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# THE EFFECT OF LOW AND MEDIUM ENVIRONMENTAL TEMPERATURES ON PHYSIOLOGICAL AND REPRODUCTIVE PARAMETERS IN SOAY RAMS

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A dissertation in partial fulfillment of the requirements for the degree of Master of Science.

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# ABSTRACT

Seven adult Soay rams, which had been cranially sympathectomised, were used to study the effect of a low (7°C) and medium (15°C) environmental temperature on three physiological parameters (body temperature, heart rate and respiration rate) and two reproductive parameters (testis diameter and plasma testosterone concentration). They were kept in a controlled temperature room with artificial lighting conditions of eight hours light and 16 hours dark (8L:16D). There was a short adaptation period, followed by three weeks at 7°C and three weeks at 15°C. Adequate food and water was present at all times.

Results showed that body temperature was lower during the 7°C period than the 15°C period ($P < 0.05$). All the other results were not significant but certain trends were noticed. The heart rate was greater during the cool period compared with the warmer period; and the respiration rate was higher during the warm period. The testis diameter increased from the beginning of the 7°C period to reach a maximum size at the end of the initial period; at the higher temperature (15°C) testis diameter gradually decreased. Testosterone concentrations were high throughout the whole experimental period but the levels were greater during the 15°C period compared with the 7°C period.

It was concluded that although light acts as the major stimulus to the onset of the breeding season in rams, low temperature alone plays a significant role, and the maximum effect is due to a combination of the two factors.

# INTRODUCTION

It is well known that sheep are seasonal breeders and that the main cue to the start of the breeding season is a decrease in daylength. In many parts of the world a decrease in daylength is accompanied by a decrease in temperature, but the importance of temperature alone is not fully understood. In the present study, ganglionectomised sheep are used so that the effect of daylength on the pineal gland is negated, and the direct effect of temperature on testis function can be ascertained.

Soay sheep are a primitive, unimproved breed of *Ovis aries* coming from the inaccessible island of Soay in the St. Kilda archipelago, off the north-west of Scotland. They are one of the most primitive domestic breeds of sheep in Europe and resemble the early neolithic sheep first brought to Britain around 5,000 BC. Very little breed improvement has been carried out by the islanders (Lincoln and Short, 1980). For this reason Soay sheep make good experimental subjects as they tend to show larger variations than the improved breeds.

Rams show a number of convenient and readily quantitated external morphological indices of their sexual state. The most important amongst these is the testis size, changes in testicular volume in rams can be accurately recorded by serial caliper measurements of testicular diameter (Lincoln and Davidson, 1977). In rams, the testosterone concentration is also reflected in the degree of cutaneous hyperaemia in the inguinal region - the 'sexual flush', and in the development of the neck muscles. Rams also show a number of readily quantifiable behavioural characteristics such as 'flehmen' (raising of the upper lip in response to olfactory stimuli) and this is exhibited only when testosterone secretion is high (Lincoln and Short, 1980).

The purpose of this study was to monitor testicle diameter, circulating testosterone levels and sexual flush in rams and to relate them to the effect of different environmental temperatures. At the same time records were made of rectal temperature, heart rate and respiration rate of each individual ram to see whether these physiological parameters changed in response to a change in ambient temperature.

# LITERATURE REVIEW

## Introduction

In the majority of mammalian species the testes do not remain uniformly active throughout the year. Instead, there is a period, usually corresponding to the mating season, when the spermatogenic and androgenic functions of the testes are maximally developed, and outside this time regression occurs (Lincoln, 1981).

It is well known that nutritional status and photoperiodic effects act as major cues to the beginning of the breeding season, but in the absence of these stimuli, what effect does temperature have on male reproductive performance? This literary review deals mainly with effects of temperature on male reproductive parameters, but in order to put these into context some work on photoperiodic and other environmental factors will be included.

The concept of the testes of the mature animals waxing and waning in activity according to season is strange to those familiar with the reproductive physiology of man or some laboratory and domesticated species (albino rat, mouse, rabbit, guinea pig, cattle, pig) in which a constant state of reproductive activity, once puberty is past, is regarded as the norm. Species such as the golden hamster, ferret, rhesus monkey, sheep and goat, in which conspicuous seasonal changes in the testes occur, appear to be the exception. Yet when a wider survey of all mammals is conducted this is clearly not the case (Sadleir, 1969). Among wild mammals there are numerous examples in which a seasonal cycle of testicular activity occurs, and in many cases the seasonal regression of the testes renders the males infertile for part of the year (Allanson, 1932; Wislocki, 1943).

Seasonal changes in daylength, temperature and food supply are important factors of the environment which dictate the seasonal changes in testicular activity. The general assumption has been that the effects of the environment on the testes are mediated through changes in the secretion of gonadotrophins by the pituitary. The only possible exception to this is where environmental factors directly influence testicular activity. For example, extremes of temperature may directly affect spermatogenesis (Lincoln, 1981).

Endocrine function relating to male reproductive behaviour

The general picture is that seasonal regression of the testes results from a decline in pituitary secretion of both lutenising hormone (LH) and follicle stimulating hormone (FSH), and the differences between species in the extent of regression reflect the degree to which the pituitary becomes 'switched off' for part of the year. In the case of the very seasonal species, complete inactivity of the pituitary occurs and the animals show the symptoms of total hypophysectomy.

The decline in gonadotrophin secretion results in regression of the testes and all the associated reproductive functions such that the animal enters a phase of reduced sexual activity. Redevelopment of the reproductive system involves a complete reversal of the changes seen during regression, and a sequence of events occurs at the level of the pituitary, testis, androgen target organs, and behaviour, ultimately resulting in the return of full sexual activity. The seasonal mammals thus provide a natural experiment in changing pituitary function (Lincoln, 1981).

## Photoperiodic effects

Before discussing the effects of temperature on reproduction, it is useful to appreciate the very important role that photoperiodism plays in regulating breeding seasons.

While seasonal changes in temperature, rainfall and food availability are the factors of the environment that dictate survival of adults and young, and are thus ultimately responsible for dictating the timing of the birth season, these are not necessarily the factors used as cues by the animals to regulate their reproductive endocrinology. This is because it is necessary to anticipate the timing of birth by dictating the time of conception, the duration of gestation in mammals usually being fixed. Since accurate timing of conception is important, animals have tended to become reliant on cues from the environment, which are best predictors of the time of year. The majority of species in the temperate and cold climates show changes in reproductive activity in response to seasonal changes in daylight length. The tilted axis of the earth that confers the annual rhythmns in daylight length are well pronounced away from the equator, and these changes provide the proximate cues from the environment that allow for the timing of the mating season. The tilted axis of the earth also provides the ultimate cause of breeding seasons via seasonal changes in temperature and thus availability of food (Baker, 1938).

The male may respond more rapidly to photoperiod than the female and is, therefore, a more suitable experimental subject for studying photoperiod effects (Lincoln and Short, 1980).

Also the male has a simpler endocrine system lacking the positive feedback response to oestradiol found in the female (Short, 1972; Karsch and Foster, 1975), and naturally no oestrous cyclicity to complicate the seasonal cycle.

In order to observe the effect of photoperiodism on the ram more accurately Lincoln (1979) has employed the technique of cranial sympathectomy. Four mature Soay rams, cranially sympathectomised by removal of their superior cervical ganglia, were housed alongside four normal rams in controlled lighting conditions of alternating 16 week periods of short days of eight hours light:16 hours darkness (8L:16D) and long days (16L:8D). The changes in the concentration of FSH, LH, prolactin and testosterone in the plasma, the size of the testes, the intensity of the sexual flush and the sexual and aggressive behaviour of the animals was recorded. While the control rams were able to respond to the artificial lighting conditions with synchronised cycles of reproductive activity, the ganglionectomised animals failed to respond. The treated rams had well developed testes and relatively high levels of gonadotrophins and testosterone in the blood throughout the experiment. It was concluded that the cranial sympathetic nervous system was involved in the photoperiodic control of seasonal breeding in the ram, probably through its role in the innervation of the pineal gland.

In the ram, the secretion of FSH, LH and prolactin by the anterior pituitary gland is influenced by changes in daylength (Pelletier, 1973; Pelletier and Ortavant, 1975; Lincoln, Peet and Cunningham, 1977). These effects are apparently due to alterations in the release of LH-releasing hormone (LH-RH) and prolactin inhibiting factor in the hypothalamus (Ravault and Ortavant, 1977; Lincoln, 1978) but the control mechanism by which the photoperiod induces these changes remains largely unknown. While the ganglionectomised rams failed to respond to the imposed lighting conditions, they nevertheless showed long-term changes in pituitary, testicular and behavioural activity; these were less pronounced than the cyclic changes occurring in the control animals. The long-term variations in concentrations of gondotrophins in the blood were conspicuous during some parts of the study; these changes resulted in alterations in testosterone secretion (as judged by the plasma levels of testosterone) and in more subtle changes in the size of the testes. The changes in the levels of androgens were associated with the changes in the sexual flush and aggressive behaviour. The long-term changes in the reproductive parameters tended to occur in a pattern inversely related to the concentration of prolactin in the plasma, but this relationship was not always apparent. There was considerable variation in the timing of the reproductive rhythms in the ganglionectomised rams. Ganglionectomised animals developed the characteristic features of Horner's syndrome including dryness of the nose and facial skin, slight ptosis and increased blood flow through certain cranial blood vessels (detected from the increased temperature of the horns on cold days).

Ganglionectomy has various effects on functions in the head which can be implicated in the alteration in the response to day-length. The effects on the vasoconstrictor control of the blood vessels in the brain and pituitary gland, and the effects of ptosis on the light intake of the eye could be suggested as explanations of some of the results. The most plausible explanation, however, involved the effects on the pineal gland.

While the ganglionectomised rams did not respond to the changes in daylength in the normal manner, they did show some long-term changes in all the parameters measured.

It was not possible during the relatively short experiment to establish whether these changes represented the effects of other environmental factors such as seasonal changes in temperature (the purpose of this study) or whether they were due to a residual annual rhythm. In support of the latter explanation, there is evidence in a variety of long-lived mammals, including sheep, that a rhythm in gonadal activity persists in the absence of any cues from the environment (Lincoln, 1978). The question remains as to whether temperature has an important role to play.

Temperature effects

The effect of heat: As early as the beginning of the present century the adverse effects of high temperature on scrotal animals appear to have been suspected in cryptorchid men (Felizet and Branca, 1898; 1902).

Since that time there have been many observations on the effects of high temperatures on the reproductive capacity of a wide group of plants, insects, lower vertebrates and scrotal mammals (Cowles, 1965).

Cowles, in his excellent review relating hyperthermia to aspermia, mutation rates and evolution has pointed out that there is a narrow temperature range which seems suitable for male gametogenesis in a wide range of plants and animals. Non-lethal temperatures above this range tend to prevent gamete formation or heighten mutation rate (Cowles suggested that increased environmental temperature may have been responsible for archosaurian extinction, due to heat-induced sterility).

In the scrotal mammals many conditions have been found to affect the spermatogenic function. Season and humidity, as they affect environmental temperature and heat regulation, are well-established factors. Hyperpyrexia due to systemic illness or local inflammation in the testis and scrotum produce degenerative effects on the spermatogenic function. Increased temperatures resulting from hot baths or scrotal insulation in man or from wool covering the scrotum in sheep produce the same effect (Dutt and Hamm, 1957; VanDermark and Free, 1970).

In contrast to the effects of heat on testicular function, extensive short-term reductions in temperature can be tolerated without serious effects on the production of sperm and fertility in a wide range of species (VanDermark and Free, 1980).

In the species with scrotal testes, testicular function may be affected not only by factors which affect whole body heat, but also by heat applied directly to the scrotum, insulation of the scrotum or even confinement of the testes to the body cavity as occurs in cryptorchidism. In one experiment, changes in body temperature were not as effective in raising the temperature of the testis as was the application of local heat (Harrison and Harris, 1956). On the other hand whole body heat is more likely to affect other body functions (endocrine, respiratory, circulatory), which may in turn affect the testes.

Seasonal changes in semen production and the occurrence of summer sterility have long been known in sheep. McKenzie and Berliner (1937) found the lowest spermatogenic activity in rams during the summer months, with spermatogenesis increasing starting in August to October and continuing through January.

High atmospheric temperatures have been shown to lead to increased testicular temperatures in the ram (Moule and Knapp, 1950). Using scrotal insulation Phillips and McKenzie (1934) found that testicular temperature was raised to 37°C compared to 34.9°C for the uninsulated testis at a room temperature between 14°C and 19°C. Removal of the insulation resulted in a return to normal temperature within 20-30 minutes. Scrotal insulation brought about a decrease in testis size and an increase in the percentage of abnormal sperm, first observed one week after insulation.

Cupps, McGowan, Rahlmann and Reddan (1960) found that semen quality in rams in a hot central valley in California during the months of July through September showed decreased ejaculate volume, decreased sperm concentration, lowered sperm motility and increased percentage of abnormal sperm. Shearing (Foote, Pope, Nichols and Casida, 1957) and air conditioning (Whiteman and Brown, 1959) improved semen quality and fertility in rams.

Similar results were obtained by Simpson, Rice, Stuckie and Dutt (1959) when two groups of crossbred rams were kept in similar surroundings with the exception that one group was allowed free access to an air-conditioned room. A 15°C temperature differential was realised in this room on hot days. Weekly semen studies revealed a higher volume, motility, concentration and percent normal cells in the treated rams. Statistically, these differences were all either significant or highly significant.

Work done by Newsome (1973) in Australia shows that high temperatures also affect animals accustomed to living in hot conditions. He found that spermatogenesis is impaired in red kangaroos during hot weather in central Australia. The incidence of impairment increases with heat load and can be quite high. Similarly, the proportion of males with reduced interstitium increased during drought. Some males suffer both disabilities concurrently. These reproductive impairments are ecologically important and pregnancy rates decline during hot, droughty weather, as male infertility and impotence increases.

The effect of cold: Much more research appears to have been done on the effect of heat on male reproductive performance than on the effect of cold. Van Dermarck (1970) states that the functional activities of the testis can be maintained over a much wider range of decreasing temperatures than is the case with increasing temperatures. However, evidence exists indicating that extremes in cold will affect not only fertility and sperm production but also the histological and physiological conditions of the testicular tissue. Compared to heat, cold poses a less severe threat to spermatogenesis in mammals. For example, male rodents can remain fertile at near zero air temperatures (Barnett, 1965).

In an experiment by Dutt and Bush (1955), they looked at the effect of low environmental temperature on initiation of breeding season and fertility in sheep. They state that although the evidence indicated that light is a major factor in regulating the seasonal incidence of oestrus in ewes, the possibility exists that other environmental factors may also have some responsibility in the regulation and expression of oestrus. Environmental temperature, which is closely associated with light changes in natural conditions suggests itself as a factor of possible importance in this respect.

Two groups of twenty ewes and three rams each were used to study the effect of low environmental temperature during the summer season on the time of onset of the breeding season and level of fertility. One group was placed in an air-conditioned room with natural light conditions where the temperature was kept at $45^{o}F$ to $48^{o}F$ on May 26 and the other group in a control room of similar size. Both groups remained in their respective rooms until October 8, and were fed on the same diet. The average date of the first oestrus was nearly eight weeks earlier for the treated ewes than for the control ewes. Regular occurrence of oestrus for the ewes which came into oestrus early but did not conceive indicates that normal season activity had been initiated in ewes kept in the air conditioned room. Fertility of the ewes in which oestrus was initiated early was apparently normal as shown by the breeding data. Semen from treated rams did not show the marked decrease in motility of cells or increase in percentage or abnormal cells which was found for control rams.

Treated rams bred to treated ewes required an average of 1.9 services per conception, while control rams required 5.3 services per conception. The average date of lambing for treated ewes bred to the two groups of rams was December 10 and February 15 respectively.

Rectal body temperature of control ewes averaged 1.4°F above those of treated ewes on days when the outside temperature was 88°F or higher. The respiration rates (breaths per min.) averaged 150 for control ewes and 28 for ewes in the air conditioned room. No differences in the pulse rate (heart beats per min.) between the two groups of ewes was found under conditions of the experiment.

Effect of temperature on endocrine function

The effect of high temperature on endocrine changes in rams has recently been studied by Byers and Glover (in press). In this study, mature Merino rams were subjected to insulation of the scrotum and each week, hourly blood samples were taken over a period of 24 hours, until the animals become azoospermic (four weeks). Weekly samples of semen were collected by electroejaculation. There was a substantial decline in testosterone levels after two weeks of scrotal insulation, and after four weeks, testosterone concentrations were only 25% of control levels.

In a previous experiment with rams, Barrell and Lapwood (1978), made an attempt to define the seasonality of reproduction in rams in the southern hemisphere by repeated measurement of semen characteristics and of plasma lutenising hormone (LH), testosterone and prolactin concentrations. These parameters were studied for 16 months in Romney rams at pasture, with Merino and Polled Dorset rams included for comparison. Semen from all three breeds showed regular seasonal changes in ejaculate volumes, with peak volume being reached in March. Plasma hormone levels also varied in a regular manner with peak levels occurring in summer and autumn, highest levels for prolactin were recorded in November to March, for LH in December to February and for testosterone in January to March. They suggested that these seasonal changes resulted primarily from changes in daily photoperiod. All three breeds exhibited a marked elevation of plasma testosterone concentrations during January, February and March, 1973, while minimum levels occurred between May and November in both years.

Other workers, in various parts of the world, have achieved similar results. Gomes and Joyce (1975), also working in New Zealand, looked at seasonal changes in semen testosterone in adult rams over a period of time. Monthly blood samples were collected from seven rams and assayed for semen testosterone concentrations. Testosterone concentrations were lowest in December (0.76 ng/ml semen), increased gradually through April (3.88 ng/ml) and reached 7.42 ng/ml in May. After a transient decrease in June (to 4.1 ng/ml), testosterone concentrations reached a peak of 8.31 ng/ml in July; concentrations then decreased in August and September. They go on to suggest that light is the major stimulus to testicular steroidogenesis where summer temperatures are moderate; in hotter climates or during acute temperature increases, thermal factors may override photoperiodic effects.

This seasonal variation in endocrine output by rams was also observed by Schanbacher and Lunstra (1976) and Katongole, Naftolin and Short (1974). The former looked at seasonal changes in semen LH, testosterone, and a libido index score in Finnish Landrace and Suffolk rams at eight week intervals from October 1974 through October 1975. Mating was highest for both breeds during the peak breeding season (October) and declined 50% by late spring and summer before it increased the next October.

Semen testosterone levels (> 6 ng/ml) and mating activity were highest during the October evaluations. Testosterone decreased gradually through the winter months and reached the lowest levels in late March (range 1.01 ng/ml to 2.06 ng/ml). Thereafter, concentrations gradually increased to levels observed the previous fall. A positive correlation ($r = 0.59$) between mean testosterone and mating scores collected across months suggested that seasonal fluctuations in semen testosterone influence the sexual behaviour of rams.

An attempt to sum up some of the factors involved in ram reproductive parameters was made by Sanford, Palmer and Howland (1974; 1977). They state that changes in libido and fertilising capability which occur from season to season appear to be linked to changes in both daylength and environmental temperature. Natural increases in daylength bring about decreases in the weight of the testes, diameter of the seminiferous tubules, number of primary spermatocytes and epididynal sperm reserves. Semen quality is adversely affected in rams if the ambient temperature is elevated to 40.5 to 41.0°C.

From mid-August through September, semen testosterone gradually increased approximately fourfold and remained at this elevated level during October and November. Concurrent with the gradual increases in the testosterone level and its maintenance for two months was a continual decrease in both environmental temperature and daylength.

Studies on other species tends to support the findings of lowered testosterone levels in male animals at high environmental temperatures. In an experiment by Bedrak, Chap and Fried (1980), male rats were exposed to temperatures of 33 to 35°C for three weeks; this led to heat acclimatisation. The semen testosterone concentration in these males was lower than that of controls maintained at 20 to 22°C (3.0 ng/ml cf 4.8 ng/ml, $P < 0.01$). The decrease in androgen was independent of major changes in semen FSH and LH concentrations as well as hypothalamic content of thyotropin - releasing hormone (THR), gonadoralin releasing hormone (GnRH) and prostaglandin $E_2$ ($PGE_2$). Similar studies have been carried out by Damber, Bergh and Janson (1980). The scrota of 40 male rats were exposed to a temperature of 33°C or 43°C for 30 minutes. Four days later there was no significant difference in basal testosterone concentration between rats exposed to the two temperatures, but the testosterone response to an injection of 30 μg LH given four days after heat treatment was significantly lower in rats exposed to a temperature of 43°C.

Eik-Nes (1965; 1968) did similar studies using the dog. He found that the rate of hormone secretion showed only slight variation at testicular temperatures between 35.5°C to 39.7°C. If any, a peak of testosterone secretion was seen at 38°C which is the average abdominal temperature of the dog.

At temperatures below 35°C (35°C is the average scrotal temperature in the canine), the ability of the infused testis to produce and secrete testosterone seemed to suffer. Since only testosterone was measured in these experiments, the mechanism by which low testicular temperature produces decreased rates of testosterone secretion is not known. At extremely low testicular temperatures, some secretion of the hormone still took place. Moreover, testes exposed to low temperatures in the course of a 90 minute experiment recovered relatively rapidly their decreased capacity to secrete testosterone when warmed to 37°C during the ensuing 30 minute period.

In an experiment involving 18 adult male rabbits (Riar, Malhotra and Bhat, 1980) six were exposed to a simulated altitude plus cold (-5°C) for the same duration, and six served as untreated controls. Body weight did not differ among the three groups, but the weights of the sex organs in the exposed groups were significantly reduced relative to those of the controls.

Minton, Wetteman and Meyerhoeffer (1979) looked at the effect of elevated temperature on endocrine functions in bulls. Eight Angus bulls were used to evaluate the effects of elevated ambient temperatures on semen testosterone concentration. Bulls were placed in temperature controlled chambers and cannulae were inserted into the jugular veins 15 hours before each of three bleeding periods. Heat stressed and control bulls were exposed continuously to $34 \pm 1$°C and $22 \pm 1$°C respectively. Respiratory rates and rectal temperatures were greater in heat stressed than control bulls. Average semen testosterone concentrations and frequency, magnitude and duration of testosterone secretory spikes were similar for both treatments. Thus, they concluded, either heat stress does not alter androgen biosynthesis on adjustments in metabolism or disposition of androgens occur during heat stress so blood concentration do not reflect testicular synthesis.

The most marked changes in testicular growth, spermatogenic recrudescence and increased androgen secretion occur in the true seasonally breeding animals, such as the reindeer and caribou (Whitehead and McEwan, 1973). They showed that the plasma testosterone level of reindeer increased from 1 ng/ml in August to 30-60 ng/ml in mid-September. By late October, when rutting activity had almost ceased, the testosterone concentration had declined to barely detectable levels. The seasonal pattern exhibited by the caribou was similar except the peak levels associated with rutting occurred about one month later than reindeer.

On the other hand, those males which are more or less fertile throughout the year exhibit fluctuations in these characteristics based on light, temperature and the interaction of these two factors.

Effect of temperature on general body functions

There are more than 200 breeds of sheep widely distributed in the world, from the Arctic circle to the Southern parts of South America and New Zealand (Mount, 1979). Sheep occur in many climates, hot and cold, wet and dry; they also live in the semi-arid regions that experience extremes of heat during the summer and of cold during the winter. The high level of tolerance of sheep to both hot and cold environments, covering most inhabited areas, constitutes their chief climatic physiological characteristic. Their adaptability is due primarily to the highly insulating fleece that gives protection both from the heat load due to solar radiation and from the effects of cold. The second physiological characteristic that is important in the animal's adaptation to the thermal environment is its economy in the use of water, and its ability to produce a highly concentrated urine.

The combination of a high thermal insulation and a high cold induced maximum metabolic rate of 25 W kg $BW^{-0.75}$ leads to a very considerable degree of resistance to cold. A consequence of the high insulation of fleece is the low critical temperature of $-20^{\circ}C$ or even lower in well fleeced animals (this depends on the level of feeding).

Respiratory and cutaneous evaporation in sheep have been calculated to share approximately equally about 25% of the total heat loss at thermal neutrality or below. Above thermal neutrality, respiratory evaporative heat loss increases relative to cutaneous evaporation, and accounts for an increasing part of total heat loss. Panting in mature sheep occurs as a rapid shallow breathing mainly through the nose at rates up to about 300 per minute; 20 per minute is the minimum resting rate.

A particular feature of the sheep's respiratory response to high temperatures is that polypnoea is readily evoked by heating the scrotum of the ram, and the high respiratory rate is maintained in spite of the concomitant fall in rectal temperature. The scrotal skin is well endowed with thermal receptors (Waites, 1962).

Sweating plays a secondary role in evaporative cooling in sheep, in contrast to its important role in cattle. Sheep sweat more than goats and characteristically display a synchronous discharge of sweat glands over the body surface. The minimum loss of water from the skin of sheep is about 8 $gm^{-2}h^{-1}$ and the maximum is from the scrotal skin that can produce sweat at 200

$gm^{-2}h^{-1}$; the local cooling so produced favours normal spermatogenesis. In this connection, panting can be induced more easily by warming the scrotal skin than other areas of the skin.

Summary

In summary, it can be seen that many environmental factors have roles to play in triggering the onset of the breeding season in male animals. Of these, photoperiod is undoubtedly the prime cue in rams, provided that nutrition is adequate, but the importance of temperature is less clear.

Most of the work that has been done reveals that high environmental temperatures have a deleterious effect on the reproductive parameters of many male animals; this effect depends on the temperature involved and the duration of the experimental period. Fewer studies have been carried out on the effect of cold temperatures on male reproductive physiology. On the whole, results from these indicate that the functional activity of the testis can be maintained over a much wider range of decreasing temperatures than is the case with increasing temperatures.

This study attempts to monitor the importance of temperature effects on the testis of the ram in the absence of any photoperiod interference or nutritional stress.

# MATERIALS AND METHODS

## Introduction

The study was carried out using eight adult Soay rams. Numbers 11, 14 and 17 were two years old, and numbers 10, 12, 13 and 15 were six years old. Rams of this breed were chosen because of their extreme seasonality in reproductive physiology and behaviour (Lincoln and Davidson, 1977).

The rams had all been operated on previously to remove their superior cervical ganglia, using the procedure described by Appleton and Waites (1955). This operation removes the sympathetic innervation to the entire head and has the effect of rendering the pineal gland functionally inactive as judged by its enzyme activity (Barrell and Lapwood, 1978). Results obtained by Lincoln (1979) have shown that cranial sympathectomy renders the ram essentially non-photoperiodic.

The rams were kept in a controlled temperature room. The room measured 3.3 m long, 3 m wide and 2.15 m high. The sheep were fed once a day on a complete experimental diet, and hay was provided in the afternoon. Water was available at all times. There were facilities for controlling both the photoperiod and temperature. Throughout the experiment the lighting regime was eight hours light (08.00 h to 16.00 h) and 16 hours dark; the lighting intensity was about 100 lux. Previously the rams had been kept separately penned in a light controlled room (8L:16D) for a period ranging from nine months (11, 14, 17) to six years (10, 12, 13, 15) but the temperature varied according to the ambient temperature. The rams were moved into the controlled temperature room on July 10th. After an initial acclimatisation period (six days at 15°C, followed by six days at 11°C) the animals were subjected to three weeks at 7°C followed by three weeks at 15°C. The temperature within the room fluctuated according to the external temperature but always remained within 2°C of the specified temperature.

One of the rams (number 16) died during the initial adaptation period leaving seven for the remainder of the experiment (numbers 10 to 15 inclusive and number 17).

Experimental technique

Throughout the experiment, measurements were made of the diameter of the testes using calipers, and the intensity of sexual skin flush on the inside of the hind leg was recorded on an arbitrary scale of 0-5 (Lincoln and Davidson, 1977). Other measurements taken included rectal temperature, heart rate and respiration rate.

All the above measurements were taken between 09.00 h and 10.00 h each day, five days a week. Firstly rectal temperature was taken using an automatic thermometer (IVAC 821), followed by heart rate and respiration rate (using a stethoscope). Each sheep was then turned over and a note made of the colour of the skin between the scrotum and the inside of the back leg. This was scored from 0-5 on a purely subjective basis. The diameter of each testis was measured through the scrotal covering, using calipers. Both testes were measured until the two diameter readings were in agreement.

Blood samples (7 ml) were collected from the jugular vein into heparinised vacutainers (between 14.00 h and 15.00 h).

Certain sheep (especially number 15) proved exceptionally difficult to bleed from and it was decided to take samples from him as often as possible but no less than twice a week, in order to decrease the amount of trauma being inflicted on the neck.

The plasma was separated immediately by centrifugation and stored at -20°C until required for the determination, by specific radioimmunoassay, of the level of testosterone (Corker and Davidson, 1978).

## Radioimmunoassay technique

Testosterone extraction

1:2 $^3H$ -testosterone (Amersham International Ltd.) (ca 1000 cpm) in 20 μl of ethanol was added to each sample (100 μl, diluted if necessary with phosphate buffered saline (PBS), containing 0.1% gelatin) and after equilibration extracted with 1 ml hexame-ether 4:1. The aqueous phase was frozen and the organic layer decanted into tubes, evaporated to dryness and the residue dissolved in 200 μl PBS. A 50 μl aliquot was used to determine the recovery and a further aliquot (100 μl) was used for radioimmunoassay.

Standards

A solution containing 1 μg testosterone/ml of ethanol was prepared, 640 μl were removed, evaporated to dryness and the residue dissolved into 100 μl of PBS giving a concentration of 640 pg/100 μl.

Suitable dilutions of this solution were then prepared to give concentrations of 320, 160, 80, 40 and 20 pg/100 μl PBS. These solutions were then aliquoted out in 100 μl quantities, covered with parafilm, and stored at 15°C. Tubes were then removed whenever a standard was required.

Radioimmunoassay

A standard curve covering the range 20-640 pg was set up with each assay. Antiserum (raised in the goat against testosterone-3-carboxymethyl oxime - BSA) was then added (100 µl of a 1/10,000 dilution) to both the standards and unknowns followed by approximately 10,000 cpm of testosterone in 100 µl PBS. Tubes were included which received 200 µl of PBS in place of the antiserum and sample, as a measure of the non-specific binding; also tubes containing 100 µl PBS, 100 µl antiserum and 100 µl tracer to be used as zeros. After whirlimixing, the tubes were incubated at room temperature for one hour or overnight at 4°C. After incubation dextran-coated charcoal suspension (1 ml) was used to separate the free and bound fraction and the bound fraction supernatant counted for 10 minutes or 10,000 counts.

Results were calculated from the standard curve using a log-logit transformation, corrected for recovery and expressed as ng testosterone/ml sample.

For the testosterone assay the detection limit was 0.2 ng/ml and the intra-assay coefficient of variation was 11.5%. The plasma samples were assayed in one batch at the end of the experiment.

# RESULTS

The results were tabulated (Appendix I) and the mean values of individual sheep for each period were used for analysis. The mean values thus obtained were used as paired comparisons for a Student's t-test.

## Body temperature

The trend in body temperature was similar for each ram (Fig. 1). After the initial acclimatisation period the body temperature dropped when the room temperature was decreased to 7°C (mean 38.7°C). The body temperature remained at a low level for the rest of the 7°C period. When the room temperature was increased the average body temperature increased and remained at a higher level for the rest of the period (mean 39.1°C). Rectal temperatures during the two periods were significantly different ($P < 0.05$).

## Heart rate

The heart rate (Fig. 2) was greater during the 7°C period (mean 131 beats $min^{-1}$) compared with the 15°C period (mean 123 beats $min^{-1}$). This difference of eight beats $min^{-1}$ was not significant.

## Respiration rate

The mean respiration rate (Fig. 3) was greater at the higher temperature than at the lower (mean at 7°C, 20.27 cf mean at 15°C, 24.33 breaths $min^{-1}$). On the whole the results for individual rams were very similar but ram 17 seemed to respond more dramatically to the higher temperature (Appendix I) and his respiration rate increased proportionately more than any of the other rams. When the mean values were subjected to the Student's t-test, the difference was not significant.

Testis diameter

All the rams showed a similar trend in their testis diameter measurements (Fig. 4). Generally, testis diameter increased gradually from the onset of the cool period (mean 51.79 mm) reaching a peak near the end of the three week period. When the temperature was increased the testis size decreased slowly over the next three weeks (mean 50.58 mm). Individually, the decline was more marked in certain rams (10, 12) but the trend was obvious in all the rams. Statistically, the difference between the mean values at the two different temperatures, was not significant.

Plasma testosterone concentration

There appeared to be no obvious trend in testosterone levels for each individual ram. The most noticeable division was between two groups of rams; the younger rams (numbers 11, 14, 17) had much higher levels of plasma testosterone than the older rams (numbers 10, 12, 13, 15) and sustained these higher levels throughout the experimental period. When the mean testosterone concentrations were compared for the two different temperatures the levels were higher during the 15$^{o}$C period (mean 13.3 ng/ml) than in the 7$^{o}$C period (mean 10.74 ng/ml). The difference was not significant using the paired comparisons.

Sexual flush

In all the rams there was a trend towards a more pronounced sexual flush at the end of the 7°C period, and this continued into the 15°C period (Appendix I). By the end of the experiment the colouration was decreasing in intensity. Rams 10 and 12 showed very little flush throughout the experimental period (Ram 10 range 0-2; Ram 12 range 0-3), whereas ram 17 showed the greatest overall increase from $\frac{1}{2}$-4. This change in intensity coincided with the increased aggressiveness observed in ram 17 towards the end of the expeimental period.

TABLE 1 (a) Collated data from all seven rams (means ± SEM)

| Date | Body temperature ($^{o}C$) | Heart rate (beats/min) | Respiration rate (breaths/min) | Testicle diameter (mm) | Plasma testosterone concentration (ng/ml) |
|---|---|---|---|---|---|
| Acclimatisation Period | | | | | |
| 15.7.81 | 39.1 ± 0.2 | 117 ± 2.7 | 23 ± 1.7 | 48.1 ± 0.8 | 13.34 ± 4.14 |
| 16.7.81 | 39.0 ± 0.2 | 138 ± 3.2 | 19.± 0.8 | 48.1 ± 1.0 | 17.12 ± 3.62 |
| 17.7.81 | 39.0 ± 0.2 | 127 ± 5 | 19 ± 1.0 | 48.6 ± 1.4 | 14.45 ± 3.86 |
| 20.7.81 | 39.1 ± 0.15 | 135 ± 5 | 21 ± 0.6 | 48.8 ± 1.1 | 9.78 ± 3.29 |
| 21.7.81 | 39.2 ± 0.17 | 116 ± 8 | 19 ± 0.5 | 49.6 ± 1.1 | 3.54 ± 0.92 |

TABLE 1 (b) Collated data from all seven rams (means ± SEM)

| Date | Body temperature (°C) | Heart rate (beats/min) | Respiration rate (breaths/min) | Testicle diameter (mm) | Plasma testosterone concentration (ng/ml) |
|---|---|---|---|---|---|
| <u>Room temperature 7°C</u> | | | | | |
| 22.7.81 | 39.1 ± 0.1 | 125 ± 5.7 | 20 ± 0.7 | 49.6 ± 0.9 | 9.16 ± 3.67 |
| 23.7.81 | 39.1 ± 0.17 | 136 ± 5 | 21 ± 1.5 | 49.3 ± 0.7 | 8.47 ± 3.48 |
| 24.7.81 | 38.9 ± 0.24 | 130 ± 5.6 | 20 ± 0.9 | 50. ± 0.8 | 7.02 ± 3.47 |
| 27.7.81 | 38.5 ± 0.27 | 140 ± 5.3 | 20 ± 1.5 | 52. ± 1.0 | 6.01 ± 3.80 |
| 28.7.81 | 38.7 ± 0.17 | 125 ± 5.2 | 20 ± 0.7 | 51.6 ± 0.9 | 9.68 ± 3.71 |
| 29.7.81 | 38.8 ± 0.24 | 120 ± 8.2 | 18 ± 0.8 | 51.6 ± 0.9 | - |
| 30.7.81 | 38.3 ± 0.18 | 131 ± 4.6 | 20 ± 0.6 | 51.8 ± 1.3 | 9.35 ± 3.50 |
| 31.7.81 | 38.5 ± 0.18 | 127 ± 4.0 | 20 ± 0.9 | 52. ± 1.5 | 20.42 ± 3.86 |
| 3.8.81 | 39.1 ± 0.29 | 136 ± 4.1 | 20 ± 1.1 | 51.9 ± 1.3 | 10.12 ± 3.63 |
| 4.8.81 | 38.5 ± 0.3 | 135 ± 6.5 | 20 ± 1.2 | 52.7 ± 1.3 | 13.59 ± 5.83 |
| 5.8.81 | 38.4 ± 0.18 | 133 ± 3.2 | 21 ± 0.6 | 53.6 ± 1.6 | 10.67 ± 4.77 |
| 6.8.81 | 38.9 ± 0.18 | 130 ± 6.2 | 20 ± 1.0 | 53.1 ± 1.2 | 12.49 ± 4.06 |
| 7.8.81 | 38.2 ± 0.23 | 132 ± 5.2 | 21 ± 1.0 | 53.3 ± 1.4 | 12.08 ± 5.06 |
| 10.8.81 | 38.6 ± 0.35 | 132 ± 5 | 22 ± 1.0 | 51.3 ± 1.7 | 11.21 ± 5.18 |
| 12.8.81 | 39.0 ± 0.24 | 138 ± 7.6 | 21 ± 1.5 | 53.1 ± 1.7 | 10.04 ± 3.95 |

TABLE 1 (c) Collated data from all seven rams (means ± SEM)

| Date | Body temperature ($^{o}C$) | Heart rate (beats/min) | Respiration rate (breaths/min) | Testicle diameter (mm) | Plasma testosterone concentration (ng/ml) |
|---|---|---|---|---|---|
| Room temperature $15^{o}C$ | | | | | |
| 13.8.81 | 39.4 ± 0.12 | 125 ± 7 | 22 ± 0.9 | 52.6 ± 1.6 | 16.48 ± 5.00 |
| 14.8.81 | 39.1 ± 0.22 | 124 ± 7.3 | 21 ± 1.6 | 51.9 ± 1.7 | 8.96 ± 3.57 |
| 17.8.81 | 39.0 ± 0.19 | 123 ± 6.5 | 23 ± 1.6 | 50.7 ± 1.5 | 15.01 ± 5.33 |
| 18.8.81 | 39.3 ± 0.25 | 121 ± 5 | 24 ± 1.4 | 50.7 ± 1.8 | 10.13 ± 4.64 |
| 19.8.81 | 38.9 ± 0.18 | 127 ± 6.2 | 25 ± 1.5 | 50.6 ± 1.5 | 12.35 ± 4.42 |
| 20.8.81 | 39.1 ± 0.1 | 133 ± 7.4 | 24 ± 2.0 | 50.7 ± 1.5 | 16.07 ± 4.37 |
| 21.8.81 | 39.1 ± 0.1 | 124 ± 8 | 26 ± 2.6 | 51.4 ± 1.8 | 12.82 ± 4.70 |
| 24.8.81 | 39.2 ± 0.1 | 133 ± 7.8 | 28 ± 2.1 | 50.3 ± 1.7 | 14.59 ± 4.92 |
| 25.8.81 | 39.1 ± 0.1 | 120 ± 7 | 24 ± 2.6 | 50.7 ± 1.7 | 15.89 ± 6.13 |
| 26.8.81 | 39.0 ± 0.1 | 119 ± 6 | 26 ± 3.5 | 50.1 ± 1.8 | 15.90 ± 5.37 |
| 27.8.81 | 39.0 ± 0.2 | 118 ± 11 | 29 ± 8.8 | 50.0 ± 1.8 | 13.12 ± 5.58 |
| 28.8.81 | 38.9 ± 0.1 | 125 ± 7 | 22 ± 2 | 50.7 ± 1.9 | 14.17 ± 5.16 |
| 31.8.81 | 39.0 ± 0.1 | 117 ± 6 | 24 ± 3 | 49.6 ± 1.8 | 9.90 ± 3.98 |
| 1.9.81 | 39.0 ± 0.1 | 118 ± 7 | 23 ± 2 | 49.3 ± 1.8 | 12.54 ± 5.48 |
| 2.9.81 | 39.0 ± 0.13 | 118 ± 6 | 24 ± 1.6 | 49.4 ± 1.8 | 11.53 ± 4.64 |

Fig. 1 Body temperature during the adaptation period, and at room temperatures of 7°C and 15°C. Each point is the mean of the seven experimental animals.

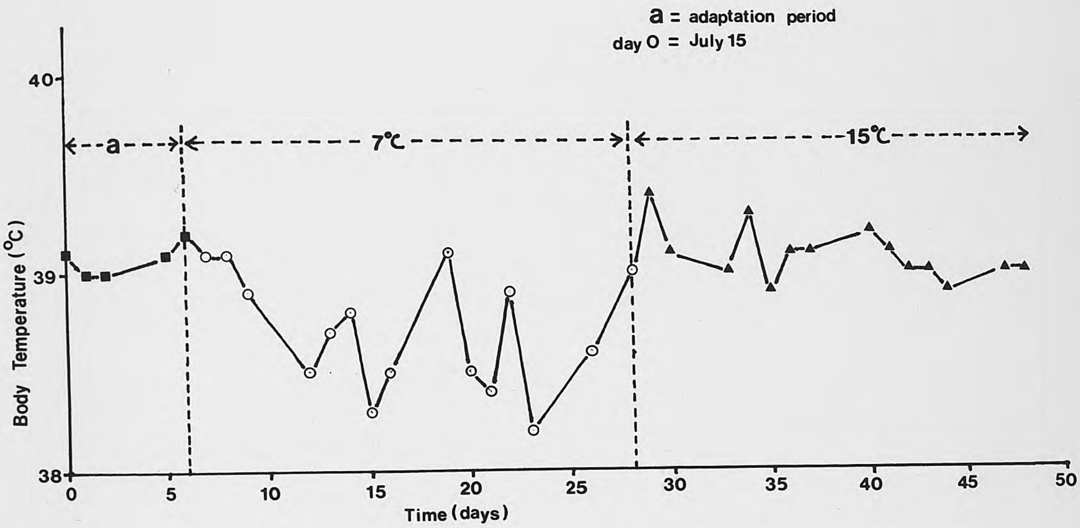

a = adaptation period
day 0 = July 15
a
7°C
15°C
Body Temperature (°C)
40
39
38
0
5
10
15
20
25
30
35
40
45
50
Time (days)

Fig. 2 Heart rate during the adaptation period, and at room temperatures of 7°C and 15°C. Each point is the mean of the seven experimental animals.

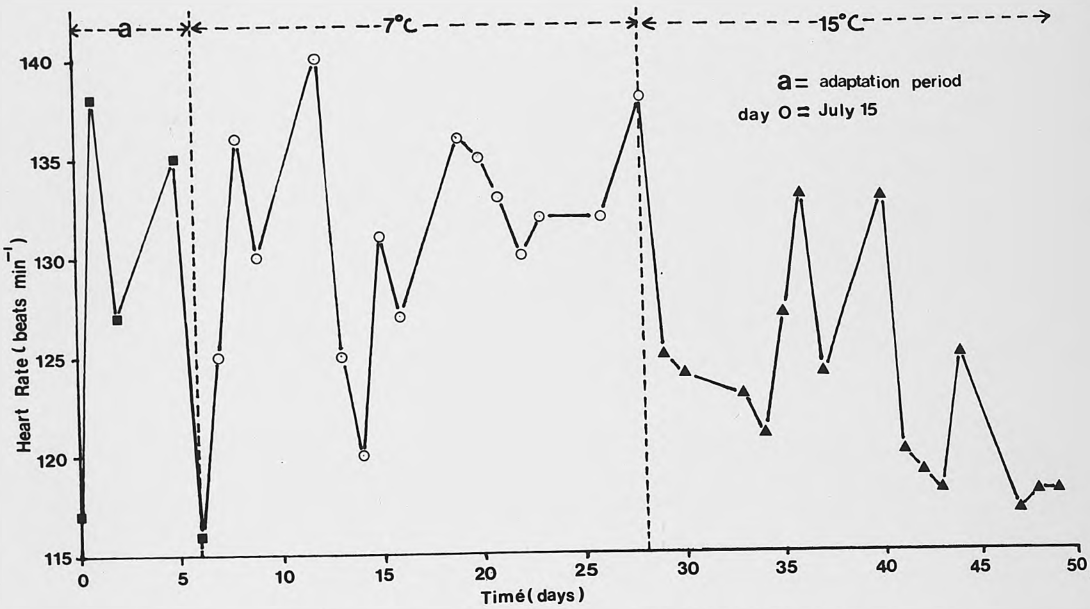

a
7°C
15°C
a= adaptation period
day 0 = July 15
Heart Rate ( beats min$^{-1}$ )
140
135
130
125
120
115
0
5
10
15
20
25
30
35
40
45
50
Timé( days )

Fig. 3 Respiration rate during the adaptation period, and at room temperatures of 7°C and 15°C. Each point is the mean of the seven experimental animals.

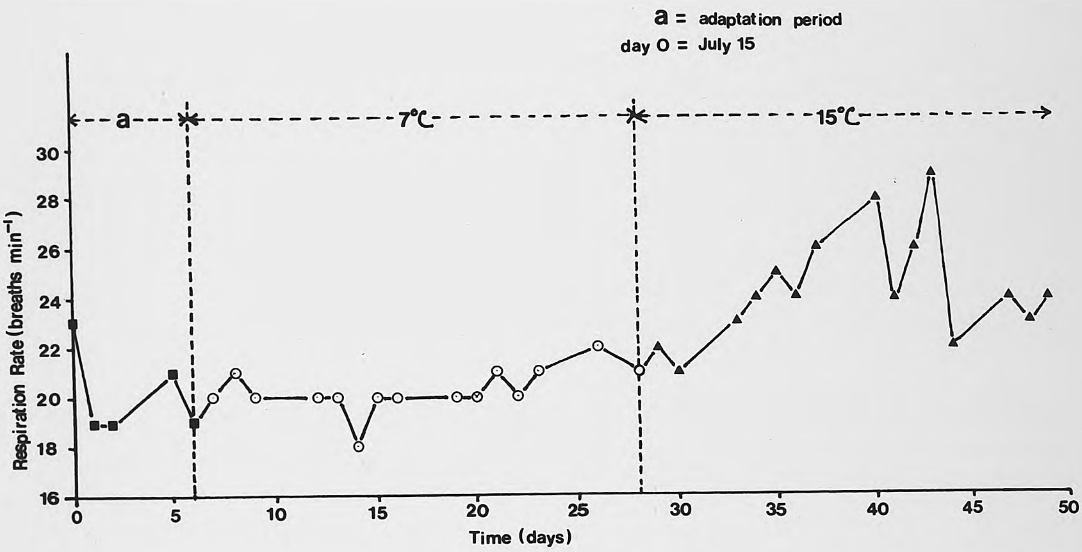

a = adaptation period
day 0 = July 15
a
7°C
15°C
Respiration Rate (breaths min$^{-1}$)
30
28
26
24
22
20
18
16
0
5
10
15
20
25
30
35
40
45
50
Time (days)

Fig. 4 Testis diameter during the adaptation period, and at room temperatures of 7°C and 15°C. Each point is the mean of the seven experimental animals.

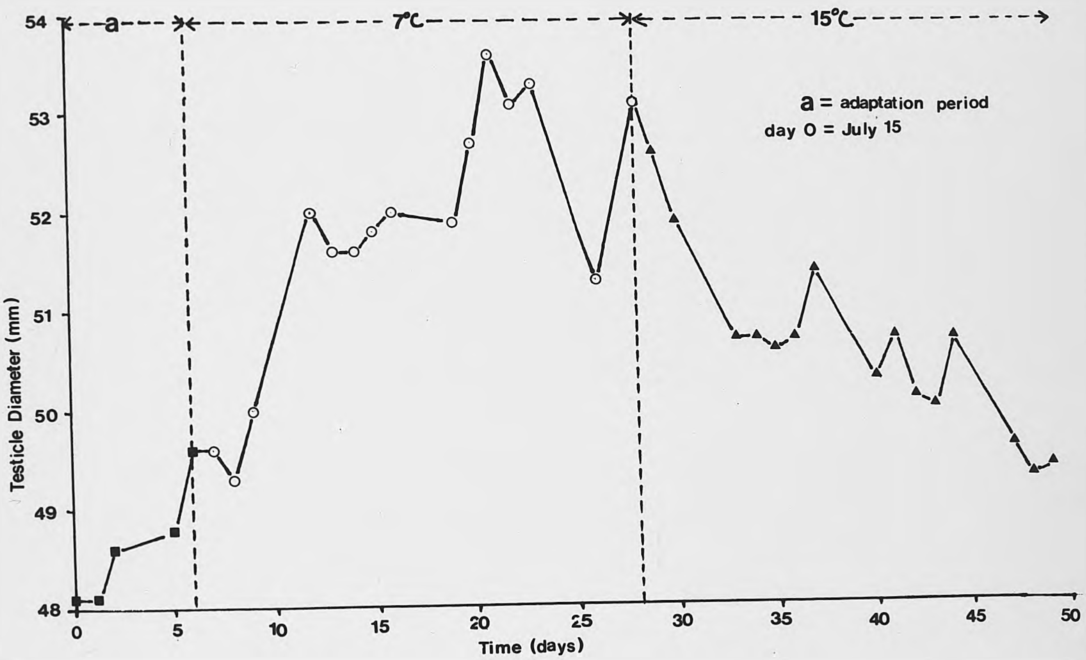
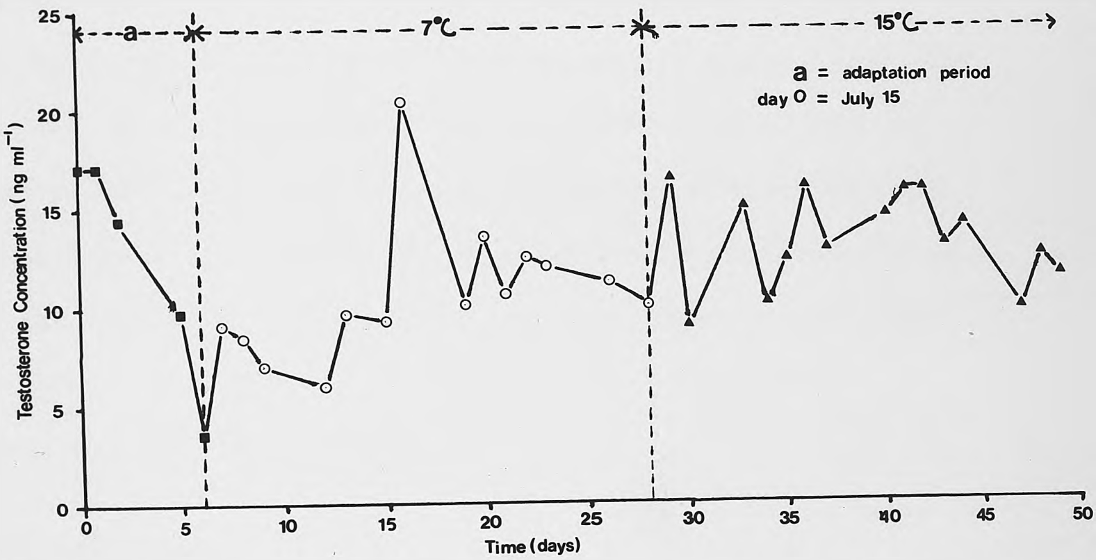

a
7°C
15°C
a = adaptation period
day 0 = July 15
Testicle Diameter (mm)
Time (days)
54
53
52
51
50
49
48
0
5
10
15
20
25
30
35
40
45
50

Fig. 5 Testosterone concentration during the adaptation period, and at room temperatures of 7°C and 15°C. Each point is the mean of the seven experimental animals.


a
7°C
15°C
a = adaptation period
day 0 = July 15
Testosterone Concentration (ng ml$^{-1}$)
25
20
15
10
5
0
0
5
10
15
20
25
30
35
40
45
50
Time (days)

# DISCUSSION

The main problem in the past when dealing with the effect of environmental temperature on ram reproductive performance has been to discount the underlying effect of photoperiod. Although superior cranial ganglionectomy does not completely prevent a natural cycle of hormone production, it does remove the very considerable effect that the pineal gland has on this cycle. It has, therefore, been possible in this study to observe the effect of temperature directly on gonad function as portrayed by testis diameter and plasma testosterone levels. At the same time various physiological parameters have been looked at in an attempt to relate these to different environmental temperatures.

## Body temperature

The average body temperature was greater at the higher environmental temperature than at the lower. This agrees with work done by other researchers in this field; Dutt and Bush (1955) observed that the average rectal temperature of ewes kept in natural conditions during hot summer weather (ambient temperature $90^{o}C$) was $1.4^{o}F$ greater than ewes kept in an air-conditioned room. Minton et al. (1979) showed similar results in heat-stressed bulls.

Exposure to elevated ambient temperatures will cause a rise in body temperature in a wide range of species (Dutt, 1960). Most mammals can maintain themselves within fairly narrow limits, provided the stress applied is not too great. In this experiment, neither of the ambient temperatures chosen (7°C and 15°C) were outside the range that Soay sheep might be expected to meet in their natural Scottish environment at some time during the year. Although their body temperature was lower in the 7°C period (mean 38.7°C) and rose during the 15°C period (mean 39.1°C), both means are well within the range that is generally accepted as a normal sheep body temperature (38°C to 40°C). Furthermore it is well known that the main function of the scrotum is to keep the testes below the average body temperature. Hence an increase in body temperature as measured rectally does not necessarily reflect a similar increase in the testis blood supply.

Heart rate

The heart rate was greater during the low environmental temperature period than at the warmer. In the experiment conducted by Dutt and Bush (1955), they found no difference in heart rate between two groups of ewes kept at 45 to 48°F and ambient temperature (up to 88°F) respectively.

Although the result obtained was not significant, the findings in this study are not what might be expected. Usually, when the blood temperature is decreased the heart rate decreases, due to the effect of the lower temperature on the pacemaker in the atrium (Burton and Edholm, 1955). The initial response of the cardiovascular system to hypothermia may well lead to an increase in heart rate; there is an initial marked peripheral vasoconstriction with a rise in blood pressure and heart rate. However, over a period of three weeks a decrease in heart rate would have been expected rather than an increase.

It is also interesting to notice that the heart rates at both environmental temperatures were on the high side of the normal range for sheep (60 to 120 beats $min^{-1}$) (Anderson, 1977). It is well known, of course, that the heart rate increases if an animal is frightened, however, these rams were quite used to being handled and the minimum amount of restraint was used during sampling times.

Respiration rate

The respiration rates were faster at the higher environmental temperature than at the lower. This result agrees well with the findings of Dutt and Bush (1955). The higher respiration rate during the 15°C period was apparently an attempt by the rams to maintain normal body temperature by heat dissipation through expelled air. As already stated an ambient temperature of 15°C would be unlikely to cause any heat stress in these rams, and so, the mean respiration rate was still very low compared with the results obtained by Dutt and Bush, where respiration rates averaged 150 breaths $min^{-1}$ during the summer period (up to 90°F).

Mount (1979) stated that at thermal neutrality, respiratory and cutaneous evaporation in sheep have been calculated to share approximately equally about 25% of the total heat loss. Above thermal neutrality, respiratory evaporative heat loss increases relative to cutaneous evaporation and accounts for an increasing part of the total heat loss. In fact, an environmental temperature of 15°C did not appear to place the rams under any undue heat stress and the respiration rate, although higher than at 7°C, was still well below the maximum rate that sheep can attain (up to 300 beats $min^{-1}$).

Testis diameter

The diameter increased from the acclimatisation period to reach a peak towards the end of the 7°C period. The diameter then decreased throughout the remainder of the experimental period. Testicular volume in sheep is closely correlated with changes in seminiferous tubule diameter and length, which in turn provide a very good index of the stage of development of the seminiferous tubules (de Reviers and Lincoln, 1978).

In the normal annual cycle the testes vary in size from a peak in the autumn breeding season to a nadir in the summer. During the regression period there is a considerable reduction in the diameter of the seminiferous tubules and in the number of germ cells completing meiosis, although a few cells complete spermiogenesis even during the period of quiescence (Lincoln and Short, 1980).

From the results in this experiment, it appears that the cooler temperature stimulated an increase in testis size. This was presumably due to an increase in the diameter and length of the seminiferous tubules in 'anticipation' of the need for sperm production. The higher temperature seemed to 'switch off' this process.

At what level does temperature have this effect on testis size? It would seem that temperature cannot function via the pineal gland (as does light) because these rams had their pineal function destroyed. A more likely explanation would be that the temperature acted locally directly on the thermoreceptors in the scrotum and on the testes themselves, or that changes in blood temperature may be monitored by the central nervous thermodetectors and the effects mediated via the hypothalamus. Waites (1962) demonstrated how sensitive the scrotal area was in relation to temperature; he found that local warming of the scrotal skin elicited polypnoea much more readily than heating any other skin area. This effect was presumably mediated via the central nervous system and it seems reasonable to assume that changes in scrotal temperature could affect other physiological parameters which are controlled by the central nervous system (such as production of FSH and LH).

Testosterone concentration

Testosterone levels as measured in peripheral plasma did not show the same pattern as testis diameter. An increase in testosterone levels would have been expected as the testes enlarged and a decrease as they regressed. In fact, the testosterone levels remained at a similar or slightly higher level while the testes were regressing. It should be noted here that testosterone levels are normally much higher in ganglionectomised animals than in normal rams (Lincoln, personal communication). From the results it can be seen that the mean testosterone levels for the $7^{\circ}C$ period were 10.74 ng $ml^{-1}$ and for the $15^{\circ}C$ period 13.3 ng $ml^{-1}$. Both these levels would be considered high in normal rams. Gomes and Joyce (1975) reported that testosterone concentrations reached 8.31 ng/$ml^{-1}$ in July in New Zealand (peak of the mating season), and Schanbacher and Lunstra (1976) considered that levels of $> 6$ ng $ml^{-1}$ corresponded to the greatest mating acitivity. These levels are below the mean values obtained for either of the two experimental periods. The ganglionectomised rams cannot, therefore, be considered 'normal' and this may account for some of the differences observed.

The main division in testosterone concentrations was seen between the young and old rams. Levels were much greater in the younger rams (11, 14, 17) and these high levels were maintained throughout the whole experimental period. It was also noticeable that the younger animals tended to be the most aggressive throughout the study, while only number 15 of the older group showed aggressive tendencies.

## Recommendations

The initial problem was that the rams had not been kept together prior to the experiment and how this would affect their behaviour was not known. Fortunately they appeared to adapt quite well, although a definite pecking order was established, of which number 13 tended to take the brunt of the aggressive behaviour. Ideally, it would have been preferable to have penned the animals individually within the chamber, but there was insufficient room to do that. It would also have been desirable to have had the lighting intensity similar to that in the first house.

Another problem was that there were no control animals in the experiment; the same animals were used for both treatments and owing to a lack of time there was no change-over period between the 7°C and 15°C periods. Also it had been hoped to put the temperature in the chamber even higher, to about 23°C, but the time available ran out. It would be useful in any further experiments on this subject to have at least three groups of rams, one group kept at a low temperature, another at an intermediate temperature (the control group) and the third at a higher temperature.

On an individual basis, ram number 10 developed a horn infection and this had to be amputated on August 4; he recovered well and did not seem to be unduly affected by the incident. Ram 13 had his wool chewed off on one side by the others. This apparently is not unusual when sheep become bored (Lincoln, personal communication) and did not appear to cause any problems for the affected ram. Taking daily blood samples from the jugular vein proved to be quite difficult as the area over the vein became thickened and scarred; this was particularly bad with ram 15, and so it was decided to bleed from him only sparingly.

## Conclusions

Animals kept at different environmental temperatures do show changes in their physiological and reproductive parameters. Body temperature decreases in a cold environment, while respiration rate increases in a warmer environment. Both of these facts have been known for a long while. In this experiment, heart rate increased at the lower temperature and this result is not easily explained.

As far as testis diameter is concerned, there appears to be an increase in size during a cold period. The effect of decreasing daylength in the Autumn is well known to act as a stimulus to the mating season in sheep; from results seen in this experiment it would appear that temperature alone also has a role to play. In the natural state the effect of both stimuli (temperature and photoperiod) probably act to reinforce one another.

The significance within sheep breeding systems is that animals are more likely to breed early in the season if they are kept at cooler temperatures. This factor may be of importance with regard to ram and ewe fertility. It may well be worthwhile keeping the rams in a cool house or under shade prior to the mating season, in the hotter regions of the world.

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APPENDIX I

## INDIVIDUAL DATA COLLECTED FROM RAM NUMBER 10

| Date | Body temperature ($^oC$) | Heart rate (beats/min) | Respiration rate (breaths/min) | Testicle diameter (mm) | Sexual Flush | Plasma testosterone concentration (ng/ml) |
|---|---|---|---|---|---|---|
| Acclimatisation Period | | | | | | |
| 15.7.81 | 40.0 | 128 | 20 | 48 | 1 | 0.69 |
| 16.7.81 | 40.1 | 149 | 16 | 46 | 1 | 0.89 |
| 17.7.81 | 39.8 | 150 | 14 | 45 | $\frac{1}{2}$ | 0.71 |
| 20.7.81 | 39.8 | 148 | 18 | 47 | 1 | 0.75 |
| 21.7.81 | 40.1 | 135 | 17 | 47 | 2 | 0.80 |

APPENDIX I

# INDIVIDUAL DATA COLLECTED FROM RAM NUMBER 10

| Date | Body temperature (°C) | Heart rate (beats/min) | Respiration rate (breaths/min) | Testicle diameter (mm) | Sexual Flush | Plasma testosterone concentration (ng/ml) |
|---|---|---|---|---|---|---|
| Room temperature 7°C | | | | | | |
| 22.7.81 | 38.9 | 132 | 18 | 46 | 2 | 1.03 |
| 23.7.81 | 39.8 | 152 | 18 | 48 | 2 | 0.96 |
| 24.7.81 | 40.2 | 152 | 17 | 50 | 2 | 1.30 |
| 27.7.81 | 39.5 | 150 | 17 | 50 | 2 | 1.77 |
| 28.7.81 | 39.5 | 134 | 20 | 50 | 1 | 0.68 |
| 29.7.81 | 39.7 | 142 | 20 | 50 | 1½ | - |
| 30.7.81 | 39.2 | 143 | 18 | 48 | 1 | 0.52 |
| 31.7.81 | 39.1 | 138 | 20 | 49 | ½ | - |
| 3.8.81 | 40.2 | 144 | 20 | 47 | ½ | 0.61 |
| 4.8.81 | 39.6 | 150 | 16 | 48 | ½ | 0.64 |
| 5.8.81 | 39.1 | 130 | 18 | 49 | 1 | 0.88 |
| 6.8.81 | 39.2 | 134 | 16 | 49 | 1 | 2.78 |
| 7.8.81 | 38.2 | 142 | 20 | 50 | 1½ | 1.29 |
| 10.8.81 | 39.2 | 136 | 22 | 45 | 1½ | 0.55 |
| 12.8.81 | 39.0 | 120 | 17 | 50 | 1½ | 0.43 |
| Means | 39.4 | 138.9 | 18.5 | 48.6 | | 1.04 |

APPENDIX I

INDIVIDUAL DATA COLLECTED FROM RAM NUMBER 10

| Date | Body temperature ($^oC$) | Heart rate (beats/min) | Respiration rate (breaths/min) | Testicle diameter (mm) | Sexual Flush | Plasma testosterone concentration (ng/ml) |
|---|---|---|---|---|---|---|
| Room temperature 15$^o$C | | | | | | |
| 13.8.81 | 39.1 | 126 | 20 | 48 | 1 | 1.09 |
| 14.8.81 | 39.7 | 126 | 20 | 48 | 1 | 4.12 |
| 17.8.81 | 39.4 | 108 | 20 | 46 | ½ | 0.63 |
| 18.8.81 | 40.0 | 101 | 22 | 44 | ½ | 0.35 |
| 19.8.81 | 39.1 | 108 | 20 | 46 | ½ | 7.41 |
| 20.8.81 | 39.2 | 120 | 20 | 47 | ½ | 0.56 |
| 21.8.81 | 39.6 | 106 | 22 | 45 | 0 | 0.64 |
| 24.8.81 | 39.2 | 114 | 24 | 44 | 0 | 0.52 |
| 25.8.81 | 39.4 | 114 | 22 | 45 | 0 | - |
| 26.8.81 | 38.8 | 117 | 18 | 43 | 0 | 1.17 |
| 27.8.81 | 39.1 | 110 | 19 | 43 | 0 | 0.77 |
| 28.8.81 | 39.1 | 110 | 16 | 44 | 0 | 4.78 |
| 31.8.81 | 38.9 | 100 | 18 | 44 | 0 | 0.37 |
| 1.9.81 | 39.4 | 110 | 18 | 44 | 0 | 0.68 |
| 2.9.81 | 39.5 | 104 | 20 | 43 | 0 | 0.55 |
| Means | 39.3 | 111.6 | 19.9 | 44.9 | | 1.69 |

APPENDIX I

## INDIVIDUAL DATA COLLECTED FROM RAM NUMBER 11

| Date | Body temperature (°C) | Heart rate (beats/min) | Respiration rate (breaths/min) | Testicle diameter (mm) | Sexual Flush | Plasma testosterone concentration (ng/ml) |
|---|---|---|---|---|---|---|
| **Room temperature 7°C** | | | | | | |
| 22.7.81 | 39.3 | 140 | 20 | 51 | 4 | 11.12 |
| 23.7.81 | 38.8 | 128 | 18 | 50 | 3 | H |
| 24.7.81 | 38.7 | 134 | 24 | 48 | 3 | 20.70 |
| 27.7.81 | 38.3 | 140 | 16 | 51 | 3 | H |
| 28.7.81 | 38.5 | 114 | 18 | 51 | 2½ | 21.17 |
| 29.7.81 | 39.1 | 116 | 17 | 50 | 2½ | – |
| 30.7.81 | 38.6 | 116 | 20 | 52 | 3 | H |
| 31.7.81 | 39.1 | 114 | 16 | 49 | 3 | 21.17 |
| 3.8.81 | 39.8 | 132 | 16 | 52 | 2½ | 19.10 |
| 4.8.81 | 38.8 | 110 | 18 | 52 | 3 | H |
| 5.8.81 | 38.8 | 134 | 22 | 51 | 3½ | 15.95 |
| 6.8.81 | 39.3 | 117 | 18 | 52 | 3 | 13.52 |
| 7.8.81 | 39.0 | 124 | 21 | 52 | 4 | 23.29 |
| 10.8.81 | 39.1 | 120 | 20 | 52 | 4 | 7.23 |
| 12.8.81 | 39.8 | 150 | 23 | 53 | 3 | 22.70 |
| Means | 39.0 | 125.9 | 19.1 | 51.1 | | 17.60 |

APPENDIX I

## INDIVIDUAL DATA COLLECTED FROM RAM NUMBER 11

| Date | Body temperature (°C) | Heart rate (beats/min) | Respiration rate (breaths/min) | Testicle diameter (mm) | Sexual Flush | Plasma testosterone concentration (ng/ml) |
|---|---|---|---|---|---|---|
| Room temperature 15°C | | | | | | |
| 13.8.81 | 39.6 | 120 | 20 | 52 | 3 | 21.17 |
| 14.8.81 | 39.7 | 112 | 19 | 50 | 3 | 14.34 |
| 17.8.81 | 39.4 | 140 | 30 | 51 | 3 | H |
| 18.8.81 | 39.7 | 116 | 24 | 52 | 4 | 15.85 |
| 19.8.81 | 39.2 | 108 | 26 | 52 | 3 | 19.29 |
| 20.8.81 | 39.3 | 112 | 22 | 51 | 3 | 23.29 |
| 21.8.81 | 39.3 | 97 | 25 | 52 | 4 | H |
| 24.8.81 | 39.5 | 111 | 25 | 50 | 3 | 22.70 |
| 25.8.81 | 39.3 | 110 | 25 | 51 | 4 | H |
| 26.8.81 | 39.3 | 102 | 31 | 51 | 3 | H |
| 27.8.81 | 39.1 | 90 | 26 | 50 | 2½ | 21.95 |
| 28.8.81 | 39.3 | 106 | 18 | 52 | 3 | 9.05 |
| 31.8.81 | 39.4 | 110 | 22 | 49 | 3 | 19.60 |
| 1.9.81 | 39.2 | 96 | 22 | 48 | 2½ | H |
| 2.9.81 | 39.4 | 110 | 24 | 49 | 3 | 21.17 |
| Means | 39.4 | 109.3 | 23.9 | 50.7 | | 18.84 |

APPENDIX I

## INDIVIDUAL DATA COLLECTED FROM RAM NUMBER 12

| Date | Body temperature ($^{o}C$) | Heart rate (beats/min) | Respiration rate (breaths/min) | Testicle diameter (mm) | Sexual Flush | Plasma testosterone concentration (ng/ml) |
|---|---|---|---|---|---|---|
| <u>Acclimatisation Period</u> | | | | | | |
| 15.7.81 | 39.2 | 116 | 30 | 46 | 1 | 7.65 |
| 16.7.81 | 39.3 | 139 | 22 | 47 | 1 | 9.87 |
| 17.7.81 | 39.0 | 135 | 22 | 46 | 2 P | 2.20 |
| 20.7.81 | 38.9 | 136 | 20 | 48 | 2 P | 1.61 |
| 21.7.81 | 39.2 | 125 | 18 | 45 | 2 | 1.38 |

APPENDIX I

## INDIVIDUAL DATA COLLECTED FROM RAM NUMBER 12

| Date | Body temperature (°C) | Heart rate (beats/min) | Respiration rate (breaths/min) | Testicle diameter (mm) | Sexual Flush | Plasma testosterone concentration (ng/ml) |
|---|---|---|---|---|---|---|
| <u>Room temperature</u> <u>7°C</u> | | | | | | |
| 22.7.81 | 39.2 | 134 | 23 | 48 | 2 P | 8.31 |
| 23.7.81 | 38.6 | 146 | 28 | 47 | 2 | 1.80 |
| 24.7.81 | 38.3 | 118 | 20 | 49 | 2 | 1.36 |
| 27.7.81 | 37.9 | 140 | 26 | 50 | 3 | 1.63 |
| 28.7.81 | 38.6 | 130 | 22 | 48 | 2 | 1.41 |
| 29.7.81 | 38.5 | 148 | 22 | 48 | $2\frac{1}{2}$ | - |
| 30.7.81 | 38.0 | 140 | 20 | 49 | 2 | 0.96 |
| 31.7.81 | 38.0 | 138 | 20 | 49 | 2 | 5.34 |
| 3.8.81 | 38.6 | 148 | 20 | 48 | 2 | 1.01 |
| 4.8.81 | 38.4 | 151 | 23 | 49 | 2 | 1.21 |
| 5.8.81 | 38.1 | 146 | 21 | 50 | 2 P | 0.64 |
| 6.8.81 | 38.2 | 152 | 22 | 51 | 2 P | 1.47 |
| 7.8.81 | 38.0 | 150 | 24 | 49 | 2 P | 1.12 |
| 10.8.81 | 38.2 | 142 | 28 | 45 | 2 P | - |
| 12.8.81 | 38.4 | 136 | 28 | 46 | 2 P | 0.67 |
| Means | 38.3 | 141.3 | 23.1 | 48.4 | | 2.07 |

APPENDIX I

## INDIVIDUAL DATA COLLECTED FROM RAM NUMBER 12

| Date | Body temperature (°C) | Heart rate (beats/min) | Respiration rate (breaths/min) | Testicle diameter (mm) | Sexual Flush | Plasma testosterone concentration (ng/ml) |
|---|---|---|---|---|---|---|
| Room temperature 15°C | | | | | | |
| 13.8.81 | 39.1 | 128 | 22 | 47 | 2 P | - |
| 14.8.81 | 38.5 | 134 | 27 | 46 | 1 P | 0.55 |
| 17.8.81 | 38.3 | 134 | 28 | 47 | 1 P | 0.87 |
| 18.8.81 | 38.4 | 124 | 21 | 45 | 1 P | 0.22 |
| 19.8.81 | 38.2 | 144 | 27 | 44 | 1 P | 0.35 |
| 20.8.81 | 38.9 | 157 | 22 | 45 | 1 P | 10.56 |
| 21.8.81 | 38.7 | 156 | 24 | 45 | 1 P | 2.68 |
| 24.8.81 | 38.8 | 160 | 23 | 45 | 1 P | 0.20 |
| 25.8.81 | 38.8 | 130 | 20 | 44 | 1 P | 0.22 |
| 26.8.81 | 38.7 | 126 | 24 | 44 | 1 | 0.32 |
| 27.8.81 | 38.7 | 114 | 19 | 44 | 1 | 0.52 |
| 28.8.81 | 38.3 | 146 | 24 | 43 | 1 | 0.51 |
| 31.8.81 | 38.7 | 131 | 23 | 42 | 1 | 7.29 |
| 1.9.81 | 38.6 | 144 | 24 | 42 | 0 | 0.24 |
| 2.9.81 | 38.6 | 140 | 26 | 43 | 1 | 1.32 |
| Means | 38.6 | 137.9 | 23.6 | 44.4 | | 1.85 |

APPENDIX I

## INDIVIDUAL DATA COLLECTED FROM RAM NUMBER 13

| Date | Body temperature ($^{o}C$) | Heart rate (beats/min) | Respiration rate (breaths/min) | Testicle diameter (mm) | Sexual Flush | Plasma testosterone concentration (ng/ml) |
|---|---|---|---|---|---|---|
| Acclimatisation Period | | | | | | |
| 15.7.81 | 38.8 | 108 | 24 | 52 | 2 | 16.00 |
| 16.7.81 | 39.0 | 136 | 20 | 53 | 2 | 26.56 |
| 17.7.81 | 38.1 | 129 | 21 | 55 | 3 | 24.45 |
| 20.7.81 | 38.8 | 138 | 22 | 53 | $2\frac{1}{2}$ | 14.05 |
| 21.7.81 | 39.0 | 120 | 20 | 53 | 3 | 3.60 |

APPENDIX I

## INDIVIDUAL DATA COLLECTED FROM RAM NUMBER 13

| Date | Body temperature (°C) | Heart rate (beats/min) | Respiration rate (breaths/min) | Testicle diameter (mm) | Sexual Flush | Plasma testosterone concentration (ng/ml) |
|---|---|---|---|---|---|---|
| Room temperature 7°C | | | | | | |
| 22.7.81 | 38.9 | 108 | 20 | 52 | 3 | 3.74 |
| 23.7.81 | 38.8 | 150 | 24 | 53 | 3 | 3.34 |
| 24.7.81 | 38.6 | 140 | 18 | 54 | 3 | 2.14 |
| 27.7.81 | 37.6 | 136 | 16 | 56 | 4 | 3.10 |
| 28.7.81 | 38.2 | 134 | 18 | 54 | 3½ | 3.50 |
| 29.7.81 | 37.8 | 128 | 17 | 54 | 4 | - |
| 30.7.81 | 37.8 | 130 | 22 | 55 | 3½ | 6.46 |
| 31.7.81 | 37.9 | 110 | 22 | 57 | 4 | 24.54 |
| 3.8.81 | 38.2 | 147 | 18 | 54 | 3 | 1.16 |
| 4.8.81 | 37.0 | 142 | 18 | 57 | 3 P | 0 |
| 5.8.81 | 37.9 | 134 | 20 | 57 | 4 | 3.14 |
| 6.8.81 | 38.3 | 148 | 20 | 56 | 3 | 27.12 |
| 7.8.81 | 37.6 | 138 | 16 | 56 | 3½P | 3.56 |
| 10.8.81 | 36.8 | 126 | 22 | 55 | 3 | 1.64 |
| 12.8.81 | 38.0 | 148 | 18 | 54 | 2 | 1.60 |
| Means | 38.0 | 134.6 | 19.3 | 54.9 | | 6.07 |

APPENDIX I

## INDIVIDUAL DATA COLLECTED FROM RAM NUMBER 13

| Date | Body temperature (°C) | Heart rate (beats/min) | Respiration rate (breaths/min) | Testicle diameter (mm) | Sexual Flush | Plasma testosterone concentration (ng/ml) |
|---|---|---|---|---|---|---|
| Room temperature 15°C | | | | | | |
| 13.8.81 | 39.0 | 160 | 22 | 59 | 3½ | 2.20 |
| 14.8.81 | 38.1 | 156 | 18 | 57 | 3 | 0.90 |
| 17.8.81 | 38.3 | 146 | 19 | 56 | 2½ | 0 |
| 18.8.81 | 38.3 | 142 | 22 | 54 | 3 | 0.42 |
| 19.8.81 | 38.3 | 146 | 22 | 54 | 1 | 0.80 |
| 20.8.81 | 38.3 | 146 | 19 | 56 | 2 P | 2.44 |
| 21.8.81 | 38.7 | 156 | 24 | 57 | 2 P | 6.20 |
| 24.8.81 | 38.8 | 154 | 24 | 54 | 1½P | 1.60 |
| 25.8.81 | 38.5 | 156 | 20 | 54 | 1½P | 1.72 |
| 26.8.81 | 38.6 | 146 | 18 | 55 | 2 P | 1.22 |
| 27.8.81 | 37.9 | 176 | 18 | 54 | 2 P | 1.54 |
| 28.8.81 | 38.8 | 160 | 23 | 55 | 2 P | 0.86 |
| 31.8.81 | 38.8 | 144 | 18 | 53 | 1 | 0 |
| 1.9.81 | 39.2 | 128 | 19 | 53 | 2½ | 0 |
| 2.9.81 | 38.7 | 134 | 24 | 53 | 1 | 0 |
| Means | 38.5 | 150 | 20.7 | 54.9 | | 1.33 |

APPENDIX I

INDIVIDUAL DATA COLLECTED FROM RAM NUMBER 14

| Date | Body temperature ($^oC$) | Heart rate (beats/min) | Respiration rate (breaths/min) | Testicle diameter (mm) | Sexual Flush | Plasma testosterone concentration (ng/ml) |
|---|---|---|---|---|---|---|
| Acclimatisation Period | | | | | | |
| 15.7.81 | 38.5 | 110 | 20 | 50 | 1 | 25.06 |
| 16.7.81 | 38.6 | 134 | 20 | 50 | 1½ | 29.05 |
| 17.7.81 | 38.6 | 111 | 20 | 52 | 2 | 17.17 |
| 20.7.81 | 38.6 | 118 | 21 | 52 | 2½ | 6.20 |
| 21.7.81 | 39.0 | 80 | 20 | 52 | 3 | 1.38 |

APPENDIX I

## INDIVIDUAL DATA COLLECTED FROM RAM NUMBER 14

| Date | Body temperature (°C) | Heart rate (beats/min) | Respiration rate (breaths/min) | Testicle diameter (mm) | Sexual Flush | Plasma testosterone concentration (ng/ml) |
|---|---|---|---|---|---|---|
| <u>Room temperature</u> <u>7oC</u> | | | | | | |
| 22.7.81 | 39.5 | 100 | 19 | 52 | 3 | 4.77 |
| 23.7.81 | 39.2 | 124 | 18 | 50 | 2½ | 4.38 |
| 24.7.81 | 39.1 | 106 | 17 | 50 | 2½ | 14.75 |
| 27.7.81 | 38.4 | 139 | 20 | 53 | 3 | 2.65 |
| 28.7.81 | 38.4 | 111 | 22 | 55 | 2 | 5.62 |
| 29.7.81 | 38.7 | 106 | 18 | 54 | 2½ | - |
| 30.7.81 | 38.3 | 112 | 17 | 56 | 2 | 4.85 |
| 31.7.81 | 38.6 | 133 | 20 | 57 | 2 | 25.06 |
| 3.8.81 | 38.7 | 118 | 20 | 57 | 2 | 22.62 |
| 4.8.81 | 38.3 | 131 | 20 | 57 | 2 | 25.62 |
| 5.8.81 | 38.3 | 125 | 21 | 60 | 3 | 12.82 |
| 6.8.81 | 38.6 | 126 | 20 | 58 | 3 | 2.25 |
| 7.8.81 | 37.4 | 111 | 20 | 60 | 4 | 12.92 |
| 10.8.81 | 38.9 | 124 | 20 | 56 | 3 | 3.55 |
| 12.8.81 | 39.4 | 126 | 18 | 60 | 3 | 9.58 |
| Means | 38.7 | 119.5 | 19.4 | 55.7 | | 10.82 |

APPENDIX I

## INDIVIDUAL DATA COLLECTED FROM RAM NUMBER 14

| Date | Body temperature (°C) | Heart rate (beats/min) | Respiration rate (breaths/min) | Testicle diameter (mm) | Sexual Flush | Plasma testosterone concentration (ng/ml) |
|---|---|---|---|---|---|---|
| Room temperature 15°C | | | | | | |
| 13.8.81 | 39.9 | 102 | 20 | 57 | 2 | 17.55 |
| 14.8.81 | 39.3 | 106 | 19 | 58 | 3 | 8.42 |
| 17.8.81 | 39.3 | 120 | 20 | 56 | 3 | 18.56 |
| 18.8.81 | 39.6 | 120 | 25 | 57 | 3 | 17.25 |
| 19.8.81 | 39.3 | 140 | 27 | 54 | 3 | 22.01 |
| 20.8.81 | 39.5 | 120 | 28 | 52 | 3 | 20.57 |
| 21.8.81 | 38.9 | 112 | 24 | 55 | 3 | 14.88 |
| 24.8.81 | 39.3 | 125 | 33 | 56 | 3 | 26.56 |
| 25.8.81 | 39.1 | 100 | 20 | 56 | 3 | 28.12 |
| 26.8.81 | 39.3 | 106 | 22 | 55 | 3 | 24.25 |
| 27.8.81 | 39.6 | 94 | 20 | 55 | 3 | 30.62 |
| 28.8.81 | 38.8 | 120 | 23 | 55 | 3 | 30.00 |
| 31.8.81 | 39.2 | 122 | 24 | 55 | 3 | 6.42 |
| 1.9.81 | 38.9 | 120 | 22 | 55 | 2 | - |
| 2.9.81 | 39.0 | 106 | 20 | 54 | 3 | 5.61 |
| Means | 39.3 | 114.2 | 23.1 | 55.3 | | 19.34 |

APPENDIX I

## INDIVIDUAL DATA COLLECTED FROM RAM NUMBER 15

| Date | Body temperature ($^{o}C$) | Heart rate (beats/min) | Respiration rate (breaths/min) | Testicle diameter (mm) | Sexual Flush | Plasma testosterone concentration (ng/ml) |
|---|---|---|---|---|---|---|
| <u>Acclimatisation Period</u> | | | | | | |
| 15.7.81 | 39.2 | 128 | 20 | 47 | 3 | 23.05 |
| 16.7.81 | 39.0 | 140 | 20 | 49 | 3 | 24.10 |
| 17.7.81 | 39.3 | 129 | 21 | 48 | 4 | 26.00 |
| 20.7.81 | 39.1 | 152 | 22 | 50 | 4 | 15.02 |
| 21.7.81 | 39.2 | 138 | 20 | 52 | 4 | 5.13 |

APPENDIX I

# INDIVIDUAL DATA COLLECTED FROM RAM NUMBER 15

| Date | Body temperature (°C) | Heart rate (beats/min) | Respiration rate (breaths/min) | Testicle diameter (mm) | Sexual Flush | Plasma testosterone concentration (ng/ml) |
|---|---|---|---|---|---|---|
| Room temperature 7°C | | | | | | |
| 22.7.81 | 38.8 | 135 | 18 | 50 | 4 | - |
| 23.7.81 | 38.7 | 129 | 20 | 49 | 4 | 17.76 |
| 24.7.81 | 39.0 | 128 | 20 | 51 | 4 | - |
| 27.7.81 | 38.2 | 160 | 20 | 55 | 4 | - |
| 28.7.81 | 38.7 | 143 | 19 | 53 | 3½ | 23.96 |
| 29.7.81 | 38.5 | 84 | 18 | 54 | 4 | - |
| 30.7.81 | 37.9 | 140 | 20 | 55 | 4 | 18.95 |
| 31.7.81 | 38.4 | 134 | 20 | 54 | 4 | - |
| 3.8.81 | 38.5 | 132 | 24 | 54 | 3 | 17.83 |
| 4.8.81 | 38.2 | 148 | 21 | 54 | 3 | - |
| 5.8.81 | 37.8 | 141 | 20 | 57 | 4 | - |
| 6.8.81 | 39.0 | 128 | 19 | 54 | 4 | 24.50 |
| 7.8.81 | 38.5 | 138 | 20 | 54 | 3 | - |
| 10.8.81 | 39.2 | 156 | 21 | 53 | 3 | 24.28 |
| 12.8.81 | 38.9 | 168 | 21 | 56 | 3 | 9.67 |
| Means | 38.6 | 137.6 | 20.1 | 53.5 | | 19.56 |

APPENDIX I

INDIVIDUAL DATA COLLECTED FROM RAM NUMBER 15

| Date | Body temperature (°C) | Heart rate (beats/min) | Respiration rate (breaths/min) | Testicle diameter (mm) | Sexual Flush | Plasma testosterone concentration (ng/ml) |
|---|---|---|---|---|---|---|
| Room temperature 15°C | | | | | | |
| 13.8.81 | 39.6 | 130 | 22 | 53 | 3 | 27.50 |
| 14.8.81 | 39.1 | 134 | 19 | 52 | 2 | 6.88 |
| 17.8.81 | 39.1 | 110 | 23 | 50 | 2 | 30.00 |
| 18.8.81 | 39.3 | 132 | 23 | 53 | 2 | - |
| 19.8.81 | 39.2 | 128 | 21 | 52 | 3 | - |
| 20.8.81 | 39.3 | 140 | 22 | 53 | 2 | 30.00 |
| 21.8.81 | 39.2 | 140 | 23 | 53 | 3 | - |
| 24.8.81 | 39.4 | 150 | 26 | 51 | 2 | 26.56 |
| 25.8.81 | 39.3 | 116 | 21 | 54 | 2 | - |
| 26.8.81 | 39.4 | 128 | 22 | 52 | 2 | 31.25 |
| 27.8.81 | 39.1 | 134 | 20 | 53 | 2 | - |
| 28.8.81 | 38.8 | 120 | 21 | 54 | 2 | 32.50 |
| 31.8.81 | 39.2 | 104 | 22 | 53 | 3 | 6.42 |
| 1.9.81 | 39.0 | 134 | 21 | 54 | 2 | - |
| 2.9.81 | 38.9 | 126 | 20 | 53 | 3 | 5.61 |
| Means | 39.2 | 128.4 | 21.7 | 52.7 | | 21.86 |

APPENDIX I

INDIVIDUAL DATA COLLECTED FROM RAM NUMBER 17

| Date | Body temperature ($^{o}C$) | Heart rate (beats/min) | Respiration rate (breaths/min) | Testicle diameter (mm) | Sexual Flush | Plasma testosterone concentration (ng/ml) |
|---|---|---|---|---|---|---|
| <u>Acclimatisation Period</u> | | | | | | |
| 15.7.81 | 38.9 | 124 | 28 | 46 | ½ | 22.42 |
| 16.7.81 | 38.4 | 122 | 18 | 46 | ½ | 1.94 |
| 17.7.81 | 39.0 | 113 | 18 | 46 | ½ | 10.50 |
| 20.7.81 | 39.4 | 122 | 23 | 46 | 1 | 5.86 |
| 21.7.81 | 39.4 | 96 | 20 | 48 | 1 | 5.30 |

APPENDIX I

# INDIVIDUAL DATA COLLECTED FROM RAM NUMBER 17

| Date | Body temperature (°C) | Heart rate (beats/min) | Respiration rate (breaths/min) | Testicle diameter (mm) | Sexual Flush | Plasma testosterone concentration (ng/ml) |
|---|---|---|---|---|---|---|
| <u>Room temperature 7°C</u> | | | | | | |
| 22.7.81 | 39.4 | 128 | 22 | 48 | 2 | 26.02 |
| 23.7.81 | 39.5 | 121 | 18 | 48 | 2 | 6.04 |
| 24.7.81 | 38.5 | 132 | 21 | 48 | 1½ | 1.90 |
| 27.7.81 | 39.4 | 114 | 24 | 49 | 2 | 1.94 |
| 28.7.81 | 39.1 | 108 | 18 | 50 | 1½ | - |
| 29.7.81 | 39.3 | 116 | 16 | 51 | 1½ | 9.87 |
| 30.7.81 | 38.4 | 134 | 21 | 48 | 1 | 8.72 |
| 31.7.81 | 38.3 | 128 | 24 | 48 | 3 | 26.00 |
| 3.8.81 | 39.7 | 132 | 24 | 51 | 4 | 8.52 |
| 4.8.81 | 39.1 | 114 | 25 | 52 | 3 | 29.06 |
| 5.8.81 | 38.7 | 122 | 23 | 51 | 4 | 30.62 |
| 6.8.81 | 39.4 | 106 | 24 | 52 | 3 | 15.83 |
| 7.8.81 | 39.0 | 120 | 23 | 52 | 4 | 30.31 |
| 10.8.81 | 39.6 | 122 | 22 | 53 | 4 | 30.00 |
| 12.8.81 | 39.5 | 112 | 23 | 53 | 4 | 25.62 |
| Means | 39.1 | 120.5 | 21.9 | 50.3 | | 17.89 |

APPENDIX I

## INDIVIDUAL DATA COLLECTED FROM RAM NUMBER 17

| Date | Body temperature ($^oC$) | Heart rate (beats/min) | Respiration rate (breaths/min) | Testicle diameter (mm) | Sexual Flush | Plasma testosterone concentration (ng/ml) |
|---|---|---|---|---|---|---|
| Room temperature 15oC | | | | | | |
| 13.8.81 | 39.4 | 110 | 27 | 52 | 4 | 29.37 |
| 14.8.81 | 39.6 | 100 | 28 | 52 | 4 | 27.50 |
| 17.8.81 | 39.4 | 102 | 23 | 49 | 3 | 30.00 |
| 18.8.81 | 39.5 | 114 | 32 | 50 | 3 | 29.68 |
| 19.8.81 | 39.3 | 118 | 31 | 52 | 3 | 24.25 |
| 20.8.81 | 39.4 | 121 | 34 | 50 | 3 | 25.06 |
| 21.8.81 | 39.3 | 119 | 42 | 43 | 3 | 27.50 |
| 24.8.81 | 39.4 | 120 | 38 | 52 | 3 | 24.00 |
| 25.8.81 | 39.1 | 116 | 39 | 51 | 3 | 24.37 |
| 26.8.81 | 39.2 | 108 | 44 | 41 | $2\frac{1}{2}$ | 28.12 |
| 27.8.81 | 39.3 | 108 | 82 | 51 | 2 | 23.31 |
| 28.8.81 | 38.9 | 115 | 32 | 52 | 2 | 21.48 |
| 31.8.81 | 39.1 | 106 | 38 | 51 | 2 | 28.75 |
| 1.9.81 | 39.1 | 94 | 34 | 51 | 2 | 23.35 |
| 2.9.81 | 38.9 | 104 | 32 | 51 | 2 | 26.06 |
| Means | 39.3 | 110.3 | 37.1 | 51.2 | | 26.19 |