

COMPARISON OF BROWN ADIPOSE TISSUE  
FROM ARCTIC AND TROPIC SMALL MAMMALS

by

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ACCEPTED  
FACULTY OF GRADUATE STUD

DATE

Sept 28, 1989


DEAN


A Thesis Submitted in Partial Fulfillment of  
the Requirements for the Degree of


MASTER OF SCIENCE

in the Department of Biology

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## ABSTRACT

The mean weights, histology and ultrastructure of the brown adipose tissue from five species of rodents from Costa Rica (tropical) were compared with the brown adipose tissue from four species of rodents from the Northwest Territories of Canada (arctic). The rate of change ( $y = 0.612 - 0.269x$ ,  $r^2 = 0.59$ ) of the relative weight of brown adipose tissue ( $y$ ) with increase in body size ( $x$ ) in the arctic rodents, compared to that of the tropic rodents ( $y = 0.965 - 0.468x$ ,  $r^2 = 0.70$ ), was statistically the same ( $P > 0.50$ ). A comparison of the total amount of brown adipose tissue, as measured by the elevations of the two lines, showed no significant difference ( $P > 0.50$ ).

In a comparison of members of the family Cricetidae from the arctic, the adult *Peromyscus maniculatus* and *Phenacomys intermedius* show remarkable consistency in the microstructure of the brown adipose tissue. The lipids are in a multilocular form with tightly packed, complex mitochondria filling most of the remainder of the cell. Sub-adult and juvenile *Peromyscus maniculatus* showed nearly identical structure. The neonates of *Phenacomys intermedius*, however, have almost no lipid in the cells while the mitochondria are more loosely packed and less complex than in the adults.

In contrast to the arctic rodents, the five tropical species show much more variation within each species and between species. *Reithrodontomys* sp. all have typical brown adipose tissue multilocular lipids in the cells, while *Mus musculus* has

unilocular cells that resemble white adipose tissue. *Heteromys desmarestianus*, although consistent within individuals, varied among individuals between a multilocular and unilocular form of the lipid. The tissue of *Liomys salvini* has a dual structure, with a number of multilocular cells on the inside and a layer of large unilocular cells on the outside. Both adults and neonates of *Oryzomys fulvescens*, have multilocular cells but with only about half of the lipid that the other species have.

In summary, it is possible that in both the arctic and the tropics, small mammals have evolved with the potential for the same amount of brown adipose tissue. In the tropics, where the cold stress on the animals is minimal, the thermogenic function of the tissue is required to a lesser extent and therefore the tissue, in some species, has transformed into what appears to be white adipose tissue by coalescing the numerous smaller lipid droplets into a single larger droplet and thus becoming more of a storage tissue. In the arctic, where the thermogenic function is essential, the cells contain multilocular lipids in a much more uniform pattern between *Phenacomys* and *Peromyscus* and between the different age groups of these two species.

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## ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. John Hayward, without whose support, both moral and financial, my work would probably not have been possible. Also, my appreciation to Dr. A.R. Fontaine, Mr. H.F. (Jack) Dietrich, and Dr. C.L. Singla for their instruction and assistance with the techniques of the Electron Microscopy Laboratory and Mr. Tom Gore for his help in the Photography Laboratory.

I gratefully acknowledge that financial support for my travel to Costa Rica and the laboratory work was provided through Dr. Hayward's NSERC Operating grants and a grant from the Northern Scientific Training Program, provided by the Federal Government of Canada, Department of Indian Affairs and Northern Development, assisted with my travel to the Northwest Territories in Canada.

## **1. INTRODUCTION**

Brown adipose tissue (BAT) is a highly vascularized and innervated form of adipose tissue that is the major contributor to a mammal's capacity for "nonshivering thermogenesis". In form, the cells of this tissue are multilocular, that is, the lipids are not found in one large droplet but in a number of smaller droplets which are surrounded by densely packed mitochondria in the cytoplasm of the cell. The close contact between the lipid droplets and the mitochondria allow for a rapid mobilization of the triglycerides in the metabolic cycle. As to the amount of BAT in any individual animal, Himms-Hagen (1986) stated that "Although no quantitative survey is available, on the basis of the fragmentary evidence available it may be summarized that the amount of brown adipose tissue is scaled to body size in an inverse fashion so that per unit of body mass there is less in large animals than in small animals."

In comparison to white adipose tissue (WAT), the nucleus in a BAT cell is more spherical in shape and is usually more centrally located within the cell. A high vascularization of the tissue and abundant cytochromes in the mitochondria gives this tissue its distinctive light brownish colour and hence its name, brown adipose tissue (Hull and Segal 1966; Daniel and Derry 1969), while a rich sympathetic innervation allows the BAT to respond rapidly to the presence of norepinephrine and induce a high level of heat production (Cottle 1970).

Brown adipose tissue was first described in 1551 by Gesner (cited in Rothwell and Stock 1985) as "a brown fatty tissue found in the interscapular region of the marmot." Since that time, BAT has been described and discussed by other

workers such as Jacobson (1817), Ehrman (1883)(cited in Rauch 1968), Hammar (1895)(cited in Rauch 1968) and Rasmussen (1923) and has variously been described as a hibernating gland and also, more accurately, as a modified form of adipose tissue. The idea that this was a discrete 'hibernating gland' was refuted by Ehrman in 1883 as he found this tissue in mammals other than hibernators at some stage in their lives. Although a thorough investigation had not been carried out, Ehrman (1883)(cited in Rauch 1968) predicted that this tissue would be found in most if not all mammals. Reviews of the literature by Johansson (1959) and Smalley and Dryer (1967) have indeed shown that this tissue is found in most mammals as juveniles and retained into adulthood, particularly by hibernators. It has also been stated by Nechad (1986) that "Although brown fat was discovered more than 400 years ago, the limits of its species distribution are still not defined with certainty." Further studies have suggested that this tissue may be found in animals other than mammals (Oliphant, 1983; Rothwell and Stock, 1985) and is not necessarily found only in the placental mammals (Rothwell and Stock, 1985).

During the 1960s, considerable work was done on the thermogenic role of BAT (Dawkins and Hull 1964; Davis 1963; Donhoffer and Szelenyi 1965). This work also included a description of the role of BAT in the maintenance of body temperature in cold-acclimated mammals (Smith 1962, 1964) and the high heat production that was measured during arousal from hibernation (Hayward and Ball 1966; Horwitz *et al.* 1968; Smith and Hock 1963). These investigators studied the increase in oxygen uptake (and therefore heat production) *in vivo* and *in vitro* for various mammals including man. This high heat production was found to occur not only during arousal from hibernation but also during moderate to severe cold stress in non-hibernators and hibernators (Donhoffer and Szelenyi 1967; Smith and

Roberts 1964) and in early, post-natal life (Napolitano and Fawcett 1958; Dawkins and Scopes 1965; Hull and Segal 1965a; Segal 1975; Gemmell and Alexander 1978).

BAT appears early in prenatal development in mammals (Barnard and Skala 1970; Barnard *et al.* 1970; Alexander *et al.* 1975; Alexander 1978). Pre-adipocytes begin to accumulate lipids as if to form white adipose tissue and then rapidly develop large numbers of mitochondria as well as increase vascularization and innervation of the tissue. At the time of birth, the quantity of BAT can be as much as 3 - 5% of the total body weight (Hull and Segal, 1965b) and under the stimulation of noradrenalin this can result in increases in oxygen consumption of up to 200%, leading directly to heat production (Cannon and Nedergaard, 1986).

In some mammals, the development and activation of BAT thermogenesis (as measured by an increase in oxygen consumption when stimulated by norepinephrine) has been described as "immature", "altricial" or "precocial" by Nedergaard *et al.* (1986). In mammals that are very "immature" at birth, such as the hamster, the activity of the BAT does not start for about 7 days and does not reach a maximum until approximately 17 days after birth. In the more-developed but still nest bound "altricial" small mammals, such as the rat, the thermogenic reaction requires 3 to 10 days after birth to develop. With "precocial" newborns such as guinea-pigs and rabbits, the thermogenic action is present at the time of birth and decreases from then on.

During the subsequent growth of the neonate, the quantity of BAT decreases rapidly with age if there is no cold stress (Nnodim and Lever 1985). In thermoneutral or warm conditions, the BAT changes to the appearance of white adipose tissue within a period of three to four weeks. This is a definite gradual change which results in a reduction in the number of mitochondria and the

coalescence of the many small lipid droplets into one large droplet (Thompson and Jenkinson 1969; Skala *et al.* 1970; Gemmell *et al.* 1972). This was also well-demonstrated by Bruck (1970) in a comparison of two 50-day-old guinea-pigs, one of which had been raised at 3<sup>0</sup>C and the other at 30<sup>0</sup>C. The warm-acclimated animal showed very little reaction to injection with noradrenaline, while the cold-acclimated animal showed an increase in oxygen consumption of just under 100%. The response of the warm-acclimated individual is a direct indication of either very little BAT or BAT that has changed to WAT characteristics as described above.

In some mammals, this transition from BAT to WAT does not occur. One case is hibernators, which will use the BAT during arousal from hibernation throughout their lives (Hayward and Lyman 1967). The quantity of BAT varies during the year but is greatest during the hibernating season. The other case is small mammals such as rodents, which, because of their small size, have very limited insulative capacity, and therefore increase the mass of BAT when cold-stressed to maintain body temperature during the winter (Didow 1968).

There have been some studies of mammals from the arctic (Grav and Blix 1976; Hart *et al.* 1961) that have addressed the problem of heat production and oxygen consumption as measures of survival capability in extreme temperatures. Grav and Blix (1976), in particular, showed that, during cold stress, BAT had the function of increasing oxygen uptake 2-fold during the first two weeks after the birth of seal pups. Beyond two weeks *post partum*, the rate of oxygen increase during cold stress decreased. This indicated that BAT was essential for the survival of seal pups during the first two weeks of life while they put on the extra layers of insulating fat.

During the last 20 years, one of the major emphases of research on brown fat has been to understand its metabolic responses and the effects of noradrenaline and

the resulting production of heat. Most workers found an increase of one- to two-fold in the oxygen consumption of various animals (Hayward 1971; Hayward and Davies 1972; Kaciuba-Uscilko and Poczopko 1973; Loudon *et al.* 1985), whereas Hayward (1968) found an increase of 10.6-fold in the oxygen consumption of bats during arousal from hibernation.

Recent work has also focused on the role of BAT in diet-induced thermogenesis (Glick *et al.* 1985) and also the role of BAT in obesity (Moore *et al.* 1986). These two closely related areas of study show that, with BAT present, the intake of "excess" carbohydrates causes an increase in the activity of the BAT, an increase in the utilization of the energy and results in an increase in the production of heat as a by-product. Meanwhile, a decrease in the intake of protein also causes an increase in the thermogenic response of the BAT (Swick and Gribskov 1983). Thus, more heat is produced while the same amount of or less food is ingested (Trayhurn and Mercer 1986; Stock and Rothwell 1986). Over-eating, as shown by Rothwell and Stock (1979) not only increased the total body weight by increasing white fat deposition, but also resulted in an increase in the oxygen uptake and the energy expenditure of the test animals. Administration of injected noradrenaline also increased the oxygen uptake by 100% over the control animals indicating that the amount and activity of BAT also increased (Rothwell and Stock 1979). Some, if not all, of this oxygen uptake increase has been implicated in some test animals as diet-induced thermogenesis involving liver metabolism (Ma *et al.* 1987).

During the period from the 1960s through to the 1980s, another research emphasis was the demonstration of the biochemical activity of the BAT (Masoro 1963; Joel *et al.* 1967), as well as the metabolic pathways of heat production and the rapid mobilization of the lipids (Hittleman and Lindberg 1970; Skala 1984).

However, as stated by Nechad (1986), "The morphological and biochemical features of brown adipose tissue development throughout the life of the animal have been characterized in detail in only a few species. The most comprehensive data concern the interscapular brown fat of the laboratory rat." As the techniques of biochemical analysis became more refined, an uncoupling protein was discovered that, in conjunction with the BAT mitochondria, was shown to release more heat than would normally be expected from cellular energetics (Bouillaud *et al.* 1983; Lean and James 1983; Nicholls and Locke 1984). This protein was found to be a 32kD protein that had a direct effect on the free fatty acids (FFA) of the BAT in the presence of noradrenaline. Some of the FFA entered the mitochondria while the remainder stayed in the rest of the cell. The FFA also acted as the principal substrate for the protein, which was found to have an uncoupling effect on the oxidative phosphorylation on the inner mitochondrial membrane, dissipating the proton gradient normally generated. This protein has also been found to bind purine nucleotides with the effect of inhibiting proton conductance and re-coupling oxidative phosphorylation (Drahota 1970; Rothwell and Stock 1985; Rothwell and Stock 1986).

Although there has been work carried on from the 1960s (Joel 1965; Roberts and Smith 1967) to the present day (Himms-Hagen 1986; Moore *et al.* 1986) on the cold acclimation mechanism and the thermogenic nature of BAT, little work has been done on a comparison of warm-adapted and cold-adapted species. The classic work in the area of arctic versus tropic metabolism of homeotherms was done in 1950 by Scholander *et al.*. Hissa (1968) did a study on the oxygen consumption differences between lemmings from the arctic and golden hamsters originally from Syria and showed that the lemmings' response of increased oxygen consumption

occurred earlier in postnatal life as a reaction to cold stress. The increased oxygen consumption response of lemmings was also about 100% greater than the response shown by the golden hamsters. The cold acclimation studies that were done were, for the most part, investigations of cold versus thermoneutral conditions and have not addressed the greater variations of temperature that small mammals have evolved to live in during expansion of their geographic distribution. The paucity of comparative and adaptive information is expressed by Himms-Hagen (1986) ". . . information currently available is mostly limited to four particular animal species, one that is always euthermic (the rat), two that become torpid (the mouse and the Siberian hamster), and one which hibernates (the Syrian hamster). Our knowledge is thus insufficient to permit any general theory about control mechanisms. Additional comparative studies of these species as well as others with similar life-styles are needed for a complete understanding of the mechanism of control of brown adipose tissue growth and thermogenesis during cold acclimation."

Therefore, with the hypothesis that there are different quantities of brown fat when comparing small mammals from the arctic with those from the tropics; and, that there are differences in the gross and fine structure of that brown fat; this study is designed to compare arctic and tropic adapted species. This study includes several species and age-groups with the intent to gain insight into the evolution of brown fat in response to geographic differences in temperature regime.

## **2. METHODS**

### **2.1. Locations of Study Areas**

In this comparison of arctic and tropic small mammals, the areas chosen were Guanacaste Province, Costa Rica, in Central America and the Northwest Territories in Canada, separated by 6,120 km.

The tropical area was located in the northwest corner of Costa Rica in Central America at 10°33' north and 84°59' west. This is an area of variable local climate. Within an 80 km radius, climatic conditions varied between dry savannah, moist grassland and dense tropical forest. The first two regions were selected as being the most suitable areas for collecting a variety of small mammals. These regions (Fig. 1a and b) were superficially similar to the northern areas in terms of gross appearance of habitats. Temperatures, cloud cover and rainfall were measured on a daily basis at the sampling sites. In Costa Rica during the last half of March and the first half of April 1988, the night time temperatures dropped down to +18°C and during the day rose up to +35°C. Very light rain fell on 4 days out of the 25 days of collecting, (Appendix 4, Table 9), making this one of their drier years.

The arctic region chosen was 20 km south of the southern shore of Great Slave Lake at 60°40' north and 115°46' west. This region consisted mostly of uniform boreal forest of aspens, black spruce and juniper bushes (Fig. 2a and b). Due to an unusually wet year, most of the land was covered with up to 6 inches of water and giving some areas, where trapping was carried out, the appearance of muskeg. During the last week of May and all of June 1988, temperatures were still low at night (down to +0.5°C) and fairly high during the days (up to +23°C) with

Figure 1. a) Trapping area near Laguna de Arenal, Guanacaste, Costa Rica. Traps set approximately 1 metre inside the edge of the brush surrounding the small grassy opening.

b) Trapping area along small river 5 kilometres north of Cañas, Guanacaste, Costa Rica. Traps set approximately 3 metres off the trail on either side.



Figure 2. a) Trapping area in a mixed spruce, aspen and juniper forest 1 kilometre north of Enterprise, Northwest Territories, Canada.

b) Typical trap-set surrounded by brush and under cover of a fallen log and low-hanging branches.



rain falling on 19 days out of the 35 days of collecting (Appendix 4, Table 10).

## 2.2. Source of Animals

Specimens were collected using Sherman and Longworth live traps set at approximately 10 m intervals along the edges of brush and fence rows. Since all animals were handled in the same manner, if some stress related to the trapping did occur, this would have been consistent in all cases and would not compromise a comparison of the results for the arctic and tropic locations. Any pregnant adult females were held for up to 2 weeks in 4-litre, plastic containers with 1/4" wire mesh tops and were provided with food and water *ad libitum*. These individuals were kept outside, sheltered from direct rainfall, with nesting material consisting of soft grasses and other plant materials obtained in the local area. It was assumed that although the adults may have been slightly stressed during the trapping and holding period, this would have had little or no effect on the neonates.

All animals were euthanized with an over-dose of diethyl ether and then the genus and species, sex, age and total body weight was recorded for each individual. Age was determined by gross external features (i.e. adult - in reproductive condition; sub-adult - slightly smaller and in non-reproductive condition; juvenile - furred but with eyes still closed (age in days estimated from overall size); and neonate - born in captivity within the previous 6 hours). Immediate removal of the brown adipose tissue reduced the possibility of weight loss in the tissue due to desiccation or due to the complications of holding the tissues or animals for even a short period of time.

### 2.3. Isolation of Brown Adipose Tissue

All possible brown adipose tissue located outside the body cavity was excised, separated into interscapular brown adipose tissue (IBAT) and extra-thoracic brown adipose tissue (EBAT - which did not include the IBAT), placed in tared Eppendorf micro test tubes and weighed to the nearest 0.1 milligram. The calculation of % IBAT is probably slightly biased in that it was not possible to remove all internal BAT for weighing. This resulted in a lower total brown adipose tissue (TOTBAT) and thus in a higher % IBAT when compared to the TOTBAT. Portions of the interscapular pad of brown adipose tissue that were to be examined further were cut into approximately 1 mm cubes and preserved in buffered 4% glutaraldehyde (Barnett *et al.* 1964). All fixed materials were then stored in sealed Eppendorf micro test tubes and returned to the University of Victoria for histological evaluation.

The interscapular pad of brown adipose tissue was chosen for detailed study as it is the largest deposit and is easily removed in its entirety. As was suggested by Barnard *et al.* (1970), the interscapular pad is the most important site during growth and as Hayward and Davies (1972) determined, the IBAT (between 40 and 45% of the total BAT) contributed approximately 40% of the total response to norepinephrine in non-shivering thermogenesis.

The percentage IBAT was calculate for each individual and a single factor analysis of variance (ANOVA (Zar 1984)) was used to compare (within each location, arctic and tropic, and between the two locations) the total body weight and percentage IBAT of the adults of all species. The same comparisons were also made of the data collected from all neonates in both locations.

#### **2.4. Light Microscopy**

Tissue samples, fixed in 4% glutaraldehyde and post-fixed in osmium tetroxide, were embedded in EPON 812, sectioned on a Reicher Om U2 ultra-microtome to thicknesses of 0.5 to 1.0 micron, and stained with Richardson's stain only or with Sudan Black B only (Appendix 1)(Humason 1967). This produced samples with the lipids and other cell components stained in various shades of blue (Richardson's stain) or with the lipids only stained a medium grey with no counterstaining of the remaining cellular components (Sudan black B).

#### **2.5. Electron Microscopy**

Tissue samples that had been fixed in 4% glutaraldehyde were post-fixed in 1% osmium tetroxide and embedded in EPON 812. Sections were cut with a Reicher Om U2 ultra-microtome to a thickness of 0.01 to 0.05 microns, stained with 2.0% uranyl acetate for 1.5 hours and 0.2% lead citrate for 6 minutes, and mounted on a #200 copper grid for viewing with a Phillips EM 300 electron microscope.

### 3. RESULTS

In Costa Rica (CR), 25 days of trapping resulted in the capture of 22 individuals from five species and three families (Table 1). These were also categorized into three age classes of adult (A), sub-adult (SA - i.e. non-breeding) and neonate (N) for a total of six different groupings of animals for consideration of the percentage interscapular brown adipose tissue.

Table 1. List of species trapped.

Family	Genus Species	Common Name	n
<b><u>Guanacaste, Costa Rica</u></b>			
Heteromyidae	<i>Liomys salvini</i>	Little Spiny Pocket Mouse	2
Heteromyidae	<i>Heteromys desmarestianus</i>	Greater Spiny Pocket Mouse	9
Cricetidae	<i>Reithrodontomys sp.</i>	Harvest Mouse	3
Cricetidae	<i>Oryzomys fulvescens</i>	Pygmy Rice Rat	5
Muridae	<i>Mus musculus</i>	House Mouse	3
			---
			22
<b><u>Northwest Territories, Canada</u></b>			
Cricetidae	<i>Peromyscus maniculatus</i>	Deer Mouse	13
Cricetidae	<i>Phenacomys intermedius</i>	Heather Vole	8
Sciuridae	<i>Tamias minimus</i>	Least Chipmunk	2
Sciuridae	<i>Tamiasciurus hudsonicus</i>	Red Squirrel	1
			---
			24

In the Northwest Territories (NWT), a total of 30 days of trapping resulted in the capture of 24 individuals from four species and two families (Table 1). These were categorized into four age classes of adult, sub-adult, juvenile (J) and neonate, for a total of seven groupings.

### 3.1. Total Content of Brown Adipose Tissue

The results of the total body weights, IBAT and EBAT are shown in Table 2 and Table 3.

The ratio of IBAT to TOTBAT in CR samples (mean = 63.96%, S.D. = 34.51, n = 22) and in NWT samples (mean = 77.89%, S.D. = 30.66, n = 24) confirmed that IBAT is the largest single deposit of BAT. Single factor analysis of variance ( $F = 2.10$ ,  $0.01 < P < 0.25$ ) showed that the two population means are not significantly different, with respect to the proportion of the TOTBAT which consists of IBAT.

In a comparison of the mean % IBAT in relation to the mean total weight of the animal (Table 4a and b) for each species and age group in each of the two locations, there is a trend of larger animals having a lower % IBAT (Fig. 3). A comparison of the slopes ( $t = -0.429$ ,  $df = 6$ ,  $P > 0.50$ ) and a comparison of the elevations ( $t = 0.259$ ,  $df = 7$ ,  $P > 0.50$ ) of the slopes shows no significant differences between the Northwest Territories and Costa Rica samples.

Table 2. Total body weights and weights of brown adipose tissue from small mammals of Guanacaste, Costa Rica.

Species Group	Age Class	Total	Weight (g)			IBAT % Body	IBAT %TOTBAT
			IBAT	EBAT	TOTBAT		
1	A	28.8828	0.0484	0.0210	0.0694	0.17	69.74
1	A	38.4582	0.0256	0.0160	0.0416	0.07	61.54
2	A	80.0360	0.0000	0.0000	0.0000	0.00	N/A
2	A	45.0493	0.0000	0.0000	0.0000	0.00	N/A
2	A	104.0000	0.0035	0.0000	0.0035	0.00	100.00
2	A	77.3766	0.0641	0.0000	0.0641	0.08	100.00
2 h	A	93.1050	0.6434	0.0754	0.7188	0.69	89.51
2	A	109.1650	0.1664	0.2496	0.4160	0.15	40.00
2	A	89.3650	0.1821	0.0063	0.1884	0.20	96.66
2	A	89.3885	0.0000	0.0000	0.0000	0.00	N/A
2	A	41.3501	0.0000	0.0000	0.0000	0.00	N/A
3 h	A	33.3432	0.4253	0.1576	0.5829	1.28	72.96
3	A	16.6610	0.0662	0.0000	0.0662	0.40	100.00
3 h	A	24.3485	0.3353	0.1001	0.4354	1.38	77.01
4	A	60.4650	0.1100	0.0265	0.1365	0.18	80.59
4	A	47.7848	0.0490	0.0000	0.0490	0.10	100.00
4	N	3.7225	0.0357	0.0080	0.0437	0.96	81.69
4	N	3.5570	0.0369	0.0133	0.0502	1.04	73.51
4	N	4.2265	0.0506	0.0316	0.0822	1.20	61.56
5	SA	28.5880	0.1512	0.0995	0.2507	0.53	60.31
5	SA	23.0890	0.0929	0.0494	0.1423	0.40	65.28
5	SA	21.2382	0.0651	0.0198	0.0849	0.31	76.68

#### Species Groups

1 Fam. Heteromyidae	<i>Liomys salvini</i>	(Little Spiny Pocket Mouse)
2 Fam. Heteromyidae	<i>Heteromys desmarestianus</i>	(Greater Spiny Pocket Mouse)
3 Fam. Cricetidae	<i>Reithrodontomys</i> sp.	(Harvest Mouse)
4 Fam. Cricetidae	<i>Oryzomys fulvescens</i>	(Pygmy Rice Rat)
5 Fam. Muridae	<i>Mus musculus</i>	(House Mouse)

h - Held alive for up to two weeks.

Table 3. Total body weights and weights of brown adipose tissue from small mammals of the Northwest Territories, Canada.

Species Group	Age Class	Weight (g)				IBAT % Body	IBAT %TOTBAT
		Total	IBAT	EBAT	TOTBAT		
11	A	23.7539	0.0842	0.0398	0.1240	0.35	67.90
11	A	24.9258	0.0381	0.0000	0.0381	0.15	100.00
11	A	21.8609	0.0699	0.0274	0.0973	0.32	71.84
11	A	25.1501	0.1627	0.1120	0.2747	0.65	59.23
11	SA	15.4113	0.0589	0.1621	0.2210	0.38	26.65
11	SA	15.7250	0.0215	0.0000	0.0215	0.14	100.00
11	SA	14.7339	0.0592	0.0340	0.0932	0.40	63.52
11	J	4.1900	0.0189	0.0256	0.0445	0.45	42.47
11	J	3.7043	0.0318	0.0000	0.0318	0.86	100.00
11	J	4.2131	0.0392	0.0000	0.0392	0.93	100.00
11	J	3.9280	0.0343	0.0000	0.0343	0.87	100.00
11	J	3.5618	0.0263	0.0000	0.0263	0.74	100.00
11	J	3.9106	0.0178	0.0000	0.0178	0.46	100.00
12 h	A	27.8836	0.0986	0.1170	0.2156	0.35	45.73
12	A	16.5909	0.0326	0.0000	0.0326	0.20	100.00
12	A	13.6833	0.0154	0.0000	0.0154	0.11	100.00
12	A	15.7738	0.0377	0.0169	0.0546	0.24	69.05
12	N	1.8460	0.0099	0.0000	0.0099	0.54	100.00
12	N	1.8976	0.0105	0.0000	0.0105	0.55	100.00
12	N	1.9815	0.0120	0.0000	0.0120	0.61	100.00
12	N	1.8731	0.0117	0.0000	0.0117	0.62	100.00
13	A	43.3848	0.0237	0.0796	0.1033	0.05	22.94
13	A	41.8285	0.0000	0.0000	0.0000	0.00	N/A
14	A	200.0000	0.0283	0.0000	0.0283	0.01	100.00

Species Groups

11 Fam. Cricetidae	<i>Peromyscus maniculatus</i>	(Deer Mouse)
12 Fam. Cricetidae	<i>Phenacomys intermedius</i>	(Heather Vole)
13 Fam. Sciuridae	<i>Tamias minimus</i>	(Least Chipmunk)
14 Fam. Sciuridae	<i>Tamiasciurus hudsonicus</i>	(Red Squirrel)

h - Held alive for 5 hours

Table 4a. Mean body weight ( $\pm$  S.D.) and mean percent IBAT ( $\pm$  S.D.) in Costa Rican species and age groups.

Species No. and Age	n	Mean Body Weight	Mean % IBAT
1 A	2	33.67 ( 6.77)	0.12 (0.07)
2 A	9	80.98 (23.69)	0.12 (0.23)
3 A	3	24.78 ( 8.35)	1.02 (0.54)
4 A	2	54.13 ( 8.97)	0.14 (0.06)
4 N	3	3.84 ( 0.35)	1.07 (0.12)
5 SA	3	24.31 ( 3.82)	0.41 (0.11)

See Table 2 for species and age codes.

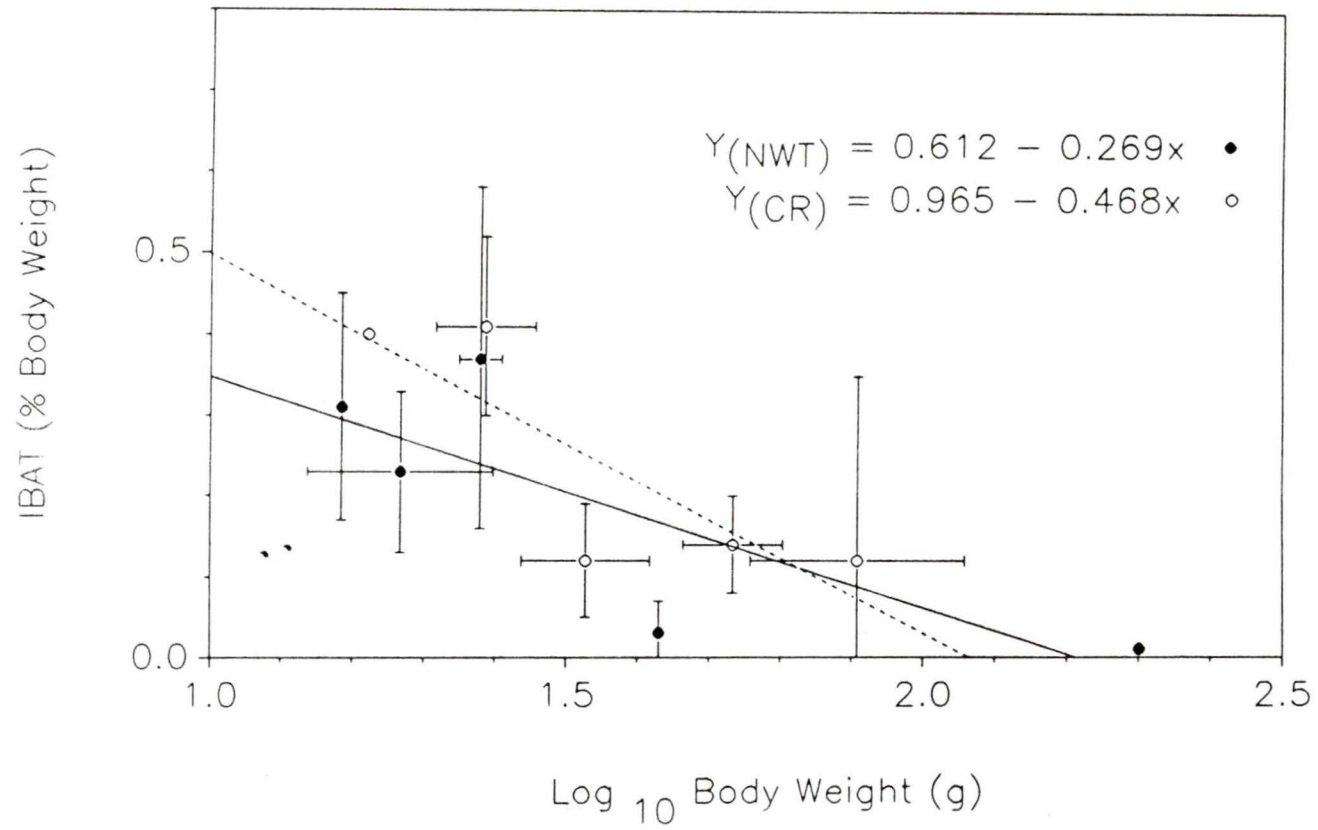
Table 4b. Mean body weight ( $\pm$  S.D.) and mean percent IBAT ( $\pm$  S.D.) in Northwest Territories species and age groups.

Species No. and Age	n	Mean Body Weight	Mean % IBAT
11 A	4	23.92 (1.50)	0.37 (0.21)
11 SA	3	15.29 (0.51)	0.31 (0.14)
11 J	6	3.92 (0.26)	0.72 (0.21)
12 A	4	18.48 (6.39)	0.23 (0.10)
12 N	4	1.90 (0.06)	0.58 (0.04)
13 A	2	42.61 (1.10)	0.03 (0.04)
14 A	1	200.00 (----)	0.01 (----)

See Table 3 for species and age codes.

An ANOVA comparison of weights (Table 5a) of all species and age groups against every other species and age group within and between locations has shown that there is considerable variation in the sizes of animals with no consistent similarities indicated. In the Northwest Territories, most of the tests indicate considerable variation in sizes. When similar ANOVA comparisons were made of the % IBAT (Table 5b) in all groups, it showed that there was almost no variation.

Figure 3. Relationship of the % IBAT ( $\pm$ S.D.) to  $\log_{10}$  mean weight ( $\pm$ S.D.) for all adults of species from arctic and tropic locations.



In comparing the Cricetids, the neonate *Phenacomys intermedius*, juvenile *Peromyscus maniculatus* (from the arctic) and neonate *Oryzomys fulvescens* (from Costa Rica), the weight (Table 5c) shows that there is significant difference between *Phenacomys* and *Peromyscus* ( $P < 0.0005$ ), and *Phenacomys* and *Oryzomys* ( $P < 0.0005$ ) but not between *Peromyscus* and *Oryzomys* ( $P > 0.25$ ). When comparing % IBAT between the same three species and age groups (Table 5d), there is a significant difference only between *Phenacomys* and *Oryzomys* ( $0.0005 < P < 0.001$ ). In the arctic, despite the significant difference in body weight between *Phenacomys* and *Peromyscus*, there is no significant difference in the percentage of IBAT ( $0.10 < P < 0.25$ ).

### 3.2. Microscopic Comparison of Samples

It is difficult to assign an absolute value to the quantity of lipid per cell in the BAT of the various species, or age groups within a species, because of the variation between individual specimens in the quantity and form of lipids found in single cells. As a result, I have decided to describe the differences in a qualitative manner.

Table 5a. Comparison of body weights within groups (Costa Rica and Northwest Territories) and between groups.

	Within (CR)				Between					
	2A	3A	4A	5SA	11A	11SA	12A	13A	14A	
1A	****	....	....	....	....	****	....	....	....	
2A		****	....	****	****	****	****	....	....	
3A			....	....	....	....	....	....	****	
4A				****	****	****	****	....	....	
5SA					....	****	....	****	****	
11A						****	....	****	****	
11SA							....	****	****	
12A								****	****	
13A									****	Within (NWT)

\*\*\*\* - indicates significant difference ( $\alpha = 0.05$ ) between tested pairs. REJECT  $H_0: \mu_1 = \mu_2$ . ( $F_{0.025(1)}$ ).

See Table 2 and Table 3 for species codes.

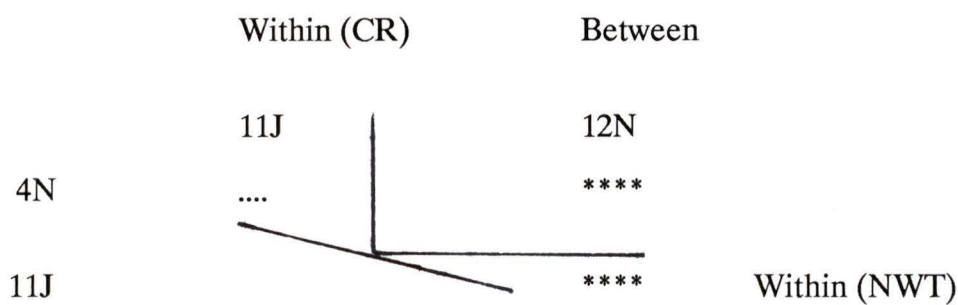
Table 5b. Comparison of % IBAT within groups (Costa Rica and Northwest Territories) and between groups.

	Within (CR)				Between					
	2A	3A	4A	5SA	11A	11SA	12A	13A	14A	
1A	....	....	....	....	....	....	....	....	....	
2A		****	....	....	....	....	....	....	....	
3A			....	....	....	....	....	....	....	
4A				....	....	....	....	....	....	
5SA					....	....	....	****	....	
11A						....	....	....	....	
11SA							....	....	....	
12A								....	....	Within (NWT)
13A									....	

\*\*\*\* - indicates significant difference ( $\alpha = 0.05$ ) between tested pairs. REJECT  $H_0: u_1 = u_2$ . ( $F_{0.025(1)}$ ).

See Table 2 and Table 3 for species codes.

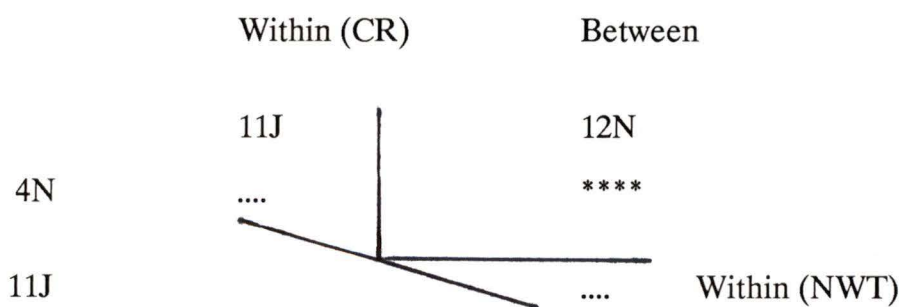
Table 5c. Comparison of neonatal and juvenile body weights within groups (Costa Rica and Northwest Territories) and between groups.



\*\*\*\* - indicates significant difference ( $\alpha = 0.05$ ) between tested pairs REJECT  $H_0: u_1 = u_2$ . ( $F_{0.025(1)}$ ).

See table 2 and table 3 for species codes.

Table 5d. Comparison of neonatal and juvenile % IBAT within groups (Costa Rica and Northwest Territories) and between groups.



\*\*\*\* - indicates significant difference ( $\alpha = 0.05$ ) between tested pairs REJECT  $H_0: u_1 = u_2$ . ( $F_{0.025(1)}$ ).

See table 2 and table 3 for species codes.

### 3.2.1. Detailed Descriptions - Costa Rica

Both specimens of *Liomys salvini* (Fig. 4) show a mixture of large and small droplets in the cells of the IBAT with the larger droplets on the outer fringes of the tissue and smaller droplets in the central region.

There is considerable variation between specimens of *Heteromys desmarestianus*. As shown in Fig. 5a, the tissue has the appearance of WAT with large unilocular droplets of lipids and the nuclei compressed on the outer fringes of the cells. As also shown in Fig. 5a, this is a well vascularized tissue. *Heteromys* BAT (Fig. 5b), is similar in some cases to that of *Liomys*, in that there is a mixture of larger unilocular droplets of lipid and smaller multilocular droplets of lipid. This tissue, however, is less well organized than that found in *Liomys*, in that the cells with the larger droplets of lipid are randomly scattered throughout the tissue (Fig. 5b).

The BAT of *Reithrodontomys* sp. contains mostly multilocular cells (Fig. 6). However, individual cells do not appear to have as much lipid as is found in BAT cell, such as the centrally-located cells of *Liomys salvini* (Fig. 4).

*Oryzomys fulvescens* deviates from the other species in that both the adults (Fig. 7a) and the neonates (Fig. 7c) have much less lipid in the IBAT cells than would be expected of typical BAT. The mitochondria in the cells of the adults (Fig. 7b), however, do not appear to be as tightly packed in the cell as the mitochondria of the neonates (Fig. 7d) but they do appear to have the same complexity of structure (Fig. 7b and 7e). The quantity and distribution of the

Figure 4. Interscapular Brown Adipose Tissue from an adult *Liomys salvini* stained with Sudan Black B to show the lipids (Typical BAT type cell outlined with arrow and hatched line).

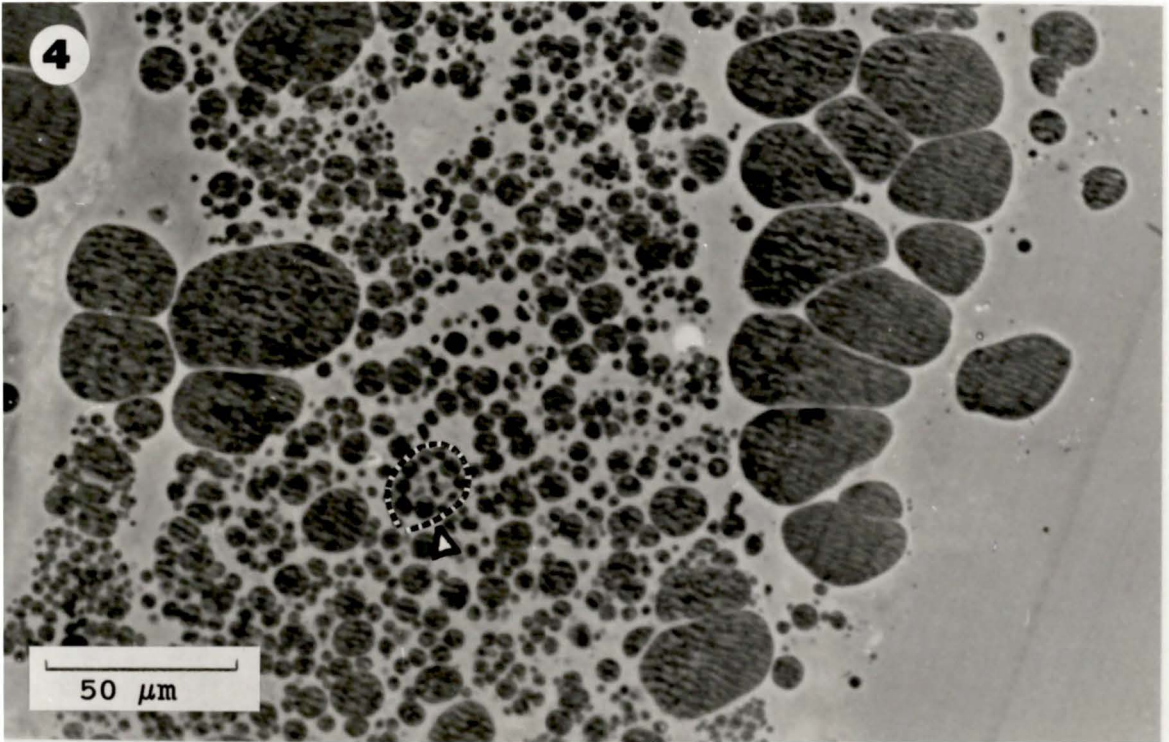


Figure 5. Interscapular Brown Adipose Tissue from *Heteromys desmarestianus*

a) Tissue stained with Richardson's Stain to show the lipids as well as other features in the tissue

(Arrows with B = blood vessels, N = nucleus).

b) Tissue stained with Sudan Black B to show lipids only and to illustrate the variation in BAT cell types

(Single cell outlined with arrow and hatched line).

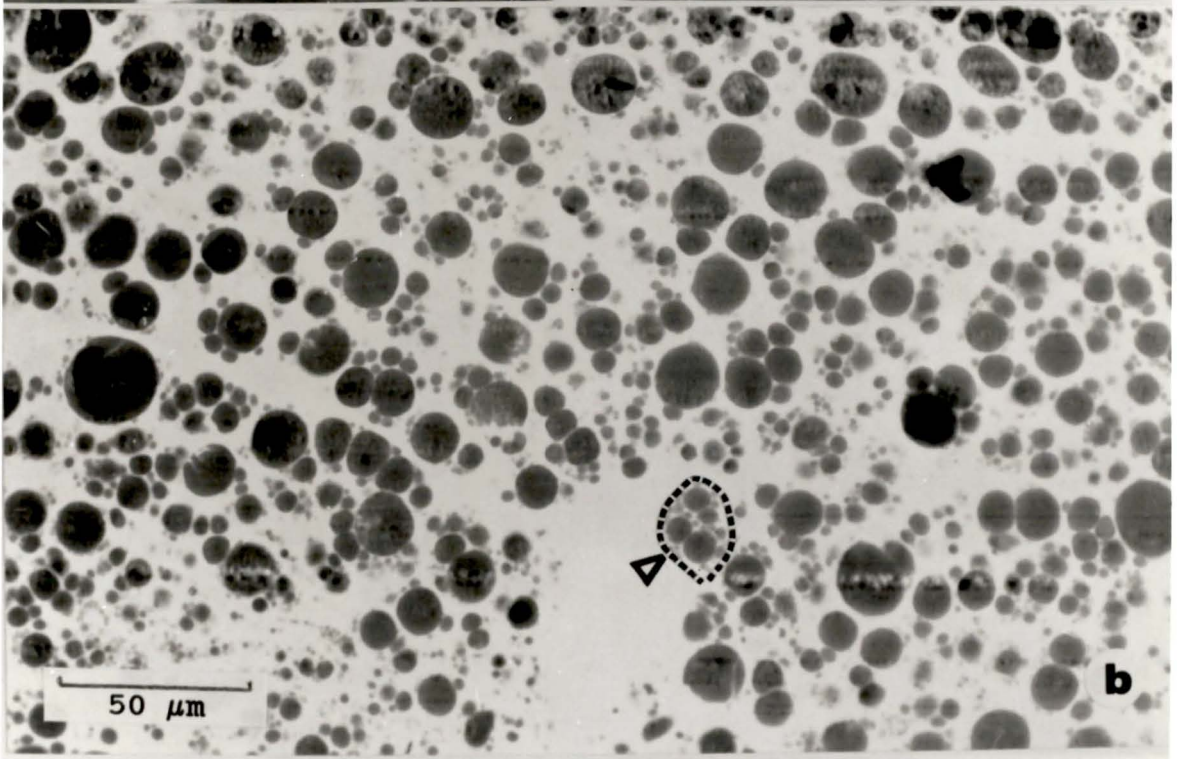
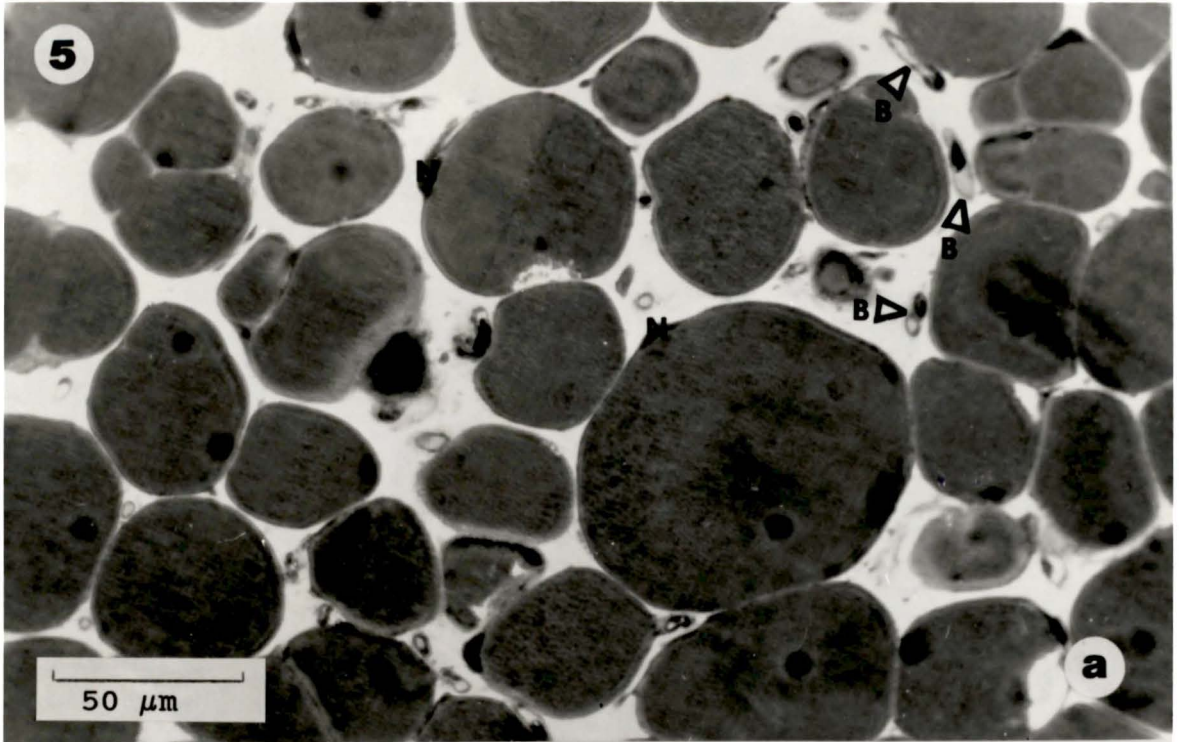


Figure 6. Interscapular Brown Adipose Tissue from *Reithrodontomys* sp. stained with Sudan Black B to show the lipids  
(Single cell outlined with arrow and hatched line).

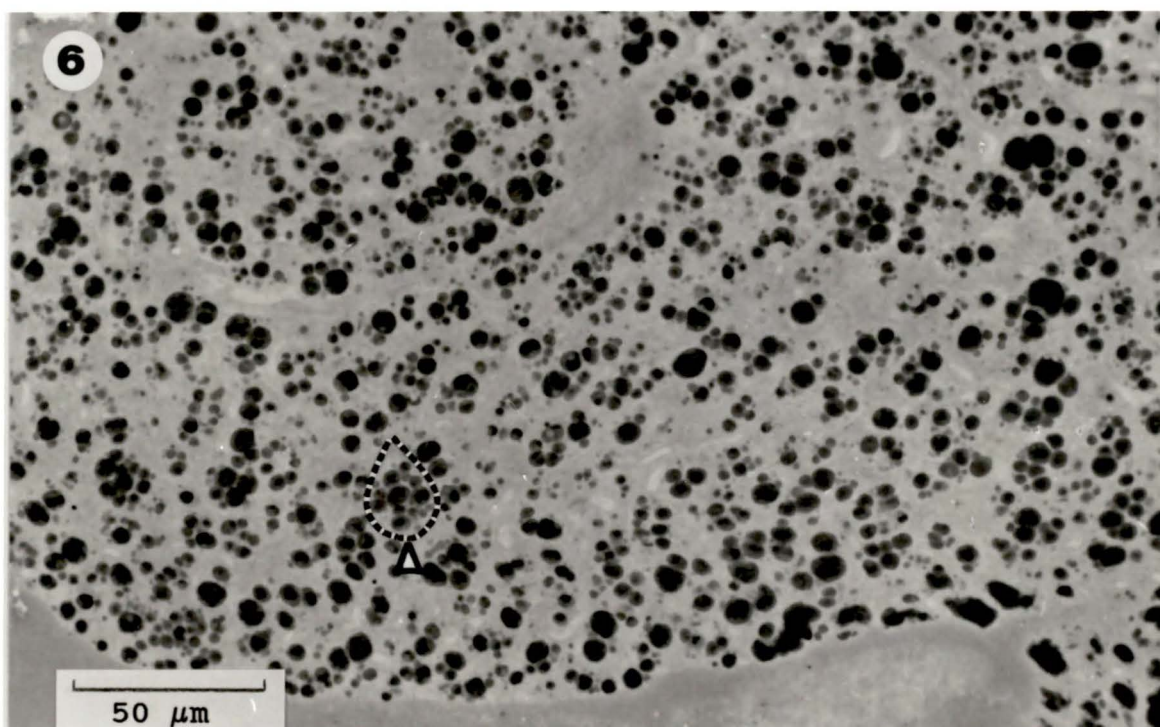
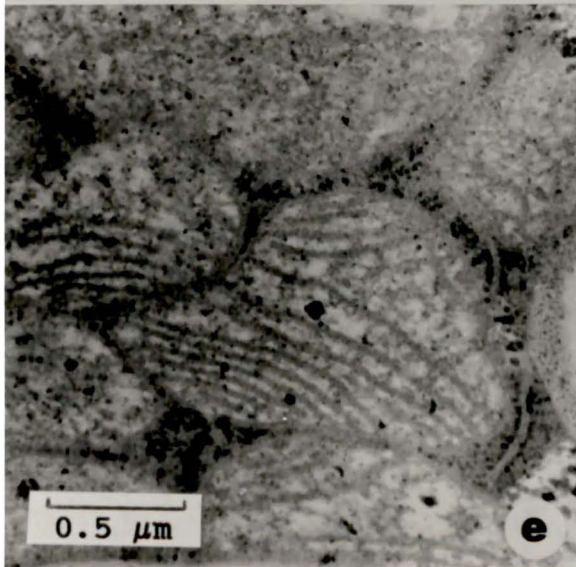
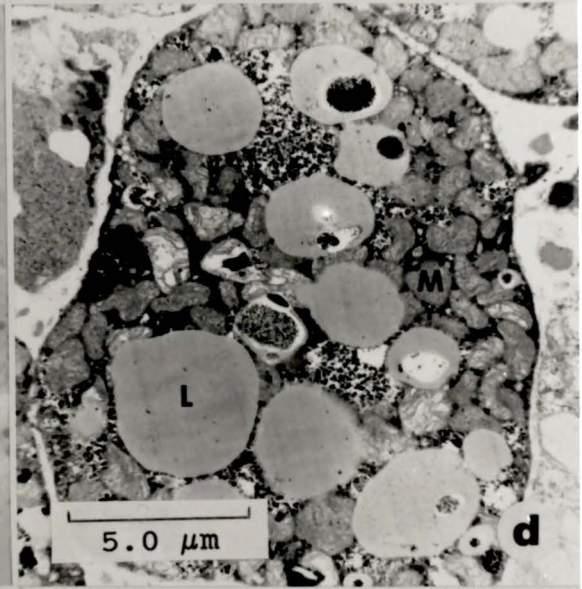
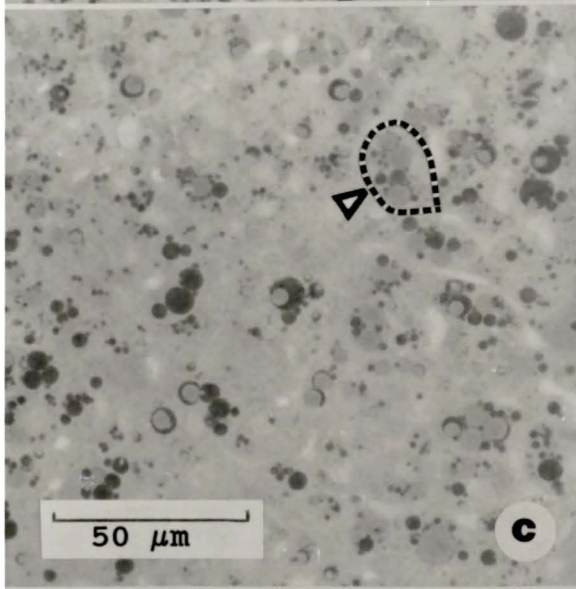
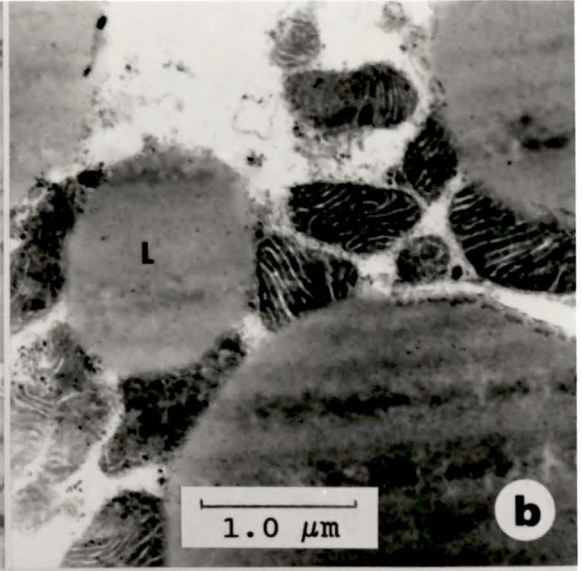
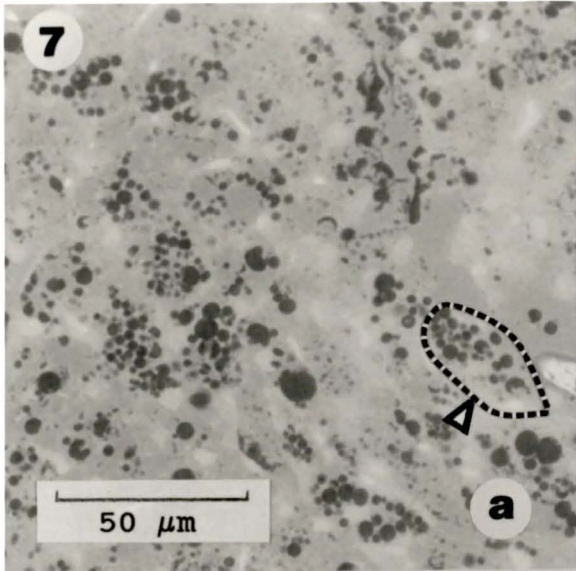


Figure 7. Interscapular Brown Adipose Tissue from *Oryzomys fulvescens*

- a) Tissue from an adult stained with Sudan Black B to show lipids (Single cell outlined with arrow and hatched line).
- b) Electron microscope view of the same individual as a) illustrating the wide spacing of mitochondria associated with the lipids (L = lipids, M = mitochondria).
- c) Sample from a neonate illustrating superficial similarity of cell structure to the adult shown in a) above (Single cell outlined with arrow and hatched line).
- d) Electron microscope photograph of a cross section of one cell showing the more compact mitochondria from the neonate shown in b) above (L = lipid, M = mitochondria).
- e) Same individual as d) showing close-up of the compact nature of the mitochondria.



lipid gives a superficial similarity to the tissue between neonate and adult *Oryzomys fulvescens*, but higher magnification of the mitochondria shows minor differences.

### 3.2.2. Detailed Description - Northwest Territories

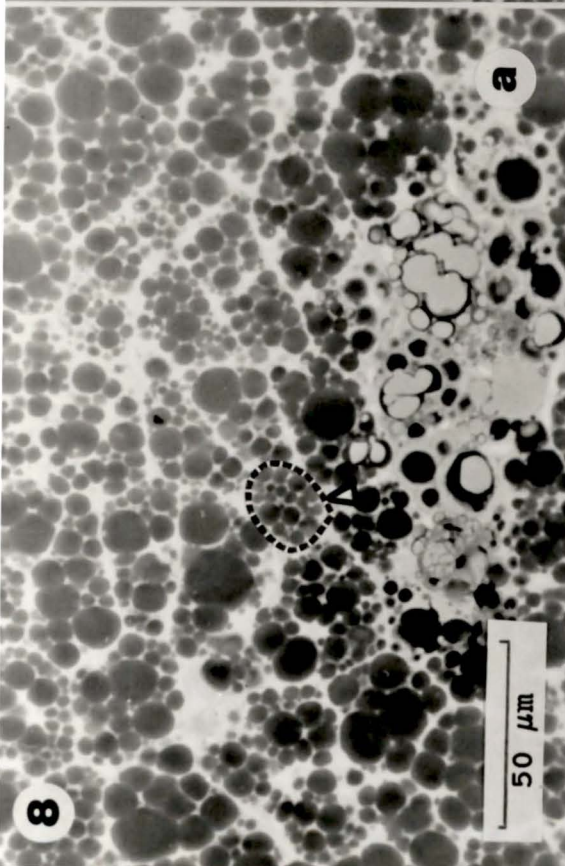
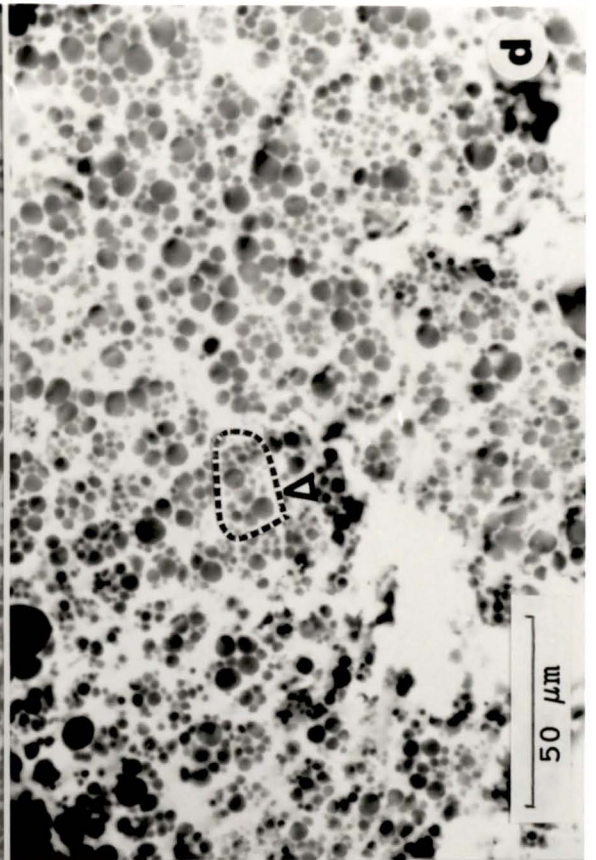
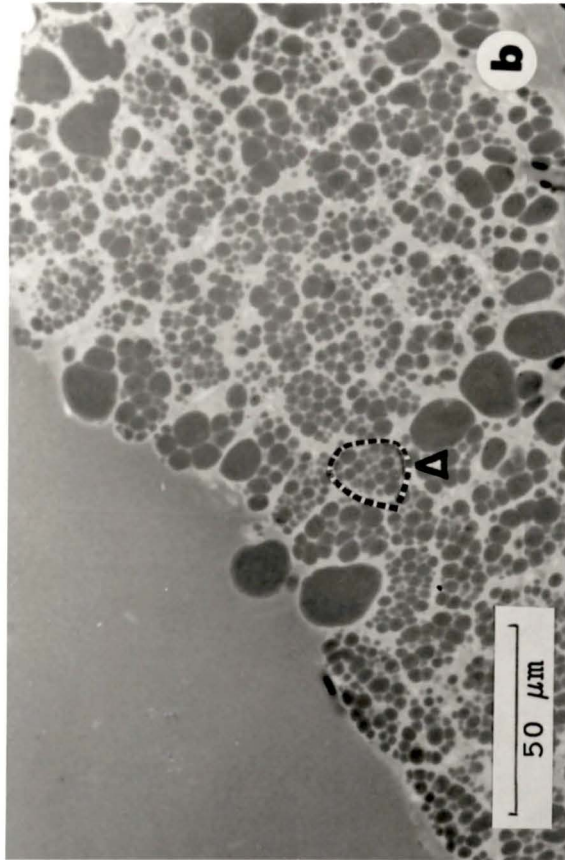
All *Peromyscus maniculatus* juveniles, sub-adults and adults have near-identical, multilocular structure of their BAT cells, typified by most of the cell being filled with small lipid droplets (Fig. 8a, b and d). The remaining volume of the cells are quite tightly packed with very complex (i.e. numerous cristae) mitochondria (Fig. 8c).

The BAT of adult *Phenacomys intermedius* has the same multilocular structure as *Peromyscus maniculatus* (Fig. 9a, Richardson's stain), including tightly-packed mitochondria (Fig. 9b). The BAT of neonate *Phenacomys intermedius* (Fig. 9c) has almost no lipid in the cells. Fig. 9d and 9e show the reduced lipid content of a single cell from a neonate *Phenacomys intermedius* and illustrate the more loosely-packed mitochondria.

*Tamias minimus* and *Tamiasciurus hudsonicus* have very few multilocular cells. The multilocular cells observed in *Tamias minimus* (Fig. 10a), have 1 to 5 large droplets of lipid per cell. The *Tamiasciurus hudsonicus* cells (Fig. 10b) have one droplet of lipid per cell; however, some cells have what appears to be coalescing lipids with 2 or 3 lobes.

Figure 8. Interscapular Brown Adipose Tissue from *Peromyscus maniculatus*

- a) Tissue from a sub-adult stained with Sudan Black B to show the lipids (Single cell outlined with arrow and hatched line).
- b) Tissue from a juvenile, stained with Sudan Black B, illustrating the similarity between individuals (Single cell outlined with arrow and hatched line).
- c) Mitochondria showing complexity of form in active tissue.
- d) Tissue from an adult for comparison to a) and b) above to illustrate the maximum amount of variation between individuals of different age groups (Single cell outlined with arrow and hatched line).



8

Figure 9. Interscapular Brown Adipose Tissue from *Phenacomys intermedius*.

a) Tissue from an adult stained with Richardson's stain to show overall structure of the tissue

(Single cell outlined with arrow and hatched line).

b) Electron microscope photograph of tissue from same individual as a) showing the compact mitochondria

(B = blood vessel, L = lipid, M = mitochondria).

c) Tissue from a neonate, stained with Sudan Black B illustrating the scant amount of lipid in the cells

(Arrow with L = lipids, Single cell outlined with arrow and hatched line).

d) Electron microscope photograph showing the loose packing of the mitochondria in cells such as seen in c)

(L = lipid, M = mitochondria, N = nucleus).

e) Higher magnification of d)

(L = lipid, M = mitochondria).

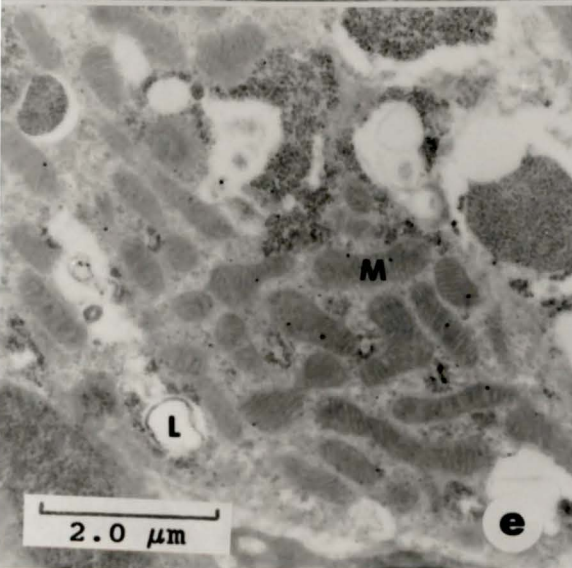
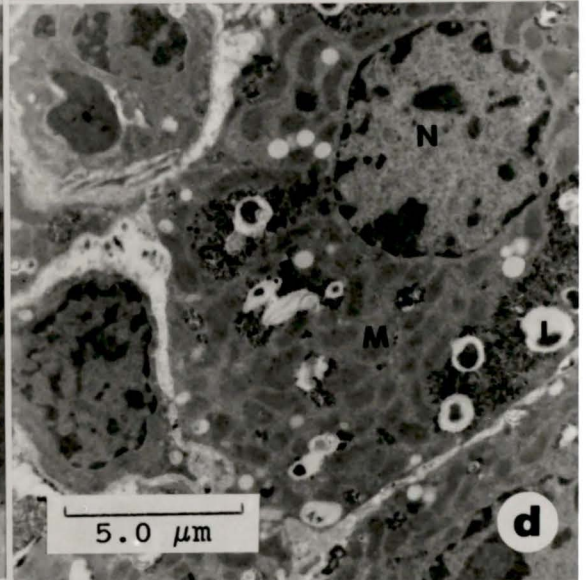
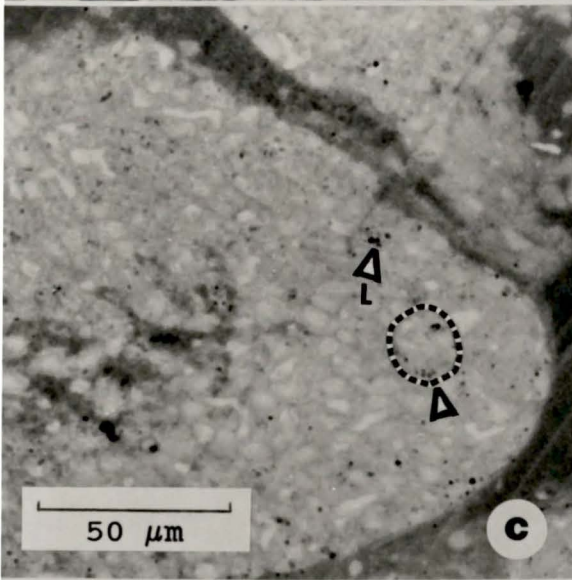
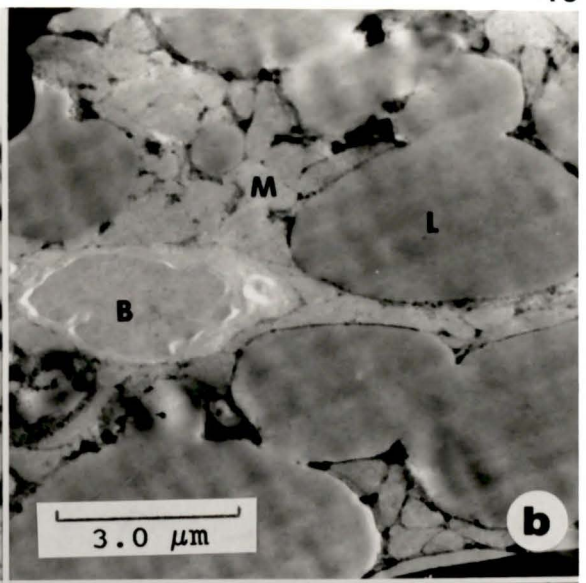
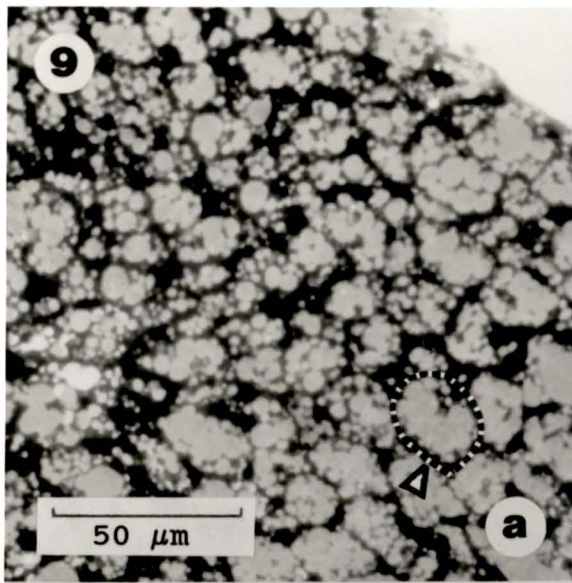
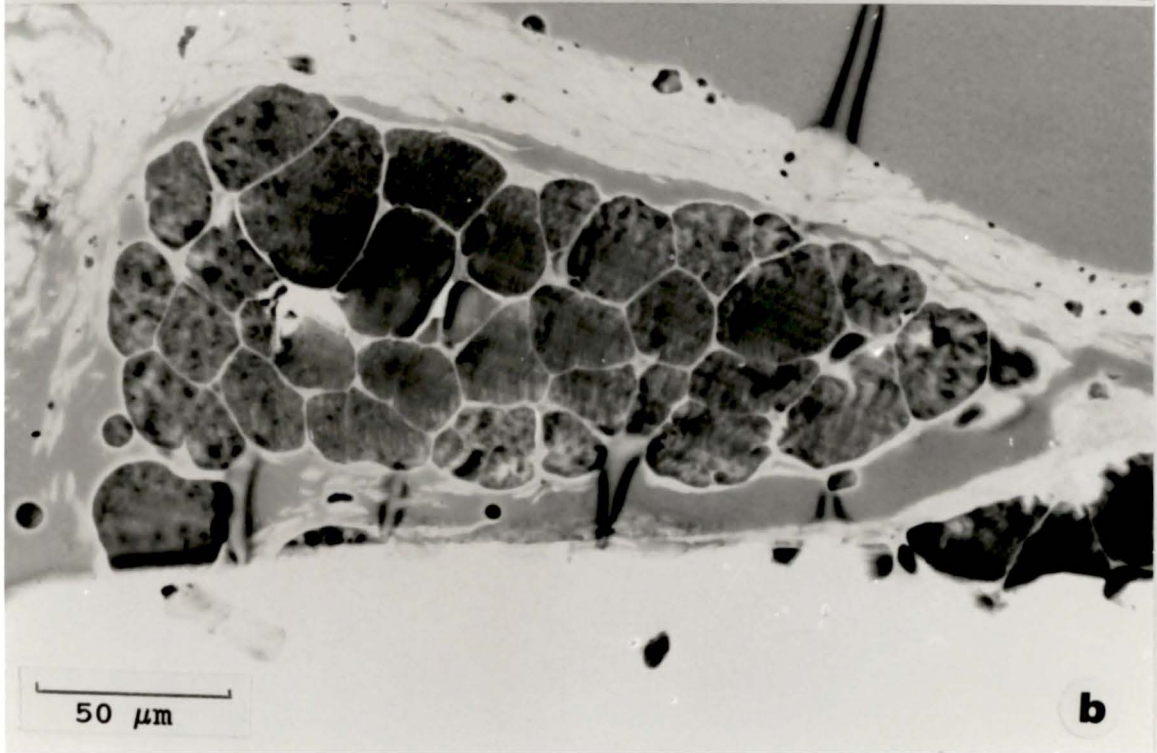
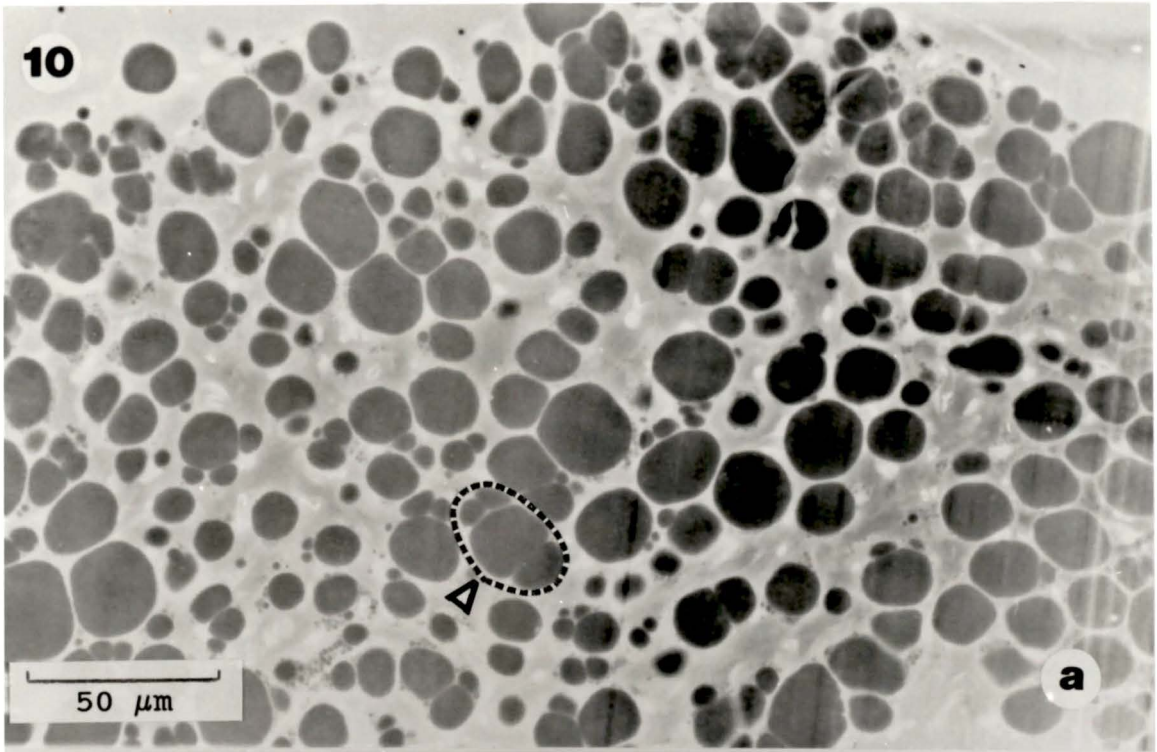


Figure 10. a) Interscapular Brown Adipose Tissue from *Tamias minimus* stained with Sudan Black B to show lipids  
(Single cell outlined with arrow and hatched line).

b) Interscapular Brown Adipose Tissue from *Tamiasciurus hudsonicus* stained with Sudan Black B to illustrate lipids.



The most noticeable feature of all the BAT samples from Costa Rican species is their considerable variation. All the specimens have varying amounts of lipid in the cells. *Mus musculus* (Fig. 11) and *Heteromys desmarestianus* (Fig. 5a) have single large lipid droplets (Fig. 12) that have the appearance of white adipose tissue. *Oryzomys fulvescens* adults (Fig. 7a) and neonates (Fig. 7c) have typically multilocular lipids in the IBAT cells, but in total have less lipid than most of the other species in this study. In contrast, the tissue samples from the Northwest Territories show remarkable similarity between *Peromyscus maniculatus* (Fig. 8a, b and d) and *Phenacomys intermedius* (Fig. 9c). The *Phenacomys intermedius* neonate has virtually no lipid in the cells (Fig. 9b).

Figure 11. Interscapular Brown Adipose Tissue from *Mus musculus* stained with Sudan Black B to show the lipids.  
(Single cell outlined with arrow and hatched line).

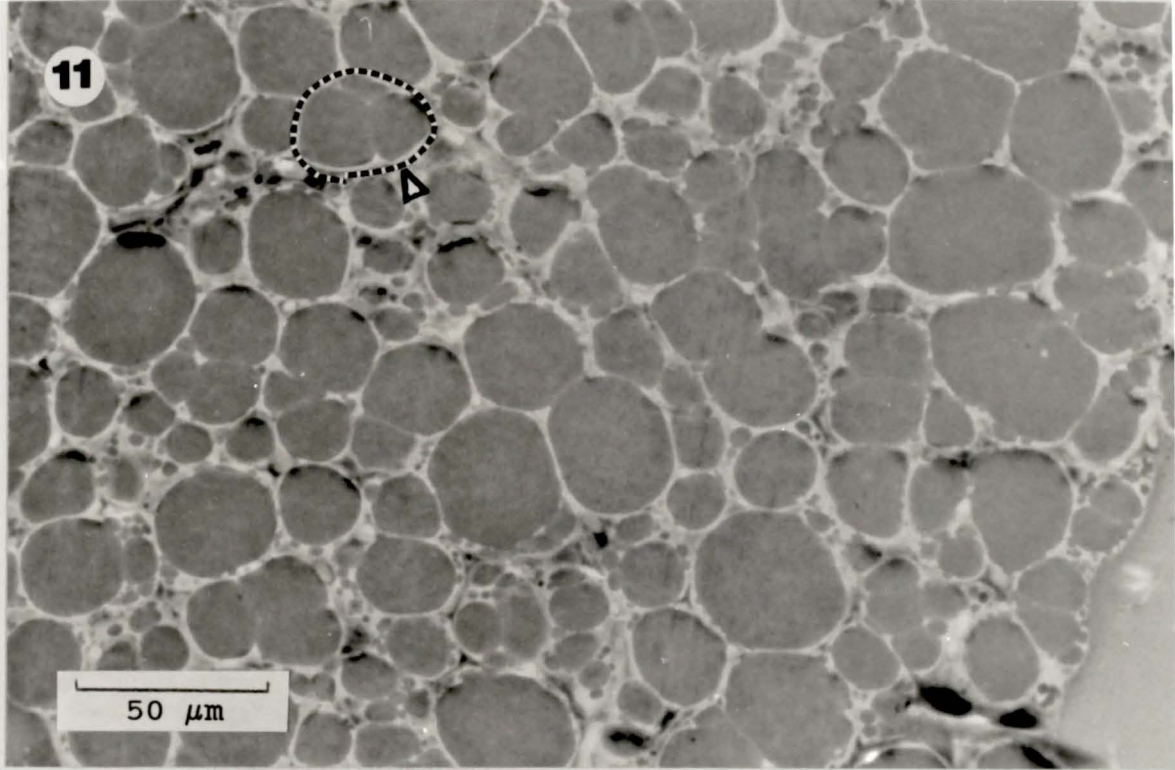
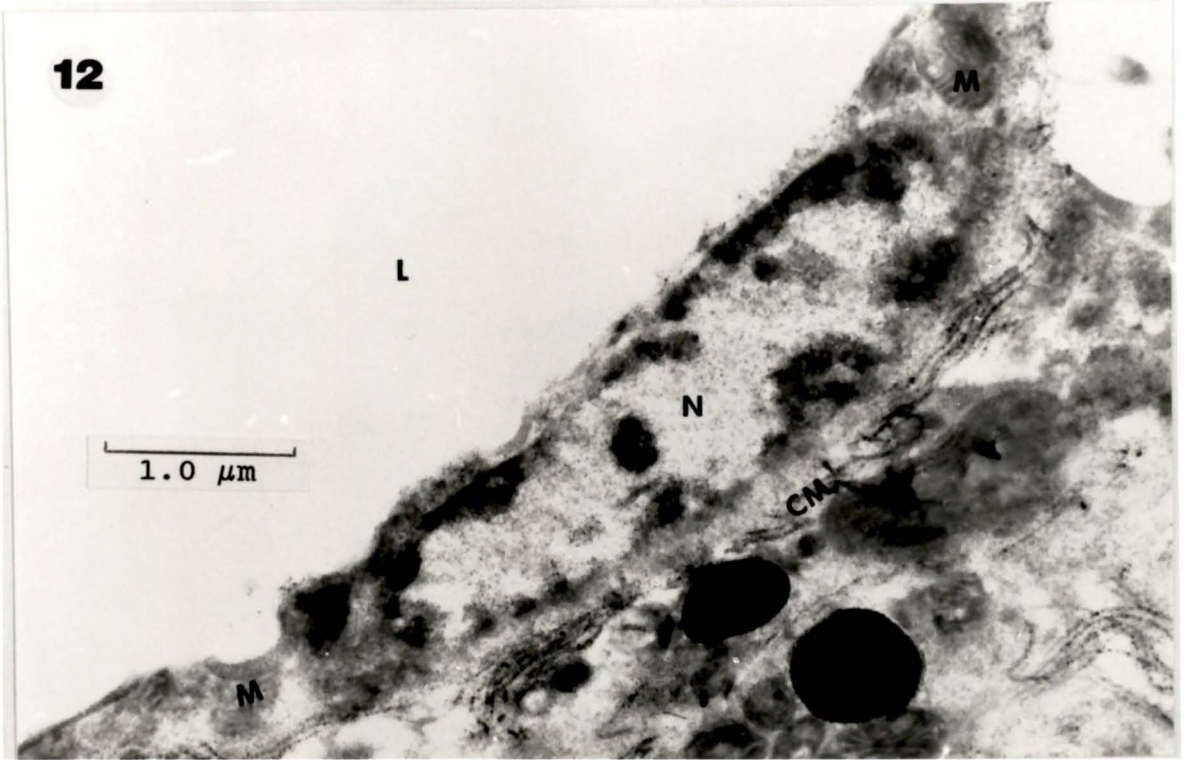


Figure 12. Interscapular Brown Adipose Tissue from *Heteromys desmarestianus* showing the similarity to White Adipose Tissue (CM = cell membrane, L = lipid, M = mitochondria, N = nucleus).



#### **4. DISCUSSION**

The original hypothesis of this thesis was that there would be a difference in the percent body weight of the BAT between individual species of rodents from the arctic and the tropics. In the tropics, small mammals were expected to have less BAT due to the constantly higher temperature of the area where they live. In the arctic, the continuous cold stress was expected to result in an increase in the BAT.

The actual results did not conform to the original expectations. Statistically comparing the two populations as a whole, calculation of the regressions of % IBAT to  $\log_{10}$  total body weight of small rodents from Costa Rica and the Northwest Territories for the means of the nine different adult species, have shown that the general trend for both populations is for increasing body size to have decreasing percent IBAT. When the regressions of the slopes and the elevations of the slopes of the Costa Rican and Northwest Territories data were compared using a t-test, no significant differences ( $P > 0.50$ ) between the slopes and the elevations were found. This indicates that the percent IBAT changes in relation to the body size at the same rate regardless of the origin of the samples and that there is the same total amount of IBAT (for similar sized animals) in both locations.

A single factor analysis of variance (ANOVA) comparing: 1.) the total body weights; and 2.) the % IBAT from the adults of each species with the adults of every other species has shown a considerable variation in the size of the rodents both within a location and between locations (Table 5a). The comparison of the % IBAT showed significant differences between *Heteromys* and *Reithrodontomys* and between *Mus* and *Tamias*. Between the remaining adults there was no significant difference

(Table 5b). This would indicate that regardless of the location, and with few exceptions, there was no difference in the quantity of brown adipose tissue in the rodents examined.

In a similar comparison of the young of Cricetid species, I found that the groups *Oryzomys* and *Phenacomys*, and *Peromyscus* and *Phenacomys* had significantly different ( $P < 0.0005$ ) total body weights while a comparison of *Peromyscus* and *Oryzomys* had no significant difference ( $P > 0.25$ ) in size. From Tables 2 and 3 in the Results, the larger of the neonates are the *Oryzomys* and they are statistically indistinguishable from juvenile *Peromyscus*. An ANOVA of the % IBAT showed a significant difference ( $0.0005 < P < 0.001$ ) between the *Oryzomys* and *Phenacomys* neonates and not between any other neonates or juveniles. Interestingly, I found that in the neonates of both *Oryzomys* and *Phenacomys*, an increase in body weight was coincident with an increase in % IBAT (Table 2 and 3). This, however, occurred only with the neonates, and did not continue into the older or larger animals. It was possible that the BAT cells were still accumulating lipids and increasing in size.

Trap stress, which is one factor that could not be eliminated, probably brought on some changes in the physiology of the animal which would result in physical changes in the BAT. I did not make any allowances for this stress assuming that, because all animals were treated the same, any changes that did occur would be similar for all specimens. Thus, there would be an absolute change in all animals but this would not result in a change in the relative differences between the different individuals.

The most noticeable difference in BAT between adult rodents from Costa Rica and the Northwest Territories was the variation in the structure of the lipids

found in the BAT cells. In Costa Rica, the variation ran from typically unilocular WAT to typically multilocular BAT with all grades of variation in-between. The BAT samples from rodents from the arctic were either typically multilocular, such as *Peromyscus* and *Phenacomys*, or typically unilocular as in *Tamias minimus* and *Tamiasciurus hudsonicus*. *Peromyscus* and *Phenacomys*, being small rodents living in a cold area, probably need the heat production of BAT to maintain their thermal balance. *Tamias* and *Tamiasciurus*, being larger rodents of a different genus, have a larger volume to surface area as well as a thicker covering of fur which reduces heat loss. This decrease in heat loss would result in a lowered need for 'extra' heat production by brown adipose tissue non-shivering thermogenesis. This would result in a reduced quantity of BAT or as found in these individual specimens, no BAT at all.

It should also be noted that the tissue samples from both arctic and tropic neonate Cricetids did not have large quantities of lipids in them. The *Oryzomys fulvescens* neonates did have more lipid per cell than the *Phenacomys intermedius* neonates but still had only approximately half as much lipid as adults other than *Oryzomys*. Under no cold stress, the *Oryzomys fulvescens* adults do not appear to retain the lipid for use in thermogenesis and therefore the tissues do not appear to have the characteristic quantity and structure of the lipids typical of BAT. In fact, in the samples examined, it was difficult to distinguish adult from neonate tissue types.

In the neonate *Phenacomys* from the Northwest Territories, the tissue samples have almost no lipids in the BAT. In the samples of tissue from adult *Phenacomys* the tissue has typically-structured, multilocular lipids as one would expect to find in BAT.

Both arctic and tropic neonates appear to fit the 'altricial' model that is described by Nedergaard *et al.* (1986) where the young do not have active BAT until 4 to 10 days after birth. This could account for the increasing % IBAT being coincident with increasing weight in the neonates. BAT function, in the absence of cold stimulus, reverts to what appears to be unilocular white adipose tissue within 60 days after birth. Under cold stress, Nedergaard *et al.* (1986) showed that the brown adipose tissue remained in a multilocular form that is typical of brown adipose tissue.

This could explain the typical multilocular form of the lipids in the BAT that occurs in *Phenacomys* when it is under cold stress. The structure of *Oryzomys* BAT, when under no cold stress, would 'revert' to the unilocular form that is typical of storage white adipose tissue.

The Lower Critical Temperature ( $CT_L$ ) of small mammals (such as those sampled in Costa Rica and the Northwest Territories), is typically in the high 20's or low 30's celsius. Ground temperatures, which probably reflect the nest temperatures of these animals, in the arctic were as much as 25°C below the  $CT_L$ . Thus, it would still be necessary to generate considerable heat for thermoregulation, most likely by the metabolic process of non-shivering thermogenesis involving BAT.

In Costa Rica, the ground temperatures (22.6 to 26.9 °C) approach the  $CT_L$ , while during the day, the air temperatures (18.4 to 33.0 °C) are also close to this  $CT_L$ . During the night, air temperatures (between 12.0 and 26.0 °C) are usually below the  $CT_L$ . Therefore, in the tropics, although temperatures are well above those of the arctic, the temperatures are still not so warm that the small rodents no longer need some capacity for non-shivering thermogenesis during periods of inactivity.

In the Northwest Territories, both day (1.6 to 27.0 °C) and night temperatures (0.5 to 11.5 °C), as well as ground temperatures (6.8 to 15.3 °C), are always well below the  $CT_L$ . Therefore, the BAT is most likely to develop in a multilocular lipid form in small rodents. Larger rodents, such as *Tamias* and *Tamiasciurus*, because they have a larger volume to surface area, thicker insulative fat and fur and perhaps have built larger nests, are warm enough for the brown adipose tissue to develop with a typically unilocular lipid structure as is found in the white adipose tissue.

In summary, in the arctic, the microclimate that the small rodents create and rest in is insufficient to raise the ambient temperature above the lower critical temperature during most of the day and thus they have well-developed multilocular brown adipose tissue. In the tropics, the ambient temperature during the night is usually slightly below the lower critical temperature. Some rodents may be able to build a nest that provides an environmental temperature that is at or above the  $CT_L$ . Other rodents may not be able to accomplish such adequacy of nest insulation and therefore retain BAT in a more 'active' form. These speculations could account for the variability of BAT lipid structure among rodents in the tropic region.

During the initial stages of life of Cricetids, interscapular brown adipose tissue increases at a greater rate than their total weight. By approximately 10 days of age, this trend reverses itself and the % IBAT decreases as the total body weight increases. This decrease in % IBAT compared to total body weight is constant through nine different species and between juvenile and adult ages in both the arctic and the tropics. Both arctic and tropic neonate Cricetids have little lipid in the BAT cells at the time of birth. During the next 10 days, in the 'altricial' model of brown adipose tissue, the tissue becomes more active and stores lipids in the cell. This

could account for the initial increase in %IBAT with increasing body weight during the first few days after parturition. After 10 days after parturition, with the BAT cells 'filled to capacity' with lipid, the expected decrease in % IBAT in relation to increasing body weight occurs as the rodents rapidly increase in size.

Although the relative weight of BAT does not appear, in this case, to be adaptive (arctic and tropic), the structure of the IBAT appears to be determined by the temperature in which the rodents live. With lower critical temperatures in the range of 25 to 30 °C, the exact microclimate that the rodents create for themselves will make a difference in the structure of the IBAT. In the tropics, where the nighttime temperatures and ground temperatures are within the range of the lower critical limits, the structure of the lipids in the IBAT in some cases are typical of brown adipose tissue, that is they are multilocular in form, whereas samples from the same area are typical of white adipose tissue in form, that is they are unilocular. In the arctic, where the daily temperatures and the ground temperatures are always below the lower critical temperature, the lipids in the IBAT are in the form of typical multilocular brown adipose tissue. The only exceptions to this are the larger rodents, *Tamias* and *Tamiasciurus*.

In conclusion, the ability of small rodents from the arctic and the tropics to produce and 'use' brown adipose tissue appears to be genotypically conserved. The environmental pressures of cold stress (mild to extreme) appear to alter the phenotypic expression of the tissue, through variation in structure and by association, thermogenic capacity.

### Possible Future Directions

In this area of study, the direction that could be pursued is further collecting of arctic and tropic specimens but concentrating on the single species of *Peromyscus* to reduce the variables being studied. By using the genus *Peromyscus*, which is found from the Canadian arctic south to Panama in Central America, the researchers would reduce the number of species to three (*P. maniculatus* in Canada and *P. mexicana* and *P. nudipes* in Central America) that are very closely related. In Costa Rica, researchers should make better connections with field stations and universities and spend more time investigating areas where *Peromyscus* mice can be found. An increased effort to collect all age groups by trapping adults for breeding and raising the young in compounds which mimic the local environment can be accomplished. This will yield definite information on the development of BAT in specimens of known ages. If possible, attempts should be made to measure the actual nest temperatures in the captive populations and compare them to the ground temperatures for future reference.

Similar collecting and rearing of young should also be carried out in the arctic, again concentrating on the species *Peromyscus*.

In both areas, lower critical temperatures should be determined for both populations. This would give a definite base line for determining the cold stress on the mice.

It may also be possible to develop a simple means of detecting the presence and possibly the concentration of the 32k D uncoupling protein as a definite indicator of BAT. Comparisons of the thermogenic capacity of BAT would be facilitated by biochemical analysis of the tissue, particularly for the concentration of the 32k D uncoupling protein or its m-RNA antecedent.

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# **APPENDICES**

## **APPENDIX 1 - Buffer, Fixatives, Embedding Material and Stains**

### Buffer

#### Millonig's Phosphate Buffer

- 11.04 g Sodium Phosphate Monobasic
- adjust to pH 7.4 with Sodium Hydroxide
- in 200 ml double distilled water

### Fixatives

#### Primary Fixative 4% Glutaraldehyde

- 2 part 50% Glutaraldehyde
- 18 parts 0.3M NaCl
- 5 parts Millonig's Phosphate Buffer

#### Post Fixative 1% Osmium Tetroxide

- 1 part 4% Osmium Tetroxide
- 1 part Millonig's Phosphate Buffer
- 2 parts 0.75 M NaCl

### Embedment

#### Luft's Epon 812 - Total Mix

- 42.6 ml EPON 812
- 43.2 ml Dodecenyl Succinic Anhydride
- 14.2 ml Nadic Methyl Anhydride
- 1.6 ml 2,4,6 Tridimethyl Amino Methyl Phenol

### Light Microscopy - Stains

#### Sudan Black B (C.I. 26150)

- saturated in 70% Ethanol

#### Richardson's Stain

- 1% Azure II  
(mixture of C.I. 52010 plus C.I. 52015)
- 1% Methylene Blue (C.I. 52015) in 1% Borax
- in double distilled water

## Electron Microscopy - Stains

Uranyl Acetate  
- 2.0% in double distilled water

Lead Citrate  
- 0.2% in double distilled water

## Fixing, Dehydrating and Embedding Sequence

Fix in 4% Glutaraldehyde	min. 2 hrs.
Rinse - 1 part Millonig's	
1 part 0.6M NaCl	3 x 5 min.
Post Fix - 1 part 4% OsO <sub>4</sub>	
1 part Millonig's	
2 parts 0.75M NaCl	2 - 4 hrs.
	@ 2 - 4°C

## Dehydration

Acetone	
30%	30 min.
50%	30 min.
70%	30 min.
95%	60 min.
95%	60 min.
100%	60 min.
100%	60 min.
100%	60 min.
Prop. Ox.	15 min.
Prop. Ox.	15 min.
Prop. Ox.	15 min.
1:1 Prop. Ox. and EPON	2 - 3 hrs.
3:1 Prop. Ox. and EPON	3 - 5 hrs.
	(overnight)
EPON	18 hrs.
	@ 60°C

## **APPENDIX 2 - Permits and Licences**

In the light of the increasing awareness of the need to conserve the wildlife of the planet and to protect the environment that they live in, most countries now require that the researcher obtain permits which outline the studies that he or she wishes to carry out and the limitations under which they must work. Permits include study permits, collection permits, export permits and import permits.

### 1. Costa Rica

Costa Rica requires a collection permit (Fig. 13) and an export permit (Fig. 14) from:

Carlos Salas Araya,  
Subdirector General,  
Servicio de Vida Silvestre,  
Ministerio de Recursos Naturales, Energia y Minas,  
Apartado: 10.104,  
San Jose, Costa Rica.

These documents outline where and when you may collect your specimens.

In the case of small mammals, the review of the permit to collect is made by:

Dr. Christopher Vaughan,  
Universidad Nacional de Heredia,  
APDO 86,  
Heredia, Costa Rica

which is located just outside San Jose. Dr. Vaughan can be of considerable assistance in making the correct connections within the Government of Costa Rica.

## 2. Northwest Territories, Canada

As in Costa Rica, permits are required in the Northwest Territories. The permits required are a Wildlife Research Permit (Fig. 15), a Licence to Capture Wildlife (Fig. 16) and the Wildlife Export Permit (Fig. 17). These are issued by:

The Government of the Northwest Territories,  
Wildlife Management Division,  
Department of Renewable Resources,  
P.O. Box 1320,  
Yellowknife, Northwest Territories, X1A 2L9.

## 3. Agriculture Canada Import Permit

In order to return biological samples to Canada from Central America, an import permit is required from:

Agriculture Canada,  
Food Production and Inspection Branch,  
Animal Health Division

(Fig. 18). This permit can usually be obtained through the local office of Agriculture Canada.

Figure 13: Collecting Permit issued by the Costa Rican Wildlife Service



SERVICIO DE VIDA SILVESTRE  
MINISTERIO DE RECURSOS NATURALES  
ENERGIA Y MINAS

TELEFONOS:  
33-81-12  
55-01-92

APARTADO:  
10.104  
San José, Costa Rica

SDVS-----049-88.

Con base en la solicitud presentada por el señor Paul Alexander Lisson, pasap. GE475535, para coleccionar con fines de investigación lo siguiente:

PEROMYSCUS, NEOTOMA Y SIGMODON (20 ejemplares de cada uno).

Se procede a conceder el permiso respectivo de acuerdo a la Ley 6919, Ley de Conservación de la Fauna Silvestre, siempre y cuando se cumpla con la demás legislación vigente y nos envíen un informe de las investigaciones realizadas.

VALIDO: A PARTIR DEL 25 DE MARZO AL 25 DE ABRIL DE 1988.

Este permiso tiene validez en todo el territorio nacional. Queda prohibida la colecta en Parques Nacionales, Reservas Forestales, Zonas Protectoras. Se debe de entregar al Servicio de Vida Silvestre un ejemplar de cada especie colectada para ser entregado al Departamento de Historia Natural del Museo Nacional.

Dado en San José a los 25 días del mes de marzo de 1988.

El presente permiso no ampara la salida del país de ningún tipo de colecta sobre Fauna Silvestre.

  
Lic. Carlos Salas Araya.  
SUBDIRECTOR GENERAL.



CSA/sab.

Figure 14: Export Permit issued by the Costa Rican Wildlife Service

SDVS-049-88



Nombre y dirección del exportador <small>Exporter's name and address</small> Paul Alexander Lisson. pasap. GE475535.		Lugar y fecha: <small>Place and date</small> 25 de marzo de 1988.		
Nombre y dirección del importador <small>Importer's name and address</small> Paul Alexander Lisson.		 REPUBLICA DE COSTA RICA  PERMISO DE IMPORTACION <input type="radio"/> EXPORTACION <input checked="" type="radio"/> ANIMALES DE VIDA SILVESTRE Y PLANTAS  <small>Importation-Exportation Permit for wild-life          animals and plants.</small>  SUBDIRECCION GENERAL DE VIDA SILVESTRE MINISTERIO DE AGRICULTURA Y GANADERIA		
Medio de transporte utilizado y ruta a seguir <small>Means and route of transport</small> Via Aérea.				
Aduana de salida <small>Customs of exit</small> Juan Santamaria.				
País de destino final <small>Country of destiny</small> Canadá.				
Centro de investigación, laboratorio de investigación, universidad, museo o parque zoológico para el que van los animales <small>Investigation Center, Investigation Laboratory, University, Museum, or zoo of destiny</small>				
Lista de especies con sus nombres vulgares científicos, número de animales a exportar <small>List of species with common and scientific names, number of animal to be exported.</small>				
NOMBRE COMUN <small>COMMON NAME</small>	NOMBRE CIENTIFICO <small>SCIENTIFIC NAME</small>	NUMERO <small>NUMBER</small>	AUTORIZADO <small>AUTHORIZED</small>	
			SI <small>YES</small>	NO <small>NO</small>
XXXXXXXXXXXXXXXXXXXX	PEROMYSCUS, NEOTOMA, veinte especímenes SIGMODON. de cada uno.	XXXXXXXXXXU.L. XXXXXXXXXXXX	X	
XXXXXXXXXXXXXXXXXXXX				
Autorización: Por cumplir a cabalidad con los requisitos del caso, seguidamente autorizamos la exportación- Importación, del producto arriba detallado.			<small>Authorization:</small> Considering that the product fulfills the requirements, we hereby authorized the Importation-exportation of the same.	
 Firma y sello de la autoridad correspondiente <small>Signature and stamp of the corresponding authority</small>				

Figure 15: Wildlife Research Permit issued by the Northwest Territories,  
Renewable Resources Branch, Wildlife Division.

Figure 16: Licence to Capture Wildlife issued by the Northwest Territories,  
Renewable Resources Branch, Wildlife Division.



GOVERNMENT OF THE NORTHWEST TERRITORIES  
CANADA

WILDLIFE RESEARCH PERMIT

FEE \$ _____	PERMIT No <b>1194</b>
PERMIT EXPIRES <b>20 Aug 88</b>	
Subject to the provisions of the Wildlife Ordinance and Regulations:	
<b>Paul Lisson</b> , _____ of <b>University of Victoria</b> is hereby permitted to conduct scientific research as approved by the Superintendent of Wildlife.	
TERMS AND CONDITIONS <b>Live white-footed deer mice, &amp; collect up to 10 individuals as per the application.</b>	
ISSUED AT <b>Yellowknife</b>	DATE <b>18 Apr. 19 88</b>
<b>Paul G. Lisson</b> PERMIT HOLDER	<b>S.G. Brynner</b> SUPERINTENDENT



GOVERNMENT OF THE NORTHWEST TERRITORIES  
CANADA

LICENCE TO CAPTURE WILDLIFE

FEE \$ _____	LICENCE No <b>1810</b>
LICENCE EXPIRES <b>20 Aug 19 88</b>	
Subject to the provisions of the Wildlife Ordinance and Regulations:	
<b>Paul Lisson</b> _____ of <b>University of Victoria</b> is hereby authorized to capture wildlife at the following location: <b>Between Ft. Providence &amp; Ft. Simpson.</b>	
TERMS AND CONDITIONS <b>Live trap white-footed deer mice, &amp; collect up to 10 individuals, and retain any non-target small mammals as per the application.</b>	
ISSUED AT <b>Yellowknife</b>	DATE <b>18 Apr 19 88</b>
<b>Paul G. Lisson</b> LICENCEE	<b>S.G. Brynner</b> AUTHORIZED OFFICER

Figure 17: Wildlife Export Permit issued by the Northwest Territories, Renewable Resources Branch, Wildlife Division.



Figure 18: Permit to Import Veterinary Biologicals issued by Agriculture Canada,  
Food Protection and Inspection Branch, Animal Health Division.



Agriculture  
Canada

Food Production and Inspection Branch      Direction générale  
Production et inspection des aliments  
Animal Health Division      Direction de la santé des animaux

Form 2001 (4-78) (50 pages) SINGLE ENTRY 1
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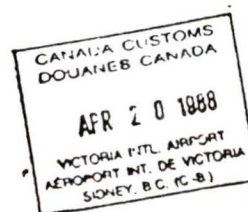
PERMIT TO IMPORT  
VETERINARY BIOLOGICS

PERMIS D'IMPORTATION DE PRODUITS  
BIOLOGIQUES VÉTÉRINAIRES

UNDER AUTHORITY OF THE ANIMAL DISEASE AND  
PROTECTION ACT AND REGULATIONS

EN VERTU DE LA LOI ET DES RÈGLEMENTS SUR LES MALADIES ET  
LA PROTECTION DES ANIMAUX

Importer - Importateur: UNIVERSITY OF VICTORIA BIOLOGY DEPT. P.O. BOX 1700 VICTORIA, B.C. V8W 2Y2 ATTENTION: PAUL A. LISSON 604-721-7117		Manufacturer - Fabricant COSTA RICA	
Establishment Licence Number or Manufacturer Numéro de permis de l'établissement du fabricant		To Clear Customs at Port(s) of Entry of - À dédouaner au(x) bureau(x) de douane: VANCOUVER, BRITISH COLUMBIA	
For the Importation of - Pour l'importation de:  PRESERVED (FIXED) BIOLOGICAL MATERIAL FROM SMALL MAMMALS.  CONDITIONS: -----  THE MATERIAL IS TO BE USED IN "IN VITRO" STUDIES ONLY AND IS NOT TO BE INTRODUCED INTO LABORATORY, DOMESTIC OR WILD ANIMALS (INCLUDING BIRDS AND FISH) UNLESS SPECIAL ARRANGEMENTS ARE MADE WITH THE HEALTH OF ANIMALS DIRECTORATE.  ALL IMPORTED MATERIAL AND DERIVATIVES THEREOF ARE TO BE AUTOCLAVED OR INCENERATED OR BOTH, ON COMPLETION OF THE EXPERIMENTS OR TESTS.  THE MATERIAL OR ANY DERIVATIVE THEREOF IS NOT TO BE DISTRIBUTED TO ANY OTHER LABORATORY OR PERSON IN CANADA WITHOUT PERMISSION FROM THE HEALTH OF ANIMALS DIRECTORATE.			
Expiry Date of this Permit Date d'expiration de ce permis		Minister of Agriculture - Ministre de l'Agriculture	
WHEN		Dated at Ottawa, Date à Ottawa (Day, Month, Year) (jour, mois, année)	
SHIPMENT CLEARS CUSTOMS		JANUARY 04, 1988	
		D.C. ALEXANDER	



**APPENDIX 3 - Weather data collected on sampling sites**

Table 6. Weather Data for the period March 25, 1988 to April 15, 1988 at various locations in Costa Rica

Date	Location	Time	Temp Air(°C)	Temp GND(°C)	Rain (0-4)	Cloud	Wind (0-4)	
25/03/88	La Georgina	16:00	20.0		0	1.0	E 1	
26/03/88	La Georgina	06:30	7.0		0	0.0	E 1	
26/03/88	La Georgina	12:00	21.0		0	0.0	E 1	
26/03/88	La Georgina	16:00	20.0		0	1.0	E 1	
26/03/88	La Georgina	23:00	2.0		0	0.2	E 1	
27/03/88	La Georgina	23:00	2.0		0	0.2	E 1	
28/03/88	La Georgina	23:00	2.0		0	0.2	E 1	
29/03/88	La Pacifica	06:00	26.0		0	0.0	E 2(3)	
29/03/88	La Pacifica	12:00	30.0		0	0.0	E 2(3)	
29/03/88	La Pacifica	15:00	31.0		0	0.0	E 2(3)	
29/03/88	La Pacifica	19:00	31.0		0	0.0	E 2(3)	
30/03/88	La Pacifica	19:00	31.0		0	0.0	E 2(3)	
31/03/88	La Pacifica	09:00	28.0	26.9	0	0.0	N/A	
01/04/88	La Pacifica	09:00	28.0	26.9	0	0.0	N/A	
02/04/88	La Pacifica	09:00	28.0	26.9	0	0.0	N/A	
03/04/88	Tilaran	09:00	22.7	22.7	0	0.2	N/A	
04/04/88	Tilaran	09:00	23.6	22.7	0	0.0	N/A	
04/04/88	L. de Arenal	07:00	23.3		0	1.0	SE 2	
05/04/88	L. de Arenal	07:15	22.6		0	0.5	SE 2	
05/04/88	La Pacifica	11:00	32.0		0	0.0	NE 2	
06/04/88	L. de Arenal	07:00	28.0	22.6	0	0.5	N/A	
07/04/88	L. de Arenal	07:00	27.3	22.6	0	0.4	N/A	
08/04/88	La Pacifica	06:00	27.0		0	0.9	NE 2	
08/04/88	L. de Arenal	07:00	23.3	22.6	0	0.8	E 1	
08/04/88	La Pacifica	12:00	30.5		0	0.0	NE 2	
08/04/88	La Pacifica	17:00	29.4		0	1.0	NE 2	
09/04/88	La Pacifica	06:00	26.0		1	1.0	E 1	
09/04/88	L. de Arenal	07:00	21.4	22.6	2	1.0	E 1	
10/04/88	L. de Arenal	07:00	23.7		0	0.9	0	
10/04/88	La Pacifica	16:30	33.0		0	1.0	W 1	
11/04/88	L. de Arenal	07:00	22.8		0	0.4	0	
11/04/88	La Pacifica	09:00	31.1		0	0.0	0	<u>Rain and Wind Codes</u>
11/04/88	La Pacifica	16:30	N/A		2	1.0	0	
12/04/88	L. de Arenal	07:00	24.7	22.6	0	0.9	0	<u>Rain</u> <u>Wind</u>
12/04/88	La Pacifica	09:00	29.0		0	0.5	E 1	0 None    Calm
13/04/88	L. de Arenal	07:00	18.4	23.9	2	1.0	E 3	1 Trace    0 - 5
13/04/88	La Pacifica	09:00	30.2		0	0.1	E 1	2 Light    6 - 10
14/04/88	L. de Arenal	07:00	23.2	23.9	2	0.8	N/A	3 Medium   11 - 15
14/04/88	La Pacifica	09:00	27.2	26.4	0	0.2	N/A	4 Heavy    16 - 20
15/04/88	La Pacifica	09:00	26.5	26.4	0	0.7	N/A	

Table 7. Weather Data for the period May 31, 1988 to June 24, 1988 at various locations in the Northwest Territories, Canada.

Date	Location	Time	Temp Air(°C)	Temp GND(°C)	Rain (0-4)	Cloud	Wind (0-4)
31/05/88	Hay River	15:45	18.0		0	1.0	E 1
31/05/88	Hay River	18:00	16.7		0	1.0	0
01/06/88	Hay River	00:01	12.0		0	1.0	0
01/06/88	Hay River	03:00	10.0		0	1.0	0
01/06/88	Hay River	08:30	9.5		1	0.7	0
01/06/88	13.1 km W Entr.	09:30	13.6		0	0.0	0
02/06/88	Hay River	09:00	14.0		2	1.0	SE 1
03/06/88	N/A						
04/06/88	13.1 km W Entr.	08:00	12.2		0	0.0	0
04/06/88	Hay River	09:00	19.3		0	0.3	E 1
05/06/88	13.1 km W Entr.	10:30	20.0	6.8	0	0.2	0
05/06/88	Hay River	15:00	22.5		0	0.2	W 1
06/06/88	13.1 km W Entr.	10:00	18.2		0	0.2	N 1
07/06/88	N/A						
08/06/88	N/A						
09/06/88	Hay River	13:00	13.6		2	1.0	N 1
09/06/88	Hay River	18:00	12.0		4	1.0	0
09/06/88	Hay River	21:00	11.5		4	1.0	0
10/06/88	1 km N Enter.	09:00	4.6		2	1.0	NW 1
10/06/88	Hay River	12:00	4.5		3/4	1.0	NW 1
11/06/88	1 km N Enter.	09:00	1.6		2	1.0	NW 3
11/06/88	Hay River	12:00	2.0		2	1.0	N 1
11/06/88	Hay River	18:00	1.9		2	1.0	N 1
11/06/88	Hay River	24:00	0.5		2	1.0	N 1
12/06/88	Hay River	09:00	3.9		0	1.0	0
12/06/88	1 km N Enter.	10:45	6.1		0	1.0	N 1
12/06/88	Hay River	21:00	5.4		0	0.8	0
13/06/88	1 km N Enter.	10:15	8.6		0	0.1	0
14/06/88	1 km N Enter.	09:30	15.4	10.5	0	0.1	0
15/06/88	Hay River	11:15	19.9		0	0.4	S 3
16/06/88	Enterprise	09:30	19.8	15.6	0	0.6	S 1
16/06/88	Hay River	15:30	27.0		0	0.7	S 1
16/06/88	Hay River	16:30	27.0		3	1.0	S 3
17/06/88	1 km N Enter.	09:30	20.2	15.1	2	0.5	0
17/06/88	Hay River	20:00			4	1.0	V 3
17/06/88	Hay River	23:59			3	1.0	V 2
18/06/88	Hay River	06:00			2	1.0	S 1
18/06/88	Enterprise	09:30	18.5	15.3	0	0.5	0
18/06/88	Hay River	16:00	22.0		0	0.9	NE 1
18/06/88	Hay River	18:30			2/3	1.0	0

(Continued)

Table 7. Weather Data for the period May 31, 1988 to June 24, 1988 at various locations in the Northwest Territories, Canada.

Date	Location	Time	Temp Air(°C)	Temp GND(°C)	Rain (0-4)	Cloud	Wind (0-4)		
19/06/88	Enterprise	09:30	11.2	13.4	0	0.8	SW 2/3		
19/06/88	Hay River	13:00			0	1.0	SW 3/4		
20/06/88	1 km N Enter	09:30	8.2	11.1	0	1.0	S 2		
20/06/88	Hay River	17:45	12.7		0	1.0	0		
21/06/88	1 km N Enter.	09:00	8.6		0	0.0	S 1	<u>Rain and Wind Codes</u>	
21/06/88	Hay River	17:30	16.1		0	0.0	0		
22/06/88	1 km N Enter.	09:00	13.3	11.6	0	1.0	N 1	<u>Rain</u>	<u>Wind</u>
22/06/88	Hay River	17:00	19.0		0	1.0	S 4	0 None	Calm
23/06/88	Enterprise	08:45	17.6	13.3	0	1.0	0	1 Trace	0 - 5
23/06/88	Hay River	14:00	19.0		3/4	1.0	V 2	2 Light	6 - 10
23/06/88	Hay River	17:00	19.0		4	1.0	S 4	3 Medium	11 - 15
24/06/88	1 km N Enter.	09:00	15.3	13.1	0	0.1	SE 2	4 Heavy	16 - 20



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ISBN 0-315-53755-8