of Ventral and Lateral Recumbent Behaviour of Lambs Castrated plus Tailed with the Knife

D. Responses to the Knife:

(i) Castration plus Tailing: Lambs castrated plus tailed with the knife showed a cortisol response that peaked at 30 minutes with a mean plasma cortisol concentration of 92 nmol/l, but the subsequent plasma cortisol concentrations remained above the pretreatment level for the whole 4 hour experimental period (Fig 3-38).

The incidence of recumbent behaviour was similar to that in control lambs, except in the periods 31 to 45 minutes and 3 to 4 hours when the incidence of recumbent behaviour was significantly lower ($p<0.01$) for castrated plus tailed lambs than for control lambs(Fig. 3-39).Apart from the first 15 minutes the recumbent behaviour was commonly ventral as it was for control lambs. In the first 15 minutes lateral recumbency made up 53% of recumbent behaviour (Fig. 3-40).

The incidence of standing/walking behaviour was significantly higher ($p<0.01$) than control lambs over the period 31 to 45 minutes (at 98%), but apart from that period it did not differ from control lambs, ranging from 74% to 95% of all observations (Fig. 3-39). Of that standing/walking behaviour a high proportion (58% to 90%) was abnormal throughout the experimental period (Fig. 3-41). This proportion of standing/walking behaviour that was abnormal was higher than that for control lambs which had a peak proportion of standing/walking behaviour that was abnormal of 29%.

Castrated plus tailed lambs showed a similar level of restlessness as control lambs, standing up of lying down 2 ± 0.7 times (mean ± s.e.m.) in 1 hour (Fig. 3-42).

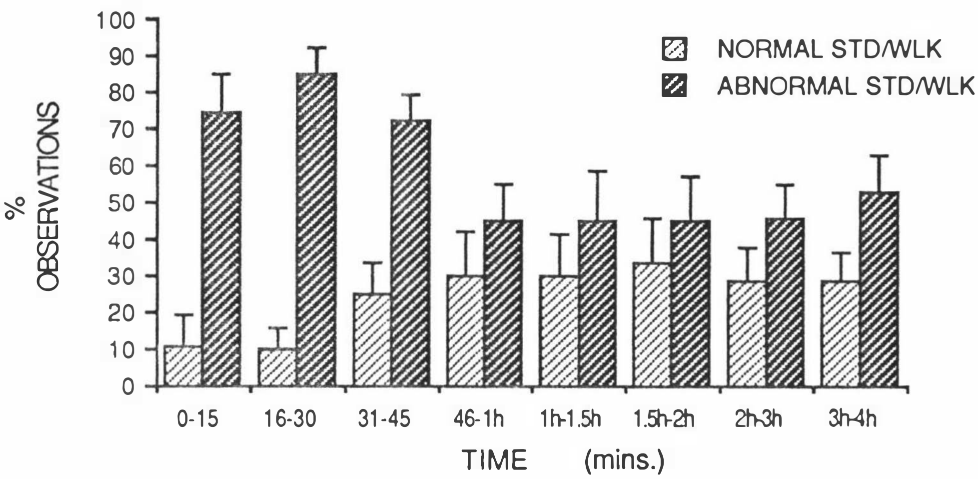


Fig 3-41: Incidence (mean ± s.e.m.) of Normal and Abnormal Standing/Walking Behaviour of Lambs Castrated plus Tailed with the Knife

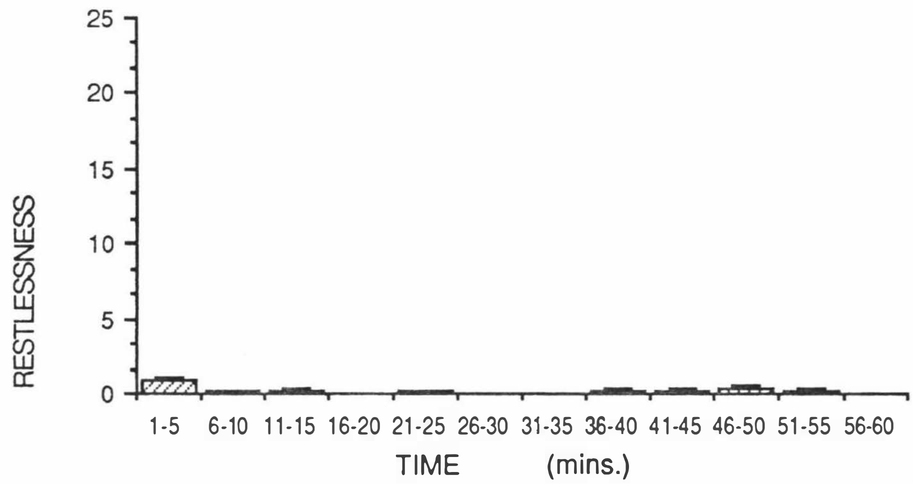


Fig 3-42 : Incidence (mean ±s.e.m.) of Restlessness of Lambs Castrated plus Tailed with the Knife

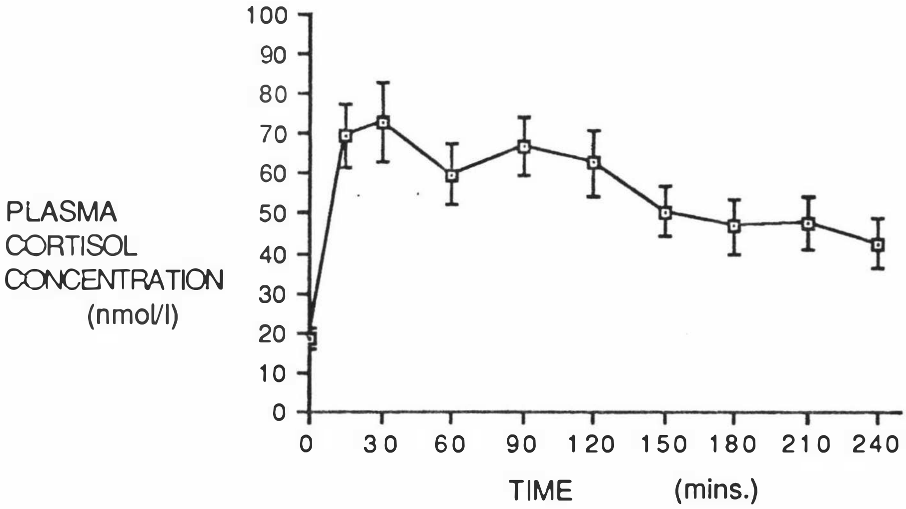


Fig 3-43: Changes in Plasma Cortisol Concentrations (mean ± s.e.m.) in Lambs Castrated with the Knife

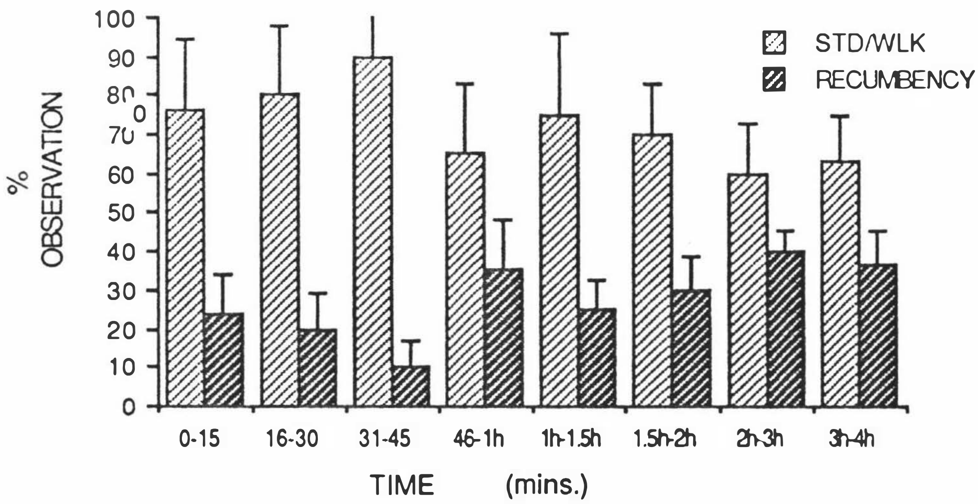


Fig 3-44: Incidence (mean ± s.e.m.) of Standing/ Walking and Recumbent Behaviour of Lambs Castrated with the Knife.

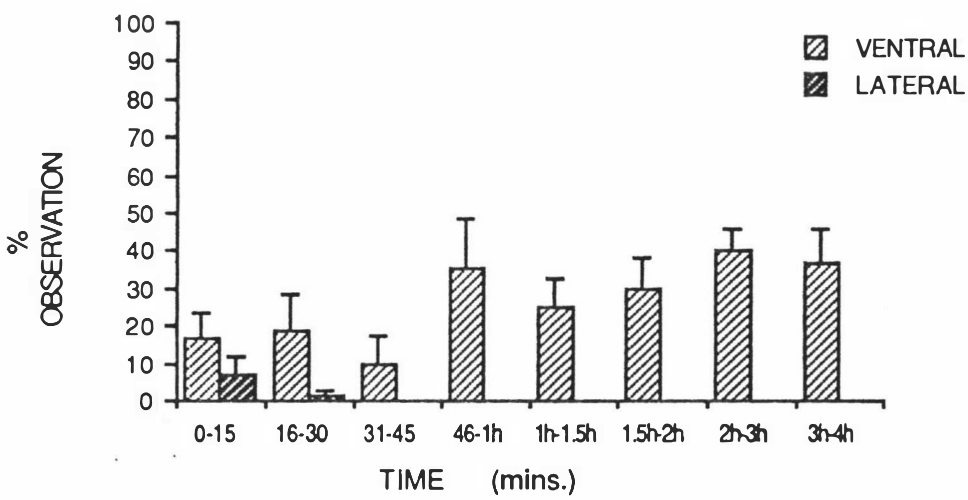


Fig 3-45: Incidence (mean ± s.e.m.) of Ventral and Lateral Recumbent Behaviour of Lambs Castrated with the Knife

D(ii) Castration Only: Lambs castrated alone with the knife showed a cortisol response that peaked at 15 to 120 minutes with mean plasma cortisol concentrations of 60 to 73 nmol/l, but the subsequent plasma cortisol concentrations remained above the pretreatment level for the whole 4 hour experimental period (Fig 3-43).

The incidence of recumbent behaviour was again not different from control lambs at any time (Fig. 3-44). However, of the recumbent behaviour a significant proportion (28%) was lateral in the first 15 minutes (Fig. 3-45). Thereafter the proportion of recumbent behaviour that was lateral was similar to control lambs, being largely ventral as it was in control lambs.

The incidence of standing/walking behaviour was not different from control lambs at any time over the 4 hour experimental period(Fig. 3-44). However, of the standing/walking behaviour a high proportion (52% to 75%) was abnormal, and this persisted throughout the experimental period (Fig. 3-46).

Lambs castrated with the knife showed a similar level of restlessness as control lambs, standing up or lying down 4.5 ± 1.6 times (mean ± s.e.m.) in 1 hour (Fig. 3-47).

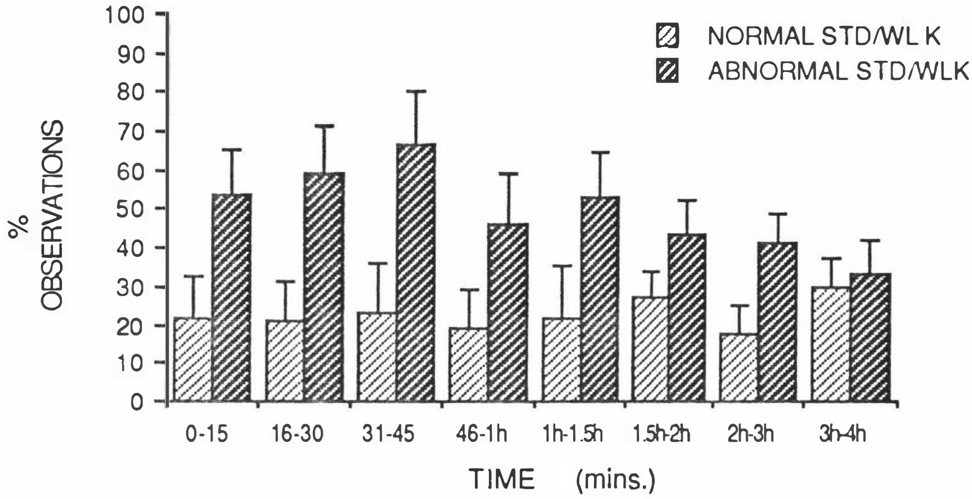


Fig 3-46: Incidence (mean ± s.e.m.) of Normal and Abnormal Standing/Walking Behaviour of Lambs Castrated with the Knife

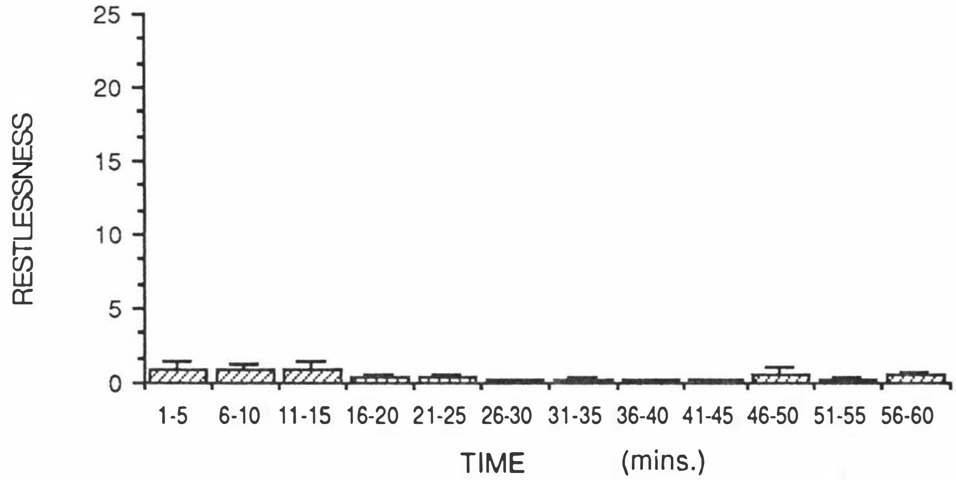


Fig 3-47: Incidence (mean ± s.e.m.) of Restlessness of Lambs Castrated with the Knife

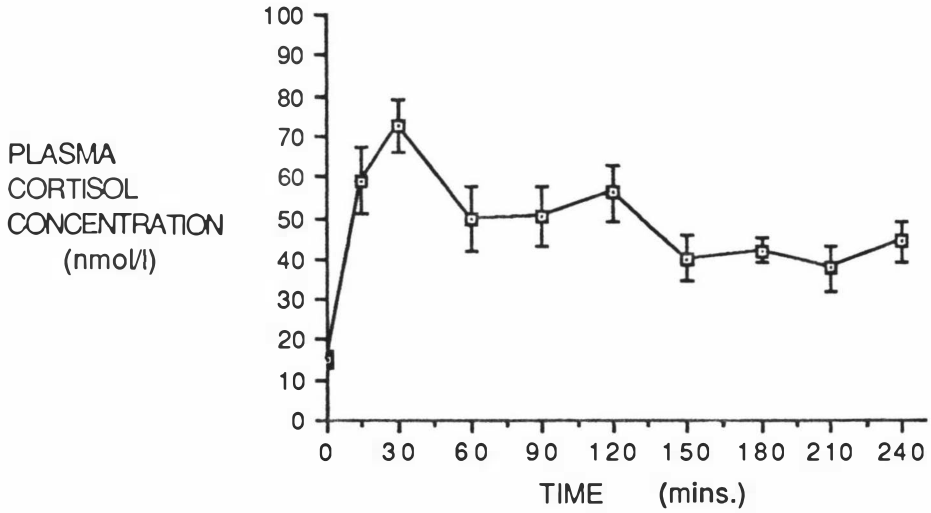


Fig 3-48: Changes in Plasma Cortisol Concentrations (mean ± s.e.m.) in Control Lambs and Lambs Tailed with the Knife

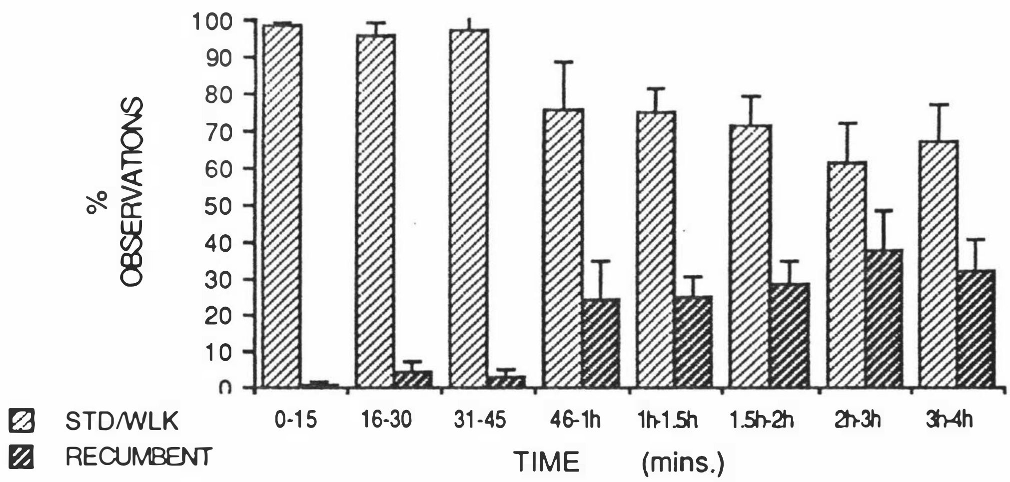


Fig 3-49: Incidence (mean ± s.e.m.) of Standing/Walking and Recumbent Behaviour of Lambs Tailed with the Knife

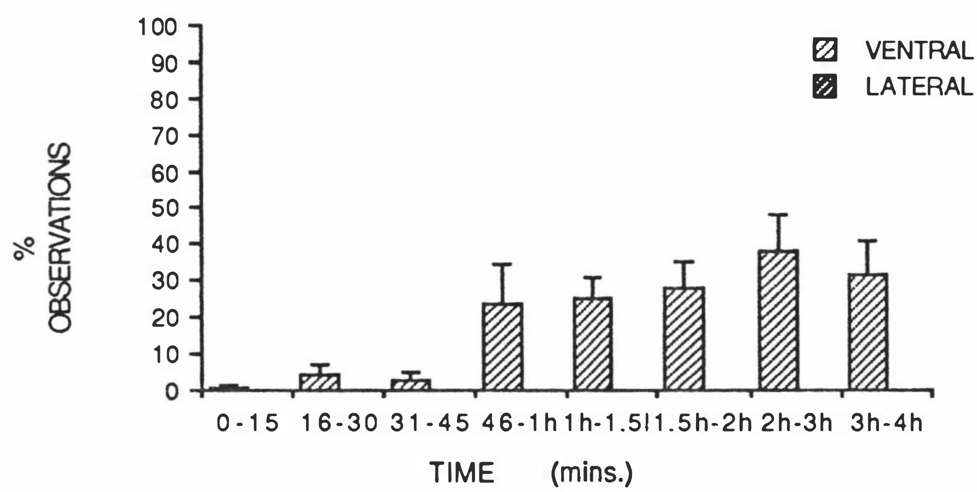


Fig 3-50: Incidence (mean ± s.e.m.) of Ventral and Lateral Recumbent Behaviour of Lambs Tailed with the Knife

D(iii) Tailing Only: Lambs tailed alone with the knife showed a cortisol response that peaked at 15 to 30 minutes with mean plasma cortisol concentrations of 59 to 72 nmol/l, but the subsequent plasma cortisol concentrations remained above the pretreatment level for the whole 4 hour experimental period (Fig 3-48).

The incidence of recumbent behaviour was lower than in control lambs only in the period 31 to 45 minutes, otherwise there were no differences in the incidences of recumbent behaviour(3-49). Also there were no differences in the proportion of recumbency that was lateral; the recumbent behaviour was almost exclusively ventral both in lambs tailed with the knife and in control lambs (Fig. 3-50).

The incidence of standing/walking behaviour was not different from control lambs at any time throughout the 4 hour experimental period (Fig. 3-49). However, of the standing/walking behaviour a significant proportion (30% to 61%) was abnormal(Fig. 3-51). This abnormal standing/walking behaviour was higher than for control lambs at all periods except from 1.5 to 2 hours after treatment.

Lambs tailed with the knife showed a similar level of restlessness as control lambs, standing up and lying down 3 ± 1.3 times (mean ± s.e.m.) in the first hour (Fig. 3-52).

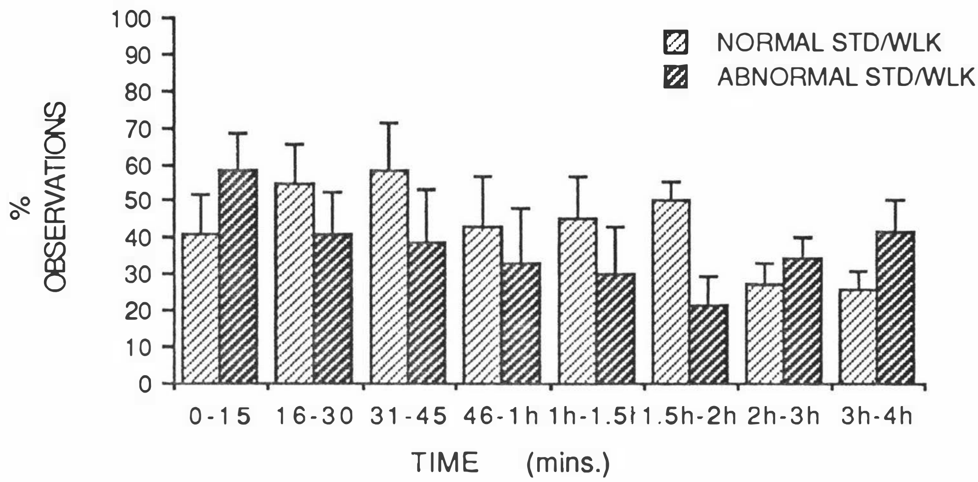


Fig 3-51: Incidence (mean ± s.e.m.) of Normal and Abnormal Standing/Walking Behaviour of Lambs Tailed with the Knife

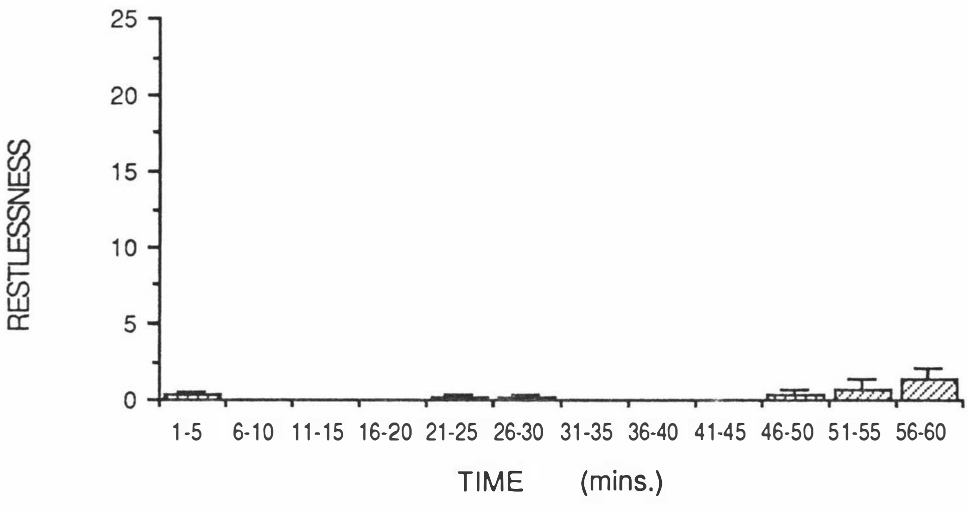


Fig 3-52: Incidence (mean ± s.e.m.) of Restlessness of Lambs Tailed with the Knife

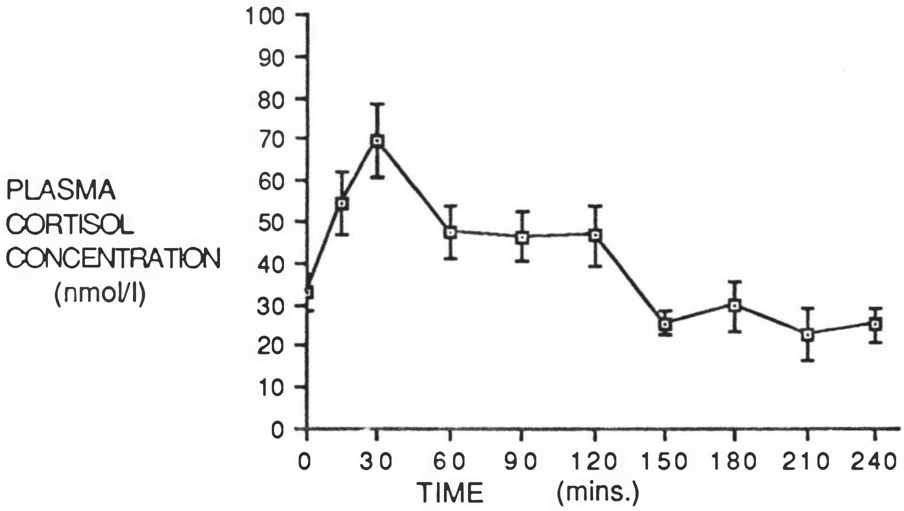


Fig 3-53: Changes in Plasma Cortisol Concentrations (mean ± s.e.m.) in Lambs Tailed with the Iron

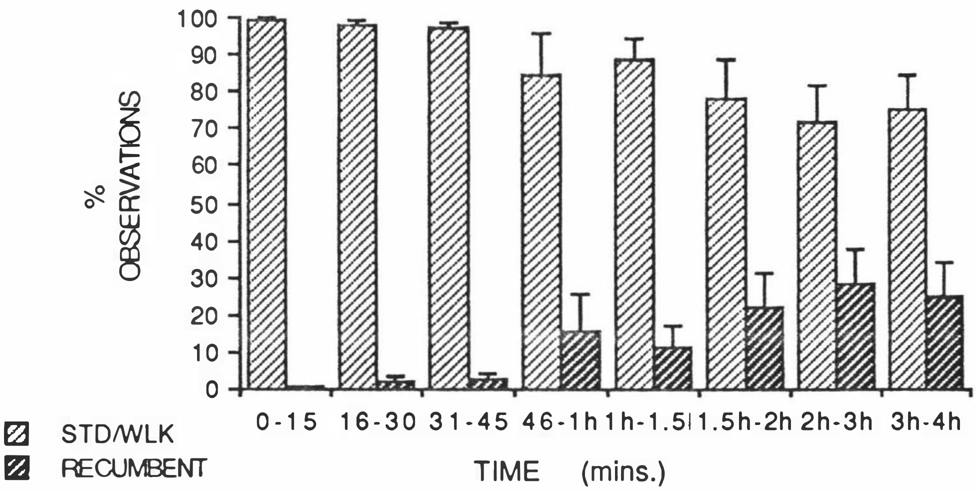


Fig 3-54: Incidence (mean ± s.e.m.) of Standing/Walking and Recumbent Behaviour of Lambs Tailed with the Docking Iron

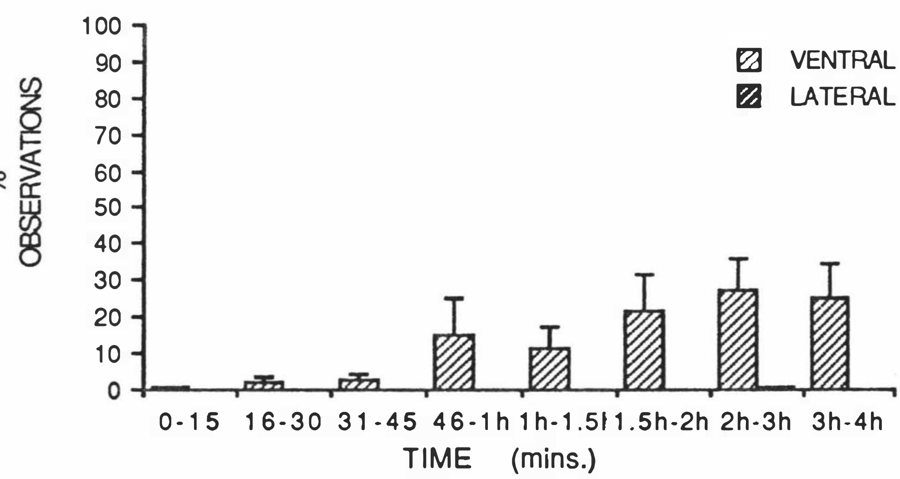


Fig 3-55: Incidence (mean ± s.e.m.) of Ventral and Lateral Recumbent Behaviour of Lambs Tailed with the Docking Iron

E. Response to the Docking Iron:

(i) Tailed Only: Lambs tailed alone with the docking iron showed a cortisol response that peaked at 15 to 30 minutes with mean plasma cortisol concentrations of 54 to 70 nmol/l. The plasma cortisol concentration remained elevated above the pretreatment level until 150 minutes after treatment (Fig. 3-53).

The incidence of recumbent behaviour was lower than in control lambs for the first 45 minutes and again from 1 to 1.5 hours, ranging from 0% to 12% (Fig. 3-54). At 46 to 60 minutes and after 1.5 hours the incidences of recumbent behaviour were similar to control lambs. Of the recumbent behaviour there was effectively no significant lateral recumbent behaviour (Fig. 3-55); the recumbency was largely ventral as it was for control lambs.

The incidence of standing/walking behaviour was not different from control lambs at any time throughout the experimental period (Fig. 3-54). However, of the standing/walking behaviour a significant proportion (28% to 50%) was abnormal in the first 1.5 hours, which was higher than the proportion of standing/walking behaviour that was abnormal for control lambs. Between 1.5 and 4 hours there were no differences in the proportion of abnormal standing/walking behaviour in lambs treated with the iron and control lambs (Fig. 3-56).

Lambs tailed with the docking iron showed a similar level of restlessness to control lambs, standing up and lying down 2.5 ±1.4 times (mean ± s.e.m.) in the first hour (Fig 3-57).

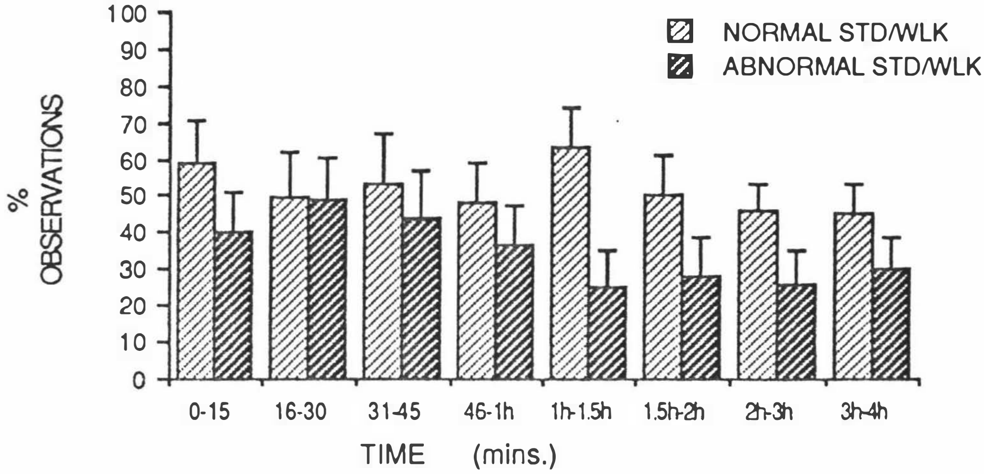


Fig 3-56: Incidence (mean ± s.e.m.) of Normal and Abnormal Standing/Walking Behaviour of Lambs Tailed with the Docking Iron

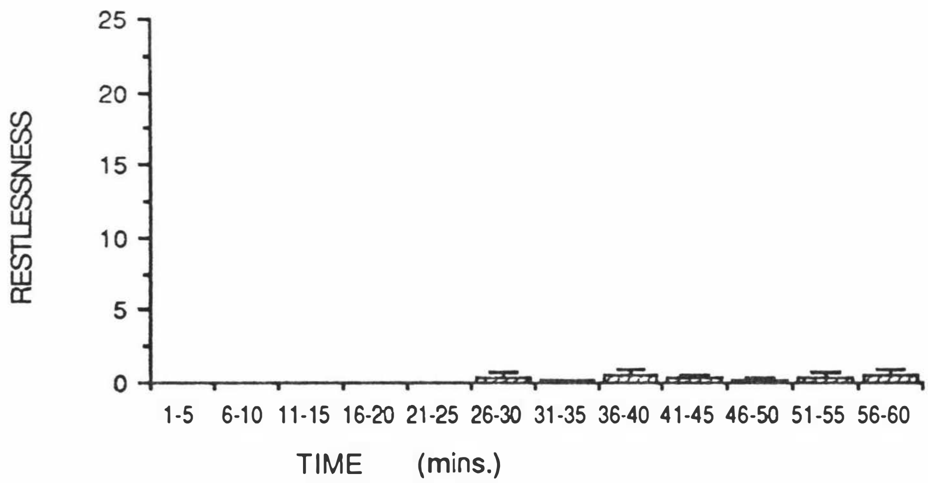


Fig 3-57: Incidence (mean ± s.e.m.) of Restlessness of Lambs Tailed with the Docking Iron

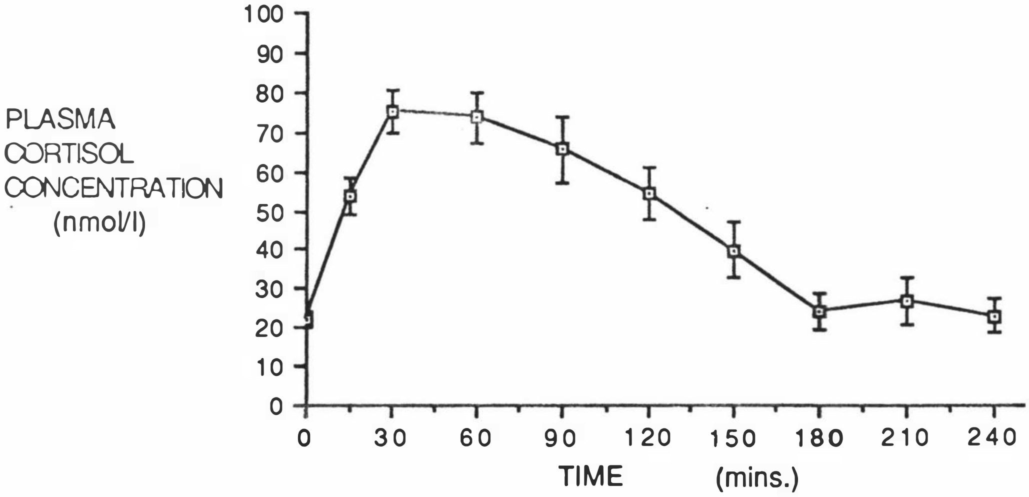


Fig 3-58 : Changes in Plasma Cortisol Concentrations (mean ± s.e.m.) in Lambs Castrated with the Ring plus Tailed with the Iron

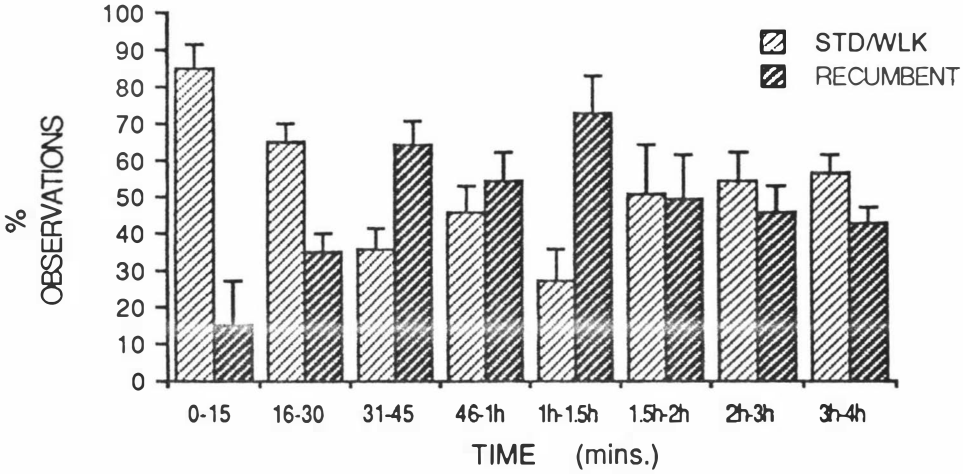


Fig 3-59 : Incidence (mean ± s.e.m.) of Standing/Walking and Recumbent Behaviour of Lambs Castratedwith the Ring and Tailed with the Iron

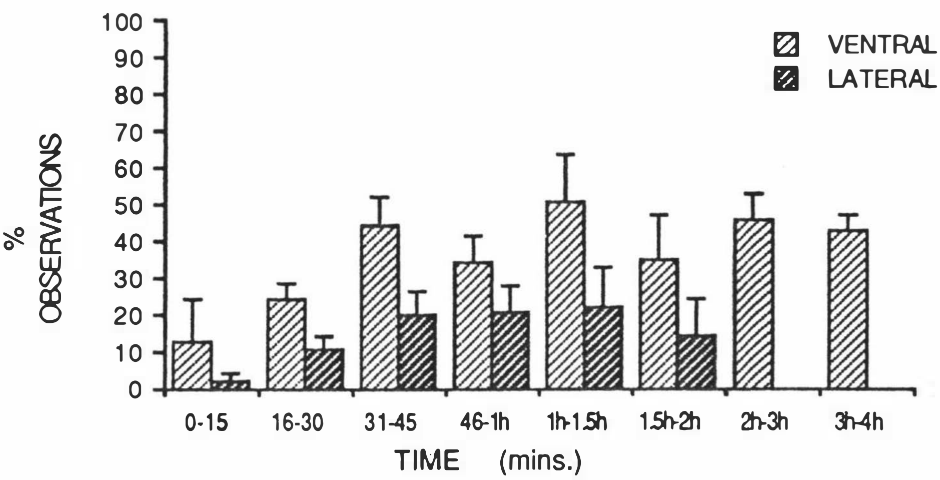


Fig 3-60 : Incidence (mean ± s.e.m.) of Ventral and Lateral Recumbent Behaviour of Lambs Castrated with the Ring and Tailed with the Iron

D(ii) Castration with the Ring plus Tailing with the Docking Iron: Lambs castrated with the ring plus tailed with the docking iron showed a cortisol response that peaked at 30 to 90 minutes with mean plasma cortisol concentrations of 66 to 75 nmol/l. The plasma cortisol concentration remained elevated above the pretreatment level until 180 minutes after treatment (Fig. 3 -58).

The incidence of recumbent behaviour was significantly higher ($p<0.05$) than in control lambs between 16 minutes and 1.5 hours; ranging from 35% to 73% (Fig. 3-59). At the other time periods there were no differences from control lambs. Of the recumbent behaviour in the period up until 1.5 hours a significantly higher ($p<0.05$) proportion (13% to 40%) than for control lambs was lateral recumbency (Fig 3-60).

The incidence of standing/walking behaviour was lower than control lambs from 16 minutes to 1.5 hours after treatment (Fig. 3-59). At the other time periods there were no differences from control lambs. Of the standing/walking behaviour higher proportions (51% to 97%) were abnormal for all time periods (Fig. 3-61) compared to those for control lambs.

The lambs showed a high level of restlessness, standing up and lying down 73 ± 8 times (mean ± s.e.m.) in 1 hour. The restlessness peaked at 21 to 45 minutes and then decreased (Fig. 3-62).

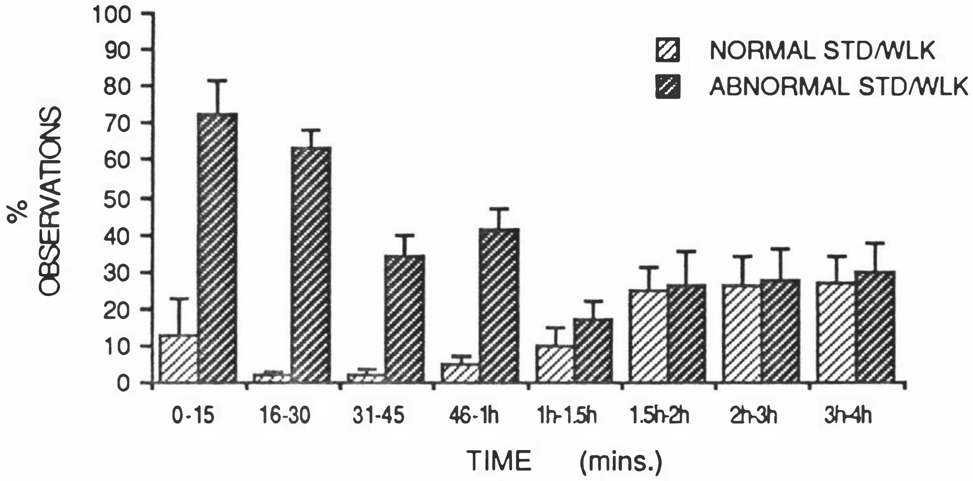


Fig 3-61: Incidence (mean ± s.e.m.) of Normal and Abnormal Standing/Walking Behaviour of Lambs Castrated with the Ring and Tailed with the Iron

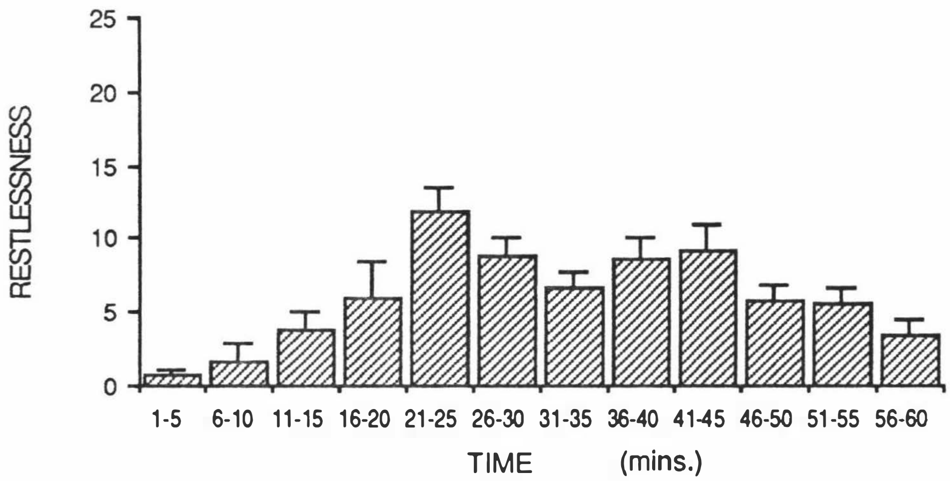


Fig 3-62: Incidence (mean ± s.e.m.) of Restlessness of Lambs Castrated with the Ring plus Tailed with the Iron

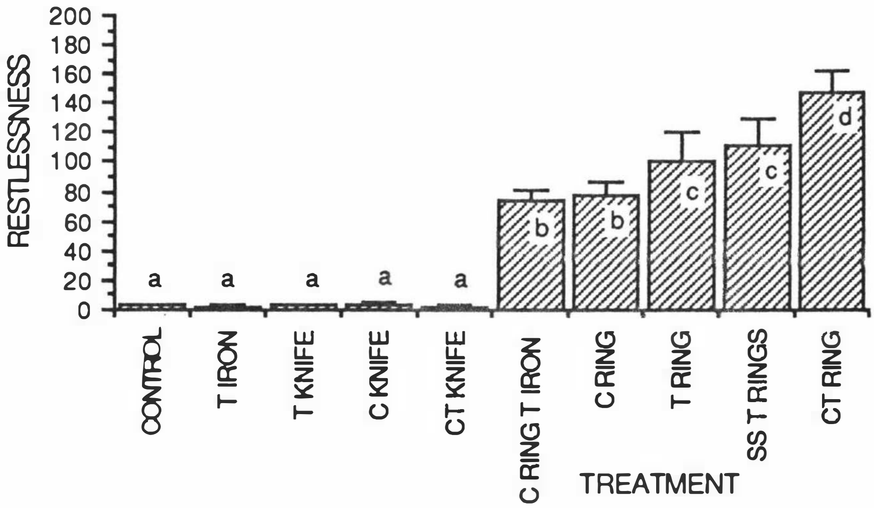


Fig 3-63: Incidence (mean ± s.e.m.) of Restlessness for all Treatments in the first 60 Minutes

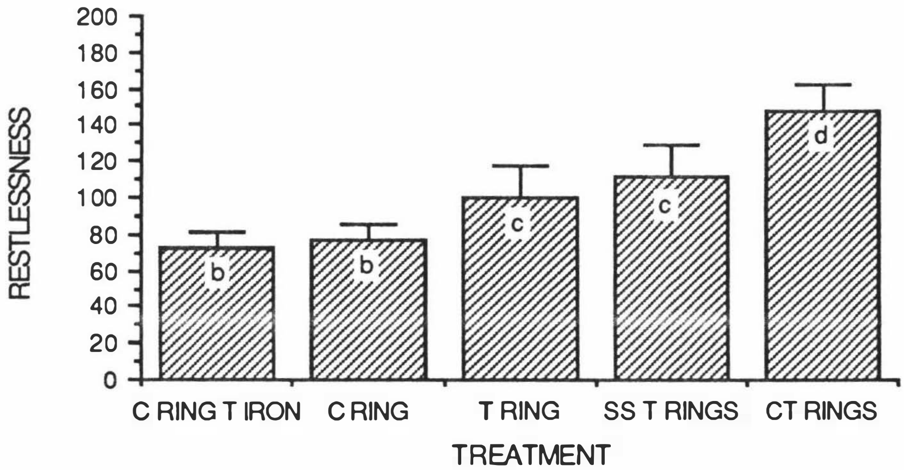


Fig 3-64: Incidence (mean ± s.e.m.) of Restlessness for all Ring Treatments in the first 60 Minutes

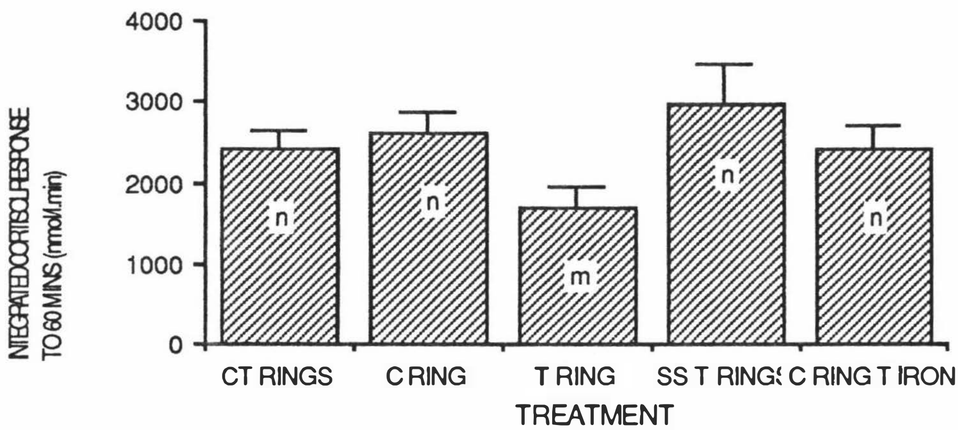


Fig 3-65: Integrated Cortisol Response (mean ± s.e.m.) for Ringed Lambs Up To 60 minutes.

Columns with different subscripts are significant at the $p<0.01$ level.

F. RESTLESSNESS:

The level of activity exhibited by lambs following treatment was quantified by the restlessness score: the number of times they stood up or lay down within the first hour after treatment.

The control lambs exhibited low restlessness and lambs that were castrated and tailed with the knife or the docking iron showed similar levels of restlessness (Fig 3-63). In comparison all lambs that had rubber rings applied to the tail and/or scrotum exhibited high restlessness scores (Fig 3-63). A ranking of the magnitude of restlessness elicited by rubber ring application shows the following hierarchy: greatest responses were caused by castration plus tailing (148 ± 14); intermediate responses by short-scrotum plus tailing (111 ± 18) and tailing only (100 ± 20); and lowest responses by castration only (77 ± 9) and castration with the ring plus tailing with the iron (73 ± 8)(Fig. 3-64).

The integrated cortisol responses up to one hour (which was the period in which restlessness was measured) for lambs that had rubber rings applied that tailing only elicited a significantly lower ($p<0.01$) response than the other treatments which were all similar (Fig 3-65).

3.3.2 INTEGRATED CORTISOL RESPONSES:

In every case of lambs handled and/or castrated and tailed there was a subsequent transient elevation of plasma cortisol concentrations, four examples are provided in Figure 3-66. In all but surgically castrated or tailed lambs the cortisol rise returned to pretreatment levels or below within the four hours of the experiment. Surgically castrated or tailed lambs still had significantly elevated plasma cortisol concentrations at the end of the four hour period so the integrated cortisol response was an underestimate of the total response for these lambs.

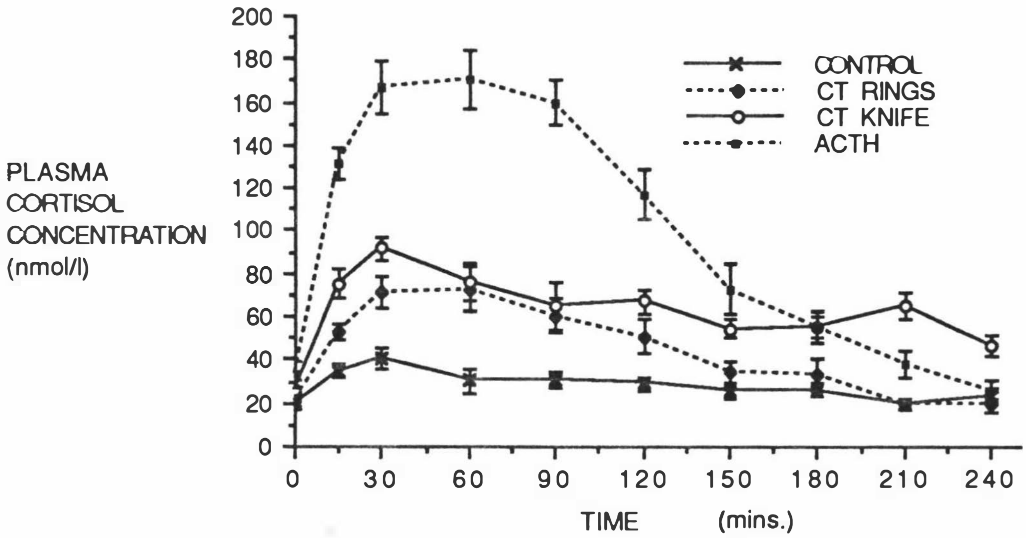


Fig. 3-66: Changes in plasma cortisol concentration (mean ± s.e.m.) in control, CT ring, CT knife and ACTH lambs.

The integrated cortisol response is an expression of the total response to the stimulus combining the magnitude of the response and the duration of the response (Mellor and Murray 1989a,b). It is represented by the area under the cortisol response curve above the level of the pretreatment concentration. The integrated cortisol responses for all groups are summarised in Table 3-2.

Table 3-2: Integrated cortisol responses (area) for lambs castrated and/or tailed by various methods.

TREATMENT GROUP	MEAN AREA (nmol/l.min)		S.E.M. (±)	n
CONTROL	3301	a	530	18
T RING	3472	a	424	10
T IRON	2828	a	510	10
T KNIFE	7940	b	1043	10
C RING	6695	c	671	10
C KNIFE	8977	b	1393	10
CT RINGS	6639	c	928	9
CT KNIFE	9808	b	1290	10
C RING T IRON	6607	c	650	9
SS T RINGS	6938	c	1138	9
ACTH	17352	d	915	10

Table of mean area, standard error of the mean (S.E.M.) and number of lambs. Means with different subscripts are significantly different ($p<0.01$ from the Student t test). Standard Deviation=S.E.M.x √mean. T=tailing, C=castration, CT=castration and tailing, SS=short-scrotum, ACTH=adrenocorticotropic injection.

A. Control Lambs :

The integrated cortisol responses for the two control groups (CT Control,2920 ± 674, n=8; T Control,3606 ± 805, n=10) were compared (Student t test). As no significant difference was found the results from the two groups were pooled. Control lambs (n= 18) had an integrated cortisol response of 3301 nmol/l.min (Fig. 3-67)

B. Tailing:

The integrated cortisol response for lambs tailed with the knife was significantly greater than that for lambs tailed with

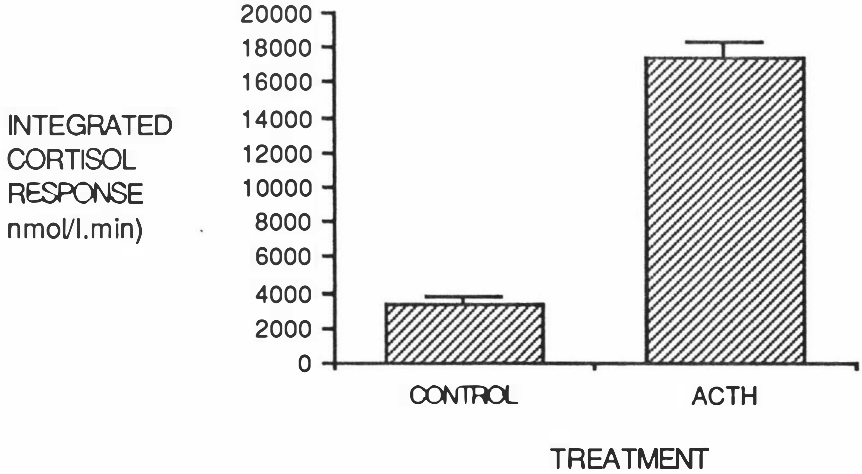


Fig. 3-67: Integrated cortisol responses(mean ± s.e.m.) of Control and ACTH injected lambs.

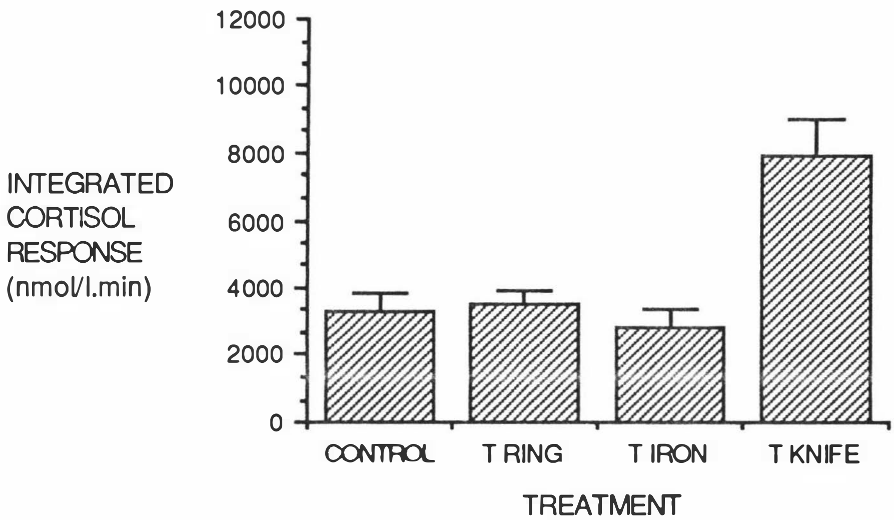


Fig 3-68: Integrated Cortisol Responses (mean ± s.e.m.) of Control and Tailed Lambs.

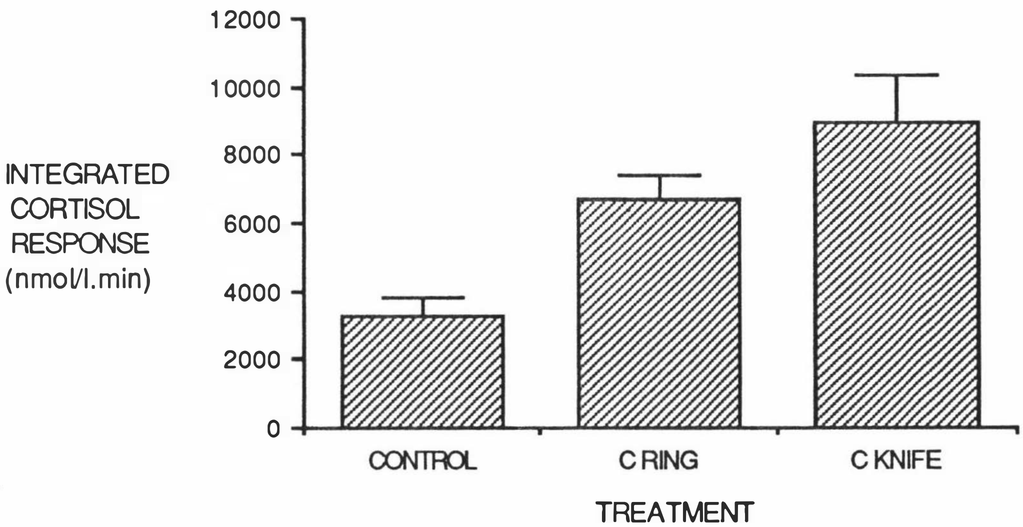


Fig 3-69: Integrated Cortisol Responses (mean ± s.e.m.) of Control and Castrated Lambs.

the rubber ring ($p<0.01$). The integrated cortisol responses for lambs tailed with the docking iron and the ring were not different from control lambs, nor from each other (Fig. 3-68).

C. Castration:

The integrated cortisol responses of lambs castrated with either the knife or the rubber ring were significantly greater ($p<0.01$) than that for control lambs. Lambs castrated with the knife had a higher ($p<0.01$) response than ring castrated lambs (Fig. 3-69).

D. Castration plus Tailing :

The integrated cortisol responses for lambs castrated plus tailed with the rings or with the knife were significantly greater ($p<0.01$) than that for control lambs. Lambs castrated plus tailed with the knife had a response that was significantly higher ($p<0.01$) than that for lambs castrated plus tailed with rings (Fig. 3-70).

E. Comparison of Tailing Only, Castration Only and Castration plus Tailing Using the Same Tool :

RINGS: Lambs castrated plus tailed had a higher integrated cortisol response than tailed only lambs ($p<0.01$), but the response of castrated plus tailed lambs was not different from lambs which were castrated only (Fig. 3-71).

KNIFE: Lambs that were castrated plus tailed, castrated only and tailed only had integrated cortisol responses greater than control lambs ($p<0.01$), but these responses were not significantly different from each other during the 4 hour period of observation (Fig. 3-72).

F. Miscellaneous Treatments :

There were no significant differences between the cortisol responses of lambs in the following groups: castration plus tailing with rings, castration with a ring plus tailing with the docking iron, and short-scrotum plus tailing with rings. Those three groups all showed greater responses ($p<0.01$) than control lambs (Fig. 3-73).

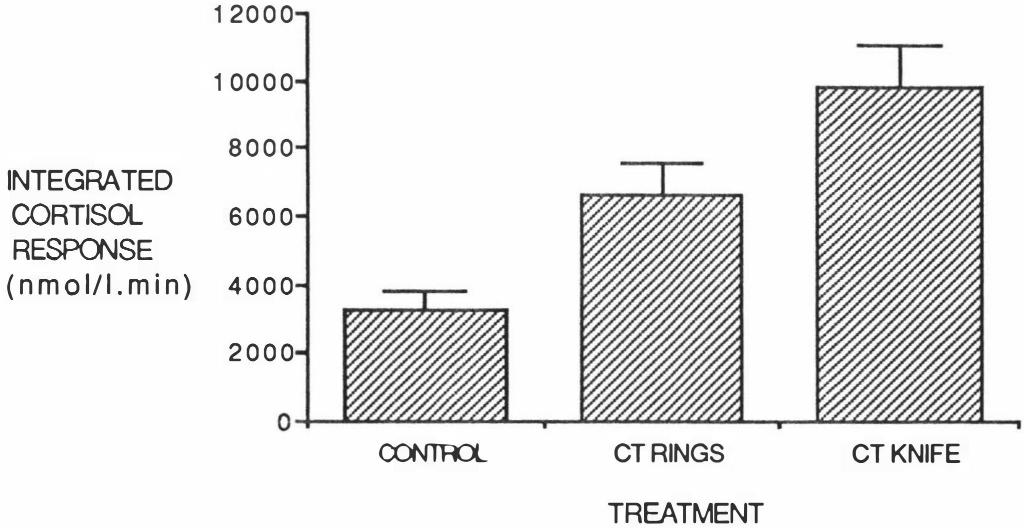


Fig 3-70: Integated Cortisol Responses (mean ± s.e.m.) of Control and Castrated plus Tailed Lambs.

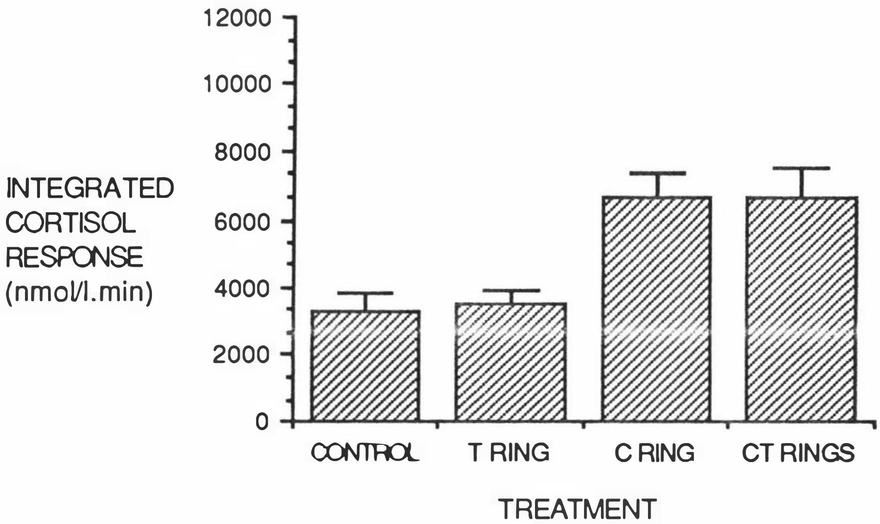


Fig 3-71: Integrated Cortisol Responses (mean ± s.e.m.) of Control Lambs and Lambs Treated with the Ring.

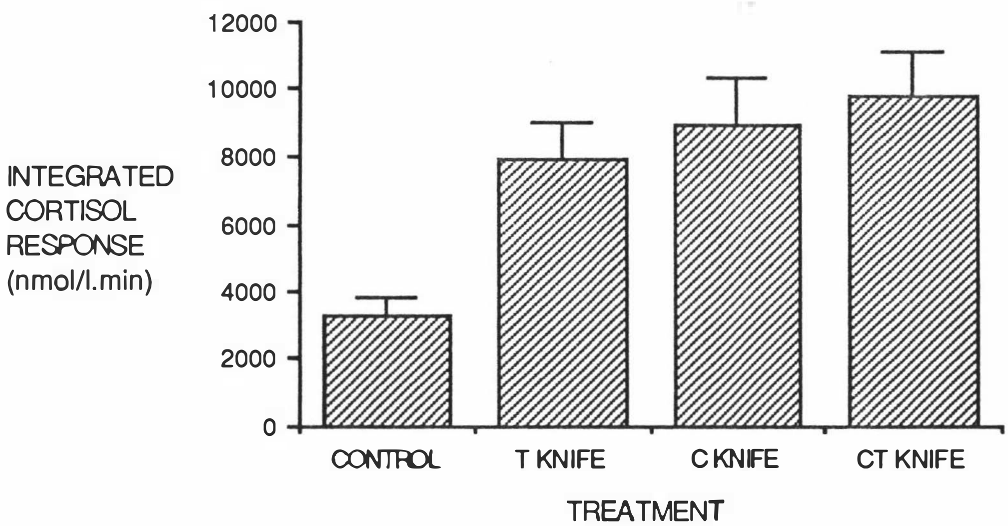


Fig 3-72: Integrated Cortisol Responses (mean ± s.e.m.) of Control Lambs and Lambs Treated with the Knife.

G. Adrenocorticotropin Injection:

Adrenocorticotropin injection caused an integrated cortisol response significantly greater than any other treatment ($p<0.01$)(Fig 3-67).

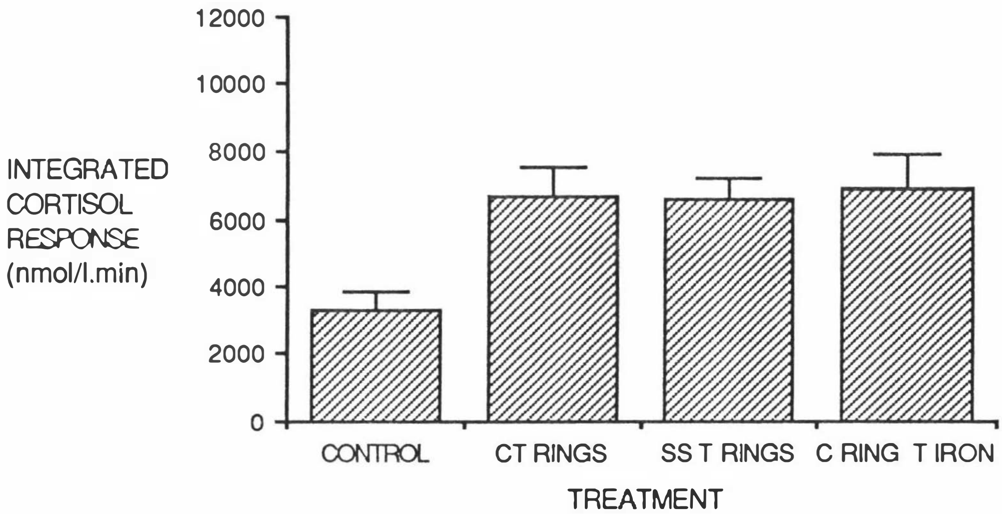


Fig 3-73: Integrated Cortisol Responses (mean ± s.e.m.) to Miscellaneous Treatments.

3.4 DISCUSSION

3.4.1 Behavioural and Cortisol Responses:

A. Responses To The Ring:

Lambs that had rings applied were characteristically active within the first hour after treatment as was shown by the restlessness results. Typical behaviours included stamping of legs, ataxia of the hind quarters, flank nuzzling and many changes of posture to and from standing or recumbency. The high level of activity of ringed lambs aged 4 to 5 weeks is in agreement with other descriptions of behaviour (Barrowman et al, 1953; Shutt et al, 1988) and similar in character to lambs less than 1 week of age (Mellor and Murray, 1989a; Wood et al, 1991).

A descriptive comparison of the behaviour of lambs less than 1 week of age with lambs of 3 to 4 weeks of age provided details of differences in the behaviours of lambs in response to castration and tailing at the two ages (Barrowman et al, 1954). According to that study lambs castrated with the ring within the first week of life apparently showed little reaction to the procedure while lambs similarly treated at 3 to 4 weeks of age showed ataxia, "great uneasiness, and recumbency with rolling". So while Barrowman et al (1954) observed no behavioural reaction to castration and little reaction to tailing in lambs less than 1 week of age, both Mellor and Murray (1989a) and Wood et al (1991) observed behavioural responses described as indicating marked distress and characterised by restlessness lasting up to 30 to 60 minutes after treatment.

The weight of evidence suggests the activity seen in lambs of less than 1 week of age and in lambs of 3 to 6 weeks of age that have rings applied is typical behaviour. The time taken for the restlessness to decrease in lambs of 3 to 6 weeks of age was less than 1 hour in the present study which is in agreement with Shutt et al (1988) but different from Barrowman et al (1953) who noted that "after 20 minutes the lambs would be lying perfectly quietly". Mellor and Murray (1989a) described restlessness as lasting only for 30 minutes in lambs less than 1 week of age with rings applied.

Although the duration of the plasma cortisol elevation from pretreatment levels can be used to compare cortisol responses from different studies, comparison of actual plasma cortisol concentrations from different laboratories cannot be made with confidence because of possible differences in assay dynamics. Lambs of 4 to 5 weeks of age exposed to experimental conditions showed plasma cortisol concentrations that were elevated longer than those of lambs less than 1 week of age (Mellor and Murray, 1989a). Control lambs in the present study had plasma cortisol concentrations that did not return to pretreatment levels until 3.5 hours after treatment while the plasma cortisol concentration of 1 week old lambs had returned by 1 hour. Similarly 4 to 5 week old lambs that were castrated plus tailed with rings had a mean cortisol response that was elevated for 3.5 hours whereas the duration of the response in 1 week old lambs was 2 hours. The plasma cortisol response to tailing only with rings was longer (3 hours) in 4 to 5 week old lambs than in lambs less than 1 week of age (1.5 hours). However the duration of the plasma cortisol response to a standard ACTH injection was relatively similar for 4 to 5 week old and 1 week old lambs (3.5 and 3 hours respectively).

These results suggest that the older lambs have an apparent distress response of greater duration than the younger lambs even though the responses to exogenous ACTH suggest similar response abilities. One could conclude from this that carrying out castration and tailing on lambs at the younger age may be less distressing. However, the relative magnitudes of the responses have not been taken into account, nor have any breed differences. A comparison between lambs of different ages will be further discussed below.

The behavioural and cortisol responses to the ring treatments will be considered in two ways; first an assessment of the behavioural responses as indices of the distress experienced and secondly a comparison of the different treatments in terms of the behavioural and cortisol responses.

The major findings were:

1. Typical behaviours elicited by rubber ring application were (i) an increase in the incidence of total recumbency, (ii) a high

proportion of recumbency that was lateral and (iii) high restlessness.

2. Lambs that were tailed only and lambs that were short-scrotumed plus tailed exhibited increased total recumbency and a high proportion of recumbency that was lateral only in the first 30 minutes. The mean plasma cortisol response for both groups of lambs peaked by 30 minutes.

3. Lambs that were castrated only exhibited increased total recumbency in the first 1.5 hours and a high proportion of recumbency that was lateral from 16 minutes to 1.5 hours. The mean plasma cortisol response peaked 1 hour after treatment.

4. Lambs that were castrated plus tailed exhibited increased total recumbency and a high proportion of recumbency that was lateral in the first 1.5 hours. The mean plasma cortisol response peaked from 30 minutes to 1.5 hours.

5. Restlessness in lambs that were castrated plus tailed was higher than in lambs that were tailed only and lambs that were short-scrotumed plus tailed and all three were higher than lambs that were castrated only and lambs that were castrated with the ring and tailed with the iron.

A(i) Validation of Behavioural Responses as Indices of Distress:

The behaviour typical of lambs with rubber rings applied was an increased incidence of recumbent behaviour in comparison to control lambs. In lambs castrated plus tailed and castrated only there was increased recumbency in the first 1.5 hours and in lambs tailed only and short-scrotumed plus tailed there was increased recumbency in the first 45 minutes. Also lambs with rubber rings applied exhibited higher proportions of recumbent behaviour that was lateral compared to control lambs which exhibited no lateral recumbency. The periods after treatment when lateral recumbency was observed varied according to the treatment: lambs tailed only showed lateral recumbency for up to 45 minutes, short-scrotumed plus tailed lambs for up to 30 minutes, castrated plus tailed for up to 1.5 hours and lambs castrated only for up to 1.5 hours.

Using the plasma cortisol concentration as an index of the distress apparently experienced, a comparison of the time scales of the high incidences of recumbent behaviour, lateral recumbency and restlessness with the plasma cortisol level will provide some indication of the distressed state the behaviours reflect.

1. Lambs that were tailed showed a plasma cortisol peak at 30 minutes to 1 hour and then a subsequent decrease. The same lambs exhibited increased recumbent behaviour and high restlessness until 45 minutes and a high proportion of lateral recumbency until 30 minutes. This parallel in the hormonal and the three behavioural activities that were different from the control lambs suggests these activities reflect the distress experienced.

2. Similarly, lambs that were castrated plus tailed showed a plasma cortisol peak at 30 minutes to 1.5 hours and then a subsequent decrease. These lambs demonstrated increased total recumbent behaviour and a high proportion of lateral recumbency until 1.5 hours and high restlessness until 1 hour. The parallel between the hormonal response and the total recumbency and the lateral recumbency suggest that these behaviours indicate a state of distress. There was in these lambs a period of high activity followed by a period of low activity which were both associated with peak cortisol levels. Thus the absence of restlessness was not associated with decreased plasma cortisol, rather there was a period of low activity that was closely associated with peak plasma cortisol levels. This was apparently similar to the observation by Mellor and Murray (1989a) of a period of distress-induced immobility in lambs of less than 1 week of age.

3. Lambs that were castrated only showed a plasma cortisol peak at 1 hour and then a subsequent decrease. These lambs demonstrated a high incidence of recumbency until 1.5 hours, a high proportion of lateral recumbency from 16 minutes to 1.5 hours and high restlessness until 1 hour. The variation in the association of the incidence of recumbent behaviour and the proportion of lateral recumbency and the hormonal response suggests that they were not as useful for determining the distress experienced after this treatment as they were for the previous treatments. Even so, the plasma cortisol peak was associated with

the three behavioural categories and thus these three behaviours appear to indicate the distress experienced.

4. Lambs that were short-scrotumed plus tailed showed a plasma cortisol peak at 30 minutes to 1 hour and then a subsequent decrease. These lambs exhibited increased recumbency until 45 minutes, a high proportion of lateral recumbency until 30 minutes and high restlessness until 1 hour. The close temporal association of the hormonal index and the behavioural activities suggests a state of distress.

Summary of Assessments:

The incidence of increased total recumbency shows close association with the achievement of the peak plasma cortisol response. For the tailing only, castration plus tailing and short-scrotum plus tailing treatments the abatement of increased total recumbency occurred immediately prior to or coincided with decreasing plasma cortisol. The castration only treatment caused increased total recumbency that extended past the onset of decreasing plasma cortisol. Based on these results it appears that the increased incidence of total recumbency above levels in control lambs indicates distress caused by the application of rubber rings.

Lateral recumbency has been previously shown to be an abnormal posture in awake lambs (Mellor and Murray, 1989a), and the present results support this because lateral recumbency was not commonly observed in control lambs but was observed in lambs that had been ringed. Also the proportion of total recumbency that was lateral was associated with the peak of the cortisol response. The association was not as close as that of increased total recumbency in that the tailing only and short-scrotum plus tailing treatments caused lateral recumbency in the early (until 30 minutes) period of the response while the castration and castration plus tailing treatments caused lateral recumbency for a period that was paralleled by the peak plasma cortisol response. These results show that the stimuli (tailing only and short-scrotum plus tailing) that caused less prolonged cortisol responses also caused immediate and less prolonged lateral recumbency than treatments involving castration. Stimuli that caused a relatively prolonged cortisol response also caused lateral recumbency to be present for a longer period. This suggests that the presence of lateral recumbency indicates distress, although the absence of lateral recumbency does not necessarily indicate an absence of distress.

In all ring treatments the high incidence of abnormal standing/walking behaviour coincided with the plasma cortisol peaks. In each case abnormal standing/walking behaviour was recorded for periods that extended until after the plasma cortisol level had peaked and was decreasing. This suggests that the presence of abnormal standing/walking behaviour indicates the existence of distress caused by the rubber ring, but gives no indication of the intensity of the distress.

A(ii) Comparison of the Behavioural Responses to Different Treatments:

The behavioural responses of lambs tailed only and lambs castrated only with the ring were similar in character but differed in timing and magnitude. Lambs tailed only showed a high incidence of recumbent behaviour in the first 45 minutes which was relatively constant (30 to 36 %), while castrated lambs exhibited high incidences of recumbent behaviour at every time period up to 1.5 hours and the incidence of recumbent behaviour increased over that time from 29% to 88%. Abnormally high proportions of the recumbent behaviour were lateral; tailed lambs exhibited significant lateral recumbency only in the first 30 minutes (39-40%), whereas castrated lambs exhibited lateral recumbency that increased in the period 16 minutes to 1.5 hours with the peak occurring in the period from 31 minutes to 1.5 hours (43-62%). Lambs castrated plus tailed showed recumbent behaviour that increased to peak at 45 minutes to 1 hour which was similar to the response to castration only but was less pronounced. Also the proportion of recumbency that was lateral peaked in the first 15 minutes (64%) and was significant until 1.5 hours. So the incidence of lateral recumbency in castrated plus tailed lambs was apparent for a similar time as in castrated lambs, but where lambs castrated only showed no significant lateral recumbency in the first

15 minutes, castrated plus tailed lambs exhibited peak lateral recumbency immediately from treatment.

It thus appears that two major patterns of behavioural responses were elicited by the stimuli of tight rings on the tail and the scrotum. Castration elicits increased total recumbency and a high proportion of lateral recumbency but the responses take time to develop to their full intensity. On the other hand, although tailing elicits similar behaviours, they are at their most intense immediately following treatment and then disappear relatively rapidly. The response to castration only was of greater magnitude at the peak than the peak response to tailing only, which suggests castration was more distressing than tailing based on the behavioural responses.

The combined situation of castration plus tailing seems to incorporate features of both of the constituent treatments in that the total recumbency was elevated until 1.5 hours and the proportion that was lateral peaked at 0 to 15 minutes and remained significantly elevated until 1.5 hours. The peaks of the lateral recumbency for lambs castrated only and lambs castrated plus tailed were similar in magnitude, but the combined treatment elicited an immediate response that persisted whereas castration only elicited a delayed response. Also the high incidence of total recumbency was present over a longer period for castrated plus tailed lambs than it was for lambs castrated only.

Lambs that were short-scrotumed plus tailed with the rings exhibited an increased incidence of total recumbency in the first 45 minutes, and a significant proportion of the total recumbency was lateral recumbency in the first 30 minutes. This was similar to the behaviour shown by lambs that were tailed only. However there was a period from 1 to 2 hours in which the incidence of recumbency was less than that of control lambs. The fact that lambs that were tailed only and lambs short-scrotumed plus tailed exhibited aberrant behaviour for only the first 30 to 45 minutes is important when considered in comparison to the behaviour of lambs castrated only and lambs castrated and tailed. Lambs short-scrotumed plus tailed had a ring applied to the neck of the scrotum without the spermatic cords included within the ring, while lambs that were castrated plus tailed had the spermatic cords included within the ring. The similarity of behaviour of tailed only and short-scrotumed plus tailed lambs suggests the constriction of the tail is the main stimulus for the behavioural responses observed, and that the constriction of the scrotum without the spermatic cords does not cause a significant additional behavioural reaction. The duration of the behavioural response was longer in lambs that were castrated plus tailed than in lambs that were short-scrotumed plus tailed. This suggests that the constriction applied to the spermatic cords was responsible for the longer term behavioural responses exhibited by castrated plus tailed lambs and lambs castrated only.

Further support for this assertion can be seen in the behaviour of the lambs that were castrated with the ring and tailed with the iron. The incidence of total recumbent behaviour increased to a peak at 1 to 1.5 hours and the proportion of recumbency that was lateral was low in the first 15 minutes and increased to a peak at 30 minutes to 2 hours. This response was similar to the response to castration only although the incidence of total recumbency and the proportion of it that was lateral was greater in lambs that were castrated only, which is consistent with the fact that the response to tailing with the iron elicits reduced total recumbency and has no significant effect on the incidence of lateral recumbency.

B. Responses to the Knife:

Lambs that were cut with the knife exhibited similar restlessness to control lambs (Fig. 3-63), they did not change position much, and usually stood still with splayed hind limbs or a 'hunched' or 'tucked up' appearance. This agrees with Shutt et al (1988) who described similar behaviour and noted difficulty in moving. Lambs that were cut showed a similar or higher incidence of standing/walking behaviour than did control lambs (Figs. 3-14, 3-39, 3-44, 3-49); lambs castrated alone and tailed alone were not different from control lambs in the incidences of standing/walking behaviour but lambs castrated plus tailed showed more standing/walking behaviour in the period between 31 and 45 minutes.

However, in all cut lambs a significant proportion of the standing/walking behaviour was abnormal. The abnormality typically involved such behaviours as splayed hind limbs, a 'hunched' or 'tucked up' appearance, standing with neck extended and head held low or moving with a shuffling, restricted gait. Abnormal standing/walking behaviour persisted throughout the 4 hour observational period in all cut lambs regardless of the site of the treatment.

The persistence of the incidence of abnormal standing/walking behaviour throughout the 4 hour observational period paralleled the plasma cortisol concentration which remained elevated for the whole 4 hours. The incidence of abnormal standing/walking behaviours coincided in their duration and magnitude with the duration and intensity of distress as indicated by the plasma cortisol concentrations.

Descriptions of the acute effects of the use of the knife as a tool for castration and tailing are sparse. In sheep Shutt et al (1988) described splayed hind limbs, reduced exploratory behaviour and slower movement in lambs castrated and tailed with the knife, but stated that "there appeared to be no behavioural response". Bennet and Beardwood (1949) stated that lambs appeared to be "crutchy" for up to 24 hours after treatment. In the studies of Garner and Sanders (1936), Filmer (1938), Ewer (1942), Sinclair, Savage and Wood (1950) and Shutt et al (1987) that looked at 'open' or surgical castration and tailing in lambs, no mention was made of the behavioural response to the surgical procedure. Indeed by describing the response to the rings as signalling appreciable pain but making little or no comment on the response to the 'open' (surgical) method, Bennet and Beardwood (1949) and Sinclair et al (1950) imply that there was little behavioral reaction to the use of the knife. Studies of the surgical method of castration in cattle also either do not describe the immediate reaction (Mullen, 1964) or describe the response as: "behaviour normal" (Fenton, Elliot and Campbell, 1958).

The present investigation using quantifiable and detailed observations has shown consistent abnormal behaviours that are different from behaviours exhibited by control lambs. The scarcity of descriptions of behavioural responses or the absence of comment on any observed behaviours by previous investigators may have been because that was outside the thrust of their study or because of the superficial similarity between surgically castrated and tailed lambs and control lambs. Without detailed observation the often subtle differences in the behaviour may not have been noted. Indeed the determination of a stance or gait to be abnormal may rely on nuances of behaviour not apparent to brief or cursory examination. Thus there seems to have been an assumption of similarity between normal lambs and lambs that are castrated or tailed with the knife. An implication of the assumption of equivalence between the behavioural responses of normal and surgically manipulated animals is the belief that the use of the knife only causes a limited intensity or duration of distress. Reinforcement of this view of surgical methods of castration and tailing can be found both in the literature (Edwards, 1985; Shutt et al, 1988; Henderson, 1990) and anecdotally.

It appears from the present results that the use of the knife causes marked distress as evidenced by plasma cortisol changes and specific behavioural reactions that are associated with the plasma cortisol elevation. Thus the preponderance of standing/walking behaviour and the high proportion of it which was abnormal, appear to indicate marked distress in cut lambs.

C. Responses to the Docking Iron:

Lambs that were tailed only with the docking iron exhibited behaviour that was similar to that exhibited by lambs treated with the knife: similar restlessness to control lambs (Fig. 3-57), a similar or higher incidence of standing/walking behaviour than control lambs (Figs. 3-14, 3-54), reduced incidence of recumbent behaviour and a significant incidence of abnormal standing/walking behaviour (Fig. 3-56). However the behaviours that were different from those in control lambs (reduced incidence of recumbency and the high proportion of standing/walking behaviour that was abnormal) disappeared by 1.5 hours after treatment. Thus the transection of the tail with the docking iron elicited behaviour which was similar initially to that elicited by transection of the tail with the knife, but the duration of the response was not as great, and by 1.5 hours the lambs tailed with the docking iron were not behaving differently from control lambs. In contrast lambs cut with the knife were still exhibiting abnormal standing/walking behaviour at 4 hours.

The plasma cortisol elevation in response to tailing with the iron was of shorter duration than that in lambs tailed with the knife (Fig. 53) a finding which is consistent with the relative durations of the behavioural responses. Both the iron and the knife transect the tissue on which they are used, however the iron as a cause of distress appears to act over a shorter period than the knife. That the behavioural responses to the iron and the knife are similar in quality is consistent with the fact that both involve transection of the tissues. This appears to be evidence to support the use of observation of abnormal standing/walking behaviour as indicative of distress in lambs that are tailed with the iron or the knife.

D. RESTLESSNESS:

Control lambs and lambs treated with the knife and iron exhibited low levels of restlessness (Fig. 3-63). Since the elevation of plasma cortisol concentrations were greater in all cut or seared lambs than in control lambs but the restlessness scores were similar it appears that restlessness did not reflect the distress experienced by lambs treated with the knife or iron.

All lambs that had rubber rings applied showed a high level of restlessness. High restlessness was always observed within the first hour after treatment with rings and this was accompanied by elevated plasma cortisol concentrations.

Tailing with the ring caused significantly greater restlessness than castration with the ring suggesting that a ring applied to the tail was more efficacious in stimulating restlessness than one applied to the neck of the scrotum (Fig. 3-64). This is supported by the fact the most restless lambs were those that at least had a ring applied to the tail (CT rings, SS T rings, T ring). However the magnitude of the restlessness response was not significantly correlated with the integrated cortisol responses up to 60 minutes for any of the ring treatment groups (Fig. 3-65). It is suggested that high restlessness indicated initial distress in lambs that were treated with the rubber ring but did not reflect the intensity of distress experienced, and that the disappearence of restlessness did not necessarily indicate a decrease in the level of distress experienced.

Since castration plus tailing caused more restlessness than short-scrotum plus tailing, the major difference between these treatments being whether the spermatic cords were included within the ring, it appears that constriction of the spermatic cords caused greater restlessness than compression of the testes between the ring and the abdominal wall.

The difference in the restlessness scores produced by: castration plus tailing with the ring (148) and castration with the ring plus tailing with the iron (73), is comparable to the difference between the restlessness scores elicited by: tailing only (100) with the ring and tailing only with the iron (5). Since the castration plus tailing with the ring and castration with the ring plus tailing with the iron differ only in the way the tail is removed it may appear that tailing with the iron inhibits restlessness. However there are several factors which do not support the concept that the iron inhibits restlessness. First tailing with the iron elicited similar low restlessness as control lambs exhibited, thus suggesting that use of the iron for tailing had no effect on restlessness. Secondly castration only with the ring and castration with the ring plus tailing with the iron caused similar levels of restlessness; if use of the iron did inhibit restlessness the restlessness score for lambs castrated with the ring plus tailed with the iron would have been expected to be lower than the restlessness score for lambs castrated only with the ring. Therefore it appears the iron does not inhibit restlessness, rather the application of rubber rings stimulates restlessness.

3.4.2 INTEGRATED CORTISOL RESPONSES

This study represents a comprehensive comparison of castration and tailing methods. The responses of lambs to the castration and tailing techniques have been more completely defined than in any previous study. The plasma cortisol concentration was measured throughout a 4 hour period after treatment, whereas Shutt et al (1987,1988) took only 3 samples: one before treatment, one at 15 minutes and one at 24 hours after treatment.

The major conclusions of this investigation based on the integrated cortisol responses were:

1. Exposure of lambs to the experimental environment, handling and blood sampling apparently caused mild distress.

2. Use of the knife for any procedure (tailing alone, castration alone, or castration plus tailing) was apparently more distressing than any other technique. The response to the knife was not fully defined, since the cortisol concentration did not return to pretreatment levels within the four hour period of observation. Thus the integrated cortisol response for all cut lambs was an underestimate.

3. Use of the knife apparently elicited similar distress during the 4 hour period of observation regardless of whether it was used for tailing alone, castration alone or castration plus tailing.

4. Use of the rubber rings for tailing alone was apparently less distressing than the use of rubber rings for castration alone or castration plus tailing. The latter two treatments caused similar

levels of distress.

5. Use of a rubber ring was apparently the least distressing method of castrating lambs.

6. Tailing with the docking iron apparently elicited a similar level of distress as tailing with a rubber ring. Both the docking iron and the rubber ring as methods of tailing apparently caused similar degrees of distress to those elicited by handling in the control group.

7. Castration plus tailing with rubber rings, short-scrotum plus tailing with rubber rings and castration with a rubber ring plus tailing with the docking iron all apparently caused similar levels of distress.

A. Tailing

Tailing with the rubber ring and with the docking iron are assumed to be painful procedures involving tissue trauma and noxious stimulation. Added to the physical component of the distress there may be a psychological component brought about by emotional arousal associated with the stimulation applied. It was unexpected that the cortisol responses to these two tailing techniques had similar magnitudes to that of handling only. Apparently handling and blood sampling were distressing experiences, and given the unfamiliar environment, lack of previous contact with humans, the handling itself and the jugular venipuncture, that is not surprising. Previous work using changes in plasma cortisol concentrations and behaviour as indices has shown that both a novel environment and restraint by humans constitute emotional stress in lambs not familiar with those treatments (Moberg et al, 1980). Furthermore, within one week of birth, in lambs used to handling and the experimental environment, the integrated cortisol responses of control lambs were less than those of lambs tailed with rings (Mellor and Murray, 1989b). Accordingly the present control results suggest that the emotional distress associated with handling and the new environment was of the same order as the physical, and any emotional, distress caused by tailing with the ring or the iron. However the type of distress differed, being almost solely emotional in control lambs and primarily physical in tailed lambs. Given that the tailed lambs were handled in the same manner as control lambs and also had tailing procedures applied, the distress of the novel environment and handling may have been over-ridden by the painful stimulation of the tailing. This concept will be further explored below.

The difference between tailing with the knife and tailing with the docking iron is in the means of severing the tissues. The knife involves a simple surgical transection of all tissues. The docking iron, however, involves pushing a heated, chiselled surface through the tissues; it is the diathermy which causes the transection more than the cutting action of the docking iron scissor. The comparative distress elicited by these two methods (Fig. 3-68) indicates that the two stimuli are disparate in their noxiousness. Since the cortisol response caused by tailing with the knife was at least 2.8 times greater than that caused by the docking iron, the diathermic stimulus was apparently less noxious than the trauma of surgical amputation. Explanation of this difference may be found by considering the effects of burns on peripheral nerves. It is widely known clinically that third degree burns do not cause as much pain as first or second degree burns (Johnston, 1985). The difference between these burns is the depth of tissue involved in the burn. Third degree burns involve the epidermal, dermal and subcutaneous tissue, and since sensory nerve receptors (including nociceptors) are in the dermis tissue they are destroyed in a third degree burn (Groer and Shekleton, 1983). Thus the symptoms of third degree burns include loss of sensation from the affected area due to the loss of peripheral nerve endings (Johnston, 1985). It may be that the same process occurs as a result of the searing effect of the docking iron, the extreme heat possibly destroying the nociceptors at the site of the burn and therefore reducing the lamb's ability to perceive noxious stimulation.

B. Comparison of Castration plus Tailing, Tailing Only and Castration Only:

Comparison of the cortisol responses to use of the ring for castration plus tailing, castration alone and tailing alone in terms of the distress apparently caused showed that the response to a combination of noxious stimuli was not additive. There was a difference in the level of distress apparently elicited by tailing alone which was lower than that apparently elicited by castration alone (Fig. 3-71). The simultaneous combination of these two techniques did not cause a level of distress that could be considered as the sum of the two component techniques. The composite technique (castration plus tailing) was equivalent in the apparent distress caused to the most noxious of the component procedures (castration). An implication of this finding is that in the presence of two or more noxious stimuli the distress apparently experienced may not be greater than the distress elicited by the single most noxious stimulus because the nociceptor input from that stimulus may be sufficient to dominate lesser inputs from elsewhere.

Further evidence to support the non-additivity of the effects of castration and tailing procedures can be seen in the comparison of distress apparently caused by handling and distress apparently caused by tailing with the docking iron or rubber ring. Handling and the associated factors of the experimental situation apparently caused distress in lambs as evidenced by a transient elevation in plasma cortisol. The combination of the handling factors and noxious stimulation (tailing with the iron or the ring) did not cause a cortisol response of significantly greater magnitude. It may be that the emotional distress of handling was overwhelmed by the painful stimulation of the tailing performed with the ring or the iron.

The relative distress apparently experienced due to tailing alone, castration alone and castration plus tailing was higher when the surgical method was used rather than the rubber rings. However, the responses to all three knife treatments were not completely defined over the 4 hours of the experiment and so a definitive comparison between the treatments with the knife cannot be made. Would the fully defined integrated cortisol responses for the knife treatments show the same relationship as did the incomplete responses up to four hours? If there were some between-treatment differences in the times taken for cortisol concentrations to return to pretreatment levels, some differences between the groups may have become significant. While there were no significant differences between the integrated cortisol responses of the 3 knife treatments during the four hour period of observation there was a trend apparent: tailing showed the lowest, castration an intermediate and castration plus tailing the highest response. It may be that measurement of the plasma cortisol responses to completion would demonstrate a significant difference between the knife treatments. Thus the complete cortisol responses to castration and tailing with the knife may or may not have been linearly additive; the present experiment could not provide definitive evidence for either possibility.

Based on the responses up to 4 hours there were no significant differences between the cortisol responses to tailing alone, castration alone or castration plus tailing with the knife (Fig. 3-72). Since the noxiousness of the stimuli in each cut group was apparently greater than that caused with rings, it appears that the cut lambs experienced higher levels of distress regardless of the site or sites cut with the knife. As with the rubber rings the responses to two different stimuli were not additive linearly within the 4 hours of observation; the cortisol response after the combined treatment was not significantly greater than the response elicited by either of the other two treatments. Thus in terms of apparently causing the most distress during the first 4 hours after treatment, it was not the infliction of several operations simultaneously but the level of distress elicited by the single most noxious technique that appeared to be the primary determinant of the cortisol response during the 4 hour period of observation.

C. Miscellaneous Treatments:

Castration plus tailing with the rings only differed from the short-scrotum plus tailing treatment in the position of the ring relative to the testes. Using a rubber ring for castration causes constriction of the scrotal tissue and the spermatic cords. When the rubber ring is used to short-scrotum a lamb the spermatic cords are not included, the ring constricts the scrotal skin and also compresses the testes between the ring and the abdominal wall.

These two treatments caused similar cortisol responses (Fig. 3-73). Since with both of these treatments constriction of the scrotal skin occurs it may be that it is the constriction of scrotal skin that is the major cause of the response. This seems unlikely; rather the fact that one treatment involved constriction of the spermatic cords and the other compression of the testes suggests that these two stimuli create similar levels of distress even though the distress may be of different quality. However from these results we cannot tell what component of the distress experienced was provided by constriction of scrotal skin or by compression of the testes/constriction of the spermatic cords.

The integrated cortisol responses to castration plus tailing with the rings and castration with the ring plus tailing with the iron were similar (Fig. 3-73). The difference in the two treatments was in the method of tailing: one group had the ring applied and one group had the docking iron applied. Given that the integrated cortisol responses to these two methods of tailing are similar (Fig. 3-68), it is consistent that the integrated cortisol responses to castration plus tailing with the rings and castration with the ring plus tailing with the iron were also similar. This result reinforces the evidence that suggests tailing with the ring and with the iron cause similar levels of distress.

D. CONCLUSIONS:

The present cortisol results suggest that use of the knife was apparently significantly more distressing than use of the rubber rings for castration and tailing. Due to the high level of distress apparently caused by use of the knife for either castration or tailing or both it is not recommended as a tool for castrating or tailing lambs.

Use of rubber rings for castration, either above or below the testes, and rubber rings or the docking iron for tailing are also recommended because of the lower levels of distress apparently caused compared to any procedure using the knife. The decision to short-scrotum lambs can be made (as previously intimated), on production criteria such as carcass configuration, body weight gain and wool growth. When castrating lambs with the rubber rings it may be convenient to tail them with rings as well. But a reason as seemingly banal as having the tails to tally, and thus accurately record lambing efficiency, may be put forward to support the use of the docking iron.

The cortisol responses suggest that it is no more distressing to castrate and tail lambs with rings than it is to only castrate them. In contrast, during the first 4 hours after treatment, castration and tailing with the knife is apparently no more distressing than only castrating or only tailing lambs with the knife.

If tailing only is to be performed then either the docking iron or the rubber rings are recommended. The present work has shown that the choice between these two methods may be made on grounds other than the acute distress caused. Such grounds would include the level of infection following the procedure, because the wounds can be a point of entry for pathogens leading to systemic bacteriaemia, and time taken for the wounds associated with the procedures to heal. Filmer (1938) demonstrated that the wound caused by the docking iron took longer to heal than the wound caused by the knife, and Ewer (1942) demonstrated that the wound caused by the docking iron was not healed in 77% of lambs with 17% having supporating wounds by three weeks after marking. The high occurence of unhealed wounds after tailing with the knife may cause higher incidences of flystrike in these lambs, but lambs that are tailed with the ring do not lose the tail until 2 to 3 weeks after application of the ring which may allow the build up of faeces and thus create conditions conducive to fly egg deposition. However in the practical situation this period of vulnerability to flystrike following tailing can be abolished by the application of antiflystrike solutions (such as Vetrazin used in the present study) to the lambs at the time of tailing. Also to be considered is the relative convenience of use and the cost effectiveness of the methods. Further investigation of the comparative levels of both systemic infection and flystrike following tailing and castration by different methods may better delineate the appropriate procedure to follow. However variations in the prevailing local conditions such as the prevalence and type of fly and climatic conditions may hamper the successful completion of such studies.

E. AGE DIFFERENCES:

Consideration of the ratios of the mean integrated cortisol responses to control handling, tailing with the ring and castration plus tailing with the ring to ACTH injection (Table 3-3) enables comparison between 4 to 5 week old lambs (present study) and lambs of 1 week of age (Mellor and Murray, 1989b). The injection of ACTH at a similar dose (14 μg/kg) to that used by Mellor and Murray (1989b) provides a standard high stimulus for cortisol response, therefore the ratio of the cortisol response for any one treatment to the cortisol response for ACTH injection will indicate the proportion of the standard response that is elicited by the treatment.

The integrated cortisol responses of lambs less than 1 week of age expressed as a proportion of the response to a standard ACTH injection differ according to the breed. Dorset lambs show responses that constitute a smaller proportion of the response to ACTH than Scottish Blackface lambs after control handling, tailing and castration plus tailing (Table 3-3). Also Dorset lambs show marked changes in the cortisol response as a proportion of the ACTH response within 5 days of birth (Mellor and Murray, 1989b). By 5 to 7 days the cortisol responses were consistent for both breeds of sheep, therefore the responses of 7 day old lambs were used for comparison with 4 to 5 week old lambs.

Scottish Blackface lambs of 1 week of age that were handled and blood sampled had a mean integrated cortisol response that was 7 % of the mean integrated cortisol response to the standard ACTH injection. Dorset lambs of the same age had a mean integrated cortisol response that was 0.1 % of the mean integrated cortisol response to the standard ACTH injection. In comparison 4 to 5 week old lambs had a mean integrated cortisol response that was 19 % of the mean integrated cortisol response to the standard ACTH injection.

TABLE 3-3: Integrated Cortisol Responses of lambs to Control Handling, Tailing with Rings and Castration plus Tailing with Rings expressed as a percentage of the mean integrated cortisol response of ACTH Lambs

TREATMENT	4-5 WEEKS OF AGE	1 WEEK OF AGE	
		SBF	DORSET
CONTROL	19 %	7 %	0.1 %
T RING	20 %	23 %	2 %
CT RING	38 %	37 %	16 %

4 to 5 week old lambs were Romney lambs used in the present study; 1 week old lambs were Dorset and Scottish Blackface (SBF) lambs used by Mellor and Murray (1989b).

The fact that the ratio of control to ACTH responses were different at the two ages suggests two possible factors; either the dynamics of ACTH clearance and action has changed causing a decrease in the response to a given dose of ACTH, or the total response of the animal, indicated by plasma cortisol, to handling and blood sampling has increased. The Scottish Blackface lambs (Mellor and Murray, 1989b) were repeatedly exposed to people and handling within enclosed pens from birth until experimental age (1 week). Lambs used in the present study were born and raised in open paddocks and so had no previous experience of enclosures, only rare exposure to people and were handled only once (for ear tagging) before castration and tailing. Therefore, it may be that the lambs that were unfamiliar with the enclosed yarding environment and human presence experienced a more profound reaction to the handling and blood sampling and thus gave a greater cortisol response than the lambs familiar with the environment and human presence. This is supported by Moberg et al (1980) who found that lambs less than 1 week of age had greater increases in corticosteroids than lambs older than 2 weeks when exposed to similar conditions of restraint and novel environment. A significant proportion of the difference in the ratio of control responses to ACTH responses at 1 week and 4 to 5 weeks of age may therefore be due to differences in the control situation rather than in the clearance and action of ACTH.

Dorset and Scottish Blackface lambs of 5 and 7 days of age show similar integrated cortisol responses to a standard exogenous ACTH stimulus (Mellor and Murray 1989b). While the ability of the two breeds to respond to a standard exogenous ACTH dose was similar, Dorset lambs showed lower integrated cortisol responses to noxious stimuli (tailing , castration plus tailing with rings) than Scottish Blackface lambs. Thus it appears the two breeds of sheep used by Mellor and Murray (1989b) experienced different levels of distress when they were tailed and/or castrated.

Romney lambs of 4 to 5 weeks and Scottish Blackface lambs of 1 week of age show similar ratios of treatment responses (tailing only, castration plus tailing with rings) to ACTH responses. Since the castration and other major stimuli did not differ between the two studies it appears that the hypothalamic-pituitary-adrenal system reacts at a similar proportion of its potential in Scottish Blackface lambs at 1 week and in Romney lambs at 4 to 5 weeks of age. However due to the demonstrated breed differences in the responses to noxious stimulation within a week of birth there would need to be an investigation of age differences using only one breed if the situation with respect to age is to be defined within each breed.

CHAPTER FOUR

EFFECT OF HANDLING ON LAMBS CASTRATED PLUS TAILED WITH THE KNIFE

4.1 INTRODUCTION

Analysis of blood samples from the first castration and tailing experiment showed that lambs castrated plus tailed with the knife had plasma cortisol concentrations that were still elevated at four hours after treatment. This elevation was also seen in lambs tailed alone with the knife and lambs castrated alone with the knife. It cannot be confidently stated that the response to surgical castration and/or tailing was still progressing four hours postoperatively, because the act of lifting and handling lambs every 30 minutes with scrotal or caudal wounds may itself be a stressor, and hence prolong elevation of the cortisol level. In order to determine whether the treatment, i.e. surgical castration and tailing, was responsible for the prolonged elevation of plasma cortisol, any effect of handling on the hormonal response must be eliminated. Accordingly a second experiment designed to remove handling as a possible stressor, and therefore determine whether the extended plasma cortisol elevation was real or an artifact of the experimental design, was performed. Also it was decided to extend the period of observation from 4 hours to 8 hours after treatment in order to more completely define the response to the castration and tailing with the knife.

The effect of handling can be eliminated by using different groups of lambs that have initial posttreatment blood samples taken at different times. A blood sample can be obtained by jugular venipuncture within 15 seconds from approaching the lamb. If the handling of the lamb did cause significant cortisol release from the adrenal gland then that cortisol would not be detectable in a blood sample taken within 15 seconds. This is because the following steps have to occur: handling has to be perceived in the CNS and cause CRF to be released from the hypothalamus, CRF then travels in the portal circulation to the anterior pituitary to induce ACTH release. ACTH then travels via the venous return system to the heart and then through the heart, the pulmonary circulation, the heart again and to the adrenal gland to effect cortisol release. Once cortisol has been released in sufficient quantities to increase the blood concentration it then has to be carried back to the heart, through the pulmonary circulation, through the heart again and then through the circulation of the head to reach the jugular vein from where blood samples were taken. Such a process can not occur rapidly enough to enable cortisol to measurable in a blood sample taken within 15 seconds of stimulation. Therefore by leaving lambs untouched after castration plus tailing for a period of time and then taking a blood sample the cortisol concentration will be due to the treatment without any detectable effect from the handling.

4.2 MATERIALS AND METHODS

Twenty five Romney x Border Leicester lambs with an average postpartum age of 39.5 days (range 37 to 42d) and an average weight of 12.5 kg (range 7.4 to 17.8) were investigated during routine castration and tailing on the Massey Sheep and Beef Cattle Research Unit. Lambs and ewes were prepared in the same way as the first trial. All lambs were castrated and tailed with a knife (see CT knife group in first trial). The lambs were separated into four groups which each had different blood sampling schedules. All lambs were blood sampled prior to treatment, the completion of the treatment was taken as time 0 for each lamb. Group A lambs (n=6) had blood samples taken at 15 and 30 minutes and thereafter one every 30 minutes until 8 hours. Group B lambs (n=7) were left undisturbed for 90 minutes after treatment; blood samples were taken at 90 minutes after treatment and every 30 minutes thereafter until 8 hours. Group C lambs (n=6) were left undisturbed for 240 minutes after treatment; blood samples were taken at 240 minutes after treatment and every 30 minutes thereafter up to 8 hours. Group D lambs (n=6) had one post-treatment blood sample taken at 8 hours. No behaviour was recorded.

The handling and blood sampling procedures were as described in the first trial. Group A lambs were treated in the same manner as CT knife lambs from experiment 1, except that blood sampling extended to 8 hours after treatment in order to more completely define the cortisol response. Groups B, C, and D were left undisturbed after the castration and tailing for increasingly greater periods before blood samples were taken. The first blood sample after treatment therefore reflected a cortisol response that was solely caused by the treatment, and had no handling component because the impact of being blood sampled and handled (if there was any) would not have had time to be measureable as a plasma cortisol increase. In this way the effect of repeated handling on lambs with scrotal and caudal wounds can be evaluated.

TABLE 4-1: Schedule of Blood Samples Taken

TIME (mins)	GROUP A	GROUP B	GROUP C	GROUP D
0	*	*	*	*
15	*			
30	*			
60	*			
90	*	*		
120	*	*		
150	*	*		
180	*	*		
210	*	*		
240	*	*	*	
270	*	*	*	
300	*	*	*	
330	*	*	*	
360	*	*	*	
390	*	*	*	
420	*	*	*	
450	*	*	*	
480	*	*	*	*

* = Blood sample taken

TABLE 4-2: Mean plasma cortisol concentrations for lambs that were castrated plus tailed with the knife and blood sampled according to the schedule in Table 4-1.

TIME	GROUP A		GROUP B		GROUP C		GROUP D	
(mins)	mean	sem	mean	sem	mean	sem	mean	sem
0	18	2.5	28	6.3	18	4.4	22	3.3
15	27	5.6						
30	55	15						
60	78	16						
90	84	17	95	6.5				
120	79	14	94	5.4				
150	71	13	91	5.6				
180	74	14	84	6.3				
210	69	12	72	5				
240	54	11	67	5.3	53	5.3		
270	41	8.4	66	6.6	56	8.8		
300	35	6.8	56	7.1	54	6.3		
330	33	5.9	46	6.3	41	7		
360	29	7.3	43	6.1	49	6.3		
390	29	7.9	42	5.2	48	7.4		
420	30	7.4	38	4.6	36	2.7		
450	27	6.3	33	3.8	31	3.3		
480	27	6.1	32	3.5	30	2	35.6	2.9

Mean plasma cortisol concentrations ± sem in nmol/l.

TABLE 4-3: Increments in plasma cortisol concentrations from pretreatment cortisol concentration (mean ± sem) for lambs that were castrated plus tailed with the knife and blood sampled according to the schedule in Table 4-1.

TIME	GROUP A		GROUP B		GROUP C		GROUP D	
(mins)	mean	sem	mean	sem	mean	sem	mean	sem
0	0	0	0	0	0	0	0	0
15	17	7						
30	57	17						
60	62	17						
90	70	19	68	4.9				
120	51	8.2	65	7				
150	55	21	62	5.9				
180	57	15	50	6.8				
210	45	16	38	7.8				
240	27	11	40	8.9	35	5.9		
270	19	10	37	11	38	11		
300	15	4.8	20	9.7	36	5.8		
330	14	9.2	16	12	23	8		
360	7	9.4	16	10	30	3.8		
390	16	10	12	11	30	4.1		
420	8	7.1	9	9.1	18	4		
450	10	7.7	3	8	13	3		
480	7	6.1	7	9	12	4.6	14	4.7

Mean plasma cortisol concentrations ± sem in nmol/l.

4.3 RESULTS

The mean plasma cortisol concentrations for lambs exposed to castration plus tailing with the knife and blood sampled according to the schedule described in the methods section above are provided in Table 4-2.

The mean plasma cortisol concentrations for the Group A lambs (those blood sampled before treatment and throughout the 8 hour period after treatment) remained significantly above pretreatment levels until 7.5 hours after treatment (Table 4-2). The mean plasma cortisol concentrations for lambs in Group B (blood sampled before treatment and from 90 minutes to 8 hours after treatment) remained significantly above pretreatment levels until 7 hours after treatment (Table 4-2). The mean plasma cortisol concentrations for lambs in Group C (blood sampled before treatment and from 4 to 8 hours after treatment) and those in Group D (blood sampled before treatment and at 8 hours after treatment) remained above pretreatment levels for the whole of the 8 hour experimental period (Table 4-2).

The mean increments in plasma cortisol concentration at the first posttreatment blood sample for each of the groups of lambs left undisturbed for a period after treatment, were not significantly different from the corresponding increment for lambs that were blood sampled throughout the 8 hour period (Table 4-3).

DISCUSSION

The results do not completely define the plasma cortisol response to castration plus tailing with the knife. The two groups that were left for the longest period before blood sampling showed mean plasma cortisol concentrations that remained above the pretreatment levels throughout the 8 hour observational period. The two groups that were blood sampled either throughout the observational period or from 90 minutes after treatment both showed mean plasma cortisol concentrations that were not significantly different from pretreatment levels by 7.5 and 7 hours respectively.

The results suggest that the lifting and handling of the lambs with wounds did not significantly affect the distress experienced by those lambs. The initial plasma samples for Groups B, C and D, which could not have had their cortisol concentrations influenced by cortisol released due to handling, were not significantly different from the corresponding mean plasma cortisol concentrations of Group A lambs. Also the plasma cortisol concentrations did not significantly increase in the samples taken after the onset of handling.

Thus it appears that handling itself was not a significant stimulus for distress in lambs that were experiencing apparent marked distress as a result of castration and tailing with the knife. Since handling had no detectable effect on plasma cortisol concentration the plasma cortisol responses to treatment with the knife in the first castration and tailing experiment apparently were due to the treatment with handling having no significant effect.

Also the duration of the plasma cortisol response to castration plus tailing with the knife was more completely defined. It appears the cortisol response was approaching completion at 8 hours but variation in duration of responses occurs.

CHAPTER FIVE

SYNOPSIS

5.1 Integrated Cortisol Responses:

The use of the knife was apparently the most distressing method for castration plus tailing, castration only and tailing only. The acute responses caused by the use of the knife appeared to approach completion at 8 hours after treatment. Importantly, in terms of this investigation, handling lambs with scrotal and caudal wounds due to castration plus tailing with the knife did not seem to significantly affect the response of the lambs.

Although both the docking iron and the knife were used to amputate the tail the responses of lambs to treatment suggested that the docking iron caused significantly less distress than did the knife. The difference in the effect of the docking iron and the knife may have been due to the destruction of nociceptors at the wound by the burning caused by the docking iron

Castration plus tailing had a similar effect on the lambs as castration only when the rubber rings were used, but tailing only with the rubber ring was significantly less distressing than either of these two treatments. This suggests that the most noxious stimulus (castration) overwhelmed the less noxious stimulus (tailing) when the two were combined. Support for this concept can be found in the responses to control handling which were similar to those following tailing only with the rubber ring or the docking iron.

Since the short-scrotum plus tailing with the rubber rings procedure elicited similar responses to castration plus tailing with the rubber rings it appeared that compressing the testes between the ring and the abdominal wall was as noxious as constriction of the spermatic cord.

5.2 Behavioural Responses:

The behaviour of lambs following castration and tailing differed according to the method used. Use of the knife and the docking iron caused no change in activity compared to control lambs, whereas lambs that had rings applied exhibited a large increase in activity. The restlessness of lambs with rings applied was greatest in those lambs with rings applied to the tail. Castration with the ring provided a greater stimulus to restlessness than the short-scrotum procedure.

However, while restlessness may have indicated initial distress in lambs treated with the rings it did not reflect the intensity of distress as indicated by the plasma cortisol response, and the disappearance of restlessness did not necessarily parallel a decrease in plasma cortisol concentrations.

The incidence of standing/walking behaviour was similar or greater in lambs treated with the knife or docking iron compared to control lambs. However, as with other behaviours and plasma cortisol concentrations the responses were shorter following treatment with the docking iron than they were following treatment with the knife. Application of rubber rings appeared to cause a decrease in the incidence of standing/walking behaviour compared to control lambs. Of the standing/walking behaviour significant proportions were abnormal in all lambs treated with any castration or tailing tools. The duration of abnormal standing/walking behaviour differed according to the treatment. Following rubber ring application abnormal standing/walking behaviour was present until after the plasma cortisol concentrations had peaked and were decreasing, and thus while such behaviours may have indicated distress they did not indicate the intensity or subsidence of distress. In lambs that were cut with the knife abnormal standing/walking behaviour persisted throughout the 4 hour observation period, as did the elevation of plasma cortisol concentrations. Similarly in lambs treated with the docking iron abnormal standing/walking behaviour was associated with elevated plasma cortisol concentrations and the disappearance of abnormal standing/walking behaviour foreshadowed the return to pretreatment levels of plasma cortisol.

As would be expected from the incidence of standing/walking behaviour, the incidence of total recumbency was significantly higher in lambs treated with the rings than control lambs and was similar or lower in lambs treated with the knife or docking iron. Following the application of rubber rings the incidence of total recumbency increased and this increase was associated with elevated plasma cortisol concentrations. Of the recumbency a significant proportion was lateral and this too appeared to parallel the plasma cortisol response. Lambs that were treated with the knife or the docking iron did not exhibit increased recumbency and only a little of the recumbent behaviour took the lateral form.

This study has shown that the behaviour exhibited after castration and tailing procedures were performed was dependent upon the method used. For lambs exposed to the rubber ring typical behaviours that appeared to indicate acute distress were high activity for up to one hour and higher than normal recumbency including significant proportions of lateral recumbency. It is thus suggested that these behaviours may be used to recognise distress caused by castration and tailing procedures using the rubber ring.

Behaviours typically demonstrated by lambs after treatment with the knife were chiefly an increase in the proportion of standing/walking behaviours that were abnormal. Such behaviours seem to indicate distress and that distress appeared to be greater than that due to rubber ring application. However the behaviour of cut lambs appeared to be more subtle and thus careful observation was required to recognise the abnormality of the postures. That is not to say that any more attention was focussed on cut lambs than ringed lambs, rather that the activity and recumbent postures adopted by lambs treated with the rings were very different from those of control lambs and were immediately obvious to even cursory observation. On the other hand cut lambs adopted behaviours that were often not markedly different from those exhibited by control lambs. It is thus suggested that the abnormal postures associated with treatment with the knife or docking iron were indicative of distress of a higher level than that of ringed lambs and that these behaviours require careful observation to be accurately detected.

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APPENDIX A :

Example of a behaviour recording sheet.

Tag No.:
Treatment:
No. In Sample:
S / T

DATE:
Weight:
Sex : M / F
Age:

Ewe Tag No.:
Birth weight:

Time of Sample	Ventral N	F	E	Sleeping?	Lateral U	D	R	F. Breathing	Std/Wlk N	Ab S	Ab W	Tremor	Restlessness	Comments
☐ 0														
1														
2														
3														
4														
5														
6														
7														
8														
9														
10														
11														
12														
13														
14														
☐ 15														
16														
17														
18														

Tag No.: ______________________ DATE: ______________________

Time of Sample	Ventral N	Ventral F	Ventral E	Sleeping?	Lateral U	Lateral D	Lateral R	F. Breathing	Std/Wlk N	Std/Wlk Ab S	Std/Wlk Ab W	Tremor	Restlessness	Comments
19														
20														
21														
22														
23														
24														
25														
26														
27														
28														
29														
☐ 30														
31														
32														
33														
34														
35														
36														
37														
38														
39														

Tag No.: ____________ DATE: ____________

Time of Sample	Ventral N	F	E	Sleeping?	Lateral U	D	R	F. Breathing	Std/Wlk N	Ab S	Ab W	Tremor	Restlessness	Comments
40														
41														
42														
43														
44														
45														
46														
47														
48														
49														
50														
51														
52														
53														
54														
55														
56														
57														
58														
59														
☐ 1:00														

Tag No.: ____________ DATE: ____________

Time of Sample	Ventral			Sleeping?	Lateral			F. Breathing	Std/Wlk			Tremor	Restlessness	Comments
	N	F	E		U	D	R		N	Ab S	Ab W			
1:05														
1:10														
1:15														
1:20														
1:25														
☐ 1:30														
1:35														
1:40														
1:45														
1:50														
1:55														
☐ 2:00														
2:05														
2:10														
2:15														
2:20														
2:25														
☐ 2:30														
2:35														
2:40														

Tag No.: ____________ DATE: ____________

Time of Sample	Ventral N	Ventral F	Ventral E	Sleeping?	Lateral U	Lateral D	Lateral R	F. Breathing	Std/Wlk N	Std/Wlk Ab S	Std/Wlk Ab W	Tremor	Restlessness	Comments
2:45														
2:50														
2:55														
☐ 3:00														
3:05														
3:10														
3:15														
3:20														
3:25														
☐ 3:30														
3:35														
3:40														
3:45														
3:50														
3:55														
☐ 4:00														

APPENDIX B :

Plasma cortisol concentrations from Trial 1.

GROUP TREATMENT : CONTROL

TAG Nº	TIME									
	0	15	30	60	90	120	150	180	210	240
368	13	38	26	44	53	34	41	41	29	26
402	11	48	42	21	40	28	28	28	14	22
426	12	30	22	39	32	36	12	26	8.6	15
467	22	25	35	13	19	22	16	13	9.6	10
475	15	27	44	35	24	37	24	26	19	10
480	30	40	39	37	21	48	25	36	27	19
529	14	31	20	15	15	14	24	24	10	22
557	18	24	22	24	18	42	31	36	18	36
372	17	16	32	34	52	34	52	17	24	16
386	20	30	34	14	48	37	47	55	29	42
393	19	28	24	29	60	28	22	13	12	9.4
399	35	80	78	10	10	9.2	9.2	25	20	18
416	20	36	31	27	28	11	16	16	14	19
419	16	14	52	16	12	13	10	9.6	8.4	19
425	28	34	40	34	32	36	42	29	28	29
445	39	42	48	16	23	25	38	36	46	64
481	11	44	96	110	26	24	14	31	17	41
489	15	40	49	34	37	46	29	28	30	19
MEAN CONC.	20	35	41	31	31	29	27	27	20	24
S.E.M. (±)	2.0	3.4	4.6	5.4	3.7	2.8	3.4	3.1	2.5	3.4

GROUP TREATMENT : CT RINGS

TAG Nº	TIME									
	0	15	30	60	90	120	150	180	210	240
380	12	39	39	26	21	14	26	22	10	33
389	20	68	80	76	52	45	41	32	28	29
418	24	54	64	51	47	32	18	25	12	20
422	31	49	109	109	54	96	51	83	24	24
427	19	49	79	85	103	76	25	14	18	10
478	30	67	88	95	78	63	70	52	20	9.2
484	14	57	53	34	83	42	24	15	33	15
533	24	38	51	78	46	34	28	27	21	20
540	12	51	78	102	58	56	24	22	12	14
MEAN CONC.	21	53	71	73	60	51	34	33	20	19
S.E.M. (±)	2.4	3.6	7.2	9.8	8.1	8.3	5.7	7.4	2.6	2.7

GROUP TREATMENT : CT KNIFE

TAG Nº	TIME									
	0	15	30	60	90	120	150	180	210	240
392	24	101	105	63	24	75	70	52	78	54
442	20	40	92	54	63	56	49	60	40	23
456	16	53	70	69	52	50	53	49	52	56
470	16	56	63	63	48	42	43	30	28	41
507	20	104	128	127	146	90	62	80	61	38
511	13	64	86	102	60	46	42	46	70	25
530	55	103	99	58	38	82	47	40	84	44
535	41	82	103	99	97	77	37	80	85	58
542	37	66	80	42	66	74	71	64	71	57
543	16	84	90	85	59	82	70	55	84	68
MEAN CONC.	26	75	92	76	65	67	54	56	65	46
S.E.M. (±)	4.4	7.2	5.9	8.3	11	5.5	4.1	5.1	6.3	4.8

GROUP TREATMENT : C RING T IRON

TAG Nº	TIME									
	0	15	30	60	90	120	150	180	210	240
397	16	26	61	63	20	39	16	9.2	8.4	12
413	24	75	109	108	59	25	22	14	10	12
431	19	49	65	67	52	43	24	25	37	18
454	15	46	72	61	64	48	27	9.4	12	15
473	22	62	78	67	86	91	73	39	17	26
477	33	56	61	50	97	51	46	37	35	38
486	31	55	65	67	52	60	57	29	64	48
544	24	63	81	88	99	80	66	44	42	26
547	17	52	84	91	61	49	27	14	15	11
MEAN CONC.	22	54	75	73	66	54	40	25	27	23
S.E.M. (±)	2.1	4.5	5.1	6.1	8.4	6.8	7.1	4.6	6.2	4.3

GROUP TREATMENT : SS T RINGS

TAG Nº	TIME									
	0	15	30	60	90	120	150	180	210	240
365	26	83	166	113	77	70	44	16	11	8.4
398	6.4	86	91	68	81	26	52	30	9.2	25
455	60	71	70	69	69	54	47	41	26	24
458	20	55	106	43	25	46	78	31	34	46
485	8	69	73	91	86	31	29	27	28	14
487	16	44	62	71	44	37	42	46	36	39
515	22	66	97	83	56	48	50	26	17	32
522	35	52	48	47	49	33	37	36	27	24
526	26	58	58	60	56	26	19	8.4	9.4	14
MEAN CONC.	20	64	88	72	59	40	44	28	21	25
S.E.M. (±)	3.2	4.9	12	7.7	6.9	4.9	5.8	3.9	3.7	4.4

GROUP TREATMENT : C RING

TAG Nº	TIME									
	0	15	30	60	90	120	150	180	210	240
421	12	16	36	64	56	12	36	15	9.2	19
452	13	44	50	63	71	35	30	11	12	9.8
459	25	41	76	97	67	59	43	44	24	15
474	36	67	67	92	94	84	53	20	16	24
503	26	52	64	92	79	50	28	24	13	12
508	8	61	95	77	26	31	18	16	10	12
518	26	58	53	109	59	46	34	13	15	12
537	30	74	67	118	75	56	55	26	14	27
545	14	59	74	92	65	34	24	20	11	9.4
549	17	35	118	112	111	95	59	35	7.4	13
MEAN CONC.	21	51	70	92	70	50	38	22	12	15
S.E.M. (±)	2.9	5.4	7.4	6.0	7.2	7.9	4.4	3.3	1.7	2.1

GROUP TREATMENT : C KNIFE

TAG Nº	TIME									
	0	15	30	60	90	120	150	180	210	240
370	32	70	84	83	71	75	86	78	54	63
373	18	92	87	46	91	108	78	48	86	51
401	23	92	35	25	38	14	33	31	29	39
435	9.2	42	33	60	49	33	39	27	25	9
453	19	27	37	54	49	47	32	13	25	13
465	13	50	52	41	44	57	24	31	31	29
468	10	62	74	68	71	70	49	54	45	54
479	10	108	124	88	109	74	54	72	51	66
495	30	77	111	98	78	70	65	70	62	57
528	24	74	89	36	68	79	46	46	71	46
MEAN CONC.	19	69	73	60	67	63	51	47	48	43
S.E.M. (±)	2.6	7.9	10	7.6	7.1	8.3	6.4	6.9	6.6	6.3

GROUP TREATMENT : T RING

TAG Nº	TIME									
	0	15	30	60	90	120	150	180	210	240
377	8.4	51	105	27	43	24	22	15	8.8	8.8
415	13	30	24	52	19	25	11	8.8	20	11
428	11	48	51	27	43	14	23	14	7.8	11
466	15	48	58	34	38	30	19	13	21	16
476	30	62	65	61	55	53	45	34	26	29
482	32	58	61	60	37	47	24	56	41	19
491	26	21	23	37	25	8.6	22	6.2	13	35
496	36	51	76	63	41	61	42	31	18	32
500	17	40	64	70	26	33	22	33	20	24
509	19	39	63	80	42	20	38	14	9.2	11
MEAN CONC.	21	45	59	51	37	32	27	22	18	20
S.E.M. (±)	3.1	4.0	7.5	5.9	3.4	5.4	3.5	4.9	3.3	3.1

GROUP TREATMENT : T KNIFE

TAG Nº	TIME									
	0	15	30	60	90	120	150	180	210	240
387	21	87	81	37	70	72	50	57	68	51
420	12	50	50	24	32	34	14	38	22	39
429	14	28	71	99	59	59	42	47	40	36
440	28	47	55	50	54	50	48	50	56	48
446	19	64	110	45	87	77	65	48	33	60
462	15	78	98	85	74	66	64	38	44	41
469	26	37	48	25	25	23	22	28	17	20
492	15	107	80	61	25	91	47	43	55	69
493	14	57	59	32	58	48	28	44	16	52
501	16	36	70	39	20	39	22	28	26	26
MEAN CONC.	18	59	72	50	50	56	40	42	38	44
S.E.M. (±)	1.7	7.9	6.6	7.9	7.4	6.6	5.6	3.0	5.7	4.7

GROUP TREATMENT : T IRON

TAG Nº	0	15	30	60	90	120	150	180	210	240
	TIME									
391	35	51	75	59	45	47	33	27	20	22
396	58	115	95	48	68	63	22	43	28	46
412	23	48	77	45	44	43	32	36	28	18
417	16	36	39	28	24	22	15	17	12	21
488	9.6	35	36	25	28	12	14	5.2	6	19
497	44	57	37	32	58	22	32	35	10	34
498	40	58	72	77	85	70	38	59	70	45
513	32	31	123	39	49	85	14	10	12	9
548	30	44	64	39	23	40	18	12	11	21
550	41	67	81	82	40	62	37	52	40	21
MEAN CONC.	33	54	70	47	46	47	26	30	23	25
S.E.M. (±)	4.4	7.7	8.8	6.2	6.3	7.5	3.1	5.8	6.3	4.0

GROUP TREATMENT : ACTH INJECTION

TAG Nº	0	15	30	60	90	120	150	180	210	240
	TIME									
331	54	135	154	195	213	139	82	34	38	18
333	50	162	222	269	156	128	43	48	30	27
335	32	154	185	150	142	129	83	52	40	37
337	25	120	126	139	172	190	123	91	52	23
338	22	105	222	177	115	62	29	24	22	16
339	66	160	163	185	184	130	83	66	44	32
341	34	142	161	180	179	132	118	91	58	59
342	13	112	194	165	195	85	36	34	17	9.6
344	13	132	129	137	125	71	23	48	9.2	12
346	27	92	113	111	121	106	108	66	70	33
MEAN CONC.	34	131	167	171	160	117	73	55	38	26
S.E.M. (±)	5.6	7.6	12	14	11	12	12	7.3	6.0	4.9

APPENDIX C :

Plasma cortisol concentrations from Trial 2.

			GROUP A						
TIME		LAMB TAG NUMBER						MEAN	SEM
	601	613	611	616	646	651		CONC.	±
0	14.4	17.2	15.9	9.6	24.4	27.2		18	2.5
15	57.8	47.2	37	11.2	26	32.8		27	5.6
30	70	50	73	58.7	36.9	164		55	15
60	62	73	154	37.3	43.8	110		78	16
90	81.2	86.6	98.8	44.2	38.1	178		84	17
120	89.8	60	66.9	80.9	72.2	47.5		79	14
150	79.4	52	154	89.4	36.1	25.3		71	13
180	79.4	55.2	112	96.3	82.9	22.3		74	14
210	90	78	111	45.1	25.9	29.3		69	12
240	81.2	38.4	57.2	39.6	19.5	32.5		54	11
270	73	35	26.2	19.1	9.9	57.7		41	8.4
300	35.6	50.4	17.2	29.5	30.9	34.2		35	6.8
330	69.6	40.6	18.2	19	30.1	17.3		33	5.9
360	64.4	21	19.5	20.7	15.9	10.2		29	7.3
390	78.4	33.6	16.3	19.1	34.9	21.7		29	7.9
420	57.6	25.2	15.2	11.4	21.4	27.5		30	7.4
450	52	13.2	9	29.7	16.9	48.8		27	6.3
480	48.2	30.2	7.8	9.6	23.3	30		27	6.1
			GROUP B						
TIME		LAMB TAG NUMBER						MEAN	SEM
	647	651	652	612	624	630	637	CONC.	±
0	39.1	32.9	28	15.3	51.2	8.7	17.7	28	6.3
15	-	-	-	-	-	-	-		
30	-	-	-	-	-	-	-		
60	-	-	-	-	-	-	-		
90	89.8	84.4	90.6	84.2	133	87.6	95.2	95	6.5
120	69.6	89	94.2	94.4	110	93.3	96.5	94	5.4
150	78.2	72.7	95.7	85.9	127	84.1	83.1	91	5.6
180	79.4	58.3	84.5	58.7	93.3	89.8	80.2	84	6.3
210	59.8	56.7	81.4	55.8	60.3	72.6	72.8	72	5
240	60.1	58.6	98.3	39.7	66.4	74.4	75.2	67	5.3
270	81.4	44.2	60.1	67.6	42.6	93.1	61.7	66	6.6
300	27.6	46.3	64.3	41	38.5	42.5	75.2	56	7.1
330	31.4	38.8	61.8	45.3	15.3	69.8	42.3	46	6.3
360	31.9	26.5	40.8	52.5	40	72.4	39.8	43	6.1
390	33.4	30.4	38.6	55.4	19.8	51.2	51.2	42	5.2
420	41.5	30.2	48.1	30.8	15.4	47.2	42.8	38	4.6
450	22.6	25.9	26.3	29.1	25.2	38.5	43.4	33	3.8
480	36.3	27.9	42.2	31.5	14.8	41.2	46.9	32	3.5

TIME								MEAN	SEM
	GROUP C								
	LAMB TAG NUMBER							MEAN	SEM
	635	639	648	627	656	638		CONC.	±
0	25.3	8.4	9.4	11.4	34.7	24.4		18	4.4
15	-	-	-	-	-	-			
30	-	-	-	-	-	-			
60	-	-	-	-	-	-			
90	-	-	-	-	-	-			
120	-	-	-	-	-	-			
150	-	-	-	-	-	-			
180	-	-	-	-	-	-			
210	-	-	-	-	-	-			
240	52.6	42.4	40.8	58.2	47.6	78.8		53	5.3
270	50.6	67.6	32.9	57.6	33.9	94.8		56	8.8
300	65.2	33.2	37.5	64.2	51.6	75.2		54	6.3
330	46.8	21.6	38.4	39.4	26.2	74		41	7.0
360	40.2	43.2	30.2	39.8	75	62.4		49	6.3
390	47.8	26.6	33.6	42.4	82.4	55.6		48	7.4
420	38.4	32.6	31.5	28	35.2	48.6		36	2.7
450	42	17.4	26.4	29.6	35.8	36.2		31	3.3
480	31.3	27.2	28	27.2	26.6	40.4		30	2.0
	GROUP D								
TIME	LAMB TAG NUMBER							MEAN	SEM
	608	609	615	619	620	655		CONC.	±
0	23.4	32	19	6.6	28.4	19.8		22	3.3
15	-	-	-	-	-	-			
30	-	-	-	-	-	-			
60	-	-	-	-	-	-			
90	-	-	-	-	-	-			
120	-	-	-	-	-	-			
150	-	-	-	-	-	-			
180	-	-	-	-	-	-			
210	-	-	-	-	-	-			
240	-	-	-	-	-	-			
270	-	-	-	-	-	-			
300	-	-	-	-	-	-			
330	-	-	-	-	-	-			
360	-	-	-	-	-	-			
390	-	-	-	-	-	-			
420	-	-	-	-	-	-			
450	-	-	-	-	-	-			
480	49	34	25.7	37.2	34.1	33.7		36	2.9

APPENDIX D

Plasma cortisol concentrations of excluded lambs.

The following lambs were excluded from the results analysis of the experiment for reasons that have been stated. In each case the pretreatment plasma cortisol concentrations were above the mean concentration of the 15 minute plasma cortisol in the treatment groups to which they belong.

The mean plasma cortisol concentration at 15 minutes for the control lambs was 35 nmol/l.

The mean plasma cortisol concentration at 15 minutes for the lambs castrated with the ring plus tailed with the docking iron was 54 nmol/l.

The mean plasma cortisol concentration at 15 minutes for lambs short-scrotumed plus tailed with the rings was 64 nmol/l.

Excluded from the control group (plasma cortisol concentrations in nmol/l):

TAG Nº	TIME									
	0	15	30	60	90	120	150	180	210	240
378	52.4	134	94.8	153	147	142	53.6	53.2	17.2	8.4
447	62	48.4	8.8	34.4	24.4	21.2	22	12.8	48.4	15.2

Excluded from the castrated with the ring plus tailed with the iron treatment group (plasma cortisol concentrations in nmol/l):

490	62	65	53	75	38	27	28	35	33	31

Excluded from the short-scrotum plus tailed with the rings treatment group (plasma cortisol concentrations in nmol/l):

451	75	87	86	96	68	55	84	53	33	32