# PHYSIOLOGICAL ASPECTS OF TORPOR IN THE FAT MOUSE

# (*STEATOMYS PRATENSIS*, DENDROMURINAE)

by

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# Preface

The experimental work described in this thesis was carried out in the Department of Biology, University of Natal, Durban, from February 1982 to May 1988 under the supervision of Professor J. Meester of the Biology Department and Professor M. Perrin of the Zoology and Entomology Department, University of Natal, Pietermaritzburg.

These studies represent original work by the author and have not been submitted in any form to another University. Where use was made of the work of others it has been duly acknowledged in the text.

Date .....  Signed .....

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## Abstract

Several aspects of the physiology of the fat mouse *Steatomys pratensis natalensis* were studied in the laboratory using standard techniques and custom-made data-logging equipment. The fat was studied both from a morphological and functional point of view.

The measurement of metabolic rates showed that euthermic *S.pratensis* have very low basal metabolic rates of 36% of expected, with torpor saving up to 69% of expended energy. Body temperatures, oxygen consumption, and activity patterns monitored over 24 hour periods with a data-logging system showed that *S.pratensis* have very low body temperatures of 31.3 to 35.0°C which fluctuate on a circadian rhythm with activity and oxygen consumption, all being lower during the day and higher at night. Torpor started very early in the morning and lasted for 5.5 to 11.7 hours. Huddling with a mate could reduce energy expenditure by 18%.

Torpid body temperatures lay just above ambient from 15 to 35°C, below which all animals tried to arouse. Forced arousal at 10 to 30°C was slow and depended on ambient temperature while no mouse could arouse at 0°C. Thermal conductance was 97.4 % of expected but cooling rates of dead *S.pratensis* were slow due to the heavy fat layer. Non-shivering thermogensis (measured after noradrenaline injection) was normal at 369% of BMR but maximum metabolism was twice as much, indicating other means of thermogenesis used additively with NST.

Dissection showed extremely heavy fat deposits in the normal mammalian positions and also three additional deposits. Histological studies revealed most deposits as white fat but there was brown fat in the interscapular region. Soxhlet analysis showed an extremely wide range of body fat content from normal mammalian levels to contents higher than in hibernating rodents.

Deprivation of food and water, or food alone, was found to induce torpor and cause the mice to become non-reproductive. Deprivation of water but not food, and deprivation of a cage mate, triggered torpor in only 40 - 44% of the cases studied. The mice took 5 to 12 days to lose 30% of their mass, but theoretically could survive longer. Weekly measurements showed no annual mass fluctuations in the laboratory but the mice became reproductively active mid-summer to early winter while torpor was at a maximum around late winter. All animals showed torpor, young more than adults and females more than males.

It is suggested that the low body temperature and metabolism of *S.pratensis* may have evolved to prevent overheating caused by their inability to lose heat through the heavy fat layer. The species could then disperse into areas where their low energetic demands would permit them to compete successfully with high metabolic rate rodents.

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# Chapter 1

## Introduction

The fat mouse *Steatomys pratensis* is a small (maximum mass 47 g (Smithers 1971)) rodent of a rusty brown colour with pure white underparts and feet. It has a characteristically short tail with four toes to the fore feet and five to the hind. The fore toes have fairly long curved claws and the body hair has a fine sheen. The name *Steatomys* is derived from the greek *Stear* meaning fat and *mys* meaning mouse.

The genus *Steatomys* belongs to the sub-family Dendromurinae which is at present included in the Muridae (Meester *et al.* 1986). The genus is confined to Africa south of the Sahara and is fairly widespread but uncommon over that area (Nel 1969). The genus occurs primarily in savanna, although it encroaches into the South West Cape, South West Arid, and Lowland Forest biomes (Nel 1969).

### 1.1 Literature pertaining to *Steatomys*

Of 114 published papers and books mentioning *Steatomys* 61 deal soley with species description, identification, taxonomy, or records of occurrence, and so are not considered here. Two mention the palaeontology of the genus and nine the parasitology. Another five deal with the identification of material from owl or black-shouldered kite pellets and one with the identification of hair (Keogh 1985). Of the rest, 12 quote other papers and do not contain original information on the genus. Papers mentioning breeding patterns only (two papers), and reproductive patterns mentioned in other papers, are covered in Appendix A.

Of the remaining 21 works only 3 (Petter 1966; Bellier & Gautun 1968; Genest-Villard 1979) deal soley with the genus, in each case *S.opimus*.

Petter (1966) discusses the circadian rhythm of torpor of several *S.opimus* from the Central African Republic. He says that the animals are fat and remain in their burrows with some food during the dry season when they show this torpor pattern.

Bellier & Gautun (1968) collected *S.opimus* by excavating their burrows and had little success catching them by trapping. They remark that *Steatomys* seems particularly sensitive to anaesthesia, although they do not say what kind of anaesthetic was used. They also report

finding a carcass at the bottom of the burrow, attributing the death to occurring during a bout of torpor without reason.

Probably the most comprehensive study undertaken on *Steatomys* is reported by Genest-Villard (1979). In this ecological study on *S.opimus* in the Central African Empire (now Central African Republic) she describes the animals as living singly in characteristic burrows and eating mainly termites. The dry season in this area was from December to February (temperatures 16 to 28°C), with the rains being at their maximum in August and September (temperatures 25 to 31°C). As many other authors do, she remarks on the extreme difficulty of trapping these animals as adults, the main trapping successes being of sub-adults. She describes the burrow structure in some detail and comments that the complexity of the burrow described by Hanney (1965) indicates a long occupation by the animal and perhaps a succession of litters in the same burrow. The burrows were clumped, generally with the females' burrows well separated and situated in the middle of the burrows of the males.

Genest-Villard's (1979) description of age classes of these animals is reported in appendix A, as are their reproductive patterns. Reproduction occurs generally in the rainy season. She describes *Steatomys* as living in a "semi-lethargie" during part of the year, coming out for the period of reproduction and burrow excavation; but also states that this "semi-lethargie" could become a true lethargy in the regions where it exists in winter. The fatness of the animals is discussed since she captured fat animals at all times of the year, and noted that even the young have an important layer of fat under the skin. Food was mainly termites but they would also take grain and other vegetable matter.

Perrin & Curtis (1980), in their study of digestive system morphology, bear out this high protein content diet as they describe *S.pratensis* as having a short gut, small caecum, and a long small intestine, with a decreased number of liver lobes and the loss of the gall bladder, although they follow Kingdon (1974) in describing *Steatomys* as a seed eater.

In a rather more comprehensive paper on the Muridae of Malawi, Hanney (1965) discusses the annual cycles of *S.pratensis* and goes into some detail on their torpor patterns. Longevity he gives as "several years", and of diet he remarks that of six stomachs examined two (July and August) were empty and three (August, October, and December) contained arthropod remains. One nest he examined in June contained a store of plant tubers and his captive animals did not take fruit. Hanney also starved an individual for 38 days without food or water and these results will be considered in chapter 7.

Of the other published accounts, most briefly cover the general ecology of members of the genus and state that these species are nocturnal, terrestrial, occur singly or in pairs, and are primarily seed-eaters, but also eat bulbs, groundnuts, insects, and grass (Copley 1950; Roberts 1951; Ansell 1960; Rosevear 1969; Smithers 1971; Kingdon 1974; Smithers 1975, 1983).

Burrow construction is described as ranging from simple and shallow (Sclater 1901; Smithers 1975) through medium deep (Smithers 1983; de Graaff 1981, p. 130) to complex and deep (Roberts 1923; Hanney 1965; Vesey-Fitzgerald 1966; Kingdon 1974; Genest-Villard 1979). This may be an indication that different species may have different burrow structures as described by Ansell (1960). These animals also burrow in the sides of anthills (Shortridge 1934) but it is not known whether these are permanent burrows or temporary ones to enable the animals to get to the termites to eat them. Smithers (1971) recounts finding an aardvark (*Orycteropus afer*) with one *S.pratensis* stuck in it's throat and another in it's mouth - this is presumably an accident owing to the habit of *Steatomys* of burrowing in termite mounds and the predilection of the aardvark for termites.

Kingdon (1974) writes that *Steatomys* are abundant in certain localities but apparently absent over large intervening areas.

Each author tends to describe the torpid state of these animals in a different way; these are discussed in chapter 8.

Sheppe & Haas (1981) and Kern (1981) both mention *S.pratensis* in ecological studies, the former trapping 35 animals in Botswana in April to June and not at any other time; and the latter trapping most of his fat mice in the mid-summer season in the Kruger National Park. Kern remarks that the high number of *S.pratensis* in his post-burn areas may have been due to a permanent resident population which implies that these animals are fairly well protected from the effects of a veld fire in their burrows. Indeed, Genest-Villard (1979) states that as long as the burrows have more than one entrance the animals can survive fire.

One unpublished work which will be referred to in some detail is an honours thesis by P.Taylor from the University of Cape Town in which he studied the physiology of *S.krebsii* from the Cape. In this study he found a higher body temperature than would be expected from Petter's (1966) work on *S.opimus*, although closer to the mammalian norm (37 - 38°C), and he found no evidence of torpor.

There is thus general agreement that certain members of this genus (particularly *S.pratensis* and *S.opimus*) become very fat and go into a state of depressed metabolism at certain times. These times seem to be the cold dry months of winter when they are found in burrows at least 200 mm below ground level.

One common remark in nearly all published references to *Steatomys* (e.g. Coetzee 1977; de Graaff 1981; Smithers 1983) is the desperate need for a full taxonomic revision of the genus. Roberts (1923) also makes the interesting observation, borne out by personal observation of this author but not mentioned anywhere else, that there are two groups within the genus, the south-western one (*krebsii*) having larger ears and longer hair than the other (*pratensis*). However, Keogh (1985) specifically refutes this. De Graaff (1981) mentions longer hair in relation to *S.parvus* but not *S.krebsii.*

For the purposes of this study it will be taken that the animal studied was *Steatomys pratensis natalensis* Roberts 1929 (considered a valid subspecies by Meester *et al.* 1986), and that *pratensis* may possibly represent a different species to the West-African *opimus* studied by Petter (1966) and Genest-Villard (1979), although Coetzee (1977) and Honacki *et al.* (1982) regard them as conspecific.

### 1.2 The Semantics of the Depressed Metabolic State

The ability to maintain a constant and high body temperature (endothermy) is very important in the mammalian way of life but is very expensive energetically. For this reason many mammals which live in environments where there is periodic energy stress show a condition known as heterothermy, which is defined by Vaughan (1972, page 365) as "the ability to maintain a constant body temperature at some times but to allow the body temperature to fluctuate at other times". This means that during an energetically stressful period the animal's metabolic rate and temperature decline to a very low level, together with a complete lack of activity.

Many words have been used to describe this state of depressed metabolism and many of the words commonly used have been defined differently by different authors.

The word 'hibernation' is generally understood as "to spend the winter in some special state suited to resist it; being said especially of animals that pass the winter in a state of torpor" (Standard Oxford Dictionary). The word 'torpor' is defined as " a condition whereby there is absence or suspension of motive power, activity, or feeling" (Standard Oxford Dictionary).

Hibernation is usually used to refer to those animals which stay torpid for at least several days and more usually several months during the winter (Garfield 1988).

The word 'aestivation' has been defined as "to spend the summer; esp. (Zool.) in a state of torpor" (Standard Oxford Dictionary); "a torpor in response to heat, drought, or food shortages" (Vaughan 1972); "a dormancy at 20°C or higher ambient temperatures" (Gunderson 1976); and "a shallow torpor" (Walker *et al.* 1979).

Because of this and because no definitions are given in field notes, it is impossible to decide which definition each author has used. The matter is further complicated by the fact that the genus *Steatomys* is found in the Northern Hemisphere as well as the Southern: winter and summer are thus at different times of the year at the limits of its range.

For these reasons the word "torpor" throughout this study will be taken to mean a state when the animal is sluggish, there are prolonged periods of apnoea, and the rectal temperature is within 1 or 2°C of ambient if below thermoneutrality. Any animal not in this state will be referred to as "euthermic". It must be stated here that 'ambient' in this case may not necessarily be the temperature of the room, as owing to a nestbox and possibly the presence of a euthermic mate, the body temperature of a torpid animal may be some degrees above the temperature of the room.

There has been much discussion in the literature as to whether torpor is an advanced or primitive mechanism. Although at one time it was considered a primitive reaction caused by the inability of the animals to keep their body temperatures stable (e.g. Cade 1964), most authors now agree that it is an advanced mechanism to reduce the amount of food needed by the animals during times of energy shortage (e.g. Hudson 1978; Vogt & Lynch 1982).

### 1.3 Aspects of torpor studied.

To have a basis on which to begin the study, metabolic rates of both torpid and euthermic animals were measured over a range of temperatures from 0 to 35°C. This forms the basis for chapter 2, while chapter 3 goes on to measure average daily metabolic rate (ADMR) both by oxygen consumption and calorifically while also giving a summary of daily activity patterns and body temperatures. To determine the insulative properties of the fat layer, thermal conductance and heating and cooling rates of the animals were determined and are reported in chapter 4.

Chapter 5 goes into greater detail on the parameters of the torpid state by covering non-shivering thermogenesis and arousal rates under stress, and chapter 6 gives detail on the anatomy, histology, and amount of fat in the fat mouse body. To ascertain if torpor could be triggered by starvation the reactions of *S.pratensis* to water and food deprivation are examined in chapter 7. Chapter 8 gives the annual cycles of weight change, reproductive condition, and torpor patterns; and also reports on an attempt to see if the animals would become torpid while pregnant. Chapter 9 synthesizes these results to give a comprehensive account of the physiology of *Steatomys pratensis*.

### 1.4 Trapping location and housing conditions of the mice.

The subjects used in this study were second, third, and fourth generation animals bred in captivity. The original stock were trapped in the Cathedral Peak area of the Natal Drakensberg during November to December 1979. Unless otherwise stated the animals used for these studies were between 9 and 24 months old at the time of experimentation.

The animals were maintained in pairs (usually male-female pairs, occasionally male-male pairs, never female-female pairs) in standard sized 'Labotec' cages 420 x 250 x 120 mm. They were fed 'Epol' mouse cubes with supplements of Brewers yeast and 'Pronutro' (a high protein content cereal), mealworms, and mixed seed. Various greens and fruit were tried but were discontinued when it was found that the animals did not eat them. Water was freely available in water bottles suspended in the cage. During any physiological trial, and for the week preceding it, the experimental animals were fed only on 'Epol' cubes and water to prevent any effect of differing diet. Shredded paper and wood shavings were used as bedding material, and each cage had two wooden nesting boxes 120 x 100 x 55 mm to enable the animals to escape any aggression from the cage-mate. Other than for the purposes of taking measurements, the animals were disturbed as little as possible.

During experimentation the cages were housed in a Rudnev constant temperature room (at a temperature of 20°C) with artificial neon lighting giving a day-length of 07h00 to 17h00. The animals were placed in this room to acclimate at least a week before any physiological measurements were taken. Other than at these times (and including the work done for chapter 7) the cages were housed in a room providing a protected environment where temperatures ranged from 17 to 28°C throughout the year and the light was natural light supplemented during the middle of the day by neon lights.

Unless otherwise stated the rectal temperatures were taken using a Digitron Model 1408 Portable Digital Thermometer with a 1 mm wide probe, accurate to ± 0.3% of reading ± 0.5°C (i.e. 0.1 resolution), coated with a thin layer of petroleum jelly and taken to a depth of 10 mm. This was found to be the best depth as it was felt that any deeper might damage the animals if they struggled and the temperature did not differ more than 0.3°C between a depth of 10 mm and a depth of 17 mm, the maximum possible. Animals were weighed on a Sartorius 1265 MP digital readout balance accurate to the nearest 0.01 g.

Many of the experiments carried out in this study were done simultaneously with white mice (*Mus musculus* var. albino). This was done as a check on the experimental procedures since the results of such experiments on the white or laboratory mouse are available in the literature. In retrospect, and in the light of Hudson & Scott's (1979) publication on torpor in white mice, and Jakobsen's (1981) descriptions of the plasticity of this species physiologically, this may not have been a wise choice, but the results found were in most cases comparable with the literature.

The white mice used in this study were originally obtained from the University of Natal Medical School's Animal Room and were maintained in exactly the same conditions as the *S.pratensis*. The ages of the white mice used ranged from 4 months to one year.

The female *S.pratensis* became perforate at various times during the study and the males either had abdominal or scrotal testes: this was always noted at the start of any experiment to ensure that if the reproductive condition of the animals had an effect on the results it would be possible to ascertain this. This is discussed more fully in Chapter 8.

### 1.5 Methods of Analysis

Most of the results in this thesis were initially analysed using the spreadsheet programme Supercalc 4 (Computer Associates). Statistical analyses were done using Epistat (Gustafson, Cap Rock, Richardson, Texas).

# Chapter 2

## Estimation of Resting Metabolic Rates

### 2.1 Introduction

The measurement of oxygen consumption is one of several standard procedures used to determine the metabolic rates of mammals. Probably the most frequently used procedure, oxygen consumption is generally reported in ml oxygen per unit mass (kilogram or gram) per unit time (minutes or hours). This measurement can be used to compare the metabolic rates of different species or taxa of mammals, and to make deductions about the energy needed for different lifestyles and/or ecological niches (see, for example, McNab 1966 and Elcar & Harvey 1987).

In mammals, the minimal rate of energy metabolism of an adult (i.e. non-growing) animal in a fasting state and at rest is known as the resting metabolic rate. The temperature range over which the amount of energy required for thermoregulation is at a minimal level and the animal does not have to use energetically expensive means of temperature regulation (sweating, panting, shivering, and non-shivering thermogenesis) is known as the thermoneutral point or zone, and the metabolic rate at this point is known as the basal metabolic rate.

Basal metabolic rate can be affected by many circumstances in an animal's life, for example annual fluctuations in weight and fat, water stress, time of the breeding cycle, past ecological history of the animal, and stress, but it remains the accepted basis upon which most predictions and interspecific comparisons are made.

It was thus decided that measurement of metabolic rates of euthermic animals over a wide range of ambient temperatures should be the first step in examining the physiology of *Steatomys pratensis*.

The white or laboratory mouse *Mus musculus* var. albino has been used for many physiological studies and is a well known species (see, for example, Jakobsen 1981). It was thought that this would be a suitable animal to use as a comparison with the fat mice.

Torpor is used by many endotherms as a mechanism for reducing energy expenditure when food is limited and it is becoming increasingly obvious that it is far more widespread than has been

assumed until recently (Hudson 1978). Unlike hibernation, torpor is often short-term (lasting a few hours) and can be difficult to determine at higher ambient temperatures which permit the animals to show normal locomotion.

Hudson (1978) discusses shallow daily torpor as a thermoregulatory adaptation used by many small mammals to overcome food shortages. Such animals are characterised by having a "critical body temperature" below which they cannot spontaneously arouse. This is in contrast to a true hibernator which can arouse if and when its body temperature falls below a set minimum (Lyman *et al.* 1982, p.66).

The species of small mammals discussed by Hudson (1978) do not assume the typical horizontal posture of the hibernator but remain upright, and if the ambient temperature is high enough show typical locomotory behaviour. This daily torpor may be seasonal and may require acclimatisation. These animals also tend to have a high fecundity.

Although a generally well-known phenomenon, torpor has not been extensively studied in *Steatomys*. Sclater (1901) was probably the first to mention its occurrence, although he ascribed it to their fatness, and it has been variously called hibernation (Roberts 1923), prolonged hibernation (Shortridge 1934), aestivation (Roberts 1951), sleep (Booth 1960), torpor (Hanney 1965), "lethargie" (Petter 1966), and inactivity (Smithers 1975).

Of the papers mentioning torpor in *Steatomys*, both Hanney (1965) and Petter (1966) are worth discussing in detail.

Hanney (1965) reports that during July captive specimens of *Steatomys pratensis* became torpid in the daytime, and at room temperatures between 18.3 and 19.4°C had body (rectal) temperatures of 19.4 to 20.5°C in the morning and early afternoon, rising to 30.5°C in the evening. He remarks that when torpid the animals could be handled "roughly" without them awaking. In torpid animals respiration was irregular, with periods of apnoea (cessation of breathing) lasting about three minutes.

Petter (1966) kept several *S.opimus* in Paris which always showed daily torpor. These animals had a period of activity in the early evening during which time their (presumably rectal) temperatures were 33 to 34.6°C, after which their body temperatures declined until they approximated the ambient temperature. They stayed near to ambient until the following afternoon when they started to rouse again. Two of Petter's animals died when left at 12°C overnight. At body temperatures of 20°C and less the animals were incapable of movement.

It thus seemed likely that *Steatomys pratensis* shows a daily torpor rather than deep hibernation with a lower critical body temperature somewhere above 12°C. As far as can be ascertained no other work has been published on their metabolic rates.

It was thus decided to measure the metabolic rates of both torpid and euthermic fat mice at a range of temperatures and to compare these to similar measurements made on *Mus musculus* var. albino.

## 2.2 Materials and Methods

All animals used in this section were acclimated in the Rudnev constant temperature room at 20°C, with day-length 07h00-17h00. Most of the resting metabolic measurements were taken in the winter with some of the runs extending into early spring. This was to ensure that both torpid and euthermic animals would be available for experimentation. The measurements were taken only in the mornings because it was thought that by midday the torpid animals might be beginning to raise their body temperatures (as found, for example, by both Petter (1966) and Vogt & Lynch (1982)) and so would be difficult to distinguish from the euthermic animals. It was also not known whether the euthermic animals had any diurnal variation in body temperature or metabolism.

Because it was impossible to decide the previous day which animals would be used for the trials, owing to their extreme flexibility in becoming torpid (see chapter 8) and because it was decided not to use semi-torpid animals for these tests, it became quite difficult to prevent the animals eating before the test. To overcome this problem, none of the tests were carried out until at least an hour after daybreak, since it was found by observation that extremely rarely were the animals found eating after the onset of daybreak. These animals were not in the strictest sense of the word post-absorptive but this was the best that could be managed under the circumstances.

All *S.pratensis* used in these experiments were in the age range 9 to 24 months, mass 23.2 g to 46.9 g with a mean of 37.5 g. All *Mus musculus* which were used for comparative studies were in the age range 2 to 12 months, mass 23.2 g to 31.6 g. No pregnant females of either species were used.

The equipment used for measuring resting metabolic rate consisted of a 1120 ml respirometer which was connected through a drying tube of silica gel to a Beckman OM-14 oxygen analyser.

The air from the analyser passed through a ball rotameter ("Lo-Flo" by Ametek) to the 0.018kW Labotec vacuum pump which drew air through the system. At 0°C the air entering the respirometer first passed through a coiled copper tube and a water trap (to collect condensation) to lower the temperature of the air entering the respirometer to 0°C.

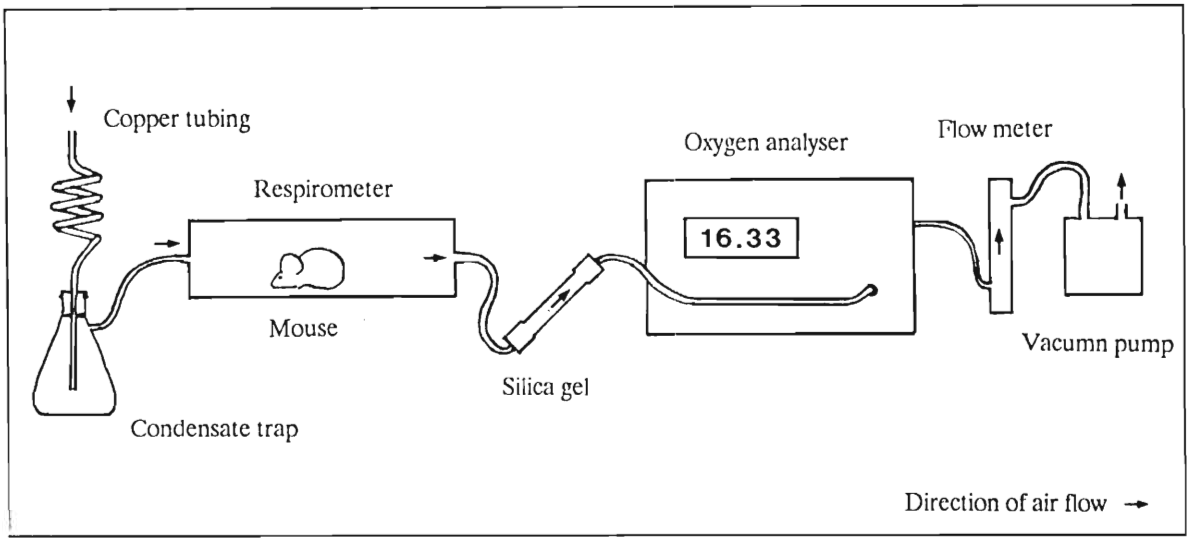


**Figure 2.1** Diagram of the system used to measure resting metabolic rate

The animal was placed in the respirometer with both clamps open and the pump running, and left to equilibrate. Equilibration time ranged from 60 minutes at 15 to 30°C (consisting of half an hour of through-flow air and a trial run of 15 minutes with 15 minutes purging time) to 30 minutes at the stressful temperatures of 0, 5, 10, and 35°C. Although the latter was short it was found that the mice became distressed easily at these temperatures (see following page). The respirometer volume (1120 ml) was such that the animal could sit up, stretch, and turn round comfortably. A piece of paper towel was folded in such a way that the animal could sit on the towel without it impeding the flow of air through the respirometer in any way. This was found to be especially important in the case of the torpid animals which tended to arouse when placed directly onto the perspex floor of the respirometer. The mice were watched throughout the experiments to check that they were resting and to see if they were showing any signs of stress.

The respirometer was then closed for 10 to 15 minutes, then opened and air pumped through at 60 ml per minute under negative pressure until the lowest reading of percentage oxygen was obtained and recorded. This was taken to be the lowest value that the oxygen in the respirometer had reached. Air was then pumped through the system until the oxygen level stabilised at or around ambient and the system closed again for another trial. Because of the problems of analyser drift (which could become quite substantial over several hours) the

analyser was left running throughout and constantly checked against ambient.

To confirm the accuracy of this method of measurement, the respirometer was purged and filled completely with calibration gas of 16.20% oxygen. When this was connected to the respirometer the lowest reading taken was 16.33%, indicating an accuracy of 97% from the 20.67% oxygen level of room air. A Metrohm recorder trace of this showed that the lowest level of percentage oxygen came through the analyser within 60 seconds of connection and thereafter gradually levelled off towards ambient.

The number of tests made depended on the behaviour of the animal concerned. If the animal was calm then more readings were taken (up to a maximum of 10), while several individuals at each temperature never settled down and had to be taken out after half an hour without any readings having been taken. At the highest and lowest temperatures (35 and 0°C respectively) care was taken not to over stress the animals. They were removed from the experiment as soon as they began to show signs of stress. Stress at low temperatures was considered to be when the mouse showed signs of exhaustion by stopping shivering and becoming unsteady on its legs. Stress at high temperatures manifested itself with the animal becoming damp around the mouth and again becoming unsteady on its legs. Generally the trials did not last for more than an hour on any one animal at these temperatures, and acclimation time was also very short. Special care had to be taken when using torpid animals at the lowest temperatures as they became distressed fairly quickly (see chapter 5).

The respirometer placing depended on the ambient temperature necessary for the trial. For 0°C the respirometer was placed in a tank of melting ice, connected to the copper tube to cool the air before it entered the respirometer; for 5 and 10°C the whole system was placed in the walk-in Rudnev fridge; and for 15 to 35°C the respirometer was placed in the Conviron.

Results were adjusted to standard temperature and pressure and calculated as ml $O_2$/g.hr. Results for each sex were first analysed separately before it was decided it would be possible to combine the results. The mean, median, and mode were calculated to test for a normal distribution, and the results were then tabulated (Tables 2.1, 2.2, and 2.3). The coefficient of variation for each data set was calculated and linear regression lines were drawn for the euthermic *Steatomys pratensis* and *Mus musculus* graphs, using only those results where the animals remained normothermic (30 - 34°C). No regression line was drawn for the torpid *S.pratensis* graph because of the nature of the data. t-Tests were run between euthermic and torpid *S.pratensis* at each temperature. Regression lines were also calculated for the ratio of

body mass to specific oxygen consumption of each animal at the 8 ambient temperatures used to check for any influence of body mass on oxygen consumption.

## 2.3 Results

### 2.3.1 Metabolic rates of *Steatomys pratensis* and *Mus musculus*

The data obtained from these experiments are shown in the following tables and figures.

**Table 2.1** Metabolic rates of euthermic *Steatomys*.

(All units in ml $O_2$/g.hr)

| Temp. | No. of animals | No. of tests | Mean | Range | 1SE | Coeff. var. |
|---|---|---|---|---|---|---|
| 0°C | 4 | 17 | 3.83 | 2.02 - 5.56 | 0.24 | 25.59 |
| 5°C | 6 | 29 | 2.79 | 1.43 - 4.53 | 0.14 | 27.60 |
| 10°C | 5 | 23 | 2.56 | 1.26 - 4.48 | 0.21 | 39.84 |
| 15°C | 8 | 29 | 2.45 | 1.80 - 3.35 | 0.07 | 15.92 |
| 20°C | 10 | 32 | 2.04 | 1.08 - 2.94 | 0.10 | 26.96 |
| 25°C | 11 | 45 | 1.04 | 0.26 - 1.92 | 0.08 | 51.92 |
| 30°C | 12 | 65 | 0.50 | 0.16 - 1.12 | 0.03 | 46.00 |
| 35°C | 10 | 28 | 1.42 | 1.05 - 1.88 | 0.05 | 17.61 |

**Table 2.2** Metabolic rates of "torpid" *Steatomys*. This table should be read in conjunction with table 4.1.

(All units in ml $O_2$/g.hr)

| Temp. | No. of animals | No. of tests | Mean | Range | 1SE | Coeff. var. |
|---|---|---|---|---|---|---|
| 0°C | 10 | 40 | 3.71 | 2.27 - 6.12 | 0.18 | 30.73 |
| 5°C | 1 | 6 | 1.66 | 0.45 - 5.36 | 0.75 | 110.84 |
| 10°C | 2 | 13 | 2.35 | 0.24 - 4.66 | 0.46 | 70.64 |
| 15°C | 3 | 16 | 1.06 | 0.25 - 2.50 | 0.17 | 65.09 |
| 20°C | 2 | 11 | 0.64 | 0.26 - 1.23 | 0.09 | 46.88 |
| 25°C | 1 | 7 | 0.59 | 0.28 - 1.01 | 0.12 | 52.54 |
| 30°C | 2 | 8 | 0.95 | 0.27 - 1.64 | 0.19 | 57.89 |
| 35°C | 2 | 12 | 0.84 | 0.36 - 1.66 | 0.13 | 53.57 |

**Table 2.3** Metabolic rates of *Mus musculus.*

(All units in ml $O_2$/g.hr)

| Temp. | No. of animals | No. of tests | Mean | Range | 1SE | Coeff. var. |
|---|---|---|---|---|---|---|
| 0°C | 3 | 15 | 6.17 | 4.28 - 8.37 | 0.34 | 21.56 |
| 5°C | 3 | 18 | 5.60 | 4.26 - 7.25 | 0.23 | 17.50 |
| 10°C | 3 | 18 | 5.18 | 3.13 - 6.80 | 0.28 | 23.17 |
| 15°C | 3 | 17 | 3.87 | 2.32 - 5.19 | 0.21 | 22.74 |
| 20°C | 3 | 20 | 3.48 | 2.15 - 4.65 | 0.16 | 20.98 |
| 25°C | 3 | 18 | 2.88 | 1.50 - 3.99 | 0.16 | 22.92 |
| 30°C | 3 | 11 | 2.19 | 0.93 - 3.28 | 0.27 | 41.10 |
| 35°C | 3 | 18 | 2.45 | 1.72 - 3.58 | 0.14 | 24.90 |

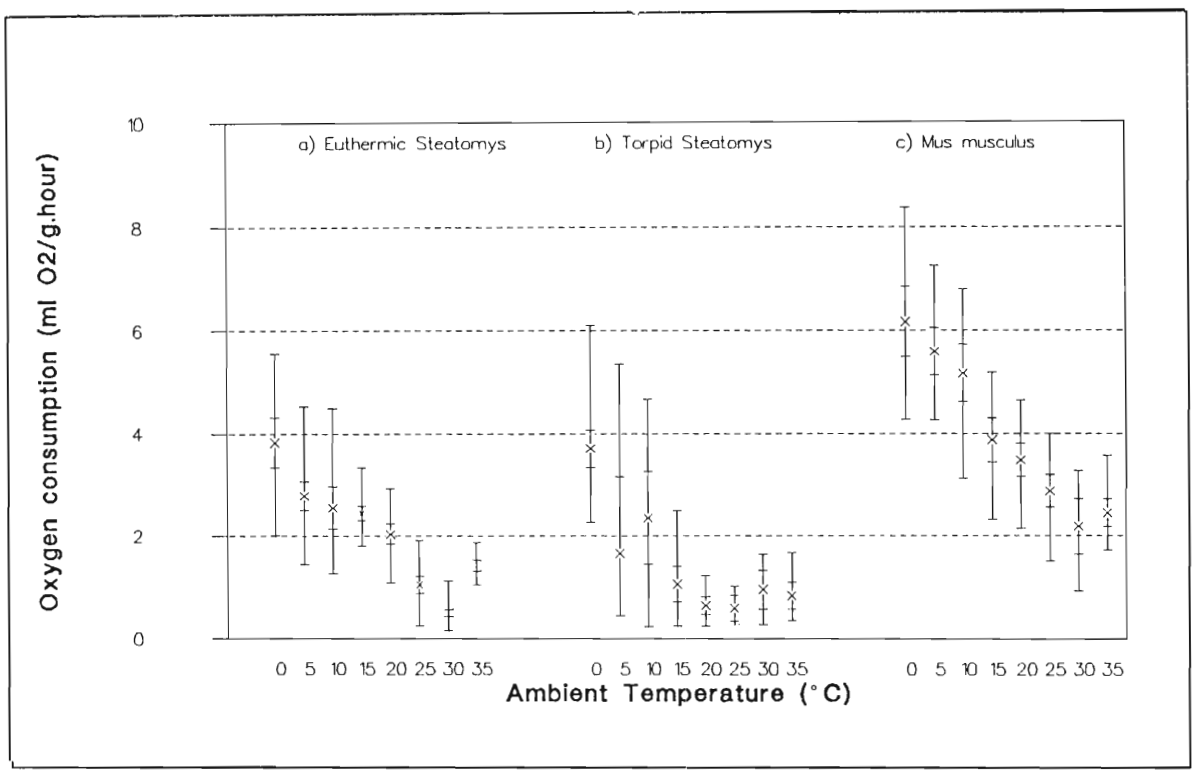


**Figure 2.2** The metabolic rates of euthermic and torpid *Steatomys pratensis*, and *Mus musculus.*
(Metabolic rates are adjusted to STP, the graph shows the range, mean, and two SE.)

From figure 2.2 it can be seen that the euthermic *S.pratensis* and the *M.musculus* show the typical pattern of oxygen consumption in mammals, being high at 0°C and gradually reducing to a low at 30°C.

Both groups of animals had their thermoneutral zones at 30°C, and both became hypothermic at temperatures lower than 15°C (figure 4.1). The regression line for the slope of the graph between 15 and 30°C for the euthermic *Steatomys* was Y = 4.59 - 0.137x and that of the *Mus* Y = 6.308 - 0.139x.

The coefficients of variation of the euthermic *S.pratensis* ranged from 15.92 to 51.92, that of *M.musculus* from 17.50 to 41.10, and that of the torpid *S.pratensis* from 17.50 to 110.84.

**Table 2.4** t-Tests between euthermic and torpid animals

| Temp. | t | df | significance |
|---|---|---|---|
| 0°C | 0.40 | 55 | P > 0.5, not significant |
| 5°C | 1.48 | 33 | P > 0.1, not significant |
| 10°C | 0.41 | 34 | P > 0.5, not significant |
| 15°C | 7.43 | 43 | P < 0.000001, highly significant |
| 20°C | 10.54 | 43 | P < 0.000001, highly significant |
| 25°C | 3.17 | 50 | P < 0.01, significant |
| 30°C | 2.29 | 71 | P < 0.05, P > 0.01, probably significant |
| 35°C | 4.20 | 38 | P < 0.01, significant |

### 2.3.2 Effect of mass on oxygen consumption

There was no effect of subject mass on oxygen consumption in either the euthermic *S.pratensis* or the *M.musculus*. At all 8 ambient temperatures the regression lines were not significantly different from zero.

### 2.3.3 Behavioural reactions

At low temperatures (0 to 10°C) the euthermic fat mice sat on their hind legs with their bodies curled up and their front feet and noses tucked into their bellies. Piloerection occurred and the ears were flat against the head. This made the body spherical and so reduced the surface area to body volume ratio, thus reducing the area for heat loss. At very low temperatures (0 and 5°C) the euthermic *S.pratensis* were sometimes active and although the body temperatures fell in nearly all cases (see chapter 4) only 2 out of the 12 animals shivered.

At high temperatures (30°C if the animal was very active when first put into the respirometer, otherwise 35°C), the *S.pratensis* lay with their underparts flat against the perspex base of the

respirometer, the legs splayed out and the head stretched out with the chin down. After a time they became wet around the mouth from licking, presumably salivating in an attempt to lose heat by evaporative cooling. The fat mice became distressed fairly quickly at 35°C, becoming exhausted and unsteady on their feet after 45-60 minutes. The length of time taken depended on whether they were active when first put into the respirometer and thus building up a heat load which could not easily be lost again. Their body temperatures were well above normal and no doubt the animals would not have survived long at this temperature.

At 0°C the *M.musculus* lay shivering with their eyes closed and their fur piloerected. At 5 and 10°C they lay curled up, still with their hair erect, and shivering. At 15°C they lay curled up but did not shiver and at 20, 25, and 30°C they tended to be active, sitting quietly in between bouts of exploring the respirometer. At 35°C the animals lay flat and very still on the bottom of the respirometer, and would become damp around the mouth, presumably from salivating in an effort to lose heat by evaporative cooling.

Torpid *S.pratensis*, at temperatures ranging from 15 to 30°C, stayed curled up and quiet; while at 35°C they lay on their stomachs, not as stretched out as the euthermic animals, and showing no salivation in an attempt to lose heat. All torpid *S.pratensis* attempted to arouse from torpor at ambient temperatures under 15°C.

In contrast to the euthermic *Steatomys* nearly all the torpid *Steatomys* (6 out of 8 animals) shivered when put in to the respirometer at 0°C. The two which did not shiver while in the respirometer went into shivering arousal when taken out into room temperature. At 5 and 10°C the animals tended to shiver less, although their body temperatures dropped.

## 2.4 Discussion

### 2.4.1 Euthermic *Steatomys pratensis*, and comparisons with *Mus musculus*

The gradient of the euthermic *S.pratensis* and *M.musculus* oxygen consumption regressions show the typical pattern of endothermic animals. The oxygen consumption of resting animals at 0°C is high, gradually reducing to a minimum at 30°C.

The amount of oxygen consumed by both species of animals at 0, 5, and 10°C is lower than would be expected by extrapolation of the regression line. This was because at these temperatures both the euthermic *S.pratensis* and the *M.musculus* became hypothermic (figure

4.1). Since the euthermic *S.pratensis* at room temperatures close to 20°C had rectal body temperatures of 30.6 - 34.3°C it was not surprising that at an ambient temperature of 35°C they became hyperthermic. The *M.musculus* also became hyperthermic at 35°C. These results are discussed more fully in chapter 4.

Although figure 2.2c shows the same basic form as the euthermic *S.pratensis* response (figure 2.2a), the actual oxygen consumption figures are higher.

Specific oxygen consumption in the thermoneutral zone as reported for *Mus musculus* in the literature varies rather widely. Several of the results from the literature are summarized in table 2.5.

**Table 2.5** Metabolic rates and thermoneutral zones as reported for *Mus musculus* in the literature.

| Author | Metabolic rate at thermoneutrality |
|---|---|
| Brody (1945, in Schmidt-Nielsen 1975) | 1.65 ml $O_2$/g.hr |
| Kruszyna & Smith (1975) | 2.12 ml $O_2$/g.hr |
| Cassin (1963, in Kruszyna & Smith 1975) | 2.80 ml $O_2$/g.hr |
| Pennycuick (1967, in Kruszyna & Smith 1975) | 2.31 ml $O_2$/g.hr |
| Mount (1971) | 1.86 ml $O_2$/g.hr |
| Eisenberg (1981, p. 496) | 3.40 ml $O_2$/g.hr |
| Gorecki & Kania (1986) | 3.40 ml $O_2$/g.hr |
| Hudson & Scott (1979) | 1.47 ml $O_2$/g.hr |
| **Author** | **Thermoneutral zone** |
| Hudson & Scott (1979) | 31 - 35°C |
| Kruszyna & Smith (1975) | 34°C |
| Gorecki & Kania (1986) | 28°C |
| Mount (1971) | 32°C |
| Jakobsen (1981) | 30 - 32°C |

Jakobsen (1981) however, shows that acclimation temperature can have a marked effect on the metabolic rate and thermoneutral zone, increasing the former by up to 20%.

Resting metabolic rate at 30°C in this study for *Mus musculus* was 2.19 ml $O_2$/g.hr at an acclimation temperature of 20°C which compares well with the oxygen consumptions from the literature.

McNab (1988) gives the equation:

$$MR = 3.45\ mass^{-0.287}$$

where MR is the mass-specific metabolic rate, to predict the metabolic rate of any mammal. Although slightly different to the original equation proposed by Kleiber (1961, in Kleiber 1972) this equation is often referred to as the Kleiber equation.

Using this equation the basal metabolic rate of *M.musculus* is calculated as 1.30 ml $O_2$/g.hr (where mean mass was 30.28 g) which is lower than the results found here or any of the results from the literature.

One of the most interesting aspects of the euthermic *S.pratensis* results is the extremely low basal metabolic rate. Using McNab's equation the fat mice used in these experiments, having a mean mass of 37.54 g, have an expected metabolic rate of 1.22 ml $O_2$/g.hr. The measured resting metabolic rate at 30°C (the temperature at which the lowest metabolic rates were recorded) was 0.50 ml $O_2$/g.hr, which is only 41% of the expected value.

Lovegrove (1986) gives two equations to calculate the resting metabolic rates of rodents:

that of Hayssen & Lacey of

$$MR = 4.98\ mass^{-0.331}$$

and that of Lovegrove for subterranean rodents of

$$MR = 3.79\ mass^{-0.322}$$

The resting metabolic rate of *Steatomys pratensis* in this study was 33% and 43% of these expected values respectively.

There have been many attempts to interpret lower or higher metabolic rates than those predicted by the metabolic equation. Factors such as climate and food habits, as well as taxonomic affinity and body mass are known to affect the relationship (McNab 1983).

In general higher metabolic rates are found in mammals which weigh more than 100g and do not burrow, but are also found in soricine shrews (McNab 1983) and vertebrate eaters (McNab

1986).

Lower metabolic rates than those predicted have been correlated with a subterranean - both burrowing and fossorial - way of life in animals weighing more than 200 g (McNab 1966, 1979b, 1986); adaptation to aridity (McNab 1974); scarce food resources (Jarvis 1978); heteromyid rodents (McNab 1979a); termite- and ant-eaters (McNab 1983); the insectivorous bats (McNab 1983); and also with the 'lower' or 'more primitive' groups of mammals, the marsupials and monotremes (McNab 1988).

There are several potential reasons for a low metabolic rate which have been postulated in the literature. McNab (1983) draws a minimal boundary curve, below which animals tend to enter torpor and above which they have high rates of metabolism.

According to McNab (1983) below a body mass of around 80 g basal rates become much higher than predicted by the Kleiber equation. The exception to this are species of Heteromyidae which do not have a high rate of metabolism although the smaller members of the family are known to enter torpor. Below McNab's boundary line come the smaller marsupials, the myrmecophagous species, the heteromyid rodents, some cricetid rodents, the insectivorous bats, the smaller frugivorous bats, the crocidurine shrews, and *Heterocephalus glaber*. McNab thus divides the smaller mammals into two groups: those which do not change the scaling rate of $mass^{-0.25}$ and enter torpor, at least at times of food shortage, and those mammals which change the scaling such that the basal rate is higher than that expected from the Kleiber equation.

McNab (1983) gives the equation:

$$MR = 15.56/mass^{0.67}$$

where MR is the mass specific oxygen consumption, to estimate the metabolic rate below which the animals show torpor, or rather above which the animals can use continuous endothermy. In this case, *Steatomys pratensis* would have to have a metabolic rate of 1.37 ml $O_2$/g.hr to show continuous endothermy, which is clearly much higher than their actual metabolic rate, and so following McNab they would be expected to show torpor. Similarly, *Mus musculus* would have to have a metabolic rate of 1.58 ml $O_2$/g.hr to remain endothermic which accords well with the results obtained here. The actual metabolic rate measured was much higher and these animals did not show any torpor.

However McNab (1983) insists that all mammals well to the left of this curve "readily enter torpor when they are quiescent", which does not apply to *S.pratensis* as these animals do not show torpor every day and seem able to remain out of torpor for months at a time.

A point that McNab (1983) raises is that small mammals with low metabolic rates can compensate for a low metabolic rate by improving thermal insulation. This is discussed further in chapter 4.

Of the various reasons put forward to explain why subterranean mammals have low metabolic rates, McNab (1979b) argues that burrowing animals, being limited in their abilities to lose heat by their burrows, have low metabolic rates, high thermal conductances, and high ranges of thermoneutrality. Vleck (1979) has suggested that lower metabolic rates compensate for the energetic demands of burrowing. Jarvis (1978) suggests that the low metabolic rate of *Heterocephalus glaber* is an adaptation to scarce food resources, and Arieli *et al.* (1977) correlate a low metabolic rate with an attempt to normalise the partial pressures of oxygen and carbon dioxide in the blood, thus enabling the animals to cope with the hypoxia and hypercapnia found in burrows.

Fat mice seem to live in fairly stable burrows but presumably do not spend a lot of time digging since they forage out of the burrow (Hanney 1965), so they are unlikely to need a lower metabolic rate to cope with the high energetic demands of burrowing. Hanney (1965) reports them to eat a varied diet of cereals, nuts, and insects, as well as hoarding tubers, and Perrin & Curtis (1980) state that they have stomach characteristics consistent with a high protein content diet, so they appear to be opportunistic feeders and can be assumed to have a food supply which is probably not scarce nor of low quality. As these animals forage out of their burrows it is also unlikely that the build up of carbon dioxide and lowered oxygen levels would cause any physiological problems as the only time at which a low oxygen level might be important would be when the animal was active, and therefore not in the burrow.

However, the suggestion by McNab (1979) that low metabolic rates could guard against overheating could have a bearing on these results. In view of the very heavy fat layer found in *S.pratensis* which covers the body completely (see chapter 6) it is not impossible that at times the animals may find themselves in thermal stress. This view will be developed later in the thesis.

Arid- or desert-adapted species of rodents generally have low basal metabolic rates and narrow thermoneutral zones (Hart 1971). Low metabolic rates are advantageous to desert animals because they can thus raise the lower critical temperature and prevent overheating (Hart 1971).

It seems unlikely however that the lower metabolic rates found in *Steatomys pratensis* have evolved as an adaptation to arid climates. Nel (1969) remarks that the genus is primarily a savanna species and in present times *S.pratensis* is found near water if in an arid area (Smithers 1983). This situation may occur since the soil near rivers is often sandy and thus easy to burrow into. Desert species also tend to be able to raise their body temperatures without raising their metabolic rate when under heat stress (Hart 1971), which *S.pratensis* is unable to do.

Myrmecophages (termite- and ant-eaters) conform to the pattern described by McNab (1986) for burrowing animals, having low basal rates down to a certain body mass below which their basal rates become higher. Although *S.pratensis* is known to eat termites (Smithers 1983) it is well under the minimum mass for any such effects to apply.

Lovegrove (1986) suggests that the combination of two selection factors for low metabolic rate would be additive, for example a subterranean species inhabiting an arid habitat would have an extra low metabolic rate. It is possible that the extremely low metabolic rate found in *S.pratensis* is a combination of two or more selection factors.

In many of the papers quoted here (e.g. McNab 1974; McNab 1983) *Heterocephalus* has stood as a lonely example of an animal with an extremely low metabolic rate. It now seems that *Steatomys pratensis*, with a metabolic rate of 0.50 ml $O_2$/g.hr approximates *Heterocephalus*'s metabolic rate of 0.55 ml $O_2$/g.hr (body mass 39 g to *Steatomys*'s 38 g). This is especially interesting in that *H.glaber* has very different characteristics from *S.pratensis* since it has no hair, is social, and has very little ability to regulate its body temperature at lower ambient temperatures (McNab 1966; Jarvis 1978). The one major difference between the two seems to be in thermal conductance (chapter 4).

None of the other dendromurine genera studied so far have a metabolic rate as low as found in *S.pratensis*, although Knight & Skinner (1981) in their study of *Malacothrix typica* found a metabolic rate of 0.95 ml $O_2$/g.hr, which was only 57.6% of the predicted value. Although they admit that more work needs to be done on this aspect, they found that these animals had body temperatures which stayed constant at 37°C, and did not exhibit torpor during the study. One reason why these animals showed such a low metabolic rate may have been that they were overweight, since animals used in this study had an average mass of 22 g while de Graaff (1981) gives the average mass as 15 to 16 g.

Taylor (1984) in his preliminary, unpublished work on *Steatomys krebsii* from the Cape, found a basal metabolic rate of 1.48 ml $O_2$/g.hr (93% of expected) at a thermoneutral zone of 30°C.

These animals had body temperatures of 37 to 38°C and were able to keep their body temperatures stable between ambient temperatures of 5 and 35°C. In general these animals were smaller (21.4 g) than the *S.pratensis* used in this study and did not show any signs of torpor. Further studies would be required to elucidate these differences.

Results seem to show that out of all the dendromurine species examined, *Steatomys pratensis* and *S.opimus* are the only known species which use torpor. It is extremely unlikely that with its high and stable body temperature *S.krebsii* would show any torpor when it did not do so in Taylor's study. This creates a fascinating problem as to why and how just these two species out of the family could have developed the use of torpor.

### 2.4.2 The effect of mass on oxygen consumption

There was no effect of mass on oxygen consumption at any temperature studied in either the euthermic *S.pratensis* or *M.musculus*. This could mean that there was too little range in the masses of the animals studied to produce a statistically realistic result but could also mean that the amount of fat in the animals affected the total mass of the animals without affecting the amount of metabolising tissue. The former possibility is thought to be the reason that the *Mus* showed no effect of mass on oxygen consumption since at no temperature did the range of masses of the animals used cover more than 3.6 g, while the maximum range at one temperature in the *S.pratensis* was 13.9 g. The effect of the amount of fat on the oxygen consumption of the *S.pratensis* is dealt with in chapter 6, although even with an adjustment for this the metabolic rate would still be a long way below expected.

McNab (1979a) reports the mass-specific basal metabolic rate of the heteromyid rodents to be nearly independent of mass, also the smallest heteromyids have the lowest metabolic rates, which is a departure from other groups of mammals. He suggests these low rates are not due to burrowing and may be to balance the water or food budget. *Steatomys pratensis* are similarly unlikely to have low metabolic rates because of the burrows.

The most likely reason for the extremely low metabolic rates found in *S.pratensis* is their extremely low body temperature. Rectal body temperatures of the euthermic animals at ambient temperatures of 15 to 30°C were 30 to 33.8°C. The *M.musculus* rectal body temperatures were 36.2 to 37.7°C at the same temperatures, which are entirely "normal" for non-burrowing eutherian mammals (McNab 1966). Not only are the *S.pratensis* body temperatures low but they were more variable (see chapters 4 and 5).

### 2.4.3 Behavioural adjustments

One factor which must be taken into account is the animals ability to conserve or dissipate body heat at low and high temperatures by postural adjustments, since without these the metabolic expenditure to keep the body temperature stable would be much greater.

At ambient temperatures from 15 to 30°C the *Mus musculus* neither shivered nor salivated, which accords well with the body temperatures obtained since at these temperatures they were neither hypo- or hyperthermic. At 15°C and below these animals were curled up (to conserve heat), at 20 to 30°C they rested lying along the bottom of the respirometer, and at 35°C they splayed themselves out onto the floor of the respirometer (to conduct heat to the perspex floor).

The euthermic *Steatomys pratensis*, however, curled up upright from 0 to 20°C and from 30°C upwards lay flat out on the respirometer floor presumably to lose heat. This suggests that even though the slope of the regression line indicates both *M.musculus* and *S.pratensis* lose heat at the same rate, *S.pratensis* is less capable of coping with extreme temperatures than *M.musculus*. This may be correlated with the fact that *S.pratensis* rarely shivered at low temperatures although its body temperature dropped (see chapter 5).

Torpid *S.pratensis* showed very few postural adjustment changes, being curled up upright from 5 to 30°C. At 0 to 10°C they went into shivering arousal and at 35°C they lay along the bottom of the respirometer. Although they would presumably not have survived at 5 and 10°C (chapter 4), and perhaps would have started arousing if their body temperatures had dropped much further, this seems to indicate that they were quite unstressed at ambient temperatures from 5 to 30°C. This has several ecological implications, probably the most important being that it may be very important for these animals to find a nest and burrow site which would not drop below their thermal minimum (see chapters 4 and 5).

### 2.4.4 Torpid *Steatomys pratensis*

The general shape of the graph for torpid *S.pratensis* (see figure 2.2b) is rather different to that of the euthermic animals. Oxygen consumption at 0°C is high, gradually reducing in value to 20°C (the 5°C result is not in line but it assumed that this was experimental error owing to using results from one animal only). The oxygen consumption rates from 15 to 35°C are all very similar (independent of ambient temperature), and since at ambient temperatures below the

thermoneutral zone heat production (metabolic rate or oxygen consumption) must be equivalent to heat loss (body temperature minus the ambient temperature, times the thermal conductance) (McNab 1974), this indicates a change in body temperature. The body temperatures of the torpid animals did tend towards ambient at these temperatures (chapter 4).

The coefficients of variation of the metabolic rates of the torpid animals were very much higher than those of the euthermic animals. It is possible that this may be due to the torpid animals undergoing periods of apnoea while their metabolic rates were being measured. Hanney (1965) reports that his animals respired 6 to 30 inspirations per minute over three minutes and then went into apnoea for three minutes. The animals in this study generally respired 5 to 7 times over a period of about 8 to 10 seconds, then went into apnoea for 20 to 35 seconds, but it is entirely likely that different animals at different temperatures had different respiration rates. It is also possible that the higher coefficients were produced by using fewer animals.

One problem experienced with trying to measure the oxygen consumption of torpid animals was that when removed from their nestboxes and put into the respirometer the animals tended to go into shivering arousal. This problem was partly alleviated by placing a piece of paper towel on the floor of the respirometer, but was a problem throughout the experiments. If the animal showed any signs of arousal (walking round, shivering, face washing) then the run was stopped and the animal returned to its cage. Since at 0, 5, and 10°C all the torpid mice tried to arouse, measurements were taken only on those animals which remained torpid for the first few minutes of acclimation, i.e. those mice which were presumably reacting to their body temperatures dropping and not to the stress of being handled when taken out of their nestboxes. This was the reason for so few results at 5°C, as after many attempts only one animal remained torpid long enough to take any measurements. Although all the animals tried to arouse at 0°C, only some were able to raise their body temperatures to above 25°C.

From table 2.4 it can be seen that at 0, 5, and 10°C there is no significant difference between the oxygen consumption of the torpid and euthermic animals. At 15, 20, and 25°C there is a strong significant difference, while at 30°C there is a possibly significant difference and at 35°C the value is again significant.

This seems to indicate that there is a metabolic saving for the animals to be torpid at 15, 20, 25, 30, and 35°C but not at other temperatures. The difference at 30°C is obviously due to a larger range of measured rates in the torpid animals than in the euthermic ones. This may be caused by apnoea or by using fewer torpid animals, but it seems unlikely to be a true difference. The difference at 35°C may be that the euthermic animals were more active when first put into the

respirometer and thus built up a heat load which they could only eliminate by using energetically expensive methods, while the torpid animals remained immobile and so did not have this problem.

The metabolic saving for an animal to be torpid at 25°C is thus 0.45 ml $O_2$/g.hr, or 43% of the euthermic value. The metabolic saving for an animal to be torpid at 20°C would be 1.40 ml $O_2$/g.hr or 69%, and the saving for an animal to be torpid at 15°C would be 1.39 ml $O_2$/g.hr or 57%. These figures show an appreciable energetic saving in being torpid at temperatures between 15 and 30°C, below which it becomes dangerously close to the lower critical temperature, to around 30°C when they approach the upper critical temperature.

In the presence of *ad lib.* food and water and at fairly high (20 to 25°C) ambient temperatures the *S.pratensis* were often found torpid outside their nestboxes, mostly curled up upright but sometimes on their sides. This seems to indicate that torpor may be a necessary physiological phenomenon for these animals since they were obviously selecting for cooler ambient temperatures which may make it easier to become torpid, thereby saving energy for the animals (Hudson 1978).

Not only does there seem to be no metabolic advantage in being torpid when the ambient temperature goes below 15°C but it seems the animals cannot survive these temperatures unless euthermic. On two occasions when the control mechanisms in the constant temperature rooms in which the animals were kept broke down and the room temperatures plummeted (once to minus 5°C and once to 7°C) only, and all of, the torpid animals died.

This lower critical temperature is corroborated by Petter (1966) who reports that *S.opimus* died when left overnight at 12°C. All his animals were torpid every day, probably due to inadequate levels of protein in their diet of apple alone; Montoya & Ambid (1978) reported torpor in the garden dormouse *Eliomys quercinus* caused by a lack of protein on a wholly apple diet.

It thus seems that *S.pratensis* and *S.opimus* show the typical "critical body temperature" response as discussed by Hudson (1978). He attributes this to a loss of adequate membrane function, particularly in the heart, as demonstrated by $K^+$ ion loss.

Hudson (1978) discusses shallow, daily torpor in many species of small mammals. A point he makes is that these mammals in general do not assume the horizontal curled position of the 'true hibernator' but instead tend to remain upright. The *Steatomys* in this study, however, were found torpid in all positions from curled up upright, to flat on their sides, to curled up on their

sides. Whether they were curled up or not seemed to depend on ambient temperature but it did not seem to make much difference to them whether they were on their sides or upright. When two animals were found torpid in a nestbox together they were most often lying with one on its side partly on its back and one lying across the others stomach. This was presumably to try and combine the two bodies so that both created as near a sphere as possible and so conserve their body heat (see chapter 3).

### 2.5 Experimental inaccuracies

Some experimental inaccuracies which may have influenced the results but which were unavoidable under the circumstances were:

The effect of the mixing of gases in the respirometer was shown to be minor since calibration with a standard gas showed a 97% accuracy.

Although the barometric pressure was measured before and after each run and the results adjusted to STP, humidity build-up in the respirometer (more of a problem at lower temperatures) may have affected the partial pressures. The air was dried before being taken through the analyser but in spite of this there may have been a slight build-up of water on the analyser sensor. A more accurate way of allowing for this humidity would have been to measure for evaporative water loss, but this was not practical while using short time closures, and it was also felt that the dry air which would have had to be pumped into the system might have unnecessarily stressed the animals.

In retrospect the respirometer should have been smaller. This would have minimised the problems of mixing and would also have reduced the time taken for the oxygen level in the respirometer to stabilise.

The Beckman OM-14 analyser used had a very slight drift of about 0.01% per minute after a one hour warm-up. This was well within the limits set by the manufacturers but had to be considered.

## 2.6 Ecological implications of these results

Since no fieldwork was done it is only possible to surmise what temperatures *Steatomys* in the field are subjected to.

As mentioned in chapter 1 there is some divergence of opinion on how deep and complex fat mice burrows are. Sclater (1901) and Smithers (1975) say that they have short burrows with a single entrance while Smithers (1971) reports the burrows to be around 200 mm deep and Roberts (1923), Hanney (1965), Vesey-Fitzgerald (1966), Kingdon (1974), and Genest-Villard (1979) say their burrows are 0.5 to 2 m deep with a nest of shredded grass and more than one entrance. This may be a problem of confusing the burrows of different species as it has been stated (Ansell 1960) that the different species dig different burrows. It can probably be taken that *S.pratensis* builds complex and fairly deep burrows extending generally 500 mm below the surface within which they also have shredded grass nests.

Above ground temperatures in the Cathedral Peak area (about 1 km from where the original animals of this colony were trapped) range from 8.4 to 28.4°C in summer and -0.8 to 19.7°C in winter with extremes of -4.5 and 32.0°C reported over a 14 year period (Weather Bureau, 1986).

In a study on rodent moles, Nanni (unpubl. MSc thesis, University of Natal, Pietermaritzburg) found that with very similar above-ground temperatures the soil temperature at 320 mm was almost constant around 17°C. It seems very likely then that the *S.pratensis* burrows also remain around 17°C with their nests being several degrees warmer (Hart 1971), making the microclimate for the animals around 20°C. This is in the middle of the temperature zone of 15 to 25°C where the animals can save energy by being torpid.

The nest and burrow system could be extremely important for the animals. Tertil (1972) shows that use of a nest by *Apodemus agrarius* reduces oxygen consumption by 8 to 31% and several authors (e.g. Genest-Villard 1979) remark on how dependent on their burrows the fat mice are.

It must also be remembered that acclimation temperature can have a fairly substantial effect on the physiological abilities of the animals (Jakobsen 1981); a lower acclimatization temperature from the acclimation temperature of 20°C used in this study could well have an effect on the wild *S.pratensis*. The most likely temperature scenario of the animals in the field would be frequent short cold shocks as the animals left the burrows to forage.

Since it is so important that the burrow temperatures do not go under 15°C it would be very important for the animals to find soil deep enough to dig their burrows. This may limit their choice of habitats and be the reason that Kingdon (1974) writes that although they may be locally abundant they are apparently absent over large intervening areas.

## 2.7 Summary

In summary, euthermic *S.pratensis* have very low basal metabolic rates (36% of expected from the Kleiber equation) of 0.50 ml $O_2$/g.hr coupled with low and variable body temperatures of 30 to 34°C. In comparison *M.musculus* had expected basal metabolic rates of 2.19 ml $O_2$/g.hr.

Torpor is an efficient method of saving 43 to 69% of metabolic energy at ambient temperatures of 15 to 25°C, below which the animals have a critical temperature from which they cannot arouse, and above which torpor does not confer additional energy savings.

# Chapter 3

## Daily patterns of body temperatures, activity, oxygen consumption and energy usage

### 3.1 Introduction

Since most rodents have particular feeding patterns and predator avoidance techniques, they also tend to have well-defined circadian cycles of activity. These cycles are reflected in their body temperatures and oxygen consumption.

Circadian rhythms have been studied in numerous rodent species, many of which show patterns of daily torpor (Hudson 1978). During this daily torpor these animals let their body temperatures approach ambient and raise them for an active period, usually in the evening (Hudson 1978). The rate of temperature loss and gain, the timing of this, and the limits of thermoregulation during torpor are highly specific to the taxon concerned.

Except for Petter's (1966) work on *S.opimus* and Hanney's (1965) on *S.pratensis* very little has been published on circadian rhythms in any *Steatomys* species. Since both these authors deal exclusively with torpid animals, although Hanney implies that not all his specimens became torpid, there has been no published work on circadian rhythms in euthermic *Steatomys*. Most authors (e.g. Ansell 1960; Vesey-Fitzgerald 1966; Genest-Villard 1979) refer to the genus as nocturnal but little more is known about their cycles either in the wild or in the laboratory. Several authors (e.g. de Graaff 1981) remark that they dug members of this genus out of their burrows while torpid but do not give the time of day this occurred. It can probably be assumed, however, that this was during daylight hours.

Petter (1966) writes that his animals were active for a short (one hour) period in the early evening, during which time their temperatures ranged from 33 to 34.6°C. These animals were torpid every day with body temperatures a few degrees above that of the holding room. These body temperatures started to rise at the end of the afternoon, reached a maximum while the animals were active, and then declined until they were just above ambient by about 03h00 or 04h00.

Hanney (1965) records *S.pratensis* body temperatures of 19.4 to 20.6°C during the daytime (ambient 18.3 to 19.4°C), rising to 30.5°C by 06h30. He also remarks that some of the wild

animals remained in their burrows for 9 to 10 days, but obviously could not say whether they were torpid for this whole time.

There were several problems associated with the energetic measurements made in this study which were known before experiments began. First, the measurement of average daily metabolic rates (ADMR) in the laboratory is not really applicable to a field situation where the animals would meet a range of environmental temperatures. However, it does give an estimate of the amount of energy needed by a fat mouse for a day, and it can be used to compare the metabolic requirements of this animal to any other. Second, the drift on the oxygen analyser (probably caused by water vapour build-up) was not known (see chapter 2), and was difficult to quantify (but was small), as was the problem of the rate of mixing of the air in the respirometer.

The terms used to express the amount of energy needed by an animal to maintain itself for a day can be confusing unless properly defined. The conditions necessary to measure basal metabolic rate have already been discussed in chapter 2. Average daily metabolic rate or ADMR is sometimes used to denote the amount of energy an animal would use during a day if it stayed in the same conditions as necessary for BMR, i.e. BMR (in ml $O_2$/g.hour) x 24 (Lindstedt & Boyce 1985) and it is sometimes used to denote the amount of energy an animal would use per day, which thus includes basal metabolism, locomotor activity metabolism, and possibly thermoregulation, pregnancy, or lactation (Randolph 1980). For this reason some authors have called the latter daily energy budget, DEB (Scheck & Fleharty 1979). It is this concept which is studied here although it will be referred to as the ADMR.

Laboratory measurements of ADMR can be done in several ways. The oxygen consumption of the animal can be measured for a day and translated into calories or joules used per day or expressed in ml $O_2$ used per g.hr. It can also be calculated by feeding the animal a particular amount of food and measuring the calorific content of the leftover food, faeces, and urine and taking the difference to be the amount of energy that animal needed to maintain it's energy balance for that day. Some authors have included in this measurement the amount of energy needed for pregnancy, lactation, or fat gain but in this case none of the animals was pregnant, lactating, or gained or lost more than 2 g mass (5% of body weight) per day.

## 3.2 Materials and Methods

### 3.2.1 Measuring system

A Rockwell AIM 65 microcomputer was connected to a customised data collection system to measure simultaneously oxygen consumption from the Beckman analyser, body temperature from an implanted mini-mitter, and activity from a monitor which worked on the basis of changing electrostatic fields when the mouse moved across it.

The mouse was housed in a plastic (3 000 ml) respirometer 65 mm x 244 mm x 190 mm. Excess food was placed each day in the respirometer itself and water was provided via the spout of a water bottle placed through a hole in the side of the respirometer. Air flow was 60 ml/minute. The air flow had not been intended to be so low but only after the results were analysed was it found that the flow regulator was reading incorrectly.

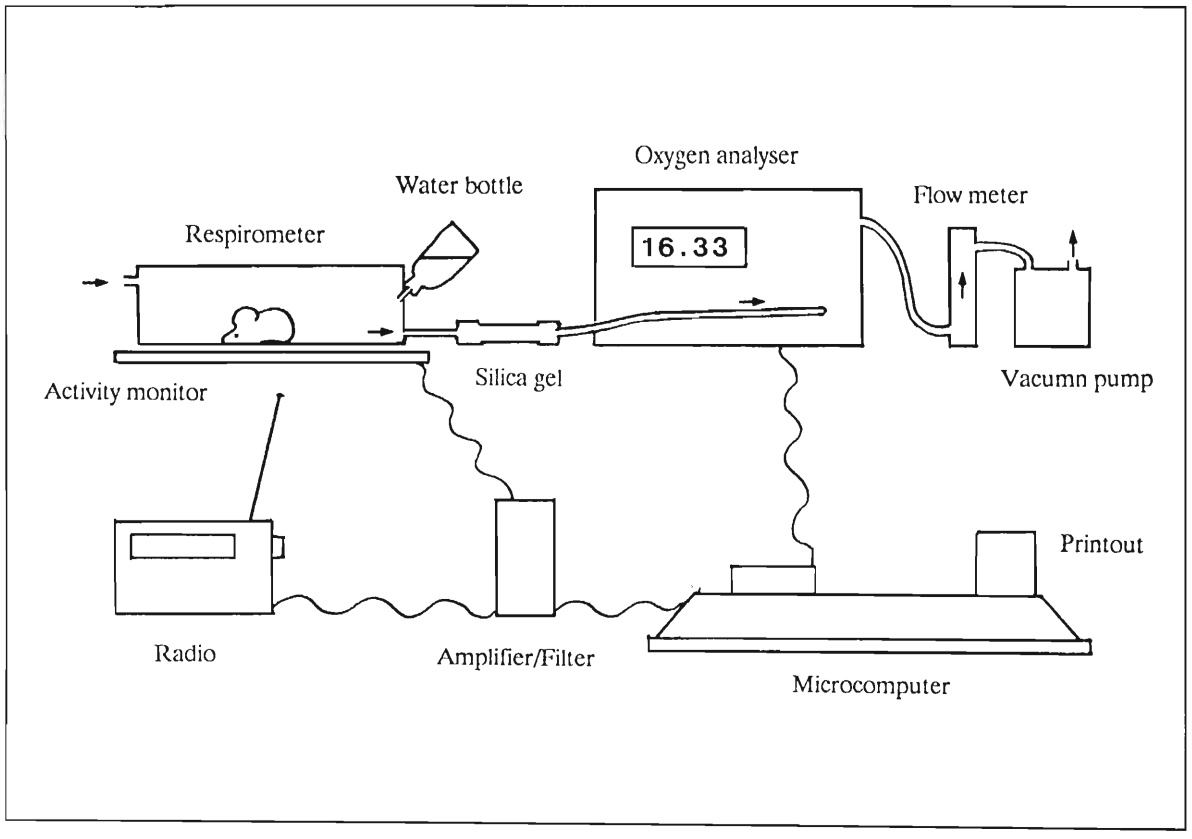


**Figure 3.1** Diagram of the system used to measure ADMR.
(See Appendix B for more detail.)

### 3.2.2 Mini-mitter type and implantation

Temperature sensitive radio transmitters "Mini-mitters" (Model X from the Mini-Mitter Co, Indianapolis) were assembled and the capsule coated with a 50:50 mixture of paraffin wax to beeswax. The low-level radio signals emitted from these transmitters were received on an ordinary AM radio, the rate of clicks produced being dependent on the ambient temperature. Each mini-mitter was calibrated by placing it in water of varying temperatures (ranging from melting ice to 40°C) and a calibration curve drawn up of the number of clicks emitted per minute at every two degrees centigrade.

Seven male *Steatomys pratensis* were anaesthetised (see chapter 5 for description of anaesthetic dosages and procedures) and a small (10 mm) cut was made in the skin and the muscle layer of the abdomen about halfway between the end of the rib cage and the penis. The mini-mitter was soaked in 100% ethanol to disinfect it and was slipped into the peritoneal cavity. The cut was sewn with resorbable suture, painted with 1% mercurochrome solution and the animal was left in a cage on its own for twenty-four hours to recover. Only one mini-mitter failed to work after implantation and all animals made a good recovery from the operation, none of them showing any signs of infection or irritation from the wounds. The mice were left for at least three days between implantation and the start of measurements.

### 3.2.3 Measuring Procedures

Experiments were conducted at two temperatures: the thermoneutral zone (30°C) and 20°C. Although it would undoubtedly have given some interesting results, no runs were made at 10°C as it was thought that this temperature would have over-stressed the animals and probably would have killed the torpid ones.

No bedding was given in the respirometer at either temperature. This may have stressed the animals but ensured that there were no micro-climatic problems and also made the collection of leftover food and faeces easier.

Every day at 13h00-14h00 the animal or animals were removed from the respirometer and weighed. The respirometer was cleaned, the leftover food and the faeces being weighed and put into an oven at 70°C to dry to constant weight. The silica gel which was used to dry the air going into the analyser was changed, fresh food was weighed and put into the respirometer and the animal was replaced or changed. The water bottle was weighed every day to ascertain how much

water the animal had drunk. The animals were weighed before and after each run to check that they had not lost or gained an excessive amount of weight which would have affected the validity of the results.

The system was run without any animals for several days to act as controls, to test for the effects of the electrical interference on the activity and temperature readings, and also to provide a control for water evaporation from the water bottle and mass changes in the food caused by humidity.

Some measurements were made on animals in their Labotec cages to get an estimate of their activity patterns when they were not disturbed, and also to get a trace of their body temperatures when in their nest boxes, with and without their mates. Obviously these runs could not include measurements of oxygen or food consumption.

Since it was realised at the end of these measurements that very few data had been collected on torpid animals, one series of measurements was taken on a naturally torpid animal (which did not have an implanted mini-mitter) to record activity patterns. Another series of measurements was taken on an animal which was fed less than its usual requirement of food to force it to become torpid. Unfortunately these experiments were terminated when the temperature in the constant temperature room dropped to 7°C owing to an equipment failure and both torpid animals died.

### 3.2.4 Analysis method

#### Computer runs

The results were transferred from the Rockwell printouts onto a spreadsheet programme (SuperCalc 4) for analysis.

Although the original readout from the Rockwell computer gave both maximum and minimum values of mini-mitter counts per minute over the ten minute periods measured, it was found in practice that only one or the other gave accurate readings. This depended on how sensitively the machine was set as it also picked up interference from the constant temperature room itself. The accuracy of the readings was determined by a manual count of the blips produced by the mini-mitter and comparing this to that counted by the machine.

The results from all the euthermic animals in the particular category studied were analysed to give a mean body temperature for each ten minute time period. Since the intention of this calculation was to show trends of body temperatures the data were smoothed using a three point running average and these results were then plotted.

After examination of the results from the torpid animals it was seen that these animals went into and aroused from torpor at varying times of the day. This rendered simple averaging of the results ineffective and since very few animals were measured while torpid these results were plotted separately.

Activity was ascertained by taking the readout from the machine, which was a measure of the difference between the lowest and the highest rate of activity for each ten minute period, and comparing this to the maximum readout obtained from the control run (no animal, and therefore due to electrical interference in the constant temperature room). Anything above this base reading was considered activity of the animal and was given a positive recording, anything under being given a negative or zero reading. The positive marks were then summed and averaged to give an estimation of the number of times that the animal was active in each time period.

After analysis of the results it was found that some animals had very low levels of activity which were difficult to separate from base levels of interference. For this reason the results were clumped together into three groups: euthermic animals at 20°C, torpid animals at 20°C, and animals at 30°C.

The results from the oxygen analyser were converted into ml $O_2$ consumed by the animal per gram hour, adjusted to STP and averaged. By using a conversion factor, analyser drift was accounted for and the results were plotted.

**Food and faeces**

Food and faeces were oven dried at 70°C, desiccated and weighed. No attempt was made to separate the leftover food from the faeces and urine as the extremely low weight of the dried faeces and urine (< 0.01 g in one case) compared to the mass of the leftover food (3.92 to 22.35 g) made the calculated difference in calorific units negligible. Kilojoules consumed by the mice were calculated by estimating the mass loss between the food put into the respirometer (calculated to dry mass) and the dry mass of the food, faeces, and urine taken out. The mass was then multiplied by the calorific value of the dried food (obtained from bomb calorimetry) to give

the total kilojoules consumed.

### 3.3 Results

The animals lost an average of 0.5 to 0.9 g on the first day or two of the runs at 20°C, but these losses were well within reasonable limits and were thought to be the animals adjusting to the lack of bedding material. These weight losses were not noticeable after the first two days and did not occur at 30°C.

The animals from which results were obtained were designated as torpid, euthermic, torpid to euthermic, or euthermic to torpid, depending on the body temperatures of the animals on consecutive days. The latter two groupings were not included in the final results as they were the same as the second day, i.e. the torpid to euthermic animals had a similar pattern to the animals which were euthermic on both days, and the euthermic to torpid patterns were the same as the animals which were torpid on both days. Although not definitive this does seem to show that there is no residual effect of the animal having been torpid or euthermic the previous day.

#### 3.3.1 Body temperatures

As a general pattern all the animals tested showed similar changes in body temperature over each 24 hour run. The body temperatures rose slowly during the afternoon until they reached a high point around the time that the lights were switched off in the constant temperature room. They remained high for a varying period of time before declining to a low level in the early to late morning hours, after which they gradually started increasing again.

**Table 3.1** Maximum and minimum temperatures of euthermic *Steatomys pratensis*

| Ta | With mate | n | resp/cage | Tmin | Tmax |
|---|---|---|---|---|---|
| 20°C | no | 22 | resp | 31.34 | 33.21 |
| 20°C | yes | 8 | resp | 31.18 | 34.19 |
| 20°C | no | 11 | cage | 32.33 | 35.00 |
| 20°C | yes | 8 | cage | 31.57 | 34.26 |
| 30°C | no | 24 | resp | 32.64 | 34.48 |
| 30°C | yes | 10 | resp | 32.55 | 34.36 |

From table 3.1 and figure 3.2 it can be seen that the body temperatures of all the fat mice in these regimes were essentially similar.

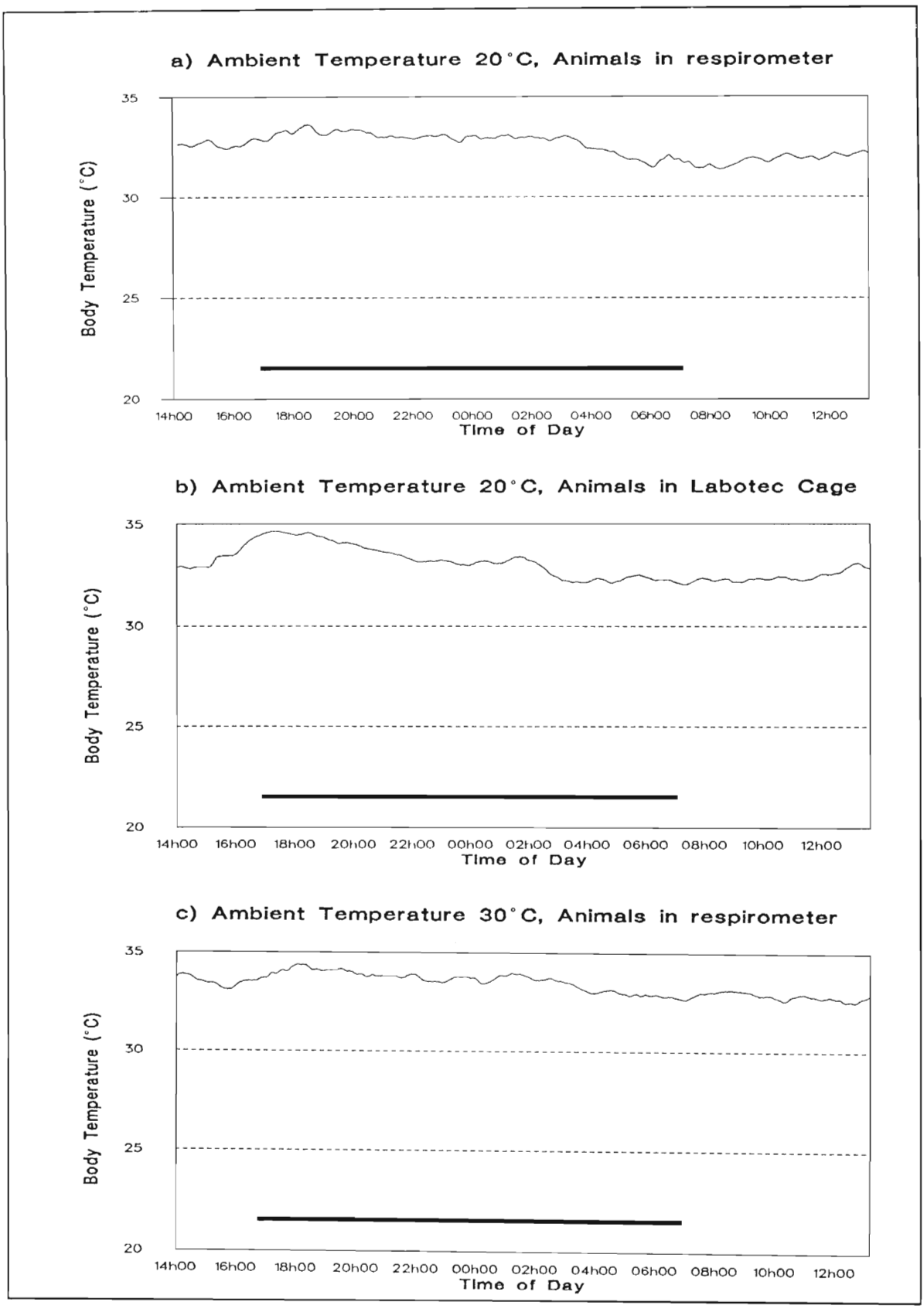


**Figure 3.2** Body temperatures of euthermic *S.pratensis*.
(All graphs are plotted on the same scale for comparison with figure 3.3.)
(Dark bar indicates duration of scotophase.)

Body temperatures of the euthermic animals ranged from 29.5 to 35°C, although measurement interference from the constant temperature room equipment may have shown the results to be slightly higher than they actually were.

**Table 3.2** Entrance to, and arousal from, torpor in *S.pratensis*.
(Ambient temperature was 20°C throughout.)

| Run | Mate | resp/ cage | Time of entrance | Time taken to drop Tb | hrs in t | Time of arousal | Time taken raise Tb |
|---|---|---|---|---|---|---|---|
| 269 | no | resp | 03h41 | 3.0 hrs | - | - | - |
| 270 | no | resp | 00h51 | 2.5 hrs | > 9 | - | - |
| 327 | no | resp | 03h01 | 1.8 hrs | 3.7 | 08h31 | 1.3 hrs |
| 271 | yes | resp | 00h31 | 3.3 hrs | 8.3 | 12h11 | - |
| 272 | yes | resp | 04h11 | 2.3 hrs | 4.7 | 11h11 | - |
| 273 | yes | resp | 09h11 | 1.5 hrs | - | - | - |
| 10.2 | no | cage | 02h01 | 7.5 hrs | 0.7 | 10h11 | 1.2 hrs |
| 13.2 | no | cage | 07h01 | 3.8 hrs | 2.0 | 12h51 | 0.7 hrs |

In table 3.2 it can be seen that entrance to and arousal from torpor is highly variable. The earliest time that these animals started to enter torpor was 00h51 and ranged to 09h11. Times taken for the body temperature to drop to ambient ranged from 1.5 to 7.5 hours, and time spent in torpor ranged from 0.7 to over 9 hours. Arousal starts ranged from 08h31 to after 13h00 and took from 0.7 to 1.3 hours. There are few results of arousals as in most cases the trial was ended before the mouse had fully aroused from torpor.

The diurnal rhythms of the torpid mice are shown in figure 3.3.

The only starving animal in these results was run 327 (figure 3.3 (a)) which had been deprived of food the previous day. Torpor in this case was entered at a normal time (03h01), lowest body temperature was achieved fairly fast (1.8 hours), and arousal time was the earliest time recorded (08h01).

Animals in nestboxes in cages took the longest times to drop their body temperatures to ambient (3.8 to 7.5 hours) and spent the shortest time with their body temperatures around ambient (0.7 to 2 hours).

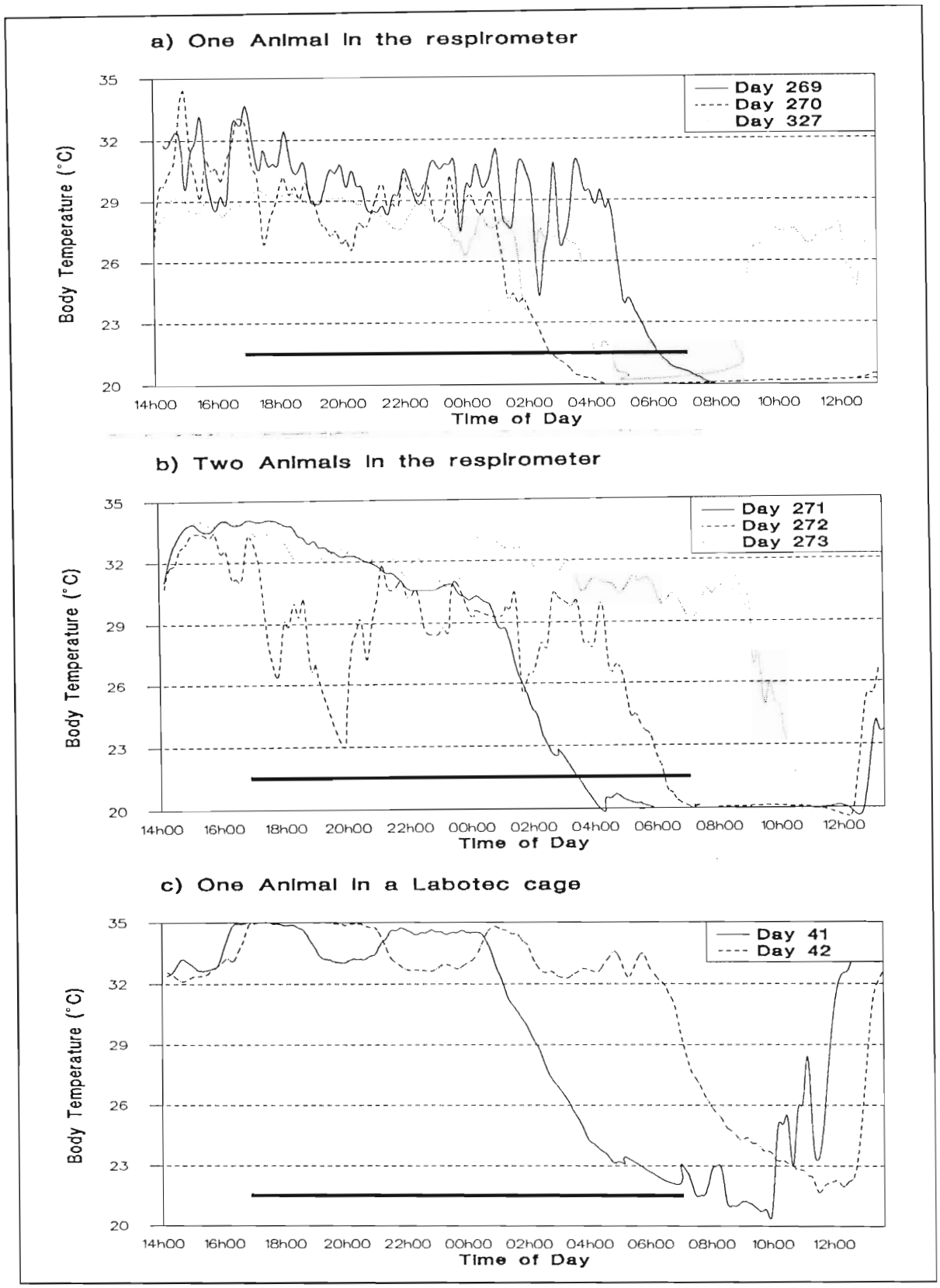


**Figure 3.3** Body temperatures of torpid *Steatomys pratensis*.
(Ambient temperature 20°C throughout.)
(All graphs plotted on the same scale as figure 3.2 for comparison.)
(Dark bar indicates duration of scotophase.)

### 3.3.2 Activity

The results from the activity pattern measurements are plotted on figure 3.4.

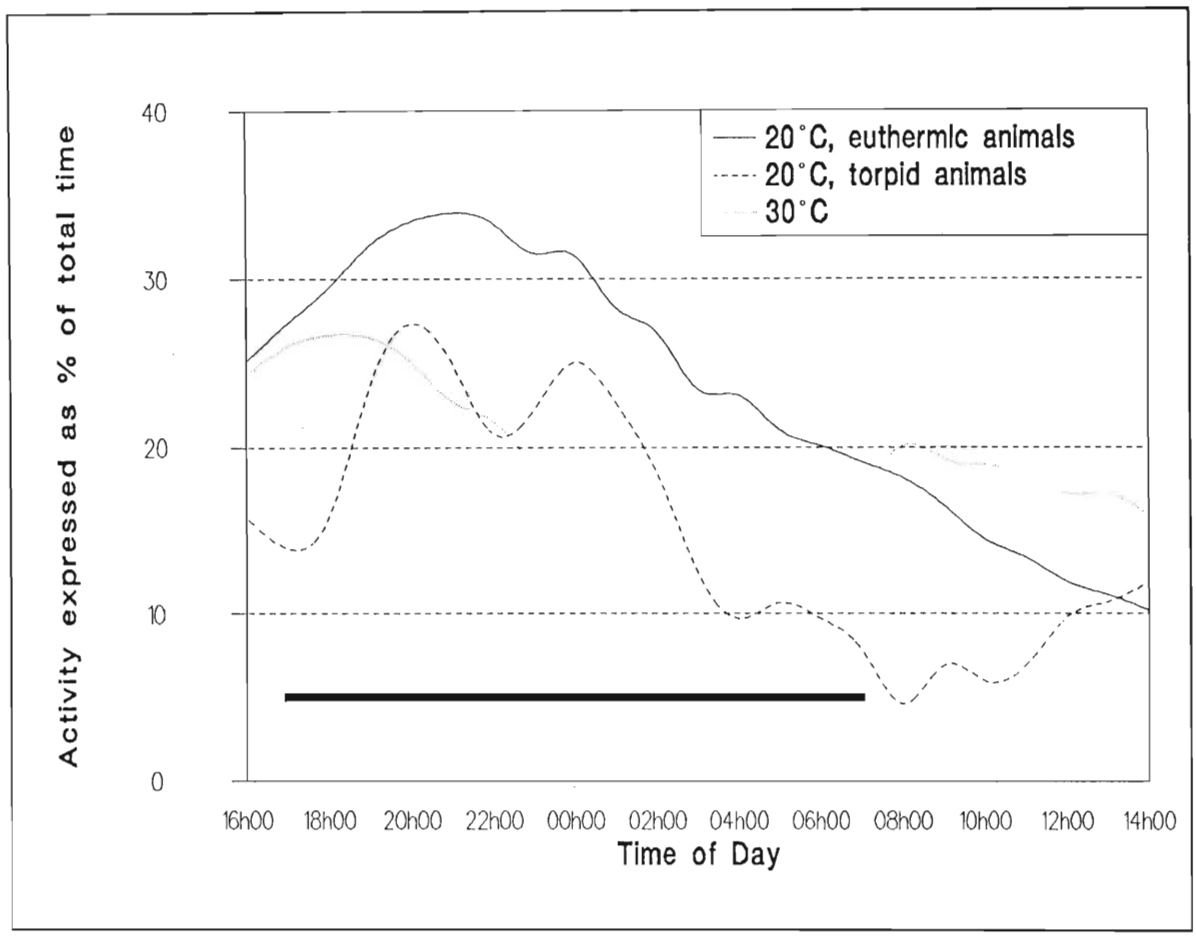


**Figure 3.4** Activity patterns of euthermic and torpid *Steatomys pratensis* at 20 and 30°C ambient temperatures.
(Dark bar indicates duration of scotophase)

In general, all the animals were active in the first part of the evening, from around 16h00 or 17h00 until the early hours of the morning, 02h00 to 03h00.

The euthermic animals were the most active, the peak of activity being 2 to 4 hours after darkness. The animals which became torpid showed a peak of activity slightly earlier (1 to 2 hours after dark) and the animals at 30°C even earlier (0 to 1 hours after dark). The activity of the torpid animals declined very rapidly after 01h00 while that of the euthermic animals at both temperatures did not.

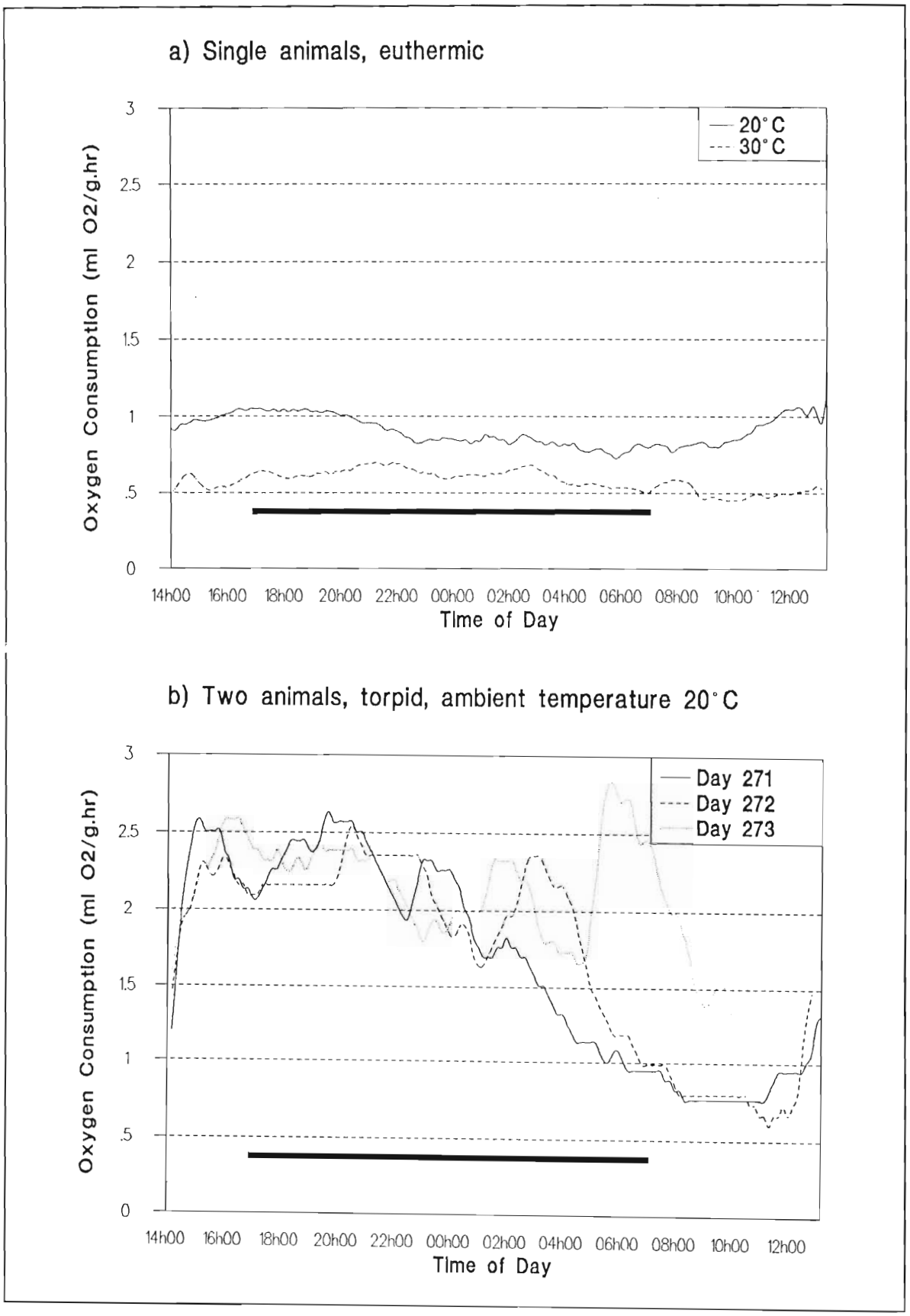


**Figure 3.5** Oxygen consumption of single euthermic and torpid pairs of *Steatomys pratensis*.
(Dark bar indicates duration of scotophase.)

### 3.3.3 Oxygen consumption

Because of the low turnover rates in the respirometer the results were combined into euthermic animals at 20 and 30°C (figure 3.5 (a)) and torpid animals at 20°C (figure 3.5 (b)). The results do show a trend in oxygen consumption in that all the animals measured had higher rates of oxygen consumption when the animals were active and had higher body temperatures (compare with figures 3.2, 3.3, and 3.4).

Total oxygen consumed by the animals at 20 and 30°C was 133.7 ml $O_2$/g.day and 84.7 ml $O_2$/g.day respectively.

### 3.3.4 Daily metabolic rates from food and faeces determination

Table 3.3 gives the metabolic rates of the animals per day determined by the calories consumed.

**Table 3.3** Daily energy consumed by *S.pratensis.*

(Units in kJ/day)

| Temp. | No. mice in resp. | euth./t | n | kJ used (mean) | kJ/ani | S.E |
|---|---|---|---|---|---|---|
| 20°C | 1 | euth. | 20 | 41.42 | 41.42 | 2.30 |
| 20°C | 2 | euth. | 8 | 68.00 | 34.00 | 3.72 |
| 20°C | 1 | torpid | 2 | 18.22 | 18.22 | 2.16 |
| 20°C | 2 | torpid | 2 | 44.64 | 22.32 | 9.58 |
| 30°C | 1 | n/a | 24 | 30.90 | 30.90 | 1.76 |
| 30°C | 2 | n/a | 10 | 56.97 | 28.48 | 2.57 |

The results of all the runs which included one animal only are significantly different from each other: ANOVA, $P < 10^{-6}$. Energy expenditure per animal per day ranged from 16.06 kJ (torpid at 20°C) to 60.10 kJ (euthermic at 20°C). The animals generally used least energy when torpid at 20°C, then when at 30°C, then huddling with a mate at 20°C, and most energy was expended by a lone animal being euthermic at 20°C.

**Table 3.4** Water consumed by *S.pratensis*.

(Units in ml/day)

| Ta | No. mice in resp. | euth/t | n | ml $H_2O$ (mean) | S.E | ml $H_2O$/g |
|---|---|---|---|---|---|---|
| 20°C | 1 | euth | 18 | 4.36 | 1.03 | 0.12 |
| 20°C | 2 | euth | 8 | 6.86 | 2.43 | 0.09 |
| 20°C | 2 | torpid | 2 | 1.60 | 1.13 | 0.02 |
| 20°C | 1 | no food | 2 | 2.17 | 1.54 | 0.06 |
| 30°C | 1 | - | 21 | 3.95 | 0.86 | 0.11 |
| 30°C | 2 | - | 10 | 5.47 | 1.73 | 0.08 |

Water consumption rates did not include the water content of the food but this was extremely small: the maximum consumed was 0.01 ml/g of animal. Water consumption of these animals ranged from a mean of 0.80 ml/animal.day (20°C, 2 animals, both torpid) to 4.36 ml/animal.day (20°C, 1 animal, euthermic). Water consumption was positively correlated with the amount of food consumed at 20°C (Pearson's correlation coefficient, $P < 0.01$) but not with the amount of food consumed at 30°C ($P > 0.50$).

There is a significant difference between the torpid and euthermic animals (t-test, $P < 0.05$).

Using Hudson's (1962) equation from Glenn (1970) to determine water consumption of $I = 0.24\ W^{-0.12}$ where I is the expected water consumption/g.day and W is the mass of the animal, these animals would be expected to use 0.16 ml $H_2O$/g.day or around 5.68 ml $H_2O$/animal.day. This is within the range of the results found here.

## 3.4 Discussion

### 3.4.1 Body temperatures

Most eutherian mammals show a slight (1 to 2°C) variation in body temperature on a circadian rhythm, the higher body temperature being found at that time of day when the animal is normally active (Hart 1971). This was found in the euthermic *S.pratensis* although the actual variation (2.32°C) was slightly larger than expected but may have been affected by activity of the animals. As noted in chapter 2 the body temperatures found here are very low compared to most eutherian mammals. This is discussed in detail in chapter 4, with the effects of ambient temperature on body temperature.

At 20°C there is essentially no difference in the minimum body temperatures of the animals with and without a mate in the respirometer, or with a mate in a cage. The mean minimum body temperature of the mouse in a cage on it's own is higher than expected, but the maximum body temperature from this run is higher as well. Since most of these runs were done on one animal (m48B1) which had a slightly higher body temperature than normal (resting diurnal body temperature of 34.1 to 34.4°C) this would have affected the mean result. Huddling with another animal can sometimes cause mammals to have higher resting body temperatures (Andrews *et al.* 1987) and sometimes does not change the body temperatures of the animals but reduces their combined oxygen consumption (Hill 1983). The animals tested here were always found huddling when the ambient temperatures were under the thermoneutral zone but did not have body temperatures higher than normal. This will be discussed further in section 3.4.4.

Minimum body temperatures of animals at 30°C are higher than those at 20°C. This was not surprising as the high ambient temperature would tend to keep the animals body temperatures slightly above normal. A certain amount of heat would be produced by activity as the animals fed and explored the respirometer.

A point to be made here is that the rectal body temperatures measured mid-morning (chapters 4 and 8) are highly indicative of the actual deep body temperatures of the animals. However, in cases where the animal had been in torpor earlier in the day and had aroused early, the rectal body temperatures may have been measured as euthermic and the animals incorrectly assigned to a euthermic status while in actual fact they had been torpid. Since nearly all the animals measured only aroused from torpor after midday it is thought that this is a minor source of error.

Genest-Villard (1979) gives the body temperature of freshly captured (presumably during the day) *S.opimus* as from 31.3 to 38.3°C. This variability is interpreted according to the amount of energy the animal exerted to try and escape. This shows that while the lower body temperature is perfectly normal according to this study, these animals can and do become hyperthermic upon over exertion. The thermal conductances and body temperatures of these animals is discussed in chapter 4.

In his study of *S.krebsii*, Taylor (1984) found that while the body temperatures of these animals were much higher than those of *S.pratensis*, there was a similar circadian cycle of body temperature with those of the *S.krebsii* being highest during their nocturnal period of activity and dropping by about 1.5°C during the day. Oxygen consumption in these animals followed a similar pattern of being highest at night and lower during the day.

The body temperature graph of the torpid animals is essentially similar to that of the euthermic animals except that the degree of temperature depression during the day is much greater. It has been suggested by Walker *et al.* (1981) and discussed by Wang & Wolowyk (1988) that there is a continuum in energy conservation between slow wave sleep, torpor, and hibernation by which torpor is simply an extension of the drop in body temperature that every mammal experiences when asleep. The data here would tend to support such a view. Hudson's (1978) view is that every young altricial mammal has the ability to re-warm from a cold body temperature, and the retention of this ability into adulthood would manifest itself as torpor. Since the fat mice are born altricially this view would be quite compatible with the development of torpor in *S.pratensis*.

Circadian body temperatures found in the torpid mice show great similarity to those published both by Petter (1966) and Hanney (1965). Both sets of animals studied by them showed body temperatures just above ambient during the daytime, rising to an evening temperature of 30.5°C (Hanney) and 33 to 34.6°C (Petter). It is possible that Hanney's animals may have had even higher body temperatures later in the evening.

Different authors have defined the amount of time spent in torpor in different ways. Probably one of the best methods is that used by Daan (1973) who considers that the onset and end of a bout is the first temperature change leading to torpor and normothermia respectively. Since by it's definition daily torpor must last less than a day and be of such a duration that the animal has time to forage for food, torpor could not last more than about 15 hours, leaving one hour for arousal and a few for foraging.

Unlike the results discussed by Tucker (1966) for *Perognathus californicus*, time spent in torpor varied greatly in the cases measured here. It is possible that had more animals been studied the variation would not have been found to be so great since this is probably a representative sample of the least and most time these animals can spend in torpor. Although time spent in torpor has been related in some species to the amount of food available (Tucker 1966; Hudson 1978; Hudson & Scott 1979) this is not an issue in this case where all the animals were fed *ad lib.*

A difference between the results found here and those of Petter (1966) is the amount of time spent in torpor by the fat mice. Petter's animals spent from 03h00 or 04h00 until the end of the next afternoon (probably 14 hours per day) in torpor while the animals studied here generally spent less time. However, whilst the times of entrance into torpor varied considerably in this study, they often (6 out of 8 cases) started to enter torpor around 03h00 to 04h00, the same

time as Petter's mice (1966). As will be discussed later (chapter 7) Petter's animals may have shown a permanent torpor due to lack of protein in their diets (Montoya *et al.* 1979) which may have contributed to the generally longer times spent in torpor.

Maximum time spent in daily torpor by the *Peromyscus* species reviewed by Hudson (1978) was 11.3 hours. *Peromyscus leucopus* as studied by Gartner *et al.* (1973) spent a minimum of 40 minutes a day in torpor but usually longer (up to 6.25 hours). These animals usually entered torpor later in the day than the *S.pratensis*. Daan (1973) found his dormice all started torpor between midnight and noon and aroused a few hours before lights out in the evening. He also found that the longer the torpid bout, the earlier in the day both onset and end of torpor occurred.

In fat mice, the length of torpor was generally shorter than that found by other authors and in other species. Further, these results could have been affected by the *ad lib.* food and the warmer ambient temperature at which they were kept.

In this study the time spent from the onset of torpor to the start of arousal was not correlated with time of start.

Time taken for the animals to drop their body temperatures to around ambient varied greatly depending on the amount of insulation available to them. There was no difference in the cooling rates of those animals in the respirometer with or without a mate, but the cooling rates of those animals in a nest box with bedding was significantly different ($P < 0.05$). Cooling rates of the animals without any bedding or nest box varied from 1.5 to 3.3 hours and is discussed in chapter 4. Cooling rates of the animals with a nest box ranged from 3.8 to 7.5 hours.

It can thus be seen that the nest must be extremely important to the animal to slow the rate of heat loss. Daan (1973) says the temperature decrease in the nest boxes of *Eliomys quercinus* can lag 0-3 hours behind the point where the animals have started to enter torpor. However, Vogt & Lynch (1982) report no difference in the time their *P.leucopus* took to drop their body temperatures within or without a nest except at extremely low temperatures of 1°C.

In certain circumstances the nest may also be extremely important to slow the rate of heat gain of the animals. According to Genest-Villard (1979) the nests of *S.opimus* may serve to reduce the temperature to which the animals are exposed by almost 5°C, from 32 to 27.1°C. In summer in some of the areas in which these animals live this may reduce the temperature from above the thermoneutral zone, thereby saving the animal energetically expensive methods of heat loss.

Similar patterns of torpor are probably to be found in wild *S.pratensis* as well. The patterns of torpor found here are very similar to those found in wild *Peromyscus leucopus* by Vogt *et al.* (1983). These animals on two occasions entered torpor at around 05h00 and were fully aroused by 15h00. These patterns were also found in captive animals by Gaertner *et al.* (1973) and Vogt *et al.* (1983) conclude that laboratory measurements of daily torpor do accurately reflect the free-ranging animals. In a similar study Wang (1973) concluded that laboratory studies on the Richardson's ground squirrel *Spermophilus richardsoni* were comparable to the natural cycles.

Arousal rates, the time taken for the animal to raise it's body temperature from around ambient to normal euthermic levels are discussed in chapter 5.

All animals measured here showed only a daily torpor but the possibility exists that they may be able to go into torpor for longer than a few hours. On only one occasion was any animal from this colony found torpid in the evening, the usual time of activity of these animals, but they were very rarely taken out of their nest boxes at these times so any torpid animals may have escaped notice. It is also possible that torpor lasting for longer than 24 hours is a reaction to a food shortage and since all these animals were fed *ad lib.* this would not easily be identified. The series of experiments designed to test this theory had to be abandoned when the temperature in the "constant" temperature room went down to 7°C and killed the torpid animals.

Hanney (1965) marked nine holes of *S.pratensis* in August and found that while four animals left every night four remained in their burrows for 9 to 10 days. Genest-Villard (1979) says that since all her animals were captured with a full stomach they must be active every day, although in some areas they could show a torpor of longer duration. Smithers (1983) says that during spells of very cold weather members of this genus remain underground and possibly stay in torpor for two to three weeks at a time.

It is not known if the animals which stay underground actually remain torpid. It is known that they hoard food (Hanney 1965) so it is possible that they may arouse from torpor to eat. Chew *et al.* (1965) say that although the *Perognathus longimembris* (which also show a daily torpor) spend several days in their burrows during inclement weather they were not continuously torpid. Several authors (Daan 1973; French 1976) have pointed out that torpor lasting more than a day is generally in multiples of 24 hours, i.e. the animals come out of torpor in time for their normal period of activity, no matter how many days they have been torpid.

### 3.4.2 Activity

The animals were active in the first part of the evening with the activity tailing off into the early hours of the morning. The euthermic animals at 20°C showed the most amount of activity.

Torpid animals showed a similar amount of activity to euthermic ones in the evening hours. This was unexpected as a pilot study had shown that torpid animals generally had earlier and reduced levels of activity (Richardson 1980). Chew *et al.* (1965) found that torpid pocket mice showed earlier activity patterns than the euthermic ones. Unlike the euthermic *S.pratensis* the activity of the torpid animals declined to almost no movement at all for the rest of the day. This was hardly surprising since torpid animals would be unlikely to move around. What movement there was undoubtedly related to those animals which came out of torpor earlier than the others.

Euthermic mice were active for a much longer period of the day than had been expected from observation of the laboratory colony. They may have been influenced by the strange surroundings and had no nest or nest box to block out any strange sights or sounds.

The high amount of activity in the animals kept at 30°C is probably an artefact caused by false positive results. It might be more accurate to consider that at certain times of the day the animals must be quiescent and non-active, thus that time of day when the results are lowest would actually be zero activity, and the whole graph could be moved down to accommodate this. This would mean that in general the animals at 30°C showed very little activity, an observation born out by the observations taken during the RMR measurements (chapter 2) when the animals made very few unnecessary movements. A lower amount of activity at higher temperatures has been noticed in several other rodent species (Hart 1971; Ross 1980).

The fat mice generally showed more low level activity throughout the day at 30°C than at 20°C, and showed an earlier peak of activity; both of these may be caused by the animals being unwilling to spend a lot of time active and so building up a heat load.

Although these results are very general and cannot be taken as absolute they do show that the animals are all active at those times of night when their body temperatures are highest: 17h00 (start of the dark phase) to 23h00. Whether this causes the higher body temperatures or whether they are independent of each other is a moot point (Hart 1971; Hill 1975). Since the day length was from 07h00 to 17h00 with no twilight zone this shows that Vesey-Fitzgerald (1966) and Genest-Villard (1979) were right in labelling these species strictly nocturnal.

### 3.4.3 Oxygen consumption

Figure 3.5 shows that all the mice consumed more oxygen during the first part of the night when their body temperatures were higher and they were more active.

The total amount of oxygen the animal used at 30°C was 84.7 ml $O_2$/g.day. This was much higher than the results from either the BMR or the calorific measurements and can only be assumed to be grossly in error. The results from the animals held at 20°C were similarly far too high (133.7 ml $O_2$/g.day).

The results shown on figure 3.5 (b) are especially interesting in that two of the runs show normal torpor patterns and the third shows the effects of involuntary hypothermia. At some time during the night (unfortunately it is not known just when) the animals managed to empty the water bottle into the respirometer and soaked themselves. From the figure it can be seen that they attempted to raise their body temperatures by consuming more oxygen (probably both shivering and non-shivering thermogenesis) from 04h50, but from 05h30 this high oxygen usage declined, presumably because they were exhausted, and from figure 3.3 (b) it can be seen that from around 08h00 the body temperatures started declining as well. The animals were checked and the run terminated early but it is possible that had they been left they would have dropped into a proper torpor.

The other two runs show normal torpor patterns in that oxygen consumption patterns closely follow body temperature patterns (compare with figure 3.3). However, there is not a great rise in oxygen consumption preceding the animals arousing from torpor as has been reported for many arousing mammals (Malan 1988), both oxygen consumption and body temperature rise at about the same time. This may be caused by the fairly mild temperature at which the animals were kept, as they only had to raise their body temperatures by around 10°C to get to a euthermic state. It may also be quite a common occurrence but most authors only measure either oxygen consumption or body temperature and so could not correlate them.

It was not the purpose of this study to make any decision as to whether these animals had any endogenous daily rhythm. It can probably be assumed that they do have some sort of a rhythm but the light regime that they were kept in would have entrained any internal rhythm.

### 3.4.4 Calorific results

There have been various attempts to predict the daily metabolic rate of a mammal by quantifying basal metabolic rate, thermoregulation, activity and other metabolic costs.

Average daily metabolic rate is approximately three times basal (Karasov 1981), although Randolph (1980) found that both *Peromyscus leucopus* and *Tamias striatus* had ADMRs approximately twice the basal metabolic rates. Chappell (1980b) gives a minimum estimate of 2.9 x BMR and a maximum of 4 x depending on the time of year. Activity costs can be a major part of ADMR - Chappell (1980b) gives these as about 3 x BMR, or a third to a half ADMR in arctic mammals which have to spend proportionately more energy on thermoregulation. Meyer & Guillot (1986) give the cost of locomotion in the laboratory mouse as 3.2 x basal, feeding 2.4 x, and grooming from 1.1 to 1.8 x. SDA, the heat increment of feeding, is small in these rodents and can therefore probably be safely ignored in these approximations.

The average daily metabolic rate determined here of 30.9 kJ for *S.pratensis* at 30°C (the thermoneutral zone) would be the equivalent of 1538 ml $O_2$ consumed (McNab 1988, 1l $O_2$ = 20.09 kJ). This would mean that the animal was using an average of 1.77 ml $O_2$/g.hr, which is 3.5 x the basal rate.

At 20°C and the average daily metabolic rate of 41.42 kJ, this would mean an average of 2.37 ml $O_2$/g.hr, or 1.2 x the resting metabolic rate. Similarly the ADMR of the torpid animals is equivalent to 1.04 ml $O_2$/g.hr or 1.63 x their resting metabolic rate.

To calculate the metabolic rate of a mammal under any circumstances the following equation from Wunder (1975) can be used:

$$\mathbf{MR = aMb + Mtr + Ma}$$

where **MR** is the total metabolic rate,
**a** is a coefficient used to correct for the change in posture associated with activity,
**Mb** is the basal metabolism,
**Mtr** is the energy used for thermoregulation when the animal is under the thermoneutral zone, and
**Ma** is the metabolism due to activity.

Mtr can be calculated as: Et (in kJ/day) = $(16\ M^{0.5})*(Tb-Ta-22.5M^{0.25})$ mass in kg, T in °C, (converted units from Lindstedt & Boyce 1985).

Basal metabolic rate at 30°C for these animals was 0.50 ml $O_2$/g.hr (chapter 2) which is the equivalent of 8.75 kJ/day. ADMR at 30°C as determined here was 30.9 kJ/day. Since at 30°C the animals were not under their thermoneutral zone Mtr is zero; a is approximately 1.7 when the animals are active and 1.0 when at rest (Wunder 1975). In this study the animals were active for about 8 hours of the day (or 30% of the time), so a in this case would be 1.23. Thus at 30°C **Ma,** the metabolism due to activity, is 30.9 minus 1.23(8.75) or 20.11 kJ/day.

At 20°C, the Mtr calculated from the Lindstedt & Boyce (1985) equation would be 6.75 kJ/day. Total MR was 41.42 kJ/day. This means that Ma would be 23.88 kJ/day. This is higher than the amount of energy used for activity at 30°C but as has been seen the animals spent more time active at 20°C. The level of activity itself produces a certain level of oxygen consumption which is independent of ambient temperature (Hart 1971).

Thus in the thermoneutral zone over 65% of the expended energy of these animals was used for activity and SDA; at 20°C this level was 57%.

The difference between the estimated amount of energy used for thermoregulation from the Lindstedt & Boyce (1985) equation and the difference between the RMR at 20 and 30°C is due to the change in thermal conductance between the two temperatures (see chapter 4) and also to activity causing postural and behavioural changes (Sun & Jing 1985).

The amount of time the animals spend active per day is very important to their energy budgets. Too much activity at 30°C may lead to an excess of heat which can only be dissipated using energetically expensive means, while too much activity at low temperatures could use too much energy in thermoregulation. Wolff & Bateman (1978) define the amount of hoarding and time of foraging in *Perognathus flavus* to be vital in maintaining the correct energy balance. Since it is possible that *Steatomys pratensis* uses the same or similar technique, this theory is discussed in some detail in chapter 7.

It is thus important that each animal maximise the time spent in the nest, which is probably close to the thermoneutral zone, and restrict foraging to a minimum. Chappell (1980b) gives limits of activity of the lemmings he studied of 30% of the time in winter and 70% in summer, the latter being much higher because it included reproduction, social interactions, etc.

The optimal way that these animals have of saving energy is by becoming torpid. From table 3.3 it can be calculated that at 20°C the fat mice can save 56% of the energy necessary to survive a day by becoming torpid and 18% by huddling with a mate while euthermic. The animals did not

save quite so much energy (46% of the euthermic rate for one animal) by huddling with a mate while torpid but these latter results are not statistically different from each other.

In chapter 2 it was seen that becoming torpid at 20°C saved the animals 69% of their expended energy at rest. Since the results of being torpid at 20°C for a day show a savings of 56% whilst the animal is only torpid for a mean of 8.84 hours (from onset to start of arousal, the energetically saving times) there must be other factors coming into play. One factor is that the animals body temperatures were not as high or as constant as the euthermic animals in the hours preceding torpor, and another factor is that the torpid animals showed much less activity in total then the euthermic animals. These lower and less stable body temperatures before the animal goes into torpor have been noted in other species, e.g. *Mus musculus* (Hudson & Scott 1979).

Gebczynski *et al.* (1972) found that the torpid daily energy budget of dormice was half that of euthermic animals, while the euthermic period of the torpid animals was 9 hours. Vogt & Lynch (1972) found a saving of 5 to 47% of daily energy expenditure of *Peromyscus leucopus* by becoming torpid. Vogt & Lynch (1982) give the saving for a torpid animal as 20% less than the euthermic animals while Wang & Wolowyk (1988) state that the savings for most mammals by going torpid is from 18 to 31% depending on the depth and duration of daily torpor. Thus *S.pratensis* can save more energy than most rodents by going torpid for part of the day.

There is no negative correlation between the amount of time the fat mice spent in torpor and the amount of energy they consumed for that day. However, again there may not have been sufficient results to show any trend. French (1976) found a correlation between the amount of energy used related to the amount of time *Perognathus longimembris* spent in torpor. These animals preferred to stay euthermic, however, and would preferentially choose higher ambient temperatures to achieve this. French explains this as a means to reduce the chance of freezing during cold spells and enhance the ability to escape from predators.

It is a moot point whether the animals in this and other studies ate less because they were torpid or whether they ate less because they "wanted" to go into torpor. In contrast to those authors who found that their animals spent an appropriate length of time in torpor depending on how much food they were given (Tucker 1966; Wolff & Bateman 1978), these animals were depending on a ration of food not as it was given but as it was eaten. This indicates a regulation of food intake by the animals, but could also be a result of having less time while normothermic to eat.

Unlike other species (e.g. Townsends vole *Microtus townsendii* as studied by Andrews *et al.* 1987) two *Steatomys* sharing a cage did not have higher body temperatures when at rest than those animals on their own (see Table 3.1). However, there is a great saving of energy from two animals sharing a respirometer, indicating that although they regulate their body temperatures quite precisely they must save on the amount of energy needed for thermogenesis, probably by huddling.

Huddling is used by many small mammals in cold climates and can reduce energy expenditure by up to 30% (Hart 1971). These savings are probably due to a decrease in the volume to surface area ratio of the mice meaning a drop in the amount of energy needed for thermoregulation but a comfort factor has been invoked (Martin *et al.* 1980). In the case of the *S.pratensis* it must be due to thermoregulation as there was no significant difference between the amount of energy used by one mouse on its own in the thermoneutral zone and by one mouse while sharing with another (Table 3.3).

Huddling by *P.leucopus* decreases energy expenditure of euthermic animals by 16 to 33% (Vogt & Lynch 1982). Indeed, Glaser & Lustik (1975) claim that huddling in a nest can give the same amount of energy savings as a nest in a nest cavity (29%). Huddling, however, only saves the animals concerned energy when the ambient temperature is well below the thermoneutral zone and the animals spend most of their time in the nest (Vickery & Millar 1984). Daily torpor and huddling together gave a 58% saving in the *P.leucopus* studied by Vogt & Lynch (1982). This was higher than the 46% found in this study.

As *Steatomys pratensis* are rarely found in pairs and never more than two adults in a nest (Smithers 1971), the question arises as to why more of these animals do not huddle to conserve energy. One reason may be that since they all have nests in nest cavities (Genest-Villard 1979; Smithers 1983) and since Glaser & Lustik (1975) show that huddling saves the same amount of energy as a nest in a nest cavity, they may have no need to huddle. Since ambient temperature is probably often not very far below the thermoneutral zone (chapter 2) it is doubtful that huddling would save these animals much energy anyway. There is also the problem of oxygen depletion in the burrow to be considered (Hill 1983).

Nests on their own, however, can provide an important source of insulation for mice at low temperatures; Vogt & Lynch (1982) cite a saving of 44% in *Peromyscus leucopus*. Chappell (1980b) says that even in the arctic rodents he studied the animals can keep their nests at such a temperature that it is within their thermoneutral zone. Beck & Anthony (1971) express the view that the nest increased the effective thermoneutral zone of the animals they studied (*Microtus*

*longicaudus*) by 5°C and Tertil (1972) says that use of the nest is the most important thermoregulating mechanism in *Apodemus agrarius*. Hart (1971) gives the savings in metabolic expenditure of *Reithrodontomys* as from 17 to 24% and Vogt & Lynch (1982) claim that adding a nest and torpidity to an animals repertoire can save 74% of the daily energy.

Although the *S.pratensis* drank a considerable amount of water (ranging from 1.33 to 8.08 ml per animal per day) this was much lower than that predicted by Hudson's (1962 in Glenn 1970) equation. These rates are at the low end of the scale of 98 to 399 ml $H_2O$/kg.day as reported for (euthermic) murids by Maiga (1984). Since water turnover is inextricably linked to metabolism it can be assumed that as *S.pratensis* has such a low metabolism it would also have a low water turnover rate and the rates measured here are actually higher than expected. Torpid animals drank a lot less water than euthermic ($P < 0.029$), which would support the view that water consumption and metabolism are linked. These laboratory reared animals were on a diet with a very low water content (12%). It is possible that in the field they would be able to make up much of this from their food. Dietary moisture can have an effect on the temperature regulation of rodents (Hart 1971) but the effect of this dry diet on *S.pratensis* is not known.

Some rodents are assumed to go into torpor in response to a lack of water; MacMillen (1965) reports that *Peromyscus eremicus* go into torpor in response to lack of water but other authors have related this to the fact that rodents will stop eating when deprived of water and so will go torpid through starvation (Hudson 1978). This is discussed in chapter 7. Evaporative water loss was not studied here.

All these measurements except for those measuring two animals were conducted on adult male *S.pratensis* so it is not known if there is any difference in the amount or level of body temperature, activity, or oxygen consumption due to sex. It can probably be safely assumed that there is none as the animals showed no difference in any of the other parameters examined (chapter 2) and rodents do not usually show such sexual differences (Hart 1971).

ADMR, however, may change with age: ADMR of *Peromyscus leucopus* decreases with age (Hill 1983), and there is some evidence that the amount of torpor in *Steatomys* changes with age (chapter 8). This was unfortunately beyond the scope of this study.

### 3.5 Experimental inaccuracies

Low turnover rates and analyser drift unfortunately caused the results from the oxygen consumption to be suspect. Because of the size of the respirometer necessary to allow the animal some freedom of movement (3 l) and the low air flow (60 ml), the air in the respirometer turned over very slowly, giving the readings an extremely long time lag. This meant that the lower rates of oxygen consumption could not be accurately correlated with any activity or rise in body temperature. Analyser drift, originally thought to be a problem, turned out to be a fairly minor source of error.

Unfortunately the activity monitor and the oxygen consumption monitor were connected to a common monitoring instrument and the results from one often tended to affect the other: the results of the activity patterns readings are thus unfortunately no better than those of the oxygen consumption.

### 3.6 Ecological implications of these results

Summer and winter metabolic rates of the same animals may be completely different as has been found in several rodent species, for example in *Mus musculus* (Jakobsen 1981) and *Phodopus sungorus* (Heldmaier & Steinlechner 1981b). Daily metabolic rates were measured here in winter to compare torpid and euthermic animals.

It is not known whether any of these results can be applied to a field situation in this species but Randolph (1980) concluded that the ADMRs measured in his animals were equivalent to those in the field providing the animals had a similar level of locomotor activity, in duration and intensity if not in specific type of behaviour, and were subjected to the same temperature regime. The *Steatomys pratensis* in this study were not subjected to changes in temperatures throughout the day but their field metabolic rates could be estimated knowing their metabolic rates in different temperatures and comparing them to those temperatures found in the field.

As mentioned earlier, torpor bouts in these experiments lasted less than a day but the possibility exists that these animals can go into torpor for longer. This may be important to the wild animals in times of extreme food shortage, which is discussed in chapter 7.

As has already been pointed out in chapter 2 the *S.pratensis* must be extremely dependent on their burrows, not only for raising their micro-climate to around a temperature at which the

animals can save energy but also for protection from predators. Spending up to 12 hours a day in torpor may save a lot of energy but it leaves the animals vulnerable to attack.

### 3.7 Summary

*Steatomys pratensis* shows a circadian rhythm in body temperature with the average temperatures in the euthermic animals being from 31.1 to 32.6°C throughout the day and from 33.2 to 35.0°C throughout the night. Activity and oxygen consumption generally follow this pattern, being lower during the day and higher at night.

Torpid animals let their body temperatures drop to around ambient from the early hours of the morning and raised it again from late morning to into the afternoon. Amount of time spent in torpor was highly variable from 5.5 to 11.7 hours.

Energy expenditure for an animal in the thermoneutral zone (30°C) was 30.9 kJ/day which is three times basal metabolic rate. Energy expenditure for a euthermic animal at 20°C was 41.4 kJ/day and for a torpid animal 18.2 kJ/day, a saving of 56%. Huddling with a mate at 20°C reduced the energy expenditure of each animal by 18%.

# Chapter 4

## Thermal conductance of *Steatomys pratensis* and *Mus musculus*

### 4.1 Introduction

The body temperature of an animal depends not only on the rate at which heat is produced (metabolism) but also on the rate at which it is lost to the environment. The rate at which body heat is lost to the environment depends both on the rate at which the fat and fur conduct the heat to the surface, and also on the rate at which the heat leaves the surface of the animal (radiation). The latter depends on the difference in temperature between the surface of the body and the ambient temperature. Thus the greater insulation, the less the temperature loss or gain per unit time.

The advantages of insulation are offset by the problems of losing heat when the body temperature is too high and the physical problems of carrying a heavy fat or fur layer. It is thus obvious that an animal which is too small to carry a large fat or fur layer must have a high rate of metabolism to keep its body temperature at a normal mammalian level. This is the basis for the scaling factor in mammalian metabolism, the actual factor of which has caused so much discussion in the literature (McNab 1988).

Thermal conductance is a phrase used specifically to denote the amount of metabolic heat needed to be produced to offset that heat lost by conductance, such that the body temperature stays constant (Bartholomew 1982, p. 359). Since the size of the body concerned is so important thermal conductance is normally expressed per unit weight. It is thus defined as:

$$C = \frac{\text{metabolic rate}}{Tb - Ta}$$

where C is the mass specific thermal conductance, Tb is body temperature, and Ta is ambient temperature.

Below the lower critical temperature (see chapter 2) thermal conductance is constant while the body temperature is constant, and above the lower critical temperature in the thermoneutral zone it increases with a constant body temperature (Hart 1971).

The term thermal conductance used here is not the true physical heat conductance which depends on surface area but a concept which has become widely used in the biological literature

(see, for example, Bradley & Deavers 1980). It has been suggested that the term heat transfer coefficient should be used instead (Thompson 1985) but thermal conductance is used here because of its widely understood meaning.

Every animal has a zone of temperature tolerance within which it can keep it's body temperature at a normal level (Hart 1971). Below this zone the amount of energy needed to keep the temperature stable becomes too much for the animal to produce. If it cannot find a way to avoid these pressures by either moving to a warmer climate or dropping it's body temperature and going into torpor it must eventually die. Since the amount of heat loss is affected by the ambient temperature the time until death is temperature dependent (Hart 1971). There is a similar zone at the other end of the ambient temperature scale: mammals that cannot offload heat over a certain temperature have a limited and temperature dependent time until death (Hart 1971).

Heat loss from an animal actually occurs through four different pathways: radiation, conduction, convection, and evaporation (McNab 1980). Radiation has been discussed above, conduction is usually negligible in small mammal studies because of the small surface in contact with the substrate, but evaporative water loss can have an effect on the results obtained. Evaporative water loss, however, can be ignored at temperatures below thermoneutrality, since the amount of heat loss through this pathway is low at these temperatures (McNab 1980). Thermal conductance which includes evaporative water loss is known as "wet" thermal conductance. Since no measurements of evaporative water loss were made in these experiments all results given here are "wet" thermal conductance.

There are two methods for determining thermal conductance in mammals (McNab 1980). The first is to measure the slope of the regression line through the metabolic rates measured at different ambient temperatures below thermoneutrality: providing the line intercepts the x-axis (when metabolic rate would be zero) at the body temperature of the animal, the slope of that line is the thermal conductance (McNab 1980). The other method of calculating thermal conductance is to calculate mean conductance from individual conductances from each measure of oxygen consumption and temperature. The differences between these methods and their results is discussed later.

It is known (Hanney 1965) that *S.pratensis* has a low body temperature. Since the body temperature has a great bearing on the thermal conductance, the body temperatures found here will be discussed in some detail.

Because *Steatomys pratensis* has a thick layer of fat under the skin, the aim of this study was to find out to what extent this fat layer affects heating and cooling of the body of both live and dead animals, and whether their thermal conductances are similar to other rodents in spite of their having the low body temperatures and metabolism reported in chapters 2 and 3.

## 4.2 Materials and Methods

### 4.2.1 Body temperatures of *Steatomys pratensis* and *Mus musculus*

These results were taken in conjunction with the measurements of chapter 2. At each ambient temperature 7-22 *S.pratensis* were taken from their Labotec cages, their rectal body temperatures measured, and placed in the respirometer (for descriptions see chapter 2). For comparison some animals were also placed in empty Labotec cages in a darkened Conviron. Ambient temperatures were variously 0, 5, 10, 15, 20, 25, 30, or 35°C. After an hour the animals were removed, their rectal temperatures taken again, and returned to their original cages.

Three *M.musculus* were run at the same temperatures in the same way for comparison.

All the animals were weighed before the experiments began and body postures and actions were noted throughout the respirometry trials. All runs were done in the morning (09h00 to 12h00): this eliminated the problems of fluctuations in body temperature caused by circadian rhythmns (chapter 3).

### 4.2.2 Thermal conductance of *S.pratensis* and *M.musculus*

Thermal conductances were calculated by two methods, firstly by calculating the slopes of the regression lines (from chapter 2), and secondly by calculating the mean of all thermal conductance results from the oxygen consumption readings at ambient temperatures of 25°C and under, using the equation C = metabolism/Tb-Ta.

Since the thermal conductances calculated here are "wet" thermal conductances, the results found here are only applicable to temperatures below thermoneutrality (25°C and below in both *S.pratensis* and *M.musculus*).

### 4.2.3 Thermal conductivity of dead *S.pratensis* and *M.musculus*

Five lengths of thermo-couple wire which could be connected to the same thermometer as was used throughout the study were inserted at several points into a freshly dead *S.pratensis* and *M.musculus*. The wires measured (i) ambient temperature (ii) the temperature at the fur/skin interface (iii) the temperature under the fat layer but above the muscle layer (iv) the temperature in the middle of the abdomen and (v) the rectal temperature.

The body was placed in a constant temperature room at an ambient temperature which was the same as the body temperature of the live euthermic animal (32°C for *S.pratensis* and 37°C for *M.musculus*), and allowed to reach a constant. It was then transferred to a colder temperature (20°C) and the temperatures at all points measured every four minutes for an hour or until the temperatures were stable. The body was then transferred back into the warmer room and the temperatures again measured for an hour or until stable.

Two *S.pratensis* and one *M.musculus* were run for comparison.

Since the temperature in the constant temperature room cycled by one or two degrees a mean of the ambient temperatures was taken for each run, and the difference between the ambient and body temperatures calculated. To allow for the fact that the heat loss was actually an exponential curve, the temperature differences were logged and plotted as a function of time. The regression lines were calculated for these slopes. The relative rate of cooling was determined from the slope of the regression line.

## 4.3 Results

### 4.3.1 Body temperatures of live animals

The first and most obvious result is that *Steatomys pratensis* has an extremely low body temperature compared to most other rodents. Although these results were all obtained using rectal temperatures, it has been seen in chapter 3 that these are indicative of the actual internal body temperatures of the animals.

The results of the body temperatures obtained after exposure to varying ambient temperatures are summarised in Table 4.1. There are fewer results from the torpid animals as at ambient temperatures from 15 to 35°C only those animals which did not arouse from torpor are included.

All animals attempted to arouse below 15°C.

The original body temperatures of the *S.pratensis* (i.e. when taken out of their nestboxes at an ambient temperature of 20°C) ranged from 29.5 to 34.4°C with a mean of 31.8°C when euthermic, 21.2 to 23.7°C with a mean of 21.7°C when torpid and on their own in the nestbox, 25.3 to 28.6°C with a mean of 27.3°C when sharing the nestbox with a euthermic mate, and the *M.musculus* temperatures ranged from 35.5 to 38.1°C with a mean of 36.7°C. Although not included in these calculations, pregnant female *S.pratensis* had generally higher body temperatures of 32.2 to 34.5°C.

**Table 4.1** Body temperatures of *S.pratensis* and *M.musculus* after a one-hour exposure

Euthermic *S.pratensis*

| Ta | n | mean | S.E. | Range |
|---|---|---|---|---|
| 0 | 4 | 27.85 | 1.97 | 23.5-32.4 |
| 5 | 6 | 28.13 | 0.94 | 23.9-30.7 |
| 10 | 5 | 29.82 | 0.27 | 28.8-30.3 |
| 15 | 8 | 31.60 | 0.28 | 30.1-32.7 |
| 20 | 10 | 32.10 | 0.23 | 31.3-33.6 |
| 25 | 11 | 31.81 | 0.28 | 30.1-33.8 |
| 30 | 12 | 32.93 | 0.14 | 32.0-33.7 |
| 35 | 10 | 38.19 | 0.10 | 37.7-38.6 |

Torpid *S.pratensis*

| Ta | n | mean | S.E | Range |
|---|---|---|---|---|
| 0 | 10 | 22.62 | 1.26 | 17.1-28.7 |
| 5 | 1 | 21.10 | | |
| 10 | 2 | 22.23 | 1.94 | 19.5-26.0 |
| 15 | 1 | 22.80 | | |
| 20 | 2 | 24.15 | 1.55 | 22.6-25.7 |
| 25 | 1 | 28.60 | | |
| 30 | 2 | 33.70 | 0.60 | 33.1-34.3 |
| 35 | 2 | 37.50 | 0.60 | 36.9-38.1 |

*M.musculus*

| Ta | n | mean | S.E. | Range |
|---|---|---|---|---|
| 0 | 3 | 32.77 | 0.72 | 31.9-34.2 |
| 5 | 3 | 32.67 | 0.09 | 32.5-32.8 |
| 10 | 3 | 33.73 | 0.32 | 33.1-34.1 |
| 15 | 3 | 36.53 | 0.49 | 35.9-37.5 |
| 20 | 3 | 36.73 | 0.38 | 36.3-37.5 |
| 25 | 3 | 37.13 | 0.47 | 36.2-37.7 |
| 30 | 3 | 37.03 | 0.38 | 36.4-37.7 |
| 35 | 3 | 39.83 | 0.19 | 39.6-40.2 |

(These results are plotted on figure 4.1)

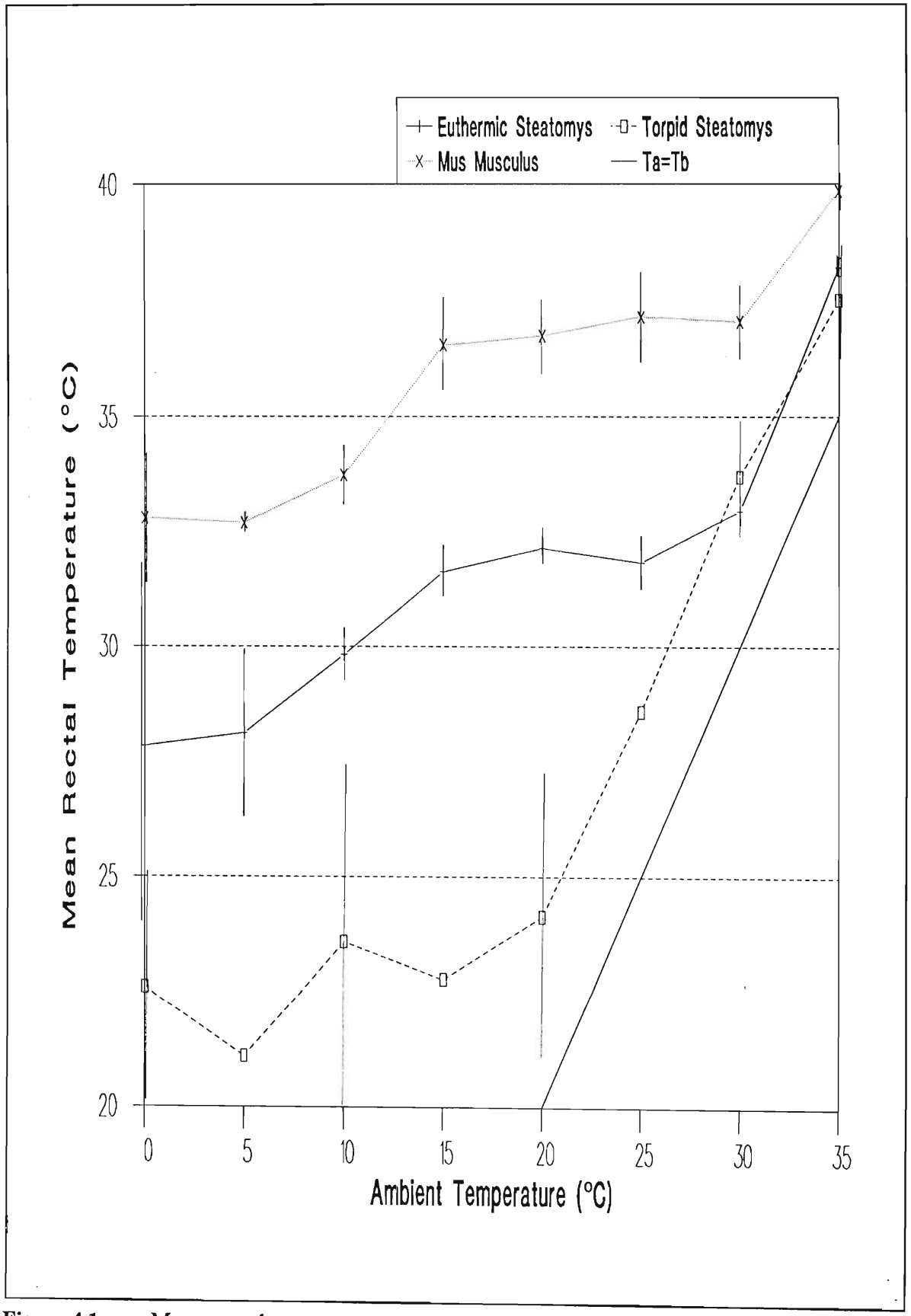


**Figure 4.1** Mean rectal temperatures of euthermic and torpid *S.pratensis* and *M.musculus* after a one hour exposure to various ambient temperatures. Vertical lines indicate 2 standard errors.

The maximum mass difference at any one ambient temperature was 6.6 g.

The behavioural reactions of both *S.pratensis* and *M.musculus* at various ambient temperatures were described in chapter 2. In summary, the animals curled up at low ambient temperatures and at high temperatures they lay flat out along the bottom of the respirometer in an obvious attempt to lose heat by conduction.

There were no differences between the body temperatures of the animals tested in Labotec cages and those in the respirometer (all results were within 1 SE of each other).

### 4.3.2 Thermal conductances of live animals

Again, there are fewer results from the torpid *S.pratensis* as only those animals which did not arouse from torpor are included. The results do not include ambient temperatures above the thermoneutral zone (30°C). The results are summarised in Table 4.2.

**Table 4.2** Thermal conductances of *S.pratensis* and *M.musculus*.
(all units in ml $O_2$/g.hr per °C)

Euthermic *S.pratensis*

| Ta | n | mean | S.E. | Range |
|---|---|---|---|---|
| 0 | 4 | 0.16 | 0.015 | 0.14-0.20 |
| 5 | 6 | 0.13 | 0.004 | 0.12-0.15 |
| 10 | 5 | 0.13 | 0.018 | 0.09-0.19 |
| 15 | 8 | 0.17 | 0.011 | 0.14-0.21 |
| 20 | 10 | 0.22 | 0.019 | 0.12-0.31 |
| 25 | 11 | 0.19 | 0.018 | 0.12-0.27 |

Torpid *S.pratensis*

| Ta | n | mean | S.E | Range |
|---|---|---|---|---|
| 0 | 10 | 0.19 | 0.013 | 0.12-0.26 |
| 5 | 1 | 0.10 | | |
| 10 | 3 | 0.21 | 0.046 | 0.12-0.26 |
| 15 | 3 | 0.14 | 0.023 | 0.09-0.16 |
| 20 | 3 | 0.26 | 0.023 | 0.22-0.30 |
| 25 | 1 | 0.17 | | |

*M.musculus*

| Ta | n | mean | S.E. | Range |
|---|---|---|---|---|
| 0 | 3 | 0.19 | 0.006 | 0.18-0.20 |
| 5 | 3 | 0.20 | 0.012 | 0.18-0.22 |
| 10 | 3 | 0.22 | 0.023 | 0.18-0.26 |
| 15 | 3 | 0.18 | 0.012 | 0.16-0.20 |
| 20 | 3 | 0.21 | 0.017 | 0.18-0.23 |
| 25 | 3 | 0.24 | 0.003 | 0.23-0.24 |

(These results are plotted in figure 4.2)

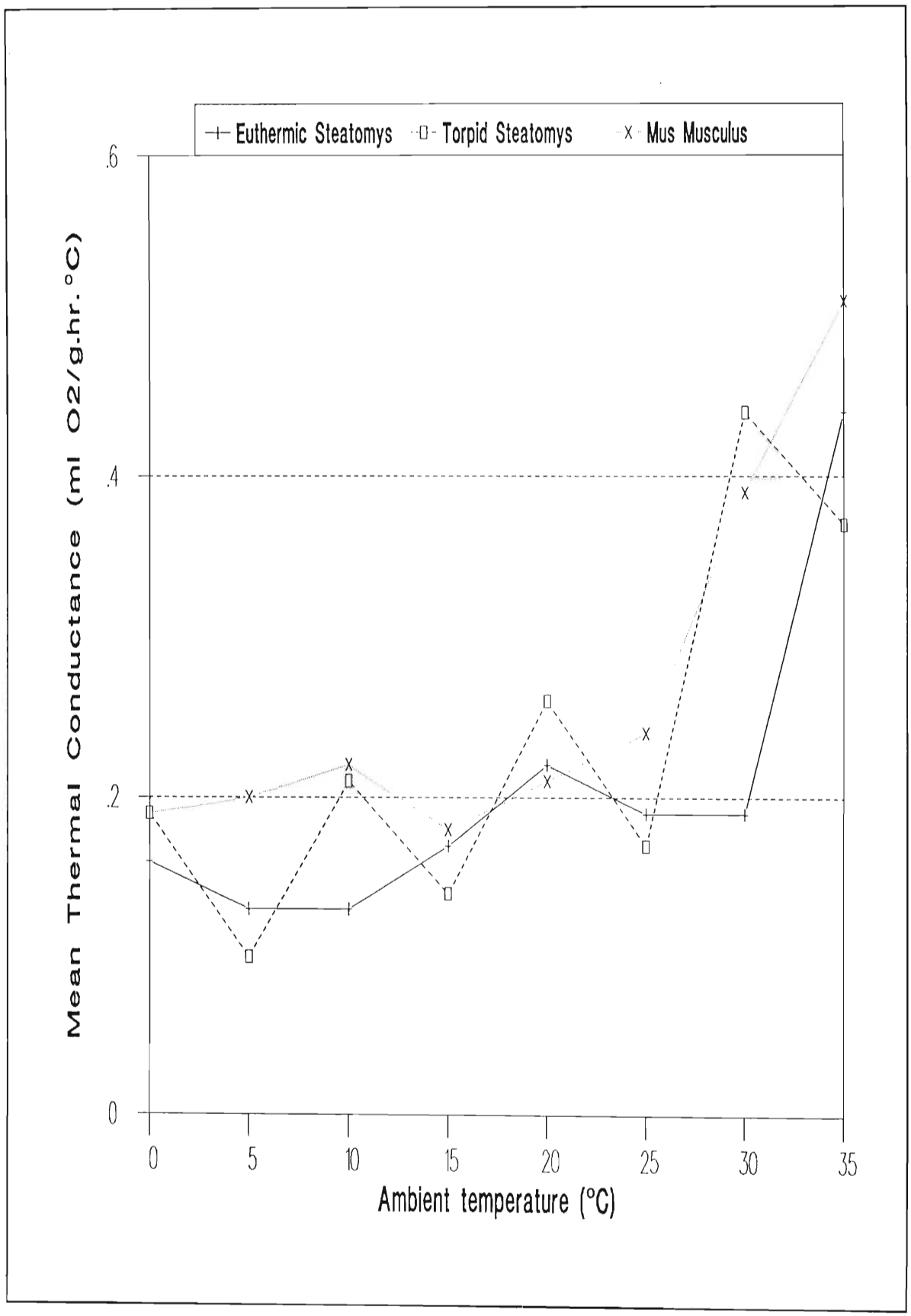


**Figure 4.2** Mean thermal conductance of euthermic and torpid *S.pratensis* and *M.musculus* at various ambient temperatures.

Combined results from both methods of calculating thermal conductances after McNab (1980) are presented in Table 4.3.

**Table 4.3** Thermal conductances
(all in ml $O_2$/g.hr.°C)

| | Slope reg. line | Mean conductance |
|---|---|---|
| *S.pratensis* | 0.137 | 0.159 (0.151) |
| *M.musculus* | 0.139 | 0.205 |

There are two mean conductances given for *S.pratensis*. The first one contains all results from the oxygen consumption readings, but since the oxygen consumption results for ambient temperature 20°C were probably high (see chapter 2) the mean conductances were recalculated without those results.

### 4.3.3 Cooling rates of dead animals

**Table 4.4** Cooling rates of *S.pratensis* and *M.musculus*. Numbers given are the slopes of the logged temperature differences.
(Confidence limits (± the given number) are given in brackets under each figure.)

| | Under fur | Under skin | Abdominal | Rectal |
|---|---|---|---|---|
| *S.pratensis* (lean) | -1.72 (0.20) | -0.69 (0.03) | -0.59 (0.02) | -0.81 (0.05) |
| *S.pratensis* (38.06 g) | -0.70 (0.10) | -0.55 (0.04) | -0.45 (0.01) | -0.66 (0.06) |
| *M.musculus* | -0.64 (0.03) | -0.63 (0.03) | -0.61 (0.02) | -0.70 (0.02) |

In each case, the higher the value here, the faster the cooling rate. Each animal shows a gradient of cooling rates. The fastest rate of cooling was either under the fur or rectal temperatures, then under the skin, and then the abdominal temperatures. The results from the white mouse are interesting in that there was very little retardation of cooling due to the body of the mouse: rates of cooling of the probe under the fur were very similar to the rates of cooling in the abdomen. In contrast even the lean *S.pratensis*, although the fur did not help in retarding cooling, cooled more slowly than the *M.musculus*. The fatter *S.pratensis* cooled most slowly of all, with the core retaining the most heat.

It must be remembered that as the mass of the animal concerned retards heat loss, it would be expected that the heaviest animal would lose heat the most slowly.

## 4.4 Discussion

### 4.4.1 Body temperatures of *S.pratensis* and *M.musculus*

As has been seen in chapter 3 the body temperatures of the fat mice range from 31.1 to 35.0°C every day in a circadian rhythmn. This variation of 3.9°C is similar to other rodents which show torpor, for example *Peromyscus leucopus* shows a range of 4°C (Vogt *et al.* 1983) but broad compared to non-torpid rodents (Hart 1971).

As discussed in chapter 3, the *S.pratensis* studied here have body temperature patterns very similar to those studied by Hanney (1965) and the *S.opimus* studied by Petter (1966) and Genest-Villard (1979).

However Taylor (1984), in his study of *S.krebsii* found that not only did his animals not show any torpor but they also had high body temperatures of 37 to 38°C.

From figure 4.1 and table 4.1 it can be seen that the body temperatures of *S.pratensis* stay almost exactly the same after exposure to ambient temperatures of 15 to 25°C. At 30°C there is a slight but not significant rise in body temperatures, and at 35°C there is a very significant rise due no doubt to the animals inability to offload excess heat. This is presumably the reason for the very high metabolic rates at this temperature.

Below 10°C there is a great drop in body temperatures. Although some (4 out of 10) of the euthermic animals did manage to keep their body temperatures up at a "normal" level, in general although the animals shivered violently they were unable to remain normothermic. At 10°C, although the fat mice did keep their body temperatures to "normal" they had to shiver violently to do so and it is doubtful that they would have managed to remain normothermic for any length of time at this temperature: 10°C thus seems to be below the lower limit of normothermia for these animals.

The results for the torpid animals closely follow the Tb = Ta line; this indicates that at each ambient temperature (15 to 35°C) the animal's body temperature was one or two degrees above ambient. These results do not extrapolate below 15°C, but in common with the euthermic

animals, the torpid animals' body temperatures did not drop so low and they tried to arouse from torpor by shivering. From the results it can be seen that these attempts were unsuccessful, torpid fat mice cannot arouse from torpor at ambient temperatures under 15°C. This is discussed further in chapter 5.

The body temperatures of the *M.musculus* were unchanged after an hour of exposure to ambient temperatures from 15 to 35°C. Gorecki & Kania (1986) found that their mice maintained their body temperatures to a "normal" level between 10 and 32°C, with the animals becoming hypothermic below these temperatures and hyperthermic above. The metabolic rate of these animals was, however, higher than those results found here so this could have had an influence, although the acclimation temperatures were the same in both cases.

Hudson & Scott (1979) also found a labile body temperature in *Mus musculus* at low temperatures, but the animals studied had body temperatures of 36.0°C at an ambient temperature of 2.5°C.

The *S.krebsii* studied by Taylor (1984) kept their body temperatures stable between 5 and 35°C, although there was some evidence of hypothermia at 5°C and hyperthermia at 35°C. This is a wider range than that found in *S.pratensis*.

Other african rodents also have wide limits of body temperature stability: *Praomys natalensis* and *Rhabdomys pumilio* as studied by Haim & Fourie (1980a) kept their body temperatures around the same at ambient temperatures of 5 to 30°C. An exception to this rule is that of *Heterocephalus glaber*, which has very little thermal tolerance (Withers & Jarvis 1980) having a body temperature incompletely regulated between 20 and 35°C and following ambient more closely at other temperatures.

The white-footed mouse *Peromyscus leucopus*, which shows many anatomical and physiological similarities to *Steatomys pratensis*, can regulate its body temperature from 10 to 30°C (Hill 1983).

Thermal limits, the time until death of the animals, were not specifically examined here. However, at ambient temperatures at which the body temperatures of the animals start to fall, the animals are losing too much heat which cannot be replaced until they move out of that temperature and so could probably be considered outside their thermal limits. The lowest body temperature to which a white mouse can be cooled before death occurs is 9.2°C, but they may

not survive re-warming (Hart 1971) and so this would not be considered a true ecological lower limit.

Thermal limits for the euthermic animals here then were 10°C for both the euthermic *S.pratensis* and the *M.musculus* at the lower end of the scale and 30°C at the higher end. Limits for the torpid fat mice were be 15 and 30°C.

The thermal responses of most murids to lower ambient temperatures is considerably influenced by their previous thermal history, especially cold acclimation (Hart 1971). In the light of this, perhaps the results found here were affected by the animals long acclimation in the laboratory. This seems unlikely, however, and it is probable that the lower limit for *S.pratensis* is about 10°C. If this is so then these animals may be unable to forage late at night at certain times of the year.

Petter's (1966) *S.opimus* probably had lower limits of around 12°C. Thus torpid *S.pratensis* and torpid *S.opimus* have similar lower body temperature limits.

Higher thermal limits are as variable as lower limits in rodents. Some rodents can let their body temperatures rise without an increase in oxygen consumption (Hart 1971). These are normally desert living rodents and since neither species studied here managed to let their body temperatures rise without increasing oxygen consumption, it suggests that neither species is adapted to desert conditions. High thermal limits are affected by humidity and the animals ability to offload heat through evaporative water loss (Hart 1971).

At high temperatures both *S.pratensis* and *M.musculus* salivated in what was taken to be an attempt to lose heat by evaporative cooling (chapter 2) although Golightly & Ohmart (1978) relate the drooling associated with high temperatures in the ground squirrels they studied as caused by higher respiratory rates (panting), not evaporative cooling.

There is some evidence that *S.krebsii* overheats at 35°C (Taylor 1984). Genest-Villard (1979) gives the body temperatures of the animals she studied as up to 38.8°C depending on how much energy they had expended trying to escape. This seems to indicate that these mice do not easily lose any heat produced by excessive exercise, but unfortunately there is no information as to whether these animals survived the high body temperatures.

Since the ambient temperature limits that both torpid and euthermic *S.pratensis* can survive are very similar, torpor is not designed to help the animals survive cold climates and therefore must

be to save energy.

### 4.4.2 Thermal conductance of *S.pratensis* and *M.musculus*

As can be seen from Table 4.3, the thermal conductances of both species of animals derived from the two different sources are somewhat different. McNab (1980) discusses both methods of deriving thermal conductances and relates the differences to the amount that the slope overestimates the body temperatures of the animals. The difference between them should be the amount of physical thermoregulation used by the animals as opposed to the chemical thermoregulation (NST).

McNab (1980) uses the equation:

$$\frac{Cm}{Cf} = 0.060 \text{ (overestimate of Tb)} + 1.0$$

where Cm is the thermal conductance calculated by the mean of all the results, and Cf is the thermal conductance from the fitted slope. The overestimation of body temperature of *S.pratensis* from this equation is 1.70°C and that of *M.musculus* 7.97°C. Since the regression equation for the fitted slope estimated the body temperature of the *S.pratensis* to be 33.5°C and that of *M.musculus* to be 45.4°C, these correction factors reduce the body temperatures to 31.8 and 37.4°C respectively. These body temperatures compare very favourably with the mean body temperatures found of 31.83 and 36.80°C respectively, so the correct thermal conductances can be assumed to be those calculated from the means of all the results (0.151 ml $O_2$/g.hr.°C in the case of *S.pratensis* and 0.205 ml $O_2$/g.hr.°C in the case of *M.musculus*).

The mean body temperatures are the means of all the fat mice after exposure to temperatures ranging from 15 to 25°C. The overestimation of body temperature in *S.pratensis* is calculated using the second determination of thermal conductance. The result of using the first is an over estimation of 2.77°C to give a body temperature of 30.73°C. This is still within reasonable limits for diurnal temperatures.

Smaller mammals tend to have higher minimum thermal conductances than larger ones owing to the greater ease with which heat leaves the body (McNab 1980). Thermal conductance scales with body mass in the same way as metabolism, the equation $C = 0.95(\text{mass})^{-0.50}$ usually being used (Hart 1971). Thus the results found here are 97.4 % of expected for *S.pratensis* (C = 0.151 ml $O_2$/g.hr.°C, mass 37.54 g), and 118.7 % of expected for *M.musculus* (C = 0.205 ml $O_2$/g.hr.°C, mass 30.28 g).

Bradley & Deavers (1980) give another equation for the calculation of thermal conductance in Muridae: $C = 0.84(mass)^{-0.47}$. Using this equation the results are 98.8 % of expected for *S.pratensis* and 121.2 % of expected for *M.musculus*.

Thus *S.pratensis* has a standard thermal conductance and *M.musculus* is a little higher. This means that in spite of having a low metabolism *S.pratensis* loses heat at a normal rate. Although the discrepancy between the expected and observed results is higher for *M.musculus*, these results still mean that the *M.musculus*, having a higher conductance, would lose heat more easily than the fat mice. Considering the heavy fat layer in the fat mice which must retard heat loss, this is entirely expected.

The measured thermal conductance for *Mus musculus* has been given by Bradley & Deavers (1980) as 0.19 but the mass of this animal was much smaller than those animals measured here. Hudson & Scott (1979) give the thermal conductance of *Mus musculus* as ranging from 0.15 to 0.26 ml $O_2$/g.hr.°C depending on the ambient temperature. The results found in this study are thus within the limits of those found by other workers.

*S.krebsii* have a low thermal conductance for their body size (0.11 ml $O_2$/g.hr, 53.6 % of expected) (Taylor 1984). One of the other dendromurine species *Malacothrix typica* also have a lower thermal conductance than expected (0.167 ml $O_2$/g.hr.°C, 83 % of expected although these results may have been affected by overweight animals) (Knight & Skinner 1981). Both these species have hair which appears longer, fluffier, and less silky than *S.pratensis*, and which may contribute to the low conductances (personal observation).

Thermal conductance at temperatures below thermoneutrality is generally only applicable when the body temperature of the animal stays constant (McNab 1980). However, assuming that the animal has not "reset" its body temperature and is trying to keep it "normal", then the thermal conductance must be at a minimum and the equation discussed above must balance: since metabolic rate is fixed at the maximum, Tb - Ta is reduced and the thermal conductance (the rate at which the body loses heat) stays the same. This is the reason the thermal conductances for both the euthermic *S.pratensis* and the *M.musculus* remain around the mean (see figure 4.2), even though their bodies actually lost too much heat and the body temperatures dropped.

Body positions of the animals can be extremely important in keeping the body temperature within a tolerable zone. As already discussed in chapter 2, the animals curled up at lower temperatures to reduce the surface area for heat loss and at high temperatures stretched out along the floor of the respirometer in an attempt to lose heat by conduction.

Lower critical temperature is that ambient temperature at which the animal is still within its thermoneutral zone but thermal conductance is at a minimum.

There are two methods of calculating the lower critical temperature. Morrison (1960) gives it as Tb - 4(body mass)$^{0.25}$ and McNab (1979a) and Haim (1981) give it as Tb-RMR/C.

The lower critical temperature of *S.pratensis* from these equations would be 29.5°C as calculated from Morrison's equation and 28.7°C as calculated from Haim's equation. The results found here are not accurate enough to distinguish between them since the closest that these results could come was 30°C. This is high considering the low body temperature in these animals: the temperature differential between the body temperature and the lower critical temperature is small: this is indicative of a small mammal with a low rate of metabolism (McNab 1979a)

The lower critical temperature of *M.musculus* as calculated from these equations would be 27.6 and 26.3°C respectively. This latter is lower than the results found in this study although this may have been influenced by the high thermal conductance.

High lower critical temperature is usually associated with small mammals with poor insulation (Morrison 1960) and nocturnal desert rodents tend to have lower lower critical temperatures (32 to 34°C) than diurnal ones (37-40°C) (Knight & Skinner 1981). None of the animals studied here have high lower critical temperatures and again are therefore probably not of desert origin.

### 4.4.3 Heat loss from dead *S.pratensis* and *M.musculus*

The rate of heat loss from the *S.pratensis* cannot be compared to that from the *M.musculus* as the bodies were different masses and thus not comparable. However, the rates of cooling within the same animal can be compared with each other.

From table 4.4 it can be seen that the amount of heat loss through the fur compared to under the skin of the *M.musculus* was 0.64 to 0.63, a ratio of 1.02 compared to a ratio of 2.49 for the leaner fat mouse and 1.27 for the fatter one. Thus, for the white mouse the skin was a relatively minor source of heat retardation, for the fatter *S.pratensis* it was more important but for the leaner *S.pratensis* it was extremely important. The fur in the leaner *S.pratensis* was in bad condition and the results from this animal thus show a great loss of heat through the fur.

The amount of heat loss from the abdomen compared to from just under the skin was a ratio of 1.03 for white mouse, 1.17 for the leaner *S.pratensis*, and 1.22 for the fatter *Steatomys*. Thus the abdomen was not a major insulator in the white mouse, was more important in the leaner *S.pratensis* and was much more important in the fatter *S.pratensis*.

From this it can be seen that while the skin and muscle of the white mouse contributed little in the way of insulation, it was much more important to the fat mice. The major insulator of the white mouse must thus be the fur while to the leaner *S.pratensis* the fur was less important than the skin and muscle layers. The conclusions about the white mouse are corroborated by Mount (1971) who says that fur contributes 30 to 40 % of the total insulation of the white mouse.

The fatter *S.pratensis* was in good condition and showed a good gradation of cooling. In this case the fur insulation could be compared to that of the leaner animal, it being assumed the fur thickness would not change much with mass of the animal but would be species specific. The fur of the fatter animal showed a much better insulation than that of the leaner animal. The skin and abdomen also showed good insulation, probably related to the amount of fat in the skin, under the skin, and in the abdomen (chapter 6).

Thus, the fat mice have a much better insulation than the white mouse, probably caused by the fat layer and causing the difference in thermal conductances of the two species. This would help to retard heat loss which could be very important in an animal having such a low metabolism: heat lost to the environment would not easily be replaced.

In spite of many authors comparison of the rates of cooling of a dead body with the thermal conductance of a live animal, these rates are not comparable, the former being expressed in degrees of heat and the latter being expressed in power units. However, Mount (1971) concludes that the rates of heat loss from live and dead *M.musculus* are similar on the basis of the similarity between his results on live animals and other workers results for skins.

One difficulty, however, of having a good insulation is getting rid of excess heat when needed. Genest-Villard (1979) gives some pertinent data when she relates the body temperature of the fat mice to the amount that the animal tried to escape before having its temperature taken. This was also noted in this colony: if an animal struggled a lot before having its temperature taken the temperature was much higher, indicating that the animals could not easily offload excess heat.

Most rodents offload heat from their tails and skin (Hart 1971), and *M.musculus* is no exception (Hart 1971). Jakobsen (1981) reports that saliva spreading is not well documented in this species although it was seen in this study. *Peromyscus leucopus* loses excess heat through the tail and also by salivating (Hill 1983).

*S.pratensis* probably has trouble losing excess heat as it has a short tail and the thick layer of fat in the skin probably retards heat loss that way. At high temperatures the animals lay flat along the floor of the respirometer, presumably to try to lose heat by conduction and they also salivated. The males showed well descended testes which probably aided them in heat loss. The ears in this species are not particularly large which means little heat could be lost by that path.

### 4.4.4 Comparison between cooling rates of dead animals and live animal rates of entry into torpor

The rate at which a dead *S.pratensis* cooled down such that their core temperatures approached ambient was 6.4°C/hour. Since this is obviously an exponential rate, it is not strictly extendible to 10°C/1.6 hours but since this measurement was taken in the middle of the exponential curve it is probably close enough. 10°C is the amount a euthermic *S.pratensis* would have to cool down to reach one or two degrees above ambient of 20°C.

In contrast, the time taken for a torpid *S.pratensis* to drop its core temperature down to ambient (a drop of about 10°C) without any bedding material or mate was between 1.83 and 3 hours (chapter 3). It thus seems that at least some of the torpid animals were deliberately slowing down their rate of body temperature drop when entering torpor. Similarly the *Mus musculus* studied by Hudson & Scott (1979) took 5 hours to drop their body temperatures, although it is not clear if they could burrow into their wood shavings and so increase their insulation.

This deliberate deceleration of the rate of heat loss has been noted in other mammals, especially those entering hibernation. Miller & South (1981) remark on this in *Marmota flaviventris* and Bartholomew & Cade (1957) in *Perognathus longimembris*. The latter authors reported that their animals were interspersing periods of great activity with periods of rest while the body temperatures dropped, a state that they put down to vasoconstriction of the locomotor muscles or general vasodilation.

### 4.5 Ecological implications of these results

In the light of the results that *S.pratensis* cannot cope with ambient temperatures under 10°C or much over 30°C, the importance of their burrows and nests becomes apparent and explains why Kingdon (1974) writes that these animals are very dependent on their burrows.

As discussed in chapter 2, extreme temperatures in the Cathedral Peak area (where the original animals were trapped) range from -4.5 to 32.0°C. It is unlikely that the ambient temperature in this region would be so high that the burrows and nests could not keep the ambient temperature under 30°C and as has been discussed (chapter 2) the burrows and nests probably do not fall below the animals' lower limits. However, in other areas the above-ground temperatures might rise above a level which would be difficult for the animals to cope with (Genest-Villard 1979) and the insulative abilities of the burrows would be just as important to keep the temperatures down.

The animals could be under a lot of cold stress while foraging. The fat mice probably have two main ways of overcoming these problems: one would be to restrict their foraging to times when the ambient temperatures outside the burrows were high enough, and the other would be to remain torpid for that day in the hope that the next would be warmer.

If the mice restricted their foraging to those times when the ambient temperatures were high enough to allow them to forage without expending too much energy in maintaining their body temperatures, they would have to forage in the early evening. To do so they might have to use a foraging strategy described by Wolff & Bateman (1978) for *Perognathus flavus* which is discussed in chapter 7.

The body temperature of the euthermic fat mice would be unlikely to change over a year but thermal conductances of rodents can fluctuate, depending on fur and fat changes (Hart 1971). *Peromyscus leucopus* as studied by Wickler (1980) changes its fur density throughout the year and also changes its thermal limits such that it becomes more tolerant of lower temperatures. *S.pratensis* might have become acclimated to the mild temperatures in the holding rooms and freshly caught animals might have larger limits of thermal endurance.

Short cold shocks while out foraging would be the most likely temperature regime the fat mice would be subjected to. This could affect their metabolism by enlarging their thermal tolerance (Jakobsen 1981).

### 4.6 Summary

*S.pratensis* has a low metabolic rate, a normal thermal conductance, and an extremely low body temperature. Mean body temperature found was 31.8°C. Body temperatures remained normal from 10 to 30°C, with the animals becoming hypothermic below this and hyperthermic above. Body temperatures of *Mus musculus* were higher and nearer the mammalian norm but this species had similar thermal limits to *S.pratensis*. Torpid *S.pratensis* body temperatures came to lie just above ambient at temperatures ranging from 15 to 35°C, below this all the animals tried to arouse.

Thermal conductance of the *S.pratensis* was 0.151 ml $O_2$/g.hr.°C, or 97.4 % of expected based on body size. *M.musculus* had a thermal conductance of 0.205 ml $O_2$/g.hr.°C, or 118.7 % of expected.

Cooling rates of the *S.pratensis* were much slower than those of the white mouse, probably due to the effects of the heavy fat layer. Although the low metabolic rate and low body temperature produce little heat, *S.pratensis* does not lose this very easily.

# Chapter 5

## Arousal patterns of torpid, and non-shivering thermogenesis in euthermic, *Steatomys pratensis*

### 5.1 Introduction

During forced arousal a torpid mammal must warm its body from close to ambient temperature to normothermic as fast as possible. Since this can entail a temperature rise of 10 to 30°C, it is obviously extremely costly energetically.

Fast arousal may play an extremely important, if rarely used, role in the life of a torpid mammal. It is probable that no torpid animal has the ability to arouse fast enough to escape predators, but it may be important in escaping other natural disasters such as burrow flooding, extreme cold, or invasion by a conspecific.

During forced arousal the animal may use all methods of heat production available to it. There are three main methods of heat production in a mammal, namely shivering, non-shivering thermogenesis, and exercise induced thermogenesis. Shivering and exercise induced thermogenesis are often not additive, with one replacing the other (Hart 1971). While the body temperature is too low to allow muscular shivering the animal will use non-shivering thermogenesis but after the body temperature has risen a little it will generally use shivering thermogenesis or vigorous exercise as well.

In recent years maximal thermogenesis, the combination of all methods of heat production, has been measured in several rodents (e.g. Gorecki & Kania 1986). Maximal thermogenesis is the peak metabolic effort that can be sustained for a short period of time before the body temperature starts to drop (Hart 1971). Maximal values are usually about 6 to 10 times the basal metabolic rate (Hart 1971; Gorecki & Kania 1986). The method commonly used to test maximal thermogenesis is to measure the animal's metabolism in a helium-oxygen mixture and by extrapolation determine the temperature at which this would have occurred naturally. However it can also be measured by simply measuring the animals metabolism at lower temperatures until the animal can no longer produce enough metabolic heat and the body temperature starts dropping.

Non-shivering thermogenesis (NST) has been described by Jansky (1973) as "a heat-production mechanism liberating chemical energy due to processes which do not involve muscular contractions". It is thus a method of heat production used by nearly all mammals, but especially young and hibernating mammals, to warm themselves when it is not possible or not desirable to use muscular shivering. NST is produced by the respiration of brown adipose tissue (BAT), muscles, and liver, with lesser amounts being produced by the heart, brain, and adipose tissue (Jansky 1973). BAT can produce up to 70% of the heat produced by NST (Jakobsen 1981). The amount of heat production by these different organs differs depending on the species.

BAT is a highly vascularised fatty tissue found in a large deposit between the scapulae, but also in smaller amounts around the heart and in several other locations. The locations and histological appearance of brown adipose tissue is discussed in chapter 6. When under nervous stimulation BAT produces heat to warm the body in specific areas, mainly the spine and chest (Hart 1971). There is some evidence that BAT is involved in stimulating other organs to produce heat by NST (Hart 1971).

NST can be measured in several different ways (Jansky 1973) but is measured here as the ratio between the minimal oxygen consumption of a quiet (generally anaesthetised) animal at thermoneutrality, and the maximal oxygen consumption of the same animal after an injection of noradrenaline.

NST has been studied by several authors who have come to conclusions about the ability of mammals to remain normothermic under particular conditions (e.g. Haim 1981; Haim & Fourie 1980a).

It seems unlikely that forced arousal is the same process as natural arousal, the former usually being in response to some strong external stimuli while the latter probably occurs due to an endogenous rhythmn. However, some authors (e.g. Cranford 1983 in *Zapus princeps*) describe arousal patterns without giving the details under which these were measured, presumably assuming that the processes are the same. The two processes in *Steatomys pratensis* will be compared.

It was not known how fast *S.pratensis* could arouse from torpor. De Graaff (1981) remarks that *S.pratensis* "soon" recovered its movement from torpidity when dug out of its nest while torpid, although Smithers (1975) writes that this takes "quite a few moments". NST in all *Steatomys* species was unknown.

## 5.2 Materials and Methods

### 5.2.1 Arousal patterns

Torpid fat mice were taken out of their nestboxes, their rectal temperatures measured, and the animals placed as rapidly as possible in a large (5 l) glass jar with 10 mm depth of wood shavings. Rectal temperatures were taken every 5 minutes up until 45 minutes, and then again at 60 minutes when the experiment was terminated. The animals were observed throughout this period, with notes being taken of whether they were active, digging, shivering, body- or face-washing, etc.

All experiments were run between the hours of 08h00 and 11h30, and were conducted at ambient temperatures of 0, 10, 20, and 30°C. Six animals were run at 0°C, and 3 each at 10, 20, and 30°C.

No animal was used for more than one of these experiments as it was felt that the previous experience could have affected their abilities. Rectal temperatures are generally indicative of internal body temperatures (chapter 3).

A few natural arousals were measured (chapter 3) which will be compared with the forced arousals.

The means of the body temperatures were plotted, but because the results from the arousals at 0°C needed special discussion, all results of the body temperatures at this temperature were plotted again on a separate figure.

### 5.2.2 Non-shivering thermogenesis

Euthermic animals were taken from their cages, weighed, and anaesthetised by injecting them intraperitoneally with 0.06 mg pentobarbitone sodium ("Sagatal", May & Baker Ltd) per gram body weight. This was much higher than the manufacturers recommendation of 0.06 mg per 2.25 g body weight but was found to be the necessary amount by experimentation. It is possible that the original solution (60 mg per 1 ml) may have been adversely affected by the 1 + 9 dilution with distilled water necessary to produce an amount which was possible to inject accurately with a tuberculin syringe. Although Bellier & Gautun (1968) remark that *S.opimus* is particularly susceptible to dying while under anaesthesia, no problems were encountered here: smaller

dosages were given to non-experimental animals to begin with and the dosages were increased until the desired effect was obtained. Bellier & Gautun (1968), however, do not give the anaesthetic used: in the light of the large amount of fat in *Steatomys* it would not be surprising if they reacted badly to ether or any other fat solvent which is used as an anaesthetic.

After injecting the anaesthetic the animals were returned to their cages until the anaesthetic had taken effect: this kept the animals calmer and thus the anaesthetic seemed to work better. When the animals were no longer reacting to any stimulus (generally 10 minutes) they were removed from their cages and placed in the respirometer under an open flow system at an ambient temperature of 31°C (the thermoneutral zone) and a flow rate of 60 ml per minute. The respirometer organisation was the same as in figure 2.1.

Readings were taken every two minutes until 4 successive readings had been recorded within 0.05 % of each other. The animal was then removed and 1.5 x $10^{-3}$ mg per g body weight noradrenaline (Merck, bitarttrate) was injected subcutaneously. The mouse was returned to the respirometer and readings taken every two minutes for the following hour.

Rates of 5 animals were measured in total. The oxygen consumption before the injection of noradrenaline and the maximum oxygen consumption after the noradrenaline injection were taken. These results were converted to ml $O_2$/g.hr, adjusted to STP, and the mean, range, and SE calculated.

## 5.3 Results

### 5.3.1 Arousal patterns

The general pattern of forced arousal from torpor was similar in the animals at all temperatures, the only difference being the timing of some of the behaviours.

At the beginning of arousal the body was flat on the ground with little or no weight resting on the hind limbs. The animal was fairly unresponsive to stimuli, the ears were held back towards the skull and the eyes were closed (plate 5.1). Occasionally the animal would try and walk around, but the hind legs were extremely unco-ordinated, and the mouse frequently fell over.

After some time the mouse began to shiver, starting almost unnoticeably and gradually becoming more energetic until the animal was shivering violently with the eyes half closed, the

ears flat back against the skull, the fur very fluffed up, and the front paws contracted and lifted off the ground (see plate 5.2).

**Plate 5.1** *Steatomys pratensis* at the start of arousal from torpor

**Plate 5.2** *Steatomys pratensis* in the shivering phase of arousal

Soon after this violent shivering started the animal began to walk around in bouts, firstly digging with the front feet alone and gradually as the hind legs became more coordinated digging with both front and hind legs. After some time (the time varying depending on the ambient temperature) of bouts of shivering interspersed with bouts of walking and digging, more weight was transferred onto the hind legs until the mouse could sit on its haunches (see plate 5.3) to wash its face, and after this to groom the rest of its body. By this time body temperature was at or above the normal euthermic level.

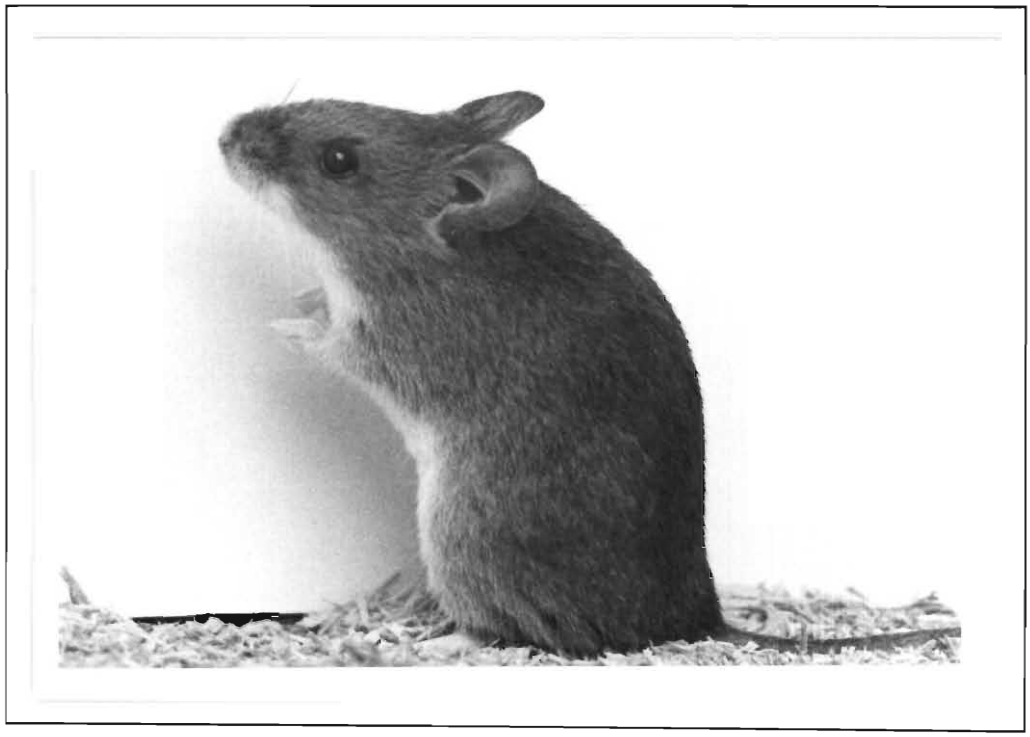

**Plate 5.3** *Steatomys pratensis* after arousal showing the ability to balance on the hind legs

Figures 5.1 and 5.2 show the body temperatures of the arousing animals. The lines on figure 5.1 are the means of the results from the animals at the four ambient temperatures, and those on figure 5.2 are the results at 0°C plotted separately. From the figures it can be seen that the time it took the animals to arouse to normothermia differed depending on the ambient temperature, with animals arousing fast at 30°C and having extreme difficulty at 0°C.

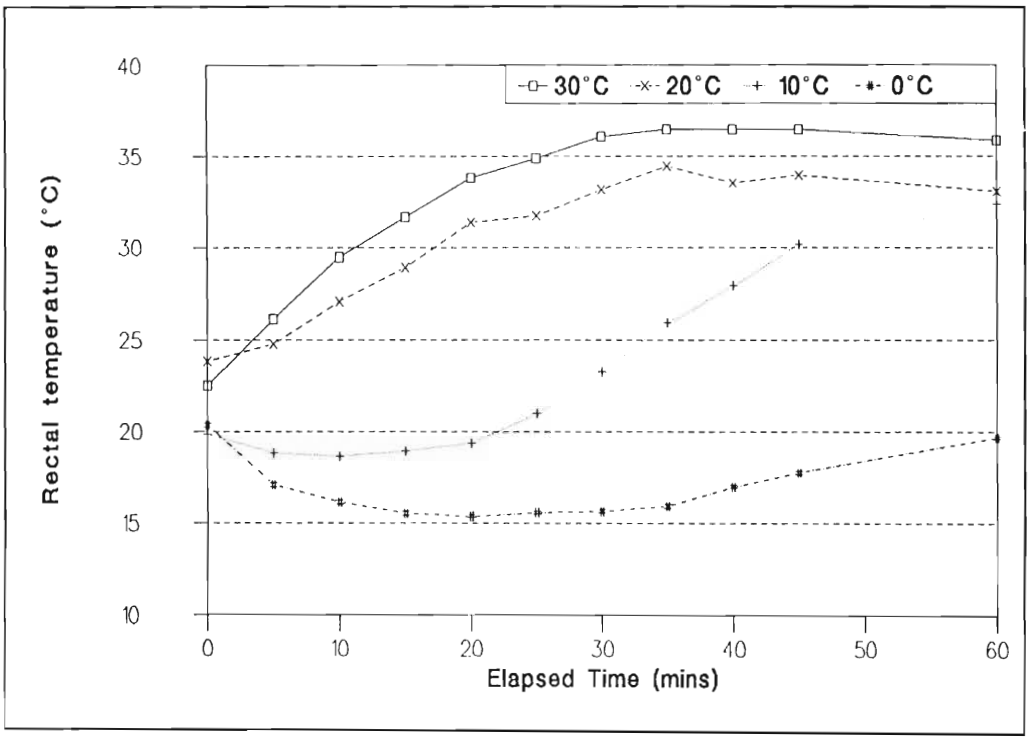


**Figure 5.1** Mean rectal body temperatures of *Steatomys pratensis* arousing from torpor at 0, 10, 20, and 30°C.

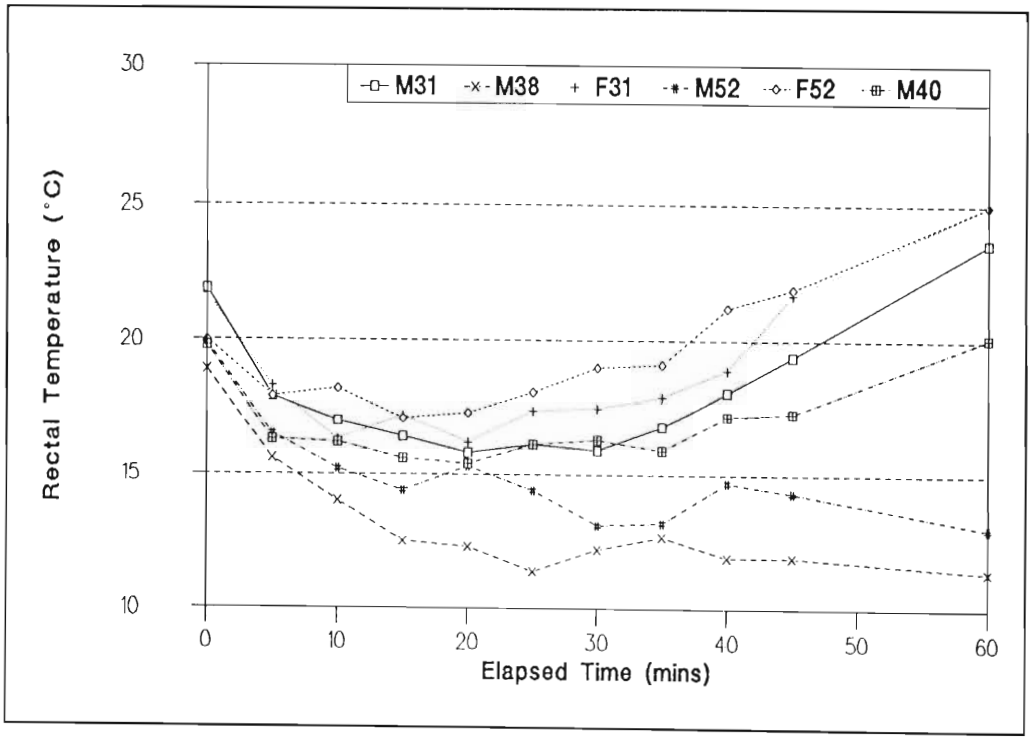


**Figure 5.2** Rectal body temperatures of individual *Steatomys pratensis* arousing from torpor at 0°C

A summary of the arousal rates is given in Table 5.1.

**Table 5.1** Arousal rates of *Steatomys pratensis*. Results given are mean (± SE).

| Ta | n | time of arousal (minutes) | rate of arousal (°C/min.) |
|---|---|---|---|
| Forced arousals | | | |
| 0 | 6 | - | - |
| 10 | 3 | 46.3 (4.1) | 0.248 (0.01) |
| 20 | 3 | 19.0 (6.6) | 0.407 (0.07) |
| 30 | 3 | 13.3 (3.3) | 0.690 (0.11) |
| Natural arousal (chapter 3) | | | |
| 20 | 3 | 70.0 (15.3) | 0.164 (0.02) |

The results from the forced and natural arousals at 20°C are statistically different from each other (t-test, $P < 0.05$).

The maximum mass difference at any one ambient temperature was 6.6 g. This was too small a difference to test for mass effect on the animals ability to arouse, but the possibility exists that greater mass differences could affect the results.

### 5.3.2 Non-shivering thermogenesis

The results from the non-shivering thermogensis experiments are summarised in Table 5.2.

**Table 5.2** Results of non-shivering thermogenesis

| Animal | Min. $O_2$ cons. pre NA | Max. $O_2$ cons. post NA | Mass | %RMR |
|---|---|---|---|---|
| m43 | 0.45 | 1.65 | 43.0 | 367 |
| m43 | 0.66 | 2.08 | 30.5 | 315 |
| m36 | 0.53 | 1.96 | 33.8 | 371 |
| f44 | 0.74 | 1.89 | 26.0 | 255 |
| f39 | 0.63 | 3.40 | 33.8 | 539 |

The mean minimum oxygen consumption was 0.602 ml $O_2$/g.hr with a standard error of 0.05, and the mean maximal oxygen consumption was 2.196 ml $O_2$/g.hr with a standard error of 0.309. These are significantly different from each other (t-test: $P < 0.01$). The mean percentage increase in RMR was 369 %.

All the animals became hyperthermic with body temperatures ranging from 35.7 to 39.8°C (mean 37.5°C).

## 5.4 Discussion

### 5.4.1 Arousal Patterns

When taken out of their nestboxes the torpid fat mice were cold to the touch, unresponsive to stimuli, and had very red feet and noses. These latter phenomena have been described by Cranford (1983) as being caused by abdominal vasoconstriction, a deduction from his observation that the animals started shivering in the front of the body and only started shivering in the posterior portion after the body temperature had reached 20°C. Since the *S.pratensis* started shivering from the front as well it is likely that they too underwent abdominal vasoconstriction during torpor.

The arousal pattern found in these animals is very similar to that of the arctic ground squirrel (*Spermophilus undulatus*), as described by Gunderson (1976). It is probable that this is a pattern common in all torpid animals under the stress of being removed from the nest and is designed to warm the anterior portion of the body first to permit the organs vital for activity (the heart, lungs, and brain), and also the sense organs to become fully functional before the rest of the body. This would ensure functional activity of the animal as rapidly as possible.

Most of the activity patterns noted in the arousing animals could be related to restoring the circulation and coordination which were absent in the torpid animal.

Face-washing may have a direct use as a grooming movement but may also be of use in restoring circulation in the facial region or circulation in the forelimbs by exercise (G.Hickman, pers. comm.). The digging observed could possibly have served to restore coordination in the hind legs and possibly also to eliminate the effects of any abdominal vasoconstriction (Cranford 1983). It may also have been muscular activity to create more exercise-induced thermogenesis to aid in raising the temperature. This pattern of activity was not taken to be an escape movement since at no time did the animals bury their noses in the sawdust, which they did while burrowing at other times. This may, however, have been a result of the sawdust layer being quite shallow.

By the time the body temperatures were 25 to 28°C the animals were showing normal movement. This normal movement while the body temperatures were still below normal is

similar to those observations made by Hudson & Scott (1979) who said that their *M.musculus* were moving normally while their rectal temperatures were 26 to 30°C.

Cranford (1983) measured both oral and rectal temperatures in his arousing *Zapus princeps* and found that while rectal temperatures closely followed oral temperatures of these animals the rectal temperatures lagged behind in the rate of temperature increase. Bartholomew & Cade (1957) recorded a similar situation in *Perognathus longimembris* but concluded that the two methods were very close. It must thus be remembered that as rectal temperatures were measured in this study the temperatures in the anterior portion of the body would quite likely be a little higher at any one time.

From figure 5.1 and table 5.1 it can be seen that the animals had no difficulty in arousing to normothermia at an ambient temperature of 30°C. This is hardly surprising since if they had remained passive their body temperatures would have risen to almost normal without any expenditure of energy. However, since they were put in a position of some stress (lights, strange surroundings, and no nesting material) the fat mice raised their body temperatures to normal with some shivering and presumably some NST within 15 minutes. There was a fair amount of "overshoot" of the body temperature at 30°C since mean body temperature went up to 36.5°C before starting to decline again. This was presumably caused by an overproduction of heat by both shivering and NST and is fairly common among arousing mammals (Cranford 1983).

At an ambient temperature of 20°C the mice took a little longer to reach normothermia (20 minutes) and did not show the same overshoot of body temperature; the maximum body temperature reached being 34.5°C. At 10°C, mean body temperatures actually decline to 18.6°C after 10 minutes and only reached normothermia after 45 minutes.

"Overshoot" does not happen at all temperatures and under all circumstances. These *S.pratensis* did not exhibit it while arousing naturally at 20°C (chapter 3) and did not do so under forced arousal when the temperatures were lower. Not all mammals show this either: *Peromyscus leucopus* studied by Vogt *et al.* (1983) did not do so while arousing naturally but the *Zapus princeps* studied by Cranford (1983) did. It is not clear, however, whether the latter animals were forcibly aroused: it seems that since they had their oral and rectal temperatures measured every minute then they probably were. Overshoot may only be a phenomenon of forced arousals.

The results from 0°C ambient temperature are especially interesting as all the mice showed a fairly substantial drop in body temperature up to 20 minutes but only some of them were able to

raise their body temperatures again after this. All these animals showed the same pattern of arousal but obviously some were more capable than others of producing heat when needed. It is unlikely, however, that many of these animals could have survived long at 0°C as no animal managed to elevate its body temperature over 25°C and only two of the six managed 25°C.

Petter (1966) reports that *S.opimus* could not survive the night at 12°C but does not say whether they were torpid or not: it is assumed that since all his other animals were torpid these two were also torpid. These animals may have tried to arouse from torpor in the same manner as the *S.pratensis* studied here but in the same way may have been unable to do so. Similarly Bellier & Gautun (1968) report finding dead *S.opimus* in burrows. They attribute this to the animals dying while torpid but could not give any indication as to whether this was due to cold.

Forced arousal from torpor in other species takes a variable amount of time depending on the species concerned, the initial body temperatures of the animals, and the ambient temperature. Bartholomew & Cade (1957) report *Perognathus longimembris* taking 20 to 30 minutes to arouse from torpor at an ambient temperature of 20-22°C. This was a rate of 0.6°C per minute and the animals did not show any overshoot. Bartholomew & MacMillen (1961) found that *Microdipodops pallidus* forcibly aroused at the same ambient temperature with different starting body temperatures had similar rates of temperature gain (0.5 to 0.8°C/min). This implies that the rate of arousal, for at least these species, is reliant on the ambient temperature at which it occurs and not on the animal's initial body temperature.

The mean result obtained here of 0.41°C/min. (table 5.1) for a fat mouse to arouse at 20°C is lower than any of the results found by the above authors for other rodent species. In the light of the low metabolism found earlier in this species this is not surprising as the animals would not be able to produce as much heat through metabolism as these other species and so would not be able to raise their temperatures as quickly.

Most of the small mammals which enter daily torpor have critical lower temperatures from which they cannot arouse if their body temperatures drop below them (Hudson 1978). This seems to be related to a problem with maintaining potassium and calcium ion levels in the cells (Wang & Wolowyk 1988).

Members of the *Peromyscus* genus die when their body temperatures drop to between 13 and 17°C, depending on the species concerned, but they can generally regulate their temperatures well above ambient without arousing (Hill 1983). One exception to this rule is that starving *P.eremicus* tend to lose control of their body temperatures at low ambient temperatures

(MacMillen 1965). Torpid *Mus musculus* have critical body temperatures from 16 to 19°C, but again they can regulate to keep their body temperatures above this (Hudson & Scott 1979). Critical temperatures for small mammals are generally 15 to 20°C (MacMillen 1965).

Unlike other small mammals which show torpor, *Steatomys pratensis* seem unable to regulate their body temperatures to remain above their thermal minimum. That they also could not arouse to normal body temperatures at ambient temperatures under 10°C was perhaps not surprising given that euthermic animals (discussed in the last chapter) were unable to remain normothermic at temperatures under 10°C.

10°C thus seems to be the limit of survival for both torpid and euthermic *S.pratensis*. Below this they cannot produce enough heat by metabolic reactions to keep their body temperatures above 30°C, even though they have normal thermal conductances (chapter 4) and are thus not losing heat any faster than any other small mammal.

Time taken until the animal's body temperature reaches critical level is presumably dependent on the original body temperature of the animal. When the temperature in the "constant" temperature room went down to -5°C and all of the torpid animals died, the euthermic ones survived presumably by having a higher initial temperature and so conserving this longer. It is also a possibility that the euthermic animals responded to the drop in temperature before the torpid ones and could therefore start high levels of metabolic heat production before the torpid ones.

**Natural arousal from torpor**

In chapter 3 it was seen that the time taken to restore body temperature to normothermia at 20°C was at least 40 minutes and more often over an hour. When under stress the animals in this experiment managed to raise their temperatures within 20 minutes.

It is thus obvious that a fat mouse can arouse from torpor almost twice as fast as it usually does; this may be very important at times in the animals life to permit a response to danger.

The speed of natural arousal (0.16°C/min., table 5.1) is about normal for an animal of this size. Hudson & Scott (1979) report a rate of 0.11 to 0.25°C/min. for *Mus musculus* which they describe as slow owing to the small amount of BAT carried by these animals. Interestingly, they also describe this as independent of the ambient temperature at which arousal took place, although the range of temperatures (12.5 to 20°C) was quite small. Wang (1973) gives the rate

of arousal of *Spermophilus richardsoni* as 0.14°C/min. and Gaertner *et al.* (1973) that of *Peromyscus leucopus* as 0.20°C/min..

Although they probably could not arouse from torpor fast enough to escape a predator, the fat mice have a high-pitched "scream" which may serve to repel predators. The captive animals hardly ever vocalised when euthermic, and only did so occasionally when torpid, but Genest-Villard (1979) remarks that this is a common happening amongst the wild *S.opimus* which she studied. Anadu (1979) also remarks on this in *S.jacksoni*. A high-pitched "scream" can help to repel mammalian predators (Baker pers. comm.) and must therefore have some survival value.

A high pitched squeak has also been noted in torpid *Perognathus longimembris* (Bartholomew & Cade 1957), which these authors suggest is an aggressive response to a conspecific to maintain its territory. *S.pratensis* certainly did use this squeal in aggressive encounters with conspecifics but never while torpid. Squealing has also been noted in torpid *Spermophilus beecheyi*, but never when the animals were euthermic (Davis & Swade 1983).

### 5.4.2 Non-shivering thermogenesis

Non-shivering thermogenesis (NST) can be affected by many factors: it is species-specific, it is highly dependent on the acclimation of the animal, and can be increased by raising the amount of protein in the diet (Jansky 1973).

The magnitude of NST in hibernators and animals showing short-term torpidity is generally much greater than in non-hibernators (Jansky 1973), and the effect of cold-acclimation is much less. In hibernating mammals NST can supply from 45 to 80% of the total heat needed to bring the body temperature to normal (Jansky 1973).

Cold-acclimation can change the amount of NST able to be produced by the animal, probably by increasing the total mass of the BAT (Jansky 1973; Hill 1983). Some species of *Peromyscus* can double the amount of NST they can produce in winter over their summer level (Hill 1983). In warm acclimated animals heat production is mostly by muscular shivering, although in very cold situations both methods of heat production are used and can be additive (Jansky 1973). A long scotophase can also increase NST, probably mediated by the pineal gland and melatonin secretion (Hill 1983; Haim & Fourie 1980b).

In his review of NST, Jansky (1973) notes that the amount of noradrenaline given the mammal can have an effect on the amount of NST measured. Each mouse in these experiments was given the same dose per body mass. Since one of these animals failed to recover it seems that this was probably the maximum dose possible.

The amount of NST a species can produce is related to the amount it would need to produce under field circumstances. If the species is subjected to wide fluctuations in ambient temperature then the animals need a high level of NST, especially if their thermal conductance is also high (Haim 1981).

NST can range from 20 to 30% of BMR in warm acclimated rats (Jansky 1973) through 125% of BMR in white mice acclimated to 28°C (Jansky 1973), 189% in white mice acclimated to 20°C (the temperature at which maximum NST is reached) to more than 300% BMR in some bats (Heldmaier 1971). Heldmaier (1971) also came to the conclusion that the magnitude of NST increases with decreasing body weight in mammals.

Typical examples of some southern African small mammals are: *Lemniscomys striatus*, a mesic species, 329% of basal metabolism (Haim 1981), *Praomys natalensis* 345% and *Rhabdomys pumilio* 520% (Haim & Fourie 1980a). The authors explain the difference in the latter two results (although the animals are roughly the same size) as *P.natalensis* having more insulation and therefore needing less NST. *Otomys irroratus* has NST 310% of basal which is high for an animal this big (Haim & Fairall 1987).

The minimum oxygen consumption of 0.60 ml $O_2$/g.hr found here (table 5.2) compares favourably with the minimum consumption of 0.50 ml $O_2$/g.hr found in chapter 2. NST of 2.20 ml $O_2$/g.hr (table 5.2) or 369% of RMR is normal for an animal this size (Hart 1971, graph on p.65). It also compares favourably with the other southern African rodents mentioned earlier. Since all the animals became hyperthermic they must have been unable to lose the excess heat produced by the NST. This lends credence to the hypothesis that the low metabolism and the low body temperature are because the animals cannot lose excess heat and have a low rate of heat production to cope with this, although the converse may also be true.

NST of a cold acclimated animal should approximate $30(\text{mass})^{-0.454}$ (Haim & Fairall 1987). For an animal the size of *S.pratensis* this should then be 6.10 ml $O_2$/g.hr (mass 33.42 g). The difference between these results is caused by the extremely low basal metabolic rate in *S.pratensis*, as well as the relatively high acclimation temperature.

**Maximal oxygen consumption**

Maximal oxygen consumption as measured during metabolic rate measurements (chapter 2) should be the resting metabolic rate at the point at which the body temperature starts declining on the RMR figure, since at this point the mice are producing heat maximally. This would be the metabolic rate at an ambient temperature of 15°C, or 2.45 ml $O_2$/g.hr, which is only slightly more than the amount measured through NST. From this, one would assume that at this temperature the animals were regulating their body temperatures with NST but perhaps with a little shivering thermogenesis. However, the fat mice managed to consume even more oxygen at 0°C (3.83 ml $O_2$/g.hr). This latter amount compares well with the amount of oxygen consumed by the torpid fat mice at 0°C: 3.71 ml $O_2$/g.hr.

It must therefore be assumed that at 0°C both the euthermic and torpid fat mice (measured in chapter 2) were producing heat through another method other than NST. It was noted that nearly all the torpid animals shivered at the lower temperatures and the euthermic animals more often were active: both are methods of producing extra heat. Neither method seemed to be more efficient as both experimental sets of animals dropped their body temperatures at 0 and 5°C.

Maximal oxygen consumption for these animals was probably thus 3.8 ml $O_2$/g.hr and was produced by both torpid and euthermic animals. This is 7.6 times the basal rate of 0.50 ml $O_2$/g.hr, according well with Hart (1971) and Gorecki & Kania (1986) who give the maximal thermogenesis as 6 to 10 times basal rate.

Maximum metabolism is also affected by seasonal acclimatisation, to a certain extent caused by the increase in NST (Hill 1983; Wickler 1980). However, Abbotts & Wang (1980) found that while the maximum metabolism did not increase throughout the year in *Spermophilus richardsoni*, NST did. They interpret these results to mean that while the animals were prepared for cold all year round, the increase in NST in winter was to warm them from torpor. Maximum metabolism is normally 4 to 8 times the minimum metabolism (Rosenmann & Morrison 1974). The maximum metabolism produced by physical effort is generally more than the maximum metabolism produced by thermogenesis (Gorecki & Kania 1986).

Although exercise can produce a lot of heat it can eliminate shivering (if the same muscle sets are used), disturb the pelage insulation, and increase the peripheral circulation such that it does not necessarily improve the heat load of the animal (Bartholomew 1982, p. 372). It is also not

clear whether exercise does increase the body temperature of the animals as it does not do so in all species (Hart 1971).

Although oxygen consumption was not measured during arousals all these animals became active as soon as they had raised their body temperatures up to a level where they could move (25°C). They interspersed bouts of shivering with bouts of activity presumably to take advantage of both methods of thermogenesis. This indicates that at least in this species these methods of thermogenesis are additive to NST and can contribute about the same amount as NST (an extra 1.60 ml $O_2$/g.hr to the 1.70 ml $O_2$/g.hr calculated from NST minus basal). Jansky (1973) gives the amount of shivering thermogenesis as about equal to NST.

Genest-Villard (1979) relates the temperature at capture of her *S.opimus* to the amount of digging they did to try and escape. Observations during this study showed that if the mouse was fighting to try and escape the body temperature went up quite dramatically.

**The thermoregulatory index**

Tomasi (1985) calculates a thermoregulatory index to indicate the ability of an animal to thermoregulate. Using the equation:

$$TI = \frac{\max MR \times \max C}{\min MR \times \min C}$$

where **'MR'** is metabolic rate and **'C'** is conductance,

*Steatomys pratensis* has a TI of 22.32 which is relatively high compared to other rodents. Tomasi (1985) gives the four small mammals he studied as from 15.6 to 23.1.

Thus although *S.pratensis* has a very low metabolic rate and cannot survive at low temperatures, it still has a relatively good ability to thermoregulate.

## 5.5 Summary

Arousal to normothermia in *S.pratensis* is accomplished within 13 minutes at 30°C, 19 minutes at 20°C, and 46 minutes at 10°C: rates of 0.69, 0.41, and 0.25°C/min respectively. In contrast, none of the animals tested could arouse from torpor at an ambient temperature of 0°C, their body temperatures rarely passing 25°C.

"Overshoot" of body temperature following arousal only occurred at 30°C and to a very much lesser extent at 20°C.

NST is produced in reaction to noradrenaline at a rate equivalent to 2.20 ml $O_2$/g.hr, or 369% of basal metabolism. Maximum metabolic rate was about twice as much (3.8 ml $O_2$/g.hr), indicating the animals ability to use other means of thermogenesis additively with NST. After injection of noradrenaline the animals became hyperthermic with body temperatures ranging from 35.7 to 39.8°C.

# Chapter 6

## Analysis of the fat deposits

### 6.1 Introduction

Although fat can account for more than half the weight of an animal, until recently it was commonly regarded as a fairly unimportant energy source (Pond 1978). Adipose tissue, the main site of fat storage in the body, is deposited in every mammal before birth, but it generally does not contain much fat until after the mammal is born and begins to metabolise fatty acids found in the mothers milk (McCance & Widdowson 1977).

There are two main kinds of adipose tissue in mammals, white and brown. Although often similar in looks to the naked eye (in many cases brown adipose tissue or BAT does look darker) both kinds can be easily distinguished histologically.

As well as the main site of energy storage in the body, white adipose tissue in the skin acts as a thermal insulator, and around the kidneys can also act as a shock absorber (Wheater *et al.* 1987). Brown adipose tissue is found mainly in newborn mammals where it seems to play a central role in temperature regulation (Wheater *et al.* 1987). Sometimes mis-called the "hibernating gland", the interscapular deposits of brown adipose tissue are responsible for raising the body temperature of a hibernating mammal from just above freezing to a temperature where the animal's muscles can assume heat production by shivering (chapter 5). It is also found in small amounts in adult mammals where it plays a role in regulating weight gain by burning off excess energy (Wheater *et al.* 1987).

In laboratory mice the main sites of white fat deposition are in the mesenteries around blood vessels, around the kidneys, adrenals, ovaries and testes, and in the axillary and inguinal region (Gude *et al.* 1982). Pond *et al.* (1984) describe 14 anatomical sites where white fat is found in guinea pigs and Pond (1986) extends these to several other species of mammal.

Brown adipose tissue is found mainly in the interscapular region but is also found adjacent to the thymus and the kidneys (Gude *et al.* 1982).

Histologically, the fat droplets in white adipose tissue accumulate and fuse to form one large droplet which in a haemotoxylin and eosin stain shows as a large empty cell with the nucleus

displaced to one side (Wheater *et al.* 1987). In brown adipose tissue the fat droplets do not fuse, there is more cytoplasm, and in haemotoxylin and eosin stained sections the cells have a frothy look while the nucleus appears in the centre of the cell (Wheater *et al.* 1987). Deposits of brown adipose tissue are usually better supplied with blood vessels than white adipose tissue (Gude *et al.* 1982).

The amount of total fat found in an adult mammal's body can vary widely depending on several factors which include season, age, sex, and available food, as well as the differences shown between species (Pond 1978). The most striking seasonal difference is the extreme amount of fat deposited by hibernators at the beginning of winter to enable them to survive until spring (e.g. McLean & Towns 1981).

There is only one reference in the literature to the amount of fat in *Steatomys* having been studied (Pond & Mattacks 1985a), but unfortunately this is only as part of a larger study and the particular species or the actual amount found is not stated. Most authors make mention of how much fat *Steatomys* has and, indeed, it is the species' most obvious characteristic, leading to its common name. The fat in these mice has been variously reported to be between the flesh and the skin (Shortridge 1934), beneath the skin and round the gut (Booth 1960), in the tissues and particularly under the skin (Smithers 1983), and all over the body (Sclater 1901), but the general concensus is that it is found mainly subcutaneously but also in the abdominal cavity. Many authors (e.g. Shortridge 1934; Smithers 1983) report on the special treatment needed for museum skins of *Steatomys* species because the fat can leach out of the skins and stain the storage drawers.

Several authors (e.g. Smithers 1975; Rosevear 1969; de Graaff 1981; Sheppe & Haas 1981) have assumed that the fat is an energy store for seasonal torpor. While this is a possibility which is discussed more fully in chapter 7, there are other explanations for the large amount of fat carried by the fat mice. The first aim was to characterise the fat by finding out where the fat deposits were, what kind of fat they were, and how much fat these animals carried.

This chapter was accordingly divided into three parts:

The first aim was to determine the gross anatomy of the fat deposits to find out if the fat in the body is an extension of normal fat deposits (in comparison to an adult *Mus musculus*) or additional deposits unique to the species.

The second aim involved histology of tissues from selected areas to determine whether the animal has brown fat as well as white fat.

The third aim was to quantify the percentage fat in *S.pratensis* and to compare it to the amount of fat in *Mus musculus*. Fat for the purposes of this thesis is that lipid which is extracted by petroleum ether.

## 6.2 Materials and Methods

### 6.2.1 Anatomy

Three deep frozen specimens of *S.pratensis* and two of *M.musculus* were skinned and the fat deposits dissected out. Annotated drawings were made and photographs (Pan F 50 ASA and Sakuracolor 100 ASA) were taken to provide the details from which the final drawings were made. Final drawings of each species are a composite of all the animals dissected.

### 6.2.2 Histology

Histological sections (2 x 3 x 3 mm) were taken from a deep frozen specimen of *S.pratensis*. The material was removed while still frozen and put straight into Zenkers-formol fixative where it was fixed while thawing. Because lipids solidify at higher temperatures than the aqueous parts of tissue, adipose tissue structure is unaltered by freezing (Pond 1986).

All sections for histology were taken from F14, an animal which died when the temperature in the animal room dropped to -5°C.

A total of 5 sections were taken. These were:

S1: a section subcutaneously in the interscapular region.
S2: a section just under this.
S3: a section just under the skin of the ventral portion of the neck.
S4 and S5: a piece of skin from the back of the animal above the flank.

After fixing, the specimens were embedded, sectioned on a microtome to 10 μm, mounted, and stained with haemotoxylin and eosin. Details of staining are given in Appendix C. Haemotoxylin and eosin were used as opposed to a lipid stain as it was thought that the fat would be just as easily identifiable with this method as with any other, and the surrounding tissues would also be easy to identify.

The sections were photographed at magnifications of 20, 100, and 200 times with a Zeiss photomicroscope. Ektachrome 64 ASA slide film was originally used, prints being made from these slides, but the results were unsatisfactory and eventually a Fuji 100 ASA print film was used to give the final results.

### 6.2.3 Total body fat levels

10 deep frozen *S.pratensis* and 7 deep frozen *M.musculus* were weighed, degutted, cut up, and freeze dried. The animals were gutted because the original plan was to include animals that had already been killed for gut analysis and consistency was necessary when comparing the specimens. There were no large fat deposits in the intestines that were removed and it was thought that the removal of them would not adversely affect the results.

The carcases from a preliminary trial were oven dried at 60°C, but it was found that the fat became liquid and probably volatile and so impossible to deal with. Freeze-drying in a Freezemobile 6 freeze-dryer (Virtis Co Inc) was then employed.

The first set of specimens were freeze-dried for two days, removed from the freeze-dryer and weighed, and replaced for a further day. When it was found that there was no difference in the weights of the specimens between the two days it was decided that two days freeze-drying would be adequate. After freeze-drying the specimens were placed in a desiccator until being ground for further analysis.

The specimens were ground in an analytical grinder and placed in dried Whatman thimbles for Soxhlet fat analysis. Petroleum ether (60 - 80°C) was used to extract the fat for eight hours, after which the amount of fat lost was ascertained both by measurement of the amount of weight lost by the dried and dehydrated thimbles, and by evaporation of the petroleum ether from the flasks and the residue measured. Evaporation from the flasks did not give as consistent or as meaningful results as the dried weights of the thimbles: the latter was thus used.

## 6.3 Results

### 6.3.1 Anatomy

The skin of the fat mice was very thick and clearly carried a large amount of fat. When the skin was removed from the body of the *S.pratensis* large amounts of fat were obvious lying on the surface of the carcase. These were relatively easily dissected into their component deposits. There were two differing groups of deposits, those on top being slightly yellower and thinner than those lying underneath, which were white and very thick.

Figure 6.1 shows the main fat deposits in *S pratensis*.

There were three overlying and thinner fat deposits: anterior to the fore leg, posterior to the fore leg, and lying lateral to the spine close to the tail.

The thicker underlying deposits lay posterior to the fore leg extending towards the spine and joining up with the deposits lying across the spine just posterior to the ears; anterior to the hind legs; posterior to the hind legs and around the tail; and along the spine from the base of the tail to the mid-dorsal region.

Internally the fat mice had two large ovoid fat deposits lying between the muscle wall of the abdomen and the intestines, and stretching along the base of the abdominal cavity. These deposits were noticeably smaller in the leanest animal dissected. The kidneys were extremely well embedded in fat, in one case to such an extent that they were hardly visible. In this animal the fat also extended down the sides of the abdomen.

In contrast, *Mus musculus* had much less visible fat in smaller and thinner deposits. The skin of the white mice did not carry any obvious layer of fat.

Fat deposits between the skin and body wall were found anterior to the fore legs; posterior to the front legs; anterior to the hind legs; posterior to the hind legs; in the muscle of the hind legs; and along the spine just posterior to the ears. This latter deposit also joined up with the deposit in front of the front legs. Figure 6.2 shows the main fat deposits in *Mus musculus*.

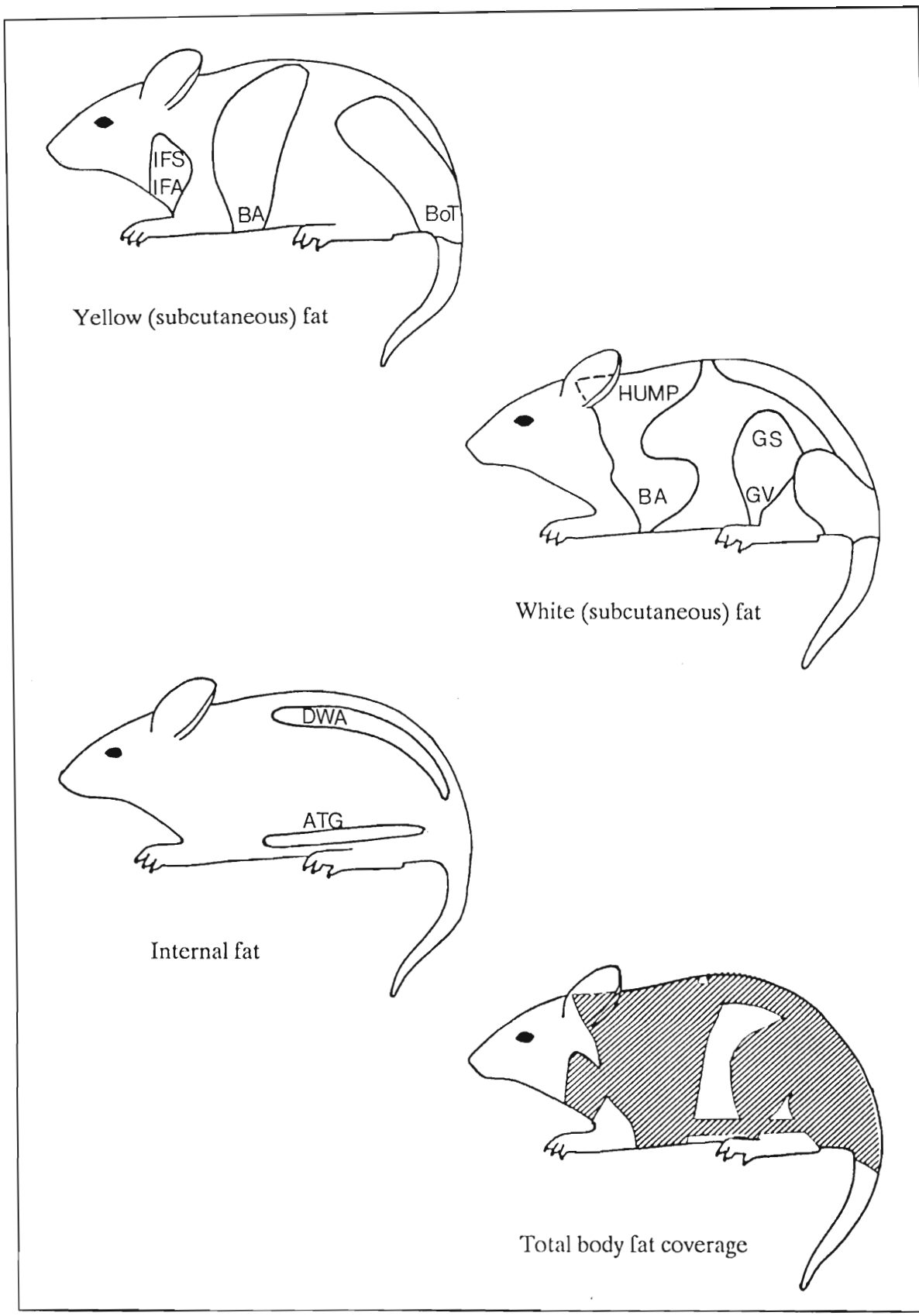


**Figure 6.1** The position and coverage of the fat deposits in *Steatomys pratensis*. (The initials refer to the fat deposits described by Pond *et al.* (1984) and are explained on pages 101 and 102.)

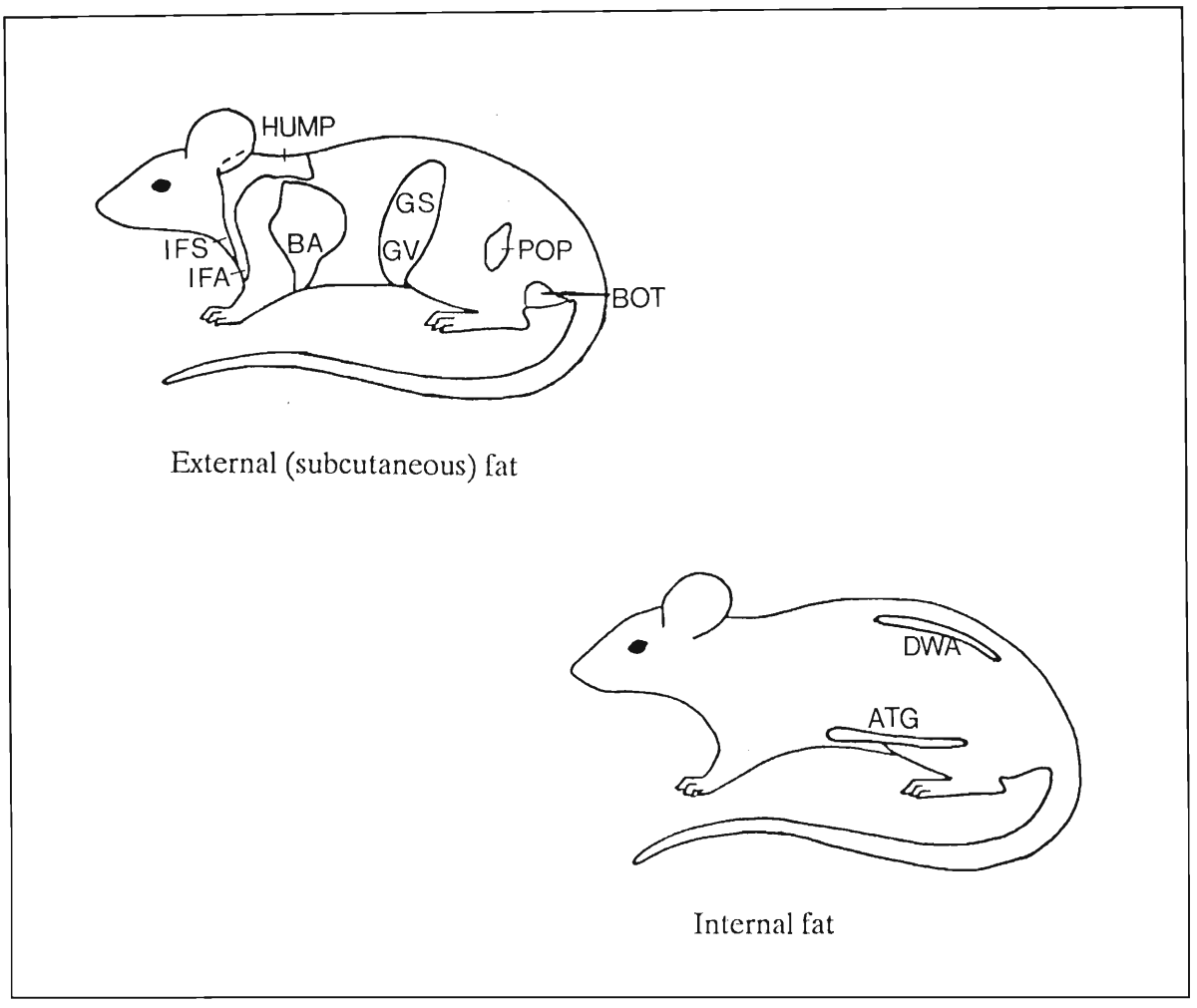


**Figure 6.2** The position and coverage of the fat deposits in *Mus musculus*.
(The initials refer to the fat deposits described by Pond *et al.* (1984), and are explained on pages 101 and 102.)

Internally the laboratory mice did not carry as much fat as the fat mice. They also had some fat in the layer between the muscle wall of the abdomen and the intestines, and the kidneys were embedded in fat, although not to the same extent as they were in *Steatomys pratensis*.

From these results it can be seen that the fat deposits in both *S.pratensis* and *M.musculus* are qualitatively similar but differ quantitatively.

### 6.3.2 Histology

All sections except S2 were found upon microscopical examination to contain white adipose tissue. Section S2 was brown adipose tissue (BAT) adjacent to the white adipose tissue. It is

thus assumed that all the fat found in discrete deposits in the fat mice, with the exception of the BAT in the interscapular region, was white fat. There was no noticeable difference at the cellular level between the slightly yellowish fat found immediately under the skin and the thicker deposits of white fat found closer to the body.

Plate 6.1 shows a section through section S2 showing the BAT and white adipose tissue separated by a blood vessel. Plate 6.2 shows a section through section S5 of the skin of *S.pratensis*, showing the extensive fat layer.

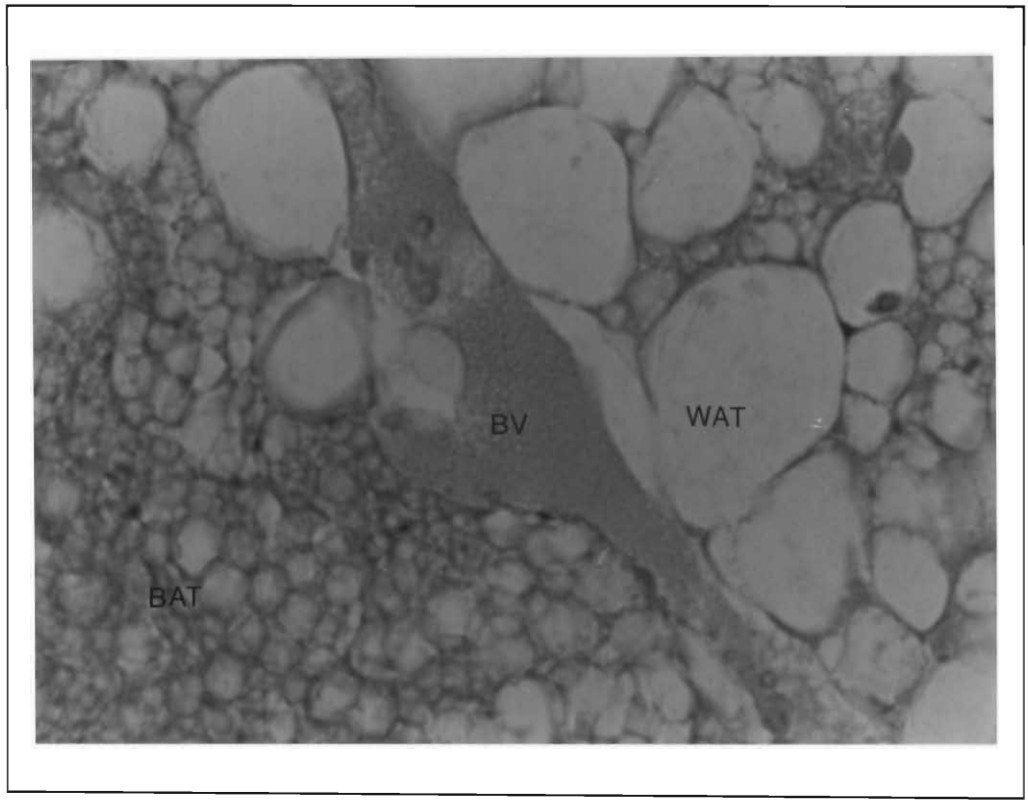


**Plate 6.1** A section through the fat of the interscapular region. BAT: brown adipose tissue; BV: blood vessel; WAT: white adipose tissue.

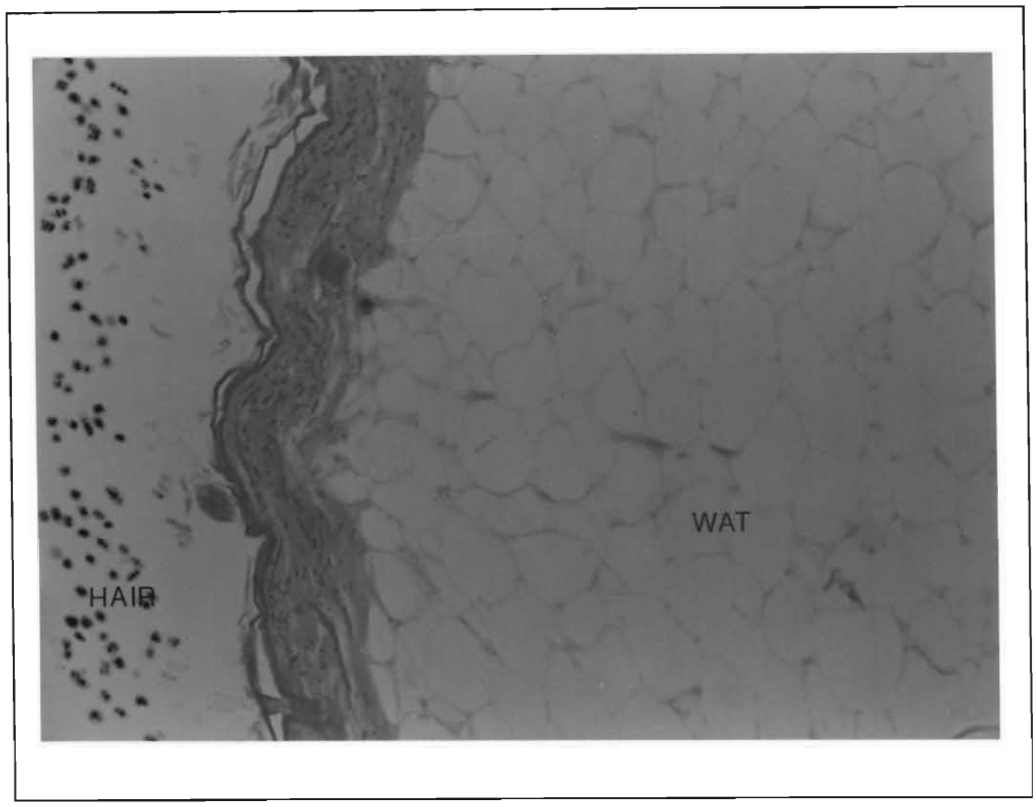


**Plate 6.2** A section through the skin showing the extensive fat layer. WAT: white adipose tissue.

### 6.3.3 Total body fat concentrations

Total amounts of fat expressed as a percentage of dry weight were calculated for each animal. The results are summarised in Table 6.1.

**Table 6.1** Amount of carcase fat found as a percentage of dry body weight

| | min | max | mean | SE |
|---|---|---|---|---|
| *M.musculus* | 24.45 | 41.53 | 34.03 | 2.15 |
| *S.pratensis* | 25.20 | 77.08 | 50.45 | 5.18 |

These results are significantly different from each other (t-test, $P < 0.05$).

Correlation coefficients were computed between the mass of the animals (both *S.pratensis* and *M.musculus*) and grams of fat, percentage fat of dry mass, lean mass, percentage water, and grams of water. A summary of the results are shown in Table 6.2.

**Table 6.2** Composition of fat and water of *S.pratensis* and *M.musculus* carcases.
(Results given here are the average results from all the animals of that species.)

| | mass | g fat | % fat dry mass | lean mass | % water | g water |
|---|---|---|---|---|---|---|
| *M.musculus* | 26.34 | 3.32 | 34.03 | 6.41* | 62.97 | 16.61* |
| *S.pratensis* | 35.85 | 9.14* | 50.45 | 7.29 | 55.19 | 19.42 |

(* denotes a statistically significant correlation with mass)

Amongst the *S.pratensis* the only statistically significant correlation was between body mass and grams of fat (Pearson's correlation coefficient = 0.755, $P < 0.05$), indicating that the larger the animal the more fat it carried.

In contrast the correlation among the laboratory mice was between body mass and lean mass (Pearson's coefficient = 0.953, $P < 0.001$), and body mass and grams of water carried (Pearson's coefficient = 0.960, $P < 0.001$). This indicates that the larger these animals become, the larger their lean body mass.

There was a negative correlation (Pearson's coefficient = -0.718, $P < 0.05$) between the percentage fat carried by the fat mice and their age in months. Animal age at death ranged from 6 to 43 months. There was no correlation between percentage fat and the sex of the animals of either species.

## 6.4 Discussion

### 6.4.1 Anatomy

Until the recent work of Pond *et al.* (1984) and Pond & Mattacks (1986), fat had been generally regarded as a fairly amorphous tissue with little structure. Since then Pond and co-workers have shown that not only is fat found in discrete and homologous areas, but different fat deposits may also have different physiological functions (Pond 1986).

Pond *et al.* (1984), Pond & Mattacks (1985b), and Pond (1986) define 11 sites of adipose tissue deposition in several different animal species. The main sites of white adipose tissue deposition in mammals, with the initials used to distinguish them, are: (i) on the dorsal wall of the abdomen, kidney and gonadal fat - DWA (ii) that attached to the guts - ATG (iii) side of the groin, left and right - GS (iv) groin ventral - GV (v) behind the arm, left and right - BA (vi) in

front of the arm, left and right - IFA (vii) in front of the shoulder, left and right - IFS (viii) under the neck muscles, left and right - UMN (ix) interscapular and dorsoscapular - HUMP (x) at the base of the tail - BOT and (xi) in the popliteal muscle - POP.

*Steatomys pratensis* has substantial amounts of fat in all these deposits except under the neck muscles and in the popliteal region, where the deposits may have been overlooked due to their small size. In addition to these deposits, the fat mice also have an extra deposit lying posterior to the fore leg, another at the base of the tail, and one lying along the spine from just above the base of the tail towards the mid-dorsal area. These mice also have an extremely thick layer of fat in the skin. It is not clear whether the second deposit of fat posterior to the fore leg is an extra deposit or an extension of the fat from the hump area, but the secondary deposits at the base of the tail and just above it are not extensions from anywhere and are extra deposits.

In general, these results confirm Shortridge (1934), Booth (1960), and Smithers' (1983) assertions that the fat is found mainly in and under the skin, but also in the body cavity. These results are also similar to those found by Taylor (1984) in *S.krebsii* where the fat was "packed around the limbs, flanks, neck, tail, and in the interscapular region. Internal fat is associated with the gonads, the kidneys and the heart."

*S.pratensis* thus has similar fat deposits to other rodents (Pond 1986) but also has extra depots which are not commonly found in small mammals. However, many larger mammals have extra fat deposits which overlie the "key" deposits, and which can carry a considerable proportion of the total body fat (Pond 1986). In particular a substantial proportion of the fat in the horses and the lion, also found in donkeys, lioness and camel, as studied by Pond (1986) was in the form of a thick layer on the inner ventral wall of the abdominal cavity. It thus seems that *S.pratensis* has characteristics of fat normally shown by larger mammals, especially with respect to the large deposits of fat on the inner wall of the abdomen.

*Mus musculus* also had deposits (i) to (vii), and (ix), although in much smaller amounts than found in *Steatomys pratensis*. Again, the fat in the neck muscles was not found but the popliteal fat was seen in these mice. There were no extra fat deposits found. These results were very similar to those results found by Wheater *et al.* (1987) in the laboratory mouse. According to Pond (1978), *Mus musculus* has seasonal fluctuations in the peri-renal fat, and the females have much greater deposits of interscapular, axillary, and visceral fat. These differences were not seen in this study, although Pond may have been referring to the wild form of *Mus musculus* which would probably be subjected to a variable diet throughout the year.

**The insulation problem**

Different species of mammals generally have their fat concentrated in different sites, for example most mammals have about half the adipose mass within the abdominal cavity but in the Carnivora intra-abdominal sites account for less than 10% of the total fat (Pond & Mattacks 1985b). These differences can be related to postural and locomotory habits (Pond & Mattacks 1985b). Since there is a limit to how much fat an animal can carry in any one deposit before it becomes unmanageable, especially when that animal might have to manoeuvre along burrows, it may be advantageous to spread the load amongst several sites. It does not necessarily follow that fat in a layer over the body is primarily for thermoregulation since if the fat is a food reserve it will function just as efficiently in a layer under the skin as anywhere else (Pond 1978).

The uses of thick layers of fat under the skin in several species of mammal are discussed later in this chapter.

The fat in the fat mice, however, is a thick layer which covers the body to such an extent that even if it had not evolved as insulation it must do so by virtue of it's thickness and position. It is thus possible that even if the fat were to be used as an energy store the animals may find themselves with a problem in heat loss at times.

### 6.4.2 Histology

From plates 6.1 and 6.2 it can be seen that while the main amount of fat in a fat mouse is white fat, used either for energy storage, for thermoregulation, or for both, the interscapular deposits of brown fat are not inconsequential.

While white fat is an energy source which can be used by any biological tissue, brown fat is only for producing heat into the blood (Jakobsen 1981).

In chapter 5 it was seen that *Steatomys pratensis* could use NST to raise the body temperature to normothermia from torpor. This comes about by using the brown fat to produce heat. However, it was also seen in chapter 5 that the fat mice could not arouse from torpor at temperatures below 10°C and could not keep their euthermic body temperatures normal at ambient temperatures under 15°C. This seems to indicate that even though there are ample deposits of brown fat in the fat mice, they cannot use them efficiently enough to survive lower temperatures.

The inability of these animals to survive low temperatures is probably partly due to the problem of the loss of $K^+$ ions when the body temperature drops too low, and partly due to inability of the animals to produce enough metabolic heat to raise the body temperatures even when their temperature is dropping below dangerous levels.

### 6.4.3 Body fat levels

Fat is known to be the most variable constituent of body tissue, ranging from undissectible levels to more than half of the live weight of the animal (Pond 1978) and is often used as an indication of the condition of the animal (e.g. Riney 1960). It has been noted that most species need a certain level of fat before they can undertake physiologically stressful activities, such as reproduction (Pond 1978) or hibernation (Phillips 1979).

Lindstedt & Boyce (1985) give the amount of fat in a eutherian terrestrial mammal to be:

$$\mathbf{Mf} = 0.075\ \mathbf{Mb}^{1.19}$$

where **Mf** is the amount of fat in kg and
**Mb** is the total body mass in kg.

Using this equation, the *S.pratensis* used here, having a mean mass of 35.85 g, would be expected to carry 5.31 g of fat. The actual mean mass of fat carried by these animals was 9.14 g, almost twice as much. Although many mammals put on an extreme amount of fat in the laboratory (McCance & Widdowson 1977; Rothwell & Stock 1986) it is unlikely that the fat mice were overfat in this study since the maximum mass ever measured was very close to the maximum mass found by Smithers (1983) in wild caught animals (see chapter 2 and appendix A).

The *M.musculus* had much less fat: with a mean mass of 26.34 g these animals should, according to Lindstedt & Boyce (1985), have carried 3.68 g of fat and actually had a mean fat mass of 3.32 g. The results found here of 9.01 to 19.53% of the fat free body mass as fat also compare well with Pitts (1978), who gives the mean percentage body fat of mice as 10 to 20%, with genetically obese (*ob ob*) mice having 45 to 60%. These latter results fall within the *S.pratensis* range of 8.73 to 98.92% of fat free body mass.

Jakobsen (1981) states that the amount of fat in a *Mus musculus* can be affected by its previous thermal history, but in this case the thermal environment is probably unimportant as neither species of rodent were kept in any extreme environment.

Sheng & Huggins (1979) give an equation of:

100-%total body water/0.732 = %fat

to calculate the amount of fat an animal should be carrying. By this equation the *S.pratensis* (with a body water of 55.19%) would have a fat content of 24.60%. This is very close to the 24.16% (of lean wet weight) actually found. Similarly the *Mus musculus* would be expected to have a fat content of 13.97% and actually had a fat content of 12.71%.

Other animals have widely varying fat levels. A selection of fat levels of rodents taken at random from the literature is summarised in figure 6.3. For this example the fat is expressed as a percentage of the lean dry mass of the animals.

From figure 6.3 it can be seen that the amount of fat in rodents varies widely. Amongst the non-hibernators and those which go into daily torpor the fat levels range from 8 to 40%, whilst amongst the hibernators the fat levels range from 125 to 212%. In this study the fat levels in *S.pratensis* ranged from 34 to 336% and those in *M.musculus* from 32 to 71%. The results found in this study are thus generally higher than those taken from the literature, but it must be remembered that these animals were laboratory-reared and were all in good condition; the other species studied in figure 6.3 were wild caught and must therefore include animals in poor condition which would lower the overall mean results.

Morrison (1960) gives the maximum fat that can be carried by any animal as 50% of the total body mass. The maximum fat found in the *S.pratensis* here was 49.73% of the fresh mass. Pond & Mattacks (1985a) give the average fatness of the non-ruminant herbivores they studied (primates, lagomorphs, rodents, and perissodactyls) as 10.21% of gross (wet) body mass: mean results found here for *M.musculus* were 12.72% and for *S.pratensis* 24.16%.

These comparisons show that while the laboratory mice have entirely normal levels of fat, the fat mice have normal to very high levels.

Since there was a positive correlation between fresh (wet) body mass and the mass of fat carried by the *S.pratensis*, the larger these animals become the more fat they carry. However, since the range of fat-free (wet) mass was from 18.16 to 34.04 g, the fat-free body mass does not seem to be a stable amount. This contradicts the work of Pitts (1978), who postulated a regulation of body size and amount of fat carried in animals. In contrast, there was a positive correlation between body size and lean mass in *M.musculus*, which combined with a positive correlation between body size and amount of water in the body does seem to indicate some regulation of the body size and amount of fat stored as postulated by Pitts (1978).

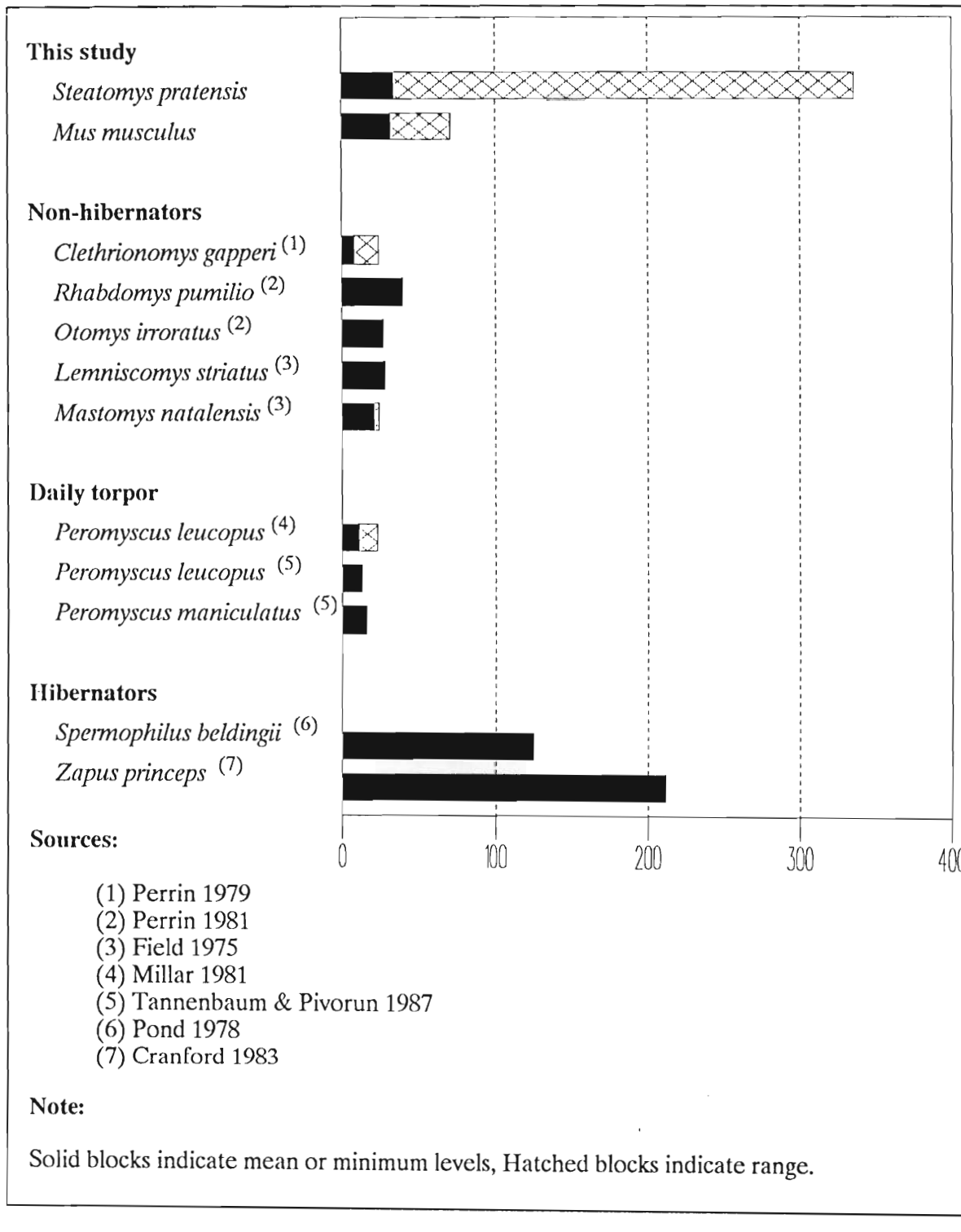


**Figure 6.3** A summary of the amount of fat in several randomly chosen rodents in comparison to *Steatomys pratensis* and *Mus musculus*. The mass of fat is expressed as a percentage of the lean dry weight of the animals. Results are rounded off to the nearest whole number and may have been calculated from the original papers.

There was an extremely wide range of results in the percentage of fat found in the *Steatomys pratensis* compared to the *Mus musculus*, and to all the other species mentioned in figure 6.3. This may have been because the fat mice encompassed a much wider range of ages than the white mice but may be simply that some adult animals may naturally be heavier than others. It is possible that this could mean that some animals could survive times of food shortage better than others in the population, but it must also be remembered that these animals can, and do, hoard food. These results indicate that the fat mice have a lower priority of regulation of fat mass as is postulated by Pitts (1978) for laboratory mice and other animals. An interesting comparison is that whilst some of the fat mice had the same amount of fat as the non-hibernating rodents and *Mus musculus*, some of them had much more fat than the hibernating rodents just before going into hibernation: this is an enormous amount of fat for such small rodents.

Genest-Villard (1979) also found a great variation in the body masses of adult *S.opimus*. Up to 100 mm head-body length the masses were proportional to the length but after this the masses varied from 30 to 50 g. Although she does not say this is directly accountable to the amount of fat the animals were carrying she remarks that all the animals caught were carrying a large amount of fat, even young animals.

Regulation of fat is generally related to the need of the animal to store fat, i.e. animals living in ecological habitats where there is an abundant and regular food supply do not need to store as much fat as animals which live in ecological niches where the food supply varies widely and at times may be absent (McNab 1968; Pond 1978; Millar 1981). Perrin (1981) relates the low level of fat in *Otomys irroratus* to its regular and abundant food supply and the higher level of fat in *Rhabdomys pumilio* to its irregular diet.

Using this hypothesis, and assuming that the fat in *S.pratensis* is used as a food reserve, then these animals may have to face conditions of extreme deprivation at times in their lives. Ability to survive food deprivation is discussed in chapter 7.

Young *S.pratensis* are born without any visible deposits of fat in their white adipose tissue, but these start to build up immediately the young begin to suckle and are noticeable within twelve hours of birth (refer to appendix A). In this respect they are similar to most other mammals, the young of which do not lay down any fat in their white adipose tissue before birth (McCance & Widdowson 1977). Youngsters of six months carry much fat and the fattest animal in this study was only 6 months old.

Percentage carcase fat and age at death were negatively correlated meaning that the younger animals carried proportionally more fat than the older ones. Perrin (1981) found a similar correlation between fat content and age in *Rhabdomys pumilio*, as did Millar (1981) in *Peromyscus leucopus*. Millar (1981) relates this to the probability that the younger animals may face a food shortage during dispersal from the nest and thus may need the extra energy. Similarly these mammals may need a higher fat reserve in winter because they are more likely to face food shortages. Indeed, Murie & Boag (1984) show that heavier *Spermophilus columbianus* juveniles last through hibernation better than their leaner relatives.

It seems probable that *Steatomys pratensis* young may face a food shortage when leaving the nest at the end of their first winter (see appendix A). The amount of fat carried by these youngsters may have ecological implications for their survival.

McNab (1968) considers the effect of fat on the metabolic rate of several desert rodents. Other than the obvious effect of much fat making the animals heavier, and so changing the mass specific metabolic rate, he finds no evidence to suggest that excess fat gained in the laboratory could affect the metabolic rate to the extent shown by several species.

### 6.4.4 Fat as a source of energy

Animals which eat perishable food (insects, greens, etc) must store their energy in the form of fat and cannot rely on hoarding to tide them over winter months.

Although under conditions of extreme starvation animals can use structural proteins for energy (Lindstedt & Boyce 1985), in general, body fat constitutes the main energy source for mammals. Indeed, the amount of fat in an animals body is often used as an indicator of the condition of the animal (Riney 1960).

Several authors (e.g. Smithers 1975; Rosevear 1969; Sheppe & Haas 1981) have assumed that fat in fat mice is an energy store for hibernation or aestivation.

Although several authors assume that a thick layer of subcutaneous fat (in, for example, marine mammals) is for thermoregulation (e.g. Schmidt-Nielsen 1975 p.324) this view is increasingly coming under critical review. Pond (1978) severely questions this assumption and points to many cases where this does not seem to apply. For example, the blubber layer of many migratory whales is thinnest when they enter the Arctic to feed for the winter and thickest when

they leave the Arctic for the warmer waters of the tropics where they do not feed. If the fat were for thermoregulation then this pattern of fat deposition would be reversed. Subcutaneous fat need not necessarily be for thermoregulation: *Castor canadensis*, the beaver, lays down a thick layer of fat uniformly over the trunk before winter which is then used as an energy source over the winter period (Pond 1978).

Gaskin (1972, p.14) claims that the fat in cetaceans is not for insulation, but for energy. However, Innes (1986) shows that the fat in a whale can certainly play an extremely important role in temperature regulation. A possible compromise has been postulated by Pond (1978) for the grey whales (*Eschrichtius robustus*) which lay down their blubber fat first but absorb the internal fat first. *Steatomys pratensis* could similarly use the skin fat layer as an energy source but not until the internal fat had been used up. Certainly the leaner animal had a smaller amount of fat between the muscle wall of the abdomen and the gut. Another way of determining the importance of the fat layer in thermoregulation would be to find out the rate of fat turnover: a high rate of turnover would indicate the fat was being used for metabolic energy while a low rate would tend towards thermoregulation. This, however, was beyond the scope of this thesis.

From the results found here it is possible to calculate a theoretical range of fat for a fat mouse to store. The minimum amount of fat carried here was 2.97 g and the maximum was 22.23 g. The minimum percentage fat that an animal needs to survive is 8% lean dry weight (Evans 1973; Perrin 1979), and using the average fat mouse value of 7.28 g fat it can be calculated that the minimum amount of fat needed by these fat mice would be 8% of 7.28 g, or 0.58 g of fat. Thus these animals have a range of 2.39 to 21.65 g of fat to survive on before dying of starvation.

Using a value of 37.66 kJ/g of fat from Perrin (1981) this means that a fat mouse could have 90.01 to 815.34 kJ of potential food stored in its fat. These results are discussed further in chapter 7.

There are thus obvious advantages and disadvantages of having a thick subcutaneous fat layer, with the advantages being increased time of survival without food and retention of body heat in times of cold, and the disadvantages being difficulty in losing heat in times of heat stress.

### 6.5 Further work to be done in the field

In retrospect it would have been interesting to have measured the amount of fat in various deposits, especially the interscapular BAT. This would have allowed more comparisons with the

work on other animals as carried out by Pond and co-workers (Pond 1978, 1986; Pond & Mattacks 1985a, 1985b, 1986; Pond *et al.* 1984). However, these measurements would be better carried out on wild-caught mice as it remains a possibility that the mice in this experiment were affected by their captive conditions, especially the feeding regime.

Unfortunately no measurements were taken of adipocyte volume. In retrospect this could have determined whether the fat mice were fat by adipocyte enlargement or proliferation (Pond & Mattacks 1985a). This information could have been used to determine whether these animals are adapted for a high protein or a high carbohydrate diet.

The low melting point of the fat in the fat mice studied here is interesting: it might be worthwhile continuing this line of research as Hill (1983) mentions that some *Peromyscus* species may accumulate subcutaneous fat of a lower melting point than that found in summer. Little more is known about the reasons for this, but changes in the liquidity or solidity of the subcutaneous fat could have a great influence on the thermal conductance of the animals and also on their ability to move at low temperatures.

### 6.6 Summary

The fat deposits in 3 *S.pratensis* and 2 *Mus musculus* were dissected out and compared with each other and with the results of Pond *et al.* (1984) and Pond (1986). Except for the deposits under the muscles of the neck both animals carried the normal deposits of fat. *S.pratensis*, however, had extremely substantial deposits, and also had three extra deposits, one posterior to the fore leg, and two at the base of the tail.

Most of these deposits consisted of white fat but there were also good deposits of brown adipose tissue in the interscapular region.

There was an extremely wide range of the amount of fat in the fat mice (25.20 to 77.07% of dry weight) compared to the white mice (24.45 to 41.53%). Body mass of *S.pratensis* is positively correlated with grams of fat carried whilst in the *M.musculus* the correlation was between body mass and lean mass. There was a negative correlation between the amount of fat carried by the fat mice and their age, but no correlation between the amount of fat carried and their sex.

The substantial deposits of fat in the fat mice meant that they have 90.01 to 815.34 kJ of potential energy in the form of fat stored in their bodies.

# Chapter 7

## The effect of food deprivation and water deprivation on *Steatomys pratensis*

### 7.1 Introduction

Starvation-induced torpor is a well-known phenomenon in many small mammals which undergo natural bouts of torpor, and indeed in many small mammmals which have not been shown to show natural torpor (Hudson 1978). Because torpor is an energy saving process (Wang & Wolowyk 1988) it is an understandable reaction for an animal deprived of food to go into torpor (at least at times of the day when it would not be feasible for it to forage, thus enabling it to preserve its energy for searching for food). Although at one time starvation-induced torpor was viewed as a thermoregulatory inadequacy, it has come to be regarded as an efficient physiological adaptation to the environment (Hudson 1978).

There is no direct evidence of food and water deprivation causing torpor in *Steatomys* but Hanney (1965) reported keeping one *S.pratensis* without food or water for 38 days: it is assumed that this animal was torpid although there is no direct statement to this effect. Montoya & Ambid (1978) reported that *Eliomys quercinus* kept on a diet of apple alone showed a torpor produced by protein starvation and comparable to the torpor provoked by complete starvation. Coupled with Petter's (1966) report that *S.opimus* were torpid every day when fed only on apple, it seems likely that *S.opimus* at least can become torpid in response to a shortage of protein in the diet, and thus probably to a shortage of food.

Hudson (1978) has shown that animals deprived of drinking water will stop eating, and so show results similar to animals deprived of both food and water. The amount of water needed varies widely from rodent species to species, some rodents being extremely dependent on water, and some desert species being able to survive on metabolic water alone and thus being completely free of the need to drink (Glenn 1970).

Although *S.pratensis* is often found near rivers and streams (Smithers 1983), this may be a reflection of the texture of the soil for digging and not necessarily an indication that these animals are dependent on the water for drinking. The response of any *Steatomys* species to a lack of water is unknown, although de Graaff (1981) records *S.krebsii* as being "more moisture tolerant" than *S.pratensis*.

Fat in rodents has traditionally been viewed as a variable energy store to tide animals over a period of reduced food availability (Pond 1978). This is especially the case in rodents which enter hibernation where fat reserves are known to be deposited before the animal enters hibernation and become depleted over the hibernation period (e.g. Murie & Boag 1984). Many authors assume that this is why *Steatomys pratensis* is very fat (Rosevear 1969; Smithers 1983; de Graaff 1981). The annual mass fluctuations in the animals studied here is discussed more fully in chapter 8.

Rodents which undergo daily torpor as opposed to hibernation do not necessarily have large fat deposits, but *S.pratensis* has a high level of fat compared to other wild rodents (chapter 6).

It was not known whether the fat in *S.pratensis* could act as an energy reserve for times of food shortage. After 38 days Hanney's (1965) *S.pratensis* had lost 32 percent of its body mass but remained in "good condition" and "rapidly recovered" when re-fed. This does seem to indicate that *S.pratensis* can last without food for longer than other (non-hibernating) rodents.

In general, rodents do not survive long without food: Willan & Meester (1987) report survival times of 5 days for *Rhabdomys pumilio* and 3 days for *Mastomys natalensis*. Several authors (e.g. Glenn 1970; Willan & Meester 1987; Buffenstein 1985) have given several rodent species restricted diets to test for mass loss rates, with varying success rates. These are discussed in section 7.4.

The aims of this study were to find out firstly if starvation induces torpor at any and all seasons and if so, how quickly it does so; secondly to find out if these animals could survive off their fat as Hanney's (1965) *Steatomys pratensis* did; and thirdly if they could regain their lost mass quickly.

### 7.2 Materials and Methods

The animals were kept under the same conditions described in chapter 1: in Labotec cages under natural conditions of light supplemented by fluorescent light during the middle of the day and under natural temperature.

At the beginning of each trial the animals were removed from the nestboxes they had been sharing with their mates, their rectal temperatures were taken, they were weighed and put into clean cages under otherwise normal conditions (sawdust, bedding, and their own nestbox).

The animals were subjected to four different regimes:

i) No food or water
ii) No food, but given water
iii) No water, but given food
iv) Given both food and water but deprived of a mate

Every morning each animal was taken out of its cage between the hours of 09h00 and 12h00, rectal body temperatures were taken and the animals were weighed. Also noted was whether the animal was torpid or not, in the nestbox or not, reproductively active or not, urinating or defaecating when picked up, and where applicable whether sharing the nestbox with its mate. When removed from their nestboxes and thus with body temperatures around 21 or 22°C the torpid mice were not comatose and seemed well aware of their surroundings. When handled gently, and especially when they became used to being handled they did not arouse if these measurements were undertaken as quickly and gently as possible. If they were handled roughly or kept out of their nestboxes for too long then they began to shiver and arouse. A torpid animal with a body temperature of around 22°C could walk unsteadily back to its nestbox; the best method of returning them to their cages was found to be to allow them to crawl back into their nestboxes on their own as this way they showed no inclination to turn around and come back out.

These measurements continued every day until the animal had lost 30% of its original mass, or if it became obviously stressed by becoming extremely lean, un-coordinated while euthermic, un-reactive to being handled while torpid, having eyes which did not look bright, or blood in the urine. The latter two problems are typical of an animal which is water stressed (Hanney 1965; Glenn 1970). Since the aim of the trial was to find out how long an animal could survive without stress, each trial was stopped immediately when the animal showed any sign of discomfort.

After being given food and water the animals were left in isolation and weighed every day for a week. After this they were reunited with their mates and measurements continued on both animals for another week.

The trials were run at four different times of the year to see if there was any difference in the animals ability to react to these strains. Trials were therefore run in November (spring), January (summer), April (autumn), and August (winter).

## 7.3 Results

### 7.3.1 Ambient temperatures at which these trials were run

Maximum and minimum temperatures were measured in the room in which the animals were kept.

| | | |
|---|---|---|
| Spring | min. 21.0°C | max. 27.6°C |
| Summer | min. 23.7°C | max. 28.8°C |
| Autumn | min. 21.3°C | max. 25.4°C |
| Winter | min. 17.1°C | max. 23.7°C |

### 7.3.2 Torpor induced by food, water, and mate deprivation

Out of a total of 64 trials, 32 animals were torpid at the start of the trial. Having torpid animals in the selection was inevitable (see chapter 8) but was also used for comparison with the euthermic animals.

The results from the euthermic animals were analysed to find out whether food, water, and/or mate deprivation would induce torpor in *Steatomys pratensis*. A summary of the results can be seen in Table 7.1. The results were originally divided into the four different seasons but after statistical tests were run between them and no differences were found, the results were pooled.

**Table 7.1** Torpor induced by deprivation

| Regime | number of euthermic mice at start | number of mice becoming torpid | percentage becoming torpid |
|---|---|---|---|
| (i) no food/water | 6 | 6 | 100 |
| (ii) no food | 7 | 7 | 100 |
| (iii) no water | 10 | 4 | 40 |
| (iv) no mate | 9 | 4 | 44 |

From table 7.1 it can be seen that all animals in regimes i and ii became torpid when deprived of food and water or when deprived of food but not water. These results came from all seasons showing that at all times of the year *S.pratensis* will become torpid when deprived of food.

Of the 13 animals in regimes i and ii, 6 became torpid within one day, 3 within 3 days, and the other 4 within 8 days. The animals which took longer than 3 days had lost 8.8, 11.3, 8.8, and 10.17 gms respectively, or 26, 26, 24, and 26% of their body mass over 5, 6, 7, and 8 days respectively. Again, these animals came from all seasons.

Out of the 10 euthermic animals which were deprived of water but not food, only 4 became torpid within the time span of the trial. These trials were generally not shorter than the other trials but more of these animals showed signs of stress: 70% (7 out of 10) of these trials were terminated because of stress reactions compared to 50% (16 out of 32) of the trials in regimes i and ii. Three of the animals which became torpid did so within 1 day, and the other in 7 days.

Of the 9 euthermic animals which were left in their cages with food and water but without their mates, 4 became torpid within the timespan of the trial: 2 within 1 day, 1 within 2 days, and 1 within 4 days.

Of the 32 animals which were torpid at the start of the trials 27 remained torpid every day until they were returned to normal conditions. Of the other 5 animals, 2 showed one day of euthermia the second day of the trial and then remained in torpor for the rest of the trial. One animal showed one day of euthermia on the fourth day of the trial before becoming torpid again; one animal became euthermic after 8 days when it started showing signs of stress (the trial was immediately terminated); and one animal became euthermic after 3 days and remained euthermic for the next 4 days of the trial until it had lost 30% of its original mass.

After being put back into normal conditions (but without their mates) most of the animals which had been torpid at the start of the trials remained torpid (a total of 23 out of 25) for at least some of the time, and only 12 out of 23 animals which had originally been euthermic showed any torpidity. After being put back with their mates 23 out of the 32 originally torpid animals showed some torpidity, and 10 out of the 32 originally euthermic animals showed torpidity. These results are summarised in Table 7.2.

**Table 7.2** Torpidity patterns after finishing deprivation trials.

| Regime | No.of mice | No.mice torpid post deprivation | No.mice torpid post feeding | Days of torpidity (n) | No.mice torpid post add mate | Days of torpidity (n) |
|---|---|---|---|---|---|---|
| Mice euthermic at start | | | | | | |
| i | 6 | 6 | 3 | 15(27) | 2 | 5(14) |
| ii | 7 | 7 | 5 | 20(40) | 3 | 11(21) |
| iii | 10 | 5 | 4 | 28(37) | 2 | 4(28) |
| iv | 9 | 4 | - | - | 3 | 7(21) |
| Mice torpid at start | | | | | | |
| i | 10 | 8 | 10 | 74(83) | 7 | 34(49) |
| ii | 9 | 9 | | 38(55) | 4 | 15(28) |
| iii | 6 | 6 | 6 | 42(43) | 5 | 28(35) |
| iv | 7 | 7 | - | - | 7 | 47(49) |

### 7.3.3 Mass loss of euthermic and torpid animals

A summary of the results can be seen in table 7.3. Again, the results were originally divided into the four different seasons but after statistical tests (ANOVA) were run between them and no differences were found, the results were pooled. The mean mass changes for each group every day were logged and a regression line calculated.

Mass changes during deprivation and after being fed were not linear: the animals tended to lose or put on a lot of mass the first day (due, no doubt, to emptying and filling the gut with food) and thereafter tended lose or gain mass in an exponential manner.

**Table 7.3** Mass losses of *Steatomys pratensis* during deprivation trials.
(The gradients given here are of the regression lines of the log of the mean mass loss per day.)

| Regime | No.of mice | Days until 30% mass lost | Gradient of mass loss/day while euthermic | Gradient of mass loss/day while torpid |
|---|---|---|---|---|
| Mice euthermic at start | | | | |
| i | 6 | 6-9 | -0.082 | |
| ii | 7 | 7-12 | -0.166 | |
| iii | 10 | 5-10 | -0.112 | |
| iv | 9 | - | N.S. | |
| Mice torpid at start | | | | |
| i | 10 | 6-11 | | -0.076 |
| ii | 9 | 8-12 | | -0.068 |
| iii | 6 | 5-12 | | -0.094 |
| iv | 7 | - | | N.S. |

Under regime i the animals took 6 to 11 days to lose 30% of their mass, while under regime ii it took 7 to 12 days, and under regime iii 5 to 12 days. Again there were no discernible differences between the animals reactions at different seasons.

There was no correlation between the mass of the animals in regimes i and ii at the start of the trial and the time taken for them to become torpid (t-test, $P > 0.10$). There were too few animals in regime iii to give a statistically valid result, but it can probably be assumed that if there was no correlation between results in regimes i and ii, then there would be no correlation in the other regime either.

As would be expected the animals lost most mass the first day they were deprived of food, and put on the most mass the first day they were again fed. This was taken to be a direct result of emptying and filling the gut with food as there was no significant difference between the mass losses of the animals which were torpid for the first two days of the trial ($n = 19$) and those which were euthermic for the first two days ($n = 6$, t-test, $P > 0.10$).

To compare the mass losses of those animals which were torpid at the start of the trials with the euthermic ones, F-tests were run between the regressions (Sokal & Rohlf 1981). The results are presented in table 7.4.

**Table 7.4** Comparison between mass loss of euthermic and torpid animals.

| Regime | F | probability |
|---|---|---|
| i | 0.025 | $P > 0.05$ |
| ii | 6.345 | $P > 0.01$, $P < 0.05$ |
| iii | 0.226 | $P > 0.05$ |

Although it can be seen that in each case the slope of the lines of the mass loss regressions are shallower for the torpid animals than for the euthermic animals, from table 7.4 it can be seen that the only statistically significant difference between the regimes was from regime ii, when the animals were allowed to drink. This implies that torpor is only of use to conserve mass in the presence of water.

After being fed and watered but without being returned to their mates, the mice put on mass in an irregular manner. Noticeable, however, was the large amount of mass put on the first day after being fed, presumably due to the animals filling their guts with food. All 64 animals gained mass between the end of the deprivation trial and the next 7 days, but the slopes of the regression lines were not significantly different from zero. Similarly, although the regression lines again were not significant, 57 out of the 64 mice gained mass after being reunited with their mates.

The mice in all these trials did not regain their original mass but generally reached an asymptote several grams below it. This was the only section of the trials which did show a statistically valid seasonal bias: although there was no other difference between the regimes, the animals lost the least amount of mass in autumn and the most in winter. These results were significantly different (ANOVA $F=7.22$, $P < 0.005$).

These results are presented graphically in figure 7.1. Although log regressions were calculated for statistical comparison, the true results are exponential curves and are plotted as such here.

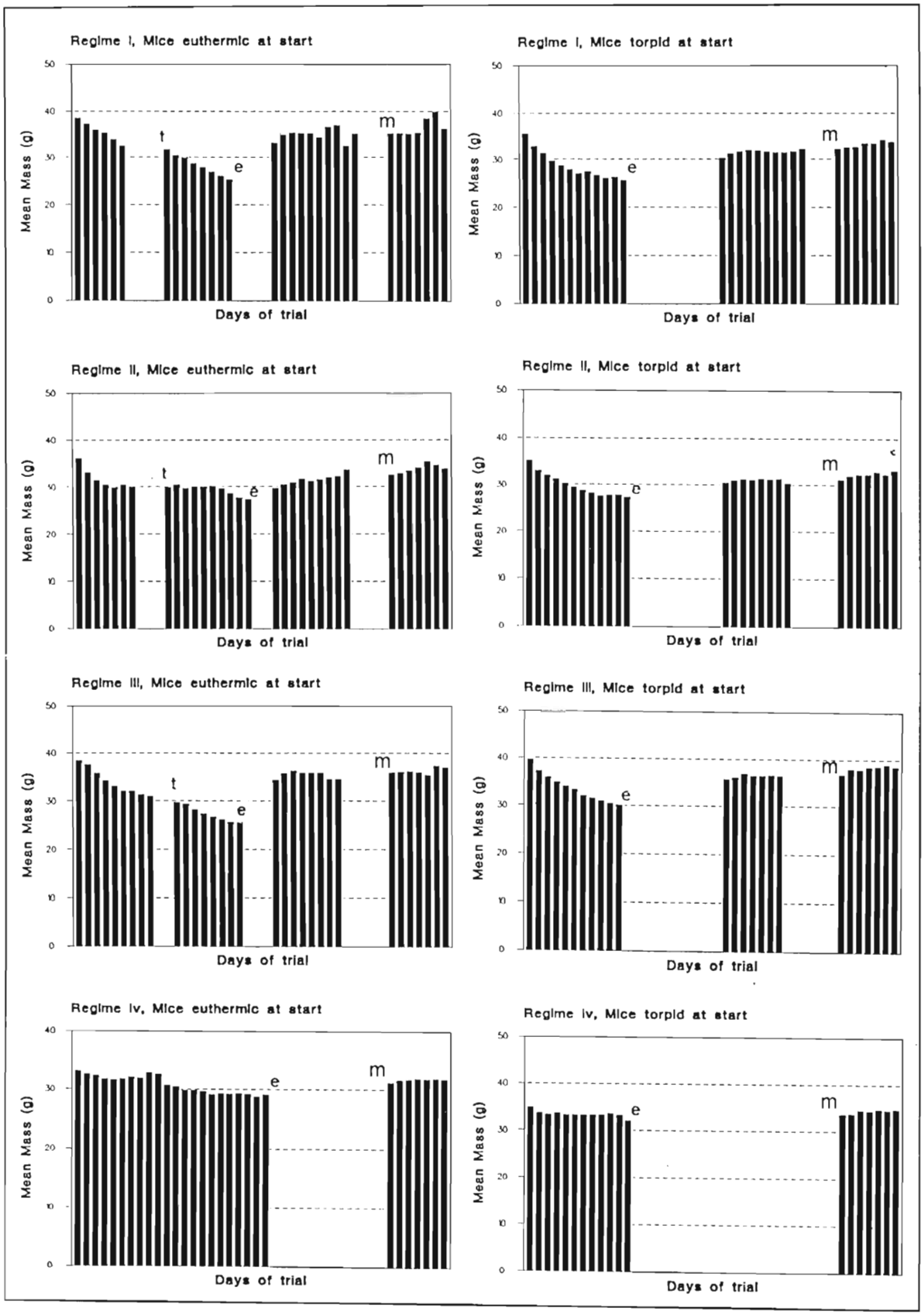


**Figure 7.1** Mass changes of *Steatomys pratensis* during and after the deprivation trials.
(In each regime t is the start of torpidity, e is the end of deprivation, and m is the addition of a mate.)

### 7.3.4 The effect of food, water, and/or mate deprivation on reproduction of *S.pratensis*

The results of the effects of deprivation on the reproductive status of *S.pratensis* are summarised in table 7.5.

**Table 7.5** The effect of deprivation on reproduction of *Steatomys pratensis*.
(The table shows the number of animals that changed reproductive condition during the trials. In each case the number of animals tested was 4.)

| Regime | spring | summer | autumn | winter | totals |
|---|---|---|---|---|---|
| i | 0 | 3 | 1 | 1 | 5 |
| ii | 0 | 1 | 0 | 1 | 2 |
| iii | 2 | 2 | 0 | 1 | 5 |
| iv | 1 | 1 | 1 | 0 | 3 |

Of these animals, 11 males regressed their testes partially or completely, 2 females became imperforate after being perforate, 1 male's testes descended, and 1 female became perforate and then imperforate again. The reason for the higher numbers in summer compared to the numbers in the other three seasons was probably that most of the animals in the other three seasons were already in a non-reproductive condition and therefore would not change. Ignoring the animals in regime (iv) which could not have been affected by lack of food, the other 12 animals included 9 males with regressed testes, 2 females which became imperforate, and the 1 female which became perforate and then imperforate again.

Other than the slight seasonal mass loss described in the previous section, none of the animals used in these experiments showed any lasting effects of being deprived of food and/or water.

## 7.4 Discussion

One of the most interesting results to come out of these experiments was that there is no difference between seasons in the animals' reactions to lack of food and water. This was unexpected as Gaertner *et al.* (1973) showed that in *Peromyscus leucopus* there was a definite difference in their ability to go torpid in response to food, the animals starving to death rather than becoming torpid during April and May but being perfectly capable of becoming torpid at other times of the year. It is possible, however, that there were too few animals used here to show any statistically valid results, and the ambient temperature difference may have been too small compared to that found in the field. It must also be remembered that the fat mice come

from areas with much milder ambient temperatures throughout the year than the *P.leucopus*. The seasonal changes in the animals are discussed further in chapter 8.

A problem throughout these experiments was the difficulty of distinguishing between torpor caused by external stress (deprivation of food, water, or mate) and that occurring naturally. At particular times of the year these animals are more prone to go into torpor (chapter 8). Unfortunately there was no way of compensating for this so the results must be interpreted in the light that some of the torpor may have occurred spontaneously.

### 7.4.1 Deprivation-induced torpor in *Steatomys pratensis*

Since all 13 of the animals which were euthermic at the start of the trials became torpid when deprived of food and water (6 animals) and when deprived of food but not water (7 animals), it is obvious that the removal of food will cause torpidity whether or not water is present. This means that all animals in the population can show torpor, a confutation of Hudson's (1978) assertion that only a fraction of the population shows torpor. However, Hudson may only have been implying natural torpor and not deliberately induced torpor. The fraction of the population showing natural torpor is discussed further in chapter 8.

Starvation induced torpor has come to be regarded as more common than was previously thought (Hudson 1978). It has been shown in *Mus musculus* (Hudson & Scott 1979), *Peromyscus maniculatus* (Howard 1951), *Peromyscus leucopus* (Hill 1975), *Apodemus sylvaticus* (Walton & Andrews 1981), *Perognathus amplus* (Reichman & Brown 1979), *Perognathus californicus* (Tucker 1966), and many others. It has also been shown in other mammalian families: Vogel (1974) describes it in *Suncus etruscus*, the smallest species of shrew; and Hudson (1978) describes several species of marsupial which show torpor probably through lack of food.

Although not reported to any great extent in southern african rodents, this may only be a reflection of the extremely small numbers of these animals which have been studied under deprivation conditions rather than an inability of these animals to become torpid.

The hypodipsia (reduced drinking) shown by *Mastomys natalensis* and *Rhabdomys pumilio* under conditions of food deprivation (Willan & Meester 1987) could be explained by the animals becoming torpid for part of the day due to starvation. In the case of *R.pumilio*, however, the animals increased their locomotory activity, indicating that they may not have been

torpid, while the *M.natalensis* decreased their activity, supporting the hypothesis that they were torpid. Similarly *Saccostomus campestris* has been reported as becoming torpid at times in the wild (Ellison 1988).

Since 9 animals out of 13 went torpid within one or two days of food and/or water deprivation it seems that torpor is a fairly rapid reaction to a post-absorptive state. This could be a natural reaction to enable the mice to become torpid on a seasonal basis to permit survival through a period of low food availability (such as would happen at the beginning of winter) as opposed to a reaction to the immediate lack of food, but considering there was no seasonal bias, this is unlikely. It thus seems that *S.pratensis* is always prepared for a withdrawal of food with the ability to become torpid.

Hill (1975) reported that when two *ad lib.* fed *Peromyscus leucopus* were deprived of food they showed torpor on the second and third days respectively. The *Mus musculus* studied by Hudson & Scott (1979) showed torpor within 24 hours of food being removed.

If those animals in regimes i and ii which became torpid within two days were reacting to a post-absorptive condition rather than starvation, then the ones which took longer than three days seemed to lose about 25% of their mass before becoming torpid (26, 26, 24, and 26% respectively). Morhardt (1970) found a mass reduction of 8.9 to 22.6% in *Peromyscus maniculatus* and 5.9 to 26.7% in *P.eremicus* before these animals became torpid.

Of the 32 animals which were torpid at the start of the trials, 27 remained torpid every day until they were fed again or given water. Three showed only one day of euthermia, and one trial was terminated due to stress. Only one mouse became euthermic and remained so for the rest of the trial. This indicates that in general not only will deprivation trigger torpor, but it will also ensure that torpid animals remain torpid every day until food or water is again available.

Since the animals were only tested for torpidity once a day to prevent over-stressing them, it was not possible to tell if any of them showed cycles of torpor longer than 24 hours. Chew *et al.* (1965) found that *Perognathus longimembris* showed longer periods of torpidity (over 24 hours) when deprived of food. Similarly, Hanney (1965) remarks that *S.pratensis* did not leave their burrows for several days at a time. It is possible that the one animal which was measured as euthermic may indeed have been torpid as it has been shown (chapter 3) that some animals may only show torpor for a few hours every day and may be normothermic by the time measurements were taken in the mid-morning. Chew *et al.* (1965) found a different rhythm of torpor in starved animals compared to the ones which were fed *ad lib.*, with the starved mice

becoming hypometabolic early in the dark part of the cycle while the fed mice only became torpid later in the night. They postulate that starvation causes an advance in the occurrence of the hypometabolic cycle.

Montoya *et al.* (1979) show that removing protein from the food of the garden dormouse (*Eliomys quercinus*) at any season by feeding them on a synthetic protein-free diet or on apple alone will cause torpor even though the animals have enough energy for their requirements. This indicates that starvation torpor may not be a simple matter of energy conservation, but may be caused by, or a side effect of, particular requirements of protein metabolism. These authors suggest that a change in diet at the beginning of the torpid season could play a role in activating hibernation or torpor in mammals.

Although the protein requirements of *S.pratensis* are not known, Petter (1966) kept *S.opimus* on a diet of apple alone and reported that they spent every night in torpor. This seems to indicate that *S.opimus* at least, and probably *S.pratensis*, will react to a lack of protein in the diet by becoming torpid. In the light of Tucker's (1966) remarks that *Perognathus californicus* will spend as little time as possible in torpor, and no time at all if they have an adequate diet (although Chew *et al.* (1965) found that 32.5% of their *Perognathus longimembris* became torpid while they had food *ad lib.*), the question is raised as to whether the *Steatomys pratensis* in this study were nutritionally stressed. This seems unlikely, however, as they had a varied diet and bred well in large cages for five years.

Hudson (1978) remarks that torpor in many small mammals is seasonal and may require acclimatisation. Although torpor shows a natural seasonal bias in *S.pratensis* (see chapter 8), starvation torpor does not seem to require acclimatisation since all the animals tested showed some torpor on the removal of their food.

Torpor could always be triggered by starvation, or rather naturally occurring torpor may be caused by self-imposed starvation as the animal could quite easily not eat for a day or two to cause itself to go torpid (Hudson 1978). This seems unlikely as many of the animals studied in different experiments did not lose a drastic amount of mass (as was found here in the first day) between being euthermic one day and torpid the next. Another reason that this is unlikely is that the animals which died when the constant temperature room dropped its temperature rapidly had stomachs full of food, indicating they had eaten the previous night before becoming torpid. Similarly Genest-Villard (1979) remarks that all the torpid *Steatomys opimus* captured in Central Africa had full stomachs.

Many authors (e.g. Bartholomew & Cade 1957) simply assume that starvation torpor is the same physiological reaction as naturally occurring torpor, although Hill (1975) showed that *Peromyscus leucopus* showed some pertinent differences in depth and duration of torpor, and Gaertner *et al.* (1973) showed that starved *Peromyscus leucopus* exhibited slower arousal times than those animals in spontaneous torpor, and had irregular heart rhythms. As heart rates were not measured in *S.pratensis* it is difficult to tell whether starvation torpor is the same as that occurring naturally, but it should be borne in mind that there may be some important differences. It is also not known if torpor occurring naturally in summer is the same as torpor occurring naturally in winter, but for the purposes of this study it is assumed they are the same.

Recovery from food and water deprivation in fat mice was generally rapid as far as torpidity was concerned although the animals took longer to regain the lost mass (see next section). After being fed or supplied with water 23 out of the 25 animals which had been torpid at the start of the trial remained torpid whilst only 12 out of the 23 originally euthermic animals showed any torpidity.

After being reunited with their mates less of the mice showed torpor than in the preceding week (151 out of 245, or 62%, compared to 217 out of 285, or 76%). This may be a result of the energetic effects of huddling with a cage mate but could also be a slow recovery from the deprivation.

### 7.4.2 Mass loss of euthermic and torpid animals

Since there was no correlation between the mass of the animals at the start of the trials and the time taken for them to become torpid, it seems that there is not a minimum mass below which the mice will become torpid. However, as mentioned previously the four animals which took longer than two days to become torpid had each lost 25% of their body mass. Although there are too few results to be statistically significant, this may be an indication that some of the mice have to lose a certain amount of mass before torpor is triggered.

There was no significant difference between the mass lost by the torpid animals and that by the euthermic animals in the first two days of the trial. The large amount of mass lost in the first day is probably a direct effect of emptying the gut of food, suggesting that torpor does not lengthen the time taken for the mice to empty their guts.

The torpid mice survived longer in the deprivation trials than the euthermic animals, although since the result was not statistically significant it is not proof that torpor could help a fat mouse survive food shortages. Similarly, it should be noted that in each comparison between euthermic and torpid animals the gradient of the regression lines were less (i.e. the animals lost mass more slowly) in the case of the torpid animals, although the results are again not statistically significant.

When comparing the results of the animals under the different regimes (table 7.4) it can be seen that only under regime ii (food deprivation, *ad lib.* water) is there any statistically significant saving in body mass when the animals are torpid. This implies that only under conditions where the animals have access to water would torpor be instrumental in aiding the mice to conserve energy.

Although all the animals gained mass after again being fed, the gradients of the regression lines were not significantly different from zero. This was probably owing to the large amount of mass gain on the first day of re-feeding, as can be seen in figure 7.1.

In the 7 days after being reunited with their mates 57 out of the 64 mice gained mass. This was likely the result of a continuance of the mass gain seen after the deprivation trials; but may have been influenced by repatriation with their mates, since separation resulted in mass loss.

It is possible that the animals would continue to gain mass but since none of the runs were significantly different from zero this is not so likely. It is thus difficult to explain the seasonal differences in mass loss at the end of the trials. One possibility is the seasons themselves: the fat mice may gain mass in autumn to survive the winter torpid months, while by the time the trials finished in winter, the animals would lose the fat they had stored. Another explanation would be that employed by Willan & Hickman (1986) to explain the non-recovery pattern in *Otomys irroratus*: animals that are habitually used to a diet with a constant energy content are less able to adjust food intake to regain lost mass. This seems unlikely for *S.pratensis* as they habitually have wide and varying diets (chapter 1), although in the laboratory they were restricted to "Epol" cubes.

Lindstedt & Boyce (1985) discuss the fasting endurance of mammals and relate it to the amount of body fat carried (and the size of the animal) and their basal metabolic rate. Unfortunately

they do not include hibernating or torpid mammals and so the results may not be applicable to *S.pratensis*. However these authors give an equation of:

$$t_s = 2948\, M_b^{1.19}/[317 M_b^{0.75} + (E_t + |E_t|)/2]$$

where $t_s$ is the survival time in days, $M_b$ is the mass of the animal in kgs, and $|E_t| = (16 M_b^{0.5})(38\text{-}22.5 M_b^{0.5} - T_a)$.

This equation assumes that the body temperature is 38°C. Since it also assumes a standard metabolic rate for a eutherian mammal (and it is shown later (chapter 9) that if the fat mice had a normal (38°C) body temperature their metabolic rates would be at normal eutherian levels) the equation is discussed anyway.

Using an average animal mass of 36.44 g and an ambient temperature of 23.6°C (average temperature of all the starvation trials), survival time from Lindstedt & Boyce's (1985) equation is 1.42 days. Even those animals which remained euthermic for the first few days of the trials survived much longer than this without ill effects. The probable reason for the anomaly between the expected and observed result would be that Lindstedt & Boyce (1985) were assuming that the amount of fat in a eutherian body is $0.075\, M_b^{1.19}$. This means that an animal of 36.44 g would possess 1.46 g of fat. It has been seen in chapter 6 that the fat mice carry a lot more fat than this.

In chapter 6 it was seen that the fat mice could store 90.01 to 815.34 kJ of fat. Using energy utilisation values from chapter 3 this means that *S.pratensis* could theoretically survive 2.9 to 26.4 days while at 30°C, 2.2 to 19.7 days euthermic at 20°C, and 4.9 to 44.8 days torpid at 20°C. The results found here are well within these limits. It must be remembered, however, that these calculations are only concerned with energy values and not with dehydration of the animals.

Therefore heavier mice have a great advantage over lean ones regarding survival during times of food shortage. This must be balanced against the disadvantages of carrying fat, i.e. overheating, lessened agility, etc.

None of the results found here support Hanney's (1965) result of 38 days to lose 32% of the animals original mass. This was a mass loss of almost 11 g from an original mass of 34 g, and 11 g of fat equates to a total energy content of 414.26 kJ. Ambient temperature can be assumed to be 19°C. Unfortunately Hanney did not give the conditions under which the animal was kept, nor did he say whether the animal was torpid or not, although it can be assumed that it was.

Assuming an ambient temperature of 20°, and if the mouse used the same amount of energy as used by the mice in this study then the fat would provide maintenance energy for 22.8 days. Since this mouse survived 15 days longer, it obviously had a much lower rate of metabolism. This can also be deduced by the slope of the graph given by Hanney (fig. 11).

The slightly cooler ambient temperatures may have aided survival, as Bartholomew & Cade (1957) have shown that *Perognathus longimembris* kept at lower temperatures lost mass more slowly than torpid individuals at higher temperatures. They attribute this to fewer arousals at the lower temperatures (8°C as opposed to 21°C). The *S.pratensis* studied by Hanney may also have had previous experience with food shortages which had accustomed it to starvation. Since the experiment was conducted on one mouse only the result is perhaps questionable.

The frequent disturbances of the torpid fat mice in this study may have added to their loss of mass, through partial arousals. Not only were the animals weighed every day but they seemed to be susceptible to any other extraneous laboratory noises. In a burrow there would be little in the way of disturbances so it is possible that their torpor can be easily disturbed.

Little information is available on mass losses of starved southern African rodents although in comparison to *Rhabdomys pumilo* which may have gone torpid and which lost almost 30% of its mass within 5 days, and *Mastomys natalensis* which probably went torpid and lost just over 20% within 5 days (Willan & Hickman 1986), *Steatomys pratensis* can survive longer than other rodents or than the time predicted by the Lindstedt & Boyce (1985) equation.

### 7.4.3 The reaction of *Steatomys pratensis* to lack of water

Only 40% (4 out of 10) of the animals in this study became torpid when deprived of water only. Since a similar number became torpid on removal of their mates, it seems unlikely that lack of water is a trigger for torpor. However, it has been shown that energy saving through becoming torpid only occurs when water is available, implying an extreme dependence on drinking water.

More of these trials (7 out of 10) were terminated because the animals showed signs of stress compared to 16 out of 32 in regimes i and ii. Of the animals which did become torpid, 3 did so within 1 day and the other within 7 days. It has been shown that depriving some animals of water leads to self-imposed starvation (Hudson 1978) but *S.pratensis* did not react as if starved, in which case they would have all become torpid.

Fertig & Edmonds (1969) show that the house mouse (*Mus musculus*) becomes torpid in response to severe water deprivation. Similarly, MacMillen (1965) shows that the cactus mouse *Peromyscus eremicus* can become torpid in summer in response to a negative water balance, but in winter animals deprived of water (but not food) died between 6 and 11 days. He suggests it is impossible for the mice to go torpid during winter for any length of time except in a shallow burrow during the day when the sun's heat can keep the temperature of the burrows above the thermal minimum. However, in summer the burrow temperatures would be high enough for the mice to become torpid without their body temperatures dropping too low. Davis (1976) writes that lack of water in ground squirrels causes torpor at any time of the year.

As mentioned in the introduction, *Steatomys* are usually found near water (Shortridge 1934), although this does not necessarily mean that they need it for drinking. However Hanney (1965) reports finding one blind *Steatomys* and quotes Neave (1907, in Hanney 1965) as noting a number of blind ones, all of which he attributes to dehydration. Although all the animals in this study group had water available at all times, on one or two occasions an animal was found to have an eye permanently shut and so to be blind in that eye. In this particular section, when the animals were deliberately deprived of water (regimes i and iii), only 4 out of the 32 animals tested had their trials terminated when they showed some eye irritation. None of these animals became permanently blind, the eyes recovering after the animals were again given water. In fact, twice as many animals (8) out of the same group had their trials terminated because of blood in the urine. However, all these details do point to the fact that *Steatomys* does need drinking water.

*Steatomys pratensis* could possibly satisfy its water requirements from the insects and plant food it eats, but laboratory animals fed on a dry diet of "Epol" cubes required 1.33 to 8.08 ml water per day. Using the equation of Hudson (1962, from Glenn 1970) it has been shown (chapter 3) that an average fat mouse would drink 5.68 ml water per animal per day. This is equal to 19% of their body mass per day. It must also be remembered that water is produced as a by-product of the metabolism of fat at the rate of 1.07 g per g fat (Schmidt-Nielsen 1975, p.418), which would aid in the water balance of the mice.

Wunder (1970) showed that the chipmunk *Eutamias merriami* drinks water equal to 12 % of its body mass/day but could reduce this to 1.5% when on water restriction.

Several authors (e.g. MacMillen 1965; Glenn 1970; McNab 1979a) have mentioned the possibility that the purpose of a low metabolic rate is to reduce the amount of water needed by the animal when water is in short supply. McNab (1968) correlates the ability of rodents to

survive on a dry diet with their adaptiveness to desert conditions. In spite of having an extremely low metabolic rate, euthermic *S.pratensis* do not need less water than other animals as defined by the Hudson equation (Glenn 1970). However in Table 3.4 it was seen that the torpid animals drank much less water than the euthermic ones. Whether this is a reason for torpidity in the wild, or a concomitant effect of becoming torpid, is not known.

Unlike food, water cannot be hoarded, so it either must be present in the animals environment (even if in the form of high water content food, snow, ice, or dew) or that animal must learn to do without it. Buffenstein (1985) showed that it is possible to deprive the gerbils (*Gerbillus pusillus*) of water gradually until they have learned to do without free water completely. Although these were desert animals and therefore probably genetically more capable of doing without water, it is possible that wild *S.pratensis* become more accustomed to having less drinking water throughout the dry season. Since the dry season where the original animals for this colony were trapped is also the colder winter, the animals would be torpid throughout most of the time of worst water stress. Several authors (e.g. Rosevear 1969) state that the fat mice aestivate in the dry season without giving any reason for this: it could be a reaction to the lack of water, lack of food, or cold.

A point to be raised here is the use of the word "estivation" in relation to *Steatomys*. In his discussion on the meanings of several words used to describe torpor of different forms, Hudson (1978) remarks that estivation is usually implied to mean a torpor in response to aridity or heat. This is undoubtedly the meaning that Rosevear (1969) attaches to the word when describing *Steatomys*, yet in this study the animals did not show any reasonable amount of torpor in reaction to the lack of water. This word would thus seem to be entirely inappropriate when discussing *S.pratensis*.

### 7.4.4 The effect of food and water deprivation on reproduction of *S.pratensis*

Since out of 48 food or water deprivation trials (i.e. not including regime iv) 46 animals were in a non-reproductive state by the end of the trial, it seems that depriving *Steatomys pratensis* of food, water, and either food or water will cause them to go into a non-breeding condition. The results are heavily biased towards summer, since in other seasons animals were already in a non-breeding condition when the trials started. Of the 2 animals which did not go into non-breeding condition, 1 male remained with descended testes throughout the trial (regime iii) and 1 female became perforate and then imperforate again (regime ii).

Merson & Kirkpatrik (1981) show that restricting food causes cessation of breeding in white-footed mice (*Peromyscus leucopus*). This cessation of breeding was not related to fat levels, nor to body mass. These authors postulate that the proportion of imperforate females in a wild population may be a more accurate reflection of the general short-term state of fitness of a population than either fat levels or body mass.

Since it would be energetically disadvantageous for any animal, especially a female, to reproduce in times of food shortage, this response is presumably a safety mechanism to prevent unnecessary wastage of energy. It may also act to halt breeding during autumn.

### 7.4.5 The effect of isolation on torpor, mass loss, and reproduction

An interesting result to arise from this work is that removing a cage mate can have an effect on the remaining animal's mass.

In table 7.2 it can be seen that while all the fat mice which were torpid at the start of the trial remained torpid when deprived of their mates, 4 out of the 9 that had been euthermic also became torpid: 2 within 1 day, 1 within 2 days, and 1 within 4 days. Out of a total of 238 trial days these mice spent 148 days torpid (62%). This is higher than that due to chance as it will be seen (chapter 8) that at any time the probability of a fat mouse being torpid is 30%. These increases in torpidity may be caused by the extra costs in thermoregulation caused by the inability to huddle with mates.

Andrews *et al.* (1987) show that the vole *Microtus townsendii* conserves energy by huddling with conspecifics. This energy conservation needed one or more days to develop or cease. *Steatomys pratensis*, with a similar energy deficit, may also became torpid.

In spite of the energy deficit of the animals that found themselves deprived of a cagemate these mice did not lose a significant amount of mass. In table 7.3 it can be seen that the slope of the graphs of mass loss are not significant in the case of regime iv. This bears out the hypothesis of Andrews *et al.* (1987) that the mice acclimatised to the loss quickly.

### 7.5 Experimental inaccuracies

Some of the animals measured as euthermic may have shown a small spell of torpor in the early morning and have raised their body temperatures again by the time the measurements were taken. Similarly, although measuring every morning was done as quickly and gently as possible it may have made some of the mice arouse. Animals not in these trials were checked some time after being taken out of their nestboxes while torpid and none had rewarmed, but starved animals might be more sensitive to such stimuli. Since it was necessary to check the animals for signs of stress it was decided not to leave any for more than a day without weighing.

All the mice used here were accustomed to having food and water available at all times, unlike the probable situation of a wild mouse. In the light of Hanney's (1965) comments on hoarding in *S.pratensis* this is perhaps not a major problem as far as food is concerned, but could have an influence on its water usage. The relatively dry atmosphere of the holding room could have affected the animals water turnover since the high humidity of a burrow would help the mouse by retarding respiratory water loss. This problem may have been partially alleviated by the increased humidity in the nestbox.

### 7.6 Ecological implications of these results, and the advantages of hoarding food

Interesting as these results are, their implications may only be important in times of extreme stress in the animal's life. Being opportunistic feeders, and probably eating a mixture of plant material, seeds, and insects (Hanney 1965; Kern 1981), food is probably available to the animals all year round, if less so in the colder winter months.

Several authors (e.g. Hanney 1965; Kingdon 1974) remark that food caches have been found in *Steatomys pratensis* burrows, e.g. Hanney (1965) reports finding 37 g of tubers in the burrow of an adult female *S.pratensis*. If this is normal for the species then it is unlikely that they have to go for any length of time without food. However, Kingdon (1974) remarks that food in the burrows is not stored long before being eaten. Assuming the 37 g of tubers found in the burrow by Hanney (1965) to have roughly the same calorific values as those reported by du Toit *et al.* (1985), who gave the total wet mass value of 3.51 to 5.90 kJ/g for the corms found in their study, this would give a total value of 130 to 218 kJ for the whole cache. At a euthermic expenditure rate of around 41 kJ/day and a torpid rate of 18 kJ/day (see chapter 3), this means that the food hoard could last 3 to 5 days for a euthermic animal and 7 to 12 days for a torpid animal.

Although these are extremely approximate figures, they do suggest that if this amount is normal for a *Steatomys* cache then they are not meant to last the winter. The food caches could be important to last the animal over a period when the weather is too cold for foraging out of its burrow or immediately after a burn when the ground cover would be extremely sparse and food would not be readily available: Kern (1981) caught *S.pratensis* in fairly high numbers after a burn and concluded that the resident population did not migrate off the burn but remained in the area.

Food shortages rather than complete deprivation of food are more likely to cause torpor in *S.pratensis* since it has been shown (Willan & Hickman 1986) that fire reduces rather than eliminates food supplies of omnivorous rodents.

Tucker (1966) and Wolff & Bateman (1978) show that the amount of food available to *Perognathus californicus* and *P.flavus* determines the amount of time spent in torpor. As temperature and food ration decreased, mass loss and time spent in torpor increased. Both authors regard food shortages as evolutionarily important in the development of torpor, although unlike *Steatomys pratensis* neither of these species go into torpor in the presence of *ad lib.* food. In a similar study Reichman & Brown (1979) show that the amount of time spent in torpor by *Perognathus amplus* is related to how deeply the animals' seed source is buried. This means that these animals are capable of assessing precisely, and responding to, variations in energy intake and expenditure.

Wolff & Bateman (1978) suggest that *P.flavus* store food in their burrows to spend the early evening hours (when the temperatures are higher) foraging for food.

Food stored in the burrows by *P.flavus* did not exceed 5 days supply, the animals were probably torpid every day and active every night, although it was possible that some of the animals could have remained torpid and underground for several days. Animals awoke from torpor in mid-afternoon, consumed the seeds which they had cached in their burrows the day before, and then foraged above ground immediately after dark, bringing the seeds back to their burrows to be stored. Wolff & Bateman (1978) explain this behaviour as adaptive since the early feeding gives the animal the energy needed to forage in the warmer part of the night.

Torpid *Steatomys pratensis* in the laboratory generally had earlier feeding times than euthermic ones. As mentioned earlier, Chew *et al.* (1965) found an earlier feeding time in *Perognathus longimembris* on the days when they were torpid. *Steatomys pratensis* could use a foraging strategy similar to *P.flavus*. The colony of *S.pratensis* did not show hoarding behaviour in their

small cages but did so in the larger ones. An advantage of bringing food back to a burrow to be eaten is that the animal would be less likely to be attacked by a predator. There would presumably be a number of invertebrates found in the burrows although it would be difficult to estimate just how much of the diet of *Steatomys* these would comprise.

In view of the comments of Gaertner *et al.* (1973) on the differences between starvation-induced and natural torpor, it may be important for mice to enter torpor early and remain in good condition rather than wait until starvation forces them into an energy imbalance. Chew *et al.* (1965) postulate that since two *Perognathus longimembris* which took longer than the others in an experiment become torpid on removal of food did not survive their torpid bouts, survival and recovery could be enhanced by early entrance into torpor.

Davis (1976) suggested that fat storage is more efficient than food storage because fat cannot be stolen or lost and is readily available when needed. There are, however, some disadvantages attached to carrying a large amount of fat (chapter 9).

Wrazen & Wrazen (1982) discuss food hoarding and torpor in the eastern chipmunk (*Tamias striatus*) and come to the conclusion that females would be at an advantage if they stored fat and became torpid as the fat would be important in the rearing of their young in the spring. However, it would be advantageous for males to cache food and remain euthermic so that they would be in breeding condition and euthermic when females came into oestrus. This is a similar system to that used by the arctic ground squirrels *Spermophilus parryii* (McLean & Towns 1981). However, *Steatomys pratensis* males probably overwinter with the females (Smithers 1983, animals found in pairs) and there is no sex difference in the animals' fat levels.

Rainfall at Cathedral Peak (where the original animals were trapped) is highly seasonal (Weather Bureau 1986) and affects grass cover, insect populations, and seed availability. The animals were trapped very close to a perennial river and probably had no problem in obtaining drinking water. It is possible that this represents an inability to exist without free drinking water.

### 7.7 Further work to be done in this area

Although the basic results seem conclusive, that depriving a *Steatomys pratensis* of food will cause torpidity, there are some interesting issues worthy of further investigation. Probably the most important would be to test water turnover of torpid and euthermic mice.

Experiments could determine how different ambient temperatures affect the time taken to lose 30% of body mass: with an optimum ambient temperature *S.pratensis* may survive as long as indicated in Hanney's (1965) result.

Since torpor may occur in response to a protein deficiency in the diet (Montoya & Ambid 1978) it would be meaningful to analyse protein requirements and assimilation in *S.pratensis*, especially when torpid. However, the mice did breed for the first five years in captivity, which demands an adequate diet, and they also showed torpor when fed extra protein in the form of "Pronutro".

### 7.8 Summary

Fat mice were deprived of food, water, food and water, or a cagemate, and their torpidity patterns and mass losses were measured every day until they had lost 30% of their original mass. Trials were run at four different seasons and the results analysed.

Deprivation of food and water, or food alone, caused torpor in *Steatomys*. Deprivation of water but not food caused torpor in 4 out of 10 individuals, and deprivation of a cage mate caused torpor in 4 out of 9 cases. Generally torpor was induced within three days. Animals lost around 25% of their body mass before becoming torpid. Animals deprived of water but not food showed more signs of stress than those deprived of food with or without water.

Mass losses when torpid mice were deprived of food, or food and water, were similar to those when euthermic animals were deprived, but there was less of a mass loss when torpid animals were deprived of water compared to euthermic mice. This implies that torpor is only of use in the presence of water.

Reproductive state changed in 15 out of the 64 mice tested; of these 13 became non-reproductive indicating that torpor may act to prevent breeding in nutritionally stressed mice.

# Chapter 8

## Annual weight, torpidity, and reproductive cycles

### 8.1 Introduction

Because of differences in food availability, temperature, and rainfall in most climates throughout the year, most small mammals show annual cycles, e.g. reproducing at a time of year when food is available for the young, or depositing fat to survive through winter. Annual cycles are particularly important for hibernating animals who must deposit enough fat or hoard enough food to last them through the winter. At one time annual cycles were thought to be wholly motivated by exogenous factors but later research has shown that most hibernators have an endogenous rhythmn which is triggered by exogenous "zeitgebers" (Muchlinski 1980). Much less work has been done on small mammals which enter daily torpor at particular times of the year, but that of Gaertner *et al.* (1973) seems to show that torpor is controlled by a mix of endogenous and exogenous factors.

Most of the published work covering the genus *Steatomys* mentions annual cycles of torpor although much of it is anecdotal.

Roberts (1923) remarks that members of this genus hibernate. Shortridge (1934) says they sleep throughout the winter, roughly April to October, and although he connects the fat with hibernation, he also says that they were found fat in all seasons. Hanney (1965) cites *S.pratensis* as spending from August to November underground, although many animals leave their burrows every night, and from owl pellets he deduces that they lead a non-fossorial life during April and May. Hanney also found torpid individuals during July. Petter (1966) kept several *S.opimus* in Paris and they showed a daily torpor cycle all year round. This has been discussed (chapter 7) and is probably related to a low protein diet and not to a natural cycle, but Petter (1966) does say that these animals show a similar lethargy in the dry season in their natural environment.

Vesey-Fitzgerald (1966) says that *S.pratensis* are "said to aestivate" during the dry season but feeding was probably continuous because specimens collected at the end of this season (in October) were still excessively fat. Rosevear (1969) gives *Steatomys* as aestivating from November to March and Kingdon (1974) found fat animals in September, November, and December. Smithers (1975) writes that *S.pratensis* in Zimbabwe accumulate fat in the latter part

of the summer and become inactive in the colder winter months, a state he describes as "not a true state of hibernation". Coetzee (1977) says that aestivation in *S.pratensis* occurs in the late winter.

Sheppe & Haas (1981) say that *S.pratensis* in Botswana aestivates in the dry season and most of the fat mice caught had "much" body fat, which they relate to the fact that most of these animals were caught before going into aestivation. In his study on the effects of burning on small mammal populations in the Kruger National Park, Kern (1981) trapped *S.pratensis* at all times of the year, although mostly in August, and describes them as migrant in autumn. His results do not seem to show any annual variation in body weight. De Graaff (1981) writes that the fat laid down by species of this genus throughout southern Africa fluctuates with season and allows the animals to "become inactive" over the unfavourable season, although he describes this as the dry summer while in most parts of the range of *S.pratensis* the dry season is actually winter. Smithers (1983) writes that the fat layer of fat mice in South Africa is built up over the summer months to enable the mice to survive the cold winter months since specimens taken from March to June are very greasy, but those taken from September to early October have "usually used up" the fat.

It thus seems that fat mice spend at least the winter and spring months from July to October in some form of torpor, although in the west they may enter torpor earlier. They build up their fat levels through the summer and although few authors actually say that the animals lose their fat again, it is implied in several publications (e.g. Smithers 1983). However, Vesey-Fitzgerald (1966), Genest-Villard (1979), and Sheppe & Haas (1981) all write that they caught fat animals at all times of the year.

Ansell (1960) gives the reproductive season of *S.pratensis* as from December to April in Zambia. Hanney (1965) says that reproduction in *S.pratensis* is between April and July in Malawi and that the males "undergo anoestrus" during the dry season. Bellier & Gautun (1968) state that the period of sexual activity of *S.opimus* is at the end of the rainy season. Smithers (1975, 1983) says that *S.pratensis* breed in the warmer and wetter months of the year while Coetzee (1977) records young from early summer to late autumn.

Genest-Villard (1979), in her extensive study of the ecology of *S.opimus*, reports that the dry season is from December to February, at the end of which time the families disperse. She established three different age classes of fat mice based on mass and body length and suggested that the young stay with their mother until the end of their first dry season when they dig their own burrows. From April all sub-adult and adult females occupy their own burrows and the sub-adult and adult males do so from March to July. Reproduction probably begins at the end of

June in the height of the rainy season and probably does not finish until the end of the rains. Sheppe & Haas (1981) say that *S.pratensis* breed from February to May, while Smithers (1983) gives the breeding season of *S.pratensis* as probably from October to May.

It can thus be inferred that *S.pratensis* in the wild start breeding from the beginning/ middle of the wet season (which would be from December in the area in which this colony was caught), to the end of the wet season (around April).

The first aim of this chapter was designed to determine whether *Steatomys pratensis* show any or all of these reproductive, torpidity, and mass annual cycles in captivity.

The second aim of the chapter was to find out if a female was made pregnant in winter whether she would become torpid while pregnant. This would also be used to ascertain if the gestation period is longer if the animals have been torpid.

There are several mammal species which can become torpid while pregnant or lactating (e.g. bears (Garfield 1988)) but no rodents are known to do so.

## 8.2 Materials and Methods

### 8.2.1 Annual weights and torpidity patterns

Every week from 22/07/81 to 19/10/83 the animals were removed from their nestboxes, each was weighed, their rectal temperatures were taken, torpidity was noted, and reproductive state (i.e. whether the females were perforate or imperforate, or whether the males had descended or undescended testes) was recorded.

All measurements were recorded for each individual in the colony every week, so although some of the animals were kept in the room lit by natural light without temperature control, some of the readings were taken from animals kept in the constant temperature room under a standard temperature and light regime. Although these readings were originally kept separate it was decided to incorporate them into the total as standard temperature and light conditions followed the natural seasons.

The only animals not included in this analysis were females with young.

The results were plotted: figure 8.1 depicts mass of both males and females, figure 8.2 shows the patterns of female reproductive states, figure 8.3 the patterns of males reproductive states, and figure 8.4 the torpidity patterns of both males and females.

### 8.2.2 Winter pregnancy

Four female *Steatomys pratensis* were put into large aquaria with very deep (c. 80 mm) sawdust and their reproductive state (perforate, imperforate, oestrus, or copulatory plug) and torpidity (body temperature) monitored for four to eight days. The mice were then injected with 10 iu (or 20 iu in one case) of Humegon (a human menopausal gonadotropin or FSH) at 16h00 on day 1. The mice were monitored for 2 days and at 10h00 on day 3 injected with 10 iu (20 iu in the same mouse as before) Pregnyl (a human chorionic gonadotropin or LH). The animals were injected intraperitoneally and immediately returned to their cages. They were monitored for the next 30 days.

Only four animals were used as the hormones were extremely expensive but also it was felt that four animals should give the results required. In three cases the male was with the female from the start of the experiment and in one case the male was added immediately after the injection of Pregnyl.

## 8.3 Results

### 8.3.1 Mass

The results originally included all animals in the colony which were measured every week but the results (when plotted on a graph) showed several times of extreme variation during the year. On closer examination of the results it was seen that these variations were caused by including the newly "adult" youngsters into the measured data set. The results were then recalculated to include only the animals that were adults at the start of the observations. The results from these animals are plotted in figure 8.1.

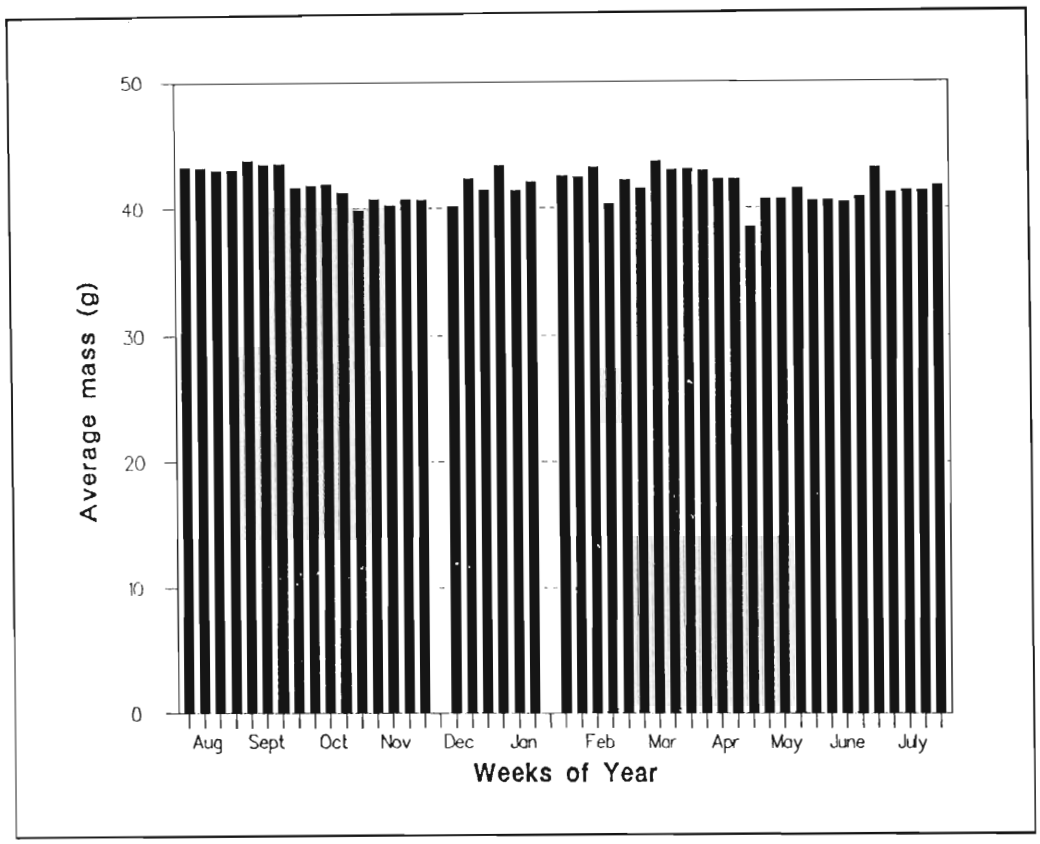


**Figure 8.1** Average mass of adult *S.pratensis* throughout the year.

From figure 8.1 it can be seen that the animals vary little in their masses throughout the year.

### 8.3.2 Reproductive condition: females

The females in the colony showed a bi-annual increase in the number of perforate animals. Nearly all females (70 to 100%) were perforate in the months January through April, and most of these (40 to 60%) were again perforate at the end of September. Nearly all females (80 to 90%) were imperforate from October to December. Females with litters were disregarded in the calculations as they remained perforate for the duration of parental care. These results are presented in figure 8.2.

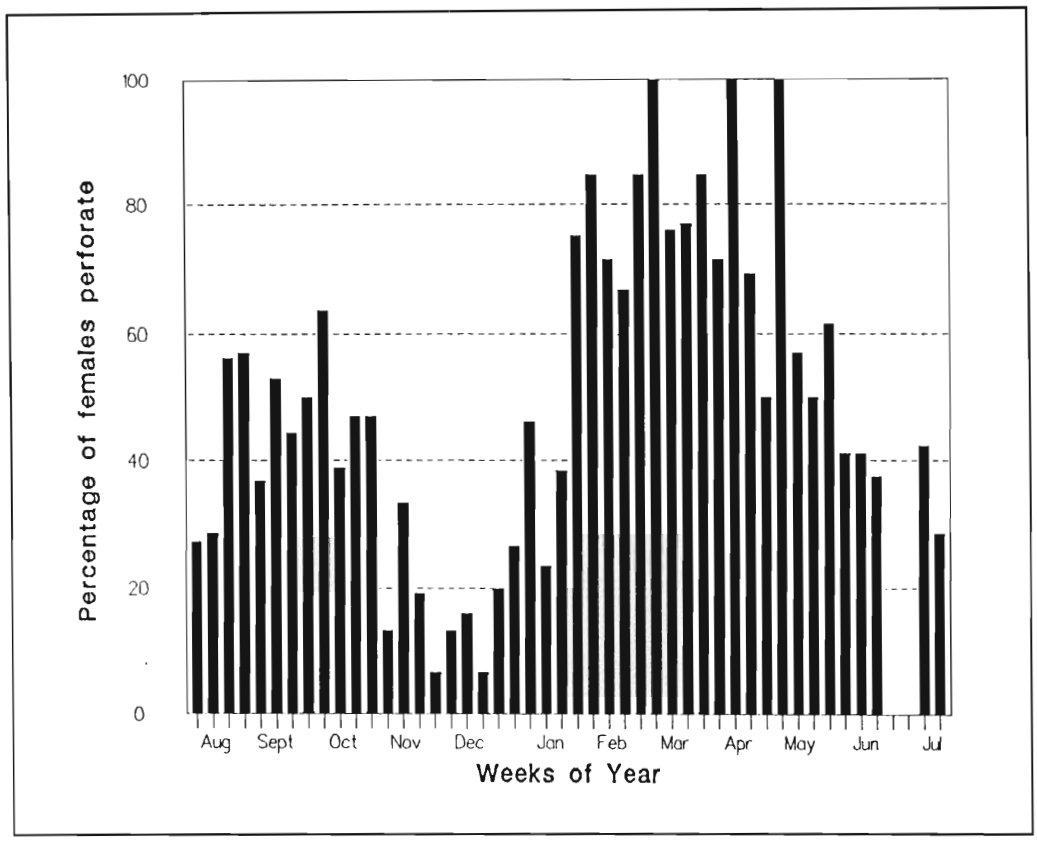


**Figure 8.2** Percentage females in the colony in reproductive condition throughout the year

### 8.3.3 Reproductive condition: males

The number of males with descended testes gradually increased from January until 80 to 100% were reproductively active, in the months February through to June. The number gradually decreased from August to October until by November less than 10% of the males were reproductively active. Results are presented in figure 8.3.

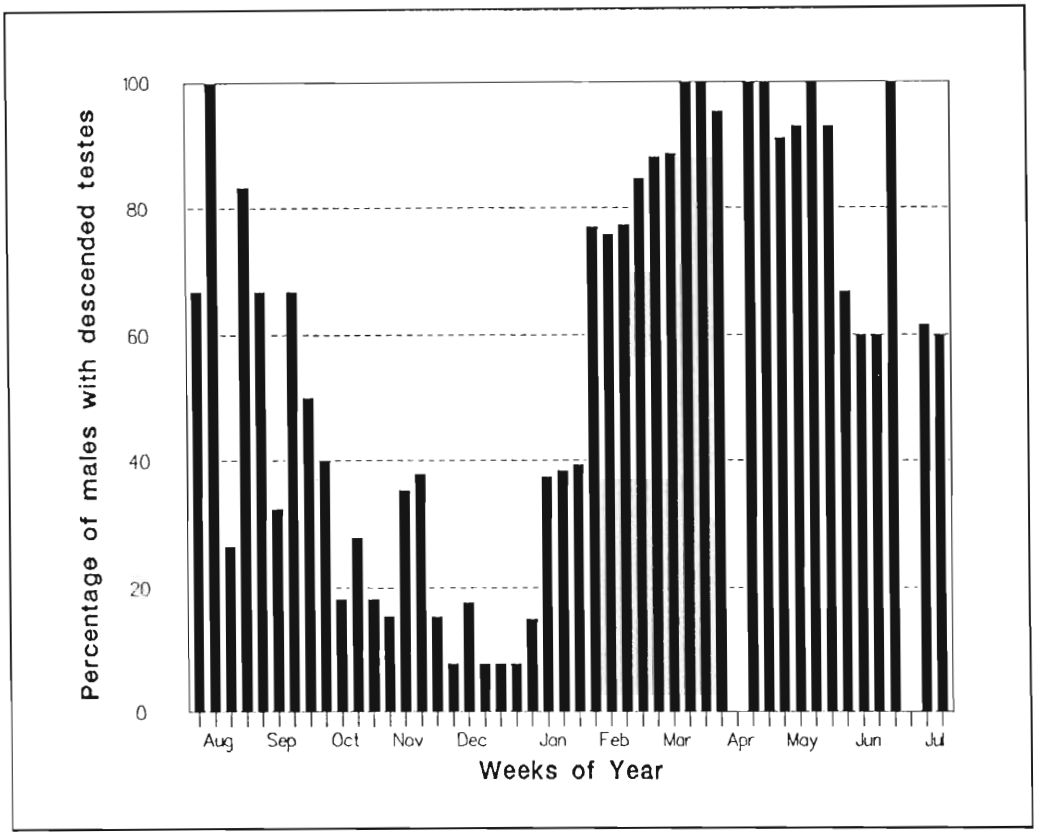


**Figure 8.3** Percentage males in the colony in reproductive condition throughout the year

### 8.3.4 Torpidity patterns

From figure 8.4 it can be seen that torpidity is definitely a winter phenomenon in *S.pratensis*. The mice show no or very little (0 to 20% of animals measured) torpidity in the summer months from December to May, with the amount of torpidity increasing gradually from then until the end of winter (October to November) when 60 to 80% of the animals measured were torpid.

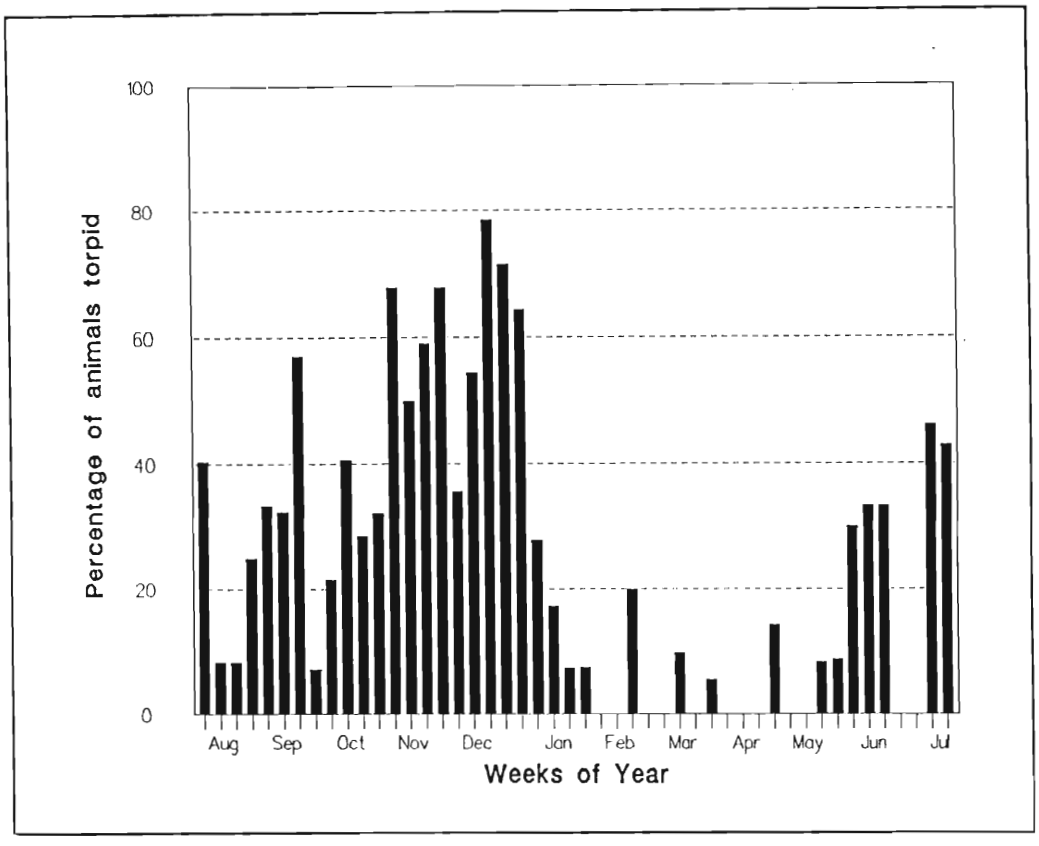


**Figure 8.4** Percentage of animals torpid throughout the year.
(Results shown are a combination of measurements taken over two years.)

All animals showed some torpor throughout the two years, although the amounts ranged from 7.1% (3 out of 42 measurements) to 63% (17 out of 23 measurements).

Younger animals spent more time torpid than older ones. These results are summarised in Table 8.1.

**Table 8.1** Percentage torpidity shown by different age groups of *S.pratensis*.
(All groups have the same number of males and females.)
(Percentage torpidity shown by females in brackets. )

| Age in years | Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| 0.5 - 1.5 | | 47.8 (47.7) | 37.2 (32.1) |
| 1.5 - 2.5 | 23.3 (31.1) | 25.9 (22.6) | |
| 2.5 - 3.5 | 15.5 (30.6) | | |

Chi-squared tests were applied between these groups on the original data of both sexes to test for differences. There was a significant difference between the two age groups in group 2 (chi-squared = 6.64, $P < 0.01$), but not among the other groups.

Females showed torpor more often than males (chi-squared = 5.36, $P < 0.05$). Although the percentage torpidity by females ranged from 48.5% in group 1 through 67.4% in group 2 to 100% in group 3, these results are not statistically significant (chi-squared tests, $P > 0.05$).

Singly-caged animals did not show more or less torpor than those kept in pairs (chi-squared = 0.29, $P > 0.5$), but animals kept with a cage mate showed the same state as the mate 91.6% of the time (i.e. either torpid or euthermic).

### 8.3.5 Pregnancy

None of the females injected produced any young. However, all animals became perforate within 2 days of the second injection and all showed a copulatory plug within 4 to 5 days, indicating that copulation had taken place.

None of the females which became naturally pregnant were found ever torpid during gestation or lactation.

## 8.4 Discussion

### 8.4.1 Mass

In the light of de Graaff's (1981) and Smithers' (1983) declarations that *S.pratensis* show a seasonal cycle of mass (and fat) gain and loss, it is surprising that the animals in this study showed little or no fluctuation in mass. Individuals showed fluctuations in mass of up to 10 g which were not related to season or other exogenous rhythms. The mean mass of the adults ranged from 38.53 to 43.80 g. Small weight losses that the animals did show occurred at the end of their torpid season, but this is not considered to be an annual cycle.

There are two possible reasons for the lack of change in body mass over the year. Firstly laboratory animals, having food always available to them, would not show the same cycles as wild animals. This is possible, but in spite of having *ad lib.* food, these same animals showed fluctuations in breeding and torpidity patterns which one would expect would also be suppressed by the conditions of captivity. Wolff & Bateman (1978) found that none of the *Perognathus flavus* they studied became torpid in the presence of *ad lib.* food.

Secondly, wild *S.pratensis* may not show the weight fluctuations claimed by de Graaff (1981) and Smithers (1983); these authors were likely catching younger and smaller animals in spring and summer. It is suggested that *S.pratensis* has a more complex life history pattern than has been previously assumed, with several cohorts of age groups. This hypothesis is supported by Vesey-Fitzgerald (1966), Genest-Villard (1979), and Sheppe & Haas (1981), all of whom found fat animals at all times of the year. Similarly Petter (1966) does not note any weight loss in *S.opimus* in spite of the small amounts of food eaten.

Most small mammals show a seasonal change in body weight which is related to body fat levels (e.g. Phillips 1979; Tannenbaum & Pivorun 1987).

*Steatomys pratensis* can enter torpor to save energy within a few days of food shortage, and provided water is available can theoretically survive for up to 48 days on their fat reserves, although the maximum managed in this study was 12 days when 30% of their weight was lost. Since they also hoard food, it seems that these animals are well equipped to survive periods of food shortage, although in the wild animals may lose weight over a severe winter.

### 8.4.2 Reproductive activity throughout the year

Females come into breeding condition by becoming perforate at the beginning of summer (December to January). Males show a similar trend, their testes descend in December to January and gradually regress from August, but their testes do not regress completely after the first breeding season. The start of breeding corresponds well with the end of topor (see the next section).

It would be expected both intuitively and from the literature that in the wild the animals would return to a non-breeding condition earlier than they did in captivity. The reproductive conditions might have been prolonged in captivity by the mild climatic conditions, the *ad lib.* food, and possibly because most of the animals did not produce any litters because they were in space restricted cages. Pengelley & Asmundsen (1975) show that the breeding females in their colony of ground squirrels (*Citellus lateralis*) hibernated with a more accurate rhythm than those females that were not allowed to breed. This assumes that breeding acts as a zeitgeber for hibernation, a possible but quite unlikely situation for *S.pratensis* as the females in the colony that did breed prolonged their reproductive season more than other females.

Although males show less time spent reproductively active during the year than females, this may be an artefact of the rather subjective way of deciding whether the males had descended or regressed testes. It would be tactical for the males to be in reproductive condition when the females are likely to come into oestrus to ensure being able to mate with them. This can be extremely important to an animal: McLean & Towns (1981) relate the earlier emergence from hibernation and the larger amount of food storage of male arctic ground squirrels *Spermophilus parryii* to the importance of the animals being euthermic when the females come into oestrus.

The *Peromyscus* studied by Lynch *et al.* (1978) showed gonadal regression at the times when the population showed the most torpor, and seasonal breeding during the rest of the year. It has been shown (chapter 7) that deprivation of food and water will cause the fat mice to enter a non-breeding condition. This is presumably a safety mechanism to prevent the mice producing young at a time when there would not be enough food and water to support them.

### 8.4.3 Torpidity

Torpor in *S.pratensis* is definitely a seasonal phenomenon (figure 8.4) with the animals showing little torpidity in the months January through May, but its probability increases from October through December when maximum torpidity of 60 to 80% of the sample occurs. There is a rapid decline in the number of animals torpid through December.

The coldest months of the year in both the field and the laboratory are May to August. This supports Coetzee's (1977) statement that *S.pratensis* go into torpor in late winter, and also Hanney (1965), Vesey-Fitzgerald (1966), and Smithers (1983) that the mice start becoming torpid in June and July. Most authors, however, say that the torpid season is finished by October (Vesey-Fitzgerald 1966) or November (Hanney 1965). The animals in this study were torpid into December although at this point they showed a very rapid decline into euthermicity presumably because of the start of the breeding season. One explanation is that the previous year's breeding season continued longer than usual and could have affected the animal's torpid season.

Results show that torpor is not an immediate response to food limitation as all animals had *ad lib.* food, although it may be used to tide the animals over a few days when the weather is too

severe to forage as stated by Smithers (1983). Protein levels of the food may change over the year which could cause a higher incidence of torpor in wild animals. This might in turn cause a genetic propensity for winter torpor over evolutionary time and the necessary selection of those mice that can show it.

Seasonal spontaneous torpor takes place in several other rodents which show daily torpor. Gaertner *et al.* (1973) report that *Peromyscus leucopus* show daily torpor on a seasonal basis during the autumn, winter and spring in Canada, with no torpor in summer. These findings are similar to those of Lynch *et al.* (1978) working with *P.leucopus* in outdoor enclosures and free-ranging in Connecticut, USA. The indoor-acclimated animals of Gaertner *et al.* (1973) did not show torpor to the same degree as those kept outdoors, which they attribute to the higher temperatures at which the indoor animals were kept. Although these animals became torpid when there was sufficient food available, these authors ascribe the torpor to an expectation of food shortages produced by the colder temperatures.

Lynch *et al.* (1978) found that mice kept in outdoor enclosures showed daily torpor during a greater portion of the year than free-ranging animals, so it is possible that the results for *Steatomys pratensis* are higher than those occurring in the field.

Unlike the animals of Gaertner *et al.* (1973), fat mice did not seem to need any acclimatisation to winter conditions to become torpid, as some of them were capable of becoming torpid even indoors in summer with *ad lib.* food. However, in a contemporaneous study of *P.leucopus* Hill (1975) found spontaneous torpor in his laboratory bred colony, indicating that it may not have been the higher temperatures which detracted from the amount of torpor in the colony of Gaertner *et al.* (1973).

All the mice in this study showed some torpor with *ad lib.* food and water which contradicts Hudson (1978) and Reichman & Brown (1979) who claim that it is advantageous for mice to show as little torpor as possible.

In spite of Hudson's (1978) claim that not all animals in a population of which some animals show short-term torpidity can become torpid, all the animals in this study showed some torpidity at one time. However, most of these animals were related, and the wild caught animals, since they were caught in the same area at the same time, may also have been related. Hill (1975) compares those animals showing torpor in his experimental group of *Peromyscus leucopus* to their relatedness to each other, i.e. he says that torpor may be only shown as a genetic trait in some animals.

At no time were all the fat mice in this study colony torpid, the maximum number torpid was 80% of the group in November. This may also be the basis for Hudson's (1978) claim. All the animals can be forced into torpidity by starvation (chapter 7).

That female *S.pratensis* showed more torpidity than males is perhaps not surprising since it would be more important for females to reach the end of winter with good fat reserves so that they could begin breeding immediately with enough reserves to maintain their young. Similarly it would be important for the males to be euthermic when the females came into oestrus.

It is difficult to explain why younger animals should show more torpidity than older ones. This is not a phenomenon which is restricted to *S.pratensis* since in a long-term study of the garden dormouse *Eliomys quercinus*, Pajunen (1981) found that the animals showed less torpor as they got older. The hibernation periods shortened with increasing age from the first to the third winter and then became stable. This was attributed not only to increasing age of the animals but also to the long rearing in the laboratory, but whether the latter was due to the steady temperature or to the environment in general was not decided. During the first two years of Pajunen's study the males exhibited longer hibernation periods but these shortened more than those of the females over the next few years so that the original difference between the sexes disappeared.

However, Davis & Swade (1983) found that the frequency of torpor in *Spermophilus beecheyi* increased with age, with the young females showing the same amount as the males but gradually showing more as they got older.

Pajunen's dormice (1981) differ from fat mice in that the female and male fat mice showed the same pattern of torpor over the first two years, but after that, the males time in torpor shortened and a difference between the sexes became apparent. The lesser amount of torpor in Pajunen's animals did not seem to affect the weight loss throughout the hibernation period since all age groups lost the same amount of weight, but the animals became heavier throughout the 6 years studied.

It is likely that young animals must put on more weight than adults to reach adult size by the end of their first winter, and becoming torpid prevents expenditure of energy which can be used for growth.

It is not known whether the differences found in dormouse and fat mice are the effect of laboratory rearing.

Since many animals taken away from their cage mates lose weight and become torpid (chapter 7), it is surprising that there was no difference in the amount of torpidity shown by singly-caged animals and those kept with mates. Obviously the animals become acclimatised to having or not having a cage mate. Although *S.pratensis* are sometimes found in pairs (Smithers 1983) it is not known whether this is related to breeding or to saving energy while torpid.

Another corollary of these results is that as the animals were torpid and not gaining weight, they must have been eating less. This raises the interesting question that either they must be eating less to go into torpor, or, torpor naturally suppresses appetite. It may also be that the mice have less time to eat as their activity periods could be shorter (Richardson 1980).

Although diet did not change over a year, the torpor patterns observed could be a genetically determined expectation of low quality food in the winter. It has been shown that a low level of protein in the diet can cause torpor (Montoya *et al.* 1979) and that diets of small mammals change throughout the year with lower levels of protein being found in winter (Perrin 1979). The animals could anticipate this and so go into torpor in the winter months.

Over 91% (481 out of 525 measurements) of the animals in this study showed the same state of torpidity or euthermicity as their cage mate. This is remarkable and seems to be caused by the animal which first became torpid, since it was noted in a preliminary study (Richardson 1980) that if an animal in any cage became torpid then its cage mate would also become torpid within a few days. This may be a physiological reaction as it would cost the euthermic animal more energy to maintain its own body temperature if it was huddling with a torpid animal. Two fat mice in a cage generally shared the same nestbox, but on the few occasions when the animals were not sharing, one was torpid and the other euthermic. This observation supports an argument for extreme plasticity in the ability to become torpid as the mice had the option of moving into another nestbox.

Lynch *et al.* note (1978) that 97% of the grouped mice studied by them were either all torpid or all euthermic. It thus seems that this is a phenomenon which also occurs in other species.

Although the number of days spent torpid increased throughout the winter, there was no discernible rhythm to any particular animal's torpor pattern. An animal would be torpid for a few days in a row and then become euthermic for a time.

### 8.4.4 Pregnancy

There are several reasons for there to have been no litters produced by the fat mice although ovulation presumably occurred (assumed from the application of the hormones and also from the external appearance of the vagina) and from the copulatory plugs it can be assumed that mating took place.

Although all the males used in this experiment had descended testes it is possible that their sperm may not have been viable as it was outside their normal breeding season. Daylength may have been too short for the females to sustain a pregnancy, but this is unlikely to affect a burrow-living animal.

## 8.5 Ecological implications of these results

One of the obvious deficiencies in this study is that it is not known how applicable these results are to a wild population. Fat mice may encounter a far wider range of temperatures, if only while foraging, in the wild than in the laboratory, and so food fluctuations would have a far greater influence on them. However, Wang (1973) has shown that while animals were kept in reasonably quiet conditions in the laboratory, there was no quantitative or qualitative difference in the measurements of body temperatures of the Richardson's ground squirrels which he studied. Similarly, Randolph (1980) showed no difference in ADMR in *Peromyscus leucopus* between free-ranging animals and those kept in laboratory cages of the same size as used in this study, although the latter were possibly under more natural conditions of temperature than the *Steatomys pratensis* used here.

Results show that under laboratory conditions *S.pratensis* has a strict seasonal cycle in respect of reproductive condition and torpidity.

It can probably be assumed that animals become torpid in winter to save energy and it is possible that they forage each evening in the same manner as the *Perognathus* described by Wolff & Bateman (1978). If they cannot find enough food that evening then they may become torpid until the next evening. This is quite possible as most of the animals showed an extreme plasticity in becoming torpid.

## 8.6 Further work

The most important result to come out of this study is that laboratory discoveries must be backed by field work, with emphasis on mass changes throughout the year. This could be done by capture-mark-release-recapture studies, but at times when the animals are in torpor, they would be almost impossible to capture without digging them out of their burrows (see Bellier & Gautun 1968). This would leave the released animal homeless which *inter alia* would naturally affect their weight.

Dietary protein levels might affect torpor patterns of wild fat mice (Montoya *et al.* 1979) but this seems unlikely because the animals went into torpor in the laboratory without any diet change. However, it would be interesting to find out if the food of fat mice changes throughout the seasons.

It was not the purpose of this chapter to explore the possibility of a "zeitgeber" for the winter torpor but only to establish if there was a strong annual cycle which could not be suppressed by laboratory holding. Having established that there is a cyclical rhythm, at least for breeding and torpor, the next (laboratory) experiment should discover just what that trigger is. However this would not be that easy: research on possible triggers for hibernation in squirrels has been ongoing for many years with only little success (Garfield 1988).

It might be possible to discover the effect of torpor on gestation length by starving a pregnant female into torpor, but it seems likely that the females would resorb or abort the foetuses if starved.

## 8.7 Summary

Adult *S.pratensis* do not show significant annual weight fluctuation after they reach adult size. They do, however, show annual fluctuations in both male and female breeding conditions, and a definite annual cycle of torpor. The animals came into breeding condition in December (mid-summer) and remained so until June and July when they went into non-breeding condition, while the amount of torpor shown increased. All animals showed torpor, young more than adults and females more than males. These can be shown to be ecologically adaptive traits. The torpor cycle and the breeding cycles are presumably linked to each other, as animals could not be made to breed during the winter months when they are usually torpid.

# Chapter 9

## Discussion

### 9.1 A possible scenario for the evolution of torpor in *Steatomys pratensis*.

The most striking physiological characteristic of these fat mice is their very low body temperature compared to most eutherian mammals. The euthermic mice regulate temperature over a 24 hour cycle, being highest in the night (33.2 to 35.0°C) when the animals are active and lowest during the day (31.1 to 32.6°C) when the animals are at rest. Activity and oxygen consumption are both higher at night and lower during the day.

Although most placental mammals have a body temperature in the range 36 to 38°C (Hart 1971), lower body temperatures are not uncommon and are found in monotremes and marsupials, and also in burrowing and desert rodents (McNab 1979b).

*Steatomys pratensis* also has a very low resting metabolic rate of 0.50 ml $O_2$/g.hr (36% of expected from the Kleiber equation), or an average daily metabolic rate of 30.9 kJ/day at 30°C (three times basal). Energetic expenditure of a euthermic animal at 20°C was 41.4 kJ/day.

*Steatomys pratensis* can only regulate it's body temperature between 10 to 30°C, becoming hypothermic below and hyperthermic above these temperatures respectively. Thermal conductance of fat mice was 0.151 ml $O_2$/g.hr.°C, 97.4% of expected based on body size.

The relation of metabolism to body temperature is always implied but rarely openly discussed in the literature (although see McNab (1984) and Gray (1981)). The low metabolic rates of desert and burrowing rodents are found in combination with very low body temperatures; it has been suggested (McNab 1966) that these are to prevent overheating and are produced by lower thyroid activity. Desert rodents often do have lower body temperatures (Hart 1971), but whether these are genetic or acquired is not known.

The difference in body temperature between *S.pratensis* and other eutherian mammals accounts for such a low metabolism. Assuming that the thermal conductance C would stay the same even if the animals had a higher body temperature, then since the metabolic rate, or MR=C(Tb-Ta), the change in metabolic rate necessary to keep the body temperature 5°C higher would be equal to the conductance times the difference between body and ambient temperature. If body

temperature was 38°C the difference would be 0.151 times 5°C or an additional 0.75 ml $O_2$/g.hr.

This would mean that at an ambient temperature of 30°C the actual metabolic rate would be the measured metabolic rate (0.50 ml $O_2$/g.hr) plus 0.75 ml $O_2$/g.hr, giving a total value of 1.25 ml $O_2$/g.hr. Since the expected metabolic rate calculated from the McNab equation in chapter 2 was 1.22 ml $O_2$/g.hr, this strongly suggests that the lower metabolic rate found in *Steatomys pratensis* is caused by the lower body temperature.

There is some disagreement in the literature as to whether the low body temperature causes low metabolism or vice versa (McNab 1984) but for the purposes of this argument it is assumed that the metabolic rate is a result of the body temperature having been "reset" to a lower level. Reasons for these animals to have such a low body temperature are now explored.

Fossorial rodents in general have low body temperatures (32 - 36°C), low metabolic rates and high thermal conductances (Lovegrove 1986). The high thermal conductance is either for the animal to lose heat, or to cope with hypoxic and hypercapnic conditions in the burrows (Lovegrove 1986). An extreme example is the naked mole rat *Heterocephalus glaber* which has a thermal conductance 125 to 250% of expected (Withers & Jarvis 1980), a very labile body temperature but a regular metabolism if the body temperature is extrapolated to 38°C (Withers & Jarvis 1980). Since *Steatomys pratensis* has a normal thermal conductance it is unlikely that the low body temperature in this species would have evolved as an adaptation to burrowing.

Desert rodents often have low metabolic rates and low thermal conductances (McNab 1979a). McNab explains this as a mechanism to prevent overheating and the loss of too much water. It seems most unlikely that a desert phylogeny could account for *Steatomys pratensis* having such a low body temperature as it is primarily a savanna species (Nel 1969; Davis 1962) and is often found near water (Smithers 1983).

However, although the fat in the mice occurs in the same deposits as other mammals, their fat is generally in thicker and heavier deposits, the layer in the skin being especially thick. Their general body shape is short and round with a short tail, and they have an inability to off-load excess heat. Cooling rates of dead fat mice were also much slower than those of the laboratory mice. Thus the basic premise that a low metabolic rate is to prevent overheating could be considered as a possible hypothesis.

*Steatomys* has been shown to be morphologically and genetically primitive (Nel 1969; Rogan 1965) but this does not necessarily mean that the genus need be physiologically primitive. In

fact, McNab (1986) proposes that morphologically conservative mammals which have low basal metabolic rates feed on invertebrates, fruits, or leaves, all food habits which he correlates with low basal metabolic rates. He suggests that because of the small amount of energy required by these animals they have survived in niches unsuitable for mammals with high metabolic rates.

Except for *S.pratensis*, *S.caurinus*, and *S.opimus* (which may all be the same species) no dendromurine has such a low body temperature. *S.krebsii* has a high body temperature of 37 to 38°C (Taylor 1984) and *Malacothrix typica* probably has a body temperature of 37°C (Knight & Skinner 1981). Since most rodents have high body temperatures it is probable that the ancestors of *S.pratensis* had high body temperatures and this species has secondarily developed a low body temperature with the associated low metabolism.

This is in direct confutation of Cade's view (1964) that torpor is a primitive mechanism retained from ancestors which were hibernators. According to Tomasi (1985), physiological deviations from a pattern are indicative of a special adaptation.

Although Hayssen & Lacey (1985) say that metabolic rates are dependent on the taxonomic affinities of the species and not on the ecology, McNab (1988) emphasises the ecological niche of the species with taxonomic affinities being of secondary importance. Since the taxonomic background of this species would probably endow it with a high body temperature it seems that the ecological niche is more important. The foods of these animals are probably insects and mixed plant materials (Genest-Villard 1979), and so therefore the metabolic rates based on food habits are likely to be medium to high (McNab 1979a).

In many ways *S.pratensis* and *S.krebsii* are very similar, having the same heavy fat layer (although *S.krebsii* may have slightly smaller deposits, see chapter 6) and probably similar ecological niches (Smithers 1983). The most obvious difference between the species is their thermal conductance. *S.pratensis* was found in this study to have a normal thermal conductance of 97.4% of expected based on body mass while *S.krebsii* was found by Taylor (1984) to have a thermal conductance of 53.6% of expected. This means that *S.krebsii* loses heat much more slowly than *S.pratensis*. However *S.krebsii* has a high body temperature; how then does it prevent itself from overheating?

Size probably plays a very important role: in spite of Smithers' (1983) assertion that *S.krebsii* are the same size as *S.pratensis* the only mass given for the former species is 24 g while adult *S.pratensis* have been seen to be almost twice that size (appendix A). Similarly, although de Graaff (1981) gives male *S.pratensis* as 22 to 30 g, and females as 34 to 48 g, in this study it was

seen that both males and females are the same size. It is possible that males are trapped at a younger age than females. *S.krebsii* studied by Taylor (1984) had a mean mass of 21.4 g and did not gain mass to any extent throughout the 55 days of the experiments. In comparison, *S.pratensis* studied here had a mean mass of 37.5 g, again almost twice the size of *S.krebsii.*

It is suggested that *S.krebsii* has a small enough body mass to lose heat without resorting to lowering either body temperature or metabolism. *S.krebsii* also has larger ears than *S.pratensis* (Roberts 1923) which must aid in heat loss, and as has been discussed in chapter 4 these mice have very long fur compared to *S.pratensis.* This longer fur may contribute to the lower thermal conductance while still giving the species the option of losing heat by "breaking" the fur in times of stress (Taylor 1984). *S.krebsii* also has a reduced distribution in a milder climate than areas occupied by *S.pratensis*, and may be limited to these areas by climate. This would account for its discontinuous distribution (Smithers 1983).

Support for this hypothesis comes from Lindstedt & Boyce (1985) who show that animals which live in highly seasonal, and generally cooler, environments tend to have greater body sizes than those animals which live in milder environments. This is a correlate of Bergmann's Rule, and is explained by Lindstedt & Boyce as larger animals having better fasting endurance than smaller ones.

But why should these animals change their fat layer rather than their metabolic rates and body temperatures? The fat layer has other uses in terms of thermal conductance and food storage, or it may have been a random event in evolution.

The low metabolism of this species predicts obligate torpidity (McNab 1983).

Hudson (1978) suggests that any mammal which is born altricially has, for the first few days of life at least, the ability to endure extreme cooling of the body and yet re-warm with no ill effects. He argues that the retention of this ability into adult life could manifest itself as torpor. This is a highly persuasive theory which would successfully explain why torpor has arisen in so many taxa independently.

*S.pratensis* can also be compared to the genetic variation of the *Mus musculus* var. albino known as the obese strain. Obesity has arisen several times independently in this species, probably the best known being the *ob/ob* strain and the viable yellow strain. These animals have a genetic propensity for putting on enormous amounts of fat. They also have a lower body temperature, lower metabolic rates, and lowered survival in the cold (Bray & York 1979). These mice appear

to have an irregulatory in the ATP-independent heat production mechanism (Jakobsen 1981). However, Seydoux (1984) showed that obese mice do not make efficient use of BAT, although the thermogenic responses could be partly restored after gradual cold acclimation or fasting.

Physiological similarity between obese *Mus musculus* and *Steatomys pratensis* may occur, but the cause is unknown.

### 9.2 Life history and ecology

Possibly the most pertinent point of any thesis of this kind is to be able to relate laboratory findings to the animal's life style in the field.

Torpor is a daily phenomenon in the fat mice studied here, the animals' body temperatures declining from the early hours of the morning and rising from late morning into the afternoon. Time spent in torpor varied from 5.5 to 11.7 hours daily. Torpor took place at those times of day when the animals would normally be asleep, and this correlates with the theory of Walker *et al.* (1981) who consider torpor to be a continuum of slow wave sleep.

Food deprivation can induce torpor, as can lack of protein in the diet (Petter 1966) but it is not known whether this is the same torpor as that which occurs naturally.

Torpor is an efficient method of saving energy: at ambient temperatures of 15 to 25°C an animal can save 43 to 69% of it's metabolic energy expenditure per hour by becoming torpid. Below 15°C the animals cannot arouse from torpor, and above 25°C they do not save energy by being torpid. Similarly, ADMR can be reduced to an average of 56% of daily expended energy by switching from euthermia into torpor (average of 18.2 kJ at 20°C).

It is most likely that during torpor, energy expended on thermoregulation is reduced to a minimum and the small amount of heat produced is a by-product of other necessary metabolic processes.

The body temperatures of torpid fat mice lay just above ambient at temperatures from 15 to 35°C, while below 15°C all animals tried to arouse from torpor.

Forced arousal from torpor at 20°C to normothermia took 13 minutes at an ambient temperature of 30°C, 19 minutes at 20°C, and 46 minutes at 10°C (0.69, 0.41, and 0.25°C/min

respectively). No animal could fully arouse from torpor at an ambient temperature of 0°C, although several attained body temperatures such that they could probably take evasive action to move from the cold environment. "Overshoot" of body temperature following forced arousal only occurred at 30°C and to a minor extent at 20°C.

Non-shivering thermogenesis utilised was 2.20 ml $O_2$/g.hr and increased basal metabolism by 369%. Maximum metabolism was about twice this value (3.80 ml $O_2$/g.hr), indicating that the animals can use other means of thermogenesis additively with NST. All animals became hyperthermic after noradrenaline administration.

Bellier & Gautun (1968) report finding dead *Steatomys opimus* at the bottom of their burrows and explain this as the animals dying during torpor: it seems likely that the mice succumbed to low ambient temperatures. When the ambient temperatures drop it seems that euthermic mice can increase their heat production sooner than torpid ones. They also take longer to lose their body heat and probably could move out of the colder areas, either by moving deeper in their burrows or increasing the nest insulation. They would thus be more likely to survive a spell of very cold weather.

While all members of the population can go into torpor, and usually do so at some time, not all mice are necessarily torpid at any one time. This may be the basis for Hudson's claim (1978) that only some of the population can become torpid. It is possible that only some of the population enter torpor at any one time, thus saving energy and so being more fit for breeding the following spring. However, should the weather become too cold the torpid mice would die, while the euthermic members of the colony may survive to re-establish the population.

Torpor is a seasonal phenomenon which the animals show even when there is adequate food, water, and high ambient temperatures. The ability to go into torpor is thus probably in "expectation" of colder temperatures, and may be induced by changes in food resources (both in quantity and quality) prior to winter. The regression of the reproductive organs is also an energy saving process, and prevents birth of young during a season in which they could not survive.

Results indicate that fat mice are energetically stressed during the winter months, and have developed torpor to survive harsh situations. The low metabolic rate and torpor of *S.pratensis* allow them to survive on less water and food; possibly permiting them to survive climates with low rainfall and productivity during winter. Having evolved these adaptations for combating food shortages, they could then colonise other areas, where their low energy demands might permit them to compete with other rodents having higher energy demands.

Both between and within species, survival time is longer in larger animals which carry more fat and thus have a longer survival time between feeding (Lindstedt & Boyce 1985). There is a large range of amount of fat stored in *S.pratensis*, and therefore a large range of times of survival during food shortages. In this study fat mice were found to take 6 to 12 days to lose 30% of their body mass, although several of these mice remained in good condition and could presumably have survived for longer. Theoretical results for these animals indicated that they should be able to survive up to a maximum of 45 days, provided water was available.

Although lack of water does not trigger torpor in *S.pratensis*, the mice are very dependent on free water since the fat in the mice can serve as a source of energy only when free drinking water is available. This may be a limiting factor to the dispersal of the species, especially in winter. During summer, lack of water is presumably not a factor in the summer rainfall areas as free and metabolic water is readily available.

Members of the *S.pratensis-S.opimus* species-complex are not particularly common but are locally abundant where they are found (Genest-Villard 1979; Kingdon 1974). There are several explanations for this, including a common food source (Smithers 1983), suitable soil for burrowing (Vesey-Fitzgerald 1966; Kingdon 1974), and available water.

Huddling with a mate reduced energy expenditure of each euthermic animal by 18% at an ambient temperature of 20°C, but did not reduce energy expenditure of torpid animals or those at 30°C. Torpor helps to reduce the energy expenditure of the animals in the wild, but if they maintain the temperature of their nests very close to the thermal neutral zone, the saving in energy would not be great.

Darling's sperm competition theory (1988) states that when males do not fight for physical dominance and numerous males mate with the same female, then the male with the biggest testes producing the most sperm, which then displaces or dilutes the sperm of rivals, would be the male whose genes survive into the next generation. This theory was used by Darling to explain the very large testes of right whales. Since male *S.pratensis* and *S.opimus* also have extremely large testes for their body size (Genest-Villard 1979), this theory should be tested. Similarly, copulatory plugs were found after mating in the fat mice. Copulatory plugs have been shown by Murie & McLean (1980) to be produced by rodents when there is pressure on the males to reduce or prevent other males from mating with the same female. Genest-Villard (1979) writes that each female's burrow is surrounded by male's burrows and furthermore the sex ratio in *S.pratensis* is highly biased towards males (appendix A) and the females in this study

were rarely kept in the same cage as they tended to fight, unlike the males. All these indicate a multiple male breeding system.

A major problem for any torpid animal is that there is little or no time to escape predators. The large and complex burrow system of *Steatomys pratensis* may reduce this problem. Being unable to arouse from body temperatures below 15°C can be overcome by burrowing and having a complex nest so that the ambient temperature does not go below the critical level. Alternatively huddling with a mate keeps the ambient temperatures above the critical level, which also has the advantage of males being near the females for breeding purposes. The restricted breeding season which results from spending a proportion of the year in a non-reproductive and torpid state is overcome by high fecundity during the breeding season (appendix A).

Fat insulation makes *S.pratensis* susceptible to overheating and it has been suggested by Sclater (1901) that the fat itself makes the animals "sluggish". Similarly, Rothwell & Stock (1986) postulate that the low metabolic rate and torpor shown by the dormice *Glis glis*, after removal of a cafeteria diet, was induced by their increased adiposity although Chew *et al.* (1965) describe obese *Perognathus longimembris* as showing less torpidity than their lean counterparts.

However, the low metabolic rate and the development of torpor which allows these mice to survive on very little energy has given *S.pratensis* an ecological flexibility which has enabled it to extend its range throughout much of sub-saharan Africa. Animals with low metabolic rates generally have longer life spans than other animals (Lindstedt & Calder 1981; appendix A), allowing the mice to live and breed for several years.

### 9.3 Restrictions on this study

The subjects were originally high altitude animals that were moved to sea level for this study. While they showed no signs of stress and bred in large cages for five years, the acclimatisation may have affected their physiological reactions.

All animals used in the study were fed the same diet (chapter 1). While they showed no signs of dietary deficiency it was obviously not the same diet as they would have had in the wild and may have affected them physiologically. The diet used here did not have any seasonal variation.

Despite two attempts to trap more animals, none were found in the area in which the original ones were caught. Not only did this mean that there was no field work to augment the laboratory results, but there was also a shortage of experimental animals.

Since these are naturally burrow-living animals they would probably be genetically adapted to high levels of $CO_2$. None of the experiments were designed to test for reactions at higher $CO_2$ levels.

### 9.4 Future work to be done

The most obvious shortcoming in this work is that there is no field work to corroborate the results. Of special interest would be the temperatures of the nests and burrows, activity times of the animals throughout the year, and body masses and reproductive status of all animals throughout the year.

From this and other studies it would seem that there are two very distinct taxa of fat mice, the *S.pratensis-opimus-caurinus* group having low body temperatures and showing torpor, and the *S.krebsii* group having higher body temperatures and not showing torpor. It would be significant to find out whether the other species (particularly *S.parvus*) align themselves with one or the other group or whether they are intermediate. The functional advantages of differences in hair structure between the two groups should be investigated since although Keogh (1985) did not find any difference in length there were other differences between the three major groups of *Steatomys*.

However, body temperatures of animals in the field would not give meaningful results as these animals can raise their body temperatures by as much as 10°C by struggling during attempts to escape. Animals tested would have to be accustomed to handling.

One major question to come out of this thesis is the extent of the fat mice's dependence on free water, either drinking water or metabolic water, since it has been shown that they cannot conserve energy by becoming torpid without drinking; their rates of water turnover in the field would be especially interesting.

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# Appendix A

## Reproduction and postnatal development of the fat mouse *Steatomys pratensis* (Rodentia: Dendromurinae) in captivity

**by E.J.Richardson and J.Meester**

### A.1 Introduction

To date no detailed description has been published of laboratory breeding patterns of *Steatomys* and the only available information comes from field notes of various workers. Shortridge (1934) states that *Steatomys pratensis* "sleeps" from April to October and gives the average number of young in a litter as probably 4 to 6. Ansell (1960) writes that in Zambia two females had six foetuses each in April and December, and that he also caught a juvenile in June and one which was estimated to have been born in February. Davis (1963) says that *S. pratensis* has "so far" failed to breed in captivity. Hanney (1965) gives reproduction in Malawi as occurring between April and July, the animals being non-fossorial during April and May. He found a male, female, and three young in a nest in July, and reports the males to become "infecund" during the dry season. Kingdon (1974) reports finding a female with three small embryos in November in East Africa and Coetzee (1977) says that the young are recorded around early summer to late autumn in temperate areas. Smithers (1971) found lactating females in February, March, April, October, and December, as well as one gravid female in February and one in December. Genest-Villard (1979) states that the dispersion of the young of *Steatomys opimus* occurs at the beginning of the rainy season and reproduction takes place during the second half of this season. Kern (1981), while trapping in the Kruger National Park, reports finding no *Steatomys pratensis* in autumn and winter although it was the dominant species in spring. Smithers (1983) gives the average number of foetuses per female of *S. pratensis* as 3.2 (n=5), range 1-9, and suggests that the young are born from October to May.

In summary, breeding in *Steatomys pratensis* seems to take place in the wet summer months, when it produces 1-9 young per litter and more than one litter per year.

The fat mouse carries a large amount of subcutaneous fat and is variously reported to eat seeds (Vesey-Fitzgerald 1966), insects (Hanney 1965), and a mixture of both (Smithers 1983). It is reported to be nocturnal (Smithers 1983). The species *Steatomys pratensis* has a low metabolic rate coupled with a pattern of torpor which is at present being studied in more detail.

Coetzee (1977) recognises three species: *S. pratensis* Peters, 1846 (including the West African *S. p. opimus* Pousargues, 1894), *S. parvus* Rhoads, 1896, and *S. krebsii* Peters, 1852. Kingdon (1974), however, regards *S. opimus* as a separate species. The animal studied here is *Steatomys pratensis natalensis* Roberts, 1929.

## A.2 Materials and Methods

The animals used in this study were first, second, and third generation captive-born stock. The original animals were trapped in the Cathedral Peak area of the Natal Drakensberg, South Africa during November-December 1979.

The animals used were originally maintained in pairs in standard laboratory cages 420 x 250 x 120 mm and fed on commercial mouse cubes but as no breeding occurred, several pairs were then housed in larger tanks (300 x 600 x 300 mm) and fed a varied diet of commercial mouse cubes, brewers yeast and a high protein dietary supplement, mealworms, and mixed seed. Greens and fruit were also offered but were discontinued when it was found that the animals did not eat them. Water was freely available in dishes. Shredded paper and wood shavings were used as bedding material, and each cage originally had two wooden nesting boxes 120 x 100 x 55 mm to serve as shelters in case of aggression between cagemates. Running wheels were also provided. Other than for the purposes of making observations and cleaning cages once a week the animals were disturbed as little as possible.

Cages were housed in a room lit by natural light supplemented by fluorescent lighting, and without temperature control. Recorded temperatures were in the range 21°C to 28°C. Both young and adults were found to be extremely docile and no form of control (e.g. anaesthetising with ether) was necessary while handling, although once animals were over 20 days of age weighing had to be done in a plastic container. Animals were weighed on a Sartorius 1265 MP digital readout balance accurate to the nearest 0.01 g.

The day when the litter was first noted (ie. when the young were less than 24 hours old, the cages being checked once a day) was designated day 0. Physical measurements were taken in the morning while observations on behavioural development were made at various times of the day. The following measurements were taken:

1. head-body length
2. tail length
3. hind foot length
4. ear length
5. litter mass

These were tabulated and graphed to ascertain at what time the animals reached adult size (see figure A.1). To determine size of adults, measurements were compared with those of all animals over one year old in the colony, as well as *Steatomys pratensis* specimens at the Transvaal Museum, Pretoria.

The following further observations were made of motor abilities and behaviour patterns (after Brooks, 1972):

1. Righting: the animal is placed on its back on a smooth surface and its ability to right itself is noted.
2. Cliff-drop aversion: the animal is placed on an elevated box, 77 mm high and its ability to move away from the edge is noted.
3. Negative geotaxis: the animal is placed facing downwards on a slope at an angle of 45° and its ability to turn and move up the slope is recorded.
4. Clinging ability: the animal is placed on a 5 mm wide bar 52 mm high and its ability to cling to and walk along the bar is noted.
5. Contact: the young from the litter are placed together and the tendency to group together or break away from the group is recorded.
6. Isolation: the young are placed at equal distances (100 mm) from their littermates and their reactions noted.

Weaning was assumed to start when the young first took solid food and to have finished when they no longer sucked from their mother.

Gestation period was estimated by observing copulatory plugs and counting days from then until parturition, and by taking daily vaginal smears. Life-span of all animals was recorded.

### A.3 Results

Figure A.1 gives the growth rates of head-body, tail, hind foot, and ear length and Figure A.2 shows the increase in mass. Figure A.3 summarises the development of physical and behavioural characteristics.

At birth young *Steatomys* were translucent pink in colour with internal organs and cranial sutures visible, and a faint groove splitting the future eyelids over the eye region. No whiskers or claws were apparent and the ear pinnae were fused to the head. The toes were also fused and

neither upper nor lower incisors had erupted. Mean body measurements at birth were: head-body 31.1 mm, hind foot 5.1 mm, tail 8.1 mm, and mass 1.96 g.

The young were born without the senses of sight or hearing. The sense of smell may have been present as the young waved their noses around in the air when removed from their parents and littermates, which is described by Williams & Scott (1953) as smelling, but since their mouths were open it is perhaps more likely that they were emitting ultrasonic distress signals in the manner described by Elwood & McCauley (1983). Indeed, when the young were returned to the parents after measuring, the mother retrieved all the young and then stopped searching, indicating that the young had some method of communicating with her which could not be perceived by the observer. The sense of touch (or at least reaction to cold) was well-developed in the young: they squeaked and wriggled away when touched by a cold metal ruler or by cold hands.

The young could not right themselves when placed on a smooth surface, move away from a cliff drop, or react to a negative slope. They could not cling to a horizontal bar and when placed in contact with each other tended to huddle. When placed in isolation from its littermates an animal could move itself only by pulling with its front legs, resulting in pivoting on the hind legs. They thus rarely met littermates but tended to huddle when they did meet.

### A.3.1 Physical development

By day two short whiskers were apparent, while small claws were visible by day three or four. Particularly obvious at this time were heavy fat deposits in the abdomen next to the hind legs.

By day four the skin was becoming grey on the dorsal surface of the head and along the back. This skin gradually became darker until day 7 or 8 when it became very dry and flaky for one or two days prior to hair appearing on the back by day 9 or 10. This hair was shorter and silkier than that of the adults but of the same brown colour. Hair appeared on the ventral surface on day 12 to 15 and grew in the same pure white as in the adults.

The upper incisors erupted on day 9 or 10 and the lower on day 12 to 15. At this time, when the mother was lifted off the litter the young tended to cling to her nipples and could be carried some distance in this manner, but this is not true nipple-clinging as found, for example, by Scott (1978) in *Mystromys albicaudatus*, as when the mother was frightened off the nest the young did not cling.

All the toes were fused at birth. The outer ones on the forefeet freed first on day 7, the outer ones on the hind feet within the next day or so. By day 12 the remaining toes were becoming looser and all were free by day 15.

The eyelid groove gradually became darker until by day 13 it was almost black. Eyes opened on day 18 to 20, although for the next few days they remained closed most of the time except when the animals were actively exploring.

Ear pinnae were folded and fused to the head at birth but had loosened by day 3 or 4, although hearing was not present until day 15 to 19.

Figure A.1 shows that hind foot length reached adult size at about 20 days, ear length at about 30 days, and tail length at around 35 days, while head-body length was only 86% of adult size by the time measurements were discontinued at 60 days.

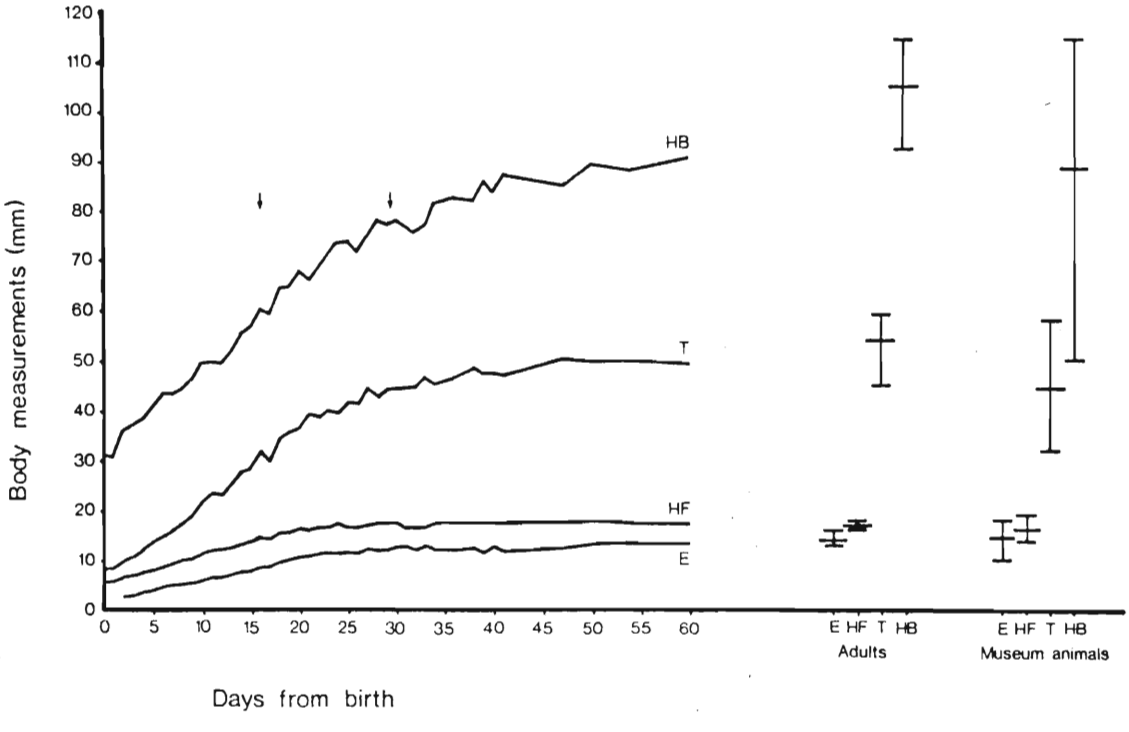


**Figure A. 1** Growth rates of the Head-Body (HB), Tail (T), Hind Foot (HF), and Ear (E) lengths of young up to 60 days. Arrows indicate start and end of weaning. Variation in laboratory adults and museum specim ns is shown in bar graphs on the right.

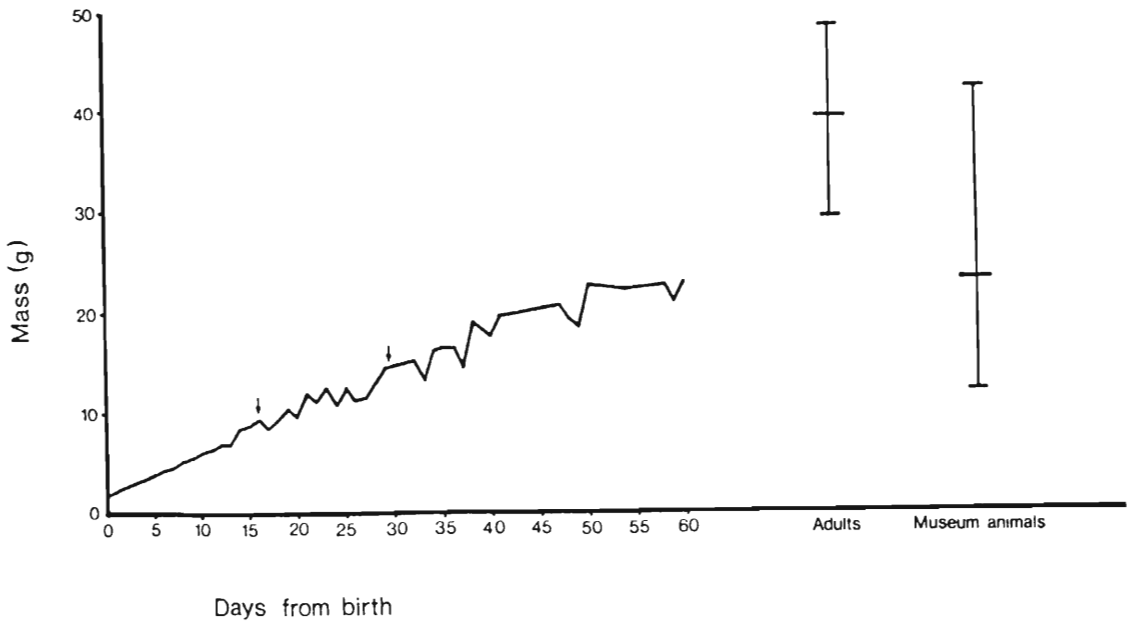


**Figure A. 2** Mass increase of young up to 60 days. Arrows indicate start and end of weaning. Variation in laboratory adults and museum specimens is shown in bar graphs on the right.

At weanin g mean mass was 15 g, 38% of the mean adult mass of 39.15 g.

### A.3.2 Behavioural development

Behavioural development of young *Steatomys* was slow with few behavioural abilities appearing before 10 days, except for strong rooting behaviour from day 3.

The ability to cling to a horizontal bar first became obvious by day 10 to 12, although the young developed the ability to manoeuvre properly on it only by day 15.

By day 9 or 10 crawling was fairly strong, although the animals tended to move backwards, and by day 11 to 13 the young managed a shaky walk with their bellies off the ground, although this was not particularly well directed because the eyes were shut.

Cliff-drop aversion developed by day 12. By day 7 or 8 the young seemed to sense a negative slope but did not have the ability to turn around on it. This is assumed from the fact that they turned their heads around to face up the slope but often lost their footing when trying to move. The ability to turn and move up the slope developed by day 14 or 15.

The ability to right themselves on a smooth surface started to develop on days 15 or 16.

Until roughly day 18 the young huddled when placed in contact with each other but after this they occasionally deliberately moved away from the group. Up to this time, when placed in isolation they tended to get up and move, still huddling when they met, but after this they did not always stay in a group after a chance encounter. After the eyes had been open for a few days the young started actively exploring when taken out of the nest, and locomotion rapidly became as competent as in adults.

For the first week or so after birth the young emitted a squeaky grizzle when disturbed, especially by cold, and towards the end of this week they were also emitting a click as described by Elwood & McCauley (1983). These squeaks and clicks gradually became less frequent, possibly due to the animals becoming used to being handled and developing thermoregulation. By day 20 vocalisations were the same squeaking as before but lasted longer, resulting in a squeal of one or two seconds duration. This was the same sound heard occasionally from the adults when they were picked up roughly when torpid, and was one of the only two types of vocalisation ever heard from the adults - the other being a longer grizzle heard during rare aggressive encounters between females. Clicking was never heard in adults.

The female was observed to lick the young in the inguinal region when they were small, presumably partly to stimulate the production of urine and faeces. No grooming movements from the young were observed until day 10, when inefficient face washing was first seen. It became more efficient within two or three days. Scratching with the hind legs followed on day 12 or 13 and around day 13 to 15, when the toes were finally separating, nibbling of the feet was frequently seen. By day 20 grooming was as in adults.

Weaning started around the time when the eyes opened, with the young sniffing at their parents' food and attempting to bite it. Interest in solid food gradually increased until by day 24 to 28 the mother of the litter would be found sleeping outside the nestbox, indicating that weaning was complete. The young were never found sucking after this, although within one or two days the female would move back into the nestbox with the young.

Young *Steatomys* were never observed to play although it is reasonable to assume that if this occurred it would happen at their most active time (late evening and early morning) when fewest observations were made. It is possible that because the behavioural observations were made at a time when the young would not normally be active the responses may have been affected and/or a biased impression of responses may have been obtained.

Around day 22 the young developed a "scatter" reaction when the nest was disturbed, running in all directions, and around day 26 they developed the adult reaction of freezing when disturbed by a strange sound. Just before and after weaning, for a period lasting two or three weeks, they showed the only aggression towards the observer recorded in this study. This aggression appeared during the time when the young were most active when disturbed, running and jumping around the cage, and when held for measuring they wriggled and bit violently until released. This aggression was never active, i.e. the young would not bite until an attempt was made to restrain them, and disappeared after one or two weeks when the animals became docile again.

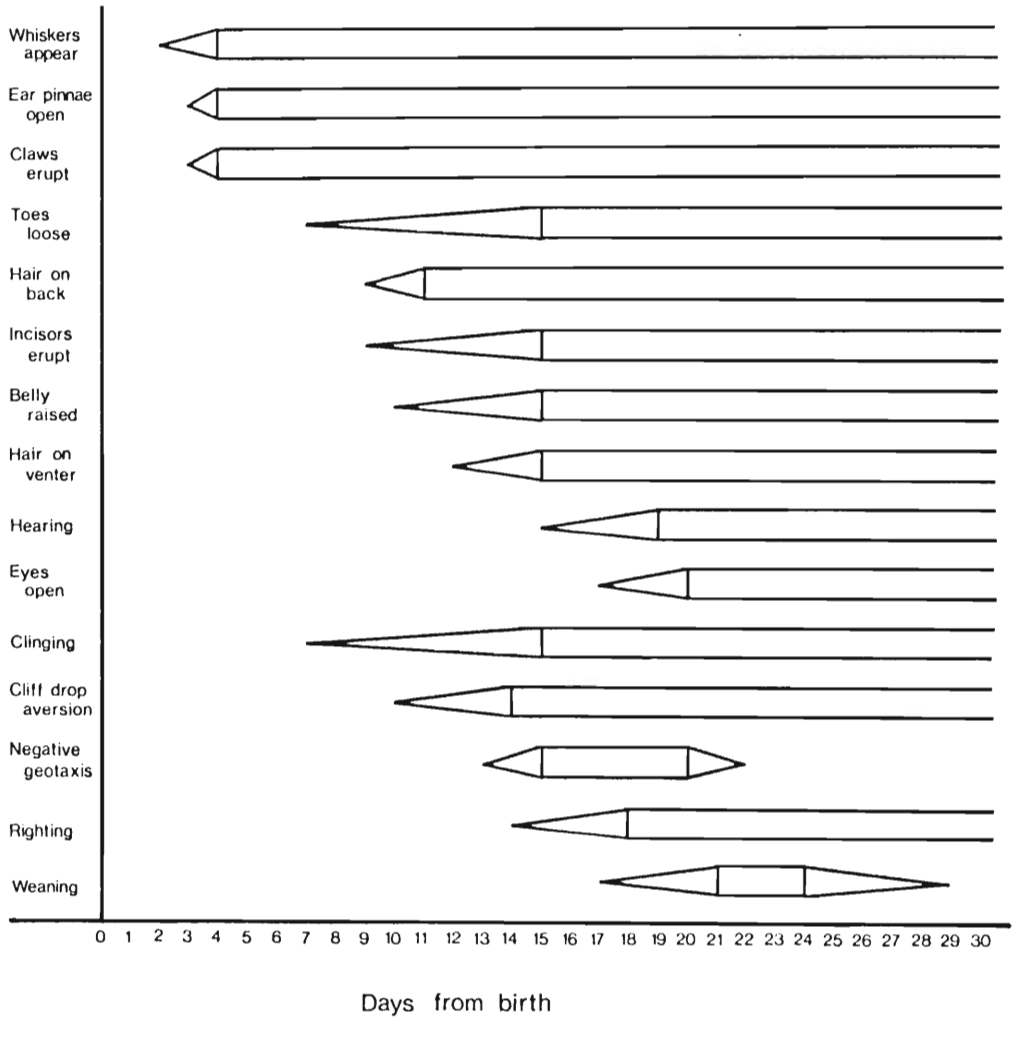


**Figure A. 3** Physical and behavioural development of young up to 30 days. Arrows show the duration of appearance and disappearance of an attribute.

### A.3.3 Parental behaviour

As would be expected from the generally calm behaviour of adult *Steatomys* towards the observer, females were very docile when their young were handled for weighing and measuring. At no time did they show any aggression towards the observer or their young when returned, although they did tend to lick them when put back in the nest. The females never carried the very small young in their mouths but, sitting up on their hind legs, rolled them with their forelegs to wherever they wanted to move them. When the young got to such a size that rolling was impossible the mother would grasp a hind leg or flank in her mouth and drag it. At weaning the females did not move the young out of the nest but themselves moved out and were found sleeping at the opposite end of the cage. If the male had been left in the cage with the female, he was always found with the young in the nestbox at this time.

Of the total of 24 litters studied, the males were left with the females in 18 cases, partly to ascertain whether or not there was a post-partum oestrus and partly to observe their reactions to the young. At no time was any aggression observed either between the males and the females or between the males and their young. In fact, barring the provoked aggression of the young towards the observer around weaning and one occasion when a female bit her mate during the day after the birth of her first litter, the only aggression ever observed in the colony was between two adult females in the same cage. Although the males did not participate in the retrieval of the young and were never seen to groom them, they generally shared the nestbox with the female and young and were often found with the young when the female was out of the nestbox. The only time when the male was never found with the female and young was on the first day after the birth of a new litter.

Sibling care was never seen in any of the litters studied, since at no time was a litter born while the previous litter was still in the cage.

### A.3.4 Litter size, sex ratio, and estimated gestation period

A total of 24 litters comprised 131 young: 19 of these died before they could be sexed and of the 112 remaining, 73 were males and 39 females. Of 90 young which survived until weaning (mortality rate = 31.3%), 56 were males and 34 were female. This gives a male ratio of 1.87 at birth and 1.65 at weaning (chi squared = 4.59, $P < 0.05$ and 2.25, $P > 0.10$ respectively). Of 75 specimens of *Steatomys pratensis* in the Transvaal Museum collections 45 are males and 30 females, a male ratio of 1.5 (chi squared = 1.14, $P > 0.25$).

Litter size varied from 4 to 9 (mean 5.6) and of the 24 litters 6 consisted of 4 young, 7 of 5 young, 8 of 6, 1 of 7, 1 of 8, and 1 of 9 (see Table A.1). The litter of 9 young did not survive, so it appears that 4 to 6 is the normal litter size as stated by Shortridge (1934).

The high mortality rate (31.3%) of pre-weaning young is affected by the loss of complete litters. Of the 24 litters studied, 5 (31 young) had 100% mortality within the first two days, 1 litter lost four animals, 2 litters lost 2 animals, 2 litters lost 1 animal each, and 14 litters had a 100% survival rate.

Of the 7 females who produced litters all except one did so in their second summer season (when they were 9 to 24 months old), the single exception being a female who produced 3 litters in her second summer and one litter the following season. One other female produced a litter in her third summer, but that litter was not included in this study as the young were born dead and were partly eaten by the parents by the following morning.

**Table A.1** Litter sizes and numbers per female per year.

| Female no. | Litter sizes 2nd summer (9-24 months old) | Litter sizes 3rd summer (19-36 months old) |
|---|---|---|
| 3 | 4 : 4 : 5 : 5 | |
| 12 | 5 : 6 : 5 : ? | |
| 13 | 5 : 6 : 8 : 5 : 9 : 4 : 6 | |
| 17(ct) | 5 : 6 : 7 | |
| 17(comp) | 6 : 6 | |
| 21 | 6 : 4 : 4 | 6 |
| 30 | 4 | |

Age at sexual maturity in *S. pratensis* differs widely between the sexes. Although by 60 days the males had scrotal testes and could be considered capable of breeding, the females did not in general become perforate until 8 months old, at the end of the first winter after birth. The minimum age at which any female became perforate was 5 months, and the animal in question was a member of a late litter from the previous year. Mean age at first litter in the females was 14 months, and the minimum age was 9 months. This latter age may reflect the fact that none of the females was placed in a large breeding tank before they were perforate - it is possible that had they been put into breeding tanks earlier, they would have become perforate at an earlier age.

At the end of the breeding season most of the females became imperforate until the next spring. The males similarly became non-reproductive as their testes regressed.

Several methods were used to estimate gestation period. A copulatory plug is present in the females of *Steatomys* for one or two days after mating. Unfortunately this is not necessarily indicative of a successful mating but on the five occasions when a plug was noticed the interval until the birth of a litter was 24, 33, 22, 23, and 33 days respectively. In none of these cases was the female suckling another litter (Elwood (1983) points out that suckling may have the effect of prolonging gestation) so it is suggested that gestation is 22 to 24 days, with another oestrus 10 days after the first if pregnancy does not occur. At no time did a female become pregnant while suckling a litter, indicating that *Steatomys pratensis* does not have a post-partum oestrus.

Vaginal smears (following the method of Honey, 1968) were taken daily during the breeding season from 4 mated mice over a total of 99 days. The results were inconclusive, the animals being mostly in metoestrus and dioestrus throughout this time and showing no discernible cycle.

The number of days from the weaning of the last litter to the birth of the next in the same season ranged from 22 to 61 and the number of days from the birth of a successfully reared litter to that of the next ranged from 46 to 86. The number of days from the birth of an unsuccessful litter (a litter which died within two days of birth) to that of the next was 31, 32, and 42 days. These times seem to bear a relationship with the time taken for a female to produce a litter after an unsuccessful oestrus, i.e. 10 days to go into oestrus again and 22 days gestation. The interval of 42 days probably indicates a second unsuccessful oestrus.

### A.3.5 Life-span

Of the animals which died of old age or unidentified reasons (i.e. all animals which did not die accidentally), the mean age at death was 39 months (range from 24 to 53 months, n=26, SD=7.2 months). There were no differences between the sexes.

## A.4 Discussion and Conclusion

The general development of *Steatomys* is similar to that of most myomorph rodents, as described by Elwood (1983).

### A.4.1 Physical development

Young fat mice are born in an altricial stage of development and have a slow growth rate to weaning at 29 days. In nearly all respects these animals show a growth pattern typical of burrowing species as described by Scott (1979): for example she gives the time that the toes separate in burrowing species as days 4-14, and in surface dwellers as days 0-8 (*Steatomys*, days 7-15); and she gives the time of eye opening in burrowing animals as days 17-23, and in surface dwellers as 14-16 (*Steatomys* 17-20). *Steatomys* also fits into the class of "slow developer" as defined by the same author. This may be a result of the fact that being a burrow-living animal enables *Steatomys* to rear its young more slowly in comparative safety, but may also be an effect of the lower metabolic rate found in *Steatomys pratensis* (Richardson, in prep.).

Case (1978) states that a species will favour altricial young if the nest site is safer than the female's body. In the complicated burrow system produced by *Steatomys* (Hanney 1965) the young have a safe nest site.

Although the young were only 38% of adult mass at weaning, the animals continued to increase in mass linearly up to 60 days when weighing was discontinued - it is probable they continue to do so up to adult size. It would be difficult, however, to extrapolate this to the field situation as it is possible that when fending for themselves they would not gain mass so rapidly. 38% of adult mass is similar to the 34.3% quoted by Nel & Stutterheim (1973, in Scott 1979) for another burrow-living, slow-developing rodent, *Desmodillus auricularis*.

These results show *Steatomys* to be a K-selected rodent, with a slow growth and development rate, and delayed reproduction in a constant climate as described by Pianka (1970).

Figures A.1 and A.2 show that preserved specimens in the Transvaal Museum include a large number of juveniles. It is entirely plausible that these are animals in their first year which have not bred as yet. The maximum mass of 42 g found in the museum specimens is lower than that of 48 g found in this study, but the maximum mass of 47 g given by Smithers (1971) is very close. One would expect that captive animals would be better fed than young in the wild and so, although some of the wild specimens weigh less than the mass at which the captive animals were weaned, it could be assumed that these wild animals were fully weaned and independent.

Genest-Villard (1979) divides *Steatomys opimus* into three age groups, which can be recognised in the animals studied here: 1) young, with mass 15-20 g and head-body length 83-90 mm (corresponding to young which have just weaned) 2) subadults with mass 20-30 g and head-body

length 90-100 mm (animals under one year old) and 3) adults with mass 30-50 g and head-body length greater than 100 mm (animals over one year old).

### A.4.2 Behavioural development

As would be expected from the slow physical development, behavioural development was also slow, with the young being unable to make any voluntary exploratory movements until they had learnt to walk (day 15) and their eyes had opened (day 20).

The overexcited behaviour of the young at the time of weaning seems to be a fairly common phenomenon in young rodents: Scudder *et al.* (1967) describes this as "hoppy" and Williams & Scott (1953) describe it as "a period ... of exaggerated escape reactions".

### A.4.3 Parental behaviour

*Steatomys* is not the only species in which the mother leaves the young at weaning. Swanson (1983) reports that females of *Microtus montanus nanus* leave the young at 15 days old and excavate a new chamber for the next litter. She suggests two advantages of this: a clean nest has no parasites and the first litter can stay in a familiar environment. She also remarks that this system may be common among small mammals and points out that in some species sexual maturation is delayed in young females in the presence of their breeding mother, while overwintering with their families - probably because the burrow cannot support more than one breeding female, and serving to prevent breeding with the father. Swanson also states that reproductive inhibition of young female gerbils may reflect an adaptation to overwintering in burrows.

In view of the fact that the males do not assist in the care of the young and there is no post-partum oestrus, it is interesting that they remained with the female and young, and since Hanney (1965) reports finding a male, female, and young in a nest together, it seems that this may occur in the wild as well. Smithers (1983) similarly reports *Steatomys* to occur singly or in pairs. Elwood (1983) remarks that paternal care may not be the primary cause of the male remaining with the female and young but rather the desire to remain near the female.

*Steatomys* does not use either mouth-carrying or nipple-clinging to transport the young, and indeed is extremely inefficient at moving the young any distance at all. The significance of this can be realised when seen in the context of the animal's burrowing existence. Although

Smithers (1983) says it burrows only during the cold dry months and Hanney (1965) says it leads a non-fossorial existence in summer, it seems unlikely that animals with good burrows would not use them for rearing the young. Indeed Hanney, in the same paper, points out that he caught a male, female, and young in a burrow. It is probable that the large number of mostly lean animals caught during the summer includes the young of the previous year leaving the parental burrow to begin their own breeding burrows, since it has been found (Richardson, in prep.) that the adults do not lose their fat in summer as assumed by Smithers (1983). Genest-Villard (1979) remarks that the young remain for a long time with their mother, which supports the idea that they stay in the parental burrow until the next summer when they leave to start their own nests.

The inconclusive results obtained from vaginal smears may be explained by the findings of Eisenberg & Isaac (1963) who write that *Liomys* and *Dipodomys*, when kept continously as pairs, went into prolonged anoestrus and did not breed. Since the vaginal smears were taken from (non-breeding) animals in the smaller cages it is possible that *Steatomys* shows a similar cessation of breeding.

### A.4.4 Species biology

The high male sex ratio in both captive and wild populations is difficult to explain. The bias towards males would seem to indicate a higher mortality rate amongst the males, which is remarkable considering that the only aggression encountered was between females. However, Pianka (1970) lists, as a correlate of being K-selected, keen intraspecific competition and directed, density-dependent mortality which may have a bearing on the sex ratio.

Litter size in this study was larger than that found by field workers. Although the maximum litter size was the same as that found by Smithers (1983), the minimum of one quoted by Smithers (1983) is smaller than found here. It may be that captive animals have a higher fecundity due to being in better condition than wild ones. For a slow-developing animal like *Steatomys* the litter size is large, although Scott (1979) points out that a burrow-living animal can afford to increase the size of the litter since it is fairly easy to keep track of young in a burrow.

Since each *Steatomys* female is multimammate, having at least 16 mammae, it is interesting that litter size seems to be limited to nine young, and most probably usually four to six.

Breeding appears to be seasonal with the reproductive organs of both sexes regressing in the cold and dry months while the adults become torpid for a time (chapter 8).

Life-span of the animals in this study was very much longer than the maximum age at death of 25 months reported by Jarvis & Morris (1961, p. 294), who also report an average age at death of 20 months in animals which have survived 12 months. However Genest-Villard (1979) reports that her *S. opimus* lived for at least two years. Breeding success of females after their first year appears erratic - of the two females in this study who produced litters in their third summer, one did so with no trouble and one failed to rear the litter.

*Steatomys* appears to be very dependent on its burrow during the breeding season, as not only are the altricial young slow developers behaviourally and physically but the adults also lack efficient methods of retrieving the young. The movement of the female out of the nest at weaning of the young may be an adaptation to burrow-living, as it would be possible in nature for her to move to another part of the burrow while still remaining in the safety that the system affords.

## A.5 Summary

Postnatal physical and behavioural development of 24 litters (131 young) of the fat mouse *Steatomys pratensis natalensis* was measured until 60 days. The young were born in an altricial state of development and grew slowly, showing the typical development pattern of burrow-living animals, until weaning at 24 to 28 days. When observations were discontinued they were still increasing in size. Both parents stayed with the young, although the males took no active part in caring for them. Gestation period was estimated at 22 to 24 days with no post-partum oestrus. Litter size was most frequently 4-6.

## A.6 Acknowledgements

Sincere thanks are due to Ms C. M. Baker for help and advice throughout this project and also for caring for the animals in the authors' absence. Mr G. F. van Wyk of the Department of Forestry provided the animals from which the breeding stock was bred and Mr D. Wolhuter assisted in obtaining Transvaal Museum data. Mr R. Peplow kindly assisted throughout the project and placed computing facilities at our disposal. Thanks are also due to Ms J. Stegeman and Mr C. W. Sapsford for assistance in caring for the animals. This project was financially supported by the Council for Scientific and Industrial Research and the University of Natal Research Fund.

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# Appendix B

## A description of the equipment used to monitor activity, body temperatures, and oxygen consumption of *Steatomys pratensis*

by E.J.Richardson and R.C.S.Peplow

### B.1 Introduction and overview

The basic data-logging system consisted of a Rockwell Aim 65 microcomputer with some custom-built electronics designed by three final year undergraduate students of the Department of Electronic Engineering in 1983.

The system provided a continuous monitor of oxygen consumption, body temperature and activity of a mouse placed in a respirometer, and recorded the results on an integral thermal printer. While the system was such that measurements could be taken every one, ten, or thirty minutes, in practise it was found that readings every ten minutes proved adequate.

The computer was calibrated by using the single line LED display which, when the computer was in test-mode, would give either the readings from the oxygen analyser, mini-mitters, or activity monitor, and would display these for a minute, allowing any adjustments to be made. At any time the computer could be asked to give a report of the latest status.

The printout gave the time of day, a hexadecimal value corresponding to the activity over the previous ten minute period, the average oxygen analyser read-out as a percentage of 255, and the maximum and minimum number of pulses from the mini-mitters over the ten minutes.

### B.2 Oxygen consumption

The 0-5 volt electrical signal from the Beckman oxygen analyser was connected into an eight bit analog to digital converter on the microprocessor.

The oxygen content of the air coming out of the respirometer was sampled every minute and these readings were averaged to give the report reading every ten minutes. The output was printed as a two-digit hexadecimal number. Calibration was performed by simultaneously reading the hexadecimal number from the LED display and the readout on the analyser and drawing up a conversion table.

The conversion table was put into the SuperCalc 4 data files and the hexadecimal numbers were thus translated into percentage oxygen readings.

### B.3 Body temperature

After every mini-mitter had been coated in wax it was calibrated by placing it into a waterbath of varying temperatures. The conversion table for each mini-mitter was again put into the SuperCalc 4 files and the correct body temperature calculated for each readout.

The radio signal from the mini-mitter was picked up on an ordinary medium wave radio, the speaker output of which was filtered to produce pulses that could be counted by the computer over a one minute interval. The computer then reported the maximum and minimum readings over the ten minute period.

A single LED was used as a monitor to ensure that the signal was being correctly picked up. The radio sensitivity (volume) was adjusted such that the computer picked up only the mini-mitter signal from the radio and not any of the extraneous signals from the constant temperature room.

### B.4 Activity

The activity monitor consisted of an etched pair of interdigitated copper foil electrodes made from a panel of electronic printed circuit board material. The monitor was placed beneath the respirometer, spaced some 5 mm above a grounded copper plate.

The capacitive coupling between the two electrodes varied as the mouse changed it's position in relation to the electrodes. This varied coupling was used to determine whether the mouse had moved within the time span measured.

With no mouse in the respirometer the activity monitor was adjusted to a base level, the mouse was then added and the sensitivity of the machine adjusted such that it recorded movement when the mouse changed position in the respirometer but recorded no movement when the mouse was stationary.

The readout from the computer printer gave the range of capacitive coupling detected (maximum and minimum) over the sample interval as a hexadecimal number. Control runs were

used to determine a base level of interference from the constant temeprature room; this base level was subtracted from the activity level reported and the resultant value was used to designate the animal as active or quiet.

### B.5 Critique

While the system itself worked well, the interference from the constant temperature room was a continual source of problems. Since they could be easily distinguished from interference the body temperature readings were accurate, but the activity monitor picked up considerable interference which made the results unreliable. For this reason the results were clumped to give a trend, with no attempt made to separate the different runs or to make any comparisons between them.

As has already been discussed the low turnover rates in the respirometer unfortunately also gave the oxygen analyser readouts a long response time lag which meant that the readings could not be closely correlated with either the body temperatures or the activity times. They were thus also clumped to give a trend without close comparisons.

In spite of these problems the system provided a method of measuring the animals' physiological patterns over 24 hours which would have been impossible to measure any other way. It thus acheived it's primary objective.

# Appendix C

## Histological Method

### C.1 Fixing, clearing, and embedding.

Fixative: Zenker-formol (Helly's fixative)
Take 1000 ml distilled water, add 50 g mercuric chloride and dissolve with heat. Add 25 g potassium dichromate (this fixes the lipids). Just before using add 5 ml 40% formaldehyde per 100 ml fixative. Fix tissue pieces c. 2 mm by 2 mm for 8 hours, then wash for 24 hours in running water. Store the pieces in 70% ethanol.

Using this fixative, care must be taken to remove the mercuric chloride from the tissue on the slide by alcohol-iodine when staining.

Clearing:
75% alcohol for two hours, changing alcohol once; 95% alcohol for two hours, changing alcohol once; 100% alcohol for two hours, changing alcohol once; Toluene for two hours, changing once.

Embedding:
Warm paraffin wax for three hours, changing twice

### C.2 Sectioning

Sections were microtomed at 10 $\mu$m.

Egg albumin adhesive:
Add 0.1 g NaCl to 200 ml distilled water, dissolve and add 1 g egg white. Shake and leave to stand for 24 hours. Filter in buchner funnel and add 200 ml glycerine and 2 ml 1:10 000 merthiolate.

### C.3 Dewaxing and Rehydrating

100% xylene for 5 minutes; 1:1 xylene:alcohol for 5 minutes; 100% ethanol for 5 minutes; 95% ethanol for 5 minutes; 80% ethanol/iodine (95 ml ethanol + 10 ml 4% tincture of iodine) for 5

minutes; 5% Na thiosulphate for 2 minutes; Rinse in running tap water; Rinse in distilled water.

### C.4 Staining

Ehrlich's Haematoxylin:
Add 3 g haematoxylin stain and 1 g Na iodate to 150 ml distilled water. Heat until just boiling and remove from heat. Add 8.1 g potassium aluminium sulphate and stir until dissolved. Mix 150 ml glycerol, 150 ml 96% ethanol, and 15 ml glacial acetic acid, and add to the above mixture when it has cooled to 50$^{o}$C.

Eosin Y:
Add 5 g Eosin Y to 100 ml distilled water and add 400 ml 95% ethanol. Take one part of this stock solution and three parts 80% ethanol. Add 0.5 ml glacial acetic acid per 100 ml stain.

Carbol-xylol:
Mix 20 ml melted phenol fused crystals with 80 ml xylene.

Method:
Ehrlich's Haematoxylin for 10 minutes; Rinse in running tap water; 1% Na bicarbonate for 30 seconds; Rinse in distilled water; Rinse in 70% ethanol; Eosin Y solution for 10 minutes; Rinse in 95% ethanol for one minute; Rinse in 100% ethanol for two minutes; Carbol-xylol for two minutes; Xylene for two minutes; Xylene for 5 minutes

Mount coverslips with DPX mountant.

### C.5 Reference

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