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BROWN ADIPOSE TISSUE METABOLISM IN RELATION TO ENERGY BALANCE

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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ABBREVIATIONS

ADP	adenosine 5'-diphosphate
ATP	adenosine 5'-triphosphate
BAT	brown adipose tissue
CNS	central nervous system
CoA	coenzyme A
cyclic AMP (cAMP)	adenosine 3',5'-monophosphate
DIT	diet-induced thermogenesis
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
GDP	guanosine 5'-diphosphate
GTG	goldthioglucose
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
NA	noradrenaline
NAD	nicotinamide adenine dinucleotide
NST	nonshivering thermogenesis
PPO	2,5 diphenyloxazole
SDS	sodium dodecyl sulfate
SE	standard error
T_3	triiodothyronine
T_4	thyroxine
VMH	ventromedial hypothalamus

SUMMARY

The expenditure of food energy for thermogenesis in brown adipose tissue (BAT) is under the control of the sympathetic nervous system and is known to be stimulated by two principal events that activate the sympathetic nervous system. These are exposure or acclimation to cold, which promotes nonshivering thermogenesis (NST) in BAT, and overeating a palatable diet, which promotes diet-induced thermogenesis (DIT) in BAT. NST and DIT are identical metabolic processes, stimulated by noradrenaline (NA) in BAT.

The major goal of the studies reported in this thesis was the elucidation of the nature of the defect in BAT thermogenesis in the genetically obese (ob/ob) mouse and the comparison of BAT function in the ob/ob mouse with BAT function in hypothalamically obese animals to find out whether they also exhibit a defect. Since the thermogenic proton conductance pathway in BAT mitochondria is known to be associated with a 32,000 molecular weight polypeptide that contains a binding site for purine nucleotides, the amount of the pathway was determined by measuring the proportion of the 32K-Da polypeptide in mitochondrial membranes and the activity of the pathway was assessed by measuring the extent of mitochondrial GDP binding. The capacity for BAT

thermogenesis *in vivo* was estimated by measuring the increase in metabolic rate after injection with noradrenaline (NA).

Results show that the genetically (ob/ob) mouse fails to activate BAT thermogenesis upon brief cold exposure, (no increase in GDP binding) despite a large metabolic and mitochondrial mass and a normal concentration of mitochondrial proton conductance pathways. The ultrastructure of BAT mitochondria of the ob/ob mouse is abnormal and does not change in the normal way with acute cold, implying that operation and/or unmasking of the pathway necessitates a structural reorganization of inner mitochondrial membranes. Thus, NST in BAT is defective in the ob/ob mouse. That BAT of ob/ob mice is refractory to NA and is therefore inherently defective is indicated by studies showing a low thermogenic response of these animals to exogenous NA. The defective initial response of BAT to cold can nonetheless be ameliorated by long term treatment with thyroxine, although survival of ob/ob mice at low temperatures is not greatly extended. Since ob/ob mice are known to have normal blood thyroid hormone levels and a permissive amount of thyroxine is necessary for the thermogenic action of NA on BAT, a refractoriness of BAT to triiodothyronine may in part explain the defect in BAT thermogenesis. Obese (ob/ob) mice can survive at low temperatures if previously acclimated to mild cold. Cold acclimation is associated with an improved

thermogenic functioning of BAT and the usual cold-induced adaptive changes in mitochondrial polypeptide composition and ultrastructure as well as tissue growth and mitochondrial proliferation. These changes may also be associated with raised blood T_3 levels. GDP binding studies of chow-fed ob/ob mice maintained at thermoneutrality show that along with defective NST they also manifest a defect in DIT. However, the adaptive response of BAT to cafeteria feeding is normal in that there is a normal activation of BAT thermogenesis and a normal increase in metabolic and mitochondrial mass. Thus, mechanisms controlling adaptive changes in BAT and mitochondria of the chow-fed ob/ob mouse are apparently normal but the peripheral switching mechanism responsible for activation of the proton conductance pathway is defective.

The studies of GTG-obese mice and VMH-lesioned rats show that hypothalamic obesity is also associated with an impaired thermogenic functioning of BAT, although the defect is not as severe as that seen in the ob/ob mouse. BAT thermogenesis is not activated by cafeteria feeding in GTG-obese mice and VMH-lesioned rats during the dynamic phase of their obesity. In contrast, the acute cold-induced activation of BAT thermogenesis occurs normally in both hypothalamically obese rodents as well as in rats made obese by parasagittal hypothalamic knife cuts. Moreover, acclimation of GTG-obese mice to cold is accompanied by the usual adaptive changes in BAT and its mitochondria. In addition,

GTG-obese mice have a greater capacity to respond thermogenically to exogenous NA than lean mice, presumably this is related to their larger than normal amount of BAT. Thus, BAT of hypothalamically obese rodents is inherently functional and has an apparently normal sympathetic innervation. The defect in DIT does not reside in BAT but rather lies with the central control mechanism responsible for the transmission of dietary signals which result in sympathetic activation of BAT thermogenesis.

The findings of the work describe in this thesis, showing defective BAT thermogenesis in genetically obese (ob/ob) mice, GTG-obese mice and VMH-lesioned rats, in association with their known high metabolic efficiency, have helped to develop and support the recent concept that the thermogenic functioning of BAT is an important factor in the regulation of energy balance. Energy balance is achieved by equilibration of ingested food energy with energy expenditure. Recent studies of lean and obese mice have shown that BAT thermogenesis can be a major factor in the overall energy expenditure of rodents and therefore play an important role in the regulation of energy balance. Accordingly, animals living at low environmental temperatures are hyperphagic and yet remain lean because the excess energy is metabolized in BAT to produce heat for maintenance of body temperature in the cold. Likewise, prolonged overfeeding does not always result in the expected deposition of energy because

of an increase in BAT thermogenesis. In contrast, a decreased energy expenditure in BAT together with the hyperphagia, contributes to obesity in several genetically obese rodents.

CHAPTER 1

LITERATURE REVIEW

PART I: GENERAL INTRODUCTION

The original objective of the work described in this thesis was to study the defective functioning of brown adipose tissue in genetically obese mice and to find out whether brown adipose tissue function might also be defective in other obese animals. At the time the work was started in 1979 only one report of defective brown adipose tissue functioning in obesity had appeared (Himms-Hagen, Desautels 1978) and the extensive current interest in diet and brown adipose tissue function, which stems largely from a report by Rothwell and Stock in 1979, had not yet developed. During the progress of this work there have been considerable advances in our knowledge of brown adipose tissue thermogenesis as an important component of energy expenditure in relation to cold (see Part II:C.2), to diet (see Part II:C.3) and to obesity (see Chapter 5), some of them as a consequence of work described in this thesis (Hogan, Himms-Hagen, 1980, 1981, 1983; Hogan, Coscina, Himms-Hagen, 1982). This introductory literature review will describe the background to present knowledge of the regulation of energy expenditure and energy intake (Part II), of brown adipose tissue metabolism and its regulation (Part III) and

of abnormalities in energy balance in animals with genetic or hypothalamic obesity (Part IV). The relation of defective thermogenic function of brown adipose tissue to obesity will be reviewed in the final discussion (Chapter 5).

PART II: ENERGY EXPENDITURE AND ENERGY INTAKE

A. The Nature and Regulation of Energy Balance

When energy intake is equal to energy expenditure a state of energy balance is said to exist and energy stores remain constant. It is obvious that a disturbance in energy balance will occur when either input or output is altered: the inevitable consequence is a gain or loss of energy stores. Aside from a small amount of glycogen, energy stores are in carcass fat which is freely available as a source of energy. Although total body fat can be measured with reasonable accuracy, determining the point of reference against which fat stores can be described as excessive or deficient is difficult and subject to a number of influences. If the point of reference is considered as that which is concomitant with longevity, then the appropriate size of fat stores will still depend on such factors as age, sex, physiological state, genetic background and environment. For example, deposition of large amounts of fat is essential for survival in some hibernating animals and migratory birds. Likewise, in geographical areas where food is sporadically available or infectious diseases are prevalent, survival may well depend on the capacity to accumulate extra fat during times of plenty. On the other hand, it is well documented in humans that obesity, a condition of "excess" fat, is associated with a number of disease states including

hypertension, adult-onset diabetes and atherosclerosis.

An extensive body of research indicates that energy stores of animals are regulated. Although constancy of body weight or fat stores is suggestive of a regulated system, more convincing evidence is provided by studies which show that animals actually defend their energy stores against perturbing influences. This is achieved not only by mechanisms controlling energy intake but also by those controlling energy expenditure. In a number of reports, regulation of body weight is the only parameter used to illustrate energy balance. This of course does not necessarily imply regulation of fat stores, since the body is comprised of other variable components; namely protein and water. Nevertheless, if the treatment is not extreme, changes in body weight in adult animals are usually due to alterations in fat content.

It is well recognized that rats are able to maintain body weight by compensatory increases or decreases in their intake of food despite variation in the energy density of regular laboratory diets (Garrow 1974). Other studies have shown that when body fat stores are manipulated by food deprivation or tube feeding, rats will spontaneously adjust food intake after withdrawal of the perturbing influence so that energy stores are returned to normal levels (Van Itallie et al. 1978; Rothwell, Stock 1981a). Alterations in energy expenditure may also occur when animals are overfed

or subjected to moderate food restriction (Rothwell, Stock 1981a). Many studies have shown that food deprived animals minimize energy loss by lowering their metabolic rate (Rothwell, Stock 1982c, Garrow 1974). Moreover, the reduced body weight (Levitsky et al. 1976; Björntorp, 1982) and body fat content (Björntorp, Yang 1982) of fasted rats return to normal when ad libitum feeding is resumed without a total net increase in food intake or a change in physical activity (Levitsky et al. 1976; Boyle et al. 1981; Björntorp, Yang 1982). This is likely due to the lower resting metabolic rate which persists during the first few days of refeeding (Boyle et al. 1981).

Since animals would appear to defend their energy reserves by coordinating energy input and output it is evident that an integrative control system is an important component for the regulation of energy stores (James et al. 1981). A 'set point' hypothesis has frequently been proposed as the mechanism responsible for energy balance regulation. This type of control system requires a sensor which accurately monitors energy content, a comparator which asssesses energy content against some ideal level and a controller which adjusts input or output so that energy reserves are precisely maintained (Garrow, 1978; Booth et al. 1981). However, there is no physiological or anatomical evidence for such a regulatory system either in animals such as the rat (Booth et al. 1981) or in man

(Garrow, 1978). Another control mechanism that has been postulated is a 'buffer' type of system which would operate by developing adaptive processes to minimize a change in energy stores (Booth et al. 1981). This simpler but less accurate mechanism more readily explains the reality of the less than precise regulation of energy balance.

B. Regulation of Energy Intake

The control of food intake is an immensely complicated area of study and the following only intends to be a brief overview describing some of the peripheral and central mechanisms which may be involved in the regulation of energy balance.

Basic to all models for the control of food intake is some form of controller which adjusts food intake in response to feedback signals generated by the immediate need for energy or the need to replenish energy stores. Overall integration for the control of energy intake resides in the central nervous system. Until recently, initiation and termination of food intake was explained by the 'dual-center' hypothesis which defined two centers in the hypothalamus: a "satiety center" in the ventromedial region and a "feeding center" in the lateral region (Anand, Brobeck 1951). Experimental evidence on which the 'dual-center' hypothesis was based were the observations that bilateral lesions of the ventromedial nuclei and surrounding area caused

hyperphagia and obesity (Hetherington, Ranson 1940; 1942) while bilateral damage in the extreme portion of the lateral hypothalamus resulted in aphagia (Anand, Brobeck 1951). This hypothesis was strengthened by the findings that electrical stimulation of the ventromedial hypothalamus suppressed eating in hungry rats and that overeating occurred in satiated rats following stimulation of the lateral hypothalamus (Morgane, 1961). However, feeding behavior has since been shown to result from the integration of internal and external stimuli in areas of the cortex, limbic system and reticular formation in addition to the hypothalamus (Morgane 1979). The current view holds that food intake may be controlled by an anatomical brain system and not centers (Lytle 1977) and that the hypothalamus acts as an important integrating and relay station (Bray 1982). Although the lateral and ventromedial hypothalamus are no longer considered to be primary centers they are still believed to be involved in modulating feeding behavior.

Recently, attention has focused on the role that various neurotransmitters and peptides play in the central feeding systems. A role for NA in the central control of feeding behavior was initially demonstrated by Grossman (1960) who found that microinjection of NA into the hypothalamus elicited feeding. The effect results from stimulation of α receptors (Leibowitz 1976) and the major site of action appears to be the paraventricular nucleus (Leibowitz 1978).

In contrast, microinjection of NA into the lateral hypothalamus suppresses food intake in hungry rats via β-adrenergic receptors (Leibowitz, Rossakis 1978). It has been suggested that 5-hydroxytryptamine is involved in satiety mechanisms (Lytle 1977) as central injection of p-chlorophenylalanine, a depleter of 5-hydroxytryptamine, results in hyperphagia (Breisch, et al. 1976). There is also evidence for the involvement of β-endorphin in the feeding system. Injection of the opiate antagonist naloxone has been shown to reduce hyperphagia in genetically obese ob/ob mice and fa/fa rats (Margules et al. 1978) and in cafeteria fed rats (Mandenoff et al. 1982).

Integrated into the central control of food intake are neural, hormonal and metabolic signals from the periphery including the gastrointestinal tract and the liver. A variety of receptors responding to intraluminal pressure, chemical properties of food and osmotic effects of gastric chyme have been described as participating in the satiety response (Van Itallie et al. 1978). Additionally, it has been shown that chemoreceptors capable of monitoring caloric content are present in the stomach of monkeys (McHugh, 1978). Cholescystokinin and bombesin, both gastrointestinal peptide hormones, have been shown to reduce meal size in experimental animals when injected both peripherally and centrally (Gibbs et al. 1979; Smith, Gibb, 1979). Observations that infusion of 2-deoxyglucose into the portal

vein of rabbits elicited feeding in normal but not vagotomized rabbits suggests that the liver also transmits satiety signals to the brain (Novin 1976). This is supported by electrophysiological studies which showed that the hepatic branches of the vagus nerve have a firing rate which is inversely proportional to glucose concentration in the portal vein (Niijima 1969). It is also likely that intermeal satiety which determines initiation of feeding is affected by the relative proportion of circulating energy nutrients and hormones (Van Itallie et al. 1978). Finally, as animals ascend the phylogenetic scale and develop more organized neural systems the physiological controls of food intake are more likely to be influenced by learned responses to external stimuli such as the texture, flavor and odor of food.

C. Regulation of Energy Expenditure

1. Classification of components of energy expenditure

Energy assimilated by animals as food is used for internal and external work and, in homeotherms, for maintenance of body temperature. A series of controlled oxidative steps have evolved which convert the chemical energy in food into ATP plus heat. Additional heat is generated when ATP is utilized so that thermal energy is the ultimate product of all chemical reactions in living organisms. Energy expenditure corresponds to heat production

and is broadly classified as obligatory thermogenesis, which is always present and facultative thermogenesis, which may or may not occur depending upon the circumstances (Himms-Hagen, 1983a). Obligatory thermogenesis is equivalent to the resting metabolic rate and is divided into essential, endothermic and postprandial compartments (Himms-Hagen 1983a). Essential heat production in animals is the consequence of those reactions necessary to maintain integrity of the cell and the organism as a whole when at rest (Girardier 1977). Such reactions include degradation and biosynthesis of macromolecules, maintenance of ion gradients across cell membranes, circulatory, respiratory and renal activity. The homeothermic state is characterized by endothermic thermogenesis. In this case, heat production results from metabolic reactions which have evolved to maintain body temperature at a level independent from that of normal ambient temperature and is controlled primarily by thyroid hormones. Post-prandial thermogenesis refers to those reactions immediately following a meal that are concerned with the processing of food for utilization by the cell i.e. digestion, absorption and storage.

Facultative thermogenesis is distinguished by its ability to switch on rapidly in response to cold, over-feeding and exercise and to quickly switch off when the stimuli are removed (Himms-Hagen, 1983a). Exercise

thermogenesis is the result of voluntary physical activity and has long been considered as the major controllable component of energy expenditure. The ability of animals to generate heat in response to low ambient temperatures is termed cold-induced thermogenesis. Diet-induced thermogenesis occurs during prolonged overfeeding and serves to limit the obesity that would otherwise develop from the hyperphagic intake.

2. Nonshivering thermogenesis

The relatively narrow range of ambient temperature within which homeothermy can be maintained with minimal metabolic activity is known as the thermoneutral zone (Girardier 1977). Below a certain critical temperature a variety of regulatory mechanisms are evoked to minimize heat loss and to increase heat production so that body temperature remains constant. The heat conserving processes such as piloerection and peripheral vasoconstriction are under sympathetic control (Himms-Hagen 1975). Two types of heat producing mechanisms enable animals to maintain body temperature in the cold. Shivering thermogenesis, which is associated with involuntary muscular activity, and nonshivering thermogenesis (NST) which refers to the heat produced from accelerated metabolic processes exclusive of those involved in shivering (Himms-Hagen 1975). The relative contribution of shivering and nonshivering thermogenesis

depends upon age, species and length of time for which animals have been exposed to the cold. Many newborn mammals, including man, possess an inherent capacity for nonshivering thermogenesis (NST) (Cannon, Johansson 1980; Smith, Horwitz 1969; Himms-Hagen 1978). The capacity for NST in non-hibernators declines with age. However, NST is an adaptive process and will develop in certain nonhibernators such as mice, rats, guinea pigs and rabbits when exposed to low ambient temperatures for long periods (Himms-Hagen 1978). Hibernators such as hamsters and ground squirrels are poikilothermic at birth (Cannon, Johanson 1980). The capacity for NST develops with increasing age, but once present, it is retained throughout life. Nonshivering thermogenesis occurs during the first phase of arousal from hibernation as well as during exposure to cold.

Initially, when a rodent is transferred from a warm to a cold environment, heat production is achieved mainly by shivering. As the duration of cold exposure increases, there is a gradual decrease in shivering and a concomitant increase in NST (Hart et al. 1956). By 2-3 weeks, the elevated metabolic rate is maintained entirely by NST and the animal is considered to be cold acclimated (Himms-Hagen 1975). Substantial evidence exists that NST is under the control of the sympathetic nervous system. This was first demonstrated by Hsieh and Carlson in 1957 who measured oxygen uptake of warm- and cold-acclimated rats at 30°C

after intramuscular injection of either noradrenaline or adrenaline. They reported that oxygen uptake of cold-acclimated rats was greater than warm-acclimated rats after injection with adrenaline but that noradrenaline was a far more potent stimulus. Furthermore, oxygen uptake of cold-acclimated rats receiving noradrenaline was similar to that of untreated cold-acclimated rats exposed to 5°C. That NA-induced thermogenesis is quantitatively equivalent to NST has been suggested by experiments which measure oxygen consumption in cold-acclimated animals at various temperatures before and after injection with noradrenaline (Janský et al. 1969; Bockler et al. 1982). The maximum thermogenic effect of NA occurs at thermoneutrality. As the ambient temperature declines to that at which the animals were cold-acclimated, resting oxygen uptake increases and the response to NA decreases by the amount of NST already elicited by cold. Ultimately, there is complete substition of NA-induced thermogenesis by NST i.e. the rate of oxygen uptake evoked by noradrenaline at thermoneutrality is the same as that observed in animals at the temperature to which they were cold acclimated.

For many years brown adipose tissue (BAT) has been recognized as a major site for heat production in the newborn of a number of species including man (Smith, Horwitz 1969; Alexander, Bell 1975; Hull, Segall 1965; Brück, 1970).

Evidence that BAT plays a prominent role during arousal from hibernation was also well established (Smith, Horwitz 1969). Until recently however, its contribution to NST in cold-acclimated nonhibernators was questionable with estimates ranging generally from 10-20% (Himms-Hagen 1978, 1975). But these values were obtained using measurements of $^{86}Rb^{+}$ uptake into tissues to determine blood flow and such studies underestimate the capacity for BAT thermogenesis (Foster, Frydman 1978a, b; Alexander, Bell 1975). This is because $^{86}Rb^{+}$ diffuses slowly from blood to tissues and as a result tissues with high rates of flow will show a low $^{86}Rb^{+}$ uptake while uptake will be high in tissues with a low flow rate (Foster, Frydman 1978a). The use of radioactively labelled microspheres to measure regional blood flow provides a more accurate evaluation of heat production in BAT (Foster, Frydman 1978a; Alexander, Bell 1975). When microspheres are introduced into the left ventricle they follow the distribution of blood and become lodged in the smallest capillaries. Blood flow can then be calculated by the cardiac output and the fractional distribution of the labelled microspheres. To estimate the oxygen consumption of any organ in vivo, measurements of the arteriovenous difference in blood oxygen concentration across the organ are required in addition to the regional blood flow. Using the microsphere method, Foster and Frydman (1978b; 1979) have conclusively demonstrated that BAT is the major site for NST in cold-

acclimated rats. When measuremnts of oxygen consumption in interscapular BAT were extrapolated to all deposits in the body, approximately 60% of the total thermogenic response to infused NA in cold-acclimated rats was attributed to BAT, 15-20% to increased mechanical activity of heart and respiratory muscles and 15-20% to the effect of an increase in body temperature on metabolic rate of tissues in general (Foster, Frydman 1978b). As well, microsphere-based estimates of blood flow indicated that BAT was the major site for NST in conscious cold-acclimated rats exposed to low ambient temperatures (6°C, -6°C, -19°C) and that BAT also plays an important thermogenic role in cold-exposed warm-acclimated rats (Foster, Frydman 1979). It has since been shown that the increase in blood flow to BAT is not mediated by the action of NA on blood vessels of the tissue but rather is secondary to the elevated metabolic activity of BAT (Foster, Depocas 1980).

Since cold-acclimated animals are extremely hyperphagic and yet remain lean (Thurlby, Trayhurn 1979; Coleman 1982; Lin et al. 1980; Hardeveld et al. 1979a) it is apparent that BAT contributes substantially to the maintenance of energy balance in animals living in the cold by utilizing the excess ingested energy for NST.

3. Diet-induced thermogenesis

Diet-induced thermogenesis (DIT) refers to the increase in heat production associated with prolonged overfeeding.

This phenomenon, initially termed "Luxuskonsumption" by Neumann (1902), is adaptive and is to be distinguished from postprandial thermogenesis which is associated with the immediate processing of food ingested. Interest in diet-induced thermogenesis (DIT) as a mechanism for disposing of excess food energy originated in 1902 with the report of a self study by Neumann, documenting weight maintenance for more than 2 years despite variable intakes of energy. Recently, Sims and coworkers (1973) have studied the effects of long-term voluntary overfeeding in man. They reported that some individuals had greater difficulty than others in depositing excess energy and required more energy from a mixed diet to maintain the elevated weight. The evidence for DIT in man is not unequivocal. However, after an extensive review of the literature Garrow (1978) concluded that experimental conditions were not appropriate for eliciting DIT in a number of the studies disclaiming its existence. Methodological difficulties are somewhat alleviated in animal studies thereby permitting a more objective assessment of the evidence for DIT and its quantitative significance.

The most dramatic demonstration illustrating the existence of DIT is probably the work of Miller and Payne (1962) in which they compared energy requirements for weight maintenance in two weanling pigs. Growth was limited by restricting either energy or protein intake. The pig

receiving the low protein diet consumed 5 times more energy than its partner, which obtained a low energy diet. This experiment has since been repeated by Gurr and associates (1980) with a larger sample size and full energy balance measurements. In this case the protein restricted pigs stored more body energy than the energy restricted pigs, but there was a threefold increase in energy intake and a twofold increase in energy expenditure. Low protein diets are also effective in eliciting DIT in rats (Miller, Payne 1962; Rothwell et al. 1982b; Tyzbir et al. 1982). However, these experiments are confounded by protein malnutrition and growth restriction and are therefore of limited value for studies on adaptive thermogenesis. Since chow-fed laboratory animals eat no more than necessary for maintenance and growth (Adolph 1947) it has been difficult to obtain an animal model which will consume excess energy without exhibiting physiological or behavioral disorders. Thus far, the most suitable method to induce voluntary hyperphagia has been the 'cafeteria' feeding regimen developed by Sclafani and Springer (1976) as modified by Rothwell and Stock (1979b). The cafeteria diet consists of a variety of palatable "human" foods which are offered to animals on a rotating basis in addition to their regular stock diet. Rats consuming this diet continue to obtain adequate nutrients for growth and maintenance (Rothwell, Stock 1981c). The ability of rats to select a nutritionally adequate diet when offered separate

sources of protein, fat and carbohydrate is well recognized (Kanarek, Beck 1980).

Rothwell and Stock have reported that the marked hyperphagia in cafeteria-fed rats is accompanied by a compensatory increase in energy expenditure which serves to limit obesity (Rothwell, Stock 1979b; 1982a; 1982b; 1982d; Rothwell et al. 1982d). For example, in one study young Sprague Dawley rats fed a cafeteria diet consumed approximately 80% more food than did their stock fed controls but increased total energy expenditure close to 100%. This resulted in a significantly lower energetic efficiency which could not be accounted for by an elevated level of activity or cost of fat synthesis (Rothwell, Stock 1979b). In the same experiment it was also observed that a spontaneous loss of body weight occurred when the cafeteria food was removed because of hypophagia and a sustained increase in oxygen consumption suggesting a metabolic adaptation to assist in recovery from obesity. These results and others have been criticized by Hervey and Tobin (1981; 1982; 1983) on the basis of methodological errors arising from the use of food composition tables to estimate metabolizable energy intake. In addition, they have also criticized the validity of estimating energy expenditure from the difference between energy intake and carcass energy gain. Additional studies conducted by Rothwell and Stock (1982a) have shown that values obtained for energy intake by bomb calorimetry agree

to within 2% with those determined by food composition tables. Moreover, energy expenditure as estimated by oxygen uptake studies was similar to that derived by the carcass balance method (Rothwell, Stock 1982d). Hervey and Tobin have also argued that feeding animals by stomach tube is a more effective method than cafeteria feeding to evaluate the presence of regulatory mechanisms for energy expenditure since intake can be accurately controlled by the experimenter. Using this technique they have concluded that overfed animals do not exhibit DIT (Hervey, Tobin 1982). However, several studies have shown that tube feeding results in large increases in body fat mass, despite a total daily intake of energy which is similar to that of control rats Cohn, Joseph 1959; 1960; Rothwell, Stock 1979a). This is likely due to the change in feeding pattern since rats are typically food nibblers and increase their metabolic efficiency when total daily intake is provided in discrete meals (Fabry, 1967). Clearly the tube fed animal is an inappropriate model to use for evaluating the effects of increased spontaneous energy intake on the regulation of energy balance. Although several experiments have shown that DIT is present in voluntary feeding situations when cafeteria food is available, the effects of cafeteria feeding on the propensity for obesity and DIT are variable and would appear to depend on age (Rothwell, Stock 1982a) and genetic background (Rothwell et al. 1982a; Rothwell, Stock 1980a).

These factors may explain some of the discrepancies between laboratories (Rolls et al. 1980; Armitage et al. 1981a; 1981b; Hervey, Tobin 1982). For example, unlike older animals, young cafeteria fed rats gain weight at the same rate as their stock fed controls (Stephans 1980; Rothwell, Stock 1982a; Brooks et al. 1982). Nonetheless, body fat content increases but strain differences are also evident (Rothwell, Stock 1982a, 1982b). Lean male Zucker rats (+/?) fed a cafeteria diet for 24 days beginning at 38 days of age increased energy intake by 73%. The percent body fat was 10.5 compared to 5.6 for the stock fed controls (Rothwell, Stock 1982a). Cafeteria fed male Sprague Dawley rats 32 days old consumed 50% more energy over the course of 82 days but fat represented only 24.9% of carcass weight compared to 21.2% for control rats (Rothwell, Stock 1982b).

That DIT is under control of the sympathetic nervous system was initially suggested by the findings of Young and Landsberg (Young, Landsberg 1977a; 1977b; 1979). By measuring NA turnover they showed that fasting suppressed sympathetic activity in the heart, liver and pancreas of rats, while sucrose supplemented chow feeding enhanced sympathetic activity in these same organs. Further evidence for sympathetic mediation of DIT was provided by Rothwell and Stock (1979b; 1982a). They reported that adminstration of propranolol suppressed the elevated resting metabolic rate of cafeteria-fed rats without affecting oxygen uptake

of chow-fed controls. Moreover, in a recent study Young and associates (1982) observed that increased NA turnover occurred in BAT of rats fed a cafeteria diet. Since ganglionic blockade exerted a greater effect on NA turnover in BAT of cafeteria fed rats than control rats they concluded the increased NA turnover in the tissue reflected an enhanced central sympathetic outflow. Rothwell and Stock (1979b; 1982a) have also shown that cafeteria feeding enhanced the capacity of rats to respond to exogenous NA by an increase in metabolic rate, suggesting that cafeteria-fed rats have a larger amount of thermogenically active BAT than their chow-fed controls. Indeed, several investigators report that both the metabolic and mitochondrial mass of BAT are greater in cafeteria-fed rats (see Part III.D.1). Blood flow studies using the microsphere method have demonstrated the quantitative importance of BAT thermogenesis in cafeteria fed rats (Rothwell, Stock 1981b). Total oxygen consumption of BAT accounted for 74% of the enhanced NA response in cafeteria fed rats compared with 42% of the smaller response in control animals. Since the increments in oxygen uptake of other tissues were similar for both groups the authors have concluded that the enhanced thermogenic capacity of overfed animals is due to increased BAT thermogenesis.

Thus, both DIT and NST are mediated by the action of NA on BAT and thus share a common heat producing mechanism.

Indeed cafeteria-fed warm-acclimated rats sustain a higher body temperature, and shiver less than controls when exposed to 5°C (Rothwell, Stock 1980b).

4. Thyroid-induced thermogenesis

Thyroid hormones are the principal hormones regulating endothermic thermogenesis (Danforth, Burger 1981; Himms-Hagen 1983a). Although the thermogenic action of thyroid hormones is not well understood two major hypothesis have been proposed to explain thyroid-induced thermogenesis. The first hypothesis was based on observations of Na^+K^+ ATPase activity in tissues of rats with altered thyroid status. These studies led Edelman and coworkers (Smith, Edelamn 1979; Ismail-Bergi, Edelman 1971; Asano et al. 1976) to suggest that an increase in Na^+K^+ ATPase activity, particularly in muscle and liver, might account for a substantial portion of the thermogenic action of thyroid hormones. However, there is disagreement of the quantitative contribution that the sodium pump makes to thyroid-induced thermogenesis. Thus, in euthyroid animals, ouabain inhibited respiration by 20-38% in liver slices of rats (Ismail-Bergi, Edelman 1970; 1971) and of mice by 30% (Guernsey, Morishige 1979) but only by 5-6% in perfused rat liver (Folke, Sestoff 1977) and 2-8% in rat hepatocytes (Clark et al. 1982). Likewise, respiration was reduced with ouabain in muscle slices of rats by 41% (Asano et al. 1976) and of mice by 58% (Guernsey, Morishige 1979), while ouabain suppressed heat production by 5-8% in

intact soleus muscle from rat (Chinet et al. 1977) and mice (Biron et al. 1979). In hyperthyroid animals, increased respiration attributed to thyroid-induced pump activity has been reported as 90% in liver slices (Ismail-Bergi, Edelman 1970; 1971) as 49% in kidney slices (Ismail-Bergi, Edelman 1971) and as 84% in muscle slices (Asano et al. 1976) from hyperthyroid rats. However, ouabain suppressible respiration and heat production was small (5-15%) in hepatocytes (Clark et al. 1982), perfused liver (Folke, Sestoff 1977) and intact muscle (Biron et al. 1979) from hyperthyroid rodents and did not significantly differ from values reported for euthyroid animals. A possible reason for the discrepancy in estimates for energy cost of active Na^+ K^+ transport has been suggested by Chinet and coworkers (1977). They argue that the high values observed with tissue slices occurs because Na^+ K^+ transport was stimulated above basal levels as a result of Na^+ leakage into cytoplasm via the cut ends. In addition, several difficulties have been raised by Himms-Hagen (1976) in using ouabain to estimate energy expenditure of the sodium pump. The major problems are that ouabain appears to have variable effects on other metabolic processes which may modify respiration and that blocking the pump will lead to an altered intracellular ionic concentration which might inhibit mitochondrial respiration. Sestoff (1980) has therefore concluded that in euthyroid and hyperthyroid tissues, Na^+ K^+ transport probably accounts for

no more than 5-10% of the total oxygen uptake and that Na^+ K^+-ATPase does not play a major role in thyroid thermogenesis.

The second hypothesis attributes maintenance of the homeothermic state to the action of thyroid hormones on mitochondria. The results from early studies suggested that uncoupling of oxidative phosphorylation was a possible mechanism of hormone action (Sterling 1979b). However, Shears and Bronk (1979) have observed increased state 4 respiration in rat liver mitochondria which cannot be due to uncoupling since it was associated with an elevated proton electrochemical gradient. That thyroid hormones exert a direct and early action on mitochondria is indicated by studies showing that the formation of ATP by isolated liver mitochondria from hypothyroid rats increased within 2 minutes when physiological amounts of T_3 were added to the incubation medium (Sterling 1977). Because T_3 receptors are present in the inner mitochondrial membrane of thyroid sensitive tissues it is possible that the direct action of the hormones is due to a conformational change induced when T_3 binds to the receptor (Sterling 1977). The extent to which the mitochondria participate in thyroid thermogenesis is not known.

PART III: BROWN ADIPOSE TISSUE AND THERMOGENESIS

A. Structure and Composition of Brown Adipose Tissue

Brown adipose tissue was first described more than four centuries ago in the marmot. Since that time many roles have been ascribed to BAT, but it was not until the early 1960's that it was identified as an important thermogenic tissue for mammals at birth, during cold-acclimation and arousal from hibernation (Smith, Horwitz 1969). At present, it is recognized as the major site for NST (Foster, Frydman 1978b; 1979) and DIT (Rothwell, Stock 1981b). In keeping with its thermogenic role, BAT has the capacity to grow or regress in accordance with the need for adaptive heat production. Thus, larger amounts of functional tissue are present in neonates, cold-acclimated animals and in animals adapted to prolonged overfeeding, while maturation of nonhibernators, reacclimation to warm temperatures, and removal of palatable foods is associated with a loss of BAT mass (Smith, Horwitz, 1969; Himms-Hagen 1978; Himms-Hagen et al. 1981).

Brown adipose tissue is a diffuse organ located in discrete areas throughout the body including the subscapular, interscapular, cervical, axillary, para-aortic and perirenal regions. The exact distribution varies from species to species and can account for 1-5% of the body mass (Afzelius 1970; Smith, Horwitz 1969). The color of the tissue is due to its rich vascularity and the high cytochrome content of

its mitochondria (Flatmark, Pedersen 1975). The tissue also receives an extensive sympathetic nervous supply. Postganglionic fibers originate within the sympathetic chain and enter BAT either via the intercostal nerves or together with the blood vessels to innervate individual adipocytes as well as blood vessels (Barnard et al. 1980).

In contrast to mature white adipocytes, which are characterized by a single large triglyceride droplet (unilocular) and few mitochondria, a typical brown adipocyte is a polygonal cell containing a high concentration of mitochondria distributed throughout the cytoplasm and in close contact with many small lipid droplets (multilocular) (Afzelius 1970; Barnard 1977; Suter 1969). The morphology of brown adipocytes, although basically similar in all species will however vary according to the developmental stage, nutritional condition and state of adaptation (Afzelius 1970; Flatmark, Pedersen 1975). For example, when brown adipocytes are in a quiescent state they become replete with fat and assume a unilocular appearance making them difficult to distinguish from white adipocytes (Cannon, Johansson 1980). The abundance of mitochondria, their large size and internal structure are clearly the most distinctive microscopic features of BAT (Flatmark, Pedersen 1975). The cristae are numerous, parallel and typically transverse the width of the mitochondrion (Suter 1969; Afzelius 1970). When isolated, the matrix appears contracted and the

intracristal space expanded (Thomson et al. 1968; Bulychev et al. 1972).

The principal fuel oxidized by BAT for thermogenesis is fatty acids (Flatmark, Pedersen 1975). This can be demonstrated by the complete inhibition of NA-stimulated respiration in the presence of 2-tetradecylglycidic acid, a specific inhibitor of mitochondrial carnitine palmitoyl-transferase (Bukowiecki et al. 1981). Brown adipose tissue mitochondria are suitably equipped for heat production. Accordingly, the rates of activation, transportation and oxidation of fatty acids are high. In BAT, acyl CoA synthetase is located exclusively on the outer surface of the mitochondrial membrane (Pedersen et al. 1975). Its activity greatly exceeds the capacity for fatty acid oxidation (Pedersen et al. 1975). Highly active "outer" and "inner" acyl CoA transferases are present along with an equally effective acyl carnitine-carnitine exchange carrier (Norman et al. 1978). There is also a very active β-oxidation system in BAT as judged by the high rate of long chain acyl carnitine oxidation (Nicholls 1979). Specialization of BAT for heat production is further reflected by the high activity of respiratory chain enzymes (Houstek et al. 1978).

In addition to an enhanced capacity for fatty acid utilization, BAT also has a substantial capacity for lipogenesis which increases during acclimation to cold (Smith, Horwitz 1969; Agius, Williamson 1981; Trayhurn 1980;

1981). In BAT of warm-acclimated rats, ketone bodies as well as carbohydrates are converted into fatty acid, suggesting that ketone bodies may be an important substrate during starvation when ketone body turnover is high and carbohydrate must be conserved (Agius, Williamson 1981). Although the primary role of BAT is to generate heat it also exports fatty acids during thermogenesis, possibly for use as a source of energy in tissues such as heart and respiratory muscles during acute cold exposure or in skeletal muscles in the later stages of arousal from hibernation (Nedergaard, Lindberg 1979; Bieber et al. 1975).

B. Control of Thermogenesis in Brown Adipose Tissue

Evidence has been presented in Part II:C to show that DIT and NST are mediated by the action of NA on BAT. The central control for BAT thermogenesis likely involves the hypothalamus. According to Brück and Zeisberger (1978) a central controller within the hypothalamus receives afferent inputs from thermal receptors located in the skin, preoptic region and spinal cord. This information is processed and used to control body temperature by regulating thermogenesis (shivering and nonshivering) and heat dissipation. Although the nature of the dietary stimulus which mediates hypothalamic control of DIT is at present unclear much information is accumulating to suggest that insulin may represent such a signal (Young, Landsberg 1980; Landsberg, Young 1981;

Rowe et al. 1981).

That there is a direct neural link between the hypothalamus and BAT is indicated by the observations that electrical stimulation of the ventromedial hypothalamus (VMH) results in an increased metabolic activity of BAT (Shimazu, Takahashi 1980) and a biphasic temperature change (Perkins et al. 1981) which is similar to that caused by stimulation of sympathetic nerves to BAT (Flaim et al. 1977). Furthermore, the hypothalamus mediates the increase in BAT temperature caused by cold exposure or stimulation of the brain stem (Brück, Hinckel 1980; Brück, Zeisberger 1978). Thus, it would appear that BAT thermogenesis is regulated in part by the hypothalamus which adjusts sympathetic outflow in response to diet or cold.

The initial step in the series of events culminating in dissipation of energy as heat is the interaction of NA released from sympathetic nerves with receptors on the plasma membrane. This is followed by depolarization of the membrane, lipolysis and an increase in mitochondrial respiration (Nicholls 1979; Horwitz 1978a; Bukowiecki et al. 1981; Flatmark, Pedersen 1975).

Radioligand binding techniques and measurements of the metabolic response to various agonists and antagonists indicate that both α-adrenergic receptors (Flaim et al. 1977; Garcia-Sainz et al. 1980; Svartengren et al. 1980) and β-adrenergic receptors

(Bukowiecki et al. 1978; 1980; Pettersson, Vallin 1976; Flaim et al. 1977; Svoboda et al. 1979; Hamilton, Horwitz 1979) are present in BAT.

Several investigators have concluded that NA exerts its thermogenic action predominately via the β adrenergic pathway (Bukowiecki et al. 1981; Cannon et al. 1978; Hamilton, Horwitz 1979). This is based on the observation that α- and β-agonists differ in their effectiveness at eliciting a thermogenic response. Concentrations in the millimolar range are necessary for α-agonists compared to micromolar levels for β-agonists (Horwitz 1978a; Bukoweicki et al. 1981; Hamilton, Horwitz 1979). Furthermore, high concentrations of α-antagonists inhibit NA-stimulated thermogenesis only partially compared to total inhibition with low concentrations of the β-adrenergic antagonist propranolol (Horwitz 1978a; Bukowiecki et al. 1981). Bukowiecki and associates (1981) have shown that β-agonist stimulated respiration in isolated BAT cells has the characteristics of a β_1 receptor subtype.

Binding of NA to the β_1 adrenergic receptor activates adenylate cyclase (Begin-Heick, Heick 1982; Skala et al. 1970) which leads to a rapid and transient rise in cAMP concentration (Pettersson, Vallin 1976; Bertin, Portet 1976). As in other tissues, cAMP exerts its effect by activating protein kinases. Nine protein kinases have been identified

in BAT, eight of which appear to be dependent on cAMP (Knight, Skala 1977). Certainly for BAT thermogenesis the most important action of cAMP protein kinase is the activation of hormone sensitive lipase.

As mentioned previously (Part II:A), fatty acids released from the breakdown of triglycerides can be exported from the cell or enter the mitochondria for combustion. In addition, fatty acids may also be re-esterified. Glycerol kinase is reported to be present in BAT of rats (Bertin 1976) and mice (Assimacopoulos-Jeannet et al. 1982) but not hamsters (Lindberg et al. 1976). However, since the ratio of exported fatty acids/glycerol is reported to be close to 3 (Nedergaard, Lindberg 1979) it has been concluded that only a small fraction of the NA-stimulated release of fatty acids is re-esterified (Nedergaard, Lindberg 1982).

C. Mechanism of Thermogenesis in Brown Adipose Tissue

Overall heat production in all cells is primarily a consequence of substrate oxidation in the mitochondria and therefore the rate of oxidation (respiration) determines the rate at which fuel energy is dissipated as heat. However, the actual site of heat evolution within a cell depends upon the extent to which mitochondrial respiration is coupled to ATP synthesis. During coupled respiration when mitochondrial substrate oxidation is obligatorily linked to ATP formation, redox reactions of the respiratory chain

are in close thermodynamic equilibrium with ADP phosphorylation and ATP formation proceeds with an efficiency of energy conservation approaching 95% (Slater et al. 1973). Thus, most of the free energy of substrate oxidation is incorporated into ATP. It is subsequently released as heat in the extramitochondrial compartments of the cells, during utilization of ATP and during degradation of products having initially utilized part of the free energy from ATP hydrolysis for their formation. Since the major portion of ATP utilized in the cytosol is generated by mitochondria, the rate of coupled respiration depends on the cytosolic demand for ATP and is therefore controlled by the phosphorylation state ratio. During loosely coupled or uncoupled respiration, which by-passes stoichiometric ATP formation, most of the chemical energy is converted into thermal energy at the level of the mitochondrion. Whether heat production is localized in the mitochondria or extramitochondrial compartments makes no difference to the overall free energy change during complete substrate oxidation (Himms-Hagen 1976). The heat generating mechanism in BAT mitochondria must however be regulated since thermogenesis in vivo can be readily switched on or off.

Various theories have been proposed to account for the extraordinary capacity of BAT to generate heat when stimulated and to revert back to basal conditions when sympathetic activity is reduced. The two most likely models

are the loosely coupled hypothesis and the ATPase hypothesis. The loosely coupled hypothesis involves the operation of a regulated proton leakage pathway which is unique to BAT mitochondria (Nicholls 1976a; Nicholls 1979). This pathway allows respiration to proceed at a rate which is no longer dependent on the availability of ADP. The ATPase hypothesis which is based on Edelman's theory for thyroid-induced thermogenesis (Edelman, 1974; Edelman 1976) postulates that BAT thermogenesis results from an increased Na^+ K^+ ATPase activity (Girardier et al. 1968; Horwitz 1978b; Horwitz, Horowitz 1977). Although present experimental evidence favours the loosely coupled hypothesis it is not necessary for both theories to be mutually exclusive.

1. Loosely coupled hypothesis

The framework for experimentation and formulation of the loosely coupled hypothesis was based on the chemiosmotic theory developed by Mitchell (Mitchell, 1966). The chemiosmotic theory proposes that the chemical potential derived from electron transfers in the inner mitochondrial membrane is used to pump protons out of the mitochndria. As the inner membrane is quite impermeable to protons an electrochemical gradient is created which is capable of doing work. Much of this electrochemical potential is used to drive the proton translocating ATP synthetase so that ATP is formed and energy is conserved. The rate of proton reentry

is automatically balanced by the rate of respiration driven proton pumping. This in turn is controlled by the proton electrochemical gradient such that the rate of coupled respiration is inversely proportional to the magnitude of the gradient (Nicholls 1979).

Nicholls and others have provided evidence for a proton conductance pathway which when operating would allow protons to reenter the matrix independent of the ATP synthetase. The redox energy stored in the proton gradient is therefore dissipated as heat rather than being conserved as ATP. That BAT mitochondria possess unusual characteristics has been known for many years. When mitochondria of BAT are freshly isolated using conditions suitable for other mitochondrial systems they are de-energized and exhibit uncontrolled respiration. This is demonstrated by their failure to maintain a proton electrochemical gradient during respiration and by their high state 4 respiration rate which shows little or no effect of ADP, uncouplers or oligomycin (Nicholls 1974a; Rafael 1978; Desautels, Himms-Hagen 1981). However, under in vitro conditions designed to mimic intracellular conditions in BAT mitochondria during NST, measurements of the adenine nucleotide potential indicate that oxidative phosphorylation still occurs (Nicholls, Berson 1977). The mitochondria are therefore not uncoupled but rather are loosely coupled. Nicholls and others (Nicholls 1974a; Locke et al 1982a; b)

have shown that the low proton electrochemical gradient in mitochondria and ensuing loss of respiratory control results from an increased permeability of the membrane to protons. This was determined by measuring proton conductance; defined as the proton current crossing the membrane per millevolt of proton electrochemical potential. Addition of purine nucleotides to isolated mitochondria decreases the proton conductance (Nicholls 1974a; Locke et al. 1982a; b). This results in an increase in the proton electrochemical gradient and a restoration in respiratory control. That the proton conductance pathway is specific to BAT is indicated by results which show that under similar isolation conditions hamster BAT mitochondria exhibit an effective proton conductance which is 100 times greater and a proton electrochemical gradient which is 20 times less than that of rat liver mitochondria (Nicholls 1974a; 1974b). Moreover, other studies which estimate proton permeability by measuring rates of mitochondrial swelling have shown that BAT mitochondria of cold-acclimated hamsters, rats and guinea pigs but not mitochondria from rat heart or liver are freely permeable to protons and become impermeable in the presence of purine nucleotides (Nicholls Lindberg 1973; Nicholls et al. 1974). The effectiveness of different purine nucleotides is similar for inducing respiratory control, inhibiting proton permeability and increasing the proton electrochemical gradient, GDP being

the most potent (Nicholls et al. 1974; Heaton, Nicholls 1977). It therefore appears that BAT mitochondria possess a unique pathway through which protons leak back into the matrix which is independent of ATP synthesis and is inhibited by purine nucleotides.

A binding site for purine nucleotides has been identified on the outer surface of the inner mitochondrial membrane which is not present in liver mitochondria and is distinct from the adenine nucleotide translocase (Nicholls 1976b; Rafael, Heldt 1976). Purine nucleotides bind without covalent modification to a single class of sites but with an apparent dissociation constant that is pH dependent such that an increased pH is associated with a reduced affinity and an increased proton conductance (Nicholls 1976b; Nicholls 1974a; Heaton, Nicholls 1977).

Using a photoaffinity analogue of ATP, Nicholls and coworkers (Heaton et al. 1978) have identified the purine nucleotide binding site as 32 000 molecular weight polypeptide. This polypeptide has been purified from hamsters, rats, guinea pigs and rabbits (Lin, Klingenberg 1980; Ricquier et al. 1982) and has been shown to exist as a dimer with a molecular weight calculated at 62 000 - 63 000 (Lin, Klingenberg 1980; Lin et al. 1980; Ricquier et al. 1982). Recently, both an enzyme-linked immunosorbent assay (Cannon et al. 1982) and a radioimmunoassay (Lean et al 1983) have demonstrated that the 32K polypeptide is exclusive to BAT

mitochondria since it was not found in mitochondria of white fat, liver or heart muscle. Further evidence that the proton conductance pathway is the molecular site for energy dissipation in BAT is obtained from studies which show a high correlation between the thermogenic state of animals, the relative amount of 32K polypeptide and the extent of GDP binding by BAT mitochondria. In newborn nonhibernators the capacity for NST is greatest and declines thereafter. Likewise, the amount of 32K polypeptide (Heaton et al. 1978) and the extent of GDP binding (Rafael, Heldt 1976; Sundin, Cannon 1980) are maximal at birth and rapidly decrease during postnatal development. However, when nonhibernators are exposed to prolonged cold at any age thermogenic activity reappears in parallel with an increase in the proportion of 32K polypeptide (Desautels et al. 1978; Ricquier, Kader 1976; Heaton et al. 1978; Lean et al. 1983) and an increase in GDP binding (Desautels et al. 1978; Sundin, Cannon 1980). The adaptive nature of BAT will be further discussed in Part III.D.1.

Although it is now generally agreed that the BAT thermogenesis involves operation of the purine-nucleotide-sensitive proton conductance pathway, there is less consensus as to how this is regulated in vivo. Purine nucleotides are present in millimolar concentrations in BAT (Petterssen, Vallin 1976), well above levels required to inhibit proton conductance in isolated mitochondria

(Nicholls 1979). Since the binding site has a high affinity for purine nucleotides (Nicholls 1976b), Nicholls (1979) has suggested that intracellular purine nucleotides are bound when the tissue is thermogenically inactive. Various possibilities have been postulated as the intracellular messenger which would displace purine nucleotides and therefore increase heat production when NA binds to the plasma membrane. Chinet (1978) and coworkers have suggested intracellular alkalinization may trigger the thermogenic process by decreasing the affinity of the 32K polypeptide for GDP (Nicholls 1976b). The experimental evidence to date is however limited. Acyl CoA has also been considered as the physiological messenger since it can competetively inhibit GDP binding (Cannon et al. 1977). This hypothesis has been criticized on the basis that levels of acyl CoA necessary to achieve effective proton conductance in BAT mitochondria will also increase membrane permeability in liver (Nicholls 1979).

Recently, several papers have been published providing evidence that fatty acids may serve as the intracellular messenger for BAT thermogenesis (Bukowiecki et al. 1981; Locke, Nicholls 1981; Locke et al. 1982a; 1982b). Bukowiecki and associates (1981) reported that exogenous fatty acids stimulate respiration of isolated BAT cells from warm-acclimated rats almost to the same extent as NA and that respiration is switched on and off in synchrony with lipolysis. The effect of fatty acids would appear to

be specific for BAT since proton conductance is thirtyfold greater in BAT mitochondria than liver for each nmol of palmitate bound (Heaton, Nicholls 1976). These experiments were however conducted under non physiological conditions. A system has now been developed whereby the effect of fatty acids on respiration, membrane potential and proton conductance of isolated mitochondria can be observed under conditions which more closely resemble those occurring in vivo (Locke, Nicholls, 1981). This is achieved by infusing physiological concentrations of fatty acids into an incubation containing pyruvate as the initial substrate. Under these conditions infusion of fatty acids but not acyl CoA completely reversed purine-induced inhibition of proton conductance in isolated hamster mitochondria (Locke, Nicholls 1981; Locke et al. 1982a). Moreover, when the infusion of fatty acids is terminated, respiration, membrane potential and proton conductance return to original levels. This effect is tissue specific as fatty acids infused into rat liver mitochondrial incubations produce only a small increase in proton conductance (Locke et al. 1982a). Finally, there is indirect evidence for a functional association between fatty acids and the 32K polypeptide. Measurements of proton conductance have shown that mitochondria from cold-acclimated guinea pigs are ninefold more sensitive to uncoupling by endogenous and infused fatty acids than those from

warm-acclimated guinea pigs (Locke et al. 1982b). Also, respiration from cold-acclimated but not warm-acclimated mitochondria increases in proportion to the rate of fatty acid infusion (Locke et al. 1982b). Although these results are suggestive of a direct interaction between the 32K and fatty acids to displace purine nucleotides, there is no evidence to negate the possibility that their action may be independent of the purine-nucleotide-sensitive pathway.

The mechanism of heat production in BAT as postulated by the Loosely Coupled Hypothesis can be summarized as follows. Noradrenaline binds to α- and β-adrenergic receptors on the plasma membrane, adenylate cyclase is activated and lipolysis ensues via a system of protein kinases and lipases to provide fuel for mitochondrial respiration. Simultaneously, an intracellular messenger, most probably fatty acids, interacts with the 32K polypeptide located on the outer surface of the plasma membrane to displace purine nucleotides. Protons leak back into the matrix via the proton conductance pathway thus bypassing ATP synthetase. This serves to lower the proton electrochemical gradient, increase proton pumping out of the matrix and stimulate loosely coupled respiration. When sympathetic activity declines, brown adipocytes function like other normal aerobic cells. The mitochondria now operate to minimize dissipation of chemical energy as heat by conserving the proton electrochemical potential in

the synthesis of ATP.

Measurements of GDP binding by BAT mitochondria and the relative amount of the 32K polypeptide are used as a quantitative index of the thermogenic state of BAT (Brooks et al. 1980; Himms-Hagen et al. 1981; Triandafillou et al. 1982; Fellenz et al. 1982; Desautels et al. 1978; Desautels, Himms-Hagen 1980; Trayhurn et al. 1982a). As mentioned previously, both parameters vary in synchrony with the thermogenic capacity of the tissue. Himms-Hagen and coworkers (Desautels et al. 1978; Desautels, Himms-Hagen 1980) have also shown that when warm-acclimated rats are initially exposed to the cold there is an altered mitochondrial ultrastructure and an increase in GDP binding which is not accompanied by a change in the relative amount of the 32K polypeptide. This was interpreted as an unmasking of binding sites already present subsequent to a structural reorganization of the inner mitochondrial membrane. As well, the unmasking of existing binding sites has been demonstrated in cafeteria-fed rats, since their BAT mitochondria exhibit an increase in GDP binding without a change in polypeptide composition (Brooks et al. 1980; 1982; Himms-Hagen et al. 1981; Stock, Rothwell 1981). The increase in GDP binding which occurs with brief cold exposure or cafeteria feeding is thought to be mediated by the action of NA on the tissue (Desautels, Himms-Hagen 1979; Brooks et al. 1982). Thus, it can be concluded that while

the 32K polypeptide represents the amount of proton conductance pathway in BAT, GDP binding reflects operation of the pathway *in vivo*.

2. ATPase hypothesis

Involvement of the sodium pump in BAT thermogenesis was initially postulated by Girardier and coworkers (1968) who observed depolarization of the plasma membrane in response to exogenous NA. Simultaneous measurment of respiration and membrane potential have shown that depolarization occurs within 1 second of NA binding to the receptor and that it always precedes the increase in mitochondrial respiration (Seydoux, Girardier 1978). The decrease in membrane potential results from an increase in membrane permeability which allows ions to move down their electrochemical gradient (Horowitz *et al.* 1971; Girardier, Seydoux 1971). This effect is mediated by α- and β-adrenergic receptors (Fink, Williams 1976; Flaim *et al.* 1977) which are equally effective in depolarizing brown adipocytes (Fink, Williams 1976). Clearly, the increase in intracellular Na^+, following the NA-induced increase in membrane permeability, will indirectly stimulate the sodium pump to restore intracellular concentrations of Na^+ and K^+ to prestimulus levels. In addition, several investigators have reported that NA directly stimulates Na^+ K^+ ATPase activity in BAT of cold-acclimated and

cafeteria fed animals (Herd et al. 1970; Horwitz, Eaton, 1975; Rothwell et al. 1981a; Rothwell et al. 1982a) via the β adrenergic pathway. An enhanced enzyme activity is achieved in the presence of cyclic AMP (Horwitz, Eaton 1975) and isoproterenol (Horwitz, Eaton 1975; Rothwell et al. 1981a) but not phenylephrine (Rothwell et al. 1981a). Moreover, NA-stimulated Na^+ K^+ ATPase is 100 times more sensitive to β than to α blockade (Herd et al. 1970).

Studies using ouabain to inhibit sodium pump activity have also implicated Na^+ K^+ ATPase in BAT thermogenesis. Addition of ouabain to isolated hamster cells depresses the magnitude of noradrenaline, isoproterenol, and phenylephrine-induced respiration by 60-70% (Horwitz 1973; Horwitz 1978a). A 10-50% reduction in NA-stimulated respiration has been reported in isolated rat adipocytes (Herd et al. 1973; Bukowiecki 1981). The extent of inhibition appears to be a function of exposure time to ouabain such that the effect progressively increases with time (Chinet et al. 1978). As reviewed by Himms-Hagen (1976) this may result from a depletion of intracellular K^+ rather than a direct inhibition of the pump by ouabain and therefore seriously questions the quantitative significance of ouabain inhibited respiration.

If Na^+ K^+ ATPase was to play a major role in BAT thermogenesis then an adequate supply of ATP would be necessary for Na^+/K^+ pumping. It is unlikely that BAT

mitochondria could provide this ATP. They have a low ATP synthetase activity (Lindberg et al. 1967; Houstek et al. 1978), insufficient to provide the necessary ATP to support thermogenesis (Cannon, Vogel 1977); this low activity is not due to impaired exchange of adenine nucleotides (Christiansen et al. 1973) or inadequate amounts of the translocator (Heaton et al. 1978). Rather, BAT mitochondria have low levels of F_1 ATPase (Cannon, Vogel 1977; Houstek et al. 1978) which appears to account for the limited rate at which BAT mitochondria can phosphorylate extramitochondrial ADP (Nicholls, Bernson 1977). On the other hand, BAT mitochondria are particularly suited for heat production in that there is a high content of respiratory chain components and a very high activity of oxidative enzymes (Houstek et al. 1978; Houstek, Drahota 1975). There is no doubt that increased Na^+ K^+ ATPase activity will contribute to BAT thermogenesis, however its significance as a major component of overall heat production is questionable (Nicholls 1979; Cannon et al. 1978; Nedergaard, Lindberg 1982). Increased sodium pumping may however play a regulatory role by generating an intracellular signal which would lead to increased mitochondrial substrate oxidation (Horwitz 1979). The NA-induced increase in intracellular Na^+ is also believed to promote Ca^{++} cycling in BAT mitochondria (Al-Shaikhaly et al. 1979; Nedergaard 1981). Nedergaard (1981) has proposed that the Na^+-induced elevation in cystolic Ca^{++}

will stimulate mitochondrial glycerol-3-phosphate dehydrogenase, known to be allosterically regulated by Ca^{++} (Bukowiecki, Lindberg 1974). This in turn would lead to a reduced availability of substrate for fatty acid re-esterification and therefore a rerouting of fatty acids toward oxidation (Nedergaard 1981). Thus, the NA-induced increase in intracellular Na^{+} can be seen to exert a regulatory influence on thermogenesis

D. Adaptive Changes in Brown Adipose Tissue in Response to Cold or Diet

1. Growth of brown adipose tissue and changes in mitochondrial composition

Adaptive modifications occur in BAT during acclimation to cold and prolonged overfeeding which serve to increase its thermogenic activity. These changes, which are more extensively documented in cold-acclimated animals, include tissue growth and structural and compositional alterations of the mitochondria.

In BAT of rats, during the first hours of cold exposure there is a substantial reduction in the number and size of triglyceride droplets (Cameron, Smith 1964; Suter, 1969), an increased dilation of blood vessels (Bukowiecki et al. 1982; Suter 1969) and an increased turnover of NA (Young et al. 1982). There is also a transient increase in the size of isolated mitochondria and a rearrangement of the

inner mitochondrial membranes into parallel cristae (Desautels, Himms-Hagen 1980). These changes in ultrastructure reappear within 7-14 days such that mitochondria from cold-acclimated rats are characterized by numerous and parallel cristae (Desautels, Himms-Hagen 1980; Suter 1969). Cold acclimation induces mitochondrial proliferation in the tissue as determined by an increase in cytochrome oxidase content (Bukowiecki et al. 1982; Triandafillou et al. 1982; Fellenz et al. 1982). As well, prolonged cold exposure increases total mitochondrial phospholipid content and decreases the phosphatidylcholine/phosphatidylethanolamine ratio (Ricquier et al. 1978). Simultaneously there are alterations in the fatty acid composition of mitochondrial phospholipids so that they become relatively more unsaturated (Ricquier et al. 1978; Mory et al. 1981). The mitochondrial polypeptide composition also changes in cold-acclimated animals and this results in an increase proportion of the 32K polypeptide (Desautels et al. 1978; Ricquier, Kader 1976; Fellenz et al. 1982; Triandafillou et al. 1982; Heaton et al. 1978; Lean et al. 1983) which is thought to be due to selective alterations in cytosolic synthesis of mitochondrial proteins, possibly combined with selective degradation or formation from larger mitochondrial polypeptides (Himms-Hagen et al. 1980). Accompanying the changes in polypeptide composition is an increase in binding of purine nucleotides which results from an increase in a

single class of binding sites and not a change in binding affinity (Desautels, Himms-Hagen 1981). Prolonged cold exposure promotes growth of active tissue mass and hyperplasia as determined by increases in protein and DNA content and total number of brown adipocytes (Desautels et al. 1978; Mory et al. 1982; 1980; Bukowiecki et al. 1982). Quantitative radioautographic studies using [^{3}H]thymidine indicate that proliferation of brown adipocytes results from differentiation of interstitital cells and preadipocytes (Bukowiecki et al. 1982). At the same time there is an outgrowth of nerve fibers to maintain sympathetic innervation with the newly formed brown adipocytes as well as the endothelial cells and a substantial increase in the total NA content of the BAT (Barnard et al. 1980).

As with chronic cold exposure, providing a cafeteria diet to rats results in mitochondrial proliferation, tissue growth and hyperplasia (Brooks et al. 1982; 1980; Bukowiecki et al. 1982; Himms-Hagen et al. 1981). These changes are associated not only with excess energy consumption but also with an altered composition of nutrient intake. That the nutrient composition of the diet is at least as important as excess energy intake in contributing to the adaptive response of BAT has been demonstrated in rats fed a low protein cafeteria diet (Rothwell et al. 1982b). Compared to stock fed controls, energy intake was similar but changes in BAT induced by the low protein diet included

increased wet weight, total protein and mitochondrial protein content. There are no other studies which have examined the effect of different nutrients on BAT growth under isocaloric conditions.

The adaptive response of BAT to cafeteria feeding is somewhat different from that of cold-acclimation. In rats, cold treatment is at least 2-3 fold more potent than cafeteria feeding as a stimulus for growth of active tissue, mitochondrial proliferation and increased cellularity (Bukowiecki et al. 1982; Himms-Hagen et al. 1981; Triandafillou et al. 1982; Fellenz et al. 1982). However, the cold-acclimated rat is hyperphagic and these effects may represent a combined stimulus of diet as well as cold. Indeed, food restriction limits the growth of BAT in rats exposed to chronic cold (Johnson et al. 1982). Hyperplasia is always observed in young cafeteria fed rats (Tulp et al. 1980; Himms-Hagen et al. 1981; Brooks et al. 1982) and may be permanent if treatment is begun in weanling rats (Rothwell, Stock 1982b) but there is no increase in DNA content when older rats (3 months old) are fed a cafeteria diet, despite their marked hyperphagia (Rothwell, Stock 1982b). Rothwell and Stock (1982b) suggest that hypertrophy of BAT occurs at all ages in cafeteria fed rats but that hyperplasia is evident only in rats overfed during early life. In contrast, cold-induced hyperplasia occurs in young and old rats alike (Mory et al. 1980). The most distinguishing

feature between the effects of diet and cold on BAT is the failure to increase the relative amount of the 32K polypeptide during prolonged cafeteria feeding (Himms-Hagen et al. 1981; Stock, Rothwell 1981). This is consistent with observed differences in GDP binding measurements in isolated mitochondria. Although elevated with cafeteria feeding, indicating thermogenic activation of the tissue, GDP binding levels are substantially less than with long term cold treatment (Triandafillou et al. 1982; Fellenz et al. 1982; Desautels et al. 1978; Himms-Hagen et al. 1981). Scatchard-plot analysis of GDP binding data for BAT mitochondria of cafeteria-fed rats are indicative of a single class of sites with no change in binding affinity (Brooks et al. 1982). The increase in GDP binding therefore results from an unmasking of existing binding sites.

The adaptive response of BAT to prolonged overfeeding or cold exposure is then characterized by an increase in active tissue mass and mitochondrial content and an enhanced capacity for thermogenesis. It would appear however that cafeteria feeding is a weaker and perhaps qualitatively different stimulus for eliciting changes in composition and function of BAT.

2. Control of adaptive changes

Three approaches are currently being used to identify the hormone(s) and/or neurotransmitter(s) responsible for

the adaptive response of BAT to diet and cold. One method is to determine whether changes in synthesis, secretion and level of circulating hormones coincide with the development and maintenance of DIT and NST. A second approach is to examine the extent to which chronic administration of hormones or neurotransmitters mimicks the cold- or diet-induced modifications in BAT composition and capacity for heat production. Subjecting animals to prolonged cold exposure or cafeteria feeding and determining the effect on BAT after removal of various hormones or neurtrotransmitters is the third technique employed to identify the mediator(s) involved in adaptation of BAT.

It is well established that NA is the extracellular mediator for controlling heat production in BAT. Observation that acclimation to cold is associated with an increased NA turnover in BAT (Young et al. 1982; Barnard et al. 1980) and that sympathetic innervation to adipocytes is maintained during growth of the tissue (Barnard et al. 1980) are consistent also with a trophic effect of NA on BAT. In this regard, long term treatment with either NA or isopropyl NA mimicks some of the characteristics of the cold acclimated state. These include an enhanced capacity of the animal for a thermogenic response to NA or isopropyl NA and a reduced sensitivity to cold (Leblanc et al. 1972; Heick et al. 1973; Leblanc, Pouliot 1964). In adult rats, there is hypertrophy and hyperplasia of BAT as determined by increases in wet

weight, total protein (Desautels, Himms-Hagen 1979; Heick et al. 1973) and DNA content (Mory et al. 1980). As well, there are increases in total homogenate activity of succinate dehydrogenase and cytochrome oxidase (Desautels, Himms-Hagen 1979; Heick et al. 1973). Young rats appear to be somewhat resistant to chronic treatment with NA in that BAT wet weight and DNA content remain the same (Mory et al. 1980). Long term administration of NA or isoproterenol does not however reproduce the effects of cold acclimation on the phospholipid changes of BAT for either young or adult rats (Mory et al. 1980). Moreover GDP binding by isolated mitochondria does not increase and the polypeptide composition of mitochondrial membranes remain unchanged (Desautels, Himms-Hagen 1979).

Chemical and surgical denervation of BAT is another approach which has been used to assess the role of NA in the adaptive response to cold. Both surgical denervation and treatment with guanethidine result in the accumulation of triglycerides and an altered mitochondrial structure in BAT of cold acclimated rats (Barnard et al. 1980; Mory et al. 1982). In addition, total tissue protein, cytochrome oxidase, DNA and phospholipid content are greatly reduced or remain unaltered when compared to cold-acclimated controls (Barnard et al. 1980; Mory et al. 1982). The cold-induced increase in GDP binding measurements and relative amount of 32K polypeptide also do not occur in guanethidine-treated rats (Mory et al. 1982). Thus, from the response of BAT

to chronic administration of NA or denervation of the tissue it can be concluded that NA plays a significant role in the control of BAT growth. Results from the guanethidine studies suggest that the cold-induced increase in the 32K polypeptide may be mediated by a factor released from sympathetic nerves innervating BAT. On the other hand, it is also possible that the mediator is released from other tissue innervated by sympathetic fibers since guanethedine destroys all peripheral adrenergic neurons (Johnson, O'Brien 1976).

Thyroid hormones are thought to be important for NST (Edelman 1976; Guernsey, Stevens 1977) and DIT (Rothwell et al. 1982d). Indeed, circulating levels T_3 are elevated in animals acclimated to cold (Reichlin et al. 1973; Rothwell, Stock 1980b; Hardeveld et al. 1979; Scammell et al. 1980), or fed a cafeteria diet (Tulp et al. 1982; Rothwell, Stock 1979b; Rothwell et al. 1982d) or a low protein diet (Tulp, Horton 1981, Tulp et al. 1979; Rothwell et al. 1982b) and thyroid hormones and catecholamines are known to interact to effect thermogenesis. For example, the thermogenic response to exogenous catecholamines is influenced by the presence of thyroid hormones such that it is increased in hyperthyroid rats (Rothwell et al 1982d; Leblanc, Villemaire 1970; Heick et al. 1973) and decreased in hypothyroid rats (Hsieh et al. 1966; Rothwell, Stock 1982d). In turn, the sympathetic nervous system appears to influence the peripheral formation of thyroid hormones,

since plasma T_3 levels are reduced in the presence of propranolol (Jung et al. 1980; Verhoeven et al. 1977) and increased with chronic administration of NA (Storm et al. 1981; Rothwell et al. 1982e). Despite the apparent interdependence of the sympathetic nervous system and thyroid hormones, accumulated evidence suggests that only a maintenance amount of thyroid hormones are required for NST and DIT. Certainly, thyroid hormones are essential for survival in the cold (Hsieh, 1962). But since hypothyroid rats can acclimate to low environmental temperatures when provided with a replacement dose of T_4 (Sellers 1974), the increase in blood T_3 levels seen in cold-acclimated euthyroid rats is presumably not essential for NST. Although no information is available regarding the effect that the diet-induced increase in T_3 has on thermogenesis, experiments with fasted animals suggest that thyroid hormones are not directly involved in DIT. The reduction in metabolic rate which occurs during fasting and is associated with a fall in circulating T_3 levels (Rothwell et al. 1982d) and a lower sympathetic activity (Landsberg, Young 1981; Young Landsberg 1979; Young et al. 1982) still is observed in hypothyroid rats treated with maintenance doses of thyroid hormones (Wimpfheimer et al. 1979; Burger et al. 1981). This indicates that a reduced sympathetic nervous activity rather than a reduced level of thyroid hormones is responsible for the lower metabolic rate during fasting and

by inference that thyroid hormones above maintenance levels are not necessary for DIT.

A limited role is also indicated for a trophic effect and a thermogenic effect of thyroid hormones on BAT. Long term treatment with physiological amounts of T_4 promote an increase in BAT wet weight which is due to an accumulation of lipid and protein (Ricquier et al. 1976; Heick et al. 1973). When pharmacological doses of T_4 are administered to rats living at thermoneutrality there are small increases in wet weight, protein and cytochrome oxidase content, but no changes are observed for either DNA or the 32K polypeptide (Triandafillou et al. 1982). Additional studies have shown that T_4 has little to do with maintenance of BAT at thermoneutrality or with the adaptive changes which occur during cold acclimation. BAT from thyroidectomized rats, acclimated to 28°C (thermoneutral) has the same total protein and cytochrome oxidase activity and the same amount of the mitochondrial 32K polypeptide as their oil-treated controls (Triandafillou et al. 1982). In thyroidectomized rats treated with a low maintenance dose of T_4 and exposed to 4°C for 2 weeks there are normal increases in cytochrome oxidase and protein content, as well as a normal increase in the relative proportion of the 32K polypeptide (Triandafillou et al. 1982). Although mitochondrial GDP binding is normal in thyrodectomized rats living at thermoneutrality, a minimal amount of T_4 is necessary for the acute cold-induced increase in GDP

binding. This requirement has been attributed to the permissive effect of thyroid hormone on the action on BAT of NA (Triandafillou et al. 1982), the mediator for unmasking of binding sites (Desautels, Himms-Hagen 1979). By analogy it is also likely that a maintenance amount of thyroid hormones is essential for DIT in BAT.

The mechanism by which thyroid hormones maintain tissue responsiveness to NA, a mixed α-, β-agonist, may in part depend on the proportion of α- and β-adrenergic receptors and their receptor affinity. Several investigators have shown that the thyroid status of animals affects the concentration of adrenergic receptors in specific tissues (Lefkowitz 1982; Kunos 1977; Gibson 1981). For example, in heart of hyperthyroid rats there is an increase in the concentration of β-receptors (Ciaraldi, Marinetti 1978) and a decrease in α-receptors (Williams, Lefkowitz 1979). Conversely, in hypothyroid rat heart there is a decrease in β-receptors and an increase in α-receptors (Ciaraldi, Marinetti 1977). Although there are no reports of changes in the α-receptor population of BAT, there is an increase in β-receptor concentration in BAT of hyperthyroid rats (Sundin 1981) and a reduced β-receptor affinity in hyperthyroid rats (Girardier 1981). Thus, it is conceivable that the permissive effect of thyroid hormones on the thermogenic responsiveness of BAT to NA involves a regulation of the concentration and/or affinity of adrenergic receptors.

In summary, these studies show that a minimal amount of thyroid hormones are necessary for NST and probably DIT but only in so far as they act in a permissive way to enhance the effectiveness of the sympathetic nervous system.

Other hormones have been suggested as mediators in growth of BAT. Doniach (1975) has proposed that TSH may exert a trophic effect on BAT since it was observed that patients with myxedema have high plasma TSH levels and enlarged fat pads in an area known to contain BAT. However, warm-acclimated thyroidectomized rats also have high circulating levels of TSH (Sellers et al. 1974) and yet have a normal amount and composition of BAT (Triandafillou et al. 1982) TSH is therefore not a likely candidate for regulating growth of BAT. A role for growth hormone as a trophic agent for BAT has also been investigated. Long-term treatment of warm acclimated rats with growth hormone does not affect BAT wet weight, total DNA, protein or cytochrome oxidase content. Nor does it affect mitochondrial GDP binding (Fellenz et al. 1982). Furthermore, BAT of hypophysectomized rats with replacement doses of T_4 and corticosterone responds normally to cold acclimation in that the usual changes in wet weight, cytochrome oxidase and protein content occur. In addition, mitochondrial GDP binding and the relative proportion of the 32K polypeptide also increase (Fellenz et al. 1982). This indicates that not only growth hormone but all pituitary hormones can be

eliminated as potential mediators for the growth of BAT and for the change in mitochondrial polypeptide composition. Although BAT is considered to be a target organ for glucocorticoids because it contains glucocorticoid receptors (Feldman 1978), the tissue grows normally in cold-acclimated hypophysectomized rats provided with replacement doses of corticosterone (Fellenz et al 1982). Thus at most, only permissive amounts of glucocorticoids are necessary for the cold-induced growth of BAT and increase in the 32K polypeptide. This also appears to be the case for insulin since mildly to moderately diabetic rats can survive at 5°C for periods of up to 3 months (Mondon 1963). Likewise, permissive amounts of insulin appear to be required for the diet-induced growth of BAT. In cafeteria-fed rats, the diminished response to NA, usually an indication of smaller amounts of thermogenically active tissue, is improved with replacement doses of insulin (Rothwell, Stock 1981d).

In conclusion, permissive amounts of several hormones are required for the thermogenic function and/or adaptive growth of BAT, but the nature of the mediator that promotes alterations in mitochondrial composition is unknown. Presumably the mediator for the increase in the 32K polypeptide would be secreted in response to cold but not in response to a cafeteria diet.

PART IV: OBESITY IN LABORATORY ANIMALS

A. Genetic Obesity

1. The ob/ob mouse

The genetically obese (ob/ob) mouse, which is used extensively as an animal model of obesity, first appeared in a non inbred stock at the Jackson Laboratories, Bar Harbor, Maine (Ingalls et al. 1950). The obese-hyperglycemic syndrome which typifies this animal is inherited as an autosomal recessive gene (ob) located on chromosome 6 (Coleman 1978) and is characterized by many aberrations including massive obesity, hyperphagia, hyperglycemia, hyperinsulinemia, insulin resistance and an extreme sensitivity to cold (Bray, York 1971a; 1979). Although the gene is maintained on several different backgrounds which serve to alter the severity of the syndrome the general phenotypic expression of the genome remains unchanged (Bray, York 1979).

Reduced energy expenditure as well as hyperphagia is implicated in the development of obesity in the ob/ob mouse. A number of studies have reported excess fat accumulation in ob/ob mice that have been pair fed to the ad libitum intake of lean littermates (Dubuc 1976; Thurlby, Trayhurn 1979; Alonso, Maren 1955; Chlouverakis 1970; Coleman 1982). Investigators using body energy balance techniques showed that young obese (ob/ob) mice living at

25-30°C and fed a diet high in fat or carbohydrate required 40% less energy for maintenance (Lin P-Y et al 1979). This increased efficiency of energy utilization is associated with a reduced metabolic rate and a lower body temperature at all temperatures below thermoneutrality (Joosten, Van der Kroon 1974a; Trayhurn, James 1978; Lin et al. 1980; Davis, Mayer 1954; Boissonneault et al.1978) as well as an inability to survive severe cold (Davis, Mayer 1954; Trayhurn et al. 1976; Mayer, Barrnett 1953; Thenen, Carr 1980) because of a failure to generate heat (Davis, Mayer 1954). These findings have suggested that the high metabolic efficiency of ob/ob mice results primarily from less energy being expended on thermoregulatory thermogenesis (Trayhurn, James 1978). Indeed, energy balance studies have confirmed this to be so. At thermoneutrality, the efficiency of energy retention for ob/ob mice, although still greater than lean mice is much less than when the animals are kept at lower temperatures (Thurlby, Trayhurn 1979; Lin P.-Y. et al. 1979; Vander Tuig et al. 1980). Moreover, maintenance energy requirements of lean mice increase as the temperature at which the animals are housed is lowered from 33°C to 25°C, whereas they remain the same for ob/ob mice (Vander Tuig et al. 1980; Lin P.-Y. et al. 1979). As ambient temperature declines, defective thermogenesis becomes significantly more important than hyperphagia in contributing toward excess energy gain (Trayhurn et al. 1981).

Although the basic genetic defect has not been identified, impaired thermoregulatory thermogenesis appears early in the development of obesity and precedes hyperphagia. Milk intake is similar in 15 day old preweanling lean and obese mice (Rath, Thenen 1979; Contaldo et al. 1981). By this time there is increased hypertrophy of epididymal fat (Joosten, Van der Kroon 1974b). increased carcass energy content (Boissonneault et al. 1978; Thurlby, Trayhurn 1978) and increased hepatic and subcutaneous adipose tissue lipogenesis (Godbole et al. 1980) which transplantation studies have shown is not the result of a defect inherent in white adipocytes (Ashwell, Meade 1978). At 5 days postnatally, oxygen uptake is lower in ob/ob mice and this coincides with the initial appearance of excess body fat (Boissonneault et al. 1978). Furthermore, unlike their lean littermates, 10 day old ob/ob mice are unable to maintain body temperature when subjected to mild cold (Thurlby, Trayhurn 1978). Thus, impaired energy expenditure and not hyperphagia is the primary causative factor of obesity in ob/ob mice. Based on a reduced metabolic response to injected NA, Trayhurn and James (1978) have concluded that defective heat production in ob/ob mice results from a reduced capacity for NST.

There is evidence that genetically obese mice may also exhibit defective thyroid thermogenesis. Their cold sensitivity, low metabolic rate and low body temperature is consistent

with a hypothyroid condition as well as with an impairment in NST. Indeed, treatment of ob/ob mice with thyroid hormones lengthens survival time at low ambient temperatures (Mayer, Barrnett 1953). At an unspecified room temperature, the metabolic rate (Otto et al. 1976) and body temperature (Joosten, Van der Kroon 1974a; Ohtake et al. 1977) of T_4 treated ob/ob mice are raised to that of lean mice. A hypersensitivity to thyroid hormones is observed in ob/ob mice for oxygen consumption (Otto et al. 1976), body temperature (Joosten, Van der Kroon 1974a), heat production (Vander Tuig et al. 1979; Lin et al. 1979a) and α glycerophosphate dehydrogenase activity (Joosten, Van der Kroon 1974a; Ohtake et al. 1977; York et al. 1978b; Otto et al. 1976). Moreover, a number of investigators have demonstrated that catecholamine-stimulated lipolysis (Begin-Heick, Heick 1977; Otto et al. 1976; York et al. 1978b) and adenylate cyclase activity (Begin-Heick, Heick 1977; Laudat, Pairault 1975; Dehaye et al. 1978) are impaired in white adipose tissue but that treatment with thyroid hormones improves the lipolytic response to adrenaline (Begin-Heick, Heick 1977; Otto et al. 1976; York et al. 1978) and increased adenylate cylase activity (Begin-Heick, Heick 1977). However, despite normal absorption of T_4 (York et al. 1978b), substantially larger amounts of thyroid hormones are necessary for ob/ob mice to achieve a catecholamine-stimulated lipolysis which is similar to lean

mice (Otto et al. 1976; York et al. 1978b). This indicates that a refractoriness to thyroid hormones exists in white adipose tissue of ob/ob mice. These results, in addition to reports that preweanling ob/ob mice have reduced blood T_4 (Mobley, Dubuc 1979) and delayed organ development which is normalized by T_4 injection (Van der Kroon et al. 1982) suggests that ob/ob mice are hypothyroid from birth on.

However, reflex development, indicative of thyroid status is normal in preweanling ob/ob mice (Joosten, Van der Kroon 1974b). Moreover, there is no histological evidence for hypothyroidism in the thyroid gland of young ob/ob mice (Joosten, Van der Kroon 1974a), blood T_3 levels are similar or slightly higher than found in lean mice (Ohtake et al. 1977; Mobley, Dubuc 1979; York et al. 1978b) and the thyroid-sensitive α glycerophosphate dehydrogenase activity in liver is normal (Bray et al. 1978; Otto et al. 1976; York et al. 1978a). After an extensive review of the literature, Bray and York (1979) have concluded that the hypothalamic-pituitary-thyroid axis is normal. Thus, it appears that ob/ob mice are euthyroid but exhibit a selective impairment in their response to thyroid hormones which may contribute to the high metabolic efficiency of these animals.

Several investigators have suggested that a defect in thyroid regulation of the Na^+, K^+ ATPase in ob/ob mice may account for the high metabolic efficiency of these animals

(York et al. 1978a; Bray et al. 1978; Bray, York 1979). Indeed, a low concentration of Na^+ K^+ ATPase enzyme units, as measured by [^{3}H] ouabain binding is observed in skeletal muscle and liver of adult ob/ob mice (Lin et al. 1978; Lin et al. 1979a). In accord with these findings, there is a reduced suppression of ouabain-inhibitable respiration in skeletal muscle and liver slices (Guernsey, Morishige 1979) and a reduced Na^+ K^+ ATPase activity in liver (York et al. 1978; Bray et al. 1978; Guernsey, Morishige 1979). Since fewer ouabain binding sites are present in skeletal muscle of 14 days old ob/ob mice (Lin et al. 1979b), reduced Na^+ K^+ ATPase activity may be a consequence of the primary biochemical lesion rather than a manifestation of the obesity per se. However, defective Na^+ K^+ ATPase is not unique to the ob/ob mouse, as reduced enzyme activity in liver homogenates has been reported for diabetic (db/db) mice (Bray et al. 1978) and a lower number of enzyme units in skeletal muscle has been noted in weanling VMH-lesioned rats (Vander Tuig et al. 1981). Thus, impaired sodium pumping is not related to the primary gene defect in ob/ob mice but is probably related to an altered regulation of Na^+ K^+ ATPase associated with the selective impairment of response to thyroid hormones.

Several reservations must be made concerning the contribution that deficient sodium pumping makes toward reduced energy expenditure of ob/ob mice. First, it is doubtful that liver plays a significant role because the

total amount of Na^+ K^+ ATPase units were similar to that of lean mice when results are expressed per organ (Lin et al. 1979a). Second, the importance of skeletal muscle in contributing to impaired thermogenesis has recently been challenged (Clausen, Hansen 1982). When whole muscle (Clausen, Hansen 1982) rather than particulate fractions from homogenates (Lin et al. 1978; Lin et al. 1979a) are used there are no significant differences between muscles from lean and obese mice in the number of specific binding sites per gram tissue wet weight. Furthermore, the ouabain-suppressible component of [^{42}K]-uptake is greater in muscles obtained from obese mice (Clausen, Hansen 1982). Third, since the quantitative significance of sodium pumping to energy expenditure is questionable (Part II.C.4.) it is difficult to discern whether a reduced activity in tissues of ob/ob mice would be a major factor in their obesity. Taken together, these observations argue against the increased metabolic efficiency of ob/ob mice being due to reduced energy cost for active Na^+ K^+ transport.

2. The db/db mouse

The diabetic (db/db) mouse, which arose from a spontaneous mutation in the C57 BL/KsJ strain, inherits an autosomal recessive gene that causes hyperphagia, obesity and eventually severe diabetes as the islets of Langerhans become atrophied (Bray, York 1979). The genetic background affects metabolic disorders arising from both the db and ob

gene such that on the same background genome (either C57 BL/KsJ or C57 BL/6J), the db/db mouse is phenotypically identical to the ob/ob mouse (Coleman 1978). It thus is not surprising that the obesity which occurs in db/db mice is due to an elevated metabolic efficiency as well as hyperphagia (Cox, Powley 1977; Coleman 1982). Like the ob/ob mouse, the activity of the thyroid-sensitive Na^+ K^+ ATPase in liver homogenates of the db/db mouse is low, implying a reduced thyroid thermogenesis (Bray et al. 1978). However, also characteristic of diabetic mice is their extreme cold sensitivity and low metabolic rate at ambient temperature below thermoneutrality (Trayhurn 1979). This impairment in thermoregulatory thermogenesis results from a defect in NST which Trayhurn (1979) has suggested is the underlying basis for the high metabolic efficiency of these animals. Further support for this proposal is obtained from pair feeding experiments which show that young db/db mice deposite 75% more fat at 23°C than their lean controls, but deposit only 25% more fat at 33°C (Trayhurn, Fuller 1980).

3. The fa/fa rat

The fatty (fa/fa) rat, first described by Zucker and Zucker in 1961, inherits its obesity as a Mendelian recessive trait. As with most obese rodents, the major characteristics associated with the obesity are hyperphagia, hyperinsulinemia, insulin resistance and hyperlipidemia (Bray, York 1971a; 1979). Several investigators report that fatty rats also

exhibit a high metabolic efficiency relative to their lean controls (Pullar, Webster 1974; Zucker 1975; Deb, Martin 1975) as well as a low body temperature (York et al. 1972; Godbole et al. 1978; Levin et al. 1980), an impaired thermogenic response to acute cold (Levin et al. 1980) and a low metabolic rate both at thermoneutrality and at ambient temperatures below thermoneutrality (Bray 1969; Kaplan 1979). Although the adult fa/fa rat has a decreased level of spontaneous activity, forced exercise is only moderately successful in alleviating the obesity (Deb, Martin 1975; Stern, Johnson 1977). Determinations of milk intake in fatty rats show that these animals are not hyperphagic at 7 days of age, despite the presence of excess body fat (Boulange et al. 1979). Moreover, the level of spontaneous activity is normal until weaning (Stern, Johnson 1977). However, preweaning fa/fa rats display both decreased rectal temperatures (Godbole et al. 1978) and reduced oxygen consumption (Kaplan 1979), suggesting that the initial development of obesity is due to a primary defect in heat generating mechanisms. Indeed, an impairment in thyroid thermogenesis is indicated by a depressed uptake and release of thyroidal ^{131}I and by low serum concentrations of protein bound iodine (Bray, York 1971b). But because serum T_3 levels (Martin et al. 1978) and hepatic Na^+ K^+ ATPase activity (Bray et al. 1978) are normal in fa/fa rats, whereas NA levels and NA turnover are low in sympathetically innervated

organs (Levin et al. 1981), it is more likely that the major thermogenic defect in these animals is an impairment in either or both DIT and NST.

4. Polygenic obesities

In contrast to the single gene or Mendelian inheritance of obesity in rodents, obesity may also be polygenic. In these animals, obesity is due to the action of several separate genes which control body fatness i.e. metabolic rate, appetite, and spontaneous activity (Festing 1979). Wide variation in the onset of obesity and severity of the syndrome are noted between the different polygenically obese animals, depending on the extreme array of 'fatness' genes that are present in the normal population (Festing 1979). Thus, the K K mouse develops only a moderate form of obesity after 4-5 months of age, whereas in the NZO mouse, excess fat accumulates before 4 weeks of age and the degree of obesity attained by these animals is comparable to that of the ob/ob mouse (Bray, York 1979; York 1979). However, both strains exhibit modest hyperinsulinemia and hyperglycemia compared to the severe hyperinsulinemia and insulin resistance observed in the recessively inherited obesities of the ob/ob mouse and the db/db mouse (Bray, York 1979; York 1979). In other polygenically obese species, such as the spiny mouse and the sand rat, obesity does not occur unless the natural diet, which is indigenous to desert regions, is replaced by

laboratory chow (Festing 1979; York 1979). Although the obesity is considered moderate in both the spiny mouse and the sand rat it is accompanied by severe diabetes and insulin resistance (York 1979). Differences in cold sensitivity are also evident in polygenic models of obesity. For example, the Djungarian hamster, which also becomes obese and diabetic under laboratory conditions (Festing 1979) is able to survive extreme cold because of an inherently high capacity for NST (Bockler et al., 1982). In contrast the spiny mouse exhibits high cold sensitivity and a very low metabolic rate at thermoneutrality in the pre-diabetic state, when compared to Swiss mice of similar weight (Wise 1977a; b). There is also some indication that the spiny mouse manifests an increased metabolic efficiency because these animals consume less food, despite a higher level of spontaneous activity, than weight-matched Swiss mice (Wise 1977b). Although there are difficulties in studying polygenic models of obesity in that there are no normal nonobese controls, they likely relate more to the human condition than the Mendelian models of obesity (Festing 1979).

B. Hypothalamic Obesity

1. The goldthioglucose (GTG)-obese mouse

In 1950, Waxler and Brecher reported that a single intraperitoneal injection of goldthioglucose (GTG) induced hyperphagia and obesity in normally lean mice. This has since

been confirmed by many investigators. Goldthioglucose localizes in the hypothalamus causing bilateral necroses of the ventromedial region (Marshall et al. 1955; Bray, York, 1979). The degree of obesity in GTG-treated mice is correlated with the extent of hypothalamic damage (Liebelt et al. 1960; Liebelt et al. 1966; Brecher et al. 1965) and is dependent upon the dose of GTG and the genetic background of mice used (Liebelt, Perry 1957; Liebelt et al. 1960).

When GTG is administrered in a dose sufficient to cause obesity there is an initial weight loss and hypophagia lasting 3-7 days which is attributed to the acute toxicity of the compound (Gray, Liebelt 1961; Debons et al. 1982). This is followed by a dynamic phase of obesity, characterized by rapid weight gain, high food intake and moderate hyperinsulinemia (Gray, Liebelt 1961; De Laey et al. 1975; Le Marchand et al. 1978). Beginning approximately 6-10 weeks post injection GTG-treated mice enter the static phase of obesity. At this time weight gain has reached a plateau and body weight stabilizes at 2-3 times that of untreated controls (Gray, Liebelt 1961). This phase is associated with hyperglycemia, marked hyperinsulinemia, insulin resistance and a return to more normal levels of food intake (Djazayery et al. 1979; Gray, Liebelt 1961; Le Marchand et al. 1978).

In addition to the hyperphagia, GTG-obese mice also manifest a high metabolic efficiency (De Laey et al. 1975;

Djazayery et al. 1979). These animals are not however cold sensitive. GTG-obese mice survive exposure to 4°C without prior acclimation to milder temperatures (Davis, Mayer 1954) and, when kept at temperature below thermoneutrality they maintain normal body temperatures (Webb et al. 1980; Joosten, Van der Kroon 1974a) and exhibit high metabolic rates (Drachman, Tepperman 1953). Furthermore, GTG-obese mice are considered to be euthyroid since they exhibit a normal thyroidal uptake and release of ^{131}I (Schindler, Liebelt 1967) and a normal hepatic Na^+ K^+ ATPase activty both before and after chronic treatment with T_3 (York et al. 1978a). Although the high metabolic efficiency of GTG-obese mice would appear not to be due to a hypothyroid condition or a reduction in energy expended for thermoregulation there is some suggestive evidence that DIT may be impaired in these animals since sympathetic activity in heart of GTG-obese mice does not respond to altered dietary stimuli (Young, Landsberg 1980).

2. The ventromedial hypothalamic-lesioned rat

Electrolytic or radiofrequency lesions of the ventromedial hypothalamus (VMH) induce hyperphagia and obesity in mature rats (Hetherington, Ranson 1940; 1942; Brobeck et al. 1943; Bray, York 1979; Frohman 1980). These alterations commonly occur in 2 successive stages; a dynamic phase manifested by rapid weight gain and overeating, followed by a static phase, lasting until senescence during which body

weight is maintained at a fairly constant percentage of control weight with near normal food intake (Bray, York 1979). Destruction of the VMH in weanling rats also results in obesity, but in contrast to the mature rats, this occurs without increases in food intake or body weight gain (Han et al. 1965; Frohman, Bernadis 1969; Vander Tuig et al. 1981; 1982). The syndrome of hypothalamic obesity in both weanling and mature VMH-lesioned rats is characterized by masssive accumulation of carcass fat, hyperinsulinemia, normoglycemia, hyperlipidemia and retardation of linear growth (Bray, York 1979; Frohman 1980). The precise anatomical locus of the lesion producing the VMH syndrome may actually involve more than one site. Lesions confined to the ventromedial nucleus produce hyperinsulinemia (Bray, York 1979) but do not produce hyperphagia or obesity (Gold, 1973). Damage to fibers running lateral to the ventromedial nucleus results in obesity and hyperphagia (Bray, York 1979) but not hyperinsulinemia or hyperlipidemia in rats pair fed to sham operated controls (Bray et al. 1982).

Accumulated evidence suggests that the obesity of VMH-lesioned rats is not only the result of hyperphagia. The presence of larger fat depots in pair-fed mature VMH-lesioned rats (Cox, Powley 1981; Han 1968) and in normophagic weanling VMH-lesioned rats (Han et al. 1965; Frohman, Bernardis 1969) is indicative of a high metabolic efficiency and presumably a decrease in energy expenditure. Indeed, oxygen

uptake of weanling VMH-lesioned rats is less than control rats both at 25°C and at 30°C (thermoneutrality is 28°C) (Vander Tuig et al. 1981). A reduction in spontaneous locomotor activity has been noted in the postlesion state in both mature and weanling rats, but this is not thought to contribute significantly to the development of obesity (Bray, York 1979). For example, in one study in which animals were housed in standard or restriction cages it was shown that activity had no effect on body fat or body weight gain for either control or VMH-lesioned rats (Han 1968). As well, it has been reported that the VMH-lesion does not impair locomotor response to a lowered ambient temperature or to changes in feeding regimen (Gladfelter 1978). The possibility that a thyroid hormone deficiency may contribute to the high metabolic efficiency and obesity has been suggested because VMH-lesions result in a reduced thyroidal uptake and release of ^{131}I (May, Beaton 1966) and a failure to increase the rate of release of thyroidal ^{125}I during exposure to cold (York et al. 1972). Furthermore, thyroid-sensitive Na^+ K^+ ATPase is present at low concentrations in skeletal muscle of weanling VMH-lesioned rats (Vander Tuig et al. 1981). However, when lesions are confined to the region of the ventromedial nucleus there are normal serum levels of thyroid hormones but obesity nevertheless occurs (Frohman, Bernardis 1968). In addition, hypophysectomized rats with VMH-lesion and replacement doses

of thyroxine still manifest the complete syndrome of hypothalamic obesity (Frohman 1980).

Recent evidence has implicated the autonomic nervous system in the development of hypothalamic obesity (Bray, York 1979). Since the VMH is considered to be in the sympathetic zone of the hypothalamus (Ban 1975), destruction of this area might therefore be expected to decrease sympathetic outflow. Indeed, VMH-lesioned rats exhibit an impaired mobilization of fatty acids when subjected to various stresses, suggestive of a reduced peripheral sympathetic activity (Nishizawa, Bray 1978). However, the VMH-lesioned rat can maintain its body temperature in the cold and survive extended periods at 3-5°C (Colby, Smith 1975; York et al. 1972; Han, Brobeck 1961, Seydoux et al. 1982).

3. The hypothalamic knife cut rat

Parasagittal knife cuts, transecting fibers which pass immediately lateral to the ventromedial nucleus produce a syndrome of hypothalamic obesity similar in many respects to that obtained with VMH lesions. Thus, knife cut rats exhibit marked body fat deposition, hyperphagia, hyperinsulinemia and normoglycemia (Tannenbaum et al. 1974; Sclafani 1981). Some differences exist however, between knife-cut and VMH-lesioned rats. For example, in contrast to the restricted growth of VMH-lesioned rats, parasagittal knife cuts actually enhance linear growth in weanling as well as adult rats (Gold, Kapatos 1975). Moreover, when knife

cut rats are prevented from overeating they do not exhibit the characteristic hyperinsulinemia of pair fed VMH-lesioned rats (Sclafani 1981; Bray et al. 1982).

Although little information is available, there is some indication that parasagittal knife cuts induce a greater metabolic efficiency in rats. Gold and Kapatos (1975) reported normal weight gain and linear growth until 8 weeks of age in weanling knife cut rats despite spontaneously suppressed food intake on an ad libitum high fat diet.

CHAPTER 2

STATEMENT OF THE PROBLEM

A connection between defective BAT thermogeneis, energy balance and obesity was first demonstrated by Himms-Hagen and Desautels (1978) when they reported that the genetically obese (ob/ob) mouse failed to activate thermogenesis in its BAT in response to cold.

The original objective of work started in 1978 and described in this thesis was to further define the nature of the thermogenic defect in BAT of ob/ob mice. Since acclimation to mild cold or chronic treatment with thyroid hormone were known to improve cold resistance of ob/ob mice, it was predicted that these procedures would alter BAT in a way that was indicative of an improved thermogenic functioning. To this end, BAT was studied in lean and ob/ob mice that had been acclimated to 14°C for 2 weeks or were treated daily for 2 weeks with thyroxine. In order to assess the size and functional capacity of BAT for thermogenesis, various characteristics were measured. Size of the tissue was estimated by the total protein content, cytochrome oxidase content and DNA content as a measure of metabolic mass, mitochondrial mass and cell number respectively. (Wet weight of BAT is not a good measure of tissue size because of its often large but variable content of stored triglycerides).

The functional capacity of the tissue was assessed by measuring the relative amount of mitochondrial polypeptides with molecular weights near 32,000 as an indicator of the concentration of proton conductance pathways. The thermogenic state of the tissue was assessed by measuring the specific binding of GDP* to isolated BAT mitochondria. The ultrastructure of BAT mitochondria was examined in lean and ob/ob mice to find out whether the low GDP binding reported by Himms-Hagen and Desautels in ob/ob mice was associated with an abnormal organization of inner membranes. To evaluate the inherent ability of BAT to generate heat, the effect of NA on oxygen uptake in lean and obese animals was studied.

Because some of the abnormalities uncovered in BAT mitochondria could possibly be secondary to the massive accumulation of triglyceride in the tissue a control was

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*Purine nucleotides, such as GDP, are known to block operation of the proton conductance pathway (Nicholls 1979) by binding to the 32K polypeptide (Heaton et al. 1978) at a site on the outer surface of the inner mitochondrial membrane (Nicholls 1976b; Rafael, Heldt 1976). When the tissue is in a quiescent state, the binding of GDP to isolated mitochondria is low. Conversely, mitochondrial GDP binding is high when the tissue is in a thermogenically active state (Himms-Hagen 1983b;c). However, the increase in GDP binding does not necessarily reflect an increase in the concentration of proton conductances pathways because binding may be elevated in the absence of a change in mitochondrial polypeptide composition. This situation is observed when the tissue is stimulated by acute cold exposure (Desautels et al. 1978), cafeteria feeding (Himms-Hagen, et al. 1981) or exogenous NA (Desautels, Himms-Hagen 1979) and is probably the consequence of a structural reorganization of inner mitochondrial membranes to expose binding sites that are already present (Desautels, Himms-Hagen 1980; Himms-Hagen 1983b;c).

sought in which BAT mitochondria would similarly be isolated in the presence of large amounts of lipid. BAT of mice made as obese as the ob/ob mouse with goldthioglucose has a large amount of lipid in its cells. The effect of cold on the thermogenic properties of BAT mitochondria was therefore assessed in GTG-obese mice during the static phase of their obesity. The capacity of GTG-obese mice for a thermogenic response to NA was also assessed.

During the course of this work the discovery by Rothwell and Stock (1979b; 1981b) that the DIT exhibited by cafeteria-fed rats was associated with an increase in sympathetic-mediated thermogenic activity and growth of BAT prompted additional studies of the effects of cafeteria feeding on BAT of ob/ob mice and of GTG-obese mice. Because GTG-obese mice manifest a high metabolic efficiency during the dynamic phase of obesity (De Laey et al. 1975; Djazayery et al. 1979), the response of their BAT to diet and cold was also studied at this time in order to determine if a defect in thermogenic functioning was present during the rapid development of their obesity.

Since a difference between genetic obesity (the ob/ob mouse) and hypothalamic obesity (the GTG-obese mouse) in the abnormality of regulation of BAT thermogenic function became apparent in the course of the work, it was of interest to study other animal models of these two types of obesity.

Therefore studies of BAT of rats with hypothalamic obesity (VMH-lesioned rats or parasagittal knife cut lesioned rats) were done in collaboration with Dr. D.V. Coscina of the University of Toronto and are described in this thesis. Studies of another genetic-obesity (the fa/fa rat) done by J. Triandafillou will be mentioned for comparison in the general discussion; Chapter 5.

CHAPTER 3

MATERIALS AND METHODS

PART I: MATERIALS

A. Animals

In all experiments, animals were housed at 28°C unless otherwise mentioned and maintained on a controlled lighting schedule (12L:12D, lights on at 6:00 a.m.) with free access to food (Purina rat chow 5012, containing 22.5% protein, 4.5% fat, 53.2% carbohydrate) and water. Except for experiments in which mice were exposed to 4°C for 3 h, all animals were killed between 7:00-8:00 a.m. either by cervical dislocation for mice or by decapitation for rats.

Female obese (ob/ob) mice and their lean littermates (+/+ or +/ob) were obtained from the C57BL/6J colony at the Jackson Laboratories, Bar Harbor, ME. At least one week was allowed for recovery before experimental procedures were initiated. Unless specified, mice were allowed to remain in groups of 3-4 for studies in which animals were acclimated to 24°C-28°C or 33°C but were placed in individual plastic cages with wood chip bedding when the ambient temperature was lowered to 14°C, 8°C or 4°C. Male and Female Holtzman rats were obtained from Canadian Breeding Laboratories, St. Constant, P.Q., and housed in single cages. Rats of the same origin with parasagittal

knife cuts or bilateral ventromedial lesions and their intact controls were obtained from D.V. Coscina at the Clarke Institute of Psychiatry in Toronto and were transported by car to Ottawa.

B. Chemicals

Biochemicals were purchased from Sigma Chemical Co. Buffers, acids and other common reagents were obtained from Fisher Scientific Co., J.T. Baker Co., BDH Chemicals Ltd. or Sigma Chemical Co. and all chemicals used for gel electrophoresis were obtained from Bio-Rad Laboratories. The NCS tissue solubilizer was purchased from Amersham Corporation. The chemicals used for electron microscopy were obtained from a variety of sources: 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. Radiochemicals were purchased from New England Nuclear: [U-^{14}C]-sucrose, 3.6 mCi/mmol; [8-^{3}H] guanosine 5'-diphosphate, trisodium salt 6.1-12.7 Ci/mmol and from Amersham Corporation: Amerlex ^{TM} T-3 RIA Kit; Amerlex^{TM} Free T-4 RIA Kit.

C. Cafeteria Diet

The cafeteria diet consisted of regular chow plus four different food items per day offered in five rotating menus. The VMH rats also received condensed milk diluted 1:1 with tap water. Food items were purchased from local supermarkets and were selected to provide a diet rich in carbohydrate and fat but also containing substantial amounts of protein. A list of the different menus and food composition is given in Appendix A-1 and Appendix A-2 respectively.

PART II: METHODS

A. Isolation of Brown Adipose Tissue (BAT) Mitochondria

Every precaution was taken to reduce the excitability of the animals prior to sacrifice so that activation of the sympathetic nervous system was minimized (Depocas, Behrens, 1977; Kvetnansky et al. 1978). Animals were killed either by decapitation (rats) or cervical dislocation (mice) and their interscapular BAT (plus subscapular for mice) removed quickly and chilled in ice cold isolation medium containing 0.25 M sucrose, 0.2 mM EDTA (free acid) and 1 mM HEPES, adjusted to pH 7.2 with 1 N KOH. Tissues were minced with scissors and then homogenized (two strokes of a teflon plunger operating at 400 rpm) in a glass homogenizer containing isolation medium and made to a known volume. Samples were removed for protein, DNA and cytochrome oxidase estimation. Homogenates from two to four similarly treated animals were pooled and made to a volume of 42 ml with isolation medium.

Mitochondria were isolated using a Sorvall RC-5B or RC2-B refrigerated centrifuge with the brake off. The procedure followed was exactly as described by Slinde, Pedersen and Flatmark (1975) except that the low speed centrifugation was carried out at 3000 rpm in an HB-4 rotor in order to increase mitochondrial yield. Mitochondria were resuspended to a final protein concentration of 10-40 mg/ml

and used immediately for the GDP binding assay.

B. Purine Nucleotide Binding Assay (GDP)

A radioactive binding assay developed by Nicholls (1976b) and modified by Desautels et al. (1978) was used to assess purine nucleotide binding sites. Immediately following isolation, mitochondria (final concentration, 0.5 - 1.5 mg protein/ml) were incubated for 1 minute at room temperature in the presence or absence of 100 µM ADP 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 of [^{14}C] sucrose pH 7.1. The reaction was started by adding [^{3}H] GDP (final concentration 10 µM, 0.65 µCi/ml), mixing rapidly and centrifuging after 30 seconds at 12,000 g for 2 minutes in an Eppendorf microcentrifuge. The supernatant was removed and the mitochondrial pellet dissolved in NCS tissue solubilizer by incubating for 3 hours at 55°C. Samples were counted with a Beckman LS6800 liquid scintillation counter in the presence of 0.05 ml 10% ascorbic acid and 10 ml toluene containing 0.7% PPO. Specific binding of GDP is the difference at 30 seconds between binding in the presence and absence of 100 µM ADP after correction for the amount of incubation medium trapped in the pellet as estimated by the values for ^{14}C sucrose. Binding of GDP is expressed as nmoles GDP bound/mg protein.

C. Protein Estimation

Protein was estimated in BAT homogenates, isolated mitochondria, and mitochondrial membranes using the Lowry method (1951) as modified by Schacterle and Pollack (1973) with bovine serum albumin as the standard. Protein was precipitated with cold 12.5% trichloroacetic acid, kept at 4°C for 30 minutes and centrifuged at 5,000 rpm in an HB-4 rotor. The pellets were dissolved in 0.5N NaOH and the samples were assayed in triplicate (Schacterle and Pollack, 1973).

D. Cytochrome Oxidase Assay

Samples of homogenate or mitochondria were prepared for cytochrome oxidase determination by activation with lubrol 0.3 mg/mg protein and dilution with 0.1M potassium phosphate buffer pH 6.6 to obtain a protein concentration of 4-8 mg protein/ml. The diluted samples plus lubrol were homogenized in a teflon-glass homogenizer at 2,000 rpm and then frozen overnight.

Cytochrome oxidase activity was measured polarographically at 37°C using a Yellow Springs Instrument YSI Model 52 biological oxygen monitor with a Clark type electrode. A 20 µl sample was added to 3 ml of medium containing 0.1M potassium phosphate buffer pH 6.6, 0.2 mM cytochrome c and 20 mM sodium ascorbate which had been stirred for 3 min (Behrens, Himms-Hagen 1977). The cytochrome c solution was prepared according to Wharton and Tzagoloff (1967) by

dissolving cytochrome c (horse heart) in 100 ml 0.01 M potassium phosphate buffer pH 7.0 containing 100 mg ascorbic acid. The concentration of cytochrome c was determined as the difference between the reduced and oxidized forms using extinction coefficients reported by Yonetani (1967). The specific activity is expressed in terms of oxygen consumed as µg atoms O_2 min^{-1} mg $protein^{-1}$.

E. DNA Estimation

DNA content of BAT homogenates was determined using diphenylamine in a colorimetric method described by Burton (1968) as modified by Giles and Myers (1965) with calf thymus DNA as the standard. The DNA was extracted from BAT homogenates by heating for 10 minutes with 5% trichloroacetic acid at 90°C. Under these conditions less than 5% of the deoxyribose is destroyed (Burton 1968). After extraction of DNA, protein was removed by filtration using a Millipore membrane filter, pore size 3.0 µM. The effect of additional impurities was eliminated by calculating the difference between optical density values at 595 and 700 mµ. This results in a zero blank and a straight line calibration (Giles, Myers 1965).

F. Polypeptide Composition

Polypeptide composition was determined by polyacrylamide gel electrophoresis in the presence of sodium

dodecyl sulfate (SDS) (O'Farrell 1975). Mitochondrial membranes were prepared by sonicating the mitochondria at a protein concentration of 2 mg/ml on ice for three 30 second intervals 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 membranes were 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 carried out with a 10% acrylamide separating gel pH 8.8, a 4.75% acrylamide stacking gel pH 6.8, and a running buffer containing 0.025 M Tris, 0.192 M glycine, and 0.1% SDS in 1.5 mm thick slab gels. Gels were run overnight at a constant current voltage of 2. Gels were stained with Coomassie blue R250, destained by diffusion and scanned at 540 nm using a Gilford spectrophotometer 2400-2. The proportion of the total area under each peak was estimated by cutting out two copies of each tracing and weighing the major peaks. The percentage of the total area represents the proportion of polypeptides in mitochondria which corresponds to the molecular weight ranges of the specified peaks. The SDS gel electrophoresis standards were obtained from Bio-Rad Laboratories and consisted of a series of proteins with molecular weights ranging from 14,000 to 92,500 (lysozyme, soybean trypsin inhibitor, carbonic anhydrase, ovalbumin, bovine serum albumin,

phosphorylase b).

G. Electron Microscopy

1. Mitochondria

Mitochondria were isolated as described in Part A except that the final concentration and resuspension were carried out in an EDTA-free medium. The mitochondria were fixed according to the procedure of Munn and Blair (1967) by adding 0.4 ml mitochondria suspension containing 4-8 mg protein to 4.6 ml EDTA-free medium plus 2.5 ml of ice cold 6% glutaraldehyde in 0.02M cacodylate buffer pH 7.0. A final concentration of 2% glutaraldehyde has been shown to stop any swelling or contraction of the mitochondria (Munn, Blair 1967). Mitochondria remained in the primary fixative solution at 4°C for 1 hour and subsequently were centrifuged for 1 hour in an HB-4 rotor at 5,000 rpm using a Sorvall refrigerated centrifuge (RC5B or RC2-B). The supernatant was replaced with 3.0 ml of 1% osmium tetroxide in 0.02M cacodylate buffer pH 7.0. The pellet was cut into small fragments with a finely drawn glass rod and kept at room temperature. After 1 hour, the solution was changed and the fragments left for a further hour in osmium tetroxide. The fragments were then successively dehydrated in 50%, 75%, 95% and 100% ethanol and progressively embedded in Vestopal, using styrene as an intermediate solvent because Vestopal is not miscible with alcohol. Two days were allowed for

infiltration with Vestopal, followed by 4 days at 60°C for polymerization. Further details of the dehydration and infiltration procedures appear in Appendix B-1. 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.

2. Tissue

Subscapular BAT was removed, cut into thin strips with a razor blade on dental wax and placed in 3 ml of primary fixative solution consisting of 3% glutaraldehyde and 5% sucrose in 0.02 M cacodylate buffer pH 7.0. BAT slices 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 3.0 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. The cubes were then washed with distilled water, slowly rotated at room temperature for 2-3 hours in 3.0 ml of saturated uranyl acetate solution in 66% ethanol and dehydrated following a modification of the procedure used

for mitochondria (Appendix B-2). Spurr embedding medium was then added and infiltration was allowed to proceed for 24 hours at room temperature before curing at 70°C for 24 hours. Further details of the dehydration and infiltration procedure appear in Appendix B-2. Tissue sections were prepared and viewed at a magnification of 8,000 x using a Siemens 101 electron microscope.

H. Estimation of Thyroid Hormone Levels in Blood

Radioimmunoassay kits containing either ^{125}I-T_3 or ^{125}I-T_4 derivative were used to estimate total T_3 and free T_4 in plasma and serum of rats and mice (Amerlex™ T-3 RIA Kit; Amerlex™ Free T-4 RIA Kit; Amersham Corporation). In both RIA Kits the antibody is bound to polymer particles of uniform diameter and separation of the antibody fraction is effected by centrifugation and decanting of the supernatant. As the kits were designed for estimation of thyroid hormones in human samples the reference standards are made up in human serum. All blood samples were collected from rats and mice after decapitation between 7-8 A.M. and kept at 4°C for 6-8 hours. The plasma or serum was then separated by centrifugation and stored at -20°C for analysis at a later date. Samples were assayed in duplicate on 25 µl aliquots for T_3 and 100 µl aliquots for free T_4 and then counted in a Beckman Gamma 4000 Counter.

I. Oxygen Consumption

Oxygen uptake was measured in an open circuit system using either a Westinghouse or modified Thermox I oxygen analyzer and a small animal chamber maintained at 31°-33°C. Mice were lightly anaesthetized with sodium pentobarbital (10 mg/ml in 0.9% sodium chloride) injected subcutaneously at a dose of 0.1 mg/g for lean and GTG-obese mice and 0.14 mg/g for ob/ob mice and oxygen uptake measured. The animals were removed from the chamber, injected with 0.1 ml of L-noradrenaline bitartrate (600 µg/kg) dissolved in 0.001 N HCl and 0.9% sodium chloride and returned to the chamber for a further determination of oxygen consumption. A dose of 600 µg/kg produced a maximal response in lean and obese mice, which usually occurred 20-35 minutes after subcutaneous injection. In the cold acclimation experiments, all mice were removed from the cold room 12 hours before oxygen uptake was measured to ensure that sufficient time had elapsed for cold-induced sympathetic activity to be switched off. As measured by direct calorimetry, Lin and coworkers (1980) report that heat production in cold-acclimated mice (14°C for 3 weeks) declines within 1 hour to that of warm-acclimated mice (25°C for 3 weeks).

J. Induction of Obesity with Goldthioglucose (GTG)

Female C57BL/6J lean (+/+) mice 8-9 weeks old were housed in groups of 4 and kept at 28°C for 1-2 weeks prior to treatment with freshly prepared goldthioglucose (aurothioglucose, Sigma). To induce obesity, mice received a single intraperitoneal injection of goldthioglucose (0.7 g/kg body weight) in isotonic saline (100 mg/ml) after an 18 hour fast. This dose usually gave a 65% incidence of obesity and a 90% survival rate. For experiments conducted during the static phase of obesity (10-13 weeks of after injection), goldthioglucose-treated mice were selected for study if they had achieved a 5 fold greater weight gain than that of lean mice. Likewise a 3 fold greater weight gain was necessary to qualify for experiments conducted during the dynamic phase of obesity (3-4 weeks after injection).

K. Statistical Analysis of the Results

Results are expressed as mean ± standard error of the mean (SE). Statistical comparisons were made using the unpaired Student's t-test.

CHAPTER 4

RESULTS AND DISCUSSION

PART I: BROWN ADIPOSE TISSUE OF GENETICALLY OBESE (ob/ob) MICE

GENERAL OBJECTIVE:

- To find out whether defective thermogenic functioning of BAT mitochondria in ob/ob mice is related to their obesity

SPECIFIC OBJECTIVES:

- To study further the defective response to cold of BAT of genetically obese mice
- To find out whether procedures known to improve cold-resistance of ob/ob mice (acclimation to mild cold; treatment with thyroid hormone) would improve the thermogenic functioning of their BAT
- To find out whether BAT of ob/ob mice can respond thermogenically to the ingestion of a cafeteria diet.

A. BROWN ADIPOSE TISSUE OF GENETICALLY OBESE (ob/ob) MICE: RESPONSE TO COLD

Objective

The purpose of these experiments was to further examine the effect of acute cold exposure (4°C for 3 hours) on BAT mitochondria of warm-acclimated lean and ob/ob mice. Another objective was to find out to what extent acclimation of ob/ob mice to mild cold (14°C) (Trayhurn, James 1978) improves thermogenic functioning of BAT mitochondria and promotes growth of the tissue.

Method

Lean (+/+ or ob/+) and obese (ob/ob) mice, 3 months old were placed in separate cages and acclimated to either 28°C (warm-acclimated) or 14°C (cold-acclimated) for 2 weeks. In later experiments, obese mice were initially kept at 21-23°C for 1 week prior to acclimation at 14°C to maximize the survival rate. Three hours before sacrifice some mice from each group were exposed to 4°C. In other experiments, 5 week old lean and ob/ob mice were kept in groups of 3-4 and maintained at 28°C for 1 week prior to sacrifice for mitochondrial GDP binding studies. In addition, 3 month old lean and ob/ob mice were maintained at either 28°C or 14°C for 2 weeks and lean mice were maintained at 4°C for 2 weeks for electron microscopy studies.

Mice were killed by cervical dislocation and subscapular BAT (3 month old) plus interscapular BAT (3 month old and 6 week old) were removed, cleaned and weighed. BAT homogenates were made up to 5 ml and samples were taken for protein and cytochrome oxidase determinations (Methods: C, D). The remainder of the homogenates from 2-3 similarly treated mice were pooled and mitochondria isolated exactly as described in Methods section A. Binding of GDP by isolated mitochondria, gel electrophoresis of mitochondrial membrane polypeptides and preparation of samples for electron microscopy of BAT mitochondria were performed as described previously (Methods: B,C,G.1,G.2). Tissue sections of subscapular BAT from warm-acclimated lean and ob/ob mice were also prepared for electron microscopy studies.

Additional mice were acclimated to either 28°C or 14°C for 2 weeks for measurements of oxygen consumption. Mice were anesthetized with pentobarbital and oxygen uptake was determined using a modified Themox oxygen analyzer in the absence and presence of injected NA (Methods: I).

Results and Discussion

1. Brown adipose tissue of warm-acclimated obese (ob/ob) mice

Obese mice gained close to 3 times more weight than did lean mice after 2 weeks at 28°C. Wet weight of BAT was substantially greater in ob/ob mice compared to lean mice (Table 1). However, this is not a true index of active tissue mass because the cells are infiltrated with large amounts of triglyceride. Nonetheless, measurements of total protein and cytochrome oxidase content did indicate a larger metabolic and mitochondria mass in ob/ob mice (Table 1). The differences between lean and ob/ob mice for protein and cytochrome oxidase content were not evident in other studies (Trayhurn et al. 1982b; Goodbody, Trayhurn 1982; Himms-Hagen, Desautels 1978). This is likely due to the inclusion in these experiments of subscapular BAT which is always greater in ob/ob mice.

GDP binding to BAT mitochondria of ob/ob mice maintained at 28°C was two-thirds lower than that of lean mice (Figure 1), signifying a lower state of thermogenic activation in the tissue of these animals and confirming the results reported by Himms-Hagen and Desautels (1978). Low binding levels were also evident in younger ob/ob mice, (6 weeks) suggesting that a low thermogenic state of BAT in ob/ob mice is an early event in the obese hyperglycemic syndrome, (GDP binding measurements for 6 mitochondrial preparations each

TABLE 1

EFFECT OF COLD ACCLIMATION (14 DAY, 14°C) ON LEAN AND OBESE (ob/ob) MICE

Temperature of Acclimation or Exposure	Warm-Acclimated		Cold-Acclimated	
	28°C	4°C	14°C	4°C
LEAN MICE	(15)	(15)	(14)	(14)
Body Weight, g				
initial	20.5 ± 0.5	19.8 ± 0.3	20.2 ± 0.4	20.3 ± 0.5
final	22.6 ± 0.8	21.6 ± 0.5	22.3 ± 0.5	22.1 ± 0.6
change	2.1 ± 0.4	1.8 ± 0.3	2.1 ± 0.3	1.9 ± 0.2
Brown Adipose Tissue				
wet weight, mg	163 ± 11	125 ± 12	274 ± 14 [b]	307 ± 17 [b]
protein, mg	10.1 ± 0.5	8.4 ± 0.6	16.1 ± 1.0 [b]	18.4 ± 0.9 [b]
cytochrome oxidase				
μg atoms $O_2 \cdot min^{-1}$	126.2 ± 9.1	108.8 ± 6.8	187.3 ± 9.8 [b]	179.9 ± 8.4 [b]
μg atoms $O_2 \cdot min^{-1} \cdot mg$ protein	13.0 ± 1.2	12.7 ± 0.7	12.4 ± 1.0	11.1 ± 0.7
OBESE (ob/ob) MICE	(12)	(12)	(17)	(17)
Body Weight, g				
initial	48.2 ± 2.4 [a]	48.9 ± 2.1 [a]	44.7 ± 1.7 [a]	43.4 ± 1.9 [a]
final	54.2 ± 1.9 [a]	54.5 ± 1.7 [a]	45.3 ± 1.5 [a,b]	44.5 ± 1.6 [a,b]
change	6.0 ± 5.0 [a]	5.5 ± 0.6 [a]	0.6 ± 0.5 [b]	1.1 ± 0.7 [b]
Brown Adipose Tissue				
wet weight, mg	1230 ±116 [a]	1291 ±121 [a]	1244 ±101 [a]	1345 ± 80 [a]
protein, mg	19.8 ± 1.5 [a]	20.3 ± 1.3 [a]	38.7 ± 2.5 [a,b]	39.2 ± 2.2 [a,b]
cytochrome oxidase				
μg atoms $O_2 \cdot min^{-1}$	158.6 ± 10.8 [a]	171.1 ± 11.1 [a]	305.5 ± 21.7 [a,b]	305.8 ± 26.1 [a,b]
μg atoms $O_2 \cdot min^{-1} \cdot mg$ protein	9.1 ± 1.4 [a]	8.5 ± 0.8 [a]	8.2 ± 0.6 [a]	8.5 ± 0.5 [a]

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

[a] Significant effect of obesity, comparing mice treated in the same way.

[b] Significant effect of cold acclimation, comparing the same ~~type of~~ mouse.

There was no significant of acute cold exposure (3 hours at 4°C) on body or tissue weights or on BAT growth.

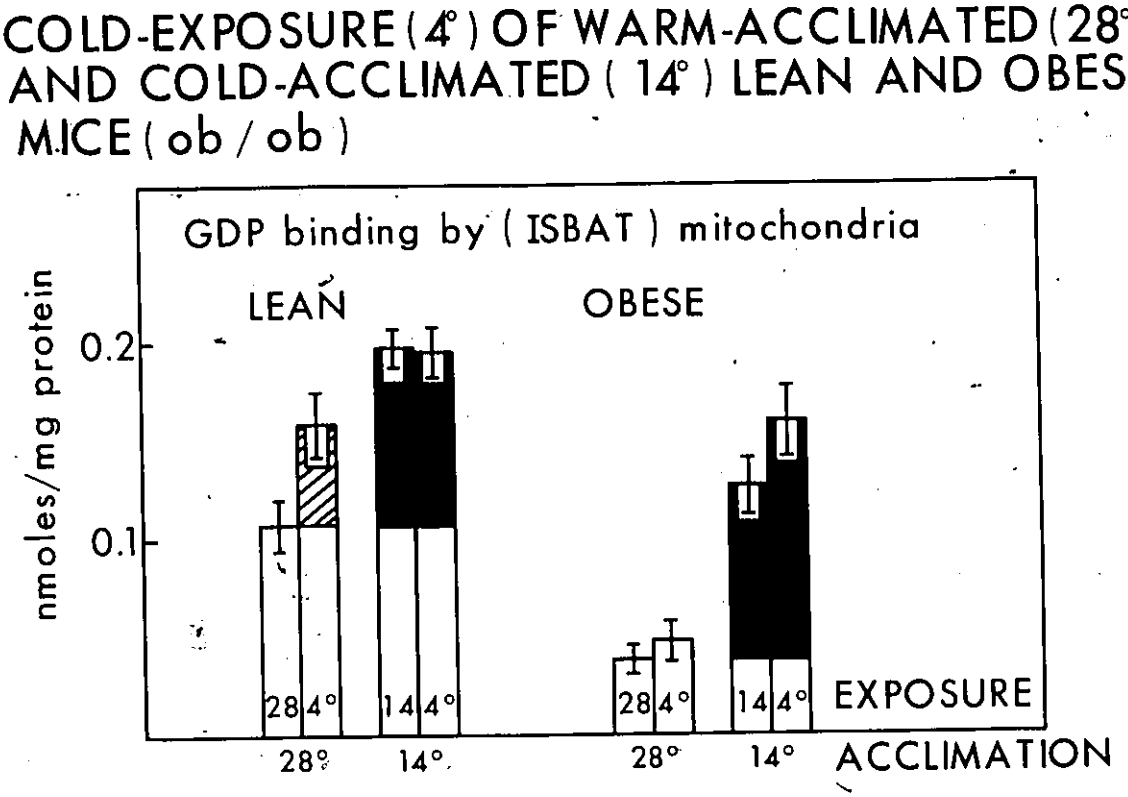

FIGURE 1. Effect of cold acclimation or acute cold exposure on GDP binding by BAT mitochondria of lean and obese (ob/ob) mice.

Bars represent the means ± SE for 5 (lean mice acclimated to 28°C, kept at 28°C or exposed to 4°C), 7 (lean mice acclimated to 14°C; kept at 14°C or exposed to 4°C), 4 (ob/ob mice acclimated to 28°C; kept at 28°C or exposed to 4°C) and 6 (ob/ob mice acclimated to 14°C; kept at 14°C or exposed to 4°C) mitochondrial preparations. Significant effect of cold exposure (3 hours at 4°C) is indicated by the cross-hatched portion of the bar. Significant effect of cold acclimation is shown by the black portion of the bars.

were: 0.049 ± 0.006 nmoles/mg protein in ob/ob mice, 0.106 ± 0.008 nmoles/mg protein in lean mice, $p < 0.001$). In subsequent studies, it was reported that GDP binding is low in BAT mitochondria of 10 and 14 day old ob/ob mice (Goodbody, Trayhurn 1982). Scatchard plot analysis showed this to be the result of a decreased number of binding sites and not a change in binding affinity (Goodbody, Trayhurn 1982).

Despite the low mitochondrial GDP binding in BAT of ob/ob mice, the relative amount of polypeptides in the 32K molecular weight region was normal, indicating a normal amount of proton conductance pathways (Table 2). Moreover, the only alteration in the mitochondrial polypeptide composition of ob/ob mice maintained at 28°C was an increase in polypeptide of molecular weights 40,700 - 44,900 (Table 2 and Figure 2). The fact that low GDP binding occurred in BAT of ob/ob mice in the presence of normal amounts of the 32K polypeptide suggests that the low thermogenic state of BAT in these animals results from a failure to unmask proton conductance pathways already present in the inner mitochondrial membrane.

Electron microscopy studies were done in order to ascertain whether low GDP binding and therefore reduced availability of proton conductance pathways was associated with changes in mitochondrial ultrastructure. Since the sections were relatively free from contamination and mitochondria were structurally intact (Figures 3 and 4)

TABLE 2

POLYPEPTIDE COMPOSITION OF BAT MITOCHONDRIAL MEMBRANES FROM LEAN AND OBESE (ob/ob) MICE

Acclimation Temperature		LEAN			ob/ob	
		28°C	14°C	4°C	28°C	14°C
Group of polypeptides mol wt range						
82,500-90,500	(1)	6.58 ±0.83	5.78 ±0.58	2.05[b] ±0.42	4.70 ±0.31	3.66[a,b] ±0.19
73,000-82,500	(2)	9.64 ±0.50	10.05 ±0.46	9.94 ±0.22	9.28 ±0.31	10.90[b] ±0.32
60,200-70,200	(3)	8.54 ±0.35	9.17 ±0.24	10.33[b] ±0.27	8.67 ±0.14	10.06[a,b] ±0.32
40,700-44,900	(4)	16.73 ±0.84	15.30 ±0.44	13.63[b] ±0.40	19.39[a] ±0.56	17.13[a,b] ±0.35
30,500-33,800	(5)	14.08 ±0.54	15.40 ±0.69	15.82[b] ±0.33	14.50 ±0.24	16.39[b] ±0.32

Values are means ± SE of the percent of the total area under the gel scan represented by peaks of the stated molecular weight ranges for 5 mitochondrial membrane preparations. Numbers of the groups correspond to numbers on the gel scans shown in Figure 2. The polypeptide composition of the mitochondria was obtained from lean and ob/ob mice acclimated to 28°C or 14°C and from lean mice acclimated to 4°C.

[a]Significant effect of obesity, comparing mice treated in the same way.

[b]Significant effect of cold acclimation, comparing the same type of mouse.

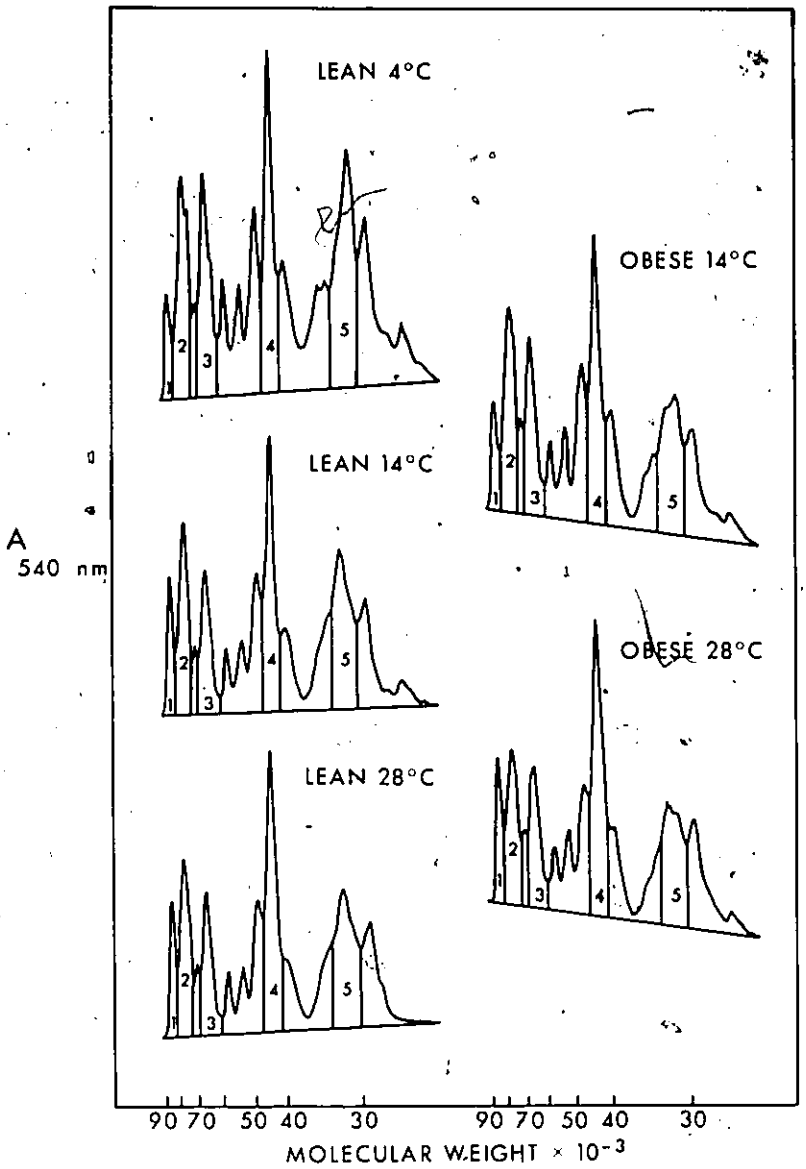

FIGURE 2. Polypeptide pattern of BAT mitochondrial membranes of warm- and cold-acclimated mice.

Left panel: typical gel scans of samples from lean mice acclimated to 28°C, 14°C or 4°C for 2 weeks.

Right panel: typical gel scans of samples from ob/ob mice acclimated to 28°C or 14°C for 2 weeks. The number of the peak corresponds to groups of polypeptides for which quantitative data and molecular weight ranges are given in Table 2.

FIGURE 3. Electron micrograph of brown adipose tissue mitochondria isolated from warm-acclimated (28°C) lean mice (x 16 000).

FIGURE 4. Electron micrograph of brown adipose tissue mitochondria isolated from warm-acclimated (28°C) obese (ob/ob) mice (x 16 000).

Fig. 3

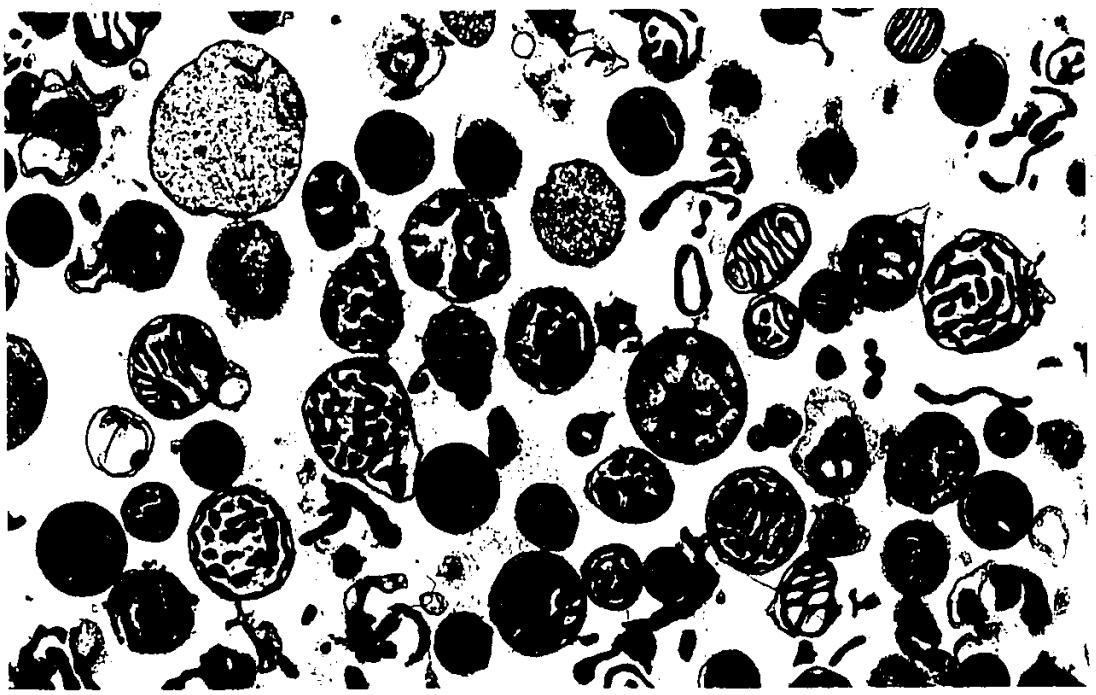

Fig. 4

the possibility that differences in binding results from coalescence of mitochondria or rupturing of membranes during the isolation procedure can be eliminated. Mitochondria from lean mice displayed the typical appearance of uncoupled mitochondria (Figure 3) which is characterized by a condensed matrix and enlarged intracristal space (Bulychev et al. 1972).

The morphology of mitochondria from warm-acclimated lean mice are of two types (Figure 3). In some mitochondria the intracristal space was enlarged and the inmerices (matrix plus inner membrane, Weber 1972) were presented in cross section as a network of circles and bands. In other mitochondria the inmerices were arranged in parallel sheets with branched and interconnecting bands. In contrast, mitochondria from warm-acclimated obese-mice displayed a large variety of structurally abnormal forms (Figure 4). The mitochondria were generally smaller with very large intracristal spaces. The majority had inmerices which appeared in cross section as circles. A few had parallel cristae and a few had an expanded matrix.

Mitochondria from lean and ob/ob mice have been isolated for electron microscopy on several occasions and sections have been cut from different portions of the mitochondrial pellet. In every case the electron micrographs have displayed a similar ultrastructure to those shown in Figures 3 and 4. Nonetheless, one cannot exclude the possibility that

morphological differences occurred because mitochondria from obese and lean mice reacted differently to the fixation procedure, although the method used for all preparations has been reported to immediately prevent swelling or contraction of the mitochondria (Munn, Blair 1967). Despite these reservations, it is probable that the low GDP binding of ob/ob mice at 28°C is due to the abnormal organization of mitochondrial membranes, such that fewer binding sites are exposed for binding of GDP.

Electron micrographs were also obtained of mitochondria in situ. Typical electron micrographs of tissue sections from subscapular BAT of warm-acclimated lean and ob/ob mice are shown in Figure 5 and 6. Because of the massive amount of triglycerides in BAT from obese mice there was considerable difficulty in locating the cyptoplasm. Glycogen particles, which appear as small electron dense granules dispersed throughout the cytoplasm were more prevalent in BAT from obese mice. The ultrastructure of mitochondria in situ from lean and ob/ob mice was different from that of sucrose isolated mitochondria in that the matrix appeared expanded, the intracristal space contracted and there was no evidence of a dotted internal structure. The cristae were parallel but often occurred in semi circular systems. Unlike the differences in ultrastructure from isolated mitochondria, tissue sections from lean and obese mice

FIGURE 5. Electron micrograph of brown adipose tissue of warm-acclimated (28°C) lean mice (x 16 000).

FIGURE 6. Electron micrograph of brown adipose tissue of warm-acclimated (28°C) obese (ob/ob) mice (x 16 000).

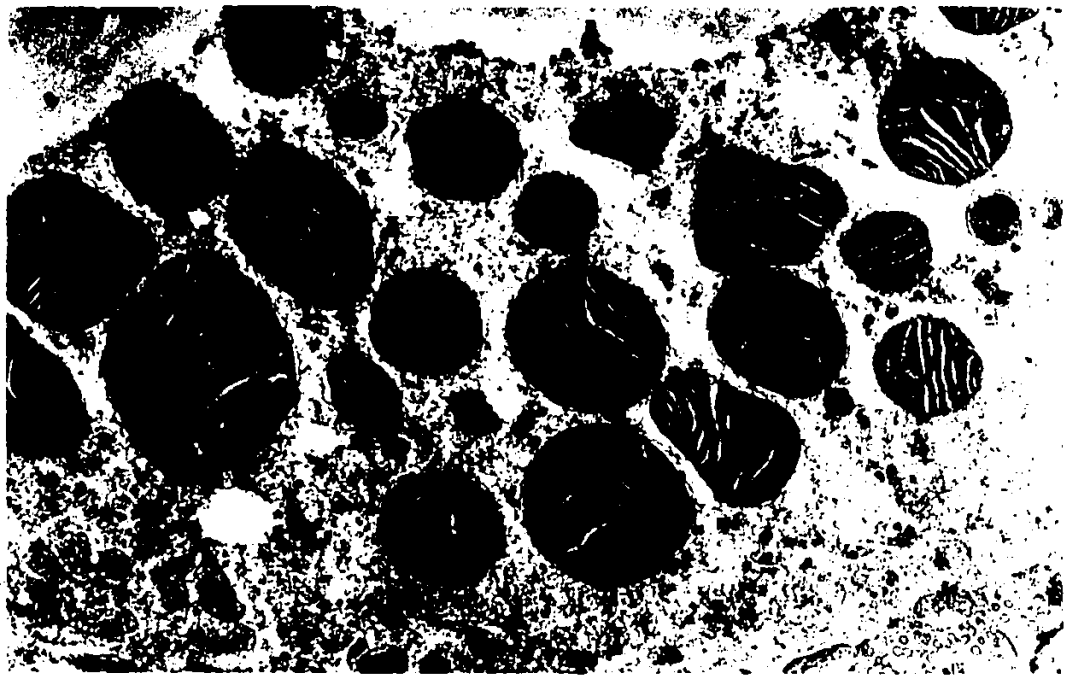

Fig. 5

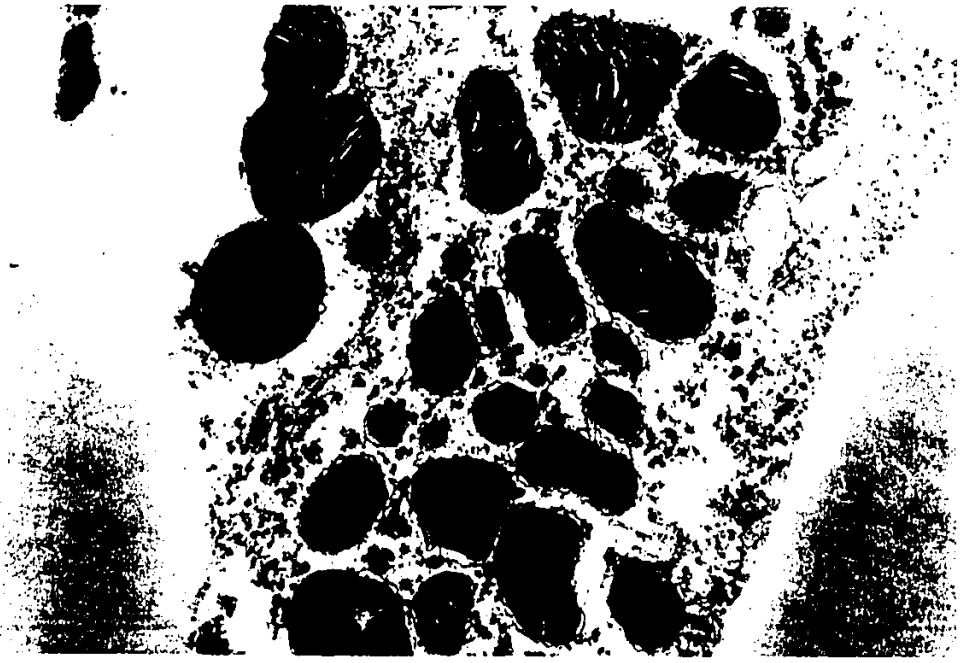

Fig. 6

displayed mitochondria which closely resembled each other, although an expanded matrix was more apparent in obese mice Greater differences might have been observed had the animals been exposed to 4°C for 3 hours (see Figures 8 and 9).

Oxygen uptake studies of anaesthetized mice showed that the resting metabolic rate of warm-acclimated ob/ob mice was slightly but significantly greater than that of lean mice ($p < 0.025$; Figure 7). These measurements were taken under thermoneutral conditions (31°C -33°C) where any effect of NST is eliminated. Body composition of ob/ob mice is very different from that of lean mice not only because of excess fat but also because the absolute amount of protein is less (Kaplan 1981; Thurlby, Trayhurn 1979; Thenen Carr 1980). For this reason, metabolic rate is best expressed in terms of the whole animal rather than as some unit of body weight. When oxygen consumption was calculated in term of ml O_2/Kg $^{0.75}$ or ml O_2/100 g body weight the values were less for ob/ob mice and the erroneous conclusion would have been drawn that the resting metabolic rate of obese mice was below normal.

When lean mice were injected with NA, oxygen uptake increased more than 200 percent above resting values. In contrast, there was no significant rise in oxygen consumption

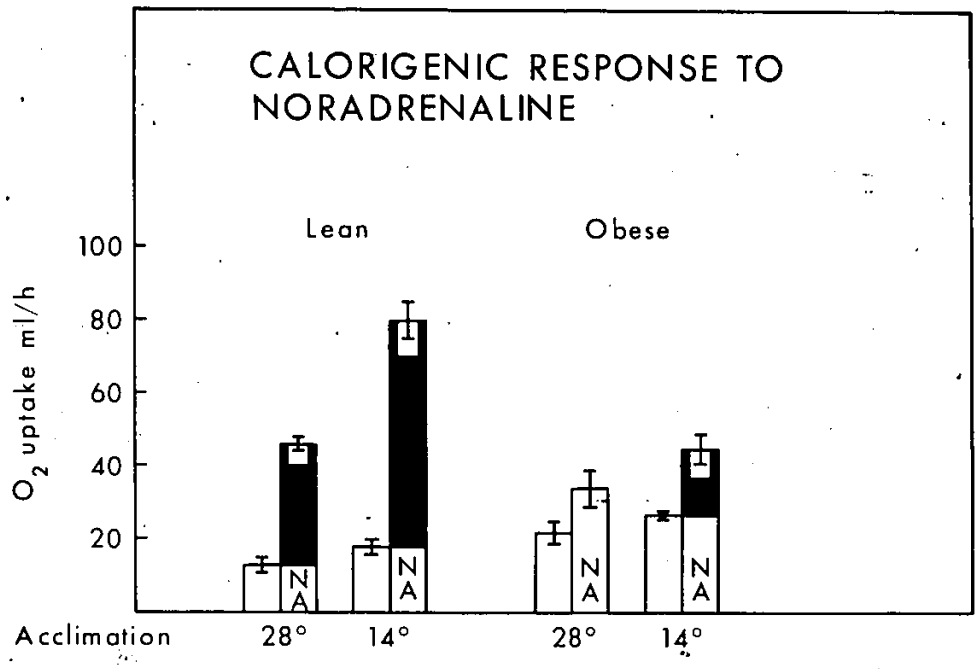

FIGURE 7. Noradrenaline (NA)-induced thermogenesis in warm-acclimated (28°C) and cold-acclimated (14°C) lean and obese (ob/ob) mice.

Bars represent resting oxygen consumption of pentobarbital anesthetized mice and maximum rate after injection of noradrenaline. Significant effects of noradrenaline are indicated by the black portion of the bars. Values are means ± SE for the number of animals shown in parenthesis with their body weights (g) which were: lean at 28°C, 22.3 ± 0.6 (6); lean at 14°C, 22.9 ± 0.4 (6); obese at 28°C, 51.0 ± 1.6 (6); obese at 14°C, 51.2 ± 1.9 (5).

for ob/ob mice, although an increase was observed for each experiment. These results show that the capacity for NST in ob/ob is much less than that of lean mice and confirms the data reported by Trayhurn and James (1978). These findings also indicate that in terms of an impaired response to NA, the ob gene is as potent on the C57BL/6J background as it is on the mixed background of the Aston colony which was used by Trayhurn and James (1978). Three facts suggest that the lower metabolic rate observed with ob/ob mice does not result from a fat impeded distribution of NA. First, there was no inverse relationship between the response to NA and the weight of ob/ob mice. Second, the amount of NA injected (600 µg/kg) was based on preliminary experiments which gave a maximal response for both lean and ob/ob mice at this dose. Third, the effect of NA was not reduced in mice made equally obese with goldthioglucose (to be discussed in Part II).

2. Effect of cold-exposure on brown adipose tissue of warm-acclimated obese (ob/ob) mice

As expected, exposure to 4°C for 3 hours did not alter the body weight or BAT wet weight of lean and obese mice (Table 1). Nor were there changes in total protein or cytochrome oxidase content of BAT in these animals (Table 1). Nevertheless, activation of BAT thermogenesis by acute cold exposure occurred in lean mice, as shown by the increase in

mitochondrial binding of GDP (Figure 1). In ob/ob mice however, BAT mitochondrial GDP binding was not altered, indicating a failure in these animals to activate BAT thermogenesis in response to acute cold (Figure 1). This defect in the unmasking of proton conductance pathways, previously reported by Himms-Hagen and Desautels (1978) in adult mice and later reported by Goodbody and Trayhurn in preweanling mice (1982), cannot be attributed to a lack of sympathetic activity in BAT. Other studies have shown that there is a similar stimulation of NA synthesis and secretion in BAT of both lean and ob/ob mice when they are exposed to 4°C for 3 hours (Zaror- Behrens, Himms-Hagen 1983). As well, the decrease in heat production in ob/ob mice is not due to an insufficient circulating substrate supply because the free fatty acid concentration in blood is known to be comparable to that of lean mice after acute exposure to 4°C (Thenen, Carr 1978; Carnie, Smith 1978). Electron micrographs of isolated BAT mitochondria from mice exposed to acute cold reinforce the suggestion that the level of GDP binding may be a consequence of the structural organization of inner mitochondrial membranes. Thus, the cold-induced increase in GDP binding observed in lean mice was associated with changes in the ultrastructure of mitochondria such that after 3 hours at 4°C all mitochondria displayed immerices which were arranged in parallel arrays (Figure 8). Likewise, the low GDP

FIGURE 8. Electron micrograph of brown adipose tissue mitochondria isolated from warm-acclimated lean mice exposed to 4°C for 3 hours (x 16 000).

FIGURE 9. Electron micrograph of brown adipose tissue mitochondria isolated from warm-acclimated obese (ob/ob) mice exposed to 4°C for 3 hours (x 16 000).

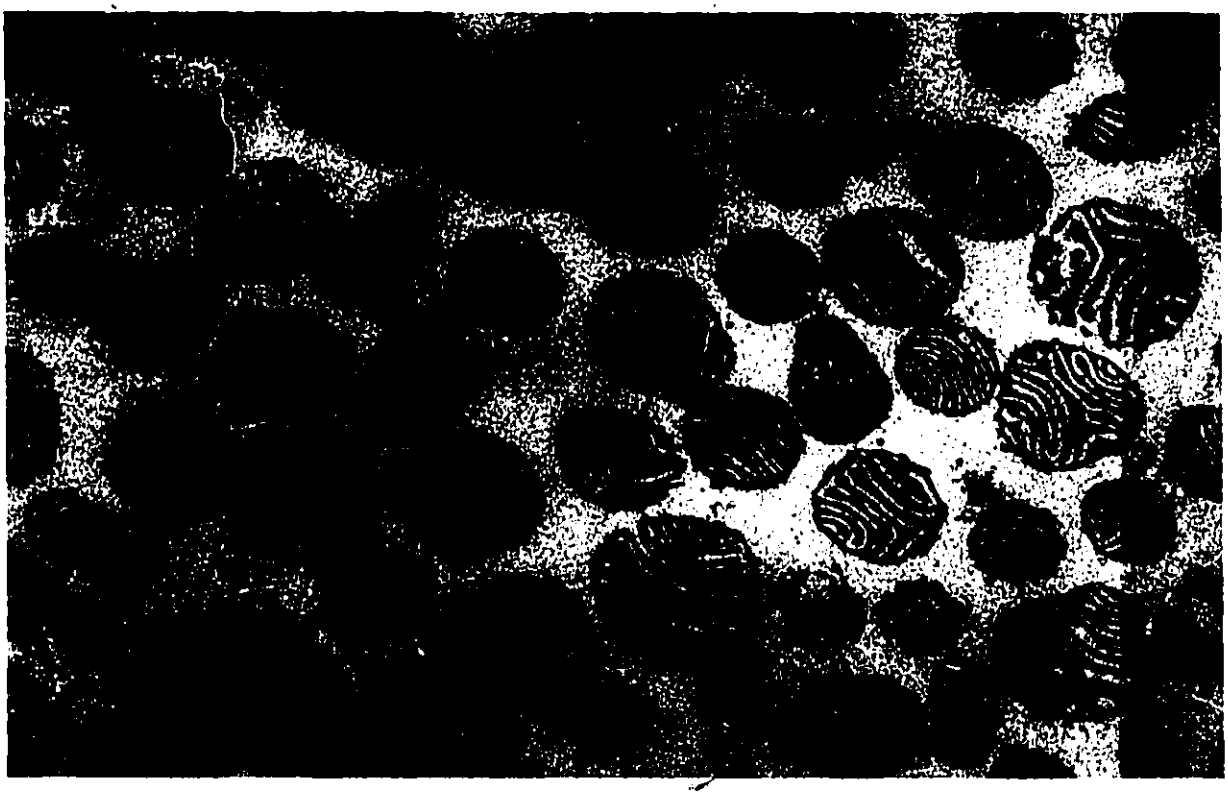

Fig. 8

Fig. 9

binding of cold-exposed ob/ob mice occurred in association with a minimal change in mitochondrial ultrastructure (Figure 9). Most mitochondria in BAT of ob/ob mice after acute cold retained the enlarged intracristal spaces with branched and circular immerices seen in mitochondria at 28°C (compare Figures 4 and 9). One unusual structure frequently observed and seen only in preparations from ob/ob mice, consisted of a collection of intact mitochondria surrounded by a membrane (Figure 9).

3. Brown adipose tissue of cold-acclimated obese (ob/ob) mice

Acclimation to 14°C for 2 weeks did not affect body weight gain in lean mice but it did prevent the gain in weight for ob/ob mice (Table 1). A large increase in thermogenic activity of BAT mitochondria occurred during cold acclimation in both lean and ob/ob mice, as indicated by the increase in mitochondrial GDP binding (Figure 1). Although a threefold increase in GDP binding occurred in ob/ob mice, the level attained was still lower than that of cold-acclimated lean mice (Figure 1).

Cold acclimation significantly altered the polypeptide composition of mitochondrial membranes from ob/ob mice (Table 2). There were increases in polypeptides with molecular weight ranges 73,000 - 82,500; 60,200 - 70,200; 30,500 - 33,800 and decreases in others, 40,700 - 44,900;

82,500 - 90,500. A similar trend was also present in lean mice acclimated to 14°C but significant changes were evident only after acclimation to 4°C. The increase in the relative amount of polypeptides near 32K represents an increase in the amount of proton conductance pathways and accounts for the large increase in GDP binding in lean and ob/ob mice.

Electron microscopy studies showed that increased binding and altered polypeptide composition were accompanied by changes in the ultrastructure of mitochondria isolated from the cold acclimated mice (Figures 10 and 11). Compared to their appearance at 28°C (Figure 3), mitochondria from lean mice maintained at 14°C were generally larger and contained numerous thin and parallel immerices (Figure 10). In contrast to the abnormal collection of mitochondria from warm acclimated ob/ob mice (Figure 4), a relatively normal ultrastructure was seen in ob/ob mice after cold-acclimation, in that the immerices frequently appeared in parallel arrays, were more plentiful and were not as distended (Figure 11). However, the mitochondria of cold acclimated ob/ob mice were smaller than mitochondria of lean mice and expanded intracristal spaces still occurred (Figure 11). These observations are in keeping with the changes in GDP binding measurements and polypeptide composition.

Cold acclimation caused an increase in BAT wet weight of lean mice but not of ob/ob mice (Table 1). However, growth of the tissue occurred in ob/ob mice as it did in

FIGURE 10. Electron micrograph of brown adipose tissue mitochondria isolated from cold-acclimated (14°C) lean mice (x 16 000).

FIGURE 11. Electron micrograph of brown adipose tissue mitochondria isolated from cold-acclimated (14°C) obese (ob/ob) mice (x 16 000).

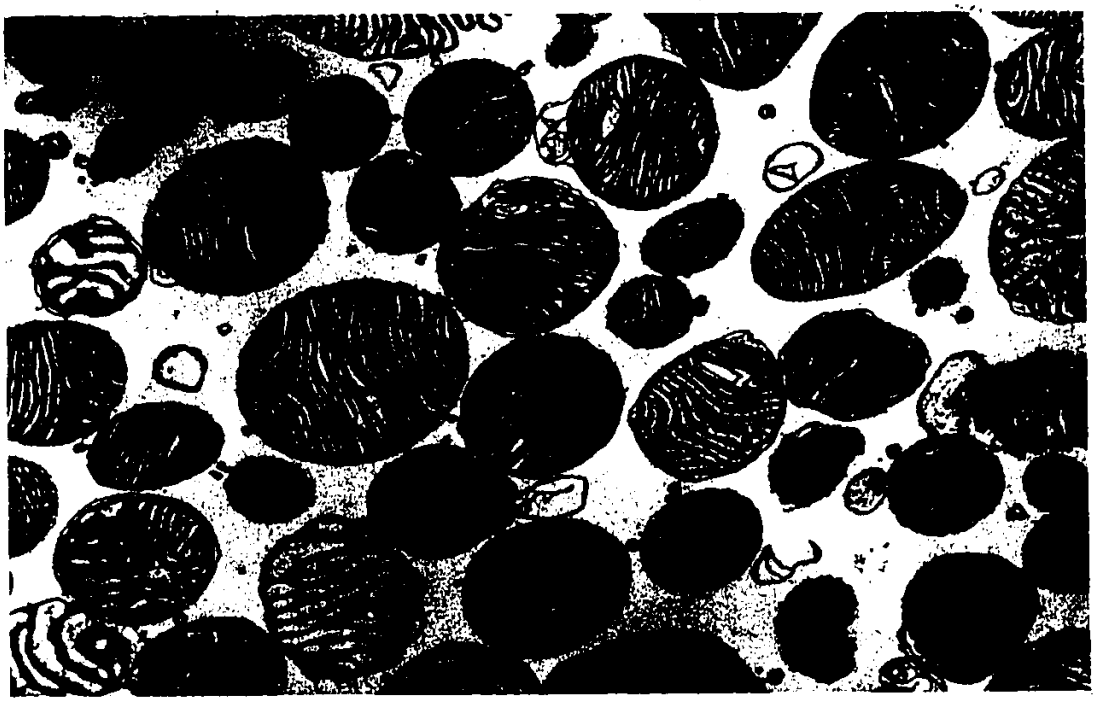

Fig. 10

Fig. 11

lean mice, as judged by the substantial increases in total protein and cytochrome oxidase content (Table 1). The increase in metabolic mass occurred in parallel with the proliferation of mitochondria since the specific activity of cytochrome oxidase in BAT homogenates did not change with cold acclimation (Table 1).

Acclimation to 14°C did not affect the metabolic rate of pentobarbital-anesthetized lean and ob/ob mice (Figure 7). The thermogenic response to injected NA was however significantly improved by cold acclimation in ob/ob mice but was still much smaller than the response observed in lean mice (Figure 7).

The enhanced capacity for NST in BAT of ob/ob mice as a consequence of cold acclimation has been substantiated by others (Seydoux et al., 1982a; Assimacopoulos-Jeannet et al. 1982). Other improvements in metabolic functioning of BAT have subsequently been reported in cold-acclimated ob/ob mice which include an increase in the basal release of glycerol and a partial restoration of the octanoate-induced increase in NAD(P)H redox state (Assimacopoulos-Jeannet et al. 1982). Furthermore, the number of Na^+ K^+ ATPase enzyme units in BAT increases normally in ob/ob mice during cold acclimation (Knehans, Romsos 1982).

4. Effect of cold exposure on BAT of cold-acclimated ob/ob mice

In these experiments, ob/ob mice acclimated to 14°C did not appear to be hypothermic and were still very active after 3 hours at 4°C, whereas ob/ob mice maintained at 28°C became extremely hypothermic and immobile during the brief exposure to cold. Presumably, the greater amount of functional BAT in cold-acclimated ob/ob mice enabled these animals to survive at lower temperatures. Acute cold exposure however, had no further effect on mitochondrial GDP binding in either lean or ob/ob mice (Figure 1). This indicates that the binding sites were completely unmasked and that a further increase in BAT thermogenesis would likely require the formation of new proton conductance pathways. As expected, there were no changes in BAT wet weight in lean and ob/ob mice after acute cold exposure, nor were there changes in total tissue protein or cytochrome oxidase content (Table 1).

Conclusions

BAT mitochondria of warm-acclimated ob/ob mice have an abnormal ultrastructure and a reduced level of GDP binding, indicating a low state of thermogenic activation of the tissue. Since mice were kept at a temperature fairly close to thermoneutrality (28°C) this would suggest a failure of the thermogenic response of BAT to the diet (chow). Exposure of the mice to cold (4°C) failed to bring about the normal thermogenic response of BAT (no increase in mitochondrial GDP binding; no ultrastructural change). Since the thermogenic response to injected noradrenaline is impaired and because normal sympathetic activation occurs in BAT of the ob/ob mouse at 4°C (Zaror-Behrens, Himms-Hagen, 1983) it is suggested that BAT of the ob/ob mouse is refractory to noradrenaline. This suggestion is now reinforced by later studies that demonstrate a reduced response of isolated BAT preparations from ob/ob mice to noradrenaline (Assimacopoulos-Jeannet et al. 1982; Seydoux et al. 1982; Begin-Heick, Heick, 1982).

Acclimation of the ob/ob mouse to cold (14°C) results in growth and improved thermogenic functioning of BAT. The defect in BAT of the ob/ob mouse thus appears to be confined to the switching mechanism responsible for the noradrenaline induced unmasking of the proton conductance pathways and thermogenesis in BAT, the slower and adaptive growth mechanisms being fairly normal.

PART 1B: BROWN ADIPOSE TISSUE OF GENETICALLY OBESE (ob/ob) MICE: RESPONSE TO THYROXINE

Objective:

The primary aim of these experiments was to determine whether the improved cold resistance of ob/ob mice chronically treated with T_4 (Mayer, Barnett 1953; Otto et al. 1976; Joosten, Van der Kroon 1974a; Ohtake et al. 1977) could be related to an increased thermogenic functioning of their BAT mitochondria. A secondary objective was to ascertain whether long-term administration of T_4, NA, or T_4 plus NA alters growth of BAT in warm-acclimated (24°C, 28°C, 33°C) lean and ob/ob mice.

Method:

Lean (+/ob or +/+) and obese (ob/ob) mice were kept in groups of 3 for at least 1 week before treatment with hormones. At 9-10 weeks of age, mice received daily subcutaneous injections for 2 weeks of either peanut oil (0.1 ml), T_4 in oil (50 µg/kg), NA in oil (400 µg/kg) or a combination of NA and T_4 in oil at doses similar to the individually injected hormones. The dose for ob/ob mice was the same as that for a lean mouse weighing 25 g. Three separate experiments were done to examine the effect of treatment with hormones on BAT composition and mitochondrial function. In the first experiment, mice were maintained at 24°C and

injected with either peanut oil, NA, T_4 or NA plus T_4. Mice were killed 16 hours after the last injection. In the second experiment, mice were maintained at 28°C and received injections of either peanut oil or T_4. Eighteen hours after the last injection, mice were placed in individual metal cages and kept at either 28°C or exposed to 4°C for 3 hours before they were killed. In the third experiment, mice were maintained at either 24°C or 33°C. They were injected with either peanut oil or T_4 and were killed 18 hours after the last injection. Immediately after sacrifice, subscapular BAT (2nd and 3rd experiment) plus interscapular BAT (all experiments) were removed, cleaned and weighed. BAT homogenates were made up to 5 ml and aliquots taken for protein and cytochrome oxidase assays (Methods C and D). The homogenates from 3 similarly treated animals were combined and mitochondria isolated for GDP binding assay as previously described (Methods A and B). Polyacrylamide-gel electrophoresis was carried out on mitochondrial membranes obtained from mice in the second experiment (Method F). Additional mice were injected with either peanut oil or T_4 and maintained at 28°C for measurement of oxygen uptake in the presence and absence of NA and for electron microscopy studies of isolated BAT mitochondria as described before (Methods G.1).

Results and Discussion

1. Effect of long-term treatment with T4 and/or NA on brown adipose tissue of warm-acclimated (24°C) lean and obese (ob/ob) mice

Chronic administration of T_4, NA, or T_4 plus NA did not affect body weight gain of either lean or ob/ob mice maintained at 24°C (Table 3). Moreover, there was no effect of hormonal treatment on the thermogenic state of BAT in lean mice as indicated by the lack of change in mitochondrial GDP binding measurements (Figure 12). In contrast, treatment with T_4 or a combination of T_4 and NA significantly increased the low GDP binding in BAT of ob/ob mice, indicating activation of BAT thermogenesis by T_4 (Figure 12). However, the thermogenic state of the tissue in T_4 treated ob/ob mice was still less than that of oil-treated lean mice, as shown in the lower level of mitochondrial GDP binding (Figure 12). Long-term treatment with NA or T_4 did not affect BAT wet weight in either lean or ob/ob mice (Table 3). Nor did it alter the metabolic or mitochondrial mass of the tissue in these animals, since total protein and cytochrome oxidase content remained unchanged (Table 3). Because subscapular BAT was not included in this experiment, all values for wet weight, protein and cytochrome oxidase content are lower for oil-treated lean and ob/ob mice than those previously shown (see Table 1).

TABLE 3

EFFECT OF LONG-TERM TREATMENT WITH T_4 AND/OR NA ON LEAN AND OBESE (ob/ob) MICE

	Control 24°C	NA 24°C	NA + T_4 24°C	T_4 24°C
LEAN MICE	(12)	(12)	(12)	(12)
Body Weights, g				
initial	22.3 ± 0.3	23.3 ± 0.3	21.5 ± 0.7	22.8 ± 0.4
final	24.5 ± 0.7	25.1 ± 0.4	24.2 ± 0.8	25.6 ± 0.6
change	2.2 ± 0.6	1.9 ± 0.5	2.7 ± 0.5	2.8 ± 0.4
Brown Adipose Tissue				
wet weight, mg	134 ± 6	143 ± 9	135 ± 9	131 ± 9
protein, mg	6.9 ± 0.5	6.8 ± 0.4	8.0 ± 0.5	6.7 ± 0.3
cytochrome oxidase				
μg atoms $O_2 \cdot min^{-1}$	53.3 ± 5.2	60.4 ± 3.1	68.0 ± 8.7	65.4 ± 4.5
μg atoms $O_2 \cdot min^{-1} \cdot mg\ protein^{-1}$	8.4 ± 1.2	9.0 ± 0.5	8.1 ± 0.9	10.0 ± 0.9
OBESE MICE	(12)	(12)	(12)	(12)
Body Weight, g				
initial	54.1 ± 1.0[a]	53.0 ± 1.1[a]	47.0 ± 0.4[a]	51.7 ± 1.2[a]
final	58.9 ± 0.9[a]	56.7 ± 1.4[a]	51.2 ± 1.1[a]	54.0 ± 1.2[a]
change	4.8 ± 0.9[a]	3.7 ± 1.2[a]	4.2 ± 1.1[a]	2.3 ± 0.8[a]
Brown Adipose Tissue				
wet weight, mg	770 ± 45[a]	755 ± 42[a]	736 ± 57[a]	722 ± 0.0[a]
protein, mg	9.9 ± 0.7[a]	10.49± 0.8[a]	11.5 ± 1.0[a]	11.0 ± 0.0[a]
cytochrome oxidase				
μg atoms $O_2 \cdot min^{-1}$	61.8 ± 7.1	73.8 ± 5.7	83.2 ± 10.0	61.3 ± 5.1
μg atoms $O_2 \cdot min^{-1} \cdot mg\ protein^{-1}$	6.5 ± 0.9[a]	7.5 ± 0.9[a]	7.4 ± 0.7[a]	5.6 ± 0.5[a]

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

[a]Significant effect of obesity, comparing mice treated in the same way. There is no significant effect of hormone treatment, comparing the same type of mouse.

PURINE NUCLEOTIDE (GDP) BINDING BY BROWN ADIPOSE TISSUE MITOCHONDRIA OF LEAN AND OBESE MICE

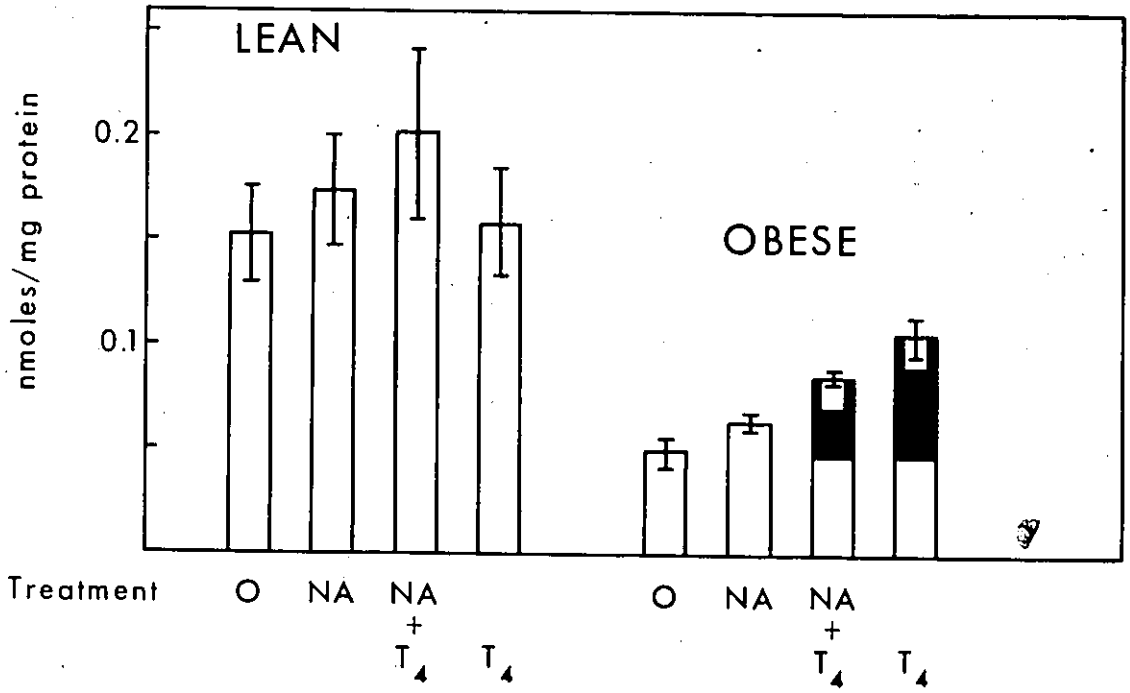

FIGURE 12. Effect of long term treatment with T_4 and/or NA on GPD binding by BAT mitochondria of lean and obese (ob/ob) mice.

Bars represent the means ± SE for 4 separate mitochondrial preparations. A significant effect of the treatment is indicated by the black portion of the bars. There are significant differences between lean and ob/ob mice chronically treated with oil ($p < 0.005$), NA ($p < 0.01$) and NA plus T_4 ($p < 0.05$).

2. Effect of long term treatment with T_4 on brown adipose tissue of lean and obese (ob/ob) mice acclimated to 28°C and exposed to acute cold

A more typical weight change for mice chronically treated with T_4 was observed in this experiment. In agreement with other studies of T_4-treated mice (Thenen, Carr 1980; Begin-Heick, Heick, 1977; Vander Tuig et al. 1979; Lin et al. 1979a), weight gain was significantly greater in lean mice and smaller in obese mice (Table 4). The effect of T_4-treatment on weight gain of ob/ob mice is likely not the result of a reduction in food intake because other studies have shown that food intake is not changed when T_4 is given at the same dose as used in this experiment (Begin-Heick, Heick, 1977). In fact, appetite is actively stimulated in ob/ob mice receiving larger amounts of T_4 (Lin et al. 1979a). The reduced weight gain of these animals is probably due to the known effect of thyroid hormones on body composition causing a substantial loss of carcass fat (York et al. 1978b; Thenen, Carr 1980) but a small gain of carcass protein (York et al. 1978b).

As in the previous experiment, chronic administration of T_4 did not affect GDP binding by BAT mitochondria of lean mice maintained at 28°C, but it did increase the low binding in ob/ob mice, such that the level of binding attained was not significantly different from either oil- or

TABLE 4

EFFECT OF COLD EXPOSURE ON OIL-AND T_4-TREATED MICE

Temperature of Exposure	Oil 28°C	Oil 4°C	T_4 28°C	T_4 4°C
LEAN MICE				
Body weights, g	(12)	(12)	(12)	(12)
initial	21.8 ± 0.7	22.0 ± 0.5	20.9 ± 0.4	21.0 ± 0.6
final	22.7 ± 0.6	23.9 ± 0.4	24.3 ± 0.5	23.2 ± 0.9
change	0.8 ± 0.6	1.9 ± 0.3	3.5 ± 0.2[b]	2.3 ± 0.6
Brown Adipose Tissue				
wet weight, mg	253 ± 14	191 ± 18	307 ± 15 [b]	209 ± 13
protein, mg	11.3 ± 0.7	10.1 ± 0.9	11.5 ± 0.5	11.2 ± 0.8
cytochrome oxidase				
μg atoms $O_2 \cdot min^{-1}$	99.9 ± 5.9	93.9 ± 7.0	122.3 ± 7.4[b]	119.8 ± 9.9
μg atoms $O_2 \cdot min^{-1} \cdot mg\ protein^{-1}$	9.4 ± 0.9	9.6 ± 0.6	11.0 ± 0.9	10.8 ± 0.7
OBESE MICE	(12)	(12)	(12)	(12)
Body weights, g				
initial	48.1 ± 1.7[a]	50.3 ± 2.2[a]	46.1 ± 1.4[a]	50.4 ± 2.5[a]
final	53.9 ± 1.7[a]	55.1 ± 2.1[a]	49.4 ± 1.4[a]	52.5 ± 1.8[a]
change	5.9 ± 0.5[a]	4.9 ± 0.6[a]	3.1 ± 0.4[b]	2.1 ± 1.0[b]
Brown Adipose Tissue				
wet weight, mg	1627 ± 84 [a]	1677 ± 61 [a]	1447 ± 96 [a]	1609 ± 73 [a]
protein, mg	19.2 ± 0.9[a]	19.2 ± 1.2[a]	21.6 ± 1.3[a]	23.9 ± 0.6[a]
cytochrome oxidase				
μg atoms $O_2 \cdot min^{-1}$	91.2 ± 5.4	103.1 ± 5.8	114.8 ± 8.9[b]	124.7 ± 3.5
μg atoms $O_2 \cdot min^{-1} \cdot mg\ protein^{-1}$	4.8 ± 0.3[a]	5.3 ± 0.3[a]	5.4 ± 0.4[a]	5.2 ± 0.5[a]

Values are the means ± SE for the number of animals given in parenthesis.

[a]Significant effect of obesity, comparing mice treated in the same way.

[b]Significant effect of T_4, comparing the same type of mouse. There is no significant effect of cold exposure, comparing the same type of mouse.

T_4-treated lean mice (Figure 13). Long term treatment with T_4 did not alter the polypeptide composition of mitochondrial membranes in BAT of lean mice (Table 5). Moreover, activation of BAT thermogenesis by T_4 treatment in ob/ob mice was not accompanied by a change in the relative amount of mitochondrial peptides with molecular weights near 32,000 (Table 5). In fact, the only significant change in T_4-treated ob/ob mice was an increase in polypeptides of molecular weight 60,000 - 70,200 (Table 5). The increase in GDP binding of T_4-treated ob/ob mice is therefore ascribed to an unmasking of binding sites already present in the inner mitochondrial membrane.

Electron micrographs of mitochondria isolated from lean and ob/ob mice maintained at 28°C are also revealing and are consistent with GDP binding measurements. The characteristic bizarre appearance of mitochondria from ob/ob mice (Figure 14) disappeared with T_4 treatment (Figure 15), to the extent that they resembled mitochondria from lean mice (Figure 16). This suggests that the T_4-induced increase in GDP binding seen in ob/ob mice results from a reorganization of mitochondrial membranes to expose more binding sites. In keeping with the lack of change in GDP binding measurements there was no detectable change, as a consequence of T_4 treatment in the ultrastructure of mitochondria isolated from lean mice (Figure 17).

PURINE NUCLEOTIDE (GDP) BINDING BY BROWN ADIPOSE TISSUE MITOCHONDRIA OF LEAN AND OBESE COLD-EXPOSED MICE

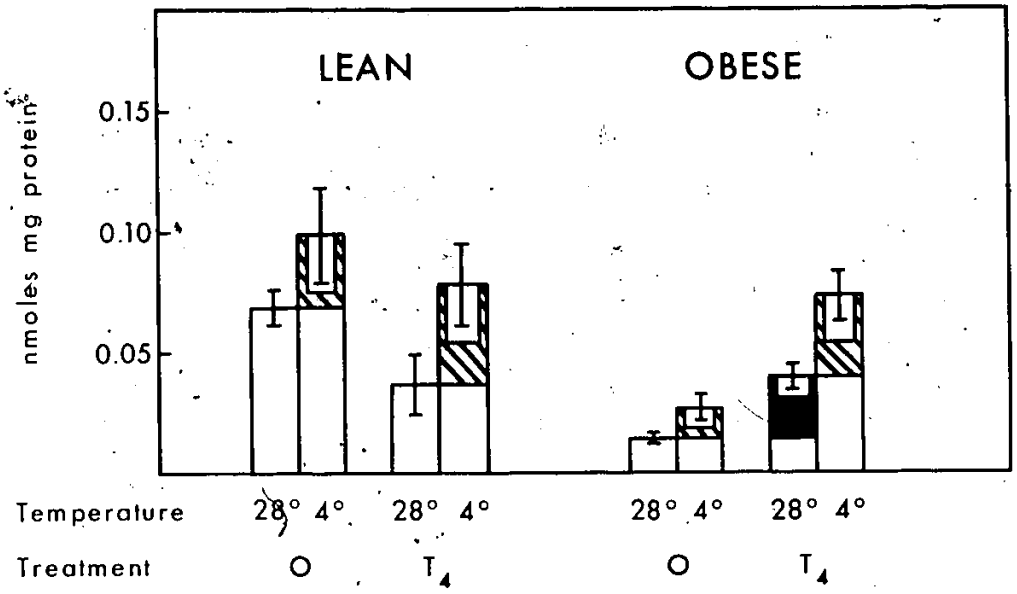

FIGURE 13. Effect of long-term treatment with T_4 on GDP binding by BAT mitochondria of lean and obese (ob/ob) cold-exposed mice.

Bars represent the means ± SE for 3 separate mitochondrial preparations. A significant effect of T_4 treatment is indicated by the black portion of the bar. A significant effect of exposure to 4°C for 3 hours is indicated by the cross-hatched portion of the bars, except for the bar representing oil-treated lean mice (NS). There are significant differences between oil-treated lean and ob/ob mice at 28°C ($p < 0.005$) and 4°C ($p < 0.05$).

TABLE 5

POLYPEPTIDE COMPOSITION OF BAT MITOCHONDRIAL MEMBRANES FROM LEAN AND OBESE (ob/ob) MICE WITH LONG-TERM T_4 TREATMENT

	Lean		Obese	
	Oil	T_4	Oil	T_4
Group of polypeptides, mol wt range				
82,500 - 90,500	4.52 ±0.44	4.50 ±0.31	4.59 ±0.31	5.04 ±0.52
73,200 - 82,500	9.71 ±0.27	9.59 ±0.49	8.20[a] ±0.18	8.91 ±0.29
60,200 - 70,200	8.57 ±0.18	8.26 ±0.20	8.44 ±0.08	9.22[a,b] ±0.27
40,700 - 44,900	19.14 ±0.82	17.84 ±1.19	18.68 ±0.51	18.97 ±0.52
30,500 - 33,800	13.63 ±0.60	13.75 ±0.53	13.93 ±0.81	13.55 ±0.60

Values are means ± SE of the percent of the total area under the gel scan represented by peaks of the stated molecular weight ranges.

[a]Significant effect of obesity, comparing mice treated in the same way.

[b]Significant effect of T_4 treatment, comparing the same type of mouse.

FIGURE 14. Electron micrograph of brown adipose tissue mitochondria isolated from oil-treated obese (ob/ob) mice (x 16 000).

FIGURE 15. Electron micrograph of brown adipose tissue mitochondria isolated from T_4-treated obese (ob/ob) mice (x 16 000).

Fig. 14

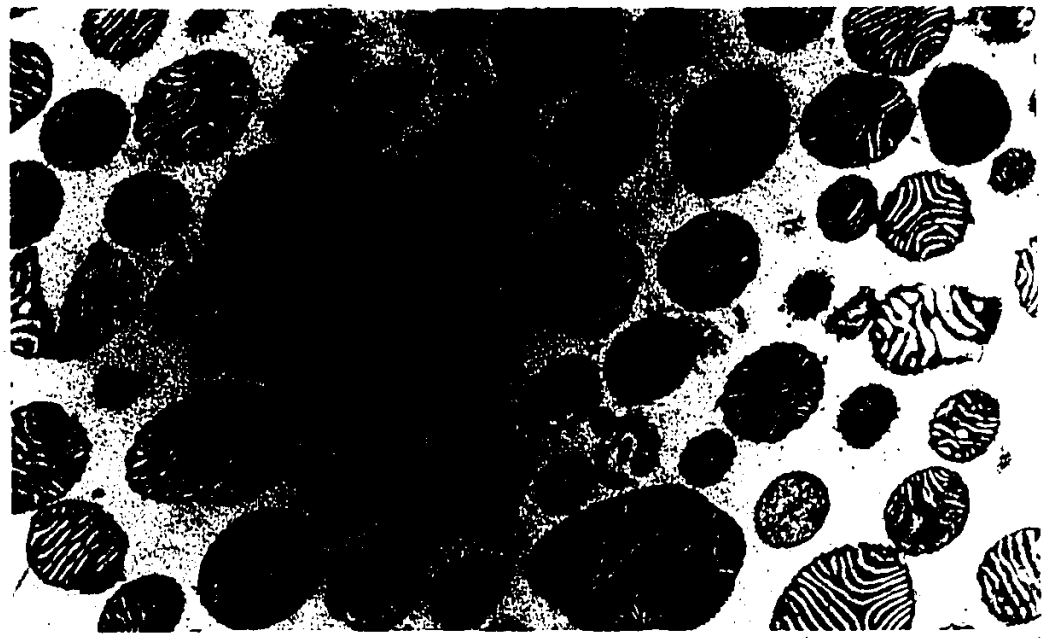

Fig. 15

FIGURE 16. Electron micrograph of brown adipose tissue mitochondria isolated from oil-treated lean mice (x 16 000).

FIGURE 17. Electron micrograph of brown adipose tissue mitochondria isolated from T_4-treated lean mice (x 16 000).

Fig. 16

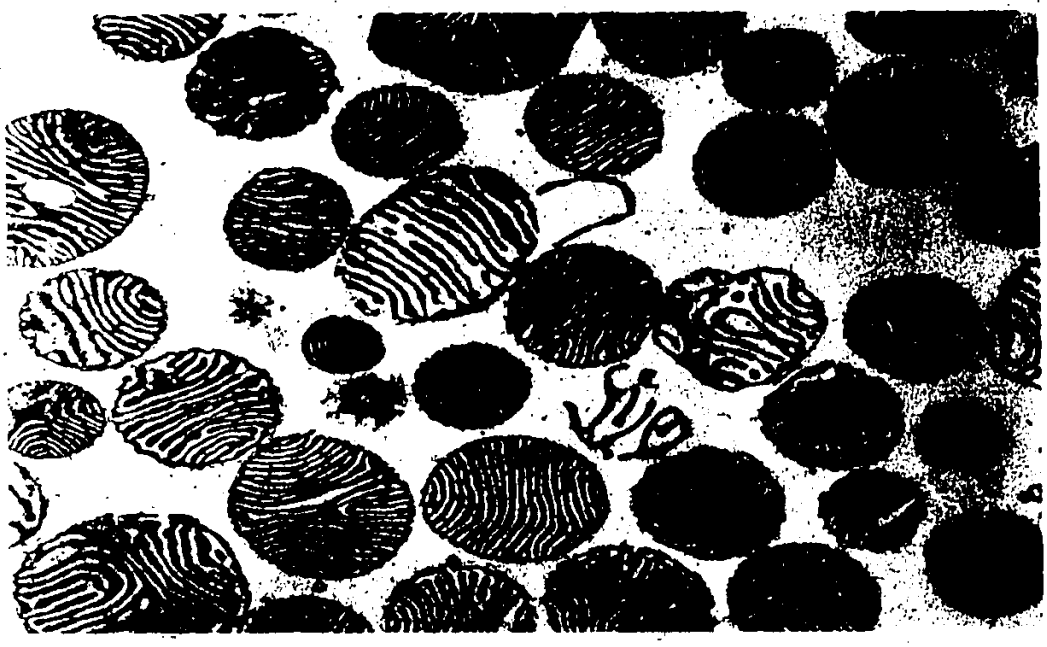

Fig. 17

A small but significant increase in BAT wet weight was observed in T_4-treated lean mice but not in T_4-treated ob/ob mice (Table 4). However, chronic administration of T_4 caused a slight increase in mitochondrial mass of BAT in ob/ob mice as it did in lean mice. This is shown by the increase in total cytochrome oxidase activity (Table 4). These results are at variance with the previous experiment (Table 3) in which no trophic effect of T_4 was observed in BAT of mice at 24°C. However, in this experiment subscapular BAT was included with the interscapular depot and the difference may therefore result from an altered response to T_4 of BAT in different locations.

Oxygen uptake was measured in pentobarbital-anesthetized mice to establish whether chronic administration of T_4 alters the metabolic rate. Although no change in oxygen uptake was observed in T_4-treated lean mice, a significant rise occurred in T_4-treated ob/ob mice (Figure 18). This is in accord with the known increase in heat production (Lin et al. 1979a; Vander Tuig et al. 1979) and body temperature Joosten, Van der Kroon 1974a) of T_4-treated ob/ob mice which is larger than that of T_4-treated lean mice. Measurements of resting metabolic rate were made at thermoneutrality (31-33°C) to eliminate the effect of cold on BAT thermogenesis. Because DIT is also known to reside in BAT (Rothwell, Stock 1979a; 1981b) and is probably defective in ob/ob mice (Part I.A), the exaggerated metabolic response of ob/ob mice

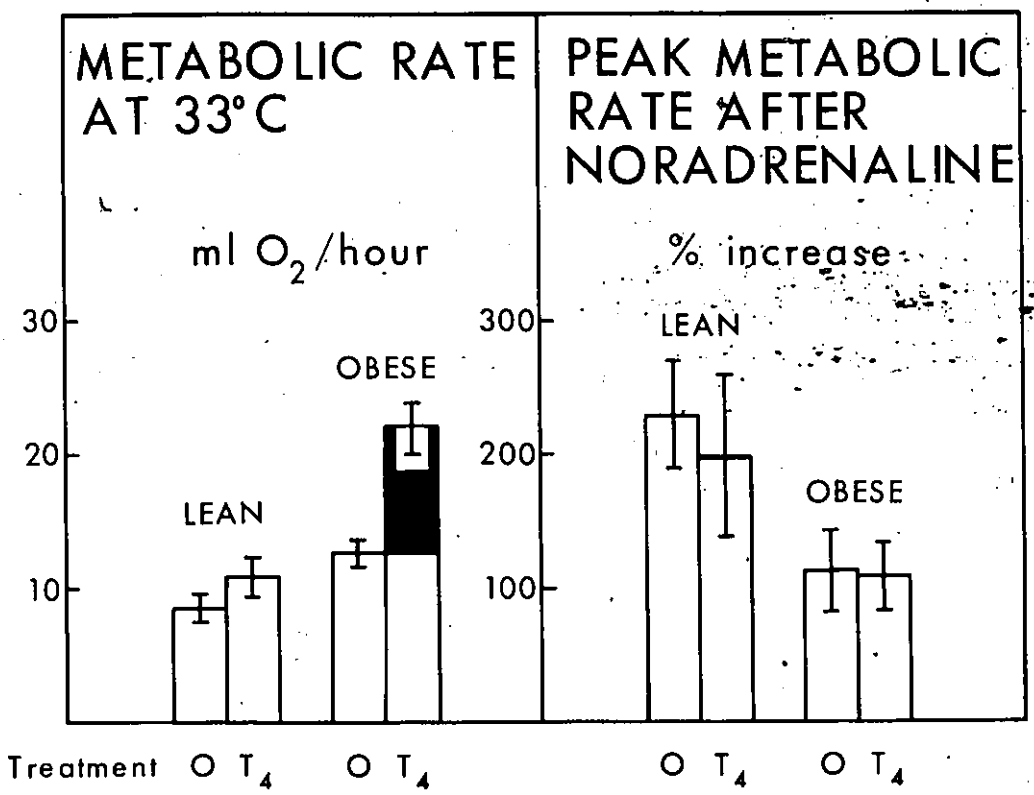

FIGURE 18. Noradrenaline (NA)-induced thermogenesis in oil-treated and T_4-treated lean and obese (ob/ob) mice.

Bars represent resting oxygen uptake (A) of pentobarbital-anaesthetized mice and maximum uptake after injection of noradrenaline (B). A significant effect of T_4 treatment is indicated by the black portion of the bar. Values are the menas ± SE for the number of animals given in parenthesis with their body weights (g) which were: lean oil-treated, 25.7 ± 0.6 (5); lean T_4-treated, 25.6 ± 0.8; ob/ob oil-treated, 58.0 ± 1.4 (6); ob/ob T_4-treated, 59.1 ± 1.3 (6).

to T_4 may in part result from an improvement in DIT. It is also possible that other tissues contributed to the enhanced oxygen uptake of T_4-treated ob/ob mice by an increase in the activity of the thyroid-sensitive Na^+ K^+ ATPase, since long term treatment with T_4 increases the number of Na^+ K^+ ATPase enzyme units in skeletal muscle, kidney and liver to a greater extent in ob/ob mice than in lean mice (Lin et al. 1979a). As seen before (Trayhurn, James 1978; Figure 7), the thermogenic response of ob/ob mice to injected NA was smaller than that of lean mice (Figure 18). Although treatment with T_4 did not alter the percent increase in metabolic rate in response to NA for lean and obese mice (Figure 18), the actual level of oxygen uptake reached in T_4-treated obese mice (44.4 ± 6.0 m/O_2/h) was significantly higher than that of oil-treated obese mice 25.2 ± 3.2 ml/h, $p < 0.001$) and T_4-treated lean mice (30.2 ± 5.7 ml O_2/h, $p < 0.001$).

Exposure of oil-treated lean mice to 4°C for 3 hours produced the usual increase in GDP binding in each experiment, but because of a large variation in binding measurements between experiments, a significant increase was not seen (Figure 13). Chronic treatment of lean mice with T_4 had no further effect on mitochondrial GDP binding (Figure 13). Exposure of oil-treated ob/ob mice to cold resulted in a slight increase in GDP binding by BAT mitochondria, although the level attained was still far less

than that of oil-treated lean mice at 28°C (Figure 13). However, T_4-treatment markedly enhanced the cold-induced increase in GDP binding such ~~that~~ binding measurements were now similar to those of cold-exposed lean mice (Figure 13). As expected, brief cold exposure had no effect on body weight, BAT wet weight or BAT contents of protein and cytochrome oxidase for either lean or ob/ob mice (Table 4).

3. Effect of long-term treatment with T_4 on lean and obese (ob/ob) mice acclimated to 33°C or 24°C

Additional experiments were done to verify that ob/ob mice exhibit defective BAT thermogenesis in response to a chow diet and to find out whether chronic administration of T_4 potentiates DIT.

As in the previous experiment (Table 4), long term treatment with T_4 had no effect on the body weight gains of lean mice, but it did prevent the weight gain in ob/ob mice at both 33°C and 24°C (Table 6). The thermogenic state of BAT is equally low in oil-treated lean and ob/ob mice maintained at 33°C compared to mice at 24°C, as shown by the equally low binding of GDP by BAT mitochondria in both genotypes (Figure 19). However, treatment of mice at 33°C with T_4 caused a similar increase in mitochondrial GDP binding in both lean and ob/ob mice, indicating that activation of BAT thermogenesis had occurred to the same extent (Figure 19). Likewise, T_4 treatment induced an

TABLE 6

EFFECT OF LONG-TERM TREATMENT WITH T_4 ON LEAN AND OBESE (ob/ob) MICE AT 33°C OR 24°C

Temperature of Acclimation	Control 33°C	T_4 33°C	Control 24°C	T_4 24°C
LEAN MICE	(15)	(15)	(14)	(14)
Body Weight, g				
initial	23.4 ± 0.5	23.5 ± 0.2	22.0 ± 0.3	22.0 ± 0.3
final	26.4 ± 0.6	27.1 ± 0.4	24.5 ± 0.3	24.8 ± 0.6
change	2.9 ± 0.3	3.6 ± 0.3	2.7 ± 0.2	2.8 ± 0.4
Brown Adipose Tissue				
wet weight, mg	433 ± 18	641 ± 36 [b]	271 ± 14 [c]	461 ± 30 [b,c]
protein, mg	11.1 ± 0.5	14.8 ± 0.8 [b]	15.2 ± 0.5 [c]	20.7 ± 1.5 [b,c]
cytochrome oxidase				
µg atoms $O_2 \cdot min^{-1}$	86.8 ± 6.0	123.6 ± 8.9 [b]	141.1 ± 9.6 [c]	217.2 ± 19.9 [b,c]
µg atoms $O_2 \cdot min^{-1} \cdot mg\ protein^{-1}$	8.1 ± 0.7	8.4 ± 0.5	9.2 ± 0.5	10.6 ± 0.7 [c]
OBESE MICE	(15)	(15)	(14)	(15)
Body Weight, g				
initial	51.9 ± 0.9 [a]	51.3 ± 0.7 [a]	51.3 ± 0.9 [a]	51.8 ± 0.5 [a]
final	56.8 ± 0.9 [a]	51.9 ± 0.6 [a,b]	54.6 ± 0.8 [a]	51.9 ± 0.7 [a,b]
change	4.8 ± 0.3 [a]	0.5 ± 0.2 [a,b]	3.3 ± 0.4	0 ± 0.5 [a,b]
Brown Adipose Tissue				
wet weight, mg	1817 ± 0.1 [a]	1731 ± 50 [a]	1628 ± 0.1 [a,c]	1816 ± 0.1 [a,b]
protein, mg	19.4 ± 1.1 [a]	28.6 ± 1.8 [a,b]	24.8 ± 2.1 [a,c]	29.2 ± 1.3 [a]
cytochrome oxidase				
µg atoms $O_2 \cdot min^{-1}$	88.1 ± 5.0	158.6 ± 8.2 [a,b]	147.5 ± 12.7 [c]	164.5 ± 10.4 [a]
µg atoms $O_2 \cdot min^{-1} \cdot mg\ protein^{-1}$	4.7 ± 0.3 [a]	5.7 ± 0.4 [a]	6.0 ± 0.3 [a,c]	5.7 ± 0.4 [a]

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

[a] Significant effect of obesity, comparing mice treated in the same way.

[b] Significant effect of T_4, comparing the same type of mouse.

[c] Significant effect of cold acclimation (24°C), comparing the same type mouse.

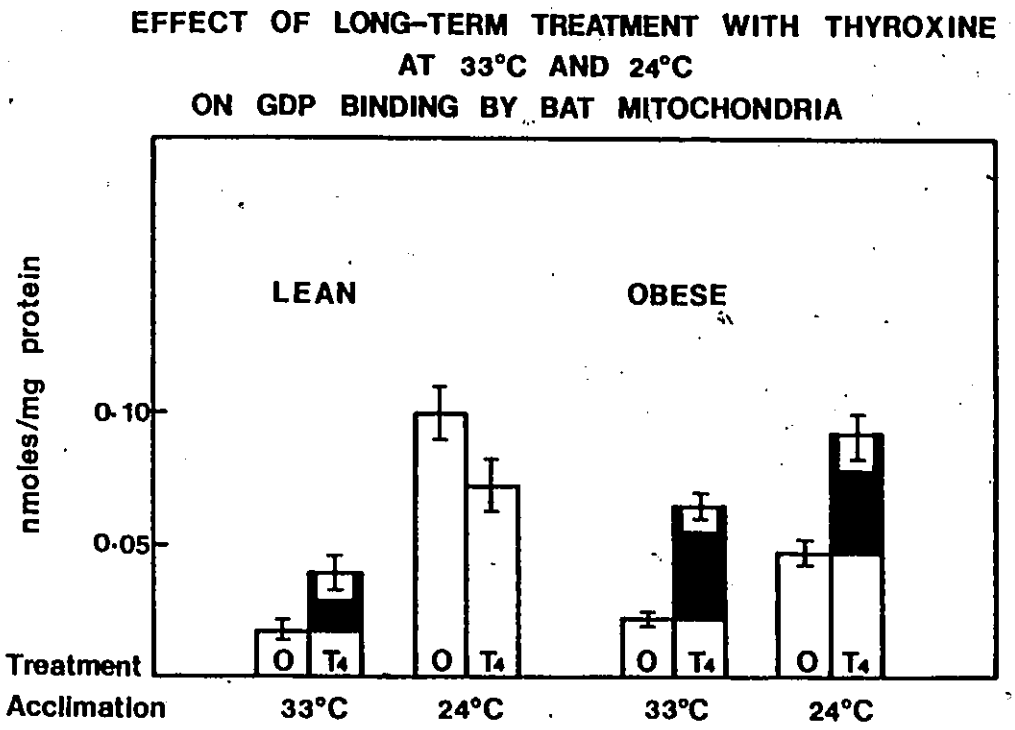

FIGURE 19. Effect of long term treatment with thyroxine on GDP binding by BAT mitochondria of lean and obese (ob/ob) mice acclimated to 33°C or 24°C.

Bars represent the means ± SE for 5 separate mitochondrial preparations. A significant effect of T_4 treatment is indicated by the black portion of the bars. There are significant differences between warm (33°C)- and cold (24°C)-acclimated mice, treated in the same way.

increase GDP binding in ob/ob mice maintained at 24°C such that the the level of binding was similar to that of lean mice at 24°C (Figure 19). No effect of T_4 treatment on mitochondrial GDP binding was seen in lean mice at 24°C (Figure 19).

As seen before (Table 4), T_4 treatment of mice at both 24°C and 33°C caused an increase in BAT wet weight for lean mice but not for ob/ob mice (Table 6). There were also increases in metabolic and mitochondrial mass of BAT in T_4-treated lean mice as well as ob/ob mice maintained at 33°C, as indicated by the increase in total protein and cytochrome oxidase content of the tissue (Table 6). A similar effect of T_4, on BAT growth was seen in lean mice at 24°C (Table 6). However, there were no changes in either cytochrome oxidase content of BAT in T_4-treated ob/ob mice at 24°C (Table 6). Overall, the results from all 3 experiments (Tables 3, 4 and 6) indicate that a trophic response of BAT to T_4 is not a consistent finding. Reasons for the discrepancy in these experiments are unknown, but it is unlikely that the differences are the result of an interaction between T_4 and ambient temperature because there does not appear to be a consistent pattern between BAT growth, T_4 and temperature. In contrast, the trophic effect of cold is already evident at 24°C as shown by the increase in protein and cytochrome oxidase content of BAT in both lean and ob/ob mice compared to their contents in BAT of mice at 33°C (Table 6).

Conclusions

Long term treatment with T_4 improves thermogenic functioning of BAT mitochondria in ob/ob mice both at their temperature of acclimation (33°C, 28°C, 24°C) and during brief exposure to cold (4°C for 3 hours). That the T_4-induced increase in GDP binding by BAT mitochondria of ob/ob mice occurred in the absence of an increase in the 32K polypeptide indicates an unmasking of proton conductance pathways already present. Since the mediator for this event is most probably NA (Desautels, Himms-Hagen, 1979), T_4 treatment likely improves BAT functioning in ob/ob mice by its well known permissive effect on the action of NA. The fact that activation of BAT thermogenesis by T_4 occurs in ob/ob mice despite a normal blood level of thyroid hormones (Mobley, Dubuc 1979;Ohtake et al. 1977) suggests that BAT in these animals may be refractory to endogenous NA because of a primary refractoriness to endogenous T_3. The mechanism whereby T_3 improves BAT responsiveness to NA, a mixed α-,β-adrenergic agonist, may involve an alteration in the balance of α and β receptors. In BAT of ob/ob mice, there is a reduced responsiveness and sensitivity of adenylate cyclase to NA (Begin-Heick, Heick 1982) and an impairment in NA-induced lipolysis (Assimacopoulos-Jeannet et al. 1982). Studies of other species show that binding of

adrenergic agonists to α_2 receptors inhibits adenylate cyclase activity and lipolysis in white adipose tissue (Wright, Simpson 1981; Aktories et al. 1980; Kather et al. 1980; Schimmel 1979) and that thyroid hormone status affects the proportion of α- and β-receptors in specific tissues (Lefkowitz 1982; Kunos 1977; Gibson 1981). It is conceivable that an excess population of α_2 receptors is present in BAT of ob/ob mice. If this is true, then treatment with T_4 might improve NA-induced adenylate cyclase activity and lipolysis in BAT of ob/ob mice as it does in their white adipose tissue (Begin-Heick, Heick, 1977; Otto et al. 1976; York et al. 1978b) by reducing the concentration of α receptors.

Other conclusions can be drawn from the experiments conducted at 33°C. At thermoneutrality (33°C), mitochondrial GDP binding reflects thermogenic activity of BAT in response to diet but not to cold. Since ob/ob mice are known to be hyperphagic at 33°C (Thurlby, Trayhurn 1979; Coleman 1982; Lin et al. 1980) and because GDP binding was similar in both lean and ob/ob mice at 33°C it is concluded that ob/ob mice exhibit defective DIT in BAT. This is in keeping with energy balance studies of mice maintained at 33°C which show that ob/ob mice pair fed to the ad libitum intake of lean mice deposit 27 percent more energy after 10 days than lean mice (Thurlby, Trayhurn 1979). Thus, chronic T_4 treatment enhances thermogenic activity in BAT of ob/ob mice in response to both cold and diet.

PART IC: BROWN ADIPOSE TISSUE OF GENETICALLY OBESE (ob/ob) MICE: RESPONSE TO CAFETERIA FEEDING

Objective:

The purpose of this experiment was to find out whether a cafeteria diet would activate BAT thermogenesis and promote growth of the tissue in genetically obese (ob/ob) mice.

Method:

Lean (+/+ or +/ob) and obese (ob/ob) mice 5-6 weeks old were kept in groups of 3 and maintained at 28°C. After 6 weeks the animals were fed either a chow diet or a cafeteria diet for 3 weeks (Materials C). Animals were killed by cervical dislocation, decapitated and blood collected in heparinized containers. Parametrial fat was removed and weighed. The proportional weight of gonadal fat pads is an index of the percentage body fat (Rogers, Webb 1980). Interscapular and subscapular BAT were combined, weighed and homogenized. The homogenates were made up to 5 ml with isolation medium for lean mice and 7 ml for ob/ob mice. Aliquots were removed for estimations of total protein, cytochrome oxidase and DNA (Methods C, D and E). The homogenates were pooled from 3 similarly treated mice and mitochondria isolated for measurement of GDP binding (Methods A and B). Plasma T_3 was determined in duplicate using the Amerlex ^{TM} T-3 RIA Kit from Amersham (Methods H).

The Kit has been evaluated with plasma samples. Estimates of T_3 in plasma samples are 3% lower than corresponding paired serum samples (Amersham Corporation).

Results and Discussion

Feeding lean mice a cafeteria diet for 3 weeks caused increases in body weight gain, gonadal fat wet weight and percent total body fat as assessed by the proportional weight of the gonadal fat pads (Table 7). No changes were seen in cafeteria-fed ob/ob mice. Although food intake was not measured in this experiment it has been measured by Trayhurn and coworkers (1982b) in young cafeteria-fed mice of the Aston strain. The digestible energy intake as measured by bomb calorimetry increased 69% in lean mice and 49% in ob/ob mice (Trayhurn et al. 1982b). These results show that ob/ob mice are capable of further exacerbating their already hyperphagic state when fed a cafeteria diet and suggest that the ob/ob mice in this experiment likely also increased their energy intake.

As expected, cafteria feeding induced activation of BAT thermogenesis in lean mice as indicated by the increase in mitochondrial GDP binding (Figure 20). Surprisingly, activation of BAT thermogenesis by cafeteria feeding also occurred in ob/ob mice (Figure 20). GDP binding values more than doubled in ob/ob mice so that the level of binding was similar in both lean and ob/ob mice. In agreement with

TABLE 7

EFFECT OF CAFETERIA FEEDING ON LEAN AND OBESE (ob/ob) MICE

	Lean		ob/ob	
	Chow (12)	Cafeteria (12)	Chow (12)	Cafeteria (12)
Body Weight, g				
initial	21.6 ± 0.5	21.9 ± 0.5	54.2 ± 1.3[a]	51.7 ± 1.0[a]
final	22.8 ± 0.7	26.3 ± 0.9[b]	61.3 ± 1.5[a]	60.0 ± 1.3[a]
change	1.2 ± 0.3	4.4 ± 0.6[b]	7.2 ± 0.5[a]	8.3 ± 1.1[a]
Gonadal Fat, mg	393 ± 47	615 ± 94[b]	5852 ±115	5989 ±234
% Gonadal Fat	1.7 ± 0.2	3.0 ± 0.4[b]	9.6 ± 0.2[a]	10.0 ± 0.3[a]
Brown Adipose Tissue				
Wet weight, mg	174 ± 8	242 ± 21	1785 ± 69[a]	1725 ± 79[a]
protein, mg	7.6 ± 0.5	10.7 ± 0.7	16.5 ± 0.9[a]	21.7 ± 1.3[a,b]
DNA µg	541.6 ± 32.5	533.7 ± 31.4	1157.4 ± 58.6[a]	1221.3 ± 79.2[a]
mg protein ·mg DNA^{-1}	14.7 ± 1.5	19.9 ± 2.3	14.5 ± 1.2	18.7 ± 1.8
cytochrome oxidase				
µg atoms $O_2 \cdot min^{-1}$	50.5 ± 3.3	68.4 ± 5.1[b]	71.1 ± 6.4[a]	106.8 ± 9.9[a,b]
µg atoms $O_2 \cdot min^{-1}$ ·mg protein	6.9 ± 0.6	6.2 ± 0.2	4.4 ± 0.4[a,b]	4.5 ± 0.4[a,b]
µg atoms $O_2 \cdot min^{-1}$ ·mg DNA	96.6 ± 8.1 (7)	135.2 ± 15.4[b] (7)	59.7 ± 5.2[a] (7)	90.0 ± 9.0[a,b] (7)
Plasma T_3, ng/dl	49.5 ± 3.3	63.1 ± 4.8[b]	85.1 ± 4.3[a]	99.6 ± 3.8[a,b]

Values are means ± SE for the number of animals given in parenthesis.

[a]Significant effect of obesity, comparing mice treated in the same way.

[b]Significant effect of cafeteria feeding, comparing the same type of mouse.

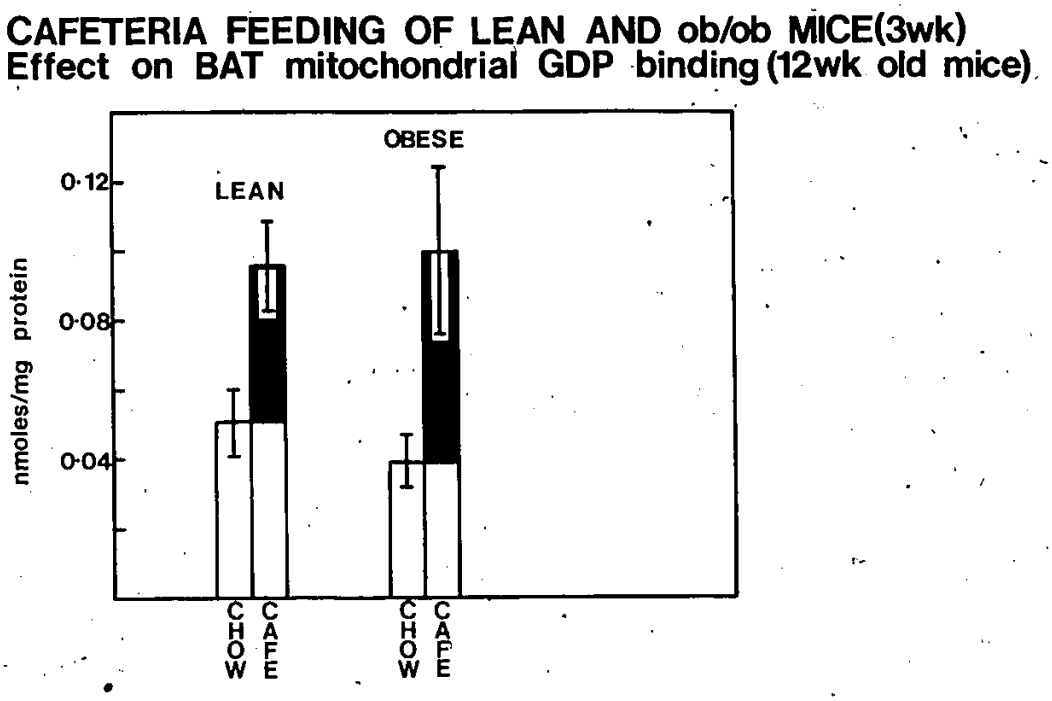

FIGURE 20. Effect of cafeteria feeding on GDP binding by BAT mitochondria of lean and obese (ob/ob) mice.

Bars represent the means ± SE for 4 mitochondrial preparations. A significant effect of cafeteria feeding (3 weeks) is indicated the black portion of the bars. There are no significant differences between chow-fed lean and ob/ob mice.

these results, Trayhurn and coworkers (1982) have subsequently reported that cafeteria feeding caused a 50% increase in mitochondrial GDP binding in lean and ob/ob mice of the Aston strain which occurred in conjunction with an increase in the apparent energy expenditure (calculated as the difference between digestible energy intake and the gain in body energy). Energy expenditure was however twice as great in the cafeteria-fed lean mice (Trayhurn et al. 1982).

As seen before, subscapular plus interscapular BAT of ob/ob mice fed a regular chow diet contained more protein and cytochrome oxidase than BAT of chow-fed lean mice (Table 7). In addition to the larger amount of metabolic and mitochondrial mass, DNA measurements indicate that twice as many cells were present in BAT of ob/ob mice (Table 7). Although calculation of total protein per unit DNA shows that the metabolic size of BAT cells was normal in these animals, the concentration of mitochondria in cells appears to be less since the total cytochrome oxidase activity in BAT homogenates per unit DNA was lower than in lean mice (Table 7), despite a normal specific activity of the enzyme in BAT mitochondria (14.5 ± 2.9 µg atoms $O_2 \cdot$ mg protein$^{-1} \cdot$min^{-1} in lean mice; 12.8 ± 2.0 in ob/ob mice, $n = 4$, NS). Cafeteria feeding for 3 weeks caused an increase in BAT wet weight, in lean mice but not in ob/ob mice (Table 7). Nonetheless, protein and cytochrome oxidase content of BAT increased in ob/ob mice as it did in lean mice (Table 7). That mitochondrial proliferation

occurred in parallel with the increase in metabolic mass in BAT of both genotypes is shown by the lack of change in the specific activity of cytochrome oxidase in BAT homogenates (Table 7). Since the DNA content was not altered by cafeteria feeding in these mice, the concentration of mitochondria in BAT cells of cafeteria-fed mice was greater than in cells from chow-fed mice (Table 7). The effects of cafeteria feeding on BAT growth in lean and ob/ob mice reported by Trayhurn and coworkers (1982) differ from this study. They measured total protein and cytochrome oxidase content and found no changes in either for ob/ob mice, while lean mice displayed increases only in cytochrome oxidase content. However, subscapular BAT was not included in the assays and the possibility exists that BAT depots in different locations respond differently to cafeteria feeding (Tulp et al. 1982).

Plasma T_3 levels were 72% higher in chow fed ob/ob mice compared to their lean controls (Table 7). Higher levels in ob/ob mice have also been noted by other investigators (Mobley, Dubuc 1979; Ohtake et al. 1977). Cafeteria feeding increased T_3 levels in the plasma of both lean and ob/ob mice. The experiments described in Part I.B. showed that long-term treatment with T_4 improved BAT thermogenic functioning in ob/ob mice. Since T_3 is required in a permissive way for the thermogenic action of NA on BAT it was concluded that BAT of

ob/ob mice is refractory to NA because it is primarily refractory to endogenous T_3 and that raising T_3 levels by T_4 treatment allowed for a more normal thermogenic response to NA. Likewise, the increased T_3 levels in plasma of ob/ob mice which occurred with cafeteria feeding, despite the already high T_3 levels, may have been sufficient to overcome BAT resistance to endogenous T_3 and account for the activation of BAT thermogenesis in cafeteria-fed ob/ob mice. A similar explanation may in part account for the increased thermogenic responsiveness of BAT to NA in cold-acclimated ob/ob mice (Part I.A). Other experiments have shown that serum T_3 levels are elevated in lean mice after 2 weeks at 8°C (serum T_3, ng/dl: 73.2 ± 4.1 at 28°C, 112.5 ± 7.7 at 8°C, n=10 $p < 0.001$). It is probable that prolonged cold exposure also increases circulating T_3 levels in ob/ob mice because activation of the pituitary-thyroid axis is normal during acute cold exposure (Ohtake et al. 1977).

Conclusions

Activation of BAT thermogenesis by cafeteria feeding occurs normally in ob/ob mice (increase in GDP binding). In contrast, DIT in BAT is defective in hyperphagic ob/ob mice fed a regular chow diet (Part I.B.3). This implies that both the nutrient composition and the energy content of the cafeteria diet are involved in stimulation of BAT thermogenesis. The results of this experiment suggest

that DIT is activated in ob/ob mice because blood T_3 levels are raised sufficiently with cafeteria feeding to overcome BAT refractoriness to endogenous T_3 thereby permitting a more normal thermogenic response to NA.

PART II: BROWN ADIPOSE TISSUE OF MICE WITH CHEMICALLY-INDUCED HYPOTHALAMIC OBESITY

GENERAL OBJECTIVE:

To find out whether BAT thermogenic function might be defective in a different, non-genetic animal model of obesity.

SPECIFIC OBJECTIVES:

To find out whether BAT thermogenic function is defective in goldthioglucose (GTG)-obese mice in the static phase of their obesity, when these animals resemble the ob/ob mouse both in the extent of their obesity and in most metabolic changes (hyperglycemia, hyperinsulinemia, insulin resistance).

To find out whether BAT thermogenic function is defective in the GTG-obese mouse in the dynamic phase of its obesity.

PART IIA: BROWN ADIPOSE TISSUE OF GOLDTHIOGLUCOSE (GTG)-OBESE MICE DURING THE STATIC PHASE OF OBESITY: RESPONSE TO DIET AND COLD

Objective:

The primary objective of these experiments was to determine whether a defect in BAT thermogenesis is present in the goldthioglucose (GTG)-obese mouse during the static phase of obesity. A secondary objective was to find out to what extent the abnormal function and ultrastructure of mitochondria isolated from BAT of ob/ob mice may be due to the massive infiltration of the tissues with triglycerides; BAT of GTG-obese mice also accumulates a large amount of triglycerides.

Method:

Lean homozygous mice (+/+) were injected intraperitoneally with either saline or GTG and maintained at 28°C for 10-13 weeks (Methods J). Mice were weighed at frequent intervals to determine the dose-efficacy and to follow the developmental pattern of obesity. In the first experiment GTG-obese and control mice were fed either a cafeteria or chow diet for 11-13 weeks starting immediately after the injection of GTG. Some of the chow-fed mice from each group as well as a number of cafeteria-fed GTG mice were placed in separate cages and exposed to 4°C for 15 1/2 hours before sacrifice. In the second experiment, some of

the chow-fed lean and GTG-obese mice were transferred to 8°C for 2 weeks starting 10 weeks after injection of GTG. Mice from both experiments were killed by cervical dislocation and naso-anal length measured. Interscapular plus subscapular BAT and in some mice parametrial white adipose tissue were removed and weighed. Aliquots were taken from BAT homogenates made to a volume of 5 ml for saline-treated mice and 7 ml for GTG-treated mice for assay of protein, cytochrome oxidase, and DNA (Methods C, D and E). Homogenates from 3-4 similarly treated mice were pooled and mitochondria isolated as described before (Methods A). Binding of GDP by isolated mitochondria and gel electrophoresis of mitochondrial membranes were performed exactly as stated in Methods section B and F. Samples for electron microscopy were prepared from BAT mitochondria of chow-fed lean and GTG-obese mice acclimated to 28°C (Methods G.1). Oxygen uptake was measured in warm- and cold-acclimated mice using a modified Thermox oxygen analyzer (Methods I).

Results and Discussion

1. Brown adipose tissue of warm-acclimated GTG-obese mice

After 11-13 weeks at 28°C, chow-fed GTG injected mice had more than doubled their body weight and gained 6 times as much weight as did the saline-treated controls (Table 8 and 9). A typical weight curve is shown in figure 21 using the means of weights recorded weekly for chow-fed lean and

TABLE 8

EFFECT OF COLD EXPOSURE AND/OR CAFETERIA FEEDING ON LEAN AND GTG-OBESE MICE IN THE STATIC PHASE OF OBESITY

Type of mouse	LEAN			GTG-OBESE			
Diet	Chow	Chow	Cafe	Chow	Chow	Cafe	Cafe
Temperature of Exposure	28°C	4°C	28°C	28°C	4°C	28°C	4°C
Number of animals	(16)	(4)	(12)	(36)	(20)	(28)	(14)
Body Weight, g							
initial	17.1±0.3		18.1± 0.3	17.9±0.4		17.6±0.2	
final	22.0±0.4		30.4± 1.4[c]	46.9±0.6[a]		49.8±0.6[a,c]	
change	4.9±0.3		12.3± 1.2[c]	29.0±0.5[a]		32.2±0.6[a,c]	
Body Length, mm	89.1±0.8		97.9± 0.7[c]	99.8±0.3[a]		99.8±0.4	
Brown Adipose Tissue							
wet weight, mg	232 ±14	221 ±18	509 ±44 [c]	1076 ±37 [a]	776 ±30 [a,b]	1215 ±40 [a,c]	914 ± 56 [b,c]
protein, mg	9.5± 0.7	-	15.3± 0.8[c]	18.9± 0.7[a]	21.5± 0.5	22.6± 1.1[a,c]	24.1± 2.2
DNA, µg	450 ±39	-	623 ±50	932 ±39 [a]	860 ±44	1108 ±53 [a]	1018 ±128
mg protein·mg DNA^{-1}	21.8± 1.1	-	26.1± 2.4	20.9± 0.7[a]	26.1± 1.3[b]	20.8± 0.7[a]	27.7± 3.7[b]
cytochrome oxidase							
µg atoms O_2·min^{-1}	61.9± 4.5	-	99.6± 6.5[c]	113.7± 4.3[a]	126.7± 4.9	139.1± 5.4[a,c]	126.2± 8.7
µg atoms O_2·min^{-1} ·mg protein^{-1}	6.7± 0.3	-	6.6± 0.3	6.2± 0.3	5.9± 0.2	6.1± 0.2	5.6± 0.5
µg atoms O_2·min^{-1} ·mg DNA^{-1}	146.0±10.3	-	167.1±13.5	126.7± 5.5	154.2± 9.8[b]	126.6± 5.9[a]	147.2± 23.4

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

[a]Significant effect of GTG, comparing mice treated in the same way.

[b]Significant effect of cold exposure, comparing the same type of mouse.

[c]Significant effect of cafeteria feeding, comparing the same type of mouse.

TABLE 9

EFFECT OF COLD ACCLIMATION (14 DAYS, 8°C) ON LEAN AND GTG-OBESE MICE IN THE STATIC PHASE OF OBESITY

Type of Mouse	Lean		GTG-Obese	
Diet	Chow	Chow	Chow	Chow
Temperature of Acclimation	28°C	8°C	28°C	8°C
Number of Animals	(20)	(19)	(19)	(17)
Body Weight, g				
initial	18.5 ± 0.3	17.7 ± 0.3	18.4 ± 0.2[a]	18.3 ± 0.2[a]
10 weeks	22.4 ± 0.4	21.8 ± 0.4	44.2 ± 0.9[a]	42.6 ± 0.9[a,b]
12 weeks	22.2 ± 0.4	23.6 ± 0.3	46.6 ± 1.1[a]	36.1 ± 0.7[a,b]
change 0-10 weeks	+3.9 ± 0.3	+4.1 ± 0.3	+25.8 ± 0.8[a]	+24.3 ± 0.8[a]
change 10-12 weeks	-0.1 ± 0.3	+1.8 ± 0.3[b]	+2.5 ± 0.6[a]	-6.5 ± 0.6[a,b]
Body Length, mm	92.5 ± 0.6	95.3 ± 0.6[b]	101.9 ± 0.4[a]	103.1 ± 0.6[a]
Gonadal Fat, mg	336 ± 27	163 ± 11	4063 ± 156	1689 ± 115 [a,b]
% Gonadal Fat	1.5 ± 0.1	0.7 ± 0.4[b]	8.6 0.2[a]	4.6 ± 0.3[a,b]
Brown Adipose Tissue				
wet weight, mg	183 ± 7	357 ± 14 [b]	806 ± 53 [a]	647 ± 21 [a,b]
protein, mg	8.7 ± 0.3	21.6 ± 0.6[b]	18.0 ± 0.9[a]	31.9 ± 1.0[a,b]
DNA, µg	445.7 ± 23.1	892.9 ± 52.9[b]	693.9 ± 47.3[a]	916.5 ± 0.7[a,b]
mg protein·mg DNA^{-1}	20.5 ± 1.2	26.3 ± 1.9[b]	27.7 ± 2.1[a]	37.7 ± 2.8[a,b]
cytochrome oxidase				
µg atoms O_2·min^{-1}	70.4 ± 2.4	134.6 ± 6.1[b]	94.1 ± 6.1[a]	203.5 ± 15.7[a,b]
µg atoms O_2·min^{-1} ·mg protein^{-1}	8.3 ± 0.5	6.2 ± 0.3[b]	5.3 ± 0.3[a]	6.0 ± 0.3
µg atoms O_2·min^{-1} ·mg DNA^{-1}	165.6 ± 10.2	155.0 ± 11.6	149.1 ± 12.5	23[illegible]4 ± 22.2[a,b]

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

[a]Significant effect of GTG comparing mice treated in the same way.

[b]Significant effect of cold-acclimation, comparing the same type of mouse.

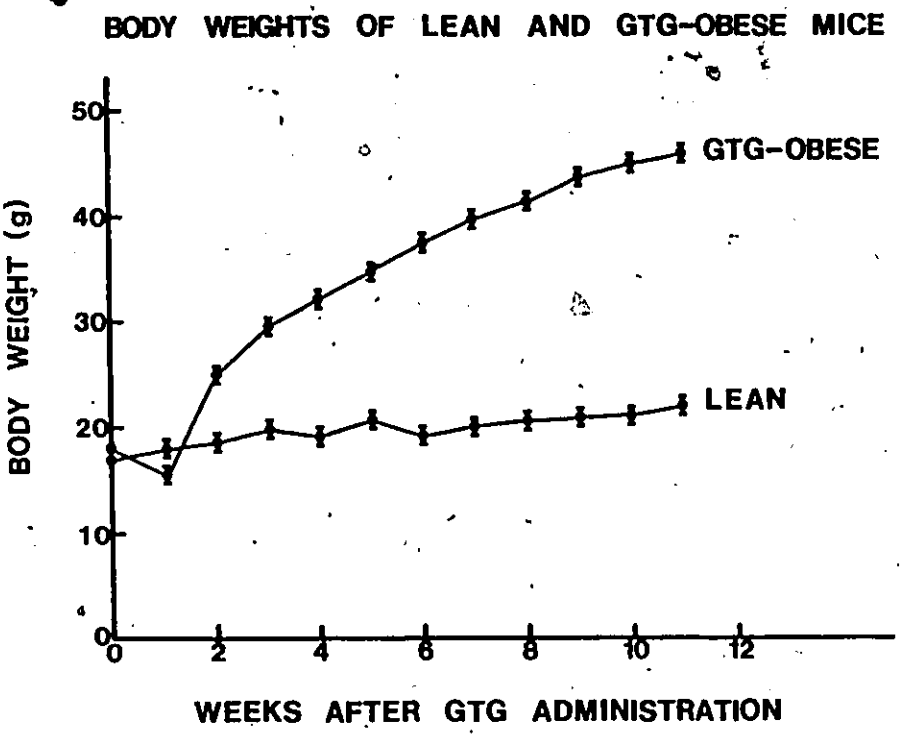

FIGURE 21. Weight curve of GTG-obese mice and lean controls.

Values are the means weights ± SE for 56 GTG-obese mice and 20 lean mice at various times after administration of GTG (0.7 g/kg) or saline.

GTG-obese mice from the first experiment. There was a transient drop in body weight during the first week after administration of GTG, followed by a rapid rise as mice entered the dynamic phase of obesity. At 3 weeks post injection, GTG-treated mice had gained 5 times more weight than their lean controls (lean mice, 2.43 ± 0.27 g per 3 weeks n = 20; GTG-obese mice, 12.25 ± 0.36 g per 3 weeks n = 56). Thereafter, a more gradual rate of gain was observed in these animals such that by 11-12 weeks GTG-obese mice had reached the static phase of the obesity gaining weight at the same rate as lean mice (lean mice, 0.11 ± 0.22 g per week n = 20; GTG-obese mice, 0.39 ± 0.25 g per week n = 56, NS).

Although GTG-treated mice were markedly obese as judged by the substantial increase in gonadal fat and proportional weight of gonadal fat pads, a measure of the percent total body fat (Rogers, Webb 1980), these animals also grew more than lean mice as shown by their increased naso-anal length (Table 8 and 9). Increased linear growth and greater bone mass in GTG-obese mice have been observed by others (Powley, Plocher 1980; DeLeeuw et al. 1981).

The wet weight of BAT from GTG-obese mice was greatly increased by the massive accumulation of lipid. However, there were also increases in cellularity and metabolic and mitochondrial mass as reflected by the larger amounts of

DNA, protein and cytochrome oxidase in the tissue (Table 8 and 9). The similar cytochrome oxidase activity per unit DNA in lean and GTG-obese mice indicates that proliferation of mitochondria occurred in parallel with the increased cellularity of the tissue. Calculations of protein per unit DNA suggests that in one experiment (Table 8) the increase in metabolic mass was achieved entirely by hyperplasia, while in the other experiment (Table 9) a slight cellular hypertrophy also occurred.

The reason for the increased growth of BAT in GTG-obese mice is not clear. It is possible that growth is secondary to the hyperphagia. Genetically obese (ob/ob) mice are also hyperphagic and chow fed ob/ob mice of comparable weight to the GTG-obese mice have a similar amount of DNA, cytochrome oxidase and protein in their BAT (see Tables 1, 4 and 7). Since GTG-obese mice also exhibited linear growth, it is conceivable that the tissue growth, which is occurring in concert with overall body growth, is mediated by growth hormone.

Binding of GDP by BAT mitochondria was normal in chow-fed GTG-obese mice in the static phase of obesity when maintained at 28°C (Figure 22 and 23). The relative amount of polypeptides with molecular weights near 32K was also normal (Legend to Pigure 22 and 23). Representative electron micrographs showed that the ultrastructure of mitochondria

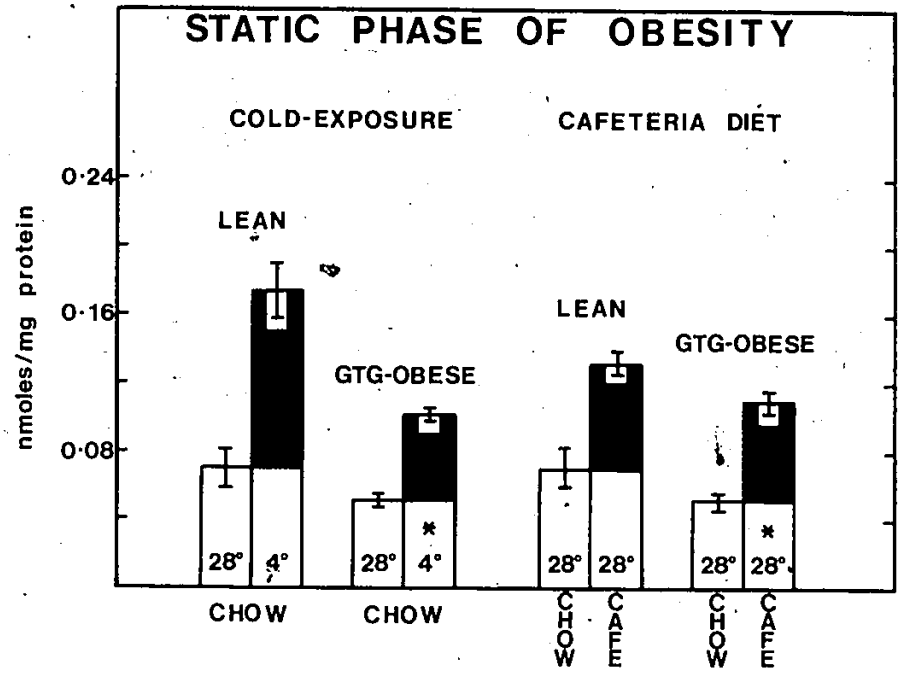

FIGURE 22. Effect of cold exposure or cafeteria feeding on GDP binding by BAT mitochondria of lean and GTG-obese mice in the static phase of obesity.

Bars are the means ± SE for 4 (lean, chow or cafeteria-fed at 28°C), 2 (lean, chow-fed, 4°C), 9 (GTG-obese, chow-fed or cafeteria-fed at 28°C), or 5 (GTG-obese, chow or cafeteria-fed 4°C) mitochondrial preparations. Significant effect of cold exposure or cafeteria feeding are indicated by the black portions of the bars. Significant differences between similarly treated lean and GTG-obese mice are indicated by asterisks. Value of mitochondrial GDP binding of cafeteria-fed GTG-obese mice at 4°C is 0.157 ± 0.011 nmoles/mg protein ($p < 0.005$ versus cafeteria-fed GTG-obese at 28°C; $p < 0.005$ versus chow-fed GTG-obese at 4°C).

continued following page

continued Legend of Figure 22

Polypeptides with Molecular Weights 29,800-34,900			
Lean.		GTG-Obese	
Chow	Cafeteria	Chow	Cafeteria
10.15 ± 1.05	11.30 ± 0.20	9.70 ± 0.50	11.40 ± 1.0

Values for the proportion of polypeptides are means ± SE for 2 mitochondrial membrane preparations (static phase) there are no significant differences.

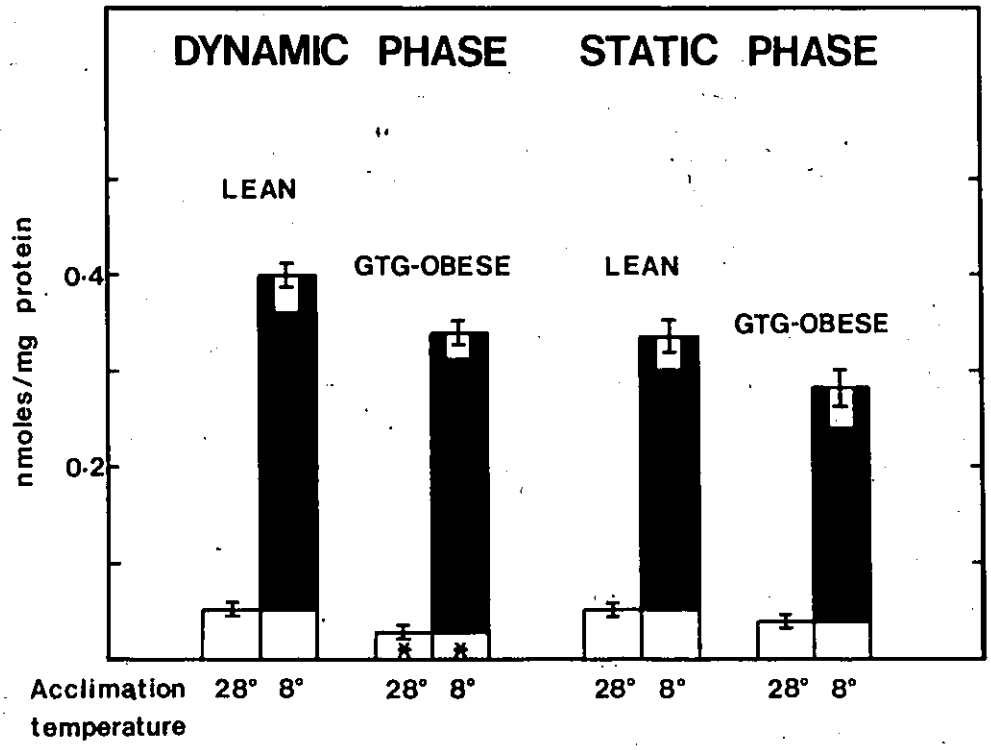

FIGURE 23. Effect of cold acclimation on GDP binding by BAT mitochondria of lean and GTG-obese mice in dynamic or static phases of obesity.

Bars represent the means ± SE for 4 (dynamic phase) or 5 (static phase) mitochondrial preparation. A significant effect of cold acclimation is indicated by the black portion of the bars. Significant differences between similarly treated lean and GTG-obese mice are indicated by asterisks.

Polypeptides with Molecular Weights 29,800-34,900

Lean		GTG-Obese	
28°C	8°C	28°C	8°C
9.12 ± 0.02%	14.18 ± 0.04%[a]	9.04 ± 0.25%	14.74 ± 0.43%[a]

Values for the proportion of polypeptides are means ± SE for 2 mitochondrial membrane preparations (static phase).
[a]Significant effect of cold acclimation.
There are no significant differences between similarly treated lean and GTG-obese mice.

isolated from GTG mice was similar to lean mice (Figure 24 and 25). These results are to be contrasted with the low GDP binding, and abnormal mitochondrial ultrastructure of similarly treated ob/ob mice (see Figure 1, 3 and 4).

Resting metabolic rates of GTG-obese mice acclimated to 28°C were more than twice those of lean mice (Figure 26), presumably due to their larger overall body size. Thermogenic response to NA was also greater in GTG-obese mice and this can be correlated with their increased amount of BAT.

2. Effect of cold exposure on brown adipose tissue of warm acclimated GTG-obese mice

Exposure of chow-fed mice to acute cold induced an increase in GDP binding to isolated BAT mitochondria from both lean and GTG-obese mice, although the level of binding attained by GTG-obese mice was less than their lean controls (Figure 22). Accordingly, unlike ob/ob mice which fail to increase GDP binding and die of hypothermia within 3-4 hours at 4°C, GTG-obese mice in the static phase of their obesity are able to activate BAT thermogenesis during acute exposure to cold. A large decrease in BAT wet weight of GTG-obese mice was also observed after 15 1/2 hours at 4°C (Table 8). Presumably this is due to a mobilization of lipid since DNA and cytochrome oxidase contents did not change and protein content was increased only slightly

FIGURE 24. Electron micrograph of brown adipose tissue mitochondria isolated from warm-acclimated (28°C) lean (+/+) mice (x 16 000).

FIGURE 25. Electron micrograph of brown adipose tissue mitochondria isolated from warm-acclimated (28°C) GTG-obese mice during the static phase of obesity (x 16 000).

Fig. 24

Fig. 25

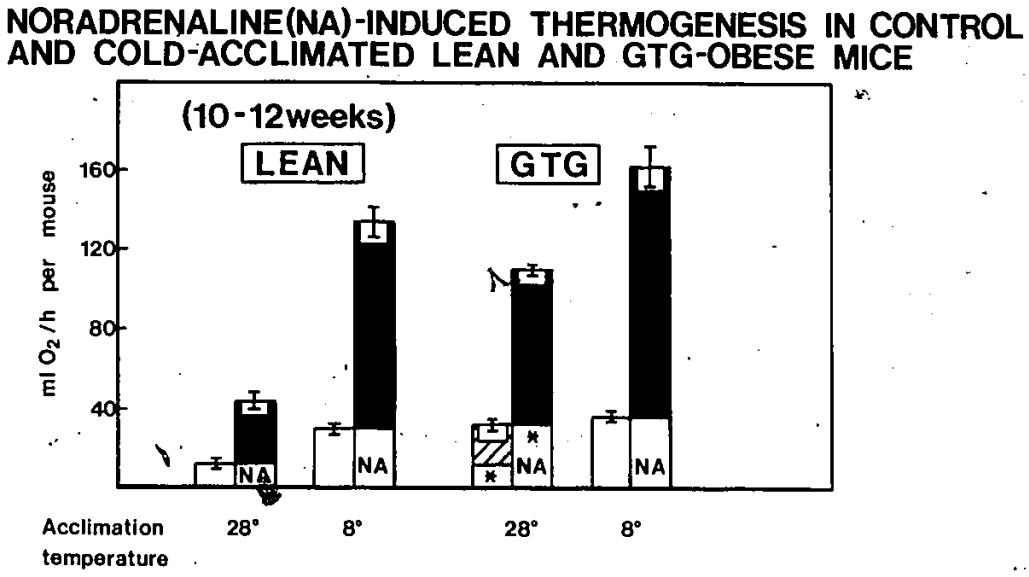

FIGURE 26. Noradrenaline (NA)-induced thermogenesis in warm-acclimated (28°C) and cold-acclimated (8°C) lean and GTG-obese mice.

Bars represent resting oxygen consumption of pentobarbital-anaesthetized mice and maximum rate after injection of noradrenaline. Significant effects of noradrenaline are indicated by the black portions of the bars. Significant differences between similarly treated lean and GTG-obese mice are indicated by asterisks. Acclimation to cold (8°C for 2 weeks) significantly increased the response to noradrenaline in both lean and GTG-obese mice. Values are means ± SE for 5 (acclimated to 28°C) or 4 (acclimated to 8°C) mice with body weights (g): lean at 28°C, 21.1 ± 0.7; lean at 8°C, 23.3 ± 0.2; GTG-obese at 28°C, 44.9 ± 1.3; GTG-obese at 8°C, 32.9 ± 1.9.

(Table 8). In addition, a slight cellular hypertrophy appears to have occurred as judged by the increased amount of protein and cytochrome oxidase per unit DNA (protein, cytochrome oxidase and DNA were not determined in BAT of lean mice at 4°C).

3. Effect of cafeteria feeding on brown adipose tissue of warm-acclimated GTG mice

Consuming a cafeteria diet for 3 months resulted in more than a twofold increase in body weight gain for lean mice and a small but significant increase for GTG-obese mice (Table 8). Skeletal growth, as determined by naso-anal length, was also greater in cafeteria-fed lean mice compared to their chow-fed controls (Table 8).

The stock diet which was used in all experiments has been formulated for rats (Purina rat chow 5012) and has a different nutrient composition from that of mouse chow. It is possible that in the quantity consumed by mice living at 28°C the rat chow diet does not provide sufficient nutrient(s) for maximal body growth. If this is indeed the case, then skeletal growth may have occurred because the cafeteria diet supplemented a low level of nutrient(s) present in the rat chow diet.

Activation of BAT thermogenesis as indicated by increased GDP binding, occurred in GTG-obese mice with cafeteria feeding but to a lesser extent than it did in

lean mice (Figure 22). Prolonged overfeeding did not however promote an increase in the proportion of polypeptides with molecular weights near 32K (Legend to Figure 22). The increase in GDP binding therefore results from an unmasking of binding sites already present. Cafeteria feeding promoted increases in BAT wet weight and in total protein, DNA, and cytochrome oxidase content for both lean and GTG-obese mice (Table 8). That the increase in mitochondrial mass parallelled proliferation of BAT cells is shown by an unchanged cytochrome oxidase activity per unit DNA (Table 8).

4. Effect of cold exposure on cafeteria-fed GTG-obese mice

A significant loss in BAT wet weight occurred during brief cold exposure of cafeteria fed GTG-obese mice which can be attributed to lipid mobilization because there were no alterations in DNA, protein or cytochrome oxidase contents (Table 8). Cafeteria feeding potentiated the thermogenic response of BAT to acute cold as shown by the higher mitochondrial binding of GDP in BAT of cafeteria-fed GTG-obese mice compared to that of their chow-fed controls (Legend to Figure 22). Since the level of GDP binding was the summation of the separate effects of cold exposure and cafeteria feeding, there would appear to be no replacement of NST by DIT. This suggests that there is no central convergence of diet- and cold-induced signals, but rather

that they act through separate and independent neural pathways to stimulate BAT thermogenesis.

5. Effect of cold acclimation on brown adipose tissue of GTG-obese mice

Exposure of GTG-obese mice to 8°C for 2 weeks resulted in a marked loss of body weight and a decrease in the weight of parametrial white adipose tissue (Table 9). That acclimation to cold serves to substantially diminish their obesity is indicated by the almost twofold decrease in percent total body fat, as estimated by the proportional weight of gonadal fat pads. Although cold-acclimated lean mice weighed the same as their warm-acclimated controls, they were in fact leaner animals since the percent total body fat was less than one-half that of mice kept at 28°C (Table 9). The naso-anal lengths of lean mice were significantly increased by prolonged cold exposure (Table 9). If as previously suggested, mice do not obtain a sufficient amount of nutrients from the rat chow diet for maximal growth at 28°C then the larger intake of food which occurs in mice living at low ambient temperatures (Lin et al. 1980; Coleman 1982; Thurlby, Trayhurn 1979) may explain the increase in skeletal growth of cold-acclimated lean mice.

The large increase in GDP binding by isolated mitochondria showed that cold acclimation stimulated BAT thermogenesis to a similar degree in both lean and GTG-obese mice (Figure 23) and to a much greater extent than either acute exposure to

cold or cafeteria feeding for 3 months (compare Figure 22 and 23). The elevated level of binding was associated with an increase in the relative amount of mitochondrial polypeptides in the molecular weight range of 29,800 - 34,900 (Legend to Figure 23) for both types of animals. In this respect cold acclimation of mice differs from cafeteria feeding in which the increase in GDP binding (Figure 22) was not associated with an increase in the 32K polypeptide (Legend to Figure 22). Despite a significantly smaller BAT wet weight, growth of the tissue, as judged by the increase in protein, DNA and cytochrome oxidase content, occurred normally in GTG-obese mice during acclimation to cold (Table 9). As the increase in protein was greater than the increase in DNA for both lean and GTG-obese mice, a slight cellular hypertrophy of metabolic mass also accompanied the hyperplasia. Cytochrome oxidase activity per unit DNA did not however change with cold acclimation suggesting parallel proliferation of mitochondria and brown adipocytes (Table 9).

The response to NA by an increase in metabolic rate was significantly enhanced by cold acclimation in both lean and GTG-obese mice compared to their controls living at 28°C (Figure 26). The substantial loss of body fat incurred by GTG-obese mice during acclimation to 8°C, in association with the increased growth of BAT and thermogenic response to NA, is illustrative of the tremendous ability of BAT for dissipation of energy as heat.

Conclusions

Both acute cold exposure and prolonged feeding of a cafeteria diet can activate BAT thermogenesis in GTG-obese mice in the static phase of their obesity (as shown by the increase in BAT mitochondrial binding), although to a somewhat lesser extent than in lean mice. This can probably be ascribed to a reduced stimulation of central sympathetic outflow as a consequence of the GTG-induced hypothalamic lesion because the thermogenic response of these mice to exogenous NA is normal. Since in GTG-obese mice there appears to be no inherent defect in activation of BAT thermogenesis or in mitochondrial ultrastructure despite an elevated lipid content in the cells, it may be concluded that the abnormal ultrastructure and functioning of BAT mitochondria of ob/ob mice are not secondary to the massive accumulation of lipid in the tissue. Furthermore, since hyperinsulinemia and insulin resistance are characteristic of both ob/ob mice (Bray, York 1979) and GTG-obese mice during the static phase of their obesity (Le Marchand et al. 1978) they can also be eliminated as contributory factors to the impaired BAT functioning of ob/ob mice. Control of BAT growth (increase in protein, cytochrome oxidase, DNA) and changes in mitochondrial polypeptides with molecular weights near 32K are normal in GTG-obese mice during the static phase of obesity.

PART IIB: BROWN ADIPOSE TISSUE OF GOLDTHIOGLUCOSE (GTG)-OBESE MICE DURING THE DYNAMIC PHASE OF OBESITY: RESPONSE TO DIET AND COLD

Objective:

The purpose of these experiments was to find out whether control of BAT thermogenesis and growth is defective in goldthioglucose (GTG)-obese mice during the dynamic phase of obesity.

Method:

Lean homozygous (+/+) mice maintained at 28°C received a single intraperitoneal injection of either saline or GTG (Methods J). Three separate experiments were done in which BAT composition and mitochondrial function were assessed in response to acute cold-exposure, cafeteria feeding, and cold-acclimation. In the first experiment, 3 weeks after injection of GTG, mice were placed in separate cages and either kept at 28°C or exposed to 8°C for 15 1/2 hours. In the second experiment, some mice were fed a cafeteria diet for 3 weeks beginning immediately after administration of GTG. In the third experiment, mice were acclimated to 8°C between 2 weeks and 4 weeks post injection. Mice were not transferred to the cold until 2 weeks after injection in order to allow sufficient time for recovery from the acute toxic effects of GTG and to permit selection of mice with

obesity inducing lesions. After sacrifice, gonadal fat pad weights, interscapular plus subscapular BAT weights and body lengths (Exp. 1 and 2) were obtained. BAT was homogenized and after samples were removed for protein, cytochrome oxidase and DNA estimations homogenates from similarly treated animals were combined and mitochondria isolated for GDP binding assay (Methods C, D and E). Metabolic rates were taken of anaesthetized chow- and cafeteria-fed mice before and after injection of NA using a modified Thermox oxygen analyzer (Methods I).

Results and Discussion

1. Brown adipose tissue of warm-acclimated GTG-obese mice

That GTG induced obesity in chow-fed mice at 28°C within 3-4 weeks after injection was clearly evident from the magnitude of weight gain, gonadal fat wet weight, and percent total body fat (proportional weight of gonadal fat; Rogers, Webb 1980) (Tables 10, 11 and 12). The growth promoting effects of GTG on body length and BAT size were also apparent at this time (Tables 10, 11 and 12). Increase in protein content of BAT occurred in each experiment, although changes in cytochrome oxidase and DNA content were not as consistently observed. Thus, the increases in mitochondrial mass and the hyperplasia seen at 3 months post injection would appear to develop more slowly than hypertrophy of the tissue. Binding of GDP to BAT mitochondria

TABLE 10

EFFECT OF COLD EXPOSURE ON LEAN AND GTG-OBESE MICE IN THE DYNAMIC PHASE OF OBESITY

Type of Mouse	Lean		GTG-Obese	
Diet	Chow	Chow	Chow	Chow
Temperature of Exposure	28°C	8°C	28°C	8°C
Number of Animals	(20)	(19)	(19)	(17)
Body Weight, g				
initial	17.9 ± 0.4	18.1 ± 0.5	18.9 ± 0.3	18.6 ± 0.4
final	19.2 ± 0.3	19.1 ± 0.3	26.9 ± 0.6[a]	25.6 ± 0.5[a]
change	+1.4 ± 0.3	+0.9 ± 0.2	+8.1 ± 0.5[a]	+7.0 ± 0.5[a]
Body Length, mm	91.9 ± 0.4		94.9 ± 0.3[a]	
Gonadal Fat, mg	225 ± 21	251 ± 24	892 ± 62[a]	802 ± 45[a]
% Gonadal Fat	1.2 ± 0.1	1.3 ± 0.1	3.3 ± 0.2[a]	3.1 ± 0.1[a]
Brown Adipose Tissue				
wet weight, mg	165 ± 5	126 ± 5[b]	412 ± 15[a]	233 ± 9[a,b]
protein, mg	8.0 ± 0.3	9.0 ± 0.4	11.3 ± 0.6[a]	12.5 ± 0.7[a]

Values are the means ± SE for the numbers of animals given in parenthesis.

[a]Significant effect of GTG, comparing mice treated in the same way.

[b]Significant effect of cold-exposure, comparing the same type of mouse.

TABLE 11

EFFECT OF CAFETERIA FEEDING ON LEAN AND GTG-OBESE MICE IN THE DYNAMIC PHASE OF OBESITY

Type of Mouse	Lean		GTG-Obese	
Diet	Chow	Cafeteria	Chow	Cafeteria
Temperature of Acclimation	28°C	28°C	28°C	28°C
Number of Animals	(20)	(20)	(20)	(20)
Body Weights, g				
initial	18.3 ± 0.3	17.4 ± 0.3[b]	18.2 ± 0.2	18.1 ± 0.3
final	19.8 ± 0.4	21.3 ± 0.3[b]	29.1 ± 0.5[a]	34.7 ± 0.8[a,b]
change	+1.5 ± 0.3	+3.9 ± 0.3[b]	+10.9 ± 0.4[a]	+16.5 ± 0.8[a,b]
Body length, mm	93.1 ± 0.6	95.1 ± 0.5[b]	96.4 ± 0.3[a]	98.3 ± 0.5[a,b]
Gonadal Fat, mg	222 ± 23	352 ± 20[b]	986 ± 42[a]	1778 ± 86[a,b]
% Gonadal Fat	1.2 ± 0.1	1.64 ± 0.1[b]	3.5 ± 0.2[a]	5.1 ± 0.2[a,b]
Brown Adipose Tissue				
wet weight, mg	192 ± 8	196 ± 8	638 ± 19[a]	906 ± 29[a,b]
protein, mg	7.3 ± 0.3	9.0 ± 0.4[b]	12.6 ± 0.6[a]	17.1 ± 0.6[a,b]
DNA, µg	531.7 ± 23.7	555.9 ± 19.3	643.6 ± 28.5[a]	733.2 ± 33.2[a,b]
mg protein·mg DNA^{-1}	14.3 ± 1.0	16.5 ± 0.9	20.2 ± 1.2[a]	24.5 ± 1.8[a]
cytochrome oxidase				
µg atoms O_2·min^{-1}	53.2 ± 2.0	46.3 ± 2.2	41.5 ± 4.4	70.1 ± 4.2[a,b]
µg atoms O_2·min^{-1} mg protein^{-1}	7.6 ± 0.5	5.1 ± 0.3[b]	3.2 ± 0.2[a]	4.1 ± 0.2[a,b]
µg atoms O_2·min^{-1} mg DNA^{-1}	104.9 ± 7.1	85.8 ± 6.0[b]	65.6 ± 7.2[a]	100.9 ± 9.3[b]

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

[a]Significant effect of GTG, comparing mice treated in the same way.

[b]Significant effect of cafeteria feeding, comparing the same type of mouse.

TABLE 12

EFFECT OF COLD ACCLIMATION ON LEAN AND GTG-OBESE MICE IN THE DYNAMIC PHASE OF OBESITY

Type of Mouse	Lean		GTG-Obese	
Diet	Chow	Chow	Chow	Chow
Temperature of Acclimation	28°C	8°C	28°C	8°C
Number of Animals	(15)	(13)	(16)	(19)
Body Weight, g				
initial	18.5 ± 0.3	18.2 ± 0.5	18.7 ± 0.3[a]	19.6 ± 0.3[a]
2 weeks	20.0 ± 0.4	19.2 ± 0.3	23.3 ± 0.7[a]	25.8 ± 0.5[a]
4 weeks	20.8 ± 0.4	21.1 ± 0.3	28.4 ± 0.8[a]	29.9 ± 0.7[a]
change 0-2 weeks	+1.5 ± 0.2	+1.1 ± 0.6[b]	+4.6 ± 0.7[a]	+6.0 ± 0.3[a]
change 2-4 weeks	+0.7 ± 0.4	+1.8 ± 0.3[b]	+5.2 ± 0.9[a]	+3.2 ± 0.7[a]
Gonadal Fat, mg	309 ± 36	202 ± 27[b]	1092 ± 66[a]	548 ± 68[a,b]
% Gonadal Fat	1.5 ± 0.2	1.0 ± 0.1[b]	4.0 ± 0.3[a]	1.9 ± 0.2[a,b]
Brown Adipose Tissue				
wet weight, mg	161 ± 7	293 ± 7[b]	381 ± 22[a]	396 ± 17[a]
protein, mg	7.8 ± 0.3	20.8 ± 0.7[b]	10.2 ± 0.3[a]	25.5 ± 0.6[a,b]
DNA, µg	423.9 ± 35.6	634.8 ± 25.8[b]	486.9 ± 37.0	686.7 ± 46.8[b]
mg protein·mg DNA^{-1}	19.9 ± 1.6	32.4 ± 1.7[b]	22.7 ± 1.9	39.4 ± 3.9[b]
cytochrome oxidase				
µg atoms O_2·min^{-1}	70.2 ± 3.2	87.4 ± 6.3[b]	84.1 ± 5.5[a]	114.8 ± 10.4[a,b]
µg atoms O_2·min^{-1} ·mg protein^{-1}	9.2 ± 0.5	4.2 ± 0.3[b]	8.3 ± 0.5	4.5 ± 0.4
µg atoms O_2·min^{-1} ·mg DNA^{-1}	178.1 ± 14.3	142.8 ± 16.5	189.5 ± 19.4	180.2 ± 25.9

Values are the means ± SE for the number of animals given in parenthesis.

[a]Significant effect of GTG, comparing mice treated in the same way.

[b]Significant effect of cold-acclimation, comparing the same type of animal.

was lower in chow-fed GTG-obese mice than in lean mice, indicating a lower state of thermogenic activation in these animals (Figure 27), despite their known hyperphagia (Gray, Liebelt 1961). This differs from GTG-obese mice during the static phase of obesity in which the level of mitochondrial GDP binding was normal. Nonetheless, the thermogenic capacity to respond to injected NA was greatly enhanced in GTG-obese mice during the dynamic phase of obesity as it was in the static phase (Figure 28). As well, their resting metabolic rates were also significantly greater then those of chow fed lean mice.

2. Effect of cold exposure on brown adipose tissue of warm-acclimated GTG-obese mice

Exposure of mice to 8°C for 15 1/2 hours did not alter body weight or body fat content (Table 10) as shown by gonadal fat pad measurements (Rogers, Webb 1980). The cold-induced increase in GDP binding to BAT mitochondria occurred in GTG-obese mice during the dynamic phase of obesity (Figure 27) as it did in the static phase (Figure 22). However, the extent of GDP binding, although large, was less than that of lean mice, indicating a lower activation of BAT thermogenesis. BAT wet weight was reduced in both lean and GTG-obese mice reflecting mobilization of triglyceride stores since the protein content of the tissue remained the same (cytochrome oxidase and DNA content of BAT were not determined in these animals).

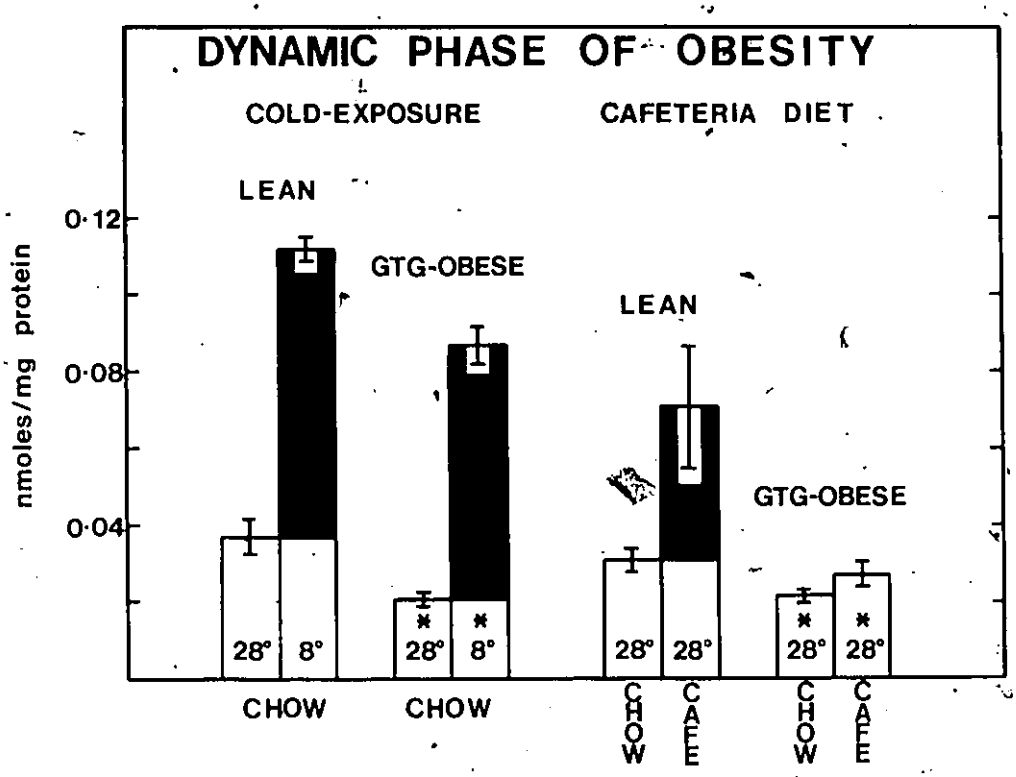

FIGURE 27. Effect of acute cold exposure or cafeteria feeding on GDP binding by BAT mitochondria of lean and GTG-obese mice in the dynamic phase of obesity.

Bars represent the means ± for 5 mitochondrial preparations. Significant effect of cold exposure (8°C for 15 1/2 hours) or cafeteria feeding (3 weeks) are indicated by the black portion of the bars. Significant differences between similarly treated lean and GTG-obese mice are indicated by asterisks.

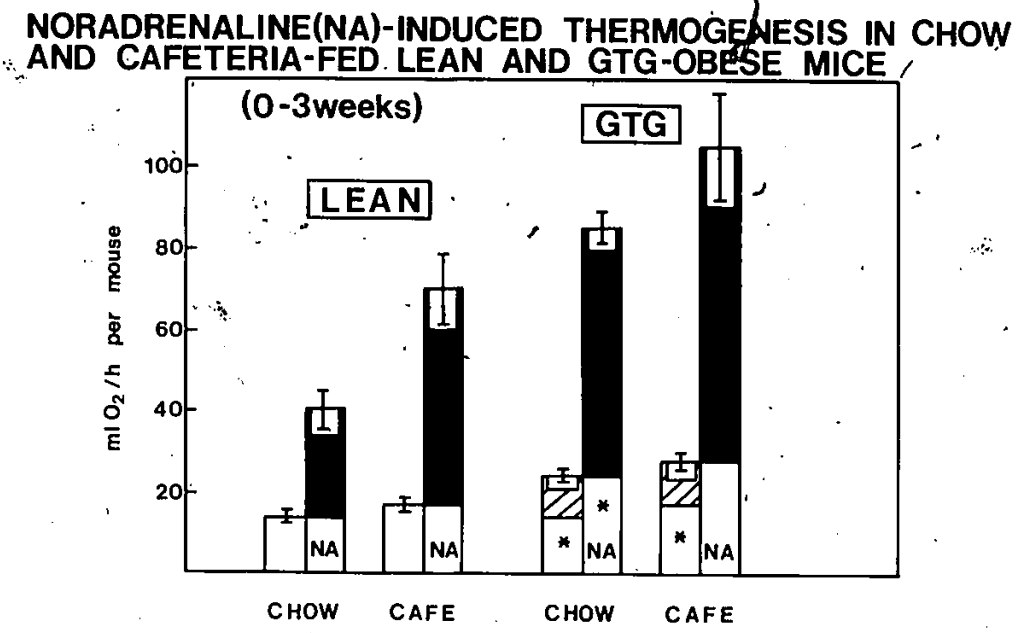

FIGURE 28. Noradrenaline (NA)-induced thermogenesis in chow-fed and cafeteria-fed lean and GTG-obese mice.

Bars represent resting oxygen consumption of pentobarbital-anesthetized mice and maximum rate after injection of noradrenaline. Significant effects of noradrenaline are indicated by the black portion of the bars. Significant differences between similarly treated lean and GTG-obese mice are indicated by asterisks. Cafeteria feeding (3 weeks) significantly increased the response to noradrenaline in lean mice ($p < 0.02$).

3. Effect of cafeteria feeding on brown adipose tissue of warm-acclimated GTG-obese mice

Cafeteria feeding for 3 weeks induced substantial increases in body weight gain, gonadal fat and total body fat in both lean and GTG-obese mice (Table 11). The increases in linear growth observed after 3 months of cafeteria feeding in lean mice were also apparent within 3 weeks in lean mice as well as GTG-obese mice (Table 11).

Activation of BAT thermogenesis by cafeteria feeding occurred in lean mice as judged by an increase in mitochondrial GDP binding (Figure 27). In contrast, GDP binding was not altered by cafeteria feeding in GTG-obese mice, indicating a lack of DIT in BAT. Thus, the thermogenic responses to diet and to cold are dissociated in BAT of GTG-obese mice during the dynamic phase of obesity. In this respect GTG-obese mice differ from genetically obese mice in which the thermogenic response of BAT to a cafeteria diet is normal (Figure 20) but to cold is defective (Figure 1). The effect of cafeteria feeding on growth of BAT was much greater in GTG-treated mice than in saline-treated controls. Thus, in GTG-obese mice increases occurred in BAT wet weight and total protein, cytochrome oxidase and DNA content while in lean mice only total protein content increased (Table 11). Calculation of protein per unit DNA suggests that cafeteria feeding did not alter metabolic cell size of BAT in GTG-obese mice and therefore that growth of the tissue was achieved

solely by hyperplasia. Proliferation of mitochondria did however outstrip growth of BAT mass since cytochrome oxidase activity per unit DNA or per unit protein was larger in cafeteria-fed GTG-obese mice than in chow-fed controls (Table 11).

Cafeteria feeding did not affect the resting metabolic rate of either lean or GTG-obese mice; however, the capacity to respond thermogenically to injected NA was increased in cafeteria-fed lean mice compared to chow-fed controls (Figure 28). Although cafeteria feeding did not enhance the already large response to NA in GTG-obese mice, the increased metabolic rate induced by NA was similar to cafeteria-fed lean mice.

4. Effect of cold acclimation on brown adipose tissue of GTG-obese mice

Acclimation to cold for 2 weeks reduced body fat content in lean and GTG-obese mice, as judged by the lower gonadal fat wet weights and the lower proportional weights of gonadal fat pads (Table 12).

A large increase in BAT mitochondrial GDP binding occurred in both GTG-obese mice and lean mice after 2 weeks at 8°C (Figure 23). Although the level of binding attained by GTG-obese mice was marginally lower than that of lean mice, the absolute increase was similar. This suggests that the slight reduction in thermogenic activity of BAT may

result from an impaired response to diet rather than to cold. Typically, acclimation of lean and GTG-obese mice to cold resulted in an increase in protein, cytochrome oxidase and DNA content of BAT (Table 12). Growth of BAT was achieved by cellular hypertrophy as well as hyperplasia as indicated by the increase in protein concentration relative to DNA. Although mitochondrial mass per cell was similar in cold-acclimated and warm-acclimated mice (cytochrome oxidase activity per mg DNA) the concentration of mitochondria in cells was less in cold-acclimated mice (lower specific activity of cytochrome oxidase in BAT homogenates).

Conclusions

The significant finding of these experiments is that the GTG-obese mouse during the dynamic phase of obesity fails to activate BAT thermogenesis in response to diet. Such a defect could underlie the high metabolic efficiency of this animal (DeLaey et al. 1975; Djazayery et al. 1979) and therefore contribute to the development of its obesity. Since thermogenic mechanisms in BAT of the GTG-obese mouse appear to be almost normal in response to cold and because this animal responds thermogenically to exogenous NA, it is probable that the defect in DIT lies with the central control of BAT thermogenesis rather than in the tissue itself. GTG is known to localize in the hypothalamus causing necrosis of the ventromedial region (VMH) (Marshall et al. 1955;

Bray, York 1979). It is therefore likely that BAT of GTG-obese mice, although inherently functional and with an apparently normal sympathetic innervation, does not respond to diet because a GTG-induced lesion(s) in the VMH, interrupts sympathetic outflow from diet-sensitive centers to BAT. That this occurs without interfering with the reception and transmission of cold-induced signals implies that the neural pathway leading from cold-sensitive receptors in the hypothalamus to sympathetic innervation of BAT must bypass the ventromedial hypothalamic region. These findings are consistent with the observation that cold-exposure but not sucrose feeding increase sympathetic activity in heart of GTG mice (Young, Landsberg 1980).

The improvement in DIT of GTG-obese mice during the static phase of the obesity may have occurred because of a central reorganization of neural pathways enabling other diet-sensitive cells to make connections with the sympathetic centers in the brain stem or because of a regeneration of nerve fibers initially damaged by GTG. As it would appear that sympathetic activity is low in BAT of cafeteria fed GTG-mice during the dynamic phase of obesity, despite normal growth of the tissue, it is further suggested that NA or any other neurotransmitter released from sympathetic nerve endings is probably not a mediator for adaptive growth of BAT during cafeteria feeding.

PART III: BROWN ADIPOSE TISSUE OF RATS WITH SURGICALLY-INDUCED HYPOTHALAMIC OBESITY

OBJECTIVE:

To find out whether defective brown adipose tissue thermogenesis might be associated with obesity in a species other than the mouse.

SPECIFIC OBJECTIVES:

To find out whether BAT thermogenic function is defective in rats with heat or knife cut lesions of the ventromedial region of the hypothalamus.

To find out whether the known sex differences in response of rats to hypothalamic lesions are associated with differences in BAT function.

PART IIIA: BROWN ADIPOSE TISSUE OF VENTROMEDIAL HYPOTHALAMIC (VMH)-LESIONED RATS DURING THE DYNAMIC PHASE OF OBESITY: RESPONSE TO ACUTE COLD EXPOSURE

Objective:

The primary purpose of these experiments was to determine whether cold-induced BAT thermogenesis is defective in mature VMH-lesioned rats in the dynamic phase of their obesity. A secondary objective was to find out if a lesion-induced sex difference exists in the thermogenic functioning of BAT.

Method:

Male and female Holtzman rats at 7-8 weeks of age were kept in single cages and maintained at 22°C for 8-11 days prior to surgery. In two separate experiments, the first with males and the second with females, bilateral radiofrequency heat lesions (55°C) of the VMH were made in 30 rats as described previously (Coscina et al. 1976; Materials A). In addition, 12 rats were sham-operated and together with 12 unoperated rats served as controls. Purina chow was placed on the floor of all cages and changed daily throughout the course of the experiments. One week following surgery, rats were transported from the Clarke Institute of Psychiatry, University of Toronto, to the University of Ottawa where they were housed under similar

conditions at 28°C. Food intake and body weight of all rats were measured regularly. Three weeks postoperatively all rats were briefly anesthetized with ether (1-2 minutes), their nasoanal length determined and the Lee Index calculated (body weight 0.33 ÷ length in mm x 10; Bernardis, Patterson 1968). The Lee Index is considered to be a sensitive indicator of the amount of carcass fat in rats with VMH lesions. A total of 4 experiments (male rats) or 3 experiments (female rats) was done beginning 6 weeks after surgery. For each experiment, 3 VMH-lesioned rats and 3 control rats were sacrificed after 24 hours at 4°C in addition to 3 VMH-lesioned rats and 3 control rats that had remained at 28°C. As there were no significant differences in food intake, body weight gain or the Lee Index between sham-operated and unoperated rats, control subgroups were combined for all analyses.

Rats were killed by decapitation 26-35 days after lesioning. Both male and female VMH-lesioned rats were extremely excitable during handling just prior to sacrifice, despite all attempts to minimize environmental disturbances. Interscapular BAT and gonadal fat were removed, cleaned and weighed. BAT homogenates were prepared and samples taken for protein, cytochrome oxidase and DNA estimations (Methods C, D and E). Homogenates from 3 similarly treated rats were pooled and mitochondria isolated for GDP binding assay (Methods A and B). Brains from all lesioned animals were

removed, placed in 10% formalin solution and sent to the Clarke Institute of Psychiatry for histological assessment of lesion size and locus (Nobrega et al. 1980). Upon histological examination, the bilateral VMH lesions were described as being generally symmetrical and centered anterior to the ventromedial nucleus. The average lesion diameter was 2.5 mm, extending rostrally from the caudal third of the optic chiasm through the ventromedial nucleus. Dorsoventrally, lesions extended from usually 0.5 mm above the base of the hypothalamus up to and at times extending into the ventral thalamus. Mediolaterally, lesions were confined within the fornices. A typical lesion at its maximum diameter is illustrated in Figure 29.

Results and Discussion

1. Brown adipose tissue of VMH-lesioned rats

At thermoneutrality (28°C), VMH-lesioned rats in both experiments exhibited hyperphagia and increased body weight gains (Tables 13 and 14) which is typical of mature rats during the dynamic phase of obesity (Bray, York 1979; Frohman 1980). These animals also became obese, as judged by the substantially larger amounts of gonadal fat and by the higher values obtained for the Lee Index (Tables 13 and 14). A greater propensity for hypothalamic obesity in female rats was indicated by their three-four fold increase in gonadal fat compared to the twofold increase in similarly treated

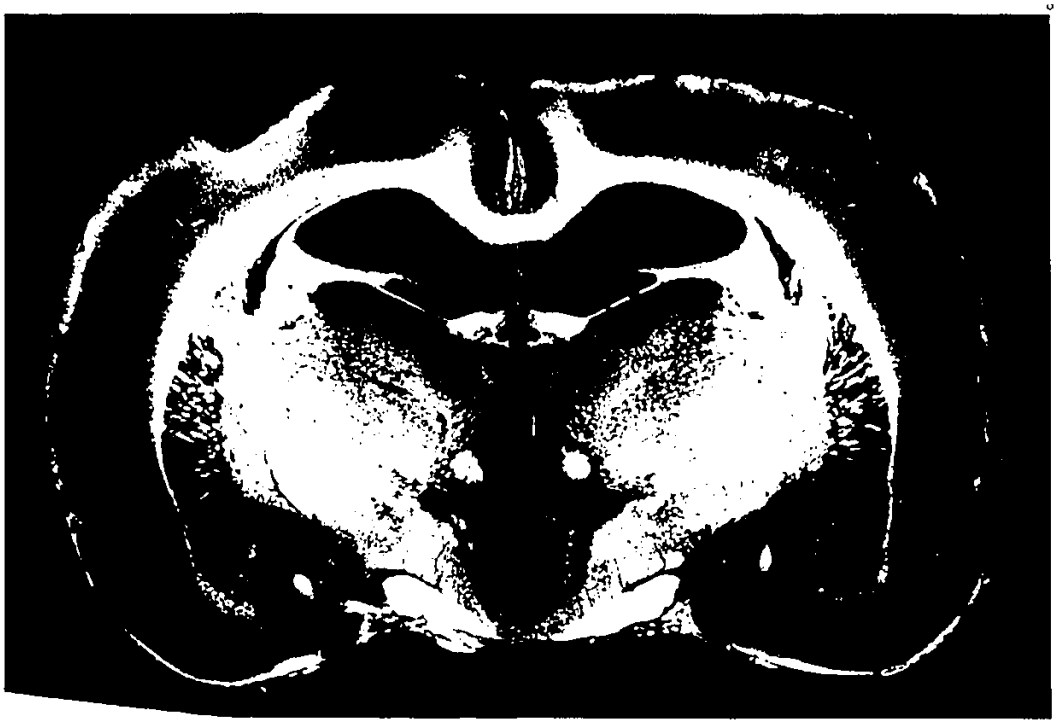

FIGURE 29. Coronal section of the VMH lesion.

TABLE 13

EFFECT OF COLD EXPOSURE ON FEMALE NONLESIONED AND VMH-LESIONED RATS

Temperature of Exposure	Nonlesioned 28°C (9)	Nonlesioned 4°C (9)	VMH-Lesioned 28°C (9)	VMH-Lesioned 4°C (9)
Body Weight, g				
initial	177.6 ± 1.9		179.1 ±2.1	
final	241.4 ± 3.5		319.2 ±4.4 [a]	
change, 26-25 days	+63.9 ± 2.7		+140.2 ±3.5 [a]	
gain, g/day	2.2 ± 0.1		4.9 ±0.2 [a]	
Body Length, mm	192.0 ± 1.0		189.0 ±1.0 [a]	
Lee Index	0.314 ± 0.002		0.346±0.002 [a]	
Gonadal Fat, g	2829 ± 409	2725 ± 464	10868 ± 621 [a]	9685 ± 518 [a]
Food Intake, kcal/day				
day 9	74.4 ± 3		88.0 ±3.8 [a]	
day 21	72.8 ± 2.6		115.6 ±3.5 [a]	
day 28	65.6 ± 3.0		113.6 ±3.7 [a]	
Brown Adipose Tissue				
wet weight, mg	379 ± 29	319 ± 24 [b]	1353 ± 117 [a]	879 ± 54 [a,b]
protein, mg	21.4 ± 1.4	26.1 ± 1.5 [b]	25.4 ± 1.9	47.8 ± 4.3 [a,b]
DNA µg	506.7 ± 52.8	426.9 ± 26.3	406.3 ± 39.5 [a]	596.0 ± 69.4 [a,b]
mg protein·mg DNA^{-1}	44.5 ± 3.7	63.1 ± 5.5 [b]	65.2 ± 6.3 [a]	87.2 ± 13.0
cytochrome oxidase				
µg atoms O_2·min^{-1}	167.5 ± 16.1	212.4 ± 15.9	176.2 ± 17.9	276.5 ± 13.0 [a,b]
µg atoms O_2·min^{-1} ·mg protein^{-1}	7.9 ± 0.6	8.1 ± 0.3	6.9 ± 0.4	6.0 ± 0.4 [a]
µg atoms O_2·min^{-1} ·mg DNA^{-1}	339.4 ± 27.6	512.3 ± 46.2 [b]	449.9 ± 45.7	499.5 ± 51.7

Values are means ± SE for the number of animals given in parenthesis.

[a] Significant effect of the lesion, comparing rats treated in the same way.

[b] Significant effect of cold exposure, comparing the same type of rat.

TABLE 14

EFFECT OF COLD EXPOSURE ON MALE NONLESIONED AND VMH-LESIONED RATS

Temperature of Exposure	Nonlesioned		VMH-Lesioned	
	28°C (12)	4°C (12)	28°C (12)	4°C (12)
Body Weight, g				
initial	268.6 ± 3.6		249.5 ± 2.9[a]	
final	406.9 ± 8.4		475.8 ± 7.6[a]	
change, 26-35 days	+138.3 ± 7.6		+226.3 ± 6.8[a]	
gain, g/day	4.9 ± 0.3		7.8 ± 0.4[a]	
Body Length, mm	232.7 ± 1.1		228.2 ± 1.3[a]	
Lee Index	0.312 ± 0.002		0.333 ± 0.002[a]	
Gonadal Fat mg	4381 ± 297	4286 ± 246	9621 ± 706[a]	9913 ± 582[a]
Food Intake, kcal/day				
day 9	111.2 ± 2.6		133.6 ± 10.4[a]	
day 14	101.2 ± 4.1		130.0 ± 7.1[a]	
day 21	111.6 ± 3.2		161.2 ± 7.2[a]	
Brown Adipose Tissue				
wet weight, mg	449 ± 29	487 ± 38	1831 ± 181[a]	1404 ± 184[a]
protein, mg	18.0 ± 1.2	22.9 ± 2.3	25.3 ± 2.1[a]	43.8 ± 2.3[a,b]
DNA, ug	546.8 ± 40.2	697.2 ± 70.8	477.8 ± 26.2	688.9 ± 62.5[b]
mg protein·mg DNA^{-1}	32.9 ± 1.8	34.2 ± 3.4	53.1 ± 3.5[a]	66.2 ± 4.2[a,b]
cytochrome oxidase				
ug atoms O_2·min^{-1}	193 ± 14.9	157.9 ± 23.2	210.5 ± 18.4	277.8 ± 27.8[b]
ug atoms O_2·min^{-1} ·mg protein^{-1}	10.7 ± 0.5	7.1 ± 0.8[b]	8.3 ± 0.4[a]	6.4 ± 0.6[b]
ug atoms O_2·min^{-1} ·mg DNA^{-1}	358.1 ± 33.4	250.0 ± 38.6[b]	447.4 ± 35.9	424.2 ± 47.1[a]

Values are means ± SE for the number of animals given in parenthesis.

[a]Significant effect of the lesion, comparing rats treated in the same way.

[b]Significant effect of cold exposure, comparing the same type of rat.

Initial weights differ because the nonlesioned rats were weighed 2-3 days after the lesioned animals.

male rats (Tables 13 and 14). In spite of accumulating more gonadal fat than male VMH-lesioned rats, daily energy intake of female VMH-lesioned rats was less, suggesting that they were metabolically more efficient than male rats (Tables 13 and 14). Stunting of linear growth (naso-anal length) occurred in both male and female VMH-lesioned rats (Tables 13 and 14). This is a characteristic feature of rats with VMH lesions and is presumably a consequence of a decrease in secretion and circulating levels of growth hormone (Bray, York 1979; Frohman, Bernardis 1968).

BAT wet weight of VMH-lesioned rats increased almost fourfold but this represents a massive infiltration of lipid because the DNA, cytochrome oxidase and protein content were near normal (Table 13 and 14). Calculation of protein per milligram DNA suggests that a slight cellular hypertrophy of metabolic mass occurred in lesioned rats, particularly in male rats (Table 14). These results are somewhat at variance with the recent report by Seydoux and coworkers (1981) which showed a marked reduction of DNA in BAT of female VMH-lesioned rats 4-5 weeks after receiving bilateral electrolytic lesions. Tissue involution was not however accompanied by a loss of either metabolic or mitochondrial mass as total protein and cytochrome content remained the same (Seydoux et al. 1981). Although the size and location of the lesions were not specified in their studies, it is possible that destruction of neurons in other areas could have resulted in an altered

secretion of hormones which are necessary to maintain normal cellularity of the tissue.

Binding of GDP to BAT mitochondria of male lesioned rats was similar to controls indicating a normal thermogenic state of the tissue at thermoneutrality (Figure 30). The thermogenic state of BAT in female lesioned rats however was low, as judged by the 50% decrease in mitochondrial GDP binding (Figure 31). Since the rats were maintained at thermoneutrality (28°C) and were also hyperphagic (Table 13) the low GDP binding suggests an impaired capacity of BAT to respond thermogenically to diet. Seydoux and coworkers (1982b) have also observed low GDP binding in BAT of female VMH-lesioned rats that were acclimated to 23°C. They also reported that the relative amount of the 32K polypeptide was only slightly less than normal (Seydoux et al. 1982b). It is therefore likely that the low thermogenic state of BAT in female lesioned rats is the consequence of a failure to unmask proton conductance pathways already present.

2. Effect of cold exposure on brown adipose tissue of warm-acclimated VMH-lesioned rats.

Exposure of VMH-lesioned rats to cold caused a marked activation of BAT thermogenesis, as judged by the large increased in mitochondrial GDP binding (Figs 30 and 31). The level of GDP binding in cold-exposed female lesioned rats, although large, was nonetheless lower than that attained by

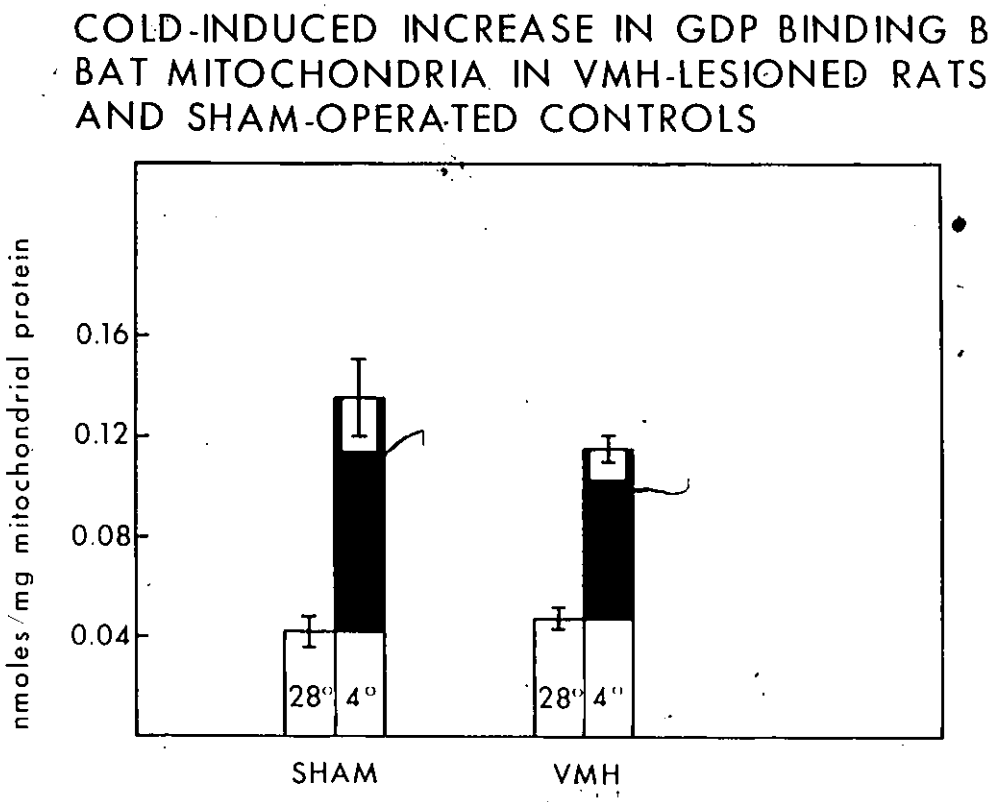

FIGURE 30. Effect of acute cold exposure on GDP binding by BAT mitochondria of male VMH-lesioned rats and controls.

Bars represent the means ± SE for 4 mitochondrial preparations. A significant effect of cold exposure (4°C for 21 hours) is indicated by the black portions of the bars. There is no significant difference between lesioned and control animals at the same temperature.

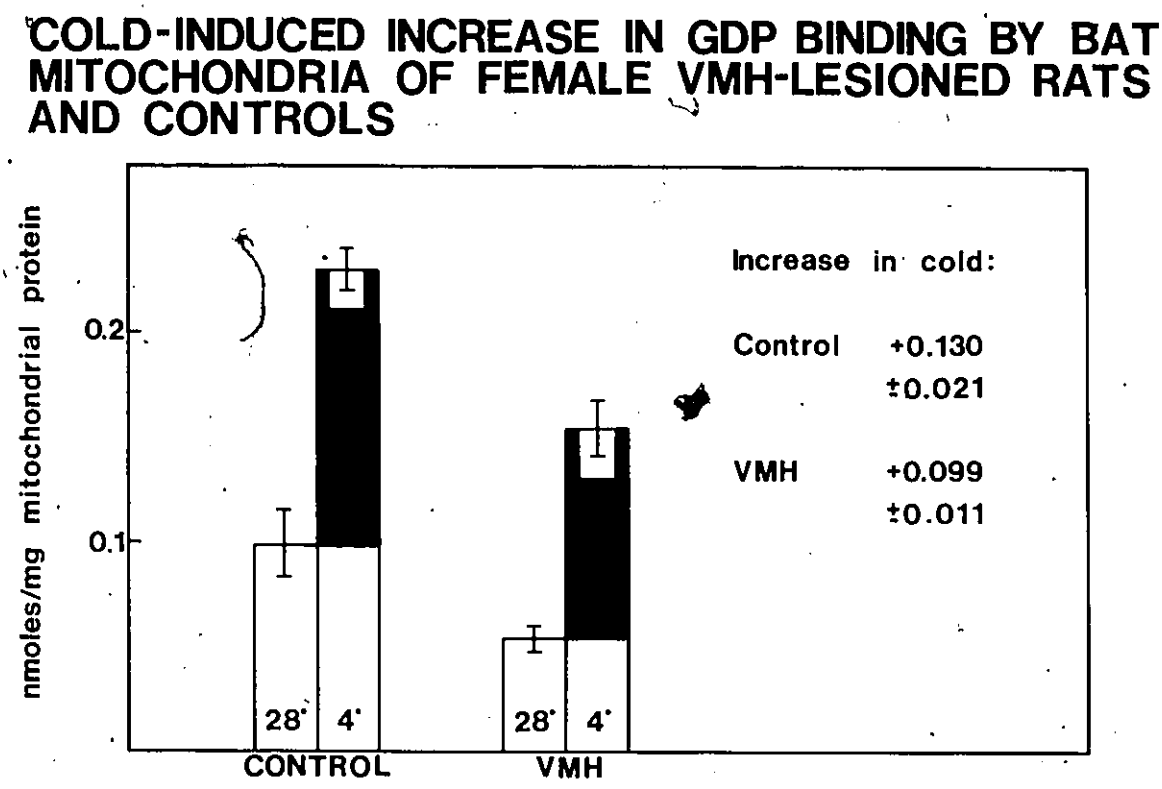

FIGURE 31. Effect of acute cold exposure on GDP binding by BAT mitochondria of female VMH-lesioned rats and controls.

BARS represent the means ± SE for 3 mitochondrial preparations. A significant effect of cold exposure (4°C for 21 hours) is indicated by the black portion of the bars. Binding by mitochondria of lesioned rats at 28°C (thermoneutrality) is significantly lower than that of control rats at 28°C.

control rats (Figure 31). This is probably due to the lower initial level of GDP binding at 28°C because the absolute increase in GDP binding was similar to that of nonlesioned rats (Figure 31).

Exposure of VMH-lesioned rats to 4°C for 24 hours caused a marked elevation in DNA, protein and cytochrome oxidase content of BAT (Tables 13 and 14) indicating an increase in cellularity and increases in metabolic and mitochondrial mass. Growth of BAT in female lesioned rats was achieved entirely by tissue hyperplasia since there were no changes in protein or cytochrome oxidase activity per unit DNA (Table 13). In BAT of male lesioned rats tissue hyperplasia was also accompanied by a slight cellular hypertrophy of metabolic mass (Table 14). Although acute cold exposure did not alter mitochondrial mass per cell in BAT of lesioned rats (no change in cytochrome oxidase per unit DNA) it did lower the concentration of mitochondria in cells (lower specific activity of cytochrome oxidase in BAT homogenates). A large decrease in BAT wet weights of lesioned rats occurred after 24 hours at 4°C, presumably the result of a massive mobilization of lipid from the tissue (Tables 13 and 14). Acute cold exposure did not initiate growth of BAT in control rats to the same extent as it did in VMH-lesioned rats. There was no significant alteration in the amount of protein, cytochrome oxidase activity or DNA in BAT of male

nonlesioned rats (Table 14). In fact, both the quantity (cytochrome oxidase activity per unit DNA) and the concentration (specific activity of cytochrome oxidase in BAT homogenates) of mitochondria in cells were decreased (Table 14). In female rats, acute cold exposure induced an increase in protein content of BAT but there were no changes in DNA or cytochrome oxidase (Table 13).

Conclusions

Activation of BAT thermogenesis by exposure to cold occurs normally in rats with radiofrequency heat lesions of the VMH (increase in GDP binding). These findings imply that critical cold-sensitive centers, responsible for the cold-induced sympathetic activation of BAT thermogenesis are not impaired by VMH lesions. In contrast, it would appear that VMH-lesioned rats may manifest an impaired response to diet since the thermogenic state of BAT in lesioned rats at thermoneutrality is low in females (low GDP binding) and normal in males (normal GDP binding) despite their hyperphagia.

PART IIIB: BROWN ADIPOSE TISSUE OF VENTROMEDIAL HYPOTHALAMIC (VMH) - LESIONED RATS DURING THE DYNAMIC PHASE OF OBESITY: RESPONSE TO A CAFETERIA DIET

Objective:

The primary objective of these experiments was to determine whether BAT thermogenesis induced by feeding of a cafeteria diet is defective in rats with lesions of the ventromedial hypothalamus (VMH). A secondary objective was to find out whether there is a lesioned-induced sex difference in the response of BAT to diet.

Method:

Male and female Holtzman rats, aged 11-13 weeks, received bilateral radiofrequency heat lesions (55°C) of the VMH as described before (Coscina et al. 1976). Non-operated rats served as controls in the following two experiments. In the first experiment, 34 female rats were kept in separate cages at the University of Ottawa and maintained at 28°C for 1 week prior to surgery. The VMH lesions were done by D.V. Coscina and upon recovery from the anesthesia, rats were fed either a cafeteria diet or a chow diet for 19-27 days along with their non-operated controls. In the second experiment, 35 male rats were lesioned at the Clarke Institute of Psychiatry, University of Toronto, after 1 week at 22°C. Lesioned rats and their controls were transported

to Ottawa 1-2 days after surgery and immediately upon arrival were fed either a cafeteria diet or a chow diet for 27-32 days. All animals were kept at 28°C and food intake and body weights were monitored regularly. Cafeteria-fed rats were offered a choice of 4 different foods from each of 5 menus rotated on a daily basis (Appendix A). As it was not possible to continuously record food intake, one menu (#5) was selected for examination of nutrient and energy intake in both experiments. Rats were killed by decapitation and in the first experiment, blood was collected for determination of serum T_3 and T_4 (Methods H). As previously observed, lesioned rats were noticeably agitated during handling before sacrifice. In both experiments interscapular BAT and gonadal fat were removed and weighed. Samples were taken from BAT homogenates for protein, cytochrome oxidase and DNA assays, and mitochondria were isolated for GDP binding assay (Methods C, D and E). Brains were preserved in a 10% formalin solution and sent to the Clarke Institute, University of Toronto for histological examination (Nobrega et al. 1980). Histological examination verified that the location and extent of the VMH lesions in both male and female rats were exactly the same as those described in the previous experiments (Figure 29).

Results and Discussion

1. Brown adipose tissue of chow-fed VMH-lesioned rats

As in the previous experiments (Part IIIA) bilateral lesioning of the VMH in mature chow-fed rats promoted increases in body weight gain, gonadal fat wet weight and energy intake (Tables 15 and 16). That female lesioned rats had become more obese than males is shown by the considerablely greater accumulation of gonadal fat. Measurements of serum T_3 and serum-free T_4 indicate that VMH-lesioned rats are euthyroid (Table 15). This is in agreement with the report that plasma protein bound iodine levels are normal in weanling VMH-lesioned rats (Frohman, Bernardis, 1968).

The wet weight of BAT was grossly elevated in both male and female lesioned rats, presumably due to a massive infiltration of lipid (Tables 15 and 16). Although lesioning induced an increase in the protein content of BAT in female rats, the DNA and cytochrome oxidase contents were not changed, indicating a normal cellularity and mitochondrial mass of the tissue but an increase in metabolic mass (Table 15). In male rats, lesioning did not affect the metabolic mass of BAT since the protein content was not different from control rats (Table 16; DNA and cytochrome oxidase activity of BAT were not measured). The binding of GDP to BAT mitochondria of male lesioned rats maintained at thermoneutrality was reduced by more than 50 percent (Figure 32). In contrast, GDP binding in female lesioned rats was significantly higher than their respective controls (Figure 33). These results

TABLE 15

EFFECT OF CAFETERIA FEEDING ON FEMALE NONLESIONED AND VMH-LESIONED RATS

	Nonlesioned		VMH-Lesioned	
	Chow (15)	Cafeteria (15)	Chow (14)	Cafeteria (15)
Body Weight, g				
initial	232.8 ± 3.3	234.9 ± 4.7[b]	235.6 ± 2.8[a]	234.7 ± 3.7[a,b]
final	270.9 ± 3.1	318.1 ± 6.5[b]	331.8 ± 8.1[a]	467.6 ± 8.2[a,b]
change, 19-27 days	+38.5 ± 3.9	+83.1 ± 6.2[b]	+88.4 ± 8.7[a]	+229.5 ± 9.6[a,b]
gain, g/day	1.6 ± 1.4	3.5 ± 0.2[b]	4.0 ± 0.3[a]	9.8 ± 0.2[a,b]
Gonadal Fat, mg	5189 ± 509	13172 ± 891	12757 ± 1459 [a]	27180 ± 1249 [a,b]
Brown Adipose Tissue				
wet weight, mg	352 ± 22	552 ± 50[b]	834 ± 85 [a]	2310 ± 139 [a,b]
protein, mg	11.7 ± 0.7	16.1 ± 1.7[b]	18.4 ± 1.9[a]	19.5 ± 1.4
DNA, µg	365.0 ± 38.8	354.9 ± 36.9[b]	382.5 ± 41.8[a]	360.4 ± 46.9
mg protein·mg DNA^{-1}	35.6 ± 3.3	48.3 ± 4.7[b]	51.0 ± 5.6[a]	61.5 ± 6.0
cytochrome oxidase				
µg atoms O_2·min^{-1}	74.2 ± 5.1	102.0 ± 8.2[b]	87.8 ± 7.2[a]	75.0 ± 8.6[b]
µg atoms O_2·min^{-1} ·mg protein^{-1}	6.3 ± 0.3	6.7 ± 0.4	4.9 ± 0.4[a]	3.7 ± 0.4[a]
µg atoms O_2·min^{-1} ·mg protein^{-1}	228.8 ± 250	312.3 ± 23.4[b]	245.7 ± 27.4	232.3 ± 26.3[a]
Serum T_3, ng/ml	(14) 0.38 ± 0.01	(14) 0.49 ± 0.03[b]	(11) 0.36 ± 0.02	(12) 0.47 ± 0.04[b]
Serum Free T_4, ng/dl	(15) 2.24 ± 0.13	(14) 2.24 ± 0.14	(14) 2.07 ± 0.13	(15) 2.34 ± 0.17

Values are the means ± SE for the numbers of animals given in parenthesis.

[a]Significant effect of the lesion, comparing rats treated in the same way.

[b]Significant effect of cafeteria feeding, comparing the same type of rat.

TABLE 16

EFFECT OF CAFETERIA FEEDING ON MALE NONLESIONED AND VMH-LESIONED RATS

	Nonlesioned		VMH-Lesioned	
	Chow (15)	Cafeteria (15)	Chow (15)	Cafeteria (15)
Body Weight, g				
initial	351.1 ± 3.7	347.9 ± 4.9[b]	347.9 ± 6.3[a]	348.4 ± 5.2[a,b]
final	440.5 ± 5.2	461.9 ± 4.5[b]	464.9 ± 9.8[a]	584.7 ± 8.4[a,b]
change, 27-32 days	+89.3 ± 3.5	+114.2 ± 3.3[b]	+116.9 ± 5.2[a]	+236.3 ± 7.9[a,b]
gain, g/day	3.0 ± 0.1	3.8 ± 0.2[b]	4.0 ± 0.2[a]	8.0 ± 0.3[a,b]
Gonadal Fat	4750 ± 200	7920 ± 480 [b]	6710 ± 610 [a]	16110 ± 840 [a,b]
Brown Adipose Tissue				
wet weight, mg	502 ± 26	1084 ± 44 [b]	1145 ± 123 [a]	2761 ± 135 [a,b]
protein, mg	22.9 ± 1.2	39.9 ± 2.4[b]	24.9 ± 1.4	34.6 ± 2.4[b]

Values are the means ± SE for the number of animals in parenthesis

[a]Significant effect of the lesion, comparing rats treated in the same way.

[b]Significant effect of cafeteria feeding, comparing the same type of rat.

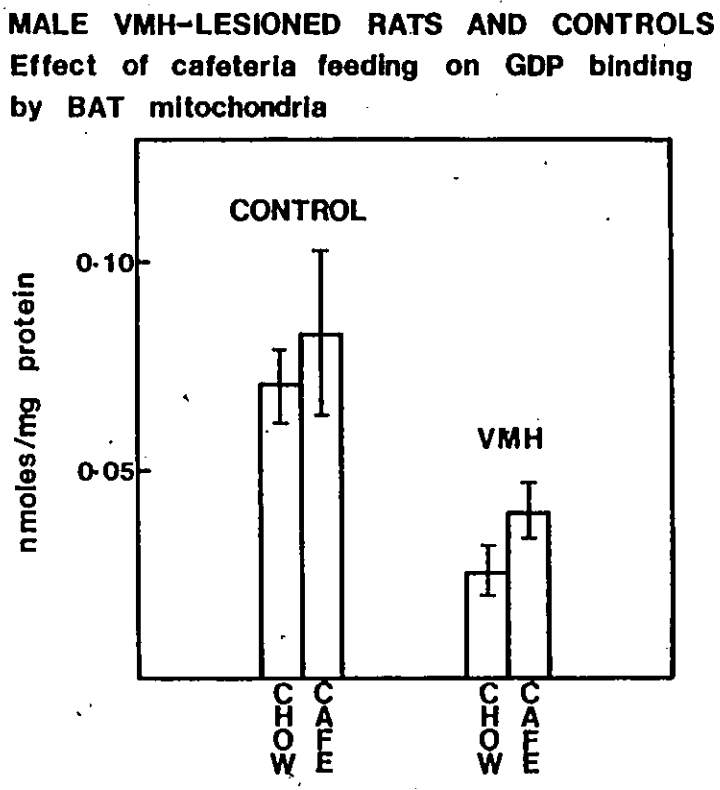

FIGURE 32. Effect of cafeteria feeding on GDP binding by BAT mitochondria of male VMH-lesioned rats and controls.

Bars represent the means ± SE for 5 mitochondrial preparations. There are no significant differences between chow-fed and cafeteria-fed rats. GDP binding is significantly lower in chow-fed and cafeteria-fed lesioned rats than their respective nonlesioned controls.

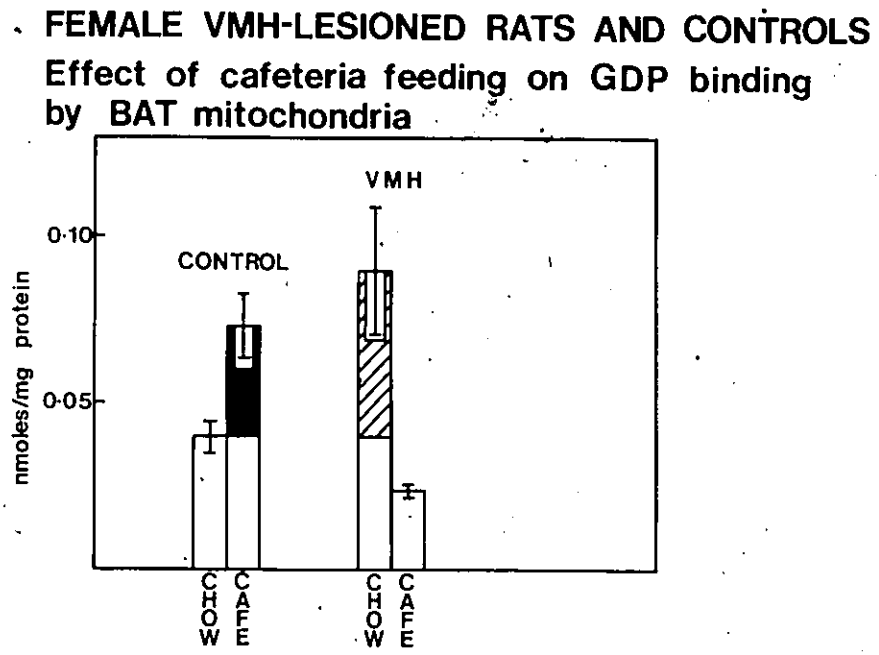

FIGURE 33. Effect of cafeteria feeding on GDP binding by BAT mitochondria of female VMH-lesioned rats and controls.

Bars represent the means ± SE for mitochondrial preparations. The black portion of the bar indicates a significant effect of cafeteria feeding (3 weeks). The cross-hatched portion of the bar shows a significant effect of the chow diet. Binding by mitochondria of cafeteria-fed lesioned rats is significantly lower than that of cafeteria-fed control rats ($p < 0.001$).

differ from the previous experiment (Figure 31) in which females displayed a low basal binding (chow-fed at 28°C). It is possible that in the present experiment female rats were more stressed at sacrifice. Handling of rats (Depocas, Behrens, 1977; Kvetnansky, 1978) or simply removing the top of the cage (Kvetnansky, 1978) is sufficient to stimulate sympathetic activity and these lesioned rats were extremely agitated.

2. Effect of cafeteria feeding on brown adipose tissue of VMH-lesioned rats

Cafeteria feeding for 19-32 days potentiated the obesity of VMH-lesioned rats during the dynamic phase and promoted an accumulation of body fat in nonlesioned controls as judged by increases in body weight gain and in wet weight of gonadal fat (Tables 15 and 16). The analysis of food intake from a single menu, measured at 3 different times in both experiments, indicates that the pattern of nutrient intake as well as energy intake changes when rats are allowed to self-select from a variety of food items (Tables 17 and 18). (Since only 1 out of 5 cafeteria menus was evaluated in terms of energy and nutrient intake (protein, carbohydrate, fat), the data can only be suggestive of overall alterations in food intake. Moreover, studies were not done to determine the metabolizable energy content of chow. Since the calculations for energy intake are based on the gross energy

TABLE 17

EFFECT OF A CAFETERIA DIET ON NUTRIENT (protein, carbohydrate, fat) AND ENERGY INTAKE OF FEMALE NONLESIONED AND VMH-LESIONED RATS

	Nonlesioned		VMH-Lesioned	
	Chow (15)	Cafeteria (15)	Chow (15)	Cafeteria (15)
Day 6				
protein, g	4.7 ± 0.2	4.0 ± 0.2[b]	5.4 ± 0.5	4.9 ± 0.4
carbohydrate, g	11.1 ± 0.5	20.3 ± 1.8[b]	12.4 ± 1.3	25.9 ± 1.2[a,b]
fat, g	0.9 ± 0	3.7 ± 0.2[b]	1.1 ± 0.1	4.8 ± 0.3[a,b]
Kcal	83.7 ± 3.5	135.0 ± 4.7[b]	95.5 ± 9.5	172.2 ± 8.2[a,b]
Day 11				
protein, g	5.5 ± 0.6	4.6 ± 0.4	8.2 ± 0.7[a]	8.3 ± 0.6[a]
carbohydrate, g	12.9 ± 1.3	20.3 ± 1.8[b]	19.4 ± 1.7[a]	35.4 ± 1.5[a,b]
fat, g	1.1 ± 0.1	3.6 ± 0.2[b]	1.6 ± 0.1[a]	6.1 ± 0.3[a,b]
Kcal	96.9 ± 9.9	147.0 ± 12.1[b]	145.8 ± 12.4[a]	241.8 ± 11.5[a,b]
Day 18				
protein, g	4.9 ± 0.3	3.6 ± 0.4[b]	7.0 ± 0.9[a]	5.2 ± 0.5[a]
carbohydrate, g	11.5 ± 0.7	15.0 ± 1.4[b]	16.4 ± 1.6[a]	28.3 ± 1.9[a,b]
fat, g	0.9 ± 0.1	2.8 ± 0.2[b]	1.4 ± 0.1[a]	5.8 ± 0.3[a,b]
Kcal	86.9 ± 5.2	105.8 ± 8.4	123.6 ± 12.3[a]	183.8 ± 11.7[a,b]

Values are means ± SE for the number of animals given in parenthesis.

Nutrient and energy intakes for the 'cafeteria' food items were estimated from food composition tables (Pennington, Church 1980).

Values for Purina chow #5012 were based on manufacturer analysis of nutrient and gross energy content.

[a]Significant effect of lesion, comparing rats treated in the same way.

[b]Significant effect of cafeteria feeding, comparing the same type of rat.

TABLE 18

EFFECT OF A CAFETERIA DIET ON NUTRIENT (protein, carbohydrate, fat) AND ENERGY INTAKE OF MALE NONLESIONED AND VMH-LESIONED RATS

	Nonlesioned		VMH-Lesioned	
	Chow (15)	Cafeteria (15)	Chow (15)	Cafeteria (15)
Day 9				
protein, g	8.7 ± 0.4	5.5 ± 0.5[b]	8.3 ± 0.4	7.9 ± 0.5[a]
carbohydrate, g	20.5 ± 0.9	22.3 ± 1.3	19.1 ± 0.7	29.5 ± 1.5[a,b]
fat, g	1.7 ± 0.1	4.6 ± 0.4[b]	1.7 ± 0.1	5.3 ± 0.4[a,b]
Kcal	154.2 ± 6.8	157.1 ± 9.9	147.0 ± 6.6	208.9 ± 10.7[a,b]
Day 16				
protein, g	7.8 ± 0.3	5.6 ± 0.9[b]	9.0 ± 0.3[a]	7.5 ± 0.6[a,b]
carbohydrate, g	18.5 ± 0.7	20.9 ± 2.5	21.4 ± 0.8[a]	36.0 ± 2.6[a,b]
fat, g	1.6 ± 0.1	4.8 ± 0.3[b]	1.8 ± 0.1[a]	6.9 ± 0.6[a,b]
Kcal	139.3 ± 5.2	153.0 ± 15.8	160.5 ± 6.11[a]	243.1 ± 17.0[a,b]
Day 23				
protein, g	6.9 ± 0.4	4.8 ± 0.2[b]	8.9 ± 1.5[a]	8.1 ± 0.6[a]
carbohydrate, g	16.4 ± 1.0	19.0 ± 1.2	21.0 ± 0.9[a]	37.9 ± 2.5[a,b]
fat, g	1.4 ± 0.1	4.5 ± 0.4[b]	1.8 ± 0.1[a]	7.1 ± 0.6[a,b]
Kcal	123.3 ± 7.1	135.0 ± 7.6	157.6 ± 6.7[a]	255.1 ± 17.4[a,b]

Values are means ± SE for the number of animals given in parenthesis.

Nutrient and energy intakes for the 'cafeteria' food items were estimated from food composition tables (Pennington, Church 1980).

Values for Purina chow #5012 were based on manufacturers analysis of nutrient and gross energy content.

[a]Significant effect of lesion, comparing rats treated in the same way.

[b]Significant effect of cafeteria feeding, comparing the same type of rat.

content of chow as provided by the Ralston Purina Co., the values are higher than the actual energy intake of chow-fed rats. Male and female cafeteria-fed lesioned rats consumed more energy, fat and carbohydrate than either chow-fed lesioned rats or cafeteria-fed nonoperated controls (Tables 17 and 18). Although protein intake was somewhat variable in cafeteria-fed VMH-lesioned rats, compared to their chow-fed lesioned controls, in 4 out of 6 measurements protein intake was greater in these animals than in cafeteria-fed nonoperated controls (Tables 17 and 18). Female nonoperated rats also increased their intake of energy, fat and carbohydrate whereas in male rats, energy intake was not affected by cafeteria feeding. Nonetheless, the pattern of food intake was altered in male rats as shown by the increased intake of fat and the decreased intake of protein. Serum T_3 levels were elevated by 29-30% in both lesioned and nonlesioned rats during cafeteria feeding, while serum-free T_4 concentrations remained unchanged (Table 15).

Activation of BAT thermogenesis by a cafeteria diet failed to occur in either male (Figure 32) or female (Figure 33) VMH-lesioned rats, as judged from the lack of increase in BAT mitochondrial GDP binding, despite the very large increase in energy intake of these animals (Tables 17 and 18). As expected, an increase in BAT mitochondrial GDP

binding occurred in cafeteria-fed female nonlesioned rats (Figure 33), indicative of a thermogenic activation by the increased energy intake (Table 17). A lack of thermogenic activation of BAT by a cafeteria diet in male nonlesioned rats (Figure 32) was correlated with a lack of any diet-induced increase in energy intake (Table 18). This contrasts with other studies (Himms-Hagen et al. 1981; Brooks et al. 1980, 1982; Triandafillou et al., 1983) in which a cafeteria diet induced both thermogenic activation of BAT and an increase in energy intake. It is possible that the age of the rats (older in these experiments) is critical in determining their responsiveness to diet (Rothwell, Stock 1982a).

BAT wet weight was markedly increased in all cafeteria-fed rats (Tables 15 and 16). In female VMH-lesioned rats, this was entirely due to an accumulation of lipid since there were no changes in the content of protein, cytochrome oxidase and DNA (Table 15). Growth of BAT did occur in nonoperated female cafeteria-fed rats as indicated by the increases in protein and cytochrome oxidase content (Table 15). That mitochondrial proliferation kept pace with the increase in metabolic mass is shown by a lack of change in the specific activity of cytochrome oxidase in BAT homogenates (Table 15). Although the DNA content remained unaltered (Table 15), this was not unexpected because cafeteria feeding is not an

effective stimulant of tissue hyperplasia in mature rats (Rothwell, Stock, 1982b). In male rats cafeteria feeding resulted in an increase in protein content of BAT in nonlesioned as well as lesioned rats (Table 16; cytochrome oxidase activity and DNA content were not measured).

Conclusions

The principal finding in these experiments is that cafeteria feeding fails to activate BAT thermogenesis in hyperphagic VMH-lesioned rats during the dynamic phase of their obesity. Furthermore, in both this experiment and in the previous one, the thermogenic state of BAT in chow-fed lesioned rats consuming excess energy at thermoneutrality is generally low (male rats, Part III B; female rats, Part III A) or normal (male rats, Part III A). The reduced ability of BAT in VMH-lesioned rats to respond thermogenically to diet may contribute to the development of their obesity. That defective DIT occurs with an apparently normal sympathetic innervation of BAT and a normal capacity of cold-induced BAT thermogenesis (Part III, A) suggests that ablation of the VMH resulted in a reduced sympathetic outflow from the diet-sensitive centers in the hypothalamus to BAT. Indeed, NA turnover studies have shown that electrolytic lesions of the VMH are associated with a low sympathetic activity in BAT of female weanling rats consuming a high carbohydrate

diet (Vander Tuig et al. 1982). Since radiofrequency lesions of the VMH did not interfere with the normal increase in protein content of BAT in cafeteria-fed male rats but did inhibit the increase in female rats, it is possible that sex hormones are involved in diet-induced growth of BAT.

PART III C: BROWN ADIPOSE TISSUE OF RATS WITH OBESITY-INDUCING HYPOTHALAMIC KNIFE CUTS: RESPONSE TO ACUTE COLD EXPOSURE

Objective:

The objective of these experiments was to find out whether cold-induced BAT thermogenesis is defective in mature rats with obesity-inducing hypothalamic knife cuts during the dynamic phase of their obesity.

Method:

Female Holtzman rats, aged 11-13 weeks, were kept in single cages and maintained at 22°C for 1 week before surgery at the Clarke Institute of Psychiatry, University of Toronto (Materials A). Twenty four rats received bilateral parasagittal knife cuts of the medial hypothalamus using a procedure developed by J. Chambers. The cuts extended 5-8 mm rostral to the interaural line, 0.7 - 0.8 mm lateral to the midline and 3 mm dorsoventrally from the bottom of the thalamus to the base of the brain (Chambers, J.; submitted for publication). An additional 12 rats were sham-operated (including lowering of the guide shaft without the knife being extended) and together with 12 unoperated rats served as controls. One week after surgery, all rats were transported to the University of Ottawa by car where

they were maintained under similar conditions but at 28°C. Food and body weights were recorded regularly and 3 weeks postsurgery all rats were briefly anesthetized with ether for measurement of nasoanal lengths. Sham operated and unoperated rats were combined for all analyses since there were no significant differences in food intake, body weights gain or nasoanal length between groups. In each of 4 experiments, 3 knife cut rats and 3 control rats were exposed to 4°C for 21 hours, whereas 3 knife cut rats and 3 control rats remained at 28°C.

Rats were killed by decapitation 28-33 days after receiving the parasagittal knife cuts. Like the VMH-lesioned rats, the knife cut rats became hyper reactive when handled. Interscapular BAT and gonadal fat were removed and weighed. BAT was homogenized and samples removed from measurements of protein, DNA and cytochrome oxidase activity (Methods C, D, E). Homogenates from 3 similarly treated rats were pooled and mitochondria isolated for GDP binding assay (Methods: A, B). Brains were placed in a 10% formalin solution and returned to Toronto for histological examination by a previously described method (Nobrega et al. 1980). A typical lesion induced by knife cuts is illustrated in Figure 34.

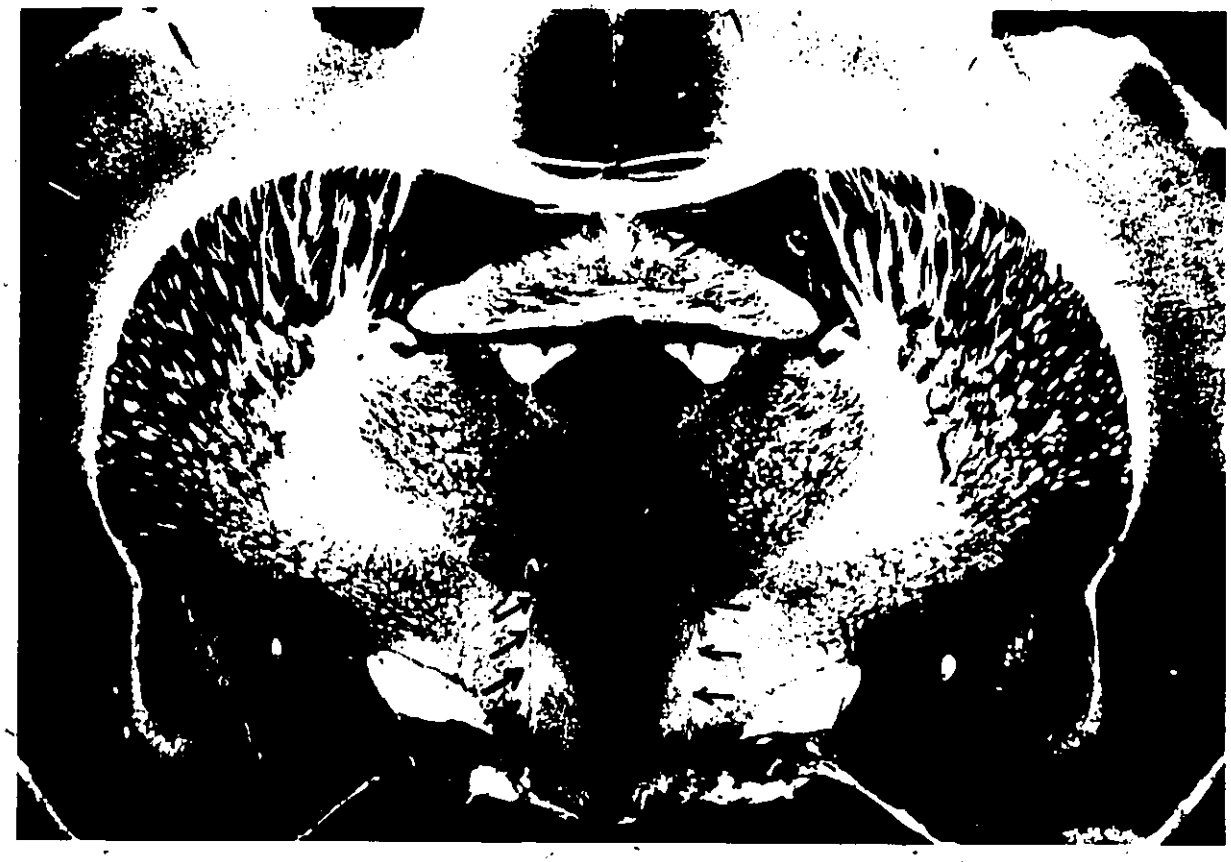

FIGURE 34. Coronal section of the obesity-inducing hypothalamic knife cut.

Results and Discussion

1. Brown adipose tissue of knife cut rats

Female knife cut rats became markedly obese at thermoneutrality as judged by the substantial increase in body weight gain and wet weight of gonadal fat (Table 19). Parasagittal knife cuts also induced skeletal growth and hyperphagia as shown by the increases in nasoanal length and daily energy intake (Table 19).

Despite a fourfold increase in BAT wet weight of knife cut rats, total DNA and cytochrome oxidase activity were normal, indicating normal cellularity and mitochondrial mass (Table 19). However, tissue hypertrophy occurred since the total protein content of BAT increased by 60 percent (Table 19). BAT of knife cut rats at 28°C exhibited a normal thermogenic state because mitochondrial GDP binding, although variable, was not significantly different from that of control rats (Figure 35). The large variation of GDP binding measurements likely reflects the excitability of knife cut rats at the time of sacrifice.

2. Effect of cold exposure on brown adipose tissue of warm-acclimated knife cut rats

Exposure of rats to cold for 21 hours activated BAT thermogenesis to an equal extent in knife cut and in control rats as judged by the similar increase in mitochondrial GDP binding (Figure 35). An increase in protein content of BAT was observed in both knife cut and control rats, but the

TABLE 19

EFFECT OF COLD EXPOSURE ON FEMALE KNIFE CUT RATS

Temperature of Exposure	Control 28°C (12)	Control 4°C (12)	Knife Cut 28°C (12)	Knife Cut 4°C (11)
Body Weights				
initial	233.1 ± 2.7		232.6 ± 1.3	
final	274.9 ± 3.1		431.3 ± 8.8[a]	
change, 28-33 days	+41.5 ± 2.5		+198.7 ± 8.7[a]	
gain, g/day	1.4 ± 0.1		6.8 ± 0.4[a]	
Body Length, mm	221.1 ± 1.0		225.6 ± 1.5	
Gonadal Fat, mg	5330 ± 300	3860 ± 430 [b]	18400 ± 1750 [a]	21400 ± 980 [a]
Food Intake, Kcal				
day 7	73.2 ± 2.3		150.4 ± 4.0[a]	
day 14	77.2 ± 2.3		126.0 ± 7.2[a]	
day 21	84.0 ± 7.0		128.0 ± 10.8[a]	
Brown Adipose Tissue				
wet weight, mg	420 ± 30	380 ± 30	1790 ± 170 [a]	1660 ± 250 [a]
protein, mg	19.0 ± 1.0	26.9 ± 1.5[b]	30.3 ± 2.4[a]	57.3 ± 5.1[a,b]
DNA, µg	372.5 ± 31.7	471.2 ± 36.5	435.9 ± 34.9	462.9 ± 34.5
mg protein·mg DNA^{-1}	53.8 ± 3.8	61.3 ± 5.5	73.2 ± 9.5	123.6 ± 8.1[a,b]
Cytochrome oxidase				
µg atoms O_2·min^{-1}	133.3 ± 6.4	196.1 ± 14.4[b]	172.4 ± 19.6	209.1 ± 13.0
µg atoms O_2·min^{-1} ·mg protein^{-1}	6.7 ± 0.7	7.3 ± 0.4	5.6 ± 0.4	3.7 ± 0.3[a,b]
µg atoms O_2·min^{-1} ·mg DNA^{-1}	384.0 ± 34.0	453.8 ± 50.0	427.2 ± 69.9	478.6 ± 39.0

Values are means ± SE for the number of animals given in parenthesis.

[a]Significant effect of knife cut, comparing rats treated in the same way.

[b]Significant effect of cold exposure, comparing the same type of rat.

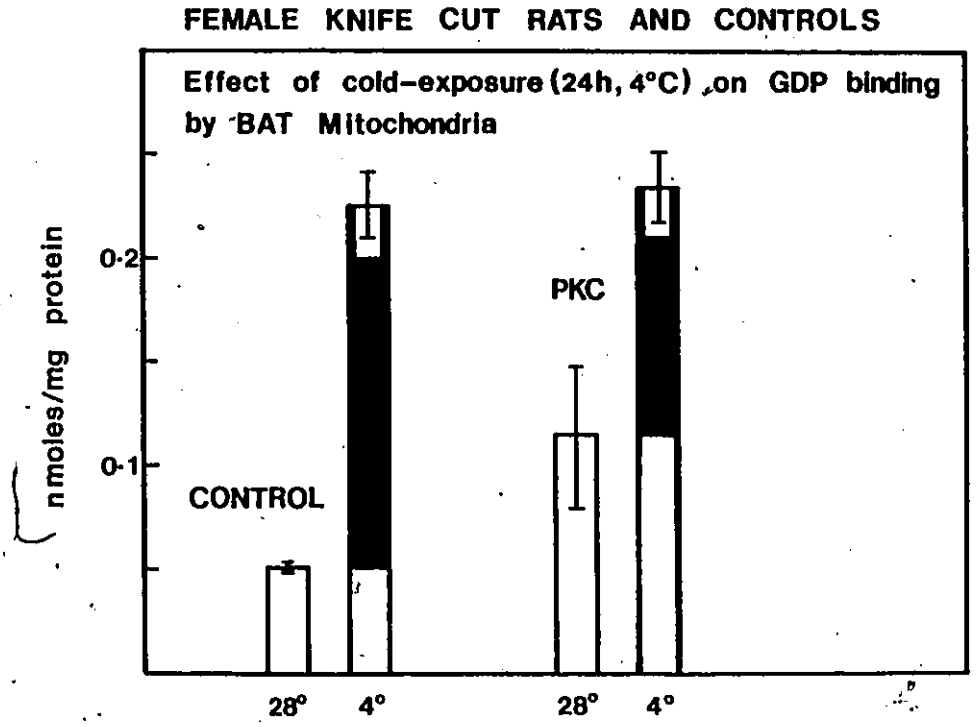

FIGURE 35. Effect of acute cold exposure on GDG binding by BAT mitochondria of female rats with hypothalamic knife cuts.

Bars represent the means ± SE for 4 mitochondrial preparations. A significant effect of cold exposure (4°C for 21 hours) is indicated by the black portion of the bars. There is no significant difference between parasagittal knife cut rats (PKC) and control rats at the same temperature.

effect of acute cold was twice as great in the knife cut rats (Table 19). However, total cytochrome oxidase activity of BAT also increased in control rats during brief cold exposure and, as shown by the lack of change in the specific activity of cytochrome oxidase in BAT homogenates, mitochondrial proliferation occurred in parallel with the increase in metabolic mass (Table 19). The hypertrophy observed was primarily due to cell enlargement since DNA content was not significantly altered by this brief exposure to cold (Table 19).

Conclusions

Results show that bilateral parasagittal knife cuts of the medial hypothalamus do not interfere with the acute thermogenic response of BAT to cold (normal increase in GDP binding).

That knife cuts may have interfered with the thermogenic response of BAT to diet at thermoneutrality is suggested by the similar GDP binding measurements in hyperphagic knife cut rats and in normophagic control rats. This possibility implies that the knife cuts severed some of the neural pathways leading either to or from the diet-sensitive centers but not the cold-sensitive centers of the hypothalamus. Centers responsible for the transmission of dietary signals to sympathetically innervated BAT may be located in the anteromedial hypothalamus (Sclafani, Berner 1977) particularly

the paraventribular nucleus (PVN) (Gold et al. 1977; Leibowitz et al. 1981). Electrolytic lesions restricted to the paraventricular nucleus cause overeating and a large increase in body weight gain (Leibowitz et al. 1981) whereas discrete lesions of the ventromedial nucleus (Gold 1973) or lesions of other areas rostral, dorsal or ventral to the paraventricular nucleus are without effect (Leibowitz et al. 1981). Moreover, knife out studies (Gold et al. 1977; Sclafani, Berner 1977) show that the damage to neutral pathway coursing through or near the VMH which results in obesity and hyperphagia either originate or terminate in the paraventricular nucleus. Since it is probable that the radiofrequency lesions of the VMH damaged the same neural pathways severed by the parsagittal knife cuts (Bray, York 1979; Powley et al. 1980), it would be predicted that knife cut rats would also display an impaired thermogenic response of BAT to cafeteria feeding.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

The major finding described in this thesis is that impaired thermogenic functioning of BAT occurs in 4 animal models of obesity: the genetically obese (ob/ob) mouse, the GTG-obese mouse, the VMH-lesioned rat and the hypothalamic knife cut rat. However, the nature of the thermogenic defect is different in animals with genetic obesity and in those with hypothalamic obesity (GTG-obese mouse, VMH-lesioned rat, hypothalamic knife cut rat). In this chapter, impaired functioning of BAT will be discussed in relation to these 4 animal models. Since the latter 3 models of obesity noted above have similarities; they will be discussed together under the heading of hypothalamic obesity. Also in this chapter, other experimental evidence supporting a role for BAT in energy balance will be presented.

The genetically obese (ob/ob) mouse

Genetically obese (ob/ob) mice, maintained at 28°C have a larger than normal amount of BAT with a normal mitochondrial polypeptide composition, but an abnormal mitochondrial ultrastructure. The thermogenic state of the tissue is less in ob/ob mice than it is in lean mice, as determined by the lower GDP binding by BAT mitochondria. Moreover, exposure of ob/ob mice to acute cold (4°C for 3

hours) does not alter mitochondrial GDP binding or ultrastructure, indicating a lack of the normal thermogenic activation of the tissue because of a failure to unmask proton conductance pathways already present. Low mitochondrial GDP binding and respiration have subsequently been reported in preweanling ob/ob mice (Goodbody, Trayhurn 1982), suggesting that the reduced activity of the thermogenic pathway is related to the primary genetic defect. It is known that the initial unmasking effect is mediated by NA (Desautels, Himms-Hagen 1979) and is therefore responsible for switching on BAT thermogenesis. Since there is normal sympathetic activity in BAT of ob/ob mice during brief cold exposure (Zaror-Behrens, Himms-Hagen 1983) and because the impaired thermogenic response to injected NA can be quantitatively accounted for by reduced heat production in BAT (Thurlby, Trayhurn 1980), it is suggested that BAT of ob/ob mice is refractory to the thermogenic action of NA. Thus, the defect appears to be inherent in the tissue and must therefore reside in the plasma membrane, the mitochondria or in the series of intracellular events which link the binding of NA with its receptor to the unmasking of purine nucleotide binding sites.

Recently, defects in NA-induced adenylate cyclase activity (Begin-Heick, Heick 1982) and lipolysis (Assimacopoulos-Jeannet et al. 1982) have been identified in BAT of ob/ob mice. The initial failure of NA to activate

BAT thermogenesis may in part result from the reduced lipolysis. Since fatty acids are the principal substrates oxidized by BAT mitochondria and respiration is critically controlled by their availability (Bukowiecki et al. 1981; Locke et al. 1982a; b), the impaired lipolytic response to NA (Assimacopoulos-Jeannet et al. 1982) could severely limit substrate availability for mitochondrial thermogenesis. Moreover, as fatty acids probably also serve as anti-nucleotides to displace purine nucleotides from the binding site on the 32K polypeptide (Locke et al. 1982a; b), it is entirely possible that diminished NA-stimulated lipolysis secondarily results in a failure to initiate loosely coupled respiration.

Treatments known to reduce the cold sensitivity of ob/ob mice can be correlated with an improvement in thermogenic functioning of BAT. Thus, acclimation to mild cold (14°C) promotes the usual cold-induced adaptive changes in mitochondria which include increases in GDP binding and in the relative amount of the 32K polypeptide and a change in ultrastructure such that the inmerices become more numerous and parallel. As well, tissue growth and mitochondrial proliferation occur normally during cold acclimation. These changes in BAT are consistent with the improved thermogenic response of cold-acclimated ob/ob mice to injected NA and are supported by the later studies of Seydoux and coworkers

(1982a) which show an increase in basal and NA-induced heat production of isolated BAT from ob/ob mice maintained at 5°C, as measured by direct microcalorimetry.

Chronic administration of T_4 to warm-acclimated ob/ob mice also causes an increase in mitochondrial GDP binding, a more normal ultrastructure and permits a further increase in GDP binding when mice are exposed to acute cold. Since untreated ob/ob mice actually have higher blood T_3 levels than their lean littermates and because thyroid hormones are necessary in a permissive way for the cold-induced activation of BAT thermogenesis (Triandafillou et al. 1982), it is proposed that BAT of ob/ob mice is refractory to endogenous T_3. Indeed, preliminary experiments using ^{125}I - T_3 show that both the number and affinity of T_3 receptors are lower in the crude membrane fraction of BAT from ob/ob mice (Himms-Hagen, J., Kates, A-L., unpublished observations). Providing these mice with exogenous T_4 at a dose that is without effect on NST in lean mice is enough to overcome BAT resistance to T_3 and allow for a more normal thermogenic response to NA. The improved BAT thermogenesis in cold-acclimated ob/ob mice may be due in part to the permissive effect of endogenous T_3, since it is known that blood T_3 levels are elevated in animals maintained in the cold Reichlin et al. 1973; Rothwell, Stock 1980b; Scammell et al. 1980; this thesis, p. 143).

Since T_3 receptors are found in the plasma membrane, nucleus and mitochondrion (Sterling 1979b), localization of the receptor defect in ob/ob mice would be useful in understanding the way in which thyroid hormones exert their permissive effect on BAT thermogenesis. The permissive effect does not appear however to involve the thyroid-sensitive Na^+K^+ ATPase, postulated as having a triggering role in thermogenesis (Chinet et al. 1978), because the concentration and total number of Na^+ K^+ enzyme units are similar in BAT of warm-acclimated (33°C, 25°C) lean and ob/ob mice and increase to the same extent with cold acclimation (14°C) (Knehans, Romsos 1982). Thyroid hormones are also known to regulate the balance of α- and β-adrenergic receptors in specific tissues (Lefkowitz 1982; Kunos 1977; Gibson 1981). Since binding of adrenergic agonists to α_2-receptors inhibits adenylate cyclase activity (Wright, Simpson 1981; Aktories et al. 1980; Kather et al. 1980), it is possible that an excess number of α_2-receptors contributes to the impaired NA-induced adenylate cyclase activity in BAT of ob/ob mice.

Studies using various adrenergic agonists and antagonists would establish whether there are differences between lean and ob/ob mice in the receptor population of BAT. If abnormalities were detected, the next step would be to find out whether the improvement in BAT thermogenesis of T_4-treated

cold-acclimated ob/ob mice could be correlated with changes in adrenergic receptor concentration and adenylate cyclase activity. Treating ob/ob mice and lean mice of altered thyroid status with various adrenergic blocking agents and then measuring mitochondrial GDP binding in response to acute cold exposure would help clarify whether an imbalance in receptor population actually affects the refractoriness of BAT to T_3. However, BAT resistance to T_3 is only part of the explanation for the defect in NST because T_4 treatment does not greatly enhance survival of ob/ob mice at 4°C.

Hyslop and York (1980) have reported an abnormal fludity in the plasma membrane of white adipocytes from ob/ob mice, which is partially corrected by acclimation to 33°C (thermoneutrality). If this represents a general membrane defect, then an altered lipid environment could impair thermogenesis by affecting transmission of the intracellular signal responsible for switching on the operation of the proton conductance pathway. It is possible that there is an abnormal membrane fluidity in BAT mitochondria of ob/ob mice which in turn might affect reception of the intracellular signal. Preliminary experiments show that the fatty acid composition of total lipids and phospholipids is different in the mitochondria of ob/ob mice with the major change being an increase in the proportion of arachidonic acid. The following values were obtained by gas-liquid chromatography and are

the means ± SE of four experiments for the percent arachidonic acid: ob/ob versus lean; 18.9 ± 0.9 versus 8.5 ± 0.6, $p < 0.001$ for total lipid, 15.9 ± 1.3 versus 5.6 ± 0.8, $p < 0.005$ for phosphatidylcholine, 26.7 ± 2.1 versus 16.2 ± 0.8, $p < 0.005$ for phosphatidylethanolamine. The phospholipid fatty acid composition of mitochondria from cold-acclimated and T_4-treated ob/ob mice would help to determine whether the increase in arachidonic acid contributes to defective thermogenic functioning of BAT.

That DIT is defective in chow-fed ob/ob mice is suggested by BAT mitochondrial GDP binding studies which show an equally low binding level in lean and ob/ob mice maintained at thermoneutrality. A higher GDP binding level would have been expected in ob/ob mice because of their known hyperphagia at this temperature (Coleman 1982, Lin et al. 1980; Thurlby, Trayhurn 1979). In contrast, cafeteria feeding of ob/ob mice promotes normal activation of BAT thermogenesis, as judged by the increase in mitochondrial GDP binding, as well as normal increases in metabolic and mitochondrial mass of the tissue. Moreover, cafeteria-fed ob/ob mice do not exhibit the lethal drop in body temperature observed in their chow-fed controls during exposure to 10°C for 24 hours (Himms-Hagen, J., unpublished observations). This is probably because sufficient heat is generated by BAT of these animals to maintain their body temperature.

The nutrient composition of the cafeteria diet is likely responsible for overcoming BAT refractoriness to NA, thereby allowing the tissue to respond to the diet-induced increase in sympathetic activity which is known to occur in BAT of cafeteria-fed ob/ob mice (Zaror-Behrens, G., Himms-Hagen, J., unpublished observations). It is conceivable that tissue responsiveness to NA is improved by a permissive effect of endogenous T_3 since the cafeteria diet causes an increase in blood T_3 levels. Other possibilities include a change in the lipid composition of mitochondrial and/or plasma membranes.

In summary, genetically obese (ob/ob) mice exhibit impaired NST and DIT (chow) because of an inherent defect(s) in the peripheral control and/or heat producing mechanisms in BAT. Acclimation to mild cold, long term T_4 treatment and cafeteria feeding activate operation of the proton conductance pathway, possibly through a permissive effect of endogenous T_3 on the thermogenic action of NA.

Hypothalamically obese rodents

In hypothalamically obese rodents (GTG-obese mice, VMH-lesioned rats, hypothalamic knife cut rats) activation of BAT thermogenesis by acute cold exposure occurs normally during the dynamic phase of obesity, as determined by the normal increase in mitochondrial GDP binding. Furthermore, cold acclimation of GTG-obese mice induces the usual changes

in BAT mitochondrial polypeptide composition and GDP binding as well as the adaptive tissue growth and mitochondrial proliferation. In addition, Seydoux and coworkers (1982b) have reported increases in mitochondrial GDP binding and the relative amount of the 32K polypeptide for cold-acclimated VMH-lesioned rats. These findings plus the observation that GTG-obese mice have a large thermogenic response to injected NA indicate that BAT of hypothalamically obese rodents is inherently functional and has an apparently normal sympathetic innervation.

However, hypothalamically-obese rodents exhibit defective DIT in BAT during the dynamic phase of obesity when hyperphagia is present. This is indicated by the low or normal mitochondrial GDP binding when they are fed a chow diet (GTG-obese mice, VMH-lesioned rats, hypothalamic knife cut rats) which is not altered by a cafeteria diet (GTG-obese mice, VMH-lesioned rats). Since peripheral control mechanisms appear to be normal in BAT of rodents with hypothalamic obesity, the most likely explanation for the failure of diet to induce thermogenesis is that hypothalamic lesions cause a defect in the central control mechanism regulating sympathetic activity in BAT. Indeed, others have shown that sympathetic activity is impaired in GTG-obese mice (Young, Landsberg 1980) and VMH-lesioned rats (Vander Tuig et al. 1982). The interruption in transmission

of dietary signals to BAT results from destruction of neural pathways in the VMH leading either to or from the diet-sensitive centers located in the CNS. At the same time, these animals retain the capacity to respond thermogenically to cold because BAT is inherently functional and the neural pathways responsible for transmission of thermal signals are not destroyed by lesions in the VMH. While the VMH has direct neural connections with BAT (Perkins et al. 1981; Shimazu, Takahashi 1980), the fact that transmission of thermal signals from cold-sensitive centers is normal indicates that other hypothalamic or extrahypothalamic regions participate in the central control of BAT thermogenesis. Whether there is an integrative center within the CNS which coordinates the input and output signals for switching on or off thermogenesis is not known.

However, an integrative center would help to explain other observations of alterations in BAT thermogenic functioning in association with changes in metabolic efficiency which are independent of the normal response to diet and cold. For example, lactating mice are hyperphagic but fail to exhibit either DIT or NST (Trayhurn et al. 1982a). Mitochondrial GDP binding by BAT of mice at mid-lactation and maintained at 21°C is more than 4 times lower than that of normophagic virgin mice at the same temperature and similar to virgin mice at thermoneutrality (Trayhurn et al. 1982a).

Similarly, although hibernators have a high capacity for NST, they can switch off BAT thermogenesis during the hibernating state, allowing their body temperature to drop to about 5°C, and switch it back on during arousal (Cannon, Johansson 1980). Moreover, hamsters appear to shut down thermogenesis in BAT when consuming a high fat diet, since body fat content increases without overeating and resting metabolic rate declines (Wade 1982; 1983). In addition, chronic food restriction of rats is associated with a decrease in mitochondrial GDP binding, resting metabolic rate and thermogenic response to exogenous NA (Rothwell, Stock 1982c) indicating a reduction in amount and activity of BAT under these conditions. It seems likely that BAT thermogenesis remains switched off during the initial period of recovery from food restriction since it has been shown that food deprived rats exhibit a high metabolic efficiency (Boyle et al. 1981; Björntorp, Yang 1982) and a low resting metabolic rate (Boyle et al. 1981) during the first few days of refeeding. Studies of BAT of animals with various brain lesions should help to identify the different regions in the CNS which participate in the central control of BAT thermogenesis as well as the network of neural pathways involved in the transmission of various signals to and from BAT.

The above discussion has described a defect in the thermogenic functioning of BAT in animals with hypothalamic and genetic obesity. Since it is clear that an impairment

in BAT thermogenesis reflects a reduction in energy expenditure and because these animals manifest a high metabolic efficiency, it may be concluded that this impairment contributes to the development and maintenance of their obesity.

Brown adipose tissue and altered states of energy balance

It is important to note that the findings of the work described in this thesis concur with research done on other animals. In recent years, studies of obese rodents have provided the major impetus for the growing recognition that BAT is involved in energy balance. In many animals the obesity develops not only because of an impairment in the control of food intake, but also because of abnormalities in energy expenditure (see literature review Part IV). Although reduced in obese rodents (Bray, York 1971a), physical activity is only a small component of the total energy expenditure (Miller, Mumford 1966). For instance, in adult lean mice living at 20-25°C, the major determinants of energy expenditure are the basal metabolic rate and thermoregulatory thermogenesis which account for approximately 36% and 38% respectively of the total energy expenditure. The amount attributed to physical activity is 10% and to DIT is 16% (Trayhurn et al. 1981). It is apparent that a reduction of energy expenditure in BAT without compensatory adjustments of food intake could contribute significantly

to positive energy balance and obesity.

In addition to the obese animals described above, impaired BAT functioning and a high metabolic efficiency have been described in the diabetic-obese (db/db) mouse and the fatty (fa/fa) Zucker rat. The db/db mouse, like the ob/ob mouse, has a low level of mitochondrial GDP binding in BAT when kept at 23°C (Goodbody, Trayhurn 1981). This low state of thermogenic activity, also observed in the preweanling animal (Goodbody, Trayhurn 1981), reflects an impairment in NST since the db/db mouse fails to survive severe cold (Trayhurn 1979). That the thermogenic defect resides in BAT is indicated by the reduced response to exogenous NA. The db/db mouse thus resembles the ob/ob mouse. In contrast, the young fa/fa rat resembles the hypothalamically obese animals, in that it manifests defective DIT in BAT, since mitochondrial GDP binding is low in this animal at thermoneutrality and fails to increase with cafeteria feeding (Triandafillou, Himms-Hagen 1983), but can activate BAT thermogenesis in response to acute cold, suggesting that the defect is located in the CNS (Triandafillou, Himms-Hagen 1983).

Just as defective BAT thermogenesis can be correlated with a high metabolic efficiency and obesity, effective BAT thermogenesis can be correlated with a low metabolic efficiency and leanness. Thus, the cold-acclimated rat approximately doubles its food intake and yet remains lean

(Hardeveld et al. 1979) because the excess food is dissipated as heat in BAT mitochondria (Foster, Frydman 1978b, 1979; Desautels et al. 1978) for maintenance of body temperature in the cold. Likewise, the hyperphagic cafeteria-fed rat does not deposit the expected excess energy (Rothwell, Stock 1979b; 1982a; 1982b; 1982d) because of an increase in BAT thermogenesis (Rothwell, Stock 1981b; Himms-Hagen et al. 1981; Brooks et al. 1980; 1982). Similar observations have been reported for the cafeteria-fed mouse (Trayhurn et al. 1982a). It is interesting to note that when interscapular BAT is removed from cafeteria-fed mice, fat deposition is no greater than their intact cafeteria-fed controls. But this is associated with a compensatory growth of BAT in other locations such that the total capacity for BAT thermogenesis likely remains the same (Connolly, Carnie 1982). Other examples can be cited which link BAT to energy balance. For instance, tumor-bearing mice become cachectic 8-9 weeks after implantation, despite normal food intake. This coincides with a dramatic increase in metabolic rate both at rest and in capacity for a response to exogenous NA. Although the metabolic mass of BAT does not change, there is an increase in mitochondrial GDP binding indicating that the tissue is in a thermogenically active state (Brooks et al. 1981). During nutritional rehabilitation, rats that had been undernourished for the first 3 postnatal weeks gain less weight than that of control rats, despite a similar intake

of food (Rothwell et al. 1982f). Since rehabilitation is associated with an increased thermogenic response to injected NA and an increase in resting metabolic rate which is almost completely blocked by propranolol (Rothwell et al. 1982f), it is possible that the low weight gain may in part result from an increase in BAT thermogenesis.

In conclusion, the functional activity of BAT in rodents can be correlated in a general way with the degree of obesity or leaness and as such must be considered important in the regulation of energy balance.

APPENDIX A-1

MENUS FOR CAFETERIA DIETS

MENU	MICE	RATS[+]
1	ham sandwich	polish sausage
	corn chips	ragatoni
	chocolate mallow cookie	peanut butter cream cookie
	licorice candy	
2	peanut butter and jam sandwich	hot dog
	bugle (snack food)	pretzle
	gum drop	licorice candy
	short bread cookie	chocolate chip cookie
3	liver and bacon spread sandwich	salami
	soda cracker	swiss cheese crackers
	Lucky Charm cereal	baby food (peaches 'n cream)
	Chocolate wafer	chocolate mallow cookie
4	cheese sandwich	chicken dog
	potato chip	tortilla chip
	chocolate chip cookie	honey donut
	marshmallow	short bread cookies
5	head cheese sandwich	cheese slice
	cheese twist	bacon dipper
	chocolate vanilla cookie	gum drop
	jujube	chocolate vanilla cookie

[+]Cafeteria fed rats also received condensed milk diluted 1:1 with tap water.

APPENDIX A-2

COMPOSITION OF CAFETERIA FOODS (MENU #5) AND CHOW (#5015)

Food[a]	Weight g	Energy[b] Kcal	Protein g	Carbohydrate g	Fat g
chocolate vanilla cookie	14	70	0.8	9.2	3.3
gum drop	28	97	tr	24.5	0.2
cheese	28	107	6.5	0.5	8.4
bacon dipper	8	43	0.6	5.1	2.3
condensed milk	100	321	8.1	54.3	8.7
chow	100	400	22.5	53.2	4.5

[a]Composition of the cafeteria foods were obtained from Bowes and Church's Food Values of Portions commonly used (Pennington, Church 1980). Values for the chow were obtained from the Ralston Purina Co.

[b]Value for the chow is the gross energy content.

APPENDIX B-1

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

APPENDIX B-2

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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