EFFECT OF FOOD RESTRICTION
ON
SERUM AND PINEAL INDOLES

By

CONSTANCE LAI-YEE CHIK, B.SC., M.D.

A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Doctor of Philosophy

McMaster University

FOOD RESTRICTION ON SERUM AND PINEAL INDOLES

DOCTOR OF PHILOSOPHY (1985) McMASTER UNIVERSITY

(MEDICAL SCIENCES) Hamilton, Ontario

TITLE: Effect of food restriction on serum and pineal indoles

AUTHOR: Constance Lai-Yee Chik, B.Sc. (University of Toronto)

M.D. (University of Toronto)

SUPERVISOR: Professor G.M. Brown

NUMBER of PAGES: x, 134

ABSTRACT

Food restriction has profound effects on various endocrine axes and on amine metabolism. In the present study, the effect of reduced food availability on pineal and serum indole was determined in adult male Wistar rats. Under a lighting regimen of 14 h light and 10 h dark, 3 weeks of 50% food restriction led to a reduction in 24 h mean serum tryptophan and serum serotonin levels but an increase in serum melatonin levels. The duration of the night-time melatonin rise was increased secondary to an earlier rise of both pineal and serum melatonin. Such changes in circulating melatonin may account for the gonadal regression observed in underfed animals. This pineal-gonadal interaction was further investigated after animals were subjected to shortened photoperiod or after pinealectomy. Shortened photoperiod failed to influence either the serum melatonin profile or the undernutrition-related gonadal regression. Pinealectomy, however, was able to reverse though incompletely the gonadal regression in underfed animals. When the pineal responsiveness to beta-adrenergic stimulation was determined in food restricted animals, both the time course and the dose responses were altered. The changes in pineal and serum melatonin post-stimulation, however, were atypical of either a sub- or supersensitive pineal gland.

Based on the present study, food availability proves to be another factor that can influence pineal activity. Its effect on the pineal, however, depends on the duration of food restriction and the environmental light/dark cycle.

ACKNOWLEDGEMENTS

I thank my family for their constant support and encouragement. The expert technical assistance given by my husband Tony and my sister Florence is greatly appreciated.

The research work presented in this thesis is made possible by a fellowship from the Medical Research Council of Canada. I am indebted to my supervisor, Dr. G.M. Brown for his guidance throughout the research. I also thank other members of the supervisory committee, Dr. E. Werstiuk, Dr. L.J. Grota, Dr. J. Laidlaw and Dr. L.P. Niles for their helpful advice. The assistance so freely given by Mrs. G.E.K. Johansson, Mr. M.G. Joshi and Ms. L. Campbell, work and otherwise, is greatly appreciated. I am also most grateful to Mrs. A. Murray for her patience and excellent secretarial skills.

Thanks are also extended to the other members of the examining committee, Dr. G. Evans, Dr. B.J. Galef, Dr. W.J. Leigh and especially to Dr. H.J. Lynch for acting as the external examiner.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

1.1 Perspectives of the present study

Food availability has profound influences on many metabolic processes. The response of the endocrine system to dietary restriction is not uniform. Any step of hormone action can be affected. Indeed, changes in hormone synthesis, degradation or tissue responsiveness have all been described (Becker, 1983). Dependent on the particular hormone axis in question, it may be suppressed, remain unchanged or be hyperactive. Such diversified responses are thought to represent adaptive mechanisms. One axis that is suppressed during dietary restriction is the hypothalamic-pituitary-gonadal axis (Howland, 1975). The suppression is believed to be at the hypothalamic level since the pituitary gland retains its ability to respond to gonadotropin-releasing hormone (Campbell et al, 1977). However, this may be simplistic since the regulation of the reproductive axis is complex. One organ that is affected by food restriction and that can influence the reproductive axis is the pineal gland. The regulating role of the pineal on the reproductive axis is linked to photoperiodism (Goldman and Darrow, 1983). In view of this connection, it is of interest to investigate the effect of food restriction on the pineal gland and its interaction with the gonadal axis under different photoperiods.

In seasonal breeding species such as hamsters and sheep, the importance of the pineal gland on the reproductive axis has been well defined (Reiter, 1980; Lincoln and Short, 1980). By contrast, the

rat reproductive axis is relatively unresponsive to the inhibitory action of the pineal and its hormone melatonin (MT). This axis, however, can be sensitized to the inhibitory action of the pineal by manipulations such as underfeeding, olfactory bulbectomy or neonatal steroid administration (Reiter, 1974). The focus of many previous studies has been on changes in sensitivity to the action of the pineal or MT in food restricted states. Few studies have determined the effect of food restriction on the activity of the pineal gland. When adult rats are subjected to chronic food restriction, pineal activity increased as determined by oxygen consumption and morphologic criteria (Walker et al, 1978). When MT is determined, short term starvation has no effect on urinary MT excretion (Lynch et al, 1975). On the other hand, when prepubertal rats are subjected to 5 weeks of protein calorie malnutrition, determination of pineal MT content reveals lower daytime and night-time levels (Herbert and Reiter, 1981). Even though MT has been accepted as the pineal hormone, the effect of food restriction on circulating MT levels has never been determined. The present study, therefore, investigated the effect of varying duration of dietary restriction on the circadian rhythm of circulating MT. Changes in pineal activity were correlated with changes in gonadal parameters. This pineal-gonadal interaction was further investigated by subjecting the animals to shortened photoperiod and pinealectomy.

Food restriction also has a profound influence on neurotransmitters which are key regulators of many hormonal axes. For instance, many aspects of the adrenergic systems are influenced by food availability. Changes in norepinephrine turnover, adrenoceptor density and tissue responsiveness have all been described (Katovich

and Barney, 1983; Landsberg and Young, 1978; Stone, 1983). Since pineal activity is intimately linked to sympathetic activity (Zatz, 1978), the effect of dietary restriction on pineal responsiveness to a beta-adrenergic agonist was determined.

Food availability also leads to changes in indole metabolism. Animals subjected to acute food deprivation have increased synthesis and turnover of cerebral serotonin (Kantak, 1977, 1978a, 1978b, 1978c). The effect of chronic food restriction, however, has not been defined. In the last section of the present study, the effect of food restriction on two circulating indoles, tryptophan and serotonin, was determined.

Since the major part of the present study is on the effect of food restriction on pineal-gonadal interaction, the subsequent section is an overview of aspects of pineal physiology.

1.2 The pineal gland and melatonin

The history of the pineal gland dates back to 325-280 BC when Herophilos of Alexandria suggested that the pineal might function as a valve controlling the "stream of thoughts" from the lateral ventricle of the brain (reviewed by Kappers, 1965; Kitay and Altschute, 1954). By contrast, Galen of Pergamon (130-200 AD) believed that the pineal organ was merely a lymph gland. In the 17th century, the renowned French philosopher Rene Descartes designated the pineal as the "seat of the soul". He also suggested that the pineal receives photic information from the eyes and thereby exercises an influence on the body which proves to be prophetic. The first endocrine effect of the pineal was described in 1898 when Heubner reported the association of

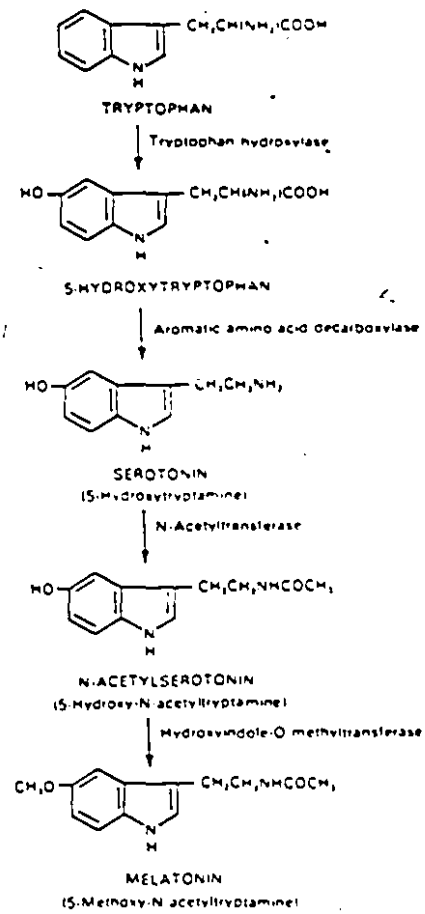
precocious puberty with a pinealoma in a four year old boy. This was followed by Marburg's hypothesis that the pineal secretes a substance that regulates the onset of puberty. This is of particular interest to the present study since dietary restriction can delay the onset of puberty. In 1918, Holmgren observed similarities between the sensory type cells in the pineal region and the cone cells of the retina in amphibia and fish. Taken together with the observation that calcification of the human pineal occurs with advancing age, this evidence led to the hypothesis that the mammalian pineal is just a vestigial remnant left behind by evolutionary progress. Of interest, in 1917, McCord and Allen found that the bovine pineal glands produced a substance that lightened the skin of frogs. The responsible compound was eventually isolated and identified as N-acetyl-5-methoxytryptamine by Lerner et al (1958, 1959). It was given the name "melatonin (MT)" because of its indole nature and its ability to lighten pigment cells.

Today, the pineal gland is recognized as an actively functioning neuroendocrine organ that responds primarily to photic stimuli. It exhibits circadian rhythms and influences the metabolic activity of a host of endocrine glands. The possible mediator is the pineal hormone melatonin.

1.3 Biochemistry of melatonin synthesis

By the use of enzyme assays (Weissbach et al, 1960; Axelrod and Weissbach, 1960) and pineal cell culture (Shein et al, 1967; Klein et al, 1970), the biosynthetic pathway of MT (Fig. 1) and its regulation in the pineal has been established.

Figure 1 The biosynthetic pathway of melatonin

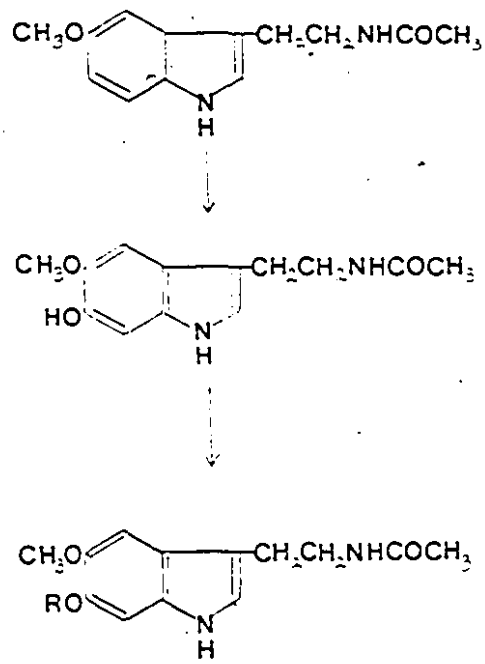


Pinealocytes possess all the enzymes that are required for MT synthesis. The indole amino acid tryptophan is the common precursor of the pineal and brain indoleamines. Uptake of tryptophan from the blood stream by pinealocytes is followed by hydroxylation at the 5-position by tryptophan hydroxylase to 5-hydroxytryptophan (5HTP) (Lovenberg et al, 1967). The 5HTP thus formed is decarboxylated to 5-hydroxytryptamine (serotonin, 5HT) by aromatic-L-amino-acid decarboxylase. Compared to other brain areas, the pineal has one of the highest concentrations and turnover rates for 5HT (Falck et al, 1966). Serotonin in the pineal has a complex fate: (a) oxidative deamination by monoamine oxidase to 5-hydroxyindoleacetic acid or 5-hydroxytryptophol (Hakanson and Owman, 1965,1966); (b) release to the extracellular space and uptake by sympathetic nerve terminals (Owman, 1965; Hakanson and Owman, 1966); or (c) N-acetylation to N-acetylserotonin (NAS) by serotonin N-acetyltransferase (NATase) with acetylcoenzyme-A serving as the acetyl donor (Weissbach et al, 1960). NAS is then O-methylated by hydroxyindole-O-methyltransferase (HIOMT) to form MT with S-adenosylmethionine providing the methyl group (Shein et al, 1967). In addition to NAS, HIOMT can also O-methylate 5-hydroxyindoleacetic acid and 5-hydroxytryptophol to form 5-methoxyindoleacetic acid and 5-methoxytryptophol respectively, both of which have been identified in the pineal (Lerner et al, 1960; McIssac et al, 1965). Although NATase is widely distributed in various tissues, in the pineal it is the key enzyme in the control of the circadian rhythm of MT synthesis (Ellison et al, 1970; Deguchi, 1975). In contrast to the widespread distribution of NATase, HIOMT is almost completely localized in the pineal gland. Outside of the

pineal, HIOMT has only been identified in the retina and the harderian gland (Cardinali and Wurtman, 1972). Using immunohistochemical techniques, MT has been localized in the same tissues, retina and harderian glands as well as the intestine (Bubenik et al, 1977, 1978). Nevertheless, the extrapineal contribution to the circulating pool of MT is small since pinealectomy in rats result in undetectable MT levels by gas chromatography mass spectrometry (Lewy et al, 1980). MT synthesized in the pineal appears to be secreted into the blood stream by simple diffusion. Whether MT is primarily secreted into the blood stream or the cerebrospinal fluid remains controversial. In the rat, the blood compartment is likely the primary site of secretion since MT concentration in the plasma from the confluence sinuum is about 8 times higher than that of trunk blood (Withyachumnarnkul and Knigge, 1980). In any case, MT crosses the blood brain barrier with ease (Anton-Tay and Wurtman, 1969).

The major metabolic pathway of circulating MT is conversion to 6-hydroxymelatonin in the liver by microsomal enzymes (Kopin et al, 1960) (Fig. 2). This is followed by conjugation with sulphate or glucuronic acid and excretion mainly in the urine (Kveder and McIsaac, 1961). Another route of metabolism is via a brain enzyme, indoleamine 2,3 dioxygenase, which cleaves the pyrrole ring of various indoleamines (Fujiwara et al, 1978). The plasma half life of MT has been estimated to be between 15-20 minutes in rats (Gibbs and Vriend, 1983). Possible changes in the production rate or metabolic clearance of MT in food restricted animals have never been determined.

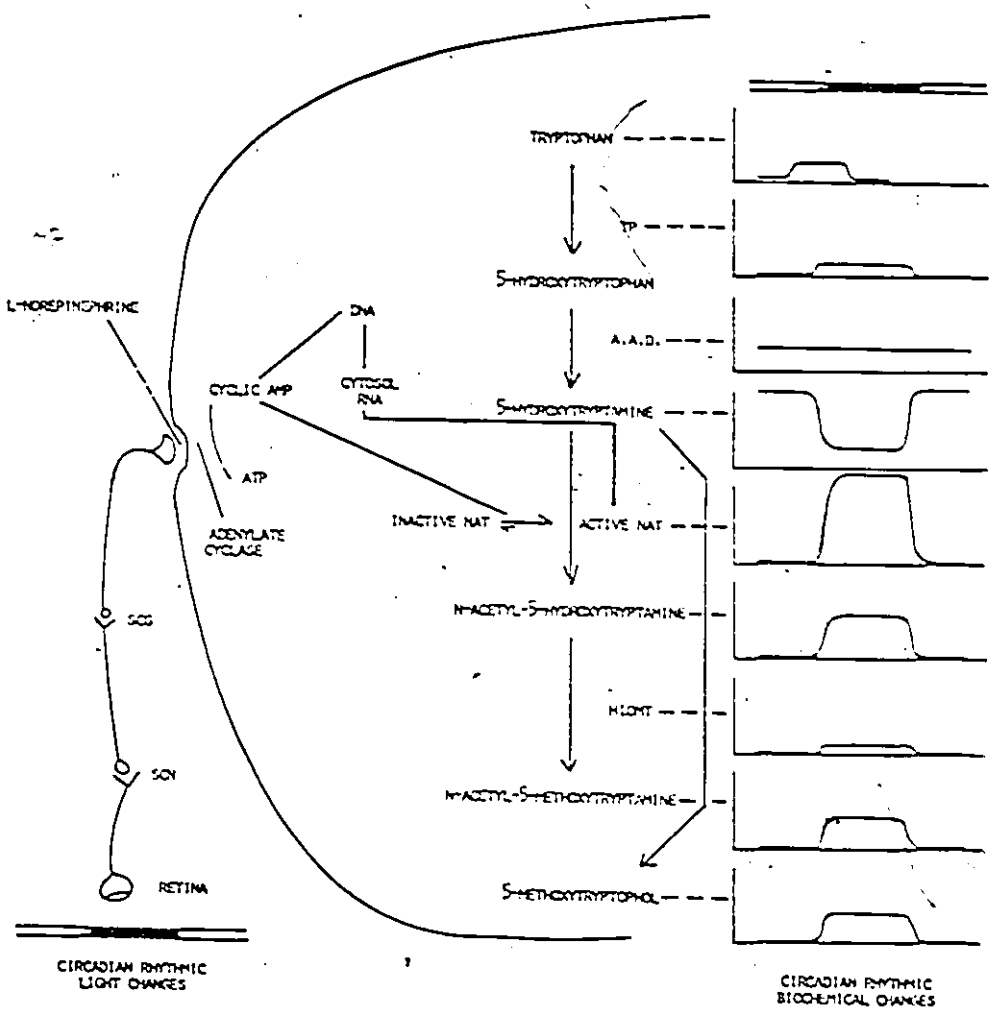
Figure 2 Metabolism of melatonin in the liver



1.4 Regulation of melatonin synthesis

A remarkable feature of the pineal is the existence of biochemical circadian rhythms and their relationship to environmental lighting (Fig. 3). The first rhythm demonstrated in the pineal was that of 5HT (Quay, 1963). Under diurnal lighting conditions, 5HT levels in the rat pineal gland are elevated during the light period and with the onset of darkness, the 5HT concentration falls gradually. This rhythm proves to be endogenous and is synchronized with environmental lighting (Synder et al, 1964, 1965, 1967). Furthermore, the 5HT rhythm is 180° out of phase with the MT and NAS rhythms in the pineal (Lynch et al, 1971; Brownstein et al, 1973). The MT rhythm persists in darkness but is suppressed by continuous illumination (Ralph et al, 1971). In 1970, Klein and Weller showed that NATase activity exhibited a marked 24 hour rhythm in the rat pineal gland. In adult rats, the daytime NATase activity is just detectable but with the onset of darkness, there is marked induction of enzyme activity. The peak night-time NATase activity is 30-100 fold higher than the daytime level (Moore and Traynor, 1976; Illnerova and Skopou, 1976). It is 180° out of phase with the pineal 5HT rhythm but in phase with the pineal MT rhythm. By contrast, the HIOMT activity has at best a small daily fluctuation (about 50%) (Quay, 1967; Klein and Lines, 1969; Lynch and Ralph, 1970). The integrity of the circadian rhythm depends upon neural impulses reaching the pineal gland via the noradrenergic post-ganglionic sympathetic fibre (pathway as shown in Fig. 4).

Figure 3 Pineal biochemical circadian rhythms and their relationship to environmental lighting



TP = TRYPTOPHAN HYDROXYLASE

AAD = AROMATIC AMINO ACID DECARBOXYLASE

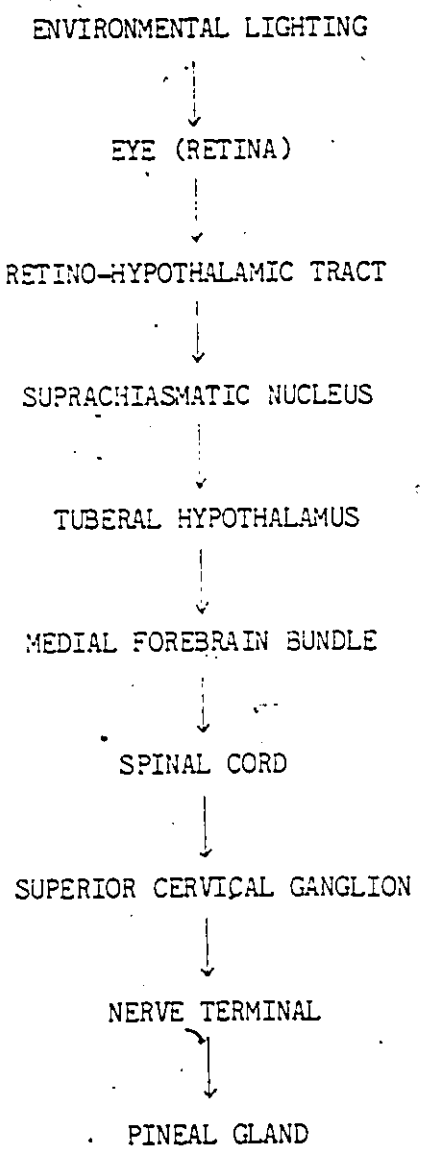
NAT = N-ACETYLTRANSFERASE

HIOMT = HYDROXYINDOLE-O-METHYLTRANSFERASE

SCN = SUPRACHIASMATIC NUCLEUS

SCG = SUPERIOR CERVICAL GANGLION

Figure 4 A schematic representation of the retinal-pineal axis



The retinal perception of darkness stimulates the sympathetic fibres innervating the pineal gland to release norepinephrine (NE) which acts upon beta-adrenergic receptors on the pinealocytes. This is of relevance to the present study since changes in sympathetic activity have been documented in the food restricted state (Katovich and Barney, 1983; Landsberg and Young, 1978).

1.5 Factors that influence pineal NATase rhythm

Other than the neural regulation of NATase activity, there are several factors endogenous to the pinealocytes which modify the ability of neural activity and environmental lighting in the control of pineal cycles. The maintenance of a high level of NATase activity at night required continuous stimulation of the β-receptor by NE (Deguchi and Axelrod, 1972b). A one minute light pulse at night leads to a rapid decline in NATase activity (Illnerova and Vanecek, 1979). The light induced decrease of NATase in darkness can be prevented by injection of catecholamine prior to light exposure. Postsynaptic stimulation of β-adrenoceptors either through neuronal release of NE or injection of isoproteronol (ISO) leads to an immediate surge in the concentration of cAMP (Deguchi, 1973). This is followed by an increase in NATase activity one hour later (Strada et al, 1972). Both increase in cAMP and NATase activity can be blocked by the β-blocker propranolol. Increase of the second messenger, cAMP initiates a sequence of events including de novo protein synthesis culminating in higher NATase activity, the rate limiting step in MT synthesis (Deguchi, 1972). The increased cAMP contents also serve to maintain the enzyme in an active form. This

activation of NATase activity leads to a parallel increase in the NAS and MT contents in the pineal gland as well as an elevation of MT level in the serum (Ho et al, 1984).

The response of NATase activity to neural inputs depends on the sensitivity of the β-adrenoceptors. Prior treatment with ISO can render the gland subsensitive to repeated ISO stimulation (Romero and Axelrod, 1974). Decreased stimulation either by denervation or treatment with 6-hydroxydopamine or reserpine leads to increased pineal β-responsiveness (Deguchi and Axelrod, 1973a, 1973b). This supersensitivity can be reversed by ISO administration. Such changes in sensitivity are rapid and can occur within a normal 24 hour day-night cycle. Pineals from rats kept in diurnal lighting condition are relatively subsensitive at the beginning of the light period but become supersensitive at the beginning of the dark period. The changes in the number of β-adrenoceptor sites undoubtedly contribute to this change in sensitivity of NATase induction in the pineal. The maximal number of specific β-adrenergic binding sites occurs at the end of the light period while during the night, the number of binding sites decreases and reaches a minimum by the end of the dark period (Romero et al, 1975). This self regulatory mechanism in the pinealocytes suggests that even though the existence of the indoleamine cycle depends on the sympathetic innervation, the degree of responsiveness is controlled within the gland itself. In dietary restricted state, changes in circulating catecholamines, adrenergic receptors and peripheral responsiveness to adrenergic stimulation have all been described (Katovich and Barney, 1983; Landsberg and Young, 1978; Stone, 1983). These in turn may influence the generation of

pineal NATase rhythm.

1.6 Hormonal regulation of melatonin synthesis

In addition to neural input, circulating hormones also influence pineal activity. This is of particular interest to the present study. In food restricted animals, gonadotropins and sex steroids are reduced while serum corticosterone is increased (Fromweiler et al, 1968; Grewal et al, 1971; Howland, 1975). Pineal HIOMT activity in mature cycling female rats is 2 fold higher during diestrus as compared to estrus (Cardinali and Vacas, 1978). Night-time urinary MT excretion has also been found to be lowest during proestrus (Ozaki et al, 1978). Similarly, the response of adenylate cyclase to NE as well as the NE-induced increase of cAMP levels in incubated pineal glands are also lowest on the day of proestrus (Davis, 1978). These changes in pineal activity likely reflect changes in gonadal steroids and other hormone levels throughout the reproductive cycle. Estradiol treatment in pharmacologic doses affect 5HT and NE turnover rates (Vacas and Cardinali, 1979a), the NE-induced increase of adenylate cyclase and cAMP contents (Davis, 1978) as well as MT synthesis and release (Wurtman et al, 1965; Cardinali and Vacas, 1978; Ozaki et al, 1978). Progesterone treatment depresses MT synthesis and release (Ozaki et al, 1978). FSH, LH and PRL also increase HIOMT activity. In male rats, testosterone accelerates pineal 5HT and NE turnover rates and MT synthesis. Testosterone treatment in castrated rats lowers pineal β-receptor concentration (Vacas and Cardinali, 1982). Indeed, binding sites for estradiol, testosterone, 5-α-dihydrotestosterone,

progesterone and PRL have been detected in the subcellular fractions prepared from rat pineal glands (Cardinali 1975, 1977; Vacas et al, 1979). These hormone binding sites in the pineal remain operative even in the absence of an intact sympathetic input as demonstrated in pineal glands in culture (Cardinali et al, 1981). However, the maintenance of this hormone responsiveness depends on the activity of the afferent sympathetic neuron (Cardinali 1975, 1977). In chronically ganglionectomized rats, there is marked reduction in estradiol and 5-α-dihydrotestosterone receptor complexes (Cardinali, 1975; 1977). These reduction in hormone receptors, however, can be reinduced by NE interaction with pineal β-receptors. Other hormones that have been reported to affect pineal activity include adrenal corticoids and endogenous opioids (Geffard 1981; Yuwiler, 1985). Increased serum corticosterone and changes in brain opioid contents have been observed in food restricted animals (Fromweiler et al, 1968; Knuth and Friesen, 1983). The physiological role of these hormones on pineal MT synthesis, however, remains to be determined.

1.7 Methods in studying pineal function

In studying the reproductive and other physiological function of the pineal gland, researchers have relied on classical methods, either by eliminating pineal secretion from the circulation through pinealectomy or immunization against MT; or by administering exogenous MT either as replacement therapy post-pinealectomy or as a drug to intact animals. Alternatively, one can study conditions associated with altered pineal function. Through these methods, an extensive amount of information about pineal function has been obtained.

Pinealectomy

Mammalian pineals are median compact organs. They lie close to their point of origin between the habenular and the posterior commissures (Quay, 1974). In laboratory rats, the pineal body is drawn out from the roof of the diencephalon during development. The detached and relatively superficial position of this organ in rats makes pinealectomy a simple operation. Pinealectomy can be performed in rats with minimal damage to the surrounding neural structures. In some rodent species, an appreciable amount of pineal tissue remains at the basal intercommissural position (Quay, 1966). In these animals, deep structures need to be removed in order to achieve complete pinealectomy. Pinealectomy removes indoles and peptides produced in the pineal. Various indoles synthesized in the pineal have been discussed earlier. Several physiologically active peptides have also been identified in the pineal. These include arginine vasotocin (Pavel, 1973), vasopressin, TRH, LHRH and GHRH (White et al, 1974; Pelletier et al, 1975).

Pinealectomy not only removes hormones produced by the pineal, the neural connection is also severed. This may be of significance since the antigonadotrophic action of MT administration may require an intact and sympathetically innervated pineal gland (Tamarkin et al, 1976). Moreover, the effect of MT administration on the thyroid gland is more pronounced in intact animals as compared to pinealectomized animals (Vriend and Reiter, 1977). The effect of pinealectomy on hormonal secretion, however, may not be permanent. The increase of adrenal aldosterone secretion post-pinealectomy returns to normal after 90 days (Kinson et al, 1968).

The efficacy of pinealectomy as a method of removing MT completely from the circulation remains controversial. Earlier reports indicated that MT rhythm persists post-pinealectomy (Ozaki and Lynch, 1976; Yu et al, 1981). This finding suggests contribution from extrapineal sources to the circulating pool of MT. Recently, using gas chromatography-mass spectrometry, circulating MT was reported to be completely abolished post-pinealectomy in rats and humans (Lewy et al, 1980; Neuwelt and Lewy, 1983). The effect of pinealectomy on laboratory rats will be discussed under the section "Pineal effects on the reproductive system".

Immunization against melatonin

Another method used for the removal of circulating MT is by immunizing animals against endogenously produced MT (Brown et al, 1976). Variable changes in endocrine parameters have been reported post immunization. Immunization against MT and N-acetylserotonin causes a significant reduction in diurnal levels of corticosterone, PRL and testosterone and elevation of TSH levels (Niles et al, 1977a, 1977b, 1977c, 1979). Plasma GH is not altered. However, in seasonal breeding animals such as hamsters, immunization against MT fails to prevent short photoperiod induced reproductive involution. (Knigge and Sheridan, 1976). Conceivably, bound MT in the circulation is still physiologically active. Alternatively, pinealectomy may remove biologically active substances other than MT. These may account for the differences between MT removal by pinealectomy versus neutralization of MT by immunization.

Melatonin administration

Exogenously administered MT has produced a wide variety of responses and reversed some effects of pinealectomy (Reiter, 1974). However, in testing the antigonadotrophic action of the compound in rats, the doses employed ranged from 1.6 µg to 5 mg per day (Rollag et al, 1980). Such a dose range is clearly supraphysiological. Serum MT response in male hamsters after a single dose of 25 µg MT (the most commonly used dose) results in serum MT over 1000 pg/ml (10 times peak nighttime levels) and remains above the highest basal levels during most of the 24 hour cycle (Brown et al, 1985). Based on a single injection given daily, the minimal amount of MT required to induce gonadal regression in female hamsters is 50 - 100 times greater than that synthesized in situ by the pineal gland (Reiter et al, 1977). The daily amount of MT synthesized in the hamster pineal gland has been estimated to be 19 ng/day (Rollag et al, 1980). The response of the reproductive axis to exogenous MT also depends on the time of administration and the route of administration. In male and female hamsters kept under diurnal cycles, MT is antigonadrotrophic only when injected late during the light phase (Tamarkin et al, 1976). Injection administered in the morning fails to inhibit sexual function. Continuous release of MT in a subcutaneous depot prevents gonadal regression due to dark exposure - a counterantigonadotrophic action (Hoffman, 1974; Reiter et al, 1974). To better understand the physiological significance of the pattern of MT secretion, Goldman and coworkers administer MT by programmed subcutaneous infusion on a daily basis to juvenile male hamsters (Goldman and Darrow, 1983). By using infusions, they could systemically vary (a) the circadian phase of MT

administration, (b) the daily duration of MT exposure, (c) the amplitude of the artificial MT peak, and (d) the total amount of MT administered. Based on these infusion studies, the duration of nocturnal pineal MT secretion has been established as the critical parameter in driving photoperiodic responses in that species (Carter and Goldman, 1983).

1.8 Pineal effects on the reproductive system

The physiological effects of the pineal on the reproductive system have been intensively investigated. In mammals, the pineal gland appears to function as a neuroendocrine transducer which converts a neural signal to a hormonal signal (Wurtman and Anton-Tay, 1969). The most investigated hormone have been melatonin. Other indoles and peptides found in the pineal such as 5-methoxytryptophol and arginine vasotocin have also been proved to be biologically active (Vaughan, 1981). Such compounds appear to participate in both neural and neuroendocrine mechanisms, among which are the control of gonadal, adrenal and thyroid function, sleep and various biological rhythms (Cardinali, 1981; Preslock, 1984). Since the present study deals mainly with the interaction between the pineal gland and the hypothalamic-pituitary-gonadal axis in rats, only the effect of the pineal on the reproductive axis will be reviewed. The relationship between the pineal and the reproductive system has attracted much attention since pineal tumors are associated with precocious puberty or delayed puberty (Kitay, 1954). The pineal seems to be an essential component of the neuroendocrine system in the regulation of photoperiodic responses. Changes in photoperiod acting via the

nervous system alter the temporal pattern of MT secretion. The changes in secretory pattern convey information about daylength from neural components of the circadian system to the reproductive system and possibly other physiological systems. Hence, hamsters subjected to constant darkness, blinding or less than 12.5 hours of light per day undergo gonadal regression within 8-10 weeks (reviewed by Reiter, 1980; Cardinali, 1981). This photoperiod related gonadal regression is associated with suppressed LH, FSH and PRL levels and is not observed in pinealectomized hamsters. The main pineal gonad-inhibiting factor is MT. MT administration late in the afternoon is capable of inducing gonadal regression. Similar injection administered in the morning is without effect. Hence, the gonadal axis appears to exhibit a diurnal variation in its sensitivity to MT action. The precise mechanism by which MT mediates its gonadal effects remains to be determined.

In nonseasonal breeding species such as the laboratory rats, the role of the pineal and its hormone is less well defined. Maintenance of animals in constant darkness leads to delay of vaginal opening in female (Fiske, 1939, 1941) and slight decrease in the weights of the testes and accessory sex organs in male rats (Itoh et al, 1962). Such changes in reproductive organs are prevented by pinealectomy. Similarly, the delayed growth of the testes, seminal vesicles and coagulating glands in blinded male rats is negated by pineal ablation (Kinson and Robinson, 1970). The blinding of females at the age of weaning usually slows the growth rate of the ovaries and uteri in intact but not in pinealectomized rats (Reiter and Ellison, 1970). In adult rats, bilateral orbital enucleation partially blocks

compensatory ovarian enlargement and the associated rise in plasma FSH after unilateral ovariectomy unless the operation is coupled with pinealectomy (Dickson et al, 1971; Sorrentino and Benson, 1970). Even though light restriction consistently impairs the development of the gonadal axis, the consequences in the rat are so insignificant that their gonadal axis remains reproductively competent (Reiter, 1972). Hence, this axis in rats has been considered to be relatively insensitive to the stimulated pineal gland. Moreover, the responsiveness of the gonadal system to pineal substances also appears to be age dependent. Typically, the reaction is more pronounced in prepubertal animals. This increased responsiveness has been attributed to the enhanced sensitivity of the hypothalamic feedback centers in the immature rats. As of yet, differences in the MT secretory profile have not been documented between immature and mature animals. In rats kept under standard laboratory conditions with 12 - 18 hour light per 24 hour period, the effect of pinealectomy on the reproductive axis has been subtle. In immature female rats, pinealectomy advances pubertal onset (Wurtman et al, 1959) without accelerating mammary gland development (Mishkinsky et al, 1966) or augmenting the compensatory ovarian growth after unilateral ovariectomy (Pites et al, 1969,1970). In immature male rats, pinealectomy leads to premature growth of the ventral prostate and seminal vesicles (Roth, 1965). In adult female rats, pinealectomy may increase the percentage of cornified vaginal smear (Gittes and Chu, 1965) but the weight of the ovaries or uteri is not altered (Bick et al, 1969). Surprisingly, in adult male rats, one study indicated a pronounced hypertrophy of the accesory sex glands 12 days

post-pinealectomy (Motta et al, 1967). The testosterone concentration in testicular vein blood has also been found to be three times higher than that in sham operated control animals (Kinson and Peat, 1971). Even though pinealectomy stimulates testosterone secretion, such animals only have slightly heavier prostatic weights. As stated by Reiter (1974), the seemingly lack of effect of pinealectomy may relate to the chronically suppressed pineal gland of laboratory rats. Rats kept in the breeding colonies are usually exposed to long photoperiod daily with at least 12 hour light per 24 hour period. Compared to their natural environment where they are only subjected to a few hours per day, these animals have already been physiologically pinealectomized. Hence, pinealectomy in rats kept under regular lighting condition has resulted only in transient endocrine changes.

Melatonin treatment counteracts many of the pinealectomy-induced changes in gonadal function. Wurtman and coworkers first demonstrated that MT injection into female rats supresses growth and functional activity of the ovary (1963). Subsequently, MT treatment has been shown to delay vaginal opening (Collu et al, 1971), and depress the proportion of vaginal smears showing estrus smears (Chu et al, 1964). When administered during proestrus, it inhibits LH release and ovulation (Ying and Greep, 1973). When given to immature female rats, it inhibits gonadotropin induced ovulation (Longenecker and Gallo, 1971). In male rats, MT treatment decreases testis weight, spermatogenesis, and plasma testosterone levels (Kinson and Peat, 1971; Konig and Rega, 1978; Mas et al, 1979). It also inhibits testicular steroidogenesis in vitro (Ellis, 1972). These changes are accompanied by depression of FSH

and LH release and variable changes in the release of PRL (Mas et al, 1979; Vaughan et al, 1978; Hanew et al, 1980). In the neonatal rat, MT administration inhibits pituitary LH and FSH responses to LHRH (Martin et al, 1977).

1.9 Mechanisms of action of pineal melatonin

Information about the sites of action and subcellular mechanisms triggered by the hormone has been fragmentary. The major site of MT action is still controversial. Most investigators agree that the brain is probably a main target site of MT activity. Injection of [^{3}H]-MT is concentrated in several brain regions particularly the hypothalamus and the mid-brain (Cardinali et al, 1973; Anton-Tay and Wurtman, 1969). Melatonin administration can affect various brain metabolic functions and constituents (reviewed by Cardinali, 1981). These include protein synthesis, serotonin and γ-aminobutyric acid content, neurotransmitter uptake and release, axonal transport, tubulin levels and prostaglandin and neurohormone release. Hypothalamic LHRH content increased post MT administration (Leonardelli et al, 1978). This is consistent with a hypothalamic rather than a pituitary site of MT action. However, in neonatal rats, a pituitary site of action is likely since MT treatment suppresses LH and FSH responses to LHRH (Martin et al, 1977, 1980). Furthermore, there are also data supportive of a peripheral action of MT at the level of the endocrine glands or hormone target tissues. MT can prevent LH from binding to its receptors within the ovary Trentini et al, 1976). Melatonin also can act directly at the gonadal level to affect steroidogenesis (Ellis, 1972).

In order to understand the mechanism of action of MT, several investigators have attempted to identify MT receptors. High affinity binding sites have been identified in the membranous fraction of bovine medial basal hypothalamus (Cardinali et al, 1979). Specific MT binding sites also occur in membranes of occipital and cerebellar cortices and pineal glands (Cardinali et al, 1979). Additionally, cytoplasmic MT binding sites have been identified in rat hypothalamus, hippocampus, striatum and midbrain (Niles et al, 1979). Indeed, cytosol binding sites have also been found in peripheral tissues such as the ovary, uterus, testes, liver and eyes (Cohen et al, 1978). Whether these binding sites for MT reflect true MT receptor sites remain controversial. The binding characteristics are compatible with those expected for a receptor. Circumstantial evidence on the receptor nature of membrane MT binding sites in the brain has been provided by studies on the correlation of such binding sites with the neuroendocrine response to the hormone. The number of total MT binding sites in hamster and rat brains is 34-56% higher at 2000 h (time of enhanced sensitivity to MT action) than at 0700 h (Vacas and Cardinali, 1979b).

The post-binding events of MT action remain ill defined. There is evidence that MT may affect contractile protein-dependent processes (Banerjee et al, 1972, 1973). Melatonin has been shown to inhibit the effect of colchicine on the movement of pigment granules in amphibian melanocytes (Malawista, 1973). The hypothesis that MT binds to tubulin to mediate its action remains to be proven. Melatonin has also been shown to influence cyclic nucleotide levels.

Intraperitoneal administration of melatonin increases cAMP levels in the rat cerebella, while levels are lowered in the midbrain and unchanged in the cerebral cortex (Anton-Tay, 1974). Injection of MT into the cisterna magna causes a significant increase in the concentration of cGMP in the CSF of the rabbit (Rudman, 1976). The mechanism involved in producing MT's effects on central cyclic nucleotide levels remains unknown. Melatonin may affect either the adenylate cyclase responsible for cAMP synthesis or phosphodiesterase, the enzyme that breaks down cAMP. The latter is unlikely since effects on adenylate cyclase persist in the presence of theophylline (Niles, 1985). Alternatively, MT may exert its effect via the action of some other hormone or neurotransmitter. MT administration has been shown to alter brain serotonin and catecholamine levels (Pagel et al, 1976; Cardinali, 1975). Since specific adenylate cyclases are coupled with serotonin, dopamine and noradrenaline receptors in the brain, MT may exert its effect via any of these cyclases.

1.10 Potentiating factors

As discussed previously, the reproductive axis of the laboratory rat is relatively unresponsive to the action of the pineal. However, under certain experimental perturbations such as neonatal steroid treatment, olfactory bulbectomy or underfeeding, the gonadal system of both prepubertal and mature rats can be sensitized to the inhibitory action of the pineal (Reiter, 1974). These treatments have been referred to as potentiating factors. Their mechanisms of action are not well understood. It has been postulated that they act either by altering the MT secretory profile or by enhancing the target

tissue responsiveness to MT action. Animals subjected to food restriction are of particular interest since changes in the quantity and quality of food availability occur normally in animals kept in their natural habitat. The existing evidence for pineal-gonadal interaction in underfed animals will be summarized in the introductory section of Chapter 2.

The purpose of the present study, therefore, is to critically examine the pineal-gonadal interaction in calorie-restricted rats. Four specific issues have been investigated.

Experiment 1: Effect of duration of food restriction on the 24 hour serum and pineal melatonin rhythms.

Experiment 2: Effect of shortened photoperiod and pinealectomy on the reproductive axis of food restricted animals.

Experiment 3: Pineal beta-adrenergic responsiveness to isoproterenol stimulation in food restricted animals.

Experiment 4: Effect of food restriction on the 24 h rhythms of circulating tryptophan and serotonin.

CHAPTER TWO

EFFECT OF FOOD RESTRICTION ON THE 24 H RHYTHM OF SERUM AND PINEAL MELATONIN

2.1 Abstract

Food restriction is known to sensitize the reproductive axis of rats to the inhibitory action of the pineal and its hormone, melatonin (MT). The present study investigated the effect of food restriction on the 24 h rhythm of serum and pineal MT. Young adult male Wistar rats were subjected to either 1 or 3 weeks of 50% food restriction under a lighting regimen of 14 h light and 10 h dark. Body weight, testicular weight, accessory organ weights, serum LH, serum testosterone and 24 h rhythm of serum and pineal melatonin were determined. Both 1 and 3 weeks of food restriction resulted in a reduction of body weight, accessory organ weights and serum LH. Serum testosterone levels were only suppressed after 3 weeks of food restriction. When the 24 h profile of serum and pineal MT was determined, 1 week of food restriction had no effect on serum or pineal MT profile. By contrast, 3 weeks of food restriction led to an earlier rise in serum and pineal MT after the onset of darkness. Peak and mean night-time serum MT levels were also significantly increased. Hence, underfed animals were exposed to a prolonged duration of higher circulating melatonin levels. Such changes in the 24 h rhythm of serum MT in the underfed rats may explain why food restriction can sensitize the reproductive axis of rats to the inhibitory action of the pineal.

2.2 Introduction

In seasonal breeding animals such as hamsters and sheep, the pineal gland and its hormone, melatonin (MT), appear to regulate changes in reproductive function (Reiter, 1980; Lincoln and Short, 1980; Goldman and Darrow, 1983). By contrast, its role in nonseasonal breeding species such as the laboratory rat has not been well defined. The reproductive axis of rats is relatively unresponsive to the action of the pineal (Reiter, 1980; Goldman et al, 1981) except when this axis is sensitized by manipulations such as underfeeding, olfactory bulbectomy or neonatal steroid administration (Reiter, 1974). The mechanism of this sensitization has not been defined. Adult rats subjected to food restriction have a reduction in serum LH, TSH, GH and PRL levels (Campbell et al, 1977). This appears to be secondary to a lack of hypothalamic stimulation rather than an inability of the pituitary gland to secrete hormones (Campbell et al, 1977). A previous study by Piacsek and Meites indicated that the low gonadotropins in underfed rats can be stimulated by constant illumination suggesting that the pineal gland may be involved (1967). Sorrentino et al (1971) subsequently demonstrated that underfed rats are more sensitive to the antigonadal effects of the pineal gland by showing an ameliorative effect of pinealectomy on gonadal regression in blinded-underfed rats. Further studies by Walker et al have provided additional support for either enhanced sensitivity to the pineal gland or increased pineal activity in underfed animals (1977a, 1977b, 1978). Indeed, underfeeding acts in a synergistic fashion with exogenously administered MT on the reproductive axis of prepubertal rats (Blask et al, 1980). Despite this known

pineal-gonadal interaction during underfeeding, the effect of food restriction on the 24 h rhythm of circulating MT has never been determined. Limited studies have shown that short term starvation has no effect on urinary MT excretion (Lynch et al, 1975) and prepubertal rats subjected to protein calorie malnutrition have lower daytime and night-time pineal MT content (Herbert and Reiter, 1981). These findings do not necessarily reflect changes in circulating MT. Levels of circulating MT can be influenced by rate of synthesis, volume of distribution, as well as rate of metabolism, all of which may be altered in the underfed animal. Furthermore, 24 h rhythm studies give a better indication of changes in the underfed animal's duration and timing of MT exposure, parameters which have been shown to be critical in mediating the inhibitory action of MT on the gonads in Djungarian hamsters (Carter and Goldman, 1983). In order to understand the mechanism through which underfeeding sensitizes the rat reproductive axis to the action of the pineal, the objective of the present study is to assess the effect of varying duration of food restriction on the 24 h rhythms of serum and pineal MT content.

2.3 Material and Methods

Male Wistar rats weighing 160-180 g on arrival from Woodlyn Laboratories, Guelph, Ontario were housed individually in a temperature (22 $\pm$ 2°C) and humidity (46 $\pm$ 4%) controlled room with 14 h of light (0600-2000 h) per day. Metabolic cages with wire meshed bottom were used. Animals were divided into two groups of comparable weight, ad libitum fed controls (C) and 50% underfed (U). During the two week adjustment period, all rats received Purina rat chow (Ralston

Purina Canada, Longueuil, Quebec) and water ad libitum. The daily food intake of the rats was determined. Animals were weighed every 3-4 days. After the adjustment period, U animals were given half the food consumed daily by C animals. The experiment was divided into three parts.

Part 1: Acute effect of food restriction

Underfed animals were subjected to one week of food restriction. At the end of the study period, both C and U animals were killed by decapitation at 1300, 1700, 2100, 2300, 0130, 0400, 0600 and 0900 h (n = 6 per time point). Trunk blood was collected for the determinations of MT, LH and testosterone (T). The blood samples were allowed to clot overnight at 4°C, centrifuged at 4000 x g for 20 minutes and the serum collected was stored at -20°C. The pineal glands were dissected immediately, frozen on dry ice and stored at -20°C until assayed for MT. Testes, seminal vesicles and ventral prostates were dissected, blotted dry and weighed using a Mettler balance (Zurich, Switzerland). A dim red light (40 watts) was used for animals killed at night since rats have no perception of red light (Cardinali et al, 1972).

Part 2: Prolonged effect of food restriction

Experiment i - Preliminary study

Underfed rats were subjected to 50% food restriction for 3 weeks while control rats continued with food ad libitum. At the end of the study period, animals were killed by decapitation at 2200, 2400, 0200, 0400 and 0700 h (n = 6 per time point). Serum, pineal,

testes and accessory glands were processed as before.

Experiment ii - Extended study

Based on the result of the preliminary study, an extended study was carried out. After 3 weeks of food restriction, both C and U rats were killed by decapitation at 1400, 1800, 2030, 2130, 2230, 2330, 0200, 0430, 0530 and 0900 h (n = 6 per time point). Serum, pineal, testes and accessory glands were processed as before.

Hormone determinations

All standards were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise specified. Solvents were obtained from Fisher Scientific Co. (Waltham, MA). Serum and pineal MT were assayed by RIA as previously described (Ho et al, 1984). The intra- and interassay variations using a serum sample of 43.9 pg/ml were 6.9% and 13.4%. Rat LH was assayed with a kit supplied by the rat hormone distribution program of NIADDK. Iodination standard, NIADDK-I-6, antibody anti rLH-S7 and reference preparation rLH-RP-2 were used. The intra- and interassay variations for LH using a serum sample of 650 pg/ml were 7.4% and 12.8% respectively. Samples from the same experiment were assayed together. Serum T was determined by RIA without chromatography using [^{3}H] testosterone from New England Nuclear Corp. (Boston, MA) and antibody to testosterone-3-BSA which recognized 100% of 5 α-dihydrotestosterone (Bubenik et al, 1975). Testosterone standard was obtained from Steraloids Inc. (Wilton, NH). Recovery was found to be 80 $\pm$ 4%. Using a serum sample of 2.5 ng/ml, intra- and interassay variations were 3% and 6.8% respectively.

Statistical analyses were performed by Student's t-test for

group comparisons and by analysis of variance for the interaction between food availability and changes in pineal and serum MT levels.

2.4 Results

Initial body weights for both C and U animals were not significantly different for the three experiments (Table 1). After one week of food restriction, the difference in body weights between C and U animals was 18% ($p < 0.001$) (Table 1). While there was no change in absolute testicular weight, both absolute seminal vesicle and prostate weights were significantly reduced by 18% and 19% respectively ($p < 0.05$) (Fig. 5). This reduction in organ weights was accompanied by a 50% reduction in 24 h mean LH levels ($p < 0.001$) (Fig. 6) but no change in mean T levels. One week of food restriction also had no effect on either serum or pineal MT contents (Fig. 7). The 24 h rhythms of serum and pineal MT for both C and U animals were virtually superimposable.

For the 3 week experiments, similar weight reduction was achieved in the preliminary and the extended studies (35% and 36%) (Fig. 5). Since neither the changes in organ weights nor the reduction in LH and T differed significantly between the two studies, the data were pooled together. After 3 weeks of food restriction, absolute testicular weight was essentially unchanged. The reduction in absolute seminal vesicle and prostate weights were similar at 40% and 39% ($p < 0.001$) (Fig. 5). Unlike the 1 week study, 24 h mean T levels were significantly reduced ($p < 0.001$) (Fig. 6) after 3 weeks of food restriction and the 24 h mean LH levels showed a reduction of 68% ($p < 0.001$) (Fig. 6).

Even though the preliminary study was limited to five time points with mainly night-time sampling, U animals had elevated pineal and serum MT levels 2 h after the onset of darkness (Fig. 8). In U animals, peak pineal MT levels were lower (1706 $\pm$ 202 vs 2520 $\pm$ 228 pg/gland, $p < 0.05$) but mean night-time pineal MT levels remain unchanged (1795 $\pm$ 191 vs 1544 $\pm$ 148 pg/gland). By contrast, both peak serum MT (90 $\pm$ 7 vs 60 $\pm$ 6 pg/ml, $p < 0.05$) and mean night-time serum MT (78 $\pm$ 8 vs 50 $\pm$ 4 pg/ml, $p < 0.005$) were significantly higher. This pattern of changes in pineal and serum MT profiles were again observed in the extended study. Underfed animals demonstrated an earlier rise in both serum and pineal MT, which occurred only 90 minutes after the onset of darkness (Fig. 9). As in the preliminary study, peak pineal MT levels were lower but mean night-time pineal MT levels remained unchanged. By contrast, both peak serum MT (98 $\pm$ 4 vs 76 $\pm$ 6 pg/ml, $p < 0.05$) and mean night-time serum MT levels (62 $\pm$ 7 vs 41 $\pm$ 4 pg/ml, $p < 0.005$) were significantly higher in U animals. The interaction between availability of food and time of sampling for pineal MT ($F = 2.80$, $p < 0.01$) and serum MT ($F = 3.18$, $p < 0.01$) were also significant.

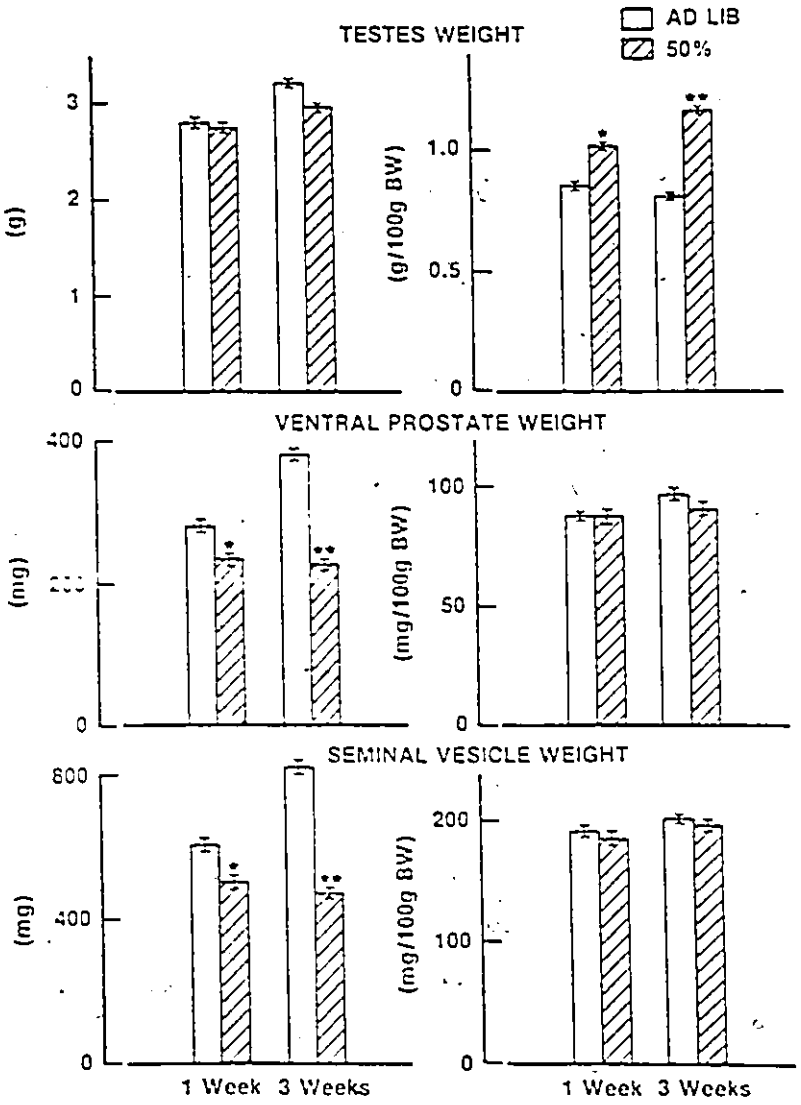
Table 1: Body weight after 1 and 3 weeks of 50% food restriction

Expt.	Group	Body Weight (g) Initial	1 week	3 weeks
1	Ad lib	280 ± 4	326 ± 10	
	50%	280 ± 4	268 ± 5*	
2(i)	Ad lib	275 ± 5	316 ± 10	380 ± 10
	50%	275 ± 5	262 ± 5*	250 ± 5*
2(ii)	Ad lib	284 ± 4	325 ± 10	393 ± 10
	50%	284 ± 4	269 ± 5*	254 ± 5*

Values are means ± SE, n = 48, 30, 60 for Expt 1, 2(i) and 2(ii) respectively.

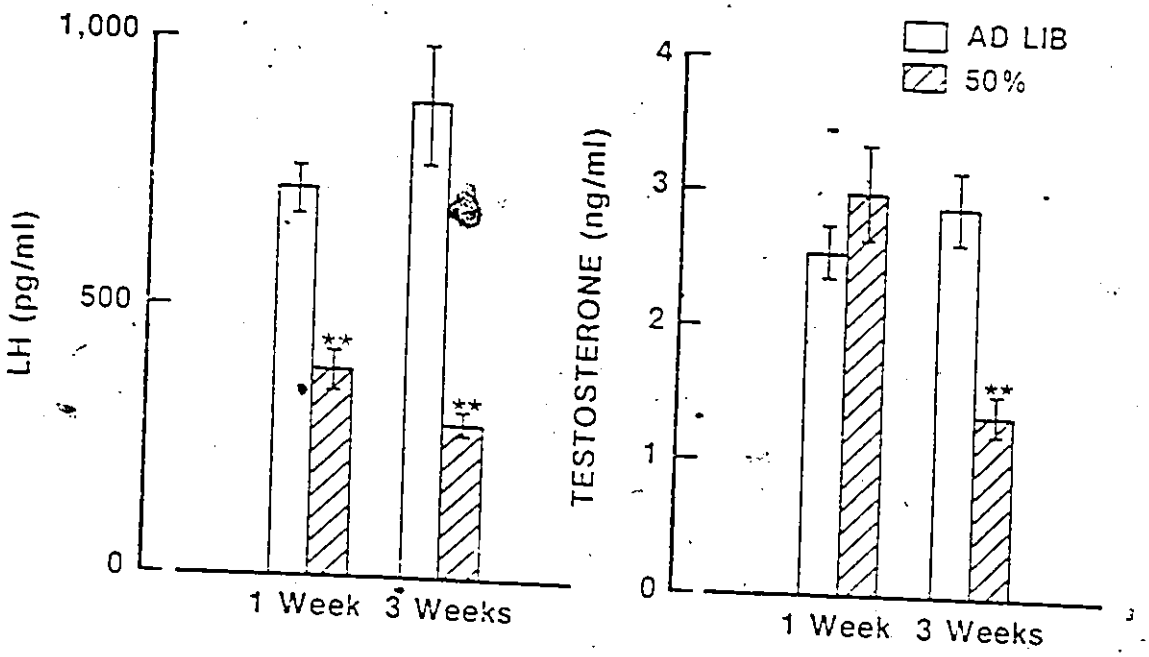
* $p < 0.001$, 50% underfed vs ad lib control

Figure 5 Effect of 1 and 3 weeks 50% food restriction on absolute and relative testicular weight, ventral prostate weight and seminal vesicle weight



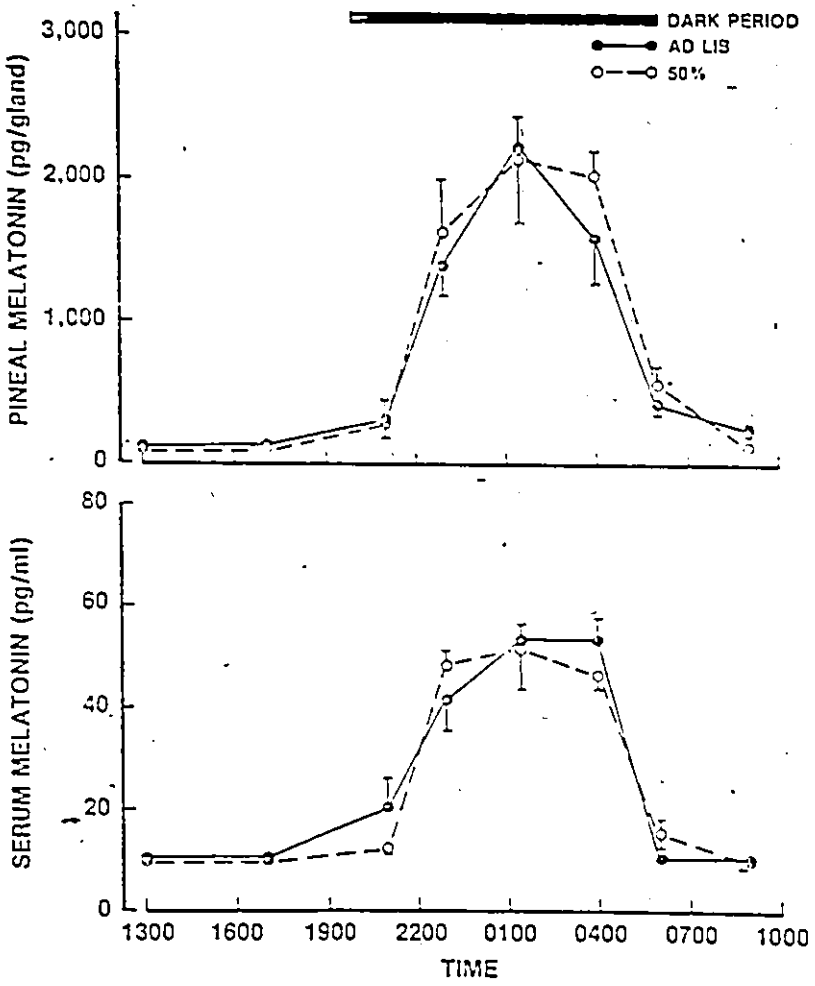
Values are means ± SE, n = 48 and 90 for 1 and 3 weeks respectively, * $p < 0.01$ and ** $p < 0.001$, 50% underfed vs ad lib control

Figure 6 Effect of 1 and 3 weeks 50% food restriction on serum LH and serum testosterone levels



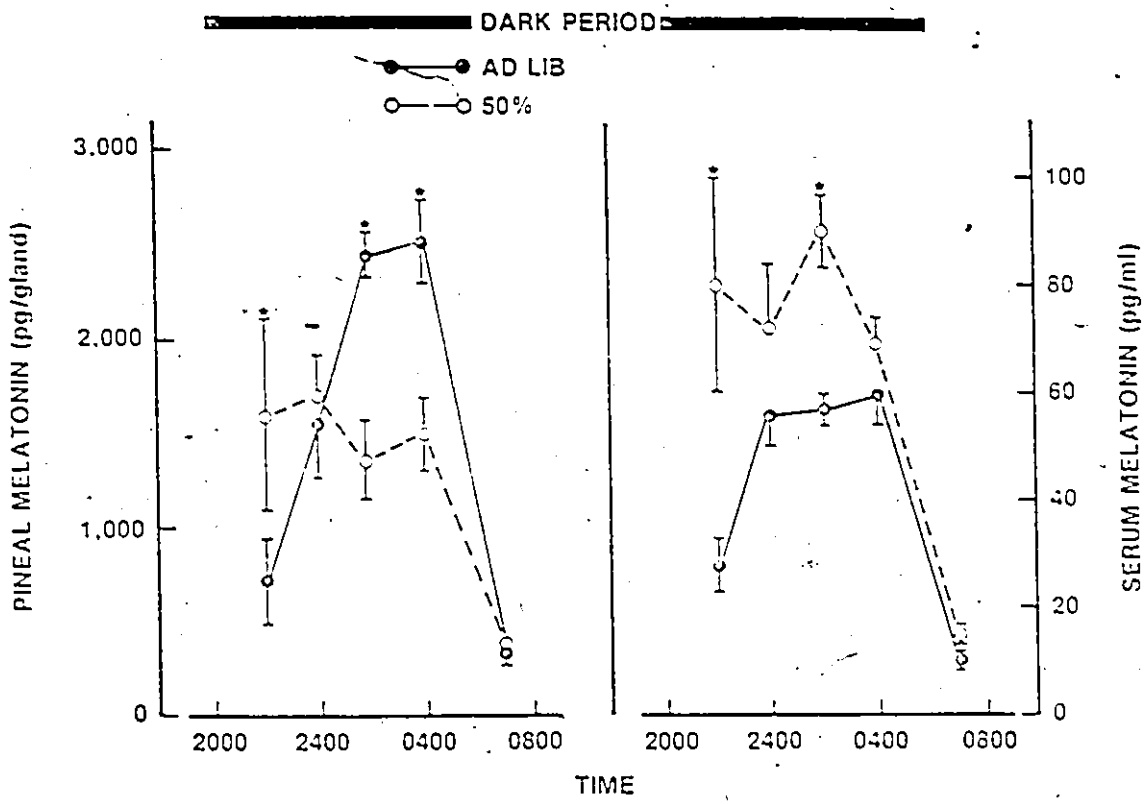
Values are means ± SE, n = 48 and 90 for 1 and 3 weeks respectively, ** $p < 0.001$, 50% underfed vs ad lib control

Figure 7 Effect of 1 week 50% food restriction on 24 h serum and pineal melatonin



Values are mean ± SE, n = 6

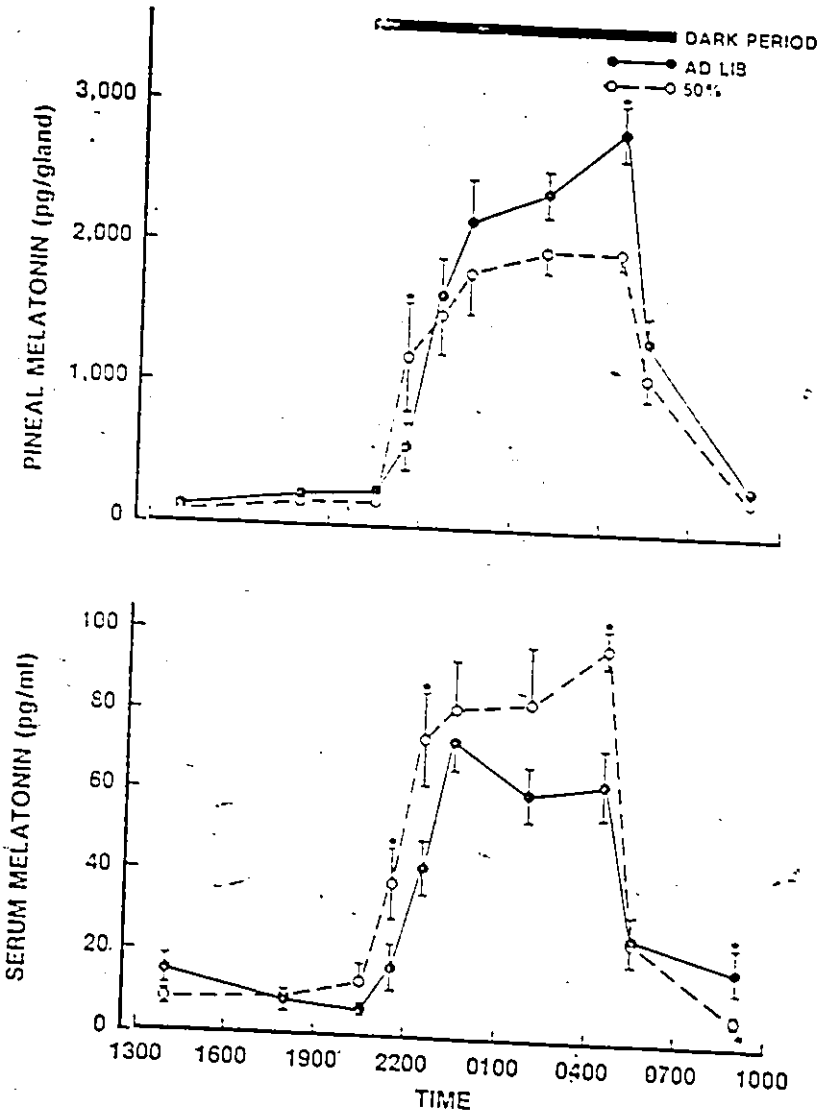
Figure 8 — Effect of 3 weeks 50% food restriction on serum and pineal melatonin (Preliminary study)



Values are means ± SE, n = 6;

* $p < 0.05$, 50% underfed vs ad lib control

Figure 9 Effect of 3 weeks 50% food restriction on 24 h serum and pineal melatonin (Extended study)



Values are means ± SE, n = 6;

* $p < 0.05$, 50% underfed vs ad lib control

2.5 Discussion

In the present study, rats were subjected to either 1 or 3 weeks of 50% food restriction. Based on preliminary studies, the duration of 3 weeks was chosen for the prolonged study since this was the period required for the body weight reduction to reach a plateau. One week was chosen for the acute study since the most dramatic weight loss occurred within 1 week of food restriction. The amount of food restriction was set at 50% based on the observation that when animals were given to 75% of food consumed by control fed animals, changes in absolute accessory organ weights were only modest and LH levels were not suppressed. By contrast, when animals were subjected to 25% of food restriction, underfed animals developed hair loss and were very lethargic. Furthermore, numerous previous studies have investigated the effect of 50% food restriction on the reproductive axis of rats (Grewal et al, 1971; Howland, 1975; Sorrentino et al, 1971).

After 1 week of food restriction, U animals had smaller seminal vesicles and ventral prostates as compared to C animals. These differences were further accentuated after 3 weeks of food restriction. The reduction in accessory organ weights however, was more a reflection of a further increment of organ sizes in C animals since the organ sizes in U animals were merely maintained at a lower level. This was confirmed by the changes in relative weights of the accessory organs. Control animals have increased relative weights whereas the relative weights of U animals remains unchanged. By contrast, similar changes were not observed in testicular weights. Neither food restriction schedule resulted in a reduction in absolute testicular size. The relative testicular weight which was already

increased after 1 week of food restriction showed a further increment after 3 weeks. Such changes in organ weights are in agreement with previously published data (Grewal et al, 1971; Howland, 1975). The changes in serum LH and T after 1 and 3 weeks of food restriction also confirmed the data previously reported by Howland (1975). While one week of food restriction had already resulted in LH suppression, T suppression required a longer period of restriction and was only observed after 3 weeks.

The effect of varying duration in food availability on pineal activity was of particular interest. After 1 week of food restriction, the 24 h rhythm of serum and pineal MT was not significantly different. Neither the amplitude nor the duration of the night-time MT peak was influenced by food restriction. This is consistent with the observation by Lynch et al (1975) that urinary MT remains unchanged after a short period of starvation in rats. Yet this brief period of food restriction has already resulted in the suppression of LH and smaller accessory glands. Taken together, these results are not supportive of circulating MT being responsible for the early changes observed above. Nevertheless, it is still possible that MT can mediate such changes at the tissue level since Cardinali and Vacas (1981) have previously demonstrated that malnourished rats have increased binding for MT sites in brain membranes after 15 days of protein restriction.

In contrast to the 1 week study, both the 24 h serum and pineal MT rhythms were significantly different when food restriction was prolonged for 3 weeks. Even though food restricted animals continued to demonstrate a nocturnal rise in both serum and pineal MT,

the amplitude and duration were significantly altered as compared to the ad libitum fed control. The duration was prolonged secondary to an earlier rise in both serum and pineal MT. In the pineal, this earlier rise was accompanied by an eventual lower peak which occurred 4 h after the onset of darkness. This earlier rise of pineal MT may reflect increased glandular activity which has previously been reported in chronically underfed rats (Walker et al, 1978). The magnitude of increase in pineal MT (five fold for U animals vs two fold for C animals) 90 min after the onset of darkness is also consistent with a hyperactive gland. With increased pineal activity, the turnover rate of pineal MT may also be increased resulting in lower pineal MT levels as observed in the present study. However, the changes in pineal activity may also relate to the chronic stress associated with food restriction. Reduced pineal MT has previously been described for animals subjected to chronic immobilization (Yocca and Friedman. 1984). A previous study on protein calorie malnourished rats by Herbert and Reiter (1981) also demonstrated pineal MT that was of lower amplitude. In that study, the rising phase of night-time pineal MT was delayed by 2 h, hence the duration of the night-time rise was shortened. The observed variation in duration may have arisen from differences in age, strain and method of food restriction (protein vs calorie).

As in pineal MT, the night-time rise in circulating MT was also prolonged in underfed animals. This is not unexpected since in rats, the 24 h rhythms of serum and pineal MT are normally highly correlated (Ho et al, 1984). However, of interest is that, in contrast to the lower amplitude of pineal MT, both peak and mean

night-time circulating MT levels were significantly higher in U animals. There are several possible explanations for this opposite trend. If the pineal gland is truly hyperactive with a higher turnover rate for MT, serum MT levels would be expected to be higher provided that there was no change in metabolic clearance. Alternatively, since the volume of distribution for MT is lower in the underfed animals with a 36% reduction in body weight, the slightly lower mean night-time MT could still account for higher circulating levels. Furthermore, the metabolic clearance of MT may be reduced during food restriction since other hormones such as corticosterone have been found to have a lower clearance rate (Fromweiler et al, 1968). The possibility of contribution to the circulating pool of MT by extra-pineal sources such as the retina and the Harderian gland also cannot be excluded (Ralph, 1981). Thus under this condition, simple measurement of pineal MT content cannot be used to represent the level of circulating MT which in any case is more relevant to its physiological function. Whether the changes in serum MT are related to the reduced quantity of food or the stress of food restriction remains to be determined.

Based on the present data, one cannot establish a causal relationship between the changes in pineal activity and the observed changes in LH, T, seminal vesicle and prostate sizes. Nevertheless, such changes in circulating night-time MT are consistent with an anti-gonadal action of MT. The enhanced amplitude of the nocturnal MT peak in the serum and the lengthening of the night-time MT rise may contribute to the lower LH and smaller accessory glands in the underfed animals. Starved rats have lower LH while the LHRH content

is increased in the median eminence (Pirke and Spyra, 1981). MT has been reported to inhibit the normal LH rise that is associated with ovulation (Reiter and Sorrentino, 1971; Longenecker and Gallo, 1971) and to increase the LHRH content in the rat hypothalamus (Leonardelli et al, 1978). In neonatal rats, Martin et al (1977, 1980) has documented an acute inhibitory effect of MT on pituitary LH and FSH responses to LHRH. Taken together, this higher circulating MT may contribute to LH suppression in the underfed animals. Furthermore, in Djungarian hamsters, Carter and Goldman have demonstrated that duration of MT exposure is the critical parameter mediating the inhibitory action of MT on the gonads (1983). Changes in the duration of MT infusion from 7 to 8 h (just 1 h difference) can account for a dramatic difference in testicular size in the hamster. In rats, MT administration to prepubertal animals whereby both amplitude and duration of the night-time MT rise are increased, can delay maturation of the gonads (Lang et al, 1983). Previously, Blask et al (1980) had also demonstrated enhanced sensitivity of the gonads of the underfed rat to MT administration in the evening and a protective effect of pinealectomy (Blask et al, 1981). Indeed, in our present study, rats subjected to 3 weeks of food restriction demonstrated an earlier activation of the pineal indicating that underfed animals were exposed to higher circulating MT levels of prolonged duration during periods of increased sensitivity.

In summary, while 1 week of food restriction had no effect on the 24 h serum and pineal MT rhythms, 3 weeks of food restriction resulted in an earlier pineal activation and higher night-time circulating MT levels. These findings suggest that in addition to

the environmental light/dark cycle, food availability is another factor that can influence the rhythm of circulating MT. Such changes in the circulating MT rhythm together with the previously demonstrated changes in MT sensitivity in malnourished rats may explain why underfeeding sensitizes the rat reproductive axis to the inhibitory action of the pineal.

CHAPTER THREE

EFFECT OF SHORTENED PHOTOPERIOD AND PINEALECTOMY ON UNDERFED RATS

3.1 Abstract

Food restriction is known to sensitize the reproductive axis of rats to the inhibitory action of the pineal gland and its hormone, melatonin. The present study investigated the effect of shortened photoperiod and pinealectomy on the undernutrition-related gonadal regression. In the first part of the study, adult male rats were subjected to 3 weeks of 50% restriction under a lighting regimen of 4 h light and 20 h dark. Body weight, testicular weight, accessory organ weights, serum LH, serum testosterone and the 24 h rhythm of serum melatonin (MT) were determined. Under shortened photoperiod, 3 weeks of 50% food restriction resulted in a reduction of body weight, accessory organ weights, serum LH and serum testosterone levels. By contrast, this treatment regimen had no significant effect on the 24 h serum MT profile based on sampling at 10 time points. In the second part of the experiment, the effect of pinealectomy was evaluated on animals subjected to 3 weeks of 50% food restriction under a lighting regimen of 14 h light and 10 h dark. Pinealectomy was found to partially reverse the gonadal inactivation observed in underfed animals. Underfed pinealectomized animals have larger accessory organs and higher serum testosterone levels as compared to underfed and underfed sham pinealectomized animals. The protective effect of pinealectomy suggests that the pineal gland is in part responsible for the undernutrition-related gonadal regression. The lack of effect of shortened photoperiod on the serum MT profile in underfed animals

indicates that the effect of food restriction on circulating MT depends on the environmental light/dark cycle.

3.2 Introduction

The reproductive axis of rats can be sensitized to the inhibitory action of the pineal and its hormone melatonin (MT) by underfeeding (Reiter, 1974). Previous studies have demonstrated that the gonadal regression and growth suppression which results from dietary restriction may in part be dependent upon the pineal gland (Sorrentino, 1971; Walker 1977a, 1977b). In the first experiment, it was found that, in addition to the environmental light/dark cycle, food availability is another factor that can influence pineal activity. Animals subjected to 3 weeks of 50% food restriction were found to be exposed to an increased duration of higher night-time circulating MT levels. Such an increased duration has previously been shown to be critically important in the photoperiod-related gonadal changes seen in seasonally breeding animals (Carter and Goldman, 1983). To further evaluate the role of the pineal during underfeeding, the effect of pinealectomy and shortened photoperiod on the undernutrition induced gonadal changes was determined. Pinealectomy is an effective way of removing circulating MT in rats (Lewy et al, 1980). In male rats, pinealectomy leads to premature growth of the ventral prostate and seminal vesicles in prepubertal animals (Roth, 1965) and enlarged accessory organs in adult animals (Motta et al, 1967). Under shortened photoperiod, the period with increased N-acetyltransferase activity lengthens (Illnerova and Vanecek, 1980) which in turn may account for the slight decrease of reproductive organ weights (Sorrentino and Benson, 1970).

3.3 Material and Methods

Part 1: Effect of photoperiod

Male Wistar rats weighing around 160 g on arrival from Woodlyn Laboratories, Guelph, Ontario were housed individually in a termperature (22 ± °C) and humidity (46 ± 4%) controlled room with 4 h of light (0800 - 1200 h) per day. Animals were divided into two groups of comparable weights, ad libitum fed controls (C) and 50% underfed (U). During the two week adjustment period, all rats received standard rat chow (Ralston Purina Canada, Longueil, Quebec) and water ad libitum. The daily food intake was determined. After the adjustment period, while the C animals continued to have food ad libitum, the U animals were given half the food consumed by the ad libitum fed animals. Underfed animals were subjected to 3 weeks of food restriction. At the end of 3 weeks, the C and U animals were alternately killed by decapitation at 1000, 1400, 1800, 2000, 2130, 2300, 0030, 0300, 0530 and 0730h (n = 7 per time point). Trunk blood was collected for the determinations of MT, LH and testosterone (T). The blood samples were allowed to clot overnight at 4 °C, centrifuged at 4000 X g for 20 minutes and the serum collected was stored at - 20 °C. Testes, seminal vesicles and ventral prostates were dissected out and weighed using a Mettler balance. A dim red light (40 Watt) was used for animals killed at night.

Part 2: Effect of pinealectomy

Three groups of male Wistar rats weighing around 200 g (control, pinealectomized, and sham pinealectomized) were obtained from Charles River Breeding Laboratories, Kingston, NY. Surgery was

performed one week prior to delivery. The animals were individually housed in a temperature and humidity controlled room with 14 h of light (0600 - 2000 h). Animals were divided into 5 groups. Group I, control, fed; Group II, pinealectomized, fed; Group III, control, underfed; Group IV, pinealectomized, underfed; and Group V, sham pinealectomized, underfed. After 2 weeks of adjustment, while fed animals continue to have food ad libitum, underfed animals were given half the food consumed by the ad libitum animals. At the end of 3 weeks, animals were killed by decapitation between 1200 and 1400 h. The following were determined: body weight, testes weight, ventral prostate weight, seminal vesicle weight, serum LH and serum T.

Hormone determinations

All standards were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise specified. Solvents were obtained from Fisher Scientific Co. (Waltham, MA). Serum MT was assayed by RIA as previously described (Ho et al, 1984). The intra- and interassay variations using a serum sample of 43.9 pg/ml were 6.9% and 13.4%. Serum LH and T were assayed by RIA as described in the method section of Chapter 2. The intra- and interassay variations for LH using a serum sample of 650 pg/ml were 7.4% and 12.8% respectively. Samples from the same experiment were assayed together. For T, using a serum sample of 2.5 ng/ml, intra- and interassay variations were 3% and 6.8% respectively.

Statistical analysis was performed by Student's t-test for group comparisons and by analysis of variance for the interaction between food availability and time of sampling for serum MT.

3.4 Results

Part 1: Effect of Photoperiod

After 3 weeks of food restriction, the difference in body weight between C and U animals was 35% (377 ± 5 vs 245 ± 3 g, $p < 0.001$). While there was no change in absolute testicular weight, both absolute ventral prostate and seminal vesicle weights were reduced by 38% in U animals (Table 2). By contrast, the relative testicular weight was increased but relative ventral prostate and seminal vesicle weights remained unchanged. Such changes in organ weights were accompanied by suppressed serum LH and serum T levels (Table 3).

Under shortened photoperiod with 4 h light and 20 h dark, serum MT increased 9 1/2 - 11 h after the onset of darkness with peak levels observed at 0300 h in C animals (Fig 10). As in C animals, serum MT also increased 9 1/2 h - 11 h after the onset of darkness with peak levels observed at 2300 h in U animals. By analysis of variance, there was no difference in serum MT profile between C and U animals. Peak MT (64.8 ± 18.4 vs 48.1 ± 8.5 pg /ml, $p < 0.1$) and mean serum MT levels of the night-time rise (49.2 ± 4.9 vs 39.0 ± 4.1 pg/ml, $p < 0.2$) were also not significantly different.

Part 2: Effect of pinealectomy

After 3 weeks of food restriction, there was no difference in body weights between Group I and II (396 ± 11 vs 398 ± 8 g) or Groups III - V (262 ± 2, 262 ± 3, 260 ± 3 g). The difference in body weight between fed and underfed animals was 34% ($p < 0.001$).

In the ad libitum fed animals, pinealectomy resulted in

In the ad libitum fed animals, pinealectomy resulted in significant increases in both absolute and relative seminal vesicle weights (Fig. 11) but had no effect on serum LH (Table 4). In food restricted animals, sham pinealectomy resulted in the greatest reduction in absolute testicular and accessory organ weights (Fig 11 - 13). By contrast, pinealectomized underfed animals had significantly higher absolute seminal vesicle weight ($p < 0.05$) and ventral prostate weight ($p < 0.01$) but these organ weights were still lower than the ad libitum fed animals. The underfed pinealectomized rats also had significantly higher serum T levels ($p < 0.05$) whereas LH remained suppressed.

Table 2: Effect of 3 weeks 50% food restriction on organ weights under a lighting regimen of 4 h light and 20 h dark

	Control	Underfed
Testicular weight (TW,g)	3.02 ± .04	2.92 ± .07
Prostatic weight (PW,mg)	351 ± 19	219 ± 13**
Seminal Vesicle weight (SW,mg)	756 ± 44	476 ± 30**
Rel TW (mg/100g BW)	804 ± 14	1191 ± 29**
Rel PW (mg/100g BW)	93 ± 5	89 ± 5
Rel SW (mg/100g BW)	199 ± 11	194 ± 12

Each value represents mean ± SEM, n = 28;

** $p < 0.001$, control vs underfed

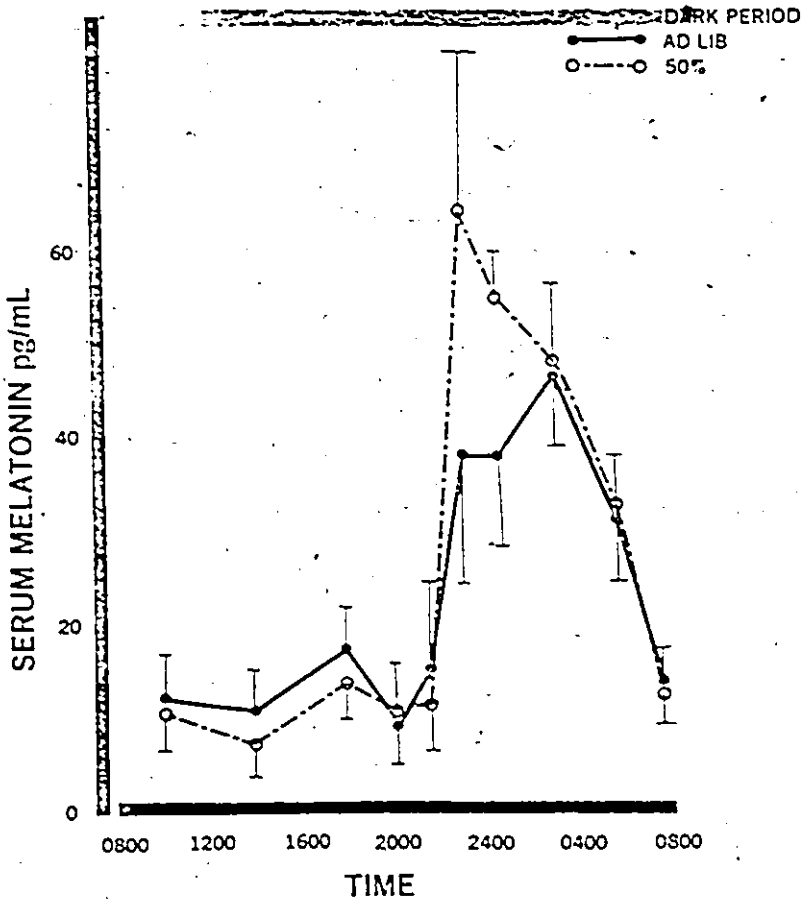
Table 3: Effect of 3 weeks 50% food restriction on serum LH and serum testosterone levels under a lighting regimen of 4 h light and 20 h dark

	Control	Underfed
LH (pg/ml)	1063 ± 192	797 ± 141*
Testosterone (ng/ml)	2.95 ± .35	1.71 ± .22**

Each value represents mean ± SEM, n = 70;

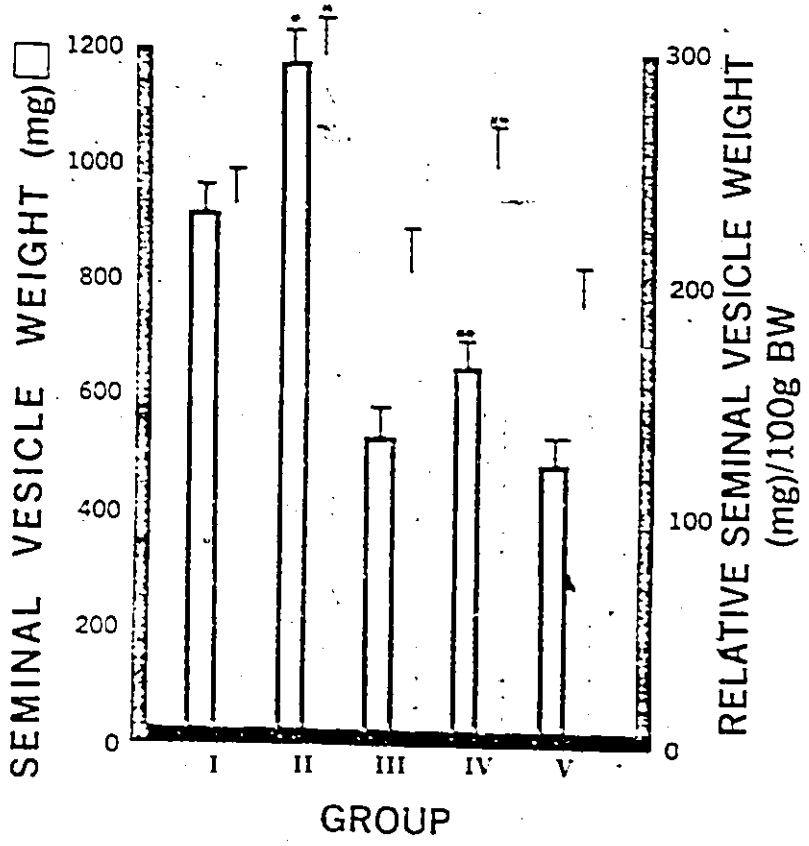
* $p < 0.05$, ** $p < 0.005$, control vs underfed

Figure 10 Effect of 3 weeks 50% food restriction on serum and pineal melatonin under a lighting regimen of 4 h light and 20 h dark



Values are means ± SE, n = 7;

Figure 11 Effect of pinealectomy on absolute and relative seminal vesicle weight after 3 weeks of 50% food restriction

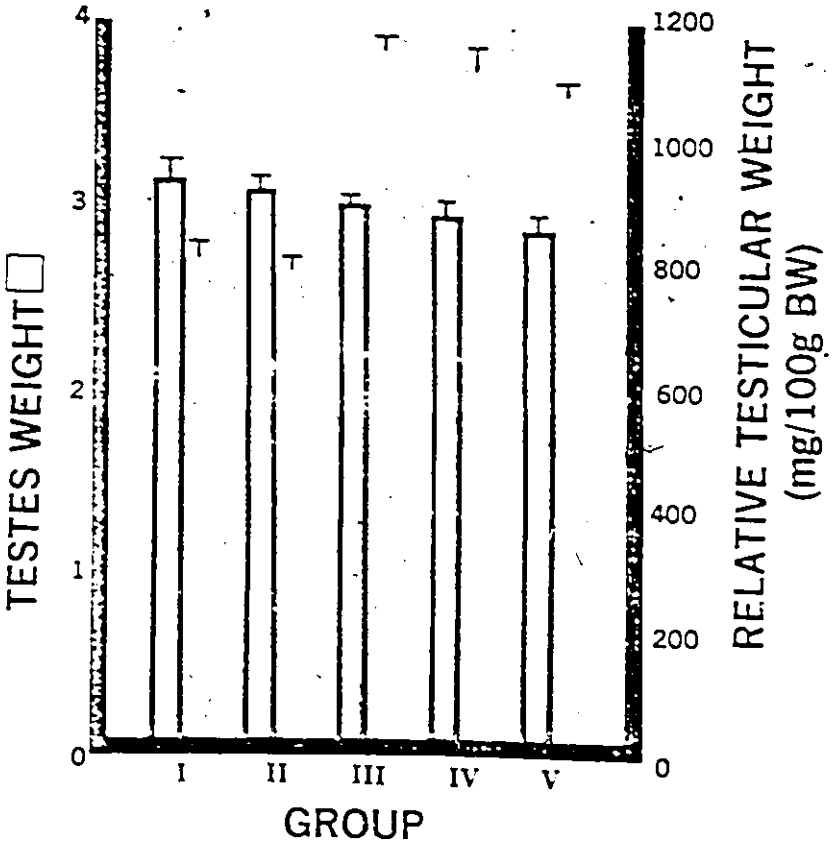


Values are means ± SE, n = 8

* $p < 0.01$, II vs I

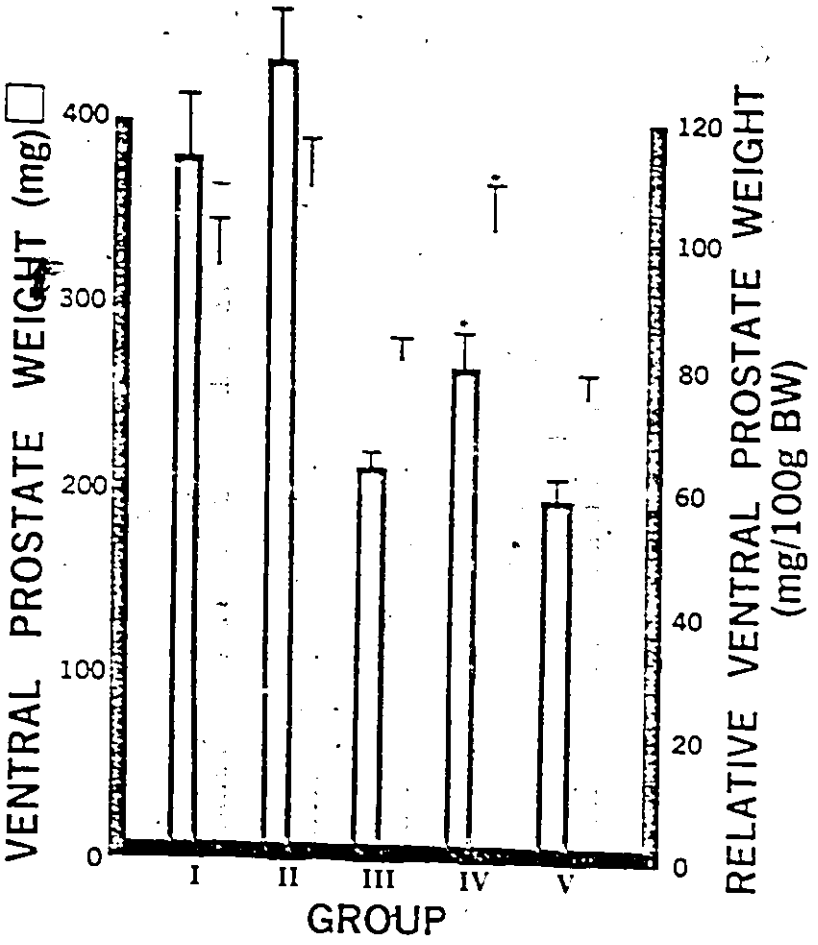
** $p < 0.05$, IV vs III and IV vs V

Figure 12 Effect of pinealectomy on absolute and relative testicular weight after 3 weeks of 50% food restriction



Values are means ± SE, n = 8

Figure 13 Effect of pinealectomy on absolute and relative ventral prostate weight after 3 weeks of 50% food restriction



Values are means ± SE, n = 8

* $p < 0.01$, IV vs III and IV vs V

Table 4: Effect of pinealectomy (Px) on serum LH and testosterone levels after 3 weeks of 50% food restriction

Groups		LH (pg/ml)	Testosterone (ng/ml)
I	Control	885 ± 145	2.03 ± .30
II	Control, Px	808 ± 171	3.48 ± .58 *
III	Underfed	425 ± 138	1.32 ± .42
IV	Underfed, Px	372 ± 34	3.33 ± .58 **
V	Underfed, sham Px	312 ± 113	1.48 ± .63

Each value represents mean ± SEM, n = 5

* $p < 0.05$, II vs I

** $p < 0.05$, IV vs III and IV vs V

3.5 Discussion

In the first part of the present study, rats were subjected to 3 weeks of 50% food restriction under a lighting regimen of 4 h light and 20 h dark (4/20). Under this treatment regimen, a 35 % reduction in body weight was accompanied by a 38% reduction in both absolute ventral prostate and seminal vesicle weight. The severity of the gonadal regression observed in U animals in this study was similar to animals subjected to 3 weeks of 50% food restriction under a lighting regimen of 14 h light and 10 h dark (14/10, Chapter 2). As in the 14/10 study, the gonadal regression was also accompanied by suppressed serum LH and serum testosterone levels. However, unlike the 14/10 study, there was no difference in serum MT profile between C and U animals. Both groups of animals demonstrated increased MT levels beginning 9 1/2 - 11 h after the onset of darkness. In C animals, peak MT levels were observed 15 h after the onset of darkness followed by a decline over the next 4 hours. The total duration of the night-time rise was approximately 10 hours. In U animals, serum MT levels increased sharply to a peak 9 1/2 - 11 h after the onset of darkness followed by a decline over the next 8 hours. As in C animals, the duration of the night-time rise was approximately 10 hours. Therefore, under shortened photoperiod, both C and U animals were subjected to a longer duration of night-time MT rise. This lengthening is similar to the previously reported increase in duration of pineal N-acetyltransferase activity under shortened photoperiod (Illnerova and Vanecek, 1980). Peak MT levels also were not significantly different between C and U animals. Hence, based on the present sampling frequency, no major differences in either amplitude

or duration of the night-time circulating MT profile were detected between C and U animals. However, since a 90 - 120 min sampling interval was used for the rise and decline phases of the night-time MT, it is possible that a subtle change in the duration of the night-time peak would be overlooked using this protocol. Moreover, the effect of varying length of food restriction was not assessed in this experiment. Therefore, it is still possible that by increasing the frequency of sampling or varying the duration of food restriction, shortened photoperiod may have an influence on the serum MT profile. Alternatively, it is possible that the changes in the serum MT profile secondary to shortened photoperiod and food restriction might be similar in nature but not be additive. Under a lighting regimen of 14 h light and 10 h dark (Chapter 2), food restriction led to an increase in duration of night-time serum MT rise by 1 hour (approximately from 8 to 9 hours). However, under the shortened photoperiod, control fed animals are exposed to a longer duration of the night-time rise of serum MT (approximately 10 hours). Therefore, it is possible that the increase in duration of serum MT secondary to food restriction which occurs under 14/10 may be negated by the effect of shortened photoperiod on serum MT profile.. This hypothesis could be tested by investigating further the effect of food restriction on serum MT profile under various lighting regimens.

In the second part of the study, the effect of pinealectomy on undernutrition-related gonadal regression was assessed. In control fed animals, pinealectomy had no effect on body weight, absolute testicular weight or serum LH levels. By contrast, absolute seminal vesicle weight and serum testosterone levels were significantly

increased ($p < 0.01$ and $p < 0.05$ respectively). Such changes in accessory organ weights and serum testosterone levels are consistent with previously reported data (Motta et al, 1967; Kinson and Peat, 1971). In underfed animals, the reduction in body weight, absolute accessory organ weights, serum LH and serum testosterone levels were not significantly different between underfed and underfed sham pinealectomized animals. By contrast, underfed pinealectomized animals had significantly larger accessory organs and higher serum testosterone levels though there was no difference in the reduction in body weight and serum LH levels. Since serum testosterone increased in the absence of any changes in the suppressed LH levels, the protective effect of pinealectomy on gonadal regression may reside at the testicular level especially in view of the previously demonstrated inhibitory effect of MT on steroidogenesis by Ellis (1972). However, pinealectomy failed to reverse completely the gonadal regression in underfed animals. These findings suggest that if the pineal is solely responsible for the gonadal regression observed in underfeeding, compounds with progonadal activities have been removed by pinealectomy.

In summary, under a shortened photoperiod with 4 h light and 20 h dark, 3 weeks of 50% food restriction had no influence on serum MT profile. The undernutrition-related gonadal regression, however, depends in part on the pineal gland since pinealectomy resulted in larger accessory organs and higher serum testosterone levels in food restricted animals.

CHAPTER FOUR

ALTERED PINEAL BETA-ADRENERGIC RESPONSIVENESS TO ISOPROTERENOL

4.1 Abstract

Adult male rats were subjected to 4 weeks of 50% food restriction under a lighting regimen of 14 h light and 10 h dark. The pineal response to isoproterenol (ISO) was determined in order to assess β-adrenoceptor-responsiveness. In a time course study, animals were injected with 0.5 mg/Kg ISO subcutaneously (SC) and killed at different times up to 180 min post injection. In a dose response study, various doses of ISO (0.2 mg/Kg to 5.0 mg/Kg) were injected intraperitoneally (IP) and animals were killed 120 min post injection. Body weight, testicular weight, accessory organ weights, pineal N-acetyltransferase (NATase), pineal and serum melatonin (MT) were determined. After 4 weeks of restricted feeding, a 40% reduction in body weight was accompanied by a 50% reduction in absolute accessory organ weights. The pineal response to ISO stimulation was altered. In the time course study, peak pineal NATase occurred 120 min post injection in the ad libitum fed animals. By contrast, the food restricted animals showed a gradual increase in pineal NATase up to 180 min post injection. In the dose response study, the ad libitum fed animals demonstrated a dose dependent increase in pineal NATase up to 5 mg/Kg dose. The food restricted animals, however, achieved their maximal pineal NATase at 1 mg/Kg dose with no further increment at 5 mg/Kg dose. These differences in responsiveness were also reflected in pineal and serum MT levels. The mechanism of this altered pineal β-responsiveness to ISO remains to be determined.

4.2 Introduction

In the pineal gland, serotonin is N-acetylated by serotonin-N-acetyltransferase (NATase) (Weissbach et al, 1960). The N-acetylserotonin (NAS) thus formed is O-methylated by hydroxyindole-O-methyltransferase to form melatonin (MT) (Axelrod and Weissbach, 1960). The formation of MT depends on the synthesis and activity of the enzyme NATase (Klein and Weller, 1970) which in turn is regulated by the sympathetic neuronal input acting on the pineal β-adrenoceptors (Deguchi and Axelrod, 1972b). Activation of the β-receptors either through neuronal release of norepinephrine or injection of the β-agonist, isoproterenol (ISO) will initiate a sequence of events culminating in the induction of NATase. Such induction leads to the production of pineal NAS and MT (Axelrod et al, 1969; Brownstein et al, 1973). Since the pineal gland is the major source of circulating MT (Lewy et al, 1980), increase in pineal MT leads to higher circulating MT. Recent evidence has indicated that food availabililty can influence sympathetic nervous activity, β-adrenoceptor properties and β-receptor mediated responsiveness (Avakian and Horvath, 1981; Burns et al, 1979; Katovich and Barney, 1983; Landsberg and Young, 1978; Stone, 1983). This, together with the known sensitizing effect of food restriction on the rat reproductive axis to the inhibitory action of the pineal gland and its hormone, MT (Reiter, 1974) indicates a possible interaction between food restriction and pineal β-receptor activity. The effect of 4 weeks 50% food restriction on pineal NATase, pineal and serum MT to ISO stimulation was therefore investigated in the present study.

4.2 Material and methods

Animals

Male Wistar rats weighing 160 - 180 g on arrival from Woodlyn Laboratories, Guelph, Ontario were housed individually in a temperature (22 ± 2°C) and humidity controlled room with 14 h of light (0700 - 2100 h) per day. Animals were divided into two groups of comparable weight, ad libitum fed controls (C) and 50% underfed (U). During the two week adjustment period, all rats received standard rat chow (Ralston Purina Canada, Longueuil, Quebec) and water ad libitum. The daily intake of the rats was determined. Animals were weighed every 3 - 4 days. After the adjustment period, U animals were given half the food consumed by C animals. At the end of 4 weeks, a dose-response and a time-course study to ISO were carried out. To maximize the response of the pineal to ISO, all the injections in the following experiments were administered at the onset of darkness (i.e. 2100 h). This time was chosen for its maximal pineal ß-adrenoceptor concentration (Romero et al, 1975) as well as maximal responsiveness in the synthesis of MT to ISO stimulation (Romero and Axelrod, 1974). After ISO stimulation, the animals were kept under continuous lighting until time for decapitation so as to eliminate the dark induced activation of pineal MT synthesis.

Part 1: Time-course study

Both C and U animals were given either subcutaneous (SC) injections of saline as control or 0.5 mg/Kg dose of ISO dissolved in saline. Saline treated animals were killed by decapitation at 0, 60

and 180 min after the injection (n = 5 for each time). The ISO treated animals were killed at 30, 60, 120 and 180 min after the injections. The pineal glands were dissected, frozen on dry ice and stored at -70°C until assayed for MT and NATase activity. Trunk blood was collected for MT determinations. The blood samples were allowed to clot overnight at 4°C, centrifuged at 4,000 X g for 20 minutes and the serum collected was stored at - 20°C until assayed for MT. The effects of food restriction on testicular weight, ventral prostate and seminal vesicle weights from time 0 and 180 min were determined.

Part 2: Dose-response study

Groups of animals (n = 5 per group) were injected intraperitoneally (IP) with either saline or different doses of ISO (0.2mg, 0.5mg, 1.0mg and 5.0mg/Kg). The method of injection was changed to IP since more animals can be injected in a shorter time period using this route of administration. The animals were killed 120 min afterwards. The collection and storage of pineal glands and serum samples were as described in the time course study.

Determination of serum and pineal MT

All standards were obtained from Sigma Chemical Co. (St. Louis, MO) and solvents from Fisher Scientific Co. (Waltham, MA). Serum and pineal MT were assayed by RIA as described previously (Ho et al, 1984). The intra- and interassay variations using a serum sample of 43.9 pg/ml were 6.9% and 13.4%.

Determination of pineal NATase activity

The NATase activity of the pineal gland was determined by a modification (Parfitt et al, 1975) of the method of Deguchi and Axelrod (1972c) within 24 h of decapitation.

Statistical analysis was performed by Student's t-test for group comparison.

4.4 Results

After 4 weeks of 50% food restriction, body weight was reduced by 40% ($p < 0.001$) (Table 5). This was accompanied by a 50% reduction in both absolute prostate and seminal vesicle weights ($p < 0.001$). The absolute testicular weight was not influenced by this duration of food restriction while relative testicular weight was increased ($p < 0.001$). Such changes in organ weights are in agreement with previously reported studies (Grewal et al, 1971; Howland, 1975).

Part 1: Time-course study

The results of the time course study of saline and ISO treated C and U animals are shown in Fig. 14 - 16. The saline treated C and U animals did not demonstrate the normal nocturnal rise in pineal NATase, pineal and serum MT since they were suppressed by continuous illumination post injection. Post 0.5mg/Kg SC ISO stimulation, the ad libitum fed animals demonstrated parallel increases in pineal NATase and pineal MT which began at 60 min post injection, peaked at 120 min and declined by 180 min. Serum MT responded similarly to ISO injection with the exception that increased serum MT ($p < 0.01$) was observed 30 min post ISO stimulation. In U animals, two major

differences in response to ISO stimulation were observed. Pineal NATase and pineal MT were both significantly lower 120 min post ISO injection ($p < 0.05$, as compared to the ad libitum fed animals). Instead of showing a decline at 180 min, pineal NATase was further increased at 180 min post ISO injection ($p < 0.05$). This higher pineal NATase activity was accompanied by persistent elevation of both pineal and serum MT in U animals.

Part 2: Dose-response study

In C animals, pineal NATase, pineal and serum MT responded in a dose dependent manner from 0.2 to 5.0 mg/Kg ISO (Fig. 17 - 19). However, in U animals, pineal NATase, pineal and serum MT responded maximally at 1.0mg/Kg and showed no further increase with 5.0mg/Kg.

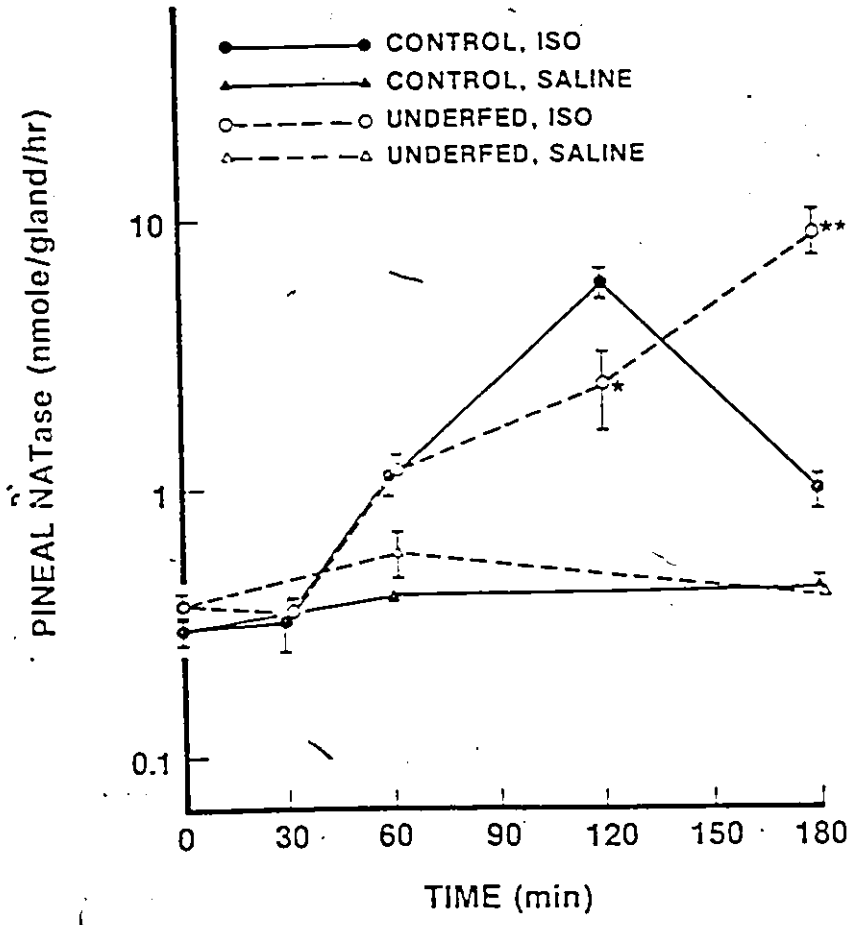
Table 5 : Effect of 4 weeks 50% food restriction on organ weights under a lighting regimen of 14 h light and 10 h dark

	Control	Underfed
Body Weight (BW,g)	430 ± 10	260 ± 10**
Testicular Weight (TW,g)	3.10 ± .06	2.96 ± .06
Prostatic Weight (PW,mg)	435 ± 20	220 ± 10**
Seminal Vesicle Weight (SW,mg)	960 ± 54	483 ± 41**
Rel TW (mg/100g BW)	700 ± 40	1150 ± 30**
Rel PW (mg/100g BW)	100 ± 6	86 ± 5
Rel SW (mg/100g BW)	216 ± 9	187 ± 14

Each value represents mean ± SEM, n = 15;

** $p < 0.001$, control vs underfed

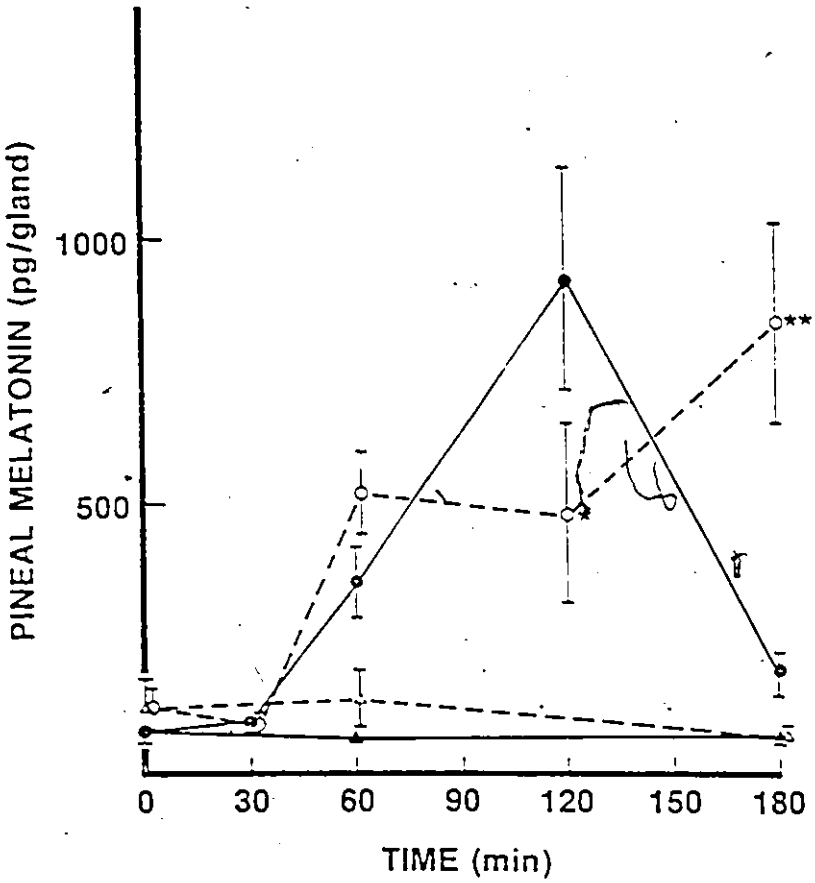
Figure 14 Time course response of pineal N-acetyltransferase to 0.5 mg/Kg isoproterenol



Values are means ± SE, n = 5,

* $p < 0.05$ and ** $p < 0.001$, 50% underfed vs ad lib control

Figure 15 Time course response of pineal melatonin to 0.5 mg/Kg isoproterenol

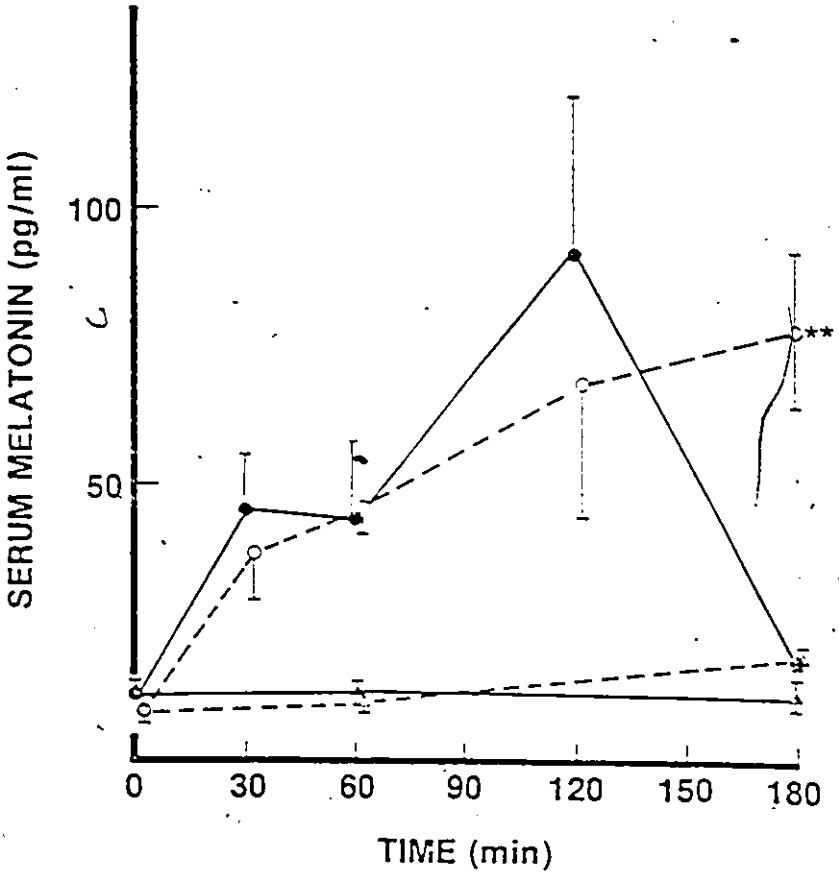


Values are means ± SE, n = 5;

* $p < 0.05$ and ** $p < 0.001$; 50% underfed vs ad lib control

Symbols as in Figure 14

Figure 16 Time course response of serum melatonin to 0.5 mg/Kg isoproterenol

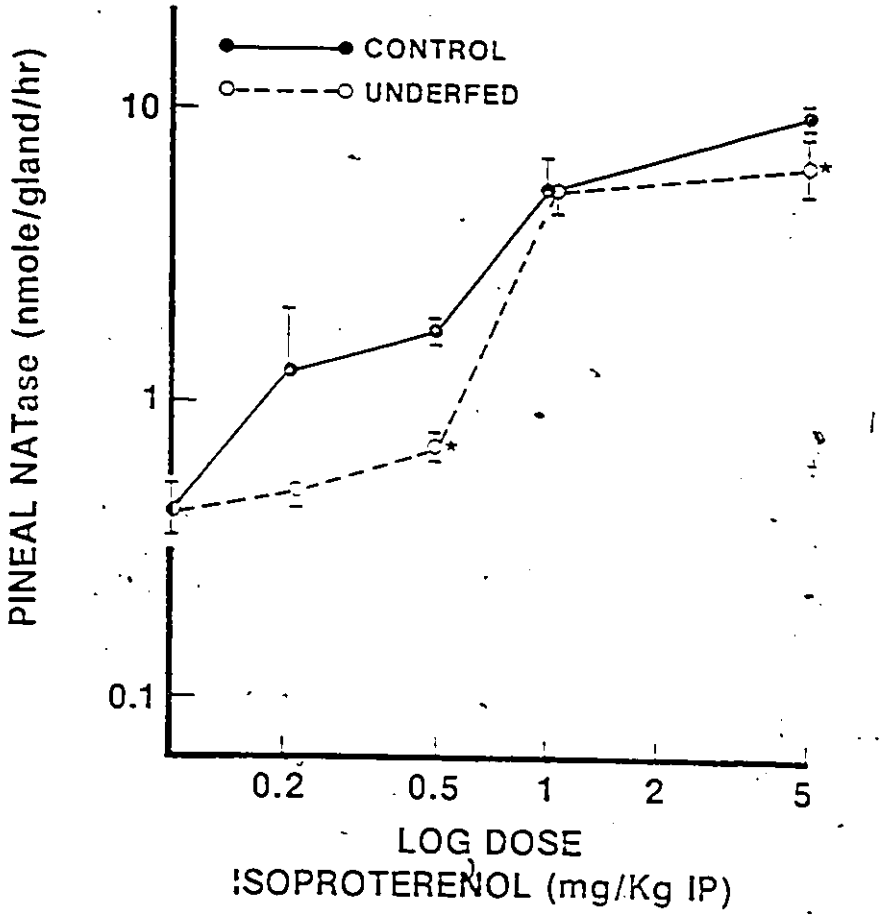


Values are means ± SE, n = 6;

** $p < 0.001$, 50% underfed vs ad lib control

Symbols as in Figure 14

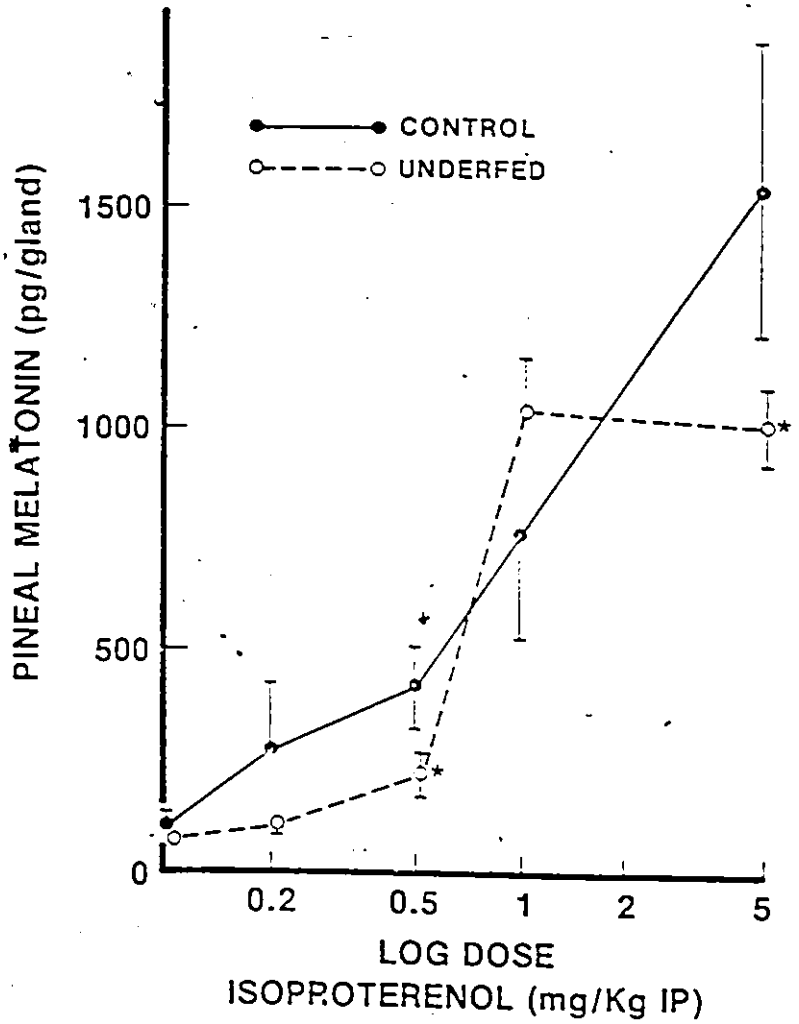
Figure 17 Dose response of pineal N-acetyltransferase 2 h post isoproterenol stimulation



Values are means ± SE;

* $p < 0.05$, 50% underfed vs ad lib control

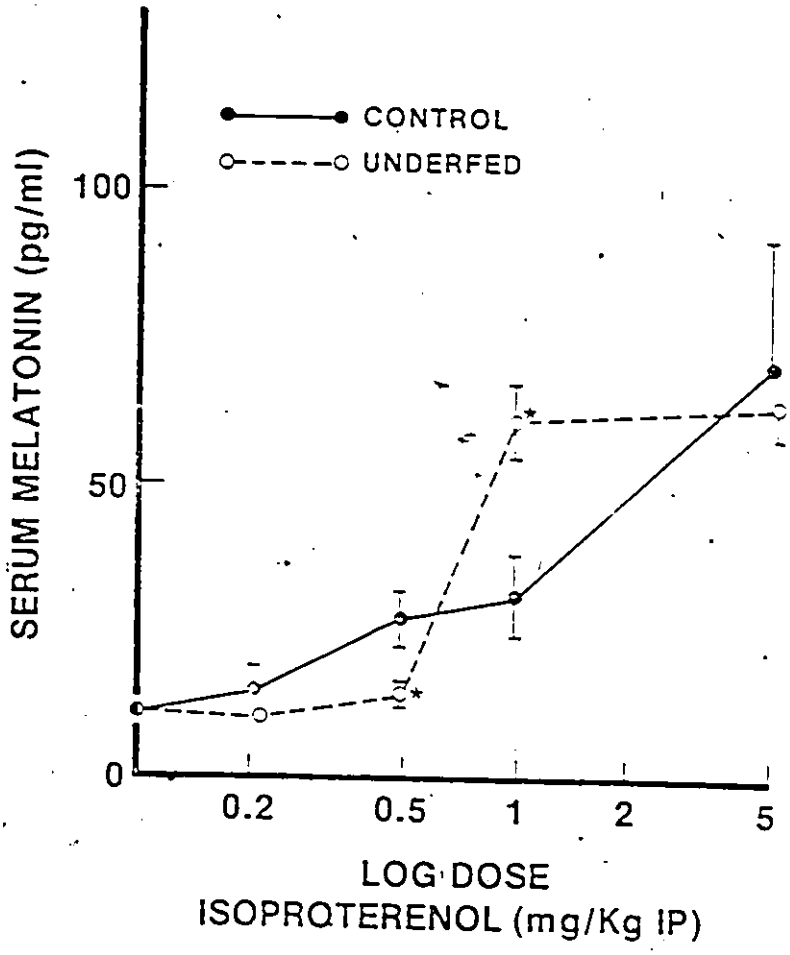
Figure 18 Dose response of pineal melatonin 2 h post isoproterenol stimulation



Values ± SE, n = 5;

* $p < 0.05$; 50% underfed vs ad lib control

Figure 19 Dose response of serum melatonin 2 h post isoproterenol stimulation



Values ± SE, n = 5;

* $p < 0.05$; 50% underfed vs ad lib control

4.5 Discussion

Pineal MT response to ISO stimulation resides in the mechanisms regulating the various steps involved in the synthesis of MT (Zatz et al, 1978). Hence, changes in the properties of the β-adrenoceptors, coupling of the receptor to adenylate cyclase or adenylate cyclase activity and other post-receptor events can lead to abnormal NATase induction. Furthermore, factors regulating HIOMT activity can also influence the ultimate pineal MT response to ISO stimulation. The present study indicates that after 4 weeks of 50% food restriction, underfed animals have an altered time-course and dose-response to ISO stimulation. The observed changes are, however, atypical for either a sub- or supersensitive pineal gland.

For C animals, both time-course and dose-responses are in agreement with previously published data (Illnerova and Vanecek, 1983; Klein et al, 1983; Romero and Axelrod, 1975; Zatz et al, 1978). A parallel relationship between pineal NATase and pineal MT was observed. This is not unexpected since these determinations are normally closely related (Ho et al, 1984). Serum MT also responded similarly with the exception that increased levels were observed 30 min post ISO stimulation in the absence of detectable increases in pineal MT content and enzyme activity. Both pineal NATase and pineal MT only increased 60 min post ISO stimulation. A similar elevation of serum MT was also evident in underfed animals 30 min post ISO injection. This increased serum MT in the absence of increased pineal NATase activity is unusual since, in rats, the pineal gland is the major source of circulating MT (Lewy et al, 1980). However, under the widespread stimulation of β-receptors by ISO, it is possible that

other tissues which contain MT and/or MT synthesizing capacity (Ralph, 1981) and are regulated similarly may respond by releasing a substantial amount of MT to the circulation.

In the time course study, pineal NATase, pineal and serum MT peaked at 120 min and declined by 180 min post ISO stimulation. By contrast, underfed animals demonstrated progressive increases in pineal NATase, pineal and serum MT and high levels were maintained 180 min post ISO stimulation. This pattern of response in underfed animals is consistent with delayed synthesis of the enzyme, NATase and a delayed night-time peak of pineal MT has also been reported by Herbert and Reiter (1981) in prepubertal rats subjected to protein restriction. This delayed response may relate to changes in the sensitivity of the pineal gland to β-adrenergic agonist. The sensitivity of the pineal gland to the stimulating effects of β-adrenergic agonists changes as a function of the prior exposure of the gland to endogenous or exogenous stimulation (Romero and Axelrod, 1975). Since food restriction leads to changes in norepinephrine turnover and brain β-receptor density (Landsberg and Young, 1978; Stone, 1983), it is possible that similar changes may also occur in the pineal gland and lead to changes in sensitivity to ISO stimulation. Although the observed delay in NATase post ISO stimulation is consistent with a hypersensitive gland with increased β-receptor activity, this may not be the explanation as discussed in the next section.

In the dose-response study, both the tendency towards a rightward shift and decreased peak pineal NATase activity in underfed animals are consistent with decreased β-receptor activity. This is

supportive of the previously described reduced cortical β-receptors (Stone, 1983) and reduced peripheral responsiveness to ISO in underfed animals (Katovich and Barney, 1983). However, reduced β-receptor activity is usually associated with a subsensitive gland and this is not supported by the finding of the time-course study. Therefore, it appears unlikely that the observed changes in pineal β-responsiveness to ISO are related to changes in pineal β-receptor activity alone. Perhaps this is not unexpected since post-receptor events that are involved in NATase induction may also be affected by food deprivation although this has never been investigated. Furthermore, pineal MT synthesis is under both neural and hormonal regulation (Cardinali, 1981; Preslock, 1984).

Various changes in hormonal parameters occur with food restriction. Reduced thyroid hormones, increased corticosterone, reduced gonadotropins and gonadal steroids have been reported in underfed rats (Burger et al, 1980; Chowers et al, 1969; Fromweiler et al, 1968; Howland, 1975). The subnormal thyroid hormones can lead to decreased β-receptor concentration and decreased receptor coupling (Bilezikian and Loeb, 1983). Higher circulating corticosterone observed in the underfed animals can contribute to the reduced peak pineal NATase (Kendall et al, 1982; Yuwiler, 1982). The effect of gonadal steroids on β-receptor activity and pineal MT synthesis have been well described (Cardinali, 1981; Preslock, 1984; Stiles et al, 1984). Conceivably, suppression of gonadal steroids in the underfed animals can influence pineal β-receptor properties, reduce pineal HIOMT and possibly pineal NATase activities. Furthermore, rats subjected to a similar degree of food restriction have altered

β-endorphin concentration in different hypothalamic areas (Knuth and Friesen, 1983). Such changes in endogenous opioid together with the known modulating effect of opioid on pineal MT synthesis (Geffard, 1982; Zatz, 1979) provide yet another possible mechanism that can influence the pineal response to ISO.

Even though the present study demonstrated altered pineal responsiveness to ISO stimulation, there were several obvious limitations to the study. The time of 120 min was chosen for the dose-response study since this was the time chosen for numerous previous reports (Illnerova and Vanecek, 1983; Klein et al, 1983). As demonstrated in the time course study, peak pineal NATase, pineal and serum MT occurred at 120 min post ISO stimulation in control fed animals. By contrast, in underfed animals, pineal NATase, pineal and serum MT increased further at 180 min post ISO injection. In order to determine the peak pineal response post ISO stimulation, the time course study should be extended to beyond 180 min post stimulation in order to characterize the temporal profile. This should be followed by a dose response at the optimal time demonstrated by the time course study. A comparative study between the two routes of administration (SC vs IP) may also characterize further the atypical responses to ISO.

In summary, 4 weeks of 50% food restriction resulted in marked reduction in both body weight and absolute accessory organ weights which was accompanied by altered pineal responsiveness to ISO stimulation. Although the changes in β-receptor activity and hormonal parameters associated with underfeeding can influence the response to ISO, the relative importance of the various mechanisms

remains to be determined.

CHAPTER FIVE

EFFECT OF FOOD RESTRICTION ON SERUM TRYPTOPHAN AND SEROTONIN

5.1 Abstract

Adult male rats, kept under a lighting regimen of 14 h light and 10 h dark were subjected to either ad libitum feeding or 50% caloric restriction. At the end of 3 weeks, body weight, serum tryptophan (TRP) and serotonin (5HT) were determined over a 24 h period. It was found that the 40% reduction in body weight was accompanied by a 10% and a 39% reduction in 24 h mean serum TRP and 5HT respectively. The timing of peak TRP and peak 5HT levels appeared to be influenced by the timing of food presentation. Reduced food consumption led to lower peak and trough 5HT levels but had no effect on peak or trough TRP levels.

5.2 Introduction

The relationship between nutritional factors and neurotransmitter metabolism has long been recognized (Landsberg and Young, 1982; Wurtman and Fernstrom, 1976). Several studies have indicated that indole metabolism in the central nervous system is abnormal in animals subjected to a short period of starvation (Curzon et al, 1972; Fuenmayor and Garcia, 1984; Kantak et al, 1977, 1978a, 1978b; Loullis et al, 1979; Perez-Cruet et al, 1972). However, the effect of food restriction on circulating levels of tryptophan (TRP) and serotonin (5HT) has not been studied in detail. This may be important since patients with anorexia nervosa have reduced plasma TRP (Coppen et al, 1976) and urinary 5-hydroxyindoleacetic acid levels (Riederer et al, 1982). Recently, we have found that the timing of feeding has a significant influence on the circadian rhythms of serum TRP and 5HT (Ho et al, 1985). To further investigate the influence of feeding on circulating indoles, the effect of caloric restriction on serum TRP and 5HT levels over a 24 h period was determined in the present study. Animals were subjected to a 3 week period of 50% food restriction since this was the duration required for the weight reduction to reach a plateau.

5.3 Material and Methods

One hundred and twenty male Wistar rats with body weights between 160-180 g were obtained from Woodlyn Laboratories (Guelph, Ontario). All animals were housed individually in metabolic cages with wire meshed bottoms. The room was temperature (22 $\pm$ 2 ^{o}C) and humidity (46 $\pm$ 4%) controlled with 14 h of light (0600 - 2000 h) per

day. Animals were divided into two groups of comparable weight, ad libitum fed controls (C) and 50% underfed (U). During the two week adjustment period, all rats received standard rat chow (Ralston Purina Canada, Longueil, Quebec) and water ad libitum. The daily food intake of each animal was determined and minimal variation between animals was observed. Animals were weighed every 3-4 days. After the adjustment period, U animals were fed daily as a group half the food consumed by C animals. In order to avoid the possible cueing effect of feeding, time of food presentation for U animals was determined by generating a random number between 1 to 24. During the 3 week of food restriction, the interval between time of food presentation varied from 12 to 32 h. After food presentation, the entire amount of food was consumed within 3 h. At the end of 3 weeks, C and U animals were alternately killed by decapitation at 1400, 1800, 2030, 2130, 2230, 2330, 0200, 0430, 0530 and 0900 h (n = 6 per time point). On the day of experiment, C animals continued with ad libitum feeding while U animals were all fed at 0300 h. For the group of U animals that was killed at 0900 h, they were fed again at 0600 h. Trunk blood was collected for the determinations of TRP and 5HT. The blood samples were allowed to clot overnight at 4°C, centrifuged at 4000 x g for 20 minutes and the serum collected was stored at -20°C. Assays were performed within ten days of sample collection.

Determination of serum tryptophan and serotonin

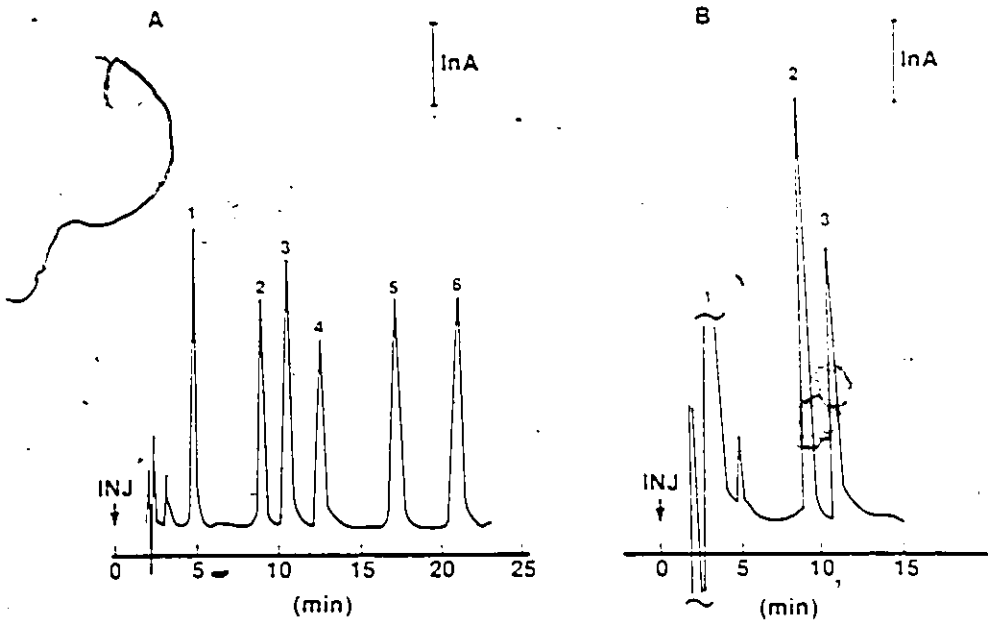
All standards were obtained from Sigma Chemical Co. (St. Louis, MO) and solvents from Fisher Scientific Co. (Waltham, MA).

Serum TRP and 5HT were determined by high performance liquid chromatography (HPLC) with an electrochemical detector. The system consisted of an Altex 210 injector with a 20 µl sample loop, a Beckman 112 pump and a Regis Hi-chrom reversed phase Spherisorb ODS column (250mm x 4.6mm ID, 5 µm particle size). The detection was by a model LC-4A electrochemical detector with a glassy-carbon working electrode from Bioanalytical Systems (West Lafayette, Ind). The potential of the working electrode was set at +0.9 V against an Ag/AgCl reference electrode. The mobile phase was 12 % methanol in 0.05 M sodium acetate buffer (pH 4.0), delivered at a flow rate of 1.5 ml/min.

To prepare the samples for analysis, 50 µl of 0.4 M perchloric acid was added to 100 µl of serum, vortex-mixed for 15 sec and centrifuged at 13,000 x g for 5 min. The supernatant was transferred into a second tube and centrifuged once more before loading into the 20 µl sample loop. A typical chromatogram of a serum sample is shown in Fig. 20. Only 5HT and TRP were detected in the serum under the above conditions. They were assayed in the linear portion of the standard curves as quantified by peak heights. The assay sensitivities for 5HT and TRP were 0.2 ng and 0.4 ng respectively. Tryptophan determined by this method was the total serum TRP content.

Student's t-test and analysis of variance were conducted as appropriate. A probability of 0.05% was taken as the point of significance.

Figure 20 Chromatograms of (A) reference standards and (B) a serum sample



A. Standard compounds: 1) 5-hydroxytryptophan; 2) serotonin; 3) tryptophan; 4) 5-hydroxyindoleacetic acid; 5) homovanillic acid; and 6) N-acetylserotonin.

B. Deproteinized rat serum: 1) solvent front; 2) serotonin; 3) tryptophan.

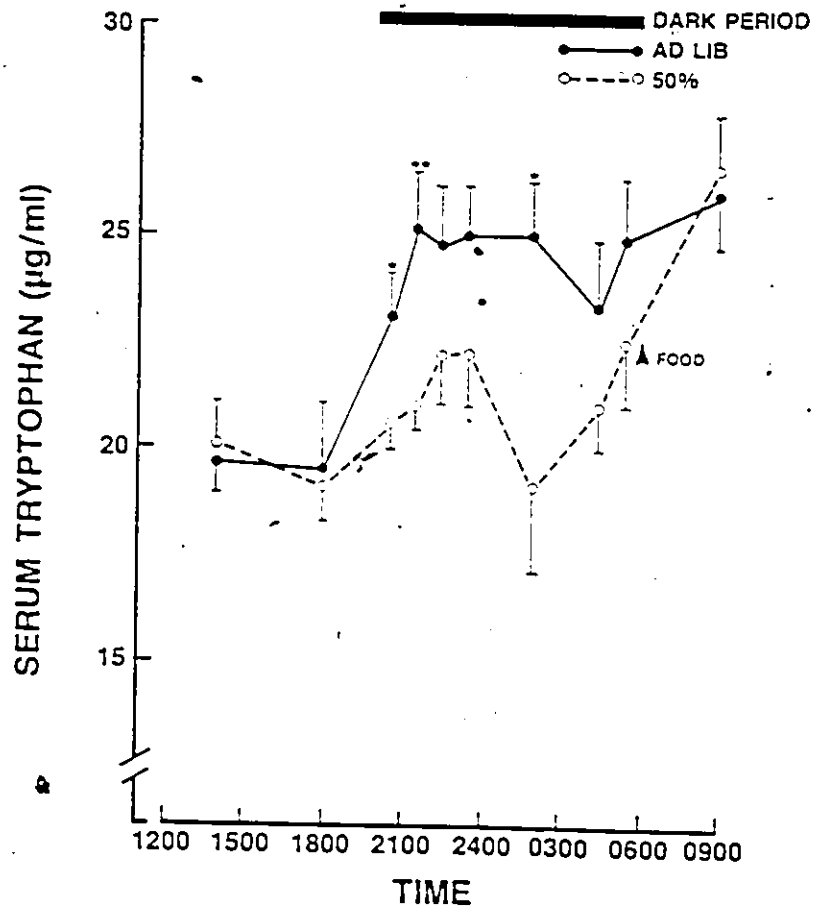
5.4 Results

After two weeks of adjustment, initial body weights for both C and U animals were 284 ± 4 g. At the end of the study period, the difference in body weight between C and U animals were 36% (393 ± 10 g vs 254 ± 5 g, $p < 0.001$).

Circadian variations of serum TRP and 5HT were demonstrated in both groups of animals ($p < 0.01$ in all cases by analysis of variance) (Figs. 21,22). In C animals, TRP levels were lowest during the light period and just before the onset of darkness. Tryptophan levels showed an abrupt increase after the onset of darkness. Elevated TRP levels were observed throughout the dark period and at the beginning of the light period. As compared to C animals, U animals demonstrated only a small increase of TRP after the onset of darkness. However, a sharp increase in serum TRP was observed 3 h after food presentation at 0600 h and the levels achieved were similar to peak levels of C animals. Mean TRP levels, however, were slightly reduced in U animals (21.5 ± 0.5 vs 23.5 ± 0.5 µg/ml, $p < 0.001$, paired t-test).

As for serum 5HT, there was a slight decline during the dark period in C animals. Peak 5HT levels were observed 3 h after the onset of light. In U animals, 5HT levels also declined after the onset of darkness and increased before the onset of light. However, the most interesting finding is the marked reduction of the 24 h mean level of serum 5HT in U animals (0.49 ± 0.03 vs 0.79 ± 0.03 µg/ml, $p < 0.001$). This reduction was reflected by lower peak 5HT (0.66 ± 0.07 vs 1.12 ± 0.11 µg/ml, $p < 0.05$) and trough 5HT levels (0.31 ± 0.05 vs 0.59 ± 0.06 µg/ml, $p < 0.05$).

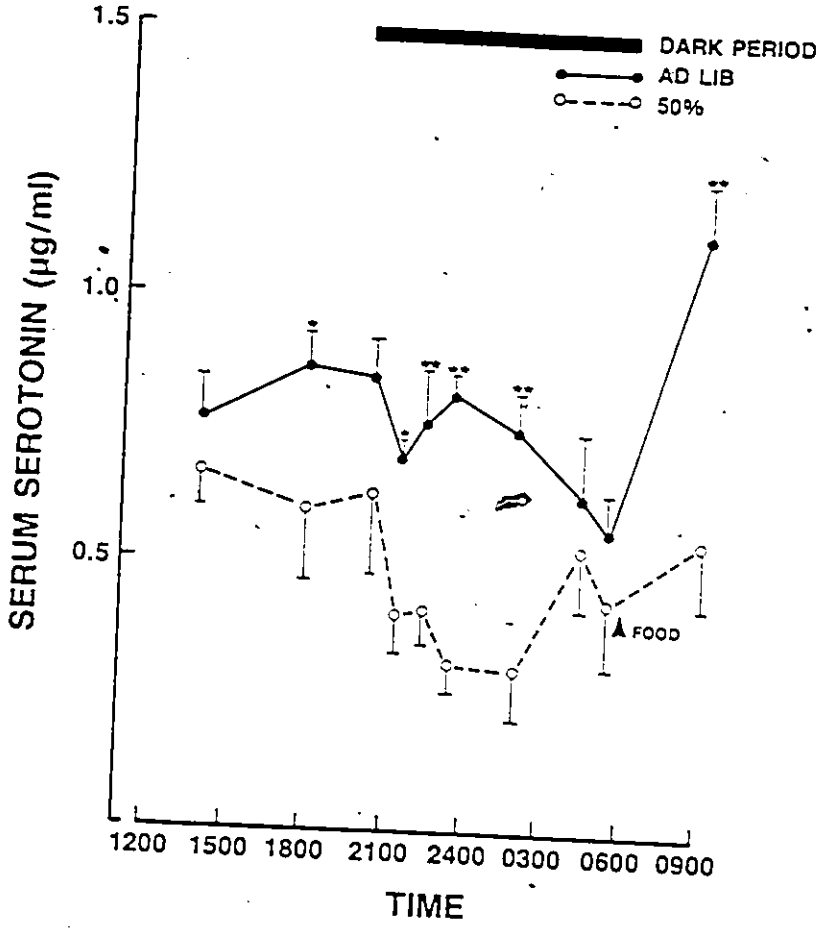
Figure 21 . Effect of 3 weeks 50% food restriction on serum tryptophan



Values are means ± SE, n = 6;

* $p < 0.05$ and ** $p < 0.01$, 50% underfed vs ad lib control

Figure 22 Effect of 3 weeks 50% food restriction on serum serotonin



Values are means ± SE, n = 6;

* $p < 0.05$ and ** $p < 0.01$, 50% underfed vs ad lib control

5.5 Discussion

The diurnal rhythm of serum TRP is a reflection of the pattern of food consumption (Fernstrom et al, 1975). In a nocturnal species such as the rat, most of the food is consumed in the early part of the dark period under ad libitum conditions (Suttie, 1968). Therefore, serum TRP levels are expected to increase after food ingestion at night. Indeed, in the present study, serum TRP levels increased shortly after the onset of darkness in ad libitum fed animals. The observed circadian variation of serum TRP in C animals are in agreement with previous reports (Fernstrom et al, 1971; Hery et al, 1977).

In U animals, serum TRP levels before feeding (at 1400 h and 1800 h) and after feeding at 0900 h did not differ from C animals. In the intervening period, TRP levels were generally increased in C animals under ad libitum feeding while TRP levels remained low in U animals until after food ingestion at 0600 h. These findings suggest that the variations in serum TRP profile between C and U animals most likely are related to the differences in the feeding regimen. This is further supported by the observation that after food presentation, peak serum TRP levels achieved in U animals were similar to those of C animals.

As for 5HT, circulating levels did not increase until 0900 h (13 h after the onset of darkness). This increase in serum 5HT may relate either to the food-related release of 5HT from the gastrointestinal tract (Bulbring and Crema, 1959; Jaffe et al, 1978) or increased peripheral 5HT synthesis after food ingestion (Nomura et al, 1977). Since rats consume most of their food in the early part of

the dark period (Suttie, 1968), the food-related release of 5HT which occurs within minutes (Kellum and Jaffe, 1976) is an unlikely explanation of the increased 5HT levels observed at 0900 h. By contrast, increased synthesis of 5HT after TRP administration only leads to elevated blood 5HT levels 6 1/2 h later (Colmenares and Wurtman, 1979). Moreover, in animals subjected to a restricted feeding schedule, serum 5HT does not increase until 10 - 13 h after feeding (Ho et al, 1985). Therefore, it is likely that the observed increase in serum 5HT at 0900 h is secondary to increased peripheral 5HT synthesis after food ingestion. However, the timing of the peak serum 5HT level in the present study, though similar to our previous finding (Ho et al, 1985), occurred earlier than that reported by Scheving et al (1972). The explanation for this difference is not clear. It is possible that this variation may be due to the different lighting regimen employed in the two studies (14 h light and 10 h dark in the present study as compared to 12 h light and 12 h dark).

In U animals, highest serum 5HT levels were observed at 1400 h with lowest 5HT levels occurring 10 - 12 h later. As U animals were fed at 0300 h the previous day (see methods), the higher 5HT levels that occurred at 1400 h (i.e. 11 h after feeding) may also relate to increased 5HT synthesis after food ingestion. Therefore, the changes in the timing of the serum 5HT peak between U and C animals may be due to an acute effect of the differences in the timing of feeding between the 2 groups. However, this difference in the timing of feeding cannot explain the reduction in mean 24 h serum 5HT levels in U animals. Apart from the general reduction of 5HT levels, regardless

of the time of sampling over the 24 h period, both peak and trough serum 5HT levels were also significantly reduced. Whether this general reduction in serum 5HT was due to a chronic or an acute effect of food deprivation cannot be concluded from this study. In our previous study (Ho et al, 1985) when serum 5HT levels were determined using the identical methodology up to 30 h after food presentation, peak and trough 5HT levels are similar to the respective 5HT levels observed in ad libitum controls of the present study but significantly higher than those of U animals. Taken these two studies together, the reduction in serum 5HT levels observed in U animals appears to be a consequence of chronic rather than acute food restriction.

The mechanism underlying the reduced circulating 5HT levels in U animals remains to be determined. Serum 5HT as determined in the present study represents the amount of 5HT released from platelets during clotting (Essman, 1978). Since platelet 5HT is not synthesized locally, the observed reduction in serum 5HT could have arisen from reduced uptake or storage, increased release, or increased degradation of platelet 5HT. Alternatively, synthesis of 5HT may be reduced since food restricted animals have reduced serum TRP as observed in this study.

The findings of the present study suppport the concept that nutritional factors can influence indole metabolism. Whether the observed food restriction-related changes in circulating TRP and 5HT also occur centrally remains to be determined. Nevertheless, the changes in peripheral indoles are consistent with several previous reports in humans. Patients with anorexia nervosa have either reduced (Coppen et al, 1976) or normal plasma TRP levels (Kaye et al,

1984; Russel, 1976) and their CSF and urinary 5-hydroxyindoleacetic acid levels are reduced (Kaye et al, 1984; Riederer et al, 1982). Such changes in indoles in turn may account for the neuroendocrine abnormalities observed in food restricted states since 5HT is a known neuroregulator of pituitary hormones (Campbell et al, 1977; Weiner and Ganong, 1978).

CHAPTER SIX

GENERAL DISCUSSION

General Discussion

In the present study, the effects of food restriction on pineal and circulating indoles were examined. The effects of food restriction on the gonadal axis have long been recognized. The involution of the gonadal organs is associated with suppressed gonadotropins (Howland, 1975). Since the suppression of gonadotropins can be reversed by constant illumination (Piacsek and Meites, 1967), it has been postulated that the pineal gland may be involved. Specifically, melatonin (MT) may be an important mediator since the nocturnal rise of MT is suppressed by constant illumination (Ralph et al, 1971). Further supportive evidence comes from studies using pinealectomy or MT administration. Pinealectomy can ameliorate the gonadal regression in prepubertal rats subjected to both blinding and dietary restriction (Sorrentino et al, 1971). In underfed ovariectomized rats, LH levels are suppressed (Walker and Frawley, 1977). When such animals are pinealectomized, LH levels increase indicating that the pineal has a definite influence on the changes of the reproductive axis (Walker and Frawley, 1977). Following MT administration, underfeeding can potentiate the inhibitory effect of MT on the reproductive axis as determined by organ weights (Blask et al, 1980). Though such data are not conclusive, the ameliorative effect of pinealectomy and the potentiating effect of MT administration on gonadal regression are suggestive of a pivotal role for the pineal.

Not only does food restriction sensitize the response of the gonadal axis to the inhibitory action of MT, food restriction also has a direct effect on pineal activity. This effect, however, depends on the duration of food restriction. Short term food restriction has no effect on pineal activity as determined by oxygen consumption, morphologic criteria or urinary MT (Lynch et al, 1975; Walker et al, 1978). However, when the duration of food restriction is prolonged, changes in pineal activity occur. Adult rats subjected to chronic food restriction have increased pineal activity as assessed by oxygen consumption and morphologic changes (Walker et al, 1978). When prepubertal rats are subjected to 5 weeks of restricted protein feeding, both daytime and night-time pineal MT contents are reduced (Herbert and Reiter, 1981). This reduction in pineal MT is not consistent with increased pineal activity unless the reduced content is secondary to a higher turnover rate. Under such conditions, simple measurement of glandular content cannot be used to represent the level of circulating MT which in any event may be more relevant to its physiological function. Yet the effect of food restriction on the serum profile of circulating MT has never been determined.

In order to examine this issue, in the first experiment (Chapter 2), two specific questions were asked.

1. Did changes in circulating MT depend on the duration of food restriction?
2. Could changes in pineal MT predict changes in serum MT in food restricted animals?

Based on preliminary studies, 1 week of 50% food restriction was chosen for the acute study and 3 weeks of 50% food restriction was

chosen for the chronic study since by 3 weeks, the reduction in body weight has stabilized in most animals. Unlike previous studies where prepubertal animals were used (Sorrentino et al, 1971; Herbert and Reiter, 1981), the present study investigated the effect of food restriction on young adult rats. Adult animals were used since during puberty, the feedback sensitivity of the reproductive axis is altered. During puberty, there are changes in circulating gonadotropins and sex steroids and changes in the MT secretory profile may also occur. These alterations in turn may interact with the effect of food restriction on the pineal-gonadal axis and complicate the interpretation of the data. The finding of experiment 1 indicated that under the lighting condition of 14 h light and 10 h dark, 1 week of food restriction had no effect on the 24 h profile of serum or pineal MT. This is consistent with the finding of Lynch et al (1975) and Walker et al (1978) that acute food restriction fails to alter pineal activity. However, when food restriction was prolonged for 3 weeks, changes in the MT profile were observed. The duration of the night-time rise was increased secondary to an earlier rise of pineal and serum MT. The amplitude of the night-time rise in serum MT was also higher. There are several possible explanations for the increase in amplitude. They may reflect changes in rate of synthesis, volume of distribution or rate of metabolic clearance. However, the concomitant reduction in peak pineal content of MT, suggests either a reduced rate of MT synthesis or increased glandular turnover of MT. A reduction in night-time pineal MT also occurs in prepubertal rats subjected to 5 weeks of protein restriction (Herbert and Reiter, 1981) and in animals subjected to chronic immobilization stress (Yocca and

Friedman, 1984). Whether the reduced pineal MT was related to changes in synthesis or turnover was not assessed in the present study. Moreover, regardless of the possible changes in synthesis or turnover, a reduction in the volume of distribution secondary to the 36% reduction in body weight might account for the discrepancy between night-time serum and pineal MT level. Furthermore, the metabolic clearance of MT may also be reduced. However, this possibility cannot be concluded from the present study. To what degree the changes in pineal activity are related to the chronic stress of food deprivation rather than the reduced quantity of food also remains to be determined.

Based on the results of experiment 1, it appears that in addition to the environmental lighting, changes in food availability is another factor that can influence pineal activity. Changes in pineal activity, however, depend on the duration of food restriction. The observed changes in circulating MT are especially of interest since they are consistent with an anti-gonadal action of the pineal. Based on the recent finding of Carter and Goldman (1983), the demonstrated modest increase in duration of night-time serum MT rise may be of significance to the antigonadal action of MT. In the study of Carter and Goldman, changes in the duration of MT infusion to Djungarian hamsters by only 1 h leads to a distinctly different response in the testes. Hence, the prolonged duration of the night-time rise of MT in the present study may account for the changes in the gonads. Furthermore, in rats, MT administration to prepubertal rats whereby both the amplitude and duration of the night-time MT rise are increased lead to delayed maturation of the gonad (Lang et al,

1983). In the present study, food restricted animals demonstrated an earlier rise of serum MT with increased amplitude.

To further investigate this pineal-gonadal interaction during underfeeding, in the second experiment (Chapter 3), the effect of pinealectomy and shortened photoperiod were determined. Pinealectomized underfed animals were found to have larger accessory organs and increased basal testosterone levels. These findings may relate to the removal of MT from the circulation since in rats, the pineal gland is the major source of circulating MT (Lewy et al, 1980). However, pinealectomy failed to reverse completely the gonadal changes. These findings suggest that the pineal is only partly responsible for the undernutrition-induced gonadal regression. Alternatively, progonadal compounds are also removed by pinealectomy. The protective effect of pinealectomy may reside at the testicular level since serum testosterone levels increase without any changes in the suppressed LH levels. An inhibitory effect of MT on steroidogenesis has previously been reported by Ellis (1972).

When animals were subjected to a shortened photoperiod of 4 h light and 20 h dark, the same dietary restriction failed to augment the gonadal suppression observed under a lighting regimen of 14 h light and 10 h dark. Under this shortened photoperiod, restricted food availability had no effect on the serum MT profile. This lack of observed effect may relate in part to the frequency of sampling. Changes in duration of less than 90 min would not be detected using this protocol. Furthermore, the effect of varying length of food restriction was not assessed in this experiment. It is possible that

under a different duration of food restriction, shortened photoperiod may have an influence on serum MT profile. Alternatively, since shortened photoperiod and food restriction usually occur at the same time in the wild, the information transduced by them may be similar. Indeed, both shortened photoperiod and food restriction lead to changes in duration of the night-time rise of serum MT. The interaction between shortened photoperiod and food restriction could be further investigated by evaluating the effect of food restriction under various lighting regimens.

The last section of the present study investigated food restriction effects on two aspects of biogenic amines. The relationship between food availability and neurotransmitter metabolism has long been recognized (Landsberg and Young, 1978; Wurtman and Fernstrom, 1976). Food restriction leads to reduced sympathetic activity as determined by circulating levels of catecholamine or norepinephrine turnover rate in various tissues such as heart cells (Landsberg and Young, 1982). Chronic food restriction leads to reduced beta-receptor density in cortical cells and reduced peripheral responsiveness to isoproterenol as determined by heart rate, blood pressure and oxygen consumption (Katovich and Barney, 1983; Stone, 1983). In the pineal gland, MT synthesis depends on the synthesis and activity of the enzyme NATase which is regulated by the sympathetic input acting on pineal beta-receptors (Zatz, 1978). Results of experiment 1 indicated that 3 weeks of 50% food restriction led to altered pineal MT secretory profile. This was characterized by an earlier rise in both pineal and serum MT accompanied by higher

night-time serum MT levels. Since the darkness induced activation of the pineal is under sympathetic control (Deguchi and Axelrod, 1972b), food restriction related changes in sympathetic activity could account for the alterations in MT profile.

To test this hypothesis, the pineal NATase, pineal and serum MT response to isoproterenol (ISO) was determined in experiment 3 (Chapter 4). The findings of experiment 3 indicated that pineal beta-adrenergic responsiveness to ISO in food restricted animals was altered. The alterations, however were atypical of either a sub- or supersensitive pineal gland. In the dose response study, peak pineal NATase, pineal and serum MT occurred at a lower dose of ISO in underfed animals. Peak levels of pineal NATase and pineal MT (but not serum MT), however, were of reduced magnitude. One mechanism for this altered pattern of responsiveness to ISO stimulation is reduced beta-adrenergic receptor activity (Deguchi and Axelrod, 1973b). This is in agreement with the previously reported reduction in beta-receptor density of cortical tissues (Stone, 1983) and reduced peripheral responsiveness to ISO (Katovich and Barney, 1983). Peak night-time pineal MT was also reduced in prepubertal rats subjected to protein restriction (Herbert and Reiter, 1981) and adult rats subjected to calorie restriction (Chapter 2). As in experiment 1, there is a discrepancy between peak serum and pineal MT responses post ISO stimulation. In underfed animals, peak pineal MT levels post ISO stimulation were reduced while peak serum MT levels remained unchanged. This discrepancy again may reflect changes in glandular turnover, or volume of distribution, or changes in peripheral metabolism of MT as discussed previously.

In the time course study, underfed animals demonstrated delayed response to ISO which is consistent with a hypersensitive gland (Romero and Axelrod, 1975). This delayed response, however, was in contrast to the earlier rise in serum and pineal MT observed in underfed animals under a lighting regimen of 14 h light and 10 h dark. This difference may have arisen because the neurotransmitter mediating the darkness induced activation of pineal NATase is norepinephrine (Axelrod et al, 1969) which could stimulate both alpha and beta adrenoceptors. Post-synaptic alpha receptors have recently been reported to potentiate the beta-adrenergic stimulation of pineal NATase (Klein et al, 1983). This possibility can be investigated by studying the pineal responses to norepinephrine in underfed animals. Furthermore, other intermediate steps involved in NATase induction may also be influenced by nutritional deprivation and account for the changes in pineal beta-adrenergic responsiveness. Alternatively, this atypical response may relate to changes in hormonal parameters since pineal MT synthesis is under both neural and hormonal regulation (Cardinali, 1981; Preslock, 1984). Potential candidates include gonadal steroids, gonadotropins, thyroid hormones, corticosterone and beta-endorphin. Food restriction leads to reduction in gonadal steroids, gonadotropins, thyroid hormones and elevation of corticosterone and beta-endorphin (Burger et al, 1980; Fromweiler et al, 1968; Howland, 1975; Knuth and Friesen, 1983). These hormones in turn have a modulating effect on the adrenergic system and pineal MT synthesis (Bilezikian and Loeb, 1983; Cardinali, 1981; Geffard et al, 1981; Preslock, 1984; Yuwiler, 1982; Zatz and Brownstein, 1979). To fully understand the underlying mechanism for the altered pineal

responsiveness to ISO in food restricted animals, the interaction between neural and hormonal factors that are involved in pineal MT synthesis need to be determined.

In chapter 5, the effect of food restriction on two precursors of MT, tryptophan and serotonin is reported. Previous studies have indicated that short term starvation led to increased synthesis and turnover of cerebral serotonin (Kantak, 1977, 1978a, 1978b). By contrast, studies on patients with anorexia nervosa have indicated reduced serum tryptophan, reduced cerebrospinal and urinary 5-hydroxyindoleacetic acid levels (Coppen et al, 1976; Kaye et al, 1984; Riederer et al, 1982). Hence, changes in indole metabolism secondary to chronic nutritional deprivation as observed in anorexic humans may not be the same as those observed after acute starvation. In the present study, after 3 weeks of 50% food restriction, there was only a modest reduction in serum tryptophan but a marked reduction in serum serotonin. The lower mean serum tryptophan levels observed may reflect the reduced amount of food consumed by underfed animals. However, peak serum tryptophan levels that occurred after food ingestion were not reduced in underfed animals. Therefore, dependent on the time of sampling and the temporal relationship to feeding, serum tryptophan levels between underfed animals and ad libitum fed controls may or may not show a difference. Indeed, reduced serum tryptophan levels were only observed in 3 of 10 sampling points. This finding may account for the variable serum tryptophan levels (reduced or unchanged) observed in anorexic humans (Coppen et al, 1976; Riederer et al, 1982; Russel et al, 1976).

By contrast, both peak and mean serotonin levels were reduced in underfed animals. Regardless of time of sampling, most underfed animals demonstrated lower serum serotonin levels. Whether similar reduction in serotonin also occurs in the central nervous system remains to be determined. This finding, however, is consistent with the reported reduced urinary 5-hydroxyindoleacetic acid levels in patients with anorexia nervosa (Riederer et al, 1982). Furthermore, such changes in serotonin, a known neuroregulator of several pituitary hormones, may account in part for the observed neuroendocrine changes in underfed animals (Weiner and Ganong, 1978). The mechanism of this reduction remains to be determined. The observed reduction in mean tryptophan levels may suggest reduced synthesis. However, changes in uptake, release and degradation cannot be ruled out.

In summary, the present study provided further information on the effect of food restriction on pineal activity, pineal response to ISO and circulating tryptophan and serotonin levels. Whereas 3 weeks of food restriction under a lighting regimen of 14 h light and 10 h dark led to a reduction in serum tryptophan and serum serotonin, serum melatonin was increased. Since the focus of the present study was on circulating levels, the underlying mechanism of these observed changes remained to be determined. To unravel these mechanisms would require studies at the cellular and molecular levels. Even though the present study failed to define the role of pineal in undernutrition-related gonadal regression, it has established that food availability is another factor that influences pineal activity. Its effect on pineal activity, however, depends on the duration of

food restriction and the environmental light/dark cycle. One conclusion that can be drawn from the present study is that in future studies on pineal and serum indoles, it is important to control for food consumption.

REFERENCES

Anton-Tay F (1974) Melatonin. Effects on brain functions. Adv Biochem Psychopharmacol 11: 315-324.

Anton-Tay F, Wurtman RJ (1969) Regional uptake of ^{3}H-melatonin from blood or cerebrospinal fluid by rat brain. Nature 221: 474-475.

Avakian EU, Horvath JM (1981) Starvation suppresses sympathoadrenal medullary response. Am J Physiol 241: E316-E320.

Axelrod J, Shein HM, Wurtman RJ (1969) Stimulation of ^{14}C-melatonin synthesis from ^{14}C-tryptophan by noradrenaline in rat pineal in organ culture. Proc Natl Acad Sci USA 62: 544-579.

Axelrod J, Weissbach H (1960) Enzymatic O-methylation of N-acetylserotonin to melatonin. Science 131: 1312.

Banerjee S, Kerr V, Winston M, Kelleher JK, Margulis L (1972) Melatonin: inhibition or of microtubule-based oral morphogenesis in Stentor coeruleus. J Protozool 19: 108-113.

Banerjee S, Margulis L (1973) Mitotic arrest by melatonin. Exp Cell Res 78: 314-318.

Becker DJ (1983) The endocrine responses to protein calorie malnutrition. Ann Rev Nutr 3: 187-212.

Bick RL, Giolli RA, Dearden LC, Stuart RR (1969) The effect of

pinealectomy and environmental lighting on gonadal, thyroid and total body weight of female Long Evans rats. Experientia 25: 531-532.

Bilezikian JP, Loeb JN (1983) The influence of hyperthyroidism and hypothyroidism on α- and β-adrenergic receptor systems and adrenergic responsiveness. Endocr Rev 4: 378-388.

Blask DE, Nodelman JL, Leadem CA, Richardson BA (1980) Influence of exogenously administered melatonin on the reproductive system and prolactin levels in underfed male rats. Biol Reprod 22: 507-512.

Blask DE, Leadem CA, Richardson BA (1981) Nutritional status, time of day and pinealectomy: factors influencing the sensitivity of the neuroendocrine-reproductive axis of the rat to melatonin. Horm Res 14: 104-113.

Brown GM, Basinska J, Bubenik G, Sibony D, Grota LJ, Stancer HC (1976) Gonadal effects of pinealectomy and immunization against N-acetylindolealkylamines in the hamster. Neuroendocrinology 22: 289-297.

Brown GM, Seggie J, Grota LJ (1985) Serum melatonin response to melatonin administration in the Syrian Hamster. Neuroendocrinology 41: 31-35.

Brownstein M, Saavedra JM, Axelrod J (1973) Control of pineal N-acetylserotonin by a beta-adrenergic receptor. Mol Pharmacol 9: 605-611.

Bubenik GA, Brown GM, Grota LJ (1975) Localization of immuno reactive androgen in testicular tissue. Endocrinology 96: 63-69.

Bubenik GA, Brown GM, Grota LJ (1977) Immunohistological localization of melatonin in the rat digestive system. Experientia 33: 662-663.

Bubenik GA, Purtill RA, Brown GM, Grota LJ (1978) Melatonin in the retina and the Harderian gland. Ontogeny, diurnal variations and melatonin treatment. Exp Eye Res 27: 323-333.

Bulbring E, Crema A (1959) The release of 5-hydroxytryptamine in relation to pressure exerted on the intestinal mucosa. J Physiol (London) 146: 18-28.

Burger AG, Berger M, Wimpfheimer K, Danforth E (1980) Interrelationship between energy metabolism and thyroid metabolism during starvation in the rat. Acta Endocrinol (Copenh) 93: 322-331.

Burns TW, Boyer PA, Terry BE, Langley PE, Robinson GA (1979) The effect of fasting on the adrenergic receptor activity of human adipocytes. J Lab Clin Med 94: 387-394.

Campbell GA, Kurcz M, Marshall S, Meites J (1977) Effects of starvation in rats on serum levels of follicle stimulating hormone, luteinizing hormone, thyrotropin, growth hormone and prolactin; response to LH-releasing hormone and thyrotropin releasing hormone. Endocrinology 100: 580-587.

Cardinali DP (1975) Changes in hypothalamic neurotransmitter uptake following pinealectomy, superior cervical ganglionectomy or melatonin administration to rats. Neuroendocrinology 19: 91-95.

Cardinali DP (1977) Nuclear receptor estrogen complex in the pineal gland. Modulation by sympathetic nerves.

Neuroendocrinology 24: 333-346.

Cardinali DP (1981) Melatonin. A human mammalian pineal hormone. Endocr Rev 2: 327-346.

Cardinali DP, Hyyppa MT, Wurtman RJ (1973) Fate of intracisternal injected melatonin in the rat brain. Neuroendocrinology 12: 30-40.

Cardinali DP, Larin F, Wurtman RJ (1972) Action spectra for effects of light on hydroxyindole-O-methyltransferase in rat pineal, retina and Harderian gland. Endocrinology 91: 877-886.

Cardinali DP, Nagle CA, Rosner JM (1975) Control of estrogen and androgen receptors in the rat pineal gland by catecholamine transmitter. Life Sci 16: 81-91.

Cardinali DP, Ritta MN (1983) The role of prostaglandins in neuroendocrine junctions: studies in the pineal gland and the hypothalamus. Neuroendocriology 36: 152-160.

Cardinali DP, Vacas MI (1978) Feedback control of pineal function by reproductive hormones. A neuroendocrine paradigm. J Neural Transm (Suppl) 13: 175-201.

Cardinali DP, Vacas MI, Estevez Boyer E (1979) Specific binding of melatonin in bovine brain. Endocrinology 103: 437-441.

Cardinali DP, Vacas MI (1981) Molecular endocrinology of melatonin: receptor sites in brain and peripheral organs. In: Adv Biosci 19: 257-261.

Cardinali DP, Vacas MI, Ritta MN (1981) A biphasic effect of estradiol on serotonin metabolism in rat pineal organ cultures. Experientia 37: 203-204.

Cardinali DP, Wurtman RJ. (1972) Hydroxyindole-O-methyltransferase in rat pineal, retina and Harderian gland. Endocrinology 91: 247-252.

Carter DS, Goldman BD (1983) Antigonadal effects of timed melatonin infusion in pinealectomized male Djungarian hamsters (Phodopus sungarus sungarus): duration is the critical parameter. Endocrinology 113: 1261-1267.

Chowers I, Einat R, Feldman S (1969) Effects of starvation on levels of corticotrophin releasing factor, corticotrophin and plasma corticosterone in rats. Acta Endocrinol 61: 687-694.

Chu EW, Wurtman RJ, Axelrod J (1964) An inhibitory effect of melatonin on the estrous phase of the estrous cycle of the rodent. Endocrinology 75: 238-242.

Cohen M, Roselle D, Chabner B, Schmidt TJ, Lippman M (1978) Evidence for a cytoplasmic melatonin receptor. Nature 274: 894-895.

Collu R, Fraschini F, Martini L (1971) The effect of pineal methoxyindoles on rat vaginal opening time. J Endocrinol 50: 679-683.

Colmenares JL, Wurtman RJ (1979) The relation between urinary 5-hydroxyindoleacetic acid levels and the ratio of tryptophan to other large neutral amino acids placed in the stomach. Metabolism 28: 820-827.

Coppen AJ, Gupta RK, Eccleston EG, Wood KM, Wakeling A, De Sousa VFA (1976) Plasma tryptophan in anorexia nervosa. Lancet 1: 961.

Curzon G, Joseph MH, Knott PJ (1972) Effects of immobilization and food deprivation on rat brain tryptophan metabolism. J

Neurochem 19: 1967-1974.

Davis GA (1978) The response of adenosine 3'5'-monophosphate to norepinephrine in the hypothalamus and pineal ogan of female rats in proestrus or diestrus. Endocrinology 103: 1048-1053.

Deguchi T (1973) Role of the beta-adrenergic receptor in the elevation of adenosine cyclic 3',5'-monophosphate and induction of serotonin N-acetyltransferase in rat pineal glands. Mol Pharmacol 9: 184-190.

Deguchi T (1975) Characteristics of serotonin-acetyl coenzyme A N-acetyltransferase in pineal gland of rat. J Neurochem 24: 1083-1085.

Deguchi T, Axelrod J (1972a) Induction and superinduction of serotonin N-acetyltransferase by adrenergic drugs and denervation in rat pineal organ. Proc Natl Acad Sci USA 69: 2208-2211.

Deguchi T, Axelrod J (1972b) Control of circadian change of serotonin N-acetyltransferase activity in the pineal organ by the β-adrenergic receptor. Proc Natl Acad Sci USA 69: 2547-2550.

Deguchi T, Axelrod J (1972c) Sensitive assay for serotonin N-acetyltransferase activity in rat pineal. Analyt Biochem 50: 174-179.

Deguchi T, Axelrod J (1973a) Superinduction of serotonin N-acetyltransferase and supersensitivity of adenyl cyclase to catecholamines in denervated pineal gland. Mol Pharmacol 9: 612-618.

Deguchi T, Axelrod J (1973b) Supersensitivity and subsensitivity

of the β-adrenergic receptor in pineal gland regulated by catecholamine transmitter. Proc Natl Acad Sci USA 70: 2411-2414.

Dickson K, Benson B, Tate G (1971) The effect of blinding and pinealectomy in unilaterally ovariectomized rats. Acta Endocrinol 66: 177-182.

Ellis LC (1972) Inhibition of rat testicular androgen synthesis in vitro by melatonin and serotonin. Endocrinology 90: 17-28.

Ellison W, Weller JL, Klein DC (1972) Development of a circadian rhythm in the activity of pineal serotonin N-acetyltransferase. J Neurochem 19: 1335-1341.

Essman WB (1978) Serotonin in Health and Disease, Vol 1, Spectrum Publications Inc, New york, pp 15-179.

Falck B, Owman C, Rosengren E (1966) Changes in rat pineal stores of 5-hydroxytryptamine after inhibition of its synthesis or breakdown. Acta Physiol Scand 67: 300-305.

Fernstrom JD, Faller DV Shabshelowitz H (1975) Acute reduction of brain serotonin and 5-HIAA following food consumption: correlation with the ratio of serum tryptophan to the sum of competing neutral amino acids. J Neural Transm 36: 113-121.

Fernstrom JD, Larin F, Wurtman RJ (1971) Daily variations in the concentration of individual amino acids in rats plasma. Life Sci 10: 813-819.

Fiske VM (1939) Effects of light and darkness on activity of the pituitary of the rat. Proc Soc Exptl Biol Med 40: 184-191.

Fiske VM (1941) Effects of light on sexual maturation, estrous cycles and anterior pituitary of the rat. Endocrinology 66:

187-196.

Fromweiler JP, Mialhe VC, Stutinsky F (1968) Demi-vie et taux de secretion de la corticosterone chez le rat. Influence du jeune. J Physiol (Paris) 60: 99-114.

Fuenmayor LD, Garcia S (1984) The effect of fasting on 5-hydroxytryptamine metabolism in brain regions of the albino rat. Br J Pharmacol 83: 357-362.

Fujiwara M, Shibata M, Watanabe Y, Nakiwa J, Harita F, Mirino N, Hayaishi O (1978) Indoleamine 2,3-dioxygenase. J Biol Chem 253: 6081-6085.

Geffard M, Gaffori O, Chaveau J, Muyard JP, Le Moal M (1981) Dramatic increase in pineal melatonin levels in the rat after subcutaneous injection of des-tyrosine gamma-endorphin. Neurosci Lett 27: 329-334.

Gibbs FP, Vriend J (1981) The half-life of melatonin elimination from rat plasma. Endocrinology 109: 1796-1798.

Gittes RF, Chu EW (1965) Reversal of the effect of pinealectomy in female rats by multiple isogenic pineal transplants. Endocrinology 77: 1061-1067.

Goldman BD, Darrow JM (1983) The pineal gland and mammalian photoperiodism. Neuroendocrinology 37: 386-396.

Goldman B, Hall V, Hollister C, Reppert S, Roychoudhury P, Yellon S, Tamarkin L (1981) Diurnal changes in pineal melatonin content in four rodent species: relationship to photoperiodism. Biol Reprod 24: 778-783.

Grewal T, Mickelsen O, Haes HD (1971) Androgen secretion and spermatogenesis in rats following semistarvation. Proc Soc

Exp Biol Med 138: 723-727.

Hakanson R, Owman C (1965) Effect of denervation and enzyme inhibition on dopa decarboxylase and monoamine oxidase activities in rat pineal gland. J Neurochem 12: 417-428.

Hakanson R, Owman C (1966) Pineal dopa decarboxylase and monoamine oxidase activities as related to the monoamine stores. J Neurochem 13: 597-605.

Hanew K, Shiino M, Rennels EG (1980) Effect of indoles, AVT, oxytocin and AVP on prolactin secretion in rat pituitary clonal (2B8) cells. Proc Soc Exp Biol Med 164: 257-261.

Herbert DC, Reiter RJ (1981) Influence of protein calorie malnutrition on the circadian rhythm of pineal melatonin in the rat. Proc Soc Exp Biol Med 166: 360-363.

Hery F, Chouvet G, Kan JP, Pujol JF, Glowinski J (1977). Daily variations of various parameters of serotonin metabolism in the rat brain. II. Circadian variations in serum and cerebral tryptophan levels: lack of correlation with 5-HT turnover. Br Res 123: 137-145.

Ho AK, Chik CL, Brown GM (1985) Scheduled feeding versus the light-dark cycle on the rhythms of circulating tryptophan, serotonin and N-acetylserotonin in rats. Life Sci 37: 1619-1626.

Ho AK, Grota LJ, Brown GM (1984) Relationship between pineal N-acetyltransferase activity, pineal melatonin and serum melatonin in rats under different lighting conditions. Neuroendocrinology 39: 465-470.

Hoffmann K (1974) Testicular involution in short photoperiods

inhibited by melatonin. Naturwissenschaften 61: 364-365.

Howland BE (1975) The influence of feed restriction and subsequent refeeding on gonadotrophin secretion and serum testosterone levels in male rats. J Reprod Fert 44: 429-436.

Illnerova AH, Skopau AJ (1976) Regulation of the diurnal rhythm on rat pineal serotonin-N-acetyltransferase activity and serotonin during ontogenesis. J Neurochem 26: 1051-1052.

Illnerova H, Vanecek J (1979) Response of rat pineal serotonin N-acetyltransferase to one min light pulse at different night times. Brain Res 167: 431-434.

Illnerova AH, Vanecek J (1980) Pineal rhythm in N-acetyltransferase activity under different artificial photoperiods and in natural daylight in the course of a year. Neuroendocrinology 31: 321-326.

Illnerova H, Vanecek J (1983) The evening rise in the rat pineal N-acetyltransferase activity under various photoperiods. Neurosci Lett 36: 279-284.

Itoh S, Takahashi H, Sakai T (1962) Atrophy of reproductive organs in male rats housed in continuous darkness. J Reprod Fert 4: 233-234.

Kantak KM, Wayner MJ, Stein JM (1978a) Effects of various periods of food deprivation on serotonin turnover in the lateral hypothalamus. Pharmacol Biochem Behav 9: 529-534.

Kantak KM, Wayner MJ, Stein JM (1978b) Effects of various periods of food deprivation on serotonin synthesis in the lateral hypothalamus. Pharmacol Biochem Behav 9: 535-541.

Kantak KM, Wayner MJ, Tilson HA, Sved A (1977) Turnover of

^{3}H-5-hydroxytryptamine to ^{3}H-5-hydroxyindoleacetic acid and the ^{3}H-5-methoxyindoles in nondeprived and 24 hr food deprived rats. Pharmacol Biochem Behav 6: 221-225.

Kappers JA (1965) Survey of the innervation of the epiphysis cerebri and the accessory pineal organs of vertebrates. Prog Brain Res 10: 87-153.

Katovich MJ, Barney CC (1983) Alteration of peripheral ß-adrenergic responsiveness in fasted rats. Life Sci 33: 1385-1393.

Kaye WH, Ebert MH, Raleigh M, Lake R (1984) Abnormalities in CNS monoamine metabolism in anorexia nervosa. Arch Gen Psychiatry 41: 350-355.

Kellum JM, Jaffe BM (1976) Validation and application of a radioimmunoassay for serotonin. Gastroenterology 70: 516-522.

Kendall DA, Duman J, Slopis J, Enna SJ (1982) Influence of adrenocorticotropin hormone and yohimbine on antidepressant induced decline in at brain neurotransmitter receptor binding and function. J Pharmacol Exp Ther 222: 566-571.

Kinson GA, Peat F (1971) The influences of illumination, melatonin and pinealectomy on testicular function in the rat. Life Sci 10: 259-269.

Kinson GA, Robinson S (1970) Gonadal function of immature male rats subjected to light restriction, melatonin administration and removal of the pineal gland. J Endocrinol 47: 391-392.

Kinson GA, Singer B, Grant L (1968) Adrenocortical hormone secretion at various time intervals after pinealectomy in the

rat. Gen Comp Endocrinol 10: 447-449.

Kitay JI, Altschule MD (1954) The pineal gland: A review of physiologic literature. Cambridge, Mass, Harvard University Press.

Klein DC, Berg GR, Weller JL (1970) Melatonin synthesis: Adenosine 3'5'-monophosphate and norepinephrine stimulate N-acetyltransferase. Science 168: 979-980.

Klein DC, Lines SV (1969) Pineal hydroxyindole-O-methyltransferase activity in the growing rat. Endocrinology 89: 1523-1525.

Klein DC, Moore RY (1979) Pineal N-acetyltransferase and hydroxyindole-O-methyltransferase: Control by the retinohypothalamic tract and the suprachiasmatic nucleus. Brain Res 174: 245-262.

Klein DC, Sugden D, Weller JL (1983) Postsynaptic α-adrenergic receptors potentiate the β-adrenergic stimulation of pineal secretion N-acetyltransferase. Proc Natl Acad Sci USA 80: 599-603.

Klein DC, Weller JE (1970) Indole metabolism in the pineal gland. A circadian rhythm in N-acetyltransferase. Science 169: 1093-1095.

Knigge KM, Sheridan MN (1976) Pineal function in hamsters bearing melatonin antibodies. Life Sci 19: 1235-1238.

Konig A, Rega R (1978) Effect of melatonin on the spermatogenesis of unilaterally orchidectomized pubertal rats. J Neural Transm [Suppl] 13: 373.

Kopin IJ, Pare CMB, Axelrod J, Weissbach H (1960) 6-hydroxylation, the major metabolic pathway for melatonin. Biochim Biophys

Acta 40: 337-378.

Kopin IJ, Pare CMB, Axelrod J, Weissbach H (1961) The fate of melatonin in animals. J Biol Chem 236: 3072-3075.

Knuth UA, Friesen HG (1983) Changes of beta-endorphin and somatostatin concentrations on different hypothalamic areas of female rats after chronic starvation. Life Sci 33: 827-833.

Kveder S, McIsaac WM (1961) The metabolism of melatonin (N-acetyl-5-methoxytryptamine) and 5-methoxytryptamine. J Biol Chem 236: 3214-3220.

Landsberg L, Young JB (1978) Fasting, feeding and regulation of the sympathetic nervous system. New Eng J Med 298: 1295-1301

Landsberg L, Young JB (1982) Effects of nutritional status on autonomic nervous system function. Am J Clin Nutr 35: 1234-1240.

Lang U, Aubert ML, Conne BS, Bradtke JC, Sizonenko PC (1983) Influence of exogenous melatonin on melatonin secretion and the neuroendocrine reproductive axis of intact male rats during sexual maturation. Endocrinology 112: 1578-1584.

Leonardelli J, Framu G, Hermand E (1978) Melatonine et cellules a gonadoliberine (LHRH) de l'hypothalamus du rat. CR Soc Biol 72: 481-484.

Lerner AB, Case JD, Heinzelman RV (1959) Structure of melatonin. J Am Chem Soc 81: 6084.

Lerner AB, Case JD, Takahashi Y, Lee TH, Mori W (1958) Isolation of melatonin, the pineal factor that lightens melanocytes. J Am Chem Soc 80: 2587.

Lerner AB, Case JD, Takahashi Y (1960) Isolation of melatonin and

5-methoxyindole-3-acetic acid from bovine glands. J Biol Chem 235: 1992-1997.

Lewy AJ, Tetsuo M, Markey SP, Goodwin FK, Kopin IJ (1980) Pinealectomy abolishes plasma melatonin in the rat. J Clin Endocrinol Metab 50: 204-205.

Lincoln GA, Short RV (1980) Seasonal breeding: nature's contraceptive. Recent Prog Horm Res 36: 1-52.

Longenecker DE, Gallo DH (1971) The inhibition of PMSG-induced ovulation in immature rats by melatonin. Proc Soc Exp Biol Med 137: 623-626.

Loullis CC, Felten DL, Shea PA (1979) HPLC determination of biogenic amines in discrete brain areas in food deprived rats. Pharmacol Biochem Behav 11: 89-93.

Lovenberg W, Jequier E, Sjoerdsma A (1967) Tryptophan hydroxylation: Measurements in pineal gland, brain stem and corticoid tumor. Science 155: 217-219.

Lovenberg W, Weissbach H, Udenfriend S (1962) Aromatic l-amino acid decarboxylase. J Biol Chem 237: 89-92.

Lynch HJ (1971) Diurnal oscillations in pineal melatonin content. Life Science 10: 791-795.

Lynch HJ, Ozaki Y, Shakal D, Wurtman RJ (1975) Melatonin excretion of man and rats: Effect of time of day, sleep, pinealectomy and food consumption. Int J Biometeorol 19: 267-279.

Lynch HJ, Ralph CL (1970) Diurnal variation in pineal melatonin and its nonrelationship to HIOMT activity. Am Zool 10: 300.

Malawista SE (1973) The effects of colchicine and of cytochalasin B on the hormone-induced movement of melatonin granules in

frog dermal melanocytes. In: Scow RO, Ebling FJG, Henderson IW (eds) Endocrinology. Excerpta, Amsterdam p 288.

Martin JE, Engel JN, Klein DC (1977) Inhibition of the in vitro pituitary response to luteinizing hormone-releasing hormone by melatonin, serotonin and 5-hydroxytryptamine. Endocrinology 100: 675-680.

Martin JE, McKellar S, Klein DC (1980) Melatonin inhibition of the in vivo response to luteinizing hormone-releasing hormone in the neonatal rat. Neuroendocrinology 31: 13-17.

Mas M, Massa R, Montagna A, Negri-Cesi P, Martini L (1979) Role of the pineal gland in the control of gonadotropins and androgen-reducing enzymes in the rat. Prog Brain Res 52: 367-371.

McIssac WM, Farrell G, Taborsky RG, Taylor AN (1965) Indole compounds: isolation from pineal tissue. Science 148: 102-103.

Mishkinsky J, Nir Z, Lajtos K, Sulman FG (1966) Mammary development in pinealectomized rats. J Endocrinol 36: 215-216.

Moore RY, Traynor ME (1976) Diurnal rhythms in pineal N-acetyltransferase and hippocampus norepinephrine: effect of water deprivation, blinding and hypothalamic lesion. Neuroendocrinology 20: 250-259.

Motta M, Fraschini F, Martini L (1967) Endocrine effects of the pineal gland and of melatonin. Proc Soc Exp Biol Med 126: 431-435.

Neuwelt EA, Lewy AJ (1983) Disappearance of plasma melatonin after

removal of a neoplastic pineal gland. New Eng J Med 308: 1132-1135.

Niles LP (1985) Effects of melatonin on adenylate cyclase activity in rat brain, pineal and retina. Adv Biosci 53: 283-288.

Niles LP, Brown GM, Grota LJ, Wilson DA, Pang SF (1977a) Antigonadal effects of blinding in the presence of antibody against circulating melatonin. Life Sci 21: 613-618.

Niles LP, Brown GM, Grota LJ (1977b) Endocrine effect of the pineal gland and neutralization of circulating melatonin and N-acetylserotonin. Can J Physiol Pharmacol 55: 537-544.

Niles LP, Brown GM, Grota LJ (1977c) Effects of neutralization of circulating melatonin and N-acetylserotonin on plasma prolactin levels. Neuroendocrinology 23: 14-22.

Niles LP, Brown GM, Grota LJ (1979) Role of pineal gland in diurnal endocrine secretion and rhythm regulation. Neuroendocrinology 29: 14-21.

Niles LP, Wong Y-W, Mishra RK, Brown GM (1979) Melatonin receptors in brain. Eur J Pharmacol 55: 219-220.

Nomura M, Colmenares JL, Wurtman RJ (1977) Effect of dietary protein on urinary 5-hydroxyindoleacetic acid levels. J Neurochem 29: 267-271.

Owman C (1965) Localization of neuronal and parenchymal monoamines under normal and experimental conditions in mammalian pineal gland. Prog Brain Res 10: 423-453.

Ozaki Y, Lynch HJ (1976) Presence of melatonin in plasma and urine of pinealectomized rats. Endocrinology 99: 641-644.

Ozaki Y, Wurtman RJ, Alonso R, Lynch HJ (1978) Melatonin secretion

decreases during the proestrous stage of the rat estrous cycle. Proc Natl Acad Sci USA 75: 531-534.

Pagel J, Christian ST, Quayle ES, Monti JA (1976) A serotonin sensitive adenylate cyclase in mature rat brain synaptic membranes. Life Sci 19: 819-824.

Parfitt A, Weller JL, Klein DC, Sakai KK, Marks BM (1975) Blockade by elevated potassium ion concentration of the adrenergic and adenosine cyclic 3'5'-monophosphate induced stimulation of pineal N-acetyltransferase activity. Mol Pharmacol 11: 241-245.

Pavel S (1973) Arginine vasotocin release into cerebrospinal fluid of cats induced by melatonin. Nature 246: 183-184.

Pelletier G, LeClerc R, Dube D, Labrie F, Puviani R, Arimura A, Schally AV (1975) Localization of growth hormone-releasing-inhibiting hormone (somatostatin) in the rat brain. Am J Anat 142: 291-401.

Perez-Cruet J, Tagliamonte A, Tagliamonte P, Gessa GL (1972) Changes in brain serotonin metabolism associated with fasting and satiation in rats. Life Sci 11: 31-39.

Piacsek BE, Meites J (1967) Reinitiation of gonadotropin release in underfed rats by constant light or epinephrine. Endocrinology 81: 535-546.

Pirke KM, Spyra B (1981) Influence of starvation on testosterone-luteinizing hormone feedback in the rat. Acta Endocrinologica 96: 413-421.

Pitis M, Nicolescu-Catangi A, Tasca C (1970) Effect of pinealectomy on the gonads in newly born and immature rats.

Rev Noum Endocrinol 7: 37-41.

Pitis M, Scherzer M (1969) The action of the pineal gland on the compensatory gonadal hyperplasia in immature rats. Rev Noum Endocrinol 6: 209-213.

Preslock JP (1984) The pineal gland: basic implications and clinical correlations. Endocr Rev 5: 282-308.

Quay WB (1956) Volumetric and cytologic variation in the pineal body of Peromyscus leucopus (Rodentia) with respect to sex, captivity and day-length. J Morphol 98: 471-495.

Quay WB (1963) Circadian rhythm in rat serotonin and its modifications by estrous cycle and photoperiod. Gen Comp Endocrinol 3: 473-479.

Quay WB (1967) Lack of rhythm and effect of darkness in rat pineal content of N-acetyl-serotonin-O-methyltransferase. Physiologist 10: 286.

Quay WB (1974) Pineal chemistry. Thomas, Springfield, Ill.

Ralph CL (1981) Melatonin production by extra-pineal tissues. Adv Biosci 19: 35-46.

Ralph CL, Mull D, Lynch HJ, Hedlund L (1971) A melatonin rhythm persists in rat pineals in darkness. Endocrinology 89: 1361-1366.

Reiter RJ (1972) Role of the pineal in reproduction. In: Reproductive Biology. Balin H, Glasser SR (eds) Excerpta Medica Foundation, Amsterdam, pp 71-114.

Reiter RJ (1974) Pineal regulation of hypothalamicopituitary axis: gonadotrophins. In: Handbook of Physiology, Vol 6, The Pituitary Gland and its Neuroendocrine Role, Part 2. American

Physiological Society, Washington DC, pp 519-550.

Reiter RJ (1980) The pineal and its hormone in the control of reproduction in mammals. Endocr Rev 1: 109-131.

Reiter RJ, Ellison NM (1970) Delayed puberty in blinded anosmic female rats: role of the pineal gland. Biol Reprod 2: 216-222.

Reiter RJ, Rudeen, PK, Sackman JW, Vaughan MK, Johnson LY, Little JC (1977) Subcutaneous melatonin implants inhibit reproductive atrophy in male hamsters induced by daily melatonin injections. Endocrinol Res Commun 4: 35-44.

Reiter RJ, Sorrentino S (1971) Inhibition of luteinizing hormone release and ovulation on PMS-treated rats by peripherally administered melatonin. Contraception 4: 385-392.

Reiter RJ, Vaughan MK, Blask DE, Johnson LY (1974) Melatonin: its inhibition of pineal antigonadotrophic activity in male hamsters. Sciene 185: 1169-1171.

Riederer P, Toifl K, Kruzik P (1982) Excretion of biogenic amine metabolites in anorexia nervosa. Clin Chim Acta 123: 27-32.

Rollag MD, Panke ES, Trakulrungsi WK, Trakulrungsi C, Reiter RJ (1980) Quantitation of daily melatonin synthesis in the hamster pineal gland. Endocrinology 106: 231-236.

Romero JA, Axelrod J (1974) Pineal β-adrenergic receptor: diurnal variation in sensitivity. Science 184: 1091-1092.

Romero JA, Axelrod J (1975) Regulation of sensitivity to β-adrenergic stimulation in induction of pineal N-acetyltransferase. Proc Natl Acad Sci USA 72: 2107-2111.

Romero JA, Zatz M, Axelrod J (1973) Beta-adrenergic stimulation of

pineal N-acetyltransferase: adenosine 3',5'-cyclic monophosphate stimulates both RNA and protein synthesis. Proc Natl Acad Sci USA 72: 2107-2111.

Romero JA, Zatz M, Kebabian JW, Axelrod J (1975) Circadian cycles in binding of ^{3}H-alprenolol to β-adrenergic receptor sites in rat pineal. Nature 258: 435-436.

Roth WD (1965) Metabolic and morphologic studies on the rat pineal organ during puberty. Prog Brai Res 10: 552-563.

Rudman D (1976) Injection of meltonin into cisterna magna increases concentration of 3',5' cyclic guanosine monophosphate in cerebrospinal fluid. Neuroendocrinology 20: 235-242.

Russell GFM (1967) The nutritional disorder in anorexia nervosa. J Psychosom Res 11: 141-149.

Scheving LE, Dunn JD, Pauly JE, Harrison WH (1972) Circadian variation in rat serum 5-hydroxytryptamine and effects of stimuli on the rhythm. Am J Physiol 222: 252-255.

Shein HM, Wurtman RJ, Axelrod J (1967) Synthesis of serotonin by pineal glands of the rat in organ culture. Nature 213: 730-731.

Snyder SH, Zweig M, Axelrod J (1964) Control of the circadian rhythm in serotonin content of the rat pineal gland. Life Science 3: 1175-1179.

Snyder SH, Zweig M, Axelrod J, Fisher JE (1965) Control of circadian rhythm in serotonin content of rat pineal gland. Proc Natl Acad Sci USA 53: 301-305.

Snyder SH, Zweig M, Axelrod J (1967) Circadian rhythm in the

serotonin content of the rat pineal gland: regulating factors. J Pharmacol Exp Ther 158: 206-213.

Sorrentino S, Benson B (1970) Effects of blinding and pinealectomy on the reproductive organs of adult male and female rats. Gen Comp Endocrinol 15: 242-246.

Sorrentino S, Reiter RJ, Schalch DS (1971) Interaction of the pineal gland, blinding and underfeeding on reproductive organ size and radioimmunoassayable growth hormone. Neuroendocrinology 7: 105-115.

Stiles GL, Caron MG, Lefkowitz RJ (1984) β-adrenergic receptors: biochemical mechanisms of physiological regulation. Physiol Rev 64: 661-743.

Stone EA (1983) Reduction in cortical beta adrenergic receptor density after chronic intermittent food deprivation. Neurosci Lett 40: 33-37.

Strada SJ, Klein DC, Weller J, Weiss B (1972) Effect of norepinephrine on the concentration of adenosine 3',5'-monophosphate of rat pineal gland in organ culture. Endocrinology 90: 1470-1475.

Suttie JW (1968) Effect of dietary fluoride on the pattern of food intake in rat and development of a programmed pellet dispenser. J Nutr 96: 529-536.

Tamarkin L, Westrom WK, Hamill AI, Godman BD (1976) Effect of melatonin on the reproductive systems of male and female Syrian hamsters: a diurnal rhythm in sensitivity to melatonin. Endocrinology 99: 1534-1541.

Trentini GP, Botticelli AR, Sannicola Botticelli C, Barbanti Silva C

(1976) Decreased ovarian LH incorporation after melatonin treatment. Horm Metab Res 8: 234-236.

Vacas MI, Cardinali DP (1979a) Effects of castration and reproductive hormones on pineal serotonin metabolism in rats. Neuroendocrinology 28: 187-195.

Vacas MI, Cardinali DP (1979b) Diurnal changes in melatonin binding sites of hamster and rat brains. Correlation with neuroendocrine responsiveness to melatonin. Neurosci Lett 15: 259-263.

Vacas MI, Lowenstein PR, Cardinali DP (1982) Testosterone decrease -adrenoceptor sites in rat pineal gland and brain. J Neural Transm 53: 49-57.

Vaughan MK, Little JC, Johnson LY, Blask DE, Vaughan GM, Reiter RJ (1978) Effects of melatonin and natural synthetic analogues of aginine vasotocin on plasma prolactin levels in adult male rats. Horm Res 9: 236-246.

Vaughan MK. (1981) The pineal gland - a survey of its antigonadotropic substances and their action. Int Rev Physiol Vol 24. McCann SM (ed), University Park Press, Baltimore, MD, pp 41-95.

Vriend J, Reiter RJ (1977) Free thyroxine index in normal, melatonin-treated and blind hamsters. Horm Metab Res 9: 231-234.

Walker RF, Bethea CL (1977a) Gonadal function in underfed rats: I. Effect of pineal gland and constant light on maturation and fecundity. Biol Reprod 17: 623-629.

Walker RF, Frawley S (1977b) Gonadal function in underfed rats:

II. Effect of estrogen on plasma gonadotropins after pinealectomy or constant light exposure. Biol Reprod 17: 630-634.

Walker RF, McMahon KM, Pivorun EB (1978) Pineal gland structure and respiration as affected by age and hypocaloric diet. Exp Gerontol 13: 91-99.

Weissbach H, Redfield BG, Axelrod J (1960) Biosynthesis of melatonin: enzyme conversion of serotonin to N-acetylserotonin. Biochem Biophys Acta 43: 352-353.

Weiner RI, Ganong WF (1978) Role of brain monoamines and histamine in regulation of anterior pituitary secretion. Physiol Rev 58: 905-976.

White WF, Hedlund MT, Weber GF, Rippel RH, Johnson ES, Wilber JF (1974) The pineal gland: a supplemental source of hypothalamic-releasing hormones. Endocrinology 94: 1422-1426.

Withyachumnarnkul B, Knigge KM (1980) Melatonin concentration in cerebrospinal fluid, peripheral plasma and plasma of the confluens sinuum of the rat. Neuroendocrinology 30: 382-388.

Wurtman RJ, Altschule MD, Holmgren V (1959) Effects of pinealectomy and of the bovine pineal extract. Am J Physiol 197: 108-110.

Wurtman RJ, Anton-Tay F (1969) The mammalian pineal as a neuro-endocrine transducer. Recent Prog Hormone Res 25: 493-522.

Wurtman RJ, Axelrod J, Chu EW (1963) Melatonin, a pineal substance: effect on the rat ovary. Science 141: 277-278.

Wurtman RJ, Axelrod J, Snyder SH (1965) Changes in enzymatic

synthesis of melatonin in the pineal gland during the estrous cycle. Endocrinology 76: 798-800.

Wurtman RJ, Fernstrom JD (1976) Control of brain neurotransmitter synthesis by precursor availability and nutritional state. Biochem Pharmacol 25: 1691-1696.

Ying SY, Greep RD (1973) Inhibition of ovulation by melatonin in the cyclic rat. Endocrinology 92: 333-355.

Yocca FD, Friedman E (1984) Effect of immobilization stress on rat pineal beta-adrenergic receptor mediated function. J Neurochem 42: 1427-1432.

Yu HS, Pang SF, Tang PL, Brown GM (1981) Persistence of circadian rhythms of melatonin and N-acetylserotonin in the serum of rats after pinealectomy. Neuroendocrinology 32: 262-265.

Yuwiler A (1982) Sympathetic innervation of the pineal gland: Effect of thyroxine and cortisol. In: Melatonin Rhythm Generating System. Klein DC (ed), Karger, Basel, pp 42-61.

Zatz M, Brownstein MJ (1979) Central depressants rapidly reduce nocturnal serotonin-N-acetyltransferase activity in the rat pineal gland. Brai Res 160: 381-385.

Zatz M, Kebabian JW, O'Dea RF (1978) Regulation of β-adrenergic function in the rat pineal gland. In: Receptors and Hormone Action, Vol 3. O'Malley BW, Birnbauer L (eds), Academic Press, New York, pp 195-219.