UNIVERSITY OF SOUTHAMPTON

REGULATION OF BROWN ADIPOSE TISSUE BY THE SYMPATHETIC NERVOUS SYSTEM IN THE GENETICALLY OBESE (fa/fa) ZUCKER RAT.

A thesis presented for the degree of
Doctor of Philosophy
by

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ABSTRACT

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NUTRITION

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The obesity of the fa/fa rat is closely linked to impaired brown adipose tissue (BAT) thermogenesis and a failure to exhibit the normal adaptive increases in BAT thermogenic function after overfeeding. The role of the sympathetic nervous system in this defect was assessed by calculating rates of noradrenaline turnover from the time-dependant decline of [^{3}H]-noradrenaline specific activity after administration of radio-labelled noradrenaline. BAT noradrenaline concentration and turnover were reduced in obese (fa/fa) rats and this was associated with reduced uptake of noradrenaline *in vivo* and *in vitro*, reduced synthesis of noradrenaline from tyrosine and a reduced central sympathetic outflow to BAT. The defect appeared to be specific to BAT, since sympathetic activity was not decreased in heart. These findings were consistent with an inability to activate the sympathetic nerve supply to BAT in response to dietary signals, since sympathetic activity in BAT of obese rats was not affected by sucrose overfeeding but increased on cold acclimatisation to the same levels found in cold acclimatised or sucrose-overfed lean rats.

Adrenalectomy increased sympathetic activity in BAT to the levels found in lean rats, but was not associated with any further increases in BAT noradrenaline turnover on sucrose overfeeding in either lean or fa/fa adrenalectomised rats. It is suggested that in these animals, sympathetic activation of BAT is required to maintain tissue sensitivity to an, as yet, unknown humoral effector of thermogenesis. This contrasted with the findings in Sprague-Dawley rats, in which increases in BAT thermogenesis, induced by cafeteria feeding and/or adrenalectomy, were closely paralleled by proportional increases in sympathetic activation of BAT.

The insulin secretory response to a glucose load was increased in obese (fa/fa) rats, suggestive of increased vagal activity. This response was reduced to normal by adrenalectomy suggesting that there is an increased parasympathetic drive in obese rats, resulting from a sensitivity to circulating corticosterone. The possible role of adrenal glucocorticoids as mediators of an imbalance between the sympathetic and parasympathetic branches of the autonomic nervous system, leading to reduced BAT thermogenesis, hyperinsulinaemia and consequent obesity, is discussed.

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ABBREVIATIONS

ABTS 2,2' azino3di [ethyl benzthiazoline sulphonate (6)] disodium salt

ACTH adrenocorticopic hormone

acyl CoA acyl coenzyme A

ADP adenosine diphosphate

ATP adenosine triphosphate

BAT brown adipose tissue

BSA bovine serum albumen (Fraction V)

cAMP adenosine 3',5'-cyclic monophosphate

COMT catechol-O-methyl transferase

DIT diet-induced thermogenesis

EDTA ethylenediaminetetraacetic acid

FAD/H_2 flavin adenine dinucleotide/reduced form

FFA free fatty acids

FMN/H_2 flavin mononucleotide/reduced form

GDP guanosine diphosphate

GTP guanosine triphosphate

i.p. intra-peritoneal

i.m. intra-muscular

k fractional turnover rate (rate constant of decline)

Kd dissociation constant

LH lateral hypothalamus

MAO monoamine oxidase

MH medial hypothalamus

NA noradrenaline

NAD/H nicotinamide adenine dinucleotide/reduced form

NST non-shivering thermogenesis

P.R.D. purina rat diet

PVN paraventricular nucleus

RNA ribonucleic acid

S.C. sub-cutaneous

SDS sodium dodecyl sulphate

$t_{\frac{1}{2}}$ half-life

T_3 triiodothyronine

T_4 thyroxine

Tris/HCl tris hydroxymethyl amino methane/hydrochloric acid

U_1 $Uptake_1$

U_2 $Uptake_2$

VMH ventromedial hypothalamus

VMN ventromedial nucleus

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CHAPTER 1.

INTRODUCTION.

1.1 Obesity

Obesity is a condition in which there is excessive storage of lipid in the adipose tissue depots of the body. What constitutes excessive varies considerably amongst different cultures but, generally, anyone 20% or more above their ideal body weight can be considered to be obese. Excessive obesity may shorten the life span, with an increased incidence of atherosclerosis, hypertension, gall bladder disease, non-insulin dependent diabetes mellitus and psychological disturbances. Even slight obesity can result in increased morbidity and mortality (Burton et al., 1985).

Obesity results from a disturbance in energy balance, where energy intake is increased relative to energy expenditure. Energy consumed as food in excess of requirements will be retained as chemical energy if not expended in any other way. In the non-growing adult triacylglycerols are the only major form of energy storage. These are stored mainly in adipose tissue. A positive energy balance may result from an increased energy intake, decreased energy expenditure, or a combination of the two. The regulation of the body's fat stores probably involves control of both energy intake and energy expenditure. In normal adults body weight remains relatively constant despite wide fluctuations in both energy intake and expenditure (Bray, 1976). In a normal population, food intake may fluctuate by up to 2-fold in subjects of the same age and weight (Rose and Williams, 1961).

1.2 Control of energy intake.

The overall control of energy intake in the form of food is integrated in the central nervous system, in particular, specific areas of the hypothalamus have an important role in the regulation of appetite. Stimulation of the lateral hypothalamus (LH) initiates feeding (Delgado and Anand, 1953)

whereas lesions produce a state of anorexia (Anand and Brobeck, 1951). Stimulation of the ventromedial hypothalamus (VMH) reduces food intake (Wyrwicka and Dobrzecka, 1960) and lesions of this area result in hyperphagia (Brooks et al., 1946). These experiments led to the dual-centre hypothesis in which it is envisaged that the VMH acts as a satiety centre, while the LH acts as a hunger or feeding centre, and a balance between the two centres controls feeding behaviour and food intake. This concept is an oversimplification. A number of nervous system tracts associated with the VMH and the LH also play important roles in the regulation of food intake. The ventral adrenergic bundle may be more important than the VMH itself in regulating food intake, since lesions of the VMH only result in hyperphagia if they also damage fibre tracts in the ventral adrenergic bundle (Gold, 1973). Similarly, the anorexia associated with lesions of the LH may result from damage to the nigro-striatal bundle (Ungerstedt, 1970). In addition, there is cross innervation between the two centres, activation of one inhibits the other, and damage to one releases inhibition of the other (Oomura et al., 1969). Lesions between the VMH and LH result in hyperphagia, suggesting that the LH is under tonic inhibition by the VMH (Albert et al., 1971). For recent reviews on the central regulation of feeding see Le Magnen (1983) and Rolls (1984).

The hypothalamic centres responsible for the regulation of food intake are controlled by neural, hormonal and metabolic signals from the periphery, including the liver and gut. Gastric distension, operating via the vagal nerves decreases feeding (Paintal, 1954) and this mechanism probably operates in conjunction with release of intestinal peptides such as cholecystokinin (Kissileff et al., 1981), Bombesin (Gibbs et al., 1979) and neurotensin (Mashford et al., 1978) to elicit the full behavioural responses associated with satiety. Glucose is important in the regulation of food intake. Insulin sensitive glucoreceptors in the VMH may be stimulated by increased plasma glucose or insulin (Anand et al., 1964; Oomura et al., 1978). 2-deoxy-D-glucose, a glucose analogue

that blocks glucose utilisation, blocks these glucoreceptors and so reduces the firing rate of ventromedial neurones, initiating feeding (Desiraju et al., 1968). It has also been suggested that variations in liver glycogen levels are communicated to the brain via the vagus (Niijima, 1969). Peripheral administration of the essential amino acid tryptophan reduces food intake and this is thought to be brought about through its uptake across the blood brain barrier and subsequent conversion to 5-hydroxytryptophan in the brain (Fernstrom and Wurtman, 1972).

The mechanism by which the body monitors its energy stores as a factor in the regulation of food intake and energy balance is not known. Some workers suggest that the hypothalamus can monitor fat depots through steroid or prostaglandin release (Hervey, 1969; Baile et al., 1973), while others suggest monitoring of intracellular hypothalamic lipid content (Van Itallie et al., 1977). Energy intake is precisely regulated and normally remains constant over a wide range of dietary energy densities (Adolph, 1947). This led to the assumption that energy intake was the major controlling factor in the regulation of energy balance and that hyperphagia was the primary cause of obesity. However, obese subects do not necessarily eat more than lean and frequently eat less (McCarthy, 1966; Maxfield and Konishi, 1966). In a normal population there is no linear correlation between energy intake and body weight and large eaters often weigh less than small eaters (Miller, 1965). Laboratory animals can easily be induced to overeat by feeding palatable diets and do not necessarily gain weight (Rothwell and Stock, 1979a). These observations suggest that a reduced energy expenditure may be of more importance than increased energy intake in the maintenance of a positive energy balance, resulting in obesity.

1.3 Energy expenditure.

Energy expenditure can be divided into several components; basal metabolic rate, physical activity and heat production induced by cold or diet. The basal metabolic rate is the energy

cost of keeping the body alive and accounts for 50-60% of daily energy expenditure. Though basal metabolic rate may be decreased in some obese subject (Miller and Parsonage, 1975), other studies have found similar or increased values (James et al., 1978; Hoffmans et al., 1979; Schutz et al., 1984). Obese subjects are often, but not necessarily, less active than lean, but the energy costs of moving are greater in the obese (Miller and Parsonage, 1975).

Cold-induced thermogenesis includes both shivering and non-shivering thermogenesis (NST). In order to maintain body temperature in an environment below thermoneutrality (about 28^{o}C for man) heat must be produced. Shivering is an acute muscular response to cold exposure which is replaced by NST during the acclimatisation to cold. Obese subjects exhibit a greater fall in core temperature (Andrews and Jackson, 1978) and a smaller increase in metabolic rate (Buskirk et al., 1963), compared to lean subjects, when exposed to reduced environmental temperatures. This suggests that the thermoregulatory response is reduced in obese subjects.

Diet-induced thermogenesis (DIT) can be divided into two components, the short term effects of a meal (the specific dynamic action or thermic effect of feeding) and long term adaptive changes to increases in food intake. The thermic effect of feeding was thought to simply comprise the energy costs of transporting and assimilating nutrients and synthesising macromolecules for storage, but there is now evidence that there is an adaptive component. The thermic effect of feeding is reduced in obese subjects (Kaplan and Leveille, 1976; Schutz et al., 1984) and increased in lean, large eaters (Morgan et al., 1982). In the long term, resting metabolic rate increases on overfeeding (Garrow, 1974) and decreases on underfeeding (Keys et al., 1950).

Dietary manipulation can produce large variations in energy expenditure in animals. Feeding of low protein diets to pigs can produce a 5-fold increase in food intake without increases in body weight, indicative of a large capacity for DIT (Gurr et al., 1980), and similar effects have been found

in rats (McCracken and Gray, 1976) and man (Miller and Mumford, 1967). If offered a highly palatable diet rats will be induced to overeat by up to 80%, without necessarily increasing body weight (Rothwell and Stock, 1979a). Work with animal models of obesity, which will be discussed in detail in section 1.5, has suggested that a reduced energy expenditure may be more important than increased food intake in the production of a positive energy balance. Overfed animals become obese when their capacity for DIT is exceeded. The development of the obese state in the hypothalamic and genetic models of obesity is not dependent upon the hyperphagia found in these animals but seems to be related in part to defects in thermoregulatory NST and/or DIT, combined with hypersecretion of insulin, resulting in increased lipogenesis and lipid deposition. Consequently much work has centred on the mechanisms and regulation of NST and DIT.

1.4 Brown adipose tissue.

Brown adipose tissue (BAT) was first demonstrated to be thermogenic in 1961 (Smith) and has now been established as the main site of both NST and DIT in the rat (Foster and Frydman, 1978; Rothwell and Stock, 1979b, 1981a). BAT has been identified in the majority of species of mammal and is most obvious in small mammals, neonates, hibernators and some non-hibernators acclimatised to cold (Rowlatt et al., 1971). BAT is located in small discrete depots at various sites in the body, in the interscapular, subscapular and axillary regions, around the heart, kidneys and aorta and between the muscles of the neck, particularly around the carotid arteries and jugular veins (Smith and Horwitz, 1969). The amount of BAT and its distribution varies considerably with species. In small rodents, BAT accounts for 1-2% of body weight, the largest and most accessible depot being in the interscapular region (Smith and Horwitz, 1969), so experimental work has tended to concentrate on this depot.

1.4.1 Composition of BAT.

BAT is characterised by multilocular adipocytes with an abundance of mitochondria, as opposed to the unilocular cells of white adipose tissue (Smith and Horwitz, 1969). BAT mitochondria have a distinctive appearance with tightly packed cristae which usually traverse the whole width of the mitrochondrion (Flatmark and Pedersen, 1975). BAT mitochondria possess a high activity of respiratory chain enzymes but only low activity of ATP synthetase (Flatmark and Pedersen, 1975). It is the high concentration of cytochromes, haems and flavins that give BAT its brownish colour, as well as its dense blood capillary network. The colour of BAT may vary from pale buff to a dark reddish brown, depending on the cellular lipid content and the state of activation and blood flow of the tissue (Afzelius, 1970).

Each BAT mass contains up to 80%, by cell number, brown adipocytes, organised into discrete lobules surrounded by connective tissue, with an extensive vascular supply and numerous sympathetic nerves terminating on the blood vessels and adipocytes (Barnard, 1977). The plasma membranes of some adipocytes may be joined to form gap junctions (Schneider-Picard et al., 1980) allowing the exchange of ions and small molecules and the spread of nerve stimulation throughout the tissue.

1.4.2 Blood supply of BAT.

After intravenous administration of noradrenaline BAT may receive up to 30% of total cardiac output (Foster and Frydman, 1978b), yet it comprises only 1-2% of body weight. The vasculature is the main extracullular compartment in BAT, being 4-6 times denser than that of white adipose tissue (Hauseberger and Widelitz, 1963). Dense capillary networks surround the cells and up to a third of the cell surface area of each brown adipocyte is in contact with capillary walls (Aherne and Hull, 1966). Interscapular BAT receives its blood from the axillaries via the cervical trunk and thoracodorsal

arteries. Venous drainage is bilateral, via the thoracodorsal veins into the subclavian and by the large central veins that empty into the inner vertebral plexus. This central unpaired venous drainage into the thorax is normally designated "Sulzer's vein" (Smith and Horwitz, 1969). Venous return in man is rather more complex, numerous veins leave the tissue and join the drainage of the back muscles (Aherne and Hull, 1963).

1.4.3 Innervation of BAT.

Anatomically, innervation to BAT appears to be predominantly sympathetic, although somatic fibres may be present (Smith and Horwitz, 1969). Experiments involving transection or stimulation of the spinal cord at various levels indicate that the spinal nerves C_3, C_4 and C_5 supply the cervical and interscapular BAT, together with medial branches from C_1 to C_5 that communicate with cranial nerves X and XII and the sympathetic system. In addition interscapular BAT may be innervated by the first five sympathetic branches of the thoracic chain (Smith and Horwitz, 1969). In the rat, five or six nerve fibre bundles containing morphologically heterogeneous fibres enter interscapular BAT bilaterally on the ventrolateral surface of each pad (Flaim et al., 1976). These bundles, which arise through the intercostal muscles beneath the pad, pass on through the pad to the skin and subcutaneous white adipose tissue dorsal and lateral to the pad (Foster et al., 1982a). Only the first four anterior bundles contribute significantly to the sympathetic innervation of the interscapular BAT pads and the fibres supplied by a given intercostal nerve are not distributed evenly throughout the tissue (Foster et al., 1982b). The nerve fibres supply both the blood vessels and the brown adipocytes. Histochemical fluorescence studies have shown that almost all the brown adipocytes are enclosed by a delicate network of adrenergic terminals (Wirsen and Hauseberger, 1967). The neurones supplying the blood vessels have long post-ganglionic fibres whereas those terminating on the brown adipocytes usually have relatively

short post-ganglionic fibres which could arise from intrinsic ganglia (Smith and Horwitz, 1969). On the basis of histochemical fluoresence studies of sympathetic fibre density, after unilateral and bilateral denervation of interscapular BAT, it has been calculated that 25% of the fibres in each pad result from cross innervation from the other pad and 15% are either intrinsic to the tissue or are supplied by nerve tracts running along the blood vessels (Seydoux et al., 1977). In denervation studies in which thermogenic activity of the pads measured *in vivo* and noradrenaline content and dopamine β-hydroxylase activity were assessed as indexes of the sympathetic innervation, Foster et al. (1982a) concluded that, functionally, the innervation was completely unilateral.

1.4.4 The mechanism of thermogenesis in BAT.

The primary stimulus for BAT thermogenesis is noradrenaline released from the sympathetic nerves in the tissue. Release from the adrenal medulla is probably not important in thermoregulation, since the concentration range of noradrenaline required at the synapse to regulate thermogenesis is 30-150 fold greater than that of circulating noradrenaline (Girardier and Seydoux, 1977). The concentration of noradrenaline required at the synapse to elicit a half-maximal respiratory response is 10^{-8}M (Pettersen and Vallin, 1976). In addition, removal of the adrenal medulla causes only a slight impairment of NST (Himms-Hagen, 1975), adrenal medullary release is probably only important in the acute response to severe cold.

The response of BAT to nerve stimulation is graded, depending on the frequency of stimulation (Flaim et al., 1976). The tissue is activated at frequencies as low as 0.1Hz. At frequencies greater than 3Hz the tissue depolarises up to a maximum response at 10Hz (Girardier and Seydoux, 1977).

On nerve stimulation of BAT there is a small transient decrease in BAT temperature, thought to be caused by an α-mediated vasoconstriction. The vasodilation that accompanies thermogenesis has been assumed to be mediated by β-receptors since blood flow is stimulated by noradrenaline administration

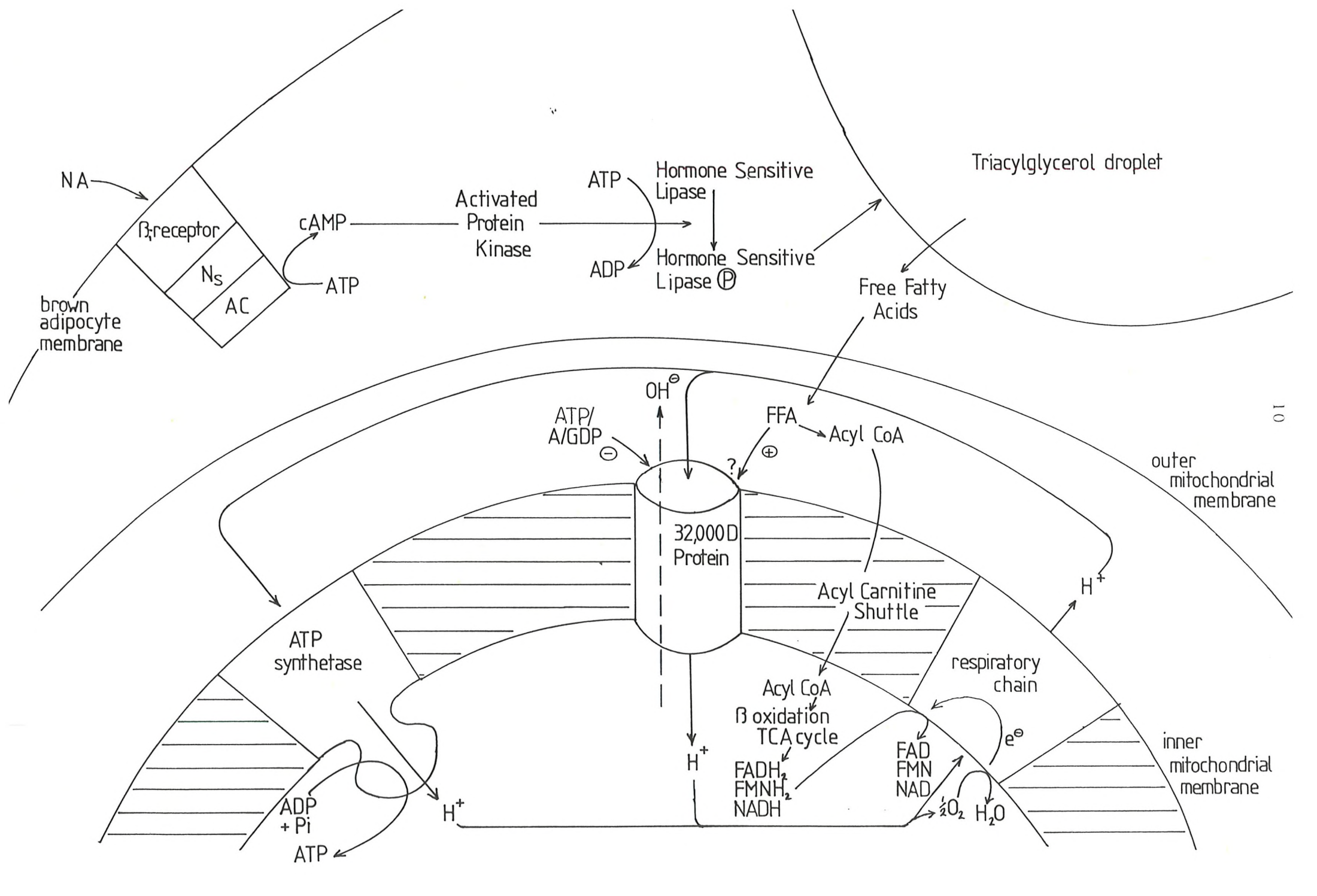
and inhibited by β-blockade (Flaim et al., 1977). However, Foster and Depocas (1981) found that blood flow in thermogenically active tissue was independant of noradrenaline concentration but was related to arterial oxygen content and concluded that intracellular oxygen tension may be linked to the production of a vasodilator. Histamine has been suggested for this role since it occurs in high concentration in BAT (Stock and Westerman, 1963) and the H_2-receptor antagonist cimetidine inhibits blood flow by 50% in stimulated BAT (Rothwell et al., 1984a).

The stimulation of thermogenesis in brown adipocytes by noradrenaline seems to be largely through interaction with β-receptors. Brown adipocytes possess a large number of specific, high-affinity, tritiated dihydroalprenalol binding sites (Svoboda et al., 1979) and binding studies indicate that these are mainly β_1-specific (Buckoweicki et al., 1980). The binding sites have a Kd of 1.08nM for dihydroalprenalol and the apparent dissociation constant for noradrenaline is 176nM. However, for half-maximal respiration to occur a receptor occupancy of less than 10% is required, indicating the presence of spare receptors. This leads to an apparent Kd for noradrenaline induced stimulation of respiration of about 6nM (Svoboda et al., 1979). Other adrenergic receptor types may have a role in BAT thermogenesis, Mohell et al. (1983) have demonstrated that only 80% of the noradrenaline stimulated thermogenesis can be accounted for by β_1-receptors and that the remainder is mediated through α_1-receptors. Neither β_1- nor β_2- antagonists completely block BAT thermogenesis in noradrenaline treated cold acclimatised rats but completely block in combination, suggesting a mixed β_1/β_2-response <u>in vivo</u> (Rothwell et al., 1982b). Recent work with a new β- agonist suggests that the brown adipocyte β-receptor may be atypical and conform to neither β-subtype (Arch et al. 1984). BAT activity could also be modified by α_2-receptors acting either presynaptically or post-synaptically. Presynaptic α_2-antagonists such as ciclazindol and mazindol inhibit noradrenaline re-uptake and stimulate metabolic rate

Fig. 1. 1 The mechanism of thermogenesis in BAT.

Noradrenaline binds to the β_1-receptor and, through dissociation of the guanine nucleotide binding protein, activates adenylate cyclase. Intracellular cAMP rises and cAMP-dependant protein kinases are activated, one of which activates the hormone sensitive lipase, and free fatty acids are released into the cytoplasm. The free fatty acids provide a substrate for respiration via β-oxidation in the mitochondria and remove the purine nucleotide inhibition of the 32000D uniport, dissipating the proton gradient and thereby uncoupling respiration from ATP synthesis.

NA = noradrenaline
N_s = stimulatory guanine nucleotide binding protein
AC = adenylate cyclase
cAMP = cyclic AMP
FFA = free fatty acids.


NA
β1receptor
Ns
AC
cAMP
ATP
Activated
Protein
Kinase
ATP
ADP
Hormone Sensitive
Lipase
Hormone Sensitive
Lipase Ⓟ
Triacylglycerol droplet
Free Fatty
Acids
brown
adipocyte
membrane
OH⊖
ATP/
A/GDP
⊖
FFA
Acyl CoA
?
⊕
outer
mitochondrial
membrane
32,000D
Protein
Acyl Carnitine
Shuttle
H+
ATP
synthetase
respiratory
chain
Acyl CoA
β oxidation
TCA cycle
FADH2
FMNH2
NADH
FAD
FMN
NAD
e⊖
inner
mitochondrial
membrane
H+
H+
ADP
+ Pi
ATP
½O2
H2O

and BAT activity in the rat (Rothwell et al., 1981). Although the relative roles of α_1, α_2 and β_2-receptors is not yet clear there is no doubt that the major effects of noradrenaline are mediated through β_1-receptors.

Within one second of noradrenaline administration and its binding to brown adipocytes there is a change in membrane potential and ion conductance (Girardier and Seydoux, 1977). When noradrenaline binds to the receptor it interacts with the guanine nucleotide binding protein, the α-subunit of which binds to GTP and dissociates from the γ- and β-subunits. The dissociated α-subunit activates adenylate cyclase in the plasma membrane and the concentration of cAMP rises (Petersen and Vallin, 1976) (see Fig. 1.1). BAT contains eight cAMP-dependant protein kinases (Knight and Skala, 1977). One of these kinases is responsible for phosphorylating inactive hormone sensitive triacylglycerol lipase and so converting it into its active form (Skala and Knight, 1977). The hormone sensitive triacylglycerol lipase hydrolyses triacylglycerols to diacylglycerols and free fatty acids. Further lipases catalyse the subsequent hydrolysis of diacylglycerols to free fatty acids and glycerol.

The substrates for the hormone sensitive lipase are the multilocular triacylglycerol droplets in the brown adipocytes. These triacylglycerols may be supplied from a number of sources. The rates of fatty acid synthesis from carbohydrate are very high in BAT, accounting for up to one third of total body lipogenesis when in a thermogenic state (Trayhurn, 1981). This process probably proceeds via an insulin mediated stimulation of glucose transport, pyruvate dehydrogenase and acetyl CoA carboxylase, thus stimulating glycolysis and the conversion of acetyl CoA into fatty acids (McCormack and Denton, 1977). Ketone bodies may also act as substrates for lipogenesis since incorporation of hydroxybutyrate into lipid is about 30 times greater in BAT than in white adipose tissue (Agius and Williamson, 1981). BAT has a high lipoprotein lipase activity, indicative of a large capacity to take up exogenous lipid from the blood, and when in a thermogenic

state imports up to 50% of its lipid (Trayhurn, 1980). Lipoprotein lipase activity varies with thermogenic state, being stimulated on cold exposure and noradrenaline administration, acting through β-receptors (Carneheim et al., 1984).

The free fatty acids released by noradrenaline stimulation of β_1-receptors provide the major substrate for brown adipose tissue respiration which proceeds via the β-oxidation pathway in the mitochondria (Nicholls, 1979). Fatty acids are transported into the mitochondria as acyl carnitine ~~acyl CoA~~, the activity of the acyl carnitine shuttle in BAT being more than sufficient to supply the required substrates for β-oxidation (Pedersen et al., 1975; Norman and Flatmark, 1978). β-oxidation yields acetyl CoA, NADH and $FADH_2$ (see Fig. 1.1).

Glucose may be an important substrate for BAT when in a thermogenic state. BAT possesses a high activity of the glycolytic enzymes hexokinase, phosphofructokinase and pyruvate kinase. Noradrenaline administration stimulates glucose oxidation by activation of pyruvate dehydrogenase, probably via α_1-receptors, yielding acetyl CoA (Gibbins et al., 1985). Noradrenaline administration also reduces acetyl CoA carboxylase activity, acting through β-receptors (Gibbins et al., 1985). Thus, fatty acid synthesis is reduced, in favour of further degradation of the acetyl CoA from glucose and free fatty acids to carbon dioxide, producing further reducing equivalents, in the tricarboxylic acid cycle which has a high activity in BAT (Cannon and Johansson, 1980). As NADH and the reduced flavoproteins are reoxidised by the respiratory chain, protons are pumped out across the inner mitochondrial membrane, which is relatively impermeable to protons, so setting up a proton electrochemical gradient which provides the motive force for ATP synthesis via ATP synthetase (Fig. 1.1). Respiration is normally controlled by the availability of ADP for ATP synthesis so dissipating the proton gradient. When ADP is limiting the proton gradient builds up and the rate of respiration decreases. In BAT mitochondria ATP synthetase activity is very low, and

rates of ATP synthesis are too low to account for the observed rates of respiration (Bulychev et al., 1972). The inner membrane of BAT mitochondria possesses a unique 32000D protein, the concentration of which varies with the thermogenic capacity of the tissue (Ricquier and Kader, 1976; Ashwell et al., 1983, 1984). This protein can act as a high conductance ion uniport, dissipating the proton gradient and uncoupling respiration from phosphorylation (Heaton et al., 1978) (Fig. 1.1). If respiration is uncoupled from phosphorylation, respiratory control is lost and the rate of respiration will be dependant upon the provision of substrates and the activity of the respiratory chain.

Purine nucleotides, particularly GDP, ATP and ADP, bind to the 32000D protein. [^{3}H]-GDP binding correlates closely with the thermogenic capacity of the tissue and is widely used to assess BAT thermogenesis (Nicholls, 1976). In isolated BAT mitochondria, the 32000D uniport is inhibited by micromolar concentrations of exogenous adenine dinucleotides (Pedersen, 1970). Since the intracellular concentration of adenine dinucleotides is in the millimolar range (Pedersen & Grav, 1972), then mitchondria in vivo would be fully coupled. The mechanism by which the uniport opens and the mitochondria become uncoupled in the thermogenic state has not been fully elucidated. When rats are briefly exposed to cold or injected with noradrenaline, isolated BAT mitochondria show a large increase in purine nucleotide binding, presumed to be associated with an ultrastructural change and unmasking of binding sites present in the membrane (Desautels and Himms-Hagen, 1980). Thus,purine nucleotide binding is not a measure of the concentration of the 32000D protein but provides a measure of the available binding sites. The unmasking response and subsequent uncoupling of the mitochondria is thought to be mediated by noradrenaline via an intracellular messenger, and a dual role of free fatty acids as both substrates and uncouplers has been proposed. Isolated mitochondria are normally uncoupled, but if fatty acids are removed

from the medium by albumin, normal respiratory control is regained (Bulychev, 1972). Free fatty acids are able to uncouple BAT mitochondria in the presence of millimolar concentrations of adenine nucleotides and mimic the respiratory effects of the normal response to noradrenaline (Locke et al., 1982). The low concentration of fatty acids required to increase proton conductance, around 0.2nM, is likely to be within the physiological range of unbound free fatty acids in the cytosol of brown adipocytes (Locke et al., 1982). The effect of free fatty acids on respiration in BAT mitochondria is readily reversible and relatively tissue specific, increasing proton conductance 30 times more in BAT mitochondria than in liver mitochondria (Heaton and Nicholls, 1976). It is not known whether free fatty acids exert their effects through direct action on the 32000D protein or through interaction with the mitochondrial membrane (Locke et al., 1982). The binding of purine nucleotides and fatty acids to BAT mitochondria are apparently independant. Fatty acid administration does not affect the affinity or capacity of the 32000D protein for purine nucleotide binding (Nicholls, 1976) and palmitate or oleate binding to BAT mitochondria is not affected by GDP (Heaton and Nicholls, 1976). It is envisaged that, in the resting state, the presence of purine nucleotides keeps the uniport blocked and hence respiration remains coupled to phosphorylation and normal respiratory control is achieved. On stimulation of lipolysis the release of free fatty acids opens the uniport, respiration is uncoupled from phosphorylation and respiratory control is lost (Locke et al., 1982) (see Fig. 1.1). It has not yet been demonstrated that intracellular free fatty acid concentrations increase and decrease along with the thermogenic response.

1.4.5 BAT as the tissue effector of NST.

NST is initiated by noradrenaline released from sympathetic nerve endings. The increase in NST in cold acclimatised rats is blocked by the β-receptor antagonist propranolol (Rothwell and Stock, 1980) and by ganglion blockers such as hexamethonium (Hsieh et al., 1957). NST may be restored in ganglion-blocked animals by noradrenaline administration (Hsieh et al., 1957). The increases in NST during cold acclimatisation are paralleled

by an increased capacity to increase metabolic rate in response to noradrenaline administration (Jansky, 1973). Thus, noradrenaline administration may be used to assess the capacity for NST.

BAT was demonstrated to be thermogenic in 1961 (Smith). However, due to its small size and apparent low blood flow during cold-induced thermogenesis, BAT was thought to be of importance only in hibernators and neonates (Jansky and Hart, 1968). In these studies, blood flow was measured by $^{86}Rb^{+}$ uptake which, due to slow rates of tissue uptake, severely underestimated blood flow to organs with a high flow rate (Foster and Frydman, 1978a). In contrast, radioactively labelled microspheres, introduced into the left atrium or left ventricle, follow the distribution of blood from the heart to the tissues, but cannot pass through the micro-circulation and are trapped in the tissue. Using this method, validated by direct measurement of venous efflux, Foster and Frydman (1978b) demonstrated that over 30% of total cardiac output goes to BAT in cold acclimatised rats treated with noradrenaline, compared with 3% at rest. Blood leaving the tissue was almost completely depleted of oxygen, and these authors calculated that more than 60% of the oxygen used during noradrenaline stimulated thermogenesis in cold acclimatised rats was consumed by BAT. The contribution of BAT to NST could be higher. Diffuse BAT may account for a proportion of the remainder and many tissues that respond to exogenous noradrenaline may not be stimulated in the physiological response to cold. Some of the remaining 40% would be accounted for by the increased mechanical work of the heart and respiratory muscles. In the newborn rabbit there is no increase in oxygen consumption on cold exposure or noradrenaline administration after excision of 80% of BAT (Heim and Hull, 1966).

The thermogenic capacity of BAT is increased in cold acclimatised rats (Foster and Frydman, 1978b) associated with up to 7-fold increases in [^{3}H]-GDP binding to BAT mitochondria (Desautels and Himms-Hagen, 1979) and increased synthesis of

the 32000D protein (Ashwell et al., 1983).

1.4.6 BAT as the tissue effector of DIT.

There are many similarities between NST and DIT. Hyperphagic rats exhibiting DIT adapt more quickly to cold exposure (Rothwell and Stock, 1980) and the onset of DIT is more rapid in previously cold acclimatised rats (Rothwell and Stock, 1981a). Oxygen consumption increases on cold exposure or noradrenaline administration at thermoneutral temperatures and this response is enhanced in both hyperphagic (Rothwell and Stock, 1979b) and cold acclimatised rats (Depocas, 1960). The increases in metabolic rate found in hyperphagic rats are inhibited by β-adrenergic blockade with propranolol, as occurs in cold acclimatised rats (Rothwell and Stock, 1980). In a similar series of experiments to those of Foster and Frydman on cold acclimatisation, Rothwell and Stock (1981a) demonstrated that 74% of the increased oxygen consumption resulting from noradrenaline administration to hyperphagic rats maintained on a cafeteria diet could be accounted for by BAT. As in cold acclimatised rats, the increased BAT thermogenesis in hyperphagic rats is associated with increases in $[^3H]$-GDP binding to BAT mitochondria (Himms-Hagen et al., 1984). However, these changes are not as large as in cold acclimatised rats.

1.4.7 Sympathetic Regulation of NST and DIT in BAT.

BAT possesses a rich sympathetic innervation, as described in section 1.4.3. The tissue content of noradrenaline is high, around 1-2 μg per gram wet weight of interscapular BAT in the rat (Barnard et al., 1980). Histochemical fluorescence studies and electron microscopy show that the noradrenaline is concentrated in the nerve terminal varicosities on blood vessels and brown adipocytes and is stored in vesicles or storage granules (Zaror-Behrens et al., 1982). BAT contains the biosynthetic and degradative enzyme pathways and noradrenaline uptake mechanisms characteristic of tissues with a functional sympathetic nerve supply (Barnard et al., 1980). BAT also contains high concentrations of dopamine, 5-hydroxytryptophan and histamine.

The histochemical fluorescence studies performed on BAT do not preclude the presence of dopaminergic nerve fibres (Barnard et al., 1980). However, BAT does not possess any dopamine uptake mechanisms so a physiological role of dopamine in the regulation of thermogenesis seems doubtful (Rothwell and Stock, 1984).

Surgical denervation of BAT reduces the tissue content of noradrenaline to less than 5% of control values (Foster et al, 1982; Stricker et al., 1984), indicating that noradrenaline is stored within the post-ganglionic fibres. Similarly, treatment with 6OHDopa, an agent that destroys sympathetic nerve endings, reduces the tissue content of noradrenaline by 91% (Young et al., 1982). Reserpine causes intraneuronal release of noradrenaline, allowing degradation by monoamine oxidase and completely depletes BAT of noradrenaline (Weiner et al., 1962). Tyramine displaces noradrenaline from storage sites within sympathetic nerves and reduces BAT noradrenaline content by 50% (Young et al., 1982).

The high tissue content of noradrenaline in BAT, while indicative of the degree of sympathetic innervation, does not provide a measure of the sympathetic activity of the tissue. Noradrenaline is stored in nerve endings in storage vesicles and the majority of noradrenaline released on nerve stimulation (up to 90%) is taken back up into the nerve ending via a stereo-specific ATP requiring active transport mechanism designated $Uptake_1$ (Von Euler et al., 1963) (see Fig. 1.2). Noradrenaline not taken back up into the storage granules and noradrenaline released spontaneously into the axoplasm are metabolised by monoamine oxidase E.C.1.4.3.4 (MAO) (see Figs 1.2 and 1.3). Some noradrenaline is taken up from the synapse via the non-stereo specific $Uptake_2$ into extraneuronal sites resulting in metabolism by catechol-O-methyl transferase E.C.2.1.1.6 (COMT) and extraneuronal MAO (see Figs. 1.2 and 1.3). A certain proportion of released noradrenaline is lost into the circulation, the amount depending upon tissue blood flow.

Increased release of noradrenaline is balanced by increased

Fig 1.2 The sympathetic nerve ending.

TOHase	=	tyrosine hydroxylase
DDC	=	dopa decarboxylase (aromatic amino acid decarboxylase)
Dβ-OHase	=	dopamine β-hydroxylase
NA	=	noradrenaline
MAO	=	monoamine oxidase
DOPEG	=	dihyroxyphenylethyleneglycol
DOMA	=	dihydroxymandelic acid
U_1	=	$Uptake_1$
U_2	=	$Uptake_2$
COMT	=	catechol-O-methyl transferase
NMN	=	normetanephrine
VMA	=	vanillyl mandelic acid
MOPEG	=	methoxhydroxyphenylethyleneglycol

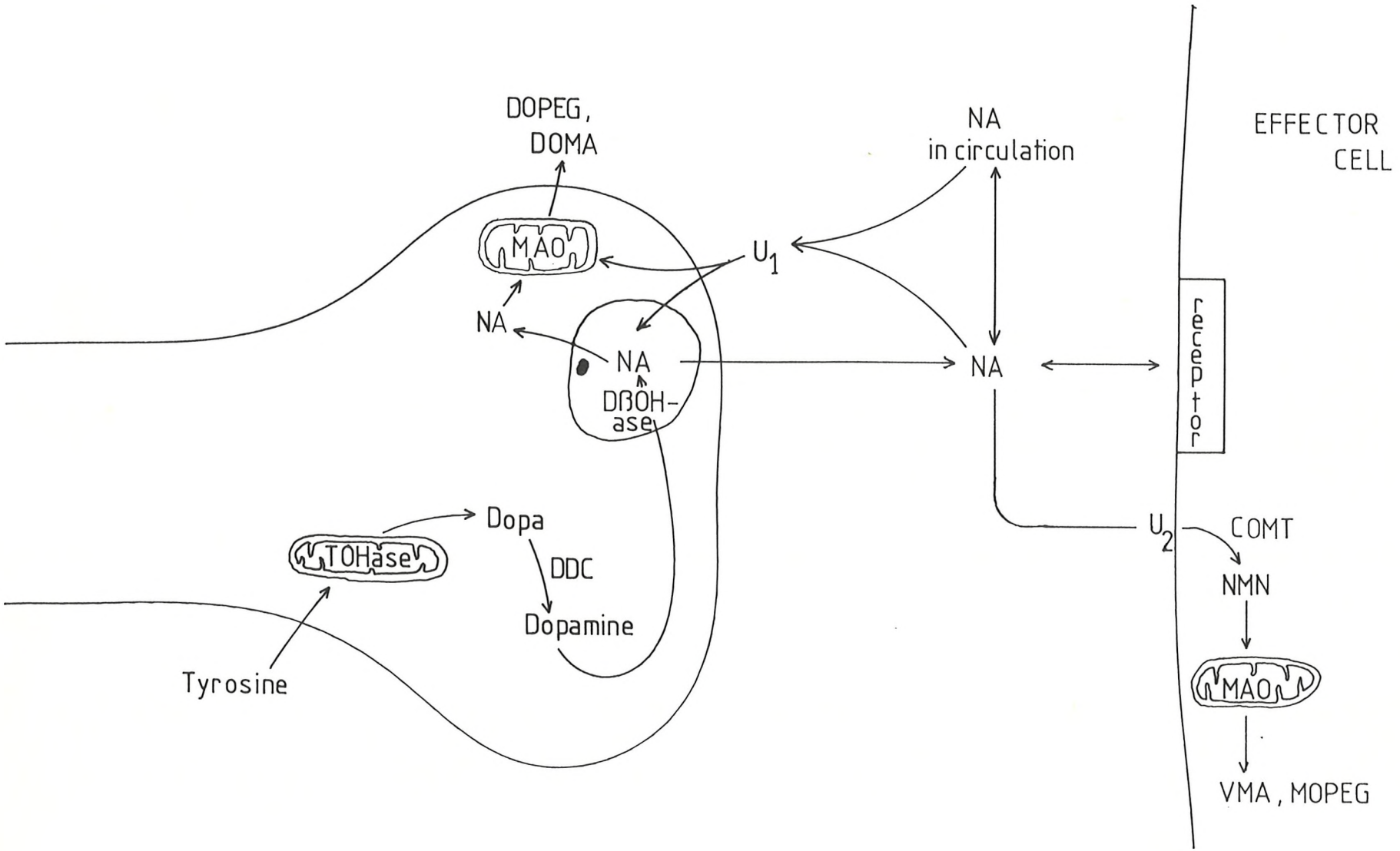
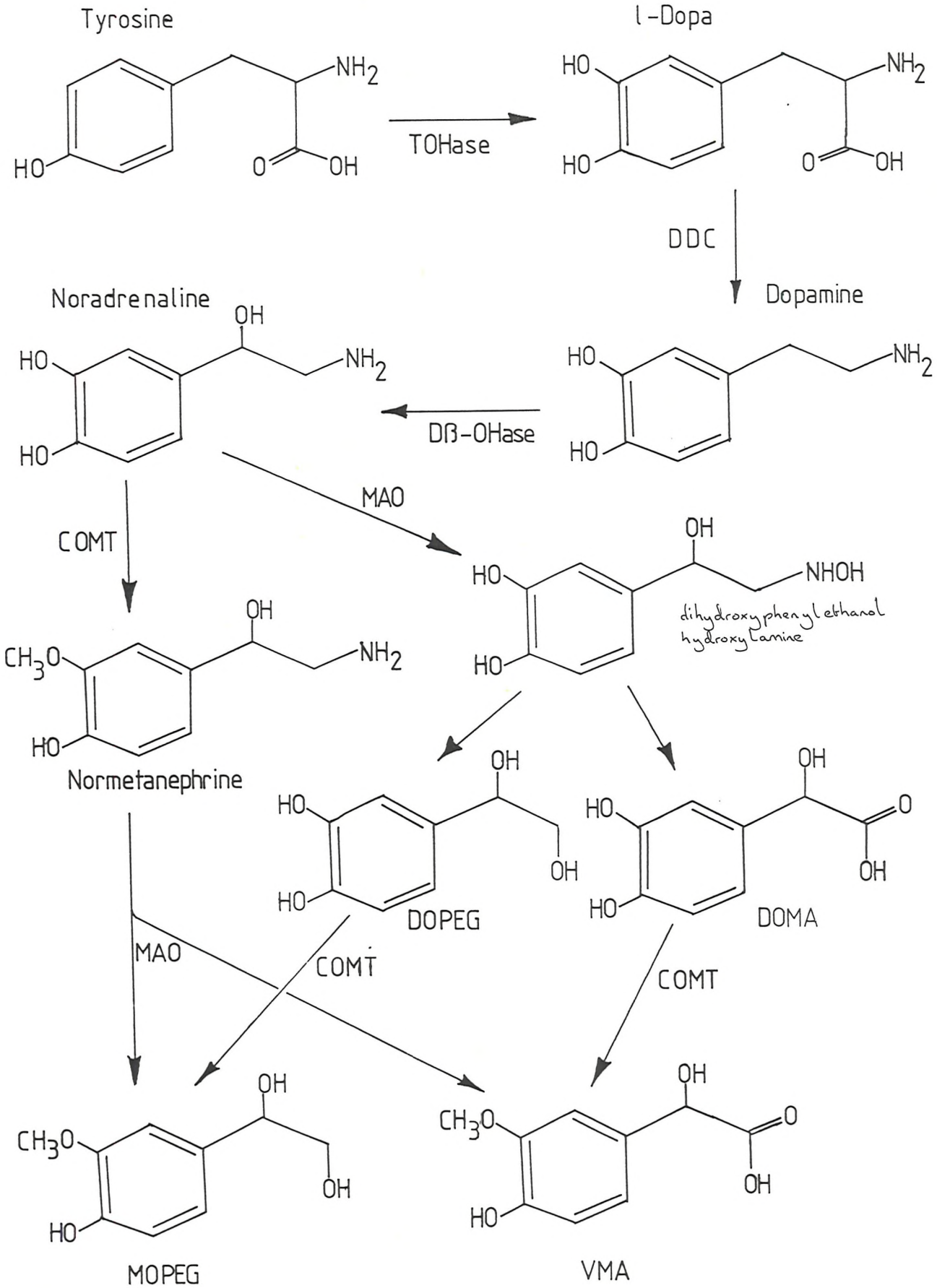

DOPEG,
DOMA
MAO
NA
NA
DβOH-
ase
U1
NA
in circulation
NA
EFFECTOR
CELL
receptor
U2
COMT
NMN
MAO
VMA, MOPEG
Dopa
DDC
Dopamine
TOHase
Tyrosine

Fig. 1.3 The pathways of noradrenaline biosynthesis and breakdown.

TOHase	=	tyrosine hydroxylase
DDC	=	dopa decarboxylase (aromatic amino acid decarboxylase)
Dβ-OHase	=	dopamine β-hydroxylase
COMT	=	catechol-O-methyl transferase
MAO	=	monoamine oxidase
DOPEG	=	dihydroxyphenylethyleneglycol
DOMA	=	dihydroxymandelic acid
MOPEG	=	methoxyhydroxyphenylethyleneglycol
VMA	=	vanillyl mandelic acid


Tyrosine
l-Dopa
TOHase
DDC
Noradrenaline
Dopamine
DBß-OHase
MAO
COMT
dihydroxyphenylethanol hydroxylamine
Normetanephrine
DOPEG
DOMA
MAO
COMT
COMT
MOPEG
VMA

synthesis from tyrosine by the enzymes tyrosine hydroxylase, dopa decarboxylase and dopamine β-hydroxylase (see Figs. 1.2 and 1.3). The rate determining step of the sequence is the conversion of tyrosine to dopa by tyrosine hydroxylase E.C.1.14.3a (Spector et al., 1969) using Fe^{2+} and tetrahydropteridine as cofactors (Ikeda et al., 1966). Dopa is converted to dopamine by dopa decarboxylase, more correctly called aromatic amino acid decarboxylase E.C.4.1.1.28 since it is relatively non-specific, utilising pyridoxal phosphate as a cofactor. Dopamine is taken up into the storage vesicles by an ATP and Mg^{2+} dependant uptake mechanism (Carlsson and Hillarp, 1963) and converted to noradrenaline by dopamine β-hydroxylase E.C.1.14.2.1 using fumurate and ascorbate as cofactors (Levin et al., 1960). Since increased release of noradrenaline is balanced by increased synthesis from tyrosine, noradrenaline is conserved in peripheral tissues and its levels remain relatively constant despite wide changes in sympathetic activity (Landsberg and Young, 1979).

An assessment of sympathetic activity can be made by measuring the rate of tissue noradrenaline turnover. There are two methods of measuring noradrenaline turnover, synthesis inhibition and isotopic labelling. If noradrenaline synthesis is blocked by inhibition of tyrosine hydroxylase with α-methyl-p-tyrosine then tissue stores of noradrenaline become depleted as noradrenaline losses are not replaced. The decline in tissue noradrenaline content with time can be used to calculate the rate of noradrenaline synthesis and hence give as assessment of sympathetic activity (Landsberg and Young, 1978). However, some α-methyl-p-tyrosine may be converted to α-methyl noradrenaline and act as a false transmitter (Maître, 1965) and so interfere with tissue function. There is also evidence that tyrosine hydroxylase inhibition may slow noradrenaline turnover (Persson and Waldeck, 1970). The second method used to assess sympathetic activity is to label the endogenous stores of noradrenaline with tracer amounts of high specific activity [^{3}H]-noradrenaline. [^{3}H]-noradrenaline is rapidly

cleared from the circulation by the axonal membrane transport system in the sympathetic nerve endings (Whitby et al., 1961). Noradrenaline is not confined to a single intracellular pool in the nerve ending. There are at least two pools of noradrenaline, a small functionally active pool with a high rate of turnover into which newly synthesised noradrenaline readily enters (Pool 1) and a large storage pool in equilibrium with Pool 1 from which noradrenaline is slowly released (Pool II), (Beaven, 1965). Pool II may represent noradrenaline bound to ATP and granular components within the vesicles (Hillarp, 1960) or may represent storage in old, small vesicles while Pool I represents storage in the smaller number of young, large vesicles rich in dopamine β-hydroxylase (Dahlström Haggendal, 1973). Very small doses of [^{3}H]-noradrenaline of less than 0.2 µg/kg body weight appear to label Pool II preferentially. The turnover rate of this stable storage pool is slow, with a half-life around 24 hours and turnover is unaffected by short term ganglionic blockade or nerve stimulation and provides an estimation of overall noradrenaline synthesis (Cottle et al., 1967; Montanari et al., 1963).

The noradrenaline stores in the nerve ending are not normally filled to capacity. This is reflected by the increased content of noradrenaline in nerve endings after preganglionic denervation or ganglionic blockade (Stjarne, 1964). [^{3}H]-noradrenaline doses of greater than 0.2 µg/kg body weight label Pool I in addition to Pool II resulting in a diphasic decline in specific activity. The first phase, up to about 12 hours, equates with loss from Pool 1. Turnover rates calculated from this phase are directly related to the rate of nerve stimulation (Hertting and Axelrod, 1961; Neff et al., 1968) and are reduced on ganglionic blockade (Hertting et al., 1962; Montanari et al., 1963; Young and Landsberg, 1979). Thus, calculation of turnover rates from this first phase of decline, give an indication of the relative rate of noradrenaline release from the sympathetic nerve ending and hence an assessment of sympathetic activity in a tissue (Young and Landsberg, 1978).

Using the methods discussed above it has been shown that noradrenaline turnover is increased in BAT when in a thermogenic state. Acclimatisation to cold (Cottle et al., 1967; Young et al., 1982), overfeeding with sucrose or fat (Schwartz et al., 1983) or a cafeteria diet (Young et al., 1982) and feeding a low protein diet (Kevonian et al., 1984; Vander Tuig and Rosmos, 1984), states in which BAT is activated, are all associated with increased noradrenaline turnover in BAT. Conversely, fasting, which reduces BAT thermogenesis, decreases noradrenaline turnover in BAT (Young et al., 1982). That changes in noradrenaline turnover do reflect changes in sympathetic activity has been demonstrated using ganglion blockers such as chlorisondamine. Chlorisondamine is a potent long acting nicotinic antagonist which reduces post ganglionic nerve impulse traffic and so decreases noradrenaline turnover where this results from sympathetic activity, resulting in increased retention of tritiated noradrenaline. Thus, in animals exhibiting increased noradrenaline turnover, while fed a cafeteria diet, there is greater retention of [^{3}H]-noradrenaline after ganglionic blockade than in animals with a lower turnover on a stock diet (Young et al., 1982).

1.4.8 Long term regulation of BAT - the adaptive response.

In addition to the acute effects of noradrenaline on [^{3}H]-GDP binding to BAT mitochondria and on brown adipocyte respiration and lipolysis there are long term changes in BAT on prolonged stimulation. Both cold acclimatisation and overfeeding lead to an increase in the wet weight and protein content of BAT, initially through hypertrophy of existing cells followed by hyperplastic growth (Buckowiecki et al., 1982). This hyperplasia involves mainly endothelial cells of the blood capilleries and small venules, interstitial mesenchymal cells and preadipocytes (Buckowiecki et al., 1982). Thermogenic capacity is increased and GDP binding to BAT mitochondria increases by up to 7-fold in cold acclimatised rats and 2-3-fold in overfed rats (Desautels and Himms-Hagen, 1979; Himms-Hagen et al., 1981). These effects are mimicked by noradrenaline

administration to warm acclimatised rats with resultant cell proliferation, increased capacity for thermogenesis and increases in mitochondrial GDP binding, comparable to those observed in overfed rats (Barnard et al., 1980). Early estimates of the amount of 32000D protein in BAT mitochondria from both overfed and noradrenaline treated rats, assessed by SDS gel electrophoresis, failed to show the increases that were found in cold acclimatised rats (Ricquier and Kader, 1976; Himms-Hagen et al., 1981). The development of a sensitive, specific radioimmunoassay to the uncoupling protein (Cannon et al., 1982) has demonstrated that both cold acclimatisation (Ashwell et al., 1983) and overfeeding (Ashwell et al., 1984) lead to increased synthesis of uncoupling protein. This increased synthesis has now been shown to be mediated by noradrenaline. The use of osmotic mini-pumps allows continuous infusion of noradrenaline which completely mimics the effects of cold acclimatisation with cell proliferation, increased GDP binding and increased synthesis of uncoupling protein (Mory et al., 1984). Ricquier et al. (1984) have demonstrated that there is an increase in uncoupling protein and its messenger RNA within one hour of cold exposure and that this mediated through β-receptors, since these effects are blocked by propranolol.

1.4.9 Central control of BAT thermogenesis.

The hypothalamus has an important role in thermoregulation (Jansky, 1973). NST is activated through thermosensors located in the preoptic and supraoptic areas of the hypothalamus and in the spinal cord (Banet et al., 1978). Thermoreceptors in the posterior hypothalamus may activate BAT and noradrenaline injected into this area stimulates BAT (Bruck and Zeisberger, 1978). The VMH is closely involved with BAT thermogenesis. Electrical stimulation of the VMH causes a sympathetically mediated rise in BAT temperature, that is blocked by propranolol, and this temperature increase is similar to that obtained on stimulation of the nerve supply of BAT (Perkins et al., 1981). The VMH seems to have a tonic stimulating effect on BAT since destruction of the VMH results in decreased spontaneous activity of the sympathetic efferents, (Niijima et al., 1984),

reduced noradrenaline turnover (Vander Tuig et al., 1982), reduced GDP binding (Seydoux et al., 1982c) and involution of the tissue (Saito et al., 1985). The hypothalamic areas involved in DIT appear to be distinct from those controlling temperature regulation since VMH-lesioned rats respond normally to cold (Hogan et al., 1982; Luboshitsky, 1984) but not to overfeeding (Hogan et al., 1982; Seydoux et al., 1982c).

1.4.10 Endocrine control of BAT thermogenesis.

Although the primary stimulus for BAT thermogenesis is noradrenaline released from sympathetic nerve endings, other processes may modulate the response of the tissue.

Thyroid hormones are required in permissive amounts for the normal BAT thermogenic response to cold. Cold exposed thyroidectomised rats cannot survive and do not exhibit the normal increases in GDP binding to BAT mitochondria (Triandafillou et al., 1982) and lipid mobilisation (Mory et al., 1981). Low maintenance doses of thyroid hormone restore the normal response (Triandafillou et al., 1982). This may partly be due to thyroid hormone mediated regulation of β-receptor population since β-receptor numbers are decreased in brown adipocytes from hypothyroid rats (Swartengren et al., 1982). However, Seydoux et al. (1982) found that the decrease in the metabolic response to nerve stimulation in BAT from hypothyroid rats was much greater than could be accounted for by the decrease in β-receptors. Sundin et al. (1984) found that the reduced respiratory and lipolytic response to isoproterenol in brown adipocytes from hypothyroid rats was brought about without changes in cAMP accumulation. Similarly, if adenylate cyclase was activated in the membrane with the diterpene forskoline cAMP accumulation increased equally in brown adipocytes from hypothyroid and euthyroid rats but the respiratory and lipolytic responses were reduced in hypothyroidism. These changes in the respiratory response occurred without changes in the amount of mitochondrial 32000D protein or GDP binding (Triandafillou et al.,1982).

Thyroid hormones have an important role in the regulation of basal metabolic rate. Hyperthyroidism increases, whereas hypothyroidism decreases, metabolic rate (Girardier, 1977; Leblanc and Villemaire, 1970). The increased metabolic rate in hyperthyroid rats seems to reduce the requirement for BAT thermogenesis via the proton conductance pathway since GDP binding to BAT mitochondria is reduced and does not increase on mild cold exposure (Sundin, 1981) or cafeteria feeding (Rothwell et al., 1983c) but does increase on cold exposure at 5°C (Sundin, 1981). Although GDP binding does not increase, hyperthyroid rats do have a potentiated metabolic response to cafeteria feeding and Na^+K^+ATPase activity may be enhanced (Rothwell et al., 1982a, 1983c). It has been suggested that while thyroid hormones may only be permissive to BAT thermogenesis on cold exposure they may play a more direct role in DIT (Rothwell and Stock, 1984).

Levels of thyroid hormones increase under conditions of increased sympathetic activity. Serum triodothyronine (T_3) levels increase in animals exhibiting NST and DIT (Scammel et al., 1981; Tulp et al., 1982) and decrease in fasting animals (Kaplan, 1979). The rate of conversion of thyroxine (T_4) to T_3 by 5' deiodinase is increased by sympathetic stimulation of BAT (Silva and Larson, 1983). Since thyroid hormones enhance the thermogenic effects of noradrenaline (Leblanc and Villemaire, 1970) a synergistic relationship exists between the sympathetic nervous system and thyroid hormones. Enhanced sympathetic activity in BAT would increase T_3, which increases the sensitivity of brown adipocytes to catecholamines with respect to lipolysis, respiration and cAMP accumulation (Sundin et al., 1984), so amplifying the thermogenic response.

Insulin enhances fat deposition and inhibits the lipolytic response to catecholamines in BAT as in white adipose tissue (Nedergaard and Lindberg, 1982). Insulin may also affect BAT thermogenesis. Insulin injections can increase metabolic rate in fasted and cafeteria fed-rats and enhance the thermogenic response to noradrenaline (Rothwell et al., 1983b). The thermogenic response to refeeding carbohydrate to fasted rats appears to be mediated by an insulin-dependant increase in

sympathetic activity since it is blocked by propranolol (Rothwell et al., 1983b). Diabetic rats fail to exhibit NST or DIT (Rothwell and Stock, 1981). These effects of insulin on BAT thermogenesis may be mediated through central regulation of sympathetic activity. Injections of insulin into the VMH increase neuronal firing rate (Oomura et al., 1978).

Glucagon has also been implicated in the regulation of BAT thermogenesis. Glucagon administration increases metabolic rate and BAT blood flow, temperature and lipolysis (Heim and Hull, 1966 ; Kuroshima et al., 1977). Long term glucagon administration improves cold tolerance and enhances the thermogenic response of BAT to noradrenaline (Yahata et al., 1981). Glucagon stimulates heat production in brown adipocytes in vitro (Kuroshima and Yahata, 1979) and the acute in vivo effects of glucagon administration are not blocked by propranolol (Heim and Hull, 1966 ; Kuroshima et al., 1977) indicating that this response is not mediated by the sympathetic nervous system. Since glucagon levels rise on cold exposure (Seitz et al., 1981), glucagon may have a physiological role in the response to cold.

Glucocorticoids are required in permissive amounts for the normal response to cold exposure (Deavers and Mussachia, 1979; Fellenz et al., 1982), largely by maintaining BAT sensitivity to noradrenaline (Maickel et al., 1967.) Chronic corticosterone treatment inhibits the thermogenic response to overfeeding but not to cold (Galpin et al., 1983), so glucocorticoids may also act centrally. Glucocorticoids may have direct actions on BAT as the tissue contains specific dexamethasone receptors (Feldman, 1978). ACTH has affects on BAT thermogenesis independent from its stimulation of glucocorticoid production. Chronic administration of ACTH increases metabolic rate and potentiates the thermogenic and lipolytic effects of noradrenaline by 50% in warm acclimatised rats without increasing plasma corticosterine concentration (Laury and Portet, 1977, 1980). ACTH treatment increases BAT blood flow (Kuroshima et al., 1968), temperature (Heim and Hull, 1966) and increases metabolic rate (Heim and Hull, 1966). The increases in metabolic rate and BAT temperature

induced by ACTH are not blocked by propranolol indicating a direct effect on the tissue (Heim and Hull, 1966). Cold exposure stimulates the release of ACTH from the pituitary (Maickel et al., 1961). However, chronic treatment of cold-acclimatised rats with ACTH inhibits the thermogenic effects of noradrenaline (Laury and Portet, 1977) so a physiological role of ACTH in the response to cold seems unlikely. Corticosterone, and presumably ACTH, increases after feeding (Brindley et al., 1979) so ACTH may modulate DIT.

1.4.11 BAT in man.

The human neonate is well endowed with BAT which is estimated to comprise 2-5% of body weight (Merklin, 1973). Babies have a high capacity for NST since the shivering response does not fully develop until one year of age (Rothwell and Stock, 1984). BAT atrophies with age and this is paralleled by a decline in the capacity for NST (Trayhurn and James, 1983). Although BAT persists throughout adult life its activity and involvement in NST and DIT are difficult to assess. In BAT from patients with phaeochromocytoma, GDP binding to BAT mitochondria and GDP sensitive respiration have been demonstrated (Ricquier et al., 1982). Skin temperature in the neck and superscapular region increases in response to sympathetic drugs (Rothwell and Stock, 1979b), indicating that BAT may be activated. However, these increases in skin temperature are probably more related to increases in subcutaneous blood flow than to BAT thermogenesis (Astrup et al., 1980). In a recent study, BAT has been shown to be responsible for, at most, only 25% of the thermogenic response to ephedrine administration, with skeletal muscle accounting for up to 50% of the increased oxygen consumption (Astrup et al., 1985). The existence of DIT in man the role of BAT, and the contributions of impaired thermogenesis to the development of obesity are controversial. The elucidation of the mechanisms that result in human obesity and the subsequent treatment of this condition are limited by the practicalities of manipulating human subjects. Consequently much work has centred on the use of animal models of obesity.

1.5 Animal models of obesity.

There are at present over fifty animal models of obesity of widely differing aetiologies available to the researcher (Sclafani, 1984). While animal models cannot necessarily represent the precise mechanisms involved in a particular human condition, an understanding of the different models of obesity could provide an important insight into the processes that are disturbed. To this end, several of these models have been extensively characterised, in particular the obesities due to hypothalamic injury, dietary manipulations and genetic lesions.

1.5.1 Hypothalamic obesity.

Lesioning of the ventromedial hypothalamus (VMH) leads to hyperphagia and obesity (Bray and York, 1979). Parasaggital knife cuts and electrolytic lesions, which do not destroy the ventromedial nucleus, but interfere with the ventral adrenergic bundle that runs through the VMH, produce a simple model of obesity in which hyperinsulinaemia, if present, is solely dependant on the increased food intake (Gold, 1973; Bray et al., 1982). A more complex state of obesity arises if the electrolytic lesions also destroy the ventromedial nucleus, resulting in severe hypoactivity, finickiness, retarded growth, disruption of ovarian function and hyperinsulinaemia, which is not dependent upon the increased food intake (Bray and York, 1979). Hypothalamic obesity may also be induced with radiofrequency lesions or injections of gold thioglucose, monosodium glutamate, bipiperidyl mustard or 5,7, dihydroxytryptamine (see Sclafani, 1984, for references). VMH-lesioned rats have a lower resting metabolic rate (Villberg and Keesey, 1984; Hustveldt et al., 1984). Noradrenaline turnover in BAT is decreased (Vander Tuig et al., 1982) and GDP binding to BAT mitochondria is reduced (Seydoux et al., 1982c) consistent with a reduced sympathetic activation of the tissue. VMH-lesioned rats have an impaired BAT thermogenic response to overfeeding (Seydoux et al., 1982c), although they respond normally to cold exposure (Luboshitsky et al., 1984). The increased food intake and

hyperinsulinaemia, coincident with a reduced metabolic rate and lack of DIT, lead to increased lipid synthesis and storage, and hypertrophy of adipocytes. In addition to reduced sympathetic activity, hypothalamic obesity has also been associated with increased parasympathetic nervous system activity, since vagotomy reverses the hyperinsulinaemia and obesity (Powley and Opsahl, 1974; Inoue and Bray, 1978; Fox and Parley, 1984). Glucocorticoids also seem to be involved, since the obesity is reversed by adrenalectomy and this reversal is prevented by corticosterone (Bruce et al., 1982; Knight et al., 1983).

1.5.2 Diet-induced obesity.

Most animal models of obesity are characterised by an increase in food intake, although the importance of this parameter in the development of the obese state varies. Obesity can often be induced in experimental animals by increasing energy intake. If the energy content of the diet is increased, as in high fat diets, then weight gain and lipid deposition can result so long as protein intake is maintained (Miller, 1979). Hyperphagia can be induced by offering sucrose in addition to the normal diet (Kanarek and Hirsch, 1977), or with a larger range of palatable items as in the so called "cafeteria" or "Junk food" diet (Sclafani and Springer, 1977). The effectiveness of these regimes in producing obesity depends upon a number of factors. Most species are capable of compensating for increased energy intake by increasing energy expenditure through thermogenesis to some extent, but this is very much dependant on the strain and age of the animal. Miller (1979) found that energetic efficiency varied considerably in different strains of rat and that lipid deposition was far more dependant on this than the level of food intake. Similarly, there is considerable variation in the ability of different strains of rat to resist obesity brought on by cafeteria feeding (Rothwell & Stock, 1982a). Most young rats can compensate for large increases in food intake (up to 80%) by increasing energy expenditure and thus gain little or no weight (Rothwell and Stock, 1979a). This ability diminishes with age (Sclafani and Gorman,

1977; Rothwell and Stock, 1983b) but may be restored in older animals by adrenalectomy (Rothwell et al., 1984b). The effects of removing cafeteria diets vary with strain, age and length of time previously on the diet. In some cases the obese body weight is maintained (Rolls et al., 1980) and in others removal of the diet results in a period of hypophagia and maintained increased energy expenditure so that body weight returns to normal (Rothwell and Stock, 1979a).

1.5.3 Genetic obesity.

There are a number of animal models of obesity in which the trait is inherited and these can be sub-divided into types according to their mode of inheritance. Polygenic inbred strains, such as the New Zealand obese mouse and Japanese KK mouse or polygenic hybrids such as the C3HF1 Wellesley mouse, probably provide the better models of human obesity from an inheritance point of view. However, most research has utilised the single gene mutants, of which the recessive mutants; the obese mouse (ob/ob), the diabetic mouse (db/db) and the Zucker fatty (fa/fa) rat are most extensively studied. Since a single gene is involved and since one gene codes for one protein, then the state of obesity must arise from a defect in a single protein. The defect may be in a structural, enzymic or regulatory protein. In practice each model of obesity is associated with so many metabolic abnormalities that it has been impossible to determine which, in each case, result from the primary lesion. Much work has centred on the obese Zucker (fa/fa) rat in which there is both hyperplasia and hypertrophy of adipocytes (Johnson et al., 1971) as found in human juvenile onset obesity (Salans et al., 1973).

1.5.4 The Zucker fatty (fa/fa) rat.

The Zucker fatty rat arose spontaneously in a 13M strain of rat that was part of a breeding programme to investigate correlations between body weight and other parameters (Zucker and Zucker, 1961). In the Zucker rat, obesity is inherited as an autosomal recessive gene designated fa for the obese allele

and Fa for the normal allele. Rats are normally bred from matings between the lean heterozygotes (Fa/fa) which yield 25% obese animals (Zucker and Zucker, 1963). Heterozygotes are phenotypically identical with homozygous lean, although they may be identified on sacrifice by an increased subcutaneous fat pad weight, and will gain weight compared with homozygous lean if fed a high-fat diet (Zucker and Zucker, 1963). It has recently been demonstrated that both the increase in oxygen consumption after feeding and $[^3H]$-GDP binding to BAT mitochondria, which are reduced in obese rats, are partially reduced in the heterozygous lean rats. The values obtained are intermediate between those of homozygous lean and homozygous obese rats (York et al., 1984). A heterozygote effect has also been shown in an increased plasma insulin and increased in vitro pancreatic insulin release on glucose perfusion of the pancreas of heterozygotes, compared with homozygous lean rats (Blonz et al., 1985). Obese rats are normally bred from lean heterozygous parents as obese females are infertile, with abnormal or absent oestrous cycles. (Saiddudin et al., 1973; Shaw et al., 1983) and obese males are only fertile if their food intake is restricted by 50% (Yen et al., 1977).

1.5.4.1 Energy balance in the obese (fa/fa) rat.

Energy intake must exceed energy expenditure if a state of obesity is to develop. The weaned obese rat consumes 40% more than its lean counterpart (Haberay et al., 1980). However, when food intake is expressed per unit body weight obese rats are only hyperphagic from 3-7 weeks of age (Dilettuso and Wangness, 1977). Feeding behaviour is disturbed in obese rats; the diurnal pattern is lost, although they still consume the larger proportion of their daily food intake at night (Haberay et al., 1980). Obese rats tend to eat fewer, larger meals (Becker and Grinker, 1977) but also nibble food between meals (Costanguay et al., 1982b).

The regulation of food intake in response to dietary changes is disturbed in the obese rat. Lean rats will increase

food intake to account for a calorically-diluted or low protein diet, but the obese rat is less able to do this (Bray and York, 1972; Young et al., 1980). If offered diets of varying protein content lean rats will regulate intake to optimise both protein and energy intake (Musten et al., 1974), while obese rats will select a diet for fat (Costanguay et al., 1982a).

The feeding responses to various stimuli are altered in the fa/fa rat. Intraventricular administration of 2 deoxy-D-glucose to lean rats induces both hyperphagia and hyperglycaemia but only the hyperglycaemia effect is observed in obese rats, suggesting an impaired glucosensitive site for food intake (Ikeda et al., 1980). Insulin administration increases food intake to a greater extent in obese than in lean rats (Ikeda et al., 1980). Naloxone, a morphine antagonist, reduces food intake in obese but not in lean rats (Margules et al., 1978). The natural opiate β-endorphin increases food intake in rats, so it is possible that the increased levels of β-endorphin found in the pituitary and hypothalamus of obese rats, contribute to their hyperphagia (Recant et al., 1983).

Hyperphagia contributes to, but is not required for, the development of the obese state. When pair-fed to lean rats the weight gain of the obese falls by 40%, but protein deposition falls by 60% (Pullar and Webster, 1974) so the obese composition is still maintained (Channusot et al., 1984), even at ?/3rds the food intake of the lean rats (Bray et al., 1973). The gain in lipid in the food restricted obese rat is largely at the expense of the lean tissue mass. Muscle protein deposition is reduced while the obese rat is suckling but recovers as it becomes hyperphagic after weaning (Reeds et al., 1982). After 34 days of age there is a clear imbalance in the partitioning of energy between fat and protein (Radcliffe and Webster, 1978). Indeed the obese rat is more efficient at utilising dietary protein as an energy source than for growth and can survive high protein, carbohydrate-free diets, that lean rats cannot tolerate (Peret et al., 1984). If given food

ad-lib the adult obese rat deposits protein at an almost normal rate (Radcliffe and Webster, 1976) and it was suggested that the obese rat overeats in order to maintain normal rates of protein deposition. This seems unlikely, however, since if allowed to select its own dietary constituents, the obese rat is still hyperphagic and the excess energy intake is in the form of fat rather than protein (Costanguay et al., 1982).

The obese rat exhibits an increased efficiency of energy utilisation, the increase in body fat per gram of food eaten and the energy deposited per unit of metabolisable energy are increased (Deb et al., 1976). The energy requirement for maintenance in obese rats has been reported as lower (Haberay et al., 1980; Mowery and Herschberger, 1982) or similar (Deb et al., 1976). Resting metabolic rate has been shown to be similar in obese and lean rats (Rothwell et al., 1981a).

The adult obese rat has a low level of spontaneous activity but this does not become apparent until the obesity is well advanced, at about 12 weeks of age (Stern and Johnson, 1977; Haberay et al., 1980). Forced exercise delays, but does not prevent, the development of obesity (Deb and Martin, 1975; Walberg et al., 1982).

The defect in energy expenditure in the obese rat appears to be in adaptive thermogenesis. Rectal temperature and oxygen consumption are reduced in obese rats at normal housing temperatures (Godbole et al., 1978; Kaplan, 1979) and GDP binding to BAT mitochondria, an index of BAT thermogenic capacity, is diminished by 50% in the fa/fa rat (Holt and York, 1982). This reduction in BAT mitochondrial GDP binding results from a reduced number of binding sites rather than any changes in affinity (Holt and York, 1982) and appears to be due to masking of the binding sites since the amount of 32000D uncoupling protein is unchanged in obese rats of 5 weeks of age (Ashwell et al., 1985). The obese rat does not exhibit the same susceptibility to cold exposure found in the obese (ob/ob) mouse (Levin et al., 1980). Young obese rats respond normally to cold exposure and noradrenaline administration (Triandafillou and Himms-Hagen, 1983; Holt et al., 1983). Older animals are

less cold tolerant and do not show the same increases in BAT mitochondrial GDP binding, oxygen consumption and BAT blood flow (Wickler et al., 1982; Holt et al., 1983; Levin et al., 1984a). In older animals BAT mitochondrial 32000D protein is reduced, which may explain the reduced thermogenic response to cold (Ashwell et al., 1985).

In contrast to their normal ability to increase BAT thermogenesis in response to cold exposure, the obese rat does not increase BAT thermogenesis in response to dietary stimuli. Obese rats do not exhibit DIT either in the thermic response to a single meal, or in long term adaptive changes to an increased energy intake. Oxygen consumption does not rise after feeding a single test meal (Rothwell et al., 1983a; Marchington et al., 1983) and BAT mitochondrial GDP binding does not increase significantly when fed additional sucrose or a cafeteria diet (Holt et al., 1983; Triandafillou and Himms-Hagen, 1983). It is possible that adrenal glucocortoids may be suppressing DIT in the obese rat, since this defect is corrected by adrenalectomy (Holt et al., 1983; Marchington et al., 1983). Although BAT is reported to possess glucocorticoids receptors (Feldman, 1978), it is unlikely that the suppression of BAT thermogenesis in response to dietary stimuli is caused by a direct effect on the tissue since glucocorticoids are normally permissive to thermogenesis (Fellenz et al., 1982). In younger obese animals acclimatisation to cold brings about a normal adaptive increase in BAT thermogenesis. Similarly BAT of obese rats responds normally to noradrenaline administration. Corticosterone administration inhibits diet-induced but not non-shivering thermogenesis (Galpin et al., 1983), which again suggests that the defect in thermogenic function in obese rats, mediated by adrenal glucocorticoids, is in the regulation of thermogenesis rather than any inherent defect in the tissue itself.

The sympathetic nervous system is thought to be the principle regulator of thermogenesis in BAT (see section 1.4). It has been suggested that the defect in energy expenditure

in the obese rat results from a decreased sympathetic activation of BAT, which may be combined with inhibition of thermogenesis by the parasympathetic nervous system (Rothwell et al., 1981a; Rothwell and Stock, 1983a). Levin et al., (1981) reported decreased concentrations of catecholamines in sympathetically innervated organs (heart, aorta, pancreas, BAT and white adipose tissue), from 3-4 month old obese rats, associated with decreased activity of dopamine β-hydroxylase, the final enzyme in the pathway of noradrenaline biosynthesis. Noradrenaline turnover was also decreased,except in heart, suggesting that sympathetic activity was reduced in obese rats (Levin et al., 1983a). Noradrenaline turnover is reduced in brown adipose tissue and other organs of the obese (ob/ob) mouse (Knehans and Rosmos, 1982, 1983; Young and Landsberg, 1983) and VMH-lesioned rats (Vander Tuig et al., 1984), so a defect in sympathetic regulation of brown adipose tissue may be common to several forms of rodent obesity. At 3-4 months of age the obesity of the fa/fa rat is well developed, the generalised decline in sympathetic activity reported in these animals could be a consequence of their obesity rather than the cause. When rats are fed a high fat, high sucrose diet for 7 days BAT noradrenaline turnover increases (Levin et al., 1983b), however, after 3 months on the diet, when the rats are manifestly obese, noradrenaline turnover and, in most cases, noradrenaline concentrations are reduced in sympathetically innervated organs (heart, pancreas, aorta, BAT and white adipose tissue) (Levin et al., 1983b).

1.5.4.2 Endocrine status of the obese (fa/fa) rat.

a. The pituitary-thyroid system.

Thyroid function may be impaired in the obese fa/fa rat. The uptake and release of ^{131}Iodine by the thyroid, serum bound iodine (PBI) and serum thyroxine (T_4) are all reduced in obese rats (Bray and York, 1971b; Autissier et al., 1980). The concentration of triodothyronine (T_3), the active hormone, appears to be normal in older animals (Autissier et al., 1980) but is depressed in younger animals and so may have some

significance in the development of the obesity (Holt et al., 1983), since the thermogenic effects of noradrenaline are reduced in hypothyroid animals (Fregly et al., 1979). The levels of thyroid stimulating hormone (TSH) in pituitary and serum are normal and rise on injection of thyroid releasing hormone (TRH), but rise only slightly in obese rats treated with propylthiouracil or a diet low in iodine, suggesting a defect in the regulation of TSH secretion by the hypothalamus (York et al., 1972). Serum T_3 levels in young obese rats are increased to normal after adrenalectomy (Holt et al., 1983). Since adrenal glucocorticoids are known to inhibit thyroid function (Chopra et al., 1975; Pamenter and Hedge, 1980), and thyroid powder treatment prevents excessive weight gain in obese rats (Levin et al., 1982) it is possible that a corticosterone suppression of thyroid function may be of importance in the development of obesity in the Zucker rat.

b. Insulin and glucagon.

Insulin is an anabolic hormone, and insulin administration can result in obesity (Chan et al., 1982). Adult obese rats are markedly hyperinsulinaemic (Zucker and Antoniades, 1972; York et al., 1972; Bazin and Lavau, 1982; Rohner-Jeanrenand, 1983), so insulin is probably important in inducing and maintaining the obese state. However, although detectable *in utero*, hyperinsulinaemia is probably not fundamental to the onset of obesity, since fa/fa rats are not hyperinsulinaemic while suckling, when the obesity starts to develop (Turkenkopf et al., 1982a). The capacity for hyperinsulinaemia does appear to be present at this time however since, if given a glucose load, pre-obese rats do show an exaggerated insulin response (York et al., 1981; Rohner-Jeanrenaud et al., 1983; Blonz et al., 1985). In adults, only severe restriction of food intake or total starvation will reduce serum insulin to normal values (Zucker and Antoniades, 1972). If lean and obese rats are made diabetic with alloxan or streptozotocin and maintained on equal doses of insulin, then weight gain and food intake as a function of body weight are similar in lean and obese rats, but the excessive hepatic lipogenesis and lipid deposition are

maintained (Stolz and Martin, 1982; Chan et al., 1982). This demonstrates that the partitioning of energy to lipid is independant of both food intake and insulin.

The response of the pancreatic β-cell to glucose is markedly influenced by the activity of the parasympathetic nervous system via the vagus nerve (Bloom and Edwards, 1980; Kaneta et al., 1967). It has been suggested that parasympathetic nervous system activity is enhanced in the fa/fa rat (Rothwell and Stock, 1983a). Obese rats are not hyperglycaemic (Bray and York, 1979) so increased vagal stimulation may be responsible for the hyperinsulinaemia. Atropine treatment prevents the normal hypersecretion of insulin in response to a glucose load in pre-obese rats and vagatomy reverses the hyperinsulinaemia in older obese rats (Rohner-Jeanrenaud et al., 1983) but does not prevent obesity in fa/fa rats (Opsahl and Powley, 1984). In addition the β-cells of the pancreas seem to be more sensitive to vagal stimulation in obese rats (Rohner-Jeanrenaud et al.,1983).

The physiological effects of insulin are partly countered by those of glucagon. In the obese rat serum glucagon levels are close to normal, although the secretory responses to arginine and hypothalamic stimulation are impaired (Bryce et al., 1970; Eaton et al., 1976a). The insulin-to-glucagon ratio is thus elevated in the obese rat. This may be responsible for much of the hyperlipogenesis (Eaton et al., 1976b).

c. Adrenal glucocorticoids.

Several of the characteristics of the obese rat seem to be indicative of raised glucocorticoids, i.e. reduced bone growth and protein deposition, fine hair and yellowish, fragile skin, hyperinsulinaemia, hyperlipogenesis and hyperphagia. These are characteristics in common with Cushings Syndrome (Hollifield, 1968). However, although one report has found increased serum corticosterone in obese rats throughout much of the day (Martin et al., 1978), other workers have failed to find any such changes (Yukimura et al., 1978; Shargill et al., 1983; Holt et al., 1983; York and Al-Baker, 1984). Although

obese rats may be more sensitive to glucocorticoids (Yukimura et al., 1978) corticosterone receptor populations, in the liver at least, seem to be normal (Shargill, 1982).

Obesity in the fa/fa rat seems to be dependant upon adrenal glucocorticoids. Bilateral adrenalectomy restores body weight, food intake, fat synthesis and deposition and serum insulin to normal (Yukimura and Bray, 1978; York and Godbole, 1978). In rats allowed to select their own dietary constituents the decrease in food intake after adrenalectomy is largely of the excess fat intake characteristic of the intact obese animals (Costanguay and Stern, 1983). Adrenalectomy has also been shown to correct the defects in BAT thermogenesis and energy balance in the obese rat (Holt and York, 1982; Marchington et al.,1983). Treatment of adrenalectomised fa/fa rats with corticosterone restores the obesity, hyperphagia and defective brown adipose tissue thermogenesis (Yukimura et al., 1978; Holt et al.,1983).

The role of glucorticoids in the development and maintenance of obesity is not necessarily a direct one. Corticosterone secretion is regulated by corticotropin (ACTH) secretion from the anterior pituitary and there is negative feedback inhibition of ACTH secretion by corticosterone. Thus, fluctuations in corticosterone levels are matched by inverse changes in ACTH secretion. ACTH secretion seems to be normal in obese rats and responds normally to adrenalectomy and corticosterone replacement (Yukimura et al., 1978). ACTH rises markedly on adrenalectomy and in intact obese rats short term treatment with ACTH, before corticosterone levels rise, is associated with increased BAT thermogenesis and decreased food intake (York and Al-Baker, 1984). Corticosterone treatment of adrenalectomised obese rats results in decreased ACTH secretion (Yukimura et al., 1978) and short term treatment of these animals with ACTH prevents the loss of thermogenic capacity normally found (York and Al-Baker, 1984).

Hypophysectomy, which removes ACTH and diminishes glucocorticoid secretion, prevents excessive weight gain in the obese rat (Powley and Morgton, 1976) so the exact significance of the pituitary-adrenal system in the obesity of the fa/fa rat remains unclear.

1.5.4.3 Development of obesity in the fa/fa rat.

Obese rats cannot be readily distinguished from their lean littermates until 3-4 weeks of age, by which time many of the abnormalities of the syndrome have already been established. Those defects most closely associated with the primary lesion must occur at a very early age before any increases in body weight and fat content are apparent.

The earliest changes which have been reported to date are the increased plasma insulin and decreased pancreatic insulin in 25% of the 21 day old foetuses resulting from matings of heterozygote parents. This 25% was assumed to represent offspring of the fa/fa genotype (Turkenkopf et al., 1982a). However, this hyperinsulinaemia is not apparent in the suckling fa/fa rat in which obesity is already developing. This may result from a relative food restriction during suckling or from the high fat, low carboyhydrate composition of the milk (Turkenkopf et al., 1982a), as a glucose load will elicit an exaggerated insulin response (York et al., 1981; Rohner-Jeanrenaud et al., 1983).

Some of the earliest defects detectable in the young pre-obese fa/fa rat are related to energy expenditure. Reduced rectal temperature may be used to identify pre-obese rats at 16 days of age (Godbole et al., 1978) and decreased oxygen consumption may be shown at 18 days (Kaplan, 1979). By housing pups just below thermoneutrality, the reduction in oxygen consumption may be demonstrated at 7 days of age (Planche et al., 1983). This has been attributed to a defect in thermoregulatory thermogenesis. It has been suggested that the reduction in metabolic rate is sufficient to account for the increased body fat (Planche et al., 1983) and increased fat cell size (Boulange et al., 1979) found at this time. At 7 days of age adipose tissue lipoprotein lipase activity is increased, indicative of an increased capacity to accumulate lipid (Boulange et al., 1979) and adipose tissue thymidine kinase and DNA polymerase, enzymes involved in cell proliferation, are also increased (Cleary et al., 1979). Thus by 7 days of age

there is evidence for a reduced metabolic rate and increased lipid deposition and fat cell proliferation.

The thermogenic capacity of BAT,as measured by GDP binding to BAT mitochondria, has been reported to be reduced at 14 days of age in fa/fa pre-obese rats identified by their reduced rectal temperature (York et al., 1984). Bazin et al., (1984) removed a small portion of BAT from 2-14 day old pups for analysis of GDP binding and subsequently identified the genotype of the animals at 7 weeks of age. GDP binding to BAT mitochondria of 2 day old fa/fa pups was reduced by 30% compared with lean and was not significantly changed at 14 days of age. In lean pups GDP binding to BAT mitochondria increased with age so that by 10-14 days GDP binding to BAT mitochondria was 50% higher than in fa/fa pups and this was not associated with any changes in affinity for GDP in fa/fa pups. Thus it seems that the defect in energy expenditure through thermogenesis is closely linked to the primary lesion, particularly since the changes in GDP binding and in the thermogenic response to a meal, show a good correlation with gene dosage (York et al., 1984).

A number of the defects in the obese Zucker rat do not manifest themselves until the pups begin to eat the high carbohydrate laboratory chow at about 17 days of age, and further develop as the pups are weaned. The hyperinsulinaemia detectable <u>in utero</u> reappears and the large increases in serum insulin are associated with hypertrophy and hyperplasia of the β-cells of the pancreas (York et al., 1972; Bazin and Lavau, 1983), along with marked hyperphagia which is not present while suckling (Boulange et al., 1979). The hyperinsulinaemia leads to increased adipose tissue and hepatic lipogenesis, as estimated by tritiated water incorporation into lipid, and are paralleled by increases in the lipogenic enzymes G6PDH and acetyl CoA carboxylase (Godbole and York, 1978). Adipose tissue hypertrophy increases and is followed by increased proliferation of adipocytes, notably in the subcutaneous and retroperitoneal depots, which continues until at least week 26, whereas in lean rats fat cell numbers stabilise at 14 weeks of age (Johnson et al., 1971).

If weaning is delayed, increases in serum insulin and lipogenesis are suppressed (Godbole et al., 1978). If pre-weaning rats are allowed early access to the maternal high carbohydrate chow, then serum insulin and adipose tissue lipogenesis rise (York et al., 1981) and food intake increases (Bazin and Lavau, 1982). Post-weaning hyperinsulinaemia is not solely due to the change in dietary constituents, as weaning on to a high fat diet of similar composition to the mother's milk, does not prevent these changes (Turkenkopf et al., 1982b; Bazin and Lavau, 1982).

As insulin levels rise after weaning there is an initial overstimulation of target organs as liver, muscle and adipose tissue respond normally to insulin(Godbole and York, 1978; Stern et al.,1975). This is followed by the development of insulin resistance, primarily in muscle and adipose tissue, but not in liver (Cushman et al., 1978; Crettaz et al., 1981), so there is a gradual switch from adipose tissue to liver as the major site of lipogenesis. Thus in the adult obese rat adipose tissue functions mainly as a storage site (Turkenkopf et al., 1980). Several defects appear to be responsible for the progressive establishment of insulin resistance in obese rats. Glucose transport is reduced in muscle as early as 5 weeks of age, sufficient to explain the reduced glucose oxidation observed (Crettaz et al., 1980). In older rats (10-11 weeks of age), insulin binding is reduced by 25-30% as a result of a reduced receptor population, glucose transport is reduced and there are additional defects in glucose oxidation, perhaps resulting from the increases in lipid utilisation (Crettaz et al., 1980, 1981).

1.6 Aims of the project.

The obesity of the Zucker fa/fa rat results from an increased energetic efficiency as a result of a reduced expenditure rather than its increased food intake. The reduced energy expenditure seems to result from an inability to activate BAT thermogenesis in response to dietary stimuli and

this defect appears to be closely linked with the primary lesion. Adrenalectomy prevents the development of obesity and restores BAT function to normal. The obese rat is able to activate BAT in response to cold exposure but not to dietary stimuli. Hence it would appear that there is no inherent defect in BAT itself. The aim of this project was to investigate the sympathetic regulation of BAT in lean and obese (fa/fa) Zucker rats in response to cold and diet and consequently to determine whether the defects in BAT thermogenesis in obese rats, and their sensitivity to adrenal glucocorticoids, would be accounted for by variations in sympathetic activation of the tissue.

The experiments described in section 3.4.1 were performed in collaboration with Dr. M. J. Stock and Dr. N. J. Rothwell, whose laboratory supplied the animals and the measurements of oxygen consumption and GDP binding to BAT mitochondria.

CHAPTER 2

MATERIALS AND METHODS.

2.1 Materials

Unless otherwise stated chemicals were of reagent grade and supplied by either BDH Chemicals, Poole, Dorset, U.K., or Sigma Chemicals, Poole, Dorset, U.K.

ABTS	Boehringer Corporation Ltd., East Sussex, U.K.
ACTH (Synacthen Depot)	Ciba Geigy Ltd., U.K.
Alumina Neutral Activity Grade 1	Woelm ICN Chemicals, W.Germany.
Chlorisondamine Chloride	Ciba Geigy Ltd., U.K.
Desmethylimipramine	Dr. D. Templeton, (Department of Physiology)
Diazepam (Valium)	Roche Products, Herts, U.K.
Fentanyl Fluanisone	Jansenn Pharmaceuticals, from Crown Chemical Co., Kent, U.K.
Glucose Oxidase (s.a.200U/mg)	Boehringer Corporation Ltd., East Sussex, U.K.
Insulin Radioimmunoassay Kit	Wellcome Reagents Ltd., Dartford,U.K.
Isotopes	Amersham Radiochemical Centre, U.K.
NSD 1055	Dr. M. Bardsley (Department of Physiology)
Peroxidase (s.a.100 U/mg)	Boehringer Corporation Ltd., East Sussex, U.K.
POPOP (dimethyl bis phenyl oxazoyl benzene)	G. & G. Chemicals Ltd., Berks. U.K.
PPO Scintillation grade (diphenylaxazole)	G. & G. Chemicals Ltd., Berks, U.K.
Rat insulin standard	Novo Laboratories, Denmark.

Table 2.1 Composition of standard P.R.D. food pellets from Christopher Hill Limited.

	% Diet by weight
Fat	3.0
Protein	20.0
Carbohydrate	54.0
Fibre	5.0
Minerals/vitamins	1.8
Metabolisable energy	9.5MJ/kg

manufacturer's data

Food pellets were sterilised before use in an autoclave.

Table 2.2

List of food items presented to cafeteria-fed rats.

Chopped ham and pork	Battenberg cake
Corned beef	Fruit cake
Liver and bacon paté	Trifle sponges
Luncheon meat	Toblerone
Pork sausages	Chocolate marshmallow
Beef sausages	Plain marshmallow
Lean bacon	Milk chocolate
Shortcake	Mars bars
Chocolate wafers	Crunchie bars
Digestive biscuits	Lasagne
Chocolate crunch cake	Popcorn
Chocolate roll	Chocolate rice crispies
Swiss roll	Cheese
Chocolate mini-roll	

under a lighting regime of lights on from 0830 hours to 1730 hours. Animals were acclimatised to this temperature for 7 days before use.

2.2.5 Adrenalectomy.

Rats were anaesthetised with fentanyl fluanisone and diazepam. Diazepam was given at a dose of 0.25 mg/100g body weight by intraperitoneal (i.p.) injection followed by fentanyl fluanisone at a dose of 1 mg/100g body weight by intramuscular (i.m.) injection (Green, 1975). Righting reflex was lost within 5 minutes and the animals were immobilised for 30-60 minutes. Recovery time was between one and three hours. Bilateral adrenalectomies were performed from the dorsal approach. Adrenals were removed through two dorsal incisions with minimal damage to the capsule. The incisions were sutured with thread and the animals allowed to recover in a quiet room at 24-26^{o}C. Adrenalectomised rats were allowed free access to laboratory chow but were maintained on a 0.9% (w/v) saline solution instead of drinking water for 7 days before experimentation.

2.2.6 Hormone administration.

a. Corticosterone.

Rats were injected sub-cutaneously (s.c.) with corticosterone in dimethylformamide, ethanol, 0.9% (w/v) saline (1:2:7 v/v/v) at a dose of 1mg/100g body weight for 7 days before use. Control animals received an equal volume of the vehicle.

b. Adrenocorticotropic hormone (ACTH)

ACTH (Synacthen Depot) was given at a dose of 25µg/100g body weight and 50µg/100g body weight intramuscularly (i.m.) 32 hours and 18 hours respectively before rats were used.

2.2.7 Glucose tolerance test.

Rats were starved for 3½ hours before the study. Glucose was administered by intraperitoneal (i.p.) injection at a dose of 150mg/100g body weight in 0.3ml 0.9% (w/v) saline. Time zero groups received an equal volume of vehicle. 3 rats were killed at each time point.

2.2.8 Collection of serum samples.

Rats were normally stunned and killed by cervical dislocation. When blood samples were required the animals were killed by decapitation and trunk blood collected. The blood was allowed to clot for 1 hour on ice, centrifuged, the serum drawn off and stored at -20°C until required for assay.

2.3 Noradrenaline turnover.

Noradrenaline turnover was estimated by measuring the time dependant decline on tissue [^{3}H]-noradrenaline specific activity after an injection of [^{3}H]-noradrenaline as discussed in section 1.4.7.

Tracer doses of [^{3}H]- noradrenaline are rapidly cleared from the circulation by the noradrenaline uptake mechanisms in sympathetically innervated tissues and taken up into the endogenous stores of noradrenaline in sympathetic nerve endings. [^{3}H]-noradrenaline is released along with endogenous noradrenaline on nerve stimulation. Noradrenaline lost on nerve stimulation is replaced by resynthesis from tyrosine, reducing the specific activity of [^{3}H]-noradrenaline in the tissue. Thus the rate of decline in tissue specific activity of [^{3}H]- noradrenaline provides a measure of the sympathetic activity of the tissue.

1-7,8[^{3}H]- noradrenaline (s.a. 36Ci/mmol) was administered at a dose of 25μCi/100g body weight (0.12μg/100g body weight) in 0.9% (w/v) saline. The volume injected was ~~either 0.3ml intraperitoneally or~~ 0.1ml intravenously into a tail vein. Rats were killed 1, 3, 5 and 8 hours after administration of [^{3}H]-noradrenaline in groups of three rats at each time point. Tissues were rapidly excised, frozen in liquid nitrogen and noradrenaline isolated as described in section 2.3.1 and assayed for noradrenaline as described in section 2.3.3. 200μl samples were assayed for noradrenaline and 400μl samples counted for tritium with 5 ml. tritoscint scintillant (see section 2.11).

The specific activity of [^{3}H]-noradrenaline was calculated from the radioactivity counted and the noradrenaline content of the sample:-

$$\text{specific activity (s.a.)} = \frac{[^3H]\text{DPM in noradrenaline}}{\mu g \text{ noradrenaline}}$$

From a semi logarithmic plot of $\log_{10}$ specific activity against time the rate constant of decline or fractional turnover rate (k) and the half-life ($t_{\frac{1}{2}}$) of noradrenaline may be calculated:-

$$k = 2.303 \times \text{slope}$$

$$t_{\frac{1}{2}} = \frac{\ln 2}{k}$$

The turnover rate is calculated as the product of the fractional turnover rate (k) and the tissue content of noradrenaline (Taubin et al., 1972):-

turnover rate (T.R.) = k x tissue content of noradrenaline

Slopes of graphs were calculated by weighted linear regression analysis.

2.3.1 Isolation of noradrenaline from tissue samples.

Noradrenaline was isolated from tissues by a modification of the method of Anton and Sayre (1962) in which noradrenaline is bound to alumina under alkaline conditions and subsequently eluted with acid.

Tissues were rapidly excised, dissected free of surrounding fat or connective tissue and frozen in liquid nitrogen. They were stored at -20^{o}C prior to analysis which was always completed within one week.

Frozen tissues were homogenised in 4 ml 0.4M perchloric acid in a glass homogeniser by 4 or 5 passes of a close fitting teflon pestle at 4^{o}C. The homogenate was allowed to stand for 10 minutes on ice and then centrifuged in an MSE bench centrifuge to remove the precipitated protein. The supernatant was divided into two aliquots each of 1.8 ml. Noradrenaline (0.2 µg in 10 µl 0.1M hydrochloric acid) was added to one aliquot to allow subsequent calculation of recovery. To each aliquot of 0.4M perchloric acid supernatant was added 3 ml 0.5M Tris/HCl, pH 8.6, 5 mg sodium metabisulphate and 4 0 mg EDTA (disodium salt). The resulting solution was brought to pH 8.6 with 5M

sodium hydroxide. 80 mg of acid washed alumina (see section 2.3.2) were added to the solution which was then shaken for five minutes by vortexing. After the alumina settled the supernatant was aspirated off and the alumina was washed three times with distilled water. Noradrenaline was eluted from the alumina by vortexing the alumina with 600 µl 0.05M perchloric acid for fifteen minutes. 200 µl samples were taken for assay of noradrenaline and in noradrenaline turnover experiments 400 µl samples were taken for counting of radioactivity.

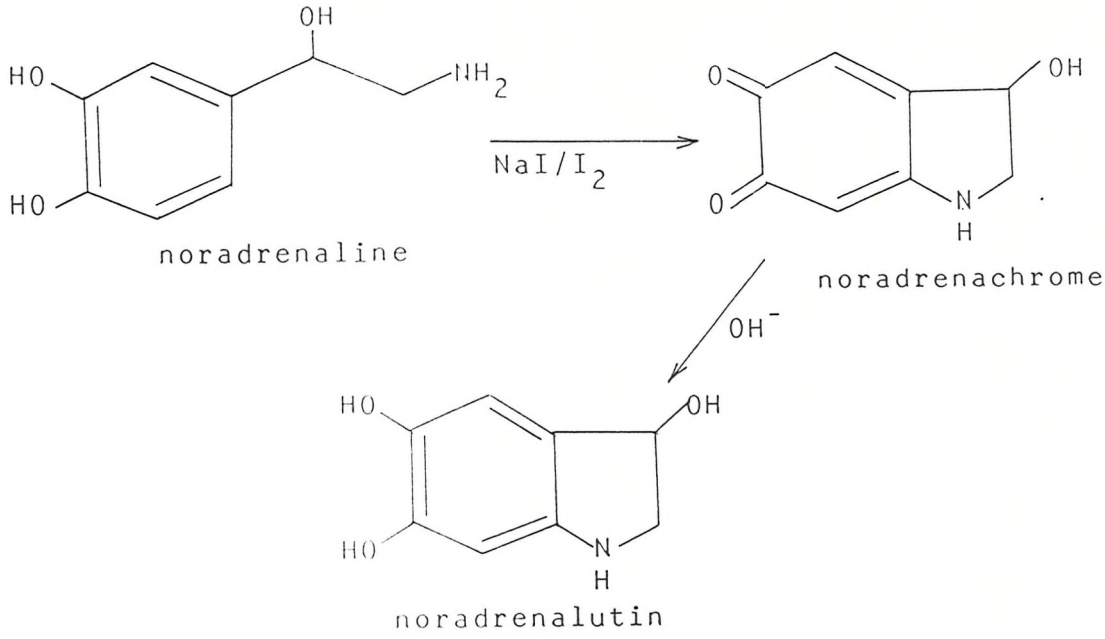
2.3.2 Preparation of acid washed alumina.

Aluminium oxide (neutral activity grade 1) was treated as follows:-

100g of alumina were added to 500 ml of 2M hydrochloric acid and heated at 95-100°C for 45 minutes with rapid and continuous stirring. The alumina was allowed to settle for 1½ minutes and the yellow supernatant and finer particles of alumina discarded. The alumina was washed twice with 250 ml portions of 2M hydrochloric acid at 70°C for 10 minutes, discarding the supernatant and finer particles of alumina after settling for 1½ minutes. The alumina was washed twice with 500 ml portions of 2M hydrochloric acid at 50°C for 10 minutes. Subsequently the alumina was washed with successive 200 ml volumes of distilled water until a pH of 3.4 was reached (20-25 washes). The finer particles of alumina which remained in suspension after allowing the alumina to settle for 1½ minutes were discarded after each wash. The washed alumina was transferred to an evaporating dish, heated at 120°C for 1 hour, heated at 200°C for 2 hours and stored in a vacuum dessicator over silica gel.

2.3.3 Assay of noradrenaline.

The procedure used was based on the method of Laverty and Taylor (1968). This method involves the oxidation of noradrenaline to noradrenachrome and subsequent rearrangement under alkaline conditions to the fluorescent indole derivative noradrenalutin:-



noradrenaline

noradrenachrome

noradrenalutin

In this reaction adrenaline is converted to its indole derivative adrenalutin so noradrenaline cannot be distinguished from adrenaline in the noradrenaline assay. However, by altering the conditions of the assay the fluorescence resulting from noradrenaline may be reduced, allowing noradrenaline to be distinguished from adrenaline.

a. Assay for noradrenaline.

0.9ml 66mM sodium phosphate, pH 6.5, was added to 200µl of 0.05M perchloric acid containing 0-0.066µg noradrenaline, 50µl 0.02M iodine in 5% (w/v) sodium iodide was added, shaken and, after 3 minutes, oxidation was stopped by the addition of 0.5ml alkaline sulphite (2.5% (w/v) sodium sulphite ($Na_2SO_3.7H_2O$) and 1% (w/v) EDTA (disodium salt) in 2.5M sodium hydroxide). Rearrangement was allowed to proceed for 5 minutes before the addition of 0.14ml glacial acetic acid to give a pH of 4.8. Fluorescence was measured after 25 minutes in an Aminco Bowman Spectrofluorimeter using excitation and emission peaks of 380 mµ and 480mµ respectively. Fluoresence was stable for 100 minutes. Reversed blanks were used, that is, reagents were added to the solution of noradrenaline in the order of acid, alkaline sulphite, iodine.

b. Assay for adrenaline.

The assay was performed as for noradrenaline but with the following modifications. 200µl 0.05M perchloric acid containing 0-0.066µg adrenaline was made up to 1.1ml with 0.9ml 0.1M sodium citrate buffer, pH 3.5. Rearrangement in alkaline sulphite was for 1 minute before 0.1ml glacial acetic acid was added to give a final pH of 5.0. Further development of fluorescence was not required and fluorescence was measured immediately using excitation and emission peaks of 410mµ and 500mµ respectively.

The fluorescence resulting from noradrenaline assayed under the conditions of the adrenaline assay was very low (Fig.2.1). By assaying samples under both assay conditions noradrenaline and adrenaline could be distinguished.

2.3.4 Assessment of noradrenaline turnover by ganglionic blockade with chlorisondamine.

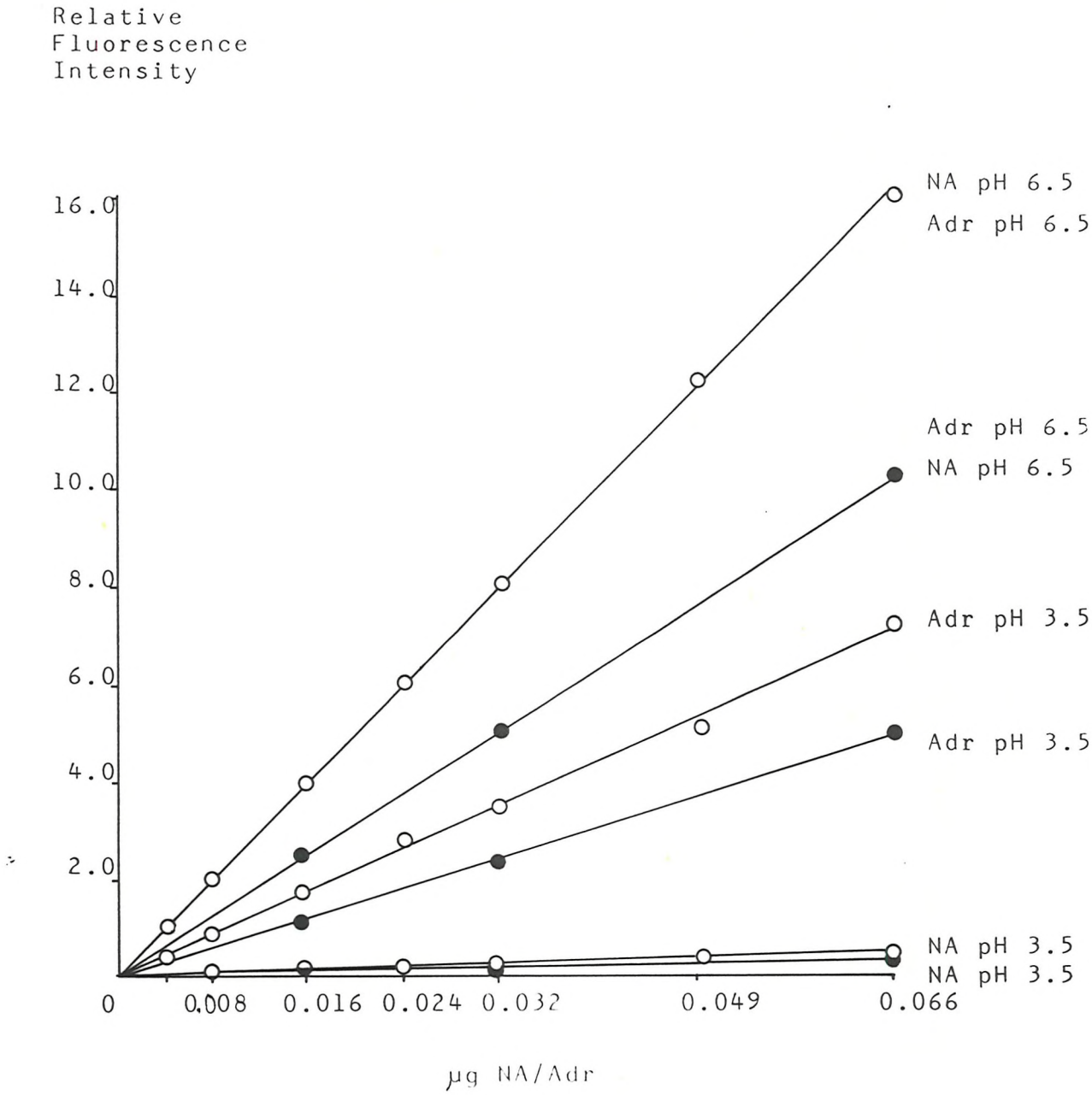
Animals were injected i.v. with 25µCi/100g body weight 1-7,8 [^{3}H]- noradrenaline in 0.1ml 0.9%/(w/v) saline. After 5 minutes and 5 hours they were given 0.5mg/100g body weight chlorisondamine chloride by i.p. injection in 0.3ml 0.9% (w/v) saline. Control animals received an equal volume of saline. All animals were killed after 10 hours. Tissues were excised and processed as in the noradrenaline turnover studies (sections 2.3.1 and 2.3.3) The extent to which the tissue specific activity of [^{3}H]-noradrenaline in chlorisondamine treated animals is maintained above that in saline treated animals is a reflection of the neuronal firing rate (see section 1.4.7). Results are expressed as the absolute and the percentage difference in [^{3}H]-noradrenaline specific activity between the two groups. If it is assumed that there is a linear decline in $\log_{10}$ [^{3}H]- noradrenaline specific activity with time then the fractional turnover rate (k) for noradrenaline turnover resulting from sympathetic nerve activity can be calculated:-

Fig. 2.1. Standard curves for the fluorimetric assay of noradrenaline and adrenaline.

Standard solutions of noradrenaline and adrenaline were assayed under the conditions of the noradrenaline (pH6.5) and adrenaline (pH3.5) assays, before (O——O) and after (●——●) extraction on alumina as described in section 2.3

NA = noradrenaline

Adr = adrenaline


Relative
Fluorescence
Intensity
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0
0.008
0.016
0.024
0.032
0.049
0.066
µg NA/Adr
NA pH 6.5
Adr pH 6.5
Adr pH 6.5
NA pH 6.5
Adr pH 3.5
Adr pH 3.5
NA pH 3.5
NA pH 3.5

$$k = \frac{\log_{10} [^3H]\text{-NA s.a. in saline treated group} - \log_{10} [^3H]\text{-NA s.a. in chlorisondamine treated group}}{10}$$

The half-life ($t_{\frac{1}{2}}$) and turnover rate are calculated as previously (section 2.3.2).

2.4 In vitro uptake of [^{3}H]-noradrenaline.

[^{3}H]- noradrenaline uptake was assessed independently from the effects of tissue blood flow and nerve stimulation, by measuring the uptake of [^{3}H]-noradrenaline in tissue slices incubated in vitro. Total uptake was measured and $Uptake_1$ and $Uptake_2$ were taken to be those proportions of total uptake blocked by desmethylimipramine and corticosterone respectively.

Tissues were excised and washed in ice cold Krebs bicarbonate. Tissues were chopped into 1mm x 0.2mm x 0.2mm slices using a McIlwain Tissue Chopper and the slices suspended in Krebs bicarbonate at 10mg wet weight/ml. All incubations were performed in triplicate. 1ml of suspended tissue slices was added to 3.4ml Krebs bicarbonate and 0.1ml 50mM Tris/HCl,pH7.4, containing 0.1μCi [^{14}C]-hydroxymethylinulin (specific activity 10mCi/mmol). Total uptake was measured in control incubations, $Uptake_1$ from incubations with 7μg desmethylimipramine and $Uptake_2$ from incubations with 70μg corticosterone. The slices were gassed with 95% O_2 5% CO_2 (v/v) and preincubated for 1 hour at 37°C in a shaking water bath. The slices were regassed and incubated for 20 minutes with 0.75μCi 1-7,8 [^{3}H]- noradrenaline (specific activity 1.5Ci/ml) in 0.5ml Krebs bicarbonate in a shaking water bath at 37°C. The final incubation volume was 5ml and the concentration of noradrenaline 10^{-7}M, of desmethylimipramine 5×10^{-6}M and of corticosterone 4×10^{-5}M.

The incubations were stopped by filtering on Whatman No. 1 filter paper and washed twice with 1ml Krebs bicarbonate. Filters were soaked for 20 minutes and counted for [^{3}H] and [^{14}C] with 5ml tritosinct scintillant. Extracellular space was

calculated from the [^{14}C]-inulin counts, so that [^{3}H] counts due to medium trapped in the tissue could be subtracted from total uptake.

2.4.1 Preparation of Krebs bicarbonate.

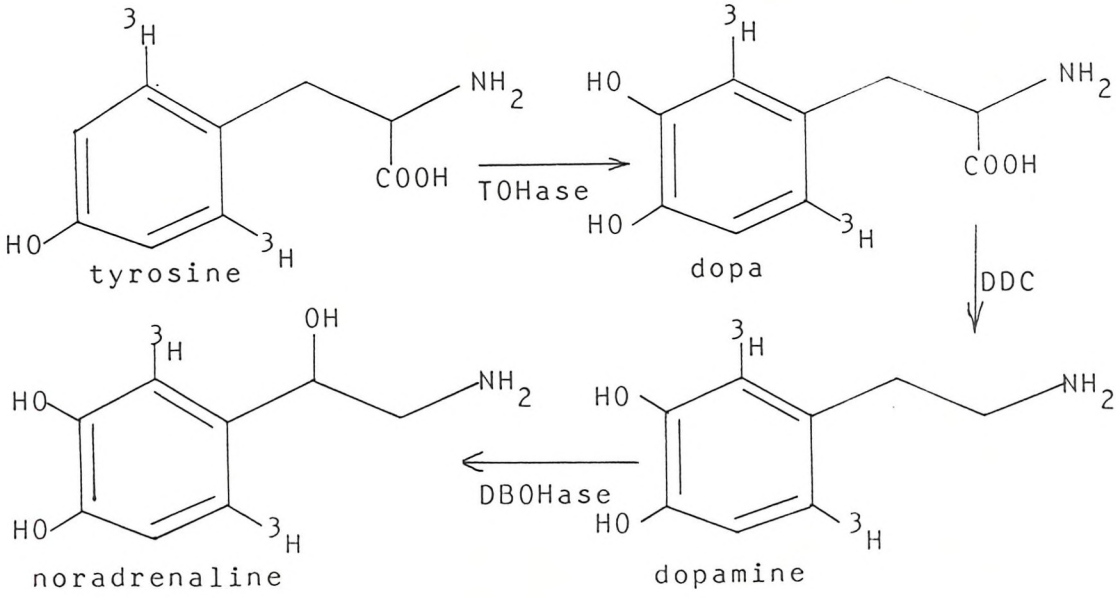
Krebs bicarbonate was made up immediately before use as follows:-

109mM NaCl
4.7mM KCl
1.2mM $MgSO_4,7H_2O$
1.2mM KH_2PO_4
11.5mM glucose
25mM $NaHCO_3$
2.5mM $CaCl_2$

The buffer was gassed for 30 minutes with 95%O_2 5% CO_2 (v/v) and brought to pH7.4 with 2M sodium hydroxide.

2.5 Noradrenaline synthesis.

Noradrenaline synthesis was estimated *in vivo* by adapting the method of Nielson (1976). 1-2,6 [^{3}H]-tyrosine was injected i.v. and the [^{3}H]-noradrenaline formed was isolated as in noradrenaline turnover experiments (section 2.3). Use of 1-2,6 [^{3}H]-tyrosine ensured that no tritium was lost in conversion to [^{3}H]-noradrenaline:-



Animals were injected i.v. with 123μCi/100g body weight 1-2,6[^{3}H]-tyrosine (specific activity 45Ci/mmol) in 0.1ml 0.9% (w/v) saline. Animals were sacrificed after 2 hours, tissues excised and the noradrenaline was extracted and assayed as described previously (section 2.3) with the exception that tissues were homogenised in 2ml 0.4M perchloric acid in these experiments. When standard solutions of [^{3}H]-tyrosine were subjected to the noradrenaline extraction procedure [^{3}H]-tyrosine was not detectable in the 0.05M perchloric acid alumina eluates. The alkaline supernatant from the alumina extraction was decanted and analysed for tyrosine.

2.5.1 Tyrosine extraction.

Amberlite CG120 columns containing 300mg resin were activated by washing with successive 2ml aliquots of 4M hydrochloric acid, distilled water, 25mM hydrochloric acid and distilled water. The alkaline supernatants from the alumina extraction of noradrenaline were brought to pH 2.0 with 2M hydrochloric acid and passed down Amberlite CG120 columns to absorb tyrosine. Columns were washed with 2.5ml distilled water to elute any unbound radioactivity and then acidified with 2ml 0.5M hydrochloric acid. Tyrosine was eluted with 10ml 4M nitric acid. 1.5ml samples of the 4M nitric acid eluate were assayed for tyrosine or partially neutralised with 250μl 10M sodium hydroxide and counted for tritium with 12ml tritoscint scintillant. When standard solutions of [^{3}H]-noradrenaline and tyrosine were subjected to the extraction procedures [^{3}H]-noradrenaline could not be detected in the 4M nitric acid eluate and tyrosine was extracted with an efficiency of 78-82% as assayed below.

2.5.2 Assay for tyrosine.

Tyrosine was estimated by the method of Udenfriend (1957) which involves the formation of a tyrosine nitrosonapthol chromophore with a characteristic absorption at 450nm.

1.5ml of Amberlite CG120 column eluate or standard solutions of tyrosine (10-200μg in 4M nitric acid) was added

to 1.5ml nitrosonapthol reagent (1% (w/v) nitroso-2-napthol in 95% (w/v) ethanol and 1.6% (w/v)sodium nitrite (fresh) and heated in a water bath at 55°C for 30 minutes. After cooling, unreacted nitrosonapthol was extracted by shaking with 10ml chloroform and the absorbance of the aqueous layer read at 450nm in glass cuvettes. Fig 2.2 shows a typical standard curve.

2.6 Protein determination using the Bradford Assay.

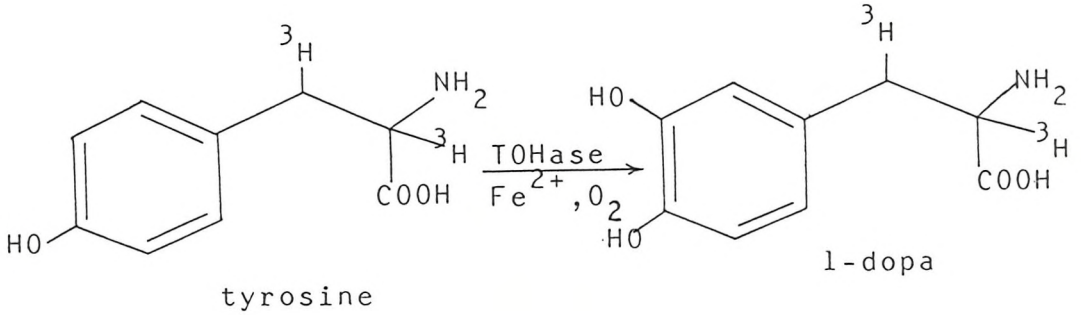
Protein concentrations were assayed using the method of Bradford (1976) which involves the binding of Coomassie Brilliant Blue G250 due to protein. When the dye binds to protein the absorption maximum of the dye shifts from 465nm to 595m.

Protein binding reagent was prepared as follows:- 100mg Coomassie Brilliant Blue G250 dye were dissolved in 50ml 95% (v/v) ethanol. 100ml 98% (v/v) orthophosphoric acid were added and the solution made up to 1 litre with distilled water. Suspended dye particles were removed by filtration and the reagent was stored in a dark glass bottle.

5ml protein binding reagent were added to 0-15μg protein sample or standard (Bovine Serum Albumen fraction V) in 100μl of the relevant buffer. The solution was mixed by vortexing, allowed to stand for 5 minutes and the absorbance read at 595nm within 25 minutes.

2.7 Tyrosine hydroxylase (E.C. 1.14.3a) assay.

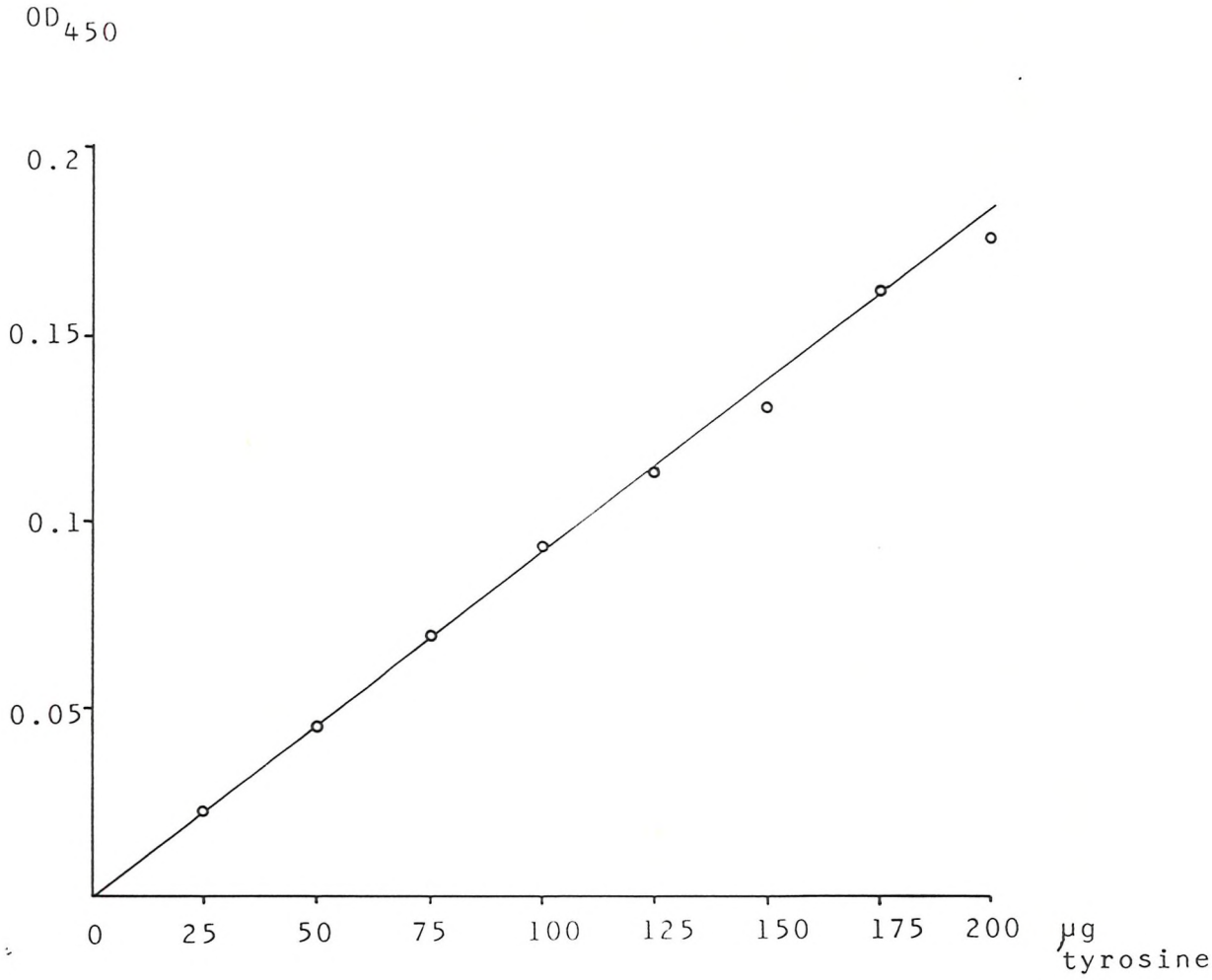
This assay is based on the method of Hendry and Iverson (1971) and involves the conversion of [^{3}H]-tyrosine to [^{3}H]-dopa utilising dimethyltetrahydropterin as the cofactor:-



tyrosine

l-dopa

Fig.2.2 Standard curve for the assay of tyrosine.


OD450
0.2
0.15
0.1
0.05
0
25
50
75
100
125
150
175
200
µg tyrosine

The pathway of noradrenaline synthesis is stopped at l-dopa by performing the assay in the present of a dopa decarboxylase inhibitor, NSD 1055. [^{3}H]-l-dopa is then separated from [^{3}H]-tyrosine by binding to alumina.

Tissues were excised and homogenised in 5-10 volumes 6mM ferrous sulphate in a glass homogeniser by 4 or 5 passes of a close fitting teflon pestle. Homogenates of BAT were centrifuged in a Beckman microfuge for 2 minutes, the fat cake discarded, and the pellet resuspended in the supernatant by vortexing. 10µl samples of tissue homogenates or, in the case of BAT, resuspended pellets, were incubated for 10 minutes with 10µl substrate/cofactor mix (section 2.7.1) at 37°C. The reaction was stopped by the addition of 200µl 0.4M perchloric acid containing 2µg/ml l-dopa as carrier. l-dopa was then separated from unreacted tyrosine.

The acidified reaction mix was added to 3.8ml extraction buffer (50mM Tris/HCl, pH8.6, 75mM sodium hydroxide and 50mM EDTA (disodium salt) and the resulting solution brought to pH 8.6 with 2M sodium hydroxide. 70mg alumina (previously washed 20 times with distilled water and dried) were added to the solution and the tubes were shaken for 5 minutes by vortexing. The supernatant was aspirated and unreacted tyrosine washed from the alumina with 4 successive 5ml aliquots of distilled water. l-dopa was finally extracted by shaking the alumina with 1.5ml 0.5M acetic acid for 10 minutes. The acetic acid eluate was counted for tritium after the addition of 12ml tritoscint scintillant.

Reaction blanks used 10µl 6mM ferrous sulphate in place of tissue homogenates. Reaction rates were linear for the time course of the assay and linear with protein concentration (see Fig.2.3).

2.7.1 Preparation of substrate/cofactor mix.

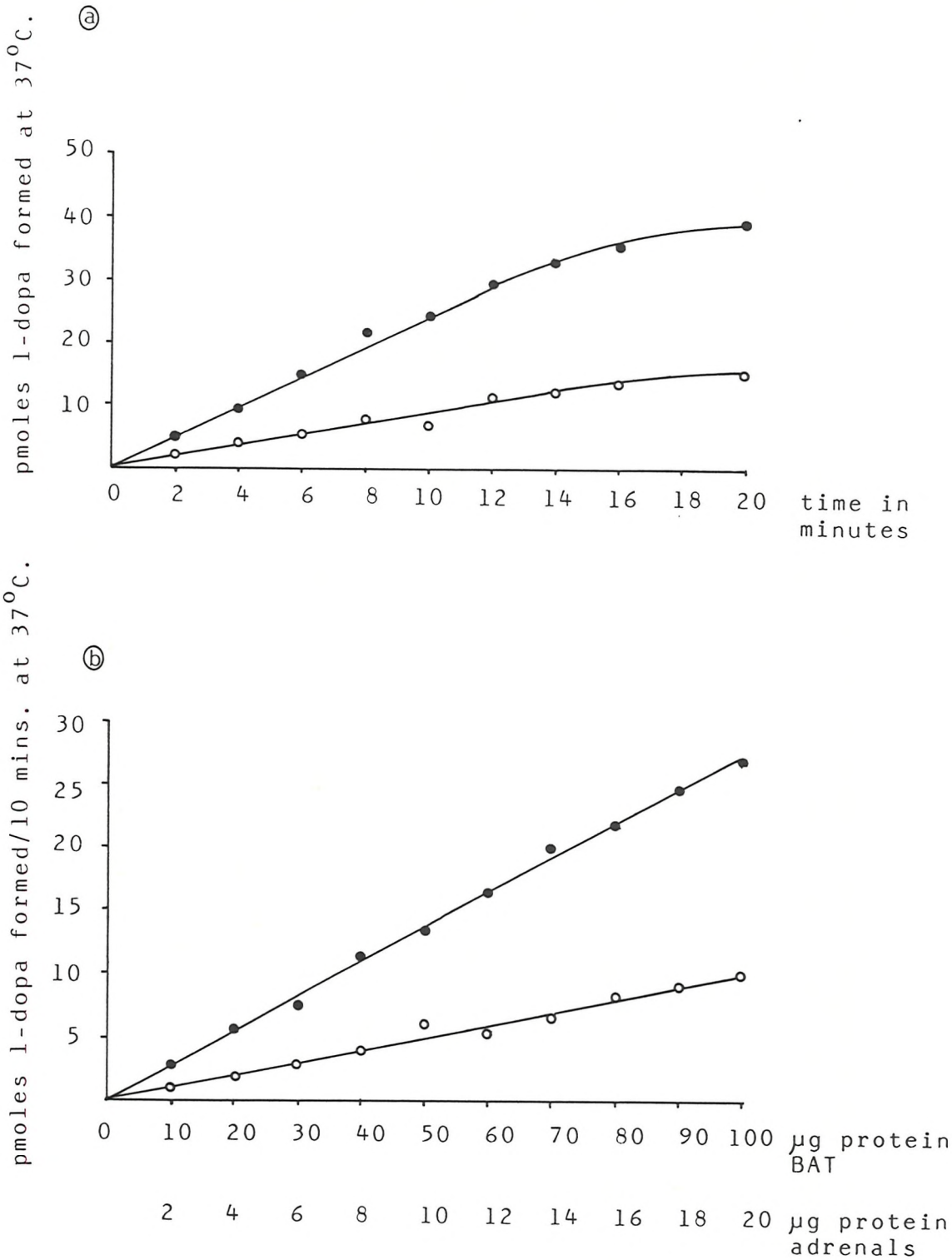
7.5µCi l-2,3 [^{3}H]-side chain tyrosine (specific activity 45Ci/mmol) in 50µl distilled water were made up to 150µl with 5mM Tris/HCl, pH8.6, and shaken with 5mg alumina at 4°C for 10 minutes. 0.5mg 6,7 dimethyl 5,6,7,8 tetrahydropterin was dissolved in 50µl mercaptoethanol and made up to 550µl with 0.8M potassium phosphate, pH6.0, containing 0.4mg/ml NSD 1055 and 0.4mM tyrosine. 150µl of this solution were mixed with 150µl of the alumina treated [^{3}H]-tyrosine solution to give the substrate/cofactor mix which was used immediately.

Fig. 2.3 Tyrosine hydroxylase assay.

a. Time course. BAT (O———O) homogenised in 5 volumes 6mM ferrous sulphate and adrenals (●———●) in 10 volumes ferrous sulphate. 10µl samples were taken and assayed as described. Incubations were stopped after 0-20 minutes.

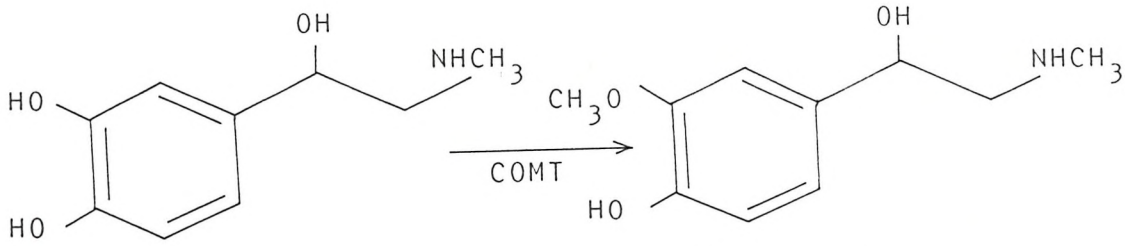
b. The effect of protein concentration on tyrosine hydroxylase activity.

Serial dilutions of tissue homogenates containing 0-100µg/10µl and 0-20µg protein for BAT (O———O) and adrenals (●———●) respectively. Incubations were stopped after 10 minutes.


ⓐ
pmoles l-dopa formed at 37°C.
50
40
30
20
10
0 2 4 6 8 10 12 14 16 18 20
time in minutes
ⓑ
pmoles l-dopa formed/10 mins. at 37°C.
30
25
20
15
10
5
0 10 20 30 40 50 60 70 80 90 100 µg protein BAT
2 4 6 8 10 12 14 16 18 20 µg protein adrenals

2.8 Catechol-O-methyl transferase (E.C.2.1.1.6) (COMT) assay.

Tissue COMT activity was assayed by the method of Axelrod and Tomchick (1958) in which adrenaline is converted to metanephrine using S-adenosyl methionine as a methyl donor:-



Adrenaline Metanephrine

The metanephrine formed is separated from unreacted adrenaline by extraction with an organic solvent and assayed fluorimetrically.

Tissues were homogenised in 10 volumes 40mM potassium phosphate, pH7.8. The homogenates were centrifuged to remove cell debris. 250µl samples of the supernatants were incubated for 30 minutes at 37°C with 750µl 53mM potassium phosphate, pH7.8, containing 0.8mM magnesium chloride, 0.05mg S-adenosyl methionine and 0.3µmoles adrenaline. Reaction blanks were incubated without S-adenosyl methionine. The reaction was stopped with 0.5ml 0.5M borate buffer, pH 10.0. Metanephrine was extracted by shaking the solution with 15ml chloroform containing 2% (v/v) amyl alcohol. Metanephrine was extracted from the chloroform by shaking with 1.5ml 0.1M HCl. The fluorescence of this solution was measured in an Aminco Bowman Spectrofluorimeter using excitation and emission peaks of 285 mµ and 335 mµ respectively. The µmolar relative fluorescence intensity of standard solutions of metanephrine and adrenaline were 126.3 and 124.5 respectively. After extraction, fluorescence was reduced to 40.0 and 2.5 for metanephrine and adrenaline respectively. Efficiency of extraction was thus 31.7% for metanephrine and only 2.0% for adrenaline. The formation of metanephrine from adrenaline was calculated from the equation:-

$$\mu\text{moles metanephrine formed} = \frac{\text{Final fluorescence} - \text{Initial fluorescence}}{\mu\text{molar fluorescence of extracted metanephrine} - \mu\text{molar fluorescence of extracted adrenaline}}$$

2.9 Insulin Radioimmunoassay.

This assay was based on the method of Hales and Randle (1963). Insulin antibody is reacted with insulin samples or standards, followed by $[I^{125}]$-insulin. The amount of $[I^{125}]$-insulin binding to the antibody is inversely proportional to the amount of insulin in the samples. Since the insulin antobody and its complex with insulin are soluble the antibody is precipitated with an antiglobulin antibody to allow its separation from unbound insulin.

The assay was performed in triplicate in 2ml glass tubes. The following tubes were set up:-

Blanks	100µl 50ml sodium phosphate, pH 7.5, containing 0.5% (w/v) BSA, and 0.025% (w/v) thiomersal and 0.9% (w/v) saline (Buffer B).
Zeroes (% bound)	100µl Buffer B
Standards	0.625µU to 10µU rat insulin in 100µl Buffer B.
Unknowns	100µl serum.

All tubes, except blanks received 100µl insulin binding reagent (section 2.9.1). Blanks received 100µl 50mM sodium phosphate, pH7.4, containing 0.5% (w/v) BSA, and 0.025% (w/v) thiomersal (Buffer A). Tubes were vortexed and left for 6 hours at 4°C. 100µl iodinated $[I^{125}]$-insulin (0.25µCi/ml) were added to all tubes and to 3 further tubes to allow counting of total added radioactivity. Tubes were vortexed and left for 18 hours at 4°C. Tubes were centrifuged at 2000g for 20 minutes at 4°C to isolate the precipitated anti-globulin antibody anti-insulin antibody complex and the supernatants discarded. Each tube was counted for one

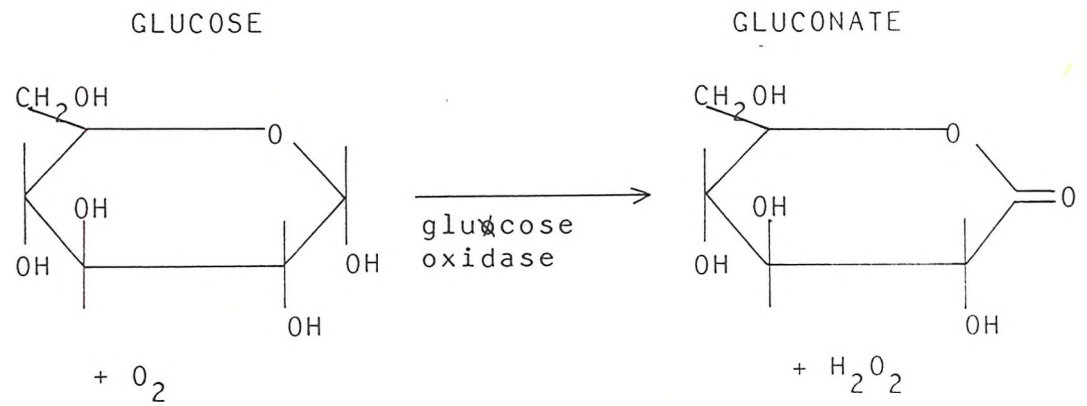
minute in a Beckman γ-counter with a counting efficiency for $[I^{125}]$ of 80%. Radioactivity bound was expressed as a percentage of the total radioactivity added after subtraction of the blank, and a standard curve plotted of percentage insulin bound against $\log_{10}$ insulin concentration. Fig. 2.4 shows a typical standard curve.

2.9.1 Insulin binding reagent.

The freeze dried reagent, obtained from Wellcome Reagents Ltd., Dartford, U.K., was reconstituted with 8ml denionised water at 4°C. The reagent contains guinea pig anti-insulin antobody, rabbit anti-guinea pig globulin antibody. 40mM sodium phosphate, pH7.4, 20mM EDTA (disodium salt), 0.1% (w/v) sodium azide and 0.5% (w/v) Bovine Serum Albumin (BSA) when reconstituted.

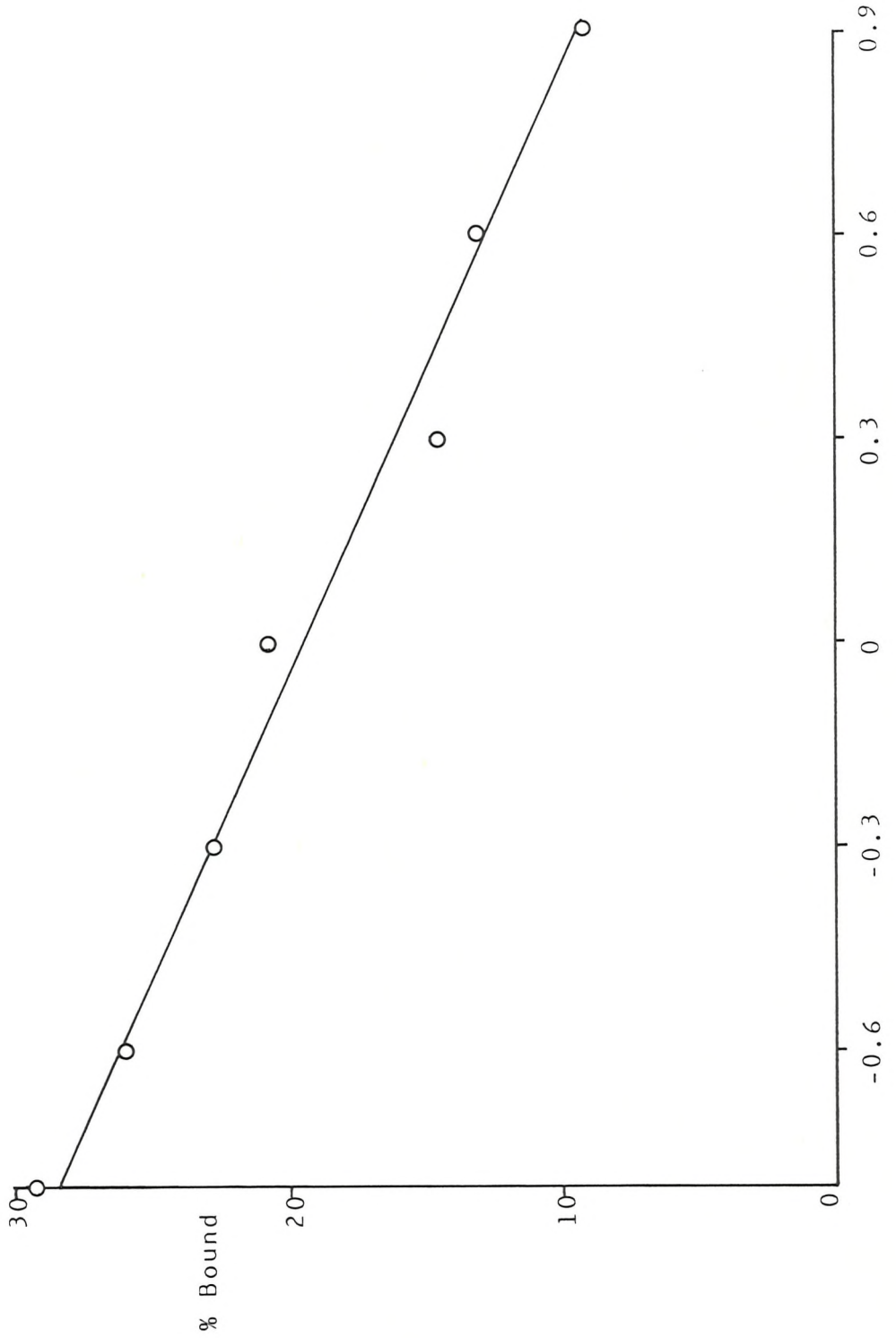
2.10 Serum glucose assay.

This assay utilises the hydrogen peroxide produced by the oxidation of glucose by glucose oxidase to oxidise the dye ABTS with peroxidase, converting the reduced dye to an oxidised form with an absorption maxiumum of 620nm:



$$H_2O_2 + ABTS_{(reduced)} \xrightarrow{\text{peroxidase}} H_2O + ABTS_{(oxidised)}$$

Fig. 2.4 Standard curve for insulin radioimmunoassay.


30
20
10
0
% Bound
-0.6
-0.3
0
0.3
0.6
0.9
log10 insulin ng

0.1ml serum was shaken with 1ml 6% (w/v) perchloric acid and precipitated protein removed, after 10 minutes, by centrifugation in an MSE bench centrifuge. 100µl samples of supernatant were added to 2.5ml glucose oxidase reagent (0.6mg peroxidase, 600mg glucose oxidase and 1.2g ABTS· dissolved in 1.2 litres 0.1M sodium phosphate, pH7.0, and stored at 4°C) and incubated for 15 minutes at 37°C. When cool the absorbance was read at 620nm. Standard curves of 0-30µg glucose were determined with each assay.

2.11 Determination of radioactivity.

Radioactivity was measured by liquid scintillation counting in tritoscint scintillant (0.05% (w/v) dimethyl POPOP, 0.4% (w/v) PPO and 33.3% Triton X100 in xylene). Samples were counted in a Philips PW4540 scintillation counter with preprogrammed quench curves. Efficiency of counting was 63% for [^{14}C] and 38% for [^{3}H].

2.12 Statistics.

Results have been expressed as means $\pm$ standard error of the mean. Statistical significance was calculated using a two-tailed unpaired Student's 't' test.

CHAPTER 3.

RESULTS AND DISCUSSION.

SECTION 3.1 Sympathetic nervous system activity in the obese (fa/fa) Zucker rat.

The increased energetic efficiency and resultant obesity of the fa/fa rat has been associated with a reduced energy expenditure which is detectable at an early age before any increases in food intake (Planche et al., 1983). This reduced energy expenditure has been linked with defective BAT thermogenesis (Holt and York, 1982), in particular with an inability to activate BAT in response to dietary stimuli. Young obese rats are able to activate BAT normally in response to cold exposure but lack the ability to increase BAT thermogenesis if overfed with sucrose or a cafeteria diet (Holt et al., 1983; Triandafillou and Himms-Hagen, 1983). In addition the short-term thermic effect of feeding is reduced in the obese rat (Rothwell et al., 1983a).

It has been suggested that a reduced sympathetic activation of BAT may be responsible for the reduced thermogenic function of the tissue in fa/fa rats. If this is the case then reduced nerve activity must be demonstrated at an early age when the obesity is developing. In the experiments reported in this thesis rats were used at 5-6 weeks of age when the obesity of the fa/fa rats was visually apparent, but body weights were still similar to lean littermates. At this age BAT in obese rats is still responsive to noradrenaline administration (York et al., 1984) and cold exposure (Holt et al., 1983; Triandafillou and Himms-Hagen, 1983), indicating that both the tissue and its nerve supply still retain a functional capacity.

As discussed in section 1.4.7, sympathetic activity may be assessed by measuring the rates of noradrenaline turnover. [^{3}H]- noradrenaline, administered by i.p. or i.v. injection, is rapidly taken up and incorporated into the endogenous stores of noradrenaline in sympathetic nerve endings. Since the [^{3}H]- noradrenaline is released along with endogenous noradrenaline on nerve stimulation, the rate of decline in tissue

[^{3}H]- noradrenaline specific activity provides an index of the activity of the sympathetic nerves of that tissue (Landsberg and Young, 1978).

Increases in BAT thermogenesis in response to cold exposure and sucrose or cafeteria overfeeding are associated with increased noradrenaline turnover in BAT (Young et al., 1982). The experiments described in this section were designed to investigate the role of the sympathetic nervous system, as assessed by measurement of noradrenaline turnover, in the response to cold and diet in the Zucker rat. Noradrenaline turnover was measured in BAT of lean and obese rats housed at 24-26^{o}C and 4^{o}C and in rats overfed with sucrose housed at 24-26^{o}C.

RESULTS.

3.1.1 Isolation and assay of noradrenaline from BAT and hearts of lean and obese (fa/fa) Zucker rats.

Noradrenaline was isolated from BAT and hearts of lean and obese rats and assayed as described in section 2.3. In brief, supernatants from 0.4M perchloric acid tissue homogenates were divided into two aliquots and 0.2 µg noradrenaline added to one aliquot to allow calculation of recovery. Noradrenaline was also recovered from standard solutions in 0.4M perchloric acid. Solutions were shaken with alumina to absorb noradrenaline, the alumina was washed and noradrenaline extracted from the alumina in 0.05M perchloric acid. Aliquots of the 0.05M perchloric acid extracts and standard solutions of noradrenaline in 0.05M perchloric acid were assayed as described in section 2.3. Table 3.1 shows the efficiency of recovery of noradrenaline isolated in this manner from standard solutions and tissue supernatants. Noradrenaline was extracted from 0.4M perchloric acid with an efficiency of 74% and extraction was unaffected by recovery from tissue supernatants.

Table 3.1 Recovery of noradrenaline from tissue supernatants.

ISOLATION SOLUTION		ASSAY SOLUTION		RECOVERY OF ADDED NORADRENALINE
Tissue supernatant	µg NA added	µg NA added	Relative fluorescence intensity	% recovery
NONE	NONE	0.2	14.1 ± 0.02	100
NONE	0.2	NONE	10.4 ± 0.03	74
lean BAT	NONE	NONE	4.2 ± 0.05	-
lean BAT	0.2	NONE	14.6 ± 0.05	74
obese BAT	NONE	NONE	2.1 ± 0.05	-
obese BAT	0.2	NONE	12.5 ± 0.05	74
lean heart	NONE	NONE	3.1 ± 0.03	-
lean heart	0.2	NONE	13.2 ± 0.04	74
obese heart	NONE	NONE	3.1 ± 0.04	-
obese heart	0.2	NONE	13.2 ± 0.04	74

Noradrenaline was isolated and assayed as described in section 2.3. Values represent means ± SEM for triplicate determinations.

Rat BAT contains dopamine and small amounts of adrenaline in addition to noradrenaline (Barnard et al., 1980). Under the conditions used, the assay for noradrenaline is not affected by the presence of dopamine but is sensitive to adrenaline (Laverty and Taylor, 1968). However, by altering the conditions of the assay, noradrenaline may be distinguished from adrenaline (see section 2.3.3).

BAT and hearts from lean and obese rats were initially assayed for noradrenaline and adrenaline, (Table 3.2). Adrenaline could not be detected in BAT or heart of lean and obese rats. Consequently in subsequent experiments tissues were assayed for noradrenaline only.

3.1.2 The effects of cold acclimatisation and sucrose overfeeding on noradrenaline turnover in lean and obese (fa/fa) Zucker rats.

In common with other workers (Young and Landsberg, 1983), i.p. administration of [^{3}H]- noradrenaline gave inconsistent results when noradrenaline turnover was measured in BAT of obese animals, so [^{3}H]- noradrenaline was administered by i.v. injection into a tail vein.

After i.v. injection of tracer doses of [^{3}H]- noradrenaline the fractional turnover rate (k) and the half-life ($t_{\frac{1}{2}}$) can be calculated from the time-dependant decline in tissue [^{3}H]-noradrenaline specific activity. Fig. 3.1 shows the graphs of $\log_{10}$ specific activity of [^{3}H] - noradrenaline plotted against time for BAT and heart of lean and obese rats. Tables 3.3 and 3.4 show the endogenous tissue content of noradrenaline in BAT and heart of lean and obese rats, the half-life and fractional turnover rate calculated from the graphs in Fig. 3.1 and the noradrenaline turnover rate and initial tissue uptake of [^{3}H] - noradrenaline calculated from these values and the endogenous tissue content of noradrenaline (see section 2.3).

The BAT noradrenaline content of obese rats housed at 24-26^{o}C was reduced by 40% compared to lean and the half-life of noradrenaline increased from 4.9$\pm$0.3 hours to 125$\pm$27 hours.

Table 3.2 Noradrenaline and adrenaline content of BAT and heart of lean and obese (fa/fa) Zucker rats housed at 25-27°C.

	BAT		HEART	
	lean	obese	lean	obese
Noradrenaline content ng/organ	275±6	130±5 ***	200±3	200±4
Adrenaline content ng/organ	0	0	0	0

Noradrenaline and adrenaline were isolated and assayed as described in section 2.3.
Values represent means ± SEM for 4 rats in each group.
*** $p < 0.001$, compared with equivalent lean group.

Fig. 3.1 Noradrenaline turnover in brown adipose tissue and heart of lean and obese rats housed at 24-26°C (●——●), 4°C (○——○) and allowed access to a 10% (w/v) sucrose solution (□——□) while housed at 24-26°C. Rats were injected i.v. with 25 μCi/100g body weight 1-7, 8 [^{3}H]-noradrenaline in 0.9% (w/v) saline at time zero (see section 2.3). Values represent means ± SEM for 3 rats at each time point.

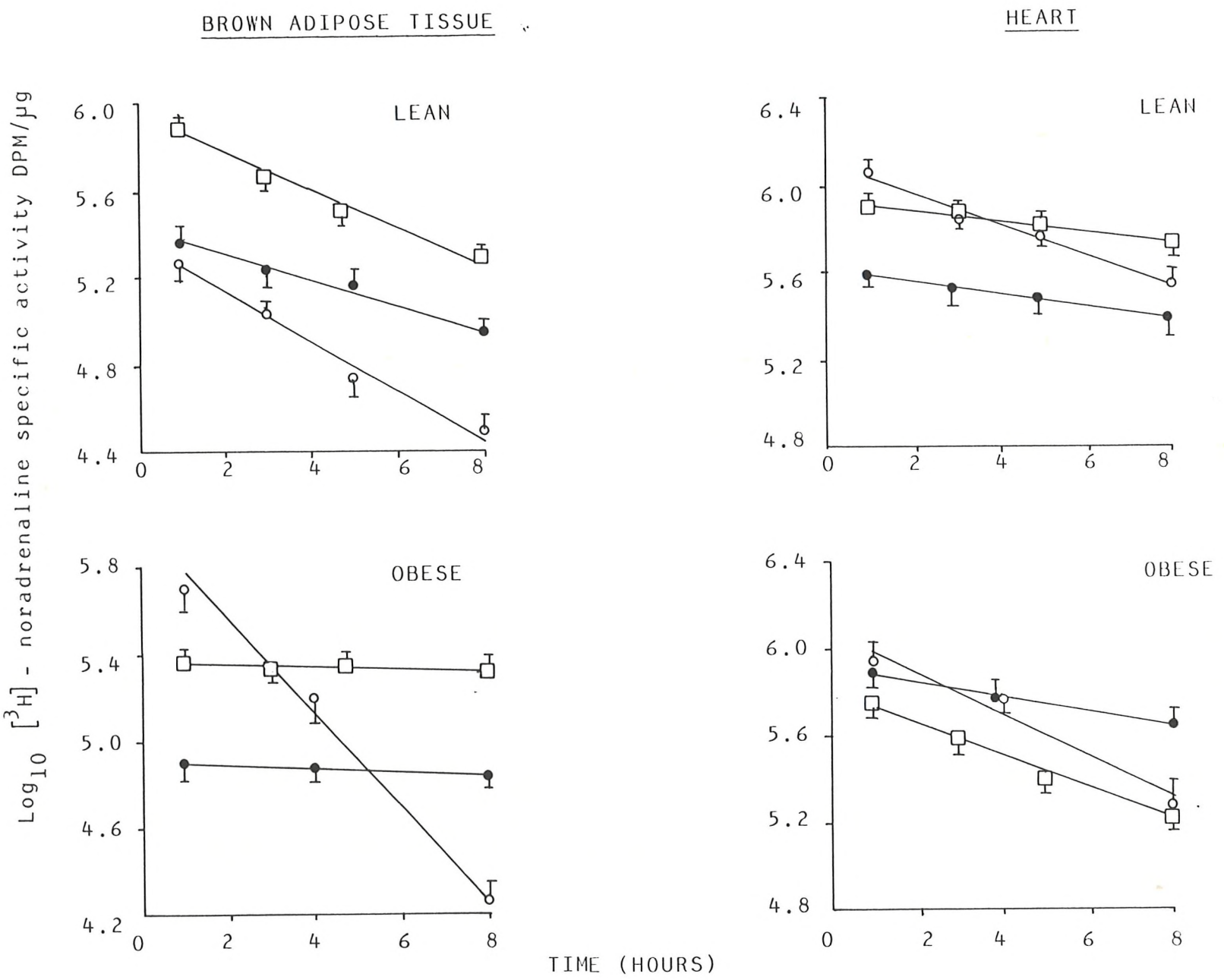

BROWN ADIPOSE TISSUE
HEART
LEAN
LEAN
OBESE
OBESE
Log10 [3H] - noradrenaline specific activity DPM/µg
TIME (HOURS)
6.0
5.6
5.2
4.8
4.4
6.4
6.0
5.6
5.2
4.8
5.8
5.4
5.0
4.6
4.2
6.4
6.0
5.6
5.2
4.8
0
2
4
6
8

Table 3.3 Legend.

4-5 week old lean and obese rats were fed chow for 7 days and housed at 24-26^{o}C or 4^{o}C or were offered a 10% (w/v) sucrose solution instead of drinking water for 7 days and housed at 24-26^{o}C. Values represent means $\pm$ S.E.M. for 9-12 rats in each group. ***, $p < 0.001$; **, $p < 0.005$; 2, $p < 0.01$. a, compared with control group; b, compared with equivalent lean group.

Table 3.3 The tissue content, fractional turnover rate, half-life, turnover rate and initial uptake of noradrenaline in BAT of lean and obese (fa/fa) rats housed at 24-26°C, rats acclimatised to 4°C and rats housed at 24-26°C and offered a 10% (w/v) sucrose solution.

		LEAN	OBESE
CONTROL 25-27°C	noradrenaline content (ng/organ)	270±30	160±20 b*
	fractional turnover (hr^{-1})	0.14±0.01	0.006±0.001 b***
	half-life (hrs)	4.9±0.3	125±27 b**
	turnover rate (ng/organ/hr)	39±6	0.9±0.3 b*
	initial uptake (pmoles/organ)	0.94±0.15	0.16±0.02 b*
COLD-ACCLIMATISED 4°C	noradrenaline content (ng/organ)	510±40 a***	340±40 a*
	fractional turnover (hr^{-1})	0.242±0.014 a**	0.488±0.051 a** b*
	half-life (hrs)	2.9±0.2 a**	1.4±0.15 a*** b**
	turnover rate (ng/organ/hr)	122±16 a*	166±37 a*
	initial uptake (pmoles/organ)	1.44±0.27	4.06±0.8
SUCROSE-FED 25-27°C	noradrenaline content (ng/organ)	510±20 a***	130±10 b***
	fractional turnover (hr^{-1})	0.198±0.020	0.012±0.006 b*
	half-life (hrs)	3.5±0.35	59±31 b***
	turnover rate (ng/organ/hr)	103±14 a*	1.5±1.0 b***
	initial uptake (pmoles/organ)	3.69±0.45 a**	0.36±0.04 b***

Table 3.4 The tissue control, fractional turnover rate, half-life, turnover rate and initial uptake of noradrenaline in heart of lean and obese (fa/fa) rats housed at 24-26°C, rats acclimatised to 4°C and rats housed at 24-26°C and offered a 10% (w/v) sucrose solution.

		LEAN	OBESE
CONTROL 24-26°C.	noradrenaline content (ng/organ)	180±8	180±5
	fractional turnover (hr^{-1})	0.07±0.003	0.09±0.01
	half-life (hrs)	9.7±0.5	7.9±1.0
	turnover rate (ng/organ/hr)	13±1	16±3
	initial uptake (pmoles/organ/hr)	0.94±0.06	1.91±0.17 b*
COLD ACCLIMATISED 4°C	noradrenaline content (ng/organ)	120±6 a***	92±14 a***
	fractional turnover (hr^{-1})	0.143±0.027 a*	0.245±0.08 a***
	half-life (hrs)	4.9±0.9 a*	2.8±0.9 a*
	turnover rate (ng/organ/hr)	17±4	22±7
	initial uptake (pmoles/organ)	1.78±0.33	1.78±0.67
SUCROSE-FED 24-26°C	noradrenaline content (ng/organ	210±13	260±25 a**
	fractional turnover (hr^{-1})	0.057±0.012	0.169±0.01 a** b**
	half-life (hrs)	12.1±2.4	4.1±0.2 a* b*
	turnover rate (ng/organ/hr)	12±3	44±6 a* b*
	initial uptake (pmoles/organ)	1.58±0.19	2.12±0.30

4-5 week old lean and obese rats were fed chow for 7 days and housed at 24-26°C or 4°C or were offered 10% (w/v) sucrose solution instead of drinking water for 7 days and housed at 24-26°C. Values represent means± S.E.M. for 9-12 rats in each group. ***, $p < 0.001$; **, $p < 0.005$; *, $p < 0.01$. a, compared with control group; b, compared with equivalent lean group.

Table 3.5 Effect of feeding additional sucrose on food intakes of lean and obese (fa/fa) rats housed at 24-26°C.

ENERGY INTAKE (kJ/24 hours)		TOTAL	CHOW	SUCROSE
Chow-fed	Lean	147.3 ± 2.9	147.3 ± 2.9	-
	Obese	192.9 ± 1.9 b***	192.9 ± 1.9 b***	-
10% (w/v) Sucrose-fed	Lean	179 ± 4.2 a***	137.8 ± 3.8	41.3 ± 2.8
	Obese	225.1 ± 5.3 a***, b***	163.9 ± 4.7 a***, b***	61.2 ± 2.1 b***

All rats were fed chow ad lib and housed at 24-26°C for 7 days. Sucrose fed rats were offered a 10% (w/v) sucrose solution instead of drinking water. Food intakes were monitored in the 24 hours preceding the study. Values represent means ± S.E.M. for 9-12 rats in each group. ***, $p < 0.001$; **, $p < 0.005$, *, $p < 0.01$, a, compared to control group; b, compared to equivalent lean group.

This resulted in a greatly reduced turnover rate in BAT of obese rats compared with lean rats housed at 24-26^{o}C. In addition the initial uptake of [^{3}H]- noradrenaline was reduced in BAT of these obese rats to 17% of that in BAT of lean rats housed at 24-26^{o}C. In contrast to the findings in BAT there were no significant differences in cardiac noradrenaline content, the half-life of [^{3}H]- noradrenaline and noradrenaline turnover between lean and obese rats housed at 24-26^{o}C, although the initial uptake of [^{3}H]- noradrenaline was increased in the hearts of obese rats.

Cold acclimatisation to 4^{o}C increased noradrenaline turnover by 3-fold in lean rats compared with rats housed at 24-26^{o}C, resulting from a reduced half-life and a 2-fold increase in tissue noradrenaline content. Obese rats exhibited a normal BAT response to cold acclimatisation. Noradrenaline turnover increased from the very low levels found in obese rats housed at 24-26^{o}C to the high levels of cold-adapted lean rats. This resulted from a 2-fold increase in tissue noradrenaline content and a decrease in half-life from 125$\pm$27 hours to 1.4$\pm$0.15 hours. The initial tissue uptake of [^{3}H]- noradrenaline also increased in BAT of cold-adapted obese rats.

In heart the half-life of noradrenaline was reduced in both lean and obese rats on cold acclimatisation, but this was associated with a decrease in cardiac noradrenaline content, so tissue noradrenaline turnover did not change on cold acclimatisation. The initial uptake of [^{3}H]- noradrenaline was not affected by cold-acclimatisation, although the differences between lean and obese rats were abolished.

In rats housed at 24-26^{o}C food intake was monitored in the 24 hours prior to the study (Table 3.5). Obese rats were markedly hyperphagic compared to lean rats. Lean rats increased their energy intake by 20% when offered a 10% (w/v) sucrose solution and this was associated with increased BAT noradrenaline content, turnover rate and initial uptake of [^{3}H]- noradrenaline. Obese rats also increased their energy intake, by 17%, when offered a 10% (w/v) sucrose solution. In contrast to lean

animals, in which BAT noradrenaline turnover was increased to the same high levels found in cold acclimatised rats by sucrose overfeeding, BAT of obese rats did not respond to the hyperphagia associated with sucrose feeding. Noradrenaline turnover remained at 3.8% of the rate found in chow-fed lean rats and only 1.5% of the rate found in sucrose-fed lean rats. Cardiac noradrenaline turnover did increase in sucrose-fed obese rats resulting from an increased noradrenaline content and decreased half-line or noradrenaline. In lean rats cardiac noradrenaline turnover was unaffected by feeding a 10% (w/v) sucrose solution.

3.1.3 DISCUSSION.

[^{3}H]- GDP binding to BAT mitochondria, which provides an index of BAT thermogenic capacity, is reduced by 50% in 5-6 week old fa/fa rats (Holt and York, 1982). The primary stimulus for BAT thermogenesis is noradrenaline released from sympathetic nerve endings (Seydoux and Girardier, 1977). Sympathetic activity may be assessed by measurement of tissue noradrenaline turnover (Landsberg and Young, 1978) and changes in BAT thermogenesis induced by cold or diet have been associated with consonant changes in BAT noradrenaline turnover (Young et al., 1982).

The data presented in this section demonstrate that the reduced thermogenic capacity of young (5-6 week old) obese (fa/fa) rats is associated with a severe reduction in BAT noradrenaline turnover to barely detectable levels, indicative of a markedly reduced sympathetic stimulation of the tissue. The defective thermogenesis of the obese rat would thus appear to result from a reduced sympathetic activation of the tissue, rather than from any defect in tissue function.

Since noradrenaline is stored within sympathetic nerve endings in BAT the endogenous tissue content of noradrenaline reflects the extent of sympathetic innervation (Barnard et al., 1980). The BAT noradrenaline content of obese rats was reduced by 40% compared with lean rats, indicating a markedly reduced sympathetic innervation of the tissue.

The initial uptake of [^{3}H]- noradrenaline into a tissue is dependent on a number of processes. Uptake varies with the number of nerve endings and hence uptake sites; tissue blood flow and the activity of the sympathetic nerves, since increased nerve activity enhances uptake (Bhagat and Zeidman, 1970). In BAT of obese rats the reduction of initial uptake to 17% of that in lean rats indicates that one or more of the above processes is reduced in BAT of obese rats.

The demonstration of a reduced noradrenaline content, half-life and turnover rate of noradrenaline in BAT of 5-6 week old obese rats reinforces the findings in older animals (Levin et al., 1983a). The turnover rate in BAT of young obese rats was very much lower than that reported in 3-4 month old obese rats. This may reflect the higher housing temperature used in this study which would reduce the requirement for non-shivering thermogenesis. As the obese rat can activate BAT in response to cold but not diet (Holt et al., 1983; Triandafillou and Himms-Hagen, 1983) the reduced noradrenaline turnover in BAT of obese rats may result from an absent diet-related sympathetic stimulation of BAT rather than any defect in response to temperature. Thus any decrease in the requirement for NST would be expected to markedly reduce noradrenaline turnover in BAT. In contrast to the obese rat, noradrenaline turnover was higher in BAT of young lean rats compared with 3-4 month old rats (Levin et al., 1983a). This may be an age-related difference since the capacity for DIT has been shown to decline with age (Rothwell & Stock, 1983b). The reduced noradrenaline turnover in BAT of older lean rats may reflect a reduction in sympathetic stimulation of BAT in response to the high carbohydrate laboratory chow.

Reduced noradrenaline turnover has been reported in other rodent models of obesity. The obese (ob/ob) mouse (Knehans and Rosmos, 1982) and weanling VMH-lesioned rats (Vander Tuig et al., 1982) have a reduced BAT noradrenaline turnover compared to their corresponding lean controls. Thus a reduced sympathetic stimulation of BAT may be common to a number of models of rodent obesity.

Noradrenaline content, half-life and turnover were normal in hearts of obese rats, indicating that the reduced values found in BAT are indicative of a specific reduction in sympathetic regulation of BAT, rather than a general reduction in sympathetic tone. A similar tissue specific defect was found in older fa/fa rats (Levin et al., 1983a), noradrenaline turnover being reduced in BAT but not heart, although cardiac noradrenaline content was slightly reduced. In the obese (ob/ob) mouse, although cardiac noradrenaline turnover was normal at 8 weeks of age (Knehans and Rosmos, 1982, 1983) it was reduced at 2, 4 and 13 weeks of age (Knehans and Rosmos, 1983; Landsberg and Young, 1983). In weanling, VMH-lesioned rats cardiac noradrenaline turnover is reduced (Vander Tuig et al., 1982). The reduced BAT noradrenaline turnover of ob/ob mice and weanling, VMH-lesioned rats may result from a general reduction in sympathetic tone.

The increased initial uptake of [^{3}H]- noradrenaline in hearts of obese rats has also been reported in older rats (Levin et al., 1983a). Since noradrenaline content and turnover are normal, indicating a normal innervation and sympathetic activity, the increased cardiac uptake may result from an increased blood flow. Although cardiac output is similar in lean and obese rats, blood pressure is higher in obese rats and blood flow to the left ventricle, as measured with radioactive microspheres, is increased by 30% (Wickler et al., 1982).

The suggestion that the observed tissue specific reduction in BAT noradrenaline turnover in the young obese rat is indicative of a reduced sympathetic stimulation of the tissue has been supported by measurements of the effects of the β-adrenergic receptor blocker propranolol on BAT thermogenesis. Noradrenaline released from sympathetic nerve endings stimulates BAT thermogenesis through receptors that are predominantly β_1-specific (see section 1.4.4). Propranolol treatment of lean rats reduces BAT mitochondrial GDP binding to the levels found in obese rats, yet propranolol treatment of obese rats does not further reduce GDP binding to BAT mitochondria

(York et al., 1985b). Since GDP binding is reduced in lean rats on β-receptor blockade, it would appear that in lean rats there is a tonic stimulation of BAT by the sympathetic nervous system, which is consistent with the high rate of noradrenaline turnover observed. In the obese rat, in which BAT thermogenesis is reduced, there would appear to be no functional stimulation of the tissue by the sympathetic nervous system, since GDP binding is not affected by β-blockade, consistent with the very low rates of noradrenaline turnover observed.

The increase in BAT noradrenaline content, fractional turnover and turnover rate in lean rats acclimatised to cold, confirms previous reports (Cottle et al., 1967; Young et al., 1982). On cold acclimatisation BAT hypertrophies (Barnard et al., 1980), the increased noradrenaline content suggests that this is accompanied by an increased sympathetic innervation of BAT. The increased BAT noradrenaline turnover is consistent with an increased sympathetic stimulation of BAT on cold acclimatisation. Obese (fa/fa) rats can tolerate reduced environmental temperatures and acclimatisation to $4^{o}C$ results in increased BAT thermogenesis, as assessed by GDP binding to BAT mitochondria (Holt et al., 1983; Triandafillou and Himms-Hagen, 1983). The increase in BAT noradrenaline turnover in cold-acclimatised obese rats from the very low rates found in obese rats housed at 24-$26^{o}C$ to the very high rates found in lean cold-acclimatised rats indicates that the stimulus of cold exposure results in a normal sympathetic activation of BAT thermogenesis in obese rats. This suggests that there is no functional defect in either BAT or its sympathetic nerve supply since both are activated on cold acclimatisation, and BAT of young rats responds normally to noradrenaline administration (York et al., 1984). Thus the reduced BAT thermogenesis of the obese (fa/fa) rat would appear to result from a defective regulation of the sympathetic nerve supply to BAT.

Cold acclimatisation has been associated with decreased noradrenaline content and increased noradrenaline turnover in heart (Young et al., 1979, 1982). Although cardiac noradrenaline content decreased in cold-acclimatised lean and obese rats cardiac noradrenaline turnover was not increased. The reason for this is not clear, but may reflect a strain difference.

In contrast to the young obese (fa/fa) rat the obese (ob/ob) mouse cannot tolerate exposure to 4°C because it is unable to activate BAT thermogenesis (Hogan and Himms-Hagen, 1980; Thurlby and Trayhurn, 1980). Although BAT noradrenaline turnover does increase to some extent on cold exposure the turnover rates obtained are not comparable with those in lean mice (Young and Landsberg, 1983; Zaror-Behrens and Himms-Hagen, 1983). In addition, the BAT of obese (ob/ob) mice appears to be insensitive to the noradrenaline stimulation of BAT blood flow and thermogenesis (Thurlby and Trayhurn, 1980; Seydoux et al., 1982a). However, this inability to respond to cold exposure seems to partly result from a reversible involution of BAT. If obese (ob/ob) mice are first acclimatised to 12°C they can survive cold exposure at 4°C, with a restoration of BAT mitochondrial GDP binding to values observed in lean cold adapted mice (Bas et al., 1983) and increased responsiveness of BAT to noradrenaline and nerve stimulation (Seydoux et al., 1982a). The role of the sympathetic nervous system in this restoration of BAT thermogenesis has not been reported.

The relatively normal response of the obese (fa/fa) rat to cold acclimatisation is similar to that of the obese VMH-lesioned rat. BAT thermogenesis is reduced in the VMH-lesioned rat, but increases on cold exposure to the values observed in sham-operated, cold-exposed rats (Seydoux et al., 1982c; Luboshitsky et al., 1984) and this is associated with a normal increase in noradrenaline turnover (Yoshida and Bray, 1984). However, a reduced sympathetic stimulation of BAT may not be as important to the development of obesity in the VMH-lesioned rat as it seems to be in the obese (fa/fa) rat. Although both

BAT thermogenesis and BAT noradrenaline turnover are decreased in weanling VMH-lesioned rats (Vander Tuig et al., 1982) the situation in adult VMH-lesioned rats is unclear. Yoshida and Bray (1984)have reported that BAT noradrenaline turnover is increased in VMH-lesioned adults compared with sham-operated rats. This could result from a normal diet-induced increase in BAT sympathetic activity in response to the hyperphagia of the adult which is not present in weanling VMH-lesioned rats (Vander Tuig et al., 1982), yet BAT noradrenaline turnover remained increased in adult VMH-lesioned rats on starvation (Yoshida and Bray, 1984). In addition to reduced thermogenesis in BAT of VMH-lesioned rats, the tissue is less sensitive and less responsive to the thermogenic effects of noradrenaline. A reduction in BAT thermogenesis, despite an increased sympathetic stimulation of the tissue, may result from a parasympathetic inhibition of BAT thermogenesis, perhaps mediated by insulin, since vagotomy reverses the obesity and hyperinsulinaemia of VMH-lesioned rats (Powley and Opsahl, 1974; Inoue and Bray, 1977). in contrast to the report of increased BAT noradrenaline turnover, Vander Tuig et al. (1985) have recently reported that BAT noradrenaline turnover is decreased in adult VMH-lesioned rats. In this study, however, the rats were all maintained on a high-fat diet, which is a potent stimulator of BAT noradrenaline turnover in intact rats (Schwartz et al., 1983). The high-fat diet would increase BAT noradrenaline turnover in the sham-operated rats, but not necessarily in the VMH- lesioned rats.

In contrast to its ability to increase BAT thermogenesis in response to cold exposure, the obese (fa/fa) rat is unable to activate BAT in response to diet-related stimuli. The obese rat is already hyperphagic, yet BAT thermogenesis is reduced (Holt and York, 1982). The thermic effect of feeding, that is, the rise in oxygen consumption after a meal, is reduced in obese rats (Rothwell et al., 1981a). In obese rats there is no increase in BAT mitochondrial GDP binding on overfeeding with sucrose or a cafeteria diet (Holt et al., 1983;

Triandafillou and Himms-Hagen, 1983), procedures normally associated with increased GDP binding. Diet-induced increases in BAT thermogenesis have been linked with increased sympathetic stimulation of BAT (Young et al., 1982), this was confirmed in the experiments reported in this section by the increase in BAT noradrenaline turnover in lean rats overfed with a 10% (w/v) sucrose solution. Both noradrenaline content and initial uptake of [^{3}H]- noradrenaline were increased, indicative of proliferation of the tissue innervation. In addition, the results presented in this section demonstrate that the reduced BAT thermogenic response to sucrose overfeeding in the obese (fa/fa) rat, results from a failure to increase sympathetic stimulation of BAT in response to increased food intake and changes in diet, since BAT noradrenaline turnover remained at the same low levels found in chow-fed obese rats.

The large increase in cardiac noradrenaline turnover on sucrose feeding of obese rats suggests that the obese rat is capable of registering diet-related signals but is unable to couple them to a specific sympathetic activation of BAT. Since the obese rat can respond to cold exposure with normal increases in BAT noradrenaline turnover and thermogenesis the defect would appear to be in those centres of the brain responsible for the regulation of the BAT thermogenesis in response to diet.

Sucrosefeeding has been associated with increased cardiac noradrenaline turnover (Young and Landsberg, 1979; Young et al., 1982). Cardiac noradrenaline turnover was not increased in lean rats fed a 10% (w/v) sucrose solution. As in the cold acclimatisation study this could represent a strain difference, however, other workers have also failed to find increases in cardiac noradrenaline turnover on overfeeding (Levin et al., 1983b,c; Fisler et al., 1984).

A defective sympathetic stimulation of BAT in response to dietary stimuli has also been reported in the Osborne-Mendel rat, which readily develops obesity when fed on high-fat or sucrose diets. BAT noradrenaline content and turnover rate are reduced in Osborne-Mendel rats compared to S5B/Pl

rats, an obesity resistant strain, and BAT noradrenaline turnover does not increase on a high-fat diet in Osborne-Mendel rats (Fisler et al., 1984). A reduced sympathetic stimulation in response to diet may explain the susceptibility to obesity in a number of strains of rat.

The obese (ob/ob) mouse and obese VMH-lesioned rat demonstrate a similar inability to respond to dietary signals, as BAT thermogenesis does not increase on overfeeding (Trayhurn et al., 1982; Rohner-Jeanrenaud et al., 1983). In contrast to the fa/fa rat, the ob/ob mouse can increase BAT noradrenaline turnover in response to sucrose feeding. However, as on cold exposure, although the magnitude of the increase in BAT noradrenaline turnover is similar in lean and obese mice, the absolute rates of noradrenaline turnover remain lower than those in lean mice (Knehans and Rosmos, 1984). There may thus be an important difference between the obese (fa/fa) rat and the obese (ob/ob) mouse. The fa/fa rat seems to have no defect in BAT, but is unable to activate the sympathetic nervous supply to the tissue in response to dietary stimuli, whereas the ob/ob mouse may be able to activate the sympathetic nerve supply to BAT, but cannot couple this to thermogenesis. If the VMH-lesioned rat is similar to the fa/fa rat, in that it is unable to increase sympathetic activity in BAT in response to dietary changes, then the discrepancies in the reports on BAT noradrenaline turnover in adult VMH-lesioned rats, could be partly resolved. BAT noradrenaline turnover in these animals has been reported to be increased (Yoshida and Bray, 1984) and decreased (Vander Tuig et al., 1985) in comparison with sham-operated rats. In the report in which turnover was decreased in VMH-lesioned adult rats, the difference could be accounted for by a stimulation of BAT noradrenaline turnover in the sham-operated rats by the high-fat diet used in the study. This would suggest that BAT noradrenaline turnover is normal or increased in VMH-lesioned adult rats, rather than as in the fa/fa rat in which turnover is severely reduced.

The results presented in this section demonstrate a reduced sympathetic stimulation of BAT in the obese (fa/fa) rat housed at 24-26^{o}C. Since the sympathetic nerve supply is activated on cold acclimatisation associated with normal increases in BAT thermogenesis (Holt et al., 1983; Triandafillou and Himms-Hagen, 1983),there is no defect in the nerve supply to BAT or the tissue itself. The defect seems to be in the regulation of sympathetic activity in response to diet since neither BAT thermogenesis nor BAT noradrenaline turnover are increased on sucrose feeding. BAT is activated by stimulation of the ventromedial hypothalamus (VMH) resulting in increased sympathetic stimulation of the tissue (Perkins et al., 1981). This response has been shown to be normal in the obese rat (Holt et al., 1985), suggesting that the defect in the obese rat may be in the recognition of dietary stimuli by the VMH or in the coupling of these signals to sympathetic nerves innervating BAT. The observation of increased cardiac noradrenaline turnover in sucrose-fed obese rats suggests that the obese rat may be capable of recognising dietary stimuli but not of coupling them to sympathetic stimulation of BAT.

SECTION 3.2 The effects of the adrenal-pituitary system on noradrenaline turnover in lean and obese (fa/fa) rats.

Obesity in the fa/fa rat is dependent upon the presence of adrenal glucocorticoids. Adrenalectomy reduces body weight, food intake, fat synthesis and deposition and serum insulin to normal (Yukimura and Bray, 1978; York and Godbole, 1978). In addition the defects in energy balance and BAT thermogenesis are corrected, whereas adrenalectomy has no appreciable effect on energy balance or BAT thermogenic function in lean Zucker rats (Holt and York, 1982; Marchington et al., 1983). Treatment of adrenalectomised fa/fa rats with corticosterone reverses the effects of adrenalectomy with a restoration of the obesity, hyperphagia and defective BAT thermogenesis (Yukimura et al., 1978; Holt et al., 1983). Treatment of intact lean rats with corticosterone decreases BAT mitochondrial GDP binding and suppresses diet-induced thermogenesis (York et al., 1985a).

ACTH has effects on BAT thermogenesis independent of its stimulation of glucocorticoid secretion. Short term treatment of lean rats with ACTH does not affect BAT thermogenesis but in obese rats, prior to increases in serum corticosterone, is associated with increased BAT thermogenic capacity and decreased food intake. Similarly, ACTH treatment prevents the decreases in BAT mitochondrial GDP binding found on corticosterone treatment of adrenalectomised fa/fa rats (York and Al-Baker, 1984).

Corticosterone and ACTH appear to have opposing effects on BAT thermogenesis in the fa/fa rat. The experiments in this section report the effects of adrenalectomy, and of corticosterone and ACTH treatments on noradrenaline turnover in BAT and heart of lean and obese Zucker rats.

RESULTS.

3.2.1 Noradrenaline turnover in intact and adrenalectomised lean and obese (fa/fa) rats.

The effects of bilateral adrenalectomy on BAT and cardiac noradrenaline turnover are shown in Fig. 3.2 and Tables 3.7 and 3.8. Adrenalectomy resulted in increased BAT noradrenaline content in lean rats, but did not significantly affect the half-life or initial uptake of [^{3}H]- noradrenaline. Although the calculated noradrenaline turnover rate was increased in BAT of lean rats by adrenalectomy this difference was not statistically significant. There was no detectable decline in [^{3}H]- noradrenaline specific activity over the time course of the study in BAT from intact obese rats so BAT noradrenaline turnover rates could not be calculated. Adrenalectomy increased the BAT noradrenaline content of obese rats to the values found in lean rats but not to the same levels as in adrenalectomised lean rats. The fractional turnover, turnover rate and initial uptake of [^{3}H]- noradrenaline were increased to lean values and were not significantly different from those found in adrenalectomised lean rats.

In contrast to the findings in BAT, cardiac noradrenaline content was unaffected by adrenalectomy. However, fractional turnover was increased resulting in increased cardiac noradrenaline turnover in both lean and obese rats. The initial uptake of [^{3}H]- noradrenaline was unaffected by adrenalectomy and did not differ between adrenalectomised lean and obese rats.

Corticosterone treatment for 7 days did not affect the noradrenaline content or initial uptake of [^{3}H]- noradrenaline in BAT from intact lean rats, but the noradrenaline turnover rate was decreased 2-fold compared with controls as a result of the increased half-life of noradrenaline in BAT of corticosterone-treated rats (Fig. 3.2 and Table 3.7). Cardiac noradrenaline content, half-life, turnover rate and initial uptake of [^{3}H]- noradrenaline were unaffected by corticosterone treatment of intact lean rats (Fig. 3.2 and Table 3.8). Corticosterone treatment of intact obese rats does not further

Fig. 3.2

Noradrenaline turnover in BAT and heart of intact (closed symbols) and adrenalectomised (open symbols) lean and obese control (◐——◑), corticosterone treated (◧——◨), and ACTH treated (▲——▲) 5-6 week old rats Rats were injected i.v. with 25μCi/100g body weight 1-7,8 [^{3}H]-noradrenaline in 0.9% (w/v) saline at time zero. Values represent means ± S.E.M. for 3 rats at each time point.

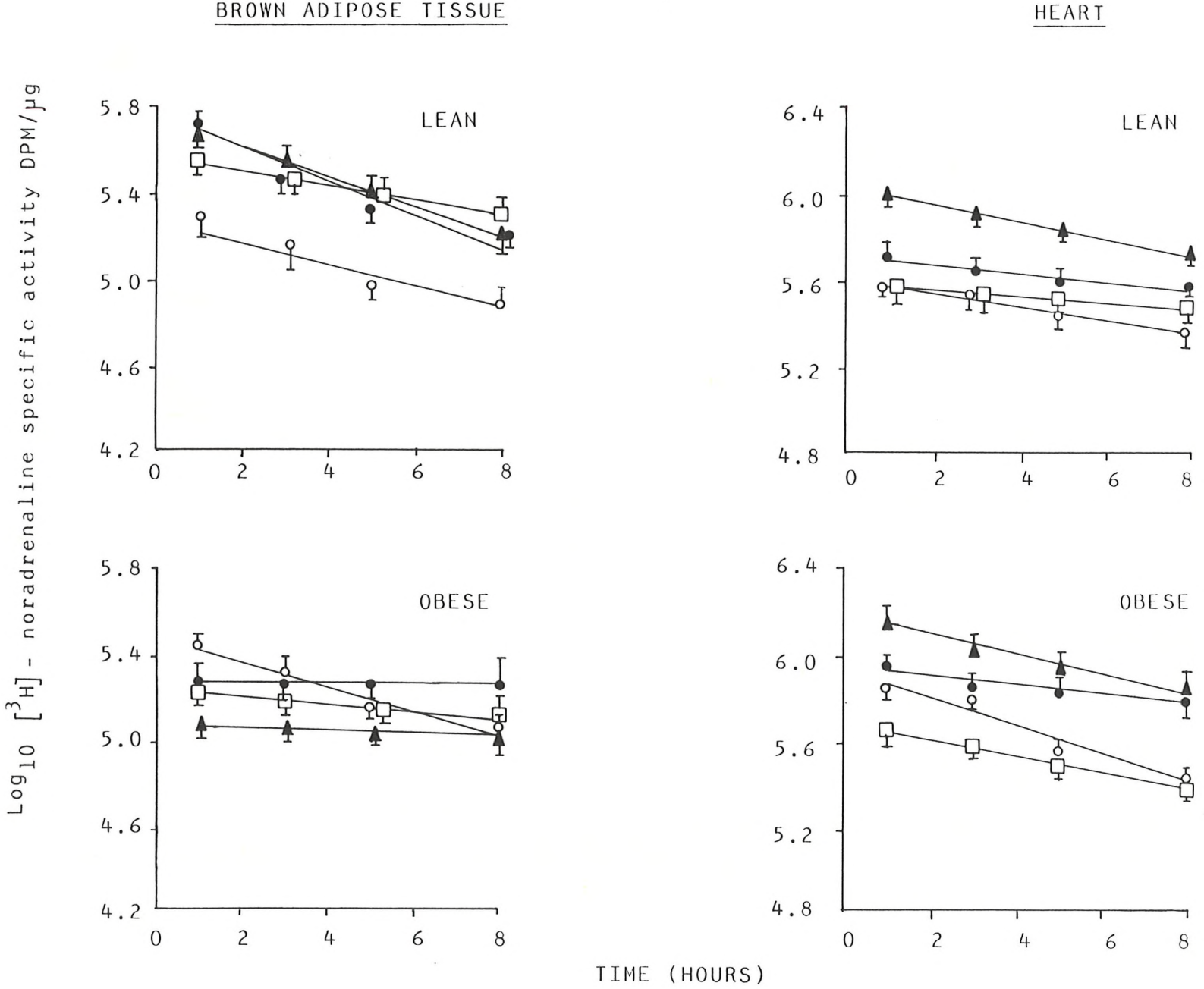

BROWN ADIPOSE TISSUE
HEART
LEAN
LEAN
OBESE
OBESE
Log10 [3H] - noradrenaline specific activity DPM/µg
TIME (HOURS)
5.8
5.4
5.0
4.6
4.2
6.4
6.0
5.6
5.2
4.8
0
2
4
6
8

Table 3.7 The effects of adrenalectomy and corticosterone and ACTH treatments on the tissue content fractional turnover rate, half-life, turnover rate and initial uptake of noradrenaline in BAT of lean and obese (fa/fa) rats.

		CONTROL		CORTICOSTERONE	ACTH	
		LEAN	OBESE	LEAN	LEAN	OBESE
INTACT	noradrenaline content (ng/organ)	$280^{+}_{-}30$	$140^{+}_{-}20$b***	$280^{+}_{-}20$	$270^{+}_{-}20$	$170^{+}_{-}4$
	fractional turnover (hr^{-1})	$0.17^{+}_{-}0.03$	-	$0.08^{+}_{-}0.003$a*	$0.145^{+}_{-}0.016$	$0.005^{+}_{-}0.002$
	half-life (hrs)	$4.0^{+}_{-}0.06$	-	$8.9^{+}_{-}0.4$a**	$4.8^{+}_{-}0.5$	$139^{+}_{-}61$
	turnover rate (ng/organ/hr)	$48^{+}_{-}12$	-	$22^{+}_{-}2.5$a*	$39^{+}_{-}7$	$0.9^{+}_{-}0.4$
	initial uptake (pmoles/organ)	$1.90^{+}_{-}0.43$	$0.31^{+}_{-}0.06$	$1.24^{+}_{-}0.1$	$1.87^{+}_{-}0.32$	$0.24^{+}_{-}0.01$
		LEAN	OBESE	OBESE		
ADRENALECTOMY	noradrenaline content (ng/organ)	$460^{+}_{-}40$a***	$240^{+}_{-}20^{a*}_{b***}$	$196^{+}_{-}20$		
	fractional turnover (hr^{-1}_{L})	$0.12^{+}_{-}0.04$	$0.15^{+}_{-}0.02$	$0.044^{+}_{-}0.006$c*		
	half-life (hrs)	$5.9^{+}_{-}1.9$	$4.5^{+}_{-}0.6$	$15.7^{+}_{-}2.0$c*		
	turnover rate (ng/organ/hr)	$54^{+}_{-}22$	$37^{+}_{-}8$	$8.6^{+}_{-}2.0$c*		
	initial uptake (pmoles/organ)	$1.03^{+}_{-}0.32$	$1.23^{+}_{-}0.29$	$0.45^{+}_{-}0.06$		

4-5 week old lean and obese rats were fed chow for 7 days or adrenalectomised and maintained on 0.9% (w/v) saline for 7 days as described in section 2.2.3. Corticosterone treated rats received 1mg/100g body weight/day by i.p. injection for 7 days. ACTH treated rats received 25 μg/100g body weight 32 hours before sacrifice and 50μg/100g body weight 18 hours before sacrifice by i.m. injection (see section 2.2.6). Values represent means $\pm$ S.E.M. for 12 rats in each group ***, $p < 0.001$; **, $p < 0.005$; $p < 0.01$. a, compared with control group; b, compared with equivalent lean group; c, compared with adrenalectomised group.

Table 3.8 The effects of adrenalectomy and coricosterone and ACTH treatments on the tissue content fractional turnover rate, half-life turnover rate and initial uptake of noradrenaline in heart of lean and obese (fa/fa) rats.

		CONTROL		CORTICOSTERONE	ACTH	
		LEAN	OBESE	LEAN	LEAN	OBESE
INTACT	noradrenaline content (ng/organ)	190±10	170±10	170±20	140±5	160±3
	fractional turnover,k (hr^{-1})	0.04±0.01	0.05±0.01	0.04±0.002	0.09±0.001	0.10±0.013
	half-life (hrs)	15.6±2.4	13.1±1.7	18.6±1.1	7.7±0.1	6.7±0.9
	turnover rate (ng/organ/hr)	8.4±1.7	9.0±1.7	6.3±1.1	13.0±6.0	16.5±2.0
	initial uptake (pmoles/organ)	1.29±0.10	2.05±0.14	0.85±0.1	2.03±0.14 a**	3.31±0.11 a**
		LEAN	OBESE	OBESE		
ADRENALECTOMY	noradrenaline content (ng/organ)	195±20	120±9	182±5		
	fractional turnover (hr^{-1})	0.14±0.01 a*	0.16±0.03 a*	0.09±0.002		
	half-life (hrs)	4.9±0.3 a*	4.4±0.8 a*	8.1±0.2		
	turnover rate (ng/organ/hr)	27±3 a*	19±3 a*	15.0±0.8		
	initial uptake (pmoles/organ)	1.24±0.14	1.28±0.3	1.18±0.05		

4-5 week old lean and obese (fa/fa) rats were fed chow for 7 days or adrenalectomised and maintained on chow and 0.9% (w/v) saline for 7 days, as described in section 2.2.3. Corticosterone treated rats received 1 ug/100g body weight/per day by i.p. injection for 7 days. ACTH treated rats received 25 ug/100g body weight 32 hours before sacrifice and 50 ug/100g body weight 18 hours before sacrifice by i.m. injection (see section 2.2.6). Values represent means ± SEM for 12 rats in each group. **, $p<0.005$, *, $p<0.01$. a, compared to control group.

reduce BAT thermogenesis (Holt et al., 1983) and BAT noradrenaline turnover was already severely reduced (Table 3.8). Therefore, the effects of corticosterone treatment on BAT noradrenaline turnover in obese rats was examined in adrenalectomised animals. BAT noradrenaline contents and initial uptake of [^{3}H]- noradrenaline were not significantly affected by corticosterone treatment but, as in intact lean rats, the fractional turnover was reduced, resulting in a 4-fold reduction in calculated noradrenaline turnover. As found in intact lean rats, cardiac noradrenaline content, half-life, turnover rate and initial uptake of [^{3}H]- noradrenaline were unaffected by corticosterone treatment of obese adrenalectomised rats.

ACTH treatment did not affect noradrenaline content, half-life, fractional turnover, turnover rate or the initial uptake of [^{3}H]- noradrenaline in BAT of intact lean rats. A very low noradrenaline turnover rate could be detected in ACTH treatment intact obese rats, but this was not significantly different from the rate obtained in the previous study in control obese rats (Section 3.1.2). Thus the reduced BAT noradrenaline content, fractional turnover, turnover rate and initial uptake of [^{3}H]- noradrenaline in BAT of obese rats compared with lean rats were maintained on ACTH treatment. Cardiac noradrenaline content, half-life and turnover rate were unaffected by ACTH treatment in both lean and obese rats although the initial uptake of [^{3}H]- noradrenaline was increased.

3.2.2 DISCUSSION.

The reduced thermogenic function of BAT in the obese rat, as assessed by GDP binding to BAT mitochondria, is restored to normal values after adrenalectomy (Holt and York, 1982) and BAT of young obese rats responds normally to cold exposure (Holt et al., 1983; Triandafillou et al., 1983) which was associated with a normal increase in sympathetic activity (Section 3.1). Consequently it has been suggested that the defective BAT thermogenesis in obese rats results from a defective regulation of the tissue and that this is dependent

upon the presence of adrenal glucocorticoids. The results presented in this section suggest that the defective BAT thermogenesis results from a glucocorticoid mediated suppression of sympathetic stimulation of the tissue.

Adrenalectomy of obese rats increased BAT noradrenaline content, fractional turnover, turnover rate and initial uptake of [^{3}H]- noradrenaline to the levels observed in lean rats. Since noradrenaline turnover is an index of sympathetic activity this indicates that the restoration of BAT thermogenic function is associated with a restoration of the sympathetic activation of the tissue. Similar findings have been reported in the obese (ob/ob) mouse. In common with the fa/fa rat the obesity of the ob/ob mouse has been associated with a reduced thermogenic capacity of BAT (Thurlby and Trayhurn, 1980; Holt and York, 1984) and reduced BAT noradrenaline turnover (Knehans and Rosmos, 1982, 1983; Landsberg and Young, 1983). Adrenalectomy abolishes the hyperphagia of these animals, reduces weight gain and restores BAT thermogenesis to normal (Holt and York, 1984; Saito & Bray, 1984) and this is associated with increased BAT noradrenaline turnover (Vander Tuig et al., 1984). Adrenalectomy also reverses the obesity in VMH-lesioned rats (Bruce et al., 1982; King et al, 1982). Thus an adrenal glucocorticoid dependant inhibition of sympathetic stimulation of BAT may be common to a number of forms of rodent obesity.

Studies with the β- receptor antagonist propranolol have supported the conclusion that the restoration of a normal BAT noradrenaline turnover in obese (fa/fa) rats is indicative of a restored sympathetic activation of the tissue. Propranolol treatment of adrenalectomised lean rats reduces BAT mitochondrial GDP binding to the values observed in intact obese rats and prevents the restoration of thermogenic function, as assesed by BAT mitochondrial GDP binding, in adrenalectomised obese rats (York et al., 1985b), suggesting that the increases in BAT thermogenesis in these animals are mediated by the sympathetic nervous system.

The restoration of BAT thermogenic function in the obese rat by adrenalectomy is blocked by administration of corticosterone but not aldosterone (Holt et al., 1983; York et al., 1985a). Chronic corticosterone treatment reduces BAT thermogenesis in lean mice and rats and impairs the thermogenic response to overfeeding (Galpin et al., 1983; York et al., 1985a). However, corticosterone is required in permissive amounts for the normal response to cold exposure (Fellenz et al., 1982) and chronic corticosterone treatment does not impair thermogenesis in response to cold exposure and noradrenaline administration in mice and rats (Galpin et al., 1983; York et al., 1985a). Chronic treatment of intact lean rats and adrenalectomised fa/fa rats with corticosterone reduced noradrenaline turnover in BAT but not heart. This suggests that the inhibition of thermogenesis in corticosterone treated lean and adrenalectomised fa/fa rats resulted from a reduced sympathetic stimulation of the tissue rather than a direct effect on BAT. It would appear that, in the intact obese rat, adrenal glucocorticoids reduce sympathetic activity in BAT and so reduce BAT thermogenesis,since adrenalectomy increases both BAT thermogenesis (Holt et al., 1982) and noradrenaline turnover and both are reduced on corticosterone replacement. Corticosterone inhibition of sympathetic activity in BAT is not restricted to the obese (fa/fa) rat since chronic corticosterone treatment of lean rats reduces BAT thermogenesis and the thermogenic response to diet but not to cold (York et al., 1985a). The defect may arise from an increased sensitivity of the obese (fa/fa) rat to circulating coricosterone (Yukimura et al., 1978). Serum corticosterone levels are similar in lean and obese rats and the ratio of bound to free corticosterone is not changed in the obese rat (Al-Baker, 1985), suggesting that the inhibition of BAT thermogenesis in the obese rat does not result from increased circulating corticosterone. At the dose of corticosterone used in these studies, BAT noradrenaline turnover was inhibited 2-fold in lean rats and 4-fold in adrenalectomised fa/fa rats, suggesting that the obese rat is corticosterone sensitive. In addition, adrenalectomised lean rats are much

less sensitive to the inhibitory effects of corticosterone replacement on BAT thermogenesis than adrenalectomised obese rats (Holt et al., 1983; York and Al-Baker, 1984).

Changes in circulating corticosterone levels induced by adrenalectomy or corticosterone administration are paralleled by reciprocal changes in ACTH levels. Serum ACTH levels are normal in the obese rat and respond normally to adrenalectomy and corticosterone replacement (Yukimura et al., 1978). Short term treatment of obese rats with ACTH is associated with increased BAT thermogenesis (York and Al-Baker, 1984). Since ACTH levels rise on adrenalectomy the restoration of thermogenic function could result from a stimulation of thermogenesis by ACTH and the effects of corticosterone replacement could reflect an inhibition of ACTH secretion. Although the initial uptake of [^{3}H]- noradrenaline was increased in heart of lean and obese rats on short term ACTH treatment, noradrenaline turnover was not increased in heart of BAT of lean and obese rats. ACTH may have a direct effect on BAT of obese rats, in contrast to the effects of corticosterone which seems to be mediated via the sympathetic nervous system. In vivo, ACTH treatment increases BAT blood flow (Kuroshima et al., 1968; Laury and Portet, 1980) and increases metabolic rate and BAT temperature (Heim and Hull, 1966). The increases in metabolic rate and BAT temperature induced by ACTH are not blocked by propranolol (Heim and Hull, 1966) indicating a direct effect of ACTH on BAT. In vitro, ACTH can stimulate lipolysis in BAT by activation of adenylate cyclase (Bertin and Portet, 1976). Since ACTH treatment did not affect BAT noradrenaline turnover in obese rats, the stimulation of BAT mitochondrial GDP binding observed in ACTH treated obese rats (York and Al-Baker, 1984) could result from a direct stimulation of the tissue. However, the ACTH stimulation of BAT mitochondrial GDP binding in obese rats can be blocked by propranolol (Al-Baker, 1985) indicating that β-receptor stimulation is involved. ACTH potentiates the effects of noradrenaline administration in warm acclimated rats (Laury and Portet, 1977),

thus the thermogenic effects of ACTH administration in obese rats may result from an increase in tissue sensitivity to noradrenaline.

In conclusion, the experiments reported in this section have demonstrated that the restoration of thermogenic capacity, brought about by adrenalectomy of obese rats, may be through two different mechanisms. The fall in serum corticosterone may restore sympathetic activation of BAT and, in addition, the increased levels of ACTH, found after adrenalectomy, may further activate the tissue by direct stimulation of the tissue or sensitisation to sympathetic stimulation.

SECTION 3.3 The effect of diet on noradrenaline turnover in adrenalectomised Zucker rats.

The intact obese rat exhibits a reduced response to the thermic effects of food (Rothwell et al., 1983) and an inability to activate BAT thermogenesis in response to overfeeding sucrose or a cafeteria diet (Holt et al., 1983); Traindafillou and Himms-Hagen, 1983). Increased BAT thermogenesis in response to overfeeding sucrose is associated with increased BAT noradrenaline turnover (Young et al., 1982) and it has been shown that BAT noradrenaline turnover was severely reduced in the obese rat and did not increase on sucrose overfeeding (Section 3.1). The defective BAT thermogenesis of the obese rat is restored on adrenalectomy (Holt and York, 1982) and was associated with increased BAT noradrenaline turnover (Section 3.2). Adrenalectomy restores the defective thermic responses to feeding (Marchington et al., 1983) and results in normal increases in BAT mitchondrial GDP binding in response to overfeeding with sucrose. The experiments described in this section report the effects of overfeeding adrenalectomised rats with additional sucrose, to determine whether the thermogenic response to overfeeding in adrenalectomised obese rats was associated with further increases in sympathetic activity in BAT,as was found on sucrose feeding intact lean rats (Section 3.1).

RESULTS.

3.3.1 Noradrenaline turnover in sucrose-fed adrenalectomised lean and obese (fa/fa) rats.

Fig. 3.3 and Tables 3.9, 3.10 and 3.11 show the effects of feeding a 35% (w/v) sucrose solution on food intake and BAT and heart noradrenaline turnover in lean and obese adrenalectomised rats. When adrenalectomised rats were allowed access to a 35% (w/v) sucrose solution in addition to chow and 0.9% (w/v) saline,energy intake was increased by 48% in lean adrenalectomised rats and 29% in obese adrenalectomised rats relative to chow-fed adrenalectomised rats. In spite of these increases in energy intake there were no significant increases in BAT noradrenaline content, half-life, turnover rate or initial uptake of [^{3}H]- noradrenaline, compared with chow-fed adrenalectomised rats, in either lean or fa/fa rats. In heart,

Table 3.9 Effect of feeding additional sucrose on food intakes of adrenalectomised lean and obese (fa/fa) rats.

ENERGY INTAKE	kJ/24 hours	TOTAL	CHOW	SUCROSE
ADRENALECTOMISED CHOW-FED	Lean	107.4 ± 6.7	107.4 ± 6.7	
	Obese	101.7 ± 2.6	101.7 ± 2.6	
ADRENALECTOMISED 35% (w/v) SUCROSE-FED	Lean	159.5 ± 1.7 b***	108.3 ± 1.0	51.2 ± 1.2
	Obese	133.4 ± 3.2 a*** b***	85.5 ± 3.8 b***	47.9 ± 0.9
INTACT 35% (w/v) SUCROSE-FED	Lean	203.3 ± 2.4 c***	140.6 ± 1.9 c***	62.7 ± 1.3 c***
	Obese	266 ± 3.7 c***	160.6 ± 3.8 c***	105.6 ± 2.6 c***

Adrenalectomised rats were maintained on 0.9% saline instead of drinking water. All rats were fed chow ad lib. Sucrose fed rats were offered a 35% (w/v) sucrose solution in addition to drinking water or 0.9% (w/v) saline as appropriate. Rats were maintained for 7 days and food intakes were monitored in the 24 hours preceding the study. Values represent means $\pm$ S.E.M. for 12 rats in each group. ***, $p < 0.001$; **, $p < 0.005$; *, $p < 0.01$. a, compared with equivalent lean group; b, compared with equivalent chow fed group; c, compared with adrenalectomised group.

Fig 3.3.

Noradrenaline turnover in BAT and heart of intact (□——□) and adrenalectomised (◐——◑) lean and obese Zucker rats fed chow ad lib (closed symbols) or offered a 35% (w/v) sucrose solution (open symbols). All rats were injected i.v. with 25μCi 1-7,8 [^{3}H]-noradrenaline in 0.9% (w/v) saline at time zero. Values represent means ± S.E.M. for 3 rats at each time point.

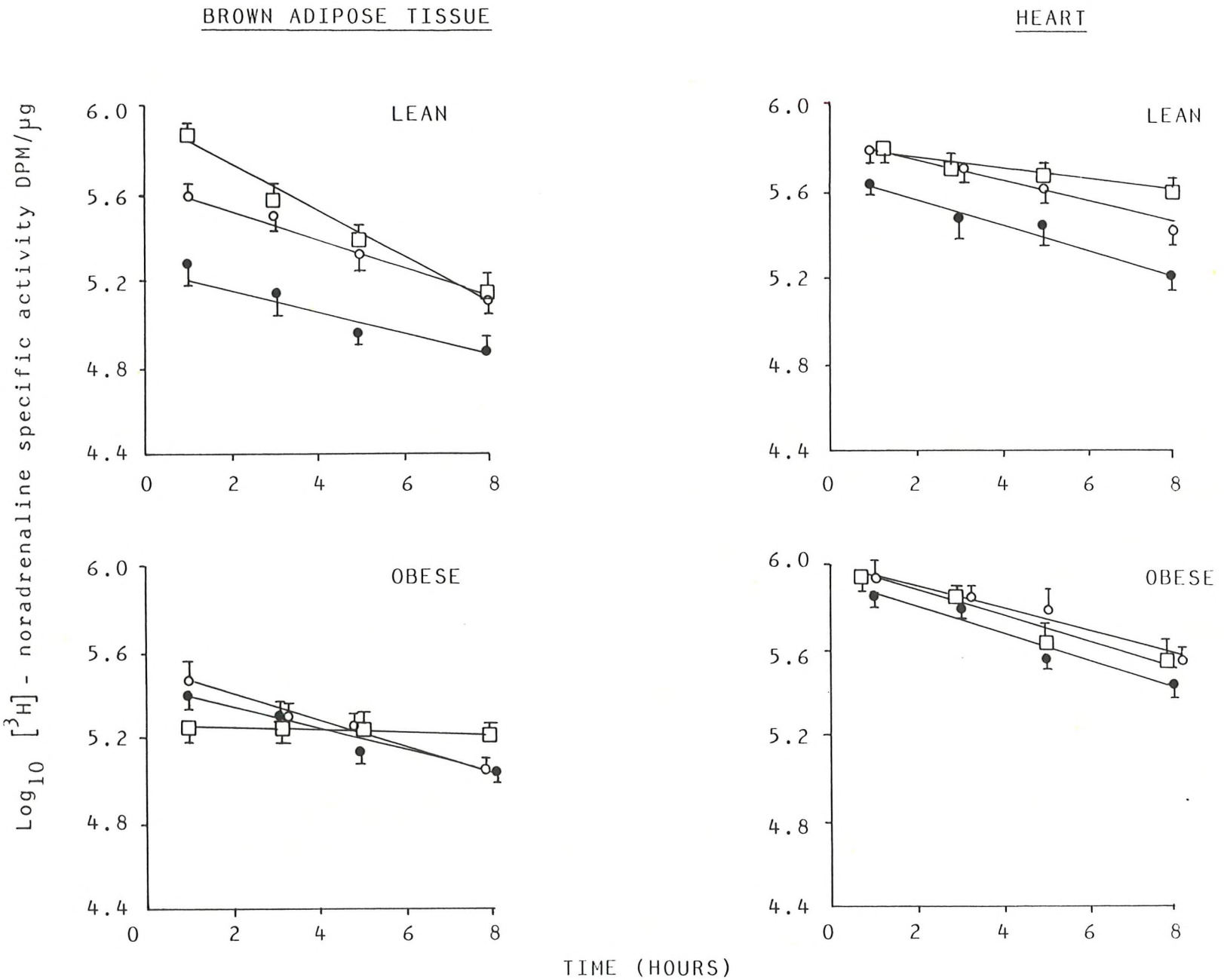

BROWN ADIPOSE TISSUE
HEART
LEAN
LEAN
OBESE
OBESE
6.0
5.6
5.2
4.8
4.4
0
2
4
6
8
Log10 [3H] - noradrenaline specific activity DPM/µg
TIME (HOURS)

Table 3.10 Legend.

Adrenalectomised rats were maintained on 0.9% (w/v) saline instead of drinking water. All rats were fed chow ad lib. Sucrose fed rats were offered a 35% (w/v) sucrose solution in addition to drinking water or 0.9% (w/v) saline as appropriate. Rats were maintained for 7 days and food intakes were monitored in the 24 hours preceding the study. Data for chow-fed adrenalectomised rats has appeared previously (section 3.2). Values represent means $\pm$ S.E.M. for 12 rats in each group. ***, $p < 0.001$; **, $p < 0.005$; *, $p < 0.01$. a, compared with equivalent lean group; b, compared with equivalent adrenalectomised group.

Table 3.10 The tissue content, fractional turnover, half-life, turnover rate and initial uptake of noradrenaline in BAT of adrenalectomised lean and obese (fa/fa) rats allowed access to a 35% (w/v) sucrose solution.

			LEAN	OBESE
ADRENALECTOMISED CHOW-FED	noradrenaline content	(ng/organ)	460 ± 40	240 ± 20
	fractional turnover	(hr^{-1})	0.12 ± 1.9	0.15 ± 0.02
	half-life	(hrs)	5.9 ± 1.9	4.5 ± 0.6
	turnover rate	(ng/organ/hr)	54 ± 22	37 ± 8
	initial uptake	(pmoles/organ)	1.03 ± 0.32	1.23 ± 0.29
ADRENALECTOMISED SUCROSE-FED	noradrenaline content	(ng/organ)	445 ± 20 a***	270 ± 7 b***
	fractional turnover	(hr^{-1})	0.159 ± 0.010	0.126 ± 0.014
	half-life	(hrs)	4.4 ± 0.3	5.5 ± 0.6
	turnover rate	(ng/organ/hr)	70 ± 8	34 ± 5
	initial uptake	(pmoles/organ)	1.87 ± 0.32	1.07 ± 0.13
INTACT SUCROSE-FED	noradrenaline content	(ng/organ)	380 ± 20	120 ± 5 a*** b***
	fractional turnover	(hr^{-1})	0.244 ± 0.025	0.017 ± 0.001 a*** b***
	half-life	(hrs)	2.8 ± 0.3	40.4 ± 1.6 a*** b***
	turnover rate	(ng/organ/hr)	94 ± 15	2.0 ± 0.2 a** b**
	initial uptake	(pmoles/organ)	4.38 ± 0.72 b**	0.29 ± 0.03 a** b**

Table 3.11 Legend.

Adrenalectomised rats were maintained on 0.9% (w/v) saline instead of drinking water. All rats were fed chow ad lib. Sucrose-fed rats were offered a 35% (w/v) sucrose solution in addition to drinking water or 0.9% (w/v) saline as appropriate. Rats were maintained for 7 days and food intakes were monitored in the 24 hours preceding the study. Dat for chow-fed adrenalectomised rats has appeared previously (section 3.2). Values represent means $\pm$ S.E.M. for 12 rats in each group. ***, $p < 0.001$; **, $p < 0.005$; *, $p < 0.01$. a, compared with equivalent lean group; b, compared with equivalent adrenalectomised group.

Table 3.11 The tissue control, fractional turnover, half-life, turnover rate and initial uptake of noradrenaline in heart of adrenalectomised lean and obese (fa/fa) rats allowed access to a 35% (w/v) sucrose solution.

		LEAN	OBESE
ADRENALECTOMISED CHOW-FED	noradrenaline content (ng/organ)	195 ± 20	120 ± 9
	fractional turnover (hr^{-1})	0.14 ± 0.01	0.16 ± 0.03
	half-life (hrs)	4.9 ± 0.03	4.4 ± 0.8
	turnover rate (ng/organ/hr)	27 ± 3	19 ± 3
	initial uptake (pmoles/organ)	1.24 ± 0.14	1.28 ± 0.3
ADRENALECTOMISED SUCROSE-FED	noradrenaline content (ng/organ)	270 ± 6 b**	250 ± 2 b***
	fractional turnover (hr^{-1})	0.116 ± 0.014	0.128 ± 0.013
	half-life (hrs)	6.0 ± 0.5	5.4 ± 0.6
	turnover rate (ng/organ/hr)	31 ± 1	32 ± 6
	initial uptake (pmoles/organ)	2.51 ± 0.12 b**	3.22 ± 0.39 b*
INTACT SUCROSE-FED	noradrenaline content (ng/organ)	290 ± 17	240 ± 6
	fractional turnover (hr^{-1})	0.069 ± 0.018	0.137 ± 0.012a*
	half-life (hrs)	10.1 ± 2.6	5.0 ± 0.4 a*
	turnover rate (ng/organ/hr)	20 ± 6	33 ± 3 a*
	initial uptake (pmoles/organ)	2.42 ± 0.29	3.21 ± 0.21a**

although noradrenaline content and initial uptake of [^{3}H]- noradrenaline increased, compared with chow-fed adrenalectomised controls in both lean and fa/fa rats, there were no significant changes in the half-life or turnover rate of noradrenaline. When intact rats were fed a 35% (w/v) sucrose solution the results obtained were similar to those obtained when rats were allowed access to a 10% (w/v) sucrose solution (Section 3.1). BAT noradrenaline turnover in lean rats allowed access to a 35% (w/v) sucrose solution was comparable to that in 10% (w/v) sucrose-fed lean rats and, as found previously, BAT noradrenaline turnover in obese rats overfed with sucrose remained at very low levels and was only 2% of that found in sucrose-fed lean rats as a result of a reduced BAT noradrenaline content and fractional turnover. Cardiac noradrenaline turnover was greater in obese rats overfed with sucrose than in sucrose-fed lean rats as a result of a reduced half-life of noradrenaline.

3.3.2 DISCUSSION.

Adrenalectomy of fa/fa rats normalises energetic efficiency and the thermogenic capacity of BAT and restores the thermic effects of meal feeding and the adaptive response of BAT to sucrose over-feeding (Holt et al., 1983; Marchington et al., 1983). The increases in BAT thermogenesis, as assessed by BAT mitochondrial GDP binding, that occur in both lean and obese adrenalectomised rats in response to overfeeding with sucrose may occur independent of changes in sympathetic activity. Adrenalectomy increases noradrenaline turnover in obese rats to the values observed in lean animals (Section 3.2). When adrenalectomised rats were allowed access to a 35% (w/v) sucrose solution there were no significant increases in BAT noradrenaline turnover in either lean or obese adrenalectomised in spite of proportional increases in energy intake that were comparable to those found in intact sucrose-fed animals which resulted in large increases in BAT noradrenaline turnover in intact sucrose-fed lean rats. This suggests that there may be a fundamental difference in the mechanism of the thermogenic response to sucrose overfeeding in adrenalectomised rats.

Increased BAT thermogenesis on sucrose overfeeding in intact lean rats was associated with increased BAT noradrenaline turnover, in contrast an increased sympathetic drive does not appear to be required for increased thermogenesis in BAT in response to sucrose feeding in either lean or obese adrenalectomised rats.

It has been demonstrated that an increased sympathetic stimulation is not a necessary requirement for increased BAT thermogenesis in adrenalectomised rats by inhibiting sympathetic stimulation of BAT with the β-adrenergic receptor blocker propranolol (York et al., 1985b). Propranolol treatment of chow-fed adrenalectomised lean and obese rats reduced BAT mitochondrial GDP binding to the low levels found in intact chow-fed obese rats. Sucrose feeding to adrenalectomised rats lead to increases in GDP binding that could not be fully blocked by propranolol (York et al., 1985b), indicating that there is a component of the increases in BAT mitochondrial GDP binding that is not sympathetically mediated. Similar results were also found in intact lean rats fed sucrose. On acute sucrose feeding (24 hours) there is a small component of the increased BAT mitochondrial GDP binding that was not blocked by propranolol. After 7 days sucrose feeding this component was increased and GDP binding was not significantly decreased by propranolol blockade (York et al., 1985b). Thus, although sympathetic activity was increased in BAT of sucrose-fed lean rats, after 7 days sucrose feeding, sympathetic stimulation is not required to maintain the increased GDP binding to BAT mitochondria. These results suggest that while sympathetic stimulation may initiate BAT thermogenesis and the long term adaptive changes in the response to overfeeding (cell proliferation, increased 32000D protein and increased thermogenic capacity), once these are established an increased sympathetic stimulation is no longer a requirement to maintain thermogenesis.

The mechanism operating in sucrose-fed adrenalectomised rats to stimulate BAT thermogenesis is unclear. Noradrenaline turnover in these animals was assessed after 7 days of sucrose feeding, at which time BAT noradrenaline turnover was not significantly increased over that in chow-fed adrenalectomised rats. There may have been an initial increase in sympathetic

activity on sucrose feeding that diminishes as the animals are maintained on the sucrose feeding regime. Another alternative is that DIT in BAT can be initiated without increased sympathetic stimulation. It is possible that the only requirement to allow a diet-induced stimulation of the thermogenesis is for a minimum sympathetic tone (presumably greater than that found in intact obese rats) to prevent involution of the tissue and maintain sensitivity to some humoral factor that can stimulate BAT thermogenesis. In normal lean rats there is a tonic stimulation of BAT by the sympathetic nervous system (Girardier and Seydoux, 1977) and denervation of BAT results in increased wet weight and fat content and reduced protein and DNA content of BAT (Mory et al., 1982; Dulloo et al., 1983) which is similar to BAT of obese rats.

Possible candidates for a humoral effector of BAT thermogenesis are catecholamines released from the adrenal medulla, insulin and ACTH. Adrenaline and noradrenaline released from the adrenal medulla are unlikely to act as humoral effectors of BAT thermogenesis in response to dietary stimuli since the concentration required at the synapse to regulate thermogenesis in BAT is 30-150-fold greater than that of circulating noradrenaline (Girardier and Seydoux, 1977). Insulin injections can increase metabolic rate in fasted and cafeteria-fed rats and enhances the thermogenic response to noradrenaline (Rothwell et al., 1983b). Diabetic rats fail to exhibit NST or DIT (Rothwell and Stock, 1981b). However, these effects of insulin may be mediated by insulin dependent increases in sympathetic activity rather than a direct effect on the tissue, since the thermogenic effect of refeeding carbohydrate to fasted rats, which appears to be insulin dependent, is blocked by propranolol (Rothwell et al., 1983b). In contrast, ACTH does have direct effects on BAT that are not blocked by propranolol (Heim and Hull, 1966). ACTH potentiates the thermogenic effects of noradrenaline (Laury and Portet, 1977), and increases BAT mitochondrial GDP binding in fa/fa rats (York and Al-Baker, 1985) without affecting

noradrenaline turnover (Section 3.2). ACTH levels are increased on adrenalectomy of lean and obese rats (Yukimura et al., 1978), further increases in response to overfeeding could stimulate BAT thermogenesis independent of the sympathetic nervous system.

SECTION 3.4 Noradrenaline turnover in rats fed a highly palatable "cafeteria" diet.

The previous sections (3.1, 3.2 and 3.3) have demonstrated that the defective BAT thermogenesis of the obese (fa/fa) Zucker rat was associated with a reduced noradrenaline turnover in BAT at an early age, indicative of a reduced sympathetic activation of the tissue. The ability of the obese rat to respond normally to cold but not to diet was associated with a normal activation of the sympathetic nerve supply to BAT in response to cold but not to dietary stimuli. Adrenalectomy, which restores BAT thermogenic capacity, and the response to diet in obese (fa/fa) rats was associated with a normal BAT noradrenaline turnover in chow-fed rats. However, the thermogenic response to overfeeding adrenalectomised rats was not associated with increases in BAT noradrenaline turnover in either lean or obese rats.

The feeding of a highly palatable diet, such as the "cafeteria" diet (see Section 2.2.3), is a potent stimulator of DIT with up to 3-fold increases in BAT mitochondrial GDP binding (Brooks et al., 1982; Himms-Hagen et al., 1983) associated with increased BAT noradrenaline turnover (Young et al., 1982). However, the ability of rats to initiate DIT in response to cafeteria feeding varies considerably. The obese (fa/fa) rat, which is unable to initiate DIT in response to cafeteria feeding or even the hyperphagia it displays on a normal chow diet (Holt and York, 1982; Triandafillou and Himms-Hagen, 1983), can be taken as an extreme case. The ability of normally lean rats to initiate DIT varies with strain (Rothwell & Stock,1982a)and age (Rothwell & Stock,1983b). The reduced ability of older rats to initiate DIT is reversed by adrenalectomy (Rothwell et al., 1984b)indicating that, as in the obese (fa/fa) rat, the thermogenic response to dietary stimuli is sensitive to adrenal glucocorticoids. Young Sprague-Dawley rats overeat by up to 80% on a cafeteria diet but are able to initiate DIT and resist obesity, gaining little or no weight (Rothwell and Stock, 1979a). However, compared with some other

rat strains and wild/hybrids the Sprague-Dawley rat displays reduced DIT and a greater tendency to gain lipid (Rothwell & Stock, 1982a). There is also a large variation in the ability of individual Sprague-Dawley rats to resist obesity on feeding a high-fat, high-carbohydrate diet, that are paralleled by variations in BAT noradrenaline turnover (Levin et al., 1983b).

The experiments described in this section report the effects of adrenalectomy on BAT thermogenesis and noradrenaline turnover in cafeteria-fed 5-6 week old Sprague-Dawley rats. The results indicate that cafeteria feeding and adrenalectomy increase BAT thermogenesis and BAT sympathetic activity and suggest that there may be differences between Zucker rats and Sprague-Dawley rats in the regulation of the thermogenic response to overfeeding.

3.4.1 The effect of adrenalectomy and diet on thermogenesis and tissue noradrenaline turnover in Sprague-Dawley rats.

The experiments described in this section were performed in collaboration with Dr. M. J. Stock and Dr. N. J. Rothwell, whose laboratory provided the animals and the measurements of oxygen consumption and BAT mitochondrial GDP binding. Table 2.12 shows the effects of adrenalectomy and diet on body, BAT and heart weights, on resting oxygen consumption before and after propranolol treatment and on BAT mitochondrial GDP binding. Sham-operated and adrenalectomised rats were fed chow or a range of palatable food items in addition to chow for 14 days. Oxygen consumption measurements were made after 10 days on the diets.

Cafeteria feeding had no effects on heart or body weight but increased the weight of interscapular BAT in the sham-operated rats. Resting metabolic rate was increased in cafeteria-fed, sham-operated rats, but propranolol treatment reduced resting oxygen consumption back to chow-fed values. The proportion of resting oxygen consumption that was propranolol sensitive was increased 4-fold by cafeteria feeding in sham-operated rats. This was associated with a 50% increase

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Table 3.12 The effects of adrenalectomy and cafeteria feeding on thermogenesis in 5-6 week old Sprague-Dawley rats.

	SHAM-OPERATED		ADRENALECTOMISED	
	CHOW-FED	CAFETERIA-FED	CHOW-FED	CAFETERIA-FED
Animal weight (g)	234 ± 5.7	231 ± 4.7	185 ± 3.8 a***	194 ± 4.7 c***
ISBAT weight (g)	0.22 ± 0.01	0.49 ± 0.02 a***	0.19 ± 0.005	0.38 ± 0.02 b*** c***
Heart weight (g)	0.76 ± 0.03	0.80 ± 0.01	0.63 ± 0.02 a**	0.74 ± 0.02
Resting VO_2 ($ml/min.W^{0.75}$)	15.07 ± 0.3	16.9 ± 0.36 a**	19.61 ± 0.23a***	20.63 ± 0.48 b**
Resting VO_2 after propranolol ($ml/min.W^{0.75}$)	14.29 ± 0.3	13.63 ± 0.54	15.83 ± 0.33	14.62 ± 0.59
ΔVO_2 ($ml/min.W^{0.75}$)	0.78 ± 0.22	3.28 ± 0.5 a***	3.78 ± 0.35 a***	6.01 ± 0.54 b*** c***
Specific GDP binding (pmol/mg protein)	51 ± 4	76 ± 7 a**	88 ± 8 a**	119 ± 11 b** c*

Animals were sham-operated or adrenalectomised and maintained on chow and water or chow and 0.9% (w/v) saline respectively. Cafeteria-fed animals were offered a range of palatable food items in addition to chow. Resting oxygen consumption was measured after 10 days and body and organ weights and BAT mitochondrial GDP binding after 14 days. Values represent means $\pm$ S.E.M. for 8 rats in each group (oxygen consumption measurements) and 16 rats in each group (weights and specific GDP binding). ***, $p < 0.001$; **, $p < 0.005$; *, $p < 0.01$. a, compared with sham-operated chow-fed group; b, compared with sham-operated cafeteria-fed group; c, compared with adrenalectomised chow-fed group.

in BAT mitochondrial GDP binding in cafeteria-fed sham-operated rats.

Adrenalectomy of chow-fed rats reduced body and heart weights but did not significantly affect interscapular BAT weight. Resting oxygen consumption was increased by adrenalectomy but was reduced to the same values as in sham-operated rats after propranolol treatment. The proportion of resting oxygen consumption that was blocked by propranolol was increased 5-fold by adrenalectomy of chow-fed rats and BAT mitochondrial GDP binding was increased by 75%. Thus the increases in propranolol sensitive resting oxygen consumption and BAT mitochondrial GDP binding were as great as those obtained by cafeteria feeding.

Cafeteria feeding of adrenalectomised rats did not increase body weight, but body weight was reduced compared to cafeteria-fed, sham-operated rats . Interscapular BAT weight was greater in cafeteria-fed, adrenalectomised rats than in adrenalectomised rats and lower than in cafeteria-fed rats. Heart weight was unaffected by cafeteria feeding adrenalectomised rats. Resting oxygen consumption was higher in cafeteria-fed, adrenalectomised rats than in cafeteria-fed rats, but no higher than in chow-fed adrenalectomised rats. Propranolol treatment reduced resting oxygen consumption to chow-fed, sham-operated values. The component of resting oxygen consumption blocked by propranolol was 8-fold greater than in chow-fed sham-operated rats and significantly greater than in either cafeteria-fed sham-operated or chow-fed adrenalectomised rats. A similar effect occurred with mitochondrial GDP binding. Binding was higher in cafeteria-fed adrenalectomised rats than in either cafeteria-fed sham-operated or chow-fed adrenalectomised rats and was elevated by 133% over the values in chow-fed sham-operated rats. Thus adrenalectomy potentiated the thermogenic effects of cafeteria feeding on Sprague-Dawley rats.

Fig. 3.4 and Table 3.13 show the effects of cafeteria feeding and adrenalectomy on noradrenaline turnover in BAT and heart of Sprague-Dawley rats. As expected from previous

Fig. 3.4

Noradrenaline turnover in brown adipose tissue and heart of chow-fed (●——●) and cafeteria-fed (○——○) sham-operated and adrenalectomised 5-6 week old Sprague-Dawley rats. Rats were injected i.v. with 25μCi/100g body weight 1-7,8 [^{3}H]-noradrenaline in 0.9% (w/v) saline at time zero. Values represent means ± S.E.M. for 4 rats at each time point.

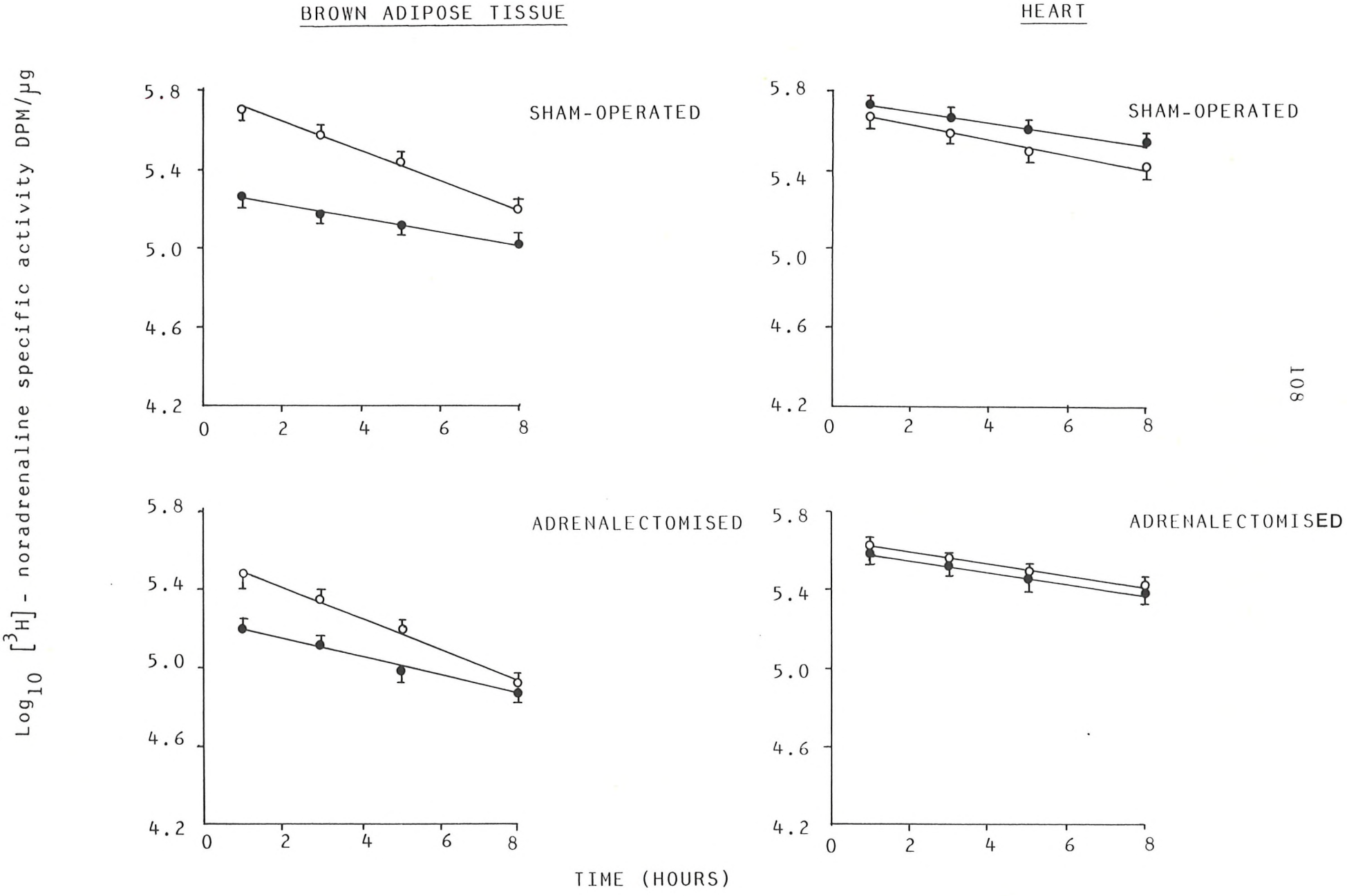

BROWN ADIPOSE TISSUE
HEART
SHAM-OPERATED
SHAM-OPERATED
ADRENALECTOMISED
ADRENALECTOMISED
Log10 [3H] - noradrenaline specific activity DPM/µg
TIME (HOURS)
5.8
5.4
5.0
4.6
4.2
0
2
4
6
8

Table 3.13 Legend.

Rats were sham-operated or adrenalectomised at 3-4 weeks of age. Adrenalectomised rats were maintained on 0.9% (w/v) saline. Rats were fed either a stock chow diet or stock chow and a range of palatable food items for 14 days prior to the study. Values represent $\pm$ S.E.M. for the 16 rats in each group. ***, $p < 0.001$; **, $p < 0.005$; * $p < 0.01$. a, compared with sham-operated chow-fed group; b, compared with sham-operated cafeteria-fed group; c, compared with adrenalectomised chow-fed group.

Table 3.13 Noradrenaline content, fractional turnover, half-life, turnover rate and initial uptake of [^{3}H]-noradrenaline in BAT and heart of sham-operated and adrenalectomised 5-6 week old Sprague-Dawley rats fed a stock chow diet or a cafeteria diet.

		BROWN ADIPOSE TISSUE		HEART	
		STOCK DIET	CAFETERIA DIET	STOCK DIET	CAFETERIA DIET
SHAM-OPERATED	noradrenaline content (ng/organ)	203 ± 5	196 ± 3	247 ± 7	284 ± 7 a**
	fractional turnover (hr^{-1})	0.077 ± 0.008	0.163 ± 0.002 a***	0.061 ± 0.009	0.087 ± 0.004
	half-life (hrs)	9.0 ± 0.1	4.3 ± 0.1 a***	11.4 ± 1.6	8.0 ± 0.4
	turnover rate (ng/organ/hr)	15.6 ± 2.0	32.0 ± 1.0 a**	15.0 ± 2.5	24.7 ± 1.7 a*
	initial uptake (pmoles/organ)	0.49 ± 0.03	1.49 ± 0.03 a***	1.73 ± 0.14	1.79 ± 0.08
ADRENALECTOMISED	noradrenaline content (ng/organ)	359 ± 16 a***	400 ± 9 b***	275 ± 10	310 ± 10
	fractional turnover (hr^{-1})	0.105 ± 0.001 a**	0.195 ± 0.01 c***	0.071 ± 0.009	0.069 ± 0.003
	half-life (hrs)	6.6 ± 0.4 a**	3.6 ± 0.2 c**	9.8 ± 1.3	10.0 ± 0.4
	turnover rate (ng/organ/hr)	37.7 ± 2.1 a**	78.0 ± 5.7 b** c**	19.6 ± 3.2	21.4 ± 1.6
	initial uptake (pmoles/organ)	0.79 ± 0.05	2.82 ± 0.22 b** c***	1.43 ± 0.11	1.75 ± 0.08

reports (Young et al., 1982) cafeteria feeding of Sprague-Dawley rats resulted in a 105% increase in calculated BAT noradrenaline turnover due to a reduced half-life rather than a change in tissue content. Initial tissue uptake of [^{3}H]- noradrenaline in BAT increased 3-fold. Cafeteria feeding of Sprague-Dawley rats resulted in a 65% increase in cardiac noradrenaline turnover resulting from an increased tissue content and decreased half-life of noradrenaline, although the change in half-life was not statistically significant.

Adrenalectomy of Sprague-Dawley rats resulted in a 142% increase in BAT noradrenaline turnover due to an increase in tissue noradrenaline content and a reduced half-life of noradrenaline. In contrast to the effects of cafeteria feeding, adrenalectomy did not significantly alter cardiac noradrenaline.

Cafeteria feeding of adrenalectomised rats leads to further increases in BAT noradrenaline turnover of 107%, resulting from an increased tissue noradrenaline content and a decreased half-life. The effects of cafeteria feeding on BAT noradrenaline turnover were increased by 143% in adrenalectomised rats compared with sham-operated rats. The initial uptake of [^{3}H]- noradrenaline was increased by 257% compared with chow-fed adrenalectomised rats and by 89% compared with cafeteria-fed sham-operated rats. In contrast to the results in sham-operated rats, cardiac noradrenaline turnover was not increased by cafeteria feeding of adrenalectomised rats.

The increases in BAT noradrenaline turnover in response to cafeteria feeding and adrenalectomy closely parallel the increases in BAT mitochondrial GDP binding and propranolol sensitive resting oxygen consumption. There were proportionally similar increases in propranolol sensitive resting oxygen consumption, GDP binding and BAT noradrenaline turnover in the order of:- chow-fed, sham-operated rats; cafeteria-fed, sham-operated rats; chow-fed, adrenalectomised rats and were greatest in cafeteria-fed, adrenalectomised rats.

3.4.2 DISCUSSION.

The results presented in this section demonstrate that in Sprague-Dawley rats changes in BAT thermogenesis and propranolol sensitive resting oxygen consumption are paralled by changes in sympathetic activity in BAT and that the responses to andrenalectomy and diet are different in Zucker and Sprague-Dawley rats.

Noradrenaline turnover in BAT of chow-fed, sham-operated Sprague-Dawley rats was more than 2-fold lower than in lean Zucker rats of the same age (Section 3.1) despite a similar cardiac noradrenaline turnover. This is consistent with the higher BAT mitochondrial GDP binding found in lean Zucker rats (Holt and York, 1982) compared with Sprague-Dawley rats and suggests that the sympathetic stimulation of BAT thermogenesis is lower in chow-fed Sprague-Dawley rats.

Cafeteria feeding of Sprague-Dawley rats increased resting oxygen consumption. This was associated with increased BAT wet weight, increased BAT mitochondrial GDP binding and increased BAT and cardiac noradrenaline turnover. These increases confirm previous reports of the effects of cafeteria feeding in Sprague-Dawley rats (Brooks et al., 1980; Young et al., 1982) and is indicative of an increased sympathetic stimulation of BAT. This is supported by the observation that the increase in resting oxygen consumption was blocked by propranolol. Although BAT mitochondrial GDP binding and BAT noradrenaline turnover increased on cafeteria feeding of Sprague-Dawley rats, the values obtained were still lower than those in chow-fed Zucker rats. However, there appears to have been a sufficient stimulation of thermogenesis to prevent the development of obesity since the cafeteria-fed Sprague-Dawley rats did not gain weight relative to chow-fed Sprague-Dawley rats.

In contrast to the findings in lean Zucker rats fed sucrose (Section 3.1, 3.3) cafeteria feeding resulted in a 65% increase in cardiac turnover in Sprague-Dawley rats, similar to that reported for Sprague-Dawley rats fed sucrose (Young et al., 1982). This suggests that the absence of any

increases in cardiac noradrenaline turnover in lean Zucker rats on sucrose overfeeding or cold exposure, may reflect a strain difference.

Adrenalectomy of Sprague-Dawley rats reduced body and heart weight and increased resting oxygen consumption and BAT mitochondrial GDP binding. BAT noradrenaline turnover increased in adrenalectomised Sprague-Dawley rats to the values observed in lean Zucker rats, as a result of an increased tissue content of noradrenaline and a decreased half-life. This suggests that there was an inhibition of sympathetic activation of BAT thermogenesis in the intact Sprague-Dawley rat that is mediated by adrenal glucocorticoids since adrenalectomy increased BAT noradrenaline turnover and the thermogenic capacity of BAT. In addition, the propranolol sensitive component of resting oxygen consumption was increased in adrenalectomised Sprague-Dawley rats. The increases in BAT noradrenaline turnover and BAT thermogenesis in Sprague-Dawley rats after adrenalectomy contrast with the findings in lean Zucker rats in which adrenalectomy was not associated with significant increases in BAT mitochondrial GDP binding (Holt and York, 1982; Holt et al., 1983; Marchington et al., 1983) or BAT noradrenaline turnover (Section 3.2).

The response of the adrenalectomised Sprague-Dawley rat to cafeteria feeding was of a greater magnitude than that of sham-operated rats. Total resting oxygen consumption and the propranolol sensitive component increased and BAT weight and BAT mitochondrial GDP binding increased. This was associated with increase noradrenaline turnover in BAT which resulted from both an increased noradrenaline content and fractional turnover rate. This was in contrast to sham-operated rats in which the increased BAT noradrenaline turnover associated with cafeteria feeding resulted from an increase in fractional turnover only. The increased noradrenaline turnover in BAT of cafeteria-fed adrenalectomised Sprague-Dawley rats was of a similar magnitude to that found in sucrose-fed, intact lean Zucker rats. As was found in sucrose-fed lean Zucker rats, the increase in BAT noradrenaline turnover in adrenalectomised

Sprague-Dawley rats induced by overfeeding was not paralleled by any increase in cardiac noradrenaline turnover.

The increases in BAT mitochondrial GDP binding and the propranolol-sensitive component of resting oxygen consumption achieved on cafeteria feeding of adrenalectomised Sprague-Dawley rats were associated with increased BAT noradrenaline turnover. This suggests that the increased DIT in cafeteria-fed adrenalectomised Sprague-Dawley rats is closely linked to increased sympathetic stimulation of BAT, in contrast to adrenalectomised Zucker rats overfed with sucrose in which BAT noradrenaline turnover was not increased. In addition, the reduced thermogenesis and thermogenic response to overfeeding in intact Sprague-Dawley rats may be associated with an inhibition of sympathetic activation of BAT, since adrenalectomy resulted in increased thermogenesis and BAT noradrenaline turnover and further increases on cafeteria feeding.

The differing response of Sprague-Dawley rats compared with Zucker rats to overfeeding may reflect differences in sensitivity to circulating corticosterone. The obese (fa/fa) rat appears to be very sensitive to corticosterone, with consequently severely reduced BAT noradrenaline turnover, reduced BAT thermogenesis and inability to respond to dietary stimuli. The Sprague-Dawley rat may show a partial sensitivity to corticosterone. BAT noradrenaline turnover and thermogenesis are reduced compared with lean Zucker rats but BAT responds sufficiently to dietary stimuli to resist obesity on a cafeteria diet. However, the Sprague-Dawley rat can rapidly become obese on high-fat, or high-fat high-carbohydrate diets. (Schemmel, 1970; Miller, 1976; Levin et al., 1983b, c). The thermogenic response of the lean Zucker rat is relatively insensitive to circulating corticosterone since BAT noradrenaline turnover and thermogenesis do not increase significantly on adrenalectomy. The heterozygous (Fa/fa) lean Zucker rat exhibits a reduced BAT thermogenic response to feeding (York et al., 1984) and, like the Sprague-Dawley rat, rapidly gains weight on a high-fat diet (Zucker and Zucker, 1963). It would be interesting to determine

whether BAT noradrenaline turnover is reduced in heterozygotes and whether these defects are abolished on adrenalectomy.

Sensitivity to circulating corticosterone seems to be linked with cardiac responses to overfeeding. In intact lean Zucker rats, which are relatively insensitive to circulating corticosterone, cardiac noradrenaline turnover did not increase on overfeeding. Cardiac noradrenaline turnover was not increased on overfeeding of adrenalectomised lean and obese Zucker rates or adrenalectomised Sprague-Dawley rats, but increased 66% in intact overfed Sprague-Dawley rats and by up to 185% in overfed intact obese Zucker rats, which shows the greatest sensitivity to circulating corticosterone.

The increased BAT thermogenic response to overfeeding in adrenalectomised Sprague-Dawley rats was associated with a 107% increase in BAT noradrenaline turnover. This is markedly different from the situation in adrenalectomised Zucker rats in which the increased BAT mitchondrial GDP binding on sucrose feeding was not associated with any changes in BAT noradrenaline turnover. This could reflect a strain difference or differences in diet composition. Feeding of a cafeteria diet results in an increased intake of fat as well as of carbohydrate. Fat is a potent stimulator of noradrenaline turnover in BAT (Schwartz et al., 1983) so the increased BAT noradrenaline turnover in adrenalectomised Sprague-Dawley rats could result from the increased fat intake rather than the increased carbohydrate intake. Cafeteria feeding of lean Zucker rats results in an increased resting oxygen consumption after 10 days on the diet that is completely blocked by propranolol treatment (Rothwell and Stock, 1982b).

SECTION 3.5 The effect of ganglionic blockade on tissue noradrenaline turnover in lean and obese (fa/fa) rats.

In previous sections measurement of noradrenaline turnover using tracer doses of [^{3}H]- noradrenaline has been used to provide an assessment of sympathetic activity. In order to demonstrate a direct relationship between noradrenaline turnover and sympathetic activity, sympathetic nerve impulse traffic may be reduced using ganglionic blockers such as chlorisondamine. Increased noradrenaline turnover could conceivably be caused by increased spontaneous release of noradrenaline or increased intraneuronal degradation Loss of [^{3}H]- noradrenaline resulting from sympathetic nerve impulse traffic is reduced by ganglionic blockade with chlorisondamine. The contribution of central sympathetic outflow to tissue noradrenaline turnover may thus be assessed from the retention of [^{3}H]- noradrenaline in tissues of chlorisondamine treated rats compared with controls. The experiments reported in this section examine the effect of ganglionic blockade on noradrenaline turnover of heart and BAT in lean and obese (fa/fa) rats. The results suggest that the diminished noradrenaline turnover observed in BAT of obese (fa/fa) rats results from a reduced central sympathetic outflow and that changes in noradrenaline turnover observed on sucrose feeding and adrenalectomy are associated with changes in sympathetic impulse traffic.

RESULTS.

3.5.1 The effect of ganglionic blockade on tissue noradrenaline content and [^{3}H]- noradrenaline specific activity in lean and obese (fa/fa) rats.

Table 3.14 shows the effects of ganglionic blockade on BAT and heart noradrenaline content in intact rats, adrenalectomised rats, sucrose-fed rats and adrenalectomised rats overfed with a 35% (w/v) sucrose solution. As expected

Table 3.14 The effect of ganglionic blockade on tissue content of noradrenaline in 5-6 week old lean and obese (fa/fa) rats.

		BROWN ADIPOSE TISSUE (ng/organ)		HEART (ng/organ)	
		LEAN	OBESE	LEAN	OBESE
INTACT	SALINE	$270^{+}_{-}30$	$130^{+}_{-}20$ b*	$180^{+}_{-}30$	$180^{+}_{-}10$
	CHLORISONDAMINE	$340^{+}_{-}20$	$160^{+}_{-}10$	$200^{+}_{-}10$	$210^{+}_{-}20$
ADRENALECTOMISED	SALINE	$390^{+}_{-}10$ a*	$220^{+}_{-}20$ b*** a*	$180^{+}_{-}40$	$190^{+}_{-}30$
	CHLORISONDAMINE	$450^{+}_{-}10$	$250^{+}_{-}10$	$210^{+}_{-}40$	$200^{+}_{-}20$
SUCROSE-FED	SALINE	$390^{+}_{-}20$ a*	$90^{+}_{-}20$ b***	$210^{+}_{-}10$	$200^{*}_{-}10$
	CHLORISONDAMINE	$470^{+}_{-}30$	$150^{+}_{-}20$	$230^{+}_{-}10$	$230^{+}_{-}10$
SUCROSE-FED ADRENALECTOMISED	SALINE	$350^{+}_{-}40$	$190^{+}_{-}20$ c*	$220^{+}_{-}30$	$180^{+}_{-}10$
	CHLORISONDAMINE	$410^{+}_{-}40$	$230^{+}_{-}20$	$230^{+}_{-}10$	$190^{+}_{-}10$

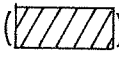
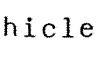
Animals received 25μCi/100g body weight 1-7,8 $[^{3}H]$-noradrenaline at time zero. At 5 minutes and 5 hours rats received 0.5mg/100g body weight chlorisondamine chloride in 0.9% (w/v) saline or vehicle by i.p. injection. Animals were sacrificed after 10 hours. Adrenalectomised animals were maintained on 0.9% (w/v) saline instead of drinking water and sucrose-fed groups received a 35% (w/v) surcrose solution in addition to drinking water or saline. Animals were kept on these regimes for 7 days prior to the study. Values represent means $^{+}_{-}$ S.E.M. for 3 rats in each group. ***, p 0.002 ; **, p 0.01; *, p 0.02.a, compared with control group; b compared with equivalent lean group, c, compared with sucrose-fed group; for comparisons of saline treated groups.

from the results presented in Sections 3.1, 3.2 and 3.3, the BAT noradrenaline content of obese rats was again very much lower than that in lean rats and was increased on adrenalectomy but not on sucrose feeding. BAT noradrenaline content was increased in sucrose-fed lean rats and in adrenalectomised lean rats. Cardiac noradrenaline content was unaffected by genotype, adrenalectomy or sucrose feeding. Ganglionic blockade increased tissue noradrenaline content in all groups but, due to the small sample size, these differences were not significantly different.

Figs. 3.5 and 3.6 and Table 3.15 show the effects of ganglionic blockade on [^{3}H]- noradrenaline specific activity in BAT and heart of lean and obese (fa/fa) rats and the fractional turnover, half-life and turnover rates of noradrenaline estimated from these data. Because chlorisondamine is administered after the injection of [^{3}H]- noradrenaline the initial uptake of [^{3}H]- noradrenaline is unaffected by the ganglionic blockade. A fractional turnover rate and half-life of noradrenaline can be estimated from the difference between $\log_{10}$ [^{3}H]- noradrenaline specific activity in the saline and chlorisondamine treatment groups. The turnover rate calculated from the fractional turnover rate and the tissue content provides an estimate of the noradrenaline turnover resulting from sympathetic impulse traffic (see Section 2.3.4).

The loss of [^{3}H]- noradrenaline from BAT and heart of lean and obese (fa/fa) rats was significantly reduced by specific activity of [^{3}H]- noradrenaline in all groups except in BAT of intact obese rats fed on either a chow diet or a sucrose supplemented chow diet. Adrenalectomy of lean rats did not affect the chlorisondamine-sensitive changes in [^{3}H]- noradrenaline specific activity in BAT but in obese rats adrenalectomy increased retention of [^{3}H]- noradrenaline to that found in lean rats. However, the estimated chlorisondamine-sensitive BAT noradrenaline turnover was not as great in adrenalectomised obese rats as in lean rats, due to the lower tissue content of noradrenaline.

Fig. 3.5 The effect of ganglionic blockade on [^{3}H] -noradrenaline specific activity in BAT of lean and obese (fa/fa) rats.

All rats received 25μCi/100g body weight 1-7,8 [^{3}H] -noradrenaline by i.v. injection in 0.9% (w/v) saline at time zero. After 5 minutes and 5 hours rats received 0.5mg/100g body weight chlorisondamine chloride in 0.9% (w/v)saline () or vehicle () by i.p. injection. Animals were sacrificed after 10 hours. Values represent means $\pm$ S.E.M. for 3 rats in each group. Number over bar represents the inhibition of the decrease in [^{3}H] -noradrenaline specific activity (DPM/μgx10^5). ***, $p < 0.001$; **, $p < 0.005$; *, $p < 0.01$ for comparisons between ganglionic blockade and saline treatment groups.

[3H] - noradrenaline specific activity DPM/µg × 10^5
4.0
3.0
2.0
1.0
0
ΔNA s.a.
1.10
0.01
0.96
1.54
2.31
0.07
1.03
1.41
**
*
*
*
**
*
LEAN
OBESE
LEAN
OBESE
LEAN
OBESE
LEAN
OBESE
INTACT
ADRENALECTOMISED
SUCROSE-FED
ADRENALECTOMISED
SUCROSE-FED

Fig. 3.6 The effect of ganglionic blockade on [^{3}H]-noradrenaline specific activity in heart of lean and obese (fa/fa) rats.

All rats received 25μCi/100g body weight 1,7,8 [^{3}H]-noradrenaline in 0.9% (w/v) saline by i.v. injection at time zero. After 5 minutes and 5 hours rats received 0.5mg/100g body weight chlorisondamine chloride in 0.9% (w/v) saline (▨) or vehicle (□) by i.p. injection. Animals were sacrificed after 10 hours. Values represent means ± S.E.M. for 3 rats in each group. Number over bar represents inhibition of the decrease in ^{3}H-noradrenaline specific activity (DPM/μgx10^5). ***, $p < 0.001$; **, $p < 0.005$; *, $p < 0.01$ for comparisons between ganglionic blockade and saline treatment groups.

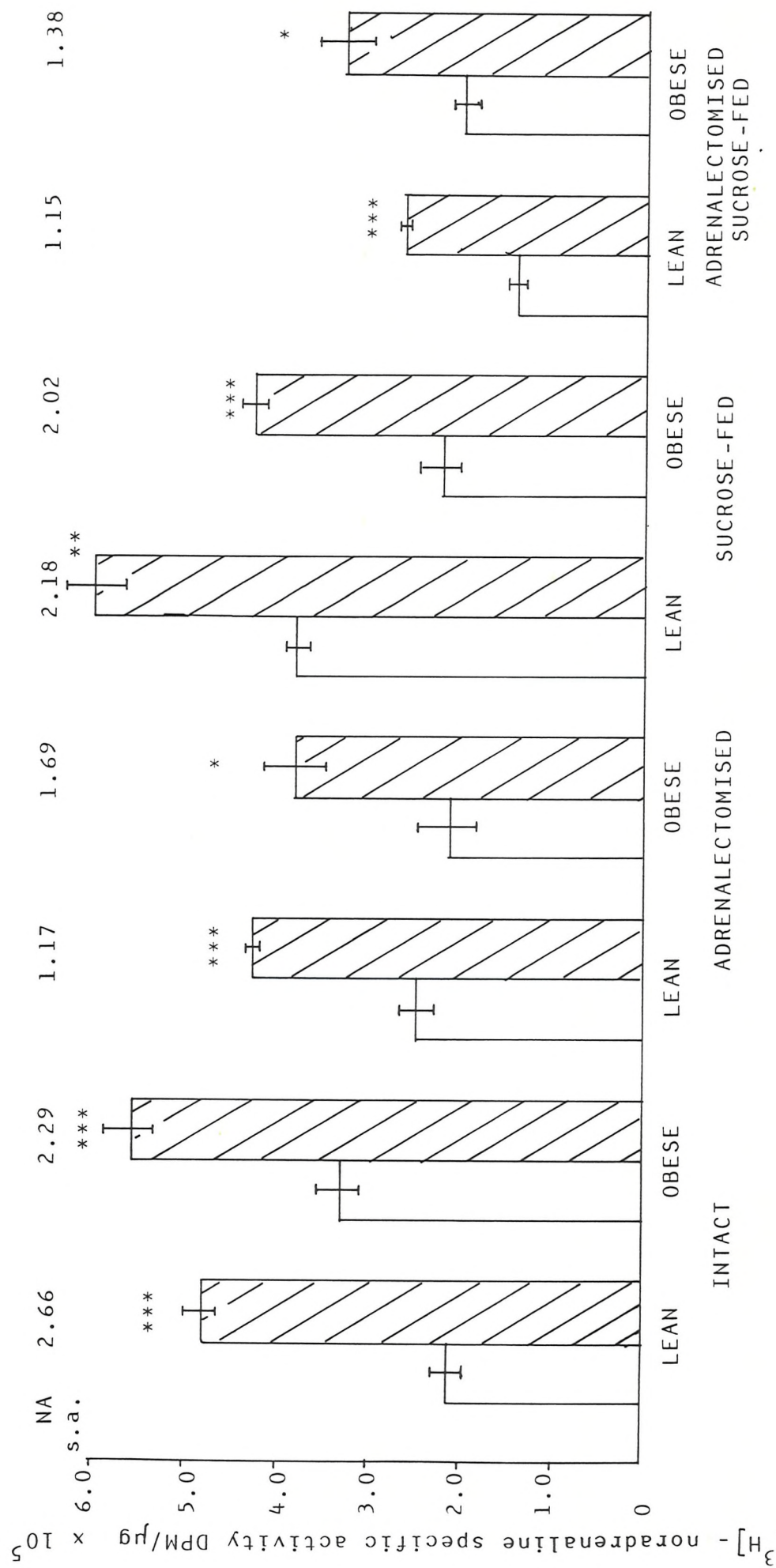

[3H]- noradrenaline specific activity DPM/µg x 10^5
6.0
5.0
4.0
3.0
2.0
1.0
0
NA s.a.
2.66
2.29
1.17
1.69
2.18
2.02
1.15
1.38
***
***
***
*
**
***
***
*
LEAN
OBESE
LEAN
OBESE
LEAN
OBESE
LEAN
OBESE
INTACT
ADRENALECTOMISED
SUCROSE-FED
ADRENALECTOMISED
SUCROSE-FED

Table 3.15 Legend.

All rats received 25μCi/100g body weight 1-7,8 [^{3}H]-noradrenaline at time zero. At 5 minutes and 5 hours rats received 0.5mg/100g body weight chlorisondamine chloride in 0.9% (w/v) saline or vehicle by i.p. injection. Rats were sacrificed after 10 hours. Adrenalectomised rats were maintained on 0.9% (w/v) saline instead of drinking water. Sucrose fed groups received a 35% (w/v) sucrose solution in addition to drinking water or saline. All rats were fed with chow ad lib and rats were kept on these regimes for 7 days prior to the study. Values were calculated from results shown in Gif.3l1 and 3.12, 6 rats in each treatment group. ***, $p < 0.01$; **, $p \leq 0.005$; *, $p < 0.01$. NS, not significant, for statistical significance of % increase in [^{3}H]-NA specific activity.

NA = noradrenaline.

Table 3.15 The effect of ganglionic blockade on tissue [^{3}H]-noradrenaline specific activity and estimated fractional turnover rate, half-life and turnover rate of noradrenaline released by sympathetic stimulation in 5-6 week old lean and obese (fa/fa) rats.

		BROWN ADIPOSE TISSUE		HEART	
TREATMENT		LEAN	OBESE	LEAN	OBESE
INTACT	increase in [^{3}H]-NA s.a.(%)	111 **	0.006 NS	124 ***	69 ***
	fractional turnover (hr^{-1})	0.075	0.0006	0.081	0.053
	half-life (hrs)	9.4	1209	8.6	13.1
	turnover rate (ng/organ/hr)	20.3	0.01	14.6	9.5
ADRENALECTOMISED	increase in [^{3}H]-NA s.a.(%)	114 *	111 *	71 ***	80 *
	fractional turnover, k(hr^{-1})	0.076	0.075	0.054	0.059
	half-life (hrs)	9.1	9.2	12.9	11.8
	turnover rate (ng/organ/hr)	29.6	16.5	9.7	11.2
SUCROSE-FED	increase in [^{3}H]-NA s.a.(%)	215 **	0.04 NS	57 **	90 ***
	fractional turnover (hr^{-1})	0.115	0.004	0.045	0.064
	half-life (hrs)	6.03	173	15.3	10.8
	turnover rate (ng/organ/hr)	44.9	0.36	9.5	12.8
ADRENALECTOMISED SUCROSE-FED	increase in [^{3}H] s.a.(%)	113 **	120 *	79 ***	70 *
	fractional turnover (hr^{-1})	0.076	0.079	0.058	0.053
	half-life (hrs)	9.1	8.8	12.0	13.1
	turnover rate (ng/organ/hr)	26.6	15.0	12.8	9.5

Sucrose feeding of lean rats resulted in a large increase in [^{3}H]- noradrenaline retention in BAT, but there was no sign of a change in [^{3}H]- noradrenaline specific activity in BAT of obese rats on chlorisondamine treatment. The estimated noradrenaline turnover rate was increased 2-fold in BAT of lean rats by sucrose feeding but was unchanged in BAT of obese rats. Sucrose feeding of adrenalectomised lean and obese rats did not change the chlorisondamine-sensitive retention of [^{3}H]- noradrenaline in BAT and estimated BAT noradrenaline turnover was unaffected by sucrose feeding of adrenalectomised rats.

In contrast to the findings in BAT there were no major changes in the chlorisondamine-sensitive retention of [^{3}H]- noradrenaline in heart of lean or obese rats on adrenalectomy or sucrose feeding, although the % retention of [^{3}H]- noradrenaline was higher in intact lean rats compared with intact obese rats. Estimates of noradrenaline turnover suggest that sympathetic stimulation of heart was unaffected by genotype, adrenalectomy or sucrose feeding.

3.5.2 DISCUSSION.

After injection of [^{3}H]- noradrenaline the specific activity of [^{3}H]- noradrenaline in a tissue declines due to the release and loss of [^{3}H]- noradrenaline and replacement by newly synthesised noradrenaline. The rate of release and hence loss is proportional to the activity of the sympathetic nerve fibres. The ganglionic blocker chlorisondamine is a potent, long acting, nicotinic antagonist. Since chlorisondamine cannot pass the blood brain barrier peripheral administration of chlorisondamine has no central effects, but blocks peripheral nicotinic receptors on skeletal muscle and on sympathetic and parasympathetic ganglia, reducing the passage of impulses through post ganglionic fibres and hence reduces the rate of decline in tissue [^{3}H]- noradrenaline specific activity (Hertting et al., 1962; Montanari et al., 1963; Young and Landsberg, 1979). The change in [^{3}H]- noradrenaline specific activity after ganglionic blockade with chlorisondamine is thus an indication of sympathetic

nervous activity.

In BAT of lean rats, sucrose feeding resulted in an increase in the chlorisondamine-sensitive retention of [^{3}H]- noradrenaline specific activity supporting previous reports in cafeteria-fed rats (Young et al., 1982) and estimated noradrenaline turnover was increased, confirming that the increases in BAT noradrenaline turnover observed on sucrose feeding lean rats (Section 3.1, 3.3) are consistent with increased sympathetic stimulation of BAT. In contrast to lean rats there was no significant retention of [^{3}H]- noradrenaline in BAT of intact obese rats after chlorisondamine treatment compared with saline treated rats, either in chow-fed or sucrose-fed rats. This supports the findings of a negligible noradrenaline turnover in BAT of intact obese rats on either feeding regime in Sections 3.1 and 3.3 and suggests that the reduced BAT noradrenaline turnover in BAT of these obese animals results from a diminished central sympathetic outflow.

Adrenalectomy increased the chlorisondamine-sensitive retention of [^{3}H]- noradrenaline specific activity in BAT of obese rats to the values found in lean rats, supporting the findings of increased BAT noradrenaline turnover in obese adrenalectomised rats and suggesting that this results from a restoration of sympathetic stimulation of BAT. Although the % retention of [^{3}H]- noradrenaline specific activity after chlorisondamine treatment was similar in BAT of lean and obese adrenalectomised rats the estimated noradrenaline turnover sensitive to chlorisondamine was not as great in BAT of adrenalectomised obese rats as in adrenalectomised lean rats due to a lower tissue content of noradrenaline. This indicates that the central sympathetic outflow to BAT may still be reduced in adrenalectomised obese rats, compared with adrenalectomised lean rats and thus suggests that restoration of a minimum sympathetic tone may be more important to the restoration of thermogenic function in adrenalectomised obese rats than the absolute level of sympathetic activity of the tissue. This observation is supported by the results obtained on sucrose feeding adrenalectomised lean and obese rats.

In Section 3.3, sucrose feeding of adrenalectomised lean and obese rats was not associated with any increases in BAT noradrenaline turnover. In the present study there were similarly no increases in the chlorisondamine-sensitive [^{3}H]- noradrenaline retention or estimated noradrenaline turnover, indicating that the increased BAT thermogenesis observed in these animals (Holt et al., 1983) was not associated with an increased sympathetic stimulation of BAT. This suggests that the restoration of BAT thermogenic function in the adrenalectomised obese rat is associated with an increased sympathetic stimulation of BAT such that the tissue is maintained in a functional state, but that the activation of thermogenesis in response to overfeeding in both adrenalectomised lean and obese rats is initiated by other, as yet unknown, factors. A possible candidate for this role is ACTH as has been discussed previously in Section 3.3.

Cardiac [^{3}H]- noradrenaline retention in chlorisondamine treated rats and estimated noradrenaline turnover rates were reduced in obese rats compared with lean rats, suggesting that there may be a small reduction in sympathetic stimulation of heart in obese rats as reported in older animals (Levin et al., 1983a). Although cardiac [^{3}H]- noradrenaline retention in chlorisondamine-treated rats was increased in intact obese rats overfed with sucrose, ~~sucrose-fed lean rats and chow-fed obese rats~~, the increases in estimated noradrenaline turnover were not as great as might have been expected from the results of the noradrenaline turnover studies (Section 3.1, 3.3).

The noradrenaline turnover rates estimated from the data in these experiments were lower than the turnover rates observed in the noradrenaline turnover studies (Sections 3.1, 3.2, 3.3). This may be due to incomplete ganglionic blockade by chlorisondamine or may reflect spontaneous release or intraneural metabolism of noradrenaline independent of sympathetic nerve activity. While measurement of noradrenaline turnover does not provide an absolute measure of

sympathetic activity these experiments have demonstrated that it does provide a valid comparative assessment of sympathetic nerve function. The experiments described in following sections further investigate sympathetic nerve function in tissues of lean and obese (fa/fa) rats.

SECTION 3.6 Noradrenaline uptake mechanisms in BAT of the obese (fa/fa) rat.

The reduced BAT noradrenaline content, increased half-life and decreased turnover rate of noradrenaline in BAT of intact obese rats was also associated with a decreased initial uptake of [^{3}H]- noradrenaline after i.v. injection of [^{3}H]- noradrenaline (Section 3.1). The initial uptake of [^{3}H]- noradrenaline after i.v. injection is dependent upon the extent of the sympathetic innervation and the activity of the sympathetic nerves since uptake is enhanced on nerve stimulation (Bhagat and Zeidman, 1970). Initial uptake is also dependent on tissue blood flow since this affects the delivery of [^{3}H]- noradrenaline to the tissue, however, resting blood flow is not significantly reduced in BAT of obese rats (Wickler et al., 1982).

Specific uptake of noradrenaline into sympathetically innervated tissues consists of two different uptake mechanisms $Uptake_1$ and $Uptake_2$. $Uptake_1$ is a high affinity, ATP and Mg^{2+} requiring,stereo specific membrane carrier system located on the nerve terminal that represents uptake into the axon. The bulk of noradrenaline released on nerve stimulation is inactivated by this re-uptake process into the nerve terminal. $Uptake_2$ is a low affinity, non-stereo specific uptake mechanism representing extra-neuronal uptake and is closely associated with metabolism of noradrenaline by catchol-O-methyl transferase. $Uptake_2$ may also have a role in inactivating noradrenaline after neural release, particularly in tissues with a low density of sympathetic innervation (Iversen, 1973). Desmethylimipramine is a potent and specific inhibitor of $Uptake_1$ while corticosterone inhibits the $Uptake_2$ mechanism (Salt, 1972). By measuring the uptake of [^{3}H]- noradrenaline into tissue slices *in vitro* in the presence of these inhibitors the relative contributions of $Uptake_1$ and $Uptake_2$ to initial uptake *in vivo* can be estimated in order to determine whether the reduced initial uptake of [^{3}H]- noradrenaline *in vivo* can be accounted for by alterations in these uptake processes.

RESULTS.

3.6.1 *In vitro* uptake of noradrenaline in BAT and brain slices from lean and obese (fa/fa) rats.

The effects of adrenalectomy, cold acclimatisation and sucrose feeding on [^{3}H]- noradrenaline uptake into tissue slices in *in vitro* incubations, in the presence and absence of desmethylimipramine and corticosterone are shown in Figs. 3.7 and 3.8 for BAT and brain respectively. $Uptake_1$ was taken to be the component of total uptake blocked by desmethylimipramine and $Uptake_2$ as the component of total uptake blocked by corticosterone. These calculated uptakes are presented in Tables 3.16 and 3.17 for BAT and brain respectively.

In BAT of obese rats total uptake, $Uptake_1$ and $Uptake_2$ were all reduced compared with BAT from lean rats, when results were expressed per mg tissue. Since BAT of obese rats contains excess lipid deposits results were also exprssed on a whole tissue basis. Total uptake and $Uptake_2$ per organ were reduced, although the difference was not statistically significant, and $Uptake_1$, calculated on a whole tissue basis was still reduced by 7-fold in BAT of obese rats compared with the values in lean rats. In BAT from obese rats $Uptake_1$ was reduced to lower values then $Uptake_2$ and there was a much greater non-specifc component to total uptake than was found in BAT from lean rats (62% compared to 10%). Thus the major decreases in noradrenaline uptake into BAT from obese rats were in $Uptake_1$. Adrenalectomy did not significantly affect the *in vitro* uptake of [^{3}H]-noradrenaline into BAT slices from lean rats. In contrast, total uptake and $Uptake_2$ were increased in *in vitro* incubations of BAT from adrenalectomised obese rats, up to the values found in lean adrenalectomised rats.

Cold acclimatisation of lean rats resulted in increased total uptake on a whole tissue basis and increased $Uptake_1$ per mg tissue and per tissue. Cold acclimatisation of obese rats resulted in large increases in total uptake, $Uptake_1$ and $Uptake_2$ per mg tissue and per tissue. The increases in $Uptake_1$ per mg tissue and per tissue, and the increase in $Uptake_2$ per

Fig. 3.7 [^{3}H]-noradrenaline uptake in brown adipose tissue from lean and obese (fa/fa) rats in vitro.

[^{3}H]- noradrenaline uptake into BAT slices in vitro was measured as described in section 2.4. Adrenalectomised rats were maintained on 0.9% saline instead of drinking water for 7 days prior to the study. Cold acclimatised rats were housed at 4^{o}C for 7 days and sucrose fed rats were offered a 35% (w/v) sucrose solution in addition to drinking water for 7 days. Values represent means $\pm$ S.E.M. for 3 rats in each group. T = total uptake, D = uptake in the presence of 5.10^{-6}M desmethylimipramine, C = uptake in the presence of $4x10^{-5}$M corticosterone (see section 2.4.)

Noradrenaline uptake pmoles/mg tissue/20 minutes.
1.0
0.8
0.6
0.4
0.2
0
T D C
LEAN
T D C
OBESE
T D C
LEAN
T D C
OBESE
T D C
LEAN
T D C
OBESE
T D C
LEAN
T D C
OBESE
INTACT
ADRENALECTOMISED
COLD
ACCLIMATISED
SUCROSE-FED

Table 3.16 *In vitro* noradrenaline uptake in BAT slices from 5-6 week old lean and obese (fa/fa) rats.

		LEAN		OBESE	
TREATMENT		pmoles/mg tissue	pmoles/organ	pmoles/mg/tissue	pmoles/organ
INTACT	Total uptake	0.59±0.03	113.0±4.9	0.23±0.03 b***	76.4±10.0
	$Uptake_1$	0.38±0.004	72.7±6.5	0.03±0.01 b**	10.0±3.3 b***
	$Uptake_2$	0.15±0.01	28.7±1.6	0.05±0.01 b**	16.5±3.3
ADRENALECTOMISED	Total uptake	0.63±0.03	95.0±9.1	0.52±0.05 a*	159.8±26.2 a*
	$Uptake_1$	0.40±0.04	60.0±12.0	0.30±0.04 a*	93.0±-20.1 a*
	$Uptake_2$	0.24±0.06	36.0±18.2	0.12±0.05	37.2±26.2
COLD ACCLIMATISED	Total uptake	0.85±0.06	300±13.6 a**	0.59±0.03 a***	410.0±47.4 a***
	$Uptake_1$	0.55±0.03 a**	194.0±6.8a***	0.36±0.01 a*** b**	250.0±15.8 a*** b***
	$Uptake_2$	0.18±0.04	63.5±9.1	0.24±0.01 a***	166.7±16.1 a*** b**
SUCROSE-FED	Total uptake	0.79±0.04	217.4±6.4a***	0.21±0.02 b***	149.4±10.6 a* b**
	$Uptake_1$	0.61±0.02 a***	167.5±3.2a***	0.06±0.03 b***	42.6±15.9 b**
	$Uptake_2$	0.20±0.02	54.9±3.0 a**	0.06±0.01 b**	42.6±5.3

Tissue slices were incubated at 37°C for 20 minutes with 10^{-7}M [^{3}H]-noradrenaline as described in section 2.4 All incubations were performed in triplicate. Values represent means ± S.E.M. for 3 rats in each group. ***, $p < 0.001$; **, $p < 0.005$; *, $p < 0.01$. a, compared with intact group; b, compared with equivalent lean group.

Fig. 3.8 [^{3}H]-noradrenaline uptake in brain from lean and obese (fa/fa) rats *in vitro*.

[^{3}H]-noradrenaline uptake into brain slices *in vitro* was measured as described in section 2.4. Adrenalectomised rats were maintained on 0.9% saline instead of drinking water for 7 days prior to the st dy. Cold acclimatised rats were housed at 4^{o}C for 7 days and sucrose fed rats were offered at 35% (w/v) sucrose solution in addition to drinking water for 7 days. Values represent means $\pm$ S.E.M. for 3 rats in each group. T = total uptake, D = uptake in the presence of 5×10^{-6} M desmethylimipramine, C = uptake in the presence of 4×10^{-5} M corticosterone, (see section 2.4).

Noradrenaline uptake pmoles/mg tissue/20 minutes.
0.8
0.6
0.4
0.2
0
T D C
LEAN
T D C
OBESE
T D C
LEAN
T D C
OBESE
T D C
LEAN
T D C
OBESE
T D C
LEAN
T D C
OBESE
INTACT
ADRENALECTOMISED
COLD
ACCLIMATISED
SUCROSE-FED

Table 3.17 *In vitro* noradrenaline uptake in brain slices for 5-6 week old lean and obese (fa/fa) rats.

		LEAN		OBESE	
TREATMENT		pmoles/mg tissue	pmoles/organ	pmoles/ gmtissue	pmoles/organ
INTACT	Total uptake	0.62±0.04	830±67	0.62±0.04	789±81
	$Uptake_1$	0.26±0.06	348±100	0.23±0.02	293±40
	$Uptake_2$	0.17±0.05	227±83	0.19±0.04	242±81
ADRENALECTOMISED	Total uptake	0.64±0.04	855±14	0.64±0.07	748±30
	$Uptake_1$	0.26±0.03	347±10	0.28±0.07	327±30
	$Uptake_2$	0.25±0.04	334±14	0.22±0.04	257±17
COLD ACCLIMATISED	Total uptake	0.62±0.03	852±25	0.63±0.05	789±79
	$Uptake_1$	0.25±0.05	344±42	0.26±0.07	326±111
	$Uptake_2$	0.21±0.01	228±8	0.22±0.06	275±95
SUCROSE-FED	Total uptake	0.78±0.02	1064±26	0.76±0.04	935±39
	$Uptake_1$	0.31±0.02	422±25	0.31±0.04	381±49
	$Uptake_2$	0.22±0.02	300±24	0.21±0.05	258±38

Tissue slices were incubated at 37°C for 20 minutes with 10^{-7}M [^{3}H]-noradrenaline as described in section 2.4. All incubations were performed in triplicate. Values represent means ± S.E.M. for 3 rats in each group. There were no significant differences between treatment groups or genotype.

tissue in BAT from cold acclimatised obese rats were greater than in lean cold acclimatised rats.

Sucrose feeding resulted in increased *in vitro* total uptake of [^{3}H]- noradrenaline, $Uptake_1$ and $Uptake_2$ in BAT from lean rats when results were expressed on a whole tissue basis and increased $Uptake_1$ per mg tissue. BAT of obese rats did not respond to sucrose feeding. Neither $Uptake_1$ nor $Uptake_2$ were significantly changed in BAT of sucrose-fed obese rats, although there was an increase in total uptake per tissue.

In contrast to the findings in BAT *in vitro* uptake of [^{3}H]- noradrenaline into brain slices was unaffected by genotype, adrenalectomy, cold acclimatisation or sucrose feeding. Brain total uptake, $Uptake_1$ and $Uptake_2$ per mg tissue were similar to the values found in BAT of lean rats.

3.6.2 DISCUSSION.

The reduced noradrenaline content and the reduction in initial uptake of [^{3}H]- noradrenaline into BAT in *vivo* in obese rats indicated that, in addition to a reduced sympathetic activity in BAT, there may be a less extensive innervation of the tissue in these animals. Measurement of [^{3}H]- noradrenaline uptake *in vitro*, which is independent of the effects of tissue blood flow and nerve stimulation, has confirmed that noradrenaline uptake is reduced in BAT from intact obese rats. This suggests that the reduced noradrenaline turnover found in BAT of obese rats could result from a reduction in the innervation of the tissue as a consequence of the reduced central sympathetic outflow to BAT. The reduced noradrenaline content and reduced uptake of [^{3}H]- noradrenaline in BAT of intact obese rats could reflect an actual reduction in the number of nerve terminals in the tissue or an impairment in the uptake mechanism or storage of noradrenaline, resulting from the reduced activity of the sympathetic nerves. This could be resolved by histochemical fluorescence studies in which the nerve terminals in the tissue can be identified by the characteristic fluorescence of the noradrenaline stored within them.

Electrical stimulation of the ventromedial hypothalamus causes a sympathetically mediated rise in BAT temperature blockable by propranolol (Perkins et al., 1981). It has recently been demonstrated that this response is not impaired in the obese rat, demonstrating the integrity of the nerve supply to BAT (Holt et al., 1985). This does not necessarily imply, however, that the sympathetic innervation of BAT is as extensive as that of BAT in lean rats. If rats are partially sympathectomised with 6-hydroxydopamine and allowed to recover, full recovery of BAT function in response to cold exposure occurs when the BAT noradrenaline content is still only 17% of normal (Depocas et al., 1984), thus a normal response can be elicited with an incomplete sympathetic innervation. This could result from a normal secretion of noradrenaline, in spite of the reduced noradrenaline content or from denervation supersensitivity. Either process could also be functioning in BAT of obese rats on nerve stimulation.

BAT from intact obese rats has a number of similarities to denervated tissue. Denervation is associated with reduced $Uptake_1$ but not $Uptake_2$ (Johnson et al.,1969). Denervation of BAT results in increased wet weight and lipid content and decreased protein and DNA content (Dulloo et al., 1984; Mory et al., 1982). In this respect BAT of obese rats would appear to be functionally denervated, which is consistent with the very low rates of noradrenaline turnover observed. However, denervation destroys the sympathetic nerve endings in BAT resulting in a 95% reduction in noradrenaline content and tyrosine hydroxylase activity (Stricker et al., 1984). Noradrenaline content of BAT from obese rats is only partially reduced and the BAT responds to nerve stimulation and cold exposure so BAT of obese rats must retain at least a partial sympathetic innervation.

Adrenalectomy and cold acclimatisation of obese rats are associated with increased BAT thermogenesis which has been linked with increased noradrenaline turnover in BAT of obese rats. The increased <u>in vitro</u> noradrenaline uptake in BAT from these animals suggests that the restoration of sympathetic activation on cold acclimatisation and adrenalectomy are

associated with a restoration of tissue sympathetic innervation and noradrenaline uptake mechanisms. However, noradrenaline content remained reduced in BAT of both cold acclimatised and adrenalectomised obese rats compared with equivalently treated lean rats. Thus it is possible that the sympathetic innervation of BAT of these obese rats, although functionally active, is still less extensive than in lean rats.

Obese rats do not respond to overfeeding with sucrose with changes in BAT thermogenesis. The absence of any increase in *in vitro* noradrenaline uptake into BAT of sucrose-fed rats is consistent with the previous data (Section 3.1, 3.3 and 3.5) which had shown that noradrenaline content, half-life, turnover rate, initial uptake of [^{3}H]- noradrenaline and chlorisondamine-sensitive noradrenaline turnover in BAT of obese rats were all unaltered by sucrose feeding. This present data further reinforces the suggestion that the defective thermogenic response to overfeeding in the obese rat is linked to an inability to activate BAT thermogenesis by sympathetic stimulation of the tissue.

SECTION 3.7 Biosynthesis and degradation of noradrenaline in BAT of the obese (fa/fa) rat.

The severe reduction in the rate of noradrenaline turnover in BAT of obese rats reflects the decreased release of noradrenaline from sympathetic nerve terminals and the consequent reduction in synthesis and degradation of noradrenaline. The pathways of noradrenaline biosynthesis and degradation have been discussed in Section 1.4.3. The rate determining step for noradrenaline biosynthesis is tyrosine hydroxylase (E.C. 1.14.3a). Tyrosine hydroxylase activity and that of the other enzymes in the biosynthetic pathway are regulated by the level of sympathetic activity. Thus, tyrosine hydroxylase activity is increased in BAT of rats exhibiting NST (Zenber et al., 1976; Kennedy et al., 1977) and DIT (Levin et al., 1983b).

Tyrosine hydroxylase activity is regulated by two processes, protein synthesis and end-product inhibition. Noradrenaline inhibits the enzyme by competing with the co-factor, tetrahydropteridine, for binding to the enzyme (Nagatsu et al., 1964). The concentration of noradrenaline in the cytoplasm of the neurone is decreased on sympathetic stimulation, reducing end-product inhibition of tyrosine hydroxylase. If sympathetic activity is reduced noradrenaline accumulates in the axoplasm, inhibiting the enzyme (Alousi and Weinder, 19866). Sympathetic stimulation also leads to increases in tyrosine hydroxylase activity as a result of synthesis of new protein in the cell ganglion. The stimulus for this protein synthesis is acetyl choline released by pre-ganglionic nerves acting on the nicotinic receptors of the ganglion, resulting in increased levels of cAMP in the cytoplasm (Theonen et al., 1973).

Since tyrosine hydroxylase activity is sensitive to changes in sympathetic activity, tyrosine hydroxylase activity was measured in BAT and other organs of lean and obese rats to determine whether the reduced turnover and synthesis of noradrenaline in BAT of intact obese rats was associated with decreased tyrosine hydroxylase.

The route of enzymic degradation of noradrenaline depends upon the site of release and uptake of noradrenaline. The majority of noradrenaline released on nerve stimulation is taken back up into the nerve ending via $Uptake_1$. Any noradrenaline not taken up by the vesicular uptake system, or any noradrenaline spontaneously released into the axoplasm, is metabolised by monoamine oxidase (E.C. 1.4.3.4) (MAO) to form dihydroxyphenylethanal which is converted to dihydroxyphenylethyleneglycol (DOPEG) or dihydroxymandelic acid (DOMA) (see Section 1.4.3). The major route of enzymic degradation of noradrenaline released by nerve stimulation is O-methylation by catechol-O-methyl transferase (E.C. 2.1.1.6) (COMT) to normetanephine. COMT is closely associated with $Uptake_2$ and extraneuronal uptake results in O-methylation and subsequent deamination by extraneuronal MAO to methoxyhydroxyphenylethyleneglycol (MOPEG) or vanillyl hydroxymandelic acid (VMA). Since COMT is located intracellularly its activity is controlled predominantly by the activity

of $Uptake_2$. $Uptake_2$ may have a role in inactivating noradrenaline release on nerve stimulation in tissues with a reduced sympathetic innervation (Iversen, 1973). $Uptake_1$ was reduced 7-fold in BAT of obese rats while $Uptake_2$, expressed on a whole tissue basis, was unaffected compared with lean rats. Thus $Uptake_2$ and O-methylation could be of importance in inactivating noradrenaline in BAT of obese rats. It has been suggested that COMT has a role in regulating the thermogenic response to noradrenaline (Chinet and Durand, 1979). However, COMT activity is regulated by sympathetic activity in a tissue and is reduced under conditions of reduced nerve stimulation such as fasting (Ismahan and Parvey, 1978). Consequently it was of interest to determine whether the reduced sympathetic stimulation of BAT in obese rats was associated with changes in COMT activity.

RESULTS.

3.7.1 *In vivo* synthesis of [^{3}H]- noradrenaline from [^{3}H]- tyrosine in lean and obese (fa/fa) rats.

Table 3.18 shows the effects of an i.v. injection of [^{3}H]- tyrosine on the *in vivo* labelling of the tissue stores of tyrosine. The tyrosine content of BAT was reduced in obese rats but the tyrosine content of heart, brain, adrenals and plasma were similar in lean and obese rats. 2 hours after the injection of [^{3}H]- tyrosine the specific activity of [^{3}H]- tyrosine was similar in BAT, heart, adrenals and plasma of lean and obese rats, indicating a uniform labelling of peripheral organs in obese rats compared with lean rats. The specific activity of [^{3}H]- tyrosine was lower in the brain than in the periphery and higher in plasma than in peripheral organs.

Table 3.19 shows the noradrenaline content, [^{3}H]- noradrenaline content and [^{3}H]- noradrenaline specific activity of tissues from lean and obese rats 2 hours after an i.v. injection of [^{3}H]- tyrosine. The noradrenaline assay used was not sufficiently sensitive to determine plasma noradrenaline content so the specific activity of noradrenaline in plasma

Table 3.18 *In vivo* labelling of tissue tyrosine with [^{3}H]-tyrosine in 5-6 week old lean and obese (fa/fa) rats.

	LEAN			OBESE		
TISSUE	tyrosine content (μg/organ)	[^{3}H]-tyrosine content (ng/organ)	[^{3}H]-tyrosine s.a. (DPM/μg)	tyrosine content (μg/organ)	[^{3}H]-tyrosine content (ng/organ)	[^{3}H]-tyrosine s.a. (DPM/μg)
BAT	173±13	12.8±1.7	52.5±3.5	110±6 b**	11.3±1.4	76.5±11.9
HEART	184±15	18.0±1.1	77.5±7.9	162±7	16.3±0.9	72.3±45
BRAIN	639±36	29.2±5.0	32.5±4.8	678±47	23.9±2.7	28.6±4.1
ADRENAL (PAIR)	125±5	10.8±1.6	62.9±11.4	130±5	9.5±0.6	53.0±5.5
PLASMA (ml^{-1})	428±16	65.1±1.3	113±5.6	417±12	63.0±3.3	109.8±3.2

5-6 week old lean and obese (fa/fa) rats were injected i.v. with 123μCi/100g body weight 1-2,6 [^{3}H]-tyrosine in 0.9% (w/v) saline. Rats were sacrificed after 2 hours, tissues excised and assayed for tyrosine as described in section 2.5. Values represent means ± S.E.M. for 4 rats in each group. **, $p < 0.005$. b, compared with equivalent lean group.

Table 3.19 In vivo [^{3}H]-noradrenaline synthesis from [^{3}H]-tyrosine in lean and obese (fa/fa) rats.

	LEAN			OBESE		
TISSUE	NA content (ng/organ)	[^{3}H]-NA content (ng/organ)	[^{3}H]-NA specific activity (DPM/μg)	NA content (ng/organ	[^{3}H]-NA content (ng/organ)	[^{3}H]-NA specific activity (DPM/μg)
BAT	250±10	1.33±0.04	10719±950	110±10 b***	0.20±0.03 b***	3103±398 b***
HEART	180±20	0.78±0.06	5961±857	160±10	0.73±0.01	6187±680
BRAIN	190±10	4.01±0.19	22797±1860	190±10	3.00±0.06 b**	15861±509
ADRENAL (PAIR)	920±30	1.90±0.30	2669±384	960±70	1.64±0.07	2249±249
PLASMA (ml^{-1})	-	0.06±.0.02	-	-	0.05±0.01	-

5-6 week old lean and obese (fa/fa) rats were injected (i.v.) with 123 μCi/100g body weight 1-2,6 [^{3}H]-tyrosine in 0.9% (w/v) saline. Rats were sacrificed after 2 hours, tissues excised and assayed for noradrenaline as described in section 2.5. Values represent means ± S.E.M. for 4 rats in each group. ***, $p < 0.001$; **, $p < 0.005$. b, compared with equivalent lean group. NA = noradrenaline.

could not be used. In this study adrenaline was not distinguished from noradrenaline so the values for adrenals represent the summation of noradrenaline and adrenaline content and synthesis. Catecholamine tissue content was greatest in the adrenal glands. There were no differences in tissue content of noradrenaline between lean and obese rats except in BAT where noradrenaline content was considerably lower in obese rats. Noradrenaline synthesis was reduced in BAT of the obese rat, both the absolute amount of [^{3}H]- noradrenaline synthesised and the specific activity of [^{3}H]-noradrenaline were reduced in BAT from obese rats in comparison with lean rats by 7.5-fold and 3.5-fold respectively. Noradrenaline synthesis was normal in hearts and adrenals of obese rats compared with lean but was slightly reduced in brain of obese rats, although the decrease in [^{3}H]- noradrenaline specific activity was not statistically significant.

3.7.2 Tyrosine hydroxylase activity *in vitro*

The effects of adrenalectomy, cold acclimatisation and sucrose feeding on *in vitro* tyrosine hydroxylase activity in tissues from lean and obese (fa/fa) rats are shown in Tables 3.20, 3.21 and 3.22 for BAT, heart and adrenals respectively. The reduced *in vivo* noradrenaline in BAT reported in the previous study was not associated with reduced *in vitro* tyrosine hydroxylase activity. Although BAT tyrosine hydroxylase activity per mg tissue was reduced compared with lean rats, this difference was not statistically significant. When tyrosine hydroxylase activity was expressed per organ or per mg tissue protein there were no differences in activity in BAT between lean and obese rats. There were no differences in tyrosine hydroxylase activity in heart (Table 3.21) and adrenals (Table 3.22) of lean and obese rats.

Adrenalectomy did not affect BAT tyrosine hydroxylase activity in lean rats but increased activity, per mg tissue, in obese rats to the levels observed in lean rats. Adrenalectomy did, however, increase tyrosine hydroxylase activity

Table 3.20 *In vitro* tyrosine hydroxylase activity in BAT of 5-6 week old lean and obese (fa/fa) rats.

	LEAN			OBESE		
TREATMENT	pmoles/hr/mg tissue	pmoles/hr/ organ	pmoles/hr/mg protein	pmoles/hr/mg tissue	pmoles/hr/ organ	pmoles/hr/mg protein
INTACT	$20.2^{+}_{-}2.9$	$3536^{+}_{-}660$	$560^{+}_{-}89$	$12.8^{+}_{-}1.3$	$3783^{+}_{-}525$	$569^{+}_{-}45$
ADRENALECTOMISED	$30.8^{+}_{-}3.8$	$3949^{+}_{-}245$	$573^{+}_{-}46$	$29.1^{+}_{-}1.9$ a***	$5676^{+}_{-}721$	$531^{+}_{-}36$
COLD ACCLIMATISED	$36.7^{+}_{-}0.9$ a***	$16340^{+}_{-}533$ a***	$693^{+}_{-}32$	$26.0^{+}_{-}2.5$ a**	$26301^{+}_{-}2367$ a***	$580^{+}_{-}81$
SUCROSE-FED	$36.2^{+}_{-}2.5$ a*	$12419^{+}_{-}848$ a***	$765^{+}_{-}50$	$10.73^{+}_{-}0.47$ b***	$7019^{+}_{-}374$ b** a**	$607^{+}_{-}98$

Adrenalectomised rats were maintained on 0.9% (w/v) saline, instead of drinking water for 7 days. Cold acclimatised rats were housed at $4^{o}C$ for 7 days. Sucrose-fed rats were offered a 35% (w/v) sucrose solution in addition to drinking water for 7 days. All animals were fed chow *ad lib*. Tyrosine hydroxylase activity was measured as described in section 2.7. Values expressed as pmoles dopa formed per hour at $37^{o}C$, pH6.0 and represent $^{+}_{-}$ S.E.M. for 4 rats in each group. ***, $p < 0.001$; **, $p < 0.0005$; *, $p < 0.01$. a, compared with control group; b, compared with equivalent lean group.

Table 3.21 *In vitro* tyrosine hydroxylase activity in heart of 5-6 week old lean and obese (fa/fa) rats.

	LEAN			OBESE		
TREATMENT	pmoles/hr/mg tissue	pmoles/hr/ organ	pmoles/hr/mg protein	pmoles/hr/mg tissue	pmoles/hr/ organ	pmoles/hr/mg protein
INTACT	12.4±1.7	4964±684	286±58	13.5±1.8	5384±720	316±38
ADRENAL-ECTOMISED	28.1±2.5 a**	12746±1371 a***	701±80 a***	26.6±1.2 a***	11230±603 a***	673±34 a***
COLD ACCLIMA-TISED	15.6±0.4	9398±456 a**	314±17.2	17.6±1.3	10429±1034 a*	344±21
SUCROSE-FED	13.28±1.1	14542±1214 a***	319±20	24.0±1.3 a** b**	19867±1245 a***	646±42 a** b***

Adrenalectomised rats were maintained on 0.9% (w/v) saline instead of drinking water for 7 days. Cold acclimatised rats were housed at 4°C for 7 days. Sucrose-fed rats were offered a 35% (w/v) sucrose solution in addition to drinking water for 7 days. All rats were fed chow *ad lib*. Tyrosine hydroxylase activity was assayed as described in section 2.7. Values expressed as pmoles dopa formed per hour at 37°C, pH6.0 and represent means ± S.E.M. for 4 rats in each group. ***, $p < 0.001$; **, $p < 0.005$; *, $p < 0.01$. a, compared to control group, b, compared to equivalent lean group.

Table 3.22 In vitro tyrosine hydroxylase activity in adrenals of 5-6 week old lean and obese (fa/fa) rats.

	LEAN			OBESE		
TREATMENT	pmoles/mg tissue	pmoles/hr/ organ	pmoles/hr/mg protein	pmoles/hr/mg tissue	pmoles/hr/ organ	pmoles/hr/mg protein
INTACT	129±14	2296±319	4806±441	152±18	2735±460	5724±882
ADRENAL-ECTOMISED	-	-	-	-	-	-
COLD ACCLIMAT-ISED	222±20 a*	7030±1079 a***	11344±641 a***	214±13 a*	6955±597 a***	12803±1413 a***
SUCROSE-FED	213±13.7	3185±290	5720±332	223±21	3138±410	6459±832

Adrenalectomised rats were maintained on 0.9% (w/v) saline instead of drinking water for 7 days. Cold acclimatised rats were housed at 4°C for 7 days. Sucrose-fed rats were offered a 35% (w/v) sucrose solution in addition to drinking water for 7 days. All rats were fed chow ad lib. Tyrosine hydroxylase activity was assayed as described in section 2.7. Values expressed as pmoles dopa formed per hour at 37°C, pH6.0 and represent means ± S.E.M. for 4 rats in each group. ***, $p < 0.001$; **, $p < 0.005$; *, $p < 0.01$. a, compared to control group.

in hearts of lean and obese rats by approximately 2-fold in both cases.

Cold acclimatisation increased BAT tyrosine hydroxylase activity expressed per mg tissue and per organ in both lean and obese rats. However, as activity was not increased per mg protein it would appear that the increases reflected the general hypertrophy of the tissue. Cardiac activity was increased when expressed per organ in both lean and obese rats whereas adrenal tyrosine hydroxylase activity was increased when the activity was expressed per mg tissue, per organ and per mg protein in both lean and obese rats.

Sucrose feeding of lean rats increased BAT tyrosine hydroxylase activity per mg tissue and per organ, but not per mg protein, suggesting that, as on cold acclimatisation, the increased activity resulted from the tissue hypertrophy. Sucrose feeding of obese rats had no effect on BAT tyrosine hydroxylase activity expressed per mg tissue or protein. However, when expressed per organ tyrosine hydroxylase activity was increased in BAT of obese rats, but not up to the levels found in lean sucrose-fed rats. Cardiac tyrosine hydroxylase activity was increased per heart in lean rats and in obese rats was increased when expressed per mg tissue or protein and per organ by sucrose feeding. The increased activity per mg tissue and protein were greater in hearts of obese rats than in lean rats. Adrenal tyrosine hydroxylase activity was unaffected by sucrose feeding.

3.7.3 Catechol-O-methyl transferase activity in lean and obese (fa/fa) rats.

Table 3.23 shows the activity of catechol-O-methyl transferase in BAT, heart and liver from lean and obese rats. There were no significant differences in COMT activity in any of the tissues between lean and obese rats.

3.7.4 DISCUSSION.

The *in vivo* synthesis of noradrenaline from tyrosine was very much reduced in BAT of obese rats compared with lean rats. The [^{3}H]- noradrenaline content of BAT reduced 7.5-fold and the specific activity of [^{3}H]- noradrenaline was reduced 3.5-fold.

Table 3.23 Catechol-O-methyl transferase activity in BAT, heart and liver from lean and obese (fa/fa) rats.

	LEAN			OBESE		
TISSUE	μmoles/g/hr	μmoles/organ/hr	μmoles/ug protein/hr	μmoles/g/hr	μmoles/organ/hr	μmoles/ug protein/hr
BAT	$1.5^{+}_{-}0.1$	$0.26^{+}_{-}0.01$	$45.0^{+}_{-}1.7$	$0.9^{+}_{-}0.2$	$0.28^{+}_{-}0.07$	$46.3^{+}_{-}1.5$
HEART	$1.4^{+}_{-}0.1$	$0.54^{+}_{-}0.04$	$46.1^{+}_{-}2.0$	$1.4^{+}_{-}0.2$	$0.54^{+}_{-}0.07$	$45.2^{+}_{-}5.6$
LIVER	$5.7^{+}_{-}0.3$	$22.3^{+}_{-}1.6$	$58.3^{+}_{-}2.9$	$5.8^{+}_{-}0.3$	$24.2^{+}_{-}1.1$	$59.7^{+}_{-}3.1$

COMT activity was assayed as described in section 2.8. Values expressed as metanephrine formed/hr at $37^{o}C$ and represent means $^{+}_{-}$ S.E.M. for 3 rats in each group.

Although the tyrosine content of BAT from obese rats was reduced the specific activity of [^{3}H]- tyrosine was similar in lean and obese rats. This indicates that the tissue stores of tyrosine were equivalently labelled in BAT of lean and obese rats. Assuming a similar subcellular distribution of [^{3}H]- tyrosine in BAT from lean and obese rats then any newly synthesised noradrenaline would have a similar specific activity in both lean and obese rats. Thus at a given synthesis and turnover rate the absolute amount of [^{3}H]- noradrenaline isolated from BAT would be the same in lean and obese rats. The amount of [^{3}H]- noradrenaline isolated from BAT of obese rats was reduced by 7.5-fold indicating a markedly reduced rate of synthesis. In addition the specific activity of [^{3}H]- noradrenaline was reduced, indicating that a much smaller proportion of the tissue stores were labelled in BAT of obese rats. Since the noradrenaline content of BAT from obese rats is lower than in lean, the specific activity of [^{3}H]- noradrenaline isolated from the tissue, at a given rate of noradrenaline synthesis, would be higher in obese rats than in lean. When allowance is made for the differences in BAT noradrenaline content, both the [^{3}H]- noradrenaline content and the specific activity of [^{3}H]- noradrenaline would have been reduced by 7.5-fold in BAT of obese rats. Thus the same proportion of newly synthesised noradrenaline in BAT was tritiated in both lean and obese rats indicating that [^{3}H]- tyrosine of a similar specific activity was available for noradrenaline synthesis in BAT of both lean and obese rats. Thus the reduced synthesis of [^{3}H]- noradrenaline in BAT of obese rats resulted from a reduced synthesis of noradrenaline rather than differences in availability of [^{3}H]- tyrosine as a substrate for synthesis. The reduced rates of noradrenaline synthesis in BAT of obese rats reported in this section are consistent with the very low rates of noradrenaline turnover found in BAT of these rats indicative of a lack of sympathetic stimulation of the tissue.

Noradrenaline synthesis was normal in heart and adrenals of obese rats. These results support the previous observations

of normal cardiac noradrenaline turnover and confirm that the defect in peripheral sympathetic function is specific to BAT in young (5-6 week old) obese rats. In the brain [^{3}H]- noradrenaline content and specific activity were reduced although the difference in specific activity was not statistically significant. The specific activity of [^{3}H]- tyrosine in the brain, although lower than in other tissues, was similar in lean and obese rats. Thus noradrenaline synthesis would appear to be reduced in brains of obese rats. These results support previous reports of reduced levels of noradrenaline in various brain regions of older obese (fa/fa) rats associated with reduced activity of tyrosine hydroxylase and dopamine β hydroxylase (Levin and Sullivan, 1979a, b). The lower specific activity of [^{3}H]- tyrosine observed in brain of lean and obese (fa/fa) rats compared with other tissues may have reflected a higher rate of [^{3}H]- noradrenaline synthesis in brain or may have resulted from a slower equilibration of [^{3}H]- tyrosine into the brain across the blood brain barrier than into other tissues.

Despite a reduced rate of noradrenaline synthesis *in vivo* tyrosine hydroxylase activity assayed *in vitro* was not reduced in BAT of obese rats. This observation supports previous findings in older animals in which the reduced BAT noradrenaline turnover was not associated with reduced tyrosine hydroxylase activity but was associated with reduced dopamine β hydroxylase activity. Tyrosine hydroxylase could be inhibited *in vivo*, however, by noradrenaline accumulating in the axoplasm of the sympathetic nerves in BAT of obese rats as a result of the reduced sympathetic stimulation of the tissue. Although tyrosine hydroxylase is the rate determining step for maximum noradrenaline synthesis dopamine β hydroxylase also has a role in the physiological regulation of noradrenaline synthesis (Molinoff and Orcutt, 1973). *In vivo* synthesis of [^{3}H]- noradrenaline from [^{3}H]- dopa is decreased in BAT of older obese rats (Levin et al., 1983) but the reduction in noradrenaline synthesis was not as great as the reduction in the synthesis from [^{3}H]- tyrosine reported here. It is thus possible that the decreased noradrenaline synthesis in BAT of the obese rat results from

both an in vivo end product inhibition of tyrosine hydroxylase and a reduced activity of dopamine β hydroxylase.

It has previously been suggested that the decrease in BAT noradrenaline content and the reduction in noradrenaline uptake into BAT might be a reflection of a reduced number of nerve terminals in BAT. The observation of a normal in vitro tyrosine hydroxylase activity argues against this hypothesis and suggests that these reductions in noradrenaline content and uptake might result from a reduced sympathetic activity in the nerves rather than a reduced number of normally functioning nerve terminals.

The increase in in vitro tyrosine hydroxylase activity observed in BAT of lean rats after cold exposure and after sucrose feeding, were consistent with previous reports (Zenber et al., 1976; Kennedy et al., 1977; Levin et al.,1983b) and both sucrose feeding and cold acclimatisation produced changes in tyrosine hydroxylase activity of a similar magnitude. Obese rats have been shown to respond normally to cold acclimatisation with a normal sympathetically-mediated increase in BAT thermogenesis. The increase in in vitro tyrosine hydroxylase activity in BAT of cold acclimatised obese rats is consistent with this response. In contrast to the effects of cold acclimatisation sucrose feeding of obese rats does not result in increases in BAT sympathetic activity or thermogenesis. In vitro tyrosine hydroxylase activity expressed per mg tissue or protein was unaffected by sucrose feeding but activity per whole tissue was increased, although the increase was nearly 4-fold lower than in cold acclimatised obese rats. These results indicate that there may be some increase in the potential capacity for noradrenaline synthesis in sucrose-fed rats that occurs independently from changes in sympathetic activity in BAT.

The changes in tyrosine hydroxylase activity in heart after adrenalectomy, cold exposure and sucrose feeding were largely consistent with the changes in noradrenaline turnover reported in previous sections (3.1, 3.2 and 3.3). Cardiac tyrosine hydroxylase activity was increased by adrenalectomy in both lean and obese rats consistent with the increased

cardiac noradrenaline turnover observed previously (Section 3.2). Cold acclimatisation resulted in increased cardiac tyrosine hydroxylase activity in both lean and obese rats, however, although fractional turnover of noradrenaline was increased in hearts of cold acclimatised rats these changes were not associated with increased noradrenaline turnover (Section 3.1). Cardiac tyrosine hydroxylase activity was increased in obese rats expressed per organ and per mg tissue and protein but was increased in lean rats only when expressed on a whole organ basis. This is consistent with the higher rates of noradrenaline turnover found in hearts of obese rats overfed with sucrose.

Adrenal tyrosine hydroxylase activity was increased by cold acclimatisation but not by sucrose feeding, confirming previous reports (Zenker et al., 1976; Levin et al., 1983b) in both lean and obese rats. The increases in plasma catecholamines on cold acclimatisation could potentially stimulate BAT thermogenesis, however, removal of the adrenals causes only slight impairment of NST (Himms-hagen, 1975) and the concentrations required for half-maximal stimulation of BAT thermogenesis are at least 30-fold greater than circulating levels.

The reduced noradrenaline turnover in BAT of obese rats was not associated with decreased activity of the degradative enzyme COMT, which exhibited a similar activity in BAT of both lean and obese rats. Since the extraneuronal noradrenaline uptake mechanism $Uptake_2$ was not reduced in BAT of obese rats, but the neuronal uptake mechanism $Uptake_1$ was severely reduced, a major route for inactivation of noradrenaline released on nerve stimulation would be expected to be extraneuronal uptake and metabolism by COMT rather than reuptake and storage in the nerve ending. Indeed, the reduced $Uptake_1$ activity in BAT of obese rats would increase noradrenaline turnover resulting from nerve stimulation since the noradrenaline released would be lost as O-methylated metabolites, rather than taken back up into the nerve ending. The observation of severely

depressed rates of noradrenaline turnover in the presence of reduced $Uptake_1$ suggest that there is minimal sympathetic activity in BAT of obese rats housed at 24-26°C. A normal activity of COMT has also been reported in heart and BAT of the obese (ob/ob) mouse (Fieldman et al., 1978) although in these animals COMT activity was reduced in liver of (ob/ob) mice in contrast to the findings here, in which liver COMT activity was similar in lean and obese rats.

SECTION 3.8 Parasympathetic nervous system activity in the obese (fa/fa) rat.

The results presented in previous sections clearly demonstrate a reduced sympathetic stimulation of BAT in the obese (fa/fa) rat. It has been suggested that this resulted from an inability to couple dietary stimuli to activation of the sympathetic innervation of BAT, perhaps at the level of the ventromedial hypothalamus (VMH), since the obese rat responds normally to electrical stimulation of the VMH (Holt et al., 1985).

Reduced activity in the VMH is associated with increased parasympathetic activity. Lesions of the VMH lead to hyperinsulinaemia as a result of increased vagal stimulation of the β cells of the pancreas. Vagotomy reverses the hyperinsulinaemia and obesity of VMH-lesioned rats (Powley and Opsahl, 1984; Inoue and Bray, 1978; Fox and Parley, 1984). In addition, since BAT noradrenaline is not decreased in adult VMH-lesioned rats it has been proposed that increased parasympathetic activity is responsible for the obesity and reduced thermogenesis of these animals (Yoshida and Bray, 1984).

Increased parasympathetic activity has been implicated in the obesity and reduced BAT thermogenesis of the obese (fa/fa) Zucker rat. Obese (fa/fa) rats are not hyperglycaemic (Bray and York, 1979; Rohner-Jeanrenaud et al., 1983) so the hyperinsulinaemia of the obese rat could result from increased vagal stimulation of the pancreas, since vagotomy or atropine treatment reverses the hyperinsulinaemia of obese rats (Rohner-Jeanrenaud et al., 1983). In contrast to VMH-lesioned rats, vagotomy does not reverse the obesity of fa/fa rats (Powley and Opsahl, 1974). Blockade of the parasympathetic nervous system with the muscarinic antogonist atropine restores the defective thermic response to feeding in obese rats (Rothwell et al., 1981a).

Atropine does not restore the thermogenic effects of over-feeding a carbohydrate diet to obese (fa/fa) rats but does increase thermogenesis in response to high-fat diets (Rothwell and Stock, 1983a). It has been proposed that feeding a high-fat diet may stimulate BAT thermogenesis directly in fa/fa rats if the inhibitory effects of the parasympathetic nervous system are removed.

The sensitivity of the β cells of the pancreas to glucose is increased on vagal stimulation (Bloom and Edwards, 1980). The exaggerated insulin response to a glucose load in obese rats (York et al., 1981; Rohner-Jeanrenaud et al., 1983) may be indicative of an increased parasympathetic activity, since vagotomy or atropine treatment block the increased insulin response in obese and pre-obese rats (York et al., 1981; Rohner-Jeanrenaud, 1983).

The exaggerated insulin response of the obese (fa/fa) rat to a glucose load appears to be indicative of an increased parasympathetic activity. Glucose and insulin levels were measured in lean and obese intact and adrenalectomised rats in response to a glucose load to determine whether adrenalectomy, which was associated with increased sympathetic activation of BAT, might also be associated with a reduced parasympathetic activity.

RESULTS.

3.8.1 The insulin response to glucose in lean and obese (fa/fa) rats.

Fig. 3.9 and Table 3.24 show the effects of a glucose load on plasma insulin and glucose in lean and obese (fa/fa) intact and adrenalectomised rats. Obese rats were slightly hyperglycaemic compared to lean rats (8.48 ± 0.17 mM and 7.54 ± 0.16 mM plasma glucose respectively) & markedly hyperinsulinaemic, basal plasma insulin was increased nearly 4-fold in obese rats. Intraperitoneal administration of glucose produced a similar level of hyperglycaemia in lean and obese rats that returned to normal levels by 30 minutes. The time course of the insulin response was similar in lean and obese rats, plasma insulin

Fig. 3.9 The effects of a glucose load on plasma insulin and glucose in lean and obese (fa/fa) rats.

All rats were fasted for 3.5 hours prior to the study. Intact (●——●) and adrenalectomised (0——0) rats were injected i.p. with 150mg/100g body weight glucose in 0.3ml 0.9% (w/v) saline. Time zero groups received an equal volume of vehicle. Serum glucose and insulin were assayed as described in sections 2.9 and 2.10. Values represent means $\pm$ S.E.M. for 4 rats in each group. ***, $p < 0.001$; **, $p < 0.005$; *, $p < 0.01$. a, compared with control group; b, compared with equivalent lean group.

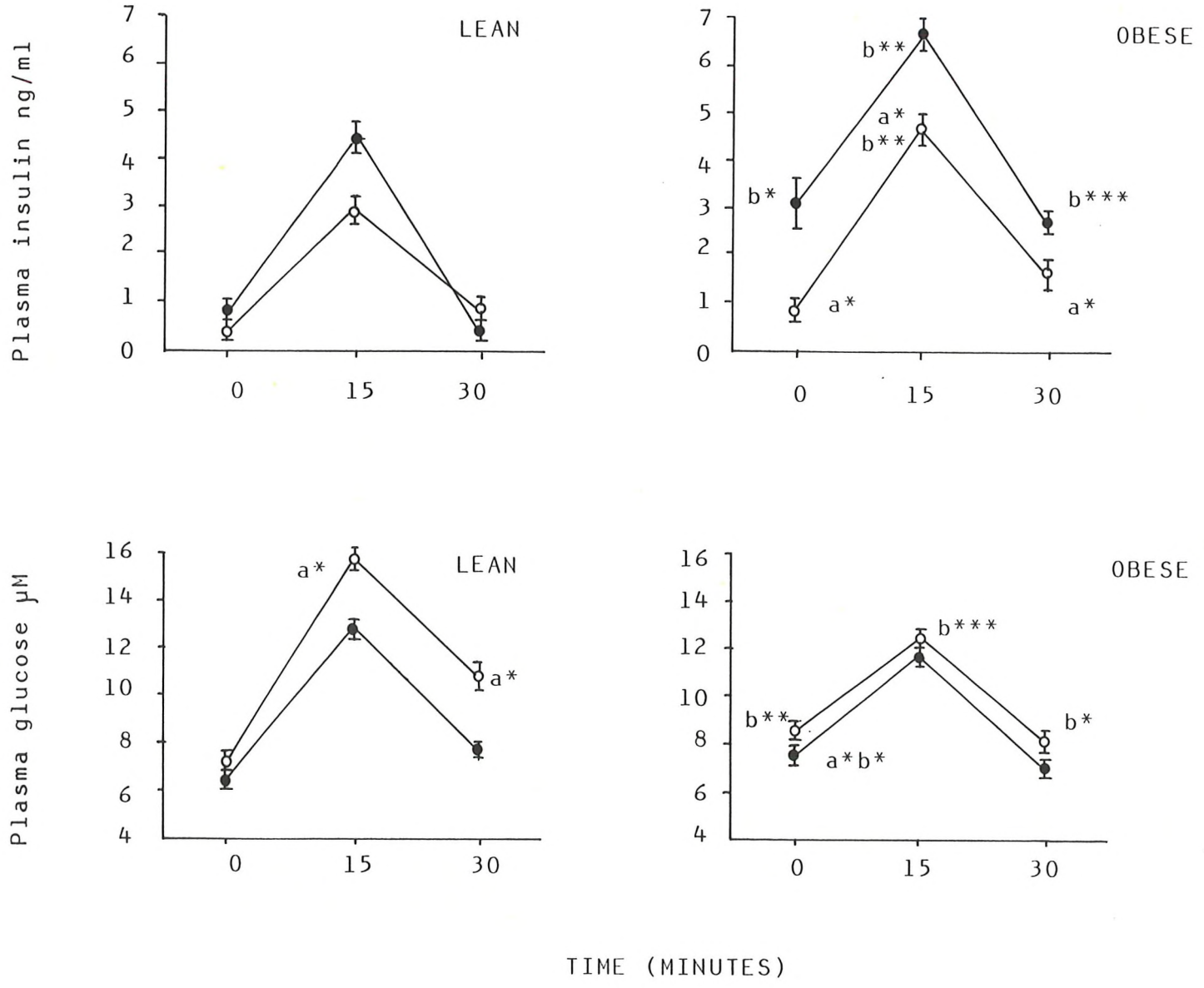

Plasma insulin ng/ml
LEAN
OBESE
b**
a*
b**
b*
b***
a*
a*
Plasma glucose µM
LEAN
a*
a*
OBESE
b***
b**
b*
a*b*
TIME (MINUTES)

Table 3.24 Effects of a glucose load on plasma glucose and plasma insulin in lean and obese (fa/fa) rats.

	Plasma insulin (ng/ml)		Plasma glucose (mM)	
	Control	Total response	Control	Total response
INTACT LEAN	0.82±0.14	51.4	6.57±0.15	98.6
INTACT OBESE	3.05±0.62 b*	56.6	7.54±0.16 b**	58.5
ADRENALECTOMISED LEAN	0.36±0.08	43.5	7.22±0.28	151.0
ADRENALECTOMISED OBESE	0.86±0.18 a*	52.2	8.48±0.17 a* b*	59.6

Adrenalectomised rats were maintained on 0.9% (w/v) saline instead of drinking water. All rats were starved for 3.5 hours prior to the study. Rats received an i.p. injection of 150mg/100g body weight glucose in 0.3ml 0.9% (w/v) saline. Time zero groups received an equal volume of vehicle. Serum glucose and insulin were assayed as described in sections 2.9 and 2.10. Values represent means ± S.E.M. for 4 rats in each group. ***, $p < 0.001$; **, $p < 0.005$; *, $p < 0.01$. a, compared with control group; b, compared with equivalent lean group.

having returned to normal after 30 minutes. However, although plasma insulin levels remained consistently higher in obese rats, the secretory response (surface area over baseline) was no greater in obese rats than in lean. The total increase in plasma glucose (surface area over base line) was reduced in obese rats to 59% of that in lean rats.

Adrenalectomised obese rats were still slightly hyperglycaemic compared to lean rats but the increased plasma insulin found in intact obese rats was reduced to that found in lean rats. Glucose administration resulted in higher plasma insulin levels, associated with a slightly smaller increase in plasma glucose compared with intact rats in both lean and obese rats. Insulin levels returned to normal after 30 minutes. The total insulin secretory response (surface area over baseline) was unaffected by adrenalectomy and was similar in lean and obese rats. Similarly, the total increase in plasma glucose was unaffected by adrenalectomy and remained lower in adrenalectomised obese rats compared with lean.

3.8.2 DISCUSSION.

Obese rats were slightly hyperglycaemic compared to lean yet markedly hyperinsulinaemic, confirming previous reports (York et al., 1972; Zucker and Antoniades, 1972; Martin et al., 1978; Bazin and Lavau, 1982; Rohner-Jeanrenaud et al., 1983). The hyperinsulinaemia did not seem to result from the increased plasma glucose directly, since adrenalectomy abolished the hyperinsulinaemia as expected, (York and Godbole, 1978) without affecting the hyperglycaemia. Although plasma insulin levels were consistently higher in obese rats the total insulin response was similar in lean and obese rats. Adrenalectomy reduced the hyperinsulinaemia of obese rats without affecting the total insulin secretory response. These results suggest that there is a defect in basal insulin secretion in the obese rat that is independent of hyperglycaemia and abolished by adrenalectomy. The results presented here differ slightly from those of Rohner-Jeanrenaud et al., (1983), in which i.v. administration of glucose to weaned anaethetised rats resulted

in a more prolonged increase in plasma insulin in obese rats resulting in a greater total insulin secretary response. Intravenous administration of glucose may provide a sharper, more potent stimulation of insulin secretion than intraperitoneal administration resulting in increased vagal stimulation of the pancreas. Acute vagal stimulation in obese rats produces larger prolonged increases in plasma insulin than in lean rats and enhances the insulin response to a glucose load compared with lean rats (Rohner-Jeanrenaud et al., 1983.) This response may have been triggered by i.v. but not i.p. administration of glucose.

Since the increased basal insulin secretion in obese rats is mediated by vagal stimulation of the pancreas (Rohner-Jeanrenaud et al., 1983), then the reversal of this hyperinsuliaemia by adrenalectomy, without affecting the total insulin secretory response, may be indicative of a reduction in parasympathetic activity to normal levels. These results suggest that the obesity of the fa/fa rat results not only from a reduced sympathetic activity in BAT but from an imbalance in the autonomic nervous system. Sympathetic activation of BAT in response to diet is reduced, resulting in a reduced energy expenditure relative to energy intake and vagal tone is increased, resulting in hyperinsulinaemia and consequent stimulation of lipogenesis and lipid deposition. Long term insulin adminstration results in an increased energetic efficiency with development of obesity (Chan et al., 1982), so the increased vagal tone and hyperinsulinaemia of the fa/fa rat may contribute to the obese state. The imbalance in the autonomic nervous system may be more in favour of increased parasympathetic activity in the VMH-lesioned rat than in Zucker rats, in which vagotomy reverses the hyperinsulinaemia but not the obesity (Powley and Opsahl, 1974). In VMH-lesioned rats, BAT noradrenaline turnover is not necessarily reduced (Yoshida and Bray, 1984) and vagotomy reverses both the hyperinsulinaemia and the obesity (Powley and Opsahl, 1974; Inoue and Bray, 1978; Fox and Parley, 1984).

Adrenalectomy corrects the imbalance in the autonomic

nervous system that is apparent in the obese fa/fa rat. Sympathetic activation of BAT is increased, vagal tone and hyperinsulinaemia is decreased and so the hyperlipogenesis, lipid deposition, hyperphagia and energy balance are restored to normal.

CHAPTER 4.

SUMMARY AND DISCUSSION.

The obese (fa/fa) rat is hyperphagic compared to its lean litter-mates yet displays an increased energetic efficiency, resulting in the development of obesity. The reduced energy expenditure of the obese rat has been linked with reduced BAT thermogenesis and, in particular, an inability to activate BAT thermogenesis in response to dietary stimuli (Holt et al., 1983; Marchington et al., 1983; Triandafillou and Himms-Hagen, 1983). This reduction in the thermogenic capacity of BAT in the obese rate has been shown to be associated with a reduced sympathetic stimulation of the tissue, as assessed by measurements of noradrenaline turnover, the effects of ganglionic blockade on noradrenaline turnover and measurements of noradrenaline synthesis rates in BAT, resulting from a reduced central sympathetic outflow.

In addition to a reduced sympathetic activation of BAT, the involution of the tissue in obese rats, that is characterised by increased lipid, was also associated with an involution of the sympathetic innervation as demonstrated by the reduced noradrenaline content of BAT from obese rats and the reduced rates of noradrenaline uptake, both *in vivo* and *in vitro*. This involution of the sympathetic nerve supply to BAT may reflect the reduced activity of the nerves rather than a reduction in the number of nerve terminals present, since the activity of tyrosine hydroxylase was not reduced in BAT of obese rats.

The reduced sympathetic activity in obese rats was shown to be specific to BAT, since noradrenaline turnover was normal in heart. This indicates that the reduced thermogenic function of BAT in obese rats results from a reduced sympathetic stimulation of this tissue in particular, rather than a general defect in the sympathetic nervous system. The generalised decline in sympathetic activity reported in older 3-4 month old obese (fa/fa) rats, with reduced noradrenaline turnover in BAT, WAT, aorta and pancreas (Levin et al., 1983a) may be a

consequence of the obesity. Rats that are allowed to eat a high-fat, high carbohydrate diet respond with increased BAT noradrenaline turnover and BAT thermogenesis (Levin et al., 1983b). Sprague-Dawley rats may become obese if maintained on this diet and, if obesity develops it is associated with reduced BAT thermogenesis and a general decline in sympathetic activity in BAT and other organs (Levin et al., 1983b, c, 1984b).

The ganglionic blocking agent chlorisondamine reduces the firing rate of sympathetic neurones and so reduces tissue noradrenaline turnover where this results from sympathetic activity in the tissue. Use of this ganglion blocking agent demonstrated that the reduced sympathetic stimulation of BAT in obese (fa/fa) rats resulted from a reduced central sympathetic outflow.

The obese (fa/fa) rat shows a normal thermogenic response to cold exposure but not to diet (Holt et al., 1983; Triandafillou and Himms-Hagen, 1983). It has been demonstrated that the thermogenic response of obese rats to cold acclimatisation was associated with a normal increase in sympathetic stimulation of BAT. This, and the normal increase in BAT thermogenic function, indicated that there was no defect in either BAT or its sympathetic nerve supply in obese rats.

In contrast to the finding of a normal sympathetic activation of BAT thermogenesis on cold acclimatisation, the inability of the obese rat to respond to overfeeding was linked to an inability to activate the sympathetic nerve supply to BAT. This suggested that the locus of the defective BAT thermogenesis in the obese rat lies in the central nervous system, perhaps in the regions of the hypothalamus associated with the regulation of feeding and diet-related BAT thermogenesis.

The hypothalamus is closely connected with the regulation of food intake and BAT thermogenesis. Electrical stimulation of the ventromedial nucleus (VMN) results in a sympathetically mediated rise in BAT temperature which may be blocked by propranolol or denervation (Perkins et al., 1981; Holt et al., 1985) and associated with increased activity of the sympathetic nerves supplying BAT (Niijima et al., 1984). Hyperphagia-inducing lesions in the VMH result in decreased BAT thermogenesis

(Seydoux et al., 1982) associated with an acute reduction in sympathetic stimulation of the tissue (Niijima et al., 1984). Long term adaptation to VMH-lesions may be complex since, in adult VMH-lesioned rats, BAT noradrenaline turnover is increased despite reduced BAT thermogenesis (Yoshida and Bray, 1984). In contrast, lesions in the lateral hypothalamus, in addition to producing aphagia, result in increased energy expenditure (Morrison, 1968) associated with increased BAT noradrenaline turnover (Yoshida et al., 1983).

The BAT thermogenic response to VMH stimulation is not impaired in the obese (fa/fa) rat (Holt et al., 1985). This indicates that the defective sympathetic regulation of BAT thermogenesis in the (fa/fa) rat lies in the afferent input to the VMH, or the transduction of these incoming signals to the efferent nerves supply of BAT. The VMH-lesioned rat responds normally to cold exposure (Hogan et al., 1982 ; Luboshitsky et al., 1984) with increased BAT noradrenaline turnover (Yoshida and Bray, 1984), but does not respond to diet (Seydoux et al., 1982c). Thus the reduced energy expenditure of the VMH-lesioned rat, like that of the obese (fa/fa) rat, seems to be associated with reduced activation of DIT in BAT.

The monitoring of central glucose and insulin concentrations have an important role in the regulation of food intake and energy balance. Insulin sensitive glucoreceptors in the VMH are stimulated by local administration of glucose or insulin (Oomura et al., 1978). 2-deoxy-D-glucose blocks these glucoreceptors and reduces the firing rate of ventromedial neurones, and results in hyperphagia (Desiraju et al., 1968), hyperglycaemia (Ikeda et al., 1980) and reduced energy expenditure (Shiraishi and Mager, 1981). Peripheral administration of 2-deoxy-D-glucose results in hyperphagia and hyperglycaemia (Smith and Epstein, 1969) and reduced cardiac sympathetic activity (Rappaport et al., 1982), reduced energy expenditure (Rothwell et al., 1981a) and reduced BAT thermogenesis (Allars and York, unpublished observations). Insulin may have a role in these responses since diabetic rats, although hyperphagic and hyperglycaemic, exhibit reduced sympathetic activation of BAT (Young et al., 1983). The responses to

2-deoxy-D-glucose are disturbed in the obese (fa/fa) rat suggesting that it is unable to monitor glucose availability. Intraventricular administration of 2-deoxy-D-glucose causes hyperglycaemia but not hyperphagia in obese rats (Ikeda et al., 1980). Peripheral administration of 2-deoxy-D-glucose does not reduce energy expenditure (Rothwell et al., 1981a) or BAT thermogenesis (Allars and York, unpublished observations) in obese rats as it does in lean rats. The hyperphagia and reduced thermogenesis of the obese rat could result from a reduced stimulation, or a reduced sensitivity, of the insulin sensitive glucoreceptors in the VMH. Although obese rats are hyperinsulinaemic, brain insulin levels are not necessarily affected by plasma insulin levels (Oomura and Kita, 1981). Although the insulin concentration of the cerebrospinal fluid is increased in obese rats (Stein et al., 1983), the brain insulin content of obese rats has been reported to be markedly reduced (Baskin et al., 1985). Reduced stimulation of the insulin sensitive glucoreceptors in the VMH by insulin in obese rats could lead to reduced neuronal activity in the VMH and consequent hyperphagia, reduced sympathetic stimulation of BAT thermogenesis and increased vagal tone resulting in hyperinsulinaemia.

The defective sympathetic activation of BAT thermogenesis in obese rats is sensitive to adrenal glucocorticoids. Adrenalectomy restores BAT thermogenesis and energy balance to normal in obese rats (Holt and York, 1982; Holt et al., 1983; Marchington et al., 1983). Adrenalectomy was associated with increased noradrenaline turnover in BAT of obese rats and chlorisondamine treatment demonstrated that this resulted from an increased central sympathetic outflow. The increased BAT thermogenesis of adrenalectomised obese rats was suppressed by corticosterone replacement (Holt et al., 1983; York and Al-Baker, 1984) and this was associated with a reduced noradrenaline turnover in BAT. Since corticosterone suppresses DIT but not NST (Galpin et al., 1983; York et al., 1985a) this suggests that the effects of corticosterone may be located centrally, in those areas of the hypothalamus concerned with the regulation of feeding and DIT.

The inhibition of sympathetic activity in BAT of obese rats may result from an increased sensitivity of an existing regulatory nervous pathway in the hypothalamus to corticosterone. Adrenalectomy does not significantly affect BAT thermogenesis in ad lib-fed lean rats (Holt and York, 1982) and was not associated with any changes in sympathetic activity in BAT. However, there may be an increase in BAT thermogenesis that is masked by the reduced food intake of adrenalectomised lean rats, since BAT thermogenesis is increased compared to intact lean rats restricted to the food intake of lean adrenalectomised rats (Holt, 1984.) Chronic corticosterone treatment does reduce BAT thermogenesis in lean rats (York et al., 1985a) and this was associated with reduced sympathetic activity in BAT. There was a greater attenuation of sympathetic activity in BAT of adrenalectomised fa/fa rats than in intact lean rats at the same dose of corticosterone. Corticosterone reduces DIT but not NST in lean rats (York et al., 1985a) and it is DIT rather than NST that is reduced in the obese rat. This suggests that since plasma corticosterone levels and the ratio of bound/free corticosterone are unchanged in obese rats (Al-Baker, 1985), the defect in obese rats involves an increased sensitivity to corticosterone in the central nervous system.

Increased sensitivity of DIT to adrenal glucocorticoids does not appear to be confined to the obese (fa/fa) rat. In lean rats the thermogenic response to diet declines with age (Rothwell and Stock, 1982, 1983) and is restored on adrenalectomy (Rothwell et al., 1984b). The strain differences that have been reported in the thermogenic response to diet (Schemmel et al., 1970; Miller et al., 1976; Rothwell & Stock, 1982a) may also reflect varying degrees of sensitivity to corticosterone. Adrenalectomy of young Sprague-Dawley rats resulted in increased BAT thermogenesis and an increased thermogenic response to cafeteria feeding, associated with increased sympathetic activity in BAT.

As in the obese (fa/fa) rat, the obesity of VMH-lesioned rats is sensitive to adrenal glucocorticoids since adrenalectomy reverses the obesity and this is prevented by corticosterone

replacement (Bruce et al., 1982; King et al., 1983). The ventromedial nucleus (VMN) was destroyed in these studies so the corticosterone inhibition of DIT and normal feeding behaviour would appear to operate through other hypothalamic centres, with increased sensitivity to corticosterone if VMH activity is reduced. Destruction of the VMN itself is not a requirement for hypothalamic obesity, which seems more dependent upon damage to the ventral adrenergic bundle (Gold 1973; Bray et al., 1982), again suggesting that a disruption in the function of other centres is of more importance than damage to the VMN itself.

The paraventricular nucleus (PVN) of the medial hypothalamus (MH) is closely associated with the regulation of feeding behaviour and energy balance. Hyperphagia-inducing lesions between the MH and LH are most effective when placed lateral to the PVN (Gold, 1970). Administration of noradrenaline to the PVN results in a stimulation of feeding (Leibowitz, 1978) and lesions in the PVN result in hyperphagia and obesity (Leibowitz et al., 1981). The inhibitory effects of noradrenaline on the PVN, which result in hyperphagia, are regulated by corticosterone. Corticosterone treatment enhances and adrenalectomy reduces the inhibitory effects of noradrenaline on the PVN (Leibowitz et al., 1976). Increased sensitivity of the PVN to these effects of corticosterone could result in an enhanced response to noradrenaline, which would be abolished by adrenalectomy, and could at least partly explain the hyperphagia and obesity. Increased inhibition of the PVN would not necessarily require an increased sensitivity of the PVN to corticosterone itself. Corticosterone could be fulfilling a permissive role, as it does in the periphery on cold exposure (Fellenz et al., 1982) and is required to maintain a normal sensitivity to an increased noradrenaline release in obese rats. Alternatively, the pathway of noradrenaline inhibition of the PVN, mediated by corticosterone, need not actually be disturbed in the obese rat, but only achieves prominance in the physiological regulation of food intake and energy balance if VMH activity is reduced, as would also occur in VMH-lesioned rats.

Adrenalectomy is associated with increased plasma ACTH levels (Yukimura et al., 1978). ACTH stimulates BAT thermogenesis in obese rats and prevents the suppression of BAT thermogenesis by corticosterone treatment of adrenalectomised fa/fa rats (York and Al-Baker, 1984). ACTH treatment did not affect sympathetic activity in lean or obese rats. In contrast to corticosterone the effects of ACTH may result from a direct interaction with the tissue. This could result from a direct stimulation of lipolysis by activation of adenylate cyclase (Bertin and Portet, 1976).

The reduced BAT thermogenic response of the obese rat has been associated with increased activity of the parasympathetic nervous system, in addition to reduced activity of the sympathetic nervous system. The parasympathetic nervous system is regulated by the same centres of the hypothalamus that regulate the sympathetic nervous system. Stimulation of the VMH results in decreased vagal activity and damage to the VMN or stimulation of the LH is associated with increased vagal activity (Oomura and Kita, 1981). The decreased sympathetic activation of BAT in obese rats may be indicative of reduced activity in the VMH and is associated with hyperinsulinaemia that is suppressed by atropine or vagotomy (Powley and Opsahl, 1974; Rohner-Jeanrenaud et al., 1983). The hyperinsulinaemia of VMH-lesioned rats is also blocked by vagotomy (Powley and Opsahl, 1974; Inoue and Bray, 1978; Fox and Parley, 1984). The stimulation of feeding initiated by noradrenaline administration to the PVN is blocked by atropine (Sawchenko et al., 1981) as is the reduced thermogenesis observed in 2-deoxy-D-glucose treatment of lean rats (Shiraishi and Mager, 1981). This suggests that these responses are mediated by the parasympathetic nervous system. Atropine treatment of obese (fa/fa) rats does not affect resting metabolic rate but increases the thermic effect of feeding up to the levels found in atropinised lean rats (Rothwell et al., 1981a). Atropine also stimulates the thermogenic effects of feeding a high-fat diet to obese rats (Rothwell & Stock, 1983a).

The reduced sympathetic activity of the obese rat has been associated with an increased vagal tone resulting in a

state of hyperinsulinaemia. The increased insulin response to a glucose load of the obese rat is abolished by vagotomy (Rohner-Jeanrenaud et al., 1983). In the present work the higher plasma insulin concentration in obese rats observed after glucose administration resulted from their elevated basal insulin concentration rather than an increase in the insulin secretory response. This suggests that there may be an increased basal vagal tone in the obese rat responsible for maintaining the hyperinsulinaemia state despite relatively normal plasma glucose levels, although in these experiments there was no increase in the magnitude of the insulin response to glucose, as would be expected if vagal activity was increased (Bloom and Edwards, 1980; Rohner-Jeanrenaud et al., 1983.) The increased basal insulin release of the obese rat was abolished by adrenalectomy, suggesting that, in addition to increasing sympathetic activity, adrenalectomy may also diminish increased parasympathetic activity of the obese rat. Thus the obesity of the fa/fa rat may result from an imbalance in the autonomic nervous system resulting in decreased sympathetic activation of BAT and increased vagal tone resulting in hyperinsulinaemia. It may be proposed that this imbalance in the autonomic nervous system results from reduced activity in the VMH. Decreased sensitivity of insulin sensitive glucoreceptors or a reduction in central glucose or insulin would reduce VMH activity. The LH is maintained under tonic inhibition by the VMH, so reduced VMH activity would result in increased activity in the LH. This imbalance would lead to increased food intake, reduced sympathetic stimulation of BAT and increased vagal tone and hyperinsulinaemia. These defects are sensitive to circulating corticosterone since they are abolished by adrenalectomy. The LH may also be held under tonic inhibition by the PVN (Gold, 1970). The sensitivity of the PVN to noradrenaline is regulated by corticosterone. Sensitivity of the PVN to corticosterone or noradrenaline, or alternatively, increased noradrenaline release would inhibit the PVN and hence increase LH activity. This in turn would lead to decreased activity in the VMH resulting in reduced thermogenesis, hyperinsulinaemia and hyperphagia that would be abolished on

adrenalectomy.

Adrenalectomy restores the thermogenic responses to feeding in obese rats (Holt et al., 1983; Marchington et al., 1983) associated with increased BAT mitochondrial GDP binding, an increased thermic effect of food and further increases in GDP binding in response to sucrose feeding. Although adrenalectomy resulted in increased BAT noradrenaline turnover in obese rats sucrose feeding did not further increase BAT noradrenaline turnover in either lean or fa/fa adrenalectomised rats and there were no changes in central sympathetic outflow. These observations were supported by the report that the increases in GDP binding on sucrose feeding of adrenalectomised rats or intact lean rats were not fully blocked by propranolol (York et al., 1985b). This is not the case in chow-fed rats in which propranolol treatment of adrenalectomised lean and obese rats and intact lean rats reduced BAT mitochondrial GDP binding to the low levels observed in intact obese rats (York et al., 1985b). This suggests that a sympathetic tone is required to maintain the thermogenic function of BAT, which is absent in intact obese rats, but that the increases in BAT thermogenesis in response to diet in Zucker rats are not necessarily dependent upon increased sympathetic stimulation of the tissue. This in turn suggests that the stimulation of DIT in these animals is brought about by a humoral effector. Adrenal medullary secretion of adrenaline or noradrenaline cannot be responsible for the stimulation of BAT thermogenesis since circulating concentrations are too low and their effects would be blocked by propranolol. It has been suggested that ACTH could be a possible candidate for this role since it can stimulate BAT thermogenesis directly and is secreted in response to feeding.

The acute effects of sucrose feeding on BAT thermogenesis in intact lean rats are more sensitive to propranolol inhibition than the chronic effects. Propranolol treatment reduces BAT mitochondrial GDP binding in intact lean rats after 24 hours sucrose feeding but not after 7 days of sucrose feeding although the decrease in the 24 hour sucrose-fed group is only down to chow-fed lean values, thus there is still a propranolol

insensitive component (York et al., 1985b). It would appear that the increased sympathetic activity of BAT of intact lean rats overfed with sucrose may be involved in the acute response to diet, leading to increased thermogenesis, proliferation of the tissue and increases in 32000D protein but that after 7 days overfeeding, the higher thermogenic capacity is not dependent upon sympathetic stimulation in the short term, and in adrenalectomised rats can be maintained under a lower sympathetic drive. It is possible that the propranolol insensitive component of the increased BAT thermogenesis in sucrose-overfed rats could be explained by an α_1-receptor mediated stimulation of thermogenesis. Mohell et al. (1983) have reported that 20% of noradrenaline stimulated thermogenesis is mediated by α_1-receptors. If this component was increased on sucrose overfeeding it would not be blocked by propranolol. However, unless this was also associated with a large increase in tissue sensitivity to noradrenaline an increased sympathetic activation of the tissue would still be required to maintain the increases in thermogenesis.

The findings in Zucker rats contrasted to those in Sprague-Dawley rats in which overfeeding of adrenalectomised rats resulted in increased thermogenesis, that was blocked by propranolol, and increased BAT noradrenaline turnover, suggesting that in these animals the increased BAT thermogenesis is entirely mediated by the sympathetic nervous system through stimulation of β-receptors. These differences could result from the difference in rat strain or from the differences in diet composition. The Sprague-Dawley rats were overfed with a cafeteria diet which is high in fat as well as in carbohydrate. In Sprague-Dawley rats, cafeteria feeding produces much greater increases in BAT noradrenaline turnover than does sucrose feeding (Young et al., 1982) so it is possible that BAT noradrenaline turnover would have increased in adrenalectomised Zucker rats offered a cafeteria diet. Cafeteria feeding of lean Zucker rats results in an increased resting oxygen consumption after 10 days on the diet that is completely blocked by propranolol treatment (Rothwell and Stock, 1982b).

There can be little doubt that the defective BAT thermogenic capacity and inability to initiate DIT in the intact obese rat is linked to a severe reduction in sympathetic activity in BAT and in inability to activate the sympathetic drive to BAT in response to dietary stimuli. However, in rats in which BAT is maintained under a higher sympathetic tone than is apparent in intact obese rats, BAT thermogenesis can be activated without necessarily further increasing BAT sympathetic activity. This suggests that the regulation of DIT may be more complex than originally supposed and may involve the integration of the sympathetic nervous system with endocrine responses. Whether this involves direct stimulation of BAT or increased sensitivity of BAT to the effects of sympathetic stimulation, perhaps through changes in receptor population, has yet to be established.

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