ROLE OF GONADAL STEROIDS IN THE MATING BEHAVIOUR OF THE RAM *Ovis aries* L.

by

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Dedication

To my parents,

Giovanna-Maria e Giovanni

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DECLARATION

This thesis contains no material which has been accepted for the award of any degree or diploma in any University and, to the best of the candidate's knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text.

Michael J. D'Occhio

Publications arising from this thesis:

Abstracts:

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D'Occhio, M.J. and Brooks, D.E. (1982). Threshold of plasma testosterone required for normal mating activity in male sheep. Hormones and Behavior (in press).

Steroid Nomenclature

Trivial Name	Systematic Name
androgens	
testosterone	4-androstene-17β-ol-3-one
5α-dihydrotestosterone	5α-androstane-17β-ol-3-one
androstenedione	4-androstene-3,17-dione
androsterone	5α-androstane-3α-ol-17-one
testosterone propionate	4-androstene-17β-ol-3-one propionate
oestrogens	
naturally occurring	
oestrone	1,3,5(10)-oestratriene-3-ol-17-one
oestradiol-17β	1,3,5(10)-oestratriene-3,17β-diol
oestradiol-17α	1,3,5(10)-oestratriene-3,17α-diol
oestriol	1,3,5(10)-oestratriene-3,16α,17β-triol
synthetic	
diethylstilboestrol	3,4,-bis(ρ-hydroxyphenyl)-3-hexene
hexoestrol	meso-3,4-bis(ρ-hydroxyphenyl)-n-hexane
progestagens	
progesterone	4-pregnene-3,20-dione
corticoids	
cortisol	4-pregnene-11β,17α,21-triol-3,20-dione

SUMMARY

The role of gonadal steroids in the mating behaviour of rams was investigated in both entire rams and castrated rams (wethers) treated with different steroids. The purpose of the initial studies in intact animals was to determine if a relationship existed between the mating drive (libido) of individual rams and their blood testosterone levels. Twenty-four hour blood testosterone profiles were used to characterize testosterone in individual rams and libido was assessed in 20 minute mating trials using hormone-treated oophorectomized ewes. Experiments in both Merino and four British breed (Border Leicester, Polled Dorset, Romney, Suffolk) rams failed to provide any evidence for a relationship between libido and blood testosterone for individual rams. Seasonal changes in the blood testosterone profile were however accompanied by parallel changes in mating activity in three of the British breeds studied (Border Leicester, Romney, Suffolk). There was also some evidence of breed differences in the seasonality of mating.

Failure to demonstrate any relationship between testosterone and mating behaviour in entire rams led to the use of the wether as an experimental model to study the finer control of behaviour by testosterone. Adult wethers which had been castrated before puberty, and which were sexually inexperienced at the time of treatment, were used in these studies. Testosterone propionate was used in place of testosterone since the former compound has a slower clearance after injection. It was found that the purely behavioural components of ram mating (sniffs, nudges, mounts) (arousal mechanisms) had a lower testosterone requirement than did the complete mating response (intromission, ejaculation)

(consummatory mechanisms). This observation suggested that brain centres associated with behaviour and the accessory sex glands have different sensitivities to testosterone. The threshold dose of testosterone required to stimulate intromission and ejaculation produced plasma testosterone levels that were lower than those normally observed in mature rams. It was concluded, therefore, that testosterone levels in rams are above the threshold required for mating and that this may explain, in part, why there is no apparent relationship between libido and circulating testosterone. Some individual differences in the response of wethers to supra-threshold doses of testosterone provided further support for the above conclusion.

Wethers could also be stimulated to show mating behaviour (but not intromission or ejaculation) by treatment with both natural (oestradiol-17β, oestrone) and synthetic (diethylstilboestrol) oestrogens. This finding was consistent with the theory that testosterone stimulates mating behaviour in males after being converted (aromatized) in the brain to oestradiol-17β. Failure of two non-aromatizable androgens, 5α-dihydrotestosterone and androsterone, to elicit mating behaviour in wethers was also consistent with the aromatization hypothesis. Although 5α-dihydrotestosterone was ineffective alone, a greater proportion of wethers treated with a combination of 5α-dihydrotestosterone and oestradiol-17β showed mating responses, compared with wethers treated only with the same dose of oestradiol-17β. Wethers receiving 5α-dihydrotestosterone and oestradiol-17β also showed intromission and ejaculation, neither of which was observed in oestrogen-treated animals. The apparent synergism between 5α-dihydrotestosterone and oestradiol-17β may have resulted from stimulation of the sex structures by 5α-dihydro-

testosterone and subsequent nervous inputs from these tissues. Alternatively, 5α-dihydrotestosterone and oestradiol-17β may have acted together within the brain. Since penile reflexes do not appear necessary for mating behaviour in males it was concluded that mating behaviour in rams may involve interactions within the brain between androgenic and oestrogenic metabolites of testosterone.

CHAPTER 1

INTRODUCTION

1.1 HISTORICAL BACKGROUND

The use of testicular tissues for the treatment of impotence in males has been practised since 1400 BC in India. However, it was not until 300 BC that Aristotle demonstrated the importance of the testes for proper development and maintenance of the secondary sex characteristics, and sexual behaviour patterns in males. By 1253 AD the Chinese were also prescribing testicular tissues of animal origin for the treatment of sexual disorders in man (for reviews see Dorfman and Shipley, 1956; Needham and Gwei-Djen, 1968; Mainwaring, 1977; Bremner, 1981).

The beginning of the modern era of research on the role of the testes in male reproduction was heralded by Bertholds' observations in 1849 on the effects of castration and testicular transplants on comb growth in roosters, which also included some observations on behaviour. Then, in 1889, Brown-Séquard claimed that he could stimulate his declining libido with glycerol extracts of testicular tissue (Brown-Séquard, 1889a,b). However, it was not until some twenty years after Brown-Séquard's report that Pezard (1911) provided the first scientific evidence, using a bioassay, that testicular extracts contained active principles which influenced comb growth in capons.

The first androgen to be identified, androsterone, was cystalized from urine by Butenandt in 1931. Four years later, David and his colleagues succeeded in isolating testosterone, now recognized as the principal androgen secreted by the testes and found in the plasma of male vertebrates, from the testes of bulls

(David, Dingemanse, Freud and Laqueur, 1935). Although the testes are the main source of plasma testesterone in males, small amounts of this steroid are also derived from the adrenals (Baird, Uno and Melby, 1969; Samuels and Nelson, 1975), and from the metabolism in various non-endocrine tissues (liver, intestine, skin, certain muscles) of weaker androgenic precursors such as androstenedione, androstenediol and dehydroepiandrosterone (see Bardin and Santen, 1975). In addition to the classical effects of testosterone on reproductive function in males (see Dorfman and Shipley, 1956; Young, 1961; Hart, 1974b), this steroid also influences metabolic activity in the liver, kidneys, lungs, pancreas, spleen, muscles and many other tissues (see Dorfman and Shipley, 1956; King and Mainwaring, 1974).

Testosterone has also been found to have both qualitative and quantitative actions in target tissues on a temporal basis. For example, during critical periods in early embryonic development (see Section 1.2.2), testosterone has "organizational" or "morphogenetic" effects on both phenotypic sex differentiation (Wilson, Griffin and George, 1980), and sexual programming of the brain (Plapinger and McEwen, 1978). These early actions of testosterone are irreversible. In adult males, on the other hand, testosterone serves "activational" (stimulation of the accessory sex glands and sexual behaviour) and "inhibitory" (negative feedback on LH secretion) roles that can be reversed by castration.

The first assays for androgens were developed during the 1930's. These were bioassays in which the response of the comb in capons and chicks (or the seminal vesicles and prostate in castrated rats) to injections of urine extracts was compared to the response of these tissues to standard preparations of androgens (for

review see Dorfman, 1969). During the 1960's a number of chemical assays based on colorimetry, fluorimetry, gas-liquid chromatography and double isotope dilution were developed for measuring testosterone in both plasma and urine (for reviews see Dorfmann, 1968; Bardin and Santen, 1975; Ismail, 1976).

The introduction of competitive protein binding (CPB) (or radioligand) assays for steroids during the late 1960's, made both the biological and chemical assays for testosterone obsolete (see Murphy, 1969). In CPB assays, steroid extracted from either plasma or urine (using various organic solvents) competes with a radiolabelled form of the same steroid for binding sites on naturally occurring steroid-binding proteins found in the blood (see Section 1.2.1). The inclusion in the assay of a series of samples containing known amounts of unlabelled steroid allows the construction of a standard curve featuring the percentage of radiolabelled steroid displaced. The amount of steroid in the test samples can be calculated from this curve by extrapolation. CPB assays for testosterone were developed using sex steroid binding globulin (SBG), which is a naturally occurring plasma protein that binds C_{18} (oestrogenic) and C_{19} (androgenic) steroids (Kato and Horton, 1968; Mayes and Nuget, 1968; Murphy, 1969; Rosenfield, Eberlein and Bongiovanni, 1969). However, because SBG binds 5α-dihydrotestosterone and androstanediols with higher affinity than testosterone, a chromatography step is required in the testosterone assay to exclude these competing steroids.

CPB assays were superseded recently by radioimmunoassays. The latter assays are the same in principle as CPB assays, except that antibodies raised against a particular steroid are used in place of the naturally occurring binding proteins (see Bardin and

Santen, 1975). Radioimmunoassays have the advantage of being more specific, sensitive and stable than CPB assays (for reviews see Bardin and Santen, 1975; Ismail, 1976). The first radioimmunoassays for testosterone were developed by Furuyama, Mayes and Nuget (1970) and Ismail, Niswender and Midgley (1972).

The advent of radioimmunoassays for steroids meant that gonadal steroid levels in plasma and urine could be measured with greater simplicity and accuracy than had been possible previously. This revolution in hormone assay technique has encouraged considerable research during the past decade on the role of gonadal steroids in reproduction in males and females. The following literature review presents a survey of studies on the role of gonadal steroids in reproduction in males, with particular emphasis on the effects of steroids on reproductive behaviour.

1.2 LITERATURE REVIEW

1.2.1 Mechanism of action of steroid hormones

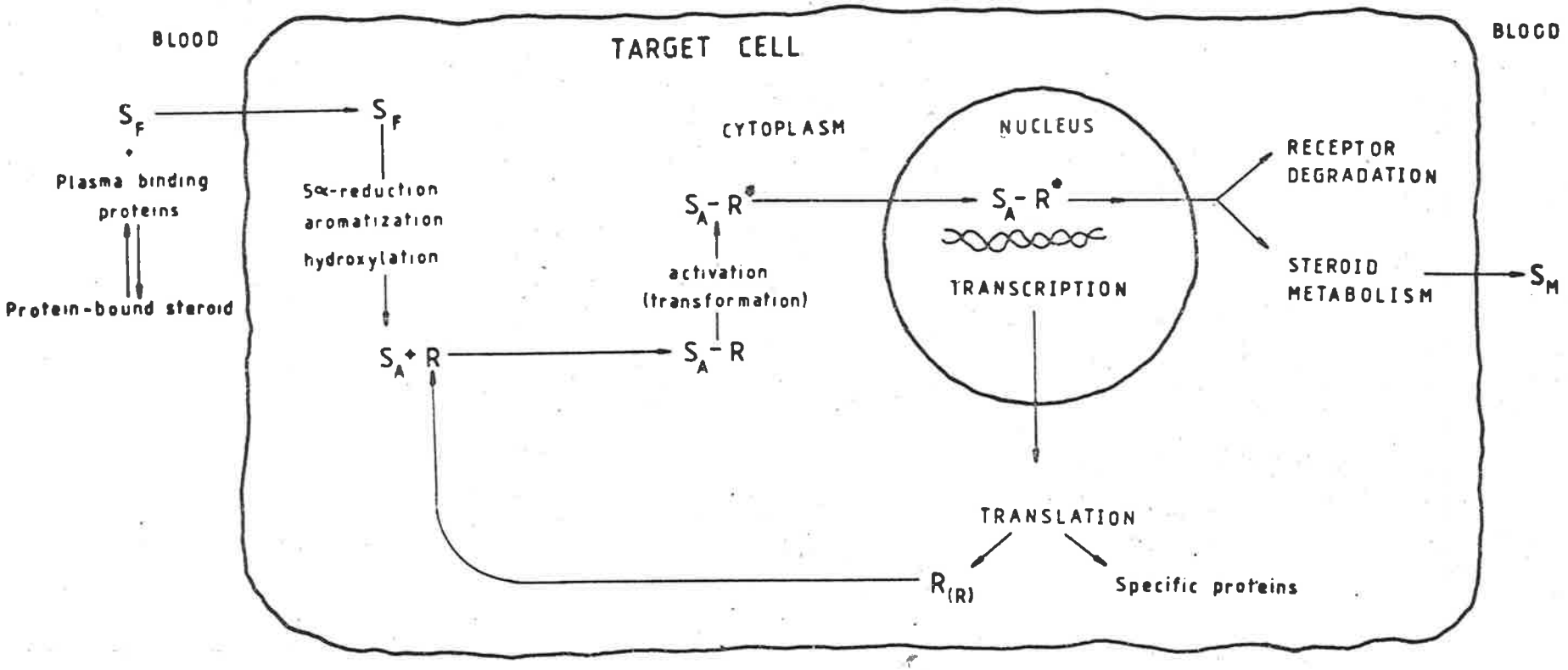
A working knowledge of the mechanism of action of steroid hormones in target cells will help in understanding some of the effects of steroids described in later sections of this literature review.

Model for the mechanism of action of steroid hormones

Figure 1.1 summarizes current thoughts on the mechanism of action of steroid hormones in target cells (see also King and Mainwaring, 1974; Pimentel, 1978a,b,c; Wagner, 1978). It is generally accepted that the capacity of cells to respond to steroids is dependent on the presence in the cell cytoplasm of specific steroid binding protein known as receptors (Wagner, 1978). The

Figure 1.1

Proposed mechanism of action of steroid hormones (see Section 1.2.1 for further details). Although the bulk of steroids found in blood are bound by both specific and non-specific binding proteins, under normal circumstances there is an equilibrium between protein-bound and free (S_F) steroid. Unbound steroids can pass freely through cell membranes and are therefore readily available to target cells. An initial step in the action of some steroids (e.g. testosterone, progesterone) in target cells involves their conversion to an active metabolite (S_A) by enzymes found in the cytoplasm. This is followed by binding of the S_A to a specific cytoplasmic receptor (R) protein, leading to the formation of the steroid-receptor (S_A-R) complex. This complex must be activated (S_A-R*), or transformed, before it can migrate into the nucleus. Within the nucleus the activated steroid-receptor complex interacts with the genome, giving rise to cellular responses to the steroid. At the same time there is replenishment of the cytoplasmic receptor (R_R). As a final step, the steroid is metabolised to an inactive form (S_M) and returned to the blood.


BLOOD
TARGET CELL
BLOOD
S_F
S_F
Plasma binding proteins
Protein-bound steroid
5α-reduction
aromatization
hydroxylation
CYTOPLASM
NUCLEUS
$S_A - R^*$
$S_A - R^*$
activation (transformation)
TRANSCRIPTION
RECEPTOR DEGRADATION
STEROID METABOLISM
S_M
$S_A + R$
$S_A - R$
TRANSLATION
$R_{(R)}$
Specific proteins

binding of a steroid to its receptor leads to the formation of the steroid-receptor complex. This complex is then transformed, or activated, in an incompletely understood fashion, and then migrates to the nucleus (Milgrom, 1981). Within the nucleus the activated steroid-receptor complex interacts with the genome giving rise to de novo protein synthesis (Pimentel, 1978b). The nature of these new proteins characterizes the response of the cell to the steroid (Williams-Ashman and Reddi, 1971).

Although there is much evidence in support of the classical steroid receptor system described above, it has also been suggested that steroids (and in particular corticosteroids) may influence cell function by binding to receptors in the cell membrane (Pietras and Szego, 1977; Baulieu, 1978; Koch, Lutz-Bucher, Briaud and Mialhe, 1978). Baulieu (1978) proposed that the two mechanisms of steroid hormone action need not be mutually exclusive, since steroids generally elicit more than one type of response in target cells.

The availability of steroids to target cells is influenced by several plasma proteins that bind circulating steroids. In man, 93-99% of plasma testosterone is protein-bound (Rivarola, Forest and Migeon, 1968; Ismail, 1976). Two of these binding proteins, sex steroid binding globulin (SBG) and corticosteroid binding globulin (CBG), bind specifically, C_{19} (androgens) and C_{18} (oestrogens) steroids within a 17β-hydroxyl group, and C_{21} (progestagens and corticoids) steroids, respectively. CBG has been found in all vertebrates studied (Seal and Doe, 1965, 1966) whereas some species lack SBG (Corvol and Bardin, 1973). Two other plasma proteins, albumin and α_1-acid glycoprotein (orosomucoid) also bind circulating steroids. However, these latter proteins are non-specific binders that have a high capacity, but low affinity, for steroids in general (Westphal, 1971).

Protein-bound steroids cannot enter cells and are therefore considered to be biologically inactive (Vermeulen, Stoica and Verdonck, 1971; Rosenfield, 1971). However, in normal plasma, there is an equilibrium between protein-bound and free steroid which means that there is always some circulating steroid available to target cells.

Although gonadal steroids influence a variety of tissues and have both quantitative and qualitative effects, their basic mechanism of action appears to be consistent with the steroid receptor model (King and Mainwaring, 1974; Mainwaring, 1977; Wilson et al., 1980). The only exceptions could be the liver and some skeletal muscles, where cytoplasmic androgen receptors have not been clearly demonstrated (Mainwaring, 1977).

1.2.2 Role of gonadal steroids on morphogenesis of the reproductive system and sexual differentiation of mating behaviour in mammals

In the absence of gonadal steroids the mammalian embryo has the inherent tendency to develop into a female (Jost, 1970; Jost, Vigier, Prépin and Perchellet, 1973). Therefore, in mammals, the female is recognized as the neutral sex and sexual differentiation involves the masculinizing actions of androgens in the male during critical periods early in life (Jost, 1970; Goy, 1970). Genotypically female embryos are susceptible to the masculinizing effects of androgens, however, since cytoplasmic androgen receptors are coded for by a gene on the X-chromosome (Lyon, 1970, 1974; Lyon and Hawkes, 1970; Price, 1970).

The indifferent embryonic gonads of mammals develop into testes under the influence of the Y-chromosome (Ohno, Nagai,

Ciccarese and Iwata, 1979; Wachtel, 1979, 1980) and begin to re-create testosterone shortly after differentiation of the Leydig cells (Wilson, 1978). During the critical period for phenotypic sex differentiation testosterone causes the development of the wolffian ducts into epididymides, vasa deferentia and seminal vesicles. At the same time the 5α-reduced metabolite of testosterone, 5α-dihydrotesterone, promotes the development of the urogenital sinus into the prostate and external male genitalia (Figure 1.2; Wilson et al., 1980).

In addition to inducing the masculine development of the sex structures, the foetal testes also provoke the regression of the Mullerian ducts in males (Jost, 1970). This regression is not dependent on androgens however, but rather on a Mullerian Duct Inhibiting Factor (a glycoprotein) that is secreted by the foetal testes (Picard, Tran and Josso, 1978; Picard and Josso, 1979).

The sexual development of the brain in mammals is also influenced by gonadal steroids during a critical period in early life (Goy, 1970), which generally occurs after phenotypic sex differentiation (Harris, 1970; Jost et al., 1973; Clarke, 1977; Wilson and Tarttelin, 1978). It has been clearly demonstrated that exposure of the undifferentiated brain of mammals to gonadal steroids during this second critical period (see Table 1.1) results in the masculinization of otherwise female patterns of sexual behaviour (Booth, 1977a,b; Jost et al., 1973; Dörner, 1976; Plapinger and McEwen, 1978). Also, males that are castrated before the critical period for sexual programming of the brain show female patterns of sexual behaviour following androgen therapy when adult (Dörner, 1975, 1979).

Beach proposed that exposure of the undifferentiated brain of mammals to androgens during the critical period ("androgeniza-

Figure 1.2

Phenotypic sex differentiation in mammals. During the critical period for differentiation of the sex organs the foetal gonads in males are actively secreting androgens. Testosterone itself appears to cause the differentiation of the Wolffian ducts into epididymides, vasa deferentia and seminal vesicles, whilst the 5α-reduced metabolite of testosterone, 5α-dihydrotestosterone seems to promote the differentiation of the urogenital sinus into the prostate and the external male genitalia. In the absence of androgens the female sex structures develop.

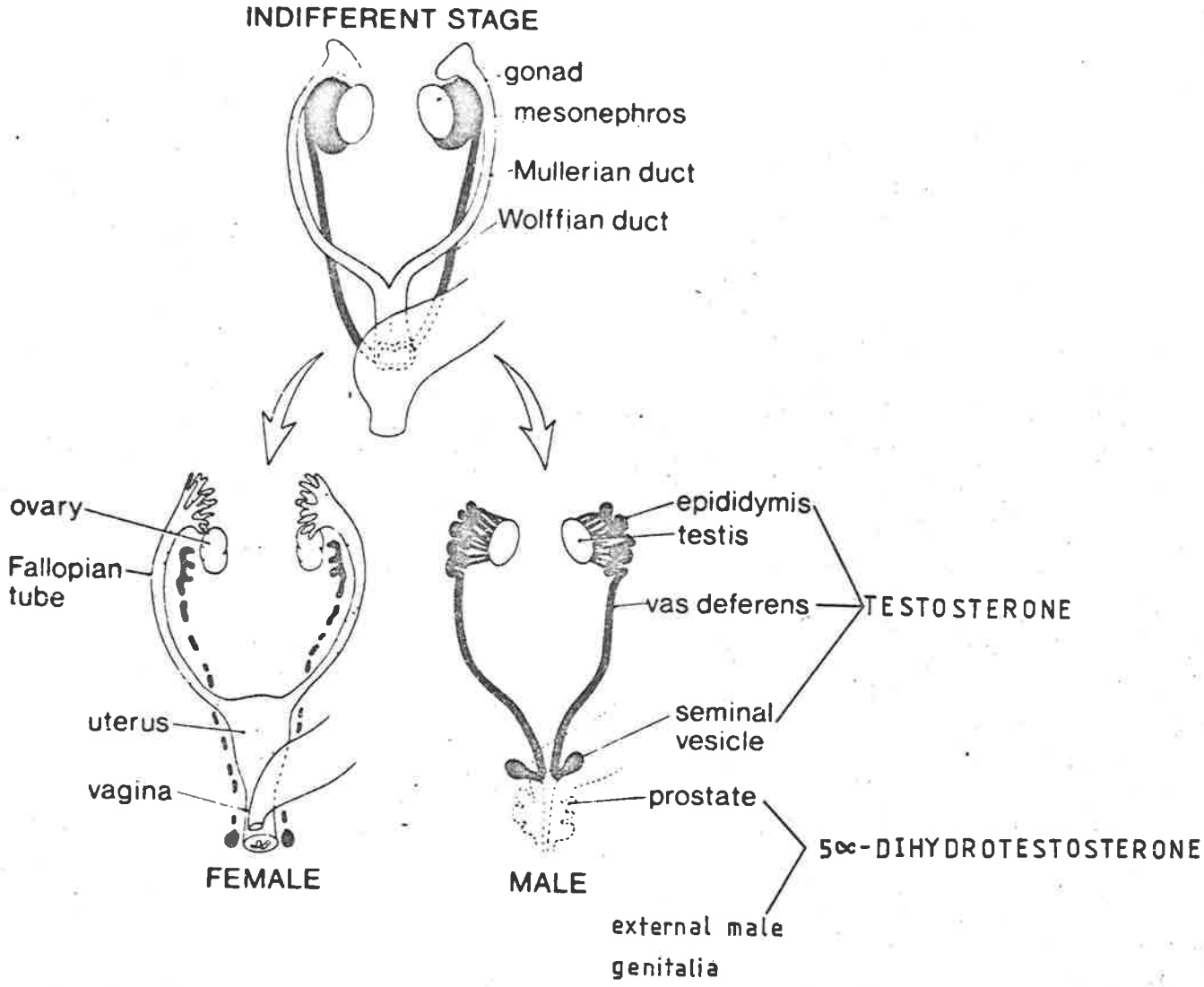

INDIFFERENT STAGE
gonad
mesonephros
Mullerian duct
Wolffian duct
ovary
Fallopian tube
uterus
vagina
FEMALE
epididymis
testis
vas deferens
TESTOSTERONE
seminal vesicle
prostate
5∝-DIHYDROTESTOSTERONE
external male genitalia
MALE

Table 1.1:

Critical period for sexual differentiation of the brain in mammals.

Timing of the critical period	Reference
PRENATAL	
guinea pig	Phoenix, Goy, Gerall and Young, 1959.
dog	Beach and Kuehn, 1970; Beach, Kuehn, Sprague and Anisko, 1972.
miniature pig	Ellendorff, Parvazi, Elsaesser, MacLeod and Reinhardt, 1979.
rhesus monkey	Eaton, Goy and Phoenix, 1973
sheep	Short, 1974; Clarke, 1977; Clarke and Scaramuzzi, 1978.
NEONATAL	
mouse	Edwards and Burge, 1971; Etgen and Whalen, 1979.
rat	Barraclough and Gorski, 1962.
hamster	Carter, Clemens and Hoekema, 1972.
ferret	Baum, 1976.
marmoset monkey	Abbott and Hearn, 1979.

tion") results in enhancement of male sexual behaviour (masculinization) and a concurrent loss of female sexual behaviour (defeminization) (Beach, 1975; Beach, Kuehn, Sprague and Anisko, 1972). This theory is supported by studies in guinea pigs (Phoenix, Goy, Gerall and Young, 1959; Goy, Bridson and Young, 1961), rats (McDonald and Doughty, 1974; Södersten, 1973; Whalen and Rezek, 1974), ferrets (Baum, 1976), hamsters (Gerall, McMurray and Farrell, 1975) and sheep (Clarke and Scaramuzzi, 1978).

Androgens also influence adult patterns of sexual behaviour in males during a postnatal maturational phase of sexual development of the brain. Thus Götz and Dörner (1976) found that rats which were castrated two weeks after birth (i.e. after the critical period and at the beginning of the prepubertal maturation phase) showed permanently reduced male sexual behaviour after androgen therapy when adult, compared with similarly treated males that had been castrated just before puberty. A similar postnatal phase of sexual development of the brain has also been demonstrated in rams (Mattner, George and Braden, 1976; Mattner, George, Wong and Cox, 1979), and proposed for primates (Abbott and Hearn, 1979).

Although testosterone has been classically recognized as the steroid responsible for sexual differentiation of the mammalian brain, more recent studies have indicated that oestradiol-17β is at least as effective as testosterone in this regard (Hendricks and Gerall, 1970; Paup, Coniglio and Clemens, 1972; Whalen and Etgen, 1978; Etgen and Whalen, 1979). The suggestion has been made, therefore, that oestradiol-17β, derived from the aromatization of testosterone, is normally responsible for "masculinizing" the brain in mammals (Naftolin, Ryan, Davies, Reddy, Flores, Petro, Kuhn, White, Takaoka and Wolin, 1975). A summary of the evidence

(and supporting articles) that is consistent with the aromatization hypothesis is given in Table 1.2.

If oestrogens are ultimately responsible for masculinizing the undifferentiated mammalian brain then it must be explained why, under normal circumstances, maternally derived oestrogens do not masculinize female foetuses. It has been suggested that α-foetoprotein, an alpha-globulin that is present in the circulation of both sexes during the foetal and neonatal periods, serves a protective function in females by binding circulating oestrogens (McEwen, Plapinger, Chaptal, Gerlach and Wallach, 1975; Vannier and Raynaud, 1975; Germain, Campbell and Anderson, 1978). α-Foetoprotein can be distinguished from the sex steroid binding globulin found in adult animals since it does not bind testosterone (Versée and Barcel, 1978). This means that in male foetuses, testosterone that is secreted by the testes is free to enter into the brain. Once in the brain, this testosterone can be converted to oestradiol-17β which in turn triggers masculinization (Lieberburg and McEwen, 1975; MacLusky, Lieberburg and McEwen, 1979; MacLusky, Chaptal and McEwen, 1979).

The conversion of testosterone to oestradiol-17β may not be an asbolute requirement for masculinization of the mammalian brain since it has been shown that nonaromatizable androgens, either alone (McDonald and Doughty, 1974; Gerall et al., 1975), or in combination with oestradiol-17β (Goldfoot and Van der Werff ten Bosch, 1975; Fox, Vito and Wieland, 1978), also have masculinizing effects.

Gonadal steroids are thought to masculinize the mammalian brain by inducing irreversible sexual dimorphisms in brain morphology (Table 1.3; for reviews see Naftolin and Brawer, 1978; Torran-Allerand, 1978; Gorski, 1979a,b; McEwen, 1980).

Table 1.2:

Evidence that oestradiol-17β mediates the effects of testosterone during masculinization of the brain in mammals.

Evidence	Reference
1. aromatase enzymes* are present in the mammalian brain during the critical period	Naftolin, Ryan, Davies, Petro and Kuhn, 1975; Reddy, Naftolin and Ryan, 1974; Lieberburg and McEwen, 1975; Naftolin, Ryan, Davies, Reddy, Flores, Petro, Kuhn, White, Takaoka and Wolin, 1975; McEwen, Liberburg, MacLusky and Plapinger, 1976; George, Tobleman, Milewick and Wilson, 1978.
2. oestrogen receptors are present in the undifferentiated brain	Kulin and Reiter, 1972; Sheridan, Sar and Stumpf, 1974; MacLusky, Lieberburg and McEwen, 1979; McEwen et al., 1976; Fox, Vito and Wieland, 1978; Vito and Fox, 1979.
3. compounds that block the aromatization of T to OE_2-17β inhibit T-induced masculinization of the brain.	Booth, 1977a, 1978; McEwen, Liberburg, Chaptal and Krey, 1977; Vreeburg, Van der Vaart and Van der Schoot, 1977; Clemens and Gladue, 1978; Södersten, 1978.
4. non-aromatizable androgens do not masculinize the brain	Luttge and Whalen, 1970; Brown-Grant, Munck, Naftolin and Sherwood, 1971; McDonald, 1971; Whalen and Luttge, 1971; McDonald and Doughty, 1972, 1974; Whalen and Rezek, 1974; Goldfoot and Van der Werff ten Bosch, 1975.

* the aromatase enzyme system is involved in the conversion of testosterone (T) to oestradiol-17β (OE_2-17β).

Table 1.3:

Sexual dimorphisms in brain morphology in mammals and birds.

Type of sexual dimorphism	Species	Reference
nuclear volume of the preoptic-anterior hypothalamic area	rat	Dörner and Staudt, 1968.
nuclear volume of the hypothalamic ventromedial nucleus	rat	Dörner and Staudt, 1969.
nuclear volume of the medial and central amygdala	rat	Staudt and Dörner, 1976.
incidence of various types of synapses in the preoptic area	rat	Raisman and Field, 1971, 1973.
dendritic brnach patterning of dorsomedial preoptic neurons	hamster	Greenough, Carter, Steerman and De Voogd, 1977.
size of a specific nucleus in the medial preoptic area	rat	Gorski, 1979a,b.
size of four brain nuclei that control singing	canary zebra finch	Nottebohm and Arnold, 1976.

1.2.3 Role of gonadal steroids in the sexual behaviour of adult males

In contrast to the irreversible morphogenic effects of gonadal steroids during early sexual development, the same hormones serve an activational role in adult animals by acting on differentiated central neural circuits. The location of brain centres that control sexual behaviour in mammals has been elucidated using lesion, electrical stimulation and hormone implant studies (Ryan and Frankel, 1978). In general, the central neural elements essential for sexual behaviour in males seem to be located in the medial preoptic-anterior hypothalamic area of the brain (Table 1.4, Fig. 1.3; Larsson, 1979), whilst the corresponding nuclei in females are found mainly in the medial basal hypothalamus (Table 1.5, Fig. 1.3). However, in females, there appear to be greater inter-species variations.

Autoradiographic mapping of the distribution in central neural tissues of systemically injected radiolabelled steroids has provided further information regarding sex steroid target centres in the brain (for the techniques involved, and reviews, see Stumpf and Sar, 1975, 1978; McEwen, 1976; Morrell and Pfaff, 1978; Kim, Stumpf, Star and Martinez-Vargus, 1978; McEwen, Davis, Parsons and Pfaff, 1979). Those areas of the brain that concentrate sex steroids are known from lesion and hormone implant studies to also be associated with sexual behaviour (Hart, 1974; Barfield, Ronay and Pfaff, 1978; Morrell and Pfaff, 1978). In addition, Krieger, Morrell and Pfaff (1978) described neuronal projections from central sex steroid concentrating centres to other areas of the brain involved in sexual responses.

Table 1.4:

Brain centres associated with sexual behaviour in males.

Species	Brain centre	Technique	Reference
rat	POA-AH	electrolytic lesions	Larsson and Heimer, 1964; Heimer and Larsson, 1966; Lisk, 1968; Dorner, Docke and Hinz, 1969; Giantonio, Lund and Gerall, 1970; Ryan and Frankel, 1978.
	POA-AH	crystalline TP implants	Davidson, 1966; Lisk, 1967; Johnston and Davidson, 1972; Davidson and Trupin, 1975.
	POA	electrical stimulation	Vaughan and Fisher, 1962; Malsbury, 1971; Van Dis and Larsson, 1971; Ryan and Frankel, 1978.
rhesus monkey	POA-AH	electrolytic lesions	Slimp, Hart and Goy, 1978.
	POA	electrolytic stimulation	Perachio, Marr and Alexander, 1979.
cat	POA-AH	electrolytic lesions	Hart, Haugen and Peterson, 1973.
dog	POA-AH	electrolytic lesions	Hart, 1974a, b.
guinea pig	MBH (AN, VN)	electrolytic lesions	Brookhart and Dey, 1941; Phoenix, 1961.

Table 1.4 (Continued)

Species	Brain centre	Technique	Reference
frog	POA-AH	electrolytic lesions, electrical stimulation	Schmidt, 1968.

POA-AH, preoptic area anterior hypothalamus;
POA, preoptic area;
MBH, medial based hypothalamus;
AN, arcuate nucleus;
VN, ventromedial nucleus.

Figure 1.3

Schematic diagram of the medial preoptic and hypothalamic areas of the brain showing the location of nuclei associated with sexual behaviour.

Brain centres which control male sexual behaviour (♂) are located primarily in the AHA-POA. The medial basal hypothalamus and mammillary body are associated with female (♀) sexual behaviour. AHA, anterior hypothalamic area; AN, arcuate nucleus; AP, anterior pituitary; DN, dorsomedial nucleus; ME, median eminence; MMN, mammillary nucleus; OC, optic chiasma; PMN, pre-mammillary nucleus; POA, preoptic area; PP, posterior pituitary; SCN, supra-chiasmatic nucleus; SON, supra-optic nucleus; T, thalamus; VN, ventromedial nucleus.

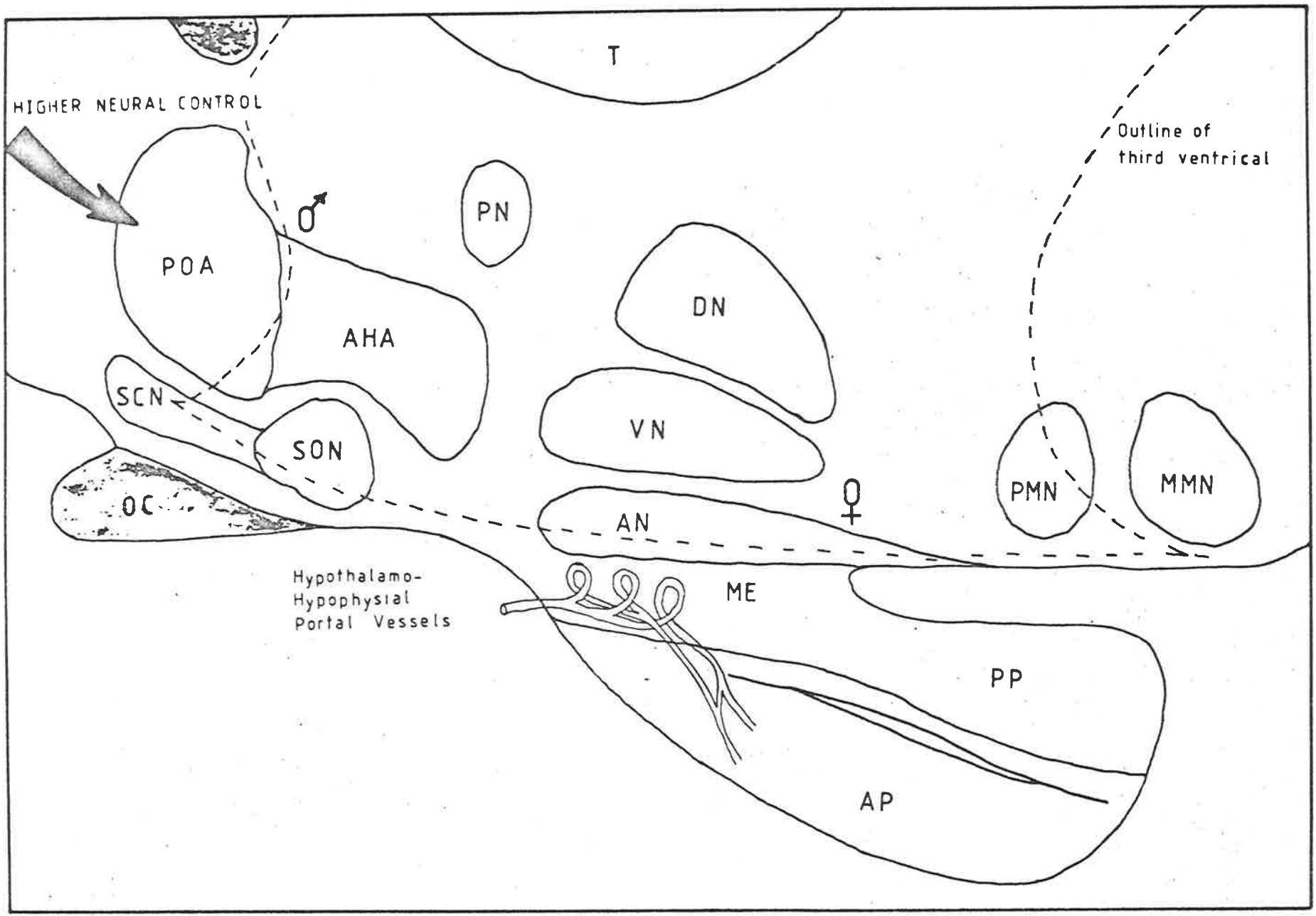

T
HIGHER NEURAL CONTROL
Outline of
third ventrical
♂
PN
POA
AHA
DN
SCN
VN
SON
♀
PMN
MMN
OC
AN
Hypothalamo-
Hypophysial
Portal Vessels
ME
PP
AP

Table 1.5:

Brain centres associated with sexual behaviour in females.

Species	Brain centre	Reference
rat	MBH	Dörner, Döcke and Moustafa, 1968a,b; Mathews and Edwards, 1977; Barfield and Chen, 1977; Davis, McEwen and Pfaff, 1979; Edwards and Pfeifle, 1981.
	POA	Lisk, 1962; Lisk and Barfield, 1975; Quadagno and Fast, 1979; Yamanouchi and Arai, 1979.
golden hamster	MBH	Malsbury, Kow and Pfaff, 1977; Malsbury and Daood, 1978.
guinea pig	MHB	Morin and Feder, 1974.
rabbit	MN PMN	Sawyer and Robinson, 1956; Sawyer, 1959; Palka and Sawyer, 1966a,b.
cat	MBH	Sawyer, 1963; Michael, 1965.
ewe	MBH	Clegg, Santolucito, Smith and Ganong, 1958; Radford, 1967; Domański, Przekop, Skubiszewski and Wolińska, 1975; Thiéry, Pelletier and Signoret, 1978.
	POA-AH	Signoret, 1970.
rhesus monkey	AH	Everitt and Herbert, 1975.

AH, anterior hypothalamus; MN, mammillary nucleus; MBH, medial basal hypothalamus (includes ventromedial and arcuate nuclei); POA, preoptic area; POA-AH, preoptic area anterior hypothalamus; PMN, premammilary nucleus.

The necessity of androgens for normal sexual behaviour in males

Although it is clear that androgens are required for normal sexual behaviour in males of all species (see Hart, 1974b), there appear to be differences between species with regard to the level of sexual activity shown by castrated animals. For example, the majority of rats (Beach and Holz, 1946), rabbits (Stone, 1932) and rams (Clegg, Beamer and Bermant, 1969) castrated before puberty do not show any mating activity when adult. On the other hand, a high proportion of dogs castrated at a similar immature age show almost normal sexual responses when adult, apart from the sexual lock (Beach, 1970; Le Boeuf, 1970).

There also seem to be differences between species with regard to the retention of the ejaculatory reflex in males castrated when adult (see references in Table 1.6). The retention of sexual responses in adult-castrated males is due to learned behaviour since steroids have a short biological half-life, and are cleared from the body within a day or so of castration (Ismail, 1976). It has also been shown in rats (Block and Davidson, 1968), hamsters (Warren and Aronson, 1956) and dogs (Schwartz and Beach, 1954), that adrenal steroids are not responsible for maintaining sexual behaviour in castrated animals.

Although mating activity following castration reflects learned behaviour, there was no relationship between the sexual experience of individual rats (Block and Davidson, 1968) and dogs (Hart, 1968), and the persistence of sexual responses after castration. In contrast to these studies, however, Rosenblatt and Aronson (1958) reported that there was a relationship between prior sexual experience and the retention of mating activity after castration in cats.

Table 1.6:

Retention of ejaculatory response in males following castration.

Animal	Period of retention of the ejaculatory response following castration	Reference
rat	1 month	Beach, 1944; Stone, 1939; Whalen, Beach and Kuehn, 1961.
	3-4 months	Davidson, 1966; Hart, 1974.
guinea pig	up to 3 months	Grunt and Young, 1952.
hamster	up to 3 months	Beach and Pauker, 1949; Whalen and De Bold, 1974.
rabbit	up to 3 months	Ågmo and Kihlström, 1974.
goat	up to 1 year	Hart and Jones, 1975.
ram	1 month to 1 year	Clegg, Beamer and Bermant, 1969.
rhesus monkey	up to 1 year	Phoenix, Slob and Goy, 1973.
	3 months	Michael and Wilson, 1974.

A positive correlation between the sex drive of individual animals at the time of castration, and the persistence of mating activity has been demonstrated in rats (Larsson, 1966), guinea pigs (Grunt and Young, 1952) and rabbits (Stone, 1932).

Beach (1958a,b) proposed that evolution has led to increased "corticalization" (cortical control) of sexual behaviour in males, and a corresponding decrease in the dependence on testicular androgens. However, Aronson (1959) argued that although this theory might prove to be correct, it was premature in view of the data on which it was based. Aronson (1959) suggested instead that testicular androgens were more important in establishing patterns of sexual behaviour in males during early development, rather than for the maintenance of these behaviours in the adult. More recently Hart (1974b) compared the results of castration studies in adult rats, cats, dogs and rhesus monkeys, and concluded that there was essentially no relationship between the degree of brain development, and retention of the ejaculatory reflex.

In summary, it is clear that males have an absolute requirement for androgens in order to show normal mating responses. Also, there appears to be some relationship between the sex drive of males at the time of castration, and the retention of sexual activity. Although higher animals (e.g. goats, rams, primates) tend to retain ejaculatory responses for longer periods after castration than lower animals (e.g. rats, guinea pigs, hamsters), there are large differences in this regard, between individuals of the same species (see references in Table 1.6). It is not possible, therefore, to conclude if there is a relationship between the degree of brain development in males, and retention of ejaculatory responses after castration.

1.2.4 Relationship between androgen status and mating activity in adult males

Although it is evident from the castration studies discussed above (Section 1.2.3) that males have an absolute requirement for androgens in order to show normal mating activity, the basis for differences in sex drive between males has not been so clearly established. Since testosterone is the principal androgen secreted by the testes and found in the circulation of male vertebrates, attempts have been made to correlate the plasma levels of this steroid with mating activity (see Young, 1961; Hart, 1974). To date, studies in adult guinea pigs (Harding and Feder, 1976), bulls (Foote, Munkenbeck and Greene, 1976) and rams (Schanbacher and Lunstra, 1976) have indicated that individual differences in libido are not directly related to differences in plasma testosterone concentration.

Castrated males serve as a useful experimental model for studying the relationship between testosterone status and mating activity since they normally have non-detectable plasma testosterone levels, and they show little sexual behaviour in the absence of hormone therapy. This makes it possible, therefore, to relate sexual responses in testosterone-treated castrates to a given dose of hormone. A summary of testosterone replacement studies in males castrated as adults is shown in Table 1.7.

Apart from the early observations of Beach and his coworkers in rats (Beach and Holz-Tucker, 1949; Beach and Fowler, 1959), it appears that above a certain threshold dose, castrates show the same level of mating activity when treated with diverse doses of testosterone.

Table 1.7:

Effect of testosterone propionate (TP) on mating activity in males.

Species	Dose of TP per day	Period of treatment (wk)	Response	Reference
rat	50-70 mg	9-10	maintenance of precastration level of mating activity	Beach and Holz-Tucker, 1949.
	100 or 500 mg	9-10	increase in mating activity above precastration level	Beach and Holz-Tucker, 1949.
	100 mg	3.5	return to precastration level of mating activity	Beach and Fowler, 1959.
	2 mg	8	return to precastration level of mating activity	Larsson, 1966.
mouse	32 or 1024 ug	5	similar response to either dose and equivalent to the mating activity of entire males	Champlin, Blight and McGill, 1963.
guinea pig	25, 50 or 100 ug	15	return to precastration level of mating activity	Grunt and Young, 1952, 1953.
rabbit	7.5, 15 or 30 mg (every 3rd day)	17	return to precastration level of mating activity	Ågmo and Kihlström, 1974.
ram	10, 500 or 1000 ug/kg body weight	4	restored sexual activty to precastration levels	Clegg, Beamer and Bermant, 1969.

Another important observation in castrates was that males classified either as high, medium or low drive before castration, returned to their respective plane of sexual behaviour after treatment with the same dose of testosterone propionate (guinea pig: Grunt and Young, 1952, 1953; rat: Larsson, 1966; bull: Blockey and Galloway, 1978).

Testosterone therapy also failed to increase mating activity in normal adult rats (Beach and Fowler, 1959), guinea pigs (Riss and Young, 1954), boars (Hupp, Andrews and Murphree, 1961), rhesus monkeys (Phoenix, 1977) and rams (Knight, 1973; Mattner and Braden, 1975; Lincoln and Davidson, 1977).

Therefore, studies in both entire and castrated males have indicated that the sex drive of individual animals is not related to absolute plasma testosterone levels. These observations suggest that libido may be dependent on the response to testosterone of central neural tissues that underlie sexual behaviour.

1.2.5 The role of oestrogens in sexual behaviour in males

The early masculinizing effects of oestradiol-17β on the undifferentiated mammalian brain were discussed in Section 1.2.2. Other studies have suggested that oestradiol-17β may also be responsible for promoting sexual responses in adult males (see references in Table 1.8).

A role for oestrogens in the sexual behaviour of males is supported by the demonstration that the enzyme system which catalyses the conversion of androgens to oestrogens (aromatase) is located in areas of the male brain known from other studies to be associated with mating activity (Ryan, Naftolin, Reddy, Flores and Petro, 1972; Naftolin *et al.*, 1975). In addition, compounds

Table 1.8:

Species in which oestrogens alone elicit mating response in the male.

Species	Reference
rat	Södersten, 1973; Davis and Barfield, 1979.
mouse	Edwards and Burge, 1971.
rabbit	Foote, Draddy, Breite and Oltenacu, 1977.
cat	Green, Clemente and De Groot, 1957.
boar	Joshi and Raeside, 1973.
red deer	Fletcher and Short, 1974; Fletcher, 1978.
ram	Mattner and Braden, 1976, 1980; Parrott, 1978; Fulkerson, Adams and Gherardi, 1981.
bull	Sawyer and Fulkerson, 1980/1981.
man	Foss, 1939.

which prevent the aromatization of testosterone to oestradiol-17β inhibit testosterone-induced mating behaviour in male rats (Christensen and Clemens, 1975; Moralí, Larsson and Beyer, 1977). This inhibition can be overcome by the concurrent administration of oestrogens (Moralí et al., 1977). Furthermore, anti-oestrogens also block the behavioural response of castrated rats to testosterone treatment (Luttge, 1975; Beyer, Moralí, Naftolin, Larsson and Pérez-Palacios, 1976). Moreover, 19-hydroxytestosterone, an intermediate metabolite in the aromatization of testosterone to oestradiol-17β, has been found to elicit some mating responses in castrated rats (Parrott, 1974; Johnston, Grunwell, Benson, Kandel and Petrow, 1975) and rams (Parrott, 1978).

The failure of the non-aromatizable androgen, 5α-dihydro-testosterone to stimulate sexual behaviour in castrated rats (McDonald, Beyer, Newton, Brien, Baker, Tan, Sampson, Kitching, Greenhill and Pritchard, 1970; Parrott, 1975), golden hamsters (Christensen, Coniglio, Paup and Clemens, 1973) and rams (Parrott, 1978), provided further evidence that oestrogens mediate the effects of androgens on sexual behaviour in males.

Evidence that the male brain has the capacity to respond to oestrogens was provided by the demonstration of oestrogen receptors in the brain of male rats (Vreeburg, Shretlen and Baum, 1975; Barley, Ginsburgh, MacLusky, Morris and Thomas, 1977; Biegl-mayer, Jettmar, Adamiker and Spona, 1978), guinea pigs (Pasqualini, Sumida, Gelly and Nguyen, 1977), bulls (Kahwanago, Heinricks and Herrman, 1969; Armstrong and Villee, 1977) and man (Davies, Naftolin, Ryan and Siu, 1975).

Collectively, the above findings provide strong support for the hypothesis that testosterone is aromatized to oestradiol-17β in

the male brain, and that the effects of testosterone on sexual behaviour in males are mediated by oestradiol-17β.

The conversion of testosterone to oestradiol-17β may not, however, be a requirement for sexual behaviour in males of all species. For example, oestrogens alone did not elicit sexual behaviour in castrated guinea pigs (Alsum and Goy, 1974) and rabbits (Beyer, de la Torre, Larsson and Pérez-Palacìos, 1975), whilst 19-hydroxy-testosterone was ineffective in male rhesus monkeys (Phoenix, 1976). Moreover, in these same species 5α-dihydrotestosterone is effective in stimulating mating activity in castrated males (see references in Table 1.9).

Testosterone may therefore elicit sexual behaviour in males after its conversion in central neural tissues to either oestradiol-17β, or 5α-dihydrotestosterone, depending on the species. Some evidence has also been provided that the complete mating response in males may involve a synergism between oestradiol-17β and 5α-dihydrotestosterone in the brain (rat: Baum and Vreeburg, 1973; Larsson, Södersten and Beyer, 1973a,b; Baum, Södersten and Vreeburg, 1974; Larsson, Södersten, Beyer, Moralí and Pérez-Palacìos, 1976; rabbit: Beyer, de la Torre, Larsson and Pérez-Palacìos, 1975; Foote, Draddy, Briete and Oltenacu, 1977; ram: Mattner, 1980).

1.2.6 A possible role for catecholoestrogens in sexual behaviour in males and females.

The major metabolic pathway for oestrogens in both the liver and the brain involves an initial hydroxylation of these steroids at the C-2 position to form catecholoestrogens (Ball, Haupt and Knuppen, 1978; Fishman, 1981). These latter compounds are structurally related to the catecholamines, which are neurotransmitters. The suggestion

Table 1.9:

Species in which 5α-dihydrotestosterone alone elicits sexual responses in the male.

Species	Reference
mouse (certain strains)	Luttge and Hall, 1973.
rat	Yahr, 1979.
guinea pig	Alsum and Goy, 1974.
rabbit	Beyer and Rivaud, 1973.
rhesus monkey	Pheonix, 1974.

has been made, therefore, that catecholoestrogens may provide the link in steroid-brain interactions (Parvizi and Ellendorff, 1975).

Since catecholamines influence sexual behaviour in both males and females (see references in Table 1.10), it is possible that the catecholoestrogens, because of their structural similarity to the former compounds, may also be involved in neural processes associated with sexual responses. In fact, the catecholoestrogens have been found to inhibit the metabolism of catecholamines in various brain tissues (Breuer and Köster, 1974), and the former compounds also bind to dopamine receptors in the anterior pituitary (Schaeffer and Hsueh, 1979). In addition, the highest concentrations of catecholoestrogens in the rat brain are found in areas known to be important for sexual behaviour (Paul and Axelrod, 1977). These preliminary data are at least consistent with a role for catecholoestrogens in central monoaminergic processes associated with sexual behaviour.

Direct studies on the effects of catecholoestrogens on sexual behaviour have been carried out in female rats (Luttge and Jasper, 1977; Marrone, Rodriguez-Sierra and Feder, 1977) and guinea pigs (Marrone et al., 1977). In both these studies, 2-hydroxy-oestradiol-17β and 2-hydroxyoestrone, had only weak or no oestrogenic effects on lordotic responses. It is unlikely that these negative findings were due to the unstable nature of catechol-oestrogens, since in a number of animals the compounds were applied directly to the hypothalamus using cannulae. However, because of the potential of catecholoestrogens to influence mono-aminergic transmission, further studies on their effects on sexual behaviour in both males (since oestrogens stimulate sexual activity in males, Section 1.2.5) and females, seem warranted.

1.2.7 Effects of various centrally located substances on sexual behaviour in males and females

Although gonodal steroids are generally recognized as the principal determinants of sexual behaviour, a number of centrally located substances also have been found to influence sexual responses in both males and females. A thorough examination of the effects of these diverse substances on sexual behaviour is beyond the intended scope of this literature review and the data are therefore presented in summary form in Table 1.10.

1.2.8 Environmental factors which influence sexual behaviour in males and in particular rams

In addition to the effects of various endogenous physiological factors (i.e. gonadal steroids, neurotransmitters, hypothalamic releasing hormones) on sexual behaviour in males and females, a number of environmental stimuli have also been found to influence sexual responses in both sexes. The following discussion will deal mainly with the male, and in particular rams. Comparative observations for females are available in the various articles cited.

Olfactory stimuli and sexual behaviour in males

Air-borne chemical substances (pheromones) that are released in the urine or faeces of animals, or secreted from cutaneous glands, have been shown to elicit both behavioural and endocrine responses in conspecifics (for reviews see Wilson and Bossert, 1963; Bronson, 1968; Whitten and Champlin, 1973; Doty, 1976).

It has been proposed that the vomeronasal organ (Jacobson's organ; see Negus, 1958; Estes, 1972), which is a component of the olfactory system in vertebrates, is involved in the perception of pheromones that influence reproductive processes, including sexual

Table 1.10:

Effects of various neurotransmitters, luteinizing hormone releasing hormone (LHRH), thyrotropin releasing hormone (TRH), adrenocorticotrophic hormone (ACTH) and opioid peptides on sexual behaviour in males and females.

Substance	Species	Effect on sexual behaviour	Reference
NEUROTRANSMITTERS MONOAMINES			
1. DOPAMINE			
male	rat	increased	Gessa and Tagliamonte, 1974, 1975; Malmnäs, 1976; Meyerson and Malmnäs, 1978.
	rhesus monkey	decreased (?)	Everitt, 1979.
female	rat	decreased	Everitt and Fuxe, 1977; Carter and Davis, 1977; Meyerson and Malmnäs, 1978.
	guinea pig	decreased	Crowley, Feder and Morin, 1976.
2. NORADRENALINE			
male	rat	increased	Malmnäs, 1973.
female	rat	increased	Everitt, Fuxe, Hökfelt and Jonsson, 1975; Nock and Feder, 1979.

Table 1.10 (Continued)

Substance	Species	Effect on sexual behaviour	Reference
3. SEROTONIN			
male	rat	decreased	Gessa and Tagliamonte, 1974, 1975; Luttge, 1975; Meyerson and Eliasson, 1977.
	cat	decreased	Hoyland, Shillito and Vogt, 1970.
female	rat	decreased	Everitt et al., 1975; Carter and Davis, 1977; Meyerson and Eliasson, 1977.
	cat	decreased	Hoyland et al., 1970.
	golden hamster	decreased	Carter, Bahr and Ramirez, 1978.
	rhesus monkey	decreased	Gradwell, Everitt and Herbert, 1975; Everitt, 1979.
4. ACETYL CHOLINE			
male	rat	increased	Bignami, 1966; Shillito, 1970; Soulairac and Soulairac, 1975.

Table 1.10 (Continued)

Substance	Species	Effect on sexual behaviour	Reference
female	rat	decreased	Lindström and Meyerson, 1967; Lindström, 1973; Clemens, Dohanich and Witcher, 1981.
	golden hamster	decreased	Lindström, 1972.
5. LHRH			
male	castrate rat	reduced intromission and ejaculation latencies	Moss, Dudley, Foreman and McCann, 1975.
	man	increased	reviewed by Moss, Riskind and Dudley, 1979.
female	rat	elicited lordosis in OVX oestrogen-primed animals	Moss and McCann, 1975; Moss et al., 1979.
	rat	no effect	Meyerson, 1979.
	golden hamster	no effect	Carter, Bahr and Ramirez, 1978.
6. TRH			
female	rat	reduced lordosis in OVX oestrogen-primed animals	Moss, 1977.

Table 1.10 (Continued)

Substance	Species	Effect on sexual behaviour	Reference
7. ACTH			
male	rabbit	decreased behaviour in intact animals; overcome by pretreatment with T	Koranyi, Endroczi and Tarnok, 1965/1966.
female	rat	elicited lordosis in OVX oestrogen-primed animals	Feder and Ruf, 1969.
	guinea pig	same response as in the rat	Feder and Ruf, 1969.
8. $ACTH_{1-24}$			
male	rabbit	increased	Haun and Haltmeyer, 1975.
	rat	increased and decreased	De Catanzaro, Gray and Gorzalka, 1981.
female	rabbit	increased	Haun and Haltmeyer, 1975.
9. $ACTH_{4-10}$			
male	rat	decreased behaviour in castrates treated with low doses of TP; no effect in castrates given high doses of TP	Bohus, Hendrickx, van Kolfschoten and Krediet, 1975.
female	rat	no effect in OVX oestrogen-primed females	Meyerson and Bohus, 1976.

Table 1.10 (Continued)

Substance	Species	Effect on sexual behaviour	Reference
OPIOID PEPTIDES			
10. β-ENDORPHIN			
male	rat	decreased behaviour when injected into the lateral ventricles	Meyerson and Terenius, 1977; Meyerson, 1978, 1979.

behaviour (Scalia and Winans, 1976). This suggestion is supported by neuroanatomical data which indicates that there are neuronal pathways linking the vomeronasal organ to the amygdala, medial preoptic area and medial hypothalamus (Winans and Scalia, 1970; Leonard and Scott, 1971; de Olmos, 1972; de Olmos and Ingram, 1972). These are areas of the brain known to be important for sexual behaviour in males (Section 1.2.3). The main olfactory bulbs, on the other hand, project to the lateral preoptic area and lateral hypothalamus (Winans and Scalia, 1970; Scott and Leonard, 1971; Heimer, 1972), areas of the brain that have not been implicated in sexual responses.

Behavioural studies in male hamsters have provided direct evidence that the vomeronasal organ is more important than the main olfactory bulbs for normal sexual activity (Powers and Winans, 1975; Winans and Powers, 1977; Powers, Fields and Winans, 1979). Deafferentation of the vomeronasal organ alone produced severe mating deficits in 30 to 40% of hamsters whereas severing of the afferent connections of the olfactory bulbs alone had no effect on mating activity. However, combined vomeronasal organ and olfactory bulb deafferentation eliminated copulation in all male hamsters. Therefore, whilst the vomeronasal organ may be more important than the olfactory bulbs for sexual responses, it appears that under normal circumstances both systems contribute to the arousal of sexual behaviour, at least in male hamsters (Powers et al., 1979).

Estes (1972) proposed that the 'flehmen' response, which is displayed by many animals after they have sniffed the urine of conspecifics, serves to facilitate the passage of airborne substances into the vomeronasal organ.

In what is generally credited as the first evidence of a behavioural pheromone in mammals, Kelley (1937) reported that Dorset and Merino rams were attracted to and attempted to mount pregnant ewes whose vulva had been smeared with vaginal mucus from oestrous ewes. Since rams do not normally show interest in pregnant ewes, these observations suggested that the rams were responding to olfactory stimuli released from the vaginal mucus taken from oestrous ewes. Bulls are also stimulated to mount non-oestrous cows whose vulva has been smeared with vaginal mucus from oestrous cows (Hart, Mead and Reagan, 1946).

In more recent studies in rams, animals rendered either temporarily (Banks, Bishop and Norton, 1963) or permanently (Lindsay, 1965) anosmic were found to show the complete mating response. The only difference in mating behaviour between normal and anosmic rams was a higher incidence of nudging in the latter animals. It would appear, therefore, that anosmic rams use nudging to compensate for the loss of smell in identifying oestrous ewes. This is consistent with observations that oestrous ewes remain still after a nudge and allow the ram to mount (see Banks, 1964).

Fletcher and Lindsay (1968) investigated the relative importance of auditory, visual and olfactory stimuli in the mating activity of rams, and concluded that whilst olfaction is important in the detection of oestrous ewes by rams, it is not essential for copulation.

Comparative data on the effects of olfactory deprivation on sexual behaviour in males of other species is shown in Table 1.11. It appears from these data that there are differences between species with regard to the extent to which mating activity in the male is

Table 1.11:

Effect of olfactory deprivation on sexual behaviour in males.

Species	Treatment	Effect on sexual behaviour	Reference
mouse	B. BLX*	complete loss of mating activity in 7/9 animals	Rowe and Edwards, 1972.
	peripherally anosmic using zinc sulphate	no effect	Rowe and Smith, 1973.
rat	B. BLX	significant decrease in sexual drive, but continued to show I & E	Beach, 1942; Bermant and Taylor, 1969.
	OB. L	same effect as B.BLX	Heimer and Larsson, 1967.
gerbil	B. BLX	no effect	Cheal and Domesick, 1979.
hamster	B. BLX**	abolished mating activity	Murphy and Schneider, 1970; Lisk, Ziess and Ciaccio, 1972.
	VO. D	severe mating deficits in 30-40% of animals	Powers and Winans, 1975; Winans and Powers, 1977.
	OB. D	no effect	Powers, Fields and Winans, 1979.
	VO. D + OB. D	eliminated copulation in all animals	Powers and Winans, 1975; Powers et al., 1979.
rabbit	B. BLX	no effect	Stone, 1925; Brooks, 1937.
cat	B. BLX	no effect	Aronson and Cooper, 1974.

Table 1.11 (Continued)

Species	Treatment	Effect on sexual behaviour	Reference
rhesus monkey	temporarily anosmic	did not lever press to gain access to receptive female	Michael and Keverne, 1968.
ram	temporarily anosmic using topical anaesthetic	no effect other than to increase the number of nudges prior to mounting	Banks, Bishop and Norton, 1963.
	B. BLX	same response as with topical anaesthetic	Lindsay, 1965.
		significant decrease in number of ewes served	Fletcher and Lindsay, 1968.

* B. BLX, bilateral olfactory bulb ablation; OB. D, olfactory bulb deafferentation; OB. L, olfactory bulb lesions; VO. D, vomeronasal organ deafferentation.

** B. BLX in this study most likely resulted in removal of the vomeronasal organ which has been shown to be important for sexual responses in hamsters (see text for details).

dependent on olfactory stimuli. However, these inter-species differences do not appear to be related to the degree of brain development since olfactory cues from the female are associated with normal sexual responses in primates as well as in rodents.

A number of compounds which seem to act as sex pheromones in mammals have been identified. Volatile, short-chain aliphatic acids (e.g. acetic, propionic, butyric) that are present in the vaginal secretions of oestrous rhesus monkeys stimulate sexual responses in the male (Michael, Zumpe, Richter and Bonsall, 1977).

In pigs, odours from the boar help to facilitate the "standing reaction" in oestrous sows (Signoret, 1976). This boar effect is thought to be mediated by two volatile androgens (5α-androst-16-ene-3-one and 3α-hydroxy-5α-androst-16-ene) which are present in the saliva and preputial fluid of boars (Melrose, Reed and Patterson, 1971; Reed, Melrose and Patterson, 1974).

Social environment during puberty and sexual behaviour in rams

It was reported in a number of studies that rams raised in all male groups tended to show less heterosexual activity as young adults compared with rams kept with cyclic ewes from an early age (Hulet, Blackwell and Ercanbrack, 1964; Pretorius, 1972; Le Roux and Barnard, 1974). The suggestion was made, therefore, that a monosexual environment during early life may lead to homosexual tendencies in rams (Hulet et al., 1964; Marincowitz, Pretorius and Herbst, 1966).

In contrast to the above reports, however, other studies in rams have indicated that the social environment during rearing does not have any long-term consequences on mating activity in young animals (Bryant, 1975; Illius, Haynes, Purvis and Lamming, 1976b).

Winfield and Makin, 1978). It has also been shown that testis size (Illius, Marx, Haynes and Lamming, 1975) and circulating testosterone levels (Illius et al., 1976b) of prepubertal rams are unaffected by social environment. Furthermore, young rams that are sexually inactive initially, tend to show normal mating activity after a period of contact with ewes (Hulet et al., 1964; Mattner, Braden and George, 1973).

Dominance and sexual behaviour in rams

In a pen mating situation dominant rams are able to suppress the mating activity of subordinate animals (Hulet, Ercanbrack, Blackwell, Price and Wilson, 1962; Banks, 1964; Pepelko and Clegg, 1965b, Marincowitz, Pretorius and Herbst, 1966). In particular, older rams tend to dominate young animals (Hulet et al., 1962). Physical contact between rams is not essential for these dominance effects since subordinant animals show reduced sexual activity when simply viewed by dominant rams (Lindsay, Dunsmore, Williams and Syme, 1976). If interactions similar to those that occur between rams in a confined area were to also operate under field conditions, then a dominant ram that was infertile, or of inferior genotype, could adversely affect the breeding potential of a flock (Hulet et al., 1962; Bourke, 1967).

In a number of field studies dominant rams were indeed found to reduce the mating activity of subordinate animals (Hulet, 1966; Bourke, 1967; Lindsay, 1966). Consequently, Fowler and Jenkins (1976) reported that the fertility of a flock could be decreased slightly if the dominant ram was sterile.

However, other field studies have indicated that subordinate rams can avoid the dominant animal, and are therefore able to express their full mating potential (Lindsay and Robinson, 1961a;

Mattner, Braden and Turnbull, 1967; Mattner, Braden and George, 1973). An important factor which contributes to the amount of interaction between rams in the field would therefore appear to be paddock size (Mattner et al., 1973).

Lindsay and Ellsmore (1968) found that the ability of dominant rams to prevent subordinates from mating was inversely related to the number of oestrous ewes present at any one time (see also Hulet et al., 1962; Mattner et al., 1967).

Nutrition and reproduction in rams

The effect of plane of nutrition on reproduction in rams was reviewed by Tassell (1967a,b), Allden (1970) and Dýrmundsson (1973). Comparative studies in other ungulates have been discussed by Moustgaard (1969) and Leathem (1970).

Nutrition and sexual development in ram lambs

An adequate plane of nutrition is essential for normal sexual development in prepubertal rams. Ram lambs subjected to under-nutrition show retarded sexual development and experience a delay in the onset of spermatogenesis (Pretorius and Marincowitz, 1968). A similar response is also observed in the field where ram lambs born at different times of the year attain puberty at different ages and body eight, due to seasonal fluctuations in pasture availability (Skinner and Rowson, 1968; Dýrmundsson and Lees, 1972).

The development of mating behaviour in young rams is also influenced by the level of nutrition. Ram lambs that received supplemental feeding were found to show sexual activity at a significantly earlier age than lambs fed on a poorer diet (Ragab, Sharafeldin and Khalil, 1966).

Nutrition and reproductive function in postpubertal rams

Tilton and coworkers reported that the libido of 14-month-old Florida native and Rambouillet rams was not adversely affected by low protein and low energy diets that resulted in body weight loses of 14% and 29% respectively over a twenty-six week period (Tilton, Warnick, Cunha, Loggins and Shirley, 1964). Although the protocol used to assess libido in this study may have been inadequate to detect treatment effects (ram libido was determined every second week during a five minute period of association with ewes) the low protein and low energy groups of rams nevertheless showed the same level of fertility as control rams during mating trials that commenced after twenty weeks of treatment.

The effects of submaintenance diets on the libido of mature Merino rams was investigated by Parker and Thwaites (1972). Rams were fed at 75% or 50% of maintenance for fifteen weeks which resulted in weight losses of 12.5% and 20.6% of the initial body weight respectively. During weeks 9 to 15 the treatment rams showed an increase in reaction time and number of mounts per ejaculation, as well as a higher incidence of failures to ejaculate during libido trials. However, this decrease in mating performance may have been due to general muscular weakness rather than to a direct effect on the sexual desire of undernourished rams (Parker and Thwaites, 1972).

Mattner and Braden (1975) reported that adult Merino rams fed a high protein diet for four to five weeks did not show any improvement in libido. On the other hand, rams that were underfed for eight weeks showed a decrease in the number of services during libido trials between weeks 6 and 8.

1.2.9 Behaviour-induced changes in plasma LH and testosterone levels in males

It has been observed in males of a number of species that sexual stimulation is associated with an increase in plasma LH and testosterone levels (see references in Table 1.12). The biological significance of this behaviour-induced change in endocrine activity in males has not been determined. A number of workers have suggested that a rise in plasma LH and testosterone in male rabbits during mating may represent a mechanism that is analogous to the neuroendocrine reflex which triggers a release of LH (and subsequently ovulation) in the doe (see references in Table 1.12).

In rats (Kamel, Mock, Wright and Frankel, 1975) and mice (Macrides, Bartke and Dalterio, 1975; Coquelin and Bronson 1979), increases in plasma LH and testosterone have been observed prior to, as well as during and after coitus. It was suggested that a pre-mating rise in plasma LH and testosterone in male rodents may serve to initiate, or facilitate, sexual activity (Kamel et al., 1975).

An increase in plasma LH and testosterone levels does not always occur during sexual activity in males (see references in Table 1.13). It was on the basis of such observations in rabbits that Hilliard and coworkers suggested that a neuroendocrine response to sexual stimulation is influenced by the plasma testosterone concentration immediately prior to mating (Hilliard, Pang, Penardi and Sawyer, 1975). For example, if the pre-mating testosterone concentration is relatively high, then there would presumably be a negative feedback effect acting on the brain-pituitary axis which would prevent an LH increase (see Figure 1.4 and Section 1.2.10). This concept was supported by two other

Table 1.12:

Increases in plasma LH and testosterone during sexual activity in males.

Species	Reference
rat	Purvis and Haynes, 1974; Kamel, Mock, Wright and Frankel, 1975; Kamel, Wright, Mock and Frankel, 1977; Kamel and Frankel, 1978; Frankel, 1981.
hamster	Macrides, Bartke, Fernandez and D'Angelo, 1974.
rabbit	Endrőczi and Lissák, 1962; Saginor and Horton, 1968; Haltmeyer and Eik-Nes, 1969.
boar	Ellendorff, Parvizi, Pomerantz, Hartjen, König, Smidt and Elsaesser, 1975; Liptrap and Raeside, 1978.
bull	Katongole, Naftolin and Short, 1971; Smith, Mongkonpunya, Hafs, Convey and Oxender, 1973.
man	Fox, Ismail, Kirkham and Loraine, 1972; Fox, 1973; Pirke, Kockott and Dittmar, 1974.
ram	Sanford, Palmer and Howland, 1974, 1977; Illius, Haynes and Lamming, 1976; Moore, Whyman and Wilson, 1978.

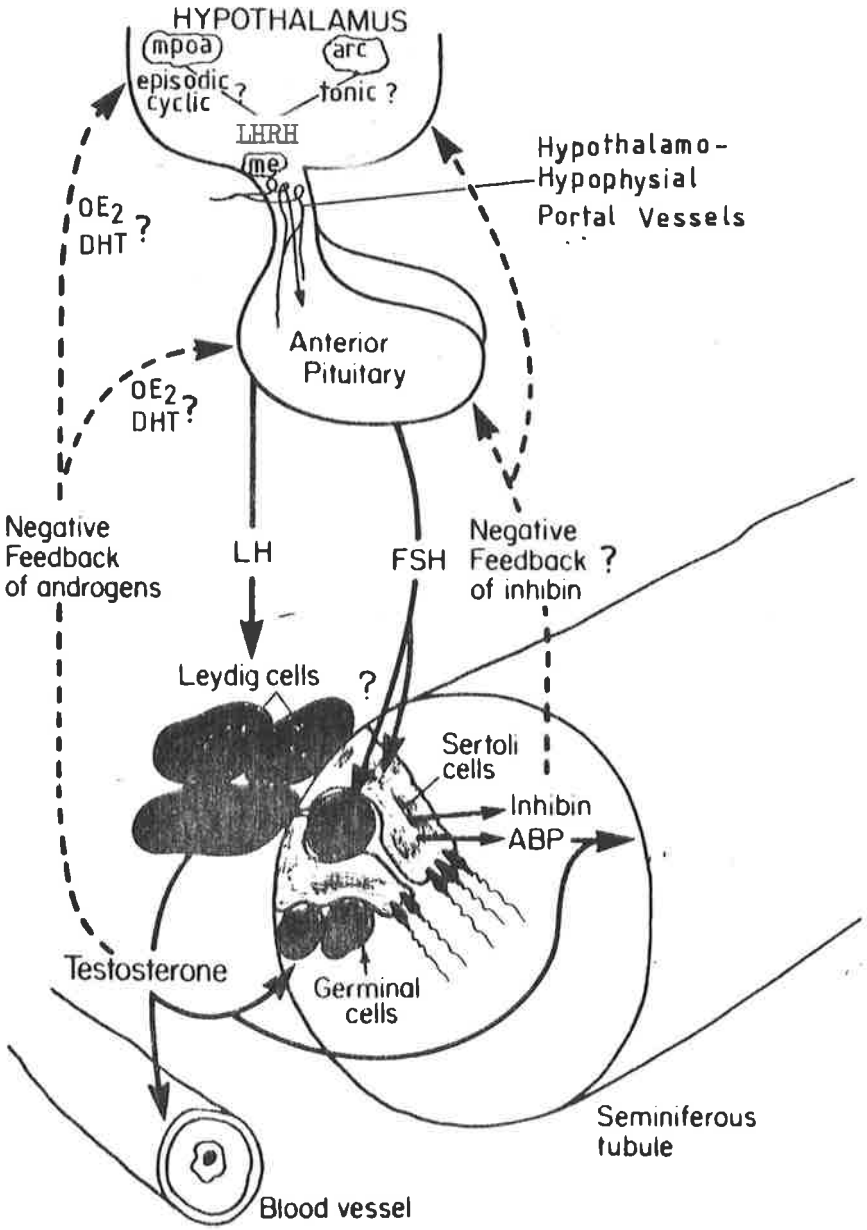
Table 1.13:

Lack of effect of sexual stimulation on plasma LH and testosterone in males.

Species	Reference
rat	Balin and Schwartz, 1976.
rabbit	Hilliard, Pang, Penardi and Sawyer, 1975; YoungLai, Moor and Dimond, 1976; Blake, Blake, Thorneycroft and Thorneycroft, 1978.
bull	Gombe, Hall, McEntee, Hansel and Pickett, 1973; Smith, Mongkonpunya, Hafs, Convey and Oxender, 1973; Short, Randel and Bellows, 1979.
ram	Purvis, Illius and Lamming, 1974; Sanford, Palmer and Howland, 1974; Illius, Haynes, Purvis and Lamming, 1976; Moore, Whyman and Wilson, 1978.
stumptail macacuque monkey	Goldfoot, Slob, Scheffler, Robinson, Wiegand and Cords, 1975.
rhesus monkey	Phoenix, Dixson and Resko, 1977.
man	Stearns, Winter and Raiman, 1973; Lincoln, 1974.

Figure 1.4

The hypothalamic-pituitary-testicular axis. Leydig cells in the testis secrete testosterone in response to luteinizing hormone (LH) which is released from the gonadotroph cells in the anterior pituitary under stimulation from luteinizing hormone releasing hormone (LHRH). LHRH is understood to be synthesized in neurons in the medial preoptic area (mpoa) and arcuate nucleus (an) which have projections to the median eminence (me). Within the me these neurons release LHRH in the vicinity of the hypothalamo-hypophysial-portal vessels. The portal vessels deliver LHRH to the site of the gonadotroph cells in the anterior pituitary which in turn secrete LH and FSH. At the testes LH stimulates the release of steroids (predominantly testosterone) from the Leydig cells whilst FSH influences the activity of the Sertoli cells and spermatogenesis. Gonadal steroids exert a negative feedback action on LH and FSH secretion. Although testosterone is the principal steroid secreted by the testes, negative feedback may be mediated by 5α-reduced (5α-dihydrotestosterone) and oestrogenic (oestradiol-17β) metabolites of testosterone. The episodic pattern of LH and testosterone secretion observed in males (Section 1.2.10 and Table 1.14) results from an interplay between an endogenous LHRH rhythm in the brain and negative feedback by gonadal steroids. The Sertoli cells secrete a putative protein, inhibin, which is thought to regulate, in part, the secretion of FSH, but not LH.



arc: arcuate nucleus

me: median eminence

mpoa: medial preoptic area

studies in rabbits (YoungLai, Moor and Diamond, 1976; Blake, Blake, Thorneycroft and Thorneycroft, 1978), and could apply to males in general. However, Katongole, Naftolin and Short (1971) reported that if the testosterone levels in bulls was relatively high at the time of sexual stimulation, then the animals showed an LH increase, but the testosterone levels remained unchanged. Other studies have indicated that there is no increase in plasma LH in males during mating, irrespective of the plasma testosterone concentration at the time of sexual stimulation (see references in Table 1.13).

Copulatory activity has also been reported to influence the activity of the brain-adrenal axis in males. Increases in plasma glucocorticoids following sexual activity were observed in boars (Liptrap and Raeside, 1978) and rhesus monkeys (Phoenix, Dixson and Resko, 1977). However, Phoenix et al. (1977) indicated that it was difficult to determine if the rise in plasma cortisol in rhesus monkeys was due to mating activity per se, or the stress associated with handling.

In contrast to the studies in boars and rhesus monkeys, sexual arousal was found to have no effect on adrenal cortical activity in rats (Szechtman, Lambrou, Caggiula and Redgate, 1974) and man (Ismail, Davidson and Loraine, 1972; Kling, Borowitz and Cartwright, 1972).

1.2.10 Temporal, circadian and circannual changes in plasma LH and testosterone levels in males. Seasonal changes in reproductive-endocrine activity in rams; role of the pineal gland

Figure 1.4 illustrates the negative feedback mechanisms which regulate the activity of the hypothalamic-pituitary-testicular

axis in males. LH secretion in males tends to remain reasonably consistent from day to day (see Purvis et al., 1974) whereas, in females, cyclic patterns of LH release occur (see Brown-Grant, 1971; Sawyer, 1975; McEwen, 1976b). This sexual dimorphism in the pattern of LH secretion is generally thought to result from sexual differentiation, early in life, of the pattern of luteinizing hormone releasing hormone (LHRH) release from the hypothalamus (see Gorski, 1973; Plapinger and McEwen, 1978). However, in gonadectomized males (rats: Gay and Sheth, 1972; ram: Riggs and Malven, 1974) and females (rhesus monkey: Dierschke, Bhattacharya, Atkinson and Knobil, 1970; ewe: Butler, Malven, Willett and Bolt, 1972), LH is released at regular intervals of around thirty to sixty minutes. These observations in castrated animals suggest, therefore, that the ultimate pattern of LH secretion in intact animals may result from an interplay between endogenous LHRH rhythms in the hypothalamus, and the negative feedback effects of gonadal steroids.

Although mean plasma LH and testosterone levels in males remain farily consistent from day to day, both hormones show episodic fluctuations during the course of a twenty-four hour period (see references in Table 1.14). As might be expected from Figure 1.4, peaks in plasma testosterone in males are generally preceded by an increase in plasma LH (see Figure 1.5, references in Table 1.14, and also Falvo, Buhl, Reimers, Foxcroft, Hunzicker, Dunn and Dziuk, 1975; Wilson and Lapwood, 1978; Toivola, Bridson and Robinson, 1978). However, Falvo et al. (1975) found that some increases in plasma testosterone in rams occurred in the absence of any apparent LH peak. The frequency of blood sampling may have accounted for the latter observation.

Table 1.14:

Episodic fluctuations in plasma LH and testosterone in males.

Species	Reference
rat, mouse	Bartke, Steele, Musto and Caldwell, 1973.
guinea pig	Moor and YoungLai, 1975.
ferret	Rieger and Murphy, 1977.
goat	Muduuli, Sanford, Palmer and Howland, 1979.
boar	Lapwood and Florcruz, 1978.
bull	Katongole, Naftolin and Short, 1971.
ram	Katongole, Naftolin and Short, 1974; Sanford, Winter, Palmer and Howland, 1974; Purvis, Illius and Haynes, 1974; Schanbacher and Ford, 1976; Wilson and Lapwood, 1978.
man	Smith, Tcholakian, Chowdbury and Steinberger, 1974; Rowe, Racey, Lincoln, Ellwood, Lehane and Shenton, 1975.

Figure 1.5:

Plasma luteinizing hormone and testosterone profiles in mature rams. The profiles illustrate the episodic nature of secretion of these two hormones and their temporal relationship.
(Sanford,L.M. et al 1974b)

SERUM LH (ng NIH-LH-S14/ml)
SERUM TESTOSTERONE (ng/ml)
LH
Testo.
Time in Hours

SERUM LH (ng NIH-LH-S14/ml)
SERUM TESTOSTERONE (ng/ml)
LH
Testo.
Time in Hours

In addition to episodic, short-term fluctuations in plasma LH and testosterone in males, circadian rhythms in the plasma levels of these hormones have also been reported in a number of species (see references in Table 1.15). The only evidence of a circadian rhythm in testosterone secretion in bulls was a slight, but consistent decline in plasma testosterone at around 1000 hours in young postpubertal animals (Thibier, 1976).

Although circadian rhythms in LH and testosterone secretion were reported for a number of species of monkeys (see references in Table 1.15), other studies in primates have failed to observe such rhythms (Wilson, Brown and Wilson, 1978; Van Horn, Beamer and Dixson, 1976). There are similar conflicting data regarding circadian rhythms in LH and testosterone secretion in boars (see references in Table 1.15, and Lapwood and Florcruz, 1978).

The majority of studies in various ram breeds have failed to demonstrate a circadian rhythm in plasma LH and testosterone similar to that reported for Finnish Landrace and Soay rams (see references in Table 1.15, and Katongole et al., 1974; Purvis, Illius and Haynes, 1974; Falvo et al., 1975; Schanbacher and Ford, 1976).

Seasonal changes in LH and testosterone secretion have also been reported for males of a large number of species (see references in Table 1.16). These circannual rhythms in the activity of the brain-gonadal axis in males are due to changes in the photoperiod. In rams, changes in daylength are thought to alter the sensitivity of the pituitary to both LHRH stimulation (Lincoln, 1977), and the negative feedback effects of gonadal steroids (Pelletier and Ortavant, 1975b; Parrott and Davies, 1979).

In most species studied, seasonal changes in LH and testosterone secretion have been related to breeding cycles (Gordon,

Table 1.15:

Circadian rhythms in plasma LH and testosterone in males.

Species	Reference
rat	Kalra and Kalra, 1977; Hostetter and Piacsek, 1977; Mock, Norton and Frankel, 1978.
rhesus monkey	Goodman, Hotchkiss, Karsch and Knobil, 1974; Michael, Setchell and Plant, 1974; Plant, 1981.
bonnet monkey	Mukku, Prahalada and Mondgal, 1976.
green monkey	Beattie and Bullock, 1978.
prosimian primates	Van Horn, Beamer and Dixson, 1976.
man	Rowe, Lincoln, Racey, Lehane, Stephenson, Shenton and Glover, 1974; Rowe, Racey, Lincoln, Ellwood, Lehane and Shenton, 1975; Gall, Glowania and Fisher, 1979.
stallion	Kirkpatrick, Vail, Devous, Schwend, Baker and Wiesner, 1976; Sharma, 1976.
pigmy goat	Muduuli, Sanford, Palmer and Howland, 1979.
boar (miniature pig)	Ellendorff, Parvizi, Pomerantz, Hartjen. König, Smidt and Elsaesser, 1975.
boar (German landrace)	Claus and Giménez, 1977.
ram (Finnish landrace)	Sanford, Winter, Palmer and Howland, 1974.
ram (Soay)	Lincoln, Peet and Cunningham, 1977.

Table 1.16:

Circannual rhythms in plasma LH and testosterone in males.

Species	Reference
rat	Mock, Kamel, Wright and Frankel, 1975; Mock and Frankel, 1978a,b.
ferret	Neal, Murphy, Moger and Oliphant, 1977.
golden hamster	Stetson and Tate-Ostroff, 1981.
bat	Gustafson and Shemesh, 1976.
stumptail macaque monkey	Slob, Ooms and Vreeburg, 1979.
crab-eating monkey	Dang and Meusy-Dessolle, 1981.
rhesus monkey	Robinson, Scheffler, Eisele and Goy, 1975; Gordon, Rose and Bernstein, 1976; Gordon, Bernstein and Rose, 1978.
squirrel monkey	Mendoza, Lowe, Resko and Levine, 1978.
red deer	Lincoln and Kay, 1979.
white-tailed deer	Mirarchi, Howland, Scanlon, Kirkpatrick and Sanford, 1978.
black bear	McMillin, Seal, Rogers and Erickson, 1976.
horse	Berndston, Pickett and Nett, 1974; Kirkpatrick, Wiesner, Kenney, Ganjam and Turner, 1977.
pygmy goat	Muduuli, Sanford, Palmer and Howland, 1979.
ram	Katongole et al ., 1974; Schanbacher and Lunstra, 1976; Schanbacher and Ford, 1976; Lincoln and Davidson, 1977; Sanford, Palmer and Howland, 1977.
man	Reinberg, Lagoguey, Cesselin, Touiton, Legrand, Delassalle, Antreassian and Lagoguey, 1978.

Rose and Bernstein, 1976; Schanbacher and Lunstra, 1976; Lincoln and Davidson, 1977; Neal, Murphy, Moger and Oliphant, 1977; Sanford, Palmer and Howland, 1977).

A seasonal shift in the timing of the circadian peak in plasma testosterone has been observed in rats (Mock, Norton and Frankel, 1978) and man (Reinberg, Lagoguey, Chauffournier and Cesselin, 1975).

Seasonal changes in LH and testosterone secretion and mating activity in rams

Sheep are recognized as short-day breeders (or negatively photoperiodic) which means that they show an increase in reproductive activity in response to decreasing or short day lengths (Yeates, 1949; Moule, 1950; Hafez, 1952; Ortavant, Mauleon and Thibault, 1964; Pepelko and Clegg, 1965). In both rams (compare Symington, 1961; Moule, 1950, with Ahmed, 1955; Lees, 1965; Pepelko and Clegg, 1965; Schanbacher and Lunstra, 1976) and ewes (Hafez, 1952) the breeding season is more clearly defined at higher latitudes. This is due to the fact that sheep at high latitudes experience greater seasonal changes in day length than those nearer to the equator.

Although rams may show some seasonal variation in libido, they will nevertheless mate throughout the year (Pepelko and Clegg, 1965; Shackell, Kelly and Allison, 1977). Therefore, it seems that the ewe is primarily responsible for determining seasonal breeding in sheep.

The principal role of photoperiod in determining seasonal changes in the activity of the hypothalamic-pituitary-testicular axis in rams has been established using artificial lighting regimes. It is now generally recognized that a decrease in day length

results in an increase in LH and testosterone levels in rams (see Pelletier and Ortavant, 1975a,b; Lincoln, 1976; Lincoln and Davidson, 1977; Sanford, Beaton, Palmer and Howland, 1976). Daylength is thought to influence LH secretion in rams by altering the sensitivity of the pituitary to both LHRH stimulation (Lincoln, 1977), and to the negative feedback effects of gonadal steroids (Pelletier and Ortavant, 1975b; Parrott and Davies, 1979). This results in changes in both the frequency and amplitude of the episodic peaks in plasma LH (Lincoln, 1978; Lincoln and Short, 1980; Lincoln, 1981).

In agreement with the controlled lighting studies above, the highest plasma concentrations of LH and testosterone in rams have generally been observed in late summer and autumn (Katongole et al., 1974; Sanford, Winter, Palmer and Howland, 1974; Schanbacher and Ford, 1976; Sanford, Palmer and Howland, 1977). However, in some studies in rams peak LH and testosterone levels were recorded during early and mid-summer (Gomes and Joyce, 1975; Wilson and Lapwood, 1978; Barrell and Lapwood, 1978/1979). An explanation of these latter observations has been provided by Lincoln. Lincoln and coworkers noted that Soay rams maintained in a natural environment showed an increase in plasma gonadotrophins and testosterone shortly before the summer solstice, when day length was still increasing, and a decline in the plasma levels of these hormones before the winter solstice (Lincoln and Davidson, 1977). They also observed that Soay rams kept under artificial lighting conditions became refractory to the effects of continuous long or short days after two to three months, and showed a spontaneous reversal in hypothalamic-pituitary-testicular activity in the absence of appropriate changes in the photoperiod. Lincoln suggested, therefore, that changes in day length are not the cause of sexual cycles in rams,

but rather, rams have endogenous sexual cycles, the timing of which can be influenced by changes in day length (Lincoln and Davidson, 1977). Thus possible breed differences in the sensitivity of rams to changes in the photoperiod (see Lincoln and Davidson, 1977; Sanford et al., 1978), together with local environmental conditions, may interact with changes in day length to determine the timing of the seasonal peak in reproductive endocrine activity in rams at a particular locality.

Since seasonal changes in plasma testosterone concentrations in rams have been correlated with changes in mating activity (see Schanbacher and Lunstra, 1976; Sanford, Palmer and Howland, 1977; Lincoln and Davidson, 1977), the suggestion has been made that changes in testosterone secretion are directly responsible for changes in sexual behaviour. However, attempts to increase the sexual drive of rams during the non-breeding season by administering testosterone (Knight, 1973; Mattner and Braden, 1975; Lincoln and Davidson, 1977), human chorionic gonadotrophin (Mattner and Braden, 1975) or gonadotrophic hormone releasing hormone (GnRH) (Schanbacher and Lunstra, 1977; Schanbacher, 1978), have been unsuccessful. For example, rams treated with GnRH had significantly higher plasma testosterone levels compared with controls, but failed to show an improvement in mating performance (Schanbacher, 1978). It would appear, therefore, that factors other than changes in the plasma testosterone concentration also contribute to seasonal changes in mating activity in rams.

The photoperiod represents an environmental factor that undergoes consistent seasonal changes and would therefore seem to be a reliable exteroceptive cue for determining seasonality. In fact, the mating activity of rams can be increased during the non-

breeding season simply by restricting their hours of daylight (Yeates, 1949; Moule, 1950; Schanbacher, 1979). Mattner (1977) suggested that changes in day length may alter the sensitivity to testosterone of central neural tissues that control sexual behaviour. Some support for this hypothesis was provided by a study in red deer. Castrated stags that were implanted with capsules containing testosterone propionate showed mating activity only during their normal breeding season (Lincoln, Guinness and Short, 1972).

Seasonal changes in the sensitivity of the brain to gonadal steroids may involve changes within central neural tissues in the numbers of gonadal steroid receptors, the activity of enzymes that convert testosterone to active metabolites (see Section 1.2.5), or the concentration of various neurotransmitters associated with sexual responses (see Section 1.2.7).

The pineal gland and seasonality in rams

The pineal gland is the only organ in mammals whose activity is known to be controlled by central mechanisms that are directly associated with visual pathways (De Groot and Gritchlow, 1960; Moore, 1969, 1973; Moore, Heller, Bhatnager, Wurtman and Axelrod, 1968; Moore and Klein, 1974: Wurtman, Axelrod and Kelly, 1968; Reiter, Sorrentino and Jarrow, 1971; Cardinali and Wurtman, 1975). Furthermore, this organ secretes a host of compounds that affect reproductive endocrine function in mammals (Cardinali, 1974; Vaughan, Reiter, McKinney and Vaughan, 1974; Benson, 1977; Lerner, 1978; Ebles, 1979; Orts, Bruot and Sartin, 1980). It has been proposed, therefore, that the pineal gland may mediate the effects of photoperiod in seasonal breeders (see Reiter, 1974).

The main compound secreted by the pineal is the indoleamine melatonin. Melatonin has been found to have both an inhibitory (Reiter and Sorrentino, 1970; Reiter, Sorrentino and Hoffman, 1970; Minneman and Wurtman, 1975; Turek, Desjardins and Menaker, 1976) and a stimulatory (Hoffman and Reiter, 1966; Van Bronswijk, Smith, Van Der Kar, Pevet and Ariens-Kappers, 1975; Reiter, Blask and Vaughan, 1975; Reiter, Vaughan, Rudeen and Philo, 1976) effect on processes associated with reproduction in mammals. The pineal gland is also regarded as a supplement source of GnRH in rats, cattle, pigs and sheep (Millar, Denniss, Tobler and Symington, 1981).

In a series of experiments using Romney rams Barrell and Lapwood (1978, 1978/1979a, 1979a) investigated the effects of removing either the superior cervical ganglia (which is effectively the same as removing the pineal since all neural afferents to the pineal pass through these ganglia (see Moore, 1973; Axelrod, 1974) or the pineal, on the neuroendocrine response of rams to changes in the photoperiod. The results of these studies suggested that the pineal gland is required for normal seasonal changes in LH, prolactin and testosterone secretion in rams. Seasonal changes in semen production were also affected by pinealectomy (Barrell and Lapwood, 1979b).

Lincoln (1979) reported that ganglionectomized Soay rams maintained under conditions of artificial lighting also failed to respond to changes in the photoperiod. However, these rams did show long-term changes in LH and testosterone secretion that were unrelated to changes in the light-dark cycle. These observations provide further support for the hypothesis that rams have an endogenous sexual cycle (Lincoln and Davidson, 1977; Lincoln

et al., 1977), and they also suggest that this cycle is entrained to changes in the photoperiod by mechanisms that involve the pineal gland (Lincoln, 1979).

1.2.11 Aims of the present study

It will have become evident from the foregoing literature review that the bulk of our understanding regarding the role of gonadal steroids in the sexual behaviour of both males and females has been derived mainly from studies in small laboratory animals, particularly rodents. This is quite understandable in view of their ease of handling, and the relatively low costs associated with working with small animals. Where larger farm animals have been used in comparative studies, the observations are limited to only a few individuals. However, since farm animals are of economic importance, a better understanding of the role of hormones in their behaviour may lead to improvements in farm management, and consequently productivity.

In the present study, the relationship between plasma testosterone levels and sex drive was investigated in adult rams. The aim of these experiments was to determine if there is any significant positive correlation between plasma testosterone levels and mating activity for individual rams. If such a relationship was shown to exist, then plasma testosterone levels may provide a convenient practical index for selecting stud rams with good libido. This could perhaps replace or be used in conjunction with the common, but unreliable practice of selecting stud rams solely on genetic background and appearance.

The effects of mating activity on plasma testosterone levels in rams were also investigated. Although it has not been clearly

established that an increase in plasma testosterone is a consistent feature of mating in males, the magnitude of the testosterone response, if it occurs, may be related to the sex drive of individual animals.

Rams also show seasonal variations in reproductive-endocrine activity that are due primarily to seasonal changes in the photoperiod. Apart from the obvious effects of latitude on the influence of photoperiod on reproduction in rams, there also seem to be breed differences in the sensitivity and therefore response of rams to changes in day length. It is important, therefore, to know the response to changes in the day length of a particular breed, in a certain locality, in order to ascertain the optimum mating period for that breed. In the present study seasonal changes in plasma testosterone concentration and mating activity were determined for Merino and four British ram breeds.

In order to obtain a better understanding of the finer control of mating behaviour by testosterone in rams, adult animals that had been castrated before puberty (wethers) were treated with graded doses of testosterone propionate, and their mating response determined. It was anticipated that this approach would also give an indication of the threshold level of plasma testosterone required for normal mating activity in adult rams. Testosterone-treated wethers also have the potential for use as "teaser" animals for synchronizing oestrus in ewes at the commencement of the breeding season. This would remove the expense and problems associated with keeping vasectomized rams which are currently used as "teasers".

The hypothesis that the effects of testosterone on sexual behaviour in males are mediated by oestradiol-17β was investigated

by administering oestradiol-17β and non-aromatizable androgens to wethers. Oestradiol-17β and 5α-dihydrotestosterone were also administered together to test the theory that the complete mating response in males involves a synergism in the brain between oestrogenic and 5α-reduced metabolites of testosterone. This type of information is necessary if hormone-treated wethers are to be used as "teaser animals". Also, if oestrogens elicit sexual behaviour in males by binding to oestrogen receptors in the brain, then a range of compounds with oestrogenic activity might be expected to stimulate sexual behaviour in males. The capacity of oestrogenic compounds to elicit sexual behaviour in males might also be expected to be related to their relative affinity for oestrogen receptors. These concepts were investigated by administering both natural and synthetic oestrogens to wethers.

CHAPTER 2

MATERIALS AND METHODS

2.1 REAGENTS

Steroids were obtained from either Sigma Chemical Co., St Louis, Missouri, U.S.A., or Steraloids Inc., Wilton, New Hampshire, U.S.A. Radiolabelled steroids were purchased from The Radiochemical Centre, Amersham, England. Other materials were obtained as follows: heparin from Commonwealth Serum Laboratories, Melbourne, Australia; sodium pentobarbital (Nembutal) from Abbott Laboratories, Sydney, Australia; xylocaine from Astra Chemicals, North Ryde, Sydney, Australia; 14 gauge (size 2) indwelling cannulae from B. Braun Melsungen, West Germany; Sephadex G25 from Pharmacia (South Seas) North Ryde, Sydney, Australia; polydimethylsiloxane (Silastic) medical grade tubing and Silastic medical adhesive (Silicone Type A) from Dow Corning Co., Missouri, U.S.A. All solvents used for the steroid assays were analytical grade, and were redistilled before use. Tincture of iodine (1% iodine, 1% iodide and 70% (by weight) ethanol in water) was used to sterilize incision sites prior to surgical procedures.

2.2 GONADECTOMIZED RAMS AND EWES

2.2.1 Castrated rams (wethers)

Ram lambs were surgically castrated within four to six weeks after birth by members of the Waite Institute farm staff as part of routine farm management. Adult rams were castrated under general sodium pentobarbital anaesthesia using aseptic conditions.

2.2.2 Oophorectomized ewes

Adult ewes were oophorectomized under general anaesthesia using a ventral approach. A 5cm longitudinal incision immediately anterior to the mammary glands, and 2cm from the midline, allowed the ovaries to be located. Ligation of the ovarian pedicle allowed the ovaries to be removed without loss of blood.

Progesterone and oestradiol-17β were used to induce oestrous behaviour in oophorectomized ewes (see Robinson, 1955). Ewes received 20, 15 and 15mg of progesterone on days 0, 2 and 4, respectively, followed by 250µg of oestradiol-17β on day 6. Oestrus behaviour occurred about 24h after oestrogen injection and lasted throughout day 7.

2.3 STEROID HORMONE TREATMENT OF MALES

2.3.1 Oestrogen-filled Silastic capsules

The rate of diffusion of steroids through Silastic membranes depends upon the total surface area and thickness of the membrane, and on the diffusion constant of each steroid (Kincl and Rudel, 1971). Using this information capsules were prepared which were calculated to release daily, either 50 or 100µg of oestradiol-17β. Silastic tubing (3.35mm ID x 4.65mm OD and either 40 or 80mm in length) was filled with an unmeasured amount of crystalline oestradiol-17β, and the ends were sealed with Silastic medical adhesive. Before implanting, the capsules were incubated for 24h at 37°C in 5% bovine serum albumin in 0.01M phosphate buffered saline, pH 7.4. To implant the capsules an area of skin (10cm x 10cm) was sterilized with tincture of iodine. Xylocaine was then injected around this site to induce local anaesthesia. After making

a 1cm incision, a surgical trocar was used to position the implant subcutaneously.

2.3.2 Hormone injections

Steroids used for injection were dissolved in absolute ethanol and then diluted in 19 volumes of peanut oil.

2.4 LIBIDO TRIAL

The mating activity of rams and hormone-treated wethers was assessed in a libido trial similar to those described by Hulet, Ercanbrack, Price, Blackwell and Wilson (1962) and Mattner, Braden and George (1971). Libido trials were carried out by introducing a male into a yard (5.0 x 8.5m) containing six oophorectomized ewes which had received progesterone and oestradiol-17β to induce oestrous behaviour. Since approximately 75% of ewes respond to hormone treatment the males had to seek out sexually receptive ewes.

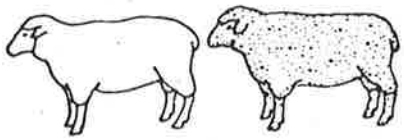
The various aspects of the mating behaviour of rams have been fully described by Banks (1964), Pepelko and Clegg (1965b) and Clegg, Beamer and Bermant (1969). In the present study those aspects of this behaviour which were recorded during a libido trial were: sniffs; nudges; mounts; intromissions; ejaculations (Figure 2.1). A description of these aspects of ram sexual behaviour is given below.

When a ram is introduced to a group of ewes, he seeks out a receptive partner by sniffing the perineum of the ewes. This sniffing affords olfactory information to the ram regarding the sexual status of individual ewes (see Section 1.2.8). If a ewe stands still after being sniffed, the next display, the nudge

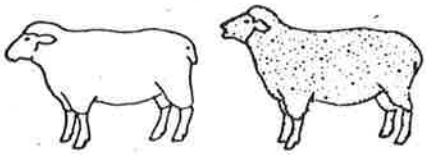
Figure 2.1:

Aspects of sexual behaviour in the ram. The motor patterns associated with sniffs, nudges, mounts and ejaculation are described in Section 2.4. Flehmen is a behaviour which generally occurs after a ram sniffs either the perineum or urine of a ewe. It is characterized by a curling back of the upper lip whilst the head is tilted back. This behaviour is thought to afford olfactory cues to the ram regarding the sexual status of the ewe (see Section 1.2.8).

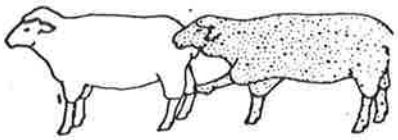
sniff



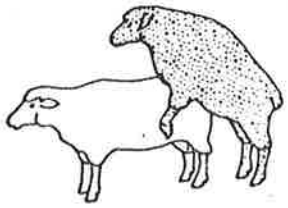
flehmen



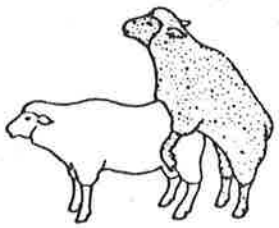
nudge



mount



ejaculation



occurs. Here the ram orients behind the ewe and makes contact with the flank of the ewe with his shoulder while the foreleg on the side of the ewe is extended and flexed in a kicking motion. At the same time, the ram tilts his head sideways towards the ewe and utters a series of grunts which are accompanied by an extension and retraction of the tongue and if the ewe remains still during and following nudge, the ram attempts a mount. During mounting, the ram straddles the rear quarters of the ewe with his forelegs and shows pelvic thrusts. The thrusts continue until intromission is achieved or the ram dismounts. Ejaculation is characterised by an especially deep thrust with the head tilted back. Following ejaculation there is usually a period of inactivity.

Some rams mount and achieve intromission and ejaculation without displaying sniffs or nudges, while others show no sexual behaviour beyond sniffs and nudges. The sequence of events culminating in ejaculation is therefore not invariable.

2.5 LIBIDO SCORE

To record mating activity data, the libido trial was divided into intervals of 15 seconds. Each aspect of ram mating behaviour observed during each 15 second interval was recorded once.

To calculate the libido score each aspect of sexual behaviour was given an arbitrary weighting as follows: sniff, 1; nudge, 3; mount, 6; intromission, 8; ejaculation (ejaculatory response), 10. For each component, the number of 15 second intervals during which it was the highest behaviour recorded were summed. This number was then divided by the length of the trial (min) and multiplied by the weighting of that particular component. The scores thus

calculated for individual components were summed to give the overall libido score (see Equation 2.1).

Equation 2.1

libido score = $1/n \; \Sigma \; (1a + 3b + 6c + 10d)$

where a = sniffs; b = nudges; c = mounts; d = ejaculatory response (intromission and ejaculation); and n (in min) = the length of the libido trial.

The equation outlined above is based on a procedure described by Grunt and Young (1951; 1952) for calculating libido scores in male guinea pigs.

2.6 BLOOD SAMPLING

Single blood samples were taken from the jugular vein by venepuncture. For serial sampling, an indwelling Braunula cannula was positioned in the jugular vein. An attempt was made to cannulate animals on the day before an experiment. After drawing a blood sample the cannula was flushed with 0.9% sterile saline containing heparin (100 units/ml) and antibiotic. Blood samples were collected into 10ml heparinized centrifuge tubes and usually centrifuged immediately. If there was an appreciable time lag between collection and centrifugation, the samples were kept on ice. The plasmas detained after centrifugation were stored at $-20^{o}C$ until required for hormone assay.

2.7 HORMONE ASSAYS

2.7.1 Phosphate buffers

(A) 0.16M, pH 7.3 (PB)

19.2g NaH_2PO_4 (anhydrous) in 800ml of glass distilled water

adjust pH to 7.3 with 1N NaOH
adjust volume to 1 litre.

(B) 0.1M, pH 7.0, 0.1% gelatin (gel PBS)

12.0g NaH_2PO_4 (anhydrous)
1.0g Sodium azide
9.0g Sodium chloride
1.0g gelatin

dissolve in 800ml of glass distilled water
adjust pH to 7.0 with 1N NaOH
adjust volume to 1 litre

2.7.2 Liquid scintillation counting

(A) Scintillation fluid

4.0g PPO (2,5-diphenyloxazole)

0.4g POPOP ($2,2^1$-p-phenylenebis (5-phenyloxazole))

dissolve in 1 litre of toluene

(B) Counting of aqueous systems

after adding aqueous sample and scintillation fluid, the scintillation vials were shaken vigorously for 10min to facilitate the transfer of radioactivity from the aqueous to organic phase.

Radioactivity was determined in a Packard liquid scintillation spectrometer Model 3380.

2.7.3 Testosterone radioimmunoassay

Plasma testosterone concentrations were determined using antisera raised in a ewe against a testosterone-3-carboxymethyl-oxime-BSA conjugate (Dr R.I. Cox, Hormone Assay Development Group, CSIRO Division of Animal Production, Blacktown, N.S.W.). The specificity of this antisera is shown in Table 2.1.

Duplicate aliquots of plasma (from 20 to 100µl depending on testosterone concentration) were added to glass extraction tubes and the final volume adjusted to 120µl with distilled water. After adding 1ml of toluene:hexane (2:1) the plasma was vigorously extracted for 5min on a mechanical shaker. Thereafter the plasma was frozen in a liquid nitrogen-ethanol bath and the solvent extract was decanted into glass incubation tubes. The solvent mix was then blown down under a stream of nitrogen gas ($40^{o}C$). The same volume of solvent mix that was used for extraction was also blown down in a series of tubes that were used to prepare standards. Standards were prepared by mixing graded amounts of testosterone (0 to 300pg) in 50µl gel PBS) with (1,2,6,7(n)-^{3}H) testosterone (approx. 20 x 10^{3}dpm in 100ml gel PBS, specific activity 93Ci/mmol) and antisera (in 100µl gel PBS; 1:20,000 final dilution. Equivalent amounts of radiolabelled testosterone and antisera were also added to dried extracts together with 50µl of gel PBS to account for the volume of the standards. Assay tubes were incubated at $4^{o}C$ for at least 12h. Free and protein-bound steroid were separated by the addition of 100µl of 1% gamma globulin followed by 900µl of 22% polyethylene glycol 6000. The tubes were allowed to stand at $4^{o}C$ for 15 to 30min and were then centrifuged at 800 x g for 10min. The supernatant (free fraction) was transferred to scintillation vials and radioactivity determined using the scintillation system described in Section 2.7.2.

Table 2.1:

Specificity data for testosterone antisera. A, cross reactivity determined by Dr M. Wong, Hormone Assay Development Group, CSIRO, Division of Animal Production; B, cross reactivity determined by author.

Steroid	% Cross reaction*	
	A	B
Testosterone	100.00**	100.00
5α-Dihydrotestosterone	31.00	35.00
4α-androstene-3β, 17β-diol	30.00	
4α-androstene-17β,19-diol-3-one	3.5	
Androstenedione	1.3	2.0
Epitestosterone (17α-hydroxy-4-androstene-3-one	0.11	
Etiocholanolone (5β-androstane-3α-ol-17-one)	0.10	
Androsterone (5α-androstane-3β-ol-17-one)	0.02	
Dehydroepiandrosterone (5-androstene-ol-17-one)	<0.01	
Oestradiol-17β	0.10	0.07
Oestrone	<0.003	
Oestriol	0.003	
Progesterone	<0.004	
Cortisol	0.003	

* calculated from the amount of steroid required to suppress maximum binding of ^{3}H testosterone by 50%.

** value for testosterone arbitrarily set at 100%.

A typical standard curve for the testosterone assay is shown in Figure 2.2. Data for recovery (efficiency of extraction), sensitivity (2 x standard deviation of the blank (5 pg)), accuracy (amount of steroid measured relative to amount added) and precisions (intra- and inter-assay coefficients of variation, $\frac{\text{standard deviation}}{\text{mean}} \times 100\%$) are given in Table 2.2 (see Abraham, 1975).

Plasma testosterone concentrations were determined without chromatography after solvent extraction. However, the only steroids which show appreciable cross reactivity with the antisera used are 5α-dihydrotestosterone and androstenediol (Table 2.1). Since the levels of these androgens in rams are very low relative to testosterone, they do not contribute significantly to the testosterone values (Falvo and Nalbandov, 1974; Schanbacher, 1976).

2.7.4 Cortisol competitive protein binding assay

The plasma concentration of cortisol was determined by competitive protein binding assay utilizing the naturally occurring corticosteroid binding globulin (CBG) of the dog (Basset and Hinks, 1969). The specificity of this CBG is shown in Table 2.3.

Preparation of CBG

To remove endogenous corticoids from dog plasma 5ml of plasma was eluted with PB (0.16M, pH 7.3) through a column (22mm x 400mm) of Sephadex G25 coarse surrounded by a water jacket of 45°C. CBG was collected in the 20ml fraction which followed the void volume. The void and bed volumes of the Sephadex column were determined using 2% blue dextran and 2% potassium ferricyanide, respectively (Figure 2.3). The CBG fraction was stored at -20°C until required for assay. The working mixture of CBG was prepared by adding 10ml of the CBG fraction, 1 ml of ethanol and 8μCi of [1,2,6,7(n) ^{3}H] cortisol (specific activity 85Ci/mmol) to 189ml PB.

Figure 2.2:

Standard curve for the testosterone radioimmunoassay; (see Section 2.7.3) and parallel curve obtained by assaying increasing volumes of plasma (O). Each point on the standard curve represents the mean of ten assays with the S.E.M. indicated by the vertical bars.

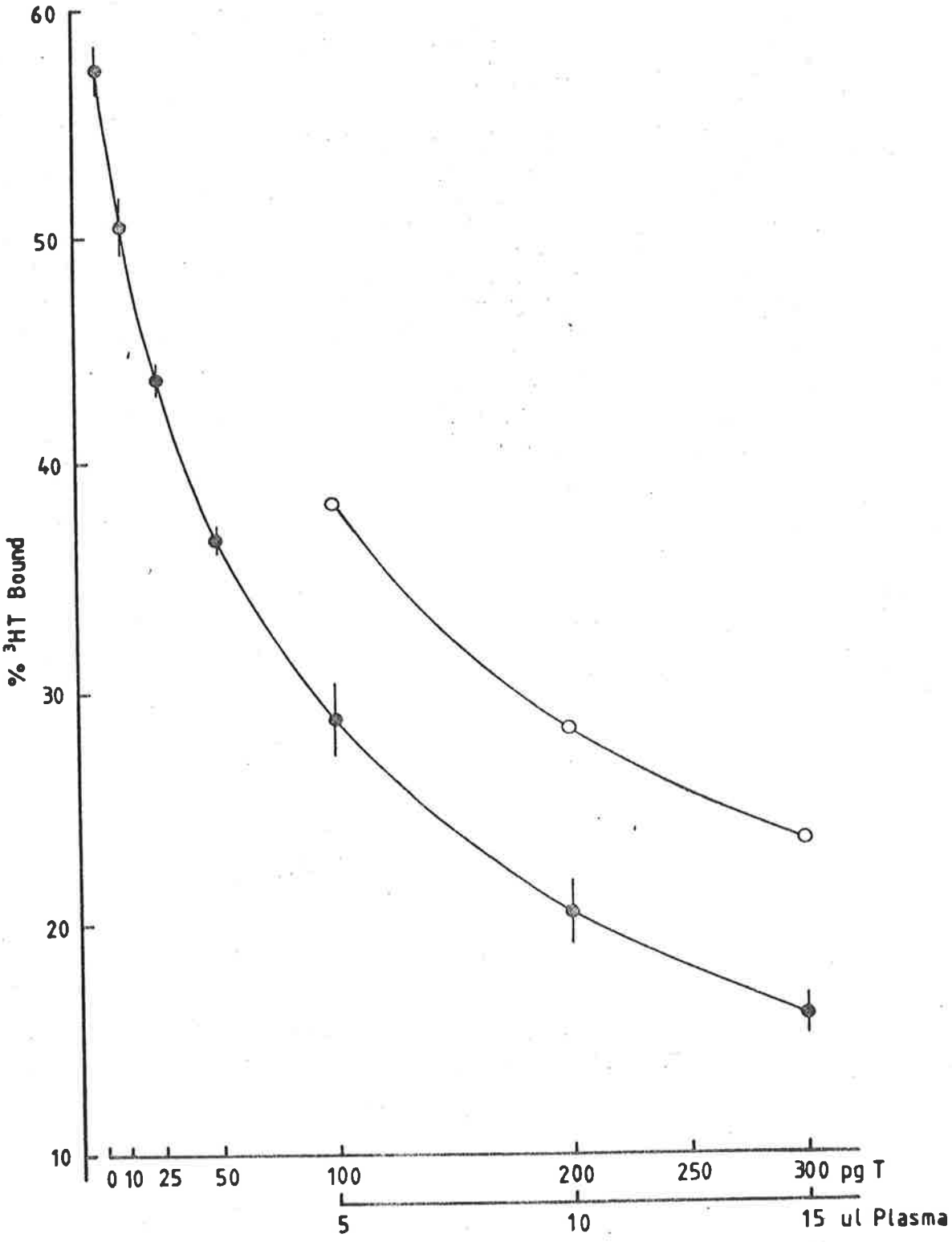

60
50
40
30
20
10
% ³HT Bound
0 10 25 50 100 200 250 300 pg T
5 10 15 ul Plasma

Table 2.2:

Validation data for testosterone radioimmunoassay and cortisol competitive protein binding assay. Values are presented as means ± S.E.M. except for sensitivity and precision.

	Testosterone	Cortisol
Recovery[a,b] (%, n = 10)	88.5 ± 0.8	84.5 ± 1.7
Accuracy[a] (n = 5) 25pg[c]	20.3 ± 0.8 (81.2)[d]	0.50ng[c] 0.44 ± 0.03 (88.0)[d]
100 "	99.2 ± 8.1 (99.2)	1.50 " 1.31 ± 0.09 (87.3)
200 "	205.2 ± 12.2 (102.6)	5.00 " 4.71 ± 0.12 (94.2)
	(slope = 0.99; r^2 = 0.99)	(slope = 0.96; r^2 = 0.98)
Sensitivity	8pg	250pg
Precision		
intra-assay C.V. (%)	6.5	8.3
inter-assay C.V. (%)	9.4	12.1

[a] recovery and accuracy were determined using oophorectomized ewe plasma treated with dextran-coated charcoal (CTP) to remove endogenous steroids.

[b] tritium labelled steroid was added to CTP and incubated at 37°C for 30min followed by 4°C for 16h before appropriate extraction. Solvent extracts were derived in scintillation vials and radioactivity determined.

[c] known amounts of cold steroid were added to CTP and treated as described above (b) before extraction and assay. The values for amount of steroid measured are corrected for recovery.

[d] values in parentheses indicate percentage accuracy.

Table 2.3:

Specificity of corticosteroid binding globulin.

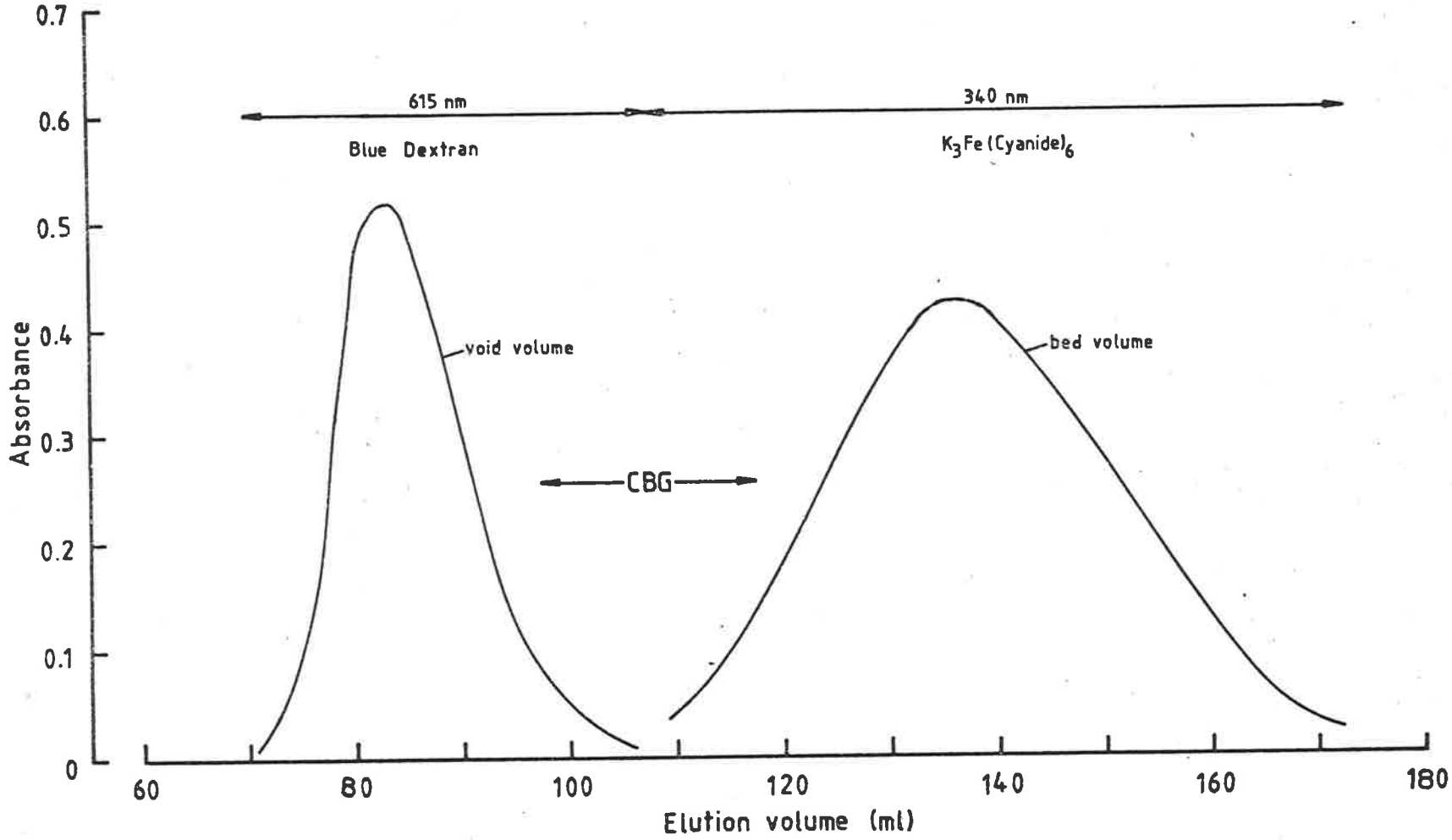
Steroid	% Cross Reaction*
Cortisol	100**
Corticosterone	43
Cortisone	20
Progesterone	22
Oestradiol-17β	8
Dehydroepiandrosterone	6
Testosterone	3

* calculated from the amount of steroid required to suppress maximum binding of ^{3}H cortisol by 50%.

** value for cortisol arbitrarily set at 100%.

Figure 2.3:

Elution profiles for blue dextran and potassium ferri cyanide ($K_3Fe(Cyanide)_6$ from a Sephadex G-25 coarse column (28mm x 400mm) described in Section 2.7.4. The elution volume for corticosteroid binding globulin (CBG fraction) is also indicated.


0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Absorbance
615 nm
340 nm
Blue Dextran
$K_3Fe(Cyanide)_6$
void volume
bed volume
CBG
60
80
100
120
140
160
180
Elution volume (ml)

Cortisol assay

Extraction: duplicate 200µl aliquots of plasma were extracted for 10min with 5ml of freshly redistilled diethyl ether. The plasma was then frozen and the ether decanted into incubation tubes and blown down under a stream of nitrogen gas (2.7.3).

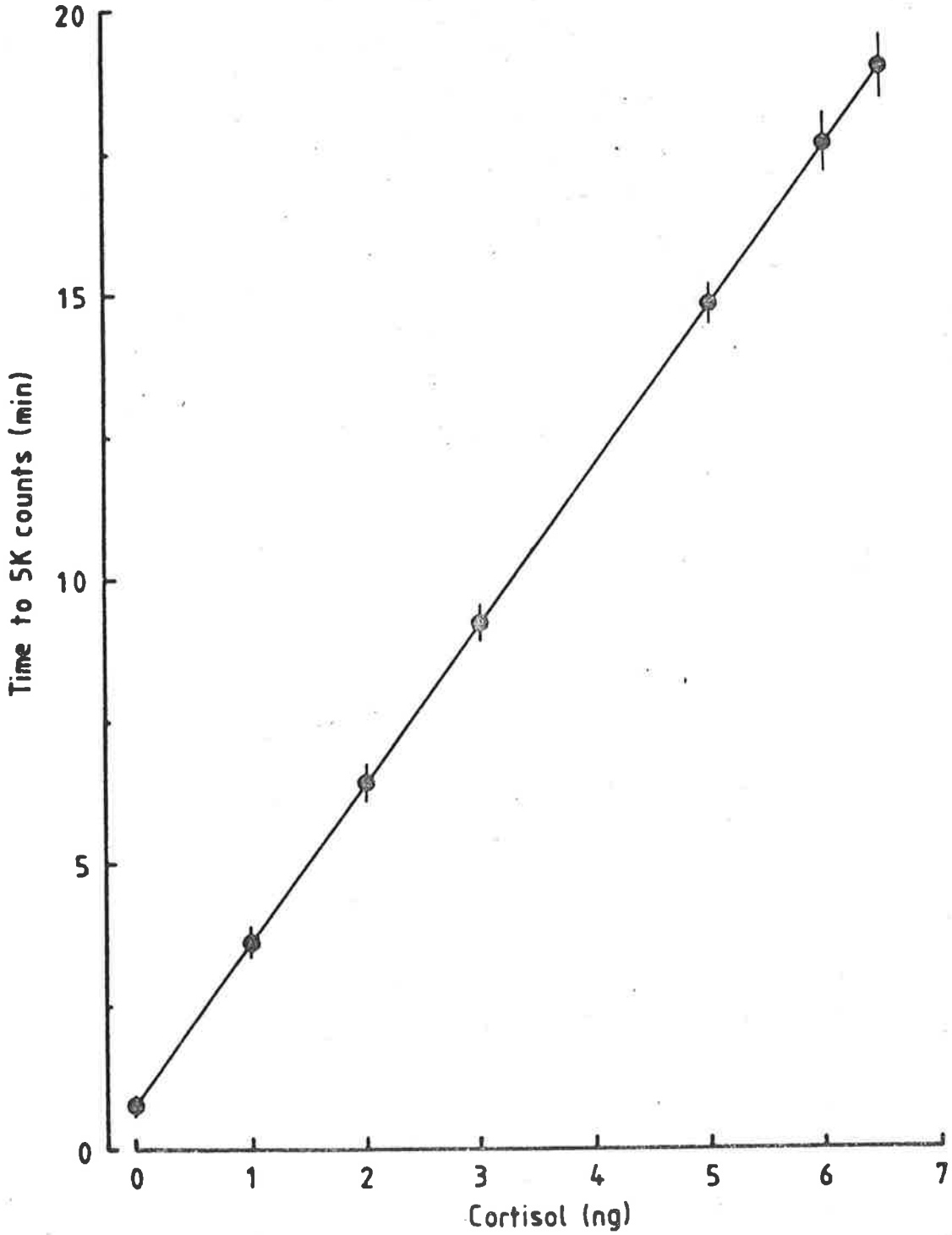
Assay: cortisol standards were prepared by mixing graded amounts of cortisol (0 to 6.5ng in 100µl PB) with 500µl of the working mixture of CBG. The same volume of CBG mixture was added to the dried extracts together with 100µl of PB. Assay tubes were incubated at 45°C for 5min and then stored at 4°C for at least 6h.

Separation of free and CBG-bound cortison: free and CBG-bound cortisol was separated on Sephadex G25-fine mini-columns (6mm x 25mm; 500mg Sephadex) at 4°C. After washing the columns with 3ml of PB, 200µl of the assay volume was transferred to the top of the column and eluted with 1.5ml of PB (bound fraction) into scintillation vials. Radioactivity was determined using the scintillation system described in Section 2.7.2.

A typical standard curve for the cortisol assay is shown in Figure 2.4. Data for recovery, sensitivity, accuracy and precision are given in Table 2.2.

Figure 2.4

Standard curve for the cortisol competitive protein binding assay (see Section 2.7.4). Each point represents the mean of ten assays with the S.E.M. indicated by the vertical bars.


20
15
10
5
0
Time to 5K counts (min)
0
1
2
3
4
5
6
7
Cortisol (ng)

CHAPTER 3

RELATIONSHIP BETWEEN PLASMA TESTOSTERONE CONCENTRATION AND MATING ACTIVITY IN ADULT RAMS: SEASONAL CHANGES IN THE TESTOSTERONE PROFILE AND MATING ACTIVITY

3.1 INTRODUCTION

The selection of rams for flock mating has historically been based on genetic background, rather than a knowledge of the mating drive of individual rams. In an effort to gauge the mating potential of individual rams before their introduction to the ewe flock, Mattner, Braden and George (1971) developed a pen mating test (libido trial) for assessing ram libido. Although libido trials seem to provide a reasonably accurate estimate of the subsequent mating performance of rams in the field (Mattner et al., 1971), they are tedious to conduct and also require specially trained observers. In recent years attention has focused on the relationship in males between the level of reproductive hormones in the blood and mating drive. If a relationship between blood hormone levels and libido could be demonstrated in rams, then plasma hormones might provide a convenient alternative index for selecting animals with high reproductive drive.

The principal androgen secreted by the testes and found in the circulation of male vertebrates is testosterone (Section 1.2.4). It is generally accepted, therefore, that this steroid (or its metabolites) is responsible for both sexual behaviour and maintenance of the sex structures in males.

In several studies in guinea pigs (Harding and Feder, 1976), bulls (Thibier, 1975; Foot, Munkenbeck and Green, 1976) and rams

(Schanbacher and Lunstra, 1976), no relationship was found between plasma testosterone levels and mating drive. However, in these studies an estimate of plasma testosterone levels was obtained from only one or two blood samples. Since it is now known that the plasma testosterone concentration in males does not remain constant, but instead shows episodic fluctuations (Section 1.2.10), a reliable estimate of the testosterone status of individual animals requires a series of blood samples to be taken at frequent intervals. Such serial sampling allows the testosterone status of males to be expressed in terms of either the frequency of testosterone peaks, mean peak height, basal testosterone levels, the mean testosterone level, or a combination of these variables.

Although testosterone is secreted episodically in rams, Purvis, Illius and Haynes (1974) found that the 24h pattern of the plasma testosterone profile (i.e. the number of episodic peaks; basal levels; mean levels) for individual rams remained consistent, not only from day to day, but also over a period of several weeks. Similar observations have been reported for the testosterone profile in bulls (Katongole, Naftolin and Short, 1971). The libido of individual rams also seems to remain reasonably consistent during a series of libido trials conducted over a relatively short period of time (Mattner, Braden and George, 1971; Wilkins and Kilgour, 1977). These observations suggest, therefore, that 24h plasma testosterone profiles, and libido trials, provide a good indication of the endocrine and reproductive status of individual rams for a particular time of the year.

Despite the apparent short-term consistency in the testosterone profile in rams, marked seasonal changes in the profile

have been reported (Section 1.1.10). These seasonal fluctuations in testosterone secretion occur in response to changes in the photoperiod, and have been associated with corresponding changes in mating drive (Section 1.2.10). However, the influence of photoperiod on seasonalality in sheep varies with latitude, and there may also be breed differences in the sensitivity (and therefore responsiveness) of rams to changes in daylength Section 1.2.10). It is imperative, therefore, to establish the seasonal changes in reproductive-endocrine activity of different ram breeds in a particular locality, in order to select the optimum mating period for each breed.

The initial experiments described in this chapter were designed to investigate the relationship between plasma testosterone concentration and mating activity in individual rams. The main purpose of these experiments was to determine if testosterone status could be used as a practical index for predicting the mating potential of a particular individual. Merino rams were used for these experiments. Merinos were also used for a preliminary investigation of seasonal changes in mating activity and in the characteristics of the plasma testosterone profile. A more detailed study of seasonal changes in reproductive-endocrine activity in rams was carried out using Border Leicester, Polled Dorset, Romney and Suffolk rams. This latter study also provided the opportunity to investigate possible breed differences in the sensitivity of rams to changes in the photoperiod.

3.2 EXPERIMENTAL PROCEDURES

3.2.1 Assessment of ram libido

A description of the libido trials is given in Sections 2.4 and 2.5 of Chapter 2, together with the procedure for calculating the libido score.

3.2.2 Blood collection and plasma testosterone assay

For 24h blood sampling the rams were brought indoors and placed in individual pens. Illumination was provided by natural lighting so that the light:dark cycle and total hours of daylight were not interrupted. Whilst indoors, the rams were fed a standard ration of lucerne-chaff between 0800 and 0900h and water was available at all times. They were allowed several days to acclimatize (except where otherwise stated) before the 24h collection period.

Indwelling jugular cannulae were used for taking hourly blood samples (Section 2.6), and the plasma testosterone concentration was determined using the testosterone radioimmunoassay described in Section 2.7.3. In experiment 3.2.3 all samples from one ram were analysed in the same assay whilst in experiment 3.2.4 all samples for each month were assayed together.

3.2.3 Relationship between the 24h plasma testosterone profile and the libido in rams

A total of eighteen adult Merino rams were used in this experiment and were maintained under field conditions except during 24h blood collection (Section 3.2.2). The rams were accustomed to handling and the experimental procedures used.

Data on plasma testosterone levels and mating drive were obtained from sixteen of the above rams during December and January. Because of the large number of rams involved in this experiment, it became logistically necessary to divide them into two groups (eight rams per group). One group was bled during the fourth week in December (see Figure 3.1A), and the other during the third week in January (see Figure 3.1B). The libido of all rams was assessed twice, during the first and second weeks in January (see Figure 3.1E).

Although the testosterone profiles and libido records were obtained on different days in this experiment, it was presumed that the two sets of data would provide an accurate indication of the testosterone status and mating drive of individual rams for that particular time of the year. This assumption was based on earlier reports which had shown that both the 24h testosterone profile and libido of individual rams remained reasonably consistent over a period of several weeks (see Introduction to this chapter).

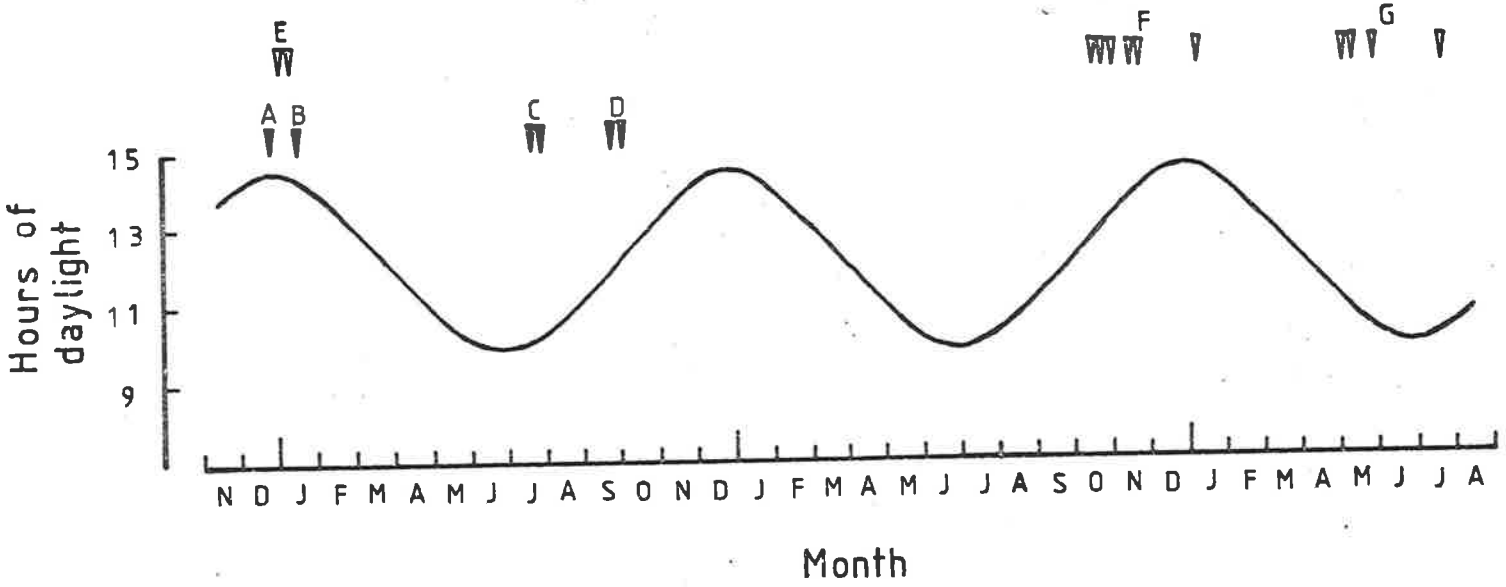
The testosterone profile of some of the rams (selected at random) was also determined later (July, September and October) in the same year (see Figure 3.1C,D). Additional libido trials on three of the rams used in December and January (plus two others from another group) were carried out a year later during October to January and during May to July (see Figure 3.1F,G).

3.2.4 Seasonal changes in plasma testosterone concentration and libido in Border Leicester, Polled Dorset, Romney and Suffolk rams

Three rams from each of the above breeds were used in this study and were maintained under field conditions as a single

Figure 3.1

Seasonal changes in daylight hours at Adelaide (35°C) not including the contribution from twilight. The data were obtained from the Smithsonian Meteorological Tables. Also indicated are the different times when serial blood samplings (▼) and libido trials (▽) were carried out using Merino rams (experiment 3.2.3).


E
A B
C
D
F
G
15
13
11
9
Hours of daylight
N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A
Month

group. The rams were around $2\frac{1}{2}$ year old at the start of the study. They were accustomed to handling and to the various experimental procedures used.

Plasma testosterone and mating activity were recorded from the rams over a period of one year, beginning in January. Each month (except April and September when the facilities required for this study were not available) the libido of the rams was assessed using the standardized libido trials. In the evening of the day of the libido trials the rams were brought indoors, placed in individual pens, and fitted with an indwelling jugular cannula. Hourly blood sampling began at 1000h the next morning and was continued for 24h. The rams were returned to their paddock within several hours after the last blood sample. The design of this experiment allowed further observations on the relationship between plasma testosterone levels and mating drive for individual rams.

Because of the large number of plasma samples generated by this latter study, it was decided to pool the plasmas for every two hours when carrying out the testosterone assays. Although this meant that some of the finer detail of the testosterone profiles was lost, the data still provided a good indication of the testosterone status of individual animals.

3.2.5 Statistical Analyses

Effects of time of year on plasma testosterone concentrations were analysed by analysis of variance techniques (Steel and Torrie, 1960). Correlation coefficients were used to determine the relationship between plasma testosterone concentrations and mating activity (Sokal and Rolf, 1969).

3.3 RESULTS

Figure 3.1 shows the annual changes in the photoperiod at Adelaide which is situated at 35°S (these data were obtained from the Smithsonian Meterological Tables).

3.3.1 Relationship between the 24h plasma testosterone profile and mating drive in Merino rams

The 24h plasma testosterone profiles for Merino rams sampled in December and January are shown in Figures 3.2 and 3.3 respectively. A characteristic feature of these profiles was the presence of episodic peaks in the plasma testosterone concentration. Although these episodic peaks were observed in all rams, there were large differences between individual animals with regard to the number of testosterone peaks that occurred during a 24h period (range 1 to 9).

There was no evidence of a diurnal rhythm in testosterone secretion during either December or January (Figure 3.4). However, there was a significant effect of month on the testosterone levels (Table 3.1), and the group mean for January (5.7ng/ml) was appreciably higher than the corresponding value for December (3.8ng/ml). This difference was due mainly to an increase in the number of testosterone peaks and higher basal levels during January, rather than a difference in peak height (Table 3.2).

Libido data obtained during the first and second weeks in January for all the above rams is presented in Table 3.3. Rams 5 and 13 failed to achieve a service during the first libido trial, but both rams served the ewes one week later. Ram 11 showed appreciably more nudges and mounts during the first libido trial

Figure 3.2:

Plasma testosterone profiles for Merino rams bled at hourly intervals for 24h in December (experiment 3.2.3, see also 3.3.1).

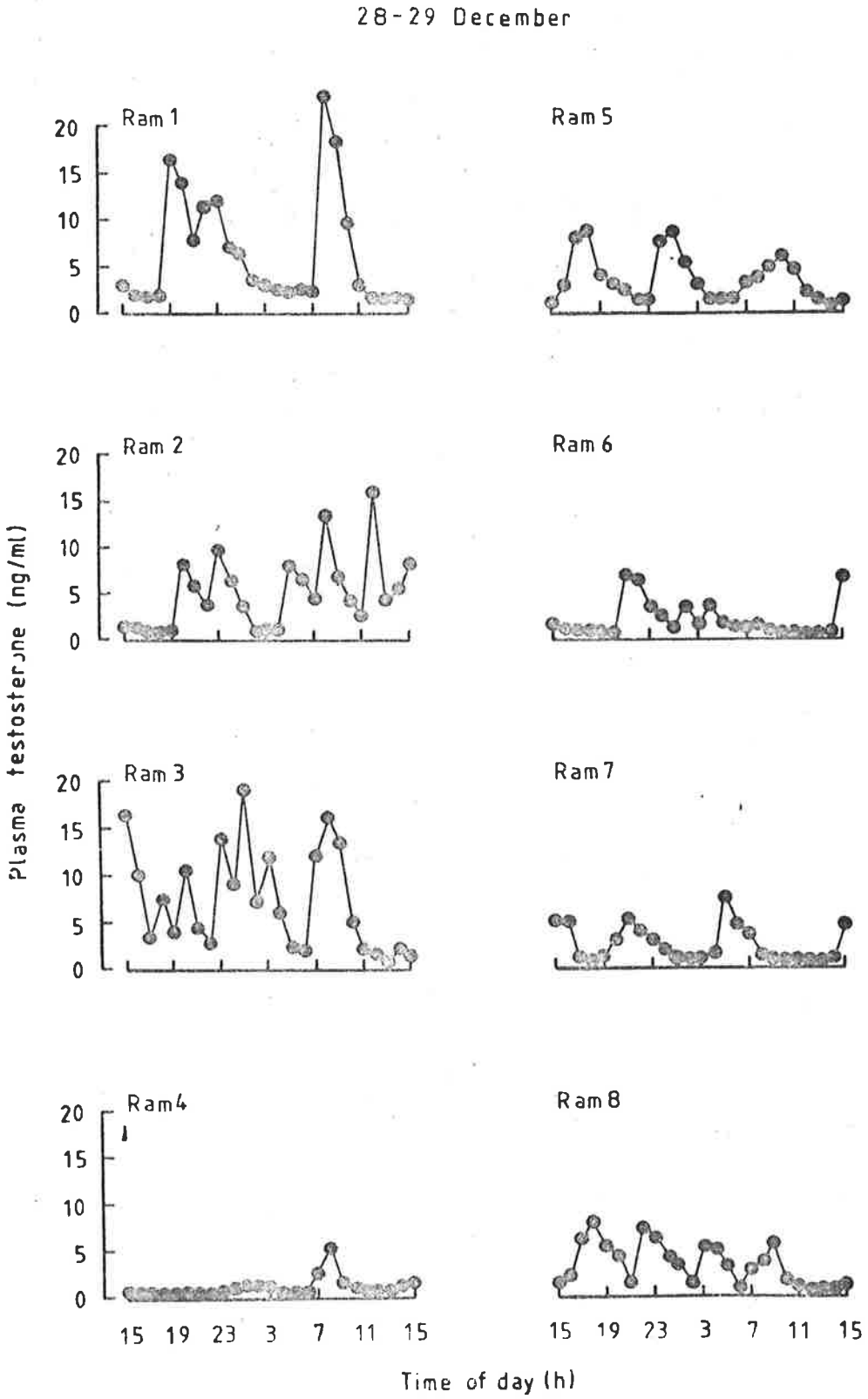

28-29 December
Ram 1
Ram 5
Ram 2
Ram 6
Ram 3
Ram 7
Ram 4
Ram 8
20
15
10
5
0
Plasma testosterone (ng/ml)
15 19 23 3 7 11 15
Time of day (h)

Figure 3.3

Plasma testosterone profiles for Merino rams bled at hourly intervals for 24h in January (experiment 3.2.3, see also 3.3.1).

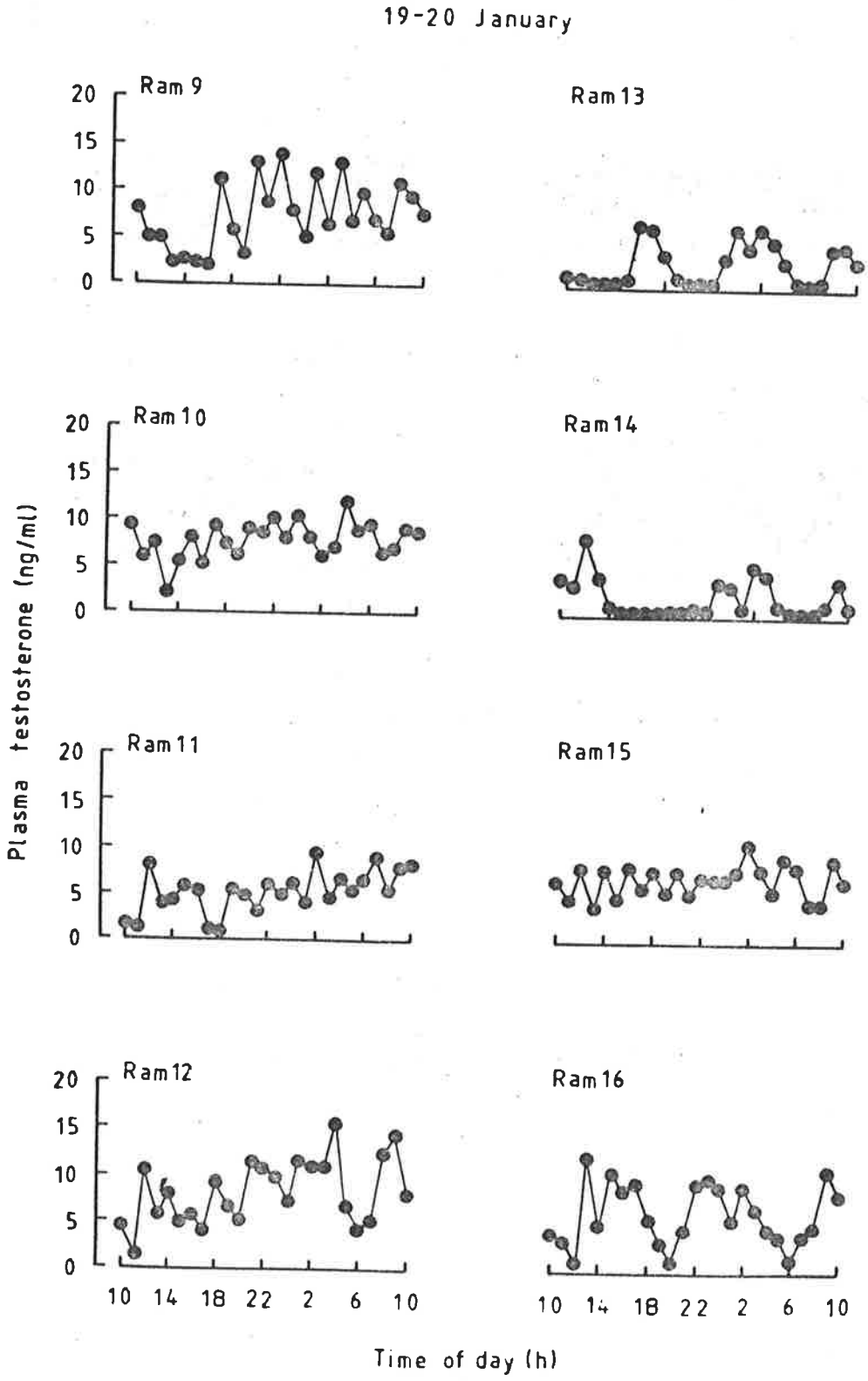

19-20 January
Ram 9
Ram 13
Ram 10
Ram 14
Ram 11
Ram 15
Ram 12
Ram 16
20
15
10
5
0
Plasma testosterone (ng/ml)
10 14 18 22 2 6 10
Time of day (h)

Figure 3.4

Mean ± 95% confidence interval for plasma testosterone in two groups of eight Merino rams bled at hourly intervals for 24h in December (a) and January (b).

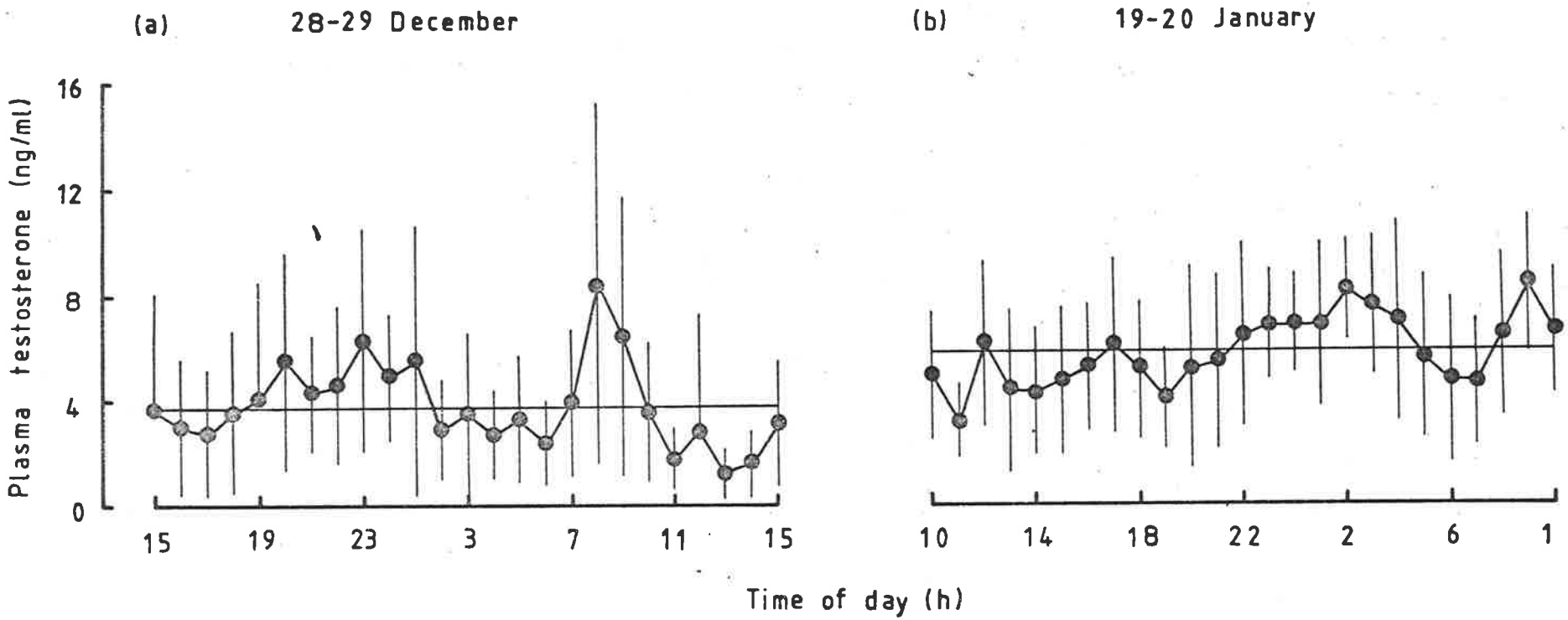

(a)
28-29 December
(b)
19-20 January
Plasma testosterone (ng/ml)
16
12
8
4
0
15
19
23
3
7
11
15
10
14
18
22
2
6
1
Time of day (h)

Table 3.1:

Analysis of variance of plasma testosterone concentrations in Merino rams sampled in December and January in experiment 3.2.3. The hormone values were analyzed on the log scale, using a generalized linear model with components for month, animals within month, and time of day.

The hormone data was transformed to $\log_{10}$ because of a log-normal distribution and heterogeneity among variances both within and between months

Source	DF	SS	MS	VR
Month	1	22.88	22.88	41.60**
Animals within month	14	145.90	10.42	18.95**
Time of day*	3	20.22	6.74	12.25**
Error	381	208.69	0.55	

* A polynomial term was included in the model for time of day.

** $p < 0.01$.

Table 3.2:

Characteristics of the plasma testosterone profiles of Merino rams sampled in December and January in experiment 3.2.3. The results are presented as means ± S.E.M. (n=3). F values were obtained from analysis of variance.

Month	Mean T (ng/ml)	Number of T peaks per 24h	Mean peak height (ng/ml)	Mean basal T level (ng/ml)
December	3.8 ± 0.8	4.0 ± 0.7	8.7 ± 1.4	1.5 ± 0.3
January	5.7 ± 0.8	6.4 ± 0.8	8.7 ± 0.9	3.3 ± 0.6
F value		5.65 P < 0.05	0.002 NS	6.42 P < 0.05

Table 3.3:

Plasma testosterone (T) concentrations and libido scores of Merino rams in experiment 3.2.3. Rams were bled during either the 4th week in December (1-8) or the 3rd week in January (9-16). The libido trials were carried out during the 1st and 2nd weeks in January.

Ram	Plasma T* (ng/ml)	Libido Score		
		Week 1	Week 2	$\bar{x}$
1	6.3	7.7 (2)**	9.9 (3)	8.8 (2.5)
2	4.9	7.5 (3)	8.1 (2)	7.8 (2.5)
3	7.3	10.3 (2)	8.9 (2)	9.6 (2)
4	1.1	6.7 (3)	4.1 (2)	5.4 (2.5)
5	3.4	8.9 (0)	6.1 (2)	7.5 (1)
6	1.9	13.9 (4)	10.6 (3)	12.3 (3.5)
7	2.3	4.3 (2)	-	4.3 (2)
8	3.4	7.1 (3)	6.3 (3)	6.7 (3)
9	7.2	10.0 (3)	7.9 (3)	8.9 (3)
10	7.7	6.8 (1)	5.5 (1)	6.2 (1)
11	5.2	8.6 (2)	3.8 (2)	6.2 (2)
12	8.2	8.8 (2)	6.1 (2)	7.5 (2)
13	2.7	3.3 (0)	4.2 (2)	3.8 (1)
14	2.1	8.9 (2)	7.1 (2)	8.0 (2)
15	6.7	8.8 (2)	11.9 (2)	10.4 (2)
16	6.4	6.5 (1)	6.8 (2)	6.7 (1.5)

* Mean ± S.E.M. of blood samples taken at hourly intervals for 24h.

** Value in parenthesis indicates the number of completed matings.

compared with the second, but despite this, achieved the same number of services on both occasions. For the remainder of the rams, the mating activity of individual animals was similar during the two trials.

Within this group of rams there was no apparent relationship between any of the various characteristics of the testosterone profile (such as number of testosterone peaks per 24h etc.) and mating activity (Table 3.4).

3.2.2 Seasonal changes in the plasma testosterone profile in Merino rams

The 24h plasma testosterone profiles of Merino rams sampled in July and September-October are shown in Figures 3.5 and 3.6 respectively. During these months the rams were bled on two occasions with an approximate interval of one week between bleeds. The profiles in Figures 3.5 and 3.6 indicate that the pattern (i.e. number of testosterone peaks per 24h) of testosterone secretion for individual rams remained reasonably constant over a short period of time.

Mean testosterone levels for rams during both July and September-October were appreciably lower than those observed in the same animals earlier in the year during December and January. This difference was due not only to fewer testosterone peaks per 24h, but also to a reduction in peak height and basal testosterone levels during July (Table 3.5) and September-October (Table 3.6). These seasonal trends were less dramatic in some rams which had relatively low plasma testosterone levels at all times studied (e.g. rams 5, 6 and 7).

Table 3.4:

Correlation coefficients between components of the plasma testosterone profile and mating activity in Merino rams (experiment 3.2.3).

Characteristic of the plasma T profile	Libido Score		Number of services	
24h mean T	0.25	NS *	-0.18	NS
T peaks/24h	0.28	NS	-0.01	NS
Mean peak height	0.30	NS	-0.02	NS
Basal T concentration	0.26	NS	-0.16	NS

* $p > 0.05$.

Figure 3.5:

Plasma testosterone profiles for four Merino rams bled at hourly intervals for 24h in Summer (December, January) and winter (July) (experiment 3.2.3, see also 3.3.2).

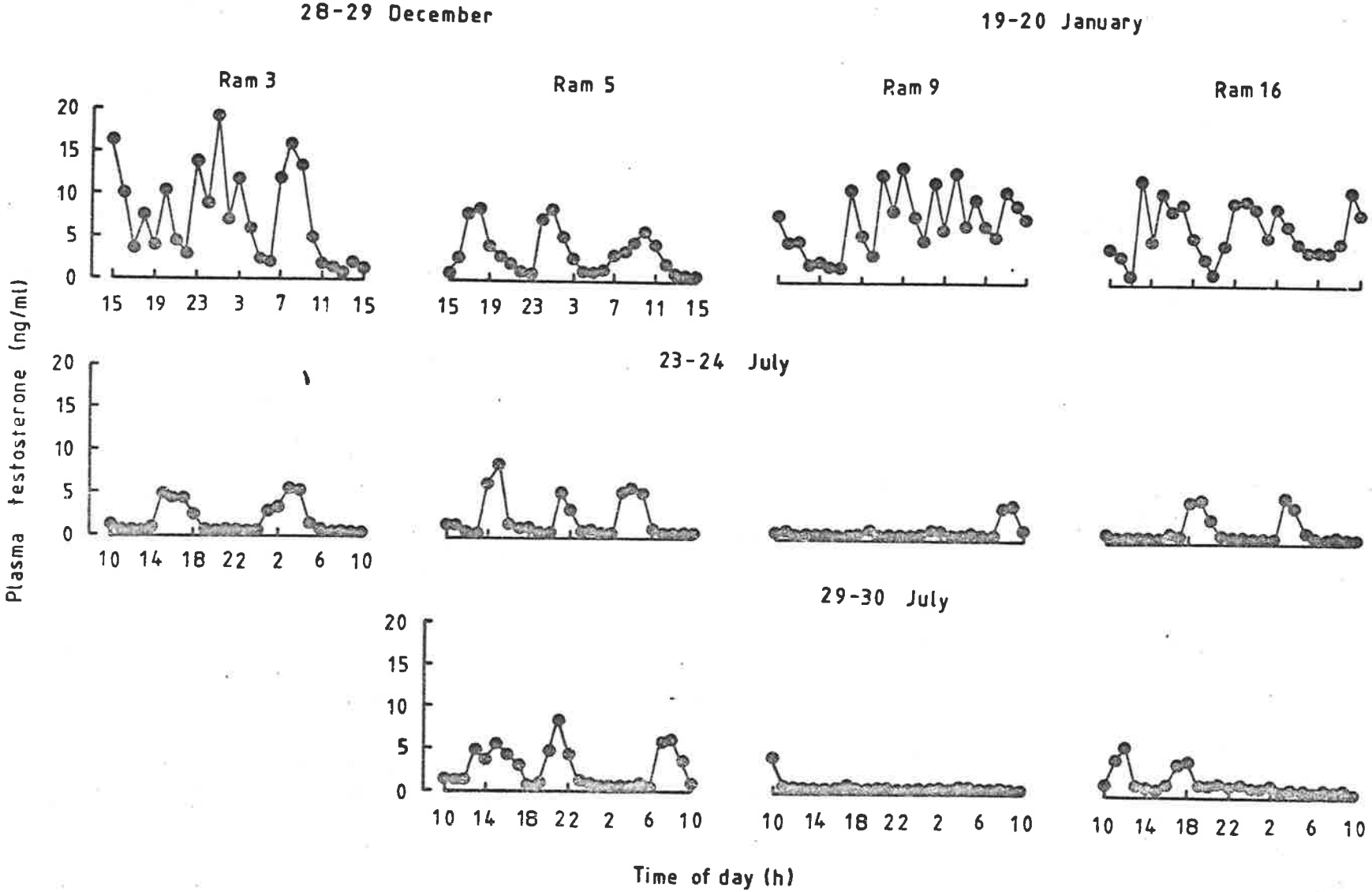

28-29 December
19-20 January
Ram 3
Ram 5
Ram 9
Ram 16
23-24 July
29-30 July
Plasma testosterone (ng/ml)
20
15
10
5
0
15 19 23 3 7 11 15
10 14 18 22 2 6 10
Time of day (h)

Figure 3.6:

Plasma testosterone profiles for three Merino rams bled at hourly intervals for 24h in Summer (December, January) and spring (September, October) (experiment 3.2.3, see also 3.3.2).

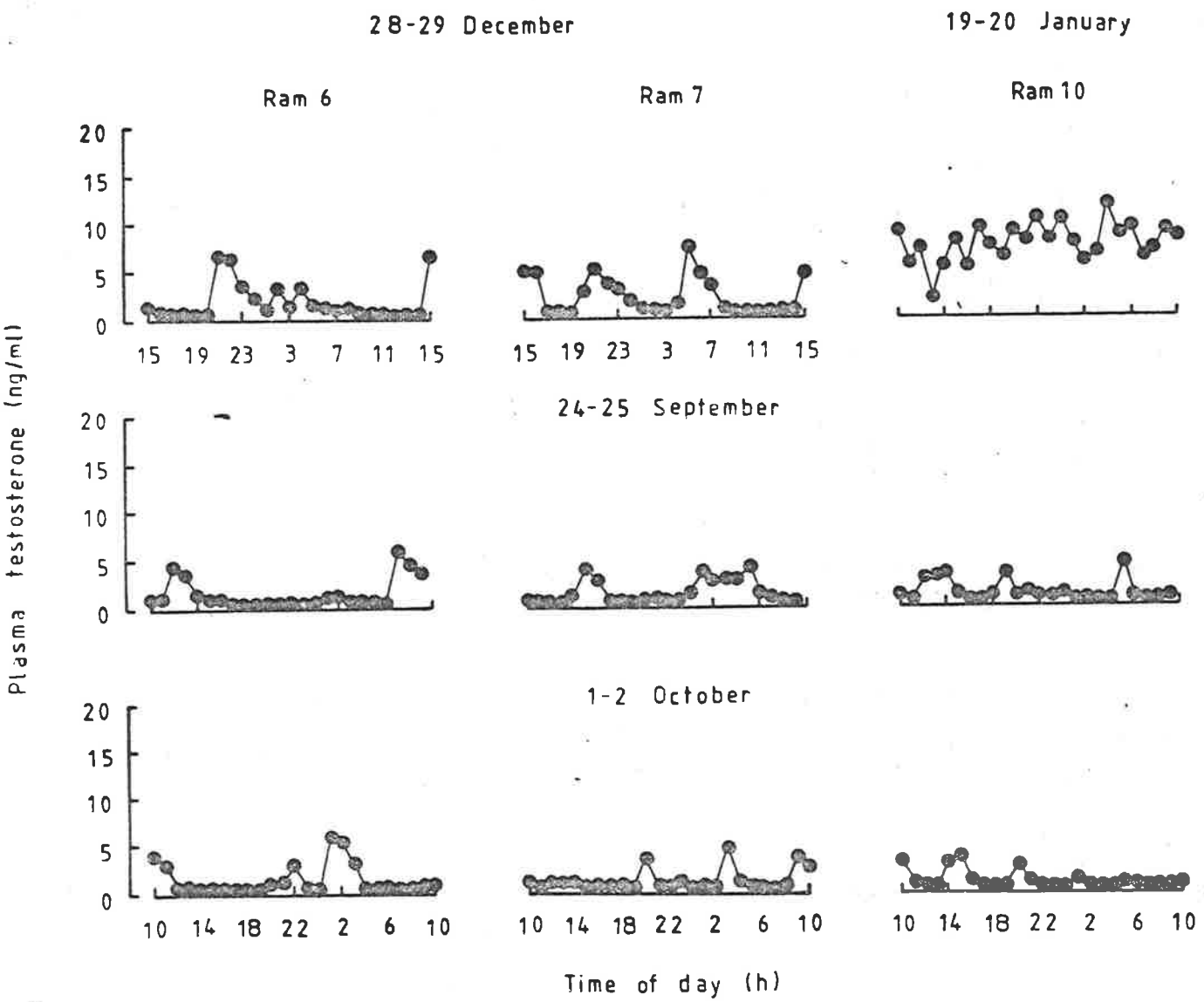

28-29 December
19-20 January
Ram 6
Ram 7
Ram 10
24-25 September
1-2 October
Plasma testosterone (ng/ml)
20
15
10
5
0
15 19 23 3 7 11 15
10 14 18 22 2 6 10
Time of day (h)

Table 3.5:

Plasma testosterone concentrations and incidence of testosterone peaks in Merino rams in summer (January-December) and winter (July) (experiment 3.2.3).

Ram	January - December		July*	
	Plasma T (ng/ml)	T Peaks/24h	Plasma T (ng/ml)	T Peaks/24h
3	7.3**	7	1.8	2
5	3.4	3	2.5	3
9	7.2	8	1.0	1
16	6.4	5	1.6	2
Mean ±S.E.M.	6.1 ± 0.9†	5.8 ± 1.1††	1.7 ± 0.3†	2.0 ± 0.4††

* Except for Ram 3, values represent the average for two 24h profiles, approximately one week apart.

** Mean (ng/ml) of blood samples taken at hourly intervals for 24h.

† $P < 0.05$ Student's t test

†† $P < 0.05$ " " "

Table 3.6:

Plasma testosterone concentrations and incidence of testosterone peaks in Merino rams in summer (January-December) and spring (September-October) (experiment 3.2.3).

Ram	January - December		September - October*	
	Plasma T (ng/ml)	T Peaks/24h	Plasma T (mg/ml)	T Peaks/24h
6	1.9**	4	1.6	2
7	2.3	4	1.5	3
10	7.7	7	1.4	3
Mean ±S.E.M.	4.0 ± 1.9†	5.0 ± 1.0††	1.5 ± 0.1†	2.7 ± 0.3††

* Values represent the average for two 24h profiles, approximately one week apart.

** Mean (ng/ml) of blood samples taken at hourly intervals for 24h.

† $P < 0.05$ Student's t test

†† $P < 0.05$ " " "

3.3.3 Seasonal changes in mating activity in Merino rams

The mating activity of five Merino rams for the periods October to January (following an increase in day length, see Figure 3.1) and May to July (following a decrease in day length), is shown in Figure 3.7. The only apparent differences in mating activity between the two periods were a decrease in the number of sniffs and a corresponding increase in nudges during May to July (Table 3.7). With regard to mounts and services there were no clear seasonal trends. Therefore, the seasonal changes in mating activity appeared less dramatic than the seasonal changes in testosterone status observed in Merinos a year earlier.

3.3.4 Seasonal changes in testosterone status and mating drive in British breed rams

Figure 3.8 shows seasonal changes in the plasma testosterone profile for Border Leicester, Polled Dorset, Romney and Suffolk rams. The plasma testosterone data are summarized in Table 3.8.

The seasonal peak in plasma testosterone concentration for Border Leicester, Romney and Suffolk rams occurred in late summer and early autumn. Testosterone levels in these breeds showed a decline before the winter solstice (22 June; see Figure 3.9), and began to increase again before the summer solstice (22 December).

The Polled Dorsets were somewhat unusual in that peak testosterone levels for this breed were observed during spring and early summer.

Mating activity data for the British breed rams are shown in Figure 3.10 and Table 3.8. The component of ram mating

Figure 3.7

Mating activity of five Merino rams during the periods October-January and May-July. Mating activity was determined in a 20min libido trial and the results are presented as means with the S.E.M. indicated by the vertical bars. The broken horizontal lines represent the overall mean for each time period (experiment 3.2.3, see also 3.3.3).

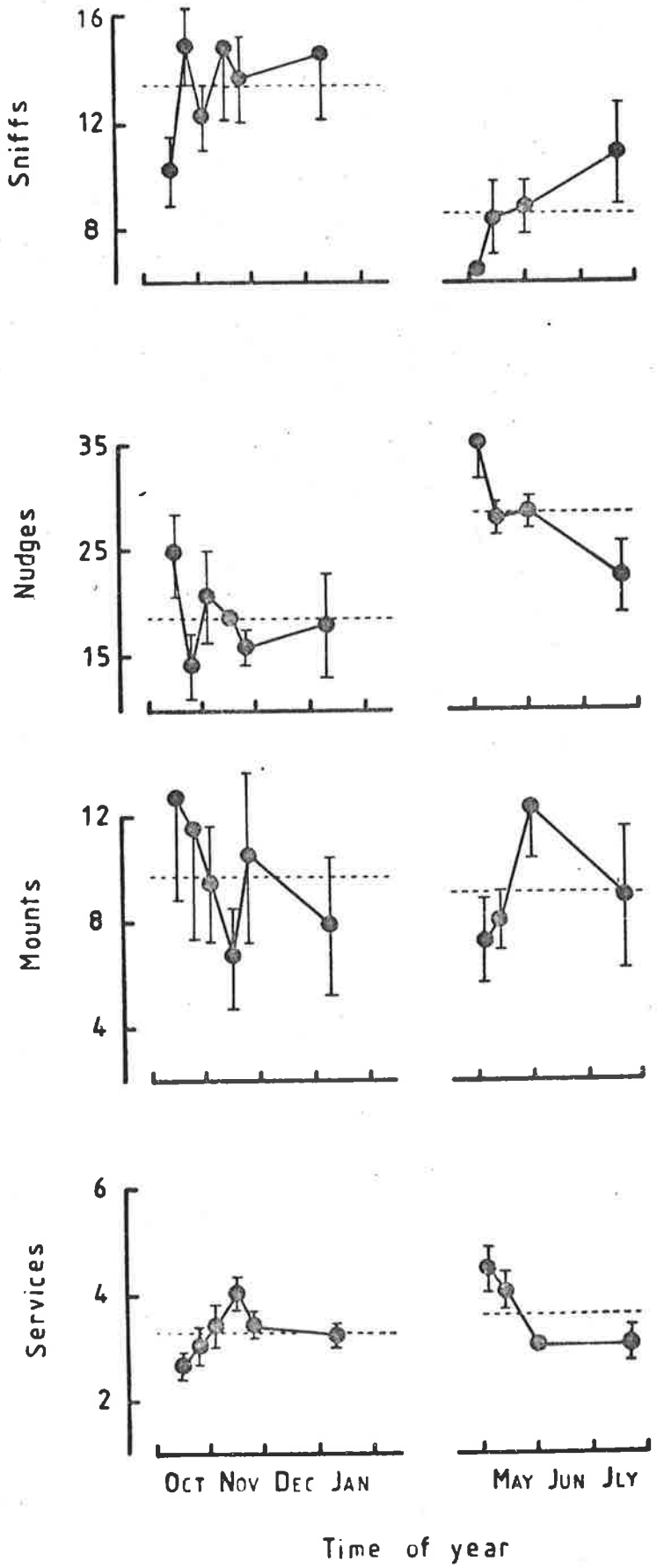


Table 3.7:

Aspects of mating activity in Merino rams during periods of increasing (October-January) and decreasing (May-July) daylength (experiment 3.2.3). The results are presented as means ± S.E.M. of six (October-January) and four (May-July) libido trials carried out during each time period (see also Figure 3.7). F values were obtained from analysis of variance.

Time of Year	Sniffs	Nudges	Mounts	Services
October-January	13.4 ± 0.8	18.5 ± 1.4	9.7 ± 1.2	3.3 ± 0.1
May-July	8.6 ± 0.7	28.4 ± 1.5	9.1 ± 1.0	3.6 ± 0.2
F Value	19.14 P < 0.01	21.18 P < 0.01	0.15 NS	1.54 NS

Figure 3.8

Seasonal changes in plasma testosterone in Border Leicester, Polled Dorset, Romney and Suffolk rams. Each month rams were bled at hourly intervals for 24h. Testosterone was determined in pooled samples for every two hours and profiles for individual rams are therefore presented as testosterone histograms (experiment 3.2.4, see also 3.3.4).


Border Leicester
Ram 6-79
Ram 6-80
Ram 6-81
Polled Dorset
Ram 6-82
Ram 6-83
Ram 6-84
Romney
Ram 6-76
Ram 6-77
Ram 6-78
Suffolk
Ram 6-98
Ram 6-99
Ram 6-100
Plasma testosterone (ng/ml)
JAN
FEB
MAR
MAY
JUN
JLY
AUG
OCT
NOV
DEC
Month

Table 3.8:

Seasonal changes in plasma testosterone (T, 24h mean) concentration and mating activity in Border Leicester (BL), Polled Dorset (PD), Romney (R) and Suffolk (S) rams. The results are presented as means ± S.E.M. except for number of services which are averages (n=3, except where indicated otherwise) (experiment 3.2.4).

Month	Breed	Plasma T (ng/ml)	Mating Activity	
			Number of Services	Libido Score
Jan	BL	3.2 ± 0.1	2.7	7.1 ± 1.0
	PD	4.1 ± 0.2	1.3	10.6 ± 1.8*
	R	3.8 ± 0.6	1.7	5.4 ± 1.8
	S	1.9 ± 0.1	0.5	5.8 ± 1.3†
Feb.	BL	5.9 ± 0.8	3.3	9.2 ± 1.3
	PD	4.5 ± 0.4	2.0	8.9 ± 1.9*
	R	7.7 ± 1.3	2.0	12.2 ± 0.9
	S	3.5 ± 0.5	1.7	7.1 ± 0.8
March	BL	6.7 ± 1.1	3.7	12.5 ± 1.4
	PD	3.8 ± 0.7	2.3	8.6 ± 1.2
	R	7.9 ± 2.1	1.7	10.1 ± 2.0
	S	6.3 ± 1.2	2.3	10.4 ± 1.7
May	BL	5.1 ± 2.0	3.7	10.9 ± 0.8
	PD	1.8 ± 0.4	2.3	12.5 ± 1.3**
	R	5.4 ± 1.8	4.3	14.1 ± 0.9
	S	4.8 ± 0.4	2.3	13.0 ± 1.0
June	BL	2.5 ± 0.9	4.0	11.2 ± 1.2
	PD	2.8 ± 0.6	1.7	12.1 ± 0.7
	R	2.4 ± 0.5	3.0	12.6 ± 1.6
	S	2.4 ± 0.4	4.7	12.4 ± 0.6
July	BL	1.4 ± 0.3	2.0	12.2 ± 2.6*
	PD	1.9 ± 0.4	2.7	10.7 ± 0.9
	R	1.0 ± 0.1	3.0	11.8 ± 0.4
	S	1.0 ± 0.3	3.3	12.1 ± 0.7
August	BL	1.7 ± 0.5	2.3	10.4 ± 1.6*
	PD	4.1 ± 0.6	2.5	10.9 ± 0.6†
	R	1.6 ± 0.1	1.3	14.9 ± 3.3*
	S	1.8 ± 0.2	3.0	11.7 ± 0.2
Oct.	BL	2.2 ± 0.5	2.7	9.3 ± 0.7
	PD	5.3 ± 1.3	2.0	8.1 ± 0.7
	R	1.8 ± 0.4	2.0	9.4 ± 0.8†
	S	1.6 ± 0.2	1.0	7.7 ††
Nov.	BL	2.3 ± 0.1	2.3	6.5 ± 1.1
	PD	6.1 ± 1.1	1.7	8.6 ± 1.5
	R	3.2 ± 0.7	1.7	9.6 ± 0.6
	S	2.3 ± 0.2†	1.0	4.0 ± 2.0†**
Dec.	BL	3.5 ± 0.7	2.3	6.4 ± 1.4
	PD	5.1 ± 1.0	1.7	9.3 ± 1.1
	R	3.7 ± 0.5	1.5	7.9 ± 0.2†
	S	2.5 ± 0.2†	1.0	4.0 ± 1.7†

* High number of abortive mounts by 1 ram
** High number of abortive mounts by 2 rams
† Data for 2 rams
†† Data for 1 ram

Figure 3.9:

Seasonal changes in plasma testosterone (●) and serving activity (O) in Border Leicester, Polled Dorset, Romney and Suffolk rams. Numbers of services were determined in a 20min libido trial and are presented as means. Plasma testosterone represents the mean of 24h means (see Table 3.8) with the S.E.M. indicated by the vertical bars (n=3, except where otherwise indicated in Table 3.8) (experiment 3.2.4, see also 3.3.4). WS, winter solstice; SS, summer solstice.

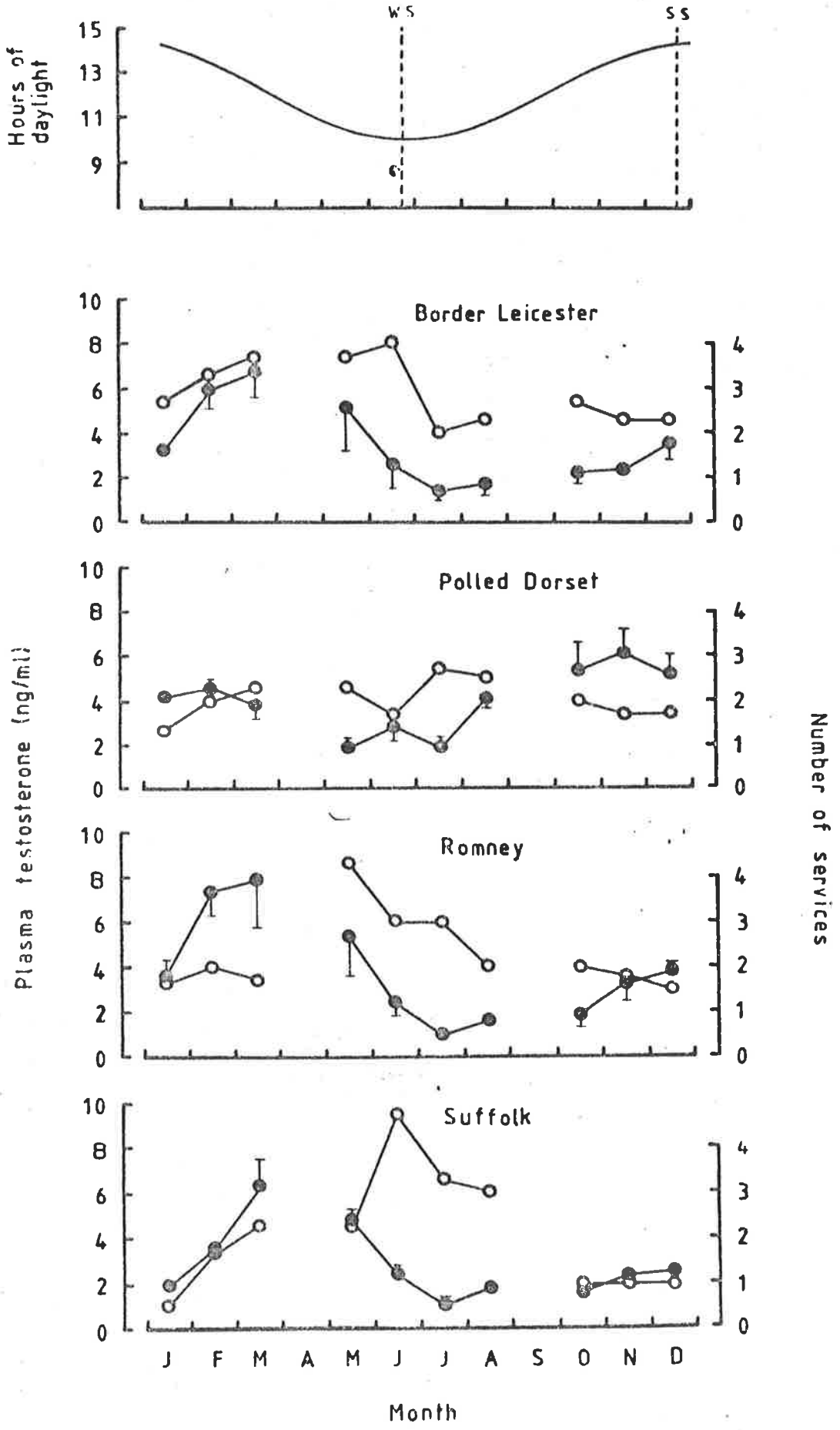
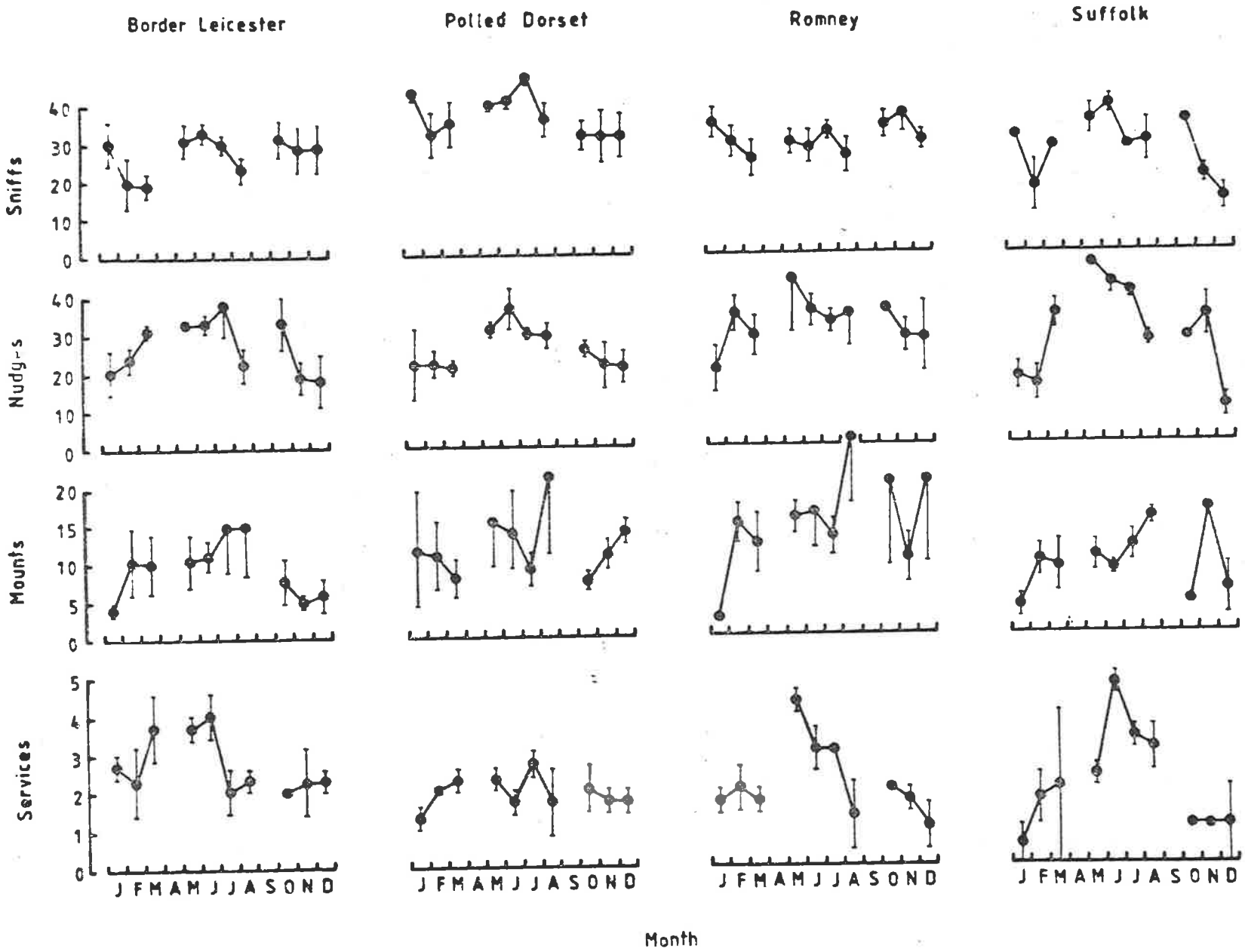

WS
SS
Hours of daylight
15
13
11
9
Border Leicester
Polled Dorset
Romney
Suffolk
Plasma testosterone (ng/ml)
Number of services
0
2
4
6
8
10
0
1
2
3
4
J F M A M J J A S O N D
Month

Figure 3.10:

Seasonal changes in mating activity in Border Leicester, Polled Dorset, Romney and Suffolk rams. Mating activity was determined in a 20min libido trial and the results are presented as means with the S.E.M. indicated by the vertical bars (n=3, except where indicated otherwise in Table 3.8) (experiment 3.2.4, see also 3.3.4).


Border Leicester
Polled Dorset
Romney
Suffolk
Sniffs
Nudg-s
Mounts
Services
0
10
20
30
40
0
5
10
15
20
0
1
2
3
4
5
J F M A M J J A S O N D
Month

activity which showed the most oustanding seasonal fluctuation, except in the Polled Dorsets, was serving capacity which was greatest in autumn and least in spring and early summer.

There did not appear to be a consistent relationship between testosterone status and mating activity for individual rams within months (Table 3.9).

There was a significant positive correlation between seasonal changes in testosterone status and number of services for Border Leicester rams, which was not apparent in the other breeds (Table 3.10). However, the Romney and Suffolk rams did show a seasonal peak in the number of services one to two months after the peak in plasma testosterone (Figure 3.9). Therefore, whilst the seasonal peak in serving capacity in these latter breeds most likely represented a response to increased testosterone levels during the preceding months, this relationship was not apparent on a monthly basis. There was no asssociation between seasonal peaks in plasma testosterone and serving capacity in the Polled Dorsets.

3.4 DISCUSSION

(a) Daily and annual changes in testosterone secretion

The episodic fluctuations in plasma testosterone concentration observed in Merino and British breed rams in this study were consistent with the pattern of testosterone secretion previously reported for rams (Katongole et al., 1974; Purvis et al., 1974; Sanford et al., 1974; Lincoln, 1976; Schanbacher and Ford, 1976; Wilson and Lapwood, 1978), and also males of other species (see references in Table 1.14).

In the present study there were large differences between individual Merino rams with regard to the number of episodic peaks

Table 3.9:

Within month correlation coefficients between plasma concentration and mating activity in British breed rams. The table represents pooled data for Border Leicester, Polled Dorset, Romney and Suffolk rams in experiment 3.2.4.

Month	Number of Services	Libido Score
Jan. (11)*	0.32 NS	0.41 NS
Feb. (12)	0.16 NS	0.70**
March (12)	-0.06 NS	0.51 NS
May (12)	0.29 NS	0.04 NS
June (12)	0.10 NS	0.29 NS
July (12)	-0.43 NS	0.36 NS
Aug. (12)	-0.17 NS	0.07 NS
Oct. (9)	0.19 NS	-0.23 NS
Nov. (11)	0.12 NS	0.48 NS
Dec. (10)	0.17 NS	0.48 NS

* Value in parenthesis indicates the number of animals.

NS Not significant ($p > 0.05$).

** $P < 0.01$.

Table 3.10:

Within breed correlation coefficients between monthly changes in plasma testosterone concentration and mating activity for Border Leicester (BL), Polled Dorset (PD), Romney (R) and Suffolk (S) rams in experiment 3.2.4.

Breed	Services	Libido Score
BL	0.65*	0.01
PD	-0.03	-0.79*
R	-0.001	-0.05
S	0.02	0.20

* $P < 0.05$

in plasma testosterone that occurred during a 24h period. Wilson and Lapwood (1978) have reported similar observations using Romney rams. It is thought that differences between rams with regard to frequency of testosterone peaks are due to differences in the pattern of luteinizing hormone releasing hormone secretion from the brain (Lincoln and Short, 1980).

There was no evidence of a diurnal rhythm in testosterone secretion in Merino rams during either December or January. This observation was consistent with the majority of studies in other ram breeds (see references above), although Lincoln, Peet and Cunningham (1977) reported that Soay rams did show a circadian rhythm in plasma LH and testosterone levels. These workers suggested that a daily rhythm in the release of LH and testosterone in Soays may be related to a 'photosensitive' phase in the 24h cycle of this breed.

The activity of the hypothalamic-pituitary-testicular axis in rams is known to increase in response to a decrease in day length (Pelletier and Ortavant, 1975a,b; Lincoln and Davidson, 1977; Lincoln et al., 1977; Sanford et al., 1978). The observation in the present study that mean testosterone levels in Merino rams were higher in January than in December was therefore consistent with this concept (see Figure 3.1). Testosterone levels observed later in the year during July (winter) and September-October (spring), were appreciably lower than the levels for both December and January. Similar seasonal changes in plasma testosterone levels have been reported for Merino rams at 33.5°S (Mattner, 1977) and 40.5°S (Wilson and Lapwood, 1978).

The Border Leicester, Romney and Suffolk rams showed peak testosterone levels during late summer and early autumn. This

was followed by a dampening of the testosterone profiles so that the seasonal nadir in testosterone secretion occurred in winter and early spring. These seasonal fluctuations in plasma testosterone levels were consistent with the circannual pattern of plasma testosterone secretion previously reported for rams (see above discussion). The seasonal peak in plasma testosterone seemed to occur earlier, and last longer, in Border Leicester and Romney rams compared with Suffolk. Suffolk rams therefore had the most restricted sexual season with regard to testosterone levels.

The seasonal increase in plasma testosterone levels in the British breed rams began towards the end of spring, when day length was still increasing, and a decline occurred before the winter solstice, when day length was still decreasing. Analogous 'out of phase' seasonal changes in testosterone secretion have been reported previously for rams maintained under field conditions (Schanbacher and Lunstra, 1976; Lincoln and Davidson, 1977; Barrell and Lapwood, 1978/1979). These findings appear contradictory to the generally accepted concept that reproductive-endocrine activity in rams is stimulated by a decrease in day length (see Section 1.2.10 and above discussion). Lincoln has attempted to explain this apparent paradox by suggesting that rams have an endogenous sexual cycle which is entrained to the seasons by changes in day length (see Section 1.2.10; Lincoln and Davidson, 1977; Lincoln et al., 1977).

The Polled Dorset rams were unusual in that they did not show a well-defined seasonal peak in plasma testosterone. Another peculiarity of this breed was that the rams tended to show an increase in testosterone levels during spring and early summer

rather than during late summer and early autumn. In contrast to these observations, however, Barrell and Lapwood (1978/1979) reported that Polled Dorsets at 40.5°S displayed a clear seasonal peak in plasma testosterone from January to March.

Based on the testosterone data for the British breed rams, it can be concluded that Suffolks are the most seasonal and Polled Dorsets the least seasonal with Border Leicester and Romney showing intermediate seasonality.

(b) Relationship between plasma testosterone and mating activity

The plasma testosterone data obtained for Merino and British breed rams was used to determine the relationship between plasma testosterone levels and mating drive for individual animals. This relationship was initially investigated in the Merino rams.

A general feature of the mating activity of the Merinos was that individual rams tended to achieve the same number of services during two libido trials conducted one week apart in January. Similar observations have been reported previously for this breed (Mattner et al., 1971; Wilkins and Kilgour, 1977). Although the number of services for individual rams remained reasonably consistent from one libido trial to the next, the rams showed a degree of variability, between trials, with regard to the number of sniffs, nudges and abortive mounts. This was reflected in the variability of the libido scores. Therefore, whilst the libido score provides a measure of the overall level of sexual activity, the number of services per libido trial perhaps best indicates the mating potential of individual rams.

There was no apparent relationship between plasma testosterone levels for individual Merino rams during December and

January, and either the libido score, or number of services during libido trials. Unfortunately, in this experiment the hormone and behavioural data were obtained one to two weeks apart. At the time it was presumed that this interval between collection of the two sets of data would not significantly affect the results. This assumption was based on previous studies in rams which had indicated that both the testosterone profile (Purvis et al., 1974) and mating activity (Mattner et al., 1971; see also Wilkins and Kilgour, 1977) of individual animals remained reasonably consistent in the short-term. However, whilst this was true for mating performance in the present study, the testosterone levels observed in rams sampled in January were generally higher than the levels observed in December. The testosterone data suggests, therefore, that the rams were going through a transition phase at this time of the year with regard to reproductive-endocrine status. It has already be mentioned that these endocrine changes were most likely due to the decrease in photoperiod which the rams experienced (see Figure 3.1 and above discussion).

Merino rams showed essentially the same level of mating activity during the periods May to July (following a decrease in day length) and October to January (following an increase in day length).

The results of the present study suggest, therefore, that Merino rams may show seasonal fluctuations in testosterone secretion in the absence of appreciable changes in mating activity.

Mattner (1977) recorded the libido of a group of adult Merino rams every second week for two years (at 33.5°S), and found that the mating drive of these animals was significantly greater in summer and autumn compared with late winter and spring. He

also found that seasonal changes in testosterone status and mating drive were positively correlated.

Data for mating activity and plasma testosterone in the British breed rams were obtained on consecutive days during each month. However, there was still no relationship, within months, between testosterone status and mating drive for individual rams. Schanbacher and Lunstra (1976) have reported similar observations using Finnish Landrace and Suffolk rams.

Seasonal changes in plasma testosterone concentration were associated with corresponding changes in serving capacity in Border Leicester, Romney and Suffolk rams. This relationship was most obvious in the Border Leicesters (correlation coefficient $r = 0.65$, $p < 0.05$). Romney and Suffolk rams displayed a peak in servicing capacity approximately one month after the peak in plasma testosterone. These latter breeds also maintained good serving capacity for a couple of months during which time plasma testosterone levels were decreasing (autumn). These observations explain, therefore, why there was no apparent correlation, within months, between changes in plasma testosterone and number of services for Romney and Suffolk rams. Lincoln and Davidson (1977) found that Soay rams also showed peak sexual activity one month after the seasonal peak in plasma testosterone. Other workers have reported correlation coefficients of 0.59 (Schanbacher and Lunstra, 1976) and 0.90 (Sanford et al., 1977) between seasonal changes in testosterone levels and mating activity in rams. The results of the present study, together with those of other workers, suggests, therefore, that there may be breed differences in the sensitivity (and therefore responsiveness) of rams to changes in their testosterone status (see also Section 1.7.2). An extreme case

may be represented by the Dorset where there was no clear association between seasonal fluctuations in plasma testosterone levels and serving capacity.

Since a seasonal increase in mating drive in rams is associated with, or occurs shortly after a rise in plasma testosterone levels, attempts have been made to improve the libido of rams during the non-breeding season with injections of testosterone, human chorionic gonadotrophin or gonadotrophic hormone releasing hormone (Section 1.2.10). Since these efforts have met with little success, it would appear that other factors (e.g. photoperiod, nutrition, environmental conditions), in addition to change in plasma testosterone levels, also contribute to seasonality of mating in rams.

In summary, the main purpose of the experiments described in this chapter was to determine if a direct relationship existed between plasma testosterone levels and mating drive for individual rams. The results obtained for both the Merino and British breed rams failed to provide any evidence for such a relationship. However, seasonal changes in plasma testosterone levels were associated with parallel changes in mating drive in several of the British breeds. This suggests that there is, nevertheless, a relationship between seasonal changes in plasma testosterone concentration and mating activity in certain (if not all) ram breeds.

In addition to long-term changes in testosterone induced by season, it has been reported that rams show short-term increases in plasma testosterone levels during copulatory activity (Sanford et al., 1974). It was decided, therefore, to investigate if an increase in plasma testosterone was a consistent feature of mating in rams, and also, if the magnitude of the testosterone increase was

related to the mating drive of individual animals. The results of this study are presented in the next chapter.

CHAPTER 4

EFFECT OF MATING ACTIVITY ON PLASMA TESTOSTERONE AND CORTISOL LEVELS IN RAMS: COMPARATIVE EFFECTS OF SHORT VERSUS PROLONGED SEXUAL STIMULATION AND THE PRESENCE OF ANOTHER RAM

4.1 INTRODUCTION

Sexual stimulation in males was reported to cause an increase in plasma LH and testosterone levels in rats, mice, rabbits, rams, bulls and men (see Section 1.2.9 and references in Table 1.12). This phenomenon may simply reflect the existence of a redundant neural mechanism in the male brain that is analogous to the neuroendocrine reflex which triggers a release of luteinizing hormone in females that ovulate in response to mating (e.g. rabbit, cat, mink; see Saginor and Horton, 1968; Schwartz, 1973). Alternatively, an increase in reproductive hormone levels in males, in response to sexual arousal, may serve an important role in initiating, or facilitating mating responses, particularly in lower animals such as rodents (Kamel et al., 1975; Macrides, Bartke and Dalterio, 1975; Coquelin and Bronson, 1979).

In rams, there does not appear to be a relationship between resting plasma testosterone levels and mating drive for individual animals (see Chapter 3; Schanbacher and Lunstra, 1976). However, there may be a relationship between the libido of individual rams, and the magnitude of the rise in plasma testosterone that has been shown to occur in rams during mating (Sanford et al., 1974a; 1977).

The experiments described in this chapter were designed (a) to investigate if an increase in plasma testosterone is a consistent feature of mating in rams, and (b) to determine if the magnitude of the testosterone rise (if it occurs) is related to the sex drive of individual animals. The effect of mating activity on plasma cortisol levels in rams was also investigated. The plasma cortisol was used as an indicator of stress.

Since rams are normally introduced into the ewe flock either in pairs, or in groups of three, the effects on mating activity, and plasma testosterone and cortisol levels, of competition between two rams for access to oestrous ewes was also investigated.

4.2 EXPERIMENTAL PROCEDURES

4.2.1 Animals

Adult Merino rams were used in the experiments described in this study and were maintained under field conditions except when required for libido trials. All animals were accustomed to handling and the procedures associated with the libido trials and blood sampling.

4.2.2 Assessment of ram mating behaviour

A description of the libido trial used to assess the mating activity of rams is given in Sections 2.4 and 2.5 of Chapter 2. In the current experiments the trials were of twenty minutes duration.

4.2.3 Blood collection and hormone assays

All blood samples were taken by the use of indwelling jugular cannulae which were secured in place the day before each experiment (Section 2.6). Blood was collected into heparinized

tubes, and then placed immediately on ice and centrifuged within one hour. Plasma testosterone and cortisol levels were determined using the appropriate assays described in Sections 2.7.3 and 2.7.4 of Chapter 2.

4.2.4 Effect of short-term exposure to sexually receptive ewes on plasma testosterone and cortisol levels in rams

Experiment 1 – Six rams were allowed to mate individually with a group of ewes for 20 minutes. Blood samples were taken from the rams immediately before, and 10 and 20 minutes after they were introduced to the ewes, and the plasmas were assayed for testosterone and cortisol.

Experiment 2 – Four rams were bled at 30 minute intervals from 0900 to 1630 hours. Starting at 1130 hours the rams were introduced individually to a group of ewes and allowed to mate for 20 minutes. The plasma testosterone concentration was determined in blood samples collected before and after the rams were allowed access to the ewes.

4.2.5 Effect of long-term exposure to sexually receptive ewes on the plasma testosterone profile in rams

Experiment 3 – Three rams were brought indoors during the first week in June and housed in individual pens (see Section 3.2.2 of Chapter 3). During the second week in July they were bled at hourly intervals for 24 hours (0900 to 0900h) to ascertain the pattern of testosterone secretion for each ram. The same bleeding schedule was repeated one week later. However, during the second bleed, each ram was allowed continuous access to two receptive ewes that were placed in the pens between the 0900 and 1000h sample.

This experiment was based on the assumption that, in the absence of ewes, the 24 hour plasma testosterone profile of individual rams remains reasonably consistent from one week to the next (see Introduction to Chapter 3 and Figures 3.5 and 3.6).

4.2.6 Effect of competition for access to oestrous ewes on plasma testosterone and cortisol levels in rams

Experiment 4 – In the first part of this experiment four rams were allowed individual access to a group of ewes for 20 minutes. The rams were bled 20, 10 and 0 minutes before being allowed access to the ewes (the zero time sample was taken immediately before a libido trial), and during the libido trial at 10 and 20 minutes. An additional blood sample was taken 30 minutes after the libido trial.

A similar procedure was followed the next day, except that on this occasion the rams were introduced to the ewes in pairs, and an additional blood sample was taken 10 minutes after the mating period. The rams were paired 20 minutes before they were allowed access to the ewes.

Plasma testosterone and cortisol levels were determined in blood samples collected on both days.

Experiment 5 – Four rams were paired in two separate sheep yards at 0800 hours. Beginning at 0900h the rams were bled at hourly intervals until 1700h. Between 1415 and 1540h each pair of rams was introduced to oestrous rams for 20 minutes. Additional blood samples were taken at 10 and 20 minutes whilst the rams were with the ewes, and all plasmas were assayed for testosterone and cortisol.

4.2.7 Statistical analysis

The effect of long-term exposure to sexually receptive ewes on plasma testosterone levels in rams was assessed by analysis of variance (Steel and Torrie, 1980).

4.3 RESULTS

4.3.1 Effect of short-term exposure to sexually receptive ewes on plasma testosterone and cortisol levels in rams

In Experiment 1 (Figure 4.1, Table 4.1) Merino rams did not consistently show a change in either plasma testosterone or cortisol levels during the twenty minutes that they were allowed to mate with oestrous ewes. There was no hormonal response in spite of the fact that all rams showed reasonably good mating drive. The two rams (2 and 4) that did show an increase in plasma testosterone displayed mating activity that was comparable to that of the other animals. Ram 2 had relatively low plasma testosterone levels when introduced to the ewes which may have allowed a neuroendocrine response in this animal (see Section 1.2.9). However, this did not appear to be the case in ram 4. Furthermore, ram 6 had lower initial testosterone levels than ram 2, but the former ram did not show an increase in testosterone during mating.

In Experiment 2 (Figure 4.2, Table 4.2), a twenty minute libido trial did not appear to influence the pattern of the testosterone profile in rams. The peak in plasma testosterone observed in ram 9 after mating may have been coincidental, since the plasma testosterone concentration in rams is known to fluctuate episodically.

Figure 4.1

Plasma testosterone (●) and cotisol (O) concentrations in Merino rams during short-term exposure to sexually receptive ewes. Rams were allowed access to ewes in a 20min libido trial and were bled via indwelling jugular cannulae (experiment 4.2.4(1), see also 4.3.1).

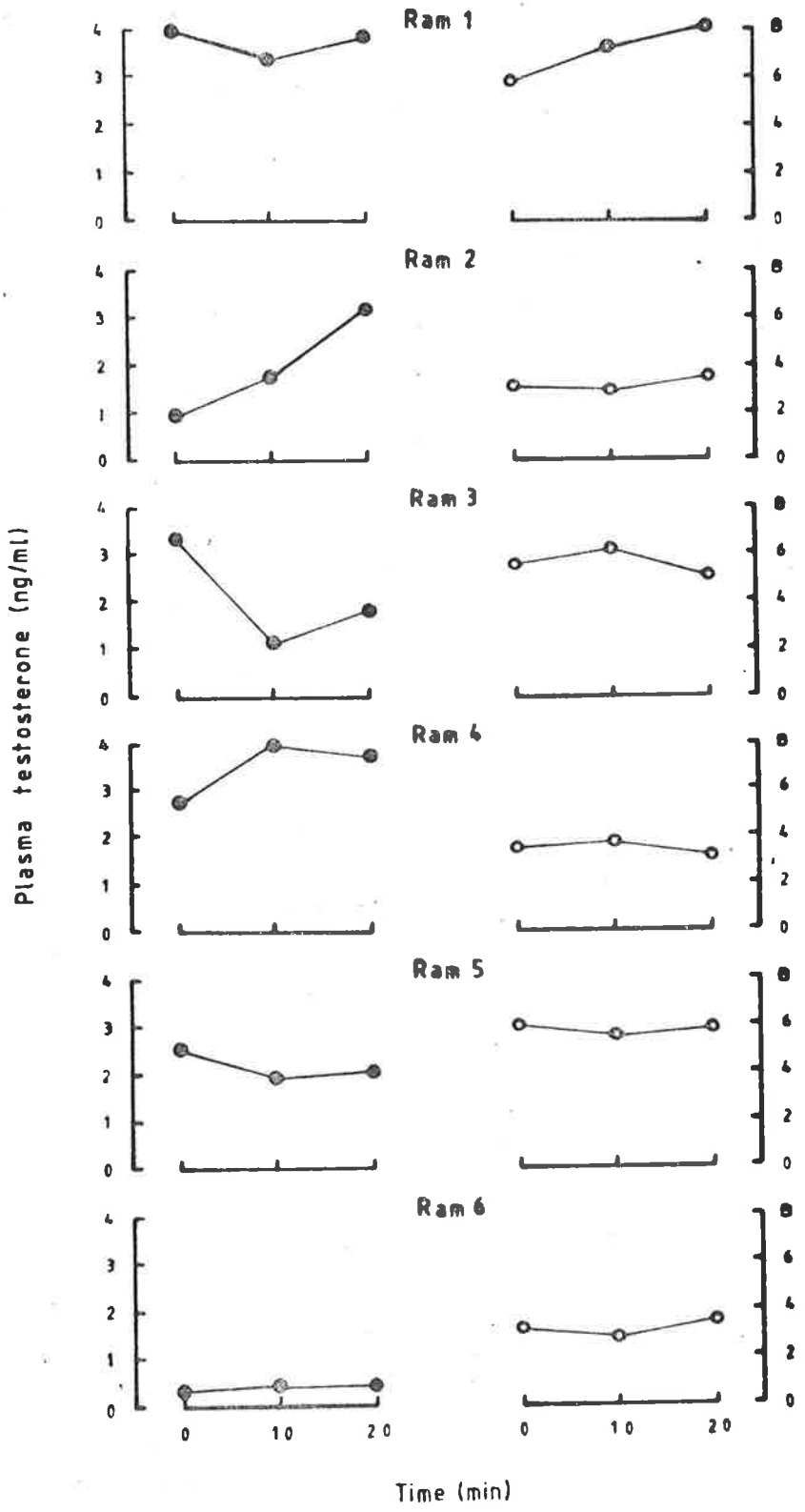

Ram 1
Ram 2
Ram 3
Ram 4
Ram 5
Ram 6
Plasma testosterone (ng/ml)
Time (min)
0
10
20

Table 4.1:

Number of services and changes in plasma testosterone and cortisol concentrations in Merino rams during a 20min libido trial (experiment 4.2.4(1)).

Ram	Number of Services	Plasma Hormone Concentration (ng/ml)					
		Testosterone			Cortisol		
		Time (min)			Time (min)		
		0	10	20	0	10	20
1	3	3.9	3.3	3.8	5.8	7.2	8.0
2	2	0.9	1.7	3.1	3.0	2.8	3.4
3	4	3.3	1.1	1.8	5.5	6.1	5.0
4	3	2.7	3.9	3.7	3.4	3.7	3.1
5	1	2.5	1.9	2.0	6.0	5.5	5.8
6	1	0.3	0.4	0.4	3.2	2.8	3.5
Mean ±S.E.M.		2.3±0.6	2.1±0.5	2.5±0.5	4.5±0.6	4.7±0.8	4.8±0.8

Figure 4.2

Plasma testosterone profiles in four Merino rams before and after short-term exposure to sexually receptive ewes. Rams were bled at 30min intervals via indwelling jugular cannulae and were allowed access to ewes in a 20min libido trial (vertical bar) (experiment 4.2.4(21), see also 4.3.1).

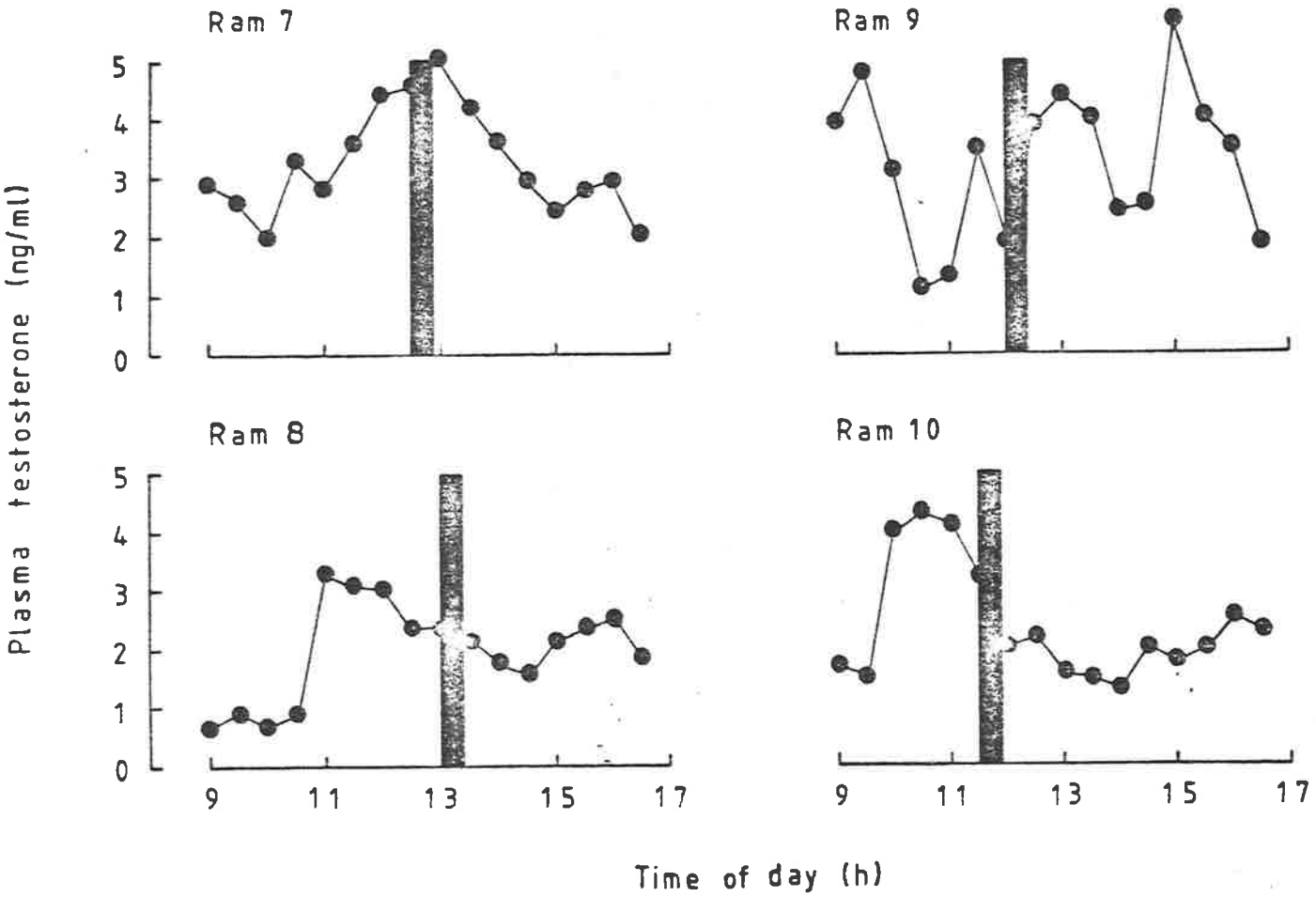

Ram 7
Ram 9
Ram 8
Ram 10
Plasma testosterone (ng/ml)
0
1
2
3
4
5
9
11
13
15
17
Time of day (h)

Table 4.2:

Plasma testosterone concentrations in Merino rams bled at 30min intervals before and after a 20min libido trial. The 20min interval during which a ram was exposed to ewes is indicated by the vertical lines (experiment 4.2.4(2)).

Time (h)	Plasma Testosterone Concentration (ng/ml)			
	Ram			
	7	8	9	10
0900	2.9	0.7	3.9	1.7
0930	2.5	0.9	4.8	1.5
1000	2.0	0.7	3.1	4.0
1030	3.3	0.9	1.1	4.3
1100	3.5	3.1	3.5	3.2
1200	4.4	3.0	1.9	2.0
1230	4.6	2.4	3.9	2.2
1300	5.0	2.4	4.4	1.6
1330	4.2	2.2	4.0	1.5
1400	3.5	1.8	2.4	1.3
1430	2.9	1.5	2.5	2.0
1500	2.4	2.1	5.7	1.8
1530	2.8	2.4	4.0	2.0
1600	2.9	2.5	3.5	2.5
1630	2.0	1.9	1.9	2.3
Mean ± SEM	3.3 ± 0.2	2.0 ± 0.2	3.2 ± 0.3	2.4 ± 0.2

4.3.2 Effect of prolonged exposure to sexually receptive ewes on the plasma testosterone profile in rams

Since Experiments 1 and 2 had indicated that a relatively short period of sexual stimulation did not affect plasma testosterone levels in rams, Experiment 3 was designed to determine if an extended period of sexual stimulation might influence the testosterone profile. However these results were negative in that rams that were allowed continuous access to oestrous ewes for twenty-four hours did not show an appreciable change in testosterone secretion (Figure 4.3, Table 4.3).

Although a detailed record of mating activity was not kept in this experiment, all three rams became sexually stimulated and served the ewes. Mating activity was particularly noticeable in all rams during the first three hours after the ewes were introduced into the pens. Thereafter, individual rams became sexually aroused at different times.

To summarize, both long- and short-term sexual stimulation does not appear to have any apparent effects on testosterone secretion in Merino rams. The increase in plasma testosterone observed in some rams during and after mating is probably coincidental due to the normal pulsatile nature of testosterone secretion in males (see Table 1.14).

4.3.3 Effect of competition for access to oestrous ewes on plasma testosterone and cortisol levels in rams

In the first part of Experiment 4 (Figure 4.4, Table 4.4) rams which were allowed to mate individually with a group of ewes did not show any changes in plasma concentration of either testosterone or cortisol. This observation was consistent with the

Figure 4.3

Plasma testosterone profiles in three Merino rams in the absence of sexual stimulation (week 1) and during continuous contact with two sexually receptive ewes (week 2). During week 2 ewes were penned with individual rams (experiment 4.2.5, see also 4.3.2).

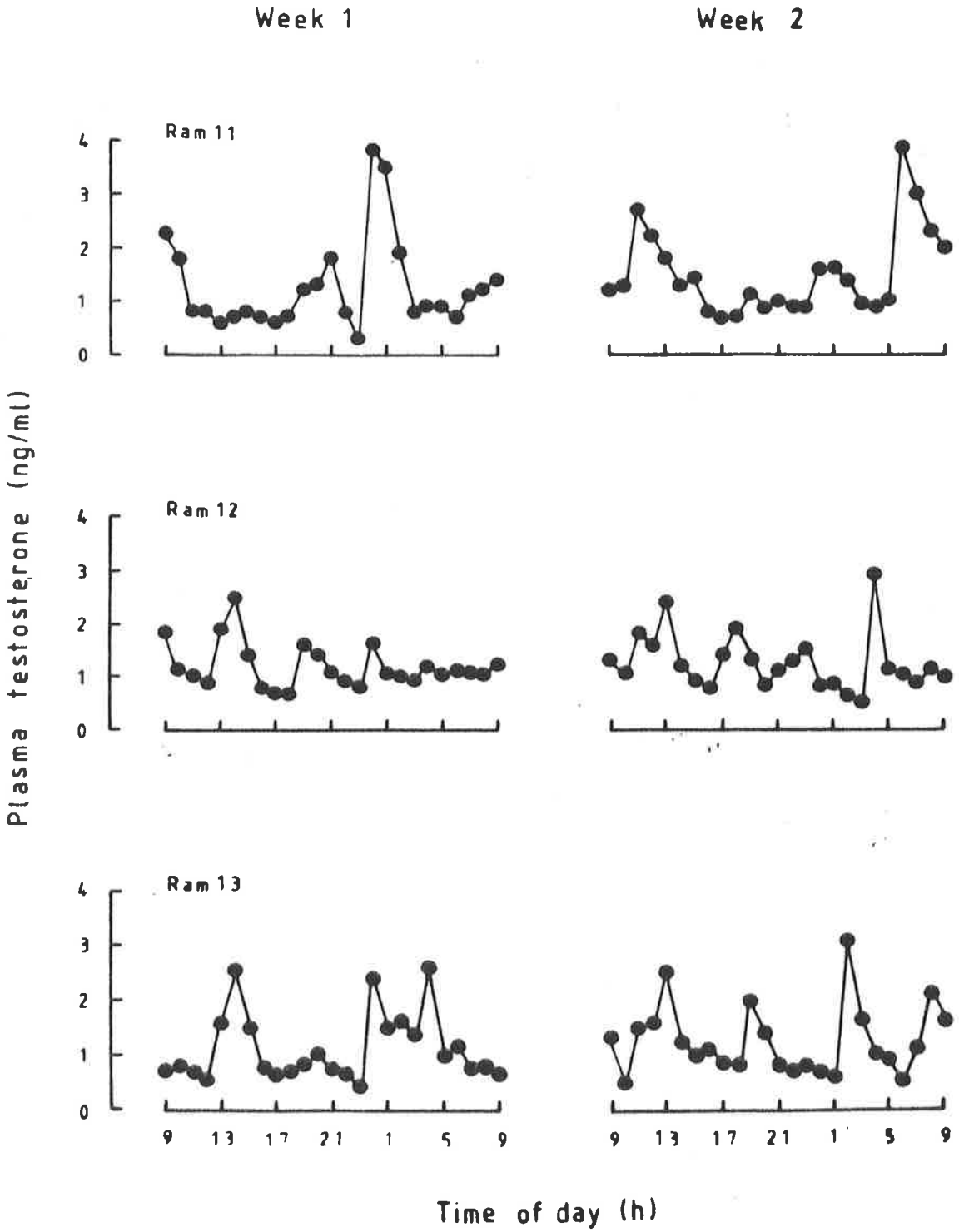

Week 1
Week 2
Ram 11
Ram 12
Ram 13
Plasma testosterone (ng/ml)
0
1
2
3
4
9
13
17
21
1
5
9
Time of day (h)

Table 4.3

Plasma testosterone concentrations in three Merino rams bled at hourly intervals for 24h in the absence of sexual stimulation (week 1), and in the presence of two sexually receptive ewes (week 2). Each ram was allowed continuous access to two ewes from 0900 to 0900h (experiment 4.2.5).

Time (h)	Plasma Testosterone Concentration (ng/ml)					
	Ram 11		Ram 12		Ram 13	
	Week 1	Week 2	Week 1	Week 2	Week 1	Week 2
0900	2.3	1.2	1.8	1.3	0.7	1.3
1000	1.8	1.3	1.1	1.0	0.8	0.5
1100	0.8	2.7	1.0	1.8	0.7	1.5
1200	0.8	2.2	0.9	1.6	0.6	1.6
1300	0.6	1.8	1.9	2.4	1.6	2.5
1400	0.7	1.3	2.5	1.2	2.5	1.2
1500	0.8	1.4	1.4	0.9	1.5	1.0
1600	0.7	0.8	0.8	0.8	0.8	1.1
1700	0.6	0.7	0.7	1.4	0.7	0.9
1800	0.7	0.7	0.7	1.9	0.7	0.8
1900	1.2	1.1	1.6	1.3	0.8	2.0
2000	1.3	0.9	1.4	0.8	1.0	1.4
2100	1.8	1.0	1.1	1.1	0.8	0.8
2200	0.8	0.9	0.9	1.3	0.7	0.7
2300	0.8	0.9	0.8	1.5	0.5	0.8
2400	3.8	1.5	1.6	0.8	2.4	0.7
0100	3.5	1.6	1.1	0.8	1.5	0.6
0200	1.9	1.4	1.0	0.6	1.5	3.1

Table 4.3 (Continued)

Time (h)	Plasma Testosterone Concentration (ng/ml)					
	Ram 11		Ram 12		Ram 13	
	Week 1	Week 2	Week 1	Week 2	Week 1	Week 2
0300	0.8	0.9	0.9	0.5	1.4	1.6
0400	0.9	0.9	1.2	2.9	2.6	1.0
0500	0.9	1.0	1.0	1.1	1.0	0.9
0600	0.7	3.8	1.1	1.0	1.1	0.5
0700	1.1	3.0	1.1	0.9	0.8	1.1
0800	1.2	2.3	1.0	1.1	0.8	2.1
0900	1.4	2.0	1.2	1.0	0.7	1.6
Mean ± SEM	1.3 ± 0.2	1.5 ± 0.2	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.3 ± 0.1

Paired t-test $P > 0.05$

Figure 4.4:

Plasma testosterone (●, ▲) and cortisol (○, △) profiles in two pairs (14 and 15, and 16 and 17) of Merino rams before (A, min), during (B, min) and after (C, min) competition for access to sexually receptive ewes. Rams were introduced to ewes in pairs in a 20min libido trial and allowed to compete for access to receptive ewes (experiment 4.2.6(4), see also 4.3.3). Also shown are the testosterone and cortisol profiles during single matings.

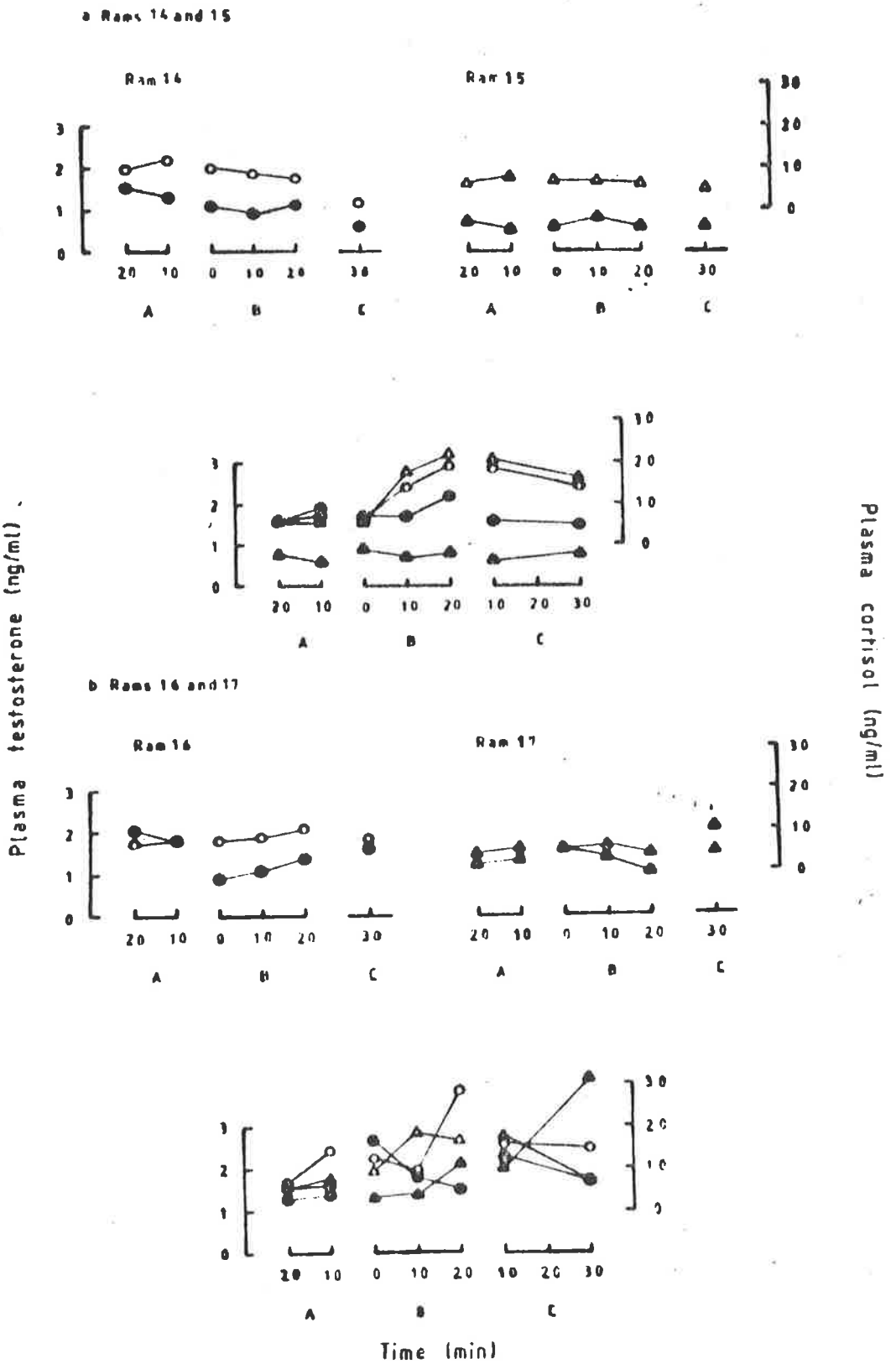

a Rams 14 and 15
Ram 14
Ram 15
b Rams 16 and 17
Ram 16
Ram 17
Plasma testosterone (ng/ml)
Plasma cortisol (ng/ml)
Time (min)
A
B
C

Table 4.4

Plasma testosterone and cortisol concentrations in Merino rams allowed to mate with sexually receptive ewes either alone (A), or in competition with another ram (P). Rams were exposed to ewes in a 20min libido trial and blood samples were taken before (-20, -10, 0), during (10, 20) and after (+10, +30) each trial (experiment 4.2.6(4)).

Time (min)	Ram 14				Ram 15			
	Plasma T		Plasma C*		Plasma T		Plasma C*	
	A	P	A	P	A	P	A	P
-20	1.5	1.6	9.5	6.8	0.7	0.8	5.8	5.6
-10	1.3	1.9	10.2	7.5	0.5	0.6	7.6	5.9
0	1.1	1.7	10.0	7.0	0.6	0.9	6.8	5.8
10	0.9	1.7	8.7	14.2	0.8	0.7	6.3	17.3
20	1.2	2.2	7.5	19.3	0.7	0.8	5.9	21.3
+10		1.6		18.5		0.6		20.6
+30	0.6	1.5	6.8	14.0	0.6	0.8	4.6	16.1

(b) Rams 16 and 17.

Time (min)	Ram 16				Ram 17			
	Plasma T		Plasma C*		Plasma T		Plasma C*	
	A	P	A	P	A	P	A	P
-20	2.1	1.3	7.5	6.5	1.2	1.5	4.6	5.3
-10	1.8	1.4	8.6	14.5	1.3	1.6	6.0	7.5
0	0.9	2.7	8.0	12.3	1.6	1.3	5.3	9.3
10	1.1	1.8	9.0	9.7	1.4	1.4	6.5	18.5
20	1.4	1.5	11.0	28.5	1.0	2.1	4.3	16.7
+10		2.3		16.0		2.0		12.9
+30	1.6	1.7	8.3	14.8	2.0	4.1	5.0	6.6

* $P < 0.05$ (Student's t test) for cortisol during and after libido trial when allowed to mate alone or paired

results obtained in Experiment 1. Testosterone concentrations were also unaffected by competition between two rams. In contrast, plasma cortisol levels showed a marked increase during the period that rams competed for access to sexually receptive ewes.

Rams 14 and 15 were equally aggressive towards each other and did not establish a dominant-subordinant relationship. The magnitude of the cortisol rise in these two animals was similar. Ram 17 was clearly dominant over ram 16, and it is interesting to note that ram 16 showed a greater increase in plasma cortisol than ram 17. Ram 16 also showed an increase in plasma cortisol during the period that the rams were paired before being introduced to the ewes. The rams could see the ewes at this time, and ram 17 began to exert his dominance about five minutes after he and ram 16 were paired. The apparent delay in the response of the brain-adrenal axis of ram 16 during the actual mating period may have been due to the cortisol response induced by the pairing of the rams shortly before contact with the ewes.

The number of services that rams achieved during single and paired libido trials is shown in Table 4.5. The most obvious feature was an overall decrease in the number of matings when rams were paired. This result was not surprising since paired rams (in particular 14 and 15) spent a proportion of the time concentrating on each other, rather than on the ewes. In particular, rams prevented each other from completing a proper mount by butting the ram which was mounting. Another interesting observation was that ram 17 appeared to be sexually stimulated by the presence of ram 16, and consequently performed better during the paired trials than when alone with the ewes. In

Table 4.5:

Number of services achieved by Merino rams during a 20min libido trial when mated alone or in pairs (experiment 4.2.6(4)).

Ram	Number of Services*	
	Alone	Paired
14	1.5	1.5
15	3.5	1.5
16	3.0	0.5
17	1.5	2.5
Mean ± SEM	2.4 ± 0.5	1.5 ± 0.4

* average of two libido trials.

contrast, ram 16 showed a dramatic decrease in the number of services in the presence of ram 17.

The design of Experiment 5 (Figure 4.5, Table 4.6) was similar to that of Experiment 4 except that paired rams were kept together for several hours before they were allowed access to the ewes. In addition, the rams were bled at hourly intervals from the time they were paired until after the mating trial. The paired mating trials were therefore superimposed on plasma testosterone and cortisol profiles.

As in Experiment 4, plasma testosterone in rams remained unaffected by competition for the oestrous ewes, whereas cortisol levels increased markedly during the same period. The increase in plasma cortisol was greater in rams 20 and 21 than in rams 18 and 19. The former pair of rams were much more aggressive towards each other than were the latter. However, there was no clear dominant-subordinant relationship established in either pair. Competition for the oestrous ewes resulted in marked decrease in the number of services achieved by the rams (Table 4.7) as was the case for Experiment 4.

4.4 DISCUSSION

In the present study mating activity in rams was rarely associated with a rise in plasma testosterone concentration. The increase in plasma testosterone observed in some rams during and after mating may have been coincidental, since plasma testosterone levels in rams are known to fluctuate episodically (Katongole, Naftolin and Short, 1974; Purvis, Illius and Haynes, 1974). Other studies in Hampshire (Purvis *et al.*, 1974), Hampshire and Clun (Illius *et al.*, 1976a), Finnish Landrace (Sanford,

Figure 4.5

Plasma testosterone (●) and cortisol (○) profiles in pairs (18 and 19, and 20 and 21) of Merino rams before and after competition for access to sexually receptive ewes. Rams were paired at 0900h and bled at hourly intervals via indwelling jugular cannulae. Paired rams competed for access to sexually receptive ewes during a 20min libido trial (▼▼). Additional blood samples were taken at 10 and 20min during the libido trial (experiment 4.2.6(5), see also 4.3.3).

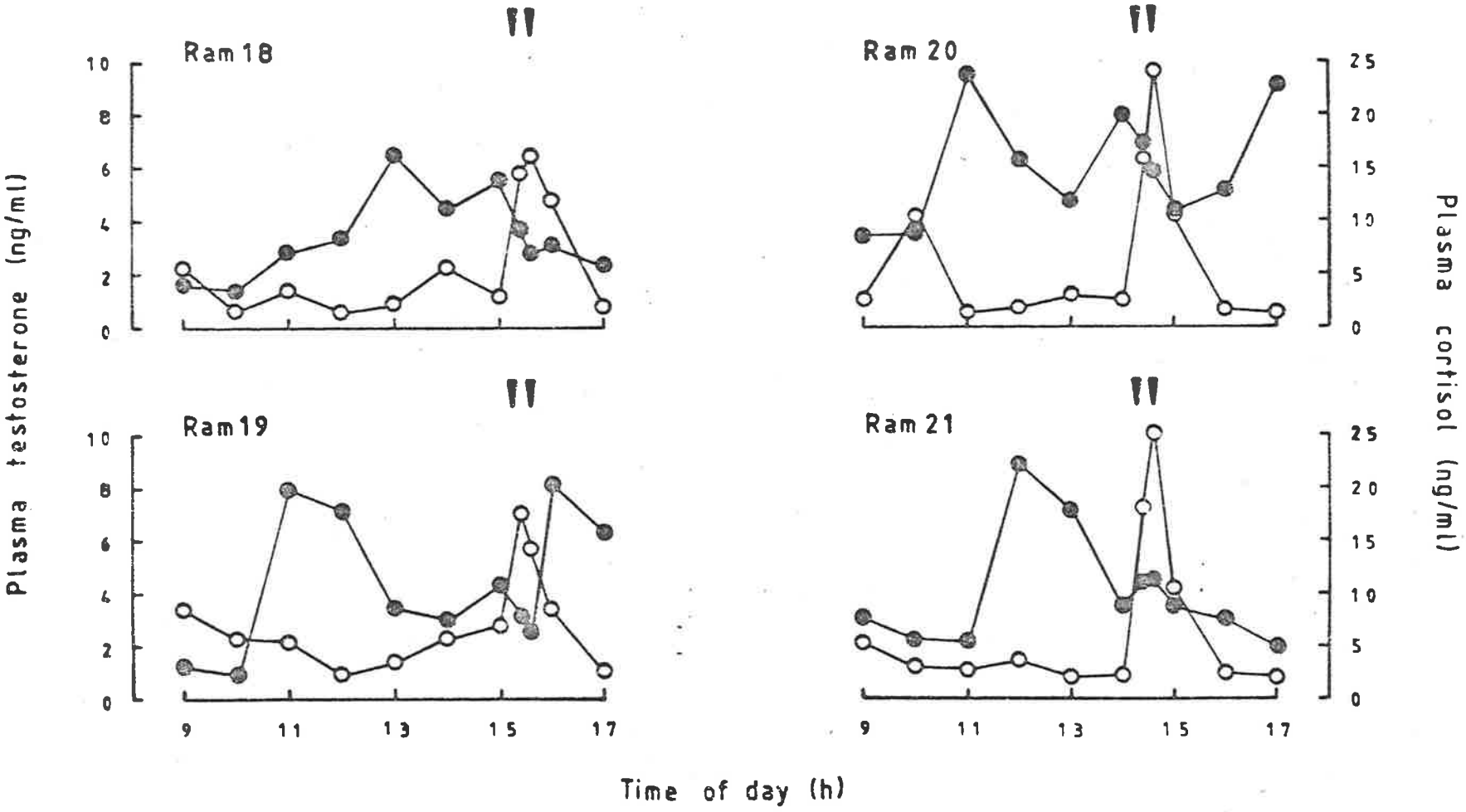

Ram 18
Ram 19
Ram 20
Ram 21
Plasma testosterone (ng/ml)
Plasma cortisol (ng/ml)
Time of day (h)
9
11
13
15
17

Table 4.6:

Plasma testosterone and cortisol concentrations in pairs of Merino rams before and after competition for access to sexually receptive ewes. Rams were paired at 0900h and exposed to ewes in a 20min libido trial between 1415 and 1435h (20 and 21) and 1515 and 1535h (18 and 19) (experiment 4.2.6(5)).

(a) Rams 18 and 19

Time (h)	Ram 20		Ram 21	
	Plasma T	Plasma C	Plasma T	Plasma C
0900	1.8	5.8	1.2	8.6
1000	1.4	1.7	0.9	5.8
1100	2.8	3.3	7.9	5.7
1200	3.4	1.6	7.1	2.3
1300	6.5	2.3	3.4	3.5
1400	4.5	5.7	3.0	5.9
1500	5.6	3.1	4.3	7.0
1525	3.7	14.6	3.2	17.6
1535	2.8	16.4	2.6	14.4
1600	3.1	12.1	8.2	8.5
1700	2.3	1.9	6.3	2.7

(b) Rams 20 and 21

Time (h)	Ram 20		Ram 21	
	Plasma T	Plasma C	Plasma T	Plasma C
0900	3.4	2.6	3.1	5.3
1000	3.5	10.2	2.3	3.0
1100	9.5	1.2	2.1	2.9
1200	6.3	2.0	8.9	3.7
1300	4.8	2.2	7.1	2.1
1400	7.9	2.0	3.5	2.3
1425	6.9	15.8	4.4	18.0
1435	5.8	24.0	4.5	25.0
1500	4.4	10.3	3.5	10.5
1600	5.2	1.6	3.0	2.4
1700	9.2	1.2	1.9	1.8

Table 4.7:

Number of services achieved by Merino rams during a 20min libido trial when mated alone or in pairs (experiment 4.2.6(5)).

Ram	Number of Services	
	Alone	Paired
18	2	0
19	1	0
20	2	0
21	2	1
Mean ± SEM	2.0 ± 0.4	0.3 ± 0.3

et al., 1974a) and Romney (Moore, Whyman and Wilson, 1978) rams have also shown that an increase in plasma LH and testosterone during mating only occurs in a proportion of animals.

Sanford et al. (1974a) suggested that repeated matings may have a different effect on the luteinizing hormone and testosterone profiles in rams as compared with single matings. These workers found that two Finish Landrace rams which did not consistently show a luteinizing hormone or testosterone response to single matings, nevertheless displayed a marked increase in the frequency of luteinizing hormone and testosterone pulses when allowed continuous contact with ewes for twenty-four hours. The increased frequency of hormone peaks (and also basal levels) corresponded to the period of maximum sexual activity, which occurred during the first twelve hours. Similar observations regarding the effects of single versus repeated matings of luteinizing hormone and testosterone secretion, have also been reported for Romney rams (Moore et al., 1978), and males of other species (Rose, Gordon and Bernstein, 1972; Bernstein, Gordon, Rose and Peterson, 1978).

Finnish Landrace rams that were allowed to mate at will with ewes for a period of eight hours during late summer (pre-mating luteinizing hormone and testosterone relatively low), and early winter (pre-mating luteinizing hormone and testosterone relatively high), showed an increase in plasma luteinizing hormone and testosterone levels during the first mating period, but not during the latter (Sanford et al., 1977). Sanford consequently suggested that rams are able to show a neuroendocrine response to mating only during periods when the hypothalamic-pituitary-testicular axis is in a naturally suppressed state (see also Moore et al., 1978). However, this did not appear to be the case in Merino rams allowed continuous contact with ewes for twenty-four hours during mid-winter (pre-mating testosterone

relatively low) in the present study. There was an important difference, however, in the timing of the present study and that of Sanford and his coworkers, relative to the breeding season in rams. Sanford et al. (1977) carried out their observations shortly before, and during the seasonal peak in reproductive-endocrine activity in rams, whereas the present study was conducted after this period (see Chapter 3). It is possible, therefore, that the neuroendocrine response of rams to sexual stimuli may vary with season.

The social environment of rams also seems to influence their ability to show a neuroendocrime response to mating stimuli. Adult rams housed next to cyclic ewes had higher plasma testosterone levels than rams kept isolated from ewes, and a greater proportion of animals in the former group showed an increase in plasma testosterone during mating compared with animals in the latter group (Illius et al., 1976b). Young rams do not show a testosterone response, either to different social environments, or during mating activity, which suggests that age also influences the neuroendocrine response of rams to sexual stimulation (Illius et al., 1976b; see Smith, Mongkonpunya, Hafs, Convey and Oxender, 1973 for comparative data in bulls).

Plasma cortisol levels remained unchanged when Merino rams were allowed to mate alone with oestrous ewes. This indicated that neither the procedures used to conduct a libido trial, nor the blood sampling, stressed the rams. With regard to the latter point, it was observed that rams resumed their interest in the ewes as soon as they were released after a blood sample had been

taken. However in some species, a rise in plasma cortisol levels is associated with mating activity (see references in Section 1.2.9).

The fact that rams did not show an increase in plasma cortisol when mated alone, meant that it was possible to assess the effect of competition between rams for access to oestrous ewes on the plasma levels of both testosterone and cortisol.

Rams that were introduced in pairs to the ewes showed varying degrees of interaction with each other. Some rams competed vigorously for the duration of the mating trial and neither was able to establish a clear dominance. In other cases one ram established his dominance early, and there was little subsequent interaction between the rams. This was because the subordinate ram stayed away from the ewes and thereby avoided the dominant animal. Other rams paid little attention to each other but instead concentrated their attention on the ewes.

Overall, rams achieved fewer services when paired, than when allowed to mate alone with the same number of ewes (see also Hulet et al., 1962). However, it was noted that some dominant rams which performed poorly when alone with ewes, could be stimulated by the presence of another ram, and actually showed an increase in mating activity.

Plasma testosterone levels in rams remained unchanged during the paired libido trials. On the other hand, cortisol levels showed a marked increase during the same period. This was in marked contrast to the single libido trials and indicates that the brain-adrenal axis of rams was activated by the social interaction imposed by the paired libido trial. Both dominant and subordinant animals responded with increased cortisol levels but the magnitude of the response appeared to be greater

in the subordinant animal. However, more replication would be necessary to establish whether this is a consistent phenomenon. Plasma cortisol data may provide an index for determining the compatibility of rams to be used for flock matings.

In summary, it appears that the brain-testicular axis of rams is not consistently activated by sexual stimulation. This observation, together with the finding that testosterone levels in rams are not related to mating drive (see Chapter 3), suggest that the plasma testosterone concentration cannot be used as an index of mating drive in rams.

Although testosterone undoubtedly plays a principal role in determining sexual behaviour in males (see Section 1.2.3), mating activity in rams is also influenced by social environment, previous sexual experience, nutrition, and other factors (see Section 1.2.8). The mating drive expressed by individual rams therefore reflects the combined effects of a number of interactions between physiological and environmental variables.

Some of the above variables can be eliminated if rams are castrated before puberty. Prepubertally castrated animals generally have non-detectable plasma testosterone levels, and they do not show sexual behaviour when adult. They therefore provide a useful model for studying the effects of testosterone on mating activity.

It was decided to investigate the finer control of mating activity by testosterone in rams by treating prepubertally castrated males with graded doses of testosterone propionate when adult. The results of this study are presented in the next chapter.

CHAPTER 5

MATING BEHAVIOUR OF CASTRATED RAMS (WETHERS) TREATED WITH GRADED DOSES OF TESTOSTERONE PROPIONATE (TP)

5.1 INTRODUCTION

The studies in adult rams reported in this thesis (Chapter 3) and by Schanbacher and Lunstra (1976), suggest that there is no relationship between plasma testosterone levels and mating drive for individual animals. However, it is clear that rams have an absolute requirement for testosterone for normal sexual behaviour since animals castrated before puberty do not, in general, show any mating activity when adult (Clegg et al., 1969). In addition, adult rams also show a marked decline in mating drive after castration (Clegg et al., 1969). Furthermore, mating responses can be elicited in rams castrated either before or after puberty by treatment with testosterone (Clegg et al., 1969; Parrott, 1978).

The experiments described in this chapter were designed to investigate the finer control of mating behaviour in rams by testosterone. Adult wethers that had been castrated before puberty were chosen as an experimental model for this study. There were two main reasons for using castrated animals. Firstly, wethers normally have non-detectable plasma testosterone levels which means that it is possible to closely manipulate their testosterone status by varying the dose of exogenous hormone. Secondly, the wethers were sexually inexperienced at the beginning of the experiments. It was presumed, therefore, that any sexual

behaviour observed after testosterone propionate (TP) treatment could be directly attributed to the effects of the hormone.

In one experiment, a twenty-four hour profile of plasma testosterone was obtained for each animal during the course of hormone therapy. It was hoped that these data would provide an estimate of the plasma testosterone threshold required for complete mating activity in normal rams. At the end of the experiment, the weight of the penis and seminal vesicles were also recorded. Additional indices of androgen status were obtained by measuring the content of fructose and citric acid in the seminal vesicles.

5.2 EXPERIMENTAL PROCEDURES

5.2.1 Animals

Adult Merino wethers which had been castrated before puberty were used in this study and were maintained under field conditions. The wethers used for Experiments 1 and 2 below were three year old animals of the Koonoona strain (body weight 51.3 ± 0.7kg (mean ± SEM; n = 36)), whilst those in Experiment 3 were eigthteen month old animals of the Bungaree strain (body weight 41.5 ± 0.5kg (n = 28)).

5.2.2 Hormone administration

Testosterone propionate (TP) was dissolved in peanut oil (see Section 2.3.2 of Chapter 2) and injected either subcutaneously or intramuscularly as indicated. During hormone treatment the wethers were yarded daily between 0800 and 0900h, injected between 0900 and 1000h, and returned to their paddock immediately thereafter.

5.2.3 Assessment of mating behaviour

The mating behaviour of control and hormone-treated wethers was assessed in a libido trial as described in Sections 2.4 and 2.5 of Chapter 2. In the current experiments the trials were of ten minutes' duration. Since the wethers had not had any previous heterosexual experience, they were allowed contact with oestrous ewes on two occasions before the start of each experiment. This allowed them to become accustomed to the libido-testing procedure, and also enabled their pretreatment mating behaviour to be determined.

5.2.4 Response of wethers to treatment with testosterone propionate

Experiment 1 — Three year old Koonoona strain wethers were randomly divided into four groups of three animals per group. Each group received one of the following treatments (intramuscular injections): oil vehicle (2ml/day); TP (1mg/day); TP (10mg/day); TP (100mg/day). The mating behaviour of these animals was assessed after 0, 2 and 6 weeks of hormone treatment.

Experiment 2 — Three year old Koonoona strain wethers were randomly divided into five groups of three animals per group. Each group received one of the following treatments (intramuscular injections): oil vehicle (2ml/day); TP (2mg/day); TP (4mg/day); TP (6mg/day); TP (8mg/day). The mating behaviour of these animals was determined after 0, 2, 6, 8 and 10 weeks of hormone treatment.

Experiment 3 — Eighteen month old Bungaree strain wethers were randomly divided into seven groups of three animals per group and each group received one of the following treatments (sub-

cutaneous injections): oil vehicle (2ml/day); TP (0.5mg/day); TP (1mg/day); TP (2mg/day); TP (4mg/day); TP (6mg/day); TP (8mg/day). The mating behaviour of these animals was assessed after 0, 2, 4, 5, 8, 10, 12 and 14 weeks of hormone treatment.

During the 7th, 10th and 11th week of hormone therapy, one animal from each treatment was bled at hourly intervals for twenty-four hours after the injection of TP, in order to follow the release of hormone into the blood. On these occasions the wethers were brought indoors, placed in individual pens, and fitted with an indwelling jugular cannula that was subsequently used for taking the blood samples (see Section 2.6 of Chapter 2). A different wether from each treatment was included in each sampling period so that eventually all animals had been sampled. The plasma testosterone concentration in these samples was determined using the radioimmunoassay described in Section 2.7.3 of Chapter 2.

After fourteen weeks of treatment the wethers were killed and their penis and seminal vesicles were dissected out and weighed. Fructose and citric acid were extracted from seminal vesicle tissue by homogenization with an Ultra Turrax homogenizer (Janke and Kundel, W. Germany). Fructose was extracted into 4ml of 80% (v/v) ethanol and citric acid into 2ml of 10% (w/v) trichloroacetic acid. The homogenates were centrifuged at 4,000xg for 10 minutes and fructose and citric acid were determined in the appropriate supernatants using slight modifications of the procedures of Lindner and Mann (1960) and Pearlman, Lardy and Johnson (1934) respectively.

5.3 RESULTS

Mating activity data for hormone-treated wethers is presented without statistical analysis because of the small number of replicates within each treatment. The results are expressed in terms of the components of ram sexual behaviour displayed by the wethers during a libido trial.

Figure 5.1

Mating behaviour of wethers receiving daily i.m. injections of either 0 (oil vehicle), 1, 10 or 100mg testosterone propionate in experiment 5.2.4(1). Mating behaviour was determined in a 10min libido trial and the number of times that a behaviour was displayed is presented as the mean with the S.E.M. indicated by the vertical bars (n=3). S, sniffs; N, nudges; M, mounts; E, ejaculatory reflexes.

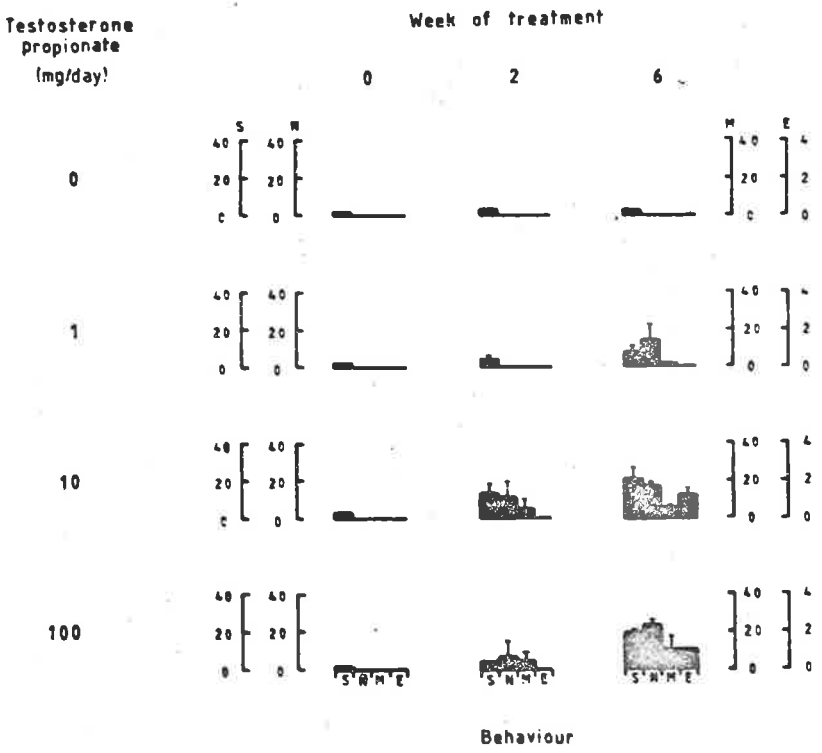

Testosterone propionate (mg/day)
Week of treatment
0
2
6
0
1
10
100
S
W
M
E
40
20
0
4
2
0
S W M E
Behaviour

Table 5.1:

Mating behaviour of wethers receiving daily i.m. injections of testosterone propionate in experiment 5.2.4(1). Mating behaviour was determined in a 10min libido trial and the results are presented as the mean ± S.E.M. of three animals. The values in parenthesis indicate the numbers of animals displaying each behaviour.

Testosterone Propionate (mg/day)	Behaviour	Week of Treatment		
		0	2	6
0	S*	2.0±0.6 (2)	4.0±0.6 (3)	2.0± 1.2 (2)
	N	0	0	0
	M	0	0	0
	E	0	0	0
1	S	2.0±1.5 (2)	3.7±2.3 (2)	7.0±4.2 (3)
	N	0	0	13.4±7.6 (2)
	M	0	0	0.7±0.3 (2)
	E	0	0	0
10	S	3.0±0.6 (3)	12.7±5.2 (3)	20.3±6.2 (3)
	N	0	10.7±8.7 (2)	17.0±2.6 (3)
	M	0	5.0±5.0 (1)	6.0±1.0 (3)
	E	0	0	1.3±0.3 (3)
100	S	2.0±0.6 (3)	3.7±0.3 (3)	18.3±2.2 (3)
	N	0	7.3±7.3 (1)	23.0±2.3 (3)
	M	0	4.7±4.7 (1)	10.0±6.5 (3)
	E	0	0	1.0±0.0 (3)

* S, sniffs; N, nudges; M, mounts; E, ejaculatory reflexes.

5.3.1 Mating activity of wethers treated with graded doses of testosterone propionate

In Experiment 1 (Figure 5.1; Table 5.1), wethers receiving 1mg TP per day (approx. 19.5µg TP/kg body weight/day) showed a greater number of sniffs compared with control animals. Two animals in the former group displayed nudging behaviour and mounted the ewes after six weeks of treatment. However, the penis of these animals was not observed to protrude from the sheath during mounting, and consequently they did not achieve intromission or ejaculation.

Both 10 and 100mg TP per day (approx. 195 and 1,950µg TP/kg body weight/day respectively) elicited the complete mating response in wethers after six weeks of treatment. This behaviour included intromission and the ejaculatory reflex which was characterized by a deep pelvic thrust and was followed by immediate dismounting. On these occasions a clear viscous fluid, presumed to be seminal plasma, was observed to drop from the penis of the wethers after they had dismounted. The results in Table 5.1 tend to suggest that after six weeks, animals that received 100mg TP showed a greater number of nudges and mounts (23.0 ± 2.3 and 10.0 ± 6.5 respectively) than animals treated with 10mg TP (17.0 ± 2.6 and 6.0 ± 1.0). However, the apparently higher activity of the 100mg group was due primarily to the behaviour of one wether that displayed numerous abortive mounts in an unsuccessful attempt to "serve" a non-receptive ewe.

The mean number of ejaculatory reflexes for the 10 and 100mg groups, after six weeks, were 1.3 and 1.0 respectively. It was concluded, therefore, that doses of 10 and 100mg TP per day produced similar behavioural responses.

The results of Experiment 1 suggested that the threshold dose of TP required to elicit the complete mating response in wethers was somewhere between 1 and 10mg per day (i.e. between

19.5 and 195µg TP/kg body weight/day). In Experiment 2, therefore, wethers received doses of TP ranging from 2 to 8mg per day.

Experiment 2 — In Experiment 2 (Figure 5.2, Table 5.2), all animals that received 2 and 4mg TP per day (approx. 40 and 80 µg/kg body weight/day respectively), showed an appreciably higher number of sniffs than the controls, and also displayed nudging behaviour and mounted the ewes during libido trials. However, these animals did not show intromission or the ejaculatory reflex.

All wethers that received 6mg TP per day (approx. 117µg TP/kg body weight/day) displayed the complete mating response which included intromission and ejaculation. In contrast, only two animals in the 8mg group (approx. 156µg TP/kg body weight/day) showed the same response. The "odd" animal in this latter group displayed some sniffs after two weeks of TP injections, but failed to show any sexual behaviour for the remainder of the treatment period. This observation highlights the fact that there are individual differences in the response of wethers to a given dose of TP.

A further general observation was that the mating behaviour of wethers which responded to 6 and 8mg TP per day was comparable to the behaviour of wethers treated with 10 and 100mg TP in Experiment 1 (compare Figures 5.1 and 5.2).

Experiment 3 — Experiment 3 (see Figure 5.3, Table 5.3) was essentially a repeat of Experiment 2 except that measurements were made of the plasma testosterone concentration in wethers undergoing TP treatment. It was hoped that this data would provide some information regarding the plasma testosterone threshold required for complete mating activity in rams.

Figure 5.2

Mating behaviour of wethers receiving daily i.m. injections of either 0 (oil vehicle), 2, 4, 6 or 8mg testosterone propionate in experiment 5.2.4(2). Mating behaviour was determined in a 10min libido trial and the number of times that a behaviour was displayed is presented as the mean with the S.E.M. indicated by the vertical bars (n=3). S, sniffs; N, nudges; M, mounts; E, ejaculatory reflexes.

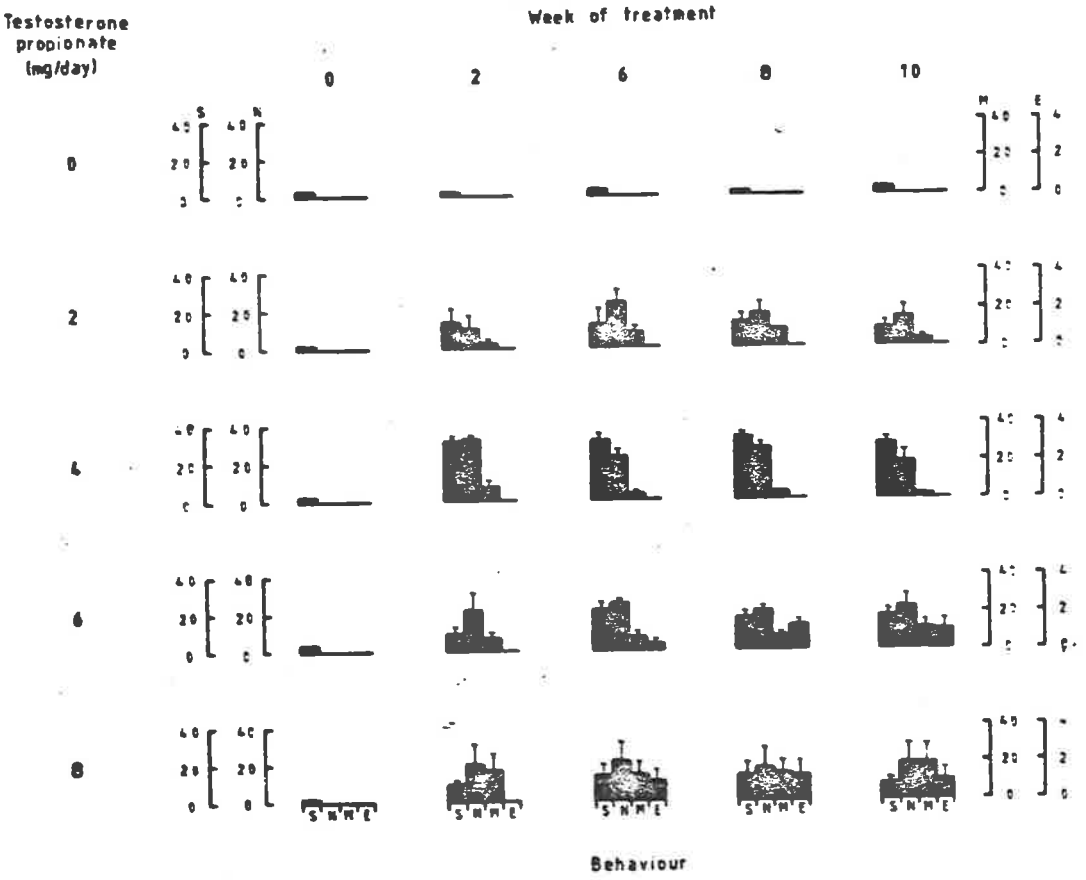

Testosterone propionate (mg/day)
Week of treatment
0
2
6
8
10
0
2
4
6
8
S
N
M
E
S N M E
Behaviour

Table 5.2:

Mating behaviour of wethers receiving daily i.m. injections of testosterone propionate in experiment 5.2.4(2). Mating behaviour was determined in a 10min libido trial and the results are presented as the mean ± S.E.M. of three animals. The values in parenthesis indicate the number of animals displaying each behaviour.

Testosterone Propionate (mg/day)	Behaviour	Week of Treatment				
		0	2	6	8	10
0	S*	3.0 ± 1.7 (2)	2.0 ± 0.6 (3)	4.0 ± 1.2 (3)	2.0 ± 2.0 (1)	4.0 ± 0.6 (3)
	N	0	0	0	0	0
	M	0	0	0	0	0
	E	0	0	0	0	0
2	S	2.0 ± 0.6 (3)	13.7 ± 7.6 (3)	12.3 ± 8.1 (3)	13.0 ± 4.0 (3)	9.0 ± 3.5 (3)
	N	0	10.3 ± 6.1 (2)	23.7 ± 6.2 (3)	12.3 ± 5.6 (3)	14.7 ± 6.1 (3)
	M	0	3.0 ± 1.5 (2)	8.0 ± 2.9 (3)	9.0 ± 0.6 (3)	3.7 ± 0.7 (3)
	E	0	0	0	0	0
4	S	3.0 ± 0.0 (3)	31.3 ± 2.4 (3)	31.3 ± 3.3 (3)	33.3 ± 1.2 (3)	28.6 ± 3.0 (3)
	N	0	32.0 ± 1.8 (3)	23.3 ± 3.5 (3)	27.0 ± 2.5 (3)	18.7 ± 6.3 (3)
	M	0	7.3 ± 3.7 (2)	3.3 ± 0.9 (3)	4.0 ± 0.6 (3)	1.7 ± 0.9 (2)
	E	0	0	0	0	0
6	S	4.0 ± 1.0 (3)	9.0 ± 3.8 (3)	21.3 ± 4.5 (3)	17.6 ± 2.3 (3)	17.7 ± 3.7 (3)
	N	0	21.7 ± 8.6 (3)	25.0 ± 2.1 (3)	20.3 ± 3.0 (3)	22.3 ± 6.0 (3)
	M	0	17.0 ± 8.6 (2)	14.3 ± 7.3 (2)	14.3 ± 7.2 (2)	18.7 ± 9.6 (2)
	E	0	0	0.3 ± 0.3 (1)	1.3 ± 0.3 (3)	1.0 ± 0.6 (2)

Table 5.2 (Continued)

Testosterone Propionate (mg/day)	Behaviour	Week of Treatment				
		0	2	6	8	10
8	S	2.0 ± 1.2 (2)	8.7 ± 2.6 (3)	12.6 ± 6.7 (2)	12.6 ± 6.7 (2)	7.0 ± 4.4 (2)
	N	0	20.8 ±10.0 (2)	21.0 ±10.5 (2)	17.7 ± 9.2 (2)	19.0 ± 9.8 (2)
	M	0	17.0 ± 8.6 (2)	14.3 ± 7.3 (2)	14.3 ± 7.2)2)	18.7 ± 9.6 (2)
	E	0	0	1.0 ± 0.6 (2)	1.3 ± 0.7 (2)	1.0 ± 0.6 (2)

* S, sniffs; N, nudges; M, mounts; E, ejaculatory reflexes.

Figure 5.3

Mating behaviour of wethers receiving daily i.m. injections of either 0 (oil vehicle), 0.5, 1, 2, 4, 6 or 8mg testosterone propionate in experiment 5.2.4(3). Mating behaviour was determined in a 10min libido trial and the number of times that a behaviour was displayed is presented as the mean with the S.E.M. indicated by the vertical bars (n=3). S, sniffs; N, nudges; M, mounts; E, ejaculatory reflexes.

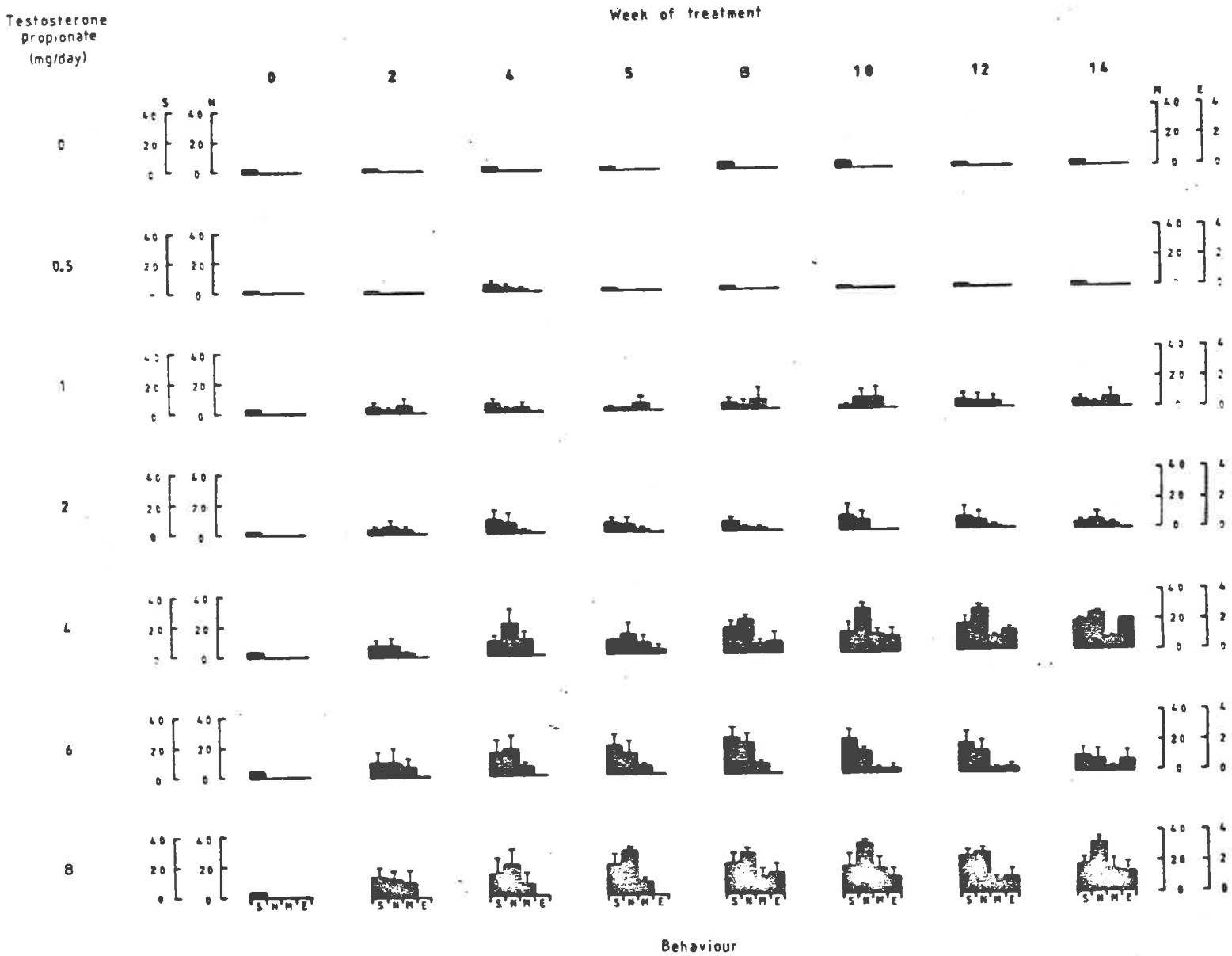

Testosterone propionate (mg/day)
Week of treatment
0
2
4
5
8
10
12
14
0
0.5
1
2
4
6
8
S
N
M
E
Behaviour

Table 5.3:

Mating behaviour of wethers receiving daily i.m. injections of testosterone propionate in experiment 5.2.4(3). Mating behaviour was determined in a 10min libido trial and the results are presented as the mean S.E.M. of three animals. The values in parenthesis indicate the number of animals displaying each behaviour.

Testosterone Propionate (mg/day)	Behaviour	Week of Treatment							
		0	2	4	5	8	10	12	14
0	S*	2.0±1.5(2)	2.0±0.0(3)	3.0 ± 1.7(2)	2.0±1.2(2)	4.0± 1.7(3)	4.0±0.6(3)	2.0±0.0(3)	2.0± 1.0(2)
	N	0	0	0	0	0	0	0	0
	M	0	0	0	0	0	0	0	0
	E	0	0	0	0	0	0	0	0
0.5	S	2.0±0.6(3)	1.0±0.6(2)	5.0 ± 2.5(3)	1.0±0.0(3)	1.0± 0.6(2)	0.7± 0.7(1)	0.3±0.3(1)	0.3± 0.3(1)
	N	0	0	2.7 ± 2.7(1)	0	0	0	0	0
	M	0	0	1.3 ± 1.3(1)	0	0	0	0	0
	E	0	0	0	0	0	0	0	0
1.0	S	3.0±0.6(3)	3.7±2.7(3)	5.7 ± 3.2(3)	1.7±1.2(2)	4.3± 3.8(2)	2.0±1.5(2)	5.0±5.0(1)	3.7±3.2(2)
	N	0	2.0±2.0(1)	2.0 ± 2.0(1)	1.0±1.0(1)	3.3± 3.3(1)	6.7±6.7(1)	4.7±4.7(1)	2.0± 2.0(1)
	M	0	5.0±5.0(1)	3.7 ± 3.7(1)	5.0±5.0(1)	7.7± 7.7(1)	7.3± 7.3(1)	4.0±4.0(1)	6.0± 6.0(1)
	E	0	0	0	0	0	0	0	0
2.0	S	2.0±1.0(2)	3.0±2.5(2)	8.7 ± 6.7(3)	6.0±2.6(3)	6.0± 2.5(3)	8.7±8.7(1)	7.7±7.7(1)	2.7± 2.2(1)
	N	0	4.7±4.7(1)	6.7 ± 6.7(1)	5.0±4.5(2)	1.7± 1.7(1)	6.0± 6.0(1)	5.7±5.7(1)	5.0± 5.0(1)
	M	0	2.7±2.7(1)	0.7 ± 0.7(1)	0.7±0.7(1)	0.7± 0.7(1)	0	1.0±1.0(1)	1.7± 1.7(1)
	E	0	0	0	0	0	0	0	0
4.0	S	2.0±0.0(3)	6.7±4.8(2)	9.3 ± 4.1(3)	8.7±1.3(3)	16.3±4.2(3)	13.3±5.8(3)	17.7±5.9(3)	18.3±1.2(3)
	N	0	6.3±6.3(1)	20.7±10.4(2)	13.7±7.5(2)	22.3±2.2(3)	29.0±3.6(3)	26.7±3.5(3)	24.3±1.5(3)
	M	0	1.3±1.3(1)	10.3 ± 6.1(2)	7.3±5.5(2)	6.0± 3.2(3)	11.3±4.3(3)	8.3±2.7(3)	6.7± 2.7(3)
	E	0	0	0	0.3±0.3(1)	0.7± 0.7(1)	1.0± 0.6(2)	1.3±0.3(3)	2.0± 0.0(3)

Table 5.3 (Continued)

Testosterone Propionate (mg/day)	Behaviour	Week of Treatment							
		0	2	4	5	8	10	12	14
6.0	S	3.7±0.9(3)	9.7±7.2(3)	14.3 ± 9.3(2)	18.3±9.3(3)	23.7±8.1(3)	22.3±6.2(3)	18.7±8.4(3)	10.0±6.1(2)
	N	0	9.7±9.7(1)	17.0 ±10.8(2)	14.0±8.7(2)	20.3±6.7(2)	13.7±3.3(3)	13.7±8.1(2)	8.0±6.6(2)
	M	0	6.0±6.0(1)	5.7 ± 3.5(2)	5.0±2.5(2)	5.7±3.2(2)	2.3±1.5(2)	2.0±1.5(2)	1.7±1.2(2)
	E	0	0	0	0	0	0.3±0.3(1)	0.3±0.2(1)	0.7±0.7(1)
8.0	S	2.0±0.6(3)	13.0±6.2(3)	14.3 ±10.3(3)	19.0±7.6(3)	19.7±7.3(3)	17.3±8.8(3)	23.0±4.9(3)	17.7±5.5(3)
	N	0	11.3 ±5.7(2)	20.3 ±10.2(2)	28.7±2.8(3)	26.3±3.7(3)	32.0±2.6(3)	26.0±3.2(3)	32.0±4.4(3)
	M	0	8.7±8.7(1)	7.7± 7.7(1)	7.7±3.9(2)	10.3±6.1(2)	15.3±9.0(2)	7.0±3.6(2)	14.0±8.0(3)
	E	0	0	0	0	1.3±0.7(2)	1.0±0.6(2)	1.0±0.6(2)	1.3±0.7(2)

* S, sniffs; N, nudges; M, mounts; E, ejaculatory reflexes.

Mating activity

One wether in the 0.5mg TP group (approx. 12µg TP/kg body weight/day) showed some nudges and mounts after four weeks of treatment. However, it is questionable whether this behaviour was due to the TP therapy since the same animal did not show any mating activity either before or after the fourth week. Also, two other wethers that received the same dose of TP failed to show any sexual responses for the duration of the experiment. Both 1 and 2mg TP per day (approx. 24 and 48µg TP/kg body weight/day, respectively) elicited consistent mating activity, which included sniffs, nudges and mounts, in one out of three animals. In Table 5.3 it can be seen that the wether which received 1mg TP showed a relatively high number of mounts during the libido trials. However, the mounting behaviour of this animal was somewhat unusual in that he repeatedly mounted and dismounted the ewes without showing pelvic thrusts.

The penis of wethers that received 1 or 2mg TP was not observed to protrude from the sheath during mounting, and hence these animals did not achieve intromission or ejaculation.

All three wethers that received 4mg TP per day (approx. 96µg TP/kg body weight/day) displayed the complete mating response which included intromission and ejaculation. In contrast, only one animal in the 6mg group (approx. 145µg TP/kg body weight/day) and two in the 8mg group (approx. 192µg/kg body weight/day) showed the complete mating response (see Table 5.3). These results suggested that the threshold dose of TP required to elicit complete mating activity in wethers was 4mg per day (i.e. 96µg/kg body weight/day). The results also indicated, as did the data in Experiment 2, that there are individual differences in the

response of animals to doses of TP above the threshold. For example, one wether in the 6mg group did not show any sexual responses for the duration of the experiment, and another in the 8mg group displayed initial mounting activity after fourteen weeks (see Table 5.3).

Regardless of some individual differences in the response of wethers to TP treatment, the mating activity of wethers that responded to 4, 6 or 8mg TP per day was, in general, comparable to the behaviour of animals treated with 10 and 100mg TP in Experiment 1, and 6 and 8mg TP in Experiment 2 (compare Figures 5.1, 5.2 and 5.3).

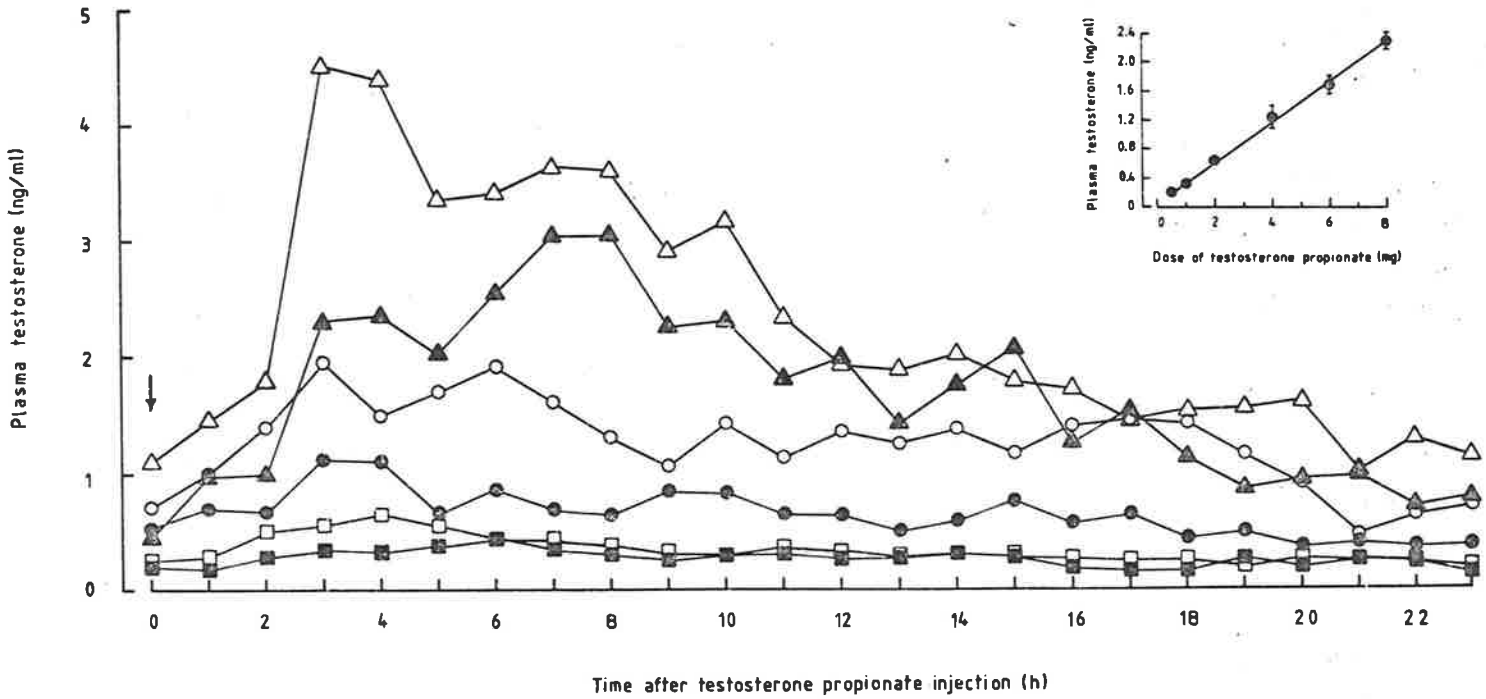
Plasma testosterone profiles

Figure 5.4 shows the plasma testosterone profiles of wethers undergoing treatment with various doses of TP (since TP does not cross-react in the assay system, it was presumed that the assay measured only free testosterone). It was found that there was a linear relationship (r^2 = 1.0) between the dose of TP and the mean plasma testosterone concentration over a twenty-four hour period (Figure 5.4 inset). The hormone data is summarized in Table 5.4. Control wethers had undetectable plasma testosterone levels.

Doses of 1 and 2mg TP per day, which elicited nudges and mounts in a proportion of the wethers, produced plasma testosterone concentrations of 0.32 ± 0.01ng/ml (24h mean ± SEM; n = 3) and 0.65 ± 0.01ng/ml respectively. These testosterone levels are much lower than those normally observed in adult rams. The threshold dose of TP required to elicit the complete mating response in wethers (96µg TP/kg body weight/day) resulted in a plasma

Figure 5.4

Plasma testosterone profiles in wethers following an i.m. injection at 0h (↓) of 0.5 (■), 1 (□), 2 (●), 4 (○), 6 (▲) or 8 (△) mg testosterone propionate. Each point represents the mean of three animals. The inset figure shows the relationship between the dose of testosterone propionate and the mean plasma testosterone concentration during the 24h after injection (mean ± S.E.M. of individual 24h means; n=3).


Plasma testosterone (ng/ml)
5
4
3
2
1
0
0
2
4
6
8
10
12
14
16
18
20
22
Time after testosterone propionate injection (h)
Plasma testosterone (ng/ml)
2.4
2.0
1.6
1.2
0.8
0.4
0
0
2
4
6
8
Dose of testosterone propionate (mg)

testosterone concentration of 1.26 ± 0.13ng/ml (24h mean ± SEM; n = 3). This was also lower than the testosterone levels normally found in rams, but approximated the levels observed during the regressed phase of testicular activity in rams (e.g. around 1.6ng/ml for Merino rams in Chapter 3).

Response of the sex organs to TP treatment

The increase in the size of the penis, and in particular the seminal vesicles, of wethers treated with graded doses of TP verified that the animals had experienced increasing hormone regimes (see Figure 5.5, Table 5.4). The dose response curves for penis length and weight tended to plateau at 4mg TP per day. Interestingly, this was also the threshold dose of TP required for intromission and ejaculation. The sizes of the penis and seminal vesicles of wethers in the 6mg group were lower than would be predicted from the respective dose response curves.

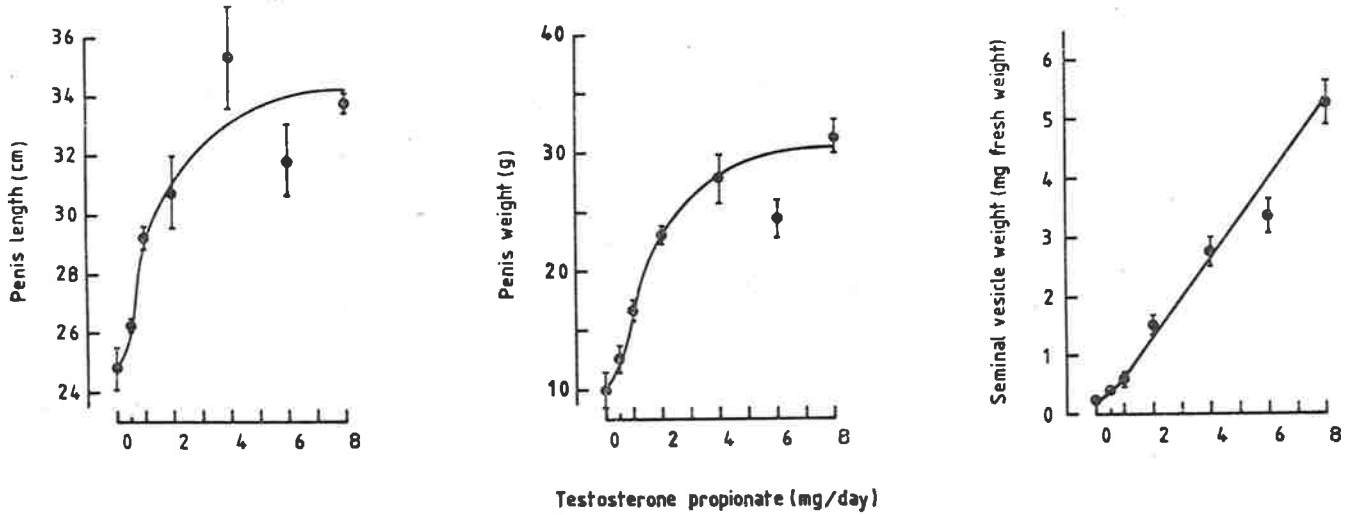
The different doses of TP used in this experiment did not appear to have an apparent effect on body weight (see Table 5.4).

5.4 DISCUSSION

Adult wethers that had been castrated before puberty showed only occasional naso-nasal and naso-perineum contacts with ewes during libido trials conducted before TP therapy. In sheep, olfactory cues are important not only in the detection of oestrous ewes by rams, but also in individual recognition and other non-sexual social interaction (Banks, 1964). It is most likely, therefore, that nosing in untreated wethers was associated with these latter behaviours, rather than with specific mating responses. Clegg and his coworkers also found that adult crossbred wethers

Figure 5.5

. Sex organ characteristics and fructose and citric acid content of seminal vesicles in wethers treated with graded doses of testosterone propionate in experiment 5.2.4(3). Organ weights were determined at slaughter after 14 weeks of treatment and the results are presented as means with the S.E.M. indicated by be vertical bars (n=3).


Penis length (cm)
Penis weight (g)
Seminal vesicle weight (mg fresh weight)
Testosterone propionate (mg/day)

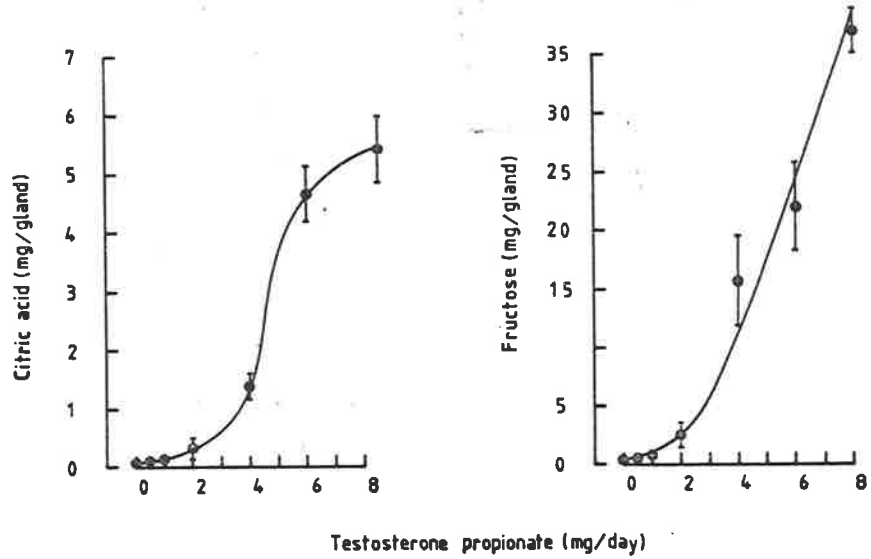

Citric acid (mg/gland)
Fructose (mg/gland)
Testosterone propionate (mg/day)

Table 5.4

Plasma testosterone concentrations and sex organ characteristics in wethers treated with graded doses of testosterone propionate in experiment 5.2.4(3). Wethers received daily i.m. injections of hormone and were killed after 14 weeks of treatment at which time sex organ characteristics were determined. Plasma testosterone concentrations were measured between weeks 7 and 11 as described in Section 5.2.4.

Testosterone Propionate (mg/day)	Body Weight (kg) Week of Treatment 0	Body Weight (kg) Week of Treatment 14	Plasma* Testosterone (ng/ml)	Penis Length (cm)	Penis Weight (gm)	Seminal Vesicles Weight (gm)	Seminal Vesicles Fructose (mg/gland)	Seminal Vesicles Citric Acid (mg/gland)	Adrenal Glands Weight (gm)
0.0	41.7±1.0	45.6±1.3	non detectable	24.8±0.7	10.0±1.5	0.2±0.01	**	**	1.42±0.07
0.5	41.3±1.7	48.3±2.6	0.25±0.02	26.2±0.2	12.7±1.1	0.4±0.02	0.02±0.0	0.34±0.13	1.60±0.08
1.0	41.2±1.1	48.8±0.8	0.32±0.01	29.2±0.4	16.6±0.8	0.6±0.1	0.05±0.02	0.56±0.11	1.56±0.13
2.0	41.6±1.3	49.9±1.7	0.65±0.01	30.8±1.2	23.3±1.0	1.5±0.1	0.31±0.15	2.65±0.96	1.69±0.08
4.0	41.7±1.9	51.4±3.7	1.26±0.13	35.3±1.7	27.7±1.9	2.8±0.3	1.39±0.21	15.76±3.83	1.77±0.09
6.0	41.6±1.5	48.1±1.7	1.69±0.10	31.8±1.2	24.4±1.5	3.3±0.3	4.70±0.44	22.07±3.71	1.40±0.08
8.0	41.4±1.8	48.7±0.3	2.25±0.12	33.8±0.4	31.4±1.4	5.3 0.4	5.49±0.52	36.86±1.85	1.50±0.08

* mean ± S.E.M. of individual 24h means.

** seminal vesicles too small for assay

that had been castrated before puberty did not show any mating behaviour towards oestrous ewes (Clegg et al., 1969). It should be pointed out, however, that in the present study three out of sixty-seven wethers were found to display nudges and also mounted the ewes without receiving hormone therapy. This observation suggests, therefore, that some elements of sexual behaviour may be present in a small proportion of pre-pubertally castrated animals.

Daily injections of TP stimulated mating responses in sexually inexperienced wethers. Doses of around 20 to 50µg TP/kg/day elicited nudging and mounting behaviour. However, the penis of these wethers was not observed to protrude from the sheath during mounting, and they did not achieve intromission or display the ejaculatory reflex. On the other hand, wethers that received close to 100µg TP/kg/day, or more, showed mating activity which was comparable to that seen in normal rams. These observations suggest that the lower doses of TP were sufficient to stimulate central neural systems associated with sexual behaviour, but were inadequate to promote the necessary development of the sex structures. Some support for this theory was obtained from actual measurements of penis size in TP-treated wethers. It would appear, therefore, that central neural tissues have a lower testosterone threshold than the sex organs.

Wethers that received daily amounts of TP of around 150, 200, or 2000µg/kg/day, displayed mating responses that were comparable to those shown by wethers treated with the threshold dose (100µg). This suggests that sex drive in rams (and possibly other males also) may be limited by the capacity of central neural tissues to respond to testosterone stimulation.

Other workers have also reported that TP therapy stimulates complete sexual behaviour in adult wethers castrated either before (Clegg et al., 1969; Parrott, 1978) or after (Clegg et al., 1969; Mattner, 1977) puberty. However, the doses of TP used in these studies have generally been in the range of 500 to 1000 µg/kg/day (see Clegg et al., 1969; Parrott, 1978). The results of the present study suggest that these doses are at least five times greater than the threshold required to elicit the complete mating response in sexually inexperienced wethers.

Wethers treated with TP displayed increased sniffing behaviour, nudges and mounts, after two weeks of therapy. intromission and the ejaculatory response, on the other hand, were not observed until five to six weeks of treatment. These findings were consistent with results previously reported by Clegg et al. (1969), and also provided further evidence that central neural tissues have a lower testosterone threshold than the sex structures. It was also noted in the present study that a proportion of wethers which received 4 or 6mg TP/day displayed their first intromission and ejaculatory reflex after 8, 10 and 12 weeks of treatment, whereas all animals that received 10 or 100mg TP/day showed the complete mating response at six weeks; therefore, although the lower doses of TP were able to stimulate the complete mating response, it appears that a longer period of treatment was required for the proper development of the sex structures, as compared with doses of 10 and 100mg TP.

Beach (1958b) has proposed that mating activity in males involves an arousal phase (sexual excitement) and a consummatory phase (ejaculation). In rams, sniffing, nudging and abortive

mounting can be considered elements of the arousal phase of copulation. The results of the present study indicate, therefore, that the arousal phase in rams has a lower testosterone threshold than the consummatory act.

Not all wethers that were treated with doses of TP above 100µg/kg/day showed the complete mating response. In fact, some animals that received around 150 to 190µg/kg/day did not show any response at all. Apparently the failure of a certain percentage of castrates to respond adequately to hormone treatment is a common occurrence since Godke and coworkers (Godke, personal communication) have found that, on average, only five out of seven steers respond to similar hormone treatments. It was also noted in the present study that those wethers which did not respond to hormone treatment were generally either very timid animals, or they had received aggressive threats from ewes during the libido trial early in the experiment.

Mattner (1977) reported that the response of wethers to TP treatment was influenced by season. He found that wethers treated with TP during the normal breeding season of rams (March, 33.5°S) showed a better response than animals treated with the same doses of hormone during the non-breeding season (October). He suggested that this was due to seasonal variations in the sensitivity of central neural tissues to testosterone.

In the present study, Experiment 1 was carried out from February to March, Experiment 2 from May to July of the same year, and Experiment 3 from February to May of the following year. Therefore, the fact that Experiments 2 and 3 were carried out at different times of the year may account for the observation that wethers which received 4mg TP/day in Experiment 3 showed the

complete mating response, whereas the corresponding animals in Experiment 2 only displayed nudges and abortive mounts. However, it must also be considered that on a body weight basis, animals in Experiment 3 received a higher dose of TP/day than their counterparts in Experiment 2 (i.e. 96µg TP/kg/day compared with 80mg/kg/day). This difference may have been sufficient to overcome the threshold required for adequate development of the sex structures in wethers in Experiment 3. There were also age and strain differences between the two groups of wethers.

In the present study TP was administered daily as a single injection and this resulted in a single peak in plasma testosterone concentration in the wethers. The plasma testosterone profile in normal rams is also characterised by episodic peaks (see Chapter 3), but the number of peaks varies with the season. In the non-breeding season 1-3 peaks/24h may be observed whereas more than 3 peaks are generally observed during the breeding season (Schanbacher and Ford, 1976; Sanford et al., 1977). Thus, the testosterone profiles produced in wethers in the present study were more comparable with regard to peak frequency, with the profiles that occur in rams during the non-breeding season. It would be of interest, therefore, to compare the behavioural response of wethers to the same dose of testosterone administered intravenously either as a single bolus injection, or as a series of injections during the day. The latter injection schedule would approximate more closely the pattern of the testosterone profile observed during the breeding season in rams.

The smallest doses of TP that elicited nudging and mounting behaviour in wethers (20 and 50µg/kg/day) produced plasma testosterone concentrations (0.32 and 0.65ng/ml (24h mean, n = 3),

respectively) that were considerably lower than those commonly observed in adult rams (see Chapter 3). Wethers that received the threshold dose of TP required for intromission and the ejaculatory reflex (100µg/kg/day) had plasma testosterone levels (1.26ng/ml; 24h mean, n = 3) that were still appreciably lower than the levels observed in adult rams during the breeding season (Chapter 3 of this thesis; Sanford et al., 1974, 1977; Schanbacher and Lunstra, 1976; Schanbacher and Ford, 1976). In fact, the testosterone levels in these wethers approximated those recorded during the non-breeding season for Suffolk (Schanbacher and Lunstra, 1976), Border Leicester (Chapter 3), Merino (Chapter 3), Romney (Wilson and Lapwood, 1978) and Hampshire-Suffolk crossbred (Schanbacher and Ford, 1976) rams, but were still lower than the corresponding testosterone levels reported for Finish-Landrace and other crossbred rams (Sanford et al., 1974, 1977). These observations indicate that plasma testosterone levels in adult rams are normally above the threshold required for complete mating activity, particularly during the breeding season. Similar conclusions have been drawn for other species (Smith, Damassa and Davidson, 1977; Damassa, Smith, Tennent and Davidson, 1977; Davidson, Stefanick, Sachs and Smith, 1978).

Since testosterone levels in adult rams appear to be above the threshold required for complete mating activity, and there is also no relationship between plasma testosterone concentration and mating drive for individual rams (see Chapter 3), then presumably, differences in libido between rams are related to differences in the response to testosterone of central neural tissues that are associated with sexual behaviour.

Testosterone is now thought to act as a prehormone that is converted to more active metabolites at its target tissues (see Baird, Horton, Longcope and Tait, 1968). For example, testosterone is converted to both 5α-dihydrostosterone and oestradiol-17β in the brain (for a review see Callard, Petro and Ryan, 1978). The observation that oestradiol-17β elicits mating responses in castrated males (Section 1.2.5) has led to the hypothesis that oestrogens may provide the final stimulus for mating behaviour in males under normal circumstances (see Ryan et al., 1972; Naftolin et al., 1975). If this theory proves to be correct, then sexual drive in males may be dependent on the activity of the aromatase enzymes in the brain which convert testosterone to oestradiol-17β (see Naftolin et al., 1975).

The influence of oestrogens on sexual behaviour in rams was investigated by administering a range of oestrogenic compounds to adult wethers. This study is discussed in the next chapter.

CHAPTER 6

EFFECTS OF OESTROGENIC HORMONES AND NON-AROMATIZABLE ANDROGENS ON MATING BEHAVIOUR IN ADULT WETHERS CASTRATED EITHER BEFORE OR AFTER PUBERTY

6.1 INTRODUCTION

Testosterone has been classically recognized as the steroid hormone responsible for sexual activity in males (see Section 1.2.4). However, during the past decade a number of workers have reported that oestrogenic hormones can also stimulate mating behaviour when administered to castrated animals (Södersten, 1973; Fletcher and Short, 1974; Gorzalka, Razek and Whalen, 1975; Fletcher, 1978) and hence it has been suggested that oestrogens, rather than androgens, are the agents responsible for the mediation of sexual behaviour in males (see Section 1.2.5; Naftolin *et al.*, 1975). This could be brought about by the conversion (by aromatization) of systemically-derived androgens to oestrogens within neural tissues (Naftolin *et al.*, 1975).

Experimental data supporting this hypothesis is reviewed in Section 1.2.5. Briefly the evidence is as follows:

(a) aromatase enzymes are located in areas of the male brain known to be associated with sexual behaviour,

(b) compounds that block the conversion (aromatization) of testosterone to oestradiol-17β inhibit testosterone-induced mating behaviour in castrates,

(c) inhibition of mating by blocking aromatization can be overcome by concurrent administration of oestrogens.

(d) androgens which cannot be aromatized to oestrogens are generally ineffective in eliciting mating behaviour in castrated males.

The experiments described in this chapter were designed to investigate if oestrogens can mediate sexual behaviour in male sheep. This was done by administering both natural and synthetic oestrogens, and also non-aromatizable androgens, to adult wethers castrated either before or after puberty. The ability of these various compounds to elicit sexual responses in wethers was assessed using libido trials.

6.2 EXPERIMENTAL PROCEDURES

6.2.1 Animals

Adult Merino wethers, castrated either before or after puberty, were used in the experiments reported in this chapter and were maintained under field conditions. The wethers castrated as adults were sexually active at the time of castration, whilst the prepubertal castrates had not had contact with oestrous ewes before their selection for the experiments (see Section 5.2.3 of Chapter 5).

6.2.2 Assessment of mating behaviour

The mating behaviour of control and hormone-treated wethers was assessed using the libido trials described in Sections 2.4 and 2.5 of Chapter 2. In the current experiments the trials were of ten minutes' duration.

6.2.3 Hormone administration

Subcutaneous implants

Silastic capsules filled with crystalline oestradiol-17β were prepared according to the method described in Section 2.3.1.

Injections

Hormones used for injection were dissolved in peanut oil (Section 2.3.2 of Chapter 2) and administered intramuscularly. During treatment the wethers were yarded daily between 0800 and 0900h, injected between 0900 and 1000h, and returned to their paddock immediately after injection.

6.2.4 Response of wethers to treatment with diverse oestrogens and non-aromatizable androgens

Experiment 1 – Silastic capsules containing crystalline oestradiol-17β were implanted subcutaneously in the neck of wethers castrated before puberty. The capsules were of two lengths calculated to release approximately either 50 or 100µg of hormone per day (Section 2.3.1). Three animals were each implanted with two of the former capsules whilst three other animals received two of the latter capsules. After twenty-five months the capsules were recovered and the amount of hormone released was determined after they had been dried to constant weight. The mating activity of the wethers was assessed one week before implantation and at 2, 85 and 87 weeks after implantation.

This experiment was intended as a pilot study to determine if a more detailed investigation of the effects of oestrogens on sexual behaviour in wethers was warranted. Since two wethers showed sexual behaviour two weeks after receiving the implants, it was decided to expand this study.

Experiment 2 — Wethers castrated before puberty were randomly divided into seven groups of three animals per group (except androsterone, n = 4) and each group received one of the following treatments: oil vehicle (2ml/day); oestradiol-17β (0.2mg/day); oestradiol-17β (1mg/day); oestradiol-17β (0.5mg/day) plus dexamethasone (8mg for the first injection followed by 2mg/day); 5α-dihydrotestosterone (10mg/day) plus oestradiol-17β (0.2mg/day); 5α-dihydrotestosterone (10mg/day); androsterone (10mg/day). The mating behaviour of the wethers was assessed after 0, 2, 4, 5, 6 and 9 weeks of treatment. The plasma concentration of cortisol in animals receiving oestradiol-17β and oestradiol-17β plus dexamethasone was determined by the method described in Section 2.7.4 of Chapter 2. Blood cortisol levels provided an index of adrenal activity in these animals.

Experiment 3 — Wethers castrated before puberty were assigned to one of the following treatments: oil vehicle (2ml/day, n = 3); oestriol (0.5mg/day, n = 3); oestradiol-17α (0.5mg/day, n = 7); oestrone (0.5mg/day, n = 3, or 1mg/day, n = 4); diethylstilboestrol (0.5mg/day, n = 3, or 1mg/day, n = 1); hexoestrol (1mg/day, n = 4). The mating behaviour of these wethers was determined after 0, 2 and 6 weeks of treatment.

Experiment 4 — Five sexually active adult rams were castrated. Mating behaviour was recorded over a period of two years after which time they were injected with either oestradiol-17β (1mg/day, n = 3) or 5α-dihydrotestosterone (10mg/day, n = 2). The mating behaviour of the animals was determined at 1, 2, 3, 4 and 12 weeks after hormone treatment. At twelve weeks, the hormone administration was terminated and mating behaviour was recorded five months later.

6.3 RESULTS

Mating activity data for hormone-treated wethers are presented without statistical analysis because of the small number of replicates within each treatment. The results are expressed in terms of the components of ram sexual behaviour displayed by the wethers during a libido trial.

6.3.1 Mating behaviour of wethers treated with various oestrogens and non-aromatizable androgens

Experiment 1 — In Experiment 1 (Figure 6.1, Table 6.1), the amount of oestradiol-17β released from the silastic capsules per day was somewhat less than the rates of 50 and 100μg that were theoretically possible. It is known, however, that the release of steroid hormones from silastic capsules decreases with time, due to the formation of fibrous connective tissue around the site of implantation and a build-up in the concentration of steroid in tissues surrounding the capsule (Smith, Damassa and Davidson, 1977). Nevertheless, one of the wethers at each level of treatment showed sexual activity during a libido trial two weeks after receiving the implants. After eighty-five weeks all wethers showed sexual responses (Table 6.1). Mounts in these animals were accompanied by pelvic thrusts but the penis was not observed to protrude from the sheath. As a result none of the wethers achieved intromission or ejaculation, nor did they display the ejaculatory reflex during mounting.

Experiment 2 — In Experiment 2 (Figure 6.2, Table 6.2) oestradiol-17β at 0.2 and 1.0mg/day elicited mating behaviour in one out of three and two out of three of the wethers respectively. These animals made pelvic thrusts during mounting but, as in

Figure 6.1

Mating behaviour of wethers implanted with Silastic capsules filled with crystalline oestradiol-17β in experiment 6.2.4(1). Mating behaviour was determined in a 10min libido trial and the number of times that a behaviour was displayed is presented as the mean with the S.E.M. indicated by the vertical bars. S, sniffs; N, nudges; M, mounts; E, ejaculatory reflexes.

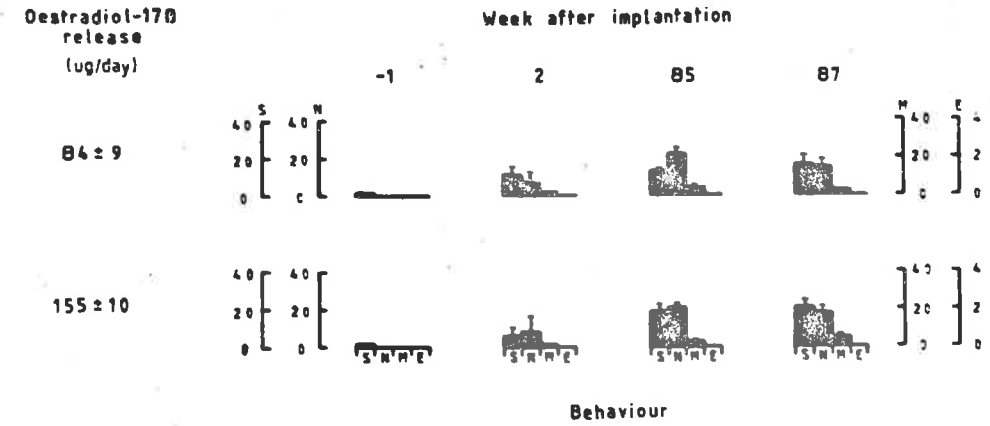

Oestradiol-17β release (ug/day)
Week after implantation
-1
2
85
87
84 ± 9
155 ± 10
S
N
M
E
40
20
0
4
2
Behaviour

Table 6.1:

Mating behaviour of wethers before and after receiving a Silastic implant containing crystalline oestradiol-17β (experiment 6.2.4(1)). Mating behaviour was determined in a 10min libido trial and results for hormone release and behaviour are presented as the mean ± S.E.M. of three animals. The values in parenthesis indicate the numbers of animals displaying each behaviour.

Oestradiol-17β Release (μg/day)	Behaviour	Weeks After Implantation			
		-1	2	85	87
84 ± 9*	S**	1.0 ± 0.6 (2)	10.7 ± 4.6 (3)	12.7 ± 1.8 (3)	16.0 ± 5.5 (3)
	N	0	6.3 ± 6.3 (1)	22.0 ± 3.8 (3)	14.7 ± 3.7 (3)
	M	0	0.7 ± 0.7 (1)	4.0 ± 1.2 (3)	2.0 ± 0.6 (3)
	E	0	0	0	0
155 ± 10	S	2.0 ± 0.6 (3)	6.0 ± 4.6 (2)	19.0 ± 2.3 (3)	21.3 ± 3.8 (3)
	N	0	8.3 ± 8.3 (1)	20.3 ± 2.7 (3)	18.0 ± 3.8 (3)
	M	0	0.3 ± 0.3 (1)	2.7 ± 0.9 (3)	5.3 ± 1.2 (3)
	E	0	0	0	0

* Total hormone released from two identical implants

** S, sniffs; N, nudges; M, mounts; E, ejaculatory reflexes.

Figure 6.2

Mating behaviour of wethers receiving daily injections of oestradiol-17β (OE_2-17β), oestradiol-17β + dexamethasone (DEX, 8mg on day 0 followed by 2mg/day), 5α-dihydrotestosterone (5α-DHT), 5α-dihydrotestosterone + oestradiol-17β or androsterone (ANO) in experiment 6.2.4(2). Mating behaviour was determined in a 10min libido trial and the number of times that a behaviour was displayed is presented as the mean with the S.E.M. indicated by the vertical bars (n=3). S, sniffs; N, nudges; M, mounts; E, ejaculatory reflexes.

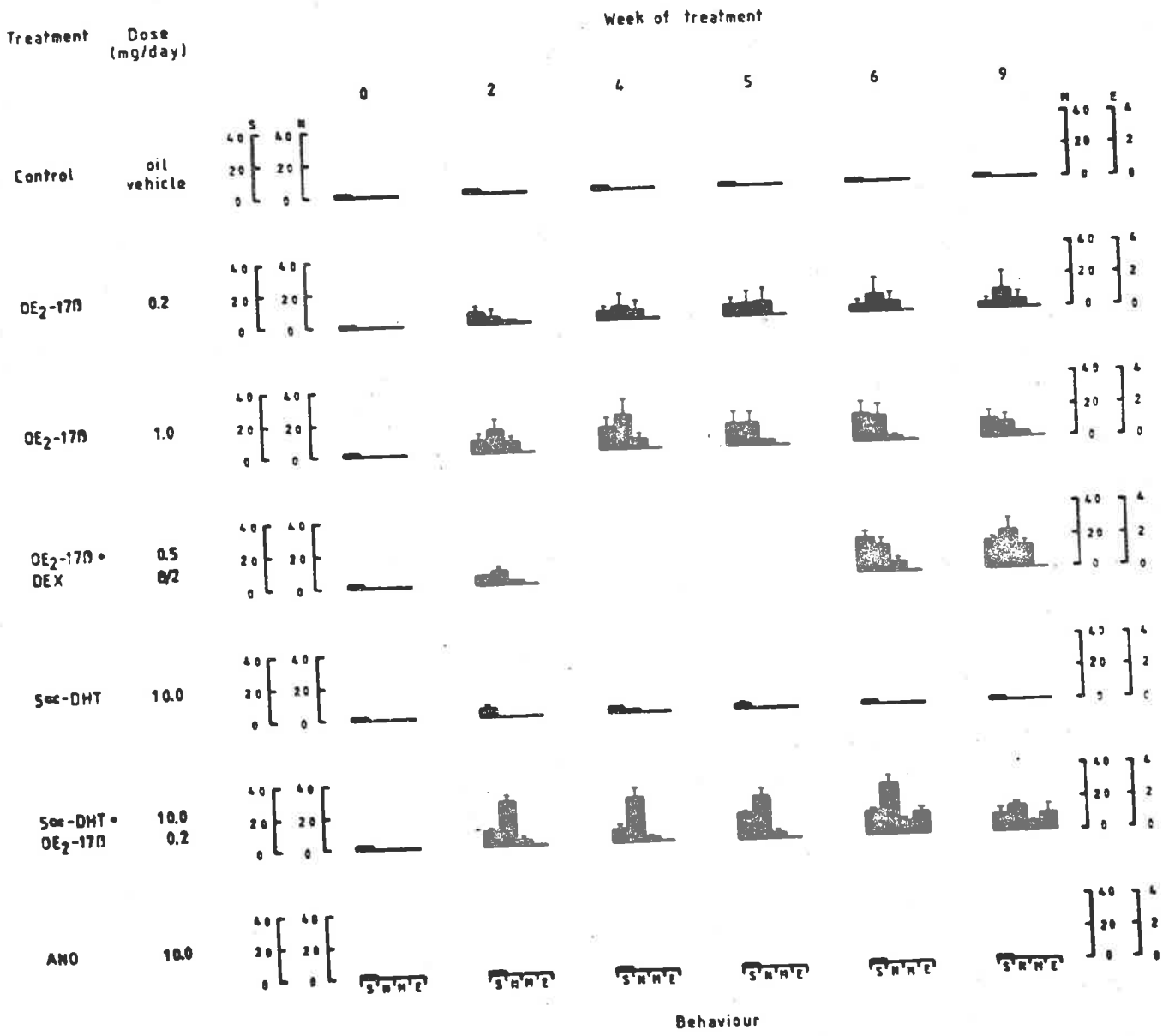

Week of treatment
Treatment
Dose (mg/day)
0
2
4
5
6
9
Control
oil vehicle
OE2-17β
0.2
OE2-17β
1.0
OE2-17β + DEX
0.5 0/2
5α-DHT
10.0
5α-DHT + OE2-17β
10.0 0.2
ANO
10.0
S
N
W
E
40
20
0
4
2
S N H E
Behaviour

Table 6.2:

Mating behaviour of wethers treated with oestradiol-17β and non-aromatizable androgens in experiment 6.2.4(2). Wethers received daily i.m. injections of hormone and mating behaviour was determined in 10min libido trials. Results are presented as the mean ± S.E.M. of three animals. Values in parenthesis indicate the numbers of animals displaying each behaviour.

Treatment	Dose (mg/day)	Behaviour	Week of Treatment					
			0	2	4	5	6	9
Control	Oil Vehicle	S*	1.0±0.6(2)	2.3±0.3(3)	0.7± 0.7(1)	1.3±0.7(2)	1.0± 0.0(3)	1.3± 0.3(3)
		N	0	0	0	0	0	0
		M	0	0	0	0	0	0
		E	0	0	0	0	0	0
Oestradiol-17β	0.2	S	0.3±03.(1)	7.7±3.3(3)	4.7± 3.2(3)	7.0±4.4(2)	3.7± 3.7(1)	3.3± 3.3(1)
		N	0	4.3±4.3(1)	8.0± 8.0(1)	7.3±7.3(1)	10.3±10.3(1)	11.3±11.3(1)
		M	0	0.7±0.7(1)	5.7± 5.7(1)	8.3±8.3(1)	6.0± 6.0(1)	5.0± 5.0(1)
		E	0	0	0	0	0	0
Oestradiol-17β	1.0	S	2.0±0.6(3)	8.3±4.1(3)	13.7± 6.4(3)	14.3±7.2(2)	16.7± 8.6(2)	11.7± 6.0(2)
		N	0	14.3±7.4(2)	20.3±10.3(2)	14.3±7.2(2)	15.7± 8.1(2)	9.0± 5.9(2)
		M	0	6.7±3.5(2)	6.0± 3.5(2)	0.7±0.7(1)	2.3± 1.2(2)	1.7± 1.7(1)
		E	0	0	0	0	0	0
Oestradiol-17β + Dexamethasone	0.5 / 8/2 **	S	2.0±0.0(3)	5.3±0.9(3)			20.7± 4.3(3)	17.0± 2.3(3)
		N	0	8.3±2.8(3)			15.7± 5.4(3)	22.7± 7.9(3)
		M	0	1.3±0.9(2)			5.7± 2.9(3)	13.7± 3.9(3)
		E	0	0			0	0
5α-Dihydro-testosterone	10	S	0.3±0.3(1)	4.7±1.8(3)	3.0± 0.6(3)	1.7±1.2(2)	1.0± 0.0(3)	0.3± 0.3(1)
		N	0	0	0.3± 0.3(1)	0	0	0
		M	0	0	0	0	0	0
		E	0	0	0	0	0	0

Table 6.2 (Continued)

Treatment	Dose (mg/day)	Behaviour	Week of Treatment					
			0	2	4	5	6	9
5α-Dihydro-testosterone + Oestradiol-17	10 + 0.2	S	2.3±0.3(3)	9.0±2.3(3)	7.7± 3.7(3)	15.3±1.5(3)	13.7± 2.3(3)	9.7± 3.7(3)
		N	0	27.7±3.7(3)	27.7± 6.1(3)	25.7±5.7(3)	31.3± 5.2(3)	15.0 ± 2.0(3)
		M	0	3.3±1.9(3)	1.7± 0.9(2)	2.7±1.7(3)	6.7± 2.7(3)	4.0± 2.1(2)
		E	0	0	0	0	1.3±0.3(3)	1.0±0.6(2)
Adrosterone	10	S	1.0±0.7(2)	0.8±0.5(2)	2.0± 0.4(4)	1.3±0.5(3)	1.8± 0.5(4)	
		N	0	0	0	0	0	
		M	0	0	0	0	0	
		E	0	0	0	0	0	

* S, sniffs; N, nudges; M, mounts; E, ejaculatory reflexes.

** 8mg on day 1 followed by 2mg/day.

Experiment 1, the penis did not protrude from the sheath. Dexamethasone did not inhibit mating behaviour induced by oestradiol-17β. However, dexamethasone did suppress the activity of the pituitary-adrenal axis. The average plasma level of cortisol over twenty-four hours as determined from blood samples taken at hourly intervals were, for the groups treated with oestradiol-17β and oestradiol-17β plus dexamethasone, 9.9 ± 1.2 (mean ± SEM of three animals) and 1.7 ± 0.4ng/ml respectively. 5α-Dihydrotestosterone (10mg/day) and androsterone (10mg/day) did not stimulate any sexual responses in wethers. However, 5α-dihydrotestosterone in combination with oestradiol-17β (0.2mg/day) elicited mating activity in all three animals. In contrast with wethers treated with oestradiol-17β alone, animals that received oestradiol-17β plus 5α-dihydrotestosterone achieved intromission and ejaculated a fluid which was presumed to be seminal plasma.

Experiment 3 — In Experiment 3 (Figure 6.3, Table 6.3) diethylstilboestrol, a potent synthetic oestrogen, elicited mating behaviour in three out of four wethers. Oestrone and oestradiol-17α elicited behaviour in four out of seven and two out of seven of the animals respectively, whilst oestriol was without effect. Hexoestrol, also a synthetic oestrogen but less potent than diethylstilboestrol did not stimulate any mating behaviour in wethers. As in Experiments 1 and 2, oestrogen-treated castrates made pelvic thrusts during mounting buy failed to achieve intromission and did not display the ejaculatory reflex.

Experiment 4 — In Experiment 4 (Figure 6.4) four out of five rams castrated when adult continued to show mounting eleven months after castration, and one ram (animal 1) still showed some mounting activity after two years (see Clegg et al., 1969).

Figure 6.3

Mating behaviour of wethers receiving daily injections of oestrone (OE_1), oestradiol-17α (OE_2-17α), oestriol (OE_3) diethylstolb-oestrol (DES) or hexoestrol (HEX) in experiment 6.2.4(3). Mating behaviour was determined in a 10min libido trial and the number of times that a behaviour was displayed is presented as the mean with the S.E.M. indicated by the vertical bars (the number of animals in each treatment is shown in Table 6.3). S, sniffs; N, nudges; M, mounts; E, ejaculatory reflexes.

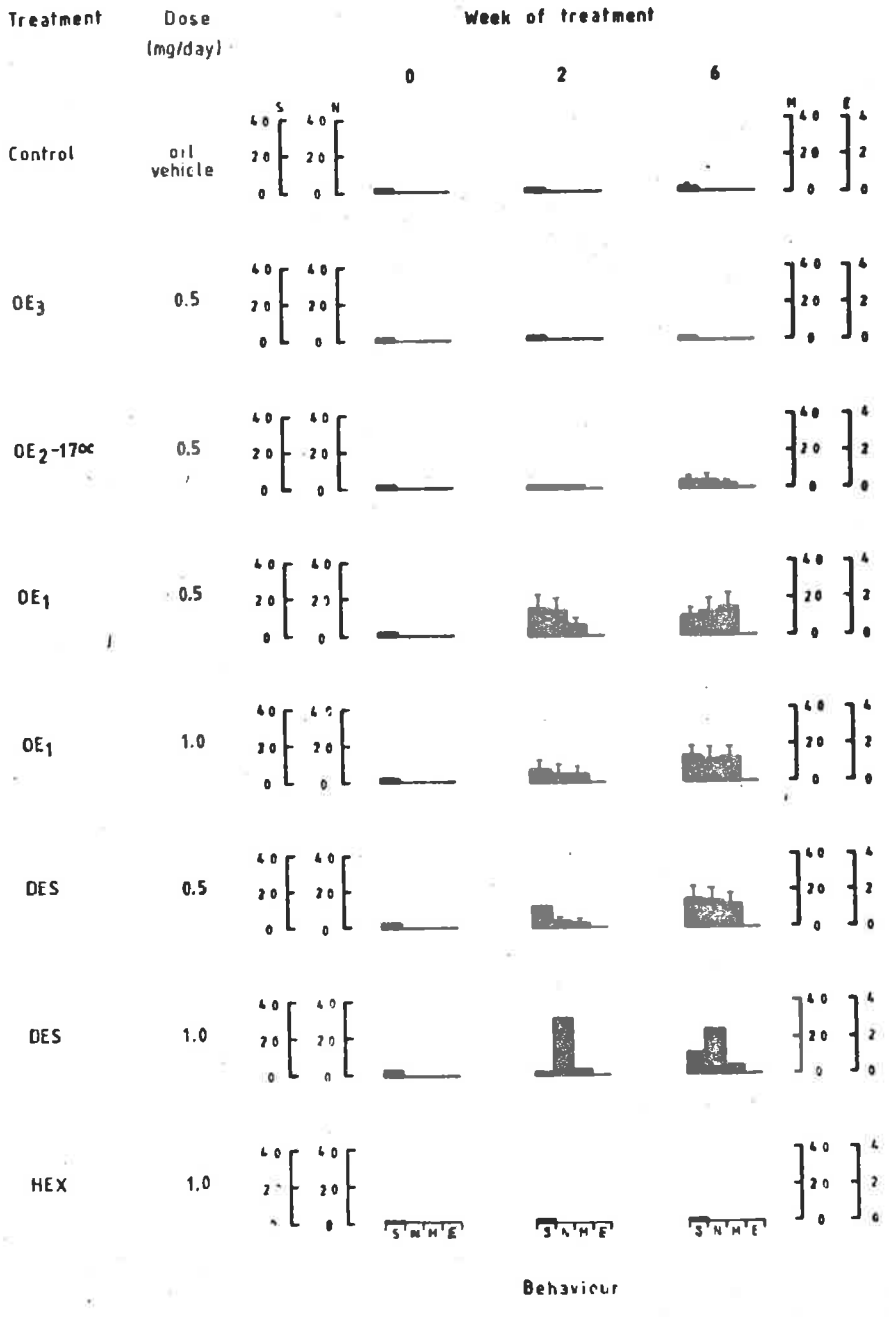

Treatment
Dose (mg/day)
Week of treatment
0
2
6
S
N
M
E
Control
oil vehicle
OE3
0.5
OE2-17oc
0.5
OE1
0.5
OE1
1.0
DES
0.5
DES
1.0
HEX
1.0
S N H E
Behaviour

Table 6.3:

Mating behaviour of wethers treated with natural and synthetic oestrogens in experiment 6.2.4(3). Wethers received daily i.m. injections of hormone and mating behaviour was determined in 10min libido trials. Results are presented as the mean ± S.E.M. of three animals. Values in parentheses indicate the numbers of animals displaying each behaviour.

Treatment	Dose (mg/day)	Number of Animals	Behaviour	Week of Treatment		
				0	2	6
Control (Oil Vehicle)		3	S*	1.3±0.3(3)	1.7±0.9(2)	2.0±1.2(2)
			N	0	0	0
			M	0	0	0
			E	0	0	0
Oestriol	0.5	3	S	0.3±0.3(1)	1.3±0.9(2)	0.7±0.3(2)
			N	0	0	0
			M	0	0	0
			E	0	0	0
Oestradiol-17α	0.5	7	S	1.4±0.5(5)	1.1±0.5(5)	3.6±2.2(3)
			N	0	0.1 0.1(1)	4.0±3.5(2)
			M	0	0.3 0.3(1)	2.0±1.3(2)
			E	0	0	0
Oestrone	0.5	3	S	2.0±1.0(3)	15.0±7.6(2)	10.0±4.5(3)
			N	0	13.7±7.1(2)	12.3±7.0(2)
			M	0	6.0±3.1(2)	14.7±8.1(2)
			E	0	0	0
Oestrone	1.0	4	S	2.0±0.7(4)	6.3±5.9(2)	13.8±5.1(4)
			N	0	4.5±4.5(1)	12.0±7.2(2)
			M	0	4.3±4.3(1)	12.3±7.2(2)
			E	0	0	0

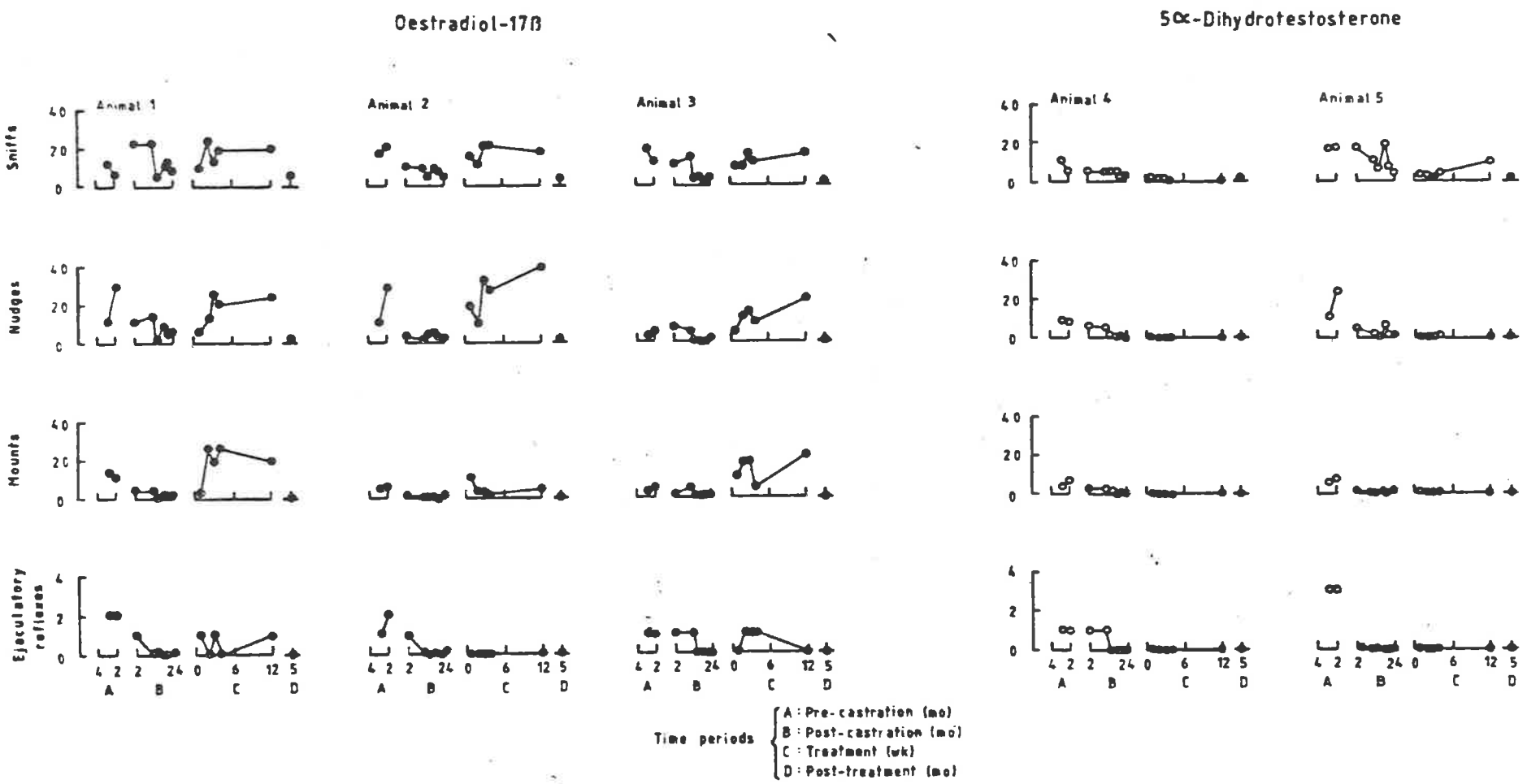
Table 6.3 (Continued)

Treatment	Dose (mg/day)	Number of Animals	Behaviour	Week of Treatment		
				0	2	6
Diethylstilboestrol	0.5	3	S	2.7±0.3(3)	11.7±0.3(3)	15.7±7.4(3)
			N	0	4.0±2.1(3)	15.0±7.5(2)
			M	0	2.7±1.5(2)	12.3±6.2(2)
			E	0	0	0
Diethylstilboestrol	1.0	1	S	3.0(1)	2.0(1)	12.0(1)
			N	0	31.0(1)	24.0(1)
			M	0	3.0(1)	4.0(1)
			E	0	0	0
Hexoestrol	1.0	4	S	0.3±0.3(1)	0.8±0.5(2)	0.3±0.3(1)
			N	0	0	0
			M	0	0	0
			E	0	0	0

* S, sniffs; N, nudges; M, mounts; E, ejaculatory reflexes.

Figure 6.4

Mating behaviour of rams castrated when adult and subsequently treated with either oestradiol-17β (1mg/day, i.m.) or 5α-dihydrotestosterone (10mg/day, i.m.) in experiment 6.2.4(4). Mating behaviour was determined in a 10min libido trial after 1, 2, 3, 4 and 12 weeks. Injections were discontinued after 12 weeks and behaviour assessed five months later.


Oestradiol-17ß
5α-Dihydrotestosterone
Animal 1
Animal 2
Animal 3
Animal 4
Animal 5
Sniffs
Nudges
Mounts
Ejaculatory reflexes
40
20
0
4
2
0
4 2 2 24 0 6 12 5
A B C D
Time periods
A : Pre-castration (mo)
B : Post-castration (mo)
C : Treatment (wk)
D : Post-treatment (mo)

Treatment with oestradiol-17β (1mg/day) restored mating responses in all three castrates one week after the commencement of therapy (see Figure 6.4). Occasionally, these animals displayed the ejaculatory reflex which characterizes a service in entire rams, and some clear viscous fluid, presumed to be seminal plasma, was observed to drip from the penis. However, the penis of these animals was too flaccid to allow intromission. Therefore, the ejaculatory response occurred without intromission.

In contrast with the castrated rams treated with oestradiol-17β, animals given 5α-dihydrotestosterone did not show an increase in sexual behaviour (see Figure 6.4, animals 4 and 5).

6.4 DISCUSSION

The results reported in this chapter support the hypothesis that androgens promote sexual behaviour in males after their conversion to oestrogens in the male brain (see Naftolin *et al.*, 1975). Thus wethers showed mating responses following treatment with diverse oestrogens, but not after treatment with 5α-dihydrotestosterone or androsterone, which are both 5α-reduced non-aromatizable androgens. Similarly Mattner (1976, 1980) and Parrott (1978) also found that oestradiol-17β and oestradiol-17β dispropionate, but not 5α-dihydrotestosterone or 5α-dihydrotestosterone propionate, respectively, can promote mating responses in castrated rams.

In addition to the presumed conversion of testosterone to oestrogens in the male brain, testosterone is also converted to 5α-dihydrotestosterone in the male accessory sex organs where it is responsible for inducing their growth and maintaining their secretory activity (Mainwaring, 1977). In the present study there was a complementary effect between oestradiol-17β and 5α-dihydro-

testosterone in that the two steroids administered together induced the complete mating response, whereas oestradiol-17β alone led only to increased sniffs, nudges and mounting behaviour. The fact that wethers treated with both steroids showed penile protrusion and ejaculated a viscous fluid suggests that 5α-dihydrotestosterone promoted the growth of the penis and initiated the production of secretions in the accessory sex glands.

Baum and Vreeburg (1973) were the first to report that a greater proportion of castrated rats treated with oestradiol-17β in combination with 5α-dihydrotestosterone showed mating responses as compared with castrated rats treated with oestradiol-17β alone. One the basis of this finding, they suggested that mating activity in male rats is dependent on the conversion of testosterone to both oestrogens and 5α-reduced androgens in the brain. This proposal has been supported by a number of subsequent studies in rats (Larsson, Södersten and Beyer, 1973; Baum, Södersten and Vreeburg, 1974; Larsson, Södersten, Beyer, Morali and Pérez-Palacíos, 1976). Mattner (1976, 1980) and Parrot (1978) have suggested that oestradiol-17β and 5α-dihydrotestosterone may also act synergistically in the brain of male sheep to elicit complete mating responses. Although the results of the present study are too few to allow definite conclusions to be drawn, they suggest that oestradiol-17β in combination with 5α-dihydrotestosterone is more effective than oestradiol-17β alone in terms of the proportion of animals stimulated to mount oestrous ewes. 5α-Dihydrotestosterone may serve a priming function in rams which is analogous to the priming role of progesterone in ewes (see Robinson, 1955).

Studies in rats have shown that the administration of oestrogens to castrated animals results in an increase in the secretion of adrenal steroids (Kitay, 1963). Furthermore, castrated rats treated with oestradiol-17β ejaculated during libido trials wheareas similarly treated castrated and adrenalectomized rats mounted but failed to ejaculate (Gorzalka et al., 1975). The difference in response to oestrogen between castrated and castrated-adrenalectomized animals was attributed to the presence of adrenal androgens in the former group (Gorzalka et al., 1975). To exclude the possibility that the behavioural response observed after oestrogen treatment in the present study was due to increased plasma androgens of adrenal origin, and not to the oestrogen as such, a group of castrated animals was given dexamethesone together with oestradiol-17β. Dexamethasone suppressed adrenal activity as assessed by plasma cortisol levels in these animals without affecting their behavioural response to oestradiol-17β. It seems unlikely, therefore, that oestradiol-17β stimulated mounting behaviour indirectly by causing the release of adrenal steroids.

Microgram quantities of oestrogenic hormones are sufficient to elicit mounting behaviour in wethers. Testosterone, on the other hand, must be given in milligram quantities for it to elicit equivalent mating responses (see Chapter 5). If oestrogens are the ultimate agents responsible for eliciting male mating behaviour, then the relatively large doses of androgen required to achieve a similar response would suggest that only a small proportion of the androgens is converted to oestrogen. This concept is supported by the low levels of oestradiol-17β measured in ram plasma (Schanbacher and Ford, 1976) and by the small amount of oestrogen

produced by aromatization of androgens in the male brain of other species (Naftolin et al., 1975).

The concentration of oestradiol-17β in the plasma of normal rams is very low (Schanbacher and Ford, 1976) and may not itself be sufficient to promote mating behaviour. However, if testosterone is aromatized to oestradiol-17β at its site of action in the brain, then the concentration of oestradiol-17β in ram plasma may bear no relationship to that within the brain where higher concentrations might be expected. An analogous situation exists for 5α-dihydro-testosterone which is the major metabolite produce from testosterone in the accessory sex organs (Mainwaring, 1977) but which is found in very low concentrations in the plasma of normal rams (Schanbacher, 1976).

Wethers castrated before puberty and treated with oestrogens when adult made pelvic thrusts during mounting but did not display the ejaculatory reflex. On the other hand, wethers that were castrated when adult and subsequently treated with oestrogen did, on occasion, show the ejaculatory response. Since the penis of these animals was too flaccid to allow intromission to occur, this suggests that the ejaculatory reflex may occur without an actual service. This concept is supported by the work of Fabre (1977) which showed that oophorectomized ewes chronically treated with TP (15mg/day) can also display the ejaculatory reflex whilst mounting oestrous ewes.

It is generally thought that the action of a steroid hormone within a tissue is dependent on the presence in the cell cytoplasm of specific receptors which bind the steroid (King and Mainwaring, 1974). The presence of oestrogen receptors has been demonstrated in the brain of the male animal in a number of species

(Kahwanago, Heinricks and Herrman, 1969; Davies, Naftolin, Ryan and Siu, 1975; Vreeburg, Schretlen and Baum, 1975) suggesting that the male brain has the capacity to respond to oestrogens.

Different steroids vary in the affinity with which they are bound by a certain receptor, and this is presumed to be one of the factors determining the biological potency of the steroid. In the present study, oestrone, oestradiol-17β and the synthetic oestrogen, diethylstilboestriol, were the most effective oestrogens in eliciting mounting behaviour. Oestradiol-17α caused mounting in a small proportion of animals whereas oestriol and hexoestrol were completely ineffective. The relative effectiveness of these oestrogens in promoting ram sexual behaviour in wethers was consistent with their relative binding affinity for the oestrogen receptor in the ram brain (Pelletier and Caraty, 1981) and also other tissues in sheep (Korenman, Tulchinsky and Eaton, 1970; Thieulant and Pelletier, 1979).

CHAPTER 7

GENERAL DISCUSSION

The importance of the testes for expression of normal mating behaviour in males has been recognized at least since the Neolithic age when animals were first domesticated (Steinach, 1940). As the result of numerous castration and hormone replacement studies, it is now clearly established that gonadal steroids are the principal determinants of mating behaviour in both males and females (Young, 1961; Hart, 1974). At the time that this thesis was conceptualized testosterone had been shown to elicit complete mating responses in wethers (Banks, 1964; Clegg et al., 1969). However, the finer control of mating behaviour in rams by testosterone remained poorly understood. One reason for this was that until the early 1970s techniques were not available for routine measurement of the minute quantities of gonadal steroids found in blood. In addition, there had been little practical demand for such information because of the age-old practice of using genetic background and body conformation as the main criteria for selecting stud rams (Ott and Memon, 1980). Unfortunately, these attributes do not always ensure that an animal will also have adequate mating drive.

Along with other efforts to maximize farm efficiency it was recognized that additional methods were needed for selecting stud rams with good libido. The development of libido trials for assessing ram mating drive (Mattner et al., 1971) provided part of the answer but unfortunately, such trials require specially trained observers and are also time consuming (Wodzicka-Tomaszewska, Kilgour and Ryan, 1981). The purpose of the initial studies described in this thesis, therefore, was to determine if a

relationship existed between the mating drive of individual rams during a libido trial and their blood testosterone concentration. It was argued that if such a relationship was shown to exist, then blood testosterone levels might provide a practical index for selecting stud rams with high libido.

If blood testosterone levels were to be of practical use in predicting the libido of individual rams, then it was obvious that a minimum number of blood samples should be required. In order for one or two blood samples to accurately reflect an individual's testosterone status, it would be necessary for circulating testosterone levels to remain reasonably constant. Unfortunately the plasma testosterone concentration in rams (and also males of other species, Table 1.14) does not remain constant but shows occasional peaks. Also, the number of peaks in circulating testosterone during a 24h period varies between rams and there is no circadian rhythm in the occurrence of peaks. These characteristics of the plasma testosterone profile made it necessary in the present studies to bleed rams at hourly intervals for 24h in order to characterize individual testosterone profiles. Even with this frequency of blood sampling it is quite likely that some testosterone peaks were missed since the half-life of testosterone in rams was reported to be around 10 minutes (Darbeïda and Brudieux, 1980).

The studies in both Merino and British breed rams failed to indicate any relationship between mating drive and various parameters of the plasma testosterone profile. These findings were in agreement with the results of Schanbacher and Lunstra (1976). The absence of a relationship between plasma testosterone and libido for individual rams suggests that mating drive is influenced by factors other than absolute blood testosterone levels. This conclusion

is supported by studies in which testosterone treatment failed to increase mating activity in rams (Knight, 1973; Mattner and Braden, 1975). However, Mittal and Ghosh (1978) reported that a single intramuscular injection of testosterone enanthate (250mg) did improve mating drive in Corriedale rams.

One factor which is known to influence the libido of rams in a pen mating situation is their position in the dominance hierarchy (Lindsay et al., 1976). Since the Merino and British breed rams were maintained under field conditions, it is quite likely that social hierarchies existed within each group. The mating drive expressed by individual rams during the libido trials may therefore have been related to their position in the respective hierarchies, a record of which was not kept. The above argument assumes that there is no relationship between dominance and plasma testosterone in rams. This remains to be determined.

Genetic background may also contribute to differences in libido between rams (Blockey and Galloway, 1975). For example, the genotype may influence the binding of testosterone in central neural tissues associated with mating behaviour (Pelletier and Caraty, 1951), or possibly even the conversion of testosterone in these tissues to more active metabolites (Naftolin et al., 1975) and their subsequent binding (Pelletier and Caraty, 1981). The action of gonadal steroids within the brain includes effects on protein synthesis (Beyer, Larsson and Cruz, 1979), the activities of enzymes which metabolize neurotransmitters (Luine, Khylchevskaya and McEwen, 1975a, b), and the electrical activity of specific neurons (Pfaff, 1981). Therefore, there are many potential loci at both the cellular and subcellular level at which subtle differences in the sequence of events initiated by gonadal steroids may underlie individual differences in mating behaviour.

There exists some controversy concerning the relationship between the libido of rams in a pen mating situation and subsequent mating performance in the field (Mattner et al., 1971; Kelly, Allison and Shackell, 1975; Walkley and Barber, 1976; Kilgour and Whale, 1980; Kilgour and Wilkins, 1980). It could be argued, therefore, that plasma testosterone profiles in rams should be related to breeding performances before concluding that there is no relationship between plasma testosterone and mating potential. However, even if such a relationship was shown to exist, the large number of blood samples required to accurately characterize individual testosterone profiles would still make this method of ram selection unattractive to the commercial stud breeder.

In a series of unique experiments, Stelmasiak (1980) investigated the relationship between mating behaviour and LH responses to luteinizing hormone releasing hormone injection in rams. Preliminary data from these studies suggests that rams with high libido show a greater LH response to luteinizing hormone releasing hormone compared with rams with relatively low libido. This approach has some promise since relatively few blood samples are needed. Future studies aimed at determining endocrine correlates to mating drive in rams should perhaps focus on younger animals. The ability to identify rams with good mating potential early in life would allow undesirable animals to be culled from the breeding stock. It may also be possible at an early age to undertake corrective procedures in suspect rams which are desired as breeders because of important attributes.

Although there was no apparent relationship between mating drive and plasma testosterone concentration for individual rams, seasonal changes in plasma testosterone were nevertheless associated with corresponding changes in mating activity in Border Leicester,

Romney and Suffolk rams. Distinct seasonal changes in the plasma testosterone profiles were also observed in Merino rams. The latter finding was consistent with results from other studies in this breed (Mattner, 1977; Wilson and Lapwood, 1978). Although the Merino is not considered a strict seasonal breeder, Mattner (1977) reported that the seasonal changes in plasma testosterone were paralleled by changes in mating activity. The observation that plasma testosterone and mating drive in rams undergo parallel seasonal fluctuations has led to the suggestion that seasonal changes in libido result directly from changes in circulating testosterone. However, a cause-and-effect relationship has not been clearly demonstrated. In fact, there is evidence to suggest that factors other than (or in addition to) changes in blood testosterone determine seasonal mating in rams. For example, increasing circulating testosterone levels in rams during the non-breeding season by hormone therapy does not result in increased mating drive (Lincoln and Davidson, 1977; Schanbacher and Lunstra, 1977; Schanbacher, 1978). In addition, the seasonal peak in mating activity in rams occurs one to two months after the peak in circulating testosterone. Environmental stimuli which may influence libido include food availability, temperature and daylength. Of these variables, daylength is the most reliable for determining seasonal breeding. In fact, photoperiod has been shown to influence the action of testosterone in central neural tissues (Horst, 1979).

The second series of studies in this thesis were designed to investigate if mating activity in rams caused an increase in plasma testosterone levels. Rams which were allowed contact with oestrous ewes in a 20 minute libido trial did not show any changes in plasma testosterone. Presumably, any increase in testosterone would

have occurred in response to stimulation by luteinizing hormone. Since luteinizing hormone was not measured in these studies it could be argued that 20 minutes may not have been sufficient time to allow a testosterone response (Lincoln, 1976). This argument would be particularly relevant if a luteinizing hormone response occurred during the latter half of the libido trials. However, rams allowed continuous access to oestrous ewes for 24 hours also failed to show any changes in their plasma testosterone profiles. It was concluded from these experiments that mating activity in rams does not induce a neuroendocrine reflex which would result in the secretion of luteinizing hormone and testosterone. Other workers have suggested that reproductive-endocrine status (Sanford et al., 1977) and social environment (Illius et al., 1976a,b) determine whether a testosterone response occurs during mating in rams.

Since rams are normally introduced into the ewe flock in pairs, or groups of three, the effects of competition for access to oestrous ewes on plasma testosterone and cortisol levels in rams were also investigated. It was found that testosterone levels remained unchanged when two rams were introduced simultaneously to a group of ewes. On the other hand, cortisol levels were elevated during the ensuing competition. Although the data are too few to allow definite conclusions, there did appear to be a relationship between the degree of competition between rams and the magnitude of the cortisol rise. These results suggest that plasma cortisol levels may provide an index for selecting compatible rams for use in flock matings.

The failure to demonstrate a relationship between plasma testosterone and mating drive in intact rams led to the use of the wether as an experimental model to study the finer control of mating behaviour in male sheep by testosterone. The ability to elicit mating

responses in adult wethers that had been castrated before puberty indicated that, in long-term castrates, brain centres associated with sexual behaviour remain sensitive to testosterone. At relatively low doses (1 to 2mg/day) testosterone propionate stimulated the behavioural components (arousal mechanisms) of mating but did not promote the necessary development of the accessory sex organs required for intromission and ejaculation (consummatory mechanisms) (Beach, 1958a,b). These findings suggested that central neural tissues associated with mating behaviour have a lower testosterone threshold than the accessory sex organs. Apparent differences in the sensitivity of tissues to testosterone may be related to the conversion of testosterone to different active metabolites at various target tissues (Naftolin et al., 1975; Mainwaring, 1977).

The threshold dose of testosterone propionate (4mg/day) required to stimulate complete mating responses in wethers, including intromission and the ejaculatory reflex, produced plasma testosterone levels that were lower than those normally seen in rams. It would appear, therefore, that circulating testosterone levels in rams are normally above the threshold required for normal mating. This observation may help to explain, in part, why there is no apparent relationship between mating drive and plasma testosterone in mature rams.

Doses of testosterone propionate that were above the threshold (4mg/day) required for complete mating responses in wethers did not stimulate any further increases in behaviour. It was also found that not all wethers which received doses of testosterone propionate above 4 mg/day showed the complete mating response. In fact, some wethers failed to respond at all to supra-threshold levels of testosterone whilst others showed only a poor response. At autopsy

it was found that the accessory sex glands in these animals had undergone normal stimulation. These observations further highlighted individual differences in mating behaviour in male sheep that are not related to differences in blood testosterone levels. The role of testosterone in male mating behaviour therefore appears to be permissive in the sense that there is an absolute requirement for testosterone for normal behaviour: however, in intact males there is no relationship between blood testosterone levels and mating drive.

The discovery of an enzyme system in the male brain that can convert androgens to oestrogens led to the suggestion that testosterone may stimulate mating behaviour in males after its aromatization to oestradiol-17β (Naftolin et al., 1975). In the final study of this thesis the role of oestrogens in the mating behaviour of male sheep was investigated by treating wethers with both natural and synthetic oestrogens and two non-aromatizable androgens. Both oestradiol-17β and diethylstilboestrol (a potent synthetic oestrogen) were found to be very effective in stimulating mating behaviour in wethers. The relative effectiveness of other oestrogens tested (oestrone, oestriol, oestradiol-17α, hexoestrol) appears to be related to their binding affinity for the oestrogen receptor identified in the brain of male sheep (Pelletier and Caraty, 1981). These findings provided strong support for the concept that oestrogens may mediate androgen-induced mating behaviour in rams. The failure of the non-aromatizable androgens, 5α-dihydrotestosterone and androsterone, to stimulate mating behaviour in wethers was also consistent with the aromatization hypothesis. Fulkerson, Adams and Gherardi (1981) have reported that oestradiol-treated wethers are possibly more effective than rams, in inducing the detecting oestrus in ewes.

The failure of 5α-dihydrotestosterone to stimulate mating behaviour in males could be due to reasons other than the fact that this androgen cannot be aromatized. For example, 5α-dihydrotestosterone injected peripherally may not cross the blood-brain

barrier at a rate sufficient to allow adequate concentration in brain centres associated with behaviour. Alternatively, the rapid conversion of 5α-dihydrotestosterone to 5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol, both relatively weak androgens, may preclude any potential behavioural effects of 5α-dihydrotestosterone (Södersten and Gustafsson, 1980a, b; Sholl and Goy, 1981). Despite these possibilities, "testosterone-sensitive" neurons in the preoptic area of the rat brain respond to oestradiol-17β, but are unaffected by 5α-dihydrotestosterone (Kendrick and Drewett, 1980).

5α-Dihydrotestosterone alone did stimulate mating behaviour in castrated males in some studies, suggesting that aromatization of androgens is not required in all species (Yahr, 1979). Yahr (1979) suggested that the apparent negative results obtained in some studies with 5α-dihydrotestosterone were due to a combination of inadequate experimental design and the bias of workers towards the aromatization hypothesis. From her review of the literature Yahr (1979) concluded that 5α-dihydrotestosterone does appear to have a role in stimulating aspects of mating behaviour in males. The structure of androgens, apart from their abilities to be aromatized, appears also to be important in determining behavioural effectiveness (Yahr and Gerling, 1978).

A synergism between oestradiol-17β and 5α-dihydrotestosterone in stimulating mating behaviour was observed in the present study and also another study in wethers (Mattner, 1980). Similar findings have also been reported in rats (Södersten and Gustafsson, 1980b). 5α-Dihydrotestosterone may synergise with oestradiol-17β by stimulating penile reflexes (Hart, 1979a, b; Södersten and Gustaffson, 1980b). However, it appears that these reflexes are not essential for intromission and ejaculation (Hart, 1979b). 5α-Dihydrotestosterone may

therefore also act directly in the brain (Baum and Vreeburg, 1973; Mattner, 1980).

In females in which both oestradiol-17β and progesterone are required for oestrous behaviour, oestradiol-17β has been shown to influence the population of progesterone receptors in the hypothalamus (Feder, Blaustein and Nock, 1979; Etgen, 1981). Progesterone, in turn, effects the metabolism of oestradiol-17β in the same tissue (Reddy, Rajan and Daly, 1980). Mating behaviour in males may therefore involve synergisms between oestradiol-17β and 5α-dihydrotestosterone which result from mechanisms similar to those demonstrated in the female. The failure of 5α-dihydrotestosterone to stimulate behaviour in several studies in males suggests that oestradiol-17β may be required for 5α-dihydrotestosterone action in some, but possibly not all, species.

The sexual differentiation of behaviour centres in the brain early in life (Section 1.2.2) might explain, in part, why oestradiol-17β stimulates appropriate mating behaviour in adult males and females. However, the hormone replacement regime in gonadectomized animals also influences their behaviour response. For example, ovariectomized ewes given a single injection of testosterone show oestrous behaviour (Lindsay and Robinson, 1961b) whereas chronic treatment with testosterone stimulates ram behaviour (Johnson, Hudson, Bogart, Oliver and McKenzie, 1956; Fabre, 1977; Marit, Scheffrahn, Troxel and Kesler, 1979). Therefore, the manner in which different hormones are delivered to the brain, in addition to the nature of these hormones, may determine whether male or female mating behaviour is stimulated.

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