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CONTROL OF BROWN ADIPOSE TISSUE GROWTH AND FUNCTION IN RATS AND HAMSTERS: ABNORMALITIES IN GENETIC MODELS OF HUMAN DISEASE (OBESITY; MUSCULAR DYSTROPHY)

by

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A thesis submitted to the School of Graduate Studies of the University of Ottawa in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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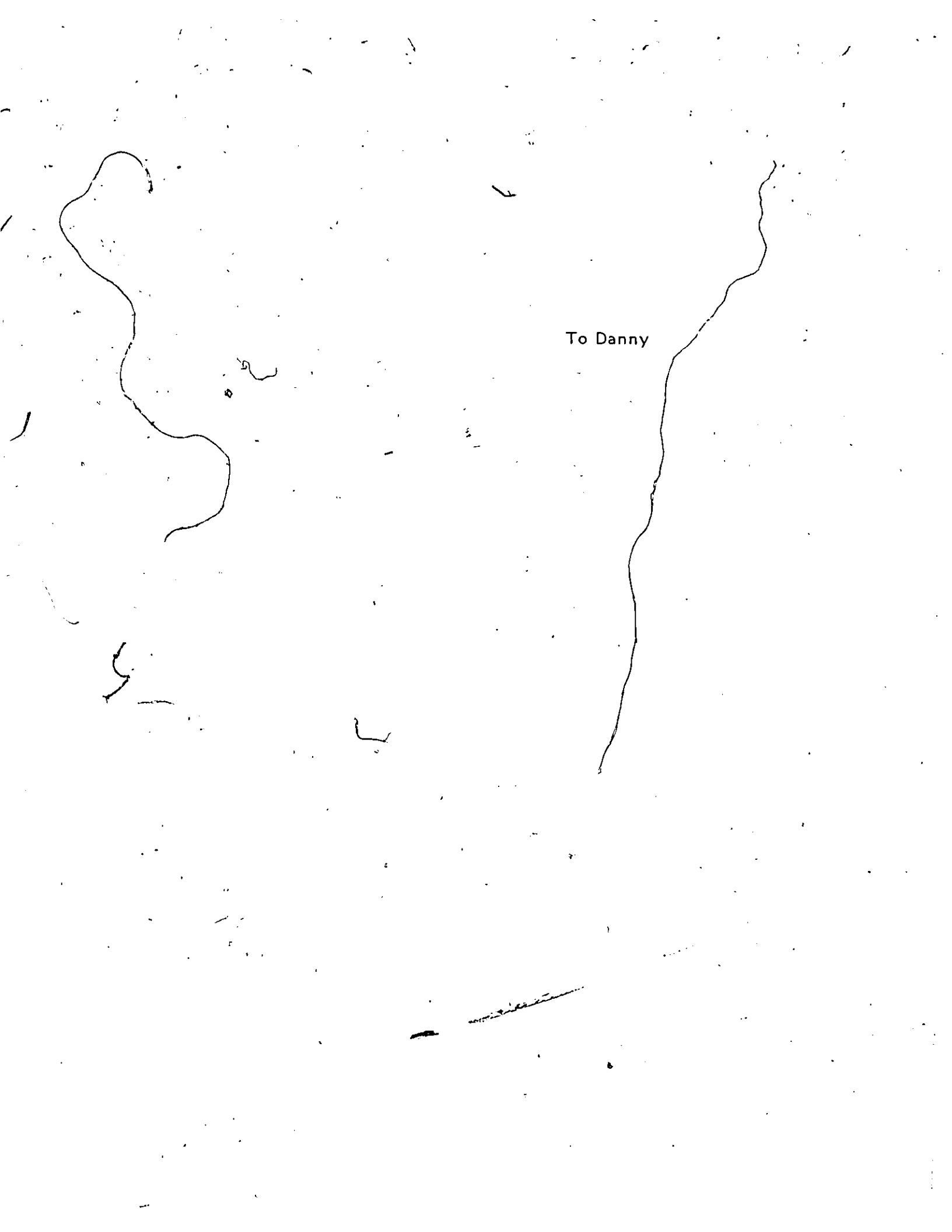
September 1984


To Danny

ACKNOWLEDGEMENTS

I wish to thank Dr. Jean Himms-Hagen for the opportunity to do this research, and for her guidance and practical assistance.

Thanks are also offered to Mrs. Cynthia Gwilliam for performing gel electrophoresis and much more. Special thanks are given to Mrs. Gisele Larose for all her help in caring for the animals.

I gratefully acknowledge assistance from Dr. Gloria Zaror-Behrens in noradrenaline turnover determinations; from Mr. Ian Park in denervation studies; and from Mrs. Misa Bosc-Davie and staff in animal surgery experiments. I am grateful also to Matthias Fellenz, University of Ottawa Summer Student, for help with hypophysectomized rats and to Wiebke Hellenbrand, University of Ottawa Summer Student, for help with dietary studies on hamsters.

I wish to thank my daughters, Joanne Bell and Margaret Triandafillou for assistance in typing the thesis, and in other ways too numerous to mention.

Financial support was provided by a studentship from the Muscular Dystrophy Association of Canada.

The thesis was printed by Sunrise Software Consulting, Kingston.

SUMMARY

The only known function of brown adipose tissue is heat production. Due to the presence of a specific proton conductance pathway in brown adipose tissue mitochondria, food energy, normally highly conserved by mitochondria, may be dissipated as heat. This heat production is controlled by the sympathetic nervous system. Under certain environmental conditions, growth of brown adipose tissue is known to occur, which in the cold-adapted rat includes hypertrophy of functional tissue, hyperplasia, increased mitochondrial content and increased proton conductance of mitochondria. Brown adipose tissue of rats grows in response to living at low temperatures or to eating a cafeteria diet.

The work described in this thesis was done to study brown adipose tissue of two mutant species, the obese Zucker (fa/fa) rat and the myopathic (BIO 14.6) hamster. Brown adipose tissue of the obese Zucker rat had not previously been studied. The goal of this part of the work was to find out if brown adipose tissue thermogenesis is defective in the genetically obese rat as had been reported in the ob/ob mouse. Proton conductance, as judged by mitochondrial GDP-binding, was found to be reduced in obese Zucker rats, but increased normally when the animals were exposed to cold. During the course of these experiments, growth of BAT was reported in cafeteria-fed rats. Experiments were included which showed that there was also an increase in proton conductance in BAT mitochondria due to cafeteria feeding in normal rats. Growth of brown

adipose tissue and thermogenic activation of its mitochondria, however, did not occur in the obese Zucker rat. Therefore, as in some other obese rodents, defective brown adipose tissue thermogenesis in response to diet is a possible cause of the development of obesity in the obese Zucker rat.

The myopathic hamster was known to have a smaller than normal amount of brown adipose tissue which grows normally in response to cold acclimation. The purpose of this second part of the work was to study growth of brown adipose tissue in myopathic hamsters. Since mediators of brown adipose tissue growth in hamsters had not previously been studied, one objective was to study the influence of the sympathetic nervous system and the pineal gland on brown adipose tissue growth in normal and myopathic hamsters. Noradrenaline injections did not cause growth of brown adipose tissue in hamsters as in rats. However, since denervation caused a large reduction in tissue size, it would appear that the sympathetic nervous system contributes to tissue growth in hamsters through a substance other than, or in addition to noradrenaline. Alpha-adrenergic injections were found to be slightly inhibitory to BAT growth in hamsters, and treatment with the α_1 blocker, prazosin resulted in significant growth of the tissue. It is not certain why prazosin has a trophic influence on brown adipose tissue of hamsters, but possibilities include increased blood flow to the tissue and increased sympathetic activation. The pineal is considered to regulate growth of BAT in hamsters due to short photoperiod. Another mechanism appears to regulate growth due to cold or a high fat diet, since pinealectomized hamsters challenged by these two conditions showed the normal amounts of tissue growth.

Growth of brown adipose tissue in the cold was previously shown to be normal in myopathic hamsters. However, in two other environmental conditions known to cause tissue growth in hamsters, short photoperiod and high-fat diet, growth was found to be defective. The small size of the tissue then may be due to lack of a signal concerning photoperiod and diet in the myopathic hamster. In myopathic hamsters, prazosin caused a greater increase in tissue growth than in normal hamsters; suggesting growth of brown adipose tissue in this animal may be restricted due to vasoconstriction, and reduced blood flow to the tissue may be secondary to hyperreactivity of vascular membranes characteristic of their disease.

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ABBREVIATIONS

ADP	adenosine diphosphate
ATP	adenosine triphosphate
ACTH	adrenocorticotropic hormone
BAT	brown adipose tissue
cAMP	cyclic adenosine monophosphate
CoA	coenzyme A
COX	cytochrome oxidase
Δ	change
DIT	diet-induced thermogenesis
DNA	deoxyribonucleic acid
db/db	diabetic mouse
2-DG	2-deoxy-D-glucose
EDTA	ethylenediaminetetraacetic acid
fa/fa	obese Zucker rat
SE	standard error of the means
GDP	guanosine diphosphate
GH	growth hormone
GTG	gold thioglucose
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
L:D	hours of light : hours of darkness
KOH	potassium hydroxide
MPT	α-methyl-p-tyrosine

NA	noradrenaline
NaOH	sodium hydroxide
NATR	noradrenaline turnover rate
NST	nonshivering thermogenesis
ob/ob	obese mouse
PPO	2,5 diphenyloxazole
PVN	paraventricular nucleus
SCG	superior cervical ganglion
SCN	suprachiasmatic nucleus
SFS	sunflower seeds
T_3	triiodothyronine
T_4	thyroxine
Tris	tris (hydroxymethyl) aminomethane
TSH	thyroid stimulating hormone
TX	thyroidectomized
VMH	ventromedial hypothalamus

CHAPTER 1 LITERATURE REVIEW

1.1 INTRODUCTION

Twenty-five years ago a review of the literature on brown adipose tissue provided a description of a tissue without a known function (Johansson, 1959). Brown adipose tissue was first associated with hibernation but later this "hibernating gland" was found in many non-hibernators as well. Although its function was unknown, many and varied functions were attributed to brown adipose tissue in relation both to hibernation and to other more common body functions (Rasmussen, 1923; Johansson, 1959).

Ten years later, brown adipose tissue had become recognized as an important site of heat production in newborn animals and in hibernators during arousal from hibernation (Smith and Horwitz, 1969). There were indications that this was also true of the cold-acclimated animal but it remained controversial until Foster and Frydman (1978a,b; 1979) identified BAT as the principal site of nonshivering thermogenesis in the cold-adapted rat. Almost at the same time, work by Himms-Hagen and Desautels (1978) on obese mice, and by Rothwell and Stock (1979) on overfed rats, suggested another function for BAT as an energy buffer in the regulation of energy balance. At present the function of this small tissue is still being elucidated, particularly in regard to its role in animal and human obesity.

When the work described in this thesis was begun, defects were known to occur in brown adipose tissue of two animal models of human diseases, the ob/ob genetically obese mouse and the myopathic hamster,

animals which have been studied extensively in relation to obesity and muscular dystrophy, respectively. At that time the ob/ob mouse was known to have reduced thermogenic activation of brown adipose tissue, both at 28°C and when exposed to cold (Himms-Hagen and Desautels, 1978). The present work on another genetically obese rodent, the obese Zucker (fa/fa) rat was undertaken to study further the possible connection between BAT thermogenesis and obesity. The myopathic (BIO 14.6) hamster was known to have a reduced amount of brown adipose tissue (Himms-Hagen and Gwilliam, 1980). In this case the objective was to find the cause of the small size of brown adipose tissue with a view to contributing to knowledge on the cause of myopathy in the hamster and to study brown adipose tissue growth by comparative studies in the hamster and the rat. Work with both the obese Zucker rat and the myopathic hamster necessitated preliminary work designed to study brown adipose tissue growth and function in normal rats and hamsters.

The introductory Literature Review and Backgrounds to individual experiments describes the state of knowledge when the experiments were initiated. Much of the work described in the thesis has been published recently (Himms-Hagen *et al.*, 1981; Triandafillou *et al.*, 1982; Fellenz *et al.*, 1982; Triandafillou and Himms-Hagen, 1983; Triandafillou *et al.*, in press, a,b) as has related work by others. The relation of this work to more recent findings will be discussed in the General Discussion and Conclusions.

1.2 THERMOGENESIS

1.2.A CLASSIFICATION

Heat production or thermogenesis in animals is accomplished by oxidation of foodstuffs. The goal of this oxidation is usually the formation of ATP, a usable form of energy for cell functions. Formation and utilization of ATP is accompanied by heat production. Oxidation can also occur for the purpose of heat production alone, with or without the intermediate formation of ATP. By whatever pathway the reaction takes place, the resulting thermogenesis is the same.

Thermogenesis occurs 1) as a result of essential body functions (essential thermogenesis), 2) to maintain body temperature (endothermic thermogenesis) and 3) in connection with the processing of food (post-prandial thermogenesis) (Himms-Hagen, 1983a). In addition to these obligatory components of thermogenesis, facultative heat production may be caused by certain conditions occurring in the life of the animal, including heat produced 1) in response to low environmental temperature (cold-induced thermogenesis, shivering or nonshivering), 2) as a result of prolonged overfeeding (diet-induced thermogenesis) and 3) as a consequence of voluntary physical activity (exercise-induced thermogenesis) (Himms-Hagen, 1983a). By regulating endothermic thermogenesis, thyroid hormone controls obligatory thermogenesis, which occurs at a fairly constant rate in most tissues of the body. In contrast, facultative thermogenesis can be turned on and off quickly by the nervous system, as required. Exercise-induced thermogenesis is controlled by acetylcholine in skeletal muscle as also is cold-induced

shivering thermogenesis. (Himms-Hagen, 1983a). In contrast, cold-induced nonshivering thermogenesis and diet-induced thermogenesis are controlled by noradrenaline in BAT.

1.2.B NONSHIVERING THERMOGENESIS

When a warm-adapted rat is introduced to a cold environment (4°C) body temperature is maintained largely by shivering. As the animal continues to live in the cold, shivering gradually diminishes until after 2-3 weeks, shivering has disappeared and is replaced by nonshivering thermogenesis (NST) as part of the process of cold acclimation (Hart *et al.*, 1956). Besides length of time in the cold, the relative proportion of shivering and nonshivering thermogenesis depends on the age and species of the animal. The capacity for NST is high in newborn mammals (non hibernators), declines with age and can be reestablished by cold adaptation in several species (rats, mice) (Himms-Hagen, 1976). In contrast, capacity for NST is generally greater in hibernators whether acclimated to cold or not (Himms-Hagen, 1976).

Nonshivering thermogenesis can be defined as a cold-induced, metabolic heat producing process that is not due to muscle activity (Himms-Hagen, 1983a). Mediated by the sympathetic nervous system (Hsieh and Carlson, 1957; Leduc, 1961), NST can be evoked by the infusion of noradrenaline (Depocas, 1960).

The thermogenic nature of brown adipose tissue (BAT) became known in the 1960's (Smith and Roberts, 1964; Smith and Horwitz, 1969). Although BAT was known to be an important source of heat in the newborn (Dawkins and Hull, 1964) and in arousal from hibernation (Smith

and Hock, 1963; Hayward and Lyman, 1967), it was generally considered too small to contribute significantly to NST in cold-adapted animals (Jansky and Hart, 1968; Himms-Hagen, 1976). The organ responsible for NST remained controversial until Foster and Frydman (1978a,b, 1979) measured blood flow to tissues of cold-acclimated rats using radioactive microspheres together with the arteriovenus differences in blood oxygen across interscapular BAT, and found that BAT was the major site of increased metabolic rate induced by infused noradrenaline or by exposure to cold. Even in warm-adapted rats BAT was estimated to contribute 37% of the increased respiration at 6°C and in cold-adapted animals this amounted to 72% (Foster and Frydman, 1979).

1.2.C DIET-INDUCED THERMOGENESIS

Diet-induced thermogenesis (DIT) is an increase in metabolic rate due to overfeeding. The term sometimes includes the rise in metabolism which immediately follows food intake (post-prandial thermogenesis) (Girardier, 1983), but as used here, refers only to the prolonged effect of diet on metabolism after food processing is complete.

The idea of energy dissipation associated with overeating is not a new one. DIT has been recognized for many years (Himms-Hagen, 1984) and the similarity of DIT in the overfed rat to NST was pointed out by Stirling and Stock in 1968. Two fairly recent events have contributed to new concepts in the mediation of DIT. First, it was found that overfeeding stimulated the sympathetic nervous system in several tissues and that fasting suppressed it, the opposite of the previously accepted idea (Landsberg and Young, 1978). Secondly, Rothwell and Stock (1979)

reported that rats offered a varied, palatable diet (cafeteria diet) overate by 80% compared to chow-fed controls, while gaining only 27% more weight. This decreased feed efficiency was accompanied by an increase in resting metabolic rate and a doubling of interscapular BAT weight and protein content, and was attributed to thermogenesis in BAT (Rothwell and Stock, 1979). The cafeteria-fed rat has been compared to the cold-adapted rat, both exhibiting enhanced cold resistance which is abolished by propranolol, and a greater respiratory response to noradrenaline (Rothwell and Stock, 1979). Like cold adaptation, cafeteria feeding is accompanied by increased noradrenaline (NA) turnover in BAT (Young *et al.*, 1982). The functional involvement of BAT in DIT was confirmed by measurements of increased blood flow to the tissue following noradrenaline infusion (Rothwell and Stock, 1981).

1.3 BROWN ADIPOSE TISSUE (BAT)

1.3.A COMPOSITION

Brown adipose tissue (BAT) is found in a very large number of mammals. Characterization of the tissue has been mainly in the laboratory rat and to a lesser degree in the Syrian hamster and other small animals (Smith and Horwitz, 1969).

BAT has a lobular appearance and can be distinguished from other tissues by its brownish colour, which is largely due to the presence of blood hemoglobin and the high level of flavins and cytochromes in its abundant mitochondria (Smith and Horwitz, 1969; Girardier, 1983).

Typically, BAT is a diffusely located tissue concentrated in the neck and thorax. The exact distribution varies with the species. In the rat, major deposits are located superficially in the interscapular and cervical regions and along the blood vessels of the neck and axillary region; in the thorax, overlaying the large vessels and nerve trunks; and caudally, extending along the aorta and other blood vessels and around the kidneys and adrenals (Smith and Roberts, 1964). BAT deposits are much more abundant in hibernators like the hamster and the ground squirrel (Joel, 1965) and in neonatal animals (Rasmussen, 1923; Cameron and Smith, 1964).

The total amount of BAT is small, approximately 0.8% and 1.4% of body weight in the warm- and cold-acclimated adult rat respectively (Foster and Frydman, 1979). Yet BAT is strategically located so as to heat organs vital to survival on the cold. By warming the blood returning to the thorax and spinal cord, both by its venous outflow of warmed blood and by its close association with vessels in more peripheral areas, this small thermogenic tissue has the potential to protect the central body core against cooling in low environmental temperatures (Smith and Roberts, 1964). In hibernators, the cervical-thoracic location of BAT is in keeping with the strategy of rewarming the anterior portion of the body first during arousal from hibernation (Joel, 1965).

A dense vascular network surrounds brown adipocytes (Smith and Horwitz, 1969). It has been estimated that in human infant BAT, one third of a fat depleted cell is in contact with the capillary wall (Aherne and Hull, 1966).

BAT has a high NA content (Young *et al.*, 1982). Fluorescence histochemical techniques have revealed a dense adrenergic innervation of BAT. Nerve fibers are found accompanying many arterial blood vessels as well as among the fat cells themselves (Cottle and Cottle, 1970; Derry *et al.*, 1969).

The predominant cell type in BAT is the brown adipocyte. Generally these cells are polygonal, fitting close together in a lobular arrangement. Individual cells are set in a fibrous network. Together with adjoining capillaries and nerve fibers they make up the lobules which in turn make up the lobes of BAT (Afzelius, 1970).

The brown adipocytes contain numerous and large mitochondria, a varying number of lipid droplets and few membranes in the cytoplasm and a nucleus which is often spherical and centrally located (Afzelius, 1970).

1.3.B FUNCTION

Thermogenesis in BAT is initiated by the interaction of noradrenaline at β-receptors on the plasma membrane, which stimulates lipolysis through activation of adenylate cyclase, increased cAMP concentration, activation of cAMP dependent protein kinases and hormone sensitive lipase. The resulting fatty acids are activated to acyl CoA and transported into the mitochondria by the carnitine shuttle and oxidized by β-oxidation, TCA cycle and electron transport (Nedergaard and Lindberg, 1982; Himms-Hagen, 1984; Nicholls and Locke, 1983). Fatty acids present in the cytoplasm also appear to uncouple mitochondrial oxidative phosphorylation by interfering with the binding of purine nucleotides to a 32 000 MW polypeptide that is part of the thermogenic mechanism (refer to Chapter

1.3.D) thereby increasing oxidation and concomitant heat production (Nicholls and Locke, 1984). The β-adrenergic receptors which interact with noradrenaline have been characterized as of the β_1 subtype (Bukowieki *et al.*, 1978; Svoboda *et al.*, 1979) but recent work involving a new β-agonist suggest these receptors are not true β_1-receptors (Arch *et al.*, 1984).

Alpha$_1$-adrenergic receptors have been identified in hamster BAT membranes and in isolated brown adipocytes (Mohell *et al.*, 1983b). Two recent reports estimate that α_1-receptors are responsible for 20% of the noradrenaline stimulated respiration in hamster BAT (Mohell *et al.*, 1983a; Schimmel *et al.*, 1983). In rat brown adipocytes very high levels of α-adrenergic agonists are required to cause an increase in respiration (Bukowiecki *et al.*, 1980). These authors concluded that α-receptors, although present in BAT, are of questionable physiological significance. Alpha$_1$-receptor stimulated phosphotidylinositol turnover has been demonstrated in rat and hamster BAT cells (Garcia-Sainz *et al.*, 1980; Mohell *et al.*, 1984). Stimulation of α-adrenergic receptors has a potentiating effect on calorigenesis and blood flow to BAT stimulated by β-adrenergic agonists (Foster, 1984).

Alpha$_2$-adrenergic receptors, generally inhibitory to the β-mediated response, have been reported to be present in rat (Sundin and Fain, 1983) and absent in hamster (McMahon and Schimmel, 1982) brown adipocytes.

Brown adipose tissue receives sympathetic innervation to both the adipocytes and the blood vessels (Cottle and Cottle, 1970; Derry *et al.*, 1969). There is an initial transient decrease in BAT temperature which

precedes the rise in temperature after nerve stimulation *in vivo* (Flaim *et al.*, 1976, 1977). This decrease in temperature has been attributed to vasoconstriction due to the action of noradrenaline on α-receptors in blood vessels serving BAT (Flaim *et al.*, 1977), since α-agonists do not have an inhibitory effect on thermogenesis of brown adipocytes (Horwitz, 1975). This biphasic response in BAT temperature has also been noted during stimulation of the ventral medial hypothalamus (Perkins *et al.*, 1981). However, the large increases in blood flow to BAT accompanying thermogenesis appear to be secondary to the increased metabolism (Foster and Depocas, 1980).

The action of noradrenaline on BAT is accompanied by depolarization of the plasma membrane which has been associated with the β-receptor (Fink and Williams, 1976; Williams and Matthews, 1974), and the α-receptor (Fink and Williams, 1976; Horwitz *et al.*, 1969). Recent studies indicate that noradenaline causes an initial depolarization via α-receptors, which is followed by repolarization and increased respiration; a second depolarization then follows the increased oxygen consumption which results from activation of β-receptors. (Girardier and Schneider-Picard, 1983).

Lipogenesis is carried out in BAT, seemingly to replenish lipids used for thermogenesis (Trayhurn, 1979b; Agius and Williamson, 1980). Glycerokinase, involved in triacylglycerol synthesis, is present in BAT of the rat (Nedergaard, 1982) but is very low in hamster BAT (Lindberg *et al.*, 1976).

In the rat, acetyl CoA oxidation in BAT is limited by availability of oxaloacetate for operation of the citric acid cycle. This seems not to be

the case in hamster BAT which is able to hydrolyse acetyl CoA to acetate (Bernson and Nicholls, 1974) and may allow fatty acid oxidation to proceed even at low temperatures when the citric acid cycle activity is very low, a distinct advantage for a hibernator experiencing low body temperature.

1.3.C CONTROL OF BAT THERMOGENESIS

Activation of the sympathetic nervous system in BAT causes NST and DIT. The regulation of this activation is not very clear in either case. NST appears to be regulated by thermoreceptors in the skin and preoptic area of the hypothalamus which relay information regarding body temperature to integrative centers in the hypothalamus (Bruck and Zeisberger, 1978) by neural pathways including the subcoeruleus region and nucleus raphe magnus of the brain (Bruck and Hinckel, 1982). The neural connections by which the hypothalamus stimulates thermogenesis in the peripheral tissues are unknown.

Neural mechanisms controlling DIT are even less clearly understood (Rothwell and Stock, 1982b). Stimulation of the ventral medial hypothalmus (VMH) elevates BAT metabolism (Perkins *et al.*, 1981; Shimazu and Takahashi, 1980; Takahashi and Shimazu, 1982). Since hypothalamic lesions in this area impair DIT but not NST in BAT (Hogan *et al.*, 1982; Seydoux *et al.*, 1982; Himms-Hagen *et al.*, 1984) it is believed that the ventral medial region of the hypothalamus may include part of the effector system regulating DIT in BAT and that either this

area is not involved in NST or that some alternate pathway is available (Himms-Hagen, 1984).

1.3.D THE THERMOGENIC MECHANISM

The relatively small size and exceptional heat producing capacity of BAT have stimulated much interest in a possible thermogenic mechanism which might be unique to this tissue.

Respiration and heat production are closely related in BAT mitochondria, brown adipocytes and whole tissue, suggesting mitochondrial respiration is responsible for the thermogenic capabilities of BAT (Nicholls, 1983). Further, no significant extramitochondrial oxygen consuming mechanism has ever been found in BAT (Lindberg *et al.*, 1981).

According to the chemiosmotic theory, the flow of electrons down the electron transport chain is coupled to the extrusion of protons from the mitochondria, across the inner mitochondrial membrane. The impermeability of the membrane to the return of these protons into the mitochondria results in a proton gradient across the membrane. This proton gradient represents the electrochemical potential which is the driving force for phosphorylation of ADP to ATP by the ATP synthase in the membrane. Synthesis of ATP results in admission of protons to the mitochondria and release of the gradient. If not released by ATP formation, the proton gradient serves to inhibit further respiration and proton release by the mitochondria. Thus oxidation is coupled to phosphorylation, and respiration is limited by the supply of ADP. The

mitochondria are considered to be "coupled" (Mitchell, 1976; Nicholls, 1982).

The search for an explanation of heat production in BAT has centered on two mechanisms whereby BAT mitochondria might be enabled to produce more heat than normal coupled mitochondria.

1. A cellular ATP hydrolysing system has been proposed which would supply ADP, allowing protons to reenter the mitochondria via the ATPase and allowing respiration to proceed.

Two ATP consuming reactions or "futile cycles" have been proposed, namely recycling of fatty acids (Lindberg *et al.*, 1967) and sodium cycling at the plasma membrane (Horwitz, 1979). Neither has been judged quantitatively capable to support more than a small fraction of the observed thermogenesis in BAT (Lindberg *et al.*, 1981; Nicholls and Locke, 1983; Girardier, 1983). Further, the obligatory coupling of mitochondrial respiration to ATP synthesis requires increased ATP synthesis with increasing BAT respiration. However, a notably low capacity for ATP synthesis has been reported in BAT mitochondria (Cannon and Vogel, 1977; Houstek and Drahota, 1977) leading to the almost universal rejection of this proposed mechanism.

2. The second mechanism would allow proton reentry into the mitochondria without the necessity for ATP synthesis, again allowing increased respiration. In the first preparations of BAT mitochondria respiration was uncoupled (Lindberg *et al.*, 1967; Smith *et al.*, 1966) but later ATP synthesis was shown to occur if fatty acids were removed by the addition of albumin, or CoA, carnitine and ATP to allow activation and

oxidation of fatty acids. Fatty acids were believed to be the substrate and uncoupling agents of thermogenesis (Lindberg *et al.*, 1981).

However low concentrations of ADP did not stimulate respiration as in other tissues while millimolar additions of ATP and GTP were found to allow mitochondria to show respiratory control with further additions of ADP. Subsequent work, largely by Nicholls (Nicholls and Locke, 1983, 1984) has developed the generally accepted concept that thermogenesis in BAT is the result of an unusually permeable mitochondrial membrane which allows protons to leak back into the mitochondria. The way in which the proton conductance mechanism is controlled is not entirely clear, but purine nucleotides and intracellular fatty acids (or their CoA derivatives) appear to be involved.

Purine nucleotides act to regulate proton conductance by binding non-covalently at the outer surface of the inner mitochondrial membrane (Nicholls, 1976a). Ricquier and Kader (1976) identified a polypeptide of molecular weight 32 000 which specifically increased in BAT mitochondrial membrane during cold acclimation of rats. The purine nucleotide binding site was identified as a 32 000 MW polypeptide (Heaton *et al.*, 1978) confirming that the 32 000 MW polypeptide and the purine nucleotide binding protein were identical. Purine nucleotides are present in millimolar concentration in cytosol of brown adipocytes (Grav *et al.*, 1970). Noradrenaline stimulation of respiration lowers ATP, but not sufficiently to explain the increased respiration through decreased purine nucleotide binding (Nicholls and Locke, 1983). Recently the action of fatty acids has been reevaluated. It is thought that fatty acids mobilized by noradrenaline stimulation of lipolysis in BAT act to uncouple the

mitochondria as well as provide substrate for respiration (Bukowieki *et al.*, 1981; Nicholls and Locke, 1983).

The 32 000 MW polypeptide appears to be specific to BAT mitochondria. BAT mitochondrial proton conductance is 100 times that of rat liver mitochondria under similiar conditions (Nicholls, 1979). The GDP-binding site is present on BAT mitochondria, not on liver mitochondria (Nicholls, 1979). The 32 000 MW polypeptide has been found in BAT mitochondria and not white fat, liver or heart muscle (Cannon *et al.*, 1982; Lean *et al.*, 1983). The 32 000 MW polypeptide has been purified and appears to exist as a dimer of 62 000 - 63 000 MW (Lin and Klingenberg, 1980; Lin *et al.*, 1980). Initial assays of the amount of this protein in BAT used polyacrylamide gel electrophoresis, a nonspecific method. More recently specific immunoassays (Cannon *et al.*, 1982; Lean *et al.*, 1983) have been developed and will doubtlessly facilitate research in this area.

The capacity for GDP-binding to BAT mitochondrial membrane varies with the thermogenic state of the animal, both being high in the newborn rat (Sundin and Cannon, 1980) and declining thereafter except when the animal is cold-adapted, when binding is again elevated (Desautel *et al.*, 1978; Sundin and Cannon, 1980). Cold-adapted rats have a greater proportion of the 32 000 MW polypeptide in the mitochondrial membrane (Heaton *et al.*, 1978; Ricquier and Kader, 1976; Desautels *et al.*, 1978) and increased synthesis of the polypeptide (Himms-Hagen *et al.*, 1980).

Purine nucleotide binding to BAT mitochondria has in practice been measured by the specific binding of GDP because ~~of its~~ high affinity for the polypeptide and because it is not translocated into the mitochondria

(Nicholls, 1976). The probable physiological regulator is ATP (Nicholls and Locke, 1983). Although GDP binds to the 32 000 MW polypeptide, the binding is not necessarily a measure of the total polypeptide in the membrane. The binding may be elevated without a change in amount of 32 000 MW polypeptide and is viewed as a measure of active polypeptide and therefore a measure of the thermogenic state of BAT. Thus the immediate increase in GDP-binding detected after 1 hour of cold exposure appears to reflect unmasking of binding sites already present in the membrane (Desautels *et al.*, 1978). The hamster has a large amount of the 32 000 MW polypeptide (Trayhurn *et al.*, 1983; Ricquier *et al.*, 1979) and a high level of mitochondrial GDP-binding (Himms-Hagen and Gwilliam, 1980; Sundin *et al.*, 1981) in BAT in the warm-adapted state, relative to warm-adapted mice or rats. The hamster also increases mitochondrial GDP-binding (Himms-Hagen and Gwilliam, 1980) and the proportion of the 32 000 MW polypeptide (Trayhurn *et al.*, 1983) when adapted to cold. These changes are however smaller than those observed in the mouse (Trayhurn *et al.*, 1983).

1.4 GROWTH OF BROWN ADIPOSE TISSUE

Growth of BAT has been observed to vary with the developmental state of the animal and three environmental conditions, temperature, dietary intake, and lighting conditions. These four modulators of growth will be discussed, followed by a consideration of some possible mediators of BAT growth. Emphasis will be on growth of BAT in rats and hamsters, where information is available.

1.4.A MEASUREMENT

Various parameters have been used to quantify growth of BAT (Cannon and Nedergaard, 1983). The most obvious and easiest measurement to make is tissue wet weight (mass). Weight is often an indication of the capacity of the tissue, but in some cases, e.g. thyroid hormone treatment, increased tissue weight is only a reflection of lipid storage and the tissue may not be more activated towards heat production (Sundin, 1981; Triandafillou *et al.*, 1982).

A better measure of functioning tissue is protein content. A change in protein content is often, but not always accompanied by a change in the level of DNA, measuring hyperplasia. Measurements of oxidative enzymes, e.g. cytochrome oxidase, reflect the mitochondrial content and capacity for oxidation.

Of critical importance in measuring growth of BAT is mitochondrial GDP-binding, a measure of the thermogenic state of the tissue. The total amount of the 32 000 MW polypeptide may also change. This is viewed as a less sensitive measure of thermogenic capacity of BAT at a given time than nucleotide binding (Nicholls and Locke, 1983), more an indicator of potential capacity for uncoupled thermogenesis. Thus after 1 hour of cold exposure, GDP-binding is known to increase without a change in the amount of the polypeptide, due to a proposed unmasking of the polypeptide binding sites (Desautels *et al.*, 1978). The amount of the 32 000 MW polypeptide has been roughly assessed by polyacrylamide gel electrophoresis (Lin and Klingenberg, 1980). The recently developed specific radioimmunoassay (Cannon *et al.*, 1982; Lean *et al.*, 1983) may reveal more about changes occurring during growth of BAT.

1.4.B DEVELOPMENTAL GROWTH

In the rat, BAT development occurs perinatally, from about 6 days before birth, when the interscapular area contains some undifferentiated cells and capillary endothelial cells with dilated rough endoplasmic reticulum, until about 2 weeks after birth. During this time the number and size of mitochondria and their respiratory activity, as well as the number and regular tight packed arrangement of their cristae increase. The lipid droplets increase in size except for a time at birth when lipids are depleted. The noradrenaline level also increases over this time except for a decrease at the time of birth. This period of differentiation is followed by a plateau and then a period of involution of the tissue, beginning around 17 to 30 weeks when all parameters gradually decline and the animal grows to adulthood (Barnard and Skala, 1970).

Differentiation of BAT in the hamster occurs postnatally. Studies of the subscapular area show no evidence of multilocularity in fetal hamsters. During the latter days of gestation and the first 2 days after birth the area contained a loose connective tissue with unilocular precursor cells and a growing number of unilocular cells. At 3 to 5 days clusters of small cells appeared among the unilocular cells closely associated with blood vessels. These immature BAT cells increased in number to become the dominant cell type by 15 days while the unilocular cells disappeared. By 30 days these cells were recognizable as multilocular (Smalley *et al.*, 1970). More recently, light microscopic radioautographic studies have indicated that the unilocular adipocytes are converted to multilocular, coinciding with parenchymal innervation. Multilocular adipocytes were also formed directly from pericytes where adrenergic

innervation was not obvious (Nèchad and Barnard, 1979). From experiments in which neonatal hamster BAT was transplanted into the denervated or innervated anterior chamber of the eye, it appears that sympathetic innervation stimulates BAT growth but that some growth occurs in the absence of the sympathetic nerves (Nèchad and Olson, 1983).

1.4.C COLD-INDUCED GROWTH

When a warm-acclimated rat is placed in the cold, changes occur in BAT during the first few hours. If the animal remains in the cold, other more gradual changes take place in BAT in connection with cold acclimation over a period of weeks. Brief cold exposure (4°C) of rats causes increased oxygen consumption (Foster and Frydman, 1979; Szelenyi, 1968), increased temperature in BAT (Smith, 1961; Szelenyi, 1968) and increased blood flow to BAT (Foster and Frydman, 1979). These changes are accompanied by depletion of lipid vacuoles (Cameron and Smith, 1964), decreased cell size (Cameron and Smith, 1964), and increased noradrenaline turnover (Cottle *et al.*, 1967; Young *et al.*, 1982) consistent with increased sympathetic stimulation of lipolysis and thermogenesis. Mitochondria are larger and there is a rearrangement of the inner mitochondrial membrane into a more parallel configuration (Desautels and Himms-Hagen, 1980). As early as 1 hour at 4°C there is an increase in GDP-binding to BAT mitochondria (Desautels *et al.*, 1978) representing an increase in thermogenic state of the tissue (Nicholls, 1979). As far as is known there is no increase in 32 000 MW polypeptide at this time.

When rats are exposed to low temperatures for longer periods (weeks), other changes occur in BAT resulting in increased BAT mass (Page and Babineau, 1950) and improved thermogenesis (Smith, 1961). The increase in tissue mass is accompanied by increased tissue protein (Smith and Roberts, 1964) and increased water and lipid free dry matter (Joel, 1965) rather than triglyceride. After an initial drop when first exposed to cold, the lipid content of BAT is almost restored by 24 hours. Droplets remain smaller and more numerous than in animals kept at room temperature (Suter, 1969).

Increased vascularity and cellularity in BAT occurs in rats exposed to 4-6°C. Proliferation of the capillary network of arterioles and especially of venules was reported by Hunt and Hunt (1967). Cell proliferation, occurring in endothelium and extravascular precursor cells was maximal at 4 days and returned to normal by 16 days (Cameron and Smith, 1964). No mitosis of mature BAT cells has been described and new cells are believed to derive from precursor cells associated with vascular endothelium (Cameron and Smith, 1964; Hunt and Hunt 1967; Bukowieki *et al.*, 1982).

Cold adaptation results in larger, more numerous mitochondria and expanded area of the inner mitochondrial membrane (Desautels and Himms-Hagen, 1980; Suter, 1969; Bukowieki *et al.*, 1982). Phospholipid content of the tissue increases, reflecting increased membrane structure (Ricquier *et al.*, 1979). Important qualitative changes occur in the mitochondria. The proportion of 32 000 MW polypeptide in the membrane increases (Desautels *et al.*, 1978; Ricquier and Kader, 1976; Heaton *et al.*, 1978) and there is increased binding of purine nucleotides to the membrane due to an increase in binding sites with no change in

affinity (Desautels *et al.*, 1978; Desautels and Himms-Hagen, 1981). During cold adaptation there is a general increase in sympathetic innervation in BAT (Barnard *et al.*, 1980). Increased sympathic innervation has been described in association with arterioles and between brown fat cells (Cottle and Cottle, 1970; Derry *et al.*, 1969), together with an increased rate of noradrenaline synthesis (Cottle *et al.*, 1967).

Lipogenesis in BAT increases in the cold-acclimated rat (Trayhurn, 1979b). In the hamster, lipogenesis is not elevated during cold adaptation and lipids are imported from other tissues, probably the liver (Trayhurn, 1980).

BAT of cold-acclimated hamsters has elevated tissue protein, and COX content. GDP-binding to mitochondria and the proportion of 32 000 MW polypeptide, both relatively high in the warm-adapted state (Himms-Hagen and Gwilliam, 1980; Ricquier *et al.*, 1979), increase when the animal is cold-adapted but to a lesser extent than in rats or mice (Himms-Hagen and Gwilliam, 1980; Trayhurn *et al.*, 1983).

1.4.D DIET-INDUCED GROWTH

When this research was begun, it was known that rats could be induced to overeat by feeding them a varied, palatable cafeteria diet, and that feeding this diet resulted in hypertrophy of BAT which was not due to lipid accumulation. It was found from experiments reported in this thesis (Experiment A-1) that this is a hyperplastic type of growth (Himms-Hagen, *et al.*, 1981). Cafeteria feeding also results in thermogenic activation of BAT (Brooks *et al.*, 1980). Noradrenaline turnover is elevated in BAT (Young *et al.*, 1982) and there is increased

noradrenaline-stimulated blood flow to BAT (Rothwell and Stock, 1981) resulting from cafeteria feeding. The effects of cafeteria feeding on BAT are similar to but less pronounced than those of cold adaptation (Bukowieki *et al.*, 1982). The 32 000 MW polypeptide was found not to increase in relative amount, as during cold adaptation (Experiment A-1) but this has not yet been confirmed by newer immunological techniques. Noradrenaline-stimulated Na^+K^+ATPase activity is elevated in BAT of cafeteria-fed rats. *In vitro* Na^+K^+ATPase activity has been correlated with *in vivo* oxygen consumption rates in control and cafeteria-fed rats (Rothwell *et al.*, 1981b) but the importance of this enzyme in BAT thermogenesis is unclear.

It is difficult to separate the effect of elevated energy content from the effect of altered composition of the diet. Rats on a high fat, low carbohydrate cafeteria diet were more hyperphagic and expended more energy in thermogenesis than rats on a high carbohydrate, low fat cafeteria diet (Rothwell *et al.*, 1983c). Low protein diets result in increased food consumption and decreased food efficiency (Young *et al.*, 1980). Since cafeteria diets have a lower protein content than chow, it has been suggested that the low protein level may be the cause of the higher metabolic rate. Rats fed cafeteria diets containing high, normal or low protein levels all had increased energy intake, elevated energy expenditure and growth of BAT. The low protein group had the lowest energy efficiency and the smallest increase in BAT while there was no difference between rats receiving normal or high levels of protein suggesting that the protein level of the diet is important, but only if it is very low (Rothwell *et al.*, 1982b).

Hamsters seem better able than rats to adjust the energy content of their diet, consuming the same number of calories or marginally overeating when fed a high fat or high carbohydrate diet (Borer, 1974; Wade, 1982). Hamsters on a high fat diet however, gain more weight and increase their lipid stores relative to chow fed animals (Borer, 1974; Wade, 1982) and this weight gain without overeating is accompanied by an increase in BAT weight, protein content and capacity of the animal for NST (Wade, 1982).

The control of food intake appears to differ between rats and hamsters in that hamsters do not increase food intake after food deprivation or in response to 2-deoxy-D-glucose (2-DG) and are less responsive than rats to the hyperphagic effects of insulin. (Silverman and Zucker, 1976; Rowland, 1983; DiBattista, 1983).

1.4.E SHORT PHOTOPERIOD-INDUCED GROWTH

Some animals e.g. the hamster and the white footed mouse, are known to be able to regulate certain body functions in response to changes in the lighting conditions in their environment. Increased BAT mass has been reported in Syrian hamsters kept in short photoperiod (Hoffman *et al.*, 1965; Reiter, 1975) and is prevented by pinealectomy (Reiter, 1975). In Siberian hamsters increased BAT mass, mitochondrial protein and cytochrome oxidase and capacity for nonshivering thermogenesis have been reported (Rafael *et al.*, 1981; Heldmaier *et al.*, 1981).

The pineal is not necessary for the growth of BAT in rats due to cold acclimation, and cold-induced growth occurred equally well in short

and long photoperiod (Kott and Horwitz, 1983). Also in the rat, Hagelstein and Folk (1979) found short photoperiod and cold had a greater effect on growth than cold alone. Rats in short photoperiod have an increased noradrenaline excretion (Haim *et al.*, 1983).

In summary, acclimation to cold or prolonged overfeeding cause changes to occur in BAT of rats, including growth of the tissue, proliferation of mitochondria and qualitative changes in mitochondria, that lead to an increased capacity for thermogenesis. Similar changes occur in response to overfeeding, but are usually smaller than those due to cold acclimation. BAT of hamsters is also known to grow in response to an additional environmental condition, short photoperiod, which has not been shown in rats. Brown adipose tissue of warm-adapted hamsters appears to have a greater thermogenic capacity than that of rats, having a greater amount of 32 000 MW polypeptide and higher GDP-binding. GDP-binding increases further when the animal is acclimated to cold.

1.5 CONTROL OF BAT GROWTH

1.5.A SYMPATHETIC NERVOUS SYSTEM

There seems no doubt that the sympathetic nervous system is involved in growth of BAT (Barnard *et al.*, 1980). BAT has been unilaterally denervated accumulates lipid and does not grow as well on the denervated side in response to cold or to cafeteria feeding (Hunt and Hunt, 1967; Himms-Hagen and Park, 1984). Although less than normal, some growth occurs in the cold, suggesting that either denervation is incomplete or that some other mediator contributes to growth of BAT in the cold. Chemical sympathectomy using guanethidine which destroys cell bodies, terminals, and axons, more completely impaires the response of the tissue to cold (Mory *et al.*, 1982) but this method also is imperfect since it is not specific for BAT.

The trophic effect on BAT of chronic sympathetic stimulation can be mimicked by the chronic administration of catecholamines. Thus, the growth of BAT has been observed in rats chronically injected with noradrenaline or isoproterenol. Wet weight, protein content, DNA and oxidative enzymes were all increased in BAT by such chronic treatments (Heick *et al.*, 1973; Desautels and Himms-Hagen, 1979; Mory *et al.*, 1980). However cold resistance of animals was not as high as in cold-adapted animals (LeBlanc and Villemaire, 1970) and there was no measurable increase in mitochondrial GDP-binding or in the proportion of the 32 000 MW polypeptide (Desautels and Himms-Hagen, 1979). In a recent study in which noradrenaline was administered by chronic infusion through osmotic minipumps, growth of the tissue and increased

GDP-binding and 32 000 MW polypeptide occurred as in the cold (Mory *et al.*, 1984). Extremely high levels of infused noradrenaline are necessary to mimic the levels of noradrenaline in BAT during cold exposure as thermogenesis is caused by noradrenaline from sympathetic nerve terminals in BAT where the concentration of noradrenaline is very high and not by circulating catecholamines (Depocas *et al.*, 1980). Growth of BAT, including increased tissue protein, GDP-binding and increased proportion of 32 000 MW polypeptides was observed in rats bearing cloned (PC 12) pheochromocytoma tumors (Ricquier *et al.*, 1983); in rats treated with fenoterol, a long acting β-adrenergic agonist, or with ephedrine, which releases noradrenaline from nerve endings (Young, P. *et al.*, 1984) and in rats with constant infusion of noradrenaline (Mory *et al.*, 1984) also suggesting sympathetic mediation of BAT growth. It may be that sustained β-receptor stimulation is necessary for these effects to occur which may not be possible with noradrenaline injections.

1.5.B THYROID HORMONE

Thyroid hormones are synthesized by and released into the blood from the thyroid gland, normally under the control of thyroid stimulating hormone (TSH) from the anterior pituitary. TSH secretion is in turn regulated by thyrotropin releasing hormone (TRH) from the hypothalamus. Blood concentrations of the two most important thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3) act by a negative feedback mechanism on TSH secretion (Nilsson and Karlberg, 1983).

T_3 is viewed as the metabolically active thyroid hormone, estimated to be 3 to 5 times more potent than thyroxine. Some is released from the

thyroid but most of the T_3 in the blood is the result of monodeiodination of T_4 in the peripheral tissues by the action of the enzyme thyroxine 5'-monodeiodinase. It is unclear what proportion of cellular T_3 originates from the plasma or is generated locally in the cell (Oppenheimer, 1983). BAT contains one form of the 5'-deiodinase and the activity of the enzyme appears to be under the control of the sympathetic nervous system (Leonard *et al.*, 1983; Silva and Larson, 1983). Stereospecific binding sites for thyroid hormone have been demonstrated in cell membrane, cytoplasm, nucleus and the inner mitochondrial membrane (Hoffenberg and Ramsden, 1983; Barsano and DeGroot, 1983). The nuclear binding sites are the best characterized and are often considered the true receptors for T_3 (Mariash and Oppenheimer, 1983). Cytoplasmic binding sites may be involved in intracellular transport of thyroid hormone. Binding sites on cell membrane may serve to recognize and admit thyroid hormone to the cell although thyroid hormone is usually thought to diffuse through the cell membrane. The function of mitochondrial binding sites is equally unclear (Hoffenberg and Ramsden, 1983; Barsano and DeGroot, 1983). Most tissues are thought to be thyroid hormone sensitive, exceptions being the spleen, testis and adult rat brain (Barsano and DeGroot, 1983).

Thyroid hormones are involved in many body functions of growth and development as well as in the control of metabolism. Only the latter will be considered here.

Endothermic thermogenesis is controlled largely by thyroid hormone (Himms-Hagen, 1983a). During cold-induced NST and DIT the level of T_3 is elevated (Scammell *et al.*, 1980; Rothwell and Stock, 1979). The possibility that thyroid hormone is responsible for the elevated metabolism

in these two conditions is complicated by the fact that the activity of the sympathetic nervous system is also elevated (Leduc, 1961; Landsberg and Young, 1983). Noradrenaline can modulate thyroid hormone secretion (Melander, 1977; Pisarev *et al.*, 1981) and promote its peripheral action by increasing conversion of T_4 to T_3 (Storm *et al.*, 1981; Nilsson and Karlberg, 1983).

In addition to the influence of catecholamines on thyroid hormone secretion and activity, evidence suggests that thyroid hormone works to turn down the activity of the sympathetic nervous system as it potentiates the action of catecholamines (Gibson, 1981). Generally noradrenaline turnover is enhanced during hypothyroidism and reduced during hyperthyroidism (Landsberg and Axelrod, 1968; Christiansen, 1972). The thermogenic effects of catecholamines is increased in hyperthyroidism and decreased in hypothyroidism (Swanson, 1956; Hsieh *et al.*, 1966; Fregly *et al.*, 1979). This "permissive" effect of thyroid hormone on catecholamine action has been variously credited to modulation of cAMP phosphodiesterase (Van Inwegen *et al.*, 1975; Lutherer *et al.*, 1978), to a change in the relative number of α- and β-adrenergic receptors (Kunos, 1977), to a change in the amount of adenylate cyclase (Krishna *et al.*, 1968) and to an altered coupling between the hormone and adenylate cyclase (Malbon *et al.*, 1980).

The prevailing concept of the mechanism of thyroid hormone action involves the action of thyroid hormone at a nuclear receptor which promotes RNA polymerase activity and cytoplasmic protein synthesis (Barsano and DeGroot, 1983). The administration of thyroid hormone to hypothyroid rats has been shown to increase their basal metabolic rate

within approximately 2 days and is correlated with increased tissue and mitochondrial oxygen consumption and increased mitochondrial enzyme activities (Barsano and DeGroot, 1983).

Increased synthesis of such proteins as malic enzyme, Na^+K^+ ATPase, and adrenergic receptors has been postulated as the way in which thyroid hormone could increase metabolism through futile cycling of substrates, ion-pumping and modulation of the response to noradrenaline (Samuels, 1983). By its "permissive" effect on the thermogenic action of catecholamines thyroid hormone can have a more immediate effect on metabolism.

Since BAT is a thermogenic tissue, there has been much interest in the effect of thyroid hormone on BAT growth and function, in cold-induced NST and in diet-induced thermogenesis.

Evidence for a role for thyroid hormone in BAT thermogenesis comes from studies of hypothyroid rats, which are reported to have reduced oxygen consumption in response to β-adrenergic stimulation (Fregley *et al.*, 1979). They are unable to survive in the cold (Sellers and You, 1950) and yet if they are provided with a low maintenance dose of T_4 they seemingly thrive in the cold as well as intact animals (Sellers *et al.*, 1974).

BAT of hypothyroid rats appears less responsive to the effects of noradrenaline than intact rats. In young hypothyroid rats BAT lacks the normal lipolytic response to noradrenaline (Hemon *et al.*, 1976). Short term cold does not mobilize lipid in BAT (Mory *et al.*, 1981) or increase blood flow to BAT (Kuroshima *et al.*, 1967) of hypothyroid rats and noradrenaline does not stimulate oxygen consumption in hypothyroid mice

(Ikimoto *et al.*, 1967). Hypertrophy of BAT has been reported in hypothyroid rats (Sellers *et al.*, 1974). Because these animals were housed at 23°C, somewhat below thermoneutral (28°C), increased sympathetic stimulation may have been manifest in a small amount of tissue growth.

Blood T_3 is raised in cold-acclimated rats (Scammell *et al.*, 1980) and the significance of this in causing cold-induced changes in BAT has received considerable attention. Rats chronically injected with T_4 develop an increased capacity to respond to the calorigenic effect of noradrenaline, accompanied by hypertrophy of BAT (Lachance and Page, 1953; LeBlanc and Villemaire, 1970). This enlargement however, is due to increased lipid deposition in the tissue without an increase in tissue protein or oxidative enzymes (Heick *et al.*, 1973; Harri, 1978).

The changes in lipids are dissimilar in cold-adapted and hyperthyroid animals. Thyroid hormone increases triglyceride while cold adaptation increases the phospholipid component (Ricquier *et al.*, 1975). Exogenous thyroid hormone may even inhibit the cold-induced changes in phospholipid (Ricquier *et al.*, 1975). Lipogenesis in BAT is reportedly not stimulated by T_3 treatment (Pillay and Bailey, 1983). However, Gnoni *et al.* (1983) found increased lipogenesis and changes in BAT mitochondria fatty acids induced by thyroid hormone somewhat similar to those induced by cold in T_3 treated rats.

Noradrenaline content and turnover in BAT are reported to be elevated in both cold-adapted and T_4 treated rats but are apparently controlled by different mechanisms in the two situations (Kennedy *et al.*, 1977).

Plasma T_3 is elevated in cafeteria-fed rats (Rothwell and Stock, 1979) the significance of which is unknown but some role in BAT thermogenesis is suggested. Fasting lowers T_3 levels (Vagenakis *et al.*, 1975). T_3 however is restored by refeeding carbohydrate or protein but not by fat (Azizi, 1978; Burger *et al.*, 1980; 1981). Increasing the fat level of the diet actually blunts the increase in T_3 in rats fed carbohydrate (Otten *et al.*, 1980). Similiarly, overfeeding a palatable mixed diet raises T_3 levels but overfeeding a high fat diet does not (Danforth *et al.*, 1979).

1.5.C PITUITARY HORMONES

When rats are exposed to cold, levels of two pituitary hormones, ACTH and TSH are raised in the blood (Usategui *et al.*, 1977; Jobin *et al.*, 1975; Hershman *et al.*, 1970). Chronic treatment with ACTH results in an increased metabolic response to noradrenaline in warm-acclimated rats (Laury and Portet, 1977) and increased basal blood flow and noradrenaline stimulated blood flow and fatty acid utilization in BAT (Laury and Portet, 1980). In cold-adapted ACTH treated rats, these parameters are reduced (Laury and Portet, 1977; 1980). ACTH treatment decreases lipid and increases protein content of BAT in cold-adapted rats only (Laury and Portet, 1980). Hypertrophy of BAT and an increase in all fractions including fat free dry matter is reported after 8 days treatment with ACTH (Lachance and Page, 1953).

A trophic effect of TSH on BAT has been suggested as an explanation of swelling of subclavicular fat pad which occurs in myxedema with high levels of TSH (Doniach, 1975).

The level of growth hormone is reduced in the cold (Eisenberg *et al.*, 1972; Kokka *et al.*, 1972). However injections of this hormone have been reported to stimulate BAT growth (Page *et al.*, 1954).

1.5.D GLUCOCORTICOIDS

Glucocorticoids are essential for maintenance of body temperature of rats during exposure to cold. In adrenalectomized, cold-exposed rats, prevention of heat loss and mobilization of fuel for thermogenesis appear to require glucocorticoids (Deavers and Musacchia, 1979). Pretreatment of hypothermic hamsters with glucocorticoids results in an improved ability to regain normal body temperature as well as maintenance of higher glucose levels (Deavers and Musacchia, 1979). It appears, however that survival in the cold does not require elevated levels of glucocorticoids, since adrenalectomized rats, given small permissive amounts of glucocorticoids, adapt to cold (Heroux, 1955).

Considering the necessity for glucocorticoids in rats living in the cold it may seem contradictory that excessive amounts of these hormones have an inhibitory action on BAT. However cortisone treatment of rats results in increased volume and weight of interscapular BAT with no evidence of cellular hyperplasia (Aronson *et al.*, 1954; Lachance and Page, 1953; Seifter *et al.*, 1951). Lipid vacuoles coalesce and the nucleus is compressed peripherally (Aronson *et al.*, 1954). After extended treatment (70 days) with cortisone, (Sala *et al.*, 1951) interscapular BAT is reduced in amount and resembles white adipose tissue. Injection of cortisone in young rats results in increased weight and fat content of BAT, reduced protein content and noradrenaline content. Noradrenaline

stimulated lipolysis is not altered, but mitochondial enzymes are reduced as well as mitochondrial oxidation of palmitate (Hahn *et al.*, 1969; Skala and Hahn, 1971). No effect on weight gain, food intake or BAT weight, protein, or GDP-binding to BAT mitochondria was noted 7 days (Holt and York, 1982) or 21 days (Marchington *et al.*, 1983) after adrenalectomy in rats. Recent studies have shown that removal of the adrenals improves the thermogenic state of BAT and reduces obesity in the obese Zucker rat (Holt and York, 1982).

Mice treated with corticosterone increase their body weight and food intake, and even when pair-fed to controls they increase energy stores (Galpin *et al.*, 1983). GDP-binding to BAT mitochondria is reduced by corticosterone treatment in both *ad lib* and pair-fed mice, but not in cold-exposed (1 hour, 4°C) mice, suggesting reduced energy expenditure in BAT in response to diet but not to cold (Galpin *et al.*, 1983).

1.5.E PINEAL GLAND

Generally, the environment of animals changes according to the season. In order to thrive many animals are able to adapt their body functions to unfavorable conditions which in temperate regions are mainly low temperature and lack of available food. One of these adaptations is illustrated by the growth of BAT in animals living in the cold in order to more favorably deal with the environment. Other strategies include migration, hibernation, molt of skins and changing reproductive function to assure that the young are born at a time of year which favors their survival. Most seasonal adaptations take time to develop and it would appear that predictive information is available to enable the animal to

synchronize its functions with abrupt changes in temperatures. The changing photoperiod is the most dependable marker of seasonal environmental changes and appears to be widely used to regulate reproductive cycles (Goldman, 1983). In this regard, certain species are considered "photosensitive", they respond to changing light schedules by altering physiological functions. Photosensitive species include the Syrian hamster (*Mesocricetus auratus*), the Djungarian hamster (*Phodopus sungorus sungorus*) and the white footed mouse (*Peromyscus leucopus*).

In the Syrian (Golden) hamster, low temperature causes growth (mass and tissue protein) of BAT and gonadal regression (Himms-Hagen and Gwilliam, 1980; Hoffman *et al.*, 1965). Short photoperiod also causes an increase in BAT mass and gonadal regression (Hoffman *et al.*, 1965; Gaston and Menacker, 1967) which are mediated by the pineal (Reiter, 1975). It is not known whether the pineal gland plays any part in cold-induced growth of BAT. It does not appear to be required for cold-induced gonadal regression in hamsters (Frehn and Liu, 1970). The role of the pineal in BAT growth has been relatively little studied and more is known about its role in control of reproductive activity.

One problem with using daylength as an indicator of season is that spring and fall have the same day length, but one predicts warm temperatures and the other cold. Evidently to solve this problem, many photosensitive species undergo a period of photorefractoriness. For example, in the Syrian hamster, gonadal regression occurs if the daylength is less than 12.5 hours (Gaston and Menacher, 1967). After 4 to 5 months the gonads regain their normal size and activity, and are again photosensitive only after a period of exposure to long days (Reiter,

1974). In this way short days cause the regression of the gonads while the resumption of reproductive activity seems to be determined by an endogenous time measuring system, the nature of which is unknown (Goldman, 1983). The mechanism which allows the animal to measure day length appears to involve the pineal gland.

Of the several products of the pineal, melatonin is considered by many as a likely mediator of photoperiod effects (Goldman, 1983). Melatonin has a trophic effect on BAT of Djungarian hamsters (Heldmaier and Hoffmann, 1974).

Melatonin production is much higher during the dark portion of the light-dark cycle in both nocturnal and diurnal species, in keeping with a role in transmitting photoperiod information. The exact mechanism by which the pineal affects the gonads is not known. Melatonin injections mimic the effects of short photoperiod in causing gonadal regression, only if given late in the day (Reiter *et al.*, 1976). It is unclear whether melatonin transmits photo information via the amplitude or duration of secretion at night, or by the total amount of the hormone produced, or its placement in the light-dark cycle. In addition, changes in sensitivity of target tissues to melatonin may alter its effects (Goldman, 1983).

The pineal makes melatonin from the amino acid tryptophan in response to sympathetic nerve stimulation, which relays photoperiodic information from the eye to the pineal via the retinohypothalamic tract, the suprachiasmatic nucleus of the hypothalamus and the superior cervical ganglion (Klein and Moore, 1979). The lack of light is stimulatory to pineal synthesis and secretion of melatonin. The rate limiting enzyme for the synthesis of melatonin is considered to be serotonin

N-acetyltransferase (NAT), the first of two enzymes responsible for the conversion to melatonin of serotonin, an intermediate in the synthesis of melatonin from tryptophan (Klein *et al.*, 1971).

The suprachiasmatic nucleus (SCN) is considered important in the maintenance of several circadian rhythms (Goldman, 1983). Light acts through the eyes to sychronize the SCN with environmental lighting and to inhibit transmission of signals from the SCN to the pineal. Stimulation of the pineal by the SCN at night serves to increase melatonin production. Lesions of the paraventricular nucleus (PVN) of the hypothalamus have been shown to reduce the night-time rise in pineal NAT activity and urinary 6-hydroxymelatonin, a metabolite of melatonin, and indicate that the SCN neurons responsible for pineal circadian rhythms project into the PVN (Klein *et al.*, 1983).

The number of β-adrenergic receptors on the pineal is lowest at the end of the night or dark period. During the day the number of receptors increases to a maximum just before lights off (Romero *et al.*, 1975; Kebebian *et al.*, 1975). This correlates with the previously observed variation in sensitivity of the pineal to β-adrenergic agonists (Deguchi and Axelrod, 1972; Romero and Axelrod, 1974). The variation in the number of β-receptors is thought to be the result of changing levels of noradrenaline resulting from lower sympathetic activity in the day and higher at night. The large increase in melatonin production at night is thought to be caused by an increase in sympathetic activity acting on the pineal at a time when it is supersensitive to its effects (Romero *et al.*, 1975).

Stimulation of melatonin production in the pineal may be protected from nonsynaptic adrenergic stimulation by neural transport mechanisms. Destruction of nerve terminals by 6-hydroxydopamine (Parfitt and Klein, 1976) and blocking of reuptake by desmethylimipramine (Lynch *et al.*, 1973) causes potentiation of increases in pineal NAT activity. However, exogenous noradrenaline or isoproterenol causes a rise in melatonin levels in the rat (Zatz, 1981). In the hamster melatonin production rose after injection of noradrenaline (Tamarkin *et al.*, 1979) but these results were not confirmed (Lipton *et al.*, 1982). Similarly, noradrenaline injections stimulates increased melatonin production in the pineal of Djungarian hamsters but not in Syrian hamsters (Steinlechner *et al.*, 1984). Propranolol supresses the nightime rise in the three species (Deguchi and Axelrod, 1972; Lipton *et al.*, 1981; Steinlechner *et al.*, 1984) suggesting that melatonin production is regulated by a β-adrenergic mechanism.

Implants of melatonin in the SCN inhibit reproductive function in the white footed mouse (Glass and Lynch, 1982), suggesting this area is involved in mediating the effects of melatonin. However SCN lesions in Syrian hamsters allow testicular regression in response to daily melatonin injections (Bittman *et al.*, 1979). Knife cuts of the anterior hypothalamus prevent regression of testes caused by melatonin in pinealectomized hamsters; this could be interpreted to mean melatonin acts on neurons in the SCN, but does not exclude other sites of action (Reiter *et al.*, 1981). The number of melatonin binding sites in the brains of rats and hamsters is higher at 2000 h than at 0700 h and may be a factor in the diurnal changes in sensitivity to melatonin (Vacas and Cardinali, 1979).

It has been pointed out that good thermoregulators have large, well developed pineal glands and that the pineal may be involved in thermoregulation (Ralph *et al.*, 1979). In white footed mice, melatonin injections cause depression of body temperature and increase in daily torpor (Lynch *et al.*, 1978). In the rat, melatonin appears to cause hyperthermia (Ralph *et al.*, 1979). Hypertrophy and hyperplasia of pinealocytes in rats chronically exposed to low temperatures demonstrate a reaction of the pineal gland to cold (Miline *et al.*, 1970). Pinealocytes of cold-acclimated Syrian hamsters exhibit membranous whorls together with increase in ribosomal density and nuclear polymorphism. These effects were augmented by exposure to a short photoperiod (Bucana *et al.*, 1973). Acute cold exposure caused an increase in granulated vesicles in mouse pinealocytes (Matsushima and Mousawa, 1981). These vesicles are also influenced by environmental lighting. Cold acclimation is affected by photoperiod. Cold tolerance of cold-acclimated and warm-acclimated mice was greater when animals were kept on short photoperiod during acclimation (Ferguson, 1979).

1.6 THE OBESE ZUCKER RAT

Several types of obesity in animals are associated with defective thermogenesis in BAT (Himms-Hagen, 1983b). The genetically obese ob/ob mouse appears to have a defect in BAT which causes a failure of cold-induced nonshivering thermogenesis and diet-induced thermogenesis, contributing to its cold sensitivity and high metabolic efficiency (Himms-Hagen and Desautels, 1978; Hogan and Himms-Hagen, 1980). In the experimentally induced hypothalamic obesity of the VMH lesioned rat

and the GTG mouse, animals lack DIT but cold-induced NST is normal (Hogan *et al.*, 1982; Himms-Hagen *et al.*, 1984; Hogan and Himms-Hagen, 1983). It is thought that the hypothalamic lesion disrupts the transmission of the dietary signal to BAT while the information on cold is normally carried by another route, or has an alternate pathway (Himms-Hagen, 1984). When the work described in this thesis was started, nothing was known about BAT of the genetically obese Zucker "fatty" (fa/fa) rat. One of the objectives was to find out whether BAT in this obese rat was also defective, and, if so, in what way the control of its function was altered.

The obese Zucker rat displays a very early onset obesity which is inherited as an autosomal recessive mutation (Zucker and Zucker, 1961). Obese animals are homozygous for the "fa" gene while heterozygotes (Fa/fa) do not differ in body weight from homozygous normals (Fa/Fa). As females are sterile, young are derived from matings of heterozygotes. The obese genotype is not visually distinguishable until 3-4 weeks, by which time the obesity is already developed, making it difficult to separate primary causal factors from abnormalities resulting from the obese state. Methods for predicting the occurence of obesity in Zucker rat pups have been developed using parameters which discriminate between the lean and obese at an earlier age. These include low oxygen consumption (Kaplan, 1979; Planche *et al.*, 1983), low body temperature (Godbole *et al.*, 1978), high lipoprotein lipase (Gruen *et al.*, 1978) and adipocyte diameter of inguinal fat pad (Boulange *et al.*, 1979).

Although abnormal weight gains are not seen until 3 weeks of age (Zucker and Zucker, 1961), overdevelopment of adipose tissue has been

detected by 1 week (Boulange *et al.*, 1979). Second to the obesity, the most striking characteristic is the extremely high blood lipid levels (Zucker and Zucker, 1961). This is in contrast to other animal models of obesity like the ob/ob mouse and is responsible for the name "fatty" rat (Zucker and Zucker, 1961). All classes of lipids in the blood are elevated, in association with excessive fatty acid synthesis (Lemonnier *et al.*, 1974; Bloxham *et al.*, 1977) and overproduction of phospholipids by the liver (Schonfeld and Pfliger, 1971). Hepatic fatty acid synthesis is not elevated in suckling rats and the elevated synthesis later in life is probably secondary to hyperphagia and hyperinsulinemia (Godbole and York, 1978). However plasma triglycerides are elevated in 2 week pups (Boulange *et al.*, 1981) and phospholipids are elevated from 8 days (Schirardin and Bach, 1981). Lipoprotein lipase activity in white adipose tissue is elevated at 1 week (Boulange *et al.*, 1981; Gruen *et al.*, 1978) while that of skeletal and cardiac muscle and BAT is reduced at 2 weeks, suggesting possible decreased clearance of blood lipids by the latter tissues (Boulange *et al.*, 1981).

Excessive food intake is an obvious cause of obesity, but is not necessary for the development of obesity in the Zucker rat. Obese rats consume more food, but also use dietary energy more efficiently (Deb *et al.*, 1976). Energy requirements for maintenance are normal (Deb *et al.*, 1976). Pair-feeding experiments have shown that although weight gain is decreased, obese rats continue to lay down more fat than controls and remain obese (Zucker, 1967; Cleary *et al.*, 1980). Food intake is elevated at 2 weeks (Bell and Stern, 1977; Stern and Johnson, 1977) but not at 1 week (Godbole *et al.*, 1981; Boulange *et al.*, 1979). At about 10 weeks,

food intake/g body weight is similar to or less than that of lean rats (Dilettuso and Wangsness, 1977). In addition to hyperphagia, obese rats have an altered food intake pattern, with larger meals and loss of the normal pattern of nocturnal feeding (Becker and Grinker, 1977; Bertin *et al.*, 1983).

Hyperinsulinaemia develops just after weaning (Zucker and Antoniades, 1972). However, in preobese suckling pups, glucose stimulated insulin secretion is larger than in lean pups and is normal after atropine treatment. Insulin release in adult animals is elevated more than that of the lean by vagus nerve stimulation and decreased by vagotomy. (Rohner-Jeanrenaud *et al.*, 1983) suggesting at least part of the hyperinsulinaemia is due to the action of the parasympathetic nervous system. Thus, while hyperphagia and hyperinsulinaemia are potentially powerful factors contributing to excess energy storage, it is clear that obesity, in the form of excess deposition of lipids in adipose tissue (Boulange *et al.*, 1979), is manifest in the Zucker rat before food intake or insulin levels are elevated.

The possibility that reduced energy expenditure is responsible for obesity in the Zucker rat has been investigated. One measure of energy expenditure is oxygen consumption. Information in this area can be confusing because of the different ways of expressing results, differences in body size and composition of animals and temperatures at which measurements were taken. Thus the obese adult has a higher whole animal oxygen consumption than its lean counterpart between 5°C and 30°C (Armitage *et al.*, 1984). Rates of oxygen consumption are lower in obese than in lean rats between 10°C and 30°C and similar at 35°C and 40°C

when expressed per g body weight (Kaplan, 1979); they are similar when adjusted to metabolic size, $g^{.7}$ (Bray, 1969) or total body protein (Kaplan, 1981). In obese pups (18-22 days at 25°C (Kaplan, 1979) and 7 days at 33°C and 28°C (Planche *et al.*, 1983)) oxygen consumption is lower than in the lean, revealing that the obese use less energy and that this defect occurs at a very early age, before the hyperphagia and the hyperinsulinemia. Body temperature is maintained at a lower level in the obese at all ages studied (at 16 days (22°C) (Godbole *et al.*; 1978), at 7 days (33°C and 28°C) (Planche *et al.*, 1983) and at 5 months (5°C to 30°C) (Armitage *et al.*, 1984)).

Studies of cold sensitivity of obese Zucker rats are inconsistent. Adult obese rats are reported to survive only 28 hours at 4°C, conditions which do not compromise the survival of lean rats (Trayhurn *et al.*, 1976) and body temperature is reduced in obese rats in the cold (York *et al.*, 1972). However some workers report normal maintenance of body temperatures at temperatures from 5° to 30°C (Armitage *et al.*, 1984) and others find variability within a group of rats (Levin *et al.*, 1980). While oxygen consumption is lower in 7 day obese pups at 33° and 28°C, the difference is greater at the lower temperature, suggesting a failure in heat production when exposed to the relatively colder conditions (Planche *et al.*, 1983). Exposure to cold resulted in a fall in rectal temperature in obese but not lean Zucker rats (Bertin *et al.*, 1983). Acclimation to 10°C appears to occur normally and rectal temperature was not reduced after cold exposure of cold-acclimated rats (Bertin *et al.*, 1983). Plasma free fatty acids do not increase when the animal is exposed to cold, as they do in the lean (York *et al.*, 1972) although epinephrine stimulated lipolysis in

white adipose tissue is normal or above normal (Zucker, 1972; Bray *et al.*, 1970) and during starvation elevated plasma free fatty acid have been observed for 79 days (Zucker, 1972). Obese Zucker rats do not increase their food intake when placed in the cold as well as lean rats (Bray and York, 1972; Armitage *et al.*, 1984; Bertin *et al.*, 1983). Another abnormal response to cold temperatures is seen in the earlier cessation of activity in the obese, as early as 3 days (Hausman *et al.*, 1983).

Abnormalities in noradrenaline levels, turnover and synthesizing enzymes are reported in the brains of obese Zucker rats (Cruce *et al.*, 1976; 1978; Levin and Sullivan, 1979a,b). Adult obese Zuckers have higher basal plasma noradrenaline levels which do not increase as much as in the lean during cold exposure or immobilization stress (Levin *et al.*, 1980). In those obese Zucker rats unable to maintain body temperature in the cold, infusion of noradrenaline did not improve cold tolerance (Levin *et al.*, 1980). A defect in stress-induced plasma noradrenaline levels was attributed to low noradrenaline levels and turnover in several sympatheticly innervated organs (Levin *et al.*, 1981). Hypothalamic stimulation caused normal sympathetic activation suggesting that efferent pathways are functioning normally although these could be modified by other factors (Levin *et al.*, 1984)

The thyroid status of the obese Zucker is variably reported to be normal or reduced. Uptake and turnover rate of radioactive iodine are reduced (Bray and York, 1971). Serum T_4 is reduced or elevated while serum T_3 is reduced or normal and peripheral conversion of T_4 to T_3 may be abnormal (Flynn *et al.*, 1983; Young, R. A. *et al.*, 1984; Durbin-Naltchayan *et al.*, 1983; Autissier *et al.*, 1980).

3,5,3'triiodothyroacetic acid, a metabolite of T_3, decreases body weight in obese only with no change in food intake and no effect on hyperlipidemia in the obese (Autissier *et al.*, 1980). Thyroid powder added to the diet reduced the weight gain of obese Zuckers without changing food intake (Levin *et al.*, 1982). Body fat was reduced in the obese while body protein was reduced in both lean and obese by thyroid hormone treatment. BAT protein was not affected. Rectal temperature was higher in the obese treated than in the lean, and fell less in the cold than the obese. The improved thermogenesis in obese Zucker treated with thyroid hormone appears to be the result of processes in tissues other than BAT (Levin *et al.*, 1983).

It has recently been found that excessive weight gains in the obese Zucker rat can be prevented by adrenalectomy (Yukimura *et al.*, 1978), and although the mechanism is not understood, both food intake and energy efficiency are affected (Yukimura *et al.*, 1978; Marchington *et al.*, 1983). Serum insulin is lowered, fatty acid synthesis in adipose tissue and liver is reduced and food intake is lowered in obese but not lean adrenalectomized rats (York and Godbole, 1979).

1.7 THE MYOPATHIC HAMSTER

The myopathic hamster appears to have a defect in the control of growth of its brown adipose tissue. Smaller than normal deposits of BAT are found in the myopathic hamster (Himms-Hagen and Gwilliam, 1980). This is consistent with the reduced calorigenic response to isoproterenol previously reported by Horwitz and Hanes (1974). Tissue protein and COX is reduced but GDP-binding and the proportion of the 32 000 MW

polypeptide in BAT mitochondria are similar to that of normal hamsters. Growth of BAT in response to low environmental temperature appears relatively normal with the usual increases in tissue protein, COX activity and mitochondrial GDP-binding occurring at 4°C (Himms-Hagen and Gwilliam, 1980).

The myopathic hamster has an inherited muscle disease which affects skeletal and heart muscle. First described in 1962 by Homberger *et al.*, the disease occurred as the result of a spontaneous mutation during the systematic inbreeding of Syrian golden hamsters. It is transmitted by an autosomal recessive gene (Homberger, 1979). The BIO 14.6 line, which is derived from the original mutant line, is the most widely used. Several other lines have been developed and have slightly different characteristics of lifespan, course of the disease, etc. The life expectancy of the BIO 14.6 line is about 12 months.

Histological studies have revealed that the progression of the disease differs in skeletal and heart muscle. In the UM.X 7.1 line early skeletal muscle lesions are visible by light microscopy between 10 and 15 days of age. The progression of the disease reaches its maximum at 120 days and then subsides. Heart lesions are histologically evident at 30 to 40 days. The development of lesions is later and more intense than in skeletal muscle. After 80 days there is healing with extensive scarring. Most animals die of congestive heart failure (Jasmin and Eu, 1979).

The lesions occur in well defined focal areas in the muscle, surrounded by areas of normal tissue. Damaged areas contain enlarged mitochondria, often clumped together and intracellular accumulation of calcium, associated with mitochondria. Muscle fibers in these foci lose

their normal striations and are eventually surrounded and infiltrated by macrophages. The degeneration of the muscle fibers occurs in small and large groups, surrounded by healthy tissue (Mendell *et al.*, 1979).

The nature of the defect which causes these changes is not known. From its beginning, the myopathic hamster appeared to be a useful model for the study of cardiomyopathy in humans (Bajusz *et al.*, 1969). Because of the potential involvement of catecholamines in human heart disease it was of interest to study the effects of these and related drugs on the development of the disease in the myopathic hamster. The severity of the lesions was found to be greatly increased by adrenaline treatment (Bajusz *et al.*, 1969). Adrenergic antagonists and calcium blocking agents have been found effective in reducing the severity of myocardial and to a lesser extent skeletal muscle lesions (Jasmin *et al.*, 1979). Lossnitzer *et al.* (1975) found that isoproterenol treatment greatly increased calcium uptake in muscle of young myopathic animals but not in that of controls.

Not only is the muscle of the myopathic hamster extraordinarily sensitive to the action of catecholamines but there are also indications of increased sympathetic nervous activity in this animal. Urinary noradrenaline is elevated (Kabara *et al.*, 1976) and noradrenaline turnover is reported to be elevated in hearts of myopathic hamsters (Angelakos *et al.*, 1973; Sole *et al.*, 1975; Jasmin and Proschek, 1983). The calcium overload hypothesis was put forward by Wrogemann to explain the muscle degeneration in the myopathic hamster (Wrogemann *et al.*, 1979). This hypothesis states that, for whatever reason, the plasma membrane of the muscle is defective and no longer regulates calcium transport in the normal way and no longer keeps intracellular calcium

levels low. This leads to overloading of intracellular calcium storage depots, in particular mitochondria become so overloaded that they become damaged and cannot supply energy to pump calcium out of the cell. A vicious cycle results in a further rise in calcium levels and death of the cell. This hypothesis points to a primary defect in the plasma membrane of muscle of the myopathic hamster.

There is, however, evidence to suggest that a defect in the microcirculation supplying heart muscle may be the cause of the cardiac lesions described in the myopathic hamster (Factor *et al.*, 1982). Silicone rubber molds of the vessels supplying the heart contain areas which are irregular and constricted in the afflicted animals. Such constrictions, it is argued, caused by vasoactive amines, could be responsible for the focal lesions in myopathic muscle by restricting the blood supply to these areas. Since a restricted blood vessel could cut the blood supply to one area of the muscle whereas a defect in the plasma membrane would be expected to cause a more generalized effect, the microvascular spasm hypothesis can explain the focal nature of the lesions whereas the calcium overload hypothesis can not. Vasoconstrictive effects were prevented by the calcium blocker, verapamil (Factor *et al.*, 1982). Treatment of young myopathic hamsters with the α_1-adrenergic antagonist prazosin resulted in a considerable improvement in cardiac lesions (Factor and Cho, 1983).

CHAPTER 2 PURPOSE OF EXPERIMENTS

To study the control of BAT growth in two disorders, obesity and muscular dystrophy.

A. A reduction in the functioning of the proton conductance pathway was shown in BAT mitochondria of the obese ob/ob mouse (Himms-Hagen and Desautels, 1978). The potential effect of this defect to reduce thermogenesis in BAT was offered as a possible explanation of the extreme cold sensitivity and obesity of these animals. The purpose of this part of the work was to determine whether a defect in BAT function and/or growth is a common feature of mutant obese animals, in particular, the obese Zucker rat.

B. The myopathic hamster has a smaller than normal amount BAT (Himms-Hagen and Gwilliam, 1980). The purpose of this part of the work was to elucidate the reason for the apparent defect in control of BAT growth in the myopathic hamster. Since BAT has been studied most in the rat, it was necessary to carry out comparative studies in the Syrian hamster to establish the environmental conditions and mediators which affect BAT growth and function.

CHAPTER 3 MATERIALS AND METHODS

3.1 MATERIALS

3.1.A ANIMALS

The following conditions apply to the treatment of all experimental animals except where specified otherwise in the methods section of individual experiments.

i. Rats

Male rats were kept at 28°C, in individual hanging metal cages with free access to food (Purina chow 5012) and water and a lighting schedule of 12L:12D. After at least one week under these conditions animals were weighed and randomly assigned to groups. At the end of the experiment animals were decapitated between 0700 and 0800 h.

ii. Hamsters

Male hamsters were kept at 24°C, in individual clear plastic cages with free access to food (Purina chow 5012) and water and a lighting schedule of 14L:10D. After at least one week under these conditions, animals were weighed and randomly assigned to groups. At the end of the experiment animals were decapitated between 0700 and 0800 h.

Handling of hamsters for injections and for killing was facilitated by putting the animals head first into a conical, open ended plastic bag.

3.1.B CHEMICALS

Biochemicals were purchased from Sigma Chemical Co. Common laboratory reagents were from Fisher Scientific Co., J.T.Baker., BDH Chemicals Ltd or Sigma Chemical Co. Chemicals used for gel electrophoresis were from Bio-Rad Laboratories. DHBH (3,4 - dihydroxybenzylamine H Br) and monochloroacetic acid were from Sigma. Alumina, neutral, Brockman Activity I was from Fisher. Sodium octyl sulfate was from Eastman Kodak Co. Chemicals for electron microscopy included absolute ethyl alcohol from the Ontario Liquor Control Board, osmium tetroxide from Electron Microscopy Sciences, styrene from Eastman Kodak Co., Spurr low viscosity embedding kit from Polysciences, Inc., glutaraldehyde 70% from Ladd Research Laboratories and Vestopal W from Mrs. Martin Jaeger, 1222 Vesenaz, Geneva, Switzerland. (-)-adrenaline bitartrate, (-)-noradrenaline bitartrate, (-)-phenylephrine HCl, (-)-isoproterenol bitartrate, yohimbine HCl and α-methyl-p-tyrosine were from Sigma. The following durgs were kindly donated by the companies indicated: Prazosin HCl from Pfizer; phentolamine HCl from Ciba-Geigy; (+)-adrenaline and (+)-noradrenaline from Stirling Winthrop. Somnitol was from MTC Pharmaceuticals, Hamilton. Radiochemicals were purchased from New England Nuclear: [U-^{14}C]sucrose, 3.6mCi/mmol; [8-^{3}H]guanosine 5'-diphosphate, trisodium salt, 6.1-8.9 Ci/mmol; [^{3}H]WB-4101 (2-(2,6-dimethoxyphenoxyethyl) aminomethyl-1, 4-benzodiooxane, [phenoxy-3-3H(N)]-) 25.7 Ci/mmol. NCS tissue solubilizer and PCS liquid scintillation counting cocktail were from Amersham Corporation.

3.1.C APPARATUS

Alzet® osmotic minipumps were from Alza, Palo Alto, California. Silastic® brand medical grade tubing (0.058 in. I.D. by 0.077 in. O.D.) and Silastic® brand medical adhesive, Silicone Type A, was from Dow Corning Corporation, Midland, Michigan. Biophase ODS 5 micron column (15 cm × 4.0 mm I.D.) (MCH-5-N-CAP) was from Bioanalytical Systems, West Lafayette, IN.

3.1.D CAFETERIA DIET

The cafeteria diet consisted of a variety of foods offered each day in addition to chow. A four day rotating menu provided each animal with a sandwich (bread, butter and a meat, cheese or peanut butter filling), a sweet food (chocolate chip cookie, shortcake, marshmallow or sweetened breakfast cereal) and a salty food (potato chips, salted crackers or pretzel).

3.2 METHODS

3.2.A ISOLATION OF MITOCHONDRIA

Interscapular BAT was quickly removed and immediately put into ice cold isolation medium (0.25 M sucrose, 0.2 mM EDTA (free acid) and 1 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), adjusted to pH 7.2 with KOH). The tissue was dissected free of adhering white adipose tissue and muscle, weighed and finely minced with scissors. Homogenization was done in a small volume of isolation mixture using a tightly fitting teflon plunger in a glass homogenizer. The homogenate was

made to a known volume and samples were removed for tissue protein, DNA and cytochrome oxidase (COX) determinations.

Mitochondria were isolated by the method described by Slinde *et al.* (1975) using a Sorval RC-2B or RC-5B refrigerated centrifuge (brake off) and HB-4 rotor. When necessary, homogenates from several animals were combined. The final mitochondrial pellet was resuspended in isolation medium (8-10 mg protein/ml) and immediately used for the GDP-binding assay.

3.2.B GDP-BINDING ASSAY

The specific binding of [H^3]GDP to BAT mitochondria was assayed by the method of Nicholls (1976b), as modified by Desautels *et al.* (1978). Mitochondria were incubated for 1 minute at room temperature in a medium containing 100 mM sucrose, 20 mM N-Tris (hydroxymethyl)-2 aminoethane sulfonate, 10 mM choline chloride, 1 mM EDTA disodium salt, 5 µM rotenone, 100 µM potassium atractyloside and 0.15 µCi/ml [^{14}C]sucrose, pH 7.1. The reaction was started by the addition of [^{3}H]GDP (final concentration 10 µM), rapidly mixed and after 30 seconds, centrifuged for 2 minutes in an Eppendorf microcentrifuge at 12 000 g. The supernatant was removed with suction and the mitochondrial pellet was dissolved in NCS tissue solubilizer by incubation for 3 hours at 55°C. Samples were counted in a Beckman LS6800 liquid scintillation counter in the presence of 0.05 ml 10% ascorbic acid and 10 ml toluene (0.7% PPO). Specific binding of [^{3}H]GDP was calculated as the difference between binding in the presence and absence of 100 µM ADP after correction for

trapped liquid by the amount of [^{14}C]sucrose. Results are expressed as nmoles GDP bound/mg mitochondrial protein.

3.2.C PROTEIN ESTIMATION

Protein was determined by the Lowry method (1951) as modified by Schacterle and Pollack (1973) with bovine serum albumin as the standard. Aliquots of homogenates or mitochondria were precipitated in ice-cold 12.5% trichloracetic acid for 30 minutes and centrifuged at 5 000 rpm in an HB-4 rotor. The pellets were dissolved in 0.5 N NaOH at 55°C.

3.2.D CYTOCHROME OXIDASE ASSAY

Samples of homogenate were activated by homogenizing with Lubrol (0.3 mg/mg protein) in a Teflon glass homogenizer at 2 000 rpm and frozen overnight. Cytochrome oxidase activity was measured polarographically at 37°C using Yellow Springs Instrument YSI Model 52 biological oxygen monitor with a Clark type electrode in a medium containing 0.1 M potassium phosphate buffer pH 6.6, 0.2 mM cytochrome c and 20 mM sodium ascorbate in a volume of 3 ml (Behrens and Himms-Hagen, 1977). The cytochrome c solution was prepared by dissolving 1 g cytochrome c (horse heart) in 100 ml 0.01 M potassium phosphate buffer, pH 7.0 containing ascorbic acid (Wharton and Tzagoloff, 1967). The concentration of reduced cytochrome c was determined by using extinction coefficients reported by Yonetani (1967). The activity is expressed in terms of oxygen consumed as µg atoms oxygen/minute.

3.2.E DNA ESTIMATION

DNA was extracted from aliquots of BAT homogenates by heating for 10 minutes with 5% trichloroacetic acid at 90°C (Burton, 1968). DNA was extimated by the technique of Burton (1968) as modified by Giles and Myers (1965), with calf thymus DNA as the standard.

3.2.F POLYPEPTIDE COMPOSITION

Mitochondrial membranes were prepared by sonicating mitochondria (2 mg protein/ml) on ice (3 x 30 seconds) at maximum intensity using the microprobe of the Bronwell Biosonik III. After centrifugation at 40 000 g for 60 minutes using a Beckman L2-65B ultracentrifuge the pellet was dissolved in 0.01 M sodium phosphate buffer pH 7.4 with 1% SDS and 10 mM phenylmethylsulfonylfluoride and heated at 100°C for 2 minutes.

Polyacrylamide gel electrophoresis was performed with mitochondrial membrane samples using 10% acrylamide separating gel (pH 8.8) and 1.5 mm thick slab gels (Method 1) or an exponential gradient acrylamide separating gel (9-15%, pH 8.35) and a 4.75% stacking gel (pH 6.8) (Method 2). The running buffer contained 0.025 M Tris, 0.192 M glycine and 0.1% sodium dodecyl sulfate (pH 8.5) Gels were run at a constant voltage, 16 hours at 35 V followed by 4 hours at 50 V; stained with 0.25% Coomassie blue R 250 and destained by diffusion. Gels were scanned at 540 nM using a Gilford spectrophotometer 2400-2 linked to a Spectra-Physics SP-4100 computing integrator programmed to calculate the relative areas under the peaks, and the molecular weights of the peaks from a standard curve were prepared on a gel run under the same conditions.

3.2.G ELECTRON MICROSCOPY

i. Mitochondria

Mitochondria were isolated as described in Part A except the final centrifugation and resuspension was done in EDTA-free isolation medium. The mitochondria were fixed according to the procedure of Munn and Blair (1967) by adding 0.4 ml mitochondrial suspension (4-8 mg protein) to 4.6 ml EDTA-free medium plus 2.5 ml ice cold 6% glutaraldehyde in 0.02 M cacodylate buffer pH 7. After 1 hour at 4°C, mitochondria were centrifuged for 1 hour at 5 000 rpm using a Sorval refrigerated centrifuge (RC-2B) and HB-4 rotor. The supernatant was then replaced with 3 ml of 1% osmium tetroxide in 0.02 M cacodylate buffer pH 7. The pellet was cut into small fragments with a finely drawn glass rod and kept at room temperature. After 1 hour, the osmium tetroxide solution was replaced and fragments allowed to stand for another hour. The fragments were then successively dehydrated in 50%, 75%, 95% and 100% ethanol, transferred to styrene and embedded in Vestopal W. Impregnation with Vestopal was continued over 2 days, followed by polymerization at 60°C for 4 days. Detailed procedure for dehydration and embedding is given in Appendix A. Thin sections were cut using a Reichert Ultramicrotome UM-2, mounted on Formvar-carbon coated 300 mesh copper grids and stained with uranyl acetate for 10 minutes followed by lead acetate for an additional 10 minutes. The sections were then washed with distilled water, allowed to dry and viewed at a magnification of 8 000 x with a Siemens 101 electron microscope.

ii. Tissue

Interscapular BAT was cut into thin strips with a razor blade on dental wax and placed in 3 ml of primary fixative solution containing 3% glutaraldehyde and 5% sucrose in 0.02 M cacodylate buffer, pH 7.2. The strips were kept at 4°C for 2 days after which the glutaraldehyde solution was removed and the tissue washed several times with 5% sucrose in 0.02 M cacodylate buffer before being cut into small cubes (1 mm) and subsequently fixed in 3 ml of 1% osmium tetroxide in 0.02 M cacodylate buffer. After 1 hour the secondary fixative solution was replaced and tissue pieces were fixed in osmium tetroxide for an additional hour. Cubes were then washed with distilled water, slowly rotated at room temperature for 2-3 hours in 3 ml saturated uranyl acetate solution in 66% ethanol and dehydrated following a modification of the procedure used for mitochondria (Appendix B). Spurr embedding medium was then added and infiltration continued for 24 hours. Tissue sections were prepared and viewed at a magnification of 8 000 x using a Siemens 101 electron microscope.

3.2.H OXYGEN CONSUMPTION

Oxygen consumption of conscious but quiet hamsters was measured in an open circuit system using a modified Thermox I oxygen analyzer. The animal was restrained in a small plastic cage at 27°C. Oxygen consumption was measured after 0.5 to 1 hour when a constant, low rate of respiration was attained and again after subcutaneous injection of 800 or 1 600 µg NA/kg (dissolved in 0.001N HCl and 0.9% sodium chloride). No difference was found between the response to the two dosages,

assumed to be maximum, occurring approximately 30 minutes after injection.

3.2.1 WB-4101 BINDING

Specific binding of [^{3}H]WB-4101 was measured in homogenates of BAT by an adaptation of the method of U'Prichard *et al.* (1977). Interscapular BAT was removed, placed in ice-cold buffer (50 mM Tris HCl, pH 7.7, 25°C), cleaned and weighed. After chopping with scissors, the tissue was homogenized on ice in 20 volumes of buffer using a Brinkman Polytron (ST-7 micro probe), setting 5 for 3 seconds and filtered through 2 layers of cheesecloth. Final protein concentration was approximately 6 mg protein/ml.

Aliquots of BAT homogenates were incubated with different concentrations of [^{3}H]WB-4101 in the absence (total binding) and in the presence of 10 µM phentolamine (non-specific binding). Specific binding was defined as the difference between total and non-specific binding. A series of 1 ml dilutions of [^{3}H]WB-4101 was made for each preparation. Each assay was done in duplicate. The reaction was started by adding 50 µl labeled compound to 50 µl buffer or phentolamine in buffer, and 50 µl homogenate (made to final volume of 1 ml with buffer) in glass tubes. Tubes were mixed well and incubated with shaking in a water bath at 25°C for 15 minutes. A preliminary experiment showed the binding was maximal by 5 minutes and did not change by 20 minutes. The solutions were then rapidly filtered under vacuum through Whatman GF/C filters followed by 2x10 ml rinse of ice cold buffer. Filters were dried and counted in 5 ml

cocktail of toluene and triton (2:1vol), 0.5% PPO using a Beckman LS 6800 scintillation counter.

The dissociation constant (K_d) and maximal binding capacity (Bmax) for specific [^{3}H]WB-4101 binding were derived from Scatchard plots of the binding data. The slope of the plot ($-1/K_d$) was determined by linear regression analysis and Bmax was obtained as the intersect with the abcissa x the protein content of the homogenate.

3.2.J NORADRENALINE ESTIMATION AND TURNOVER

Noradrenaline content of tissues was estimated either by (i) a radioenzymatic method or by (ii) high pressure liquid chromatography (HPLC).

i. Noradrenaline Estimation Method 1

The tissue was removed, and placed in ice-cold medium (0.25 M sucrose, 0.2 mM EDTA and 1 mM HEPES, pH 7.2). After cleaning on a watchglass over ice, the tissue was blotted dry, immediately frozen on dry ice, weighed and stored at -80°C. Homogenization of the tissue was done in 3 ml of 1.0% perchloric acid (except for BAT of obese rats, which was homogenized in 6 ml) using Brinkmann Polytron (PTA-10 probe, maximum speed, 10 seconds). The homogenate was centrifuged at 5 000 rpm for 40 minutes in a Sorvall RC-2B refrigerated centrifuge (HS-4 rotor). The acid extract was collected after filtering through two layers of glass wool.

Noradrenaline was assayed in the perchloric acid extract by a radioenzymatic method (Depocas and Behrens, 1977). The acid extract

was incubated with S-[^{3}H]-adenosylmethionine and phenylethanolamine N-methyltransferase in the presence of EDTA and dithiothreitol at pH 8.6 and 37°C. The [^{3}H]adrenaline formed was extracted by adsorption on alumina and eluted with perchloric acid. The [^{3}H]adrenaline was then extracted into toluene and the radioactivity was measured by counting in a Beckman liquid scintillation counter.

ii. Noradrenaline Content Method 2

The tissue was removed and placed in ice-cold medium (0.25 M sucrose, 0.2 mM EDTA and 1 mM HEPES, pH 7.2). After cleaning on a watchglass over ice, the tissue was blotted dry, immediately frozen on dry ice, weighed and stored at -80°C.

Homogenization of tissue was done on ice in 4 ml of 1% cold perchloric acid containing 150 ng 3,4 dihydroxybenzylamine HBr (DHBH) as internal standard and using Brinkmann Polytron (PTA-10 probe), maximum speed, 10 seconds. The homogenate was then centrifuged at 10 000 rpm for 30 minutes in a Sorvall RC-2B refrigerated centrifuge (SM 24 rotor). The acid extract was collected after filtering through 2 layers of glass wool. To the acid extract was added sodium bisulfite solution, to final concentration of 0.1%, 1.8 ml 2 M Tris with 5.2% EDTA, pH 8.6 and 100 g alumina to adsorb the NA. Tubes were mixed on a vortex for 10 minutes at low speed and centrifuged at 2 000 rpm in a bench centrifuge for 2 minutes. The alumina was washed three times with glass distilled deionized water, extracted with 0.5 ml 0.2 N perchloric acid to release the NA and centrifuged again in an Eppendorf microcentrifuge 5412 for 15 minutes and the extract frozen at -80°C.

NA content was measured by high pressure liquid chromatography (HPLC) using Varian 5000. The mobile phase was 0.15 M monochloroacetic acid containing 2 mM Na_2EDTA and 60 mg/l sodium octyl sulfate in glass distilled and deionized H_2O. The stationary phase was a reverse phase column (Biophase ODS 5 micron column, 15 cm x 4.0 mm I.D., (MCH-5-N-CAP)). A potential of 0.75 volts was applied between detector (electrochemical detector with glassy carbon electrode) and the $Ag/AgCl_2$ reference electrode. The flow rate was 1.5 ml/minute at room temperature (22°C).

iii. Noradrenaline Turnover

Noradrenaline turnover in BAT and heart was measured by inhibition of NA synthesis by α-methyl-p-tyrosine (MPT) (Brodie *et al.*, 1966). The dose level of 400 µg/kg was chosen for hamsters after trials showed about 30% noradrenaline remained in BAT after 3 hours with no change in body temperature. A preliminary experiment showed that the dose of MPT used did not modify the maintenance of body temperature in either normal or mypoathic hamsters during this time. MTP or saline was injected intraperitoneally and at the designated time animals were killed and the interscapular BAT and heart were quickly removed.

Noradrenaline content of the tissues was measured by the HPLC method (J ii).

The noradrenaline turnover rate (NATR) was then calculated. The rate constant (k) for the decline in NA content of the tissues was calculated from the slope (K) of a plot of the logarithm of NA content versus time, using regression analysis (Costa and Neff, 1970) (rate

constant $k=K_{,} \times 2.303)$. For each experiment the NA turnover rate (NATR) was calculated as the product of the rate constant and the initial NA content. The half life was obtained for each experiment by dividing 0.693 by the rate constant. A correlation coefficient was obtained for each regression line. An average NATR was then calculated from values from 4 individual experiments.

3.2.K STATISTICAL ANALYSIS OF THE RESULTS

Results are expressed as means ± standard error of the mean (SE). Differences between means were tested for significance by using the unpaired Student's t-test.

CHAPTER 4 RESULTS AND DISCUSSION

SECTION A BROWN ADIPOSE TISSUE IN THE OBESE ZUCKER RAT

Overall Objective:

The overall objective of this section was to study the control of thermogenic function and growth of BAT in the rat and to find out whether defective BAT function is associated with the obesity of the obese Zucker rat. More specifically the objectives were:

1. To find out whether cafeteria feeding stimulates BAT thermogenesis and growth in normal rats (Experiment A-1).

2. To find out whether thyroid hormone is involved in cold-induced BAT thermogenesis and growth in normal rats (Experiment A-2).

3. To find out whether any of the pituitary hormones is involved in cold-induced BAT thermogenesis and growth in normal rats (Experiment A-3).

4. To find out whether diet- and cold-induced BAT thermogenesis and growth occur normally in the obese Zucker rat (Experiment A-4).

EXPERIMENT A-1 THE EFFECTS OF CAFETERIA FEEDING ON BAT THERMOGENESIS AND GROWTH

BACKGROUND:

Rats fed a palatable high energy (cafeteria) diet overeat, but gain less than the expected amount of weight (Rothwell and Stock, 1979). Cafeteria-fed rats have a higher resting metabolic rate which is apparently mediated by the sympathetic nervous system, a greater thermogenic response to noradrenaline and increased size of BAT. Thermogenesis in BAT was suggested as a cause of the reduced feed efficiency of the cafeteria-fed rats (Rothwell and Stock, 1979). Feeding is known to cause an elevation, and fasting a reduction, of the sympathetic nervous system in several tissues (Landsberg and Young, 1978).

It was not known if there was an increase in purine nucleotide binding and in the 32 000 MW polypeptide in BAT mitochondria in cafeteria-fed rats similar to changes occurring during cold adaptation (Desautels *et al.*, 1978; Ricquier and Kader, 1976). These changes in cold-adapted rats are believed to be mediated by elevated sympathetic nervous activity (Barnard *et al.*, 1980).

OBJECTIVE:

The objective was to find out whether cafeteria feeding stimulates BAT thermogenesis and growth in normal rats.

METHOD:

Male Holtzman rats (Charles River) were purchased from Canadian

Breeding Laboratories (St. Constant, Quebec) at a weight of 120 g and initially kept in groups in large cages. In Part 1 of the experiment, when rats were approximately 140 g, they were placed in individual wire mesh cages where half were fed a cafeteria diet plus chow for 2 weeks. In Part 2, rats were placed in individual wire mesh cages when they were approximately 190 g. These rats were fed chow for 2 weeks, then half were fed a cafeteria diet for 2 weeks, and then all rats were fed only chow for a further 2 weeks. Groups of rats were killed after 2 weeks (control-2), after 4 weeks (control-4 and cafeteria) and after 6 weeks (control-6 and recovery). This experiment was repeated at a later date and the results were pooled.

The cafeteria diet is described in Methods C. All animals were weighed daily, and the control rats were handled as much as the cafeteria-fed rats because of the known effect of handling on plasma noradrenaline levels (Depocas and Behrens, 1977).

Rats were killed and interscapular BAT was removed, cleaned and weighed. Homogenization of the tissue and isolation of mitochondria were done and tissue protein, COX activity and mitochondrial GDP-binding were estimated (Methods A, C, D, B). In Part 1 of the experiment, tissues were homogenized in 0.25 M sucrose with a Polytron homogenizer (Brinkmann Instruments) (setting 5 for 10 seconds), filtered through a coarse cheesecloth, and made to a volume of 10 ml. Samples for protein estimation were dissolved directly in 0.5 M sodium hydroxide. Tissue protein and DNA were then estimated (Methods C, E). The proportion of 32 000 MW polypeptide in the membrane was measured by both methods of gel electrophoresis described in Methods F. (In this experiment only the

proportion of the total area under each scan was assessed by cutting out two copies of each tracing and weighing the major peaks.)

RESULTS AND DISCUSSION:

In Part 1, 140 g rats were fed a cafeteria diet for 2 weeks. The average weight gain was the same as in control rats; wet weight, total protein, and total DNA of BAT were almost doubled (Figure 1). The concentration of protein in terms of DNA was unaltered.

In Part 2, somewhat older rats (290 g) were fed a cafeteria diet (Table 1). These rats gained more weight (compare groups 2+4 with groups 3+5 in Table 1). There was considerable variation between rats. Whereas in 24 control animals the range of weight gain during the 2 weeks was 55-98 g, in the cafeteria-fed animals the range was 41-132 g. Young rats (140 g) gained no extra weight during 2 weeks of cafeteria feeding (Figure 1), while older rats (290 g) gained a small amount (Table 1). Since this experiment a similar age-related effect of cafeteria feeding on weight gain has been reported by others (Rothwell and Stock, 1982a). During the 2 week recovery from cafeteria feeding, the rats gained less weight than the control animals (Table 1).

During 2 weeks of cafeteria feeding, growth of functional interscapular BAT took place (increase in tissue protein, Table 2) as well as a large increase in the weight of the tissue (Table 2). These findings agree with previous work (Rothwell and Stock, 1979). Hyperplasia, as shown by the increase in tissue DNA content, occurred. Such hyperplasia has since been confirmed by others (Tulp *et al.*, 1982; Bukowiecki *et al.*, 1982). Proliferation of mitochondria occurred, as judged by the increase

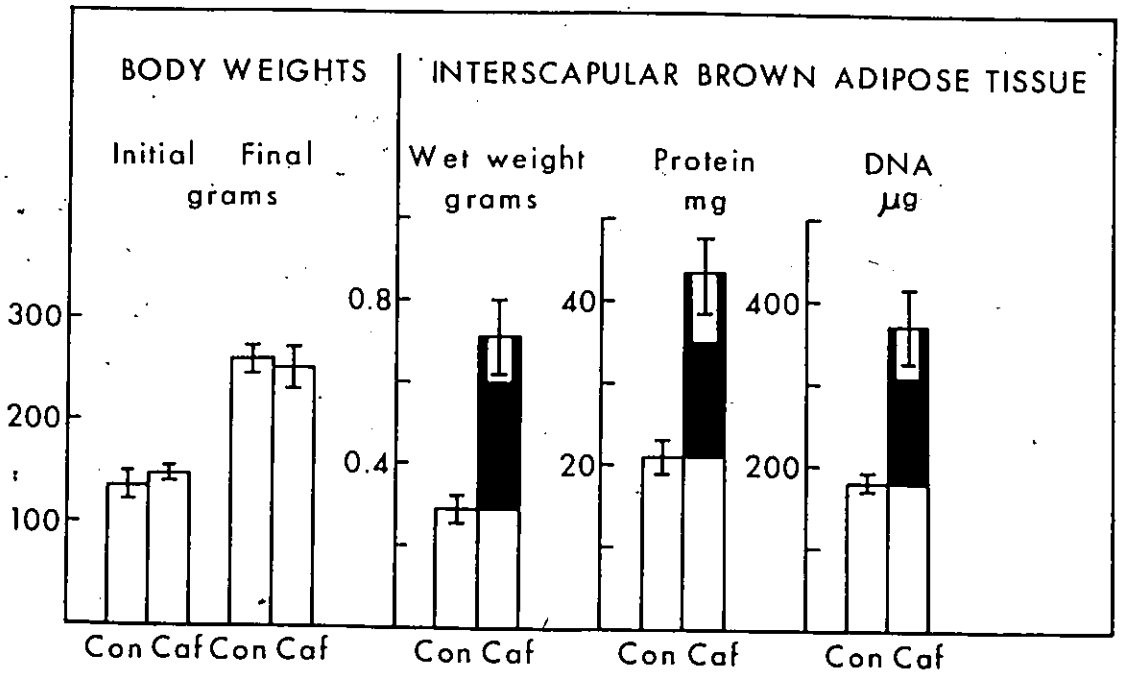

Figure 1. Growth of interscapular brown adipose tissue in cafeteria-fed rats.

Bars represent initial and final body weights and wet weight, total protein, and total DNA of interscapular brown adipose tissue of control (Con) and cafeteria-fed (Caf) rats (n = 4). Black portions of bars denote significant differences ($p < 0.01$) between control and cafeteria-fed animals. Mean gain in body weight was +118.5 ± 8.4 g in control rats and +113.5 ± 9.3 g in cafeteria-fed rats. Concentration of DNA in terms of protein content was 117.4 ± 7.0 mg/g in control rats and 116.4 ± 9.1 mg/g in cafeteria-fed rats.

TABLE 1

Body weights of rats fed a cafeteria diet

	Group	Initial wt, g	Before Cafeteria Diet		After Cafeteria Diet (2 wk)		Recovery from Cafeteria Diet (2 wk)	
			Wt,g	Gain,g	Wt,g	Gain,g	Wt,g	Gain,g
1	Control-2 (6)	189.3 ±5.3	303.2 ±5.0	+113.8 ±3.6				
2	Control-4 (12)	180.3 ±4.7	291.6 ±6.3	+111.3 ±3.3	372.0 ±6.1	+79.8* ±2.8		
3	Cafeteria (12)	184.0 ±6.0	291.8 ±7.1	+107.8 ±3.7	387.2 ±12.2	+95.5* ±6.6		
4	Control-6 (12)	192.7 ±4.5	299.6 ±6.0	+106.9 ±2.9	376.0 ±8.7	+76.4 ±3.9	434.8 ±10.5	+58.8†† ±1.0
5	Recovery (12)	179.8 ±1.1	288.9 ±4.3	+108.9 ±3.5	378.4 ±8.7	+89.7 ±6.1	426.6 ±9.4	+48.2†† ±1.0
2 + 4	Control (24)	186.5 ±3.5	295.6 ±4.3	+109.1 ±2.2	373.7 ±5.2	+78.1† ±2.4		
3 + 5	Cafeteria (24)	181.9 ±3.5	290.3 ±4.1	+108.3 ±2.5	382.8 ±7.4	+92.6† ±4.4		

Values are means ± SE of the number of experiments shown in parentheses.

Statistically significant differences between group 2 and group 3, group 4 and group 5, and groups 2 + 4 and groups 3 + 5 are shown by the similar symbols (*$P < 0.05$; †$P < 0.01$; ††$P < 0.001$).

TABLE 2

Interscapular brown adipose tissue of rats before, after, and after recovery from a cafeteria diet

Group			Wet wt, g	Protein		Cytochrome Oxidase	
				mg	mg/g	μg atoms O_2/min	μg atoms O_2 · min^{-1} · mg protein^{-1}
1	Before	Control-2 (6)	0.363 ±0.024	24.4 ±2.7	68.9 ±9.3	136.6 ±13.9	5.65 ±0.36
2	After	Control-2 (12)	0.547* ±0.023	23.0* ±1.1	42.7 ±2.5	176.4† ±11.8	7.79† ±0.54
3		Cafeteria (12)	1.125* ±0.070	40.9* ±1.9	37.7 ±2.5	244.0† ±16.8	6.03† ±0.36
4	After recovery	Control-6 (12)	0.636 ±0.051	25.2 ±3.0	39.2 ±2.1	161.9 ±15.4	6.62 ±0.47
5		Recovery (12)	0.625 ±0.020	24.8 ±1.9	39.9 ±2.9	175.0 ±9.9	7.23 ±0.34

Values are means ± SE of the number of experiments shown in parentheses.

Values in the same column in groups 2 and 3 with the same symbol are significantly different (*$P < 0.001$; †$P < 0.02$).

in COX activity, but did not quite keep pace with overall tissue growth shown by the slight decrease in activity of this enzyme per unit of homogenate protein (Table 2). Cafeteria feeding caused an almost three fold increase in GDP-binding to mitochondria of BAT (Table 3). During the course of the experiment an increase in GDP-binding in cafeteria-fed rats was also reported by Brooks *et al.* (1980). No change in the proportion of polypeptide in the region 32 000 MW could be detected in either separating system used (Table 3).

The changes seen here were reversible. After 2 weeks of recovery from cafeteria feeding, body weight (Table 1), BAT wet weight, total protein and total COX activity (Table 2) as well as GDP-binding had returned to the level of the controls.

Although food intake was not measured in this experiment, from casual observation intake was considered to be increased. The diet was similar to that used by others, which resulted in hyperphagia and BAT growth (Rothwell and Stock, 1979). Considerable attention was given to the taste preferences of the rats to ensure overeating.

Changes seen as a result of cafeteria feeding resemble those which occur in BAT during cold acclimation in the rat. Tissue protein, DNA, COX activity and GDP-binding increased. However the composition of the mitochondria was not altered, in that there was no increase in the proportion of the 32 000 MW polypeptide, such as is seen in cold acclimation (Desautels *et al.*, 1978). The changes seen here resemble those in the rat treated for 2 weeks with noradrenaline (increase in total protein and COX activity, no change in polypeptide composition of mitochondria) in combination with those due to the acute effect of

TABLE 3

Effect of cafeteria feeding on brown adipose tissue mitochondria

	Group		Binding of GDP, nmol/mg protein	Polypeptide Composition	
				First method, 30,400-35,000 mol wt	Second method 31,680-33,470 mol wt
1	Before	Control-2 (2)	0.029 ±0.0044		
2	After	Control-4 (4)	0.034* ±0.0056	17.90 ±0.96	4.71 ±0.14
				0.41 ±0.53	-0.09 ±0.46
3		Cafeteria (4)	0.099* ±0.0110	18.32 ±0.71	4.62 ±0.32
4	After recovery	Control-6 (4)	0.026 ±0.0038		
5		Cafeteria (4)	0.035 ±0.0062		

Values are means ± SE for the number of mitochondrial preparations shown in parentheses.

Values in the same column in groups 2 and 3 with the same symbol are significantly different ($P < 0.005$).

Values between the rows under Polypeptide Composition are the means of the differences in polypeptide composition in each individual experiment.

noradrenaline (an increase in GDP-binding) (Desautels and Himms-Hagen, 1979). A very recent report has shown that noradrenaline infusion with minipumps produces an increase in GDP-binding as well as a change in the BAT 32 000 MW polypeptide (Mory *et al.*, 1984). It may be that the stimulus caused by cold is stronger than that of either cafeteria feeding or noradrenaline injections causing GDP-binding to be increased much more and causing an increase in the proportion of the 32 000 MW polypeptide, or that these changes are the result of a qualitative difference in the stimulation of BAT by cold, involving the sympathetic nervous system or some other effector.

EXPERIMENT A-2 THE EFFECTS OF THYROID HORMONE ON BAT THERMOGENESIS AND GROWTH

BACKGROUND:

The role of thyroid hormone in the regulation of endothermic thermogenesis is well known (Denckla, 1973). The mechanism of the thermogenic action of thyroid hormone is not completely understood (Himms-Hagen, 1983a). Thyroid hormone appears to be essential for cold-induced thermogenesis since thyroidectomized rats die when exposed to cold (Sellers *et al.*, 1974). Whether it has a role in bringing about the adaptive changes in BAT of cold-acclimated rats is unknown, in particular, the increase in the proportion of the 32 000 MW polypeptide, which was believed not to be mediated by noradrenaline (Desautels and Himms-Hagen, 1979).

BAT of hypothyroid rats appears less responsive than normal rats to the lipogenic and thermogenic effects of noradrenaline (Hémon *et al.*, 1976; Mory *et al.*, 1981; Kuroshima *et al.*, 1967). Since in the rat, BAT is a major source of NST (Foster and Frydman, 1978a,b) which is turned on by noradrenaline released from sympathetic nerve endings, a defect in BAT thermogenesis may explain why these rats cannot survive in the cold.

In addition, blood levels of T_3 are elevated in cold-acclimated animals (Scammell *et al.*, 1980). Although thyroid hormone treatment does not increase tissue oxidative capacity (Heick *et al.*, 1973), elevated levels of thyroid hormone may be responsible for other changes in BAT, in

particular mitochondrial GDP-binding, an index of thermogenic capacity, which may contribute to elevated thermogenesis.

OBJECTIVE:

The objective was to find out whether the thyroid hormone is involved in cold-induced brown adipose tissue thermogenesis and growth in normal rats. This involved studying whether normal changes in BAT occur in response to cold in hypothyroid rats, and secondly to determine whether hyperthyroidism achieved by treatment of rats with thyroid hormone over a period of time would simulate the effects of low environmental temperature on BAT.

METHOD:

Male Holtzman rats were obtained from Canadian Breeding Laboratories (St. Constant, P.Q.) at an initial body weight of 180-200 g. Thyroidectomized (TX) rats were obtained from the same supplier. All rats had access to food (Purina rat chow 5012) and water and were kept on a 12 hours light:12 hours dark schedule at 28°C unless otherwise stated. TX animals were given 0.85% calcium chloride in drinking water.

Since thyroidectomized rats do not survive prolonged cold exposure, some rats were treated with a low maintenance dose of thyroxine (25 µg/kg in peanut oil, subcutaneously, 3 times weekly) known to permit acclimation to cold (Sellers *et al.*, 1974).

a) In the first experiment intact or thyroidectomized (TX) rats were placed in individual wire mesh cages as soon as they were received. Half of each group received the low maintenance dose of thyroxine, the other

half received oil only. After 2 weeks, rats from each group were either placed at 4°C for 15 hours or remained at 28°C.

b) Intact and TX rats were placed in individual cages as soon as they were received. For an initial 2 weeks, 2 groups of TX rats received the low maintenance dose of thyroxine while a third group of TX rats and all intact rats received injections of oil. One group of TX rats receiving thyroxine and one group of intact rats were then placed at 4°C for 2 weeks while the other group of TX rats receiving thyroxine and the TX and intact rats remained at 28°C. Injections continued during this period.

c) A group of normal, intact rats were held for two weeks in individual cages and then received daily injections of a large dose of thyroxine (1 mg/kg in oil subcutaneously) or oil.

At the end of the treatment periods rats were sacrificed and interscapular BAT was quickly removed, cleaned and weighed. The tissue was homogenized and tissue protein, cytochrome oxidase activity, DNA, mitochondrial GDP-binding and polypeptide composition were determined as outlined in Methods C, D, E, B, and F.

RESULTS AND DISCUSSION:

As shown in Tables 4 and 5, thyroidectomized rats were smaller than intact rats. However when pretreated with a low maintenance dose of thyroid hormone, body weights and weight gain were indistinguishable from those of intact rats.

The wet weight of BAT was increased in thyroidectomized rats in the first experiment (Table 4) and not altered in the second (Table 5), although the amount per 100 g body weight was increased in both. The

TABLE 4

Effect of acute cold exposure (15 h) on brown adipose tissue of thyroidectomized rats

	Intact				Thyroidectomized			
	Oil at		25 µg/kg T_4[a] at		Oil at		25 µg/kg T_4[a] at	
	28°C	4°C	28°C	4°C	28°C	4°C	28°C	4°C
Body weights (9)								
g	335.0 ±14.3	324.6 ±12.6	351.1 ±15.2	323.0 ±10.8	244.1* ±7.8	236.6* ±11.7	341.1# ±10.7	320.3# ±12.5
Δ weight, g	89.7 ± 7.7	80.6 ±5.5	106.8 ±4.6	82.3† ±7.2	32.1* ±6.6	13.8*† ±4.9	87.6# ±6.7	67.6#† ±4.4
IBAT weight (9)								
g	0.36 ±0.03	0.33 ±0.1	0.44 ±0.05	0.34 ±0.02	0.48* ±0.04	0.34† ±0.03	0.35# ±0.03	0.33 ±0.02
g/100 g	0.11 ±0.01	0.10 ±0.003	0.12 ±0.01	0.11 ±0.005	0.20* ±0.02	0.14*† ±0.01	0.11# ±0.01	0.10#† ±0.004
Protein (9), mg	24.0 ±1.7	27.6 ±0.9	26.8 ±2.5	25.4 ±3.2	28.0 ±1.9	24.1 ±3.0	26.0 ±2.3	24.1 ±1.7
Cytochrome oxidase (9)								
µg atoms O/min	135.6 ±18.2	136.4 ±15.4	171.2 ±24.9	167.8 ±19.0	142.8 ±21.2	156.5 ±24.1	152.4 ±27.3	132.2 ±19.5
µg atoms O/min per milligram homogenate protein	5.9 ±0.8	4.9 ±0.4	6.6 ±0.8	6.9 ±0.8	5.0 ±0.6	6.6 ±0.7	5.7 ±0.7	5.5 ±0.8

Values are means ± SE for the number of animals indicated in parentheses.

[a] T_4 injected 3 times a week.

*Significant effect of thyroidectomy, comparing animals at the same temperature.

#Significant effect of T_4 treatment, comparing the same type of animal treated with oil only.

†Significant effect of cold exposure, comparing the same type of animal at 28°C.

TABLE 5

Effect of cold acclimation (2 weeks) on brown adipose tissue of thyroidectomized rats

	Intact			Thyroidectomized		
	Oil at		1000 µg/kg[a] T_4 at	Oil	25 µg/kg T_4[b] at	
	28°C (6)	4°C (6)	28°C (4)	28°C (7)	28°C (7)	4°C (6)
Body weights, g						
Initial	187.5 ±14.6	199.5 ±18.3	199.5 ±19.7	163.6 ± 4.9	173.4 ± 5.8	161.3 ± 3.1
End pretreatment	298.8 ±10.8	327.7 ±12.5	324 ±17.6	184 ± 7.3*	276.6 ± 5.9#	262 ± 3.8*
Final	380.5 ±10.5	377.2 ±12.7	316.8 ± 5.9#	210 ± 9.1*	360 ± 9.4#	300.5 ±10.8*,†
Δ weight	81.7 ± 5.2	49.5 ±10.9†	-7.3 ±14.4#	26.3 ± 2.2*	83.4 ± 5.7#	38.5 ± 8.8*
IBAT weight						
g	0.36 ± 0.2	0.71† ± 0.07	0.64# ± 0.04	0.38 ± 0.04	0.37 ± 0.04	0.71 ± 0.04†
g/100 g	0.10 ± 0.01	0.19 ± 0.02†	0.20 ± 0.01#	0.19 ± 0.02*	0.10 ± 0.01#	0.24 ± 0.01*,†
Protein, mg	23.5 ± 2.1	89.4 ±12.4†	30.6 ± 4.3	28.3 ± 2.4	28.1 ± 2.4	89.7 ± 7.2†
Cytochrome oxidase						
µg atoms O/min	65.5 ± 6.8	220.6 ±55.1†	77.6 ±14.9	120.1 ± 8.7*	74.2 ±18.1#	266.7 ±36.3†
µg atoms O/min per milligram homonenate protein	2.95 ± 0.43	2.46 ± 0.49	2.55 ± 0.39	4.42 ± 0.43*	2.62 ± 0.46#	3.03 ± 0.42
Mitochondrial polypeptides 31,200-34,400 (3), % of total	14.09 ± 0.332	18.14 ± 0.147†		14.74 ± 0.3111	15.21 ± 1.05	19.52 ± 0.32†

Values are means ± SE for the number of animals indicated in parentheses.

[a] T_4 injected daily.

[b] T_4 injected 3 times per week.

*Significant effect of thyroidectomy, comparing animals at the same temperature ($p < 0.05$).

#Significant effect of T_4 treatment, comparing the same type of animal treated with oil only ($p < 0.05$).

†Significant effect of cold exposure, comparing the same type of animal at 28°C ($p < 0.05$).

total protein content of BAT was unchanged and the COX content was slightly increased in one experiment but not in the other. Mitochondrial GDP-binding was slightly but significantly increased in thyroidectomized rats (Figure 2). Polypeptide composition was not altered (Table 5). All the above changes were reversed by treatment with a low maintenance dose of thyroxine. Similar treatment of the control group had no effect on any of the parameters measured.

Short term exposure of thyroidectomized animals to cold resulted in a significant reduction in weight gain (measured over the 2 weeks of pretreatment plus 15 hours of cold exposure) and thus it would appear then that without thyroid hormone rats lost weight in the cold. Weight gain in cold-exposed animals was improved somewhat by thyroxine pretreatment (Table 4). BAT weight was reduced to normal by cold treatment. There was a small increase in mitochondrial GDP-binding in TX rats (Figure 2). The increase was much smaller than in intact rats. Treatment of thyroidectomized rats with thyroxine restored the cold-induced increase in GDP-binding to the normally high levels seen in intact rats held in similar conditions (Figure 2). Treatment of intact rats with thyroxine had no effect. Thus there is a defect in the response of BAT to acute cold exposure and this defect is reversed by thyroxine treatment.

Thyroidectomized rats, pretreated with and maintained on a maintenance dose of thyroxine, were able to tolerate the cold for 2 weeks with almost normal weight gain and BAT growth (Table 5). BAT wet weight, protein content and COX activity were the same as in intact rats and all were considerably greater than in rats at 28°C. The increases in

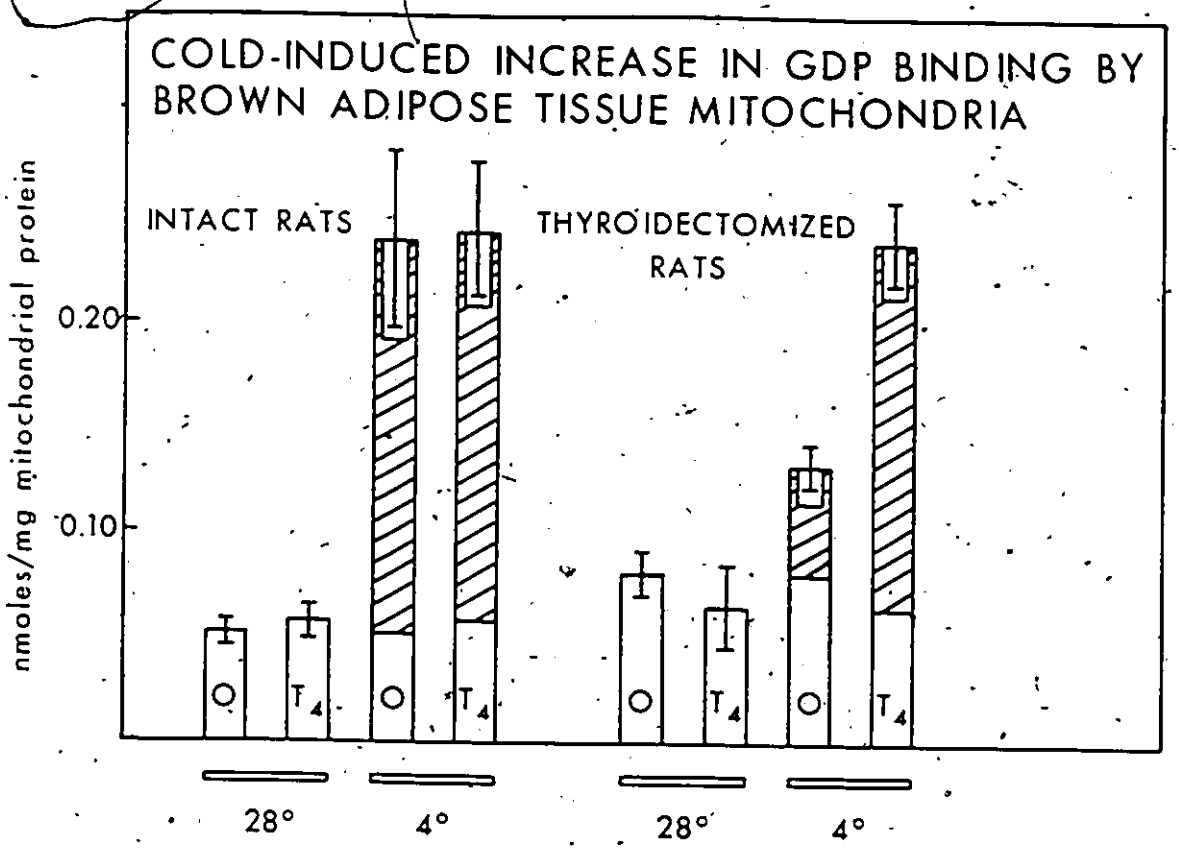

Figure 2. Cold-induced increase in GDP binding by brown adipose tissue mitochondria of intact and thyroidectomized rats.

Bars are the means ± SE of values from three mitochondrial preparations each derived from three rats. O, oil-treated rats. T_4, rats treated with thyroxine in oil. Rats were maintained at 28°C and exposed to either 4 or 28°C for 15 h, as shown beneath the figure. The value for binding in thyroidectomized rats at 28°C (0.089 ± 0.010 nmol/mg protein) is significantly greater than the value for intact rats at 28°C (0.051 ± 0.005, $p < 0.05$). The crosshatched portions of the bars denote significant differences from the corresponding values for the same type of animal at 28°C. Other data for these rats are in Table 4.

GDP-binding and in the proportion of polypeptides of molecular weight range 31 200 - 34 400 were normal (Table 5, Figure 3).

Thyroidectomy had little effect on BAT or on its metabolism at thermoneutral temperatures within the context of this experiment and may not be important in the normal functioning of the tissue. When, however, the animal was challenged by low temperature, a defect was uncovered in the thermogenic capacity of BAT as measured by mitochondrial GDP-binding. Thus, a certain amount of thyroid hormone appears to be essential for stimulated BAT metabolism. A small maintenance dose of thyroxine allowed the normal increase in GDP-binding to occur in the cold, indicating that elevated thyroid hormone levels in plasma of cold-exposed rats is not necessary for the acute thermogenic response of BAT to cold or for the long-term adaptive changes that occur. The increase in GDP-binding during acute cold exposure is thought to be mediated by noradrenaline (Desautels and Himms-Hagen, 1979). The additional requirement for thyroid hormone may be a case of the permissive effect on the actions of catecholamines and may be an important factor in the inability of the hypothyroid rat to survive in the cold.

Thyroid hormone does not seem to be involved in BAT growth. The absence of thyroid hormone had no effect on BAT size at thermoneutrality. Normal growth in the cold, including increased tissue protein, mitochondrial GDP-binding and the proportion of polypeptides in the 32 000 MW region, occurred with a low maintenance dose of thyroid hormone, showing that elevated levels are not required for adaptive growth of BAT in the cold. Whether low levels of thyroid hormone are necessary in this case is not apparent from these experiments, since

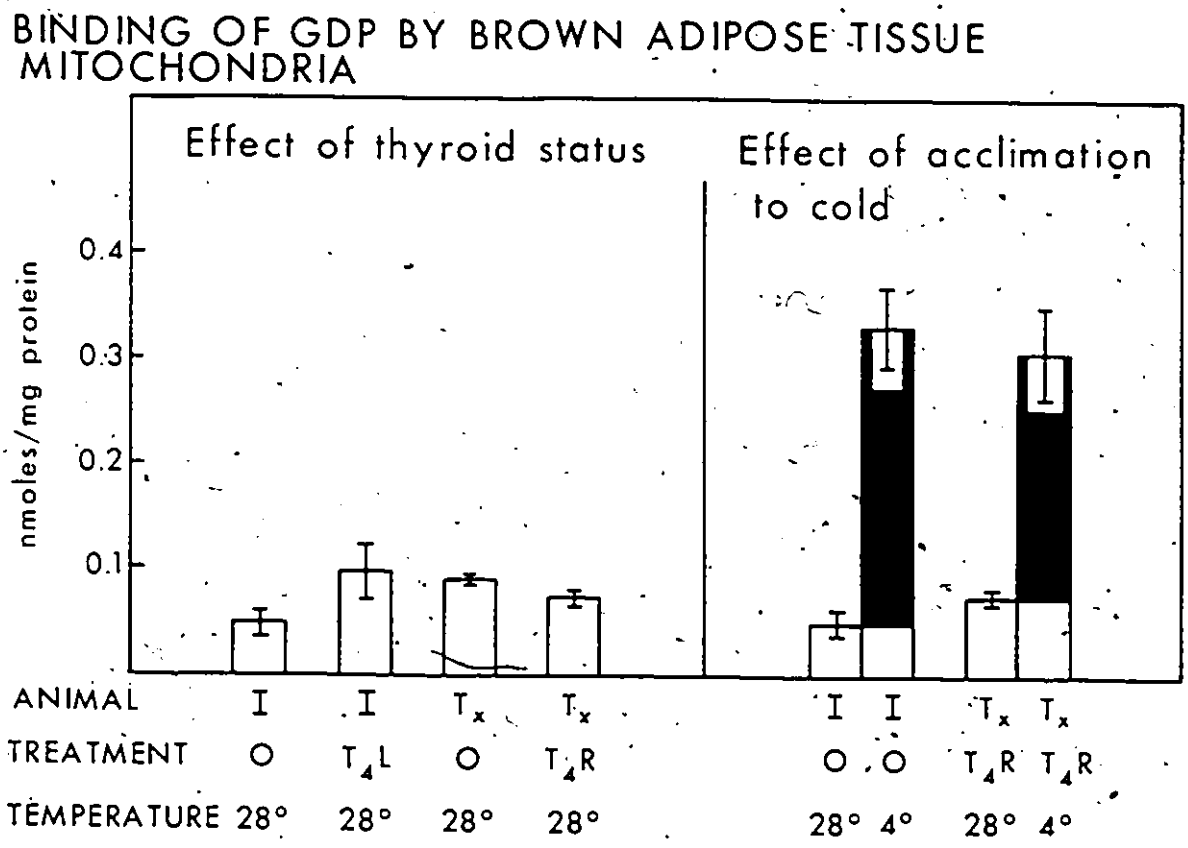

Figure 3. Binding of GDP by brown adipose tissue mitochondria of warm-acclimated and cold-acclimated intact and thyroidectomized rats.

Bars are the means ± SE of values from two mitochondrial preparations. O, oil treated rats. T_4R, rats treated with a replacement dose of thyroxine. T_4L, rats treated with a large dose of thyroxine. I, intact rats. T_x, thyroidectomized rats. The temperature of acclimation (2 weeks) is shown below the figure. The black portions of the bars denote significant differences between rats acclimated to 4°C and rats of the same type acclimated to 28°C. Polypeptide composition and other data for these rats are in Table 5.

without thyroid hormone treatment, rats do not survive prolonged cold exposure.

In the study of the effect of hyperthyroidism on BAT preliminary experiments showed that a daily dose of 50 µg/kg of T_4 for 3 weeks did not increase the capacity of BAT to respond to cold and did not mimic the effects of cold acclimation. Therefore a larger dose (1 mg/kg) was chosen.

Because of high mortality, animals were sacrificed at 10 days (Figure 3) and a second experiment lasting only 7 days was necessary to get adequate results. In both cases treatment of rats with a large dose of thyroxine resulted in a large increase in wet weight with no (Table 5) or slightly increased (Table 6) BAT protein and COX activity, suggesting that tissue lipids were responsible for the increase in tissue weight. DNA was not altered. Mitochondrial GDP-binding was either unchanged (Figure 3) or decreased in thyroxine treated animals (Table 6) and polypeptide composition was not altered (Table 6).

Treatment of rats with a large dose of thyroxine resulted in increased BAT mass due to an increase in tissue lipids as noted by others (Ricquier *et al.*, 1975; Heick *et al.*, 1973; Sundin, 1981; Gnoni *et al.*, 1983; Pillay and Bailey, 1983). One possible explanation for this finding might be increased lipogenesis. T_4 injections have been reported to increase (Gnoni *et al.*, 1983) or not to increase (Pillay and Bailey, 1983) BAT lipogenesis. In hyperthyroid animals, lipolysis in white adipose tissue is elevated and it has been postulated that BAT picks up fatty acids from the blood and increases its fat stores at the expense of white adipose tissue (Pillay and Bailey, 1983).

TABLE 6

Effect of large dose of T_4 (1000 µg/kg)

	Oil, 28°C	T_4, 28°C
Body weights, g		
Initial (12)	285.8 ± 3.4	293.0 ± 4.5
Final (12)	332.2 ± 3.4	306.4 ± 6.4*
Δ weight	+46.3 ± 1.8	+13.4 ± 3.2*
IBAT weight, g (12)	0.42 ± 0.02	0.61 ± 0.02*
Protein, mg (12)	20.7 ± 1.1	25.0 ± 0.7*
DNA, mg (12)	0.408 ± 0.039	0.329 ± 0.020
Cytochrome oxidase		
µg atoms O/min (12)	243.0 ± 11.3	297.4 ± 12*
µg atoms O/min per milligram homogenate protein	12.16 ± 0.73	11.92 ± 0.56
GDP binding (4), nmol/mg protein	0.093 ± 0.017	0.043 ± 0.004*
Polypeptides 30 200-33 200, % of total (4)	11.84 ± 0.50	11.81 ± 0.33

Values are means ± SE for the number of animals indicated in parentheses.

*Significant effect of T4 treatment.

Accumulation of lipid, together with reduced GDP-binding could also reflect reduced lipolysis due to reduced sympathetic activation. At 28°C (thermoneutrality for rats) there should be no cold-induced thermogenesis, but dietary-induced thermogenesis may have been replaced by thyroid-induced thermogenesis. As in this experiment, a reduction in GDP-binding has been observed by Sundin (1981) in hyperthyroid rats at lower temperatures, suggesting a lesser stimulation by endogenous catecholamines. This effect has caused thyroid hormone to be named "the anti brown fat hormone" (Nedergaard and Lindberg, 1982).

Since thyroid hormone treatment only slightly increased BAT protein and COX activity and had no effect on DNA or the 32 000 MW polypeptide, it is unlikely that it is the mediator of adaptive changes in BAT during adaptation to cold. T_3 is elevated in the blood of cafeteria-fed rats and a role in dietary-induced thermogenesis in BAT has been suggested (Rothwell and Stock, 1979). Na^+K^+ATPase activity is elevated in BAT of cafeteria-fed rats and in T_3 treated rats (Rothwell *et al.*, 1982a, 1983b). Mitochondrial GDP-binding, a measure of the active state of BAT, was however not elevated (Rothwell *et al.*, 1983b). The function of the Na^+K^+ATPase is not known but it is thought not to contribute significantly to BAT thermogenesis (Himms-Hagen, 1983a). It would appear that, as in the cold-acclimated animal, elevated T_3 levels are not necessary for the improved function and growth of the tissue resulting from cafeteria feeding.

EXPERIMENT A-3 THE EFFECTS OF HYPOPHYSECTOMY ON BAT THERMOGENESIS AND GROWTH

BACKGROUND:

Levels of pituitary hormones ACTH (Usategui *et al.*, 1977) and TSH (Sellers *et al.*, 1974) are elevated in the blood of rats as a result of cold exposure. Treatment of rats with ACTH is reported to cause an increased weight of BAT (Lachance and Pagé, 1953), but this finding has not been confirmed (Laury and Portet, 1980). It has also been suggested that TSH may have a trophic effect on BAT since myxedema with high TSH levels is sometimes accompanied by swelling of the subclavian fat pad (Doniach, 1975). The level of another hypophyseal hormone, growth hormone, is decreased in blood of rats in the cold but has been found to induce BAT growth (increase in wet weight) when injected into rats (Pagé *et al.*, 1954).

OBJECTIVE:

The objective was to find out whether any of the pituitary hormones is involved in cold-induced brown adipose tissue thermogenesis and growth in normal rats.

METHOD:

Male Holtzman rats, intact, hypophysectomized, or sham-operated and weighing 120-180 g were obtained from Canadian Breeding Laboratories, St. Constant, P.Q.

In the first part of the experiment, intact rats were treated with growth hormone (25 mIU of bovine growth hormone, NIH-GH-B18, 0.81 IU/mg per 100 g subcutaneously, daily for 7 days; the growth hormone was dissolved in 0.1 M glycine buffer (pH 8.4) and was injected 3.5 hours after the start of the light period).

In the second part of the experiment, hypophysectomized (weight range 123-151 g) and sham-operated rats of the same age (weight range 145-186 g) were treated with thyroxine (2.5 µg/100 g) and corticosterone (3 mg/rat), subcutaneously in oil three times per week (Tuesday, Thursday, and Saturday) throughout the experiment. These doses were chosen on the basis of their provision of an amount adequate for survival of thyroidectomized (Sellers *et al.*, 1974; Experiment A-2) or adrenalectomized (Steele, 1975; Maickel *et al.*, 1967) rats. After 6-13 days, on a Monday, Wednesday, or Friday, some rats were placed in individual wire mesh cages at 4°C; on that day an extra injection of thyroxine and corticosterone was given before exposure to cold and rats remained at 4°C for 2 weeks. The rest of the rats remained in individual wire mesh cages at 28°C; these rats also received the extra injection.

Immediately after rats were killed interscapular BAT was removed, cleaned and weighed. Tissue was homogenized, and protein, COX activity and DNA were estimated (Methods C, D, E). Mitochondria were isolated, GDP-binding was measured, and polypeptide composition was estimated (Methods A, B, F.2).

RESULTS AND DISCUSSION:

Chronic treatment of rats with growth hormone, at a dose shown by

others to promote growth in hypophysectomized rats (Cotes *et al.*, 1980), had no effect on body weight gain, BAT size (protein, DNA), mitochondrial content (COX activity) or thermogenic activity (GDP-binding of BAT mitochondria) (Table 7).

Hypophysectomized rats, although maintained on thyroxine and corticosterone, lost weight (Table 8). The weight loss was greater in the initial period; after they were put in the cold body weight was relatively stable and there was no difference between weight gain at 28°C or 4°C. Sham-operated animals showed the usual reduction of weight gain in the cold (Table 8). In keeping with their small size, the BAT of hypophysectomized rats was smaller than that of intact rats (Table 8). When expressed in terms of body weight BAT of hypophysectomized rats was larger than that of sham-operated controls (Figure 4). BAT cells were smaller in hypophysectomized rats as indicated by reduced protein and unchanged wet weight and COX activity (per mg DNA) (Figure 5). GDP-binding to BAT mitochondria was slightly higher in hypophysectomized rats but mitochondrial polypeptide composition was normal (Figure 6), indicating some activation of BAT thermogenesis in these animals.

Acclimation of hypophysectomized rats to cold resulted in growth of BAT (Table 8 and Figure 4), which, in proportion to body weight, was similar to that seen in sham-operated controls. Loss of lipid in the cold was indicated by a reduction in wet weight with no change in protein content (Figure 5). A large increase in BAT mitochondrial GDP-binding occurred in both hypophysectomized rats and in sham-operated control rats and an increase in the relative proportion of mitochondrial

TABLE 7

Effect of treatment with growth hormone on brown adipose tissue and its mitochondria

	Control	Growth hormone
Body weights, g	(12)	(16)
Initial weight	235.6 ± 6.7	246.5 ± 3.7
Final weight	285.8 ± 5.7	298.9 ± 4.0
Change in weight	+50.3 ± 2.3	+52.4 ± 2.1
Brown adipose tissue	(12)	(16)
Wet weight		
mg	322 ± 11	341 ± 12
mg/mg DNA	544 ± 47	591 ± 33
Protein		
mg	16.2 ± 0.7	14.6 ± 0.6
mg/mg DNA	27.1 ± 2.0	25.0 ± 1.0
Cytochrome oxidase		
µg atom O/min	85.3 ± 5.6	80.2 ± 5.0
µg atom O/min milligram DNA	143.4 ± 15.0	136.1 ± 7.9
DNA, µg	623 ± 48.8	597 ± 27.5
Brown adipose tissue mitochondria	(4)	(4)
GDP binding, nmol/mg protein	0.053 ± 0.013	0.039 ± 0.003

Values are means ± SE of the number of experiments shown in parentheses.

There are no significant differences between values for growth hormone - treated rats and values for control rats.

TABLE 8

Effect of acclimation to cold on brown adipose tissue of hypophysectomized or sham-operated rats maintained on thyroxine and corticosterone

	Temperature of acclimation			
	Sham-operated rats		Hypophysectomized rats	
	28°C (12)	4°C (13)	28°C (15)	4°C (11)
Body weights, g				
(a) Initial (arrival)	153.7 ± 9.8	160.7 ± 1.0	135.0 ± 2.5*	134.9 ± 2.2*
(b) Initial (put at 4°C or at 28°C	230.3 ± 7.2	220.5 ± 6.2	123.2 ± 2.3*	121.3 ± 2.3*
(c) Final	321.2 ± 5.3	269.8 ± 7.0†	120.0 ± 3.4*	119.7 ± 2.0*
Weight change, g				
b - a	+64.5 ± 7.3	+59.6 ± 6.7	-11.7 ± 2.5*	-13.6 ± 2.3*
c - b	+91.0 ± 4.9	+49.2 ± 5.2†	-3.2 ± 2.4*	-1.5 ± 1.9*
Brown adipose tissue				
Wet weight, mg	518 ± 35	709 ± 26†	392 ± 27*	464 ± 13*†
Protein, mg	21.4 ± 1.5	60.7 ± 1.6†	13.8 ± 0.8*	30.6 ± 1.9*†
DNA, µg	546.7 ± 20.6	1513.3 ± 82.7†	445.0 ± 31.3*	960.5 ± 60.5*†
Cytochrome oxidase				
µg atom O/min	148.4 ± 25.4	287.4 ± 25.7†	92.5 ± 6.0*	138.5 ± 12.2*†

*Significant effect of hypophysectomy, comparing rats at same temperature.

†Significant effect of acclimation to cold, comparing same type of rat.

Values are means ± SE of the number of experiments shown in parentheses.

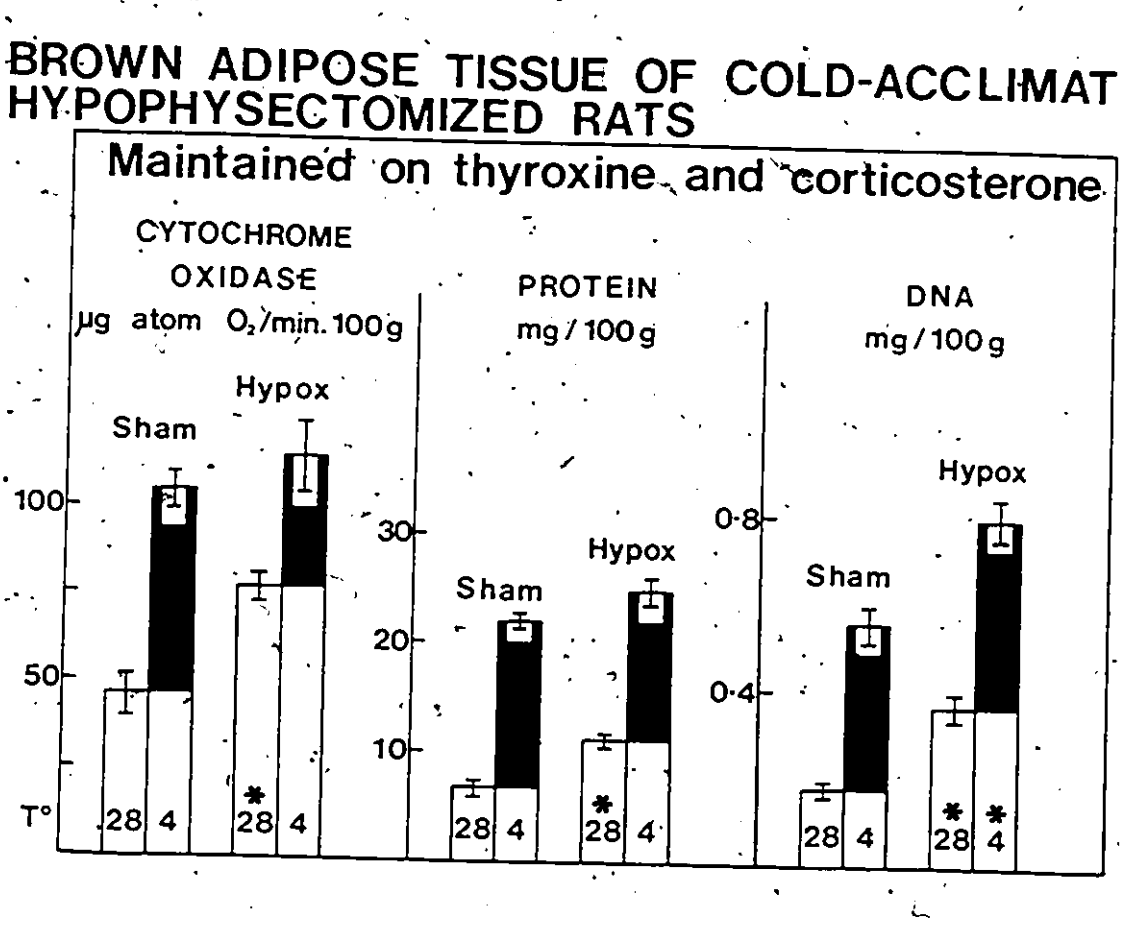

Figure 4. Brown adipose tissue of hypophysectomized rats and sham-operated rats, expressed in terms of body weight.

Acclimation temperatures were 28 or 4°C as shown in the figure. Bars are the means ± SE for the numbers of animals shown in Table 2. Black portions of the bars denote significant differences between cold and 28°C acclimated rats of the same type. Asterisks denote significant differences between hypophysectomized rats and sham-operated control rats at the same temperature of acclimation. Maintained on thyroxine and corticosterone.

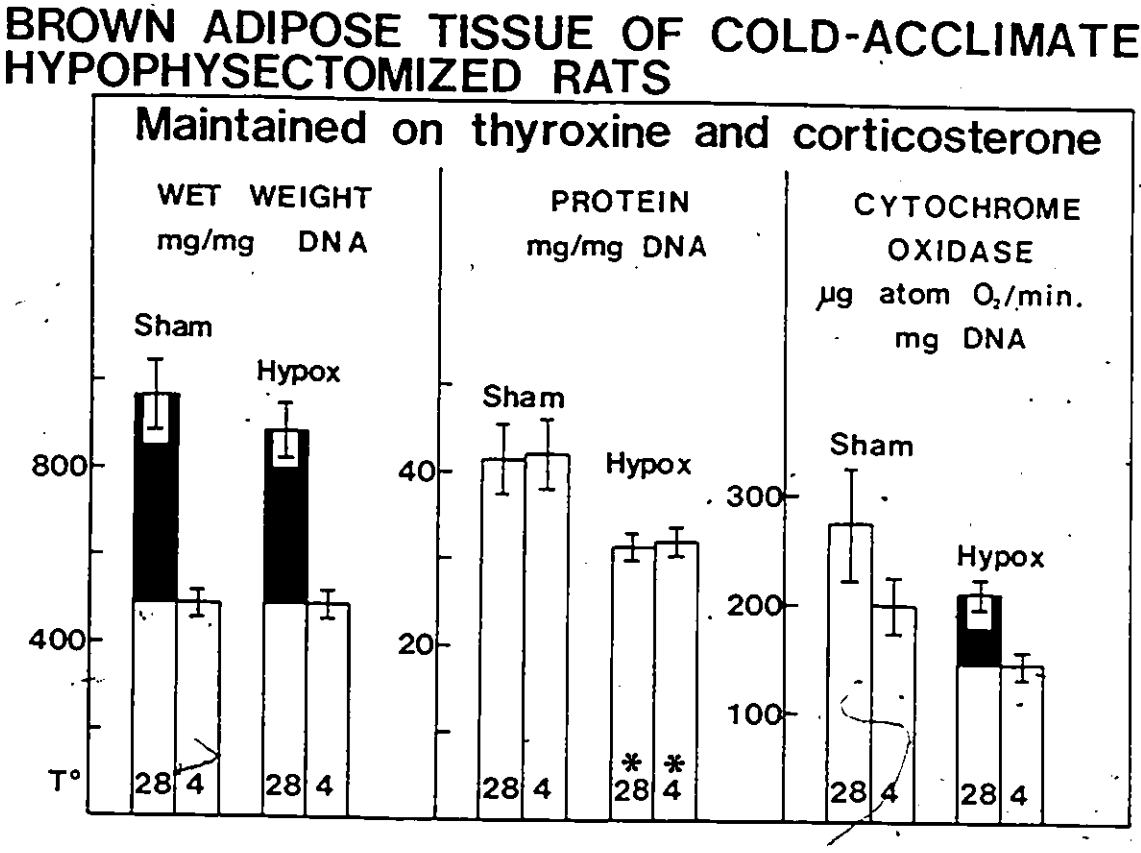

Figure 5. Amount of wet weight, protein and cytochrome oxidase per cell of BAT of hypophysectomized and sham-operated control rats.

For further information see Legend to Figure 4.

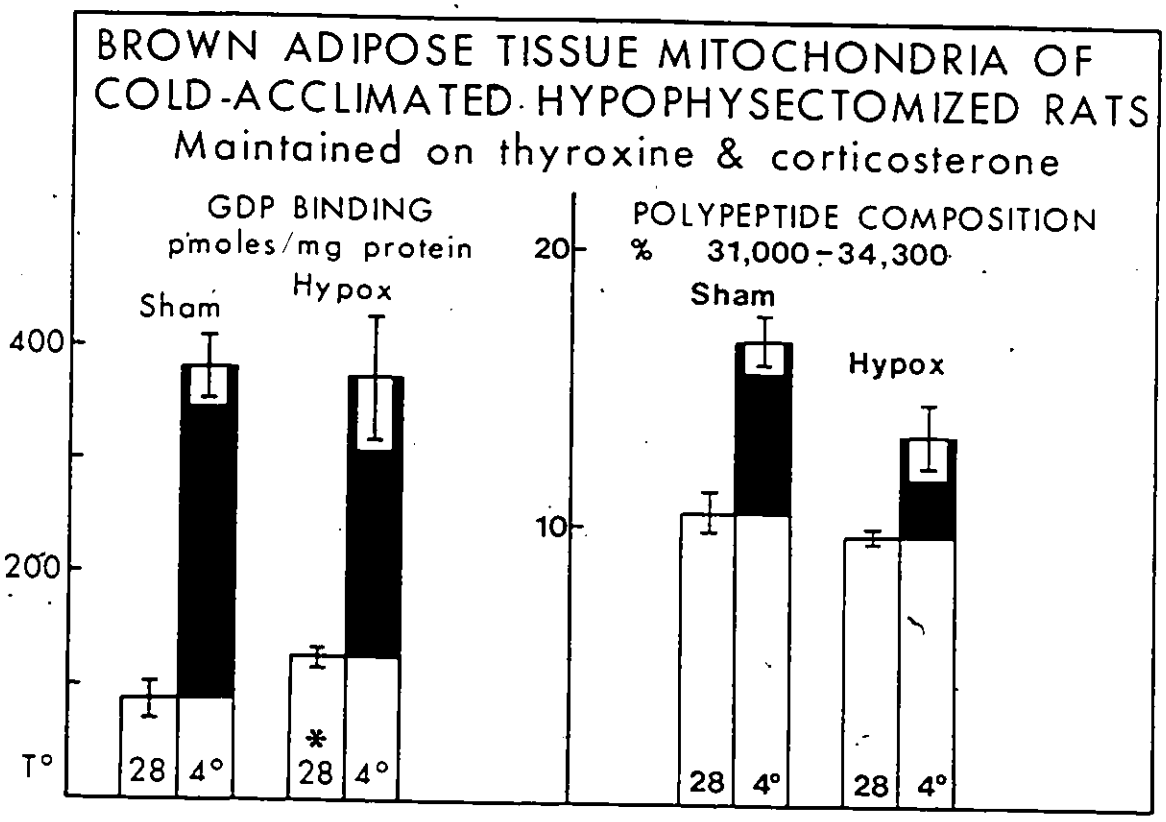

Figure 6. Binding of GDP and polypeptide composition of BAT mitochondria of hypophysectomized and sham-operated control rats.

Values are the means ± SE for four mitochondrial preparations derived from the number of animals shown in Table 8. For further information see legend to Figure 4.

polypeptides in a molecular weight range of approximately 32 000 also occurred in both groups of animals (Figure 6).

Pituitary hormones then are not necessary for growth of BAT in the cold and for the normal increases in mitochondrial GDP-binding and 32 000 MW polypeptide.

Pituitary hormones are essential for survival of rats in the cold. This is probably because they stimulate thyroid hormone and glucocorticoid secretion. Rats without thyroid hormone (Sellers *et al.*, 1974) or glucocorticoids (Maickel *et al.*, 1967; Heroux, 1955) do not survive in the cold and hypophysectomy is known to reduce the thermogenic response of rats to cold in a way that is reversed by administration of thyroxine plus corticosterone (Denckla, 1973). The thermogenic effect of noradrenaline is absent in hypophysectomized rats (Muller *et al.*, 1975) and in BAT cells isolated from hypophysectomized rats (Reed and Fain, 1970). In the present experiment, hypophysectomized rats treated with thyroxine and corticosterone were able to survive in the cold and to adapt by growing more BAT.

The reason for the larger amount of BAT (per unit body weight) in hypophysectomized rats is not clear; these animals were noticeably leaner than the controls and expression of results in terms of lean body mass may have been more appropriate. The animals may also have been cold stressed, even at 28°C, causing growth of BAT and the increase in GDP-binding which also occurred to a slight extent. A relatively large amount of BAT (protein, DNA) has since been reported in hypophysectomized rats receiving no hormone treatment (Laury *et al.*, 1984). These animals had a reduced body temperature and above normal

increases in BAT protein, DNA and phospholipids occurred during acclimatation to 15°C. Rats did not survive temperatures below 15°C without supplemental hormone treatment (Laury *et al.*, 1984). This would suggest that thyroxine and corticosterone are not required for acclimation at 15°C or, more likely, that hormone production without stimulation from the pituitary is adequate for acclimation to 15°C but not for temperatures below this. It is not known if corticosterone is required for BAT activation, as is thyroid hormone (Experiment A-2), or whether it is required for some other aspect of cold acclimation. Some action on BAT is suggested by the presence of glucocorticoid receptors (Feldman, 1978). If glucocorticoids are required for activation of BAT in the cold, then permissive amounts are adequate to allow this to occur.

EXPERIMENT A-4 THE EFFECTS OF COLD AND CAFETERIA DIET ON BAT GROWTH AND THERMOGENIC STATE IN THE OBESE ZUCKER RAT

BACKGROUND:

Defective BAT thermogenesis has been associated with obesity in two models of inherited obesity in mice. In the obese ob/ob mouse (Himms-Hagen and Desautels, 1978; Hogan and Himms-Hagen, 1980; Himms-Hagen, 1983b) there is a failure of both cold- and diet-induced thermogenesis in BAT thought to be due to a reduced capacity of the tissue to respond to endogenous noradrenaline. The diabetic db/db has a low thermic response to noradrenaline (Trayhurn, 1979a). Reduced energy expenditure due to a failure in BAT thermogenesis appears to contribute to the increased metabolic efficiency and the resulting obesity in these animals (Himms-Hagen, 1983b).

OBJECTIVE:

The objective was to find out whether diet- and cold-induced BAT thermogenesis and growth occur normally in the genetically obese Zucker rat.

During the course of this experiment it was reported that sympathetic innervation was drastically reduced in BAT of 6-8 month old obese Zucker rats (Levin *et al.*, 1981). To check the sympathetic innervation of the tissue, noradrenaline content of BAT was measured in cafeteria-fed and cold-exposed Zucker rats.

METHOD:

Zucker (fa/fa) rats (male or female as specified in results) and their lean controls (Fa/Fa or Fa/fa) were purchased from the Harriet Bird Memorial Laboratory, Stow, MA or from Dr. P. R. Johnson, Dept. of Biology, Vassar College, Poughkeepsie, NY. The cafeteria-fed animals were kept in individual plastic cages. Some obese and lean rats were exposed to 4°C for 24 hours. Some obese and lean rats were fed a cafeteria diet for 14-24 days.

After sacrifice interscapular BAT was removed, cleaned and weighed. Because of the large amount of lipid in the homogenates and its unusually high melting point, preparation of homogenates on ice was not possible. As a result, in this experiment all tissues were homogenized at 37°C to keep lipids fluid. Separate experiments demonstrated that this procedure did not alter the mitochondrial properties measured. Mitochondria were isolated and tissue protein, COX activity, DNA and mitochondrial GDP-binding were estimated (Methods A, C, D, E, B). Polyacrylamide gel electrophoresis of mitochondrial membrane samples was done (Methods F.2).

BAT from lean and obese animals which had been cold-exposed, cafeteria-fed, or chow-fed was removed and the noradrenaline content measured (Methods J.i).

RESULTS AND DISCUSSION:

Preliminary experiments with old (3-5 months) fatty rats showed that BAT had a markedly elevated wet weight, but a relatively normal protein content (Table 9). Tissue protein tended to be lower in the older obese

TABLE 9

Preliminary experiments with brown adipose tissue of lean and fatty Zucker rats

Male rats aged 10-12 weeks	Lean (6)	Fatty (6)
	28°C	
Body weight, g	282.2 ± 6.8	395.3* ± 20.0
BAT		
Wet weight, mg	374 ±30	1,676* ±120
Protein, mg	17.8 ± 2.2	25.5 ± 3.6
BAT mitochondria		
No. of preparations	3	3
GDP binding, nmol/mg protein	0.0675 ± 0.0189	0.0036* ± 0.0036

Male rats aged 19-21 weeks	Lean (4)		Fatty (4)	
	28°C	4°C	28°C	4°C
Body weight, g	433.8 ± 9.2	439.5 ±17.2	651.4* ±13.8	563.8*† ±30.6
BAT				
Wet weight, mg	602 ±70	751 ±200	2,549* ±210	2,864* ±200
Protein, mg	23.8 ± 2.2	28.1 ±1.6	17.9 ±2.6	17.0* ±2.0
BAT mitochondria				
No. of preparations	1	1	1	1
GDP binding	0.049	0.211	0	0

Values are means ± SE, with numbers of rats given in parentheses.

Rats were housed at 28°C or exposed to 4°C for 6 h.

*Significant effect of obesity comparing animals treated in the same way.

†Significant effect of cold exposure, comparing the same type of rat.

rats, but was significantly so only in the cold-exposed group (Table 9). Binding of purine nucleotides was extremely low or, in some experiments, undetectable in obese rats and the normal cold-induced increase in binding did not occur. The usual cold-induced hyperemia of BAT was also not observable in the cold-exposed obese rat. Because of the technical difficulties in isolating mitochondria from tissues with such a large amount of lipid, further experiments were done with younger rats.

In the first experiment to study the effect of cold exposure, 7-8 week old obese and lean Zucker rats were used. The body weights of obese rats at this age were only about 40% above those of lean rats, but they were markedly obese, as shown by the four to five fold increase in weight of their gonadal white adipose tissue (Figure 7). BAT wet weight was also markedly increased (Figure 7). Despite the elevated wet weight, the protein and COX activity of BAT of the obese rats was normal, but a smaller amount of DNA was present (Figure 8) indicating a cellular hypertrophy due to a small increase in mitochondrial and other proteins and a large accumulation of lipid (Figures 7 and 8). Exposure to cold produced hyperemia of the tissue in both lean and obese animals and a decrease in wet weight in the obese only (Figure 7). Protein and DNA content increased in cold-exposed animals of both genotypes, without a change in COX activity (Figure 8).

Activation of BAT thermogenesis by cold, as indicated by an acute increase in mitochondrial GDP-binding, occurred normally in obese rats (Figure 9). The polypeptide composition of polypeptides with a molecular weight near 32 000, was normal in the obese rats (Figure 9). The effect of cold exposure on polypeptide composition was not studied because

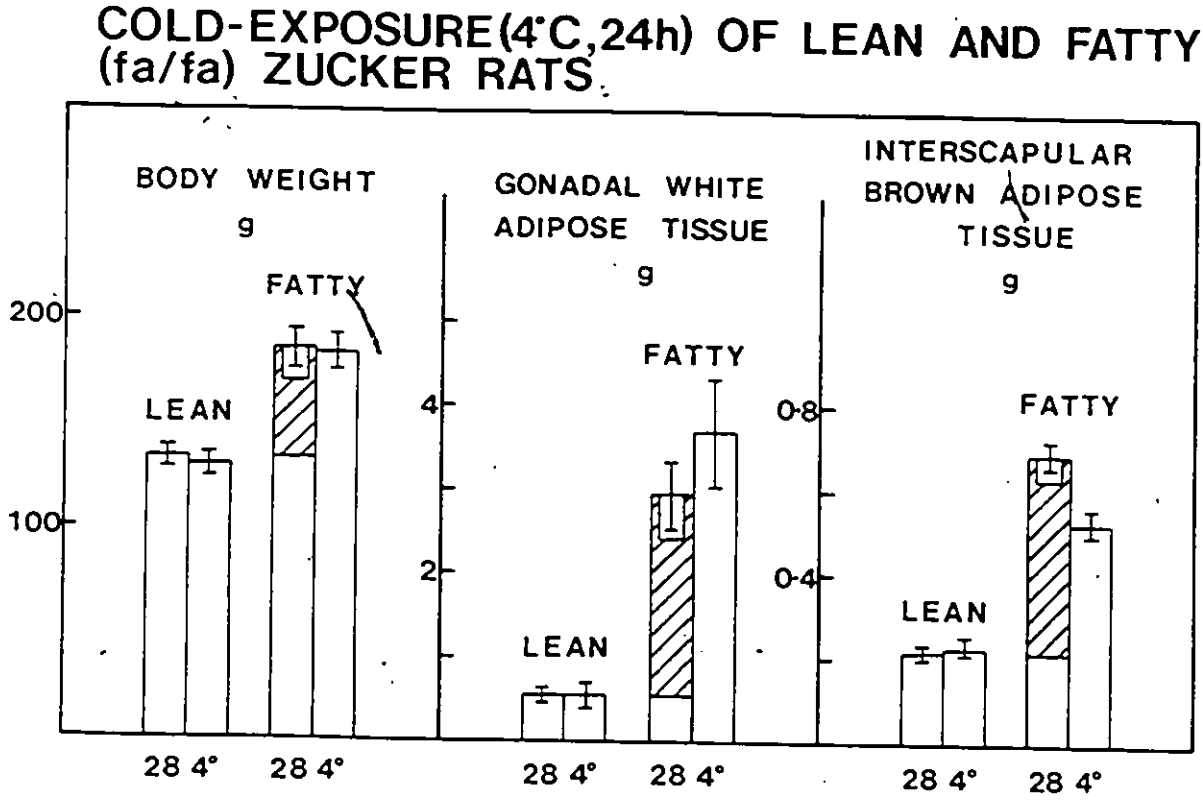

Figure 7. Effect of cold-exposure (4°C for 24 h) on body and organ weights of lean and fatty female Zucker rats.

Bars are means ± SE of 24 (body weight, interscapular brown adipose tissue weight) or 12 (gonadal white adipose tissue weight) observations. Body, gonadal white adipose tissue, and interscapular brown adipose tissue weights are all significantly greater in fatty rats at 28°C than in lean rats at 28°C (denoted by cross-hatched portions of bars). Exposure to cold had a significant effect only on brown adipose tissue weight.

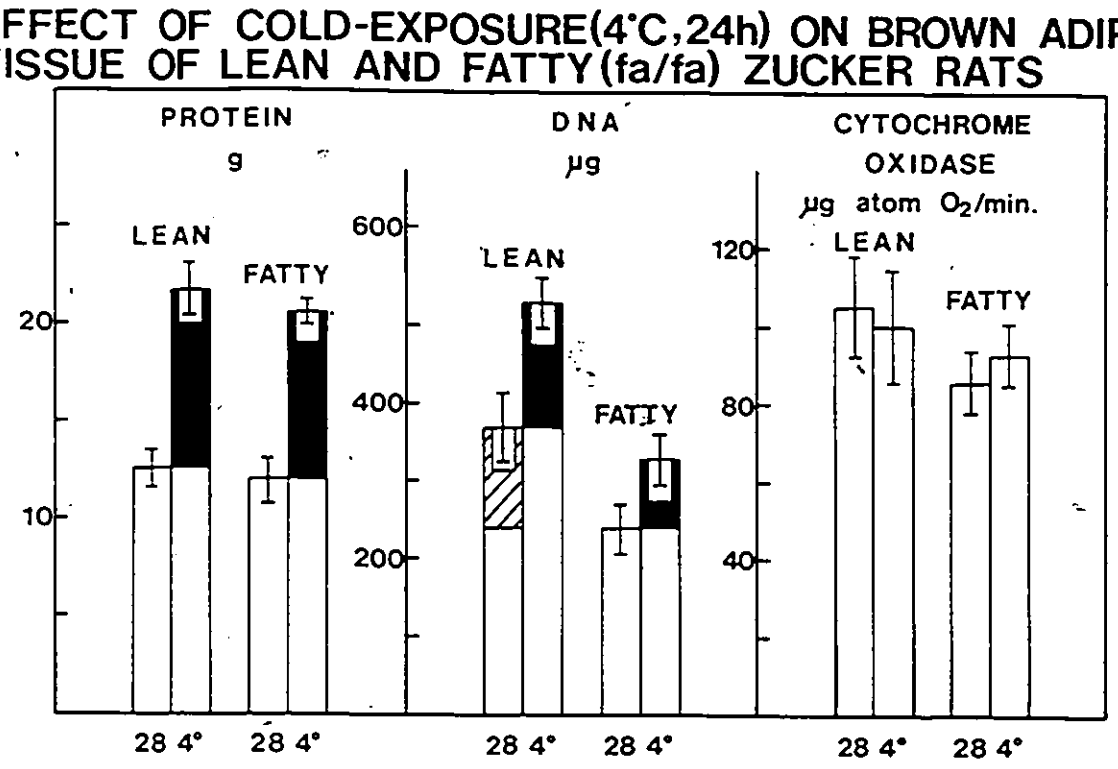

Figure 8. Effect of cold-exposure (4°C for 24 h) on brown adipose tissue of lean and fatty Zucker rats.

Bars are means ± SE of 24 (protein, cytochrome oxidase) or 12 (DNA) observations. Protein and cytochrome oxidase contents are not significantly different between lean and fatty rats at 28°C. DNA content is significantly lower in fatty rats at 28°C than in lean rats at 28°C (denoted by cross-hatched portion of bar). Exposure to cold caused significant increases in protein and DNA contents in both lean and fatty rats (denoted by solid portions of the bars).

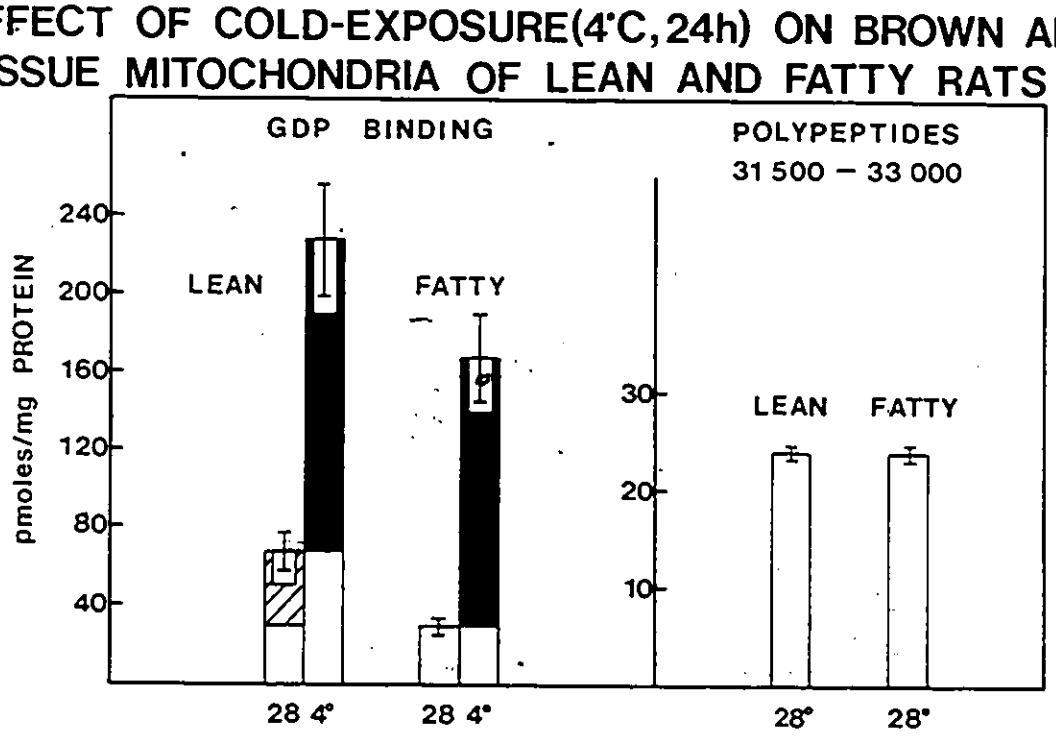

Figure 9. Effect of cold-exposure (4°C for 24 h) on BAT mitochondria of lean and fatty Zucker rats.

Values are means ± SE for 7 or 8 mitochondrial preparations. Binding of GDP is significantly lower in fatty rats at 28°C (denoted by cross-hatched portion of bar). Exposure to cold caused a significant increase in GDP binding in both lean and fatty rats (denoted by solid portions of bars). Means of increases in each experiment were +0.160 ± 0.027 nmol/mg protein in lean rats and +0.146 ± 0.021 in fatty rats (NS). Values for polypeptides of molecular weight range 31,500-33,000 are means ± SE for 4 mitochondrial preparations.

previous work showed this to be without effect during brief cold exposure (Desautels *et al.*, 1978). The binding of GDP by BAT mitochondria of obese rats was low (Figure 9) despite the normal polypeptide composition, suggesting a reduced thermogenic activation of the tissue in these animals living at thermoneutrality, possibly by diet.

In the second experiment obese and lean Zucker rats (6 to 8 weeks old at the end of the experiment) were fed either chow or a cafeteria diet plus chow. The body weight gain was greater in obese rats and was not altered by cafeteria feeding in either lean or obese (Figure 10). Body fat accumulation (as indicated by the weight of the white gonadal fat) was however much greater in the obese rats and was increased by cafeteria feeding in both lean and obese rats (Figure 10). Accumulation of lipid in BAT was greater in the obese (Figure 10) and as in the first experiment, BAT of the obese chow fed rats at 28°C had less DNA and a normal amount of protein (Figure 11). COX activity was lower (Figure 11), although it was not significantly lower in the previous experiment. Cafeteria feeding increased protein content of BAT in lean rats but not in the obese (Figure 11). COX activity and DNA did not change with cafeteria feeding in either type of rat (Figure 11).

Activation of BAT thermogenesis by diet, as indicated by an increase in BAT mitochondrial GDP-binding, occurred in lean rats (Figure 12). In the obese rats, however, the binding of GDP was low in the chow fed controls, as seen in the first experiment, and was not altered by the cafeteria feeding (Figure 12). No effect of cafeteria feeding on polypeptide composition of the inner mitochondrial membrane could be detected in either lean or obese rats (Figure 12, legend).

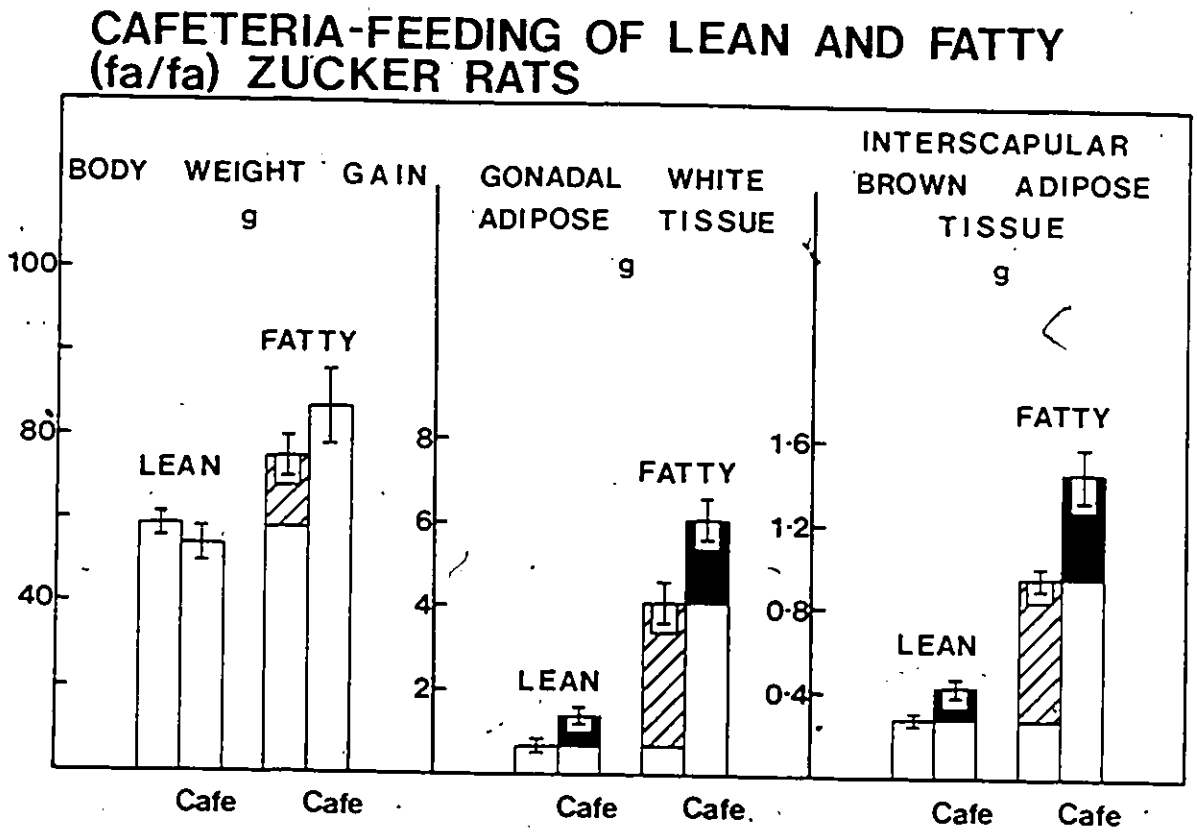

Figure 10. Effect of cafeteria feeding on body weight gain and organ weights of lean and fatty Zucker rats.

Bars represent means ± SE for 15 (lean control), 14 (lean cafeteria), or 12 (fatty control and fatty cafeteria) animals. Body weight gain during feeding period, gonadal white adipose tissue weight, and interscapular brown adipose tissue weight are all significantly greater in fatty rats fed chow than in lean rats fed chow (denoted by cross-hatched portions of bars). Actual initial body weights (in grams) were 71.9 ± 4.0 (lean control), 72.0 ± 4.2 (lean cafeteria), 100.3 ± 6.7 (fatty control), and 97.7 ± 6.8 (fatty cafeteria). Final body weights (in grams) were 130.5 ± 3.1 (lean control), 126.1 ± 4.5 (lean cafeteria), 175.8 ± 8.3 (fatty control), and 180.0 ± 14.2 (fatty cafeteria). Significant effects of cafeteria feeding (denoted by solid portions of bars) are on weight of gonadal white adipose tissue and weight of interscapular brown adipose tissue in both lean and fatty rats (solid portion of bar).

EFFECT OF CAFETERIA-FEEDING ON BROWN ADIPOSE TISSUE OF LEAN AND FATTY(fa/fa) ZUCKER RATS

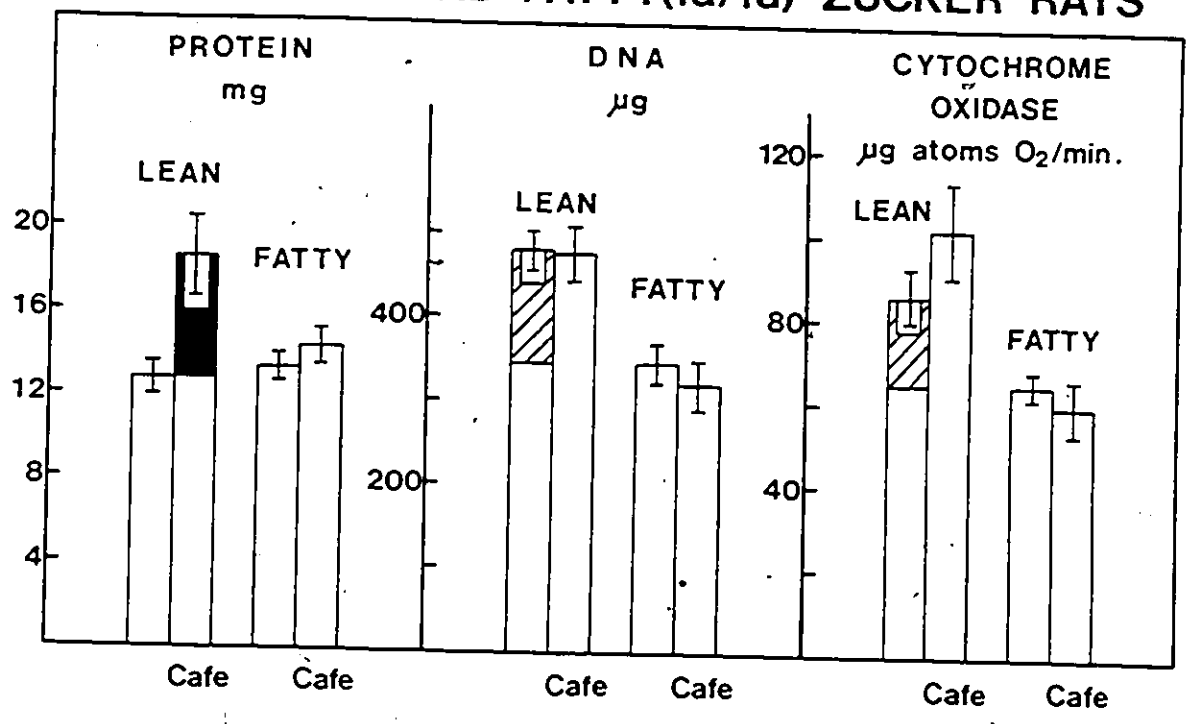

Figure 11. Effect of cafeteria feeding on interscapular brown adipose tissue of lean and fatty Zucker rats.

Bars are means ± SE for number of animals described in legend to Fig. 4. DNA and cytochrome oxidase contents are significantly smaller in control fatty rats than in control lean rats (denoted by cross-hatched portions of bars). Only significant effect of cafeteria feeding is an increase in protein content in lean rats (solid portion of bar).

EFFECT OF CAFETERIA-FEEDING ON BROWN ADIPOSE TISSUE MITOCHONDRIA OF LEAN AND FATTY RATS

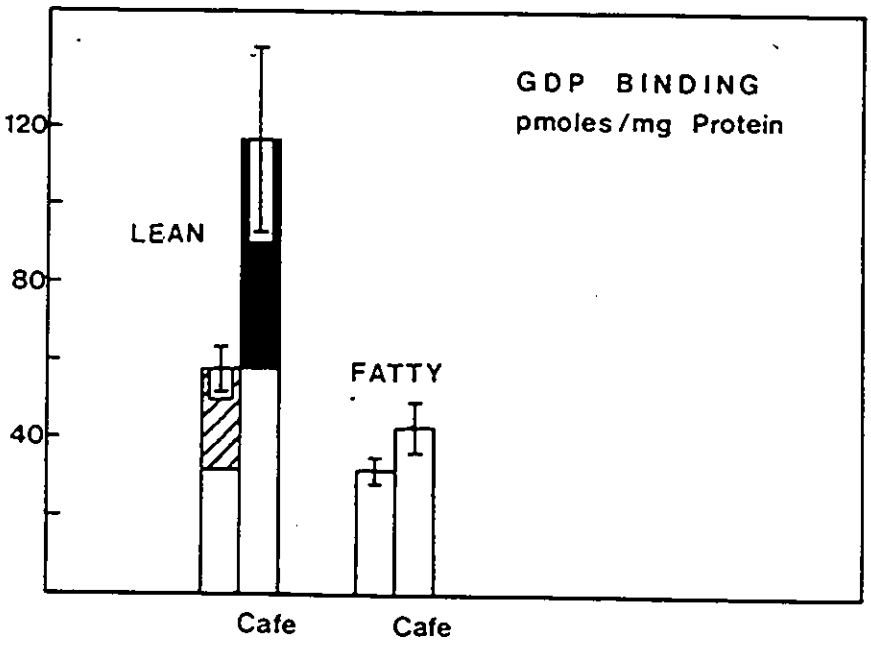

Figure 12. Effect of cafeteria feeding on GDP-binding by brown adipose tissue mitochondria of lean and fatty Zucker rats.

Bars are means ± SE for 5 (lean rats) or 4 (fatty rats) mitochondrial preparations. Binding is significantly lower in control fatty rats than in control lean rats (denoted by cross-hatched portion of bar). Cafeteria feeding caused a significant increase in binding in lean rats only (solid portion of bar). Proportion of mitochondrial membrane polypeptides in molecular weight range of 31,500-34,000 was not altered by cafeteria feeding in either lean or fatty rats (lean 19.57 ± 0.34%; lean, cafeteria-fed 20.10 ± 0.52%; fatty 19.57 ± 0.12%; fatty, cafeteria-fed 20.58 ± 0.07%).

The noradrenaline content of BAT, heart and spleen of 6 to 8 week old obese Zucker rats was normal (Table 10); there was however considerable variation in the noradrenaline content of BAT in the obese animals, with two of the three having values below the normal range. In cafeteria-fed obese rats the noradrenaline content of BAT was not significantly different from that of lean rats fed a cafeteria diet (Table 10), but again there was variation among the obese rats with three of four having values below the normal range. Exposure to cold caused a depletion of noradrenaline content in both lean and obese rats (Table 10).

As in Experiment A-1 food intake was not measured. The lack of effect of diet on BAT growth and activation in the obese might be attributed to lack of hyperphagia, especially since Zucker rats have been reported not to increase their food intake in the cold as do lean rats (Bray and York, 1972; Armitage *et al.*, 1984; Bertin *et al.*, 1983). However, by daily observation of the rats it was obvious that the human foods at least were consumed to a greater extent by the obese compared to the lean. Subsequent studies by others showed that, when overfed with sucrose, Zucker obese rats did not increase GDP-binding in BAT mitochondria as did lean rats (Holt *et al.*, 1983).

The main findings of this experiment are that BAT of the young obese Zucker rat, although normal in amount, has reduced thermogenic activation (mitochondrial GDP-binding). The tissue can be activated by cold but not by diet. The reduced GDP-binding to BAT mitochondria is unexpected since rats of this age are known to be hyperphagic (Bell and Stern, 1977). The higher than normal activation of BAT in cafeteria-fed rats (Experiment A-1) did not occur in the obese Zucker rat. The lack of

TABLE 10

Noradrenaline content of organs

	BAT	Heart	Spleen
Lean	ng/ organ		
Control	(4) 385.0 ± 25.4 [332.8-454.3]	(4) 339.3 ± 35.2	(4) 255.1 ± 34.7
Cold-exposed control	(4) 215.7 ± 6.9* [198.0-226.9]	(4) 254.4 ± 39.2	(4) 261.2 ± 53.9
Cafeteria-fed	(4) 434.1 ± 27.1 [386.4-480.2]	(3) 310.2 ± 36.4	(3) 231.7 ± 27.4
Fatty			
Control	(3) 314.6 ± 75.1 [233.2-464.5]	(4) 328.6 ± 42.1	(4) 333.4 ± 75.4
Cold-exposed control	(3) 144.2 ± 46.4 [66.8-227.1]	(3) 118.5 ± 28.4*+	(3) 298.8 ± 68.2
Cafeteria-fed	(4) 292.8 ± 52.3 [229.0-449.4]	(4) 197.9 ± 34.8	(4) 135.9 ± 23.4*+

Values are means ± SE of the number of experiments shown in parentheses.

Values in brackets are ranges.

*Significant effect of obesity, comparing rats treated in the same way.

+Significant effect of treatment, comparing the same type of rat.

diet-induced thermogenesis in BAT would suggest that sympathetic activity in BAT is reduced due to a defect in the signal concerning diet. This defect may be responsible for the reduced tissue activation at thermoneutral and may also lead to the eventual degeneration with age of the ability of the tissue to respond to cold.

Reduced GDP-binding to BAT mitochondria has also been reported by Holt and York (1982) in young Zucker rats, and is present as early as the first few days of life (Bazin *et al.*, 1984). GDP-binding has been found to increase normally after short term cold exposure in young but not older obese Zucker rats (Holt *et al.*, 1983) as in the present experiments. The response to sucrose overfeeding was reduced in the young animals (Holt *et al.*, 1983) confirming the lack of dietary effect in the present experiments. Other feeding experiments have also indicated that there is a reduced thermogenic response to diet in the obese Zucker rat. A low protein, high carbohydrate diet causes normal rats to overeat and increase their metabolic rate; but while they overeat, obese Zucker rats do not increase their metabolic rate on a low protein diet (Young *et al.*, 1980). The thermic response to a single meal (Rothwell *et al.*, 1983a) and to an intragastric tube feeding (Rothwell *et al.*, 1981) is also smaller in obese rats than in lean.

Adrenalectomy causes normalization of the low GDP-binding in the obese Zucker rat which is suppressed by corticosterone treatment (Holt and York, 1982). Normal growth of BAT and increase in GDP-binding to mitochondria occurs in sucrose overfed obese rats after adrenalectomy (Holt *et al.*, 1983) and the thermic response to a single meal is also normalized (Marchington *et al.*, 1983). How adrenalectomy works to

improve BAT activation is not known, but is in keeping with the repressive action of glucocorticoid treatment on BAT (refer to Chapter 1.5.D), in spite of the fact that weight gain, food intake or BAT weight, protein or mitochondrial GDP-binding was not affected by adrenalectomy of normal rats (Holt and York, 1982; Marchington *et al.*, 1983). Since BAT of young obese rats seems to respond normally to cold, glucocorticoids appear to affect specifically the response of BAT to diet, perhaps by an effect on the control of the sympathetic nervous system.

There are indications of abnormalities in the response of BAT of the obese Zucker rat to catecholamines, particularly in older rats. Basal and noradrenaline stimulated palmitate oxidation are not reduced in BAT of 5 week obese Zuckers (Kasser and Martin, 1982). At 2 months noradrenaline stimulated respiration in brown adipocytes is reported to be lower in the obese than lean rats (Goldberg and Morgan, 1982). A normal response to noradrenaline was reported in obese Zucker rats at 12 weeks (Bertin *et al.*, 1983) and at 3-4 months (Rothwell *et al.*, 1981) and is thought to indicate that peripheral thermogenic mechanisms are capable of functioning and the reduced response to food is probably due to reduced activation of the sympathetic nervous system. However isoproterenol-induced blood flow to BAT was reduced in the obese Zucker rat (Wickler *et al.*, 1982) and isoproterenol infusions showed a reduced metabolic response in 3-4 month obese rats (Milam *et al.*, 1982) suggesting sensitivity of BAT may be reduced. Age of the animal, dosage and method of administration of noradrenaline would seem to influence the level of response to catecholamines.

Noradrenaline turnover in BAT was somewhat reduced at 3-4 months, while content and turnover of noradrenaline were drastically reduced at 7-8 months in obese Zucker rats (Levin *et al.*, 1981). In the younger rats studied in the present experiments, noradrenaline content was normal. Obese rats (5-6 months) have a reduced number of ß-adrenergic receptors on BAT compared to the lean and adipocytes are unilocular. Lipolysis is not reduced, leading the authors to conclude there is either infiltration with white adipose tissue or a defect in fatty acid stimulated respiration (Levin *et al.*, 1982). Brown adipose tissue, while capable of responding to noradrenaline in the young animal, because of reduced activation by the sympathetic nervous system may lose its capacity to respond with age. In both the present experiments and those of Holt *et al.* (1983), young but not older animals had the normal increase in GDP-binding in the cold. Although the thermogenic response to catecholamines is variable, as is the response to cold (refer to Chapter 1.6) an early defect in diet-induced thermogenesis may be responsible for the reduced activation of BAT and the low sympathetic activity in BAT, and may lead to more serious deficiencies in BAT thermogenesis including a reduced response to cold in older animals.

SECTION B BROWN ADIPOSE TISSUE IN THE MYOPATHIC HAMSTER

Overall Objective:

The overall objective of this section was to find out why BAT fails to grow to a normal size in the myopathic hamster. More specifically the objective was to study the effect on BAT of myopathic hamsters of various stimuli known to promote growth of BAT in normal rats and/or hamsters.

EXPERIMENT B-1 THE EFFECT OF NORADRENALINE ON BROWN ADIPOSE TISSUE OF NORMAL AND MYOPATHIC HAMSTERS

BACKGROUND:

Brown adipose tissue growth in rats appears to be, at least partially, under the control of the sympathetic nervous system (Barnard *et al.*, 1980), and chronic treatment of rats with NA promotes growth of BAT (Heick *et al.*, 1973; Desautels and Himms-Hagen, 1979). The influence of the sympathetic nervous system on BAT growth in hamsters has not been studied, although it is known that noradrenaline turnover in BAT increases when Syrian hamsters are exposed to low temperatures (Feist, 1970). Increase in mass of BAT is also reported in Syrian hamsters kept in short photoperiod (Hoffman *et al.*, 1965; Reiter, 1975). This increase is prevented by pinealectomy (Reiter, 1975) and the pineal hormone, melatonin, has a trophic effect on BAT of Djungarian hamsters (Heldmaier and Hoffmann, 1974). Since melatonin is produced in the pineal in response to noradrenaline from sympathetic nerve endings acting at β-adrenergic receptors (Klein and Moore, 1979), the possibility exists that noradrenaline might stimulate BAT growth in hamsters by a direct action on the tissue itself, as seems to occur in rats, and/or indirectly through stimulation of melatonin production in the pineal.

OBJECTIVE:

The objective of this experiment was to find out whether there was any abnormality in the response of BAT of myopathic hamsters to the supposed trophic effect of noradrenaline.

METHOD:

Male Syrian hamsters were purchased from either Bio Research Consultants, Cambridge, MA, or from Charles River Company, Boston as noted for each experiment. Myopathic hamsters (BIO 14.6) were purchased from Bio Research Consultants. Pinealectomized and sham pinealectomized hamsters were from Charles River Company.

In four separate experiments, 5-6 week old hamsters were injected daily with noradrenaline in peanut oil or with oil alone for two weeks. In these experiments the time of day the injections were given, the season of the year, the dosage of noradrenaline and the presence or absence of the pineal were varied, as described for each experiment.

After hamsters were sacrificed, interscapular BAT was removed, cleaned and weighed. Homogenization of the tissue and isolation of mitochondria were done as outlined in Methods A. Tissue protein, COX activity, DNA and mitochondrial GDP-binding were assayed (Methods C, D, E, B). Epididymal white adipose tissue and testes were removed and weighed.

RESULTS AND DISCUSSION:

In the initial experiment noradrenaline was injected at about 1300 h at a level of 400 µg/kg/day which is known to cause growth of BAT in rats (Desautels and Himms-Hagen, 1979). A reduction in metabolic size of BAT (protein content, COX) occurred in both normal and myopathic hamsters, the opposite of the expected hypertrophy (Table 11).

TABLE 11

Effect of chronic treatment with noradrenaline (injections at 1100 - 1300 h) on brown adipose tissue of normal or myopathic hamsters in October-November

	NORMAL		MYOPATHIC	
	Control (12)	Noradrenaline (12)	Control (12)	Noradrenaline (12)
Body weights, g				
Initial	70.7 ± 1.6	68.7 ± 1.0	62.1 ± 1.4$^{\#\#\#}$	64.7 ± 1.3$^{\#}$
Final	88.7 ± 1.9	82.2 ± 1.7*	75.9 ± 1.2$^{\#\#\#}$	75.5 ± 1.4$^{\#\#\#}$
Change in 2 wk	+17.9 ± 0.9	+13.4 ± 1.0**	+13.8 ± 0.7$^{\#\#}$	+11.0 ± 0.9*
Brown adipose tissue				
Wet wg, mg	364 ± 18	314 ± 19	207 ± 7$^{\#\#\#}$	183 ± 9$^{\#\#\#}$
Protein, mg	19.6 ± 1.8	13.3 ± 1.1**	10.7 ± 0.5$^{\#\#\#}$	6.8 ± 0.7$^{****\#\#\#}$
Cytochrome oxidase ug atoms O_2/min	69.0 ± 5.4	30.5 ± 5.7****	34.5 ± 1.5$^{\#\#\#}$	19.0 ± 4.1***
Mitochondrial GDP binding pmoles/mg protein	(4) 119.7 ± 4.3	(4) 81.7 ± 14.3*	(4) 85.5 ± 11.6$^{\#}$	(4) 86.8 ± 18.2

Values are means ± SE for the number of animals or of mitochondrial preparations indicated in parentheses.

Noradrenaline, 0.4 mg/kg, injected daily for 2 wk.

Hamsters from Bio Research Consulstants.

Significant effect of noradrenaline, comparing the same type of hamster, indicated by *($p < 0.05$), **($p < 0.01$), ***($p < 0.005$) or ****($p < 0.001$).
Significant effect of myopathy, comparing hamsters treated in the same way, indicated by #($p < 0.05$), ##($p < 0.005$) or ###($p < 0.001$).

The production of melatonin by the pineal is stimulated by noradrenaline acting at β-adrenergic receptors and is many times higher in the hours of darkness (Klein and Moore, 1979). This has been attributed to "up regulation" of β-receptors during the day when sympathetic activity is low. In the evening sympathetic activity rises at a time when the pineal is maximally sensitive to its effects, causing a large increase in melatonin production in the dark (Romero *et al.*, 1975). In the present experiments noradrenaline was injected in the early afternoon, possibly interfering with the sensitivity of the pineal to noradrenaline and the production of melatonin. It was thought that this effect might be avoided if noradrenaline was injected just at lights on, when the pineal is reportedly least sensitive to noradrenaline.

A second experiment with injections given in the early morning also resulted in a noradrenaline-induced reduction in metabolic size (COX only) of BAT (Table 12).

In searching for a possible cause of this unexpected effect of noradrenaline on BAT growth, it was thought that some insight might be gained by differentiating the putative direct and indirect (pineal) effects of noradrenaline on BAT growth. Therefore, in a third experiment noradrenaline was administered to pinealectomized normal hamsters and to sham-operated normal controls (at 1300 h). No effect of treatment on the size of BAT could be detected in either type of animal and there was no effect of pinealectomy on the size of BAT (Table 13). Thus it seemed that the action of noradrenaline on the pineal was not a factor in inhibiting growth of BAT in response to noradrenaline and also it also appears that

TABLE 12

Effect of chronic treatment with noradrenaline (injections at 0930 h) on brown adipose tissue of normal or myopathic hamsters in September

	NORMAL		MYOPATHIC	
	Control (12)	Noradrenaline (12)	Control (12)	Noradrenaline (12)
Body wts, g				
Initial	78.8 ± 2.0	79.7 ± 2.8	74.3 ± 1.5	77.3 ± 1.7
Final	95.7 ± 2.0	94.0 ± 2.9	83.3 ± 1.3$^{\#\#\#}$	83.8 ± 1.3$^{\#\#\#}$
Change in 2 wk	+16.9 ± 1.2	+14.3 ± 1.2	+9.0 ± 0.6$^{\#\#\#}$	+6.5 ± 1.5$^{\#\#\#}$
Brown adipose tissue				
Wet wt, mg	327 ± 16.9	329 ± 27.0	204 ± 10.1$^{\#\#\#}$	195 ± 6.5
Protein, mg	19.0 ± 1.7	15.2 ± 2.0	9.3 ± 1.0$^{\#\#\#}$	6.6 ± 0.4$^{*\#\#\#}$
Cytochrome oxidase μg atoms O_2/min	57.2 ± 9.4	25.7 ± 4.3**	21.7 ± 3.6$^{\#\#}$	14.4 ± 3.0$^{\#}$
Mitochondrial GDP binding pmoles/mg protein	(4) 263.2 ± 23.2	(4) 215.3 ± 14.6	(4) 211.9 ± 39.4	(4) 167.2 ± 21.9

Values are means ± SE for the number of animals or of mitochondrial preparations indicated in parentheses. Noradrenaline, 0.4 mg/kg, injected daily for 2 wk. Hamsters from Bio Research Consultants.

Significant effect of noradrenaline, comparing the same type of hamster, indicated by $^{*}(p < 0.05)$ or $^{**}(p < 0.01)$.

Significant effect of myopathy, comparing hamsters treated in the same way, indicated by $^{\#}(p < 0.05)$, $^{\#\#}(p < 0.005)$ or $^{\#\#\#}(p < 0.001)$.

TABLE 13

Effect of chronic treatment with noradrenaline (injections at 1300 h) on brown adipose tissue of sham-operated or pinealectomized hamsters in November

	SHAM-OPERATED		PINEALECTOMISED	
	Control (12)	Noradrenaline (12)	Control (12)	Noradrenaline (12)
Body wts, g				
Initial	82.5 ± 1.9	81.5 ± 0.8	83.0 ± 2.0	83.5 ± 1.7
Final	98.0 ± 2.3	93.8 ± 1.0	95.2 ± 2.2	94.9 ± 2.0
Change in 2 wk	+13.8 ± 1.2	+12.3 ± 0.9	+12.3 ± 0.9	+11.4 ± 0.9
Organ wts, g				
Testes	2.65 ± 0.06	2.52 ± 0.06	2.72 ± 0.05	2.55 ± 0.06*
Epididymal adipose tissue	1.64 ± 0.09	1.60 ± 0.07	1.67 ± 0.08	1.61 ± 0.10
Brown adipose tissue				
Wet wt, mg	345 ± 11.5	303 ± 15.0*	315 ± 16.3	308 ± 12.7
Protein, mg	14.0 ± 0.8	15.2 ± 2.1	11.5 ± 1.0	12.7 ± 1.0
Mitochondrial GDP binding pmoles/mg protein	(4) 220.3 ± 22.4	(4) 232.5 ± 16.5	(4) 200.5 ± 18.3	(4) 207.9 ± 29.4

Values are means ± SE for the number of animals or of mitochondrial preparations indicated in parentheses. Noradrenaline 0.4 mg/kg, injected daily for 2 wk. Hamsters from Charles River Company.

Significant effect of noradrenaline, comparing the same type of hamster indicated by *($p < 0.05$).

There are no significant effects of pinealectomy, comparing hamsters treated in the same way.

the pineal contributes little to the maintenance of normal BAT size under these conditions.

Growth of BAT is seasonal in Djungarian hamsters kept in natural photoperiod and such hamsters are refractory to short photoperiod-induced growth in early summer (Heldmaier and Steinlechner, 1981). It seemed possible that seasonal refractoriness to noradrenaline injections may also occur. The first three experiments were done in fall and early winter. Noradrenaline injections were then studied in pinealectomized hamsters in early spring. The dose of noradrenaline was doubled (800 µg/kg/day) in this experiment. This dose of noradrenaline was sufficient to prevent body weight gain and to deplete fat stores of white adipose tissue but still no trophic response of BAT could be detected (Table 14).

In many hamsters treated with noradrenaline, particularly at the higher dose level, small yellow patches, resembling fat necroses (Lee and Howard, 1979) were seen in BAT. Cell damage caused by excessive accumulation of products of lipolysis may explain the regression of the tissue in 2 of the 4 experiments.

Thus, no evidence could be found for a trophic response of hamster BAT to noradrenaline.

TABLE 14

Effect of chronic treatment with noradrenaline (injections at ~1300 h) on brown adipose tissue of sham-operated or pinealectomised hamsters in March-April

	SHAM OPERATED		PINEALECTOMISED	
	Control (12)	Noradrenaline (12)	Control (12)	Noradrenaline (12)
Body wts, g				
Initial	112.3 ± 2.5	112.6 ± 2.4	110.9 ± 3.0	108.0 ± 3.3
Final	119.7 ± 2.8	110.8 ± 2.3*	116.0 ± 3.3	109.3 ± 2.6
Change in 2 wk	+7.4 ± 1.3	-1.8 ± 0.8****	+5.1 ± 0.7	+1.3 ± 1.1**
Organ wts, g				
Testes	2.78 ± 0.05	2.83 ± 0.08	3.06 ± 0.07#	3.13 ± 0.18
Epididymal adipose tissue	2.40 ± 0.14	1.82 ± 0.09***	2.40 ± 0.21	1.83 ± 0.16***
Brown adipose tissue				
Wet wt, mg	386.2 ± 13.5	409.8 ± 19.1	359.6 ± 23.6	391.2 ± 34.0
Protein, mg	18.4 ± 0.9	17.1 1.4	18.4 ± 0.9	17.5 ± 1.7

Values are means ±SE for the number of animals indicated in parentheses. Noradrenaline, 0.8 mg/kg, was injected daily for 2 wk. Hamsters from Charles River Company.

Significant effect of noradrenaline, comparing the same type of hamster indicated by *($p < 0.05$), **($p < 0.01$), ***($p < 0.005$) or ****($p < 0.001$).

Significant effect of pinealectomy, comparing hamsters treated in the same way indicated by #($p < 0.005$).

EXPERIMENT B-2 ALPHA- VS BETA-ADRENERGIC EFFECTS ON GROWTH OF BROWN ADIPOSE TISSUE IN NORMAL AND MYOPATHIC HAMSTERS

BACKGROUND:

Noradrenaline acts at α- and β-adrenergic receptors. Alpha-adrenergic receptors have been subdivided into α_1, the action of which is thought to involve calcium mobilization and phosphatidylinositol turnover, and α_2 which act by inhibiting adenylate cyclase (Fain and Garcia-Sainz, 1980). Hamster white adipose tissue contains α_2-receptors which inhibit β-adrenergic stimulated lipolysis (Hittelman and Butcher, 1973; Schimmel *et al.*, 1981). Alpha$_2$-receptors and hence the inhibitory effects on lipolysis are lacking in white adipose tissue of the rat (Fain and Garcia-Sainz, 1980). It was thought that if a similar species difference occurred in BAT it might contribute to the lack of trophic effect of noradrenaline on hamster BAT seen in Experiment B-1.

During the course of the experiment it was learned that α_2-receptors probably did not exist in BAT (McMahon and Schimmel, 1982). Since a small inhibitory effect of α-adrenergic agonist phenylephrine had been observed, it was decided to study this effect further using the α_1-antagonist prazosin.

OBJECTIVE:

The objective was to find out whether the pure β-agonist,

isoproterenol or the pure α-agonist, phenylephrine, has a trophic effect on BAT of hamsters. The effect of the α_1-antagonist, prazosin was also studied.

METHOD:

Male Syrian hamsters were purchased from Charles River Company, Boston, except for the prazosin experiment where they were from Canadian Hybrid Farms, Halls Harbour, N.S. Myopathic hamsters (BIO 14.6) were from Canadian Hybrid Farms.

The drugs were used in the following dosages -

isoproterenol bitartrate	400 µg (free base)/kg/day
phenylephrine HCl	400 µg (free base)/kg/day
prazosin	25 mg/kg/day

All drugs were injected as a suspension in peanut oil in a volume of 0.1 to 0.3 ml, depending on the weight of the animal. Animals 5-6 weeks old were injected with drugs or oil at approximately 1300 h for 14 consecutive days.

Immediately after sacrifice interscapular BAT was removed, cleaned and weighed. Homogenization of the tissue and isolation of mitochondria were done as outlined in Methods A. Tissue protein, COX activity, DNA and mitochondrial GDP-binding were assayed (Methods C, D, E, B). Epididymal white adipose tissue and testes were removed and weighed.

RESULTS AND DISCUSSION:

Injection of isoproterenol had no effect on body weight, IBAT

weight, total protein, COX activity, DNA, mitochondrial GDP-binding or testes weight (Table 15).

The results of the phenylephrine experiment were complicated by the fact that the drug-treated normal animals were initially larger than the controls injected with oil (Table 16). The results in this case have been adjusted to the body weight of the animal. This causes the significantly heavier IBAT weight of the phenylephrine treated normal hamster to become not different from the oil treated control, while the tissue protein content, similiar in absolute amount, is significantly lower in phenylephrine treated animals when related to body weight. The decrease in tissue protein in phenylephrine treated myopathic hamsters was not statistically significant, but the tissue protein was 15% lower in both normal and myopathic drug treated animals (Table 16). No effect of phenylephrine on mitochondrial GDP-binding was seen. The phenylephrine injected was the HCl derivative expected to have a shorter biological half life than the bitartrate salt used for noradrenaline and isoproterenol injections. A longer acting α-agonist may have given more conclusive effects.

Prazosin injections caused a dramatic increase in BAT wet weight and total protein in normal and myopathic hamsters (Figure 13). The proportional increase in metabolic size (total protein) of BAT was much greater in the myopathic hamsters (+67.7%) than in the normal hamsters (+26.9%). The weight gain and gonadal fat were significantly lower in normal hamsters treated with prazosin (Figure 13, Table 17), which might not be unexpected in animals with more BAT. Myopathic animals did not adjust their weight gain or fat stores (Figure 13). Because of their

TABLE 15

Effect of chronic treatment with isoproterenol on brown adipose tissue of normal or myopathic hamsters

	NORMAL		MYOPATHIC	
	Control (12)	Isoproterenol (11)	Control (12)	Isoproterenol (10)
Body weights, g				
Initial	75.9 ± 2.3	73.2 ± 2.3	50.7 ± 0.9***	49.9 ± 0.9***
Final	92.7 ± 2.2	91.5 ± 2.3	62.5 ± 1.8***	63.0 ± 0.9***
Change in 2 weeks	16.7 ± 0.9	18.3 ± 1.2	11.7 ± 1.4*	13.4 ± 0.9**
Organ weights				
Testes, g	2.38 ± 0.06	2.27 ± 0.05	1.78 ± 0.08***	1.73 ± 0.05***
Brown adipose tissue				
Wet weight, mg	328.4 ± 17.4	351.5 ± 25.7	120.2 ± 7.1***	126.7 ± 5.1***
Protein, mg	18.8 ± 1.3	20.8 ± 1.9	7.7 ± 0.6***	8.6 ± 0.5***
DNA, µg	774.4 ± 41.3	811.0 ± 67.0	299.2 ± 21.8***	346.5 ± 17.9***
Mitochondrial GDP binding p moles/mg protein	(4) 242.8 ± 8.07	(4) 234.8 ± 23.9	(4) 223.7 ± 24.3	(4) 244.8 ± 15.1

Season: Spring

Values are means ± SE for the number of animals or of mitochondrial preparations indicated in parentheses.
Isoproterenol, 0.4 mg/kg, injected daily for 2 weeks.

Significant effect of myopathy comparing hamsters treated in the same way indicated by *($p < 0.01$), ** ($p < 0.005$) or *** ($p < 0.001$).

TABLE 16

Effect of chronic treatment with phenylephrine on brown adipose tissue of normal or myopathic hamsters

	NORMAL		MYOPATHIC	
	Control (12)	Phenylephrine (12)	Control (11)	Phenylephrine (12)
Body weights, g				
Initial	75.3 ± 2.4	83.7 ± 2.4**	65.3 ± 2.1#	63.5 ± 1.3###
Final	90.4 ± 2.0	98.2 ± 2.4**	76.6 ± 1.7###	74.7 ± 1.0###
Change in 2 weeks	15.1 ± 0.9	14.2 ± 1.3	11.4 ± 0.8#	11.2 ± 0.8
Organ weights				
Gonadal fat, g	1.45 ± 0.09	1.69 ± 0.11	0.78 ± 0.03###	0.75 ± 0.02###
Testes, g	2.70 ± 0.05	2.78 ± 0.05	2.52 ± 0.08	2.57 ± 0.05###
Brown adipose tissue				
Wet weight, mg	299.9 ± 13.6	350.0 ± 18.2*	128.6 ± 6.7###	130.6 ± 5.8###
Wet weight, mg/100 g body weight	331.7 ± 13.7	355.0 ± 14.1	168.4 ± 8.5##	174.6 ± 7.0###
Protein, mg	14.1 ± 0.5	12.8 ± 0.8	8.5 ± 0.7###	7.1 ± 0.7###
Protein, mg/100 g	15.6 ± 0.6	13.2 ± 0.8*	11.2 ± 0.9##	9.5 ± 0.9#
Mitochondrial GDP binding pmoles/mg protein	(4) 310.6 ± 43.0	(4) 276.1 ± 29.0	(4) 318.9 ± 44.8	(4) 323.0 ± 71.1

Season: Summer

Values are means ± SE for the number of animals or of mitochondrial preparations indicated in parentheses.
Phenylephrine, 0.4 mg/kg, injected daily for 2 weeks

Significant effect of phenylephrine, comparing the same type of hamster indicated by * ($p < 0.05$), ** ($p < 0.025$).

Significant effect of myopathy, comparing hamsters treated in the same way indicated by # ($p < 0.01$), ## ($p < 0.005$), ### ($p < 0.001$).

EFFECT OF CHRONIC TREATMENT WITH PRAZOSIN ON BROWN ADIPOSE TISSUE OF NORMAL OR MYOPATHIC HAMSTERS

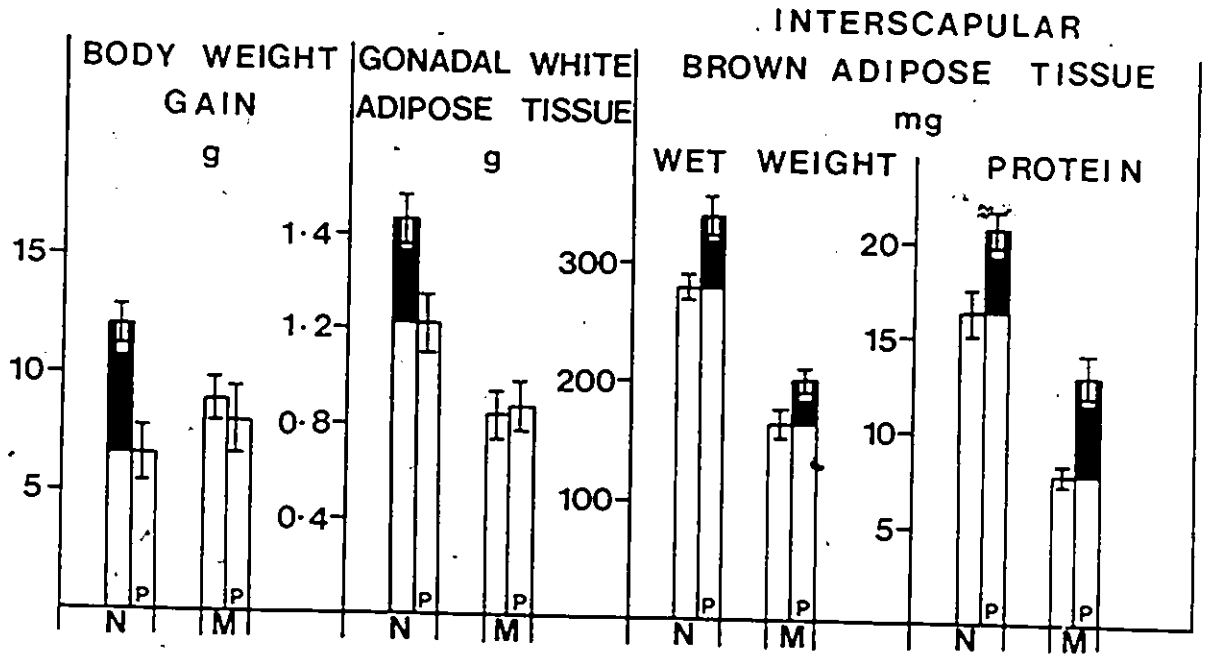

Figure 13. Effect of chronic treatment with prazosin on brown adipose tissue of normal or myopathic hamsters.

Bars are means ± SE of body weight gain, gonadal white adipose tissue and wet weight and total protein of interscapular brown adipose tissue of normal (N) or myopathic (M) hamsters (n = 12). Black portions of bars denote significant differences between oil treated hamsters and prazosin treated (P) hamsters. Further data are in Table 17.

TABLE 17

Effect of chronic treatment with prazosin on brown adipose tissue of normal or myopathic hamsters

	NORMAL		MYOPATHIC	
	Control (12)	Prazosin (12)	Control (12)	Prazosin (12)
Body weights, g				
Initial	89.6 ± 2.3	90.3 ± 1.4	81.3 ± 1.1$^{\#\#}$	81.2 ± 0.9$^{\#\#\#}$
Final	101.5 ± 2.5	97.0 ± 1.4	90.2 ± 1.4$^{\#\#\#}$	89.3 ± 1.7$^{\#\#}$
Change in 2 weeks	11.9 ± 0.8	6.7 ± 1.2*	8.9 ± 0.9$^{\#}$	8.1 ± 1.4
Organ weight				
Testes, g	2.90 ± 0.08	2.74 ± 0.07	2.83 ± 0.08	2.78 ± 0.08
Brown Adipose Tissue				
Mitochondrial GDP binding pmoles/mg protein	(4) 188.1 ± 6.5	(4) 191.8 ± 11.7	(4) 202.4 ± 24.6	(4) 194.3 ± 13.5

SEASON: FALL

Values are means ± SE for the number of animals or mitochondrial preparations indicated in parentheses.

Prazosin, 25 mg/kg, injected daily for 2 weeks.

Significant effect of prazosin, comparing the same type of hamster indicated by $^{*}(p < 0.005)$.

Significant effect of myopathy, comparing hamsters treated in the same way indicated by $^{\#}(p < 0.025)$, $^{\#\#}(p < 0.005)$ or $^{\#\#\#}(p < 0.001)$.

relative leaness, they may already be close to a minimal level of fatness. Alternatively, the differential response to prazosin treatment in normal and myopathic hamsters (larger increase in BAT., smaller weight gain and white adipose tissue in the myopathic) may indicate that prazosin is exerting its effects by different mechanisms in the two types of animals. GDP-binding to BAT mitochondria was similar in all animals, as was testes weight (Table 17).

The yellow spots mentioned in the preceding experiment after injection of noradrenaline were completely absent when isoproterenol, phenylephrine or prazosin was used.

EXPERIMENT B-3 THE SYMPATHETIC INNERVATION OF BROWN ADIPOSE TISSUE OF NORMAL AND MYOPATHIC HAMSTERS.

BACKGROUND:

Since the effect of prazosin on BAT metabolic size is much larger in the myopathic animals than in the normals (Experiment 2-B), it may be that an inhibitory α_1 effect on BAT growth in hamsters is responsible for the small size of BAT in myopathic hamsters. Two possible causes for this effect were considered. First, if noradrenaline turnover in BAT of myopathic hamsters were elevated as it is in the heart (Angelakos *et al.*, 1973; Sole *et al.*, 1975; Jasmin and Proschek, 1983) then the higher sympathetic tone may cause greater stimulation of α-receptors and increased inhibition of growth. Second, a greater inhibitory effect might be expected if there were a greater number of α-receptors on BAT of the myopathic hamsters. Moreover, since there is some doubt concerning physiological importance of α-receptors in BAT of rats (Bukowieki *et al.*, 1980), a potential cause of the difference in response of BAT growth to noradrenaline injections in rats and hamsters might be a difference in occurrence of α-receptors. Studies were therefore carried out on the turnover of noradrenaline (Experiment B-3) and the occurrence of α_1-adrenergic receptors (Experiment B-4).

OBJECTIVE:

The objective was to study the sympathetic innervation of brown adipose tissue of normal and myopathic hamsters.

METHOD:

Male Syrian hamsters and myopathic hamsters (BIO 14.6) were purchased from Canadian Hybrid Farms, Halls Harbour, N.S.

Twelve week old hamsters were injected intraperitoneally with α-methyl-p-tyrosine (MPT) at a dose of 400 mg/kg at 0900 h. Hamsters were killed by decapitation 45, 90, or 150 minutes later. Hamsters for 0 time were injected with saline. Interscapular BAT and heart were removed and assayed for noradrenaline and the noradrenaline turnover rate (NATR) and half life was calculated as outlined in Methods (J.iii).

RESULTS AND DISCUSSION:

There was an exponential decrease in NA content of BAT in both normal and myopathic hamsters between 45 and 150 minutes after injection of MPT (Figure 14). Rate constants, calculated half lives and concentration of NA were not different in the two types of animals (Table 18). However, because the total NA content was less in the myopathic hamster, the calculated NATR for BAT of this animal was less than one half the normal rate (Table 18). The elevation of the sympathetic nervous system activity observed in hearts of myopathic hamsters reported by others (Angelakos *et al.*, 1973; Jasmin and Proschek, 1983; Sole *et al.*, 1975) was not seen in this experiment probably because the times chosen as suitable for measurement of NATR in brown adipose tissue, appropriate for the rapid rate in this tissue, were too short to give reliable values for the slower NATR in the heart (Figure 14).

Thus no evidence could be found for increased NATR in BAT of myopathic hamsters.

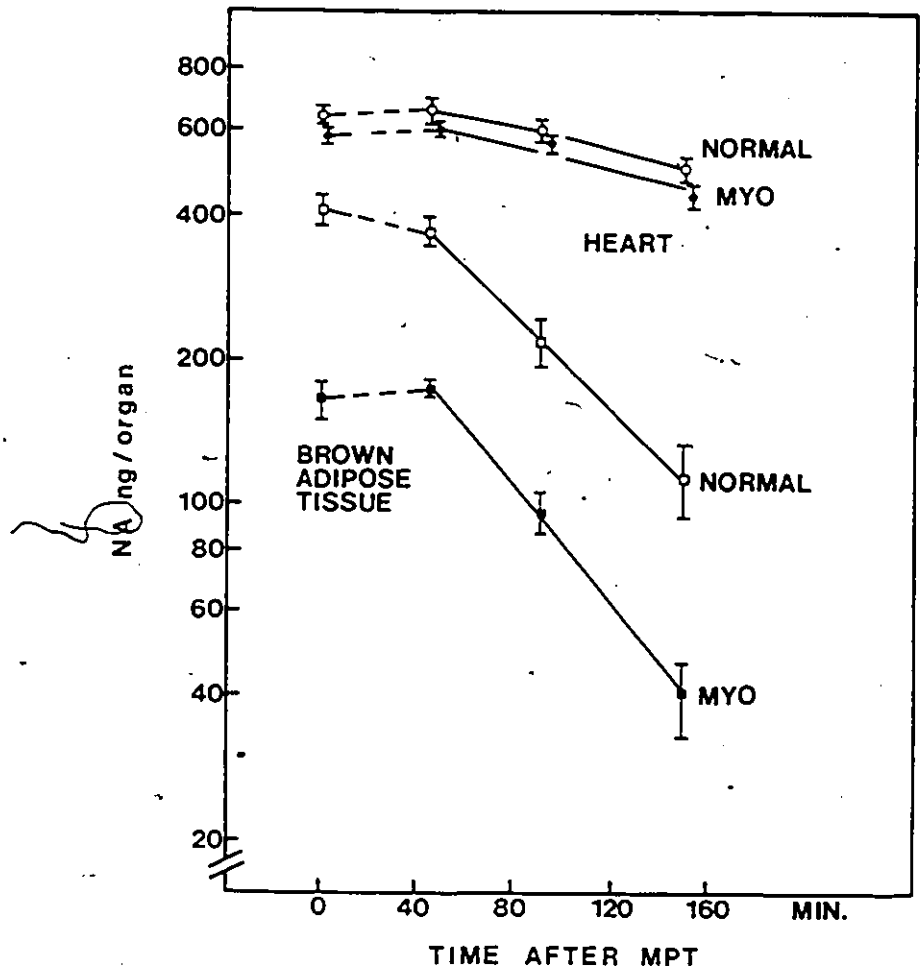

Figure 14. Turnover of noradrenaline in brown adipose tissue and heart of normal and myopathic hamsters.

The decrease in noradrenaline content after administration of MPT is shown. Half-lives and turnover rates (see Table 18) were calculated from the 45 - 150 min period. These times were chosen as suitable for brown adipose tissue. They were not suitable for accurate measurement of turnover rate in heart, where this rate is much lower than in brown adipose tissue. Experiments were done in April.

TABLE 18

Turnover of noradrenaline in brown adipose tissue and heart of normal or myopathic hamsters

		NORMAL	MYOPATHIC
Body wts, g	(16)	102.1 ± 2.6	81.3 ± 1.3***
Brown adipose tissue			
k	(4)	0.6070 ± 0.0325	0.8664 ± 0.1318
$t^{\frac{1}{2}}$, h	(4)	1.15 ± 0.06	0.86 ± 0.12
NATR, ng/h	(4)	249.3 ± 21.65	144.0 ± 22.23*
NA content, ng (initial)	(4)	410.6 ± 28.14	167.3 ± 12.47***
Wet wt, mg (initial)	(4)	303.5 ± 27.50	134.8 ± 8.30**
NA concentration ng/mg (initial)	(4)	1.37 ± 0.077	1.24 ± 0.065
Heart			
k	(4)	0.1590 ± 0.0507	0.1921 ± 0.0315
$t^{\frac{1}{2}}$, h	(4)	5.84 ± 1.61	3.86 ± 0.56
NATR, ng/h	(4)	100.3 ± 29.60	114.6 ± 21.8
NA content, ng (initial)	(4)	646.4 ± 25.95	586.6 ± 14.76
Wet wt, mg (initial)	(4)	326.5 ± 10.0	278.3 ± 4.75**
NA concentration ng/mg (initial)	(4)	1.91 ± 0.068	2.11 ± 0.081

Values for k, $t^{\frac{1}{2}}$ and NATR were obtained from the average of the slopes obtained in 4 experiments (12 animals). Values for NA content and concentration and for tissue wet weight are the means for 4 saline-injected hamsters.

Significant effect of myopathy is indicated by *($p < 0.02$), **($p < 0.005$) or ***($p < 0.001$).

EXPERIMENT B-4 ALPHA-ADRENERGIC RECEPTORS IN BROWN ADIPOSE TISSUE OF NORMAL AND MYOPATHIC HAMSTERS AND OF RATS

BACKGROUND:

Refer to BACKGROUND, Experiment B-3.

OBJECTIVE:

The objective was to study α_1-adrenergic receptors in BAT of normal and myopathic hamsters and of rats.

METHOD:

Male myopathic hamsters (BIO 14.6) and normal controls were purchased from Canadian Hybrid Farms, Halls River, N.S. Young Holtzman rats were purchased from Canadian Breeding Laboratories and acclimated either to 24°C or 5°C for 3 weeks.

After sacrifice of hamsters at eight weeks of age, interscapular BAT was dissected from 2-3 normal hamsters or 4-5 myopathic hamsters for each tissue preparation. Interscapular BAT from 2 rats or half the pad of the cold-acclimated rats was used. The specific binding of [^{3}H]WB-4101 to tissue homogenates was measured by an adaptation of the method of U'Prichard *et al.* (1977) as described in Methods I.

The binding of WB-4101 was characterized by measuring competition with the binding of [^{3}H]WB-4101 (1.25 nM) by several concentrations of adrenergic agonists, (-)-adrenaline, (-)-noradrenaline, (+)-adrenaline,

(+)-noradrenaline, (-)-phenylephrine and (-)-isoproterenol bitartrate; and antagonists prazosin and yohimbine.

RESULTS AND DISCUSSION:

Specific WB-4101 binding to BAT. homogenates was saturable and of high affinity. A saturation curve of an individual binding assay is shown in Figure 15. The Scatchard plot derived from this binding data (Figure 16) resulted in a linear plot for specific WB-4101 binding. The dissociation constant (K_d) was 0.18 nM and the number of binding sites (Bmax) was 49.3 fmol/mg protein in this experiment. Results representing five individual experiments in both normal and myopathic hamsters are presented in Table 19. The K_d was 0.19 nM and 0.15 nM; and the Bmax was 43.0 fmol and 42.3 fmol/mg protein in normal and myopathic hamsters respectively. There was no significant difference in affinity or maximum binding capacity between the two types of animals. Adrenergic agonists competed for [^{3}H]WB-4101 binding with the order of potency adrenaline > noradrenaline > phenylephrine >> isoproterenol (Figure 17.A), the order ascribed to α-receptors. The binding of [^{3}H]WB-4101 was inhibited more by prazosin than by yohimbine (Figure 17.B) indicating binding was to α-receptors of the α_1 subtype. Stereospecificity of [^{3}H]WB-4101 binding was established by the competitive binding of (+)-stereoisomers of adrenaline and noradrenaline which were much less potent than (-)-adrenaline or (-)-noradrenaline (Figure 17.A). The larger response in growth of the tissue when animals are treated with prazosin (Experiment B-2) could not then be attributed

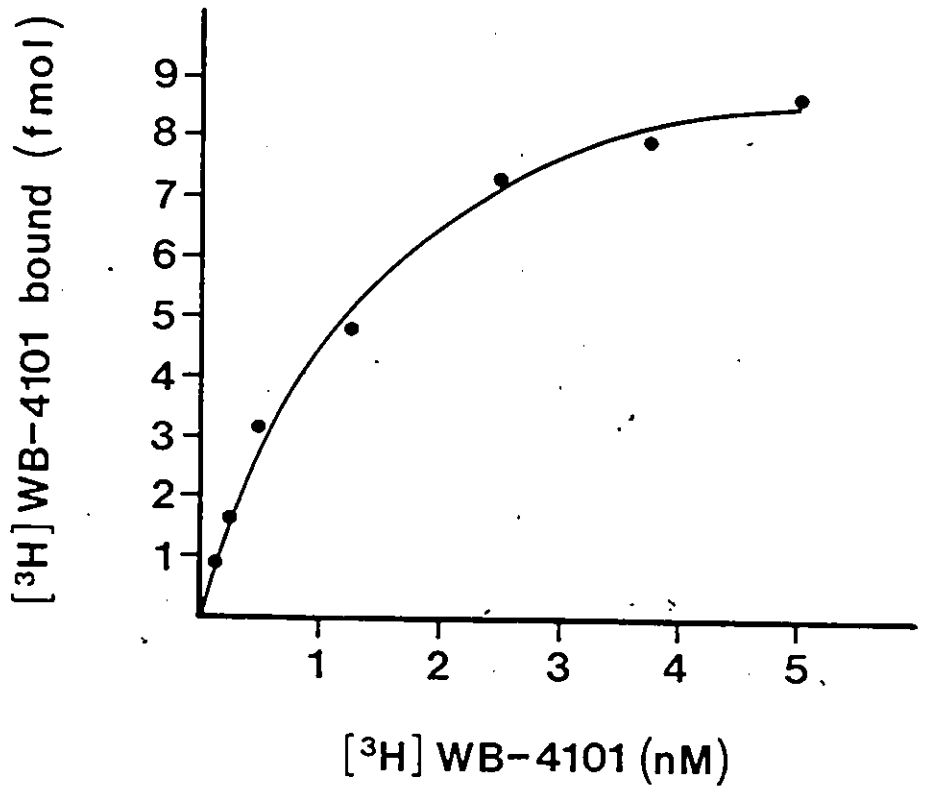

Figure 15. [^{3}H]WB-4101 binding to BAT homogenates.

Hamster brown adipose tissue homogenates were incubated for 15 minutes at 25°C as described in Methods I in the presence of various concentrations of [^{3}H]WB-4101. Non specific binding was determined in the presence of 10 µM phentolamine. Points shown are those obtained in a single experiment, performed in duplicate and represent specific binding (total - non specific binding).

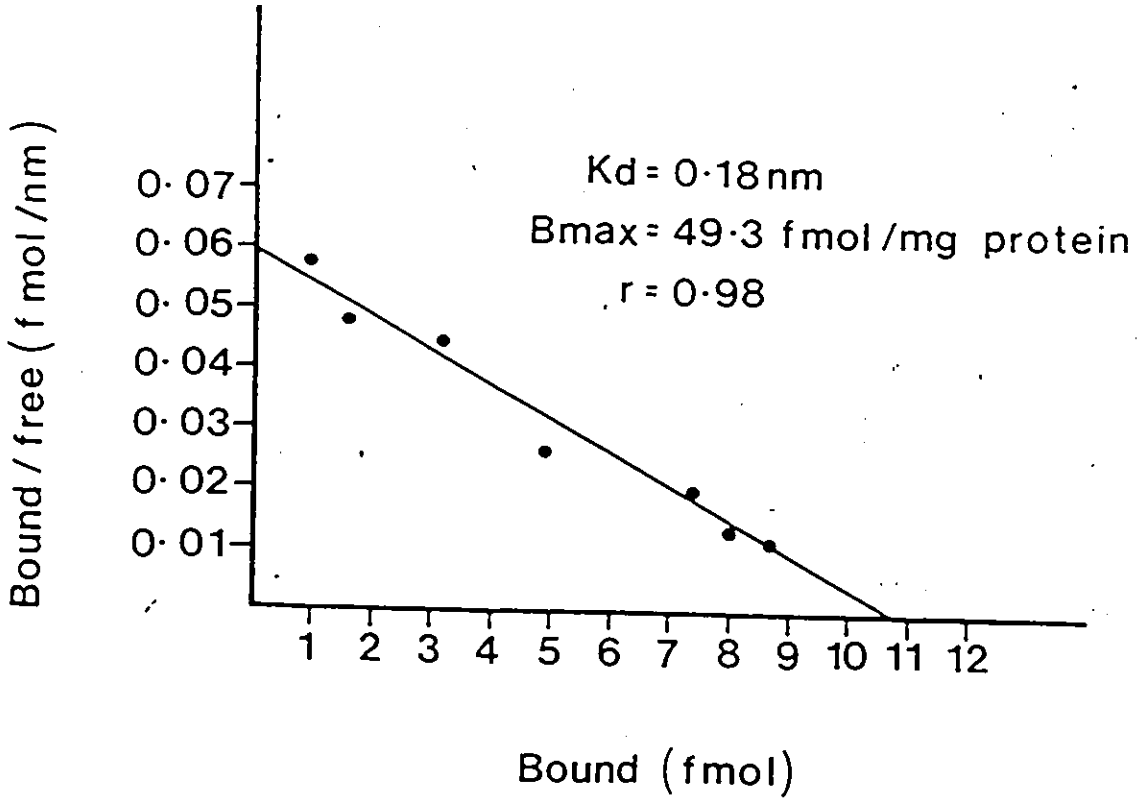

Figure 16. Scatchard plot of [^{3}H]WB-4101 binding to BAT homogenates of hamsters.

Details on binding experiments are given in Legend to Figure 15. The Scatchard plot was derived from linear regression of the binding data. K_d is determined from the slope of the line and the maximum binding B_{max} is obtained as the intercept with the abscissa. Correlation coefficient = 0.98. Protein in this individual assay = 0.22 mg.

TABLE 19

WB-4101 binding to BAT homogenates of hamsters and rats

		Kd nm	B max fmol/mg protein
Hamsters	normal (5)	0.19 ± 0.03	43.0 ± 2.5
	myopathic (5)	0.15 ± 0.01	42.3 ± 3.9
Rats	warm-adapted (3)	0.26 ± 0.04	52.1 ± 5.6
	cold-adapted (3)	0.14 ± 0.005*	57.7 ± 7.4

Values are means ± SE for the number of preparations indicated in parenthesis.

* Significant difference between warm- and cold-adapted rats ($p < 0.05$)

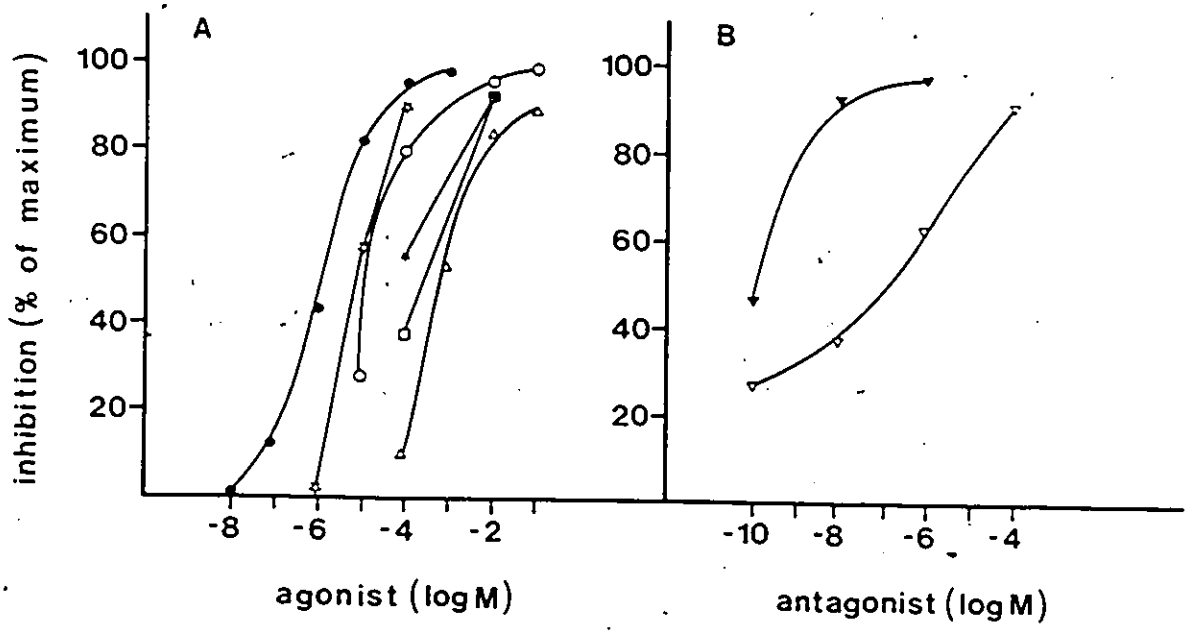

Figure 17. Inhibition of [^{3}H]WB-4101 binding by adrenergic agonists and antagonists.

Brown adipose tissue homogenates were incubated with 1.25 μM [^{3}H]WB-4101 in the presence and in the absence of the indicated concentrations of agonists (A) and antagonists (B) as described in Methods I. Binding is expressed as a percentage of specific binding. Each point represents an average of two determinations from at least two different homogenate preparations.

A. antagonists: ● (-) - adrenaline, ☆ (-) - noradrenaline
o (-) - phenylephrine, ★(-) - isoproterenol, □ (+) - adrenaline,
Δ (+) - noradrenaline.

B. agonists: ▼ prazosin, ▽ yohimbine

to a larger number of α_1-receptors on BAT of myopathic hamsters or to a change in their affinity.

Two recent reports estimate α-receptor affinity and number of hamster BAT in the same general range (K_d, 0.49nM; Bmax, 72.2 fmol/mg protein) (Mohell *et al.*, 1983) and (K_d, 0.27; Bmax, 33 fmol/mg protein) (Raasmaja *et al.*, in press). Both the above used the radioactive ligand [^{3}H]prazosin in crude membrane preparations. A lower concentration of binding sites would be expected in the total homogenates, as used in the present experiment, due to the non-membrane protein present. The receptor number reported here was however between the estimates of the two groups. Affinity of α_1-receptors was also found to be higher in the present experiments. This may be due to the different ligand used, since WB-4101 has a slightly greater affinity than prazosin for [^{3}H]prazosin binding sites (Mohell *et al.*, 1983b). Experiments by Mohell *et al.* (1983b) show that α-receptors were similar in crude membranes and in isolated brown adipocytes suggesting a post synaptic location. The similarity of data found here using [^{3}H]WB-4101 would indicate that the same receptors are being studied and these receptors are also situated on the brown adipocytes.

The WB-4101 binding in rats was in the same general range as that in hamsters. The K_d in warm- and cold-acclimated rats was 0.26 and 0.14 nM ($p<0.001$) and the Bmax was 52.1 and 57.7 binding sites/mg protein (not significantly different). The density of α-receptors in BAT remained the same while the affinity increased during cold acclimation. This experiment has shown that α_1-receptors in BAT of the rat and the hamster are quite similar in number and affinity. The increase in affinity

after cold acclimation is consistent with a particular role for these receptors in BAT at that time.

Alpha$_1$-receptors in warm- and cold-acclimated rats have also been studied using [^{3}H]prazosin (Raasmaja *et al.*, in press). These workers found no change in affinity and an increased density of α_1-receptors in crude membranes of BAT after 3 weeks cold acclimation. Since Bmax is related to tissue protein content, the lack of increase in receptor number in the present experiments, using total tissue homogenates may reflect a greater increase in non-membrane protein compared to membrane protein due to cold acclimation. The cause of the increased affinity after cold adaptation is not known, but may be due to strain differences or to some component in the homogenate which is removed during isolation of the membranes.

EXPERIMENT B-5 DENERVATION OF BROWN ADIPOSE TISSUE OF NORMAL AND MYOPATHIC HAMSTERS

BACKGROUND:

Contrary to the effect in rats, injected noradrenaline did not cause growth of brown adipose tissue in hamsters and at times was inhibitory to BAT growth (Experiment B-1). The role of the sympathetic innervation of the tissue, as opposed to the noradrenaline secreted, was therefore studied.

OBJECTIVE:

The objective was to study the effect of denervation of brown adipose tissue in normal and myopathic hamsters.

METHOD:

Male myopathic (BIO 14.6) and normal Syrian hamsters were purchased from Canadian Hybrid Farms, Halls Harbour, N.S.

BAT of 12 week old normal and myopathic hamsters was unilaterally denervated by cutting 4 of the 5 nerves supplying one side of the interscapular BAT while the hamsters were anaesthetized with ether. Three weeks later the hamsters were killed and the left and right sides of the pad dissected separately into ice-cold medium (mitochondria isolation medium). Half pads were cleaned, frozen on dry ice and stored at -80°C. Tissues were weighed, homogenized and assayed for protein and NA. (Methods C, J.ii)

RESULTS AND DISCUSSION:

Denervation of BAT resulted in a marked decline in protein content in normal hamsters to a level not significantly different from that of myopathic hamsters (Figure 18). A much smaller decrease in protein content occurred in denervated BAT of myopathic hamsters (Figure 18). Noradrenaline content of denervated tissue was reduced by 68% in normal hamsters and by 84% in myopathic hamsters (legend to Figure 18). Total noradrenaline content of intact tissue was smaller than normal in the myopathic hamster but its concentration was normal and similar to that seen before (Table 18). It would appear that the lesser trophic influence of the sympathetic innervation on the tissue cannot be attributed to noradrenaline since both the concentration and turnover of noradrenaline were found to be normal in BAT of myopathic hamsters (Experiment B-3).

It can be concluded that the sympathetic innervation of BAT does exert a trophic influence in normal hamsters and that this influence may be lesser in the myopathic hamster.

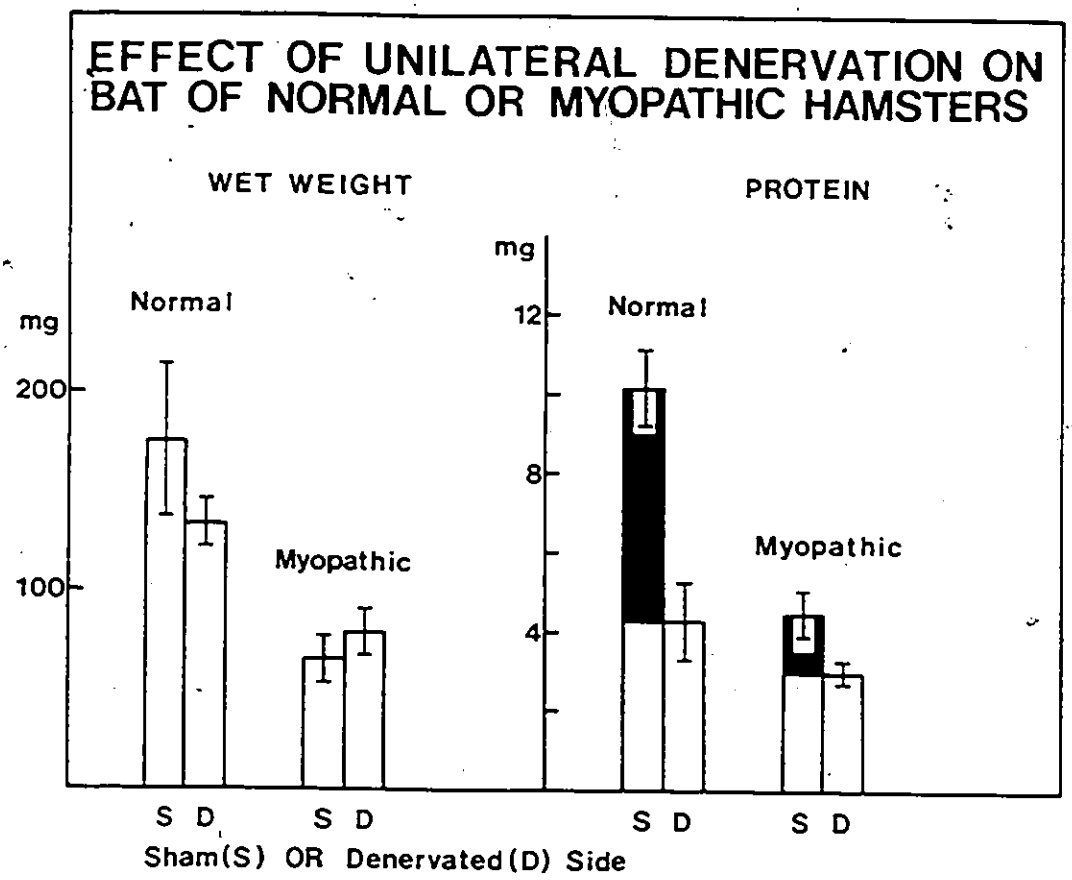

Figure 18. Effect of unilateral surgical denervation on brown adipose tissue of normal or myopathic hamsters.

Values shown are for half-pads (denervated on one side, sham-operated on the other) 3 weeks after operation (October-November). Noradrenaline contents (total ng) were: for normal hamsters, intact side 182.2 ± 23.7, denervated side 58.8 ± 9.4 ($n = 4$, $p < 0.005$); for myopathic hamsters, intact side 79.4 ± 12.7, denervated side 12.4 ± 2.55 ($n = 5$, $p < 0.001$).

EXPERIMENT B-6 THE EFFECT OF SHORT PHOTOPERIOD ON BROWN ADIPOSE TISSUE OF NORMAL AND MYOPATHIC HAMSTERS

BACKGROUND:

Although BAT of myopathic hamsters is smaller than that of normal hamsters the tissue appears to grow normally when animals are exposed to low temperatures (Himms-Hagen and Gwilliam, 1980). Two other environmental conditions known to stimulate growth of brown adipose tissue in hamsters are short photoperiod (Hoffman *et al.*, 1965) and a high fat diet (Wade, 1982). The effect of photoperiod on BAT growth in rats was unknown when these experiments were started.

OBJECTIVE:

The objective was to to study the effect of short photoperiod on BAT growth in normal and myopathic hamsters. A secondary objective was to compare short photoperiod-induced growth of BAT in hamsters and rats.

METHOD:

Male myopathic (BIO 14.6) and normal Syrian hamsters were purchased from Bio Research Consultants, Cambridge, MA and were 20 weeks old at the beginning of the experiment.

Groups of normal and myopathic hamsters were exposed to lighting schedules of 14L:10D (14 hours of light daily) or 2L:22D (2 hours of light daily) for periods of 10, 16 and 26 weeks. Additionally isolated mitochondria and BAT slices were studied by electron microscopy. (Methods G)

Young male Holtzman rats (Canadian Breeding Laboratories) were housed under the same conditions as the hamsters (Materials A ii) and kept for 16 weeks in either 14L:10D or 2L:22D lighting conditions.

After sacrifice interscapular BAT was removed, cleaned and weighed. Homogenization of the tissue and isolation of mitochondria were done as outlined in Methods A. Tissue protein, COX activity, DNA and mitochondrial GDP-binding were assayed (Methods C, D, E, B). Testes were removed and weighed.

RESULTS AND DISCUSSION:

A preliminary experiment in which groups of normal and myopathic hamsters were adapted to a short photoperiod (2L:22D) for 10 weeks resulted in the expected hypertrophy of BAT in normal hamsters; the protein content of the tissue almost doubled without any change in wet weight (Table 20). No change in protein content of BAT occurred in myopathic hamsters, even though there was a small increase in wet weight of the tissue. Regression of the testes occurred in both normal and myopathic hamsters that were adapted to a short photoperiod for 10 weeks (Table 20).

After 16 weeks in short photoperiod, BAT in normal hamsters showed an almost 2.5 fold increase in total protein without a change in wet weight of the tissue (Figure 19, Table 21). There was no change in DNA and total COX activity was increased (Figure 19). A small increase in wet weight of BAT of the myopathic hamster (Table 21) was accompanied by small increases in total protein and COX activity (Figure 19). Regression

TABLE 20

Effect of adaptation to short photoperiod for 10 weeks in September-November on normal or myopathic hamsters

	NORMAL		MYOPATHIC	
Photoperiod L:D	14:10	2:22	14:10	2:22
	(6)	(6)	(9)	(9)
Body wts, g				
Initial	110.7 ± 5.5	119.7 ± 3.8	104.7 ± 3.0	106.0 ± 2.4**
Final	117.2 ± 6.0	113.0 ± 6.4	108.4 ± 3.2	101.3 ± 2.7
Change in 10 wk	+6.5 ± 1.1	-6.7 ± 4.8$^{\#}$	+3.7 ± 1.7	-4.7 ± 2.8$^{\#}$
Organ wts, g				
Testes	3.39 ± 0.16	0.65 ± 0.30$^{\#\#\#}$	2.81 ± 0.15*	1.28 ± 0.39$^{**\#\#}$
Brown adipose tissue				
Wet wt, mg	433 ± 49	497 ± 24	181 ± 5****	213 ± 8$^{****\#\#}$
Protein, mg	18.0 ± 1.3	38.0 ± 4.7$^{\#\#}$	10.3 ± 0.7**	12.6 ± 0.9****
Cytochrome oxidase μg atoms O_2/min	43.6 ± 9.7	40.3 ± 5.1	16.2 ± 1.9***	18.4 ± 2.7***
Mitochondrial GDP binding	(3)	(3)	(3)	(3)
pmoles/mg protein	209.8 ± 15.3	274.5 ± 18.3	155.7 ± 19.2	216.0 ± 14.4

Values are means ± SE for the number of animals or of mitochondrial preparations indicated in parentheses.

Significant effect of myopathy, comparing hamsters treated in the same way, indicated by *($p < 0.05$), **($p < 0.01$), ***($p < 0.005$) or ****($p < 0.001$).

Significant effect of short photoperiod, comparing hamsters of the same type, indicated by #($p < 0.05$), ##($p < 0.005$) or ### ($p < 0.001$).

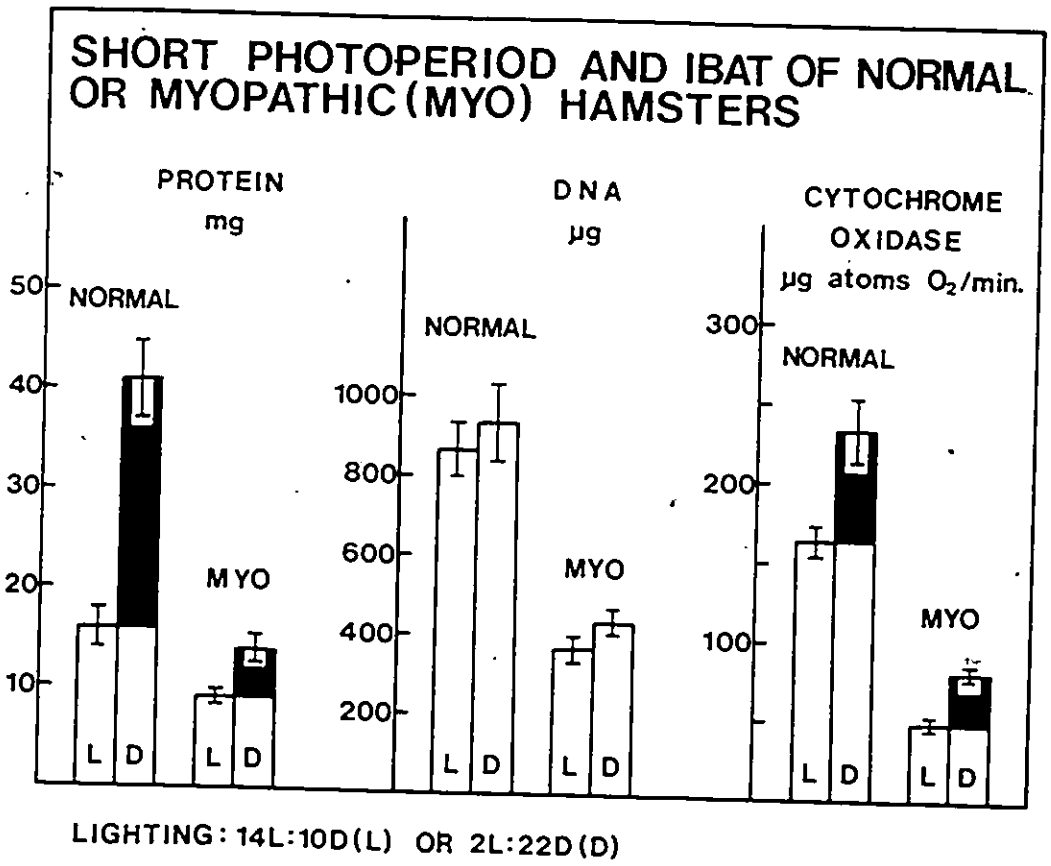

Figure 19. The effect of short photoperiod (16 wk in 2L:22D) on brown adipose tissue of normal or myopathic hamsters.

For further data see Figure 20 and Table 21.

TABLE 21

Effect of adaptation to short photoperiod for 16 weeks in January-May on normal or myopathic hamsters

	NORMAL		MYOPATHIC	
	14:10	2:22	14:10	2:22
	(8)	(8)	(12)	(12)
Body wts, g				
Initial	116.3 ± 1.8	123.0 ± 3.6	105.7 ± 3.1*	105.3 ± 3.1**
Final	124.0 ± 2.3	96.4 ± 4.1$^{\#\#\#}$	108.2 ± 2.5***	95.5 ± 2.8$^{\#\#}$
Change in 16 wk	+7.7 ± 1.3	-26.6 ± 4.1$^{\#\#\#}$	+2.5 ± 2.0	-9.7 ± 1.1$^{***\#\#\#}$
Brown adipose tissue				
Wet wt, mg	501 ± 13	490 ± 21	177 ± 4***	200 ± 10$^{\#***}$
	(4)	(4)	(4)	(4)
Mitochondrial GDP binding, pmoles/mg	269.6 ± 14.9	283.3 ± 6.5	243.6 ± 20.5	310.1 ± 9.8$^{\#}$

Values are means ± SE for the number of animals or of mitochondrial preparations indicated in parentheses. Other information for these hamsters is in Figs 19 and 20.

Significant effect of myopathy, comparing hamsters treated in the same way, indicated by *($p < 0.05$), **($p < 0.005$) or ***($p < 0.001$).

Significant effect of short photoperiod, comparing the same type of hamster, indicated by #($p < 0.05$) or ##($p < 0.005$).

of the testes occurred in short photoperiod-adapted normal and myopathic hamsters (Figure 20).

There was little effect of short photoperiod on the thermogenic state of BAT mitochondria, as evidenced by lack of change in mitochondrial GDP-binding, in normal hamsters and a small increase in the myopathic at 16 weeks only (Tables 20 and 21).

Both normal and myopathic animals lost weight during 10 and 16 weeks in short photoperiod (Tables 20 and 21). This is in contrast to weight gain reported by Wade and Bartness (1984) in hamsters kept in short photoperiod. The more severely restricted lighting conditions in the present experiments (2 hours of light compared to 8 hours daily) may be responsible for the difference in weight gain.

Continuation of the adaptation to short photoperiod for 26 weeks resulted in a reversal of changes in BAT and testes seen at 16 weeks. BAT weight and protein were similar to those of animals kept in long photoperiod and the testes recovered completely in size in the normal and partially in the myopathic animals (Table 22). The weight loss was less pronounced at 26 weeks than at 16 weeks in both normal and myopathic animals (Tables 21 and 22).

Thus in BAT of hamsters, growth is stimulated by short days. After a time, BAT seems to become photorefractory and the tissue resumes its normal size in much the same way as the gonads spontaneously regain normal size and function after being regressed by short photoperiod, although the photoperiod does not change (Goldman, 1983). That there might be a seasonal effect of the response of BAT growth to short photoperiod is indicated by a much larger increase in BAT protein

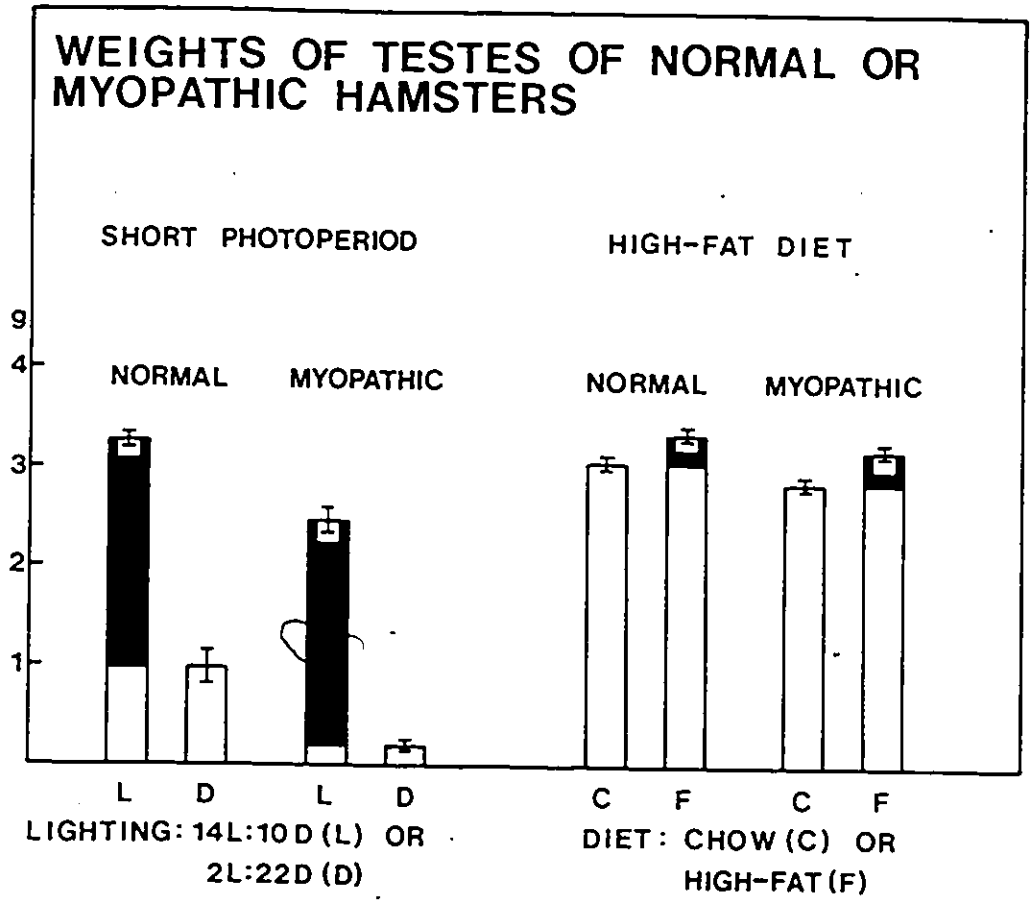

Figure 20. Effect of short photoperiod (16 wk in 2L:22D) or high-fat diet (sunflower seeds, 5-6 wk) on weights of testes of normal or myopathic hamsters.

Adaptation took place during January-May (short photoperiod) or May-June (high-fat diet). Further data for these experiments are in Tables 21 and 24 and Figures 19 and 25.

TABLE 22

Effect of adaptation to short photoperiod for 26 weeks in January-July on normal of myopathic hamsters

Photoperiod (L:D)	NORMAL		MYOPATHIC	
	14:10 (6)	2:22 (3)	14:10 (2)	2:22 (2)
Body wts, g				
Initial	120.8 ± 3.0	121.3 ± 2.6	114.5 ± 4.5	96.5 ± 11.5
Final	127.5 ± 2.6	118.0 ± 2.3	119.5 ± 5.5	90.5 ± 14.5
Change in 26 wk	+6.7 ± 2.2	-3.3 ± 0.3#	+5.0 ± 1.0	-6.0 ± 3.0
Brown adipose tissue				
Wet wg, mg	493.8 ± 24.1	499.0 ± 50.2	166.0 ± 26.5***	213.5 ± 6.5*
Protein, mg	17.1 ± 1.9	20.1 ± 3.9	14.4 ± 1.0	17.0 ± 2.0
Testes wts, g	3.47 ± 0.13	3.53 ± 0.06	2.67 ± 0.23*	1.49 ± 0.21**

Values are means ± SE for the number of animals indicated in parentheses.

Significant effect of myopathy, comparing hamsters treated in the same way, indicated by *($p < 0.05$), **($p < 0.005$) or ***($p < 0.001$).

Significant effect of short photoperiod, comparing the same type of hamster, indicated by #($p < 0.02$).

when hamsters were kept for 16 weeks at 2L:22D in the fall (data not shown) than in similar experiments conducted in the spring (this experiment).

Electron micrographic studies revealed no differences between tissue slices or mitochondrial preparations of normal and myopathic hamsters or between animals kept for 16 weeks in 14L:10D and 2L:22D lighting conditions. Figures 21-24 show typical electron micrographs of BAT and BAT-mitochondria from normal and myopathic hamsters.

Rats kept for 16 weeks under reduced lighting conditions did not differ from controls in body weight gain, BAT weight, COX activity or testes weight (Table 23). BAT protein was slightly but significantly increased (Table 23). Although short photoperiod does not affect BAT growth in rats to the same extent as in hamsters, there seems to remain nevertheless a tendency toward this response. As in the hamster, GDP-binding to BAT mitochondria did not increase in rats kept in a short photoperiod (Table 23). Earlier experiments by Hagelstein and Folk (1979) suggested that short photoperiod alone did not produce cold acclimation in rats, but did seem to enhance the effect of low temperatures on BAT weight. However, pinealectomy of rats or short photoperiod did not alter their ability to increase BAT in the cold (Kott and Horwitz, 1983). The pineal was also not necessary for growth of BAT in rats due to cafeteria feeding (Viswanathan and George, 1984).

Figure 21. Electron micrograph of brown adipose tissue mitochondria isolated from normal Syrian hamsters (X 16 000).

Figure 22. Electron micrograph of brown adipose tissue mitochondria isolated from myopathic hamsters (X 16 000).

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Figure 23. Electron micrograph of brown adipose tissue of normal Syrian hamsters (X 16 000).

Figure 24. Electron micrograph of brown adipose tissue of myopathic hamsters (X 16 000).

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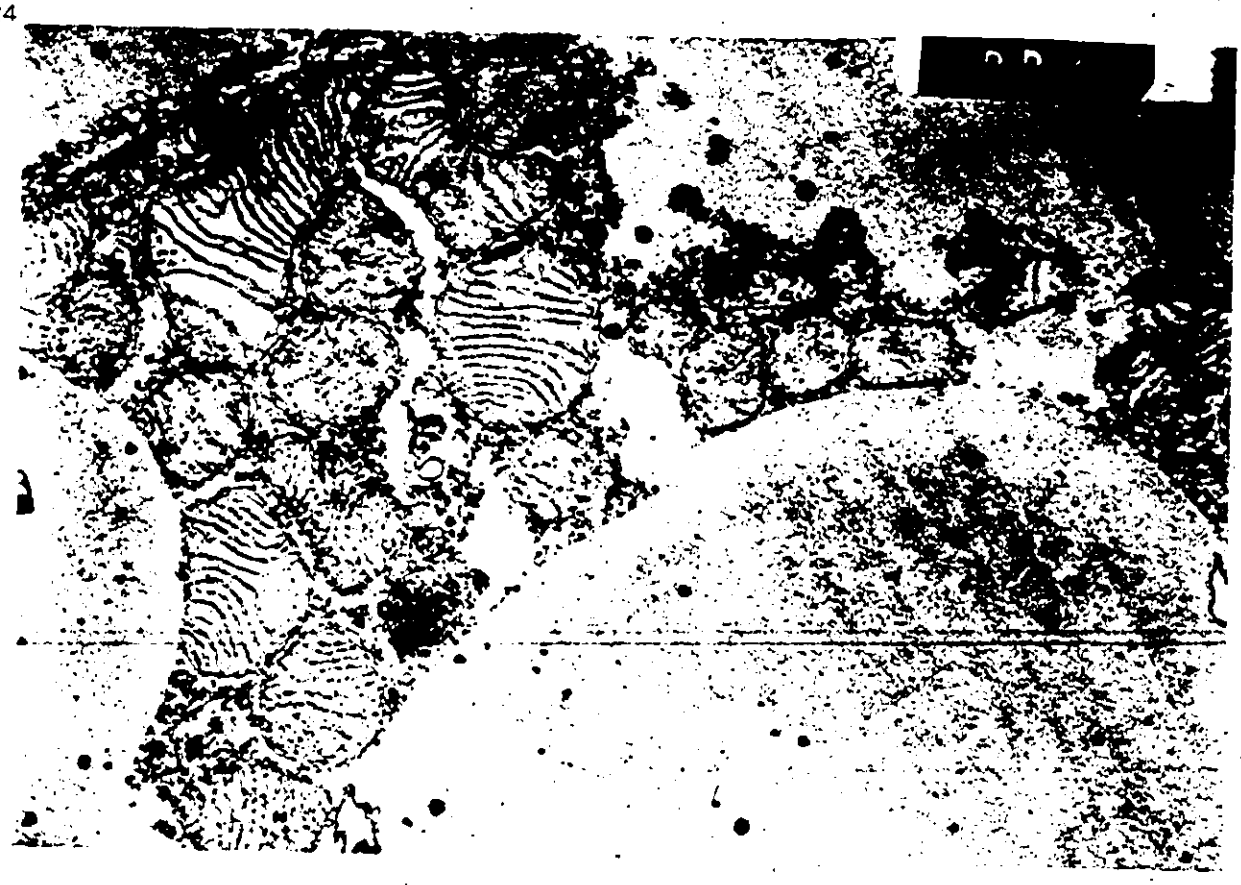

TABLE 23

Effect of adaptation to short photoperiod for 16 weeks on rats

Photoperiod L:D	14:10 (12)	2:22 (12)
Body weights, g		
Initial	223.3 ± 10.3	219.1 ± 10.2
Final	579.6 ± 15.3	566.4 ± 14.8
Change in 16 weeks	357.2 ± 18.8	347.5 ± 15.1
Organ weights		
Testes, g	3.51 ± 0.08	3.61 ± 0.10
Brown adipose tissue		
Wet weight, g	811 ± 49	909 ± 51
Protein, mg	22.5 ± 1.3	27.3 ± 1.8*
Cytochrome oxidase g atoms O_2/min	306.6 ± 12.7	325.0 ± 21.9
DNA, µg	670.6 ± 46.1	700.8 ± 67.3
Mitochondrial GDP binding pmoles/mg protein	(4) 36.7 ± 6.0	(4) 43.8 ± 9.0

Values are means ± SE for the number of animals or mithochondrial preparations indicated in parenthesis.

*Significant effect of photoperiod ($p < 0.05$).

EXPERIMENT B-7 THE EFFECT OF HIGH-FAT DIET ON BROWN ADIPOSE TISSUE OF NORMAL AND MYOPATHIC HAMSTERS

BACKGROUND:

Refer to BACKGROUND, Experiment B-6.

OBJECTIVE:

The objective was to study the effect of a high-fat diet on BAT growth in normal and myopathic hamsters.

METHOD:

Male myopathic (BIO 14.6) and normal Syrian hamsters were purchased from Canadian Hybrid Farms, Halls River, N.S. and were 6 weeks old at the beginning of the experiment.

After sacrifice of hamsters, interscapular BAT was removed, cleaned and weighed. Homogenization of the tissue and isolation of mitochondria were done as outlined in Methods A. Tissue protein, COX activity, DNA and mitochondrial GDP-binding were assayed (Methods C, D, B). White epididymal adipose tissue and testes were removed and weighed.

Groups of normal and myopathic hamsters were fed either chow or chow plus sunflower seeds (hulled, not salted or roasted).

Oxygen uptake of conscious, unrestrained hamsters was measured as in Methods H.

RESULTS AND DISCUSSION:

Adaptation of hamsters to a diet of sunflower seeds plus chow

resulted in the expected increase in the proportion of energy intake derived from fat; from 12% in the chow-fed hamsters to 62.5% (normal) or 60.5% (myopathic) in the sunflower seed fed hamsters (Table 24). No change in total energy intake occurred in either group of hamsters, but body weight gain and body fat (wet weight of white epididymal adipose tissue) were both greater in hamsters on the high-fat diet (Table 24). Wet weight of the testes increased in both groups of hamsters fed the high-fat diet (Figure 20). Hypertrophy of BAT in normal hamsters fed the high-fat diet was due to increases in both wet weight and protein content, without any change in DNA content (Table 24 and Figure 25). There was no change in protein or DNA content of BAT of myopathic hamsters fed a high-fat diet, although a small increase in wet weight occurred (Table 24 and Figure 25).

The myopathic hamsters gained much less weight than the normal hamsters during the 5-6 weeks of the experiment and their body fat was less, regardless of the diet (Table 24). Their total energy intake was, however, only slightly less than that of the normal hamsters, being 86.6% of normal on the chow diet and 88.8% of normal on the high-fat diet. The composition of their energy intake was the same as that of normal hamsters, regardless of diet (Table 24). Thus body weight gain per unit of food eaten was only 70.3% and 70.6% of that of normal hamsters, when the diet was chow or high-fat respectively (Table 25). This apparently lower metabolic efficiency cannot be explained by thermogenic action of BAT or by a change in metabolic rate, since both GDP-binding to BAT mitochondria (Table 24) and resting metabolic rate (Table 25) were similar in normal and myopathic hamsters. The increase in metabolic rate after

TABLE 24

Effect of a high-fat diet on normal or myopathic hamsters.

	NORMAL		MYOPATHIC	
	CHOW (11)	CHOW + SFS (12)	CHOW (12)	CHOW + SFS (12)
Body wts, g				
Initial	53.0 ± 0.9	53.8 ± 0.8	52.7 ± 1.1	52.0 ± 1.4
Final	108.2 ± 1.7	126.3 ± 2.1##	86.2 ± 1.9*	97.7 ± 1.5*##
Change in 5-6 wk	+55.2 ± 2.0	+72.5 ± 2.0##	+33.5 ± 1.7*	+45.7 ± 1.5*##
Food intake kcal/d	28.3 ± 0.54	27.7 ± 0.45	24.5 ± 0,49*	24.6 ± 0.29*
Wt gain/food eaten g/(kcal/d)	1.95 ± 0.054	2.62 ± 0.065##	1.37 ± 0.070*	1.85 ± 0.047*##
Organ wts				
Epididymal adipose tissue, g	1.62 ± 0.10	3.11 ± 0.14##	0.82 ± 0.06*	1.30 ± 0.05*##
Brown adipose tissue, mg	266 ± 9	405 ± 17##	120 ± 6*	145 ± 8*#
Brown adipose tissue				
Mitochondrial GDP binding	(4)	(4)	(4)	(4)
pmoles/mg protein	336.2 ± 19.7	401.3 ± 20.2	357.0 ± 14.1	409.2 ± 116.5
Composition of food intake, kcal	(11)	(12)	(12)	(12)
Chow	28.3 ± 0.54	3.9 ± 0.29## (14%)	24.5 ± 0.49*	4.3 ± 0.28## (17.1%)
SFS		23.8 ± 0.36## (86%)		20.3 ± 0.42*## (82.9%)
Composition of energy intake, kcal				
Carbohydrate	17.6 ± 0.33 (62%)	5.6 ± 0.18## (20%)	15.2 ± 0.30* (62%)	5.4 ± 0.19## (22%)
Fat	3.3 ± 0.06 (12%)	17.3 ± 0.26## (62.5%)	2.9 ± 0.06* (12%)	14.9 ± 0.31*## (60.5%)
Protein	7.4 ± 0.14 (26%)	4.8 ± 0.09## (17.5%)	6.4 ± 0.13* (26%)	4.3 ± 0.09*## (17.5%)

Values are means ±SE for the number of animals or of mitochondrial preparations indicated in parentheses. Hamsters from Canadian Hybrid Farms were fed chow or chow plus sunflower seeds (SFS) for 5-6 weeks in May-June. Values in parentheses in the lower part of the Table indicate the proportion of total energy intake derived from the component listed.

Significant effect of the high-fat diet, comparing the same type of hamster, indicated by #($p < 0.025$) or ##($p < 0.001$).

Significant effect of myopathy, comparing hamsters fed the same diet, indicated by *($p < 0.001$).

Other information for these hamsters is in Figures 20 and 25.

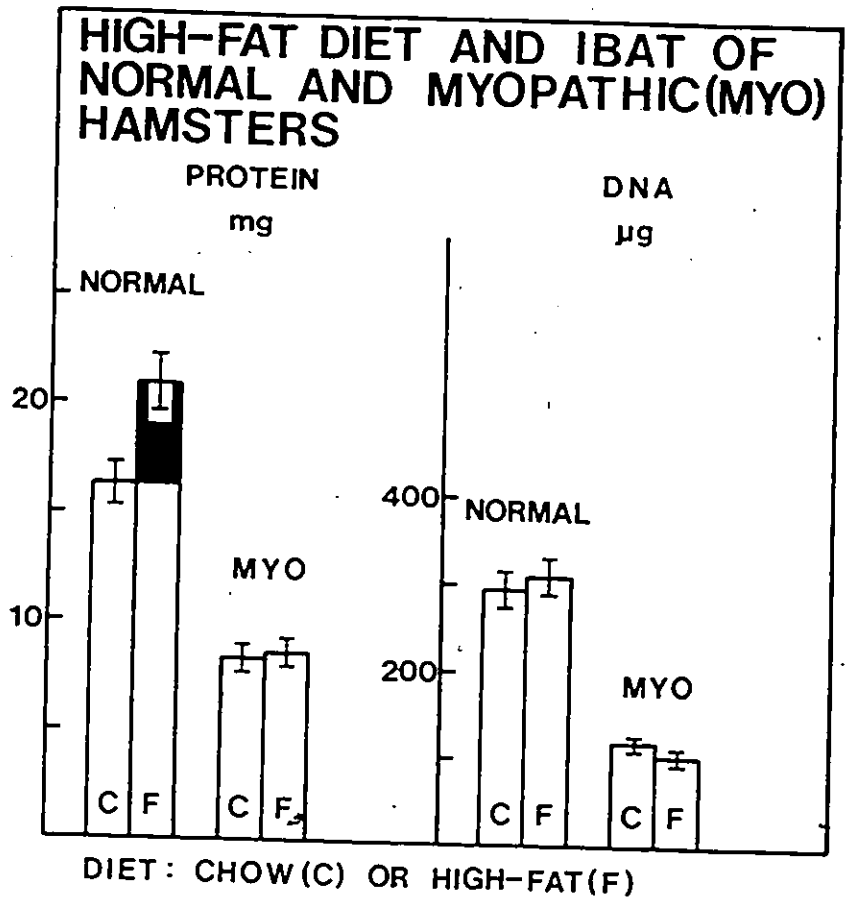

Figure 25. Effect of high-fat diet on brown adipose tissue of normal or myopathic hamsters.

For further data see Figure 20 and Table 24.

TABLE 25

Effect of noradrenaline on resting metabolic rates of conscious normal or myopathic hamsters

	NORMAL (4)	MYOPATHIC (4)
Body wts, g	122.8 ± 1.0	103.3 ± 1.4***
Metabolic rate, ml O_2/h. g		
Resting	0.424 ± 0.037	0.395 ± 0.025
Increase after NA	+0.494 ± 0.032##	+0.339 ± 0.021**##
% increase	+118.6 ± 10.0#	+86.4 ± 5.9*##

Values are means ± SE for the number of animals indicated in parentheses. Noradrenaline (NA) was administered subcutaneously, 0.8 or 1.6 mg/kg.

Significant effect of myopathy is indicated by *($p < 0.05$), **($p < 0.01$) or ***($p < 0.001$).

Significant effect of noradrenaline (paired t-test) is indicated by #($p < 0.005$) or ##($p < 0.001$).

injection of noradrenaline was lower in the myopathic hamsters agreeing with others (Horwitz and Hanes, 1974), and probably caused by the smaller amount of BAT.

It can be concluded that myopathic hamsters respond normally to the availability of a more palatable high-fat diet in that they select a higher proportion of fat yet maintain a normal energy intake. The normal trophic response of BAT to the change in diet is, however, lacking in the myopathic hamster.

EXPERIMENT B-8 THE EFFECT OF PINEALECTOMY ON GROWTH OF BROWN ADIPOSE TISSUE IN HAMSTERS

BACKGROUND:

To this point the purpose of the experiments was to study the role of the sympathetic nervous system in the growth of BAT in normal and myopathic hamsters. However, since noradrenaline did not appear to mediate BAT growth, a possible role for another mediator was studied. Growth of BAT in short photoperiod-adapted hamsters appears to be mediated by the pineal gland (Reiter, 1975) and the pineal hormone, melatonin, is known to have a trophic effect on BAT (Heldmaier and Hoffmann, 1974). Myopathic hamsters did not grow more BAT when adapted to short photoperiod (Experiment B-6). This might suggest that either their BAT did not respond to the pineal-mediated mechanism or that their pineal glands were not functioning effectively. However, the normal regression of their testes, known also to be mediated by the pineal (Reiter *et al.*, 1976) suggests that their pineal glands were functioning and a refractoriness to melatonin seems a more likely possibility. Myopathic hamsters, however, also did not grow more BAT in response to a high-fat diet (Experiment B-7). It seems unlikely that this could be due to a refractoriness to melatonin. However, the possible participation of the pineal in the trophic effect of a high-fat diet could not be excluded.

OBJECTIVE:

The objective was to find out whether the pineal gland is involved in the trophic response of brown adipose tissue to cold or to a high fat diet.

METHOD:

Pinealectomized and sham-operated hamsters were purchased from Charles River Company, Boston.

Pinealectomized and sham-operated hamsters were (1) acclimated to 24°C or 4°C for three weeks or (2) exposed to 4°C or 24°C for 17 hours (1400 to 0700 h) or (3) fed either chow or a high-fat diet of sunflower seeds (hulled, not salted or roasted) and chow.

After sacrifice of animals (7-8 weeks old) interscapular BAT was removed, cleaned and weighed. Homogenization of the tissue and isolation of mitochondria were done as outlined in Methods A. Tissue protein, COX activity, DNA and mitochondrial GDP-binding were assayed (Methods C, D, E, B). Epididymal white adipose tissue and testes were removed and weighed.

RESULTS AND DISCUSSION:

Pinealectomized or sham-operated hamsters living in the cold for 3 weeks increased their food intake, reduced their rate of body weight gain and white adipose tissue (Table 26). Cold-induced gonadal regression occurred in both sham-operated and pinealectomized animals (Table 26). The wet weight of BAT was slightly increased in sham-operated and not altered in pinealectomized hamsters by acclimation to cold, but large increases in tissue protein and DNA and increased GDP-binding (Figures 26 and 27) indicate normal tissue hypertrophy and activation in the cold in the absence of the pineal. The only differences between pinealectomized and sham-operated animals was a

TABLE 26

Effect of pinealectomy on the response of hamsters to acclimation to cold

Temperature of acclimation	SHAM-OPERATED 24°C (12)	SHAM-OPERATED 4°C (12)	PINEALECTOMISED 24°C (12)	PINEALECTOMISED 4°C (12)
Body wts, g				
Initial	95.5 ± 2.0	91.5 ± 1.8	90.1 ± 2.0	89.9 ± 2.5
Final	106.7 ± 2.2	91.3 ± 2.0**	106.2 ± 3.6	84.0 ± 4.0**
Change in 3 wk	+11.2 ± 0.8	-0.2 ± 1.1**	+16.1 ± 2.2#	-5.9 ± 3.7**
Food intake				
kcal/d	24.8 ± 0.75	35.4 ± 1.65**	29.6 ± 1.30##	36.6 ± 2.49*
Organ wts				
Testes, g	3.06 ± 0.09	2.43 ± 0.21*	3.12 ± 0.11	2.19 ± 0.23**
Epididymal adipose tissue, g	2.03 ± 0.09	0.94 ± 0.08**	2.01 ± 0.16	0.83 ± 0.11**
Brown adipose tissue, mg	324 ± 13.0	378 ± 19.0*	338 ± 18.0	363 ± 17.0

Values are means ± SE for the number of animals indicated in parentheses. Hamsters from Charles River Company were adapted to 24°C or to 4°C for 3 wk in July-August.

Significant effect of cold, comparing the same type of hamster, indicated by *($p < 0.05$) or **($p < 0.001$).

Significant effect of pinealectomy, comparing hamsters treated in the same way, indicated by #($p < 0.05$) or ##($p < 0.005$).

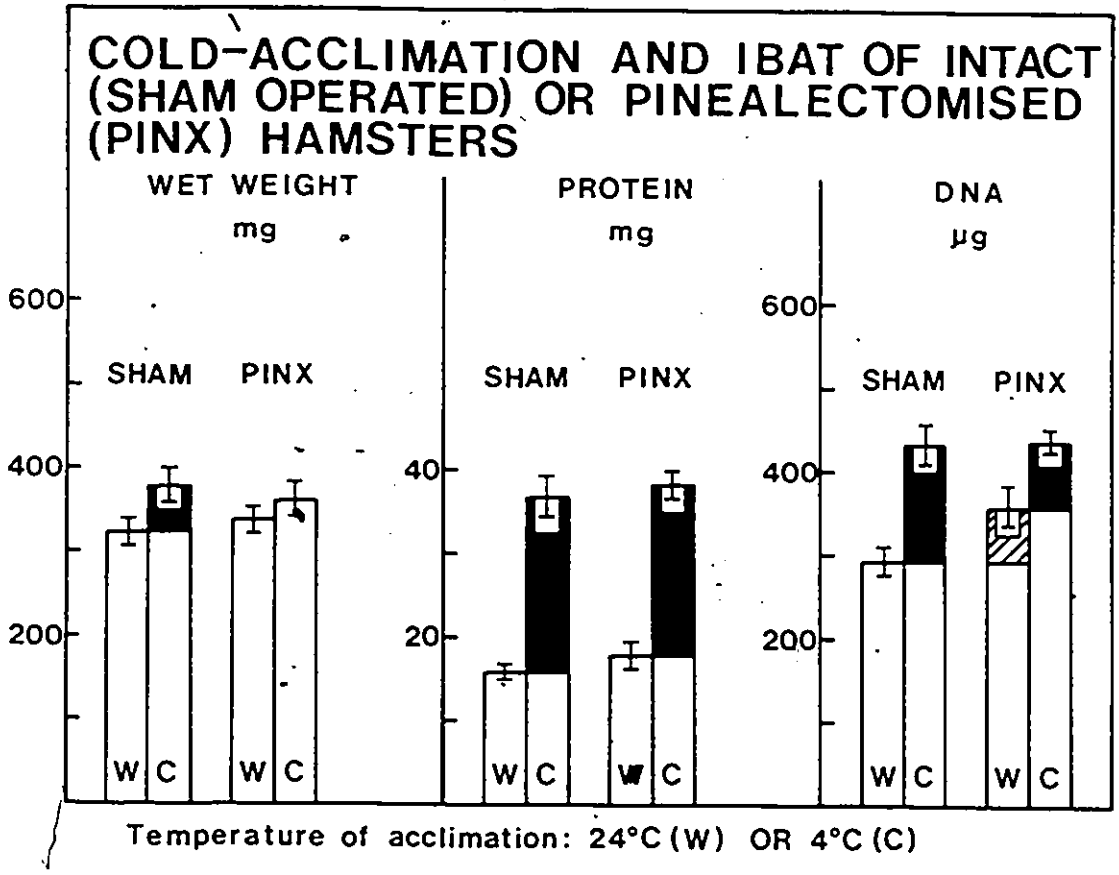

Figure 26. Effect of acclimation to cold on brown adipose tissue of pinealectomized or sham-operated hamsters.

Further data are in Table 26. Significant differences between warm- and cold-acclimated animals are indicated by the black portions of the bars. Significant differences between pinealectomized and sham-operated animals are shown by cross-hatched portions of the bars.

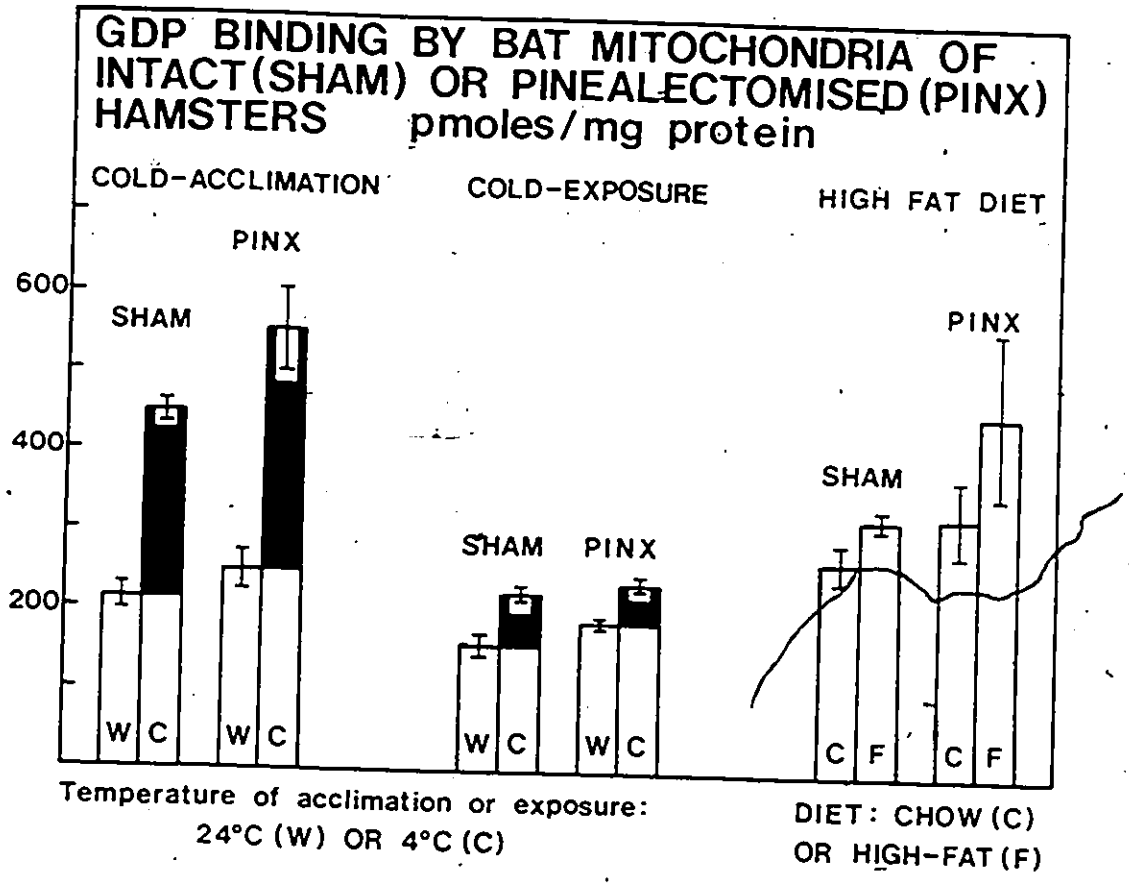

Figure 27. Effect of acclimation to cold, acute exposure to cold, or adaptation to a high-fat diet on the thermogenic state (level of GDP-binding) of mitochondria isolated from brown adipose tissue.

Bars are the means ± SE of values for 4 (cold-acclimation, high-fat diet) or 3 (cold exposure) mitochondrial preparations. For further data and information see Tables 26-28 and Legend to Figure 26.

slightly greater food intake, weight gain and greater cellularity (DNA) in BAT of pinealectomized animals at 24°C (Table 26, Figure 26).

Tissue protein (Table 27) and GDP-binding (Figure 27) was significantly increased in sham and pinealectomized animals exposed to 4°C for 17 hours indicating that early activation of the thermogenic response and growth of the tissue also does not require the pineal.

Sunflower seeds added to the diet of hamsters resulted in a very large increase in the proportion of energy derived from fat, a drastic reduction in the proportion of carbohydrate and a smaller but significant decrease in protein (Table 28). This diet either did not change the total energy intake (pinealectomized) or increased it slightly (sham) (Table 28). Despite the almost unchanged energy intake, a large increase in body weight gain and white adipose tissue occurred in both groups, suggesting an increase in metabolic efficiency, as described previously (Wade, 1982). The high-fat diet caused increased wet weight of BAT and increased protein without a change in DNA (Figure 28) or in GDP-binding (Figure 27) and occurred in the absence of the pineal gland (Table 28, Figures 27 and 28).

It is concluded that the pineal gland is not required for either cold- or diet-induced growth of BAT in the hamster.

TABLE 27

Effect of pinealectomy on the response of hamsters to acute cold exposure

Temperature	SHAM-OPERATED		PINEALECTOMISED	
	24°C	4°C	24°C	4°C
	(4)	(5)	(5)	(6)
Body wts, g	126.3 ± 4.1	121.4 ± 3.8	124.0 ± 4.4	119.5 ± 5.0
Brown adipose tissue				
Wet wt, mg	488.3 ± 22.3	312.0 ± 26.3*	486.0 ± 12.5	303.2 ± 18.1**
Protein, mg	14.4 ± 2.0	31.4 ± 2.1**	17.9 ± 1.4	31.7 ± 2.1**

Values are means ± SE for the number of animals indicated in parentheses. Hamsters from Charles River Company were placed at 4°C for 17 h (1400 h to 0700 h next day) or left at 24°C.

Significant effect of cold-exposure, comparing the same type of hamster, indicated by *($p < 0.005$) or **($p < 0.001$).

There are no significant effects of pinealectomy.

TABLE 28

Effect of pinealectomy on the response of hamsters to a high-fat diet.

	SHAM-OPERATED		PINEALECTOMISED	
	CHOW (12)	CHOW + SFS (12)	CHOW (12)	CHOW + SFS (12)
Body wts, g				
Initial	62.8 ± 0.9	63.9 ± 1.1	58.6 ± 1.1	57.7 ± 1.3
Final	107.3 ± 1.9	132.8 ± 3.1**	102.2 ± 2.4	125.6 ± 3.0**
Change in 5-6 wk	+44.4 ± 2.1	+68.8 ± 2.4**	+43.6 ± 2.0	+67.9 ± 2.1**
Food intake				
kcal/d	26.23 ± 0.65	29.56 ± 0.82**	26.72 ± 0.78	28.94 ± 0.85
Wt gain/food eaten				
g/(kcal/d)	1.70 ± 0.07	2.33 ± 0.07**	1.63 ± 0.05	2.35 ± 0.06**
Organ wts				
Testes, g	2.96 ± 0.05	3.30 ± 0.10**	2.93 ± 0.09	3.10 ± 0.07
Epididymal adipose tissue, g	1.92 ± 0.12	3.83 ± 0.24**	1.77 ± 0.12	3.84 ± 0.17**
Brown adipose tissue, mg	305 ± 10	411 ± 24**	276 ± 12.5	420 ± 19.3**
Composition of food intake, kcal				
Chow	26.23 ± 0.65 (100%)	3.53 ± 0.37** (12%)	26.72 ± 0.78 (100%)	3.71 ± 0.30** (12.4%)
SFS	-	26.03 ± 0.77** (88%)	-	25.23 ± 0.72** (87.6%)
Composition of energy intake, kcal				
Carbohydrate	16.26 ± 0.40 (62%)	5.65 ± 0.24** (19.1%)	16.57 ± 0.49 (62%)	5.63 ± 0.23** (19.5%)
Fat	3.10 ± 0.08 (11.8%)	18.85 ± 0.54** (63.8%)	3.15 ± 0.09 (11.8%)	18.30 ± 0.52** (63.2%)
Protein	6.87 ± 0.17 (26.2%)	5.06 ± 0.17** (17.1%)	7.00 ± 0.21 (26.2%)	5.01 ± 0.16** (17.3%)

Values are means ±SE for the number of animals indicated in parentheses at the head of each column. Hamsters from Charles River Company were fed chow or chow plus sunflower seeds (SFS) for 5-6 wk in May-June. Values in parentheses indicate the proportion of total energy intake derived from the component listed.

Significant effect of the high-fat diet, comparing the same type of hamster, indicated by *($p < 0.005$) or **($p < 0.001$).

There are no significant effects of pinealectomy.

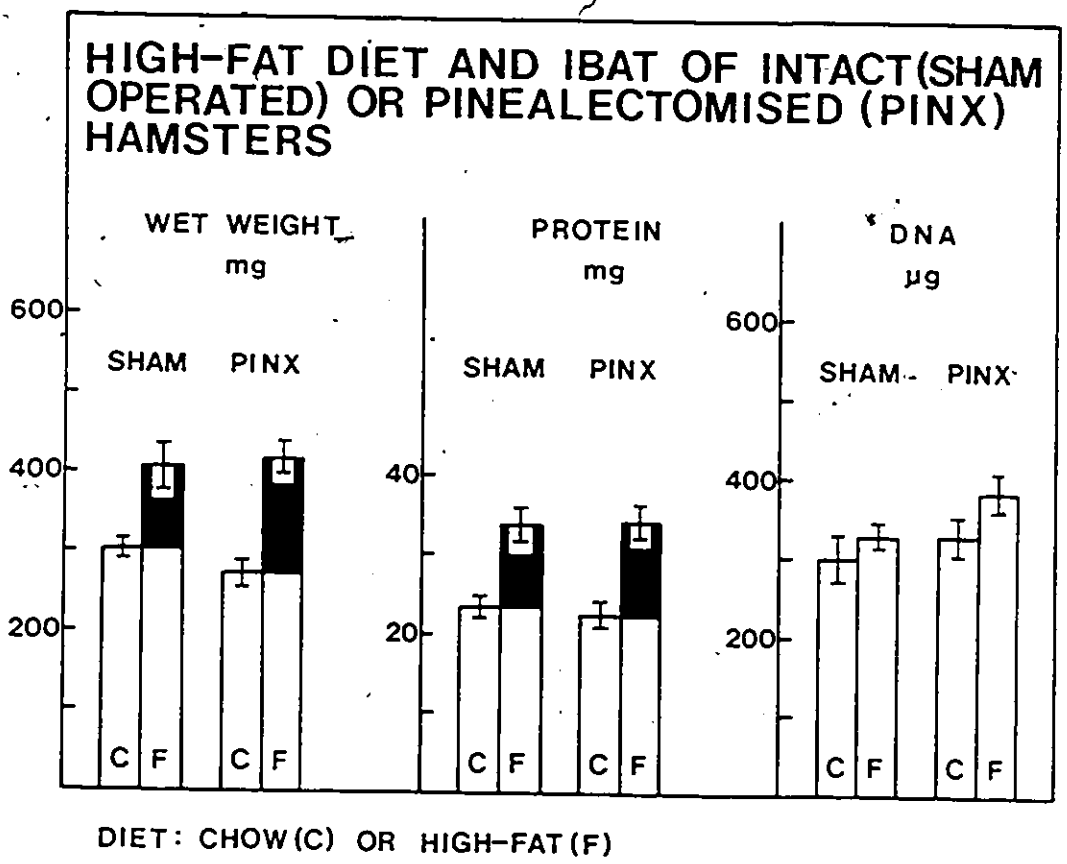

Figure 28. Effect of adaptation to a high-fat diet on brown adipose tissue of pinealectomized or sham-operated hamsters.

For further data and information see Table 28 and Legend to Figure 27.

EXPERIMENT B-9 THE EFFECTS OF MELATONIN TREATMENT ON GROWTH OF BROWN ADIPOSE TISSUE

BACKGROUND:

Melatonin treatment has been shown to cause growth of BAT in hamsters. Melatonin in beeswax implants (Heldmaier and Hoffmann, 1974) and injected late in the day (Wade and Bartness; 1984) results in growth of BAT, in Djungarian and Syrian hamsters respectively. Implants of melatonin in silastic capsules cause gonadal regression in Syrian hamsters (Turek *et al.*, 1975).

To test the possibility that BAT of myopathic hamsters may be unresponsive to the trophic effects of melatonin and that this may be the reason for the reduced size of the tissue, both normal and myopathic hamsters were treated with melatonin, first by continuous infusion with minipump and then by melatonin implanted in silastic capsules.

OBJECTIVE:

The objective was to study the growth of BAT in normal and myopathic hamsters treated with melatonin.

METHOD:

Male hamsters, myopathic (BIO 14.6) and normal, were purchased from Bio Research Consultants, Cambridge, MA (Group I) or from Canadian Hybrid Farms, Halls Harbour, N.S. (Groups II-IV)

Four groups of normal and myopathic hamsters were implanted with devices which allow continuous release of melatonin. They were

anaesthetized with Somnitol (0.35 ml Somnitol/saline (1:1) for normal hamsters and 0.30 ml for myopathic hamsters).

Group 1- At 11 weeks of age, melatonin or vehicle was infused over a period of six weeks by osmotic minipumps, implanted under the skin through a small dorsolateral incision. Minipumps were replaced after 2 weeks and 4 weeks alternating the side of placement. Melatonin was dissolved in propylene glycol (0.76% w/v) to deliver 100 µg/hamster/day. The experiment was carried out from October to December, for a total of 6 weeks.

Group 2 Hamsters in this group were 22 weeks old at the beginning of the experiment. Melatonin was implanted in two, 50 mm capsules of silastic tubing (0.058 in.I.D. by 0.077 in. O.D.) sealed with Silicone Type A Adhesive and placed beneath the skin of the back as described by Turek *et al.* (1975). Empty capsules were placed in control hamsters. The experiment was done during July to October for 8 weeks and 13 weeks.

Group 3 Twenty-two week old hamsters were implanted with two 50 mm silastic capsules containing melatonin or empty capsules for 10 weeks during September to December.

Group 4 Fourteen week old hamsters were implanted with two, 50 mm silastic capsules containing melatonin or empty capsules for 8 weeks during February and March.

At the end of the treatment period hamsters were sacrificed and interscapular BAT was removed, cleaned and weighed. Homogenates were made, tissue protein and COX activity were measured (Methods A, C, D). Testes and epididymal white adipose tissue were removed and weighed.

RESULTS AND DISCUSSION:

In Group 1, interscapular BAT weight, protein and epididymal fat pad weight were significantly reduced in myopathic hamsters. Melatonin had no effect on any of the parameters measured (Table 29).

At the end of 8 weeks of treatment, animals in Group 2 had interscapular BAT which was slightly, although not significantly, larger in melatonin treated animals, and testes of some animals were smaller, suggesting an effect of melatonin on these tissues. Melatonin implants were left in another five weeks at which time no difference was seen between treated and non-treated groups (Table 30).

In Groups 3 and 4, treatment for 8 weeks and 10 weeks produced no significant growth of BAT (Tables 31 and 32).

It was then impossible to judge whether BAT of myopathic hamsters is less responsive to melatonin because of the failure to demonstrate the trophic effect of melatonin in normal hamsters. Since the effect of photoperiod on BAT growth may depend on the season of the year (Experiment B-4) hamsters were treated with melatonin at different seasons, fall, summer and early spring. However, no response was detected at any time of year.

All four experiments failed to reproduce the gonadal regression of the testes of Syrian hamsters with melatonin implants as seen by Turek *et al.* (1975). To my knowledge there are no other reports of effects on gonads on Syrian hamsters due to continuously available melatonin although afternoon injections of melatonin cause gonadal regression and growth of BAT in Syrian hamsters (Wade and Bartness, 1984).

TABLE 29

Effect of treatment with melatonin implants on brown adipose tissue of normal or myopathic hamsters

(Experiment 1: minipumps, 6 weeks)

	NORMAL		MYOPATHIC	
	Control (4)	Melatonin (4)	Control (4)	Melatonin (4)
Body weights, g				
Initial	82.0 ± 2.0	78.8 ± 2.5	75.5 ± 3.3	73.5 ± 2.2
Final	102.0 ± 1.9	99.0 ± 4.7	93.5 ± 2.9	94.0 ± 3.1
Change in 6 weeks	20.0 ± 1.1	20.3 ± 2.6	18.0 ± 1.6	20.5 ± 1.8
Organ weights				
Gonadal fat, g	1.19 ± 0.05	1.28 ± 0.10	0.62 ± 0.07#	0.54 ± 0.05#
Testes, g	3.26 ± 0.05	3.14 ± 0.03	3.17 ± 0.09	3.16 ± 0.13
Brown adipose tissue				
Wet weight, mg	333.8 ± 12.0	318.3 ± 8.9	148.8 ± 10.5#	163.5 ± 12.1#
Protein, mg	31.9 ± 0.9	28.7 ± 1.5	12.5 ± 0.9#	14.1 ± 1.3#

Season: Fall

Values are means ± SE for the number of animals indicated in parentheses.

Significant effect of myopathy, comparing hamsters treated in the same way indicated by #($p < 0.001$).

TABLE 30

Effect of treatment with melatonin implants on brown adipose tissue of normal or myopathic hamsters

(Experiment 2: silastic capsules, 8 and 13 weeks)

	NORMAL		MYOPATHIC	
	Control	Melatonin	Control	Melatonin
8 weeks	(4)	(4)	(4)	(4)
Body weights, g				
Initial	103.5 ± 1.8	115.5 ± 7.3	102.5 ± 0.5	95.8 ± 3.2
Final	108.0 ± 2.1	119.8 ± 9.1	104.8 ± 2.1	100.3 ± 3.3
Change in 8 weeks	4.5 ± 2.3	4.3 ± 1.8	2.3 ± 1.7	3.0 ± 2.5
Organ weights				
Gonadal fat, g	1.50 ± 0.41	1.55 ± 0.32	0.72 ± 0.01$^{\#\#\#}$	0.69 ± 0.05$^{\#}$
Testes, g	2.74 ± 0.12	1.94 ± 0.68	2.48 ± 0.05	2.08 ± 0.13**
Brown adipose tissue				
Wet weight, mg	350.3 ± 17.7	427.0 ± 57.6	179.3 ± 5.5$^{\#\#\#}$	154.3 ± 4.7$^{*\#\#}$
Protein, mg	15.6 ± 1.4	21.3 ± 2.4	7.04 ± 0.4$^{\#\#\#}$	5.93 ± 0.4$^{\#\#\#}$
13 weeks	(3)	(3)	(3)	(3)
Body weights, g				
Initial	130.7 ± 10.3	124.3 ± 10.4	96.7 ± 4.5	104.8 ± 6.5
Final	133.7 ± 10.4	123.3 ± 9.0	100.0 ± 1.2$^{\#}$	110.0 ± 6.5
Change in 13 weeks	3.0 ± 2.1	-1.0 ± 1.5	3.3 ± 4.9	5.5 ± 2.3
Organ weights				
Gonadal fat, g	2.00 ± 0.23	1.45 ± 0.07	0.66 ± 0.04$^{\#}$	0.59 ± 0.10$^{\#}$
Testes, g	3.40 ± 0.15	2.86 ± 0.24	2.08 ± 0.14$^{\#\#}$	1.55 ± 0.47
Brown adipose tissue				
Wet weight, mg	489.3 ± 38.7	453.3 ± 49.8	160.0 ± 11.5$^{\#\#}$	160.0 ± 14.7$^{\#\#}$
Protein, mg	21.4 ± 2.1	22.3 ± 2.4	10.3 ± 1.2$^{\#\#}$	8.7 ± 1.1$^{\#\#}$

Season: Summer-Fall

Values are means ± SE for the number of animals indicated in parentheses.

Significant effect of melatonin, comparing the same type of hamster indicated by *($p < 0.02$) or **($p < 0.05$).

Significant effect of myopathy comparing hamsters treated in the same way indicated by #($p < 0.05$), ##($p < 0.005$) or ###($p < 0.001$).

TABLE 31

Effect of treatment with melatonin implants on brown adipose tissue of normal or myopathic hamsters (Experiment 3: silastic capsules, 10 weeks)

	NORMAL		MYOPATHIC	
	Control (8)	Melatonin (9)	Control (8)	Melatonin (9)
Body weights				
Initial	150.4 ± 2.5	151.4 ± 1.7	109.6 ± 2.2#	110.1 ± 2.6#
Final	145.3 ± 4.0	148.4 ± 4.2	105.3 ± 2.4#	104.1 ± 2.1#
Change in 10 weeks	-5.1 ± 4.2	-3.0 ± 4.3	-4.4 ± 1.5	-6.0 ± 1.9
Organ weights				
Gonadal fat, g	2.96 ± 0.28	3.33 ± 0.40	0.66 ± 0.04#	0.62 ± 0.04#
Testes, g	3.45 ± 0.11	3.10 ± 0.31	2.42 ± 0.11#	1.58 ± 0.20**#
Brown adipose tissue				
Wet weight, mg	530.3 ± 21.7	629.1 ± 29.6*	165.8 ± 5.1#	163.3 ± 5.3#
Protein, mg	17.1 ± 1.5	19.2 ± 1.3	9.2 ± 0.4#	9.4 ± 0.7#

Season: Fall

Values are means ± SE for the number of animals indicated in parentheses.

Significant effect of melatonin, comparing the same type of hamster indicated by *($p < 0.02$) or **($p < 0.005$).

Significant effect of myopathy, comparing hamsters treated in the same way indicated by #($p < 0.001$).

TABLE 32

Effect of treatment with melatonin implants on brown adipose tissue of normal or myopathic hamsters (Experiment 4: silastic capsules, 8 weeks)

	NORMAL		MYOPATHIC	
	Control (6)	Melatonin (6)	Control (4)	Melatonin (4)
Body weights, g				
Initial	107.0 ± 3.2	105.2 ± 2.8	104.3 ± 1.2	102.3 ± 1.3
Final	121.2 ± 3.6	120.8 ± 4.7	114.5 ± 4.3	110.0 ± 3.2
Change in 8 weeks	14.2 ± 0.9	15.7 ± 2.7	10.3 ± 3.6	7.8 ± 2.4
Organ weights				
Gonadal fat, g	2.03 ± 0.08	2.23 ± 0.28	0.98 ± 0.14##	0.91 ± 0.09#
Testes, g	2.60 ± 0.14	2.82 ± 0.10	2.88 ± 0.11	2.69 ± 0.09
Brown adipose tissue				
Wet weight, mg	418.0 ± 18.4	484.5 ± 42.5	183.5 ± 14.9##	173.8 ± 20.7##
Protein, mg	19.4 ± 1.2	22.8 ± 1.2	11.2 ± 1.7#	11.1 ± 2.1##

Season: Spring

Values are means ± SE for the number of animals indicated in parentheses.

Significant effect of myopathy, comparing hamsters treated in the same way indicated by #($p < 0.005$) or ##($p < 0.001$).

CHAPTER 5 GENERAL DISCUSSION AND CONCLUSIONS

Growth of BAT in Rats

Growth of BAT due to cafeteria feeding of rats, previously found to be an increase in functional tissue (Rothwell and Stock, 1979), was found to be a hyperplastic type of growth (Experiment A-1). This was confirmed by Tulp *et al.* (1982) and Bukowiecki *et al.* (1982). Cell number in BAT did not increase after cafeteria feeding of Zucker lean and obese rats (Experiment A-4) so this may be a strain specific characteristic. BAT was thermogenically activated by cafeteria feeding (Experiment A-1), as reported by others (Brooks *et al.*, 1980).

Thermogenic activation of BAT in the cold was reduced in thyroidectomized rats (Experiment A-2). BAT of hypothyroid rats is reportedly unresponsive to the effects of noradrenaline (Hemon *et al.*, 1976; Mory *et al.*, 1981; Kuroshima *et al.*, 1967). Noradrenaline is seen as the physiological activator of BAT thermogenesis (Himms-Hagen, 1984) and it now appears that activation of the tissue by noradrenaline also requires thyroid hormone. Growth of BAT in hyperthyroid rats was found to be due to lipid accumulation and not to an increase in functional tissue, agreeing with Heick *et al.* (1973). Tissue activation was lower in thyroid hormone treated animals and it would appear that increased cold resistance and noradrenaline sensitivity seen by others after thyroid hormone treatment (LeBlanc and Villemaire, 1970; Heick *et al.*, 1973) is due to the action of thyroid hormone in tissues other than BAT, possibly due to the increase in oxidative capacity in heart and skeletal muscle seen

by Harri (1978). Reduced activation of BAT was also seen by Sundin in hyperthyroid rats at low temperatures suggesting that thyroid hormone treatment lessens the need for cold-induced nonshivering thermogenesis in BAT (Sundin, 1981).

Experiments on hypophysectomized rats given maintenance amounts of thyroxine and cortisone showed that growth and activation of BAT at 5°C does not require any of the hormones of the pituitary (Experiment A-3). It is not known if glucocorticoids are necessary for activation of BAT in the cold as are thyroid hormones (Experiment A-2) but it appears that the function of the pituitary during cold acclimation may be to stimulate production of these two hormones. Some role for glucocorticoids is suggested by the presence of specific glucocorticoid receptors on BAT (Feldman, 1978). It had been suggested that TSH (Doniach, 1975) and growth hormone (Pagé *et al.*, 1954) might have a trophic effect on BAT. Growth hormone injections did not stimulate BAT growth (Experiment A-3). Elevated levels of TSH in hypothyroid rats (Experiment A-2) also did not cause growth of BAT. ACTH injections were earlier reported to increase BAT protein (Lachance and Pagé, 1953) but have since been found not to cause growth of BAT in warm-acclimated animals (Laury and Portet, 1977; 1980). Although TSH and ACTH are elevated during acclimation to cold in the rat, they were not found to be necessary for activation or growth of BAT at low temperatures. Hypophysectomized rats, without any replacement therapy, could adapt to mild cold (Laury *et al.*, 1984). Their adrenals hypertrophied in the normal way. They therefore seemed to have adequate amounts of glucocorticoids despite lack of ACTH. Thus, the role of glucocorticoids remains uncertain.

Growth of BAT in Obese Zucker Rats

As in the obese ob/ob mouse, BAT has been found to be defective in the obese Zucker rat (Experiment A-4). Both obese species have reduced thermogenic activation of BAT at thermoneutral temperatures (Desautels and Himms-Hagen, 1979; Experiment A-4). The obese Zucker rat has normal activation of BAT when acutely exposed to low temperatures but a reduced response when fed a cafeteria diet (Experiment A-4), differing from the obese mouse which does not activate BAT in response to cold or to diet (Himms-Hagen and Desautels, 1978; Himms-Hagen, 1983b). In this respect, the obese Zucker rat resembles two other obese rodents, the GTG mouse and the VMH lesioned rat, in both of which BAT is thermogenically activated by cold but not by diet (Hogan and Himms-Hagen, 1983; Hogan *et al.*, 1982). In the GTG mouse, obesity is induced by administration of goldthioglucose, which damages parts of the hypothalamus, particularly in the ventromedial area (Himms-Hagen, 1984). In the VMH rat the ventromedial region is destroyed. It has been concluded that diet sensitivity in BAT requires the functioning of cells or fibers in the ventromedial region of the hypothalamus (Himms-Hagen, 1984). Moreover, stimulation of this region promotes BAT thermogenesis and lipogenesis (Perkins, *et al.*, 1981; Shimazu and Takahashi, 1980). The defect in the obese Zucker rat responsible for the suppression of diet-induced thermogenesis in BAT is not known but, because of similarities with the GTG mouse and the VMH lesioned rat, a hypothalamic defect would be a likely possibility.

More recent work has verified that the obese Zucker rat has a reduced thermogenic response to diet (Rothwell *et al.*, 1981a; 1983a; Marchington *et al.*, 1983), and a reduced activation of BAT by diet (Holt *et al.*, 1983). A "gene dosage" effect was seen in that the heterozygote (Fa/fa) has GDP-binding and thermic response to a meal, intermediate between those of fa/fa and Fa/Fa rats (York *et al.*, 1984). BAT activation in response to cold in younger but not older obese Zucker rats has been found to be normal (Holt *et al.*, 1983) agreeing with results presented here. Recent work also shows that obesity and the reduced thermogenic state of BAT and the reduced activation of BAT by diet are all improved by adrenalectomy (Yukimura *et al.*, 1978; Holt and York, 1982; Holt *et al.*, 1983) and would seem to substantiate that a defect in BAT diet-induced thermogenesis may be responsible for obesity in this animal. Central modulation of sympathetic stimulation by glucocorticoids or ACTH may be an important factor in the regulation of BAT growth in cafeteria feeding and also in the defective response of BAT to diet in the obese Zucker rat.

Growth of BAT in Hamsters

Two mediators were investigated for the growth of BAT in hamsters, noradrenaline and melatonin. When studies were begun it seemed that in rats, growth of BAT was under the control of the sympathetic nervous system. Noradrenaline injections in rats result in increased functional size of the tissue (Desautels and Himms-Hagen, 1979; Mory *et al.*, 1980). Growth of BAT (tissue mass) in hamsters had been observed after treatment with the pineal hormone melatonin (Heldmaier and Hoffmann, 1974). Melatonin production by the pineal is controlled by noradrenaline acting at receptors on the pineal (Klein and Moore, 1979). To solve the problem of whether noradrenaline acted to cause BAT growth in hamsters directly as it appears to do in rats, or indirectly by acting on the pineal to produce melatonin, the strategy was to observe BAT growth in both intact and pinealectomized hamsters injected with noradrenaline. Noradrenaline treatment failed to cause BAT growth in hamsters (Experiment B-1) either at the dose level used to stimulate growth in rats, or at double this dose, which resulted in depletion of lipid from white adipose tissue (Experiment B-1). Since there appears to be a diurnal sensitivity in the pineal for production of melatonin (Romero and Axelrod, 1974; Romero *et al.*, 1975) and in the target tissues for melatonin receptors (Vacas and Cardinali, 1979), noradrenaline injections were repeated at different times of the day and in pinealectomized animals, and repeated at different times of the year, since the reproductive effects of photoperiod vary according to the season (Goldman, 1983). In all experiments noradrenaline injections failed to

cause growth of BAT, and in some experiments a significant reduction of the oxidative capacity of the tissue was seen. The effect of noradrenaline on BAT growth in hamsters has not been studied previously. Noradrenaline turnover in BAT, however, is known to be increased in cold-exposed hamsters (Feist, 1970). Noradrenaline probably mediates the acute thermogenic response of the tissue since injection of noradrenaline into hamsters results in a rise in metabolic rate (Experiment B-7) and in the temperature of BAT (Horowitz *et al.*, 1982), and isolated cells from hamsters give a good thermogenic response to noradrenaline *in vitro* (Nedergaard, 1982). The increase in GDP-binding to BAT mitochondria in acutely cold-exposed hamsters seen in Experiment B-8 is probably in response to noradrenaline.

White adipose tissue of hamsters contains α-receptors which are inhibitory to lipolysis in that tissue and which are not present in white adipose tissue of rats (Fain and Garcia-Sainz, 1980). To investigate whether BAT of hamsters and rats might vary in the same way, resulting in a different response to noradrenaline injections in the two species, the β-adrenergic agonist, isoproterenol, which also causes growth of BAT in rats (Heick *et al.*, 1973) and the α-adrenergic agonist, phenylephrine, were injected into hamsters. The former was without effect on any of the parameters measured, but there was a marginal decrease in BAT protein with phenylephrine (Experiment B-2). Because of the short time that phenylephrine would be expected to be available to the animal, and since phenylephrine is a mixed α_1- and α_2-agonist, prazosin, a specific α_1-antagonist was injected at a high dosage level (Experiment B-2). Prazosin treatment resulted in considerable growth of BAT in hamsters

and a reduction in body weight gain and fat accumulation (Experiment B-2).

Prazosin is a hypotensive agent which acts by decreasing vascular resistance as a result of blockade of α_1-adrenergic receptors with little or no increase in heart rate or circulating catecholamine levels (Colucci, 1983). Prazosin also lowers triglyceride and increases HDL (high density lipoproteins) serum levels in humans (Leren, 1984) and rats (Dall'Agilio *et al.*, 1983). The mechanism of these metabolic effects is not understood but in patients on prazosin therapy for 3 months no change in body weight was reported (Rouffy and Jaillard, 1984).

It is not possible to satisfactorily explain the effects of prazosin injections on BAT growth. One possibility is that noradrenaline, acting on α_2-adrenergic receptors, exerts a restraining influence on growth that is removed by the action of prazosin. Two alternatives can also be proposed. First, prazosin may cause BAT growth by increasing blood flow to BAT. Dilation of blood vessels in BAT has been noted in cold-exposed and cold-acclimated rats (Bukowiecki *et al.*, 1982). Although blood flow in BAT has not been studied in relation to tissue growth, blood flow to the tissue in rats is increased with noradrenaline infusion, cold exposure and cafeteria feeding (Foster and Frydman, 1978; 1979; Rothwell and Stock, 1981), all conditions resulting in tissue growth. Although the increased blood flow to BAT caused by noradrenaline is secondary to the elevated metabolism of the tissue (Foster and Depocas, 1980), as is also considered to be the case in exercising skeletal muscle (Thompson and Mohrman, 1983), the possibility exists that by manipulating blood flow per se, tissue growth might be affected.

Secondly, prazosin may cause BAT growth by increasing sympathetic stimulation. Although it was not measured, a fall in blood pressure almost certainly occurs with prazosin treatment. Elevation of blood pressure by phenylephrine infusion causes a suppression of BAT thermogenesis by the baroreceptor reflex which is attributed to a decrease in sympathetic nervous activity in BAT (Shibata, 1982). High doses of prazosin have been found to increase noradrenaline turnover in the brain and the periphery (Cavero and Roach, 1980). Prazosin given to normal human subjects increases the plasma noradrenaline levels and free fatty acid levels during exercise (McLeod *et al.*, 1984). It may be then, that by increasing sympathetic activity in BAT, possibly as a result of lowering of blood pressure, prazosin treatment stimulates growth of BAT.

Thus, the way in which prazosin acts to cause growth of BAT in hamsters is not clear. It would be of interest to compare the response to prazosin injections in rats and hamsters. This may be a general characteristic of BAT or like the response to noradrenaline injections may be peculiar to hamsters.

When these experiments were begun, BAT was known to grow (functional tissue) in hamsters exposed to cold temperatures (Hoffman *et al.*, 1965; Himms-Hagen and Gwilliam, 1980) and the weight of BAT was increased in hamsters kept in short photoperiod (Hoffman *et al.*, 1965; Reiter, 1975). Experiments were done showing that the growth of BAT due to short photoperiod resulted in a large increase in tissue protein (functional tissue) (Experiment B-6). Curiously wet weight of BAT was not increased by short photoperiod as in the above experiments and in those of Bartness and Wade (1984) although the photoperiod is similar to

that used by Reiter (1975). The hypertrophic effect of short photoperiod was not seen in castrated hamsters (Kott and Horwitz, 1984). Growth of BAT in rats kept in short photoperiod was minimal although statistically significant. Hamsters fed a high-fat diet also grew more BAT and increased their weight gain without increasing food intake, confirming the work of Wade (1982). Hamsters fed a high fat diet, whether supplemented with sunflower seeds (this experiment) or with lard (Wade, 1982), increased their feed efficiency and growth of BAT. It should also be mentioned that in BAT growth due to short photoperiod or to a high fat diet, thermogenic activity (GDP-binding to mitochondria) did not increase as during cold acclimation. This somewhat different type of BAT growth can be seen as useful when the purpose of the tissue growth is for heat production in the future and not for immediate use as during cold acclimation. Hamsters, being hibernators, could then prepare for hibernation by growing BAT before the arrival of low temperatures, under the influence of shorter days in the fall and a high-fat diet of seeds and nuts, without increasing their dissipation of energy.

Rats fed a high-fat diet also develop obesity (Schemmel *et al.*, 1970). The extent of overeating probably depends on the length of the feeding period (Kanarek and Hirch, 1977) but in at least two instances, rats consumed significantly less or the same amount as chow fed controls, while gaining more weight and increasing fat stores (Kuroshima *et al.*, 1977; Lemonnier, 1972). Increase weight of BAT, accompanied by increased cold tolerance and increased thermogenic response to noradrenaline is reported in rats fed a high-fat diet (LeBlanc, 1957;

Kuroshima *et al.*, 1977) suggesting similarities with hamsters fed a high-fat diet.

BAT of hamsters grows in response to cafeteria feeding as well as to a high-fat diet but the tissue is not thermogenically activated as occurs in cafeteria-fed rats (Schimmel and McCarthy, 1984; Himms-Hagen, unpublished; Experiment A-1). Although a cafeteria diet is a high-fat diet, it is not the equivalent of a diet made high in fat by the addition of a fat like lard. In the latter case, animals become obese even when they do not overeat (Wade, 1982; Kuroshima *et al.*, 1977; Experiment B-7). On a cafeteria diet, animals overeat but do not become as obese as would be expected from the degree of overeating (Rothwell and Stock, 1979). Variety and palatability of foods would appear to be important in stimulating animals to overeat on a cafeteria diet. The qualities of the diet which trigger increased thermogenesis are not known.

A high-fat diet causes BAT growth in hamsters seemingly without activating it. Animals however have increased thermogenic potential when challenged with noradrenaline or cold exposure (Wade, 1982). Animals are energy efficient, probably because of the reduced energy costs of storing fats from dietary fats compared to dietary carbohydrate, as shown by Pullar and Webster (1977).

Since the growth of BAT in short photoperiod is reportedly under the control of the pineal (Reiter, 1975) and since the mediation of BAT growth by the sympathetic nervous system was in question (Experiment B-1, noradrenaline injections did not cause tissue growth in hamsters as in rats), it was of interest to study the influence of the pineal and melatonin on BAT growth. Acute exposure to cold stimulates pineal

activity (Ralph *et al.*, 1979) and cold acclimation causes atrophy of the testes in hamsters (Hoffman *et al.*, 1965) as does melatonin (Reiter *et al.*, 1976). In Experiment B-8 growth of BAT was found to occur equally well in intact or pinealectomized animals in the cold or when fed a high-fat diet and it would appear the pineal is not involved in growth due to cold or high-fat diet.

In hamsters fed a high-fat diet, weight gain and BAT growth are considerably enhanced when animals are kept in short photoperiod (Wade, 1983; Bartness and Wade, 1984). Melatonin injections also cause an exaggeration of the effects of a high-fat diet (Bartness and Wade, 1984). At low levels of melatonin, an effect on weight gain could be seen without a change in BAT (Wade and Bartness, 1984) suggesting weight gain and BAT growth may be regulated independently. Pinealectomy did not prevent the increased weight gain and BAT weight in short photoperiod on a high fat diet (Bartness and Wade, 1984).

The effect of melatonin on BAT growth was studied by continuous infusion from melatonin implants (Experiment B-9). Melatonin implanted in beeswax resulted in growth of BAT (tissue mass) (Heldmaier and Hoffmann, 1974) and recently experiments by Heldmaier *et al.* (1981) have shown increased respiratory capacity of BAT of Djungarian hamsters implanted with melatonin. Melatonin implants did not result in gonadal regression as reported by others (Turek *et al.*, 1975) or stimulate BAT growth (Experiment B-9). Where hypertrophy of BAT has been seen after continuous infusion of melatonin the animal used was the Djungarian hamster and not the Syrian hamster as in these experiments. Experiments showing growth of BAT in Syrian hamsters have used afternoon injections

of melatonin (Wade and Bartness, 1984). It may be that the Syrian hamster does not respond to continuously elevated levels of melatonin as does the Djungarian hamster.

The mediator of BAT growth in hamsters remains unknown. The pineal gland does not mediate the hypertrophy of BAT that occurs in hamsters that are acclimated to cold or fed a high-fat diet. Noradrenaline injections did not cause growth of BAT in hamsters, although the same dose was given as resulted in tissue growth in rats (Experiment B-1; Desautels and Himms-Hagen, 1979; Mory *et al.*, 1980). Recently, constant infusion of noradrenaline (Mory *et al.*, 1984) or injection of long acting catecholamines (Young, P. *et al.*, 1984) in rats has been found to result in tissue growth, increased GDP-binding and increased proportion of 32 000 MW polypeptide, suggesting that noradrenaline is able to fully reproduce the effects of cold acclimation on BAT growth and function in this species and that the sympathetic nervous system controls growth of BAT.

Denervation, however, caused the tissue to regress (Experiment B-5) suggesting that tissue size is indeed regulated by the sympathetic nervous system and that some mediator other than or in addition to noradrenaline coming from the sympathetic nerves may be responsible for growth of BAT. A trophic effect of the sympathetic nerves in BAT of hamsters is also indicated by implantation studies (Néchad and Olson, 1983).

The growth of BAT resulting from minipump infusion of noradrenaline is thought to be the result of noradrenaline acting directly on BAT, since it occurred when the minipump was placed near BAT and

not when implanted 6 to 9 centimetres away (Ricquier and Mory, in press). However, the same group (Mory *et al.*, 1980) previously reported hypertrophy of BAT (increased protein, DNA) after noradrenaline injections in the scapular and lumbar region. This suggests that noradrenaline, when injected, could affect BAT growth indirectly and that the lack of growth seen in hamsters injected with noradrenaline could be due to a metabolic peculiarity of this species. It would then be useful to study BAT growth in hamsters after noradrenaline infusion or after long acting catecholamine treatment to establish whether noradrenaline is involved at all in BAT growth in the hamster.

Growth of BAT in the Myopathic Hamster

Because the myopathic hamster was found to have a reduced amount of BAT (Himms-Hagen and Gwilliam, 1980), a series of experiments was done to study growth of BAT in normal and myopathic hamsters.

Brown adipose tissue growth in hamsters was known to be stimulated by cold acclimation and this response was normal in myopathic hamsters (Himms-Hagen and Gwilliam, 1980). In two other situations found to stimulate BAT growth in hamsters, short photoperiod and feeding a high-fat diet, the response of the myopathic hamster was found to be defective (Experiments B-6 and B-7). Since cold-induced growth is near normal, it would appear that the smaller size of BAT in myopathic hamsters may be due to lack of a signal relaying information about lighting and dietary conditions, rather than to a fault in the tissue itself.

Growth of BAT in short photoperiod is thought to be controlled by the pineal since increased BAT weight due to short photoperiod was prevented by pinealectomy (Reiter, 1975). However, increased BAT weight due to short photoperiod in hamsters fed a high-fat diet was not prevented by pinealectomy (Bartness and Wade, 1984). The pineal hormone, melatonin has been shown to have a trophic effect on BAT of hamsters (Heldmaier and Hoffmann, 1974; Bartness and Wade, 1984; Rafael *et al.*, 1981). Because of failure to demonstrate growth of BAT by melatonin infusion, it was not possible to study a possible defect in the response of myopathic hamsters to this hormone. Since testes regression was normal in myopathic hamsters in short photoperiod, it would appear that the pineal is functioning normally. It is not known if BAT is a target

tissue for melatonin. The antigonadal action of melatonin is thought to occur primarily as a result of action at the hypothalamus (Reiter *et al.*, 1981; Rusak; 1980; Turek *et al.*, 1980). Implants of melatonin in beeswax in the anterior hypothalamus of the white footed mouse cause an increase in interscapular BAT (fat free dry matter) and suggests a hypothalamic site of action for melatonin in stimulating BAT growth (Glass and Lynch, 1982).

The mechanism regulating growth of BAT by a high-fat diet is also not known, but may involve a hypothalamic center as has been proposed for growth induced by cafeteria feeding (Landsberg and Young, 1983; Rothwell and Stock, 1983a); it does not require the pineal gland (Experiment B-8). Regulation of energy intake seems to function normally in myopathic hamsters in that energy intake did not change in response to a high fat diet and a diet of similar composition was selected. The myopathic hamster had a lower metabolic efficiency (smaller weight gain per unit of food eaten). This agrees with the small amount of white adipose tissue as well as BAT in these animals. The relative leanness does not seem to be due to increased protein turnover known to occur in their diseased muscles (Li, 1980; Nicholls *et al.*, 1980) since no increase in metabolic rate was detected (Experiment B-7). However, the existence of an increased metabolic rate at other times of the day cannot be excluded, for example, during the dark phase.

A defect then has been observed in the myopathic hamster which interferes with the recognition of photoperiod and of a high-fat diet by BAT but does not interfere with cold-induced hypertrophy of the tissue. This defect may be on a pathway common to both photoperiod and diet,

before the signal derived from cold interacts and may be located in the hypothalamus. The mediator of BAT growth in hamsters is not known but seems to come from the sympathetic innervation (refer to DISCUSSION, Growth of BAT in Hamsters). There appears to be an abnormality in the control of sympathetic nervous activity in the heart of the myopathic hamster (Angelakos *et al.*, 1973; Sole *et al.*,.1975; Jasmin and Proschek, 1983) and the high levels of excretion of catecholamines in the urine (Kabara *et al.*, 1976) suggest altered sympathetic function. However, noradrenaline turnover in BAT was found to be normal (Experiment B-3), ruling out the hypothesis that excessive sympathetic nervous activity is responsible for the small size of the tissue. Denervation of BAT had a smaller effect in the myopathic hamster (Experiment B-5) as would be expected if a trophic factor associated with the sympathetic nerves were lacking.

Prazosin causes a much greater increase in BAT growth in myopathic hamsters than in normal hamsters (Experiment B-2). Alpha$_1$-receptors were found to be equally abundant in BAT of the two types of animals (Experiment B-4) suggesting the growth inducing effect of prazosin is due to its action at α_1-receptors in the blood vessels, rather than those on brown adipocytes. Adrenergic receptors of blood vessels in BAT have not been studied. Lesions in heart and skeletal muscle may be caused by excessive vascular reactivity to sympathetic agents (Factor *et al.*, 1982). Prazosin treatment has been reported to greatly improve the cardiac lesions of myopathic hamsters (Factor and Cho, 1983) supporting this idea. Similarly, growth of BAT in myopathic hamsters resulting from prazosin treatment is greater than in normal hamsters and may be due to

the release of vasoconstriction and increased blood flow. Also supporting the vascular hypothesis is a report by Hunter and Elbrink (1983) of increased contractility of aortic strips from myopathic hamsters in response to noradrenaline, phenylephrine, isoproterenol, histamine or 5-HT. This would agree with the hypothesis that the permissive effect of catecholamines and alterations in calcium movements as suggested by Jasmin and Proschek (1983), and Wrogemann *et al.* (1979) are responsible for the increase contractility of blood vessels in the myopathic hamster. Stimulation of α_1-receptors is associated with calcium movements which may be altered with prazosin treatment.

Elevated metabolism in BAT is thought to increase blood flow (Foster and Depocas, 1980). When stimulated by cold, the increased metabolism in BAT may be enough to overcome vasoconstriction due to the disease allowing growth of the tissue. BAT growth is normal in cold-acclimated myopathic hamsters (Himms-Hagen and Gwilliam, 1980). Since growth of the tissue due to short photoperiod or a high-fat diet is not associated with increased metabolism, the tissue may continue to be inhibited by constriction of its blood supply, thus inhibiting growth. Resting blood flow to BAT is greatly reduced in myopathic hamsters reflecting the small size of the tissue (Wickler and Horwitz, 1984). When expressed per gram of tissue, blood flow was found to be lower, but not significantly so, in three BAT deposits (Wickler and Horwitz, 1984). Isoproterenol-stimulated blood flow per gram of BAT is normal in myopathic hamsters (Wickler and Horwitz, 1984). The positive effects of prazosin on BAT growth could also be due to to improvements in the myocardium, improving blood circulation to the body as a whole.

Increased blood flow may explain the improved condition of muscle and of BAT in the myopathic hamster after prazosin treatment. This explanation is also compatible with the therapeutic effect of isoproterenol when given at low dosage levels (Jasmin and Proschek, 1983). At high dosage levels, isoproterenol promotes the development of lesions in the myopathic hamster (Lossnitzer, 1975). At these levels, isoproterenol may act as an α-adrenergic agonist, as suggested by the work of Hunter and Elbrick (1983) promoting damage to muscle possibly by a mechanism involving calcium. At low levels, isoproterenol may stimulate muscle metabolism enough to dilate blood vessels and improve the blood supply leading to amelioration of muscle lesions.

BAT of myopathic hamsters, although reduced in amount, appears to be relatively normal. Cold-induced growth and activation is normal (Himms-Hagen and Gwilliam, 1980) as is depolarization of plasma membrane of BAT by noradrenaline (Horowitz *et al.*, 1982). Some abnormalities however, have been reported. The rise in temperature over BAT in response to noradrenaline *in vivo* is lower (Horowitz *et al.*, 1982), brown adipocytes from myopathic hamsters have a reduced thermogenic response to isoproterenol (Horwitz and Wickler, 1983) and the activity of some oxidative enzymes is reduced (Wickler and Horwitz, 1983). Studies on very young myopathic hamsters would indicate whether or not these abnormalities are of primary or secondary origin since BAT of the myopathic hamster is reduced as early as 4 weeks of age (Himms-Hagen and Gwilliam, 1980).

APPENDIX A. PROCEDURE FOR DEHYDRATION OF MITOCHONDRIA AND EMBEDDING IN VESTOPAL-W

Mitochondrial fragments are in 3.0 ml osmium tetroxide solution

50% ethanol	add equal volume, remove half wash 2 times more wait 3 minutes wash once more wait 5 minutes repeat above steps with 75% and 95% ethanol
100% ethanol	add equal volume, remove half wash 4 times more wait 15 minutes wash 10 times more, rapidly
styrene	transfer to clean oven dry vials using 100% ethanol add equal volume, remove half wash 4 times more wait 5 minutes wash 5 times more wait 5 minutes
styrene + fresh Vestopal (1:1 mixture)	remove as much styrene as possible add styrene-Vestopal mixture on rotator for 10 minutes
Vestopal	remove mixture add Vestopal on rotator for 10 minutes change solution and transfer mitochondrial fragments to clean oven dry vials on rotator for 10 minutes change solution on rotator for 2 days

- transfer mitochondrial fragments to oven dry gelatin capsules, add Vestopal.

- cure samples for 4 days in a 60°C oven.

APPENDIX B. PROCEDURE FOR DEHYDRATION OF TISSUE AND EMBEDDING IN SPURR

Tissue pieces are in 3.0 ml uranyl acetate solution

50% ethanol	add equal volume, vortex, remove as much as possible
	wash 3 times more
	leave tissue in 3.0 ml ethanol 3 min.
	wash once more
	leave tissue in 3.0 ml ethanol 5 min.
	wash once more
	leave tissue in 3.0 ml ethanol 5 min.
	repeat above steps with 75% and 95% ethanol.
100% ethanol	add equal volume, vortex, remove as much as possible
	wash 3 times more
	leave tissue in 3 ml ethanol 15 min.
	wash 6 times more
	leave tissue in 3 ml ethanol 15 min.
	wash 10 times more, rapidly
Spurr	remove ethanol
	add 3 ml Spurr (standard medium) on rotator for 10 min.
	change solution
	on rotator for 24 hours
	change solution 6 times more during this period.

- transfer tissue pieces to oven dry gelatin capsules, add Spurr medium

- cure samples for 24 hours in a 70°C oven.

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