A STUDY OF THE EFFECT OF PROGESTERONE ON THE BODY WEIGHT REGULATION IN INTACT FEMALE RATS

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ABSTRACT

It is the aim of this study to elucidate the influence of progesterone on body weight regulation in intact female rats.

A study of the literature includes a description of the body weight regulation and the effects of ovarian hormones on it. The controlled-system approach tries to link behavioral and physiological factors altering energy balance.

The experimental study is subdivided into food-intake - and food-selection studies, a locomotor activity study, a study eliciting a possible role of thermogenesis, and finally rat liver studies which consist of a gas chromatography analysis of hepatic fatty acids and an electron microscopy study examining the ultrastructure of hepatocytes.

It can be concluded that the effect of progesterone treatment on the body weight of intact female rats depends on the route of administration. There is a significant increase in body weight after subcutaneous progesterone injections without changes in total caloric intake and nutrient selection habits, indicating the importance of energy expenditure. But changes in spontaneous activity make no contribution in the progesterone-induced energy storage. It is also concluded that peripherally located brown adipose tissue thermogenesis is not changed, without ruling out the effect of more centrally located thermogenic organs as the liver. In this organ, small but significant changes in the fatty acid profile occur during the subcutaneous progesterone treatment.

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LIST OF ABBREVIATIONS

AA = amino acid(s)
AT = adipose tissue
BAT = brown adipose tissue
BW = body weight
CA = catecholamine(s)
CCK = cholecystokinin
CH = carbohydrate
CI = caloric intake
CNS = central nervous system
CRF = corticotropin releasing factor
DIT = diet-induced thermogenesis
E = estrogen
EI = energy intake
ER = endoplasmatic reticulum
Es = estradiol
FA = fatty acid(s)
FAME = fatty acid methyl ester(s)
FFA = free fatty acid(s)
FI = food intake
GC = gas chromatography
GH = growth hormone
GI = gastrointestinal
HL = hepatic lipase
IL = intracellular lipase
IP = intraperitoneal(ly)
LH = lateral hypothalamus
LPL = lipoprotein lipase
NA = noradrenalin
OH = ovarian hormone(s)
OV = oily vehicle
OVX = ovariectomy
P = progesterone
PVN = paraventricular nucleus
prot = protein
RER = rough endoplasmatic reticulum
SC = subcutaneous(ly)
SER = smooth endoplasmatic reticulum
SNS = sympathetic nervous system
sug = sugar(s)
TG = triglyceride(s)
TMS = trimethylsilylation
VMH = ventromedial hypothalamus
WAT = white adipose tissue

CHAPTER I

LITERATURE REVIEW

I.1 BODY WEIGHT REGULATION

Body weight (BW) is carefully regulated in most laboratory animals and in human beings through a variety of physiological and behavioral mechanisms. It is obvious that careful BW regulation is essential to the health and well-being of these organisms.

BW in rats (and other species) is a product of the energy balance, the balance between energy intake and energy expenditure. If intake exceeds expenditure, calories are stored and 90% is stored as fat; when output is chronically higher than caloric consumption, the body's energy stores are mobilized, and BW drops.

Caloric intake (CI) is simply determined by food consumption. Since various foodstuffs vary in caloric content and nutritive value, what a rat eats may be as important for BW regulation as how much it eats.

Animals expend calories in a wide variety of ways and for the sake of convenience, energy expenditure is often divided into obligatory and facultative components. Obligatory energy

expenditure includes both the basal metabolic rate (the cost of maintaining minimum body functions, growth and reproduction) and the thermic effect of feeding (the cost of assimilating and storing of ingested nutrients). Facultative energy expenditure includes behaviors such as voluntary exercise and thermoregulatory behavior (nest-building) in addition to controllable metabolic heat production as in thermoregulatory or diet-induced thermogenesis.

There are many physiological and behavioral factors influencing CI and caloric expenditure. So the constancy of BW can only be achieved by a complex series of interactions that relate short-term feeding behavior to long-term caloric storage. Despite extensive study an understanding of the specific control mechanisms leading to this regulation has remained elusive and no unified mechanism has been developed and agreed upon. Perhaps one of the most important factors is the highly redundant nature of these control mechanisms (Anderson et al. 1984).

I.1.1 THE CONSTANCY OF AN ACCURATELY REGULATED VARIABLE : BW

During the adult life of most species, BW remains relatively constant over substantial periods of time (Anand 1961). Forbes et al. (1970) show that for human beings there is a small rise in absolute BW of both males and females between 20 and 50 years of age. Hervey (1969) concludes that adults of most mammalian species gain weight only very slowly with age. Although short-term errors do occur in this regulation, long-term BW

regulation is an amazingly accurately regulated variable.

Many authors use the term BW set-point to describe the level at which rats maintain their BW. Although some believe that rats really regulate their BW about a neurally determined set-point, it is more likely that the set-point is a product of a number of forces acting upon regulatory behaviors and metabolic processes. The set-point may just be a compromise among these various forces. The concept of a BW set-point includes the idea that alterations in the quantity of stored calories (and consequently BW) initiate changes designed to restore the calorie storage to its original level (= set-point). Thus, when one factor of the energy balance changes, it automatically involves an opposite adjustment of another one.

During a single year, an average adult will ingest more than one million calories (Bray 1975); however BW usually remains essentially unchanged. Storage of only 1% of this total caloric intake would result in a considerable weight gain. For BW to remain stable over a period of time, all ingested calories must be expended.

There seems to be a mechanism by which energy intake (EI) is monitored. Data of Mc Hugh (1978) show that the monkey has a highly responsive caloric meter for all the major nutrients. So volume and nutrient composition of one meal will influence subsequent food intake (FI).

Caloric expenditure declines progressively with age (Bray 1979). A gradual decline in CI is essential for prevention of obesity.

Ambient temperature also influences FI (Bray 1980). When goats are placed in a high environmental temperature, the rectal temperature rises slightly, and FI declines. And cold exposure will bring about a quantitative increase in FI of rats (Sellers et al. 1954).

There is clear evidence that under most conditions high levels of physical activity are associated with an increase in EI and a shift towards more muscular tissue and less fat (Bray 1979).

Further evidence supporting the concept of a regulated total caloric storage comes from experiments in which caloric storage was disturbed either by restricting calories (Boyle et al. 1978, Levitsky 1970, Levitsky et al. 1976) or feeding excess calories through a stomach tube (Cohn et al. 1962). When caloric restriction or tube feeding was stopped, the animals returned to the initial BW. Moreover, many workers have observed that during a refeeding period following restriction and weight loss, rats gain more weight per unit of food ingested than do control animals and an increase in FI is not essential to BW restoration (Boyle et al. 1978, Meyer et al. 1964, Meyer et al. 1956, Borer et al. 1979, Levitsky et al. 1976, Szepesi et al. 1976). In an attempt to control BW during fasting, a substantial reduction in the capacity for nonshivering thermogenesis occurs (Trayhurn et al. 1988) and there is a slow recovery during the refeeding period.

So energy balance is physiologically controlled and it is assumed that total caloric storage -and thus BW- is the regulated variable in the calorie intake-output balance. It is now important to

clarify the behavioral, hormonal and metabolic signals and their impact on the interaction of energy expenditure with the mechanism regulating energy intake.

Many experiments in which these signals have been manipulated show that the BW set-point is indeed a compromise maintained by making adjustments in the intake and/or expenditure of energy. Studies of animals with injury to the ventromedial nuclei or the paraventricular nucleus (Fukushima et al. 1987, Brooks et al. 1946, Leibowitz et al. 1981, Tokunaga et al. 1986) of the hypothalamus show that the animals go through an initial transient phase of weight gain with marked hyperphagia. This is followed by a reduction in FI and the establishment of an elevated steady-state BW. The lesions have apparently changed the forces and thus consequently the compromise set-point. That the body is now protecting this new point is demonstrated after a distrurbance of this new plateau by over- or underfeeding, as it is followed by a return to the new level of BW.

BW is also influenced by photoperiod, e.g. in Syrian hamsters (Bartness et al. 1984, Hofmann et al. 1982, Campbell et al. 1983) and this supports the idea of BW as an accurately regulated variable. Animals are not only able to respond to metabolic perturbations with corrective behavioral and physiological adjustments so that supplies of metabolic fuels to crucial tissues are not compromised, but metabolic challenges may also be predicted well in advance of their occurrence. Animals have a selective advantage if they are able to anticipate these demands and begin making physiological and behavioral adjustments before there is a metabolic emergency. A naturally occurring example of

predictable metabolic challenge is winter and the increase in BW and carcass lipid during this short photoperiod represent adaptive preparatory responses that enhance winter survival.

Besides the overall control of energy balance, there are probably some other important feeding control mechanisms as nutrient specific appetites and their regulation and role in feeding (Anderson et al. 1984).

I.1.2 BODY WEIGHT & THE CONTROLLED-SYSTEM APPROACH

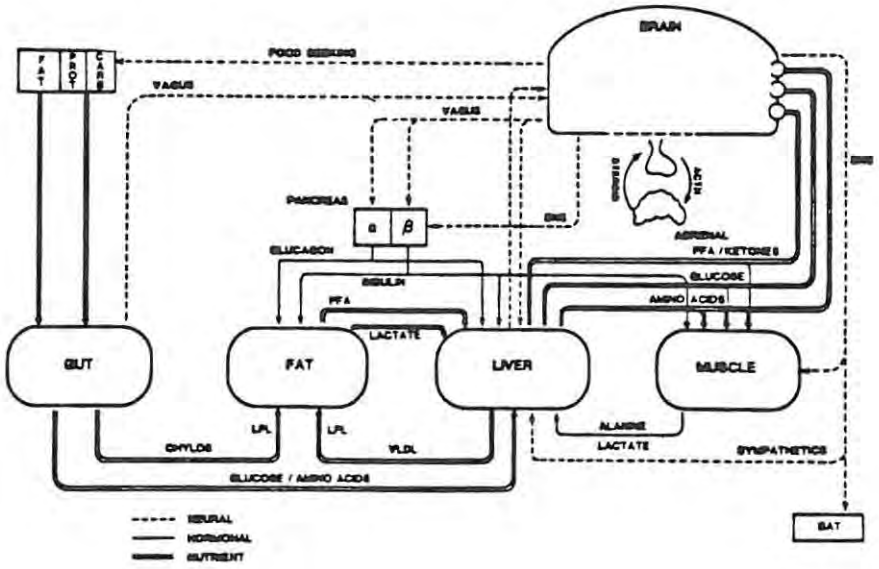
In a recent article Bray (1989) analyses the concept of BW control and its disturbance (obesity), using a controlled-system approach and tries to link some behavioral and physiological factors altering energy balance. A regulated system has several features. First, there is a controller located in the brain; second, there is a controlled system consisting of FI, its storage and metabolism; third, there are feedback signals that inform the controller about the controlled system, and, finally, there are the efferent control mechanisms.

I.1.2.1 THE CONTROLLED NUTRIENT SYSTEM

In order to maintain a relatively constant level of total energy stores, BW, energy intake and energy expenditure are matched. The quantity and quality of intake at each meal must be related by some mechanism(s) to "body needs", as subsequent meals are adjusted to changes in diet and caloric stores. This regulated system for nutrient balance is concerned with maintenance of appropriate nutrient stores of each macronutrient in addition to water. It consequently includes intake, storage and disposal of protein, carbohydrate, and fat. Most calories which are not used after intake are stored as fat. This occurs either by making bigger fat cells, or more fat cells (Hirsch et al. 1976). The rest of the consumed and not expended energy is stored as protein and carbohydrate (glycogen). Stored calories are mainly distributed among adipose tissue (AT), muscle and liver.

Comparing the nutrient intake in relation to quantities of each nutrient already in body stores, the daily intake of protein and fat is only a small percentage of total stores, while carbohydrate intake per day is equal to 100 % of the body stores of carbohydrate (Flatt 1987). Therefore it is not surprising that changes in carbohydrate balance from day-to-day influence changes in carbohydrate intake on the subsequent day, while daily protein and fat balance have much less effect on subsequent FI (Flatt et al. 1985).

The flow of nutrients through the controlled system is presented in the figure below (fig I.1) (Bray 1989).



Nutrient intake is shown on the left, with the various components of tissue metabolism shown below. Food entering the gut can send signals to the brain. Within the gut, food is digested and absorbed into the portal circulation directly to the liver (pathway for carbohydrate, amino acids and short-chain fatty acids). The other lipids are packed in chylomicrons that are cleared by means of lipoprotein lipase (LPL) prior to reaching liver or other tissues. Glucagon and insulin act on adipose tissue and on the liver, an organ which receives nutrients from the fat cells and the circulation. Amino acids, glucose, free fatty acids (FFA), and ketones from the liver are utilized by muscle and other tissues, including the brain. In general, the brain utilizes only carbohydrate, although, in some cases ketone bodies are utilized as well. One major limitation in fatty acid oxidation by the brain is the transport of FA across the blood-brain barrier.

In his review, Bray (1989) indicates that the balance of each major nutrient may be regulated separately. Achievement of nutrient balance requires that the net oxidation of each nutrient equals the average composition of the nutrients in the diet. If the system is disturbed, it will attempt to return to its original output level and will do so successfully if the disturbance does not force the system out of its range of operation. Obesity occurs when the controlled system fails to maintain balance and excess fat is stored. Excess carbohydrate can be converted to fatty acids, although this is an energetically expensive transformation. To avoid obesity, the ingestion of a high-fat diet requires greater fat oxidation than a low-fat one.

Nutrient intake plays a variable role in the development of experimental forms of obesity and probably in human obesity as well. It appears that hyperphagia is essential for the development of obesity in animals with lesions in the paraventricular nucleus (Fukushima et al. 1987, Leibowitz et al. 1981, Tokunaga et al. 1986, Weingarten et al. 1985), while in another form of hypothalamic obesity, produced by lesions in the ventromedial hypothalamus, hyperphagia is not essential for the development of this syndrome (Cox et al. 1981, Frohman et al. 1969, Han et al. 1970). Most genetic obesities are hyperphagic, but this is not essential (Coleman 1982, Bray et al. 1979, Cox et al. 1977). Among syndromes caused by peripheral manipulations, ovariectomy results in modest hyperphagia, though this is not essential (Slafani 1984) but these animals are more susceptible to weight gain from a high-fat diet.

It is now well recognized that dietary obesity can be produced in

at least three ways. The first of these is to feed animals a high-fat diet (Bray 1980). The second is to offer a sucrose solution in addition to their normal diet (Kanarek et al. 1977). The third is to provide animals with a variety of "snack" foods in addition to their regular diet (Sclafani et al. 1976). In each instance the animal fails to compensate for the novel foods or changed fat content by adjusting the CI to maintain energy balance. There seems to be a loss of the caloric monitoring mechanism. The animal appears not to know that it has eaten large amounts of energy in form of fat, while it does seem to know if it is in the form of pellets or powdered diet. In contrast, Wade (1983) shows that golden hamsters fed a high-fat diet do not increase their CI, but they become obese because of decreases in energy expenditure and this effect is exaggerated by reduction of the day length. This decrease in actual energy expenditure is accompanied by increases in thermogenic capacity and brown adipose tissue (BAT) mass. So, this apparent loss of BW control mechanism could be explained as a regulated BW gain, as some animals (hamsters and voles) use environmental cues, such as photoperiod and diet quality, to make their anticipatory changes in weight, adiposity and thermogenic capacity to enhance winter survival (Bartness et al. 1984, Dark et al. 1984). A prewinter change in diet composition from grasses to seeds could provide the natural source of lipid-rich diet (Morton 1975, Murphy 1971).

Another important dietary component which influences FI is the amino acid composition of the food (Harper 1976). If diets are imbalanced in the content (high or low levels of single amino acids) of amino acids, FI is reduced.

I.1.2.2 THE CONTROLLER

The controller, integrated by the CNS, consists of several components, some of which are neural and others humoral, that control the intake of calories in the form of food, the storage and the disposal of those calories.

a/ Anatomy :

Several anatomic regions of the brain appear to play an important role in the control of energy balance (hippocampus, amygdala, striatum,...) (Luiten et al. 1987), but the hypothalamus is known to play a major role (Leibowitz 1987). Stellar (1954) and Ungerstedt (1971) present the dual-centre hypothesis. Experiments show reciprocal electrical activity of the ventromedial hypothalamus (VMH) and lateral hypothalamus (LH) (Steffens 1975). During periods of hunger, the VMH is inhibited, the LH activated; but with satiety the VMH is electrically active and the LH area less active (Anand et al. 1964). So, the LH, through which dopaminergic fibers run, is involved in the activation which leads to feeding ; the VMH involved in satiety.
Destruction of the lateral hypothalamus (LH) reduces or abolishes FI (aphagia) and reduces BW and body fat in all mammalian species (Keesey et al. 1986). On the other hand, destruction of the VMH of experimental animals (including mice, rats, ground squirrels, monkeys, dogs, cats, as well as human beings) is associated with hyperphagia and obesity (Bray 1979). The anatomic substrate in the hypothalamus which controls feeding is thus common to all of the mammalian species which have been studied.

Lipid metabolism is synchronized to the circadian fluctuation in FI (LeMagnen et al. 1970) and appears to be under the influence of the VMH. Stimulation of the VMH enhances lipolysis in white AT (Shimazu 1981). Electrical activity of the VMH (the satiety centre) but not the LH (the feeding centre) is enhanced in certain types of animals during the light period when the animals are not eating but is suppressed during the dark hours when they are eating (Schmitt 1973). Thus, neural control of FA metabolism may be part of the intrinsic mechanism which governs the animal's circadian feeding behavior.

The hypothalamus not only influences the intake of energy, but also the expenditure. Brooks (1946) indicates that locomotor activity behavior is reduced by destroying the VMH. Hypothalamic obesity is reversed when quinine is added to the diet (Grossman 1966). This suggests that taste perceptions influence the VMH.

b/ Neurotransmitters :

Noradrenaline (NA), serotonin, histamine (Sakata et al. 1988), gamma-aminobutyric acid, and a number of peptides may be involved in the transmission of information that regulates FI and nutrient stores (Baile et al. 1986, Morley 1987).

Infusion of noradrenaline and adrenaline into the VMH can increase FI and fat stores (Shimazu et al. 1986, Leibowitz 1970) and also when injected peripherally (Stevens et al. 1967, Russek et al. 1968). Injection of the alpha-agonist NA into the VMH of the rat increases FI, even in the satiated animal. This effect is blocked

by phentolamine, an alpha-adrenergic blocking drug (Bray et al. 1975). Thus, the effect of the VMH on FI can be inhibited by alpha-adrenergic catecholamines, i.e. , FI can be activated. In contrast FI can be suppressed in hungry rats by injection into the LH of a beta-agonist such as isoproterenol and the beta-blocker propanolol will overcome this suppression.
Thus adrenergic mechanisms for FI involve an alpha-adrenergic hunger and a beta-adrenergic satiety system (Leibowitz 1970).
What is also striking about these neurotransmitter effects on eating is that they are characterized by a specific change in macronutrient selection, besides an increase or decrease in total FI. This specificity argues for a physiological function in regulation of diet composition. In particular, PVN injection of noradrenaline in the rat causes a selective increase in carbohydrate ingestion, in association with little or no change in fat and in some cases a suppression of protein (Leibowitz 1986). A constant carbohydrate craving can be seen in animals receiving chronic stimulation with noradrenaline.
The brain monoamines, which act to suppress eating, may also participate in this process of controlling diet composition. This is indicated by the evidence that peripheral and hypothalamic injection of the CA-releasing drug amphetamine, and lateral hypothalamic administration of CA, cause a preferential decrease in protein consumption, whereas dopamine-receptor blockade preferentially stimulates protein ingestion (Leibowitz et al. 1986).

Serotonin also plays an important role in the regulation of food intake and nutrient stores (Blundell 1984) and appears to be most effective in the medial hypothalamus. Tryptophan and

5-hydroxytryptophan, two precursors of serotonin, both decrease FI. Drugs that block the effect of serotonin can increase BW and those that stimulate release of serotonin or inhibit its re-uptake from nerve endings will result in a reduction in BW (Wong et al. 1987). While noradrenaline in the LH produces satiety for protein, it is observed that serotonin in the VMH selectively suppresses carbohydrate intake leaving protein intake unchanged (Blundell 1984, Shor-Posner et al. 1986, Wurtman 1984).

Thus, both noradrenaline and serotonin play important, but usually reciprocal roles in the regulation of FI through structures located in the medial and lateral hypothalamus (Leibowitz 1986). Leibowitz (1987) also suggests that under conditions involving energy expenditure, for example, during food deprivation, stress, and at the end of the active period of the diurnal cycle, the neurotransmitter system in the hypothalamus becomes physiologically activated. At this particular time, hepatic glycogen stores are low, blood glucose levels decline (LeMagnen 1981) and in the paraventricular nucleus noradrenaline is called upon to initiate the feeding process and thereby restore carbohydrate reserves. This is found to be associated with a sharp peak of circulating corticosterone.

c/ Peptides :

Several peptides also modulate FI (Morley 1987). Neuropeptide-Y (NPY), beta-endorphin, dynorphin, growth hormone-releasing hormone, and galanin all stimulate FI and BW (Lichtenstin et al. 1984, Stanley et al 1985) when applied to the VMH (Leibowitz 1986, Morley 1987) and GABA, which mediates the effects of some

of those endogenous peptides, may be an important transmitter in the feeding system (Powley 1977). On the other hand, a variety of other peptides, including bombesin, cholecystokinin, anorectin, calcitonin, neurotensin, thyrotropin-releasing hormone, and corticotropin-releasing (CRF) factor can inhibit feeding when injected topically in the region of the ventromedial nucleus or when infused into the third ventricular system (Arase et al. 1987, Morley 1987). In addition to these peptides, there also is evidence to suggest that infusing either insulin or glucagon into the ventricular system of the brain can decrease FI in several species, but the physiological importance of this remains unclear (Brief et al. 1984, Inokuchi et al. 1984).

Neuropeptide Y produces a pattern of diet preference, similar to noradrenaline (Stanley et al. 1985). This raises the possibility that noradrenaline and NPY may be acting through the same VMH system that specifically controls carbohydrate intake (Everitt et al. 1984). Interestingly, this contrasts with galanin, which stimulates fat intake to a greater extent than carbohydrate intake (Tempel et al. 1986), and also the opioid peptides which enhance ingestion of fat and, to some extent, of protein, while actually suppressing the relative proportion of carbohydrate ingested (Leibowitz 1986).

I.1.2.3 EFFERENT CONTROL MECHANISMS

These mechanisms include mainly the efferent effects produced by

the autonomic nervous system and circulating hormones, in addition to behaviors involved in seeking and ingesting of food.

a/ Autonomic nervous system:

Both the efferent sympathetic and parasympathetic nervous systems are involved in the control of energy balance. In animals where obesity follows hypothalamic lesions, there is evidence for increased activity of the efferent vagus nerve (Cox 1981, Inoue et al. 1983). Reduction in sympathetic activity is characteristic of the obese state and there is an inverse relationship between the activity of the sympathetic nervous system and FI (Arase et al. 1988, Arase et al. 1987, Sakaguchi et al. 1988). Experimental situations that increase (VMH-lesions, genetic obesity) or decrease (LH-lesions, fenfluramine) FI and BW, result in a decrease or increase in sympathetic activity respectively (Sakaguchi et al. 1988, Arase et al. 1987, Lupien et al. 1985, Yoshida et al. 1983). Destruction of the VMH is also associated with hyperinsulinemia and hypertriglyceridemia. These changes appear to be partly the result of changes in the autonomic nervous system (Nishizawa et al. 1978). The vagus nerve becomes hyper-reactive and the sympathetic nervous system is reduced in activity. In a series of studies, Powley et al. (1974) transect the vagus nerve of rats below the diaphragm and show that the obesity of animals with VMH-lesions is abolished and that the animals return to normal weight.

Cold exposure and fasting, as well as overfeeding with sucrose, fat, or other palatable foods, are conditions known to alter the

functional state of the sympathetic nervous system (SNS) (Avakian et al. 1981, Rothwell et al. 1980, Young et al. 1977) and so energy expenditure. Cold exposure and overfeeding, which result in increased heat production and O^2 consumption (Foster et al. 1978), activate the sympathetic nervous system in the pancreas, liver, heart and BAT (Young 1981). Fasting, on the other hand, appears to reduce the activity of the SNS in all tissues. Genetically-obese rats (Osborne-Mendel rats) may have a less active functional state of SNS (Fisler et al. 1984).
In contrast to other researchers, Peterson et al. (1988) note that depression in parasympathetic activity also may occur with increasing percentages of body fat. A disordered homeostatic mechanism may promote excessive storage of energy by decreasing sympathetic activity, while defending against weight gain by decreasing parasympathetic activity.

b/ Insulin & Glucagon :

As mentioned above, increased levels of insulin are characteristic of obesity. Moreover insulin injections can increase FI (LeMagnen 1983, Hoebel et al. 1966), due to an increase in carbohydrate intake (Kanarek et al. 1980). This is probably the result of a decreased glucose concentration in the blood. An anatomic locus for this effect is suggested by the studies on LH lesioned rats (Epstein et al. 1967). In these animals insulin-induced hypoglycemia does not increase FI. Leibowitz (1987) indicates that insulin, just like corticosterone, appears to function synergistically with the central neurotransmitters for their effects on FI. The strongest evidence has been obtained for eating elicited by NA injection into the PVN, which is abolished

by adrenalectomy and attenuated by dissection of vagal fibers to the pancreas (Roland et al. 1986). It is also indicated by Friedman et al. (1987) that insulin counteracts the satiating effect of a fat meal in rats.

Injections of glucagon into rats and humans diminish FI.

c/ Adrenal Steroids :

The development or progression of experimental obesity is reversed or attenuated by adrenalectomy (Bruce et al. 1982, Debons et al. 1982, Mook et al. 1972, Saito et al. 1984). Addison's disease with adrenal insufficiency is associated with leanness, whereas Cushing's syndrome is associated with obesity. The fact that almost all defects in the genetically obese animal are reversed by adrenalectomy (Holt et al. 1982, Marchington et al. 1983) suggests that glucocorticoids play a key role in the development of this syndrome. Adrenalectomy produces two effects on FI (Fukushima et al. 1985). First, FI returns to nearly normal levels after adrenalectomy in genetically obese animals (Saito et al. 1984, Marchington 1983), in animals with ovariectomy-induced obesity (Mook et al. 1972), and in animals with hypothalamic obesity (Bruce et al. 1982). Feeding patterns also revert to normal. Leibowitz (1986) has shown that adrenalectomy almost completely abolishes the stimulation of FI caused by NA injection into the PVN. Treatment with corticosterone rapidly reverses this effect.

Energy expenditure (stimulation of thermogenesis and BAT activity) is also increased and energetic efficiency decreased after adrenalectomy (Fukushima et al. 1985, van der Tuig et al. 1984).

Many researchers have shown increased activity of the sympathetic nervous system following adrenalectomy (Holt et al. 1982, Marchington et al. 1983, van der Tuig et al. 1984). After adrenalectomy, the negative feedback signal produced by corticosterone is absent and CRF will be synthesized in the PVN and released into the hypothalamic portal circulation to stimulate increased ACTH output from the pituitary. This increased CRF also may reduce FI and stimulate sympathetic activity following adrenalectomy. Injections of CRF into the cerebral ventricle of the rat decrease FI and BW (Levine et al. 1983) and increase circulating levels of adrenaline and noradrenaline (Brown et al. 1982).

d/ Gonadal Steroids :

Gonadal steroids also play a role in the modulation of energy balance. In the rodent and primate there is an association between the estrous cycle and the intake of food and physical activity (Bray 1974). When estradiol (Es) rises during the estrous cycle locomotor activity increases and FI shows a reciprocal decrease. During diestrus, on the other hand, estrogen (E) is low, locomotor activity is decreased, FI increases and BW rises. Castration in the female rat abolishes this cyclic pattern and administration of Es will restore it. As might be expected from this cyclic pattern of FI and estrous cycles, castration with removal of Es is followed by increased FI and obesity (Sclafani et al. 1981). This obesity is not reversed by vagotomy (Eng et al. 1979) whereas hypothalamic obesity is. Adrenalectomy does reverse the obesity induced by castration (Mook et al 1972). The potent effect of Es on energy storage is modulated both through direct

action of the hormone on the VMH and through the peripheral effects of Es. Progesterone (P) is thought to antagonise the E effects on BW (Wade 1976).
Testosterone, one of the most potent androgens, increases FI and lean tissue and decreases total body fat of weanling rats (Nunez et al. 1982).

e/ Hypophysis Hormones :

The pituitary, through its secretion of growth hormone (GH) and control of other endocrine glands, is also involved in control of energy storage. In hypophysectomized rats, growth ceases and body fat content increases relative to lean mass. Chronic GH therapy causes lean mass to increase and fat to decrease. Thus GH has a role in facilitating the transfer of calories from fat to lean tissues (Woods et al. 1974).

f/ Thyroid Hormones :

Thyroid hormones modulate the basal metabolic rate and thus modify caloric disposal. Although catabolism of protein, carbohydrate and fat are all accelerated by administration of thyroid hormones, enhanced lipolysis is among the earliest changes and fat stores can be severely depleted. Conversely, deficiency of thyroid hormones is accompanied by increased body fat. These modifications in caloric stores indicate that FI is not always adjusted appropriately to altered caloric disposal. Circulating levels of triiodothyronine are usually elevated in overfed animals and depressed by food restriction.

I.1.2.4 AFFERENT OR FEEDBACK SIGNALS

Several mechanisms measure the quantity of calories stored as triglycerides (TG), glycogen and protein and send this information about the state of peripheral metabolism to the brain. These afferent signals can be transmitted over the somatic sensory system, through the bloodstream, or via the autonomic nervous system.

a/ Sensory Signals:

The sight and smell of food are important signals for initiating food-seeking behavior and identifying potential sources of food. Along with the taste and texture of the food in the mouth, these sensory cues about the availability and quality of food can serve both as positive feedback signals, initiating continued food ingestion (Powley 1977), or negative signals to slow down or terminate eating. The ability of most animals to avoid foods that previously have made them sick, is an example of these afferent sensory signals, integrated with a central learning system.

Also ambient temperature and heat production play a role in regulation of FI (Brobeck 1960). High ambient temperature tends to reduce FI via a rise in rectal temperature and low temperature to increase it. It has also been suggested that endogenous heat production from BAT or other sites may act as an afferent satiety signal (Glick et al. 1981), as increases in heat production occur

following ingestion of food. It is called "diet-induced thermogenesis " (DIT) and provides a mechanism for adjusting energy balance to counteract errors in appetite control (Stock et al. 1986). Increases in DIT in response to overfeeding have been demonstrated in several species and these increases in energy expenditure appear to be mediated by the SNS, which causes activation of heat production in BAT (Rothwell et al. 1981).

b/ Gastrointestinal Signals :

Information from food in the gastrointestinal (GI) tract can be initiated by one of two mechanisms. The first is gastric distension (Jordan 1973) and the second is release of GI hormones by nutrients acting directly on neural receptors in the richly innervated enteric plexus.

Gastric and intestinal distension are both mechanisms for terminating meals by negative neural feedback systems. The vagus nerve is the principal afferent sensory relay for this type of information.

Signals generated by the digestion and/or absorption of food from the gut may also participate in producing satiety, a phenomenon which is shown in stomach - and intestine - cannula experiments (Smith et al. 1976). This effect could be mimicked by the peptide cholecystokinin (CCK), which is a GI hormone that reduces FI by constricting the pylorus and delaying gastric emptying; thus increasing the duration and intensity of signals from gastric distension (McHugh et al. 1986). In addition to CCK, a bombesin-like peptide, also released from the GI tract, may serve as an

important signal for the presence and/or quantity of food that is being ingested resulting in a inhibition of FI (Gibbs et al. 1986).

LeMagnen (1959) demonstrated that in rats the interval between meals was related to the size of the preceding meal. If the stomach is experimentally emptied shortly after eating, the interval between meals is shortened. Conversely, vagotomy which delays gastric emptying, prolongs the intermeal interval (Snowdon 1970).

The humoral mechanism of termination of food ingestion is indicated by experiments in which blood from satiated rats was cross-transfused into starved rats and vice-versa (Davis et al. 1971). Up to 3 hours after eating there is a substance in the circulation of the fed animals that reduces FI in the hungry rats.

c/ Nutrient & Hormonal Signals :

Since total energy storage is the end result of the intake, distribution, and disposal of the "independent" units, carbohydrate, fat and protein, there are several metabolic feedback signals that provide a measure of total stored calories, e.g. plasma glucose, FFA, glycerol and amino acids, etc., all of which provide a measure of the peripheral metabolic state that can be detected in the circulation. From this information brain feeding control systems are able to respond and direct the animal to make appropriate food choices so that both quantitative

(energy) needs as well as the qualitative (nutrient) needs are met.

When a normal diet is diluted with indigestible material, such as cellulose, the mass of food ingested is adjusted to provide maintenance of total CI (Bray et al. 1972). Compensatory mechanisms independent of GI distension lead to adjustment of the mass of food ingested to maintain a constant CI. When foods with an increased caloric density are made by increasing the percentage of fat, some strains of rats will show precise adjustment of consumption and will maintain BW in spite of the increased caloric density of the diet (Schemmel et al. 1970). However most strains of rats are unable to make a complete adjustment and will store excessive quantities of calories and become obese.

Nutrient and hormonal signals may act on both the liver and the brain. The hepatic mechanisms clearly involve the vagal afferent nerves, which mediate the signals originating from hepatic oxidation of metabolic fuels (Langhans et al. 1985). Niijima (1984) shows that glucose injected into the hepatic circulation decreases vagal afferent firing rate, probably through hepatic glucose receptors. The sensitivity of the hepatic glucoreceptors also appears to be influenced by the state of energy balance. A decrease of FI after glucose infusion into the portal vein occurs only in food-deprived rabbits, suggesting that liver energy reserve (glycogen) may signal energy balance. The significance of hepatic glucoreceptors in maintaining body energy balance is questioned however by the observation that denervation of the liver in dogs and rats does not affect FI (Bellinger et al. 1981, Louis-Sylvestre et al. 1980).

Glucagon may also act on the liver and intestinal receptor systems to initiate satiety, and the effect is partially abolished by vagotomy (Geary et al. 1986).

The possibility that glucose was a major feedback signal was supported originally by Mayer in what has come to be known as the glucostatic theory (Mayer et al. 1967). According to this theory, control of FI is based on the rate of utilization of glucose. When plasma concentrations of glucose and insulin are high, the system recognizes a state of caloric storage and hunger is diminished. The glucostatic hypothesis however is unable to account for food regulation in ruminants that have low levels of glucose but can nonetheless maintain normal levels of body energy stores. Tordoff et al. (1988) indicate that neither the glucose blood levels nor plasma insulin concentration are important in signaling the metabolic state of the body, but the glucose taken up by the liver. When glucose availability is reduced, hunger occurs and food-seeking is initiated.

In addition to the effects of glucose and glucagon it is clear that fatty acid (FA) metabolism (Friedman et al. 1986) and hepatic redox potential may also serve as afferent vagal signals. Injection of 3-hydroxybutyrate or lactate will change the redox potential in the liver and produce satiety.

Also glycerol may serve as a feedback signal indicating AT mass. Glycerol has some characteristics of an ideal indicator of calories stored in AT. Alpha-glycerophosphate is the source of the glycerol used for TG synthesis in AT and is produced by metabolism of glucose. When TG are hydrolyzed to form FA, the

glycerol moiety is released into the circulation because the levels of glycerol kinase in AT are too low to allow significant conversion of free glycerol. Thus basal lipolysis is accompanied by the production of a substance that cannot be reutilized in AT and is thus a marker for the rate of TG hydrolysis and is a function of the size of the adipocyte (Smith 1970). The circulating glycerol is primarily metabolized in the liver to form new glucose. Thus it may be that the rate of gluconeogenesis from glycerol in liver can be transmitted to the brain as an index of peripheral metabolism of TG.

Increased fatty acid oxidation by the liver (for instance during insulin deficiency (Ramirez et al. 1983)) is associated with a decrease in FI (Langhans et al. 1985, Friedman et al. 1985). This idea of an energostatic control of feeding has led to the suggestion that the rate of energy production is inversely related to feeding (Nicolaïdis et al. 1976). But, FI of hamsters is not enhanced during inhibition of FA oxidation, which is in contrast to rats (Schneider et al. 1988).

Increasing the percentage of fat in the diet will increase body fat stores in most species (Donato et al. 1985, Schemmel et al. 1970). Within each species, however, there are some members that are considerably more resistant to developing obesity when eating a high-fat diet than others. Fisler et al. (1985) observed important differences between sensitive and resistant rats in their response to a high-fat diet. Blood levels of ketones were increased in the resistant rats and may be a reflection of changes in insulin concentration (Yoshida et al. 1987). Ketones are the metabolic product of FA metabolism by the liver, and are secreted

for transport to other tissues, where they provide an important metabolic fuel. Ketones also may provide a signal to the liver or brain about the state of fatty acid oxidation. Bray et al. (1987) observed that the transport of 3-hydroxybutyrate across the blood-brain barrier is significantly higher in the rats that are resistant to dietary obesity than in those that are sensitive to high-fat diets. When animals are fed a high-fat diet, there is an increase in blood-brain barrier transport mechanisms but the resistant animals still transport significantly more than the sensitive ones. Moreover, infusion of ketones into the ventricular system of the brain enhances sympathetic activity, as reflected by the increased thermogenic properties of BAT (Bray 1989) and there is an increased sympathetic firing rate of nerves to BAT when ketones are microinjected into the VMH (Sakaguchi et al. 1988). So the VMH might play a pivotal role in producing resistance to obesity in rats eating a high-fat diet (Davis et al. 1981, Oku et al. 1984).

Circulating nutrients and hormones also may act directly on the brain in those areas not protected by the blood-brain barrier, on receptors in the blood-brain barrier, or after transport across this barrier.

The role of blood glucose concentration in providing signals directly to the brain is uncertain. A 7% decrease in blood glucose concentration occurs prior to the initiation of meals in the rat (Louis-Sylvestre et al. 1980) and with food consumption, blood glucose increases (Strubbe et al. 1977). From these results it has been hypothesized that the brain directly responds to changes in blood glucose concentration. Hypoglycemia, however,

does not always elicit eating. For example, infusion of fructose, a sugar which does not cross the blood-brain barrier (Rapoport 1976), to insulin-treated rats eliminates the feeding response despite hypoglucosemia (Stricker et al. 1977).
As indicated before, the uncertainty of the role of blood glucose concentration in controlling feeding probably arises from the fact that blood concentration does not necessarily indicate the rate of glucose utilization by tissues. Thus, brain cellular glucose utilization appears to be more closely related to feeding (Glick et al. 1968). Furthermore, feeding after insulin treatments may not be purely a consequence of hypoglycemia but due to other metabolic effects of insulin. Insulin infusion also reduces the plasma concentration of ketone bodies, amino acids (AA) and liver glycogen, while gastric emptying increases (Friedman et al. 1982).

The concept that plasma AA influence feeding behavior was first introduced by Mellinkoff et al. (1956). They observed an inverse relationship between serum AA concentration and appetite in man and proposed that shifts in the plasma AA pattern are monitored by the brain (the aminostatic control of feeding). High protein or imbalanced diets inhibit FI and are associated with large shifts in the plasma and brain AA patterns in rats (Harper et al. 1970). Changing AA concentrations, particularly the AA that are precursors for neurotransmitters, such as tyrosine or tryptophan, may play an important role in signaling the adequacy of protein intake. The synthesis of at least five brain neurotransmitters is influenced by precursor availability related to the degree of unsaturation of the enzymes involved in converting the precursor to the neurotransmitter (Anderson et al. 1983). So, diet-induced

fluctuation in precursor availability may provide signals which are used by the brain to direct feeding behavior so that the animal's intake of both quantity and quality of food becomes appropriate.

The possible role for serotonin, which has tryptophan as precursor, in the regulation of protein intake and/or carbohydrate balance is suggested by the relationship between tryptophan and FI (Blundell 1984, Wurtman 1984). Tryptophan cannot be synthesized within the body, and appears in the blood as a result of protein ingestion and body protein breakdown. Along with tyrosine, it is transported across the blood brain barrier via a carrier mechanism specific for this large neutral AA (NAA) . Due to the competitive nature of AA transport, the effect of food ingestion on brain tryptophan is not simply related to the tryptophan content, but also to the other specific AA. Carbohydrate (CH) ingestion increases the ratio of plasma tryptophan/NAA and therefore brain tryptophan uptake (Fernstrom et al. 1971). This is due to CH induced-insulin release. Insulin increases the uptake of all AA into tissues, but has a minimal effect on tryptophan. So, because of the reduced level of other AA in the blood, more tryptophan enters the brain relative to competing AA. Thus, protein ingestion decreases brain serotonin level whereas tryptophan administration and CH ingestion will increase the concentration of brain tryptophan and serotonin (Wurtman et al. 1976).

The relationship between diet composition, brain tyrosine and brain catecholamine metabolism is not well defined. Single meals both of protein and CH increase brain tyrosine (Fernstrom et al. 1978) but chronic high-protein feeding decreases brain tyrosine

(Anderson et al. 1984).

Besides an over-all inhibitory effect of serotonin on FI, serotonin's primary role may be to regulate the composition of food consumed in such a way as to achieve adequate intake and balance of protein and CH. This controlling role is possible because brain serotonin synthesis is under precursor control and levels can be modulated in opposite directions by CH and protein consumption. Ingestion of a high-CH diet will raise brain serotonin and will cause the animal to seek a high-protein diet and high AA levels decrease brain serotonin (Li et al. 1982).

Fenfluramine inducess rats to select more protein relative to CH in the subsequent hour of feeding (Li et al. 1983). However, in the next hours, the rats reverse the feeding preference showing greater relative preference for CH. This is consistent with the known action of fenfluramine, which causes an initial release of serotonin into the synaptic cleft and hence increases activity in serotonergic neurons (Fuxe et al. 1975). Because of the release, the neurons become depleted of serotonin and activity in the neurons is eventually decreased.

I.2 OVARIAN HORMONES AND BODY WEIGHT REGULATION

Animals are able to respond to metabolic perturbations with appropriate corrective behavioral and physiological adjustments so that supplies of metabolic fuels to crucial tissues and organs are not compromised. However some metabolic challenges may be predicted well in advance of their occurrence, so that adjustments can be made before the metabolic emergency. For example, two obvious and naturally occurring examples of predictable metabolic challenges are lactation and winter.

Lactation places enormous energy demands upon the mother (Ota et al. 1967). Female rats anticipate the demands of lactation by overeating, gaining weight, and storing fat during pregnancy (Steingrimsdottir et al. 1980). During lactation these lipid stores are mobilized and lactating rats may shed in excess of 80 % of their carcass lipids (Steingrimsdottir et al. 1980).

Winter, with its increased thermoregulatory demands and diminished food supplies, is also energetically challenging. Many species prepare for the rigors of winter with anticipatory changes in BW and fatness. Some species, such as guinea pigs and ground squirrels, use endogenous circannual rhythms to gain weight in autumn (Davis 1976). Other species, including some hamsters and voles, use environmental cues, such as photoperiod and diet quality, to make their anticipatory changes in BW and adiposity (Bartness et al. 1984, Dark et al. 1984).

One of the internal signals which animals may use in making these

anticipatory adjustments in behavior and energy metabolism is changes in the circulating titres of sex steroids. Also experimentally induced fluctuations of ovarian hormones (OH) alter BW and composition as well as a number of behaviors thought to control BW (e. g., FI and voluntary exercise) in a variety of mammalian species (Wade 1976). Many species with seasonal BW changes are also seasonal breeders, and their circulating levels of sex hormones fluctuate accordingly (Turek et al. 1979, Zucker et al. 1980). But what are the physiological or biochemical mechanisms by which ovarian hormones act to influence BW and composition and the related behaviors ?

I.2.1 EFFECTS OF OVARIAN HORMONES ON BODY WEIGHT REGULATION

The effects of progesterone are closely related to and dependent upon estrogen, as will be discussed later . So, the activity of progesterone, as well as that of estrogen will be discussed.

In adult female rats, withdrawal of OH by ovariectomy (OVX) induces a hyperphagia and rapid weight gain which last for approximately one month. Then the hyperphagia subsides and BW is maintained at 20-25% above the level of sham-operated controls (Gentry et al. 1976, McElroy et al. 1987). Running-wheel activity is permanently suppressed by OVX (Wang 1923). These effects of OVX can be reversed by treatment with Es alone. When ovariectomized (OVX) rats are treated with physiological doses of Es there is a transient hypophagia and loss of BW. BW curves of Es-treated rats are then parallel to, but lower than, those of OVX

controls (Mook et al. 1972). Es treatment does not affect eating and BW of OVX rats that are already regulating at a low BW because of prior adrenalectomy (Redick et al. 1973) or because of neonatal underfeeding (Zucker 1972), but Es does decrease BW in OVX-adrenalectomized rats that have been made mildly obese by giving them an especially attractive diet.

Es stimulates voluntary exercise and reduces thermoregulatory nest-building (Mook et al. 1972). Thermoregulatory nest-building is a behavior related to the control of heat loss to the environment. If given the opportunity to do so, rats build nests to conserve heat (e.g. in cold environment).

Although all carcass components may be affected by E withdrawal and replacement, by far the most striking changes are seen in carcass fat content (Gray et al. 1981). Leshner et al. (1973) found that ovariectomy doubled carcass fat content in female rats. Treatment with Es reverses the increase in adiposity caused by OVX (Roy et al. 1977). McElroy et al. (1987) show that dynamic changes in carcass composition (further increase in total carcass lipid) continue to occur in OVX rats even after BW has stabilized.

The BW promoting actions of P are expressed in lean animals only (Jankowiak et al. 1974). P has no effect on FI, voluntary exercise, BW, or carcass composition in OVX (overweight) rats (Galletti et al. 1964, Hervey et al. 1966, Rodier 1971, Ross et al. 1974). Even in OVX rats whose BW had been reduced by food restriction, treatment with P does not increase BW (Ross et al. 1974). However, adrenal removal, which prevents the BW gain that

typically follows OVX, does permit exogenous P to increase feeding (Roberts et al. 1972). And in the presence of E (in gonadally-intact females or in OVX rats given Es), treatment with high doses of P (5mg/day) depresses activity and increases BW and FI (Galletti et al. 1964, Hervey et al. 1967, Wade 1975). The hyperphagia and increased BW-gain during P treatment are transient just as is the case after OVX (Roberts et al. 1972).

Carcass analyses indicate that the BW-gain during P treatment (in the presence of E) is primarily due to increased fat deposition (Galletti et al. 1964, Hervey et al. 1967), similar to ovariectomy. The fact that the effects of OVX and P treatment appear to be similar and nonadditive may suggest that they cause essentially the same physiological response, which can only occur once or act through the same mechanism. So, E cannot depress BW in already lean rats, as P cannot raise BW above the obesity induced by OVX (Roberts et al. 1972).

The fluctuations in eating and BW during estrous cycles, pregnancy, and pseudopregnancy are consistent with this hypothesis. During estrous cycles, the proestrous peak in plasma E is followed by a decrease in FI and nest-building and increase in activity (Butcher et al. 1974, ter Haar 1972). Since energy expenditure (wheel-running activity and heat loss) increases and intake decreases, proestrous rats lose BW. These behaviors are highly correlated with the occurrence of estrous behavior. Changes in eating, activity and nest-building occur almost exclusively during the 8-12 hours when females are sexually receptive (Wade 1976), which suggests a common endocrine basis for all of the behavioral changes.

At diestrus, when E titers are reduced, FI and BW-gain are elevated. Thus during the estrous cycle the rat oscillates about a state of energy balance rather than preserving it all the time.

In addition to changes in nest-building behavior, the body temperature of female rats fluctuates with the estrous cycle. Brobeck et al. (1947) and McLean et al. (1971) reported that body temperature was lowest at proestrus in cycling rats. However Marrone et al. (1976) indicate a significant increase in the colonic temperatures of female rats at proestrus. These investigators remark that it is not likely that the proestrus hyperthermia is entirely due to increased locomotor activity, since there is not a good correlation between steroid treatments that increase body temperature and those that stimulate running wheel activity in ovariectomized rats. P, which has no effect on locomotor activity in OVX rats (Rodier 1971), seems to raise body temperature significantly (Freeman et al. 1970). Proestrus hyperthermia may explain the drop in nest-building seen at this time. If the rats are hyperthermic (because of hormonal changes), a drop in nest-building would allow the rats to lose some of this heat.

There are a lot of contradictory data available on these hormonal factors affecting thermoregulation and it seems to be difficult to replicate older work in this field. Work done primarily in human beings has led to the widely accepted hypothesis that P is thermogenic (Rothchild 1969, Freeman et al. 1970), although there is little evidence explaining how P raises body temperature. However, it is unlikely that P alone accounts for the fluctuations in colonic temperature during estrous cycle. Marrone et al.

(1976) have found that treatment of OVX rats with Es benzoate also raises colonic temperature and this is consistent with the finding that colonic temperature rises at proestrus. This finding contradicts with the earlier suggestions that Es lowers body temperature in rabbits (Brown et al. 1970). In contrast, Laudenslager et al. (1980) see no effect of Es treatment on rectal temperature in OVX rats. But Yochim et al. claim that OVX reduces mean core temperature in rats.

During pregnancy and pseudopregnancy, when plasma P levels are elevated, there is an exaggeration of the behavior seen at diestrus; eating, BW-gain and fat deposition are also elevated (Knopp et al. 1973, Wade et al. 1969), as they are during exogenous P treatment.

Wade (1976) hypothesizes that Es is the principal ovarian steroid affecting the regulation of body weight in female rats and that the principal role of P is simply to attenuate the actions of Es:
1/ Es alone reverses the OVX-induced changes in eating, activity and BW, whereas P has no effect on any of these measures in OVX rats;
2/ P inhibits activity and stimulates eating and BW-gain in intact or spayed, Es-treated rats;
3/ P and ovariectomy cause quantitatively and qualitatively similar changes in behavior and in BW and composition;
4/ the effects of P and OVX are not additive, suggesting a common mode of action.

It is suggested that high plasma P titres functionally ovariectomize female rats by inhibiting the actions of Es in the

brain or by inhibiting Es secretion in intact females (Wade 1976). But, P may also act via independent pathways and mechanisms to counteract the actions of Es. Although the sites of P action have not been identified, nearly all of the central and peripheral tissues that contain E receptors also have P receptors (MacLusky et al. 1978, Gray et al. 1979, Gray et al. 1980, Demanes et al. 1982, Pirovino et al. 1986). To show the dependence of P on Es and to explain why P has minimal effects on energy balance in the absence of Es, it should be noted that in all these tissues Es priming is necessary to induce high levels of progesterone receptors (Gray et al. 1979).

I.2.2 SITES OF OVARIAN HORMONE ACTION

As noted above, sex steroids are considered to be efferent signals in the controlled-system approach, used by Bray (1989). They are one of the several endocrine control mechanisms directed by the controller in the brain. Gonadal steroids affect energy balance and adiposity in a variety of mammalian species and there are several hypothesis about the mechanisms by which OH influence BW.

The steroid can act directly in the brain to provoke behavioral (FI and activity) and neuro-hormonal alterations, resulting in changes in BW and carcass composition. However, several lines of evidence indicate that the behavioral changes are neither necessary nor sufficient to produce the hormone-induced BW shifts.

Sex hormones may also act on non-neural peripheral tissues to alter metabolic processes. Es and P have profound effects on the disposition and utilization of circulating and intracellular metabolic fuels and changes in the availability of these metabolic fuels may contribute to the changes in FI. From this perspective, OH-induced behavioral changes are viewed as consequences, rather than causes of changes in peripheral metabolism.

Finally, OH probably act concurrently in the brain and in peripheral tissues. So, it is quite possible that gonadal hormones affect BW and eating behavior via multiple, redundant mechanisms, so that elimination of any one of them may not be sufficient to block responsiveness to the hormones. Redundant mechanisms may have evolved because the consequences of a failure to regulate one's energy balance for a few days are certainly more serious than for example, a failure to mate, especially in small, polyestrous homeotherms.

I.2.3 BEHAVIORAL EFFECTS OF OVARIAN HORMONES ON BW-REGULATION

It has been widely assumed that the changes in BW and adiposity which follow fluctuations in ovarian hormone secretions are due to changes in behavior. It was assumed that ovarian hormones act in the brain to alter FI and voluntary exercise and these behavioral changes could then influence adiposity. Indeed, during estrous cycles, the estrus decrease in BW is accompanied by an increase in voluntary exercise and a decrease in FI (Wade

1976). Following mating there is an immediate decrease in running-wheel activity and an increase in FI and BW-gain (Wang 1924).

Es implants in the vicinity of the medial preoptic area, a region with a high density of E receptors, stimulate running wheel activity in ovariectomized rats. Lesions in this area block the effects of systemic Es treatments on activity without causing a general debilitation (King 1979). Concurrent treatment with E antagonists also blocks Es-induced activity in neurologically intact rats (Roy et al. 1975). These findings are certainly consistent with the possibility that Es acts via E receptors in the medial preoptic area to stimulate voluntary exercise.

Wade et al. (1970) indicate that Es implants in the vicinity of the VMH, another region with a high density of E receptors, decrease FI & BW in OVX rats. These effects are achieved in the absence of direct effects on peripheral tissues (Nunez et al. 1980). These findings are consistent with a VMH-receptor-mediated action of Es on FI and the possibility that Es exerts its effects on eating by exciting, either directly or indirectly, hypothalamic neurons that normally restrain FI and/or limit BW. The anorexia of proestrus (or other periods of high Es availability) could be explained by estrogenic stimulation of the VMH area. Conversely, perhaps the overeating after E withdrawal is a result of decreasing VMH activity.

However, the VMH is not the sole site of E action on eating or BW, because lesions of the VMH do not block the effects of Es and OVX on either measure (Beatty et al. 1975). One possibility is

that Es could act at additional neural loci to affect FI and BW (Donohoe et al. 1981).

There are several lines of evidence which suggest that changes in BW which follow fluctuations in OH are not just due to changes in behavior.
First, after experimental manipulations of ovarian hormone levels the time course of the transient changes in FI and BW correspond quite closely (Gentry et al. 1976, Mook et al. 1972), but the changes in running-wheel activity are permanent (as long as hormonal status is held constant).

Hormone-induced changes in BW need not be accompanied by changes in FI. Hervey et al. (1968) found that P treatment significantly increased BW in intact female rats even when the normally occurring hyperphagia was prevented by restricting their FI to pretreatment levels. Similarly, the obesity following OVX is seen whether or not the animals are hyperphagic (Roy et al. 1977, Mueller et al. 1980). Spayed female rats had to be restricted to about 80% of their preovariectomy ad lib food consumption in order to prevent OVX-induced BW-gain. Thus, hyperphagia is not necessary for either P- or OVX-induced obesity.

In weanling rats, ovarian hormones can alter BW without affecting either FI or running-wheel activity. Es treatment depresses BW in ovariectomized weanlings but has no effect on eating or activity (Porterfield et al. 1974, Ross et al. 1974, Schwartz et al. 1981). Similarly, P increases BW of intact weanling females without increasing FI (Ross et al. 1974).

Roy et al. (1977) have also shown that the hypophagia seen during Es treatment is not sufficient to produce the observed weight loss. When ovariectomized, vehicle-treated rats were pair-fed with ovariectomized, Es-treated animals, they lost no BW. The BW losses of the Es-treated rats could not be attributed to Es-induced hyperactivity, because essentially the same results were obtained when the drug, MER-25, was substituted for Es (Roy et al. 1977). MER-25 mimics the effects of Es on FI and BW (Roy et al. 1976) without stimulating running-wheel activity (Roy et al. 1975).

Similar results are often seen during naturally occurring changes in hormone levels. For example, pregnant hamsters lose substantial amounts of energy (body fat) without changing their FI (Wade 1986). Thus, there are significant hormone induced shifts in energy balance without changes in FI, indicating that Es and P must also affect energy expenditure.

Thus, it appears as though OH affect BW & adiposity both by altering regulatory behavior and via non-behavioral (i.e., metabolic) actions and it can be suggested that the multiple and diverse effects of sex hormones on various metabolic processes may be more important than behavior in determining BW and composition. In fact, changes in the availability of metabolic fuels, induced by hormonal actions on metabolic processes, could be responsible for some of the behavioral responses to ovarian steroids.

It is important to keep in mind that OH could also act in the brain to influence peripheral fuel metabolism. So, one should

not necessarily equate neural sites of hormone action with purely behavioral effects. It should be possible to dissociate central and peripheral hormone actions by using intracerebral hormone implants which minimize leakage to the periphery (Nunez et al. 1980).

I.2.4 METABOLIC EFFECTS OF OVARIAN HORMONES ON BW-REGULATION

OH can act in the brain to influence both behavior and peripheral metabolic processes; at the same time they can act on nonneural peripheral tissues to affect the distribution and utilization of metabolic fuels which may then feed back to alter regulatory behaviors.

I.2.4.1 SITES OF OH ACTION ON METABOLIC PROCESSES

It is likely that the important target sites for gonadal actions on metabolism will share at least two attributes :
1/ they will have the characteristics of a classical steroid hormone target tissue;
2/ they will be of importance in the control of body's metabolic processes.

A/ Hypothalamus :

Besides its importance in behavior, the hypothalamus is one potential target site for the action of OH on metabolism. The hypothalamus contains cytoplasmic E (Eisenfeld 1970) and P (MacLusky et al. 1978) receptors. These hormones are concentrated in hypothalamic cell nuclei (Blaustein et al. 1978, Lieberburg et al. 1977), and they alter brain RNA and protein metabolism. The hypothalamus appears to influence metabolism via two principal efferent pathways : the autonomic nervous system and the anterior pituitary gland. Although direct hypothalamic effects, via autonomic nerves, have been demonstrated in pancreas, liver, adipose tissues (Wade et al. 1979) and indirect effects mediated via hypothalamic control of pituitary prolactin, growth hormone, adrenocorticotropic hormone and thyrotropic hormone secretion have been shown, little is known about the role of the hypothalamus in gonadal effects on metabolism. In any case, the hypothalamus cannot be the sole site of action, because ovariectomy increases, and Es treatment decreases both FI and BW in rats with large hypothalamic lesions (Beatty et al. 1975).

B/ Pituitary :

The anterior pituitary gland may be another site of OH action on metabolism. Like the hypothalamus, the anterior pituitary contains cytoplasmic E and P receptors (Eisenfeld 1970, Kato et al. 1977). The polypeptide hormones secreted by the anterior pituitary affect metabolism in numerous ways, either directly (e.g., growth hormone in AT or prolactin in mammary gland) or indirectly by stimulating hormone secretion by other glands (e.g.

ACTH in the adrenal cortex or TSH in the thyroid). OH can alter secretion of these metabolic hormones by the pituitary (Chen et al. 1970, Dickerman et al. 1972), but it is not always clear whether these OH act directly on the pituitary or the effects are mediated via the hypothalamus. As with the hypothalamus, the anterior pituitary cannot be the sole site of sex steroid action, because Es treatment decreases FI and BW in hypophysectomized rats (Wade 1974).

E mimetics can cause decreases in FI and BW when given in doses which are too low to affect hypothalamic and pituitary E receptors, suggesting that nonneural, peripheral actions alone are sufficient (although perhaps not necessary) to influence energy balance (Nunez et al. 1980, Bowman et al. 1981).

C/ Liver :

The liver is perhaps the most important organ for the regulation of energy metabolism. It plays a central role in the body's synthesis and metabolism of carbohydrates, lipids and proteins. Anything which alters handling of the various metabolic fuels by the liver could affect adiposity and eating behavior (see I.1.2.4, Sawchenko et al. 1979).

Human liver, as well as rat and turtle liver, contain cytoplasmic E and P receptors (Eisenfeld et al. 1976, Pirovino et al. 1986, Riley et al. 1988, Trueba et al. 1990, Demanes et al. 1982). OH treatments have been shown to alter hepatic RNA and protein synthesis (Kurtz et al. 1976), possibly including enzymes involved in TG production and transport.

D/ Adipose tissue :

Although AT is dramatically affected by manipulations of OH (Leshner et al. 1973, Hervey et al. 1967), it is often only regarded as a potential site for steroid storage or metabolism (Twombly et al. 1967, Bleau et al. 1974). However, OH might be able to act directly upon AT to influence metabolic activity. There are high-affinity, hormone-specific cytoplasmic E (Gray et al. 1981) and P (Gray et al. 1979) receptors in rat AT. Although there are significant variations in receptor concentration from one fat pad to another, there are both E and P receptors in all fat pads, including abdominal, subcutaneous and brown fat (Wade et al. 1978).

Es can increase the concentration of at least one protein in AT : P receptors. In ovariectomized rats there is almost no P receptor in AT, but a single injection of Es benzoate induces the appearance of cytoplasmic P receptors (Gray et al. 1979). It is also possible that Es and P might act on AT to alter synthesis of the enzyme, lipoprotein lipase (Hamosh et al. 1975, Kim et al. 1975).

E/ Skeletal muscle :

Androgens can stimulate protein synthesis in a number of tissues, including skeletal muscles. Dubre et al. (1976) indicate that there are also cytoplasmic E receptors in skeletal muscles.

I.2.4.2 METABOLIC EFFECTS OF OVARIAN HORMONES WITH POSSIBLE RELEVANCE FOR BW CONTROL

A/ Carbohydrate metabolism :

OH influence several aspects of CH metabolism in rat including hepatic gluconeogenesis and glycogen content, plasma insulin levels, glucose tolerance, pancreatic islet size and insulin production and basal glucose levels. Es and P, given singly or in combination in doses sufficient to alter FI and BW, increase liver glycogen content, decrease hepatic gluconeogenesis, and improve glucose tolerance (Bailey et al. 1972, Matute et al. 1973). Increases in endogenous Es and/or P secretion during estrous cycles and pregnancy produce similar changes in these measures (Sladek 1974). It has been suggested that these changes in CH metabolism are due to elevated pancreatic insulin secretion, because Es and P, given singly, in combination, or secreted endogenously, significantly increase plasma insulin levels (Matute et al. 1973). There are some data which suggest that OH could act directly on pancreatic islets to stimulate insulin production (Costrini et al. 1971). These data raise the possibility that OH could modulate adiposity and FI by altering pancreatic insulin secretion and perhaps tissue responsiveness to insulin (Salans 1971).

However, there are a number of reasons why this hypothesis is unlikely to account for the observed effects of OH on behavior and BW :

1/ Es and P have very similar effects on insulin and CH metabolism

and they tend to be more effective when given together than when given singly (Matute et al. 1973). On the other hand, Es and P have opposing effects on FI and adiposity.

2/ P increases plasma insulin levels and improves glucose tolerance in ovariectomized rats (Bailey et al. 1972), but P has no effect on FI or adiposity in ovariectomized rats (Ross et al. 1974).

3/ The increases in OH secretion preceding estrus and during pregnancy cause similar changes in insulin secretion (Sladek 1974, Costrini 1971), but rats undereat and lose BW at estrus and overeat and gain BW during pregnancy.

4/ The effects of ovariectomy and Es replacement on FI and BW are not attenuated in streptozotocin-diabetic rats maintained with constant daily insulin therapy (Dudley et al. 1979), indicating that OH affect eating and BW in absence of changes in pancreatic insulin output.

5/ Es treatment does not alter FI or BW responses to either acute or chronic insulin treatment in ovariectomized rats (Dudley et al. 1979), suggesting that altered tissue responsivenes to insulin cannot be the basis for the BW- and appetite-suppressing actions of Es.

These comparisons indicate that changes in CH metabolism do not provide a ready explanation for Es and P effects on adiposity, but it would certainly be incorrect to rule out any such participation in these effects. However, there is no obvious causal relation between altered CH metabolism and ovarian actions on FI and BW in rats.

B/ Fat metabolism :

In contrast, there are some rather striking parallels in ovarian effects on BW and on certain aspects of lipid metabolism.
Long chain fatty acids are an important metabolic fuel which can be used by a variety of tissues as a source of energy or stored in AT until needed. These FA are transported in the blood as FFA or esterified with glycerol to form TG. FA are stored in fat cells as TG. After a meal or a direct intravenous fat injection, FA are rapidly cleared from the blood. FFA readily enter tissues, and the rate of uptake is proportional to plasma FFA concentration. Before the FA in TG can be cleared from the blood the TG must be hydrolyzed. Two different TG lipases, present in or near the vascular wall, are the rate limiting factors in clearance of TG from the blood. One is found in the liver (hepatic lipase), and the other (lipoprotein lipase) is found in other tissues which utilize TG (e.g. heart, lung, skeletal muscle, mammary gland, AT). A third lipase (intracellular lipase, referred to as "hormone-sensitive lipase") breaks down the stored TG (lipolysis) into glycerol and FFA, which are then released into the bloodstream.

There are a few critical control points which govern the availability of FA as metabolic fuels or for storage in AT :
1/ Dietary intake : the FA available for oxidation or storage is determined in large part by the amount of fat and CH (CH can be converted to FA in several tissues) consumed in the diet.
2/ Liver TG production : the other important source of TG is endogenous synthesis in the liver.
3/Lipolysis : the rate of lipolysis and release of FFA into the blood by AT is determined by the activity of the intracellular

lipase (IL).

4/ Plasma clearance : removal of TG from plasma is limited by the extracellular hepatic lipase (HL) and lipoprotein lipase (LPL).

There are coordinated changes in these factors which serve to maintain a continuous supply of oxidizable fuels during various metabolic states. For example during a meal increased circulating insulin and glucose levels raise AT LPL activity and lower IL, increasing storage of TG. During a fast AT LPL activity decreases and IL activity increases, so that less FA is stored and more is released for oxidation. During lactation decreased LPL in AT and increased activity in mammary gland may serve to divert dietary lipid from storage in AT to mammary glands for milk production (Hamosh et al. 1970).

It is possible that Es and P exert their effects on carcass adiposity by altering the balance between LPL and the IL in AT, which leads to an altered TG clearance and consequently influencing the blood level of the fuel. In female rats OVX doubles AT LPL activity (Hamosh et al. 1975) and increases body fat storage (Leshner et al. 1973). Both of these effects are reversed by treatment with physiological doses of Es alone (Hamosh et al. 1975, Roy et al. 1977, Gray et al. 1982, Ramirez 1981, Schwartz et al. 1981, Steingrimsdottir et al. 1980). This Es induced decrease in white adipose tissue (WAT) LPL activity is accompanied by a significant reduction in tritium incorporation (from tritiated water) into newly synthesized FA in WAT (Bartness et al. 1981).

In OVX rats, large doses of P (5mg/day) affect neither AT LPL nor

carcass fat content (Hervey et al. 1966). However, in gonadally-intact rats P treatment approximately doubles AT LPL activity (Kim et al. 1975, Xu et al. 1987) and increases fat storage (Hervey et al. 1966). Steingrimsdottir et al. (1980) show that the rise in FI appears 12 h after the increase in WAT LPL activity, which is consistent with the idea that increased LPL activity may contribute to P-induced hyperphagia, as the rate of metabolite clearance may be monitored by the liver (see I.1.2.4 c).

The amount of fat stored in the body is dependent on both the rate of storage (limited by LPL) and the rate of lipolysis (IL). These two TG lipases appear to be reciprocally regulated (Patten 1970); increases in the activity of one enzyme are associated with decreases in the other. Hansen et al. (1980) and Benoit et al. (1982) suggest that Es increases IL activity while decreasing LPL activity (Wade et al. 1985). P (in the presence of Es) should lower IL activity as it increases LPL activity. In this way OH could alter body adiposity by inducing coordinated changes in the activities of these enzymes in AT.

How might Es and P act to produce such changes in enzyme activities? Es and P may act directly on adipocytes to modulate the rate of enzyme production.

1/ Rat AT contain specific, high-affinity E and P receptors whose affinity for various hormones and analogs is correlated with their effectiveness in altering BW (Wade et al. 1978).

2/ In the absence of P receptors there is no AT response to P. In ovariectomized rats, the level of AT P receptor is very low (Gray et al. 1979), and these animals show no changes in AT LPL activity

or BW in response to P treatment. Es treatment induces a rapid increase (6-12hr) in AT P receptors (Gray et al. 1979) and in the presence of Es, P increases both BW and AT LPL activity.
3/ Es is able to increase the level of at least one protein in rat AT : the P receptor.
4/ Patten (1970) indicates that it is very likely that LPL is formed in the adipocytes themselves.

In his article Ramirez (1980) refuses the hypothesis that Es induced hypophagia and BW decline could be due to hyper-triglyceridemia. The time course of Es effects on BW and FI differs from the time course of TG elevation (Es-induced hyperlipemia only appears at a time when Es effects on FI & BW have begun to disappear) and the antiestrogen, nafoxidine, which is estrogenic as far as BW is concerned, blocks the hyper-triglyceridemia, provoked by Es treatment. Moreover, the rate of plasma TG clearance seems not to be impaired by Es.
So, neither hyperlipemia, nor impaired removal of TG can be regarded as the cause of reduced FI resulting from Es.

Also, P administration in nafoxidine-treated OVX rats, has no effect on WAT LPL activity, but P does increase FI, BW and carcass fat content (Gray et al. 1981). So, P-induced BW increase is not entirely due to changes in WAT LPL activity. The same is seen by Schwartz et al. (1981). P treatment increases BW & FI in OVX, Es-primed weanling rats, without increasing WAT LPL activity.
Thus, although increased WAT LPL activity probably contributes to P-induced obesity (Steingrimsdottir et al. 1980), there are clearly other metabolic responses that probably require some

induction of P-receptor, because in the absence of any Es-priming, P does not increase adiposity in OVX rats (Gray et al. 1981).

So, this hypothesis certainly does not preclude the concept of OH acting on other tissues to alter AT enzyme activities indirectly. For example, Kim et al. (1978) suggest that Es can act on the liver to increase the production and circulating levels of certain apoproteins which serve as cofactors for AT LPL. These apoproteins alter AT LPL activity.

Es and P could also act on the hypothalamus and/or pituitary to alter synthesis and release of other hormones which affect lipid metabolism (Kastin et al. 1975). For example, Es increases plasma prolactin levels (Chen et al. 1970), and prolactin decreases AT LPL activity as Es does (Zinder et al. 1974).
It is also conceivable that OH could act in the hypothalamus to modulate sympathetic activity in AT (Wade et al. 1970), however, as already noted, central functions cannot entirely be responsible for those OH effects, since these OH effects on BW are not prevented by hypophysectomy or large hypothalamic lesions.

As already indicated, Es- or P-induced changes in BW need not be accompanied by changes in either FI or voluntary exercise (Roy et al. 1977, Hervey et al. 1968), but these behavioral responses to OH could contribute to the changes in enzyme activity. For example, either fasting or exercise can decrease AT LPL activity, as Es does (Borensztajn et al. 1970), and Es induces a hypophagia and stimulates running-wheel activity in ovariectomized rats.

While hormone-induced changes in FI cannot explain these fluctuations in adiposity or enzyme activity, perhaps the metabolic shifts induced by Es and P could contribute to the changes in food consumption during OH treatment. FI is sensitive to changes in circulating metabolic fuels (see I.1.2.4 c), so that OH could modulate FI by altering the availability of oxidizable substrates.

Treatment with Es and P is associated with significantly altered levels of circulating TG in several species. Es significantly raises plasma TG, while P exerts a protective effect on Es-induced hyperlipemia. These effects of OH on circulating TG are due to alterations in both TG clearance and hepatic TG production. Es also increases tritium incorporation into hepatic FA, a finding which is consistent with its effect on liver TG production and, according to Lorenzo et al. (1986), P seems to increase lipogenesis in the liver too.

Es raises blood TG levels in rats by reducing AT LPL activity and hepatic TG lipase activity and by stimulating hepatic TG synthesis (Watkins et al. 1972, Ferreri et al. 1978, Kim et al. 1975). While P affects none of these measures in ovariectomized rats, it significantly lowers serum TG in Es-treated rats by increasing mammary gland and AT LPL activity and HL activity and by lowering hepatic production (Kim et al. 1975, Hamosh et al. 1975). These hormones could act directly on the liver to modulate TG production and lipase activity, as they may act directly on AT to alter LPL activity. Thus, Es may decrease FI in female rats by increasing the availability of TG as a metabolic fuel. The components or

break-down products of TG are oxidized in a wide variety of tissues (e.g. long-chain FA in muscle, FA and glycerol in liver, ketone bodies in brain). Increased use of TG as fuel can also spare other substrates such as glucose for oxidation elsewhere.

This hypothesis does not explain the transience of the changes in FI and BW following ovariectomy or hormone treatment. After a couple of weeks the change in FI subsides and normal rate of weight gain is resumed (Wade 1979). Ferreri et al. (1978) have shown that during Es treatment the hyperlipemia, too, is transient. Although this supports the idea that the changes in FI may be due to changes in TG availability, it still does not explain the transience of the fluctuations in TG storage. But Ramirez (1980) shows that the time course of Es effects on FI and BW differs from the time course of TG elevation. According to his data, Es-induced hyperlipemia only appears at a time when the Es effects on FI and BW begin to dissipate or disappear entirely.

If, as noted before, Es & P can decrease or increase the storage of metabolic fuels without changing caloric intake, then it can be suggested that OH may initiate some additional process(es) that change(s) metabolic efficiency by altering energy expenditure. Although OH-induced changes in activity may have an effect on energy balance, Himms-Hagen (1981) & Girardier et al. (1983) indicate that by far the most significant means of energy expenditure is obligatory and facultative heat production, and brown adipose tissue (BAT) is a major site of this metabolic heat production (nonshivering thermogenesis) in mammals. The

principal effector of BAT thermogenesis is believed to be noradrenaline released from sympathetic nerve terminals innervating this tissue. A unique proton short-circuit pathway located in the inner membranes of BAT mitochondria permits oxidation of metabolic fuels (principally FA) without formation of ATP, thus releasing large amounts of heat.

In OVX rats, treatment with physiological doses of Es increases resting thermogenesis (oxygen consumption) and heat loss in rats (Bartness et al. 1984, Laudenslager et al. 1980, Laudenslager et al. 1982). In addition, Es decreases thermoregulatory nest-building, which would also facilitate heat loss. This hypothesis that Es influences energy storage in part by way of changes in energy expenditure is supported by Schneider et al. (1986). The normal nightly peak in resting oxygen consumption is severely depressed on the night of the estrus, following the precipitous drop in plasma Es levels (Saidapur et al. 1978), and concurrent with a dramatic BW gain.

One possible site for Es-induced thermogenesis is BAT, an important locus for both diet- and cold-induced thermogenesis in rats (Rothwell et al. 1979). Es treatment increases interscapular BAT wet weight (Kemnitz et al. 1983), protein content (Bartness et al. 1984), and in situ FA synthesis (Edens et al. 1983). An increased rate of lipogenesis in BAT is shown to be closely associated with increased BAT thermogenesis (Kemnitz et al. 1983). These changes are all seen in BAT of cold-exposed rats and treatments that reduce energy expenditure (such as food restriction) suppress thermogenesis in BAT (Hayashi et al. 1983). Surgical denervation of interscapular BAT enhances OVX-induced BW

gain (Wade et al. 1985) and prevents the Es-induced increases in resting oxygen consumption completely (Bartness et al. 1984).

It is not clear where Es acts to stimulate BAT thermogenesis ; it could act directly on the fat pad, because BAT does contain E receptors (I.2.4.1 D), or in the hypothalamus to influence BAT via sympathetic nerves.

McElroy et al. (1987), on the other hand, claim that the OVX-induced BW gain in rats cannot, even in part, be explained by a reduction in BAT thermogenesis. It is noted that OVX fails to suppress either the growth or the thermogenic activity of BAT in rats. So, some other tissue(s) capable of altering energy expenditure must be conserving sufficient energy to account for the increase in stored carcass energy. It is demonstrated that metabolic heat production in liver, but not in BAT (Schneider et al. 1986), is significantly deppressed in Syrian hamsters after OVX, suggesting that hepatic mechanisms for thermogenesis may play a role in the OVX-induced BW gain in hamsters. It is also stated that Es-induced changes in energy storage may be mediated by changes in the daily rhythm of energy expenditure, which are not dependent on alterations in BAT thermogenesis.

I.2.4.3 DO OVARIAN HORMONES ACT & INTERACT TO PARTITION METABOLIC FUELS AMONG DIFFERENT TISSUES?

OH-induced changes in WAT LPL activity may function to divert circulating metabolic fuels away from AT storage and into other

metabolically active tissues (expending energy), possibly including skeletal muscle (Ramirez 1981), uterus (Gray et al. 1983) and BAT (Edens et al. 1983). A concurrent increase in IL activity would also divert lipid fuels away from storage depots to sites of oxidation (e.g. BAT).

It is possible that in cycling rats, at estrus, elevated levels of Es act to direct TG away from WAT and liver so that they can be used as fuel by striated muscles. Cycling female rats (particularly at estrus) are substantially more active in and out of running wheels than are pregnant ones (Brobeck 1947). It is likely that Es acts directly on the medial preoptic area to stimulate running-wheel activity (Colvin et al. 1969). While Es is acting on the brain, it could be acting simultaneously in various peripheral tissues to increase circulating fuel supplies for use by striated muscles. TG are an important substrate for oxidation by skeletal muscles during exercise, and exercise increases LPL activity in muscle (Borensztajn et al. 1970).

In pregnant rats, the high P/low Es levels could prepare the rats for lactation by increasing TG storage in WAT. Indeed, running wheel activity drops and both maternal fat storage and WAT LPL are increased then (Hamosh et al. 1970). Perhaps the decreased activity and elevated P secretion of early and mid gestation serve to divert circulating TG away from muscles and into AT storage. The extra TG stored during pregnancy could be used for milk production during lactation. At the end of pregnancy and during lactation, elevated prolactin titres act to divert long chain FA away from WAT and liver and into mammary glands (Zinder et al. 1974, Lorenzo et al. 1986). Relatively little TG should be

oxidized by striated muscles during lactation, because post-parturient rats are inactive until their pups are weaned (Wade 1979).

Thus, it is possible that during various naturally occurring reproductive states Es, P and prolactin may interact to partition available TG among striated muscles, mammary glands and AT.

I.3 DIETARY SELF-SELECTION IN RATS

I.3.1 INTRODUCTION

Animals must eat to survive and, in addition to the problem of finding a sufficient quantity of food, they must also obtain a nutritionally adequate diet. Of the many materials which serve as food for animals, few are capable of supplying all of the nutrients essential for survival, growth and reproduction. Similarly, many materials are toxic or harmful if eaten (in large quantities). Animals must therefore exhibit some degree of selection in their ingestion of foodstuffs.

Numerous investigators (Richter *et al.* 1938, Collier *et al.* 1969, Theall *et al.* 1984) have shown that, when animals are given the opportunity to select the components of their diets, they do so in a way which results in normal growth and the proportions of protein, CH and fat selected are more or less constant. In their article, Collier *et al.* (1969) suggest that not growth considerations but dietary and caloric balance appear to be the prime determinants of intake in *ad libitum*-fed animals.

Manipulations of physiological parameters or environmental conditions, for the most part, result in nutritionally appropriate alterations in diet selection. For example, during both pregnancy and lactation, when protein requirements are increased to sustain the growth of the offspring as well as the

mother, rats increase the percentage of their diets taken as protein (Leshner et al. 1972). When energy requirements are increased by prolonged exposure to cold temperatures or exercise (Collier et al. 1969, Leshner et al. 1971) rats, offered a choice between a high-prot and high-CH diet, increase the proportion of the diet selected as CH. The result of these types of experiments have led to the hypothesis that self-selection patterns may reflect underlying metabolic requirements of the animal (Leshner et al. 1972).

This ability of animals to select food in accordance with bodily needs is also proved in other experiments. Water-restricted rats take a larger proportion of their food from diets lower in protein content and higher in fat content and in this way they adapt to water deficiency through selection of foods metabolically less demanding on water regulation mechanisms (Overmann et al. 1973). There also seems to occur a shift of preference following starvation towards an increased caloric consumption by selecting more fat and decreasing the consumption of CH (Andik et al. 1952). In contrast, Schutz et al. (1954) find large individual differences.

One mechanism through which the body and the brain determine how much of a nutrient an animal should choose to eat requires some input about the nature and content of what has recently been eaten and/or metabolized. One possible type of input is the pattern of amino acids in the plasma. This pattern changes characteristically depending on the proportions of protein and CH in a meal, causing parallel alterations in brain levels of the amino acid, tryptophan, and the synthesis and release of

tryptophan's neurotransmitter product, serotonin (Fernstrom et al. 1972). Moreover, the enhanced release of serotonin (as occurs after consumption of a CH-rich, protein-poor meal or after administration of fenfluramine), causes animals to diminish their elective consumption of CH, thereby increasing the protein:CH ratio in the next meal (see I.1.2.4 c, Wurtman et al. 1983).

So, diet selection patterns might provide information on the metabolic alterations associated with obesity, physiological, pharmacological or environmental manipulations. A few studies on dietary self-selection in obese animals show that obesity does lead to modifications in selection patterns. In comparison with lean controls, animals made obese by lesioning of the VMH select greater proportions of their diets as fat, and lesser proportions as protein (Kanarek et al. 1980). Destruction of the VMH does not, however, alter percent CH selected. In contrast to the choices made by animals with VMH destruction, genetically obese mice (ob/ob) select lower proportions of their diets both as protein and as CH, and higher proportions as fat than do lean controls (Mayer et al. 1951). Additionally, rats made obese by neonatal administration of monosodium glutamate display patterns of diet selection different from those of VMH-lesioned or genetically obese animals. They select a greater proportion of their diet as CH than do controls (Kanarek et al. 1980). Experimental obesity also may be induced by surgical removal of the ovaries from female rats and probably by P administration to intact females too (see I.2.1).

I.3.2 OVARIAN HORMONES & DIETARY SELF-SELECTION

As OH seem to have quite an important effect on BW and FI (total caloric intake), it would be very interesting to investigate their effect on the selection of the diet. Unfortunately, rather few and contradictory data are available on this matter. Leshner et al. (1972) did self-selection studies through the course of the estrous cycle and during pregnancy and lactation. FI decreased during the estrous phase, but there were no changes in the dietary selection pattern. Thus, there is a general reduction in appetite rather than a change in the requirements for specific nutrients. In contrast, during both pregnancy and lactation protein intake increased while CH intake was maintained at a level equal to that in non-impregnated controls. It is concluded then that OH have a minor influence on the energy selection pattern and that the self-selection follows the varying nutritional requirements of the organism. Indeed, in both of these situations (lactation and pregnancy) protein is needed to sustain the growth of the offspring. Quite similar results are reported by Richter et al. (1938), who find that, during both pregnancy and lactation, rats increase their intake of both protein and fat, while keeping CH intake constant. In contrast, Scott et al. (1948) find no variation in selection patterns during these periods and Tribe (1955) finds no change in either protein or CH intake during pregnancy and lactation but does observe an increase in fat intake during these periods.

A more recent study on female humans (Cohen et al. 1987) documents positive changes in food consumption and CH craving during the

menstrual cycle. In the luteal phase (when Es levels are low and P levels high) both amount eaten and CH craving are greater than in the follicular phase. Also in the studies of Dalvit (1981) and Pliner et al. (1983) comparable changes in CH intake are reported in relation to the human menstrual cycle. Moreover, cycling female rats show heightened taste sensitivity and a marked preference for sweet substances compared to males and to non-cycling females (Wade 1976).

Kanarek et al. (1980) examined the role of OH in diet selection and FI in female rats using the OVX-animal model. BW and FI increase significantly following OVX, but these animals show no changes in diet selection, which is different from other forms of experimentally-induced obesity. This suggests that distinct metabolic patterns may underlie various forms of obesity. In contrast, a study of Leshner et al. (1973) finds increased protein intake after OVX, but here fat is not presented as a separate dietary fraction, but only as a component (5% by weight) of the other two diets (protein and CH). This may be the reason for the different results.

As OVX and P treatment to intact female rats have previously been shown to have some similar effects on BW and FI, it would be interesting to examine the effect of P treatment on food selection.

CHAPTER II

BODY WEIGHT & FOOD INTAKE STUDIES

II.1 INTRODUCTION

As mentioned in the literature review (I.1), the BW of most animals is carefully regulated and often remains relatively constant over substantial periods of time. Then the energy equation is zero and balanced. Environmental, physiological, pharmacological, etc. factors can temporary or permanently disrupt this energy balance.

Administration of P to intact female rats is thought to increase BW and adiposity (Hervey et al. 1967), and Roberts et al. (1972) compare those P-effects with OVX and suggest that they cause essentially the same physiological response. The experiments presented in this thesis examine the effects of P on BW regulation in intact female rats and attempt to determine its effect on FI and food selection.

II.2 MATERIALS AND EXPERIMENTAL PROCEDURE

II.2.1 Chemicals and Reagents

Progesterone was purchased from Sigma Chemicals Co., USA and Ethyl Oleate, which serves as its solvent was purchased from BDH Chemicals. To dissolve P and make a solution of 20mg/ml the oily vehicle (OV) was slightly warmed.

II.2.2 Animals

In all studies female rats of the Wistar strain were used. Rats were housed either individually or grouped in cages in a temperature-controlled environment (21-23°C), on a regulated 12-hour light : 12-hour dark cycle (exceptionally this cycle was interrupted, see II.2.3 D). Throughout all studies, the injected dose was kept at 20mg/kg BW, which is in agreement with similar studies in the literature. This P dosage suppresses the estrous cycle and reproduces the physiological situation of pregnancy (Hervey et al. 1967).

II.2.3 Procedure

A/ Experiment 1

Two groups of six rats were used (weighing between 250 and 310 g), and from the 7th day of experiment on they were injected every day (around midday) with a dose of 20 mg/kg BW or with a corresponding volume of ethyl oleate, the oily vehicle (OV) (= 1 ml/kg BW). Initially, the rats were injected intraperitoneally (IP), but from day 27 on, the injection was given subcutaneously (SC). The rats were housed singly in cages and had access to food and water ad libitum. They were weighed daily to the nearest 0,1 g at about 11h00.

B/ Experiment 2

Four groups of 5 female rats (weighing between 230 and 290 g) were injected daily near 13h00. The rats were housed 5 per cage and had access to food and water ad libitum. Group 1 and 2 were injected IP with the P-solution (1 ml/kg BW) and the same volume of the OV respectively. The 3th and the 4th group were injected SC with the same P-solution and OV respectively. They were weighed to the nearest 0,1 g daily near midday.

C/ Experiment 3

Three groups of 4 rats were used, which were all injected SC daily (near midday), two groups with P and the last group with the corresponding volume of the OV. From day 14 to day 22 of the experimental period, one P-group and the OV-group received a daily additional IP injection of fenfluramine (5 mg/kg BW) (Wurtman et al. 1977) at the end of the light cycle (at about 17h00). The rats were housed 4 per cage and they had access to food and water ad

libitum. Every day the BW was measured to the nearest 0,1 g near 11h00.

D/ Experiment 4

This study maked use of three groups of 6 female rats (with a BW between 170 and 220 g). During the experiment one rat died and the experiment was continued, omitting the data of that one rat. Rats of group 1 (5 rats) were injected (IP) daily with P, group 2 with the OV (IP) and group 3 was not injected at all. The rats were weighed daily (near 16h00).

In addition to determining mass changes an attempt was made to measure the daily caloric intake and especially the composition of the ingested food : the amounts of protein, carbohydrate (CH) and fat. This self-selection method is often used to test the nutrient requirements of the animals (see I.3.1). A method was devised to give the rats the opportunity to select and compose their own daily diet. The animals in this experiment were offered an array of purified foodstuffs and allowed to choose their diet completely (cafeteria-system). Only rats that are fully adequate (that select a well-balanced diet and have a normal BW gain) were used in this study. The presentation of the diets is very important since each energy source should be presented in a similar way to avoid differences which make one more attractive and easier to ingest. Moreover, it must be possible to pick up the spilled food and adjust the appropriate amount of ingested protein, CH or fat. This was quite a difficult task as spillage of the three food choices was usually mixed and was often contaminated with droppings and urine of the rat.

As rodents like to gnaw and in order to produce a food somewhat like the normal laboratory chow food it was attempted to make protein and sugar pellets with a tabletting machine. An edible colouring agent was added to the protein and sugar powder in order to produce a red protein pellet and a yellow CH one. The tablet had a thickness of 1 mm and was compressed moderately hard. This set-up didn't seem to be the right solution as spillage was enormous and, although the pellets were differently coloured, it was almost impossible to separate and pick up the spilled pieces and powder.

Another possibility was to prepare liquid formulations (Rozin 1968) : a sugar solution, corn oil, and soluble casein as protein source. But drinking is not the most natural way of FI and special bottles (nondripping, even in accidental contact with rat's fur) should be at one's disposal.

Eventually, it was decided to follow the example of Theall et al. (1984) and to incorporate the three energy sources into gels. This had several advantages :
1/ it helps to minimize appearance and texture differences among the preparations (a CH -, protein - & fat - gel).
2/ it allows the diets to be coded with food dyes (added to the water) which facilitates recognition of spillage.
3/ the texture of a gel limits spillage, and if rats do spill, those pieces can be picked up easily.
It is necessary to make the gels isocaloric so as to preclude the possibility that the rats simply select the gel with the highest

caloric value. Protein and sugars are almost isocaloric and have a caloric value of 4 Kcal/g, but fat is twice as caloric (9,2 Kcal/g). So fibers (caloric value is zero) had to be added in order to make isocaloric diets.

It was also decided to add to each gel the same vitamin and mineral mixture, so that consumption of the diets in any proportion results in an adequate intake of all dietary essentials, other than those under investigation.

Gelatine was the basis of the gels. As gelatine is a protein source, all gels contained a certain amount of protein, and this had to be taken into account.

The principal problem was to make three gels with a good consistency (not too hard, dry or gritty, not too liquid and sticky) which incorporate the vitamins, minerals, fiber, food dye and either protein, CH or fat and which are isocaloric. Especially the corn oil, which serves as fat source, seemed difficult to incorporate permanently into the gel. Only when great amounts of methylcellulose, which helps to make a stable emulsion, and a solubilising machine were used, was the gel with the corn oil stable and no longer felt and appeared greasy. Furthermore, this solubilizing agent, methylcellulose, served as fiber source and replaced bran, which is not pure enough according to Martindale.

For each preparation, several concentrations of gelatine gel were tested in various ratios of gel/product to be incorporated, and eventually it was found that the best results were obtained using

70 g gel to incorporate 30 g ingredients.
The complete composition of the three isocaloric formulations is presented below.

	protein gel	CH gel	fat gel
gelatine	2,8g	4,2g	2,8g
casein	27g	-	-
sucrose	-	25,6g	-
corn oil	-	-	12g
min. mixt.	1,2g	1,2g	1,2g
vit. mixt.	0,3g	0,3g	0,3g
methylcell.	1,5g	2,9g	16,5g
methylparaben	100mg	100mg	30mg
propylparaben	-	-	70mg
food dye	q.s.	q.s.	q.s.
water	ad 100g	ad 100g	ad 100g

Diets were stored in a cold room at 4 °C in capped jars. The use of casein, sucrose and corn oil as energy sources was based on the results of feeding experiments (Richter et al. 1938) in which several protein, CH and fat sources were tried out.

To prepare the gels, start to boil the water, add the water-soluble products (preserving agents, food dyes, gelatine, sucrose) and stir until well dissolved. Continue on stirring and add the rest, which is well mixed in advance (casein or corn oil, mineral mixture, methylcellulose). The vitamin mixture is added after cooling down a little and the solution is poured into 50-ml ointment jars just before the stiffening of the gel.
To prepare a good, well solubilized fat gel, it is necessary to pass the warm mixture through the solubilizing machine.

Per kg mineral mixture there was 300g Ca(CO3), 214.2g NaCl, 220g K2(HPO4), 110g Ca(HPO4), 125g Mg(SO4), 25g Fe(SO4), 3.2g Mn(SO4), 1.8g Cu(SO4), 5.45g Zn(SO4), 0.8g KI.

To obtain 1kg vitamin mixture 890g methylcellulose was mixed with 0.03g folic acid, 0.5g riboflavin, 1.5g nicotinic acid, 1.5g thiamin.HCl, 2.5g pantothenic acid, 25g inositol, 700.000 IU vit A, 100.000 IU vit E, 0.0125g biotin, 0.5g p-aminobenzoic acid, 1.25g menadion, 1.5g pyridoxine.HCl, 1.5g of a 0.1% trituration of vit B12, 5g vit C, 50g choline chloride, 36.000 IU vit D. The vitamin mixture was stored at 4 °C.

After weighing, the feeding jars were fixed in the cage next to each other with an iron wire and the positions of the jars in the cage was alternated daily when the jars were refilled (near 17h00).

Rats are active at night and the major part of the daily FI takes place during the dark. That is why the rats were trained to consume all of their daily caloric intake during the 12-hour dark phase and to select their food from three jars containing the three different gels. In the morning, each rat was put into another cage and starved (water ad libitum) during the light phase (until 18h00). One could measure the amount from each jar by weighing the feeding jars and collecting the spillage and returning it to the appropriate diet jar.

The main difficulty of the experimental set-up was to separate the three different gel pieces from each other and from the droppings

and urine of the rats. In order to reduce contamination of the gel in the tray, the rats were given only 4 hours a day to eat and were starved for the rest of the day. The lights went out at 18h00 and the rats had access to food until 22h00. Then, light was switched on again and rats were changed to another cage and starved (water ad libitum) for the next 20 hours. Unfortunately, this reduced feeding time did not prevent spillage and cleaning the cages (separation of spilled gel pieces) remained difficult. So, reducing the feeding time did not really improve the situation. Next, the use of bigger wire-meshed cages (40x40 cm) seemed to help a lot. Instead of putting the three feeding jars together, they were fixed in each corner of the cage. The spilled gel pieces were caught on a plate 10 cm under the grid. Once the rat dropped a piece, it could not be touched anymore. So, the spilled pieces were automatically separated. To drain the urine during the 12-hour feeding time at night, the plate was inclined. In the morning rats were moved to other cages (water ad libitum) and the exact amount of FI was determined. Using these data, the amount of protein, CH and fat consumed per rat overnight was calculated daily (taking the gelatine gel as protein source into account).

E/ Experiment 5

This study was very similar to the previous one, and made use of 12 female rats assigned on the basis of BW to two groups (BW between 200 and 240 g), but the P solution (group 1) and the OV (group 2) were injected SC instead of IP. Rats were given the opportunity to get used to the food selection rations for 1 week and only rats able to compose a well-balanced diet were included

in the experiment. In the same way as in previous experiment daily recordings were taken of BW and caloric intake from each food jar. In contrast to the previous experiment, the measured amount of each gel consumed was corrected with a factor, indicating the degree of desiccation overnight. This factor was slightly different for each preparation and for each night. So, it was decided to put control jars of the three gels in the animal room every night during the feeding period to obtain values with which to adjust the measurements.

II.3 STATISTICAL ANALYSIS OF RESULTS

All data were statistically analyzed using one way analysis of variance followed by Scheffe's test for multiple range comparisons, or the Student-t-test. Results were expressed as means +/- SEM and reported as significantly different at the 5% level or less.

II.4 RESULTS AND DISCUSSION

A/ Experiment 1 (fig II.1)

The mean BW of the 2 groups changed in a very similar way, even when daily IP injections (P and OV) were given. Only when the rats were injected SC, did a remarkable increase in BW occur for the P-group. The mean BW gain of the P-group was significantly

different from that of the control group (OV) as early as the second day after the first SC injection ($p < 0,05$). Although the rate of BW gain varied, in no instance did an individual treated SC-P-rat fail to show a gain of weight.
Although with IP-injections, the mean BW of the P-group increased slightly, it was not statistically different from the OV-control group. This slow increase in BW of the P-group should be interpreted as normal growth.

It is not clear why the OV rats stopped growing during treatment (IP and SC) although they grew normally before the first IP injection and continued growing when experiment was ended (results not shown). During treatment the mean BW of the OV-group fluctuated without any overall increase. One would expect different result as a certain amount of extra calories was injected daily (OV), and consequently a constant BW gain would be expected. It is not impossible that the OV, ethyl oleate, on its own, influences physiological processes related to BW regulation. A quite similar ethyl oleate effect seems to occur in the study of Hervey et al. (1967). Some of their animals, besides losing weight, developed lesions at injection sites, although rats given P in ethyl oleate as solvent gained as much weight as did matched rats receiving other preparations, and rarely developed lesions. The authors concluded that dissolved P apparently protects against the toxic effects of this solvent. They continued the use of P in ethyl oleate, but control rats received olive oil. Since in the present study no lesions were observed, it was decided to continue to treat the control rats with ethyl oleate, because ethyl oleate, unlike olive oil, is the only real control. Moreover, it is also the solvent employed by most other researchers.

The main conclusion is that the P-induced effect on BW depends on the route of administration. SC-P-injections produce an immediate BW gain; however the same dose, given IP, fails to elicit any weight response, although the characteristic effects on the reproductive system, namely suppression of estrus and vaginal mucification, occur after IP injection (Ho-Yuen 1977).
It is possible that the weight increasing effect of P depends on a fairly sustained active level of the hormone and that an intermittent, fluctuating level, such as may be produced by daily injections at sites giving relatively rapid absorption (IP), is ineffective.

B/ Experiment 2 (fig II.2)

These results are in conformity with those of the previous experiment. Only SC-P-treatment showed a statistically significant influence on BW gain. From day 4 on, the cumulative BW change was statistically different ($p < 0,05$).
Again, the same ethyl oleate effect was observed for the SC-OV group. During the 9 days of experiment, BW fluctuated without really increasing, even though rats normally reach the maximum adult BW at a much higher level. The animals of the OV-IP group did grow, albeit slightly, over this period of time, as did the P-IP group. The similarity of growth pattern in these two groups makes it quite clear that IP-administration is not the ideal route of administration for investigation of the P-induced effects on BW.

fig II.1 Influence of progesterone treatment on BW.

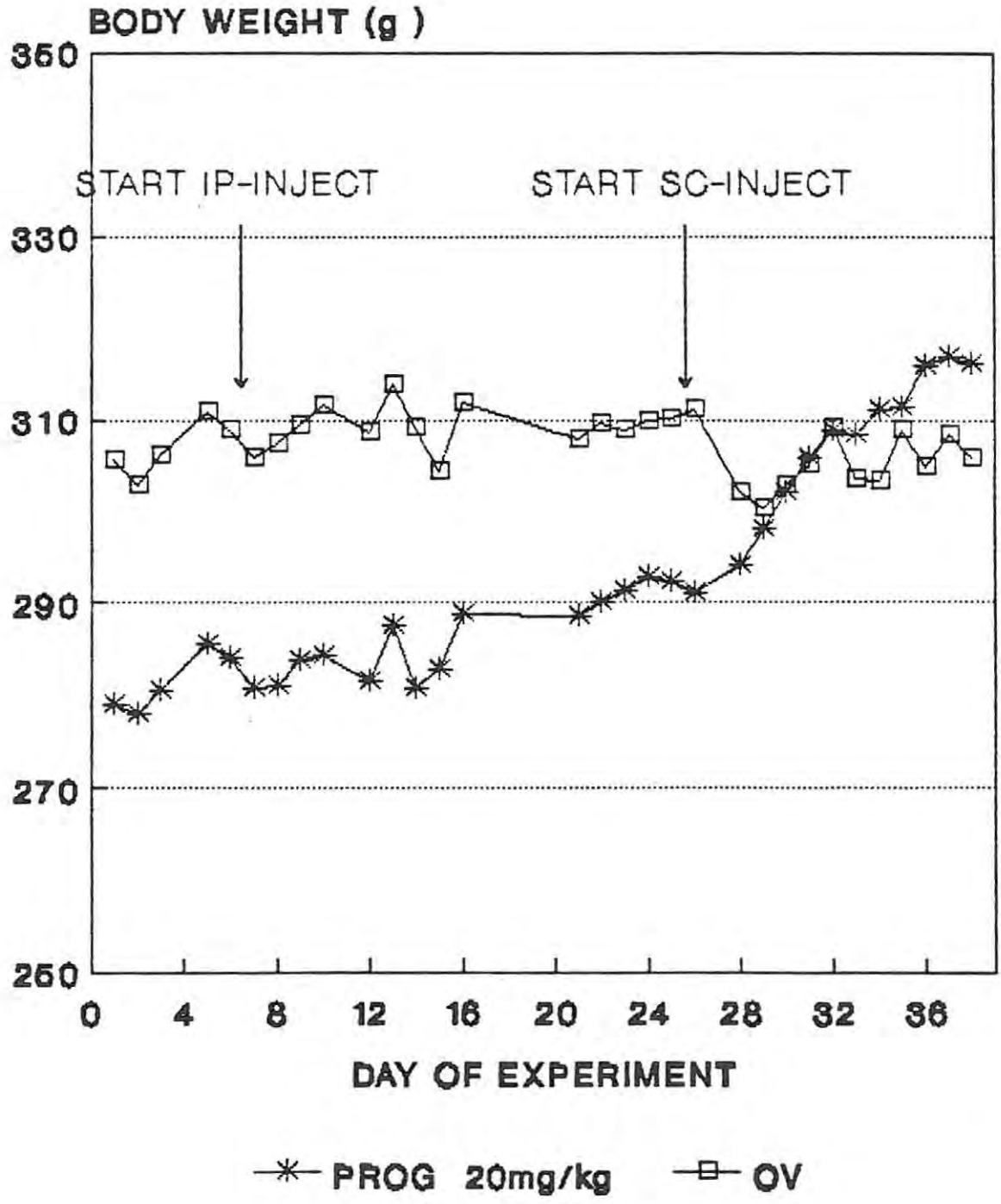
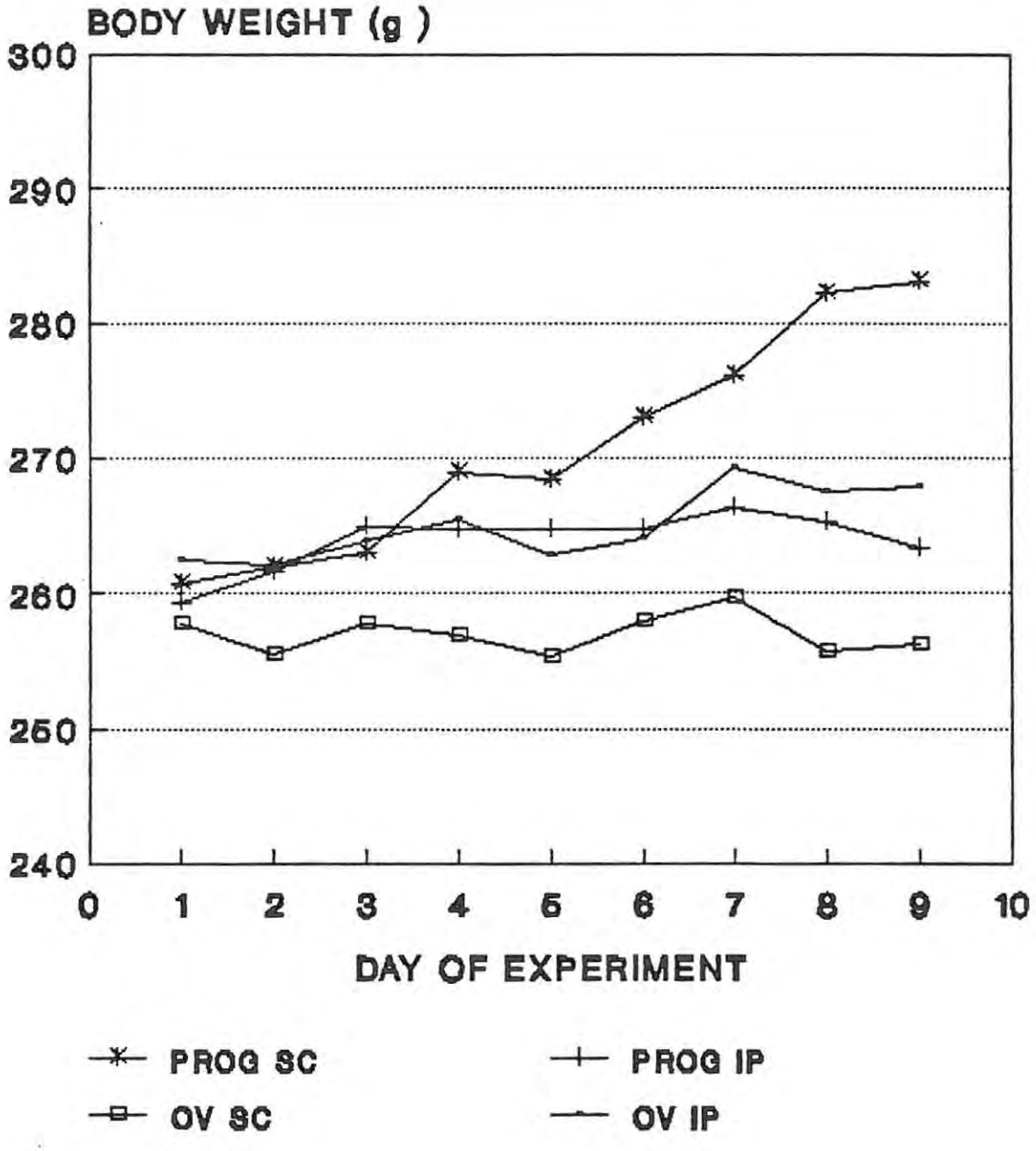


fig II.2 Influence of route of administration on P-induced increase in BW.



C/ Experiment 3 (fig II.3 & fig II.4)

As noted above, fenfluramine is an appetite-suppressant, probably exerting its effect through its serotonin-releasing action. It is known that the anorectic action of fenfluramine is only temporary due to the eventual depletion of serotonin.
During the fenfluramine administration, a remarkable decline in BW was observed in both the SC-P group and the SC-OV group (although to a lesser extent). During the last couple of days of fenfluramine administration, rats probably acquired resistance and the fenfluramine effect disappeared both in the P- and in the control-group. The mean BW's of the two groups increased, although slightly, during the last days. Then, when the fenfluramine administration was stopped, the mean BW's of both groups rapidly returned to original level and after three days, the original less steep slope of the BW curves re-established itself.

So, the fenfluramine intervention had a very similar effect on the mean BW of both groups. There was statistically no difference between the slopes of the two curves. Again, the result of this experiment is an indication that the P-induced BW effect is not based on an appetite-activating principle, since both groups reacted similarly to treatment with the appetite suppressant fenfluramine. Moreover, the typical fenfluramine effect (a rapid BW decline and development of resistance after about a week) was observed in the P group as well as in the control group, a finding which probably excludes an interaction between P and fenfluramine.

Furthermore, it is also likely that the P effect is not a serotonin-mediated one as P induced a BW increase while fenfluramine induced a BW decline.
Mueller et al. (1980) found that, although OVX rats gain more weight than do controls on the same amount of food, during 33 hours of food deprivation, OVX and control animals lose BW at the same rate, effects which also indicate that this BW gain is probably a process independent of FI.

Experiment 4 and 5 further examined the role of FI, especially food selection, in the P-induced BW effect.

In figure II.4, one can see that the steady BW gain during SC-P treatment is interrupted and reversed by a concurrent fenfluramine treatment. After the last fenfluramine injection, BW rose very quickly and levelled off to fit the original slope of the mean BW curve.

D/ Experiment 4 (fig II.5 - fig II.9)

Intraperitoneal injections are the usual way of administering P in enzyme-induction studies and this treatment is known to suppress estrus (Ho Yuen 1977).
Experiment 1 shows that IP administered P fails to elicit the BW response, indicating that a sustained level of the hormone is required, a level which however may not be achieved after daily IP injections (fig II.6).
This means that the enhanced activity of the hepatic microsomal enzyme system seen by Ho Yuen (1977) after IP-P treatment may not be related the solely to its effect on the reproductive

fig II.3 Influence of fenfluramine on P-induced BW-increase.

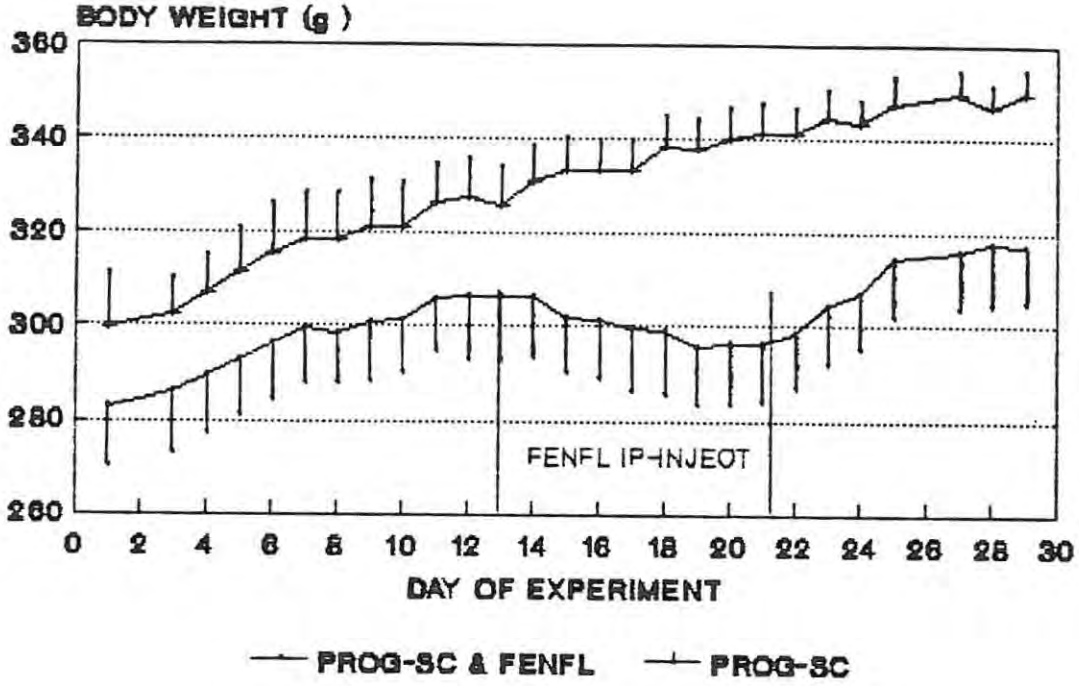
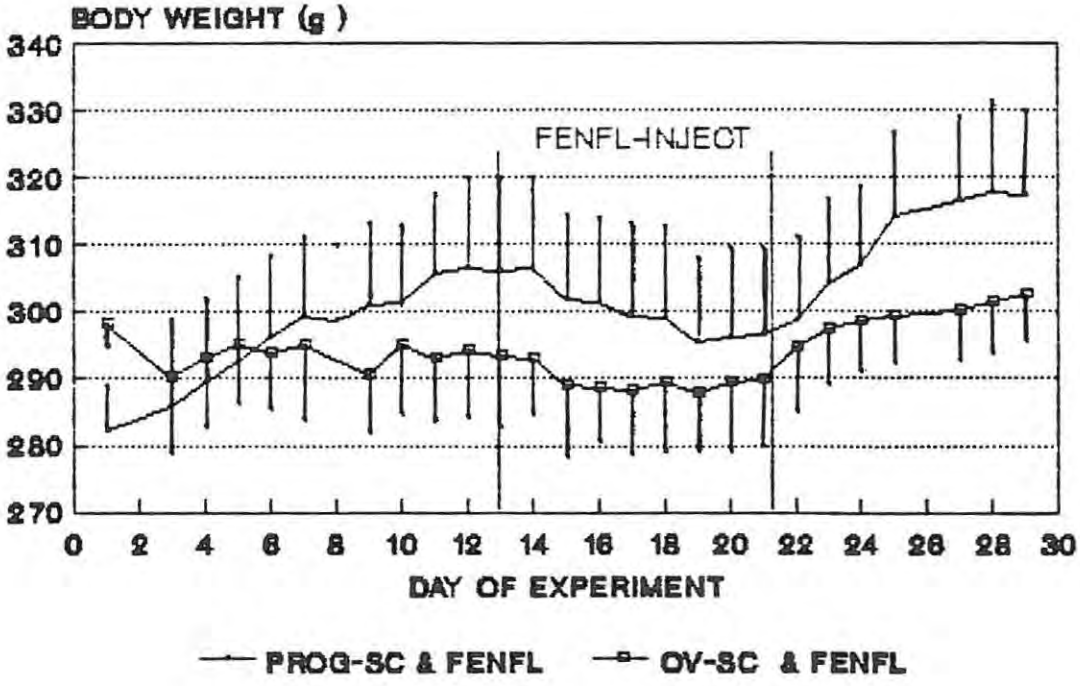


fig II.4 Influence of fenfluramine on P-induced BW-increase.



system.

Several authors support the idea that FI plays an important role in the P-induced BW increase (I.2.1).
It would be interesting to discover if the route of administration of P affects FI and food selection. In accordance with the effects on the reproductive system, IP-injected P may influence caloric intake or food selection habits without showing any BW response.

IP-injected P failed to provoke a clear BW gain, or to cause a significant effect on the daily caloric intake (fig II.5).
Both during the 4-hour feeding period and during the 12-hour one, the increased amount of ingested food was too small to be significantly different. The rats had to adjust to the 4-hour feeding time and they lost weight in the beginning. A rat normally eats 8 to 10 times for about 10 minutes each, mainly during the dark phase, and it was quite difficult for it to adapt to this 4-hour feeding period. The P rats seemed to adapt a little more easily than the two other groups but, again the difference was not statistically significant. With the 12-hour feeding time allowed during the whole dark phase, all rats gain weight very quickly to reach the normal level after a few days.

All rats selected a well-balanced diet of protein, sugar and fat (fig II.7-II.9). Although there were considerable differences, none of the rats refused to eat anyone of the three rations, and pronounced inter-animal differences dominate the statistics. It is mainly the appetite for fat which differs so markedly. Only a minority of the rats choose approximately an equal caloric intake

from each of the three diet sources as in the experiment of Kanarek et al. (1980). In our work, between 50-60 % of the calories are taken as sugar, 30-35 % as protein and 15-25 % as fat for the 4-h feeding period and 40-50 % protein, 40-50 % sugar and 15 % fat for the 12-h one.

In the 4-h feeding study (which is in fact a study with starvation conditions) one can see some results similar to those observed by Schutz et al. (1954). Although there are large individual differences (which prevent clear results), an increase in fat consumption can be seen. The P group tends to follow the pattern of the control group but the the OV group shows no difference in fat preference between the 4-h and 12 h-feeding time. The increased fat selection by the P-treated rats during the 4-h feeding time is totally at the expense of protein intake and this observation contrasts with the study of Andik et al. (1952), in which the increased fat intake is accompanied by decreased CH-intake.

The often contradictory results during the 2 feeding periods make the whole experiment even harder to interpret. For example, the OV group select more food in the form of CH during the 4-h feeding time while during the 12-h period, it is the P group which exhibits CH craving. The opposite can be said about the appetite for lipids. The only conclusion one can make is that during a fasting situation, all animals start to ingest an increased percentage of directly available energy (CH) and P treatment does not really change this reaction.
As could be expected, there is no effect of P on FI and on food selection habits after daily IP treatment.

fig II.5 Daily caloric intake during IP-treatment.

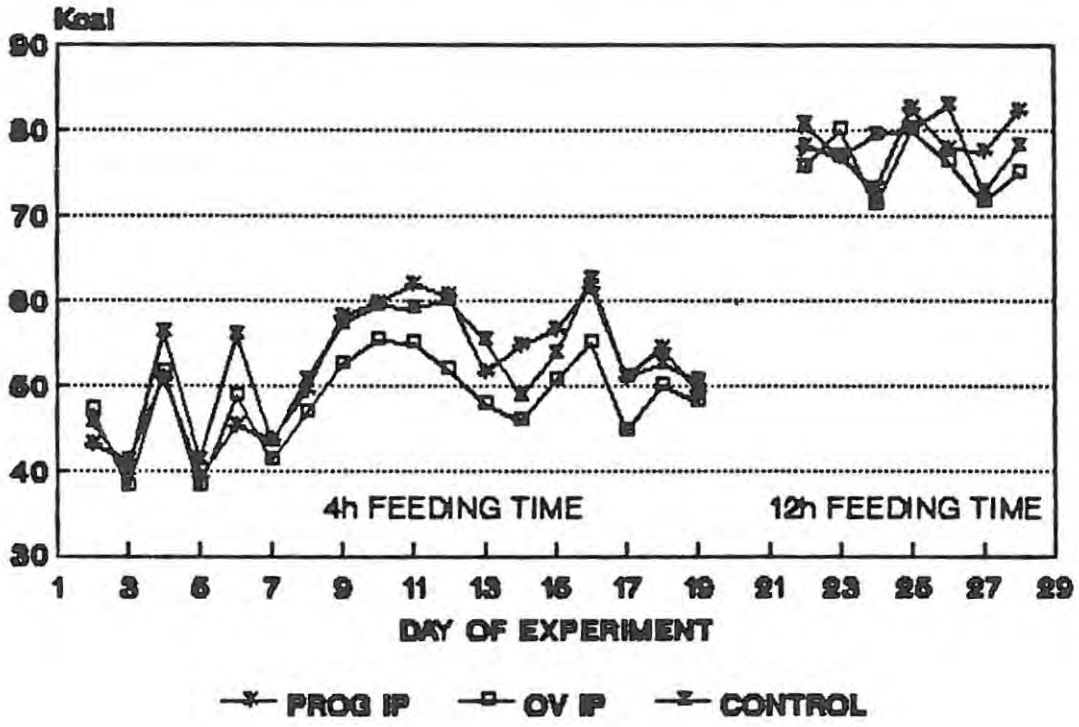


fig II.6 Body weight and IP-treatment.

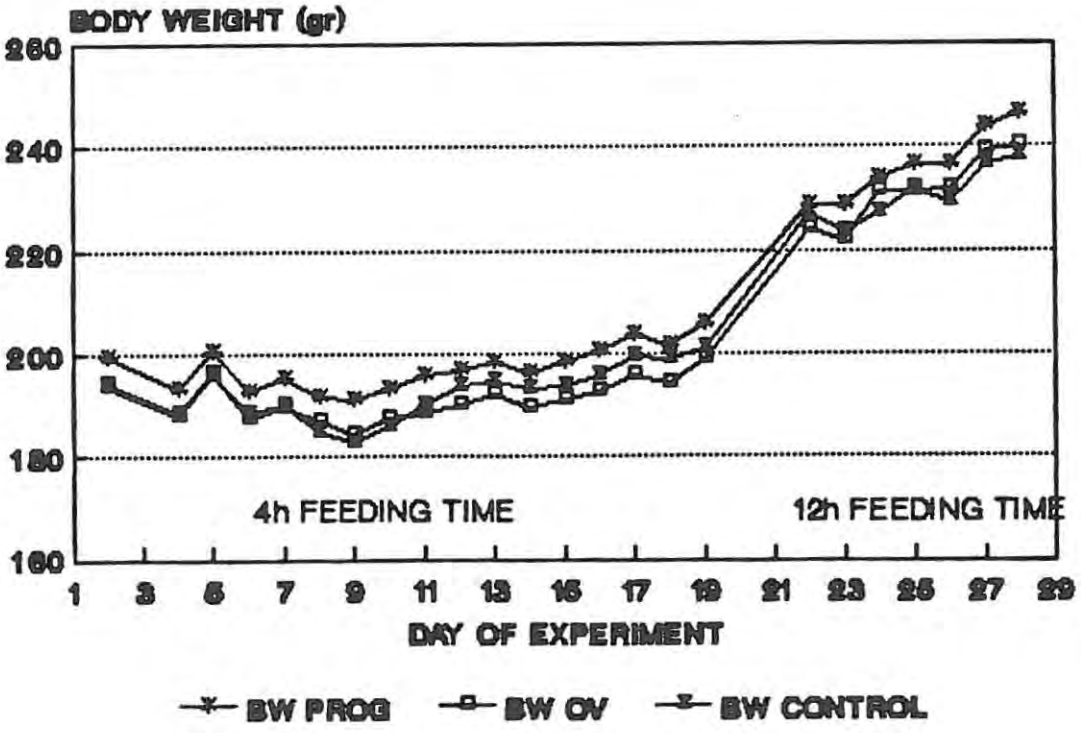


fig II.7 % of daily caloric intake as protein / IP-treatment.

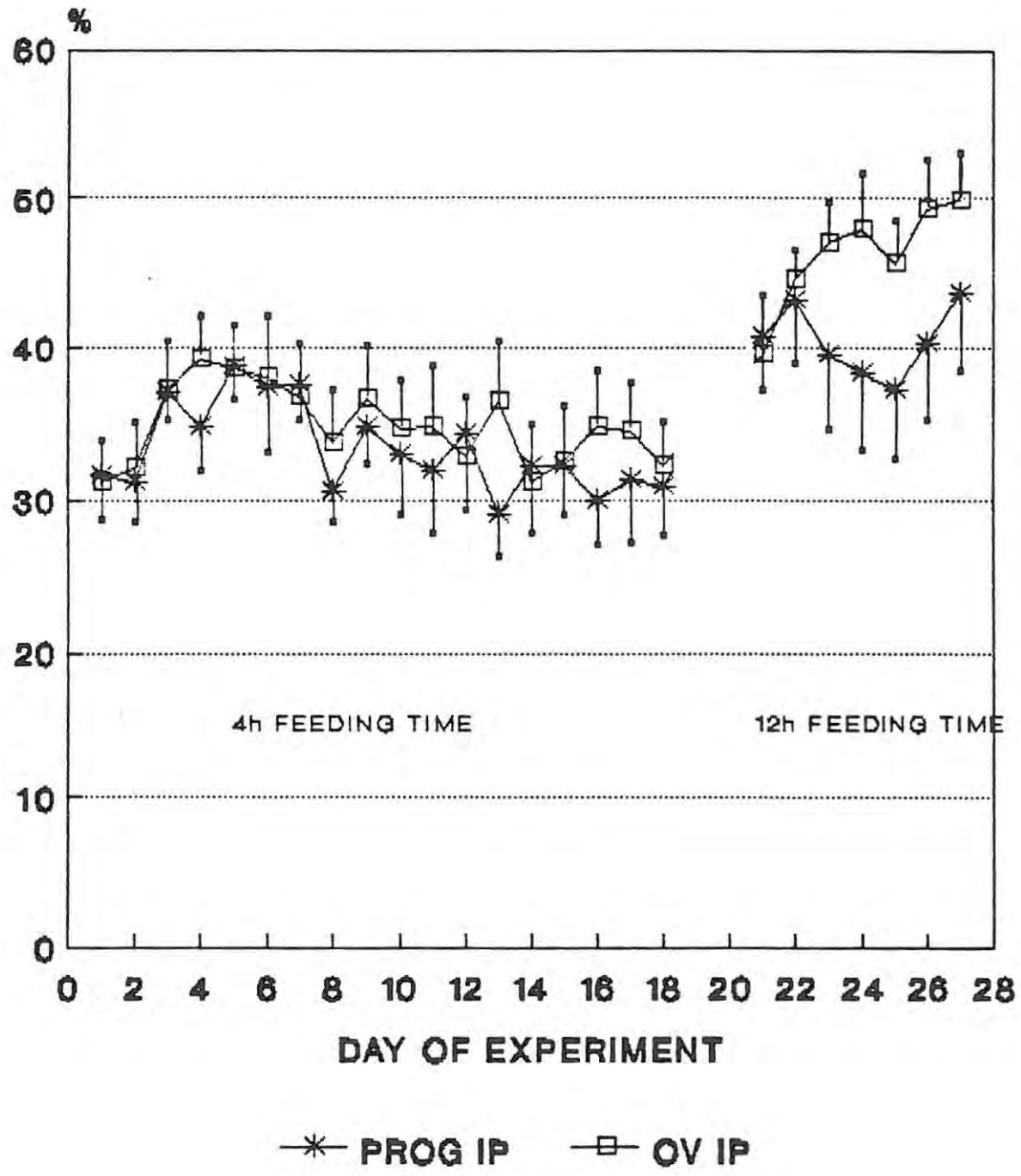


fig II.8 % of daily caloric intake as sugar / IP-treatment.

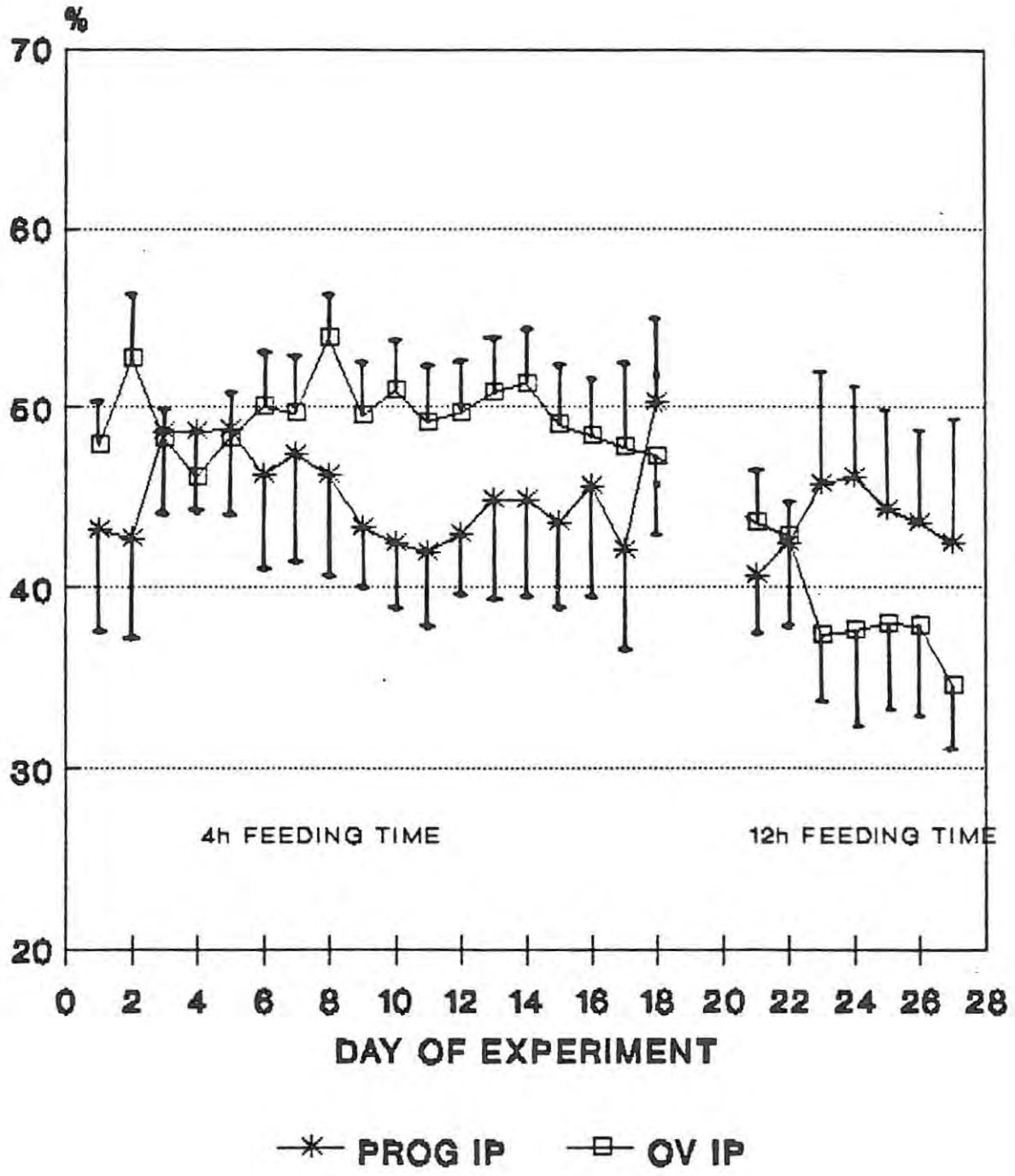
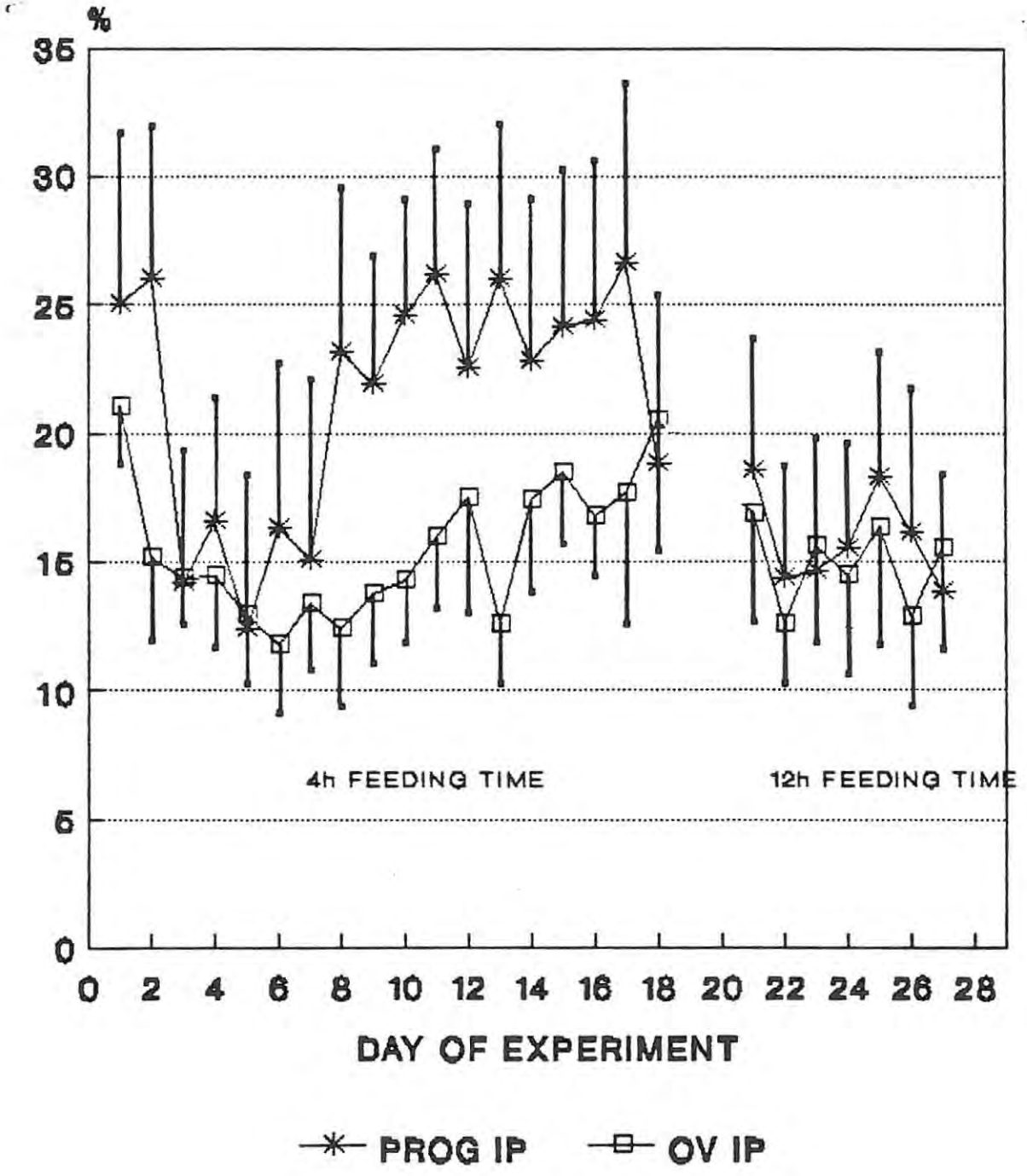


fig II.9 % of daily caloric intake as fat / IP-treatment.



E/ Experiment 5 (fig II.10-II.14)

In comparison with the finding in the preceding experiments, much clearer conclusion can be drawn. While daily SC-P-injections raised the mean BW significantly (cumulative BW gain statistically different, $p<0,05$, on day 7 of the study = 2 days after first injection), daily caloric intake remained almost unaltered (fig II.10 & fig II.11). So, the P-treated animals gained more weight per kilocalorie consumed than OV-treated ones. The cumulative caloric intake over the whole period of the study was not significantly different from that of the control group (fig II.11). This result is in contrast with the results of Galletti et al. (1964), Hervey et al. (1967), Wade (1975) which show a parallel rise in FI and BW. It is in agreement with a study of Ross et al. (1974) where P induces an increase in BW of intact weanling female rats without increasing FI, but the rats used in the present experiment were much older. Also, Schneider et al. (1986) demonstrated a highly significant BW gain in Syrian hamsters during the estrous cycle, but no change in FI.

In all those experiments showing increased FI, rats were not offered a "cafeteria diet", but the normal rodent chow and this may be one reason for the difference. In our work, the rats had to select among three preparations in order to compose a balanced diet. So, it is possible that an increased requirement for one energy source due to P is translated into an increased food intake when rats do not have the chance to select. That is why it is interesting to examine the self-selection pattern of the two groups.

This result of unaltered total caloric intake is in agreement with the idea that P-induced BW gain needs not to be accompanied by changes in FI. Hervey et al. (1968) found that when the normally occurring hyperphagia is prevented by restricting the FI to pretreatment levels, BW still significantly increases during P treatment. Similarly, the obesity following OVX is seen whether or not the animals are hyperphagic (Mueller et al. 1980, Roy et al. 1977), and these spayed female rats have to be restricted to about 80 % of their pre-OVX ad libitum food consumption in order to prevent OVX-induced BW gain. Thus, hyperphagia is not necessary for P-induced obesity.

The self selection patterns are slightly different from those seen in previous experiments, and this is partially due to the more accurate way of measuring the weight of the three gels. During this experiment, the amount of each gel consumed is corrected by use of a factor which indicates the degree of desiccation. This adjustment gives quite different results for the diet which is only consumed in small quantities (fat preparation). The protein and CH results are less sensitive to desiccation as they are consumed in larger quantities.
Between 50 and 60 % of the diet is taken as protein, circa 40 % as sugar and approximately 5 % as fat (fig II.12-II.14).
Again, the individual difference in appetite for lipids is very big, although no rats are used in this study which refuse to ingest one of the three diets. The individual differences in protein and CH intake are reasonable, but the selection habits of the two groups are too similar to see a significant difference.

The results of this self-selection experiment are in agreement

with those of Kanarek et al. (1980), although these authors made use of the OVX-rat-model. P treatment and OVX do not change the selection habits of female rats and are consequently considered to provoke no or a minor change in the requirements for a specific nutrient. Our results do not support the hypothesis of some workers (Cohen et al. 1987, Dalvit 1981, Pliner et al. 1983) which states that an increased CH craving during the follicular phase of the human menstrual cycle is due to high P levels.

Thus, there are significant P-induced shifts in energy balance and BW, without changes in FI and food selection, indicating that P must also affect energy expenditure. The present data on changes in food utilization following SC-P-treatment provide support for the hypothesis that OH may act directly on metabolic tissues, particularly AT, and that possible changes in FI following the manipulation of hormonal condition may be a reflection of changes in fat metabolism (Wade et al. 1979).

fig II.10 Influence of SC-P-treatment on BW.

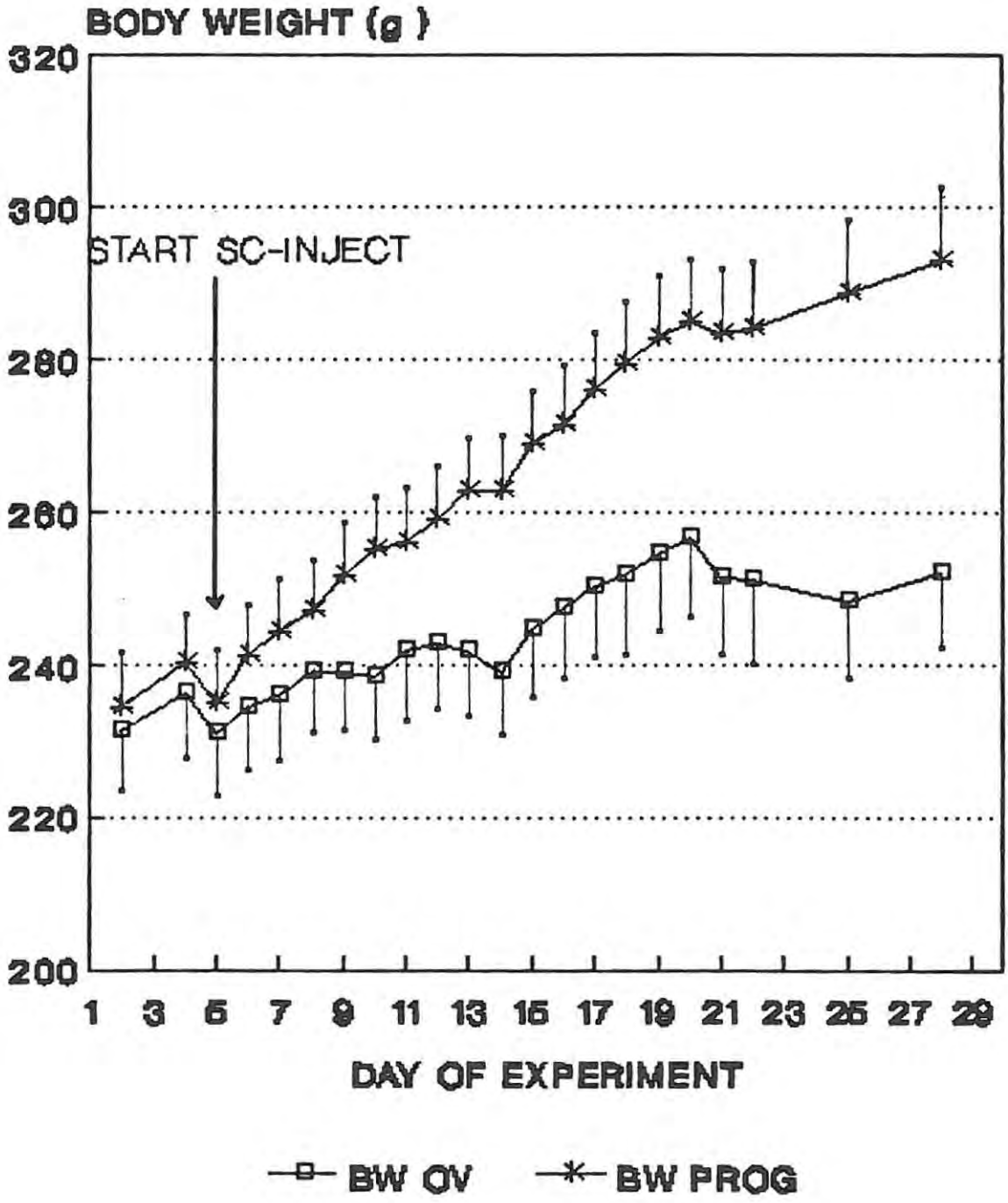


fig II.11 Influence of SC-P-treatment on caloric intake & BW.

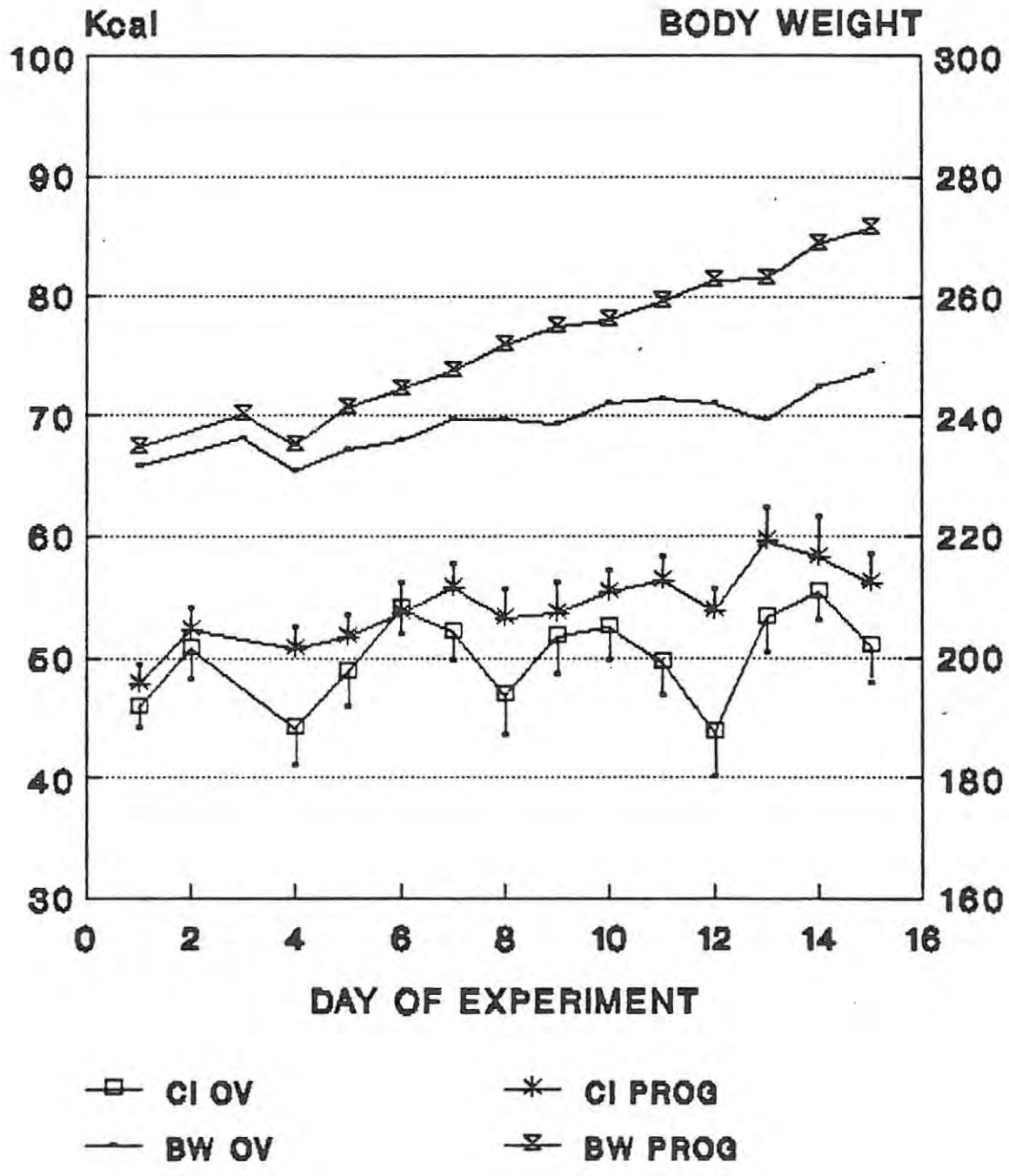


fig II.12 % of daily caloric intake as protein / SC-treatment.

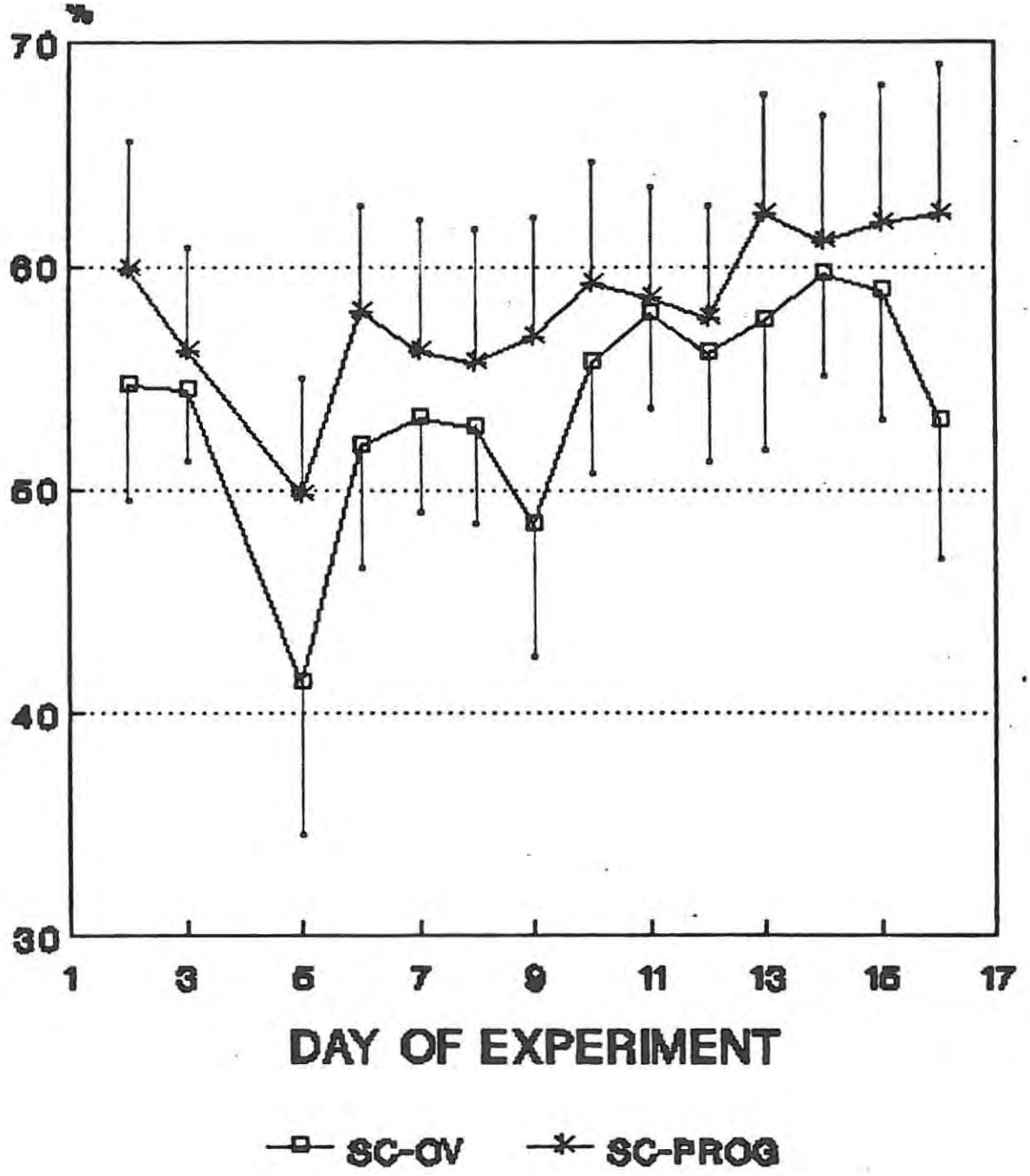


fig II.13 % of daily caloric intake as sugar / SC-treatment.

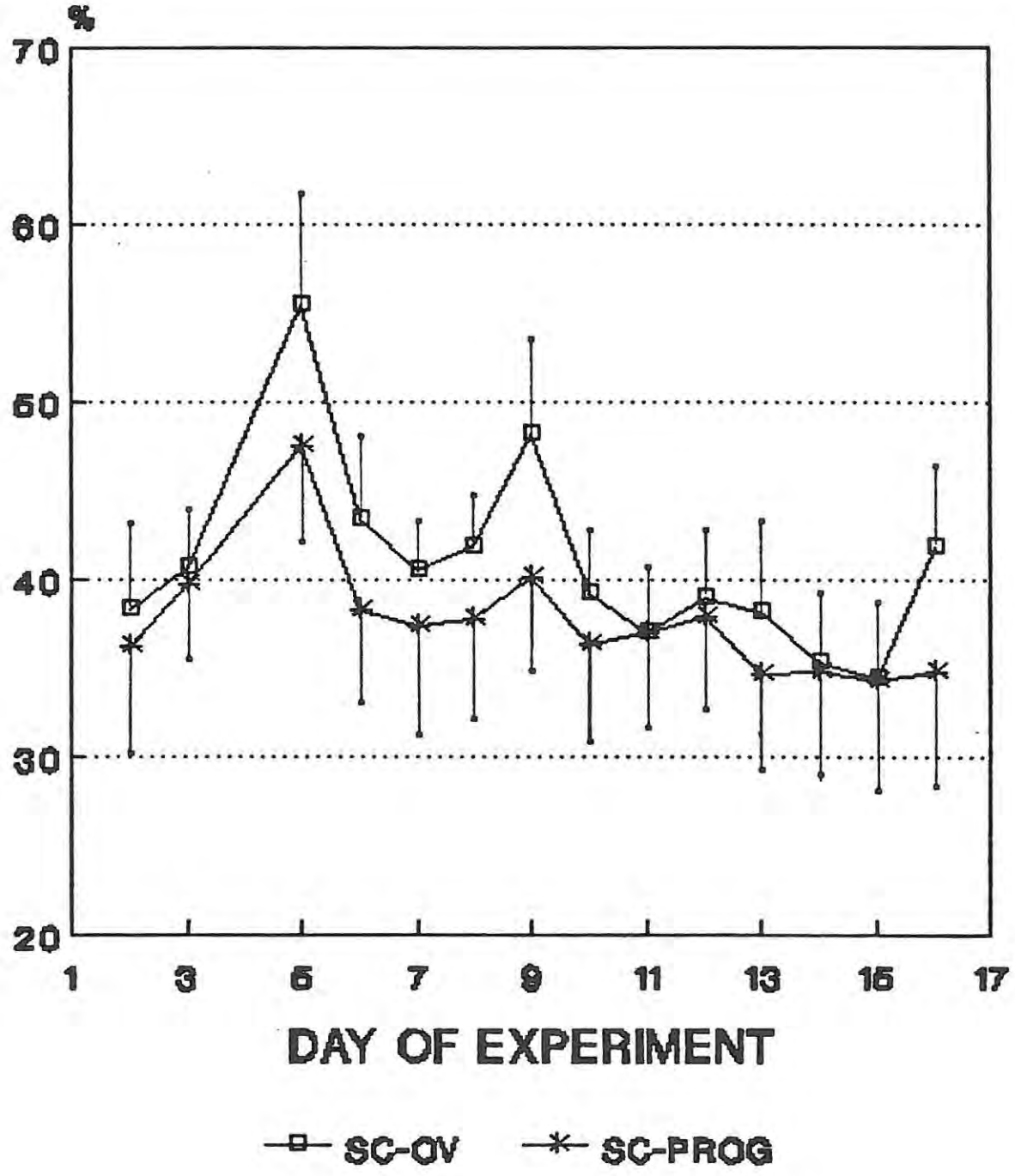
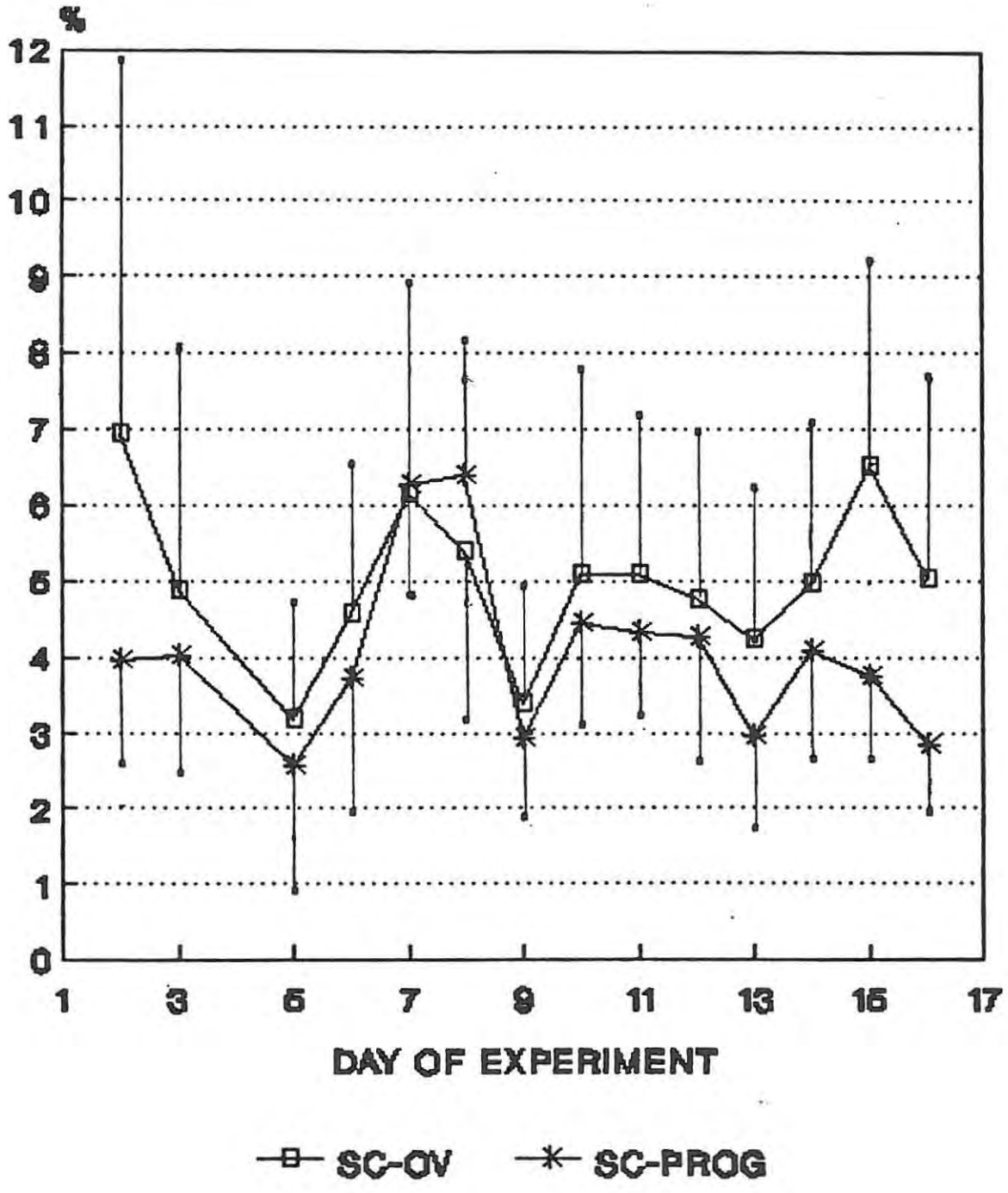


fig II.14 % of daily caloric intake as fat / SC-treatment.



CHAPTER III

LOCOMOTOR ACTIVITY STUDY

III.1 INTRODUCTION

In the past, several experiments have been undertaken to investigate the effects of OH on spontaneous activity and to estimate its contribution to OH induced BW changes. In 1923 Wang asserted that OVX suppresses permanently running-wheel activity. Whereas Es stimulates voluntary exercise (Mook et al. 1972), P seems to depress activity, (Galletti et al. 1964, Wade 1975). Very few data are available on the influence of P on spontaneous activity in intact female rats and most experiments use a running-wheel set-up, which is often considered as an exaggerated measure of day-to-day exercise (Jennings 1971).

Locomotor activity is generally regarded as horizontal or lateral, forward directed movements of the animal, such as walking or running. This study made use of a photocell activity cage which may monitor a slightly different aspect of voluntary activity. It operates on a photocell system in which interruptions of one or more light beams produced by lateral movements of animals are converted to electrical impulses, which are transferred to computerized counters. Locomotor activity of the animal is thus reflected as the number of infra-red light beam interruptions during a specific time period.

III. 2 MATERIALS AND EXPERIMENTAL PROCEDURE

III.2.1 Animals

Intact female rats of the Wistar strain were used, with masses between 210 and 250 g. Prior to testing, rats were housed three to a cage, in a temperature-controlled environment (21-23 °C), on a regulated 12-h light : 12-h dark cycle. The rats had access to water and food ad libitum both in the home cage and in the locomotor-activity cage. Rats were injected SC daily (around midday) with either P solution (20mg/kg BW) or the same volume of the OV, ethyl oleate.

III.2.2 Apparatus

Locomotor activity was monitored in three photocell activity cages constructed by the Physics Department, Rhodes University.
The cages, black painted aluminium boxes measuring 500 mm square by 200 mm high, are mounted on 800-mm-high bases, which carry the sensors and associated electronics. Activity is monitored by computer recording of the interruptions of infra-red (i-r) light beams produced by lateral movements of the animals in the cages.

The light sources comprise eight high-power (243 mW/steradian), narrow-beam (4 deg. half-angle), type ME124 i-r-light-emitting

diodes, situated equidistantly (100 mm apart), in two adjacent walls of the cages, opposite to which are placed eight type-MT2 broad-band phototransistors. The effect of the light beams on behaviour is reduced by the use of i-r beams, to which the retina of the rat is relatively insensitive. The use of i-r light beams also permits the use of the cages under conditions of normal ambient lighting or in total dark.

The transmitter-receiver pairs are so positioned that if a rat breaks a beam, a second rat moving past will break another circuit and, thus, be counted. Moreover, since the computer only measures changes in the status of the beams, a rat sitting in front of a beam is only counted once when it enters and once when it leaves the beam.
When a rat interrupts an i-r beam, the voltage level at the phototransmittor rises and this rise is detected by an analog-voltage comparator. The comparator delivers a logic signal (beam intact/beam broken) to one of an array of buffers. These buffers are interrogated by the data-logger computer and the current beam status recorded. The three cages share a common data bus and are individually interrogated by the computer. The computer interrogates each cage about 30 times every minute, limiting the maximum rate of counting to about 30 counts every minute per cage.

The data logging program provides opportunity for the user to pretest the state of individual beams, and the integrity of the computer to record breaks in individual beams. The program also allows input by the user of information such as name, date and drug treatments, which allows easy identification of the data

set.

In order to monitor gross movements and to avoid "nuisance counts" resulting from a rat grazing a beam and causing many rapid interruptions, the data logging program can be instructed to record only breaks which are sustained for a certain time period. The computer asks the user to indicate a "Filter Factor", which is then multiplied by a predetermined constant to yield the required period for which breaks must be sustained. Typically a constant of 0,1 is used. A filter factor of 2 is used, which means that all breaks must be sustained for 0,2 s before they are eligible for recording.

Data are recorded on disk at user selectable intervals and appear as ASCII records of upper breaks together with the time at which the record is made and the identification code of the tank which supplied the data.

III.2.3 Procedure

In conformity with the procedures of Burton (1989) and Khan (1989), the activity of groups of three rats per photocell cage was monitored because marked differences in the individual baseline activity of rats and, thus, large variances in activity counts occur. The combined recording of the activity of three rats per activity cage greatly reduces this variance in counts. Thus, the locomotor activity of three rats was used as one reading and 12 rats were used to give four readings (n=4).

The photocell cages were housed in a separate room. The background-noise level produced by ventilation fans (necessary to control temperature and humidity in the room) was high enough to mask extraneous noise. Lights were switched off at 18h00 and switched on at 6h00 manually.

Since three activity cages were used, which may show small differences in sensitivity, rats of various treatments were tested in each cage.

A/ Experiment to determine baseline locomotor activity

Initially it is necessary to ascertain the level of activity of animals in control conditions. Three groups of three rats each were housed in the activity cages for about 2 days during which locomotor activity was recorded by the computer every 30 minutes. Food and water was available ad libitum.

B/ Experiment to determine the effect of SC administered P on locomotor activity

The activity of five groups of three rats (n=5) under SC-P treatment and of four groups (n=4) SC-OV rats was monitored overnight. During the light phase the rats were housed in the home cages with access to food and water ad libitum. At 17h00 they were transferred to the activity cages and they had a habituation time of 1 hour. Readings commenced at 18h00 after switching off the lights.
Treatment had started 1,5 week earlier to ascertain that the mean

BW gain of the P rats was statistically different from the OV rats.

III.3 STATISTICAL ANALYSIS OF THE RESULTS

Statistical analysis was performed using one way analysis of variance, followed by Scheffe's multiple range test. Results were expressed as means +/- SEM and reported as significant different at the 5 % level or less.

III.4 RESULTS AND DISCUSSION

Initially it was necessary to ascertain the level of activity shown by animals in control conditions (fig II.1). Rats were active during the dark phase and the activity was even increased when they were not well habituated to the activity cage. The first night the overall activity was higher than the second one. Although rats remained active throughout the dark phase, the activity fluctuated.

During the light phase rats were very sedate and they "woke up" when lights were switched off at 18h00. Here, we were looking for a possible sedating effect of P. Therefore it was advisable to do the experiment under conditions when rats are very active (Robbin 1977). That is why it was decided to do the study overnight during the dark phase following a habituation period of 1 hour.

Recordings were taken for the whole dark phase to minimize big individual temporary fluctuations.

Although the P rats seemed to be a bit less active during the 12-hour dark phase, again this difference was not significant and the mean overnight activity of both the P rats and OV rats was very similar to that of non-treated rats. Also, the activity pattern of those three groups was similar; most active at the start of the dark phase, less at 4h00. This is in agreement with the results of Fraile et al. (1988), although they made use of running wheel cages. P was found not to depress the general locomotor activity of rats (fig II.2).

So, we can conclude that spontaneous activity has little or no contribution in the P induced energy storage. In any case, to account for such an obvious BW gain, one would expect to see a remarkable drop in activity.
In an attempt to answer the question as to the role of the preovulatory P peak in the control of spontaneous activity, Stern et al. (1972) showed that OVX of cycling female rats just after the proestrus Es peak, but prior to the preovulatory P surge, does not alter their activity peak. These data suggest that Es, and not P influences activity.

It can be concluded that, if any, behavioral effects of P (on FI and activity) have a minor importance in P-induced BW gain and the increase in caloric efficiency probably has a metabolic origin. As there is no apparent difference between caloric intake and use in activity, energy must be saved somewhere else in the body in order to be stored as TG in WAT.

fig III.1 Locomotor activity of control female rats.

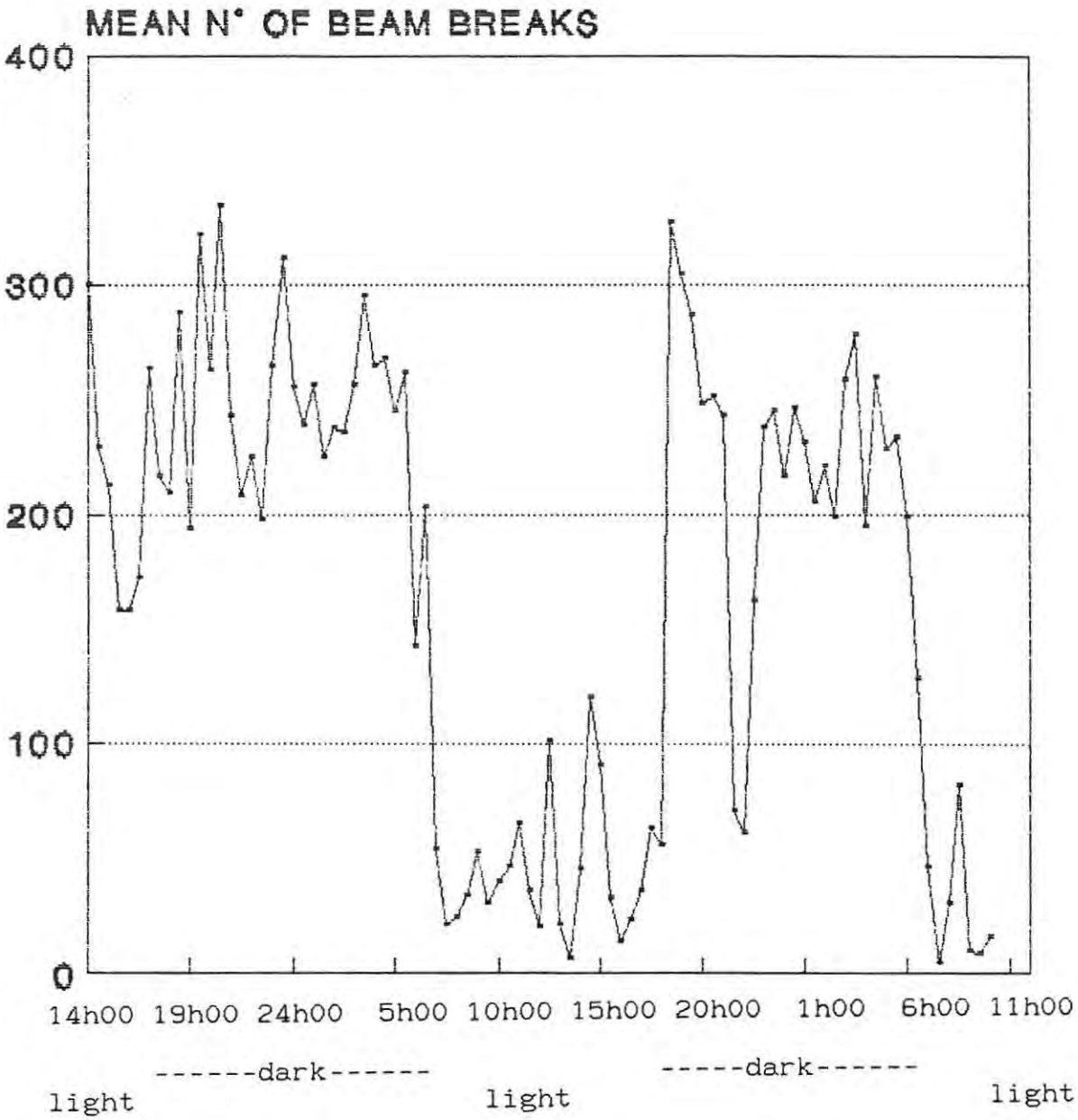
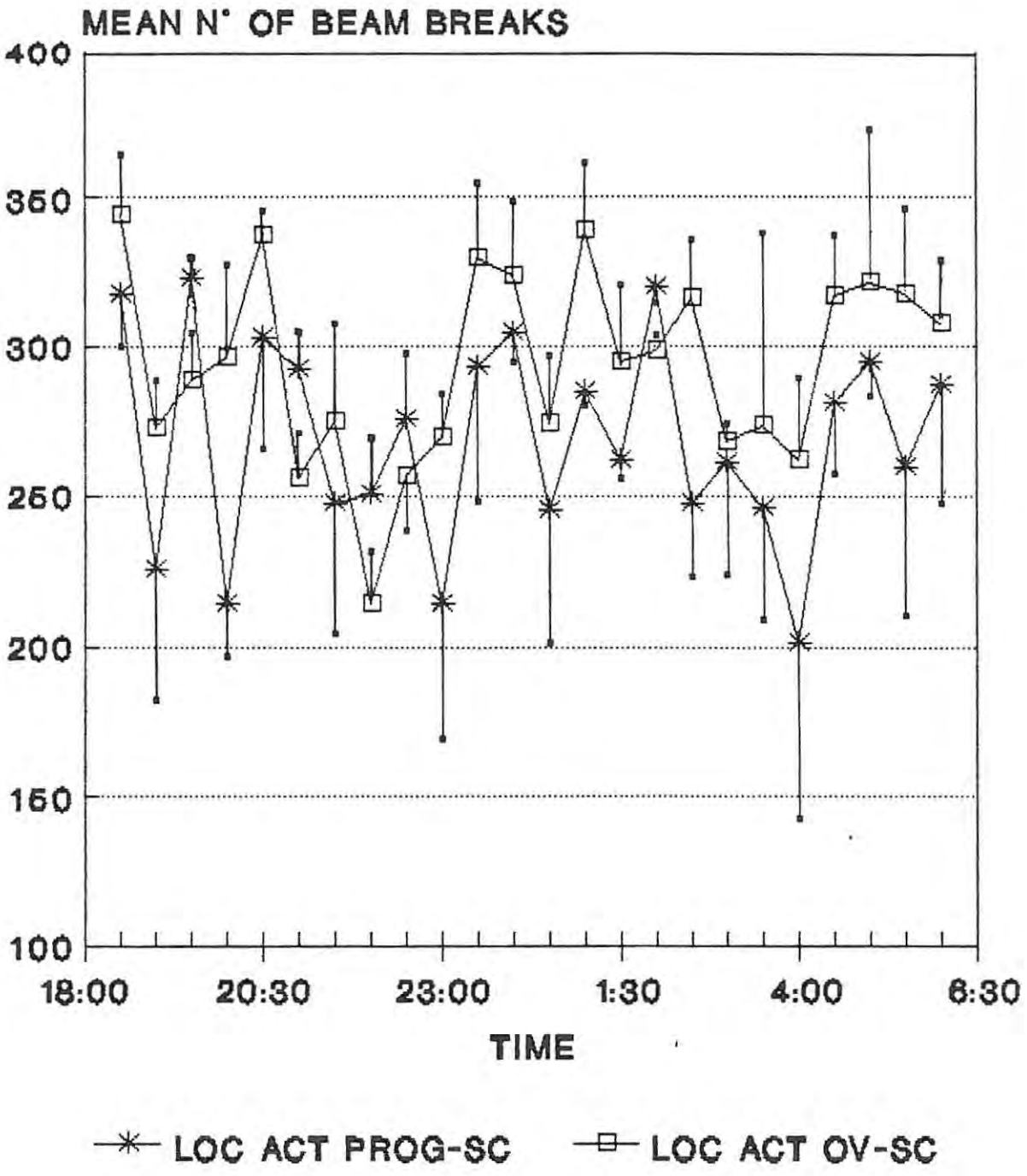


fig III.2 Locomotor activity during SC-P-treatment.



CHAPTER IV

PROGESTERONE & CORE TEMPERATURE

IV.1 INTRODUCTION

If OH can decrease or increase the storage of metabolic fuels without changing caloric intake and voluntary exercise, they must necessarily initiate some other processes which contribute to the BW gain and adiposity by reducing energy expenditure.

To examine a possible role of thermogenesis in the P-induced increase in metabolic efficiency, the core temperature of P- and OV-treated rats was measured with a thermocouple. A marked decrease in body temperature enables hibernating animals to save a lot of energy in order to survive the rigours of winter.
If thermogenesis is the sole system behind OH-induced changes in energy expenditure, one would expect obvious changes in body temperature to account for these marked drops and gains in BW, provoked by OVX, Es and/or P treament. So, P is thought to have a temperature lowering effect.

As mentioned in the literature review, one possible site of OH-induced thermogenesis is BAT, as it is also an important locus for both diet- and cold-induced thermogenesis in rats. Lots of contradictory results are obtained in experiments examining the role of BAT in the OH influence on BW regulation (I.2.4.2).

Also the peak in core temperature during the night of ovulation which coincides with high P levels in cycling females, has led many to believe that P is thermogenic. This temperature raising effect of P, which seems to oppose its overall energy saving effect, has also been examined by many researchers, using different methods and animals and giving different results.

IV.2 MATERIALS AND EXPERIMENTAL PROCEDURE

IV.2.1 Animals

Two groups of 6 and 7 intact female rats (Wistar strain) were used with masses between 220 and 260 g. They were housed one per cage in a temperature-controlled environment (21-23 °C) on a regulated 12-h light : 12-h dark cycle. Rats had access to food and water *ad libitum* and were treated daily (around 12h00) with a SC injection of either P solution or the OV (as in previous experiments). Body temperature was evaluated 2 weeks after the first SC P injection when rats were rapidly gaining weight relative to control animals.

IV.2.2 Apparatus

The core temperature of the rats was measured via the insertion of

a thermocouple into the rectum. To avoid injuries in the rectum wall, the probe was made smooth by covering the top with a bulb of solder, which conducts heat very well and quickly. The probe was connected with a recording instrument which reads the temperature to 0,1 °C.

IV.2.3 Procedure

It is very important to measure the core temperature as quickly as possible because the insertion of the probe stresses the animals and stress also may influence the body temperature. The probe was coated with vaselin as lubricant and then inserted into the rectum to a depth of 3 cm, as indicated by a mark on the probe (Lomax 1966). A period of 10 to 15 s was allowed for meter stabilization before the temperature was recorded. Readings were done in the morning and in the afternoon and rats were hand-held by the experimenter.

IV.3 STATISTICAL ANALYSIS OF THE RESULTS

Statistical analysis was performed using the Student t-test and the results were expressed as means +/- SEM and reported as significantly different at the 5 % level or less.

IV.4 RESULTS AND DISCUSSION

It can be concluded that in this study no significant influence of P on core temperature was detected. There was no drop in body temperature which would explain the increased metabolic efficiency and there was no increase in core temperature, which would correspond with the so-called thermogenic capacities of P around ovulation.

treatment	N° of animals	temperature* (°C) mean +/- SEM
P	7	36,16 +/- 0,07
OV	6	36,25 +/- 0,08

* values are means +/- SEM of 4 observations on different days and different times during light phase. No measurements are done during the dark phase.

This finding differs from that of Wrenn et al. (1959) and Rothchild (1969), who observed a body temperature rise. The difference is perhaps due to the use of other test animals: cows (Wrenn et al. 1959) and humans (Rothchild 1969).

This result also contrasts with that of Marrone et al. (1976) who demonstrated that P elevates body temperature, but they made use of OVX rats. As P-treated OVX rats do not show any BW change, this observed body temperature change has no importance in BW regulation. It also contrasts with the work of Freeman et al. (1970) in which a mean rise of 0,4 °C at a depth of 2 cm was monitored in both intact and OVX female rats. Moreover this

temperature effect seemed to be transient and returned to baseline within 2 weeks in spite of continued P treatment. On the other hand, the P-induced increase in BW lasts about 5 weeks before it stabilizes about 20 % above the control level. So, it is possible that in the present experiment the body temperature has returned to normal before the first measurement was done (after 2 weeks).

In contrast, core temperature measurements, using female guinea pigs (Czaja et al. 1986) showed a marked drop in rectal temperature following P injection. Rectal and core temperatures at both 4 cm and 8 cm sites drop significantly.

Several matters should be considered :
1/ There is as yet no real evidence that the P peak around ovulation and the temperature rise after ovulation is fully correlated. The luteinizing hormone surge preceding ovulation may also be responsible for the temperature-peak (de Mouzon et al. 1984). Moreover, Marrone et al. (1976) show that Es also raises colonic temperature, and both Hashimoto et al. (1968) and Yoshinaga et al. (1969) claim that the raise in colonic temperature in rats around estrus occurs when both Es and P are still high.

2/ Wade (1976) points out that labeling P thermogenic may be somewhat premature, since there is little evidence suggesting just how P raises body temperature. It could just as well be acting to decrease heat loss rather than increasing heat production. If decreased heat loss is involved, the seeming contradiction between the P-induced BW gain and the rise of body temperature after ovulation thought to be due to P would be

explained. So, perhaps metabolic heat production is unchanged or even reduced and the rate of heat loss reduced to such an extent that both the thermal and caloric balance are positive.

Although BAT is accepted to be the major site of nonshiver thermogenesis in mammals, other tissues too, may have an important role in metabolic heat production. Schneider et al. (1986) find that Es-induced changes in energy storage are mediated by changes in the daily rhythm of energy expenditure, which are not dependent on alterations in BAT thermogenesis. This is in agreement with the results of Mc Elroy et al. (1987) who claim that OVX-induced BW gain in rats, cannot be explained by reduction in BAT thermogenesis. It is demonstrated that metabolic heat production in liver, and not in BAT, is significantly suppressed in Syrian hamsters after OVX, suggesting that hepatic mechanisms for thermogenesis may play a role in the OVX induced BW gain in hamsters (Schneider et al. 1986).
This confirms the findings of Wade (1986) who indicates that OH do not affect BAT thermogenesis in rats and hamsters.

The liver also seems to have a significant role in diet induced thermogenesis since a fall in metabolic efficiency (augmented cellular heat production) is observed in hepatocytes from cafeteria-fed rats (Berry et al. 1985).

Moreover, BAT is scattered all over the body of the rat and one BAT-pad may have different effects on core temperature than another. The anatomical location of a thermogenetic organ or BAT pad may be very important. Interscapular BAT is the most peripherally located BAT depot and is consequently ideally suited

for heat dissipation to the external environment, but may have less of a contribution to the heating of the core of the animal than, for example, periaortic or perirenal BAT or the liver.

So, OH may activate or inhibit one organ or BAT pad more than another and this may result in changes of both thermal and caloric balance. It is not unlikely that heat production in the liver provokes a bigger increase in core temperature than interscapular BAT, but less heat will be dissipated to the external environment. Thus, increased hepatic importance in thermogenesis after OVX as indicated by Schneider et al. (1986), may play an important role in decreasing heat loss instead of decreasing heat production, as indicated by Wade (1976). So, temperature homeostasis probably involves a multifaceted control system which depends upon a variety of mechanisms involved in heat production and in heat dissipation. It is likely that OH simultaneously affect many of these mechanisms, and that the overall effect of OH on body temperature depends upon the summation of these actions plus characteristics and conditions of the species being studied.

3/ To examine the hypothesis that the liver is indeed a crucial organ in P-induced increase in metabolic efficiency, the same experiment should be repeated inserting the thermocouple approximately 7 to 8 cm into the rectum of the rat. Post mortem examination (Lomax 1966) shows that, at this distance, the tip of the probe is lying behind the liver. This researcher explains that the temperature of the liver represents the best approximation to the body core temperature and that measurements of temperature at more distal points in the gastro-intestinal

tract will be variably affected by changes in local blood flow. He also suggests inserting the probe and leaving it in position chronically throughout the experiment. Experience has shown this technique to be very difficult.

Czaja et al. (1986) also stress the importance of the depth to which the probe is inserted. Es significantly increases rectal temperature measured at a depth of 4 cm, but has no significant effect on measurements taken at a depth of 8 cm. So, as indicated by Czaja et al. (1986) a temperature measurement at a depth of 3 cm is more likely to represent surface temperature than core temperature and, if the statement of Wade (1986) that interscapular BAT thermogenesis is not changed during P treatment is correct, then it is to be expected that no temperature effect is observed at a depth of 3 cm.

It can be concluded that the wide variety of techniques for measuring body temperature, of the experimental conditions and of test animals is probably the most serious cause of contradicting results.

CHAPTER V

RAT LIVER STUDIES

V.1 INTRODUCTION

The liver is perhaps the most important organ for the regulation of energy metabolism. It plays a central role in the body's synthesis, metabolism and interconversion of CH, lipids and protein.
Anything which alters handling of the various metabolic fuels by the liver could affect adiposity and eating behavior (I.1.2.4 & I.2.4.1 C)

There are several indications that the liver plays a crucial role in the P-induced effects on adiposity and BW.

1/ Rat liver contains cytoplasmic E receptors (Eisenfeld et al. 1976) and the activity of P on BW is dependent on and closely related to the presence of Es. In a number of experiments the response of a target organ to P is enhanced by previous exposure to Es (Chan et al. 1977).

2/ P treatment alters hepatic RNA and protein synthesis, possibly

including enzymes involved in TG production and transport (Kurtz et al. 1976). Also, P has been shown to increase both AT and hepatic LPL activity in rats (Kim et al. 1975).

Lorenzo et al. (1986) state that P increases lipogenesis (expressed in terms of tritium incorporation into FA) in rat liver but decreases plasma FFA concentrations. It is further indicated that the stimulatory effect of P on the rates of hepatic lipogenesis is probably due to an enhanced enzymic capacity. A similar conclusion is made by Dahm et al. (1978), using medroxyprogesterone acetate. The lipogenic stimulation by this progestagen is due to the elevated activity of certain enzymes providing lipid precursors. Also P activates those hepatic lipogenic enzymes (Dahm et al. 1977) providing precursors for either FA or cholesterol.

Chan et al. (1977) say that P exerts a protective effect on Es-induced hyperlipemia which, in their opinion, is primarily a result of increased TG production. P alone is found not to change the rate of VLDL biosynthesis and does not provoke hyper-triglyceridemia (Kim et al. 1978).

3/ It is demonstrated that metabolic heat production in the liver, and not in BAT, is significantly suppressed in Syrian hamsters after OVX, suggesting that hepatic mechanisms for thermogenesis may play a role in the OVX induced obesity in hamsters (IV.2.4). As indicated before (I.2.1) OVX induced obesity seems to be very similar as P induced obesity. Moreover, changes in the activity of hepatic mechanisms for thermogenesis have been associated with genetic obesity in rats (Litterst 1980).

Without ruling out possible significant contributions of other metabolic changes, P-induced changes in lipid metabolism are obviously very important in the P-induced BW effect. Since the liver is a crucial organ in fat metabolism, it can be expected that the liver plays a principle role in the P-induced obesity. That is why some studies have been undertaken which analyse P-induced modifications in hepatic FA synthesis. Moreover the ultrastructure of hepatocytes from P-treated female rats are examined via electron microscopy.

V.A FA COMPOSITION OF RAT LIVER : GC ANALYSIS

V.A.1 MATERIALS AND EXPERIMENTAL PROCEDURE

V.A.1.1 Animals

This experiment also made use of intact female rats of the Wistar strain weighing between 220 and 270 g. Rats were housed in a temperature-controlled environment (21-23 °C) on a regulated 12-hour light : 12-h dark cycle and they had access to water and food *ad libitum*.

V.A.1.2 Chemicals and Reagents

All solvents used in this study were of analytical grade. Hexamethyldisilazine and trimethylchlorosilane used for the trimethylsilylation of cholesterol was purchased from Pierce.

V.A.1.3 Procedure

This experiment examines a possible effect of P on rat liver FA metabolism profiling the principle FA's in rat liver. Total lipids were extracted from liver tissue, the FA transesterified and analyzed with gas chromatography (GC). The extraction procedure was based on the method of Folch et al. (1957).

Rats were sacrificed by neck fracture, the liver was rapidly removed and 1 g chopped liver tissue was homogenized with 2:1 chloroform - methanol mixture (v/v) and diluted to a volume of 20 ml. The homogenation was carried out in a Thomas tissue homogenizer, which consists of a teflon pestle and a borosilicate glass tube. The steel rod of the teflon pestle was attached to an electric drill which allows the teflon pestle to be rapidly rotated while in the glass homogenizer. To homogenize the liver tissue, the glass homogenizer tube was moved up and down relative to the pestle. Rapid rotation of the pestle and the vertical movement of the glass tube cause disintegration of the tissue and disruption of the cells. Then, the homogenate was filtered

through a fat-free paper into a Büchner filter under low pressure. Now the filtered solution was divided in two equal parts, which were treated in exactly the same way and the second sample served as a spare sample in case of an accident. The crude extract was mixed thoroughly with one-fifth its volume of water (=2 ml per testtube) and the mixture was to separated into two phases by centrifugation. The volumes of the upper and lower phases, were respectively 40 and 60 per cent of the total volume of the system. As much of the upper phase as possible was removed by pipetting, and removal of its solutes was completed by rinsing the interphase three times with small amounts of the pure solvents of the upper phase in such a way as not to disturb the lower phase. Finally, the clean lower phase is the total pure lipid extract and it was evaporated to dryness at 40°C under nitrogen stream before trans-esterification.

Pure solvents of the upper phase were obtained in the following way. Chloroform, methanol and water were mixed in a separatory funnel in the proportions 8:4:3 by volume. When the mixture was allowed to stand, a biphasic system was obtained. The two phases were collected separately. It has been found (Folch et al. 1957) that the approximate proportions of chloroform, methanol and water in the upper phase are 3:48:47 by volume. In the lower phase, the respective proportions are 86:14:1. The upper phase used in this experiment as rinsing fluid was prepared directly by making use of the above proportions.

Before the principal FA and cholesterol in the extract were profiled, using capillary GC with flame ionization detection, they had to be converted to volatile derivatives (fatty acid methyl

esters & trimethylsilylated cholesterol). This was done in two steps according to Muskiet et al. (1983).

Transesterification of FA and hydrolysis of cholesterol esters :

This method was preferred to saponification, followed by esterification by heating with borotrifluoride - methanol reagent as it is shorter and gives similar results. 2 ml of a methanol-hydrochloric-acid solution (prepared by adding 50 ml of methanol to 10 ml of a 6 mol/l hydrochloric acid solution in water) were added to the lipid extract. The tubes were flushed with a stream of nitrogen, tightly capped and heated in an oil bath at 90°C for 4 h. After cooling, the samples were extracted twice (shaking vigorously for 2 min each time) with 2 ml of hexane and the combined hexane layers were dried with anhydric sodium sulfate. After filtering, the hexane was evaporated at 40 °C under a stream of nitrogen. So, the test tube contained the FA - methyl esters and cholesterol.

Trimethylsilylation (TMS) :

To the dry residue 1 ml of pyridine was added, 0,2 ml of hexamethyldisilazine and 0,1 ml of trimethylchlorosilane. The capped tube was heated at 80 °C for 30 min in a heating block. After the addition of 4 ml of water, FA - methyl esters (FAME) and trimethylsilylated sterols were extracted into 3x2 ml of chloroform. Impurities in the chloroform extract were eliminated by treating it with a 10 % w/w sulfuric acid solution, rinsing with distilled water, then with saturated sodium bicarbonate and finally rinsing with water again.

Again the chloroform layer was dried with anhydric sodium sulfate, filtered and evaporated to dryness at 40 °C under a stream of nitrogen. The residue was redissolved in the desired volume of chloroform (150 µl) and stored at -20 °C under nitrogen and protected from light until FAME separation was performed by GC.

Separation of FAME and TMS cholesterol by GC :

Profiling of FA and cholesterol was achieved by GC in a Hewlett Packard model 5890 A gas chromatograph. Operation conditions employed were : oven temperature program was 1 min at 170 °C, 3 °C/min to 230 °C, 1 min at 230°C, 10 °C/min to 280 °C, 15 min at 280 °C; injector temperature is 250 °C; detector (flame ionization) temperature is 250 °C. Individual FAME and TMS cholesterol were identified by their retention time, using standard solutions.

In contrast to the method of Muskiet et al. (1983) the total FA profile and cholesterol was measured simultaneously in a single gas-chromatographic run. Therefore, a non-polar Durabond DB1 column (J&W Scientific, Rancho Cordoba, California, USA) of film thickness 0,25 µm was used, as TMS cholesterol shows poor gas chromatographic properties on a polar stationary phase, such as the Durabond DB 225, which is, a better column to separate the FAME. Comparative tests, using a DB1 and a DB225 column, have shown the same FA profile of our samples. Although an apolar DB1 column is not the ideal one for separation of FAME, it gave satisfying results for the analysis of female rat liver FA's and it allowed a simultaneous analysis of FA and cholesterol.

In this experiment, no attempt was made to quantify the FA and cholesterol in a sample, but FA and cholesterol peaks were compared to examine a possible change in FA and cholesterol profile following P treatment. That is why no internal standard was added to the sample. Peak areas of one sample were added up and the contribution (percentage) of each peak was calculated. Peaks smaller than 1 % were not taken into account.

A/ Experiment 1

Phenobarbitone is an inducer of drug-metabolizing enzymes of hepatic microsome (Eling et al. 1970). Okabe et al. (1986) examined FA and cholesterol levels in plasma of patients receiving phenobarbitone and found that phenobarbitone also affects lipid metabolism. It causes a substantial induction of the cytochrome P-450 family isoenzymes, members of which catalyse the n and n-1 hydroxylation of FA (Gibson et al. 1985, Wen et al. 1987). It also increases hepatic synthesis of TG (Goldberg et al. 1987).

The present study examined the FA and cholesterol profile of female rat liver after a prolonged phenobarbitone treatment (1 month). Sodium phenobarbitone (1 mg/ml) was dissolved in water and the water bottles were filled with this solution.

This experiment made use of 11 rats which were divided in a group (n=6) which drank the sodium phenobarbitone solution ad lib. and a control group (n=5) which had access to plain water.

B/ Experiment 2

In this study 16 female rats were divided into 4 groups of 4 rats. Group 1 and 2 were injected daily SC either with the P solution or the OV respectively and group 3 and 4 were injected IP either with P or ethyl oleate respectively. This treatment lasted for 2 weeks prior to the trial. This period was long enough for the P-induced BW gain to occur.

C/ Experiment 3a

Rats, which had been treated daily IP with the oily P solution or with the OV for a certain period (3 weeks or more) accumulated a white fat-like deposit on the liver surface and between the lobes.
Rats injected SC however did not have this deposit. Again, this shows the importance of the route of administration. SC injected rats apparently metabolise the ethyl oleate load in a different way. Electron microscopy studies (V.B) and two dimensional thin-layer chromatography of this material can elucidate the nature and composition of this deposit and consequently the corresponding metabolism.

The identification presented here is based on the method of Hubmann (1973). It is a two dimensional thin-layer chromatography of lipids on a microscale. The fatty deposit from the liver was homogenised in chloroform : methanol (2:1 v/v), centrifuged and a drop of the supernatant lipid extract was applied to one corner of a micro TLC plate (45 x 25 mm; silica gel 60 F254, Merck). The spot (diameter 1mm) was applied by means of a self-drawn glass

capillary. A distance of 1,5 cm below the upper margin was clearly marked. The spot was then developed with solvent A (chloroform - methanol - distilled water, 65:25:4), and the chromatography was terminated when the solvent front had reached the marking. After drying the plate, a second development with solvent system B (n-hexane -diethylether -glacial acetic acid, 85:20:2) in the same direction was carried out, and the solvent front was allowed to reach the top edge of the plate. The chromatogram was then turned through 90°, and a third development was allowed to proceed using solvent system B. The lipids were rendered visible as yellow spots in an iodine atmosphere.

D/ Experiment 3b

The lipid deposit was also analysed via GC in order to investigate the FA and cholesterol profile and elucidate a possible P influence. Five rats were injected daily IP with the P solution for 1 month prior to the trial. The control group (n=6) was treated with the same volume of ethyl oleate.

E/ Experiment 4

Some hamsters use an environmental cue such as photoperiod to make anticipatory changes in BW and adiposity (I.2). Body fat accumulates when hamsters are switched to a short light period, as in winter (Bartness et al. 1984, Hamilton et al. 1988). Decreasing day lengths trigger changes in energy metabolism that result in accumulation of body fat without requiring increased food intake, which is a similar situation as our P-induced BW increase. Hoffman et al. (1982) show the importance of the pineal

gland in this effect, as no photoperiod induced BW effects are seen in pinealectomized rats. This is contradicted by Bartness et al. (1984).

Changes in lipid metabolism in the liver may be a cause of important changes leading to these photoperiod-triggered fluctuations in body fat.

In this study, 12 female rats received a fat load via daily IP injections (2 ml ethyl oleate/kg BW) for a period of 2 weeks. After the last injection, one group (n=6) was kept in a dark room for 3 days (water and food ad libitum). The control group (n=6) stayed in the animal room on a regulated 12-h light : 12-h dark cycle. After 3 days, all rats were sacrificed and the liver FA and cholesterol profiles were determined.

V.A.2 STATISTICAL ANALYSIS OF THE RESULTS

Statistical analysis is performed using one-way analysis of variance, followed by Scheffe's multiple-range test. Results are expressed as means +/- SEM and reported as significantly different at the 5 % level or less.

V.A.3 RESULTS AND DISCUSSION

A/ Experiment 1 (fig V.1)

The eight most prominent FA's and cholesterol (peak area > 1%) are reported here. These represent about 95 % of the total lipids. Analysis of the multiple minor FA's is difficult due to their relatively low concentrations and therefore they are not taken into account.

As could be expected the FA profile of the phenobarbitone group is obviously different from the control group. Phenobarbitone is known to induce some hepatic enzymes and Okabe et al. (1986) find a changed FA and cholesterol profile in the serum of phenobarbitone treated humans. Higher blood concentrations of cholesterol together with higher concentrations of palmitic, stearic and oleic acid (relative to linoleic acid) are observed in patients treated with phenobarbitone.

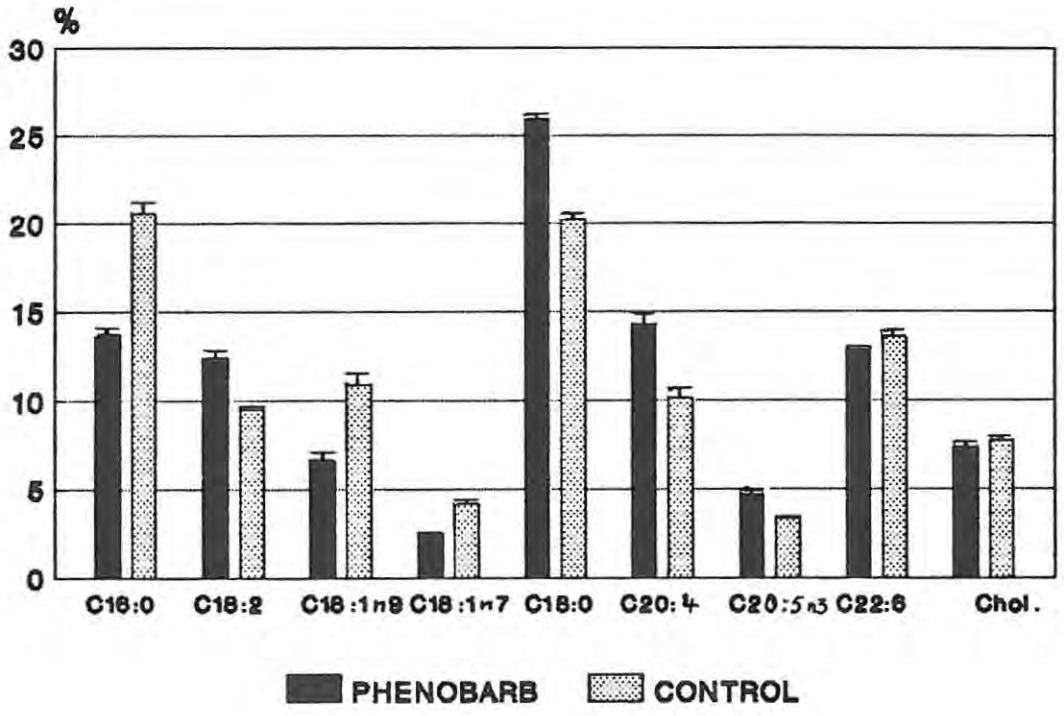
The analysis of rat liver gives totally different results from those reported for humans. In addition, the FA profile has also changed significantly following phenobarbitone treatment (7 of the 8 FA peaks are significantly changed). The cholesterol proportion is unchanged. Thus, phenobarbitone is more likely to affect the conversion of FA's than to influence cholesterol synthesis. The percentages of palmitic, oleic and vaccenic acid drop while the others increase significantly. It is very difficult to interpret these results in terms of increased or decreased activity of enzymes involved in elongation or (de)saturation reactions. But

the drop in the proportion of palmitic acid and the increase in the proportions of the longer FA's stearic, linoleic and arachidonic acid might be related to changes in levels of enzymes catalyzing elongation reactions.

Besides FA synthesis, the excretion of lipids may be influenced by phenobarbitone. Consequently it is possible that selective changes of transport of lipid species occur, depending on FA composition.

Although the specific changes in profile between the present results and those obtained in human serum are very different, the two studies agree that phenobarbitone influences FA metabolism.

Fig V.1 FA profile of rat liver during phenobarbitone treatment.



B/ Experiment 2 (fig V.2-V.5)

Again, minor peaks (peak area < 1%) are not taken into account and the nine prominent peaks (representing about 95 % of the total lipids) of this trial correspond to those of the phenobarbitone experiment.

Before considering the effect of P, it is interesting to look at the importance of the route of administration. The liver lipid profile of IP OV rats differs from that of SC OV rats in the proportion of oleic acid (fig V.2). SC OV rats have a liver profile very similar to non-treated rats (the control group of the phenobarbitone experiment)(fig V.3). This means that the resorption of the SC injected ethyl oleate happens in such a way as not to influence the liver FA and cholesterol metabolism. The same volume of OV, injected IP, provokes a remarkable increase in the proportion of oleic acid (it rises to more than 15%) while the proportions of the most other FA's consequently drop slightly.

Moreover, it appears that oleic acid, in the SC OV rats, has already been converted to linoleic acid when taken up by the liver. There is little or no trace left of the daily SC ethyl oleate load (fig V.3).

This FA analysis gives only proportional and no quantitative results. So, it is possible that the liver is more saturated with lipids without showing any change in the profile.

It is probable that IP injected oil is transported directly to the

liver and deposited there while SC OV is metabolized peripherally. Moreover, a fat deposit is formed on the liver after IP injections and not after SC treatment.

It is not surprising then that the route of administration is crucial for the resorption, metabolism and activity of the steroid, progesterone.

The results of this lipid analysis for the IP treated rats (P and control) are in conformity with the BW experiments : there is no statistical difference between the two groups in FA and cholesterol profile (fig V.4).
On the other hand, when rats are injected SC, there is a small but significant increase in the proportion of stearic acid and C20:5 n3 at the cost of most other FA's although the individual decreases are not statistically significant (fig V.5). It is difficult to understand the real meaning of this result. The changes are not as obvious as in the phenobarbitone experiment and there, the proportional increase of stearic acid is accompanied by increases of other long chain FA's.

But if P, as shown in the work of Dahm et al. (1977), is a stimulator of lipogenic enzymes, it may stimulate the synthesis of certain FA's without provoking an overall equal increase. So, P may cause an increase in synthesis of stearic acid.
Also, it is possible that P influences not so much FA synthesis as lipid transport and excretion out of the liver and that the FA composition of the lipids is a determining factor : i.e. these may be a selective impairment of transport of certain lipid species, depending on FA composition.

An analysis of the distribution of FA's among the different separated lipid species (triglycerides, cholesterolesters and phospholipids) would probably give more information about the P induced alterations.

Another possibility is that the activity of peroxisomal beta-oxidazing enzymes, a chain-shortening system that primarily handles long-chain FA's (e.g. C18, C20, C22) which are poor substrates for mitochondrial oxidation (Bremer 1977), is reduced after P treatment. Moreover, this hypothesis is in line with the induced energy saving since the first dehydrogenation step of peroxisomal FA oxidation is neither directly nor indirectly coupled to an energy conserving system, implicating its role in thermogenesis. On the other hand, it seems that the contribution of the peroxisomes to overall FA oxidation remains minor and consequently too small to contribute significantly to hepatic heat production (Hue et al. 1981).

As far as cholesterol is concerned, the present results are in contrast to the work of Panini et al. (1987) which showns that P provokes an inhibition of cholesterol biosynthesis. The unchanged proportion of cholesterol in the results presented here leads us to conclude that under our experimental conditions cholesterol synthesis is either not changed or that the synthesis and excretion of cholesterol has increased simultaneously.

C/ Experiment 3 (fig V.6 & V.7)

IP injected ethyl oleate must be transformed in the liver or must provoke other metabolic changes as it is injected as an oil and

gives rise to the development of a solid deposit on the liver. This happens to IP-P-treated and IP-OV-treated rats alike. This deposit does not develop during SC treatment.

Thin-layer chromatography shows the composition of the extract of the deposit. All lipids are nicely separated : cholesterol esters, triglycerides, FFAs, free cholesterol and phospholipids (fig V.7). Probably WAT has developed as a result of the ethyl oleate load. Electron microscopy studies (V.B) confirm this statement.

The analysis of the FA and cholesterol profile of this deposit again confirms this statement (fig V.6). About 70 % of the FA is oleic acid; which means that oleic acid after transport to the liver has not been metabolised but transformed to TG and excreted as LDL, which apparently leads to the formation of a WAT around and on the liver lobes.

The profile of the IP-P-rats is not different from the IP-OV-group.

D/ Experiment 4 (fig V.8)

This experiment examines the ability of rats to dispose of IP-injected fat loads while kept in a dark room for three days. Rats kept in the dark have the same liver FA and cholesterol profile as control rats. It must be mentioned that the individual differences are quite large. In both groups, there are "quick-disposers" (rats with lower proportion of oleic acid) and "slow-disposers" (rats with higher proportion of oleic acid and lower

proportion of most other FAs). It is important to note that rats were injected the double amount of ethyl oleate (2ml/kg BW) and that this probably provoked the high proportion of oleic acid, even after three days since the last load.

Legend :

C16:0 = palmitic acid
C18:0 = stearic acid
C18:1n9 = oleic acid
C18:1n7 = vaccenic acid
C18:2n6 = linoleic acid
C20:4n6 = arachidonic acid
C22:6n3 = docosahexaenoic acid
Chol = cholesterol

Fig V.2 FA profile of rat liver during SC & IP OV treatment.

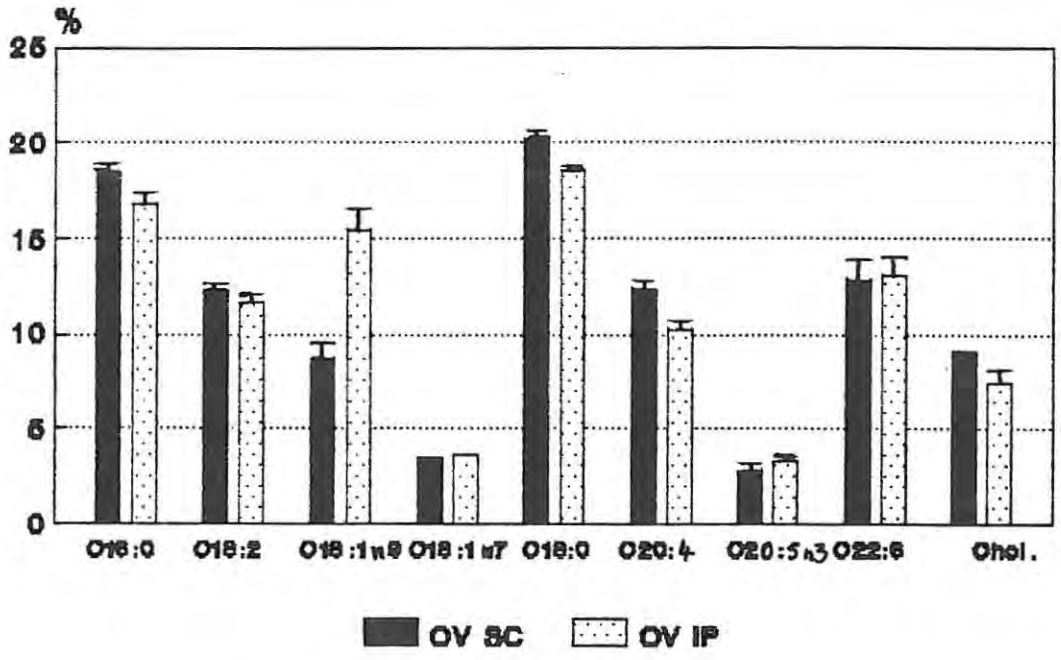


Fig V.3 FA profile of rat liver during SC-OV treatment.

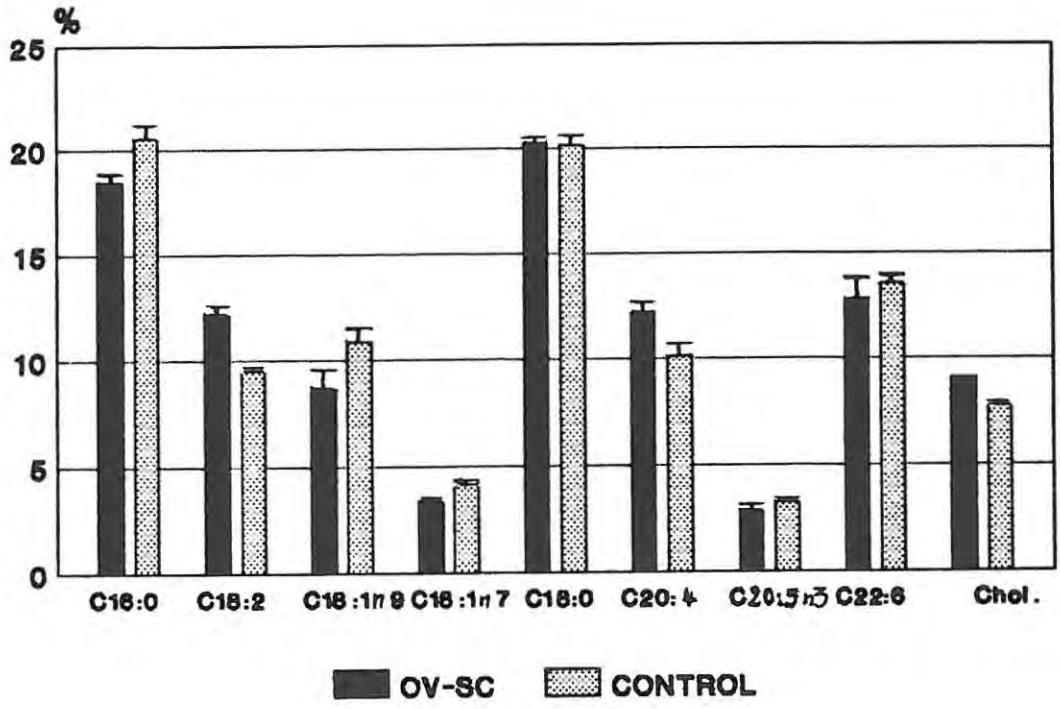


Fig V.4 FA profile of rat liver during IP-P treatment.

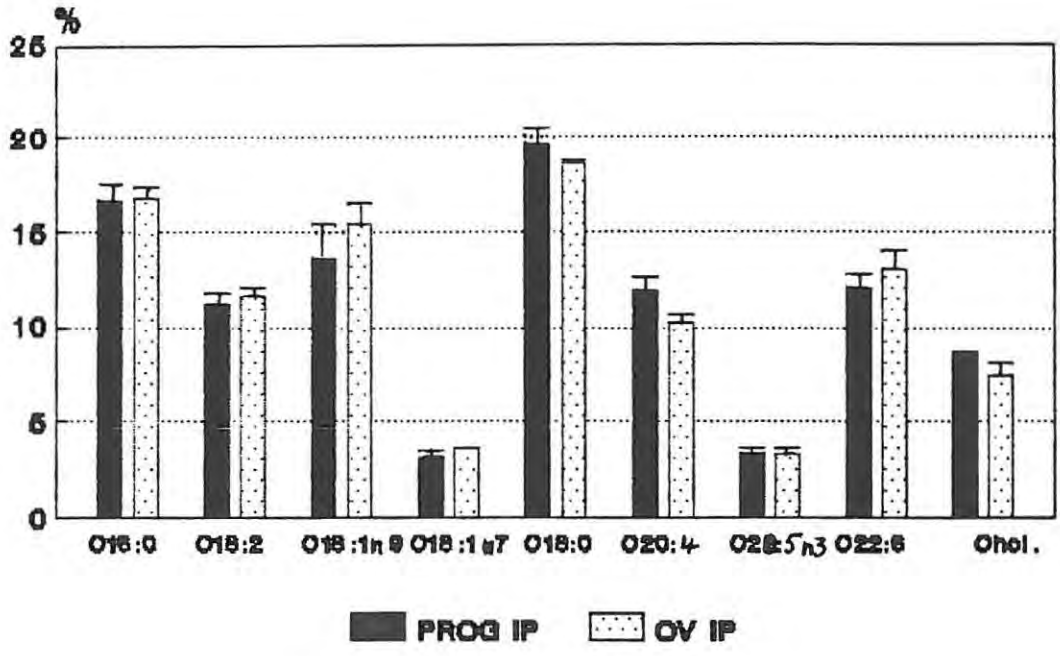


Fig V.5 FA profile of rat liver during SC-P treatment.

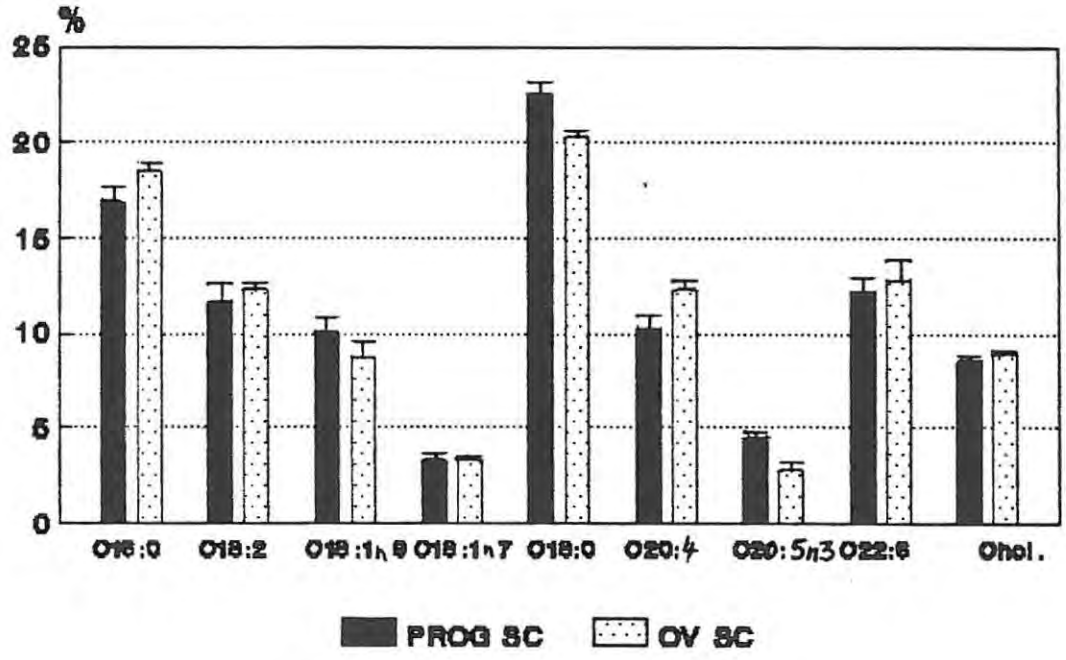


fig V.6 FA profile of fat deposit on liver after IP-P treatment.

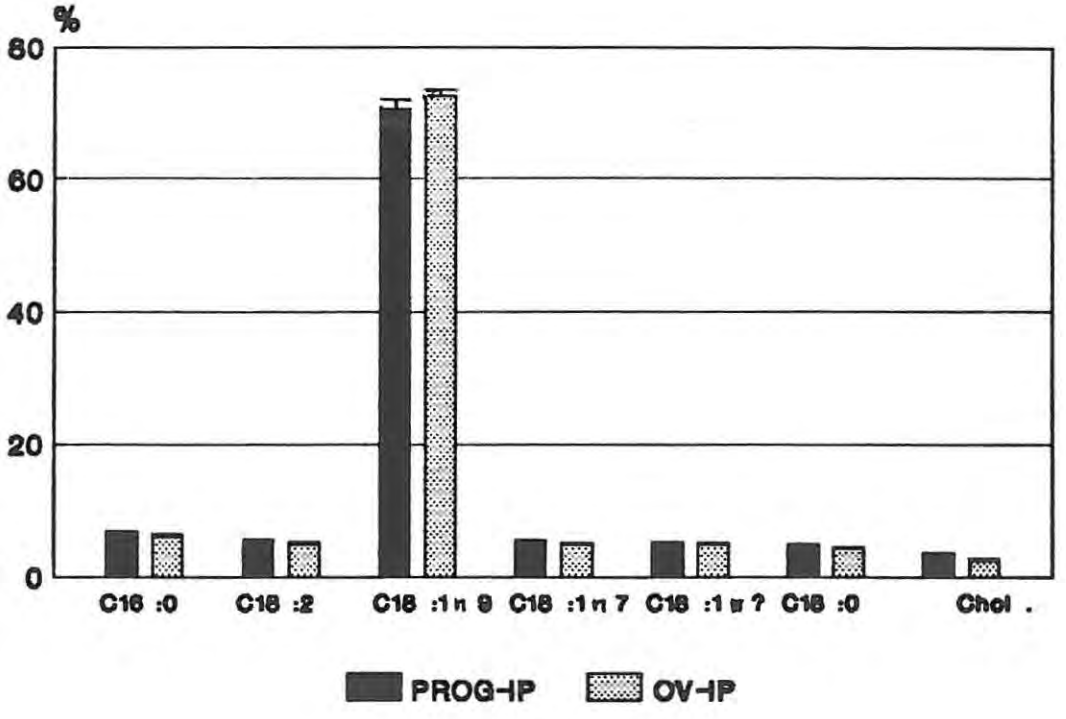


fig V.7 Two-directional development thin-layer chromatograph of fat deposit on the liver of IP (P &OV) treated female rats. This map shows the localization of the spots : 1 = front, 2 = cholesterol esters, 3 = triglycerides, 4 = free fatty acids, 5 = cholesterol, 6 = phospholipids.

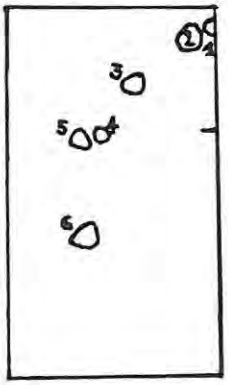
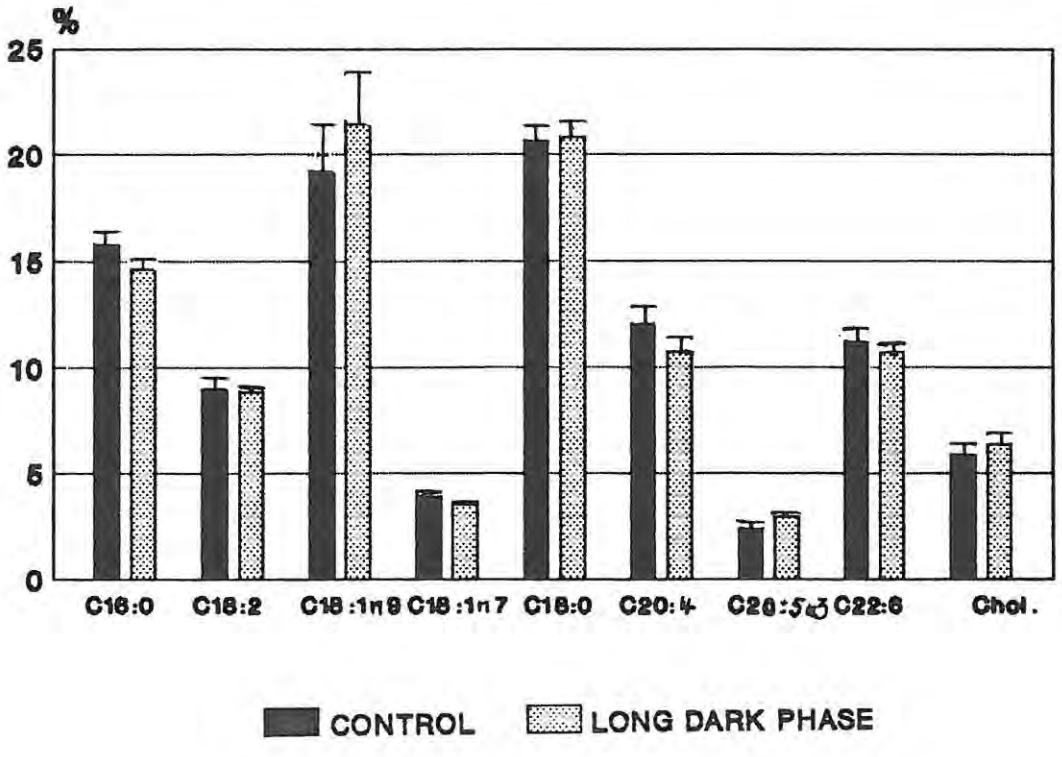


fig V.8 FA profile of rat liver after IP-OV load and long dark period



V.B ELECTRON MICROSCOPY STUDY

V.B.1 INTRODUCTION

SC-P-treatment provokes a small, but significant change in liver total FA distribution. So does phenobarbitone, a drug known to induce hepatic enzymes some of which are important in lipid metabolism (Goldberg et al.) and to induce a prominent proliferation of hepatic smooth endoplasmatic reticulum (SER) (Kanai et al. 1986). There seems to be a close relationship between these two effects as most enzyme activity in the liver is localized in the SER of hepatocytes.

Ho Yuen (1977) states that the synthetic progestin, norethynodrel, has several similarities with phenobarbitone as far as enzyme-induction, SER hypertrophy and glycogen depletion are concerned. A SC treatment of P which induces a change in hepatic total lipid FA profile and a concomitant BW gain, may also alter the ultrastructure of the hepatocyte.

V.B.2 EXPERIMENTAL PROCEDURE

The procedure followed here is the one of Cross (1987). Rats (V.A.1.1) were sacrificed by neck fracture and the liver was removed as soon as possible and placed in cold 5 % phosphate

buffered, glutaraldehyde fixative and some small pieces (1 cub mm) were cut. Those small pieces of liver tissue were placed in specimen tubes and allowed to fix in buffered glutaraldehyde for 12 hours at 4°C. Then they were transfered to 0,1 M phosphate buffer and kept in the refrigerator overnight.

Next, the buffer was decanted off and replaced with fresh buffer and allowed to wash for 10 minutes. Secondary fixation was carried out for 90 min in 1 % osmium tetroxide. This process was followed by two further 10 min washes in phosphate buffer. Dehydration was achieved by passing the tissues through a series of ethanol concentrations (5 min each); 30%, 50%, 70%, 80%, 90%. The tissues were finally dehydrated by immersing them in absolute ethanol twice for 5 min each. The fixation and dehydration were carried out at 4 °C up to the 80% ethanol stage. The dehydration agent, ethanol, is not miscible with the embedding medium, araldite, and consequently a transitional solvent (propylene oxide) was used between the dehydration and embedding stage of this process. So the absolute ethanol was decanted off and the tissues were washed twice in propylene oxide for 15 min. The penetration of the araldite resin was achieved by transferring the tissue from 100% propylene oxide to mixtures; 75:25 propylene oxide : araldite, 50:50 propylene oxide : araldite, 25:75 propylene oxide : araldite and by allowing infiltration for 60 min each time. Finally, the tissue pieces were transferred to pure resin and allowed to stand overnight. The next morning, the resin was allowed to polymerise in an oven at 60°C for 36 hours.

In order to obtain satisfactory ultra-thin sections from the polymerised resin block, it must be first trimmed to the correct

shape : the optimum shape and size of the block face being a trapezium with sides about 0,2 mm long. Sections of approximately 50 nm were cut on an LKB UM3 ultra-microtome, using glass knives. Glass knives were manufactured with a LKB knifemaker. The sections were collected on 300 mesh uncoated copper grids.

Staining was carried out in 5% freshly made aqueous uranyl acetate followed by lead citrate (Reynolds formulation). The grid was inverted and placed on the surface of a drop of uranyl acetate stain and allowed to stain for 30 min. After removing the grid with a pair of forceps, it was washed allowing a stream of distilled water to flow over the surface. The excess water was removed and the grid placed onto a drop of lead citrate stain for 5 min. Again this was followed by the washing procedure with distilled water. Standard precautions were taken to avoid contamination of grids by lead carbonate precipitation during staining.

The sections were viewed in a Hitachi HU II B transmission electron microscope.

In this experiments 5 groups of 4 female rats were used. Two groups were injected SC either with P solution or OV and an other two IP with P solution and OV respectively. Rats injected SC (both with P or with OV) did not have a fat deposit on the liver. The IP treated rats did have such deposits. Besides cutting pieces of liver tissue, some pieces of this deposit were taken as well. The last group of rats were untreated ones.

V.B.3 RESULTS AND DISCUSSION

The morphological structure of the liver of all experimental rats is preserved throughout the experiment; there is no evidence of necrosis, inflammation or fibrosis. Moreover, little or no cytologic changes occur in P (SC and IP injections) treated rats.

An electron micrograph of a section through a hepatic parenchymal cell from untreated animals is shown in fig V.9.

In contrast to norethynodrel (Ho Yuen 1977), progesterone (SC or IP administered) does not provoke an apparent increase in SER. As in untreated (fig V.9) and OV treated (fig V.11) rats, the SER in hepatocytes is localized in small patches, distributed at random throughout the cytoplasm. The RER retains its lamellar appearance and seems to be as abundant in P treated as in OV treated animals.

The mitochondria show no alteration in size or shape and retain the same general distribution throughout the cytoplasm. There is no depletion of liver cell glycogen as observed in electron micrographs of cells from rats treated with norethynodrel (Ho Yuen, 1977) or phenobarbitone. Glycogen occurs in close association with the SER and depletion of glycogen may give the impression that the SER is more compact and more clearly visualized and consequently increased in amount. The electron micrographs of all animals (P-, OV- and un-treated rats) show that glycogen lakes are abundant.

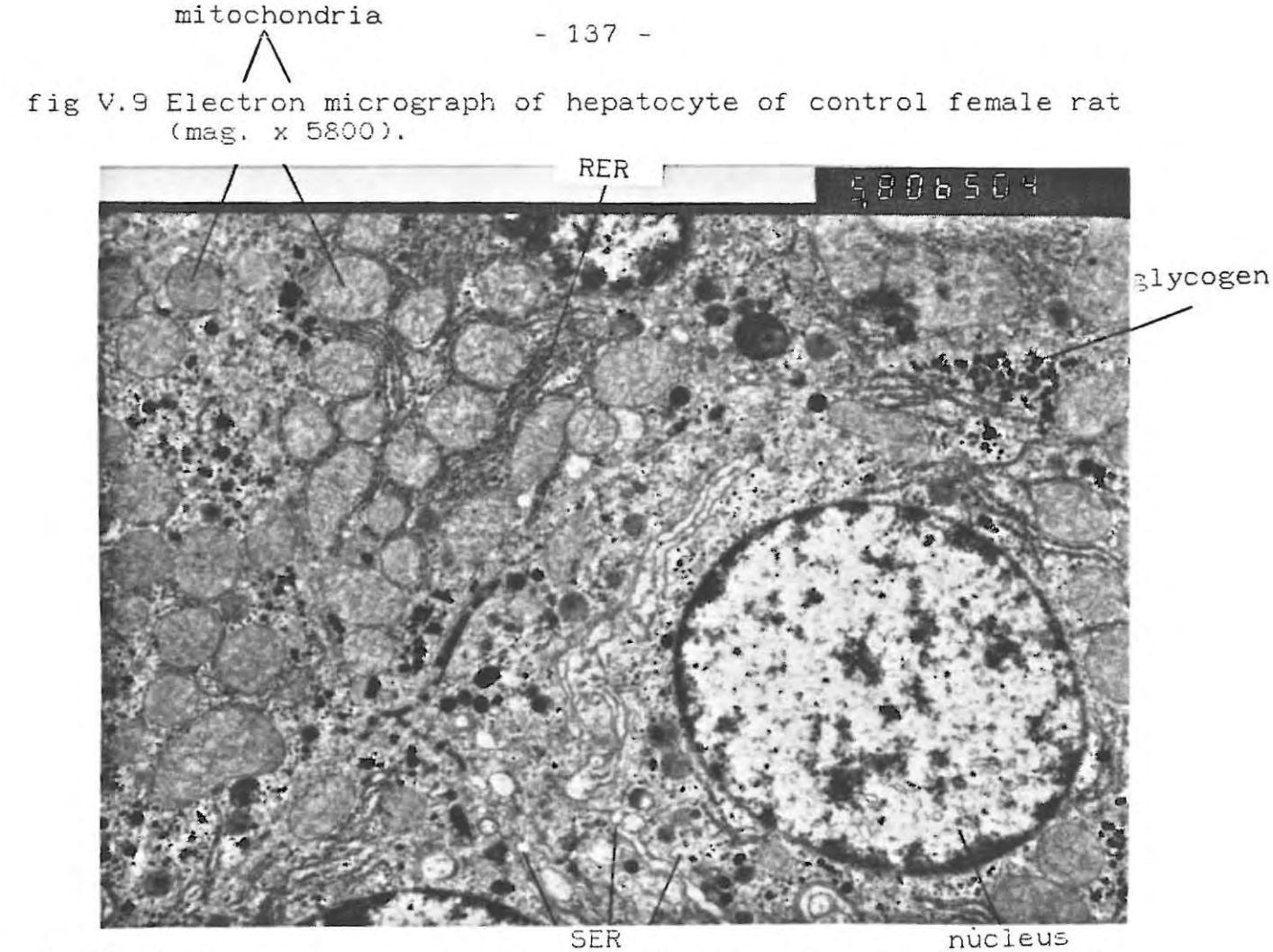


fig V.9 Electron micrograph of hepatocyte of control female rat (mag. x 5800).

fig V.10 Electron micrograph of hepatocyte of control female rat (mag. x 5800).

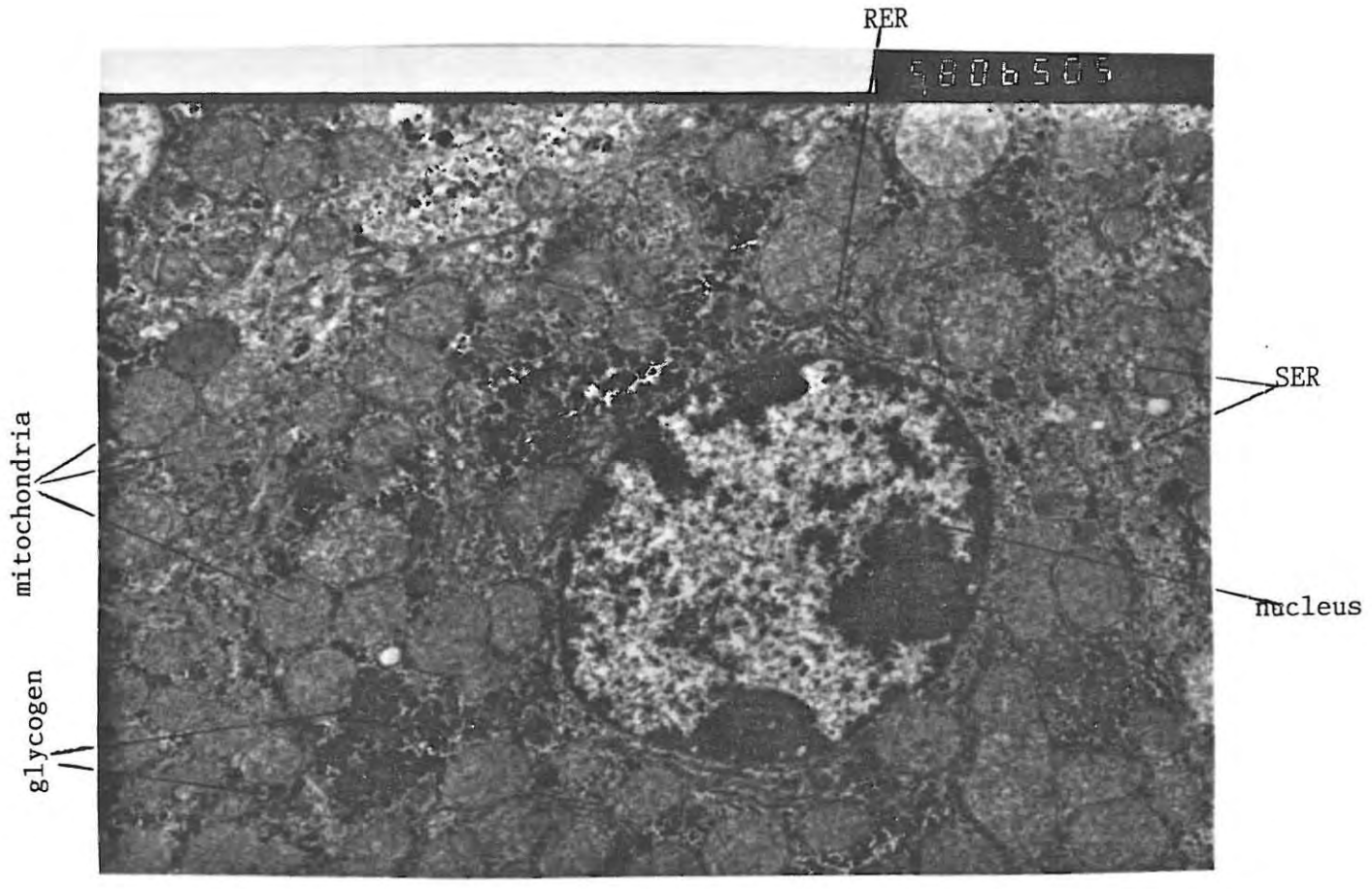


fig V.11 Electron micrograph of rat liver after 2 weeks of daily IP-OV treatment (mag. x 5800).

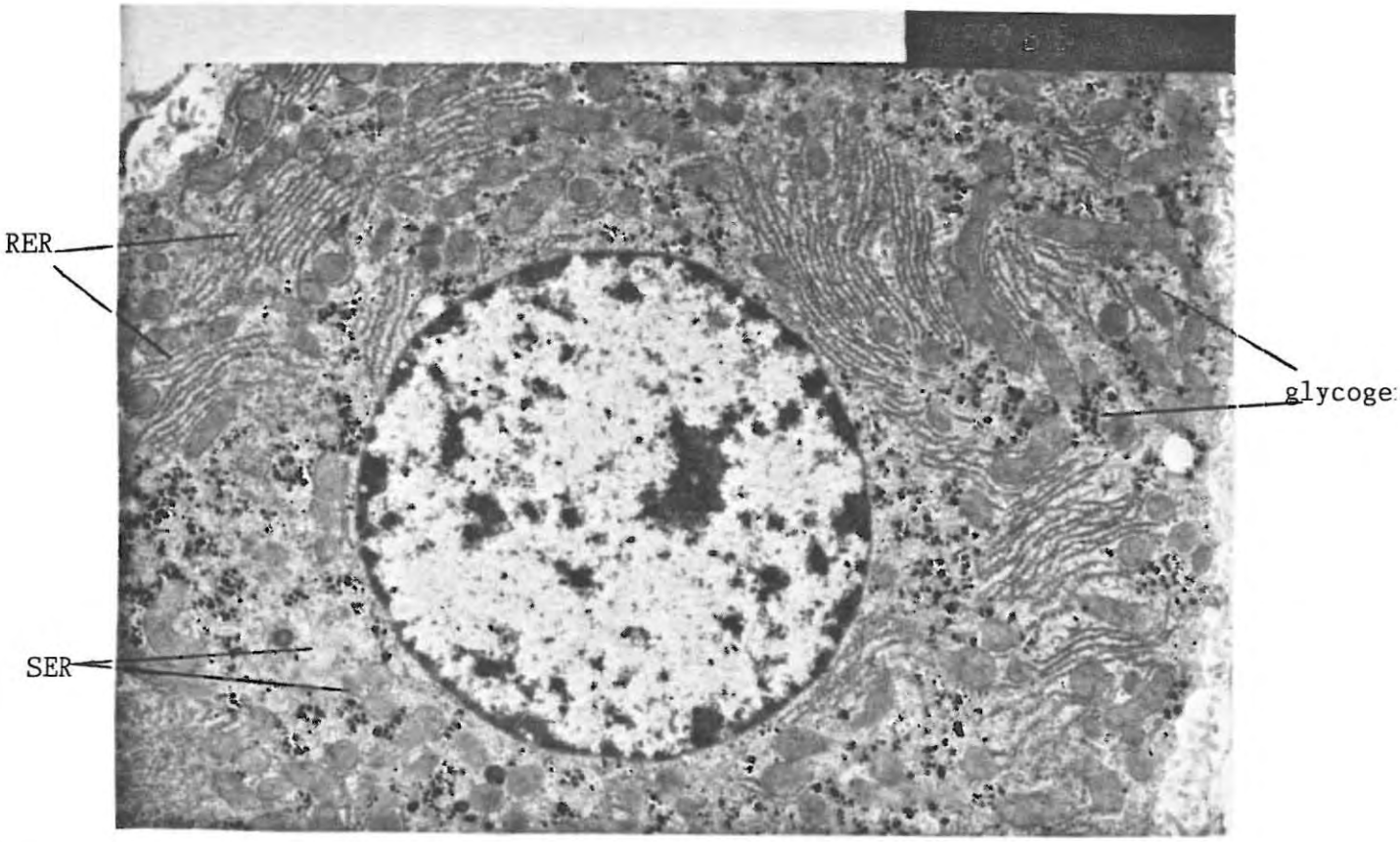


fig V.12 Electron micrograph of rat liver after 2 weeks of daily IP-OV treatment (mag. x 7200).

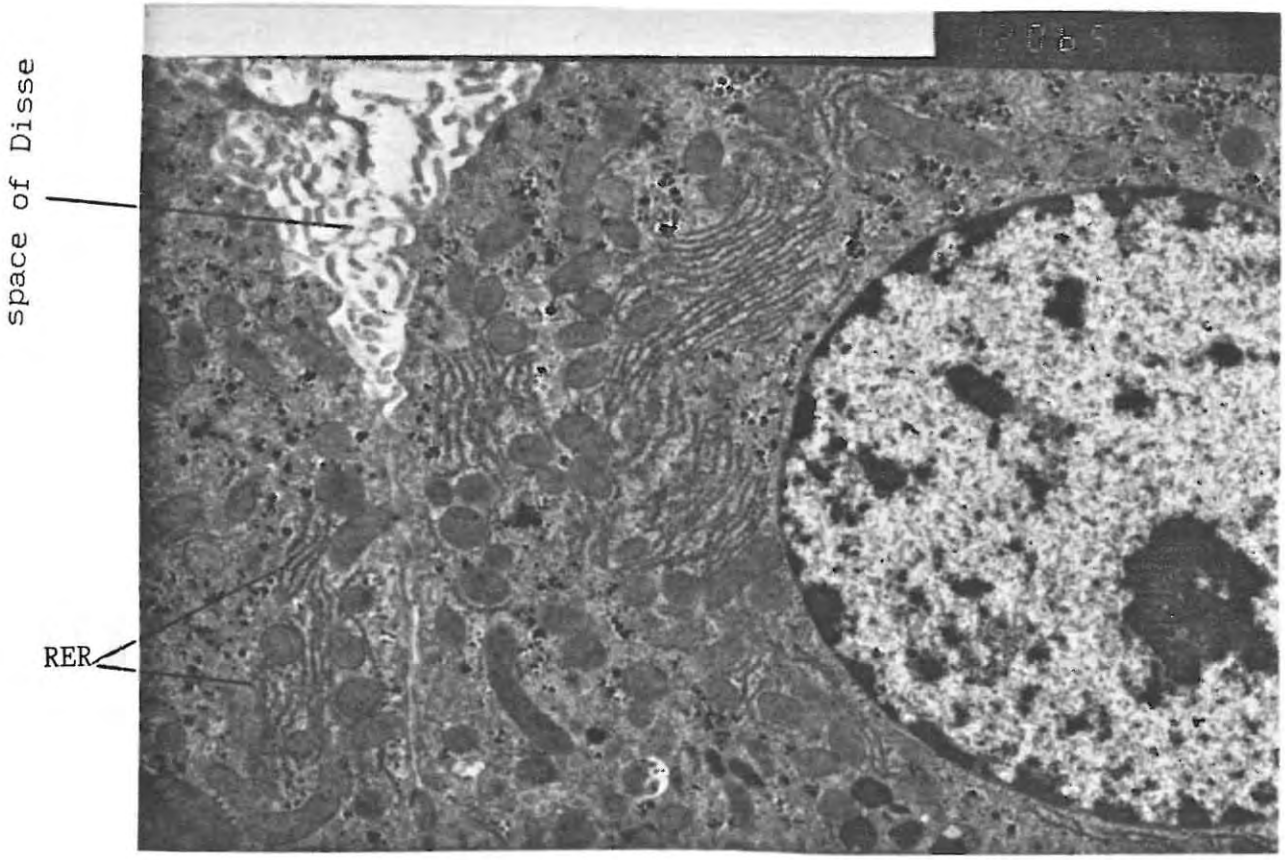


fig V.13 Electron micrograph of rat liver after 2 weeks of daily IP-P treatment (mag. x 5800).

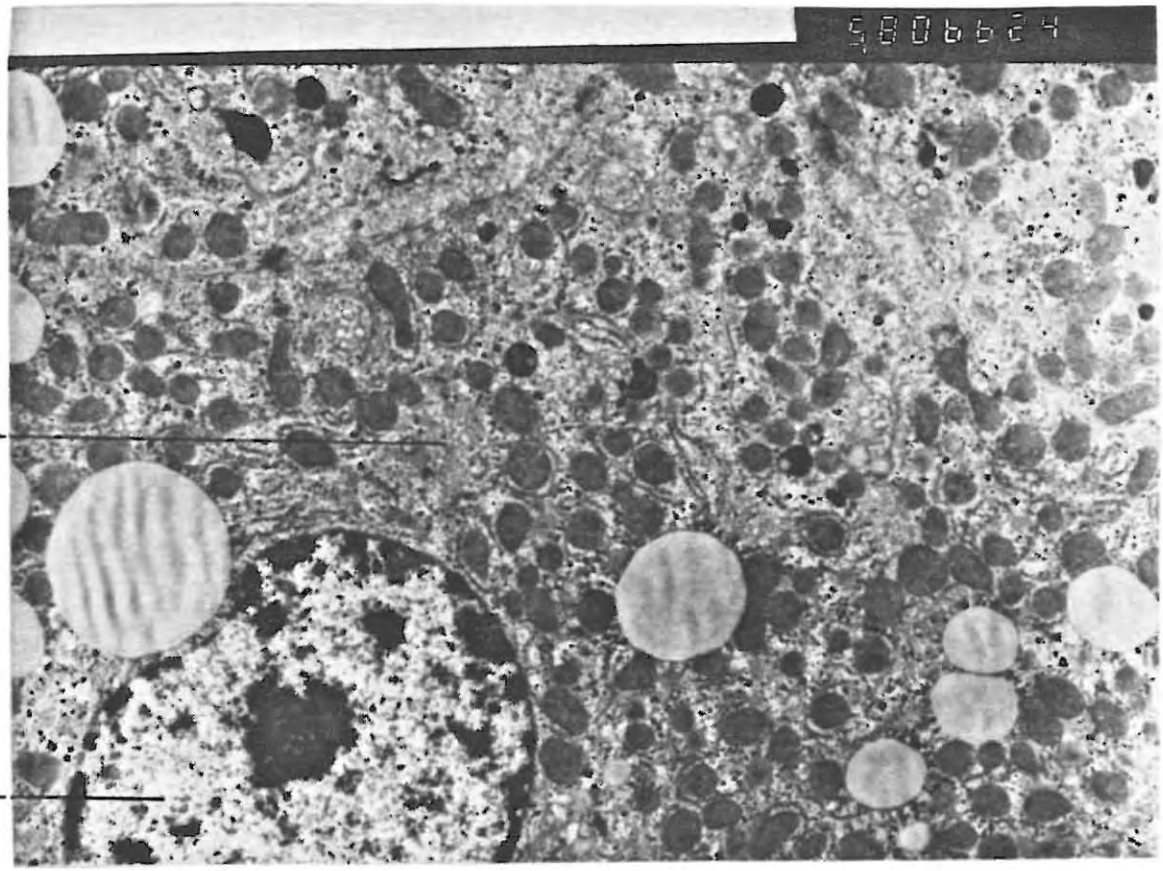


fig V.14 Electron micrograph of rat liver after 2 weeks of daily SC-OV treatment (mag. x 10000).

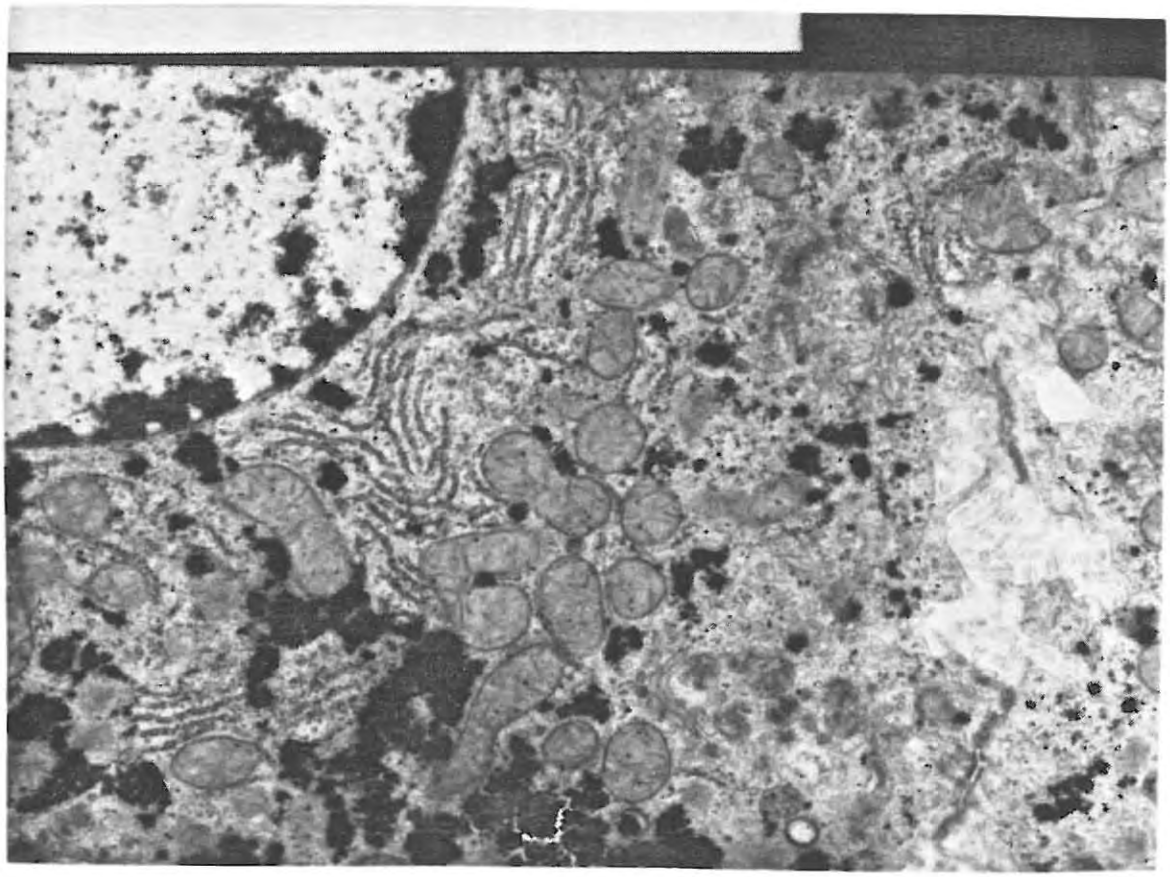


fig V.15 Electron micrograph of rat liver after 2 weeks of daily SC-P treatment (mag. x 14000).

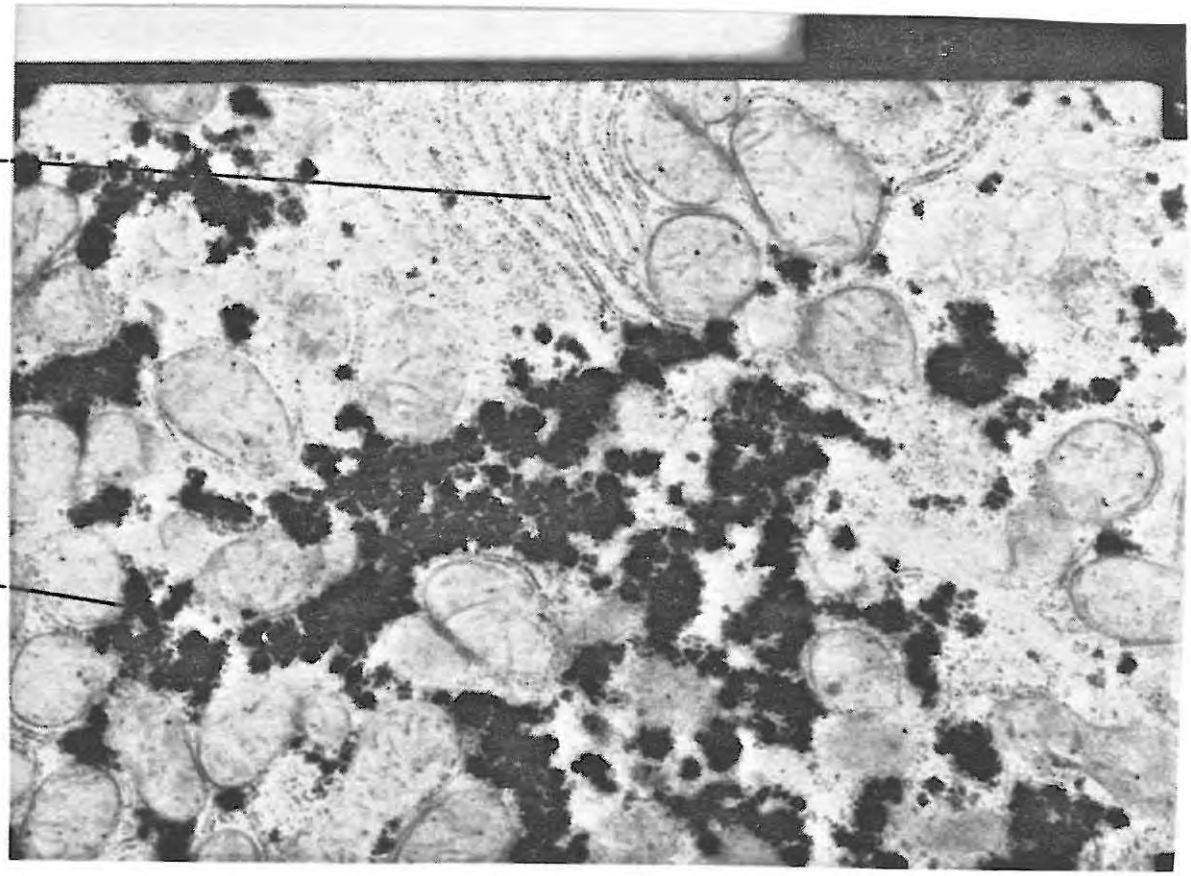


fig V.16 Electron micrograph of rat liver after 2 weeks of daily SC-P treatment (mag. x 10000).

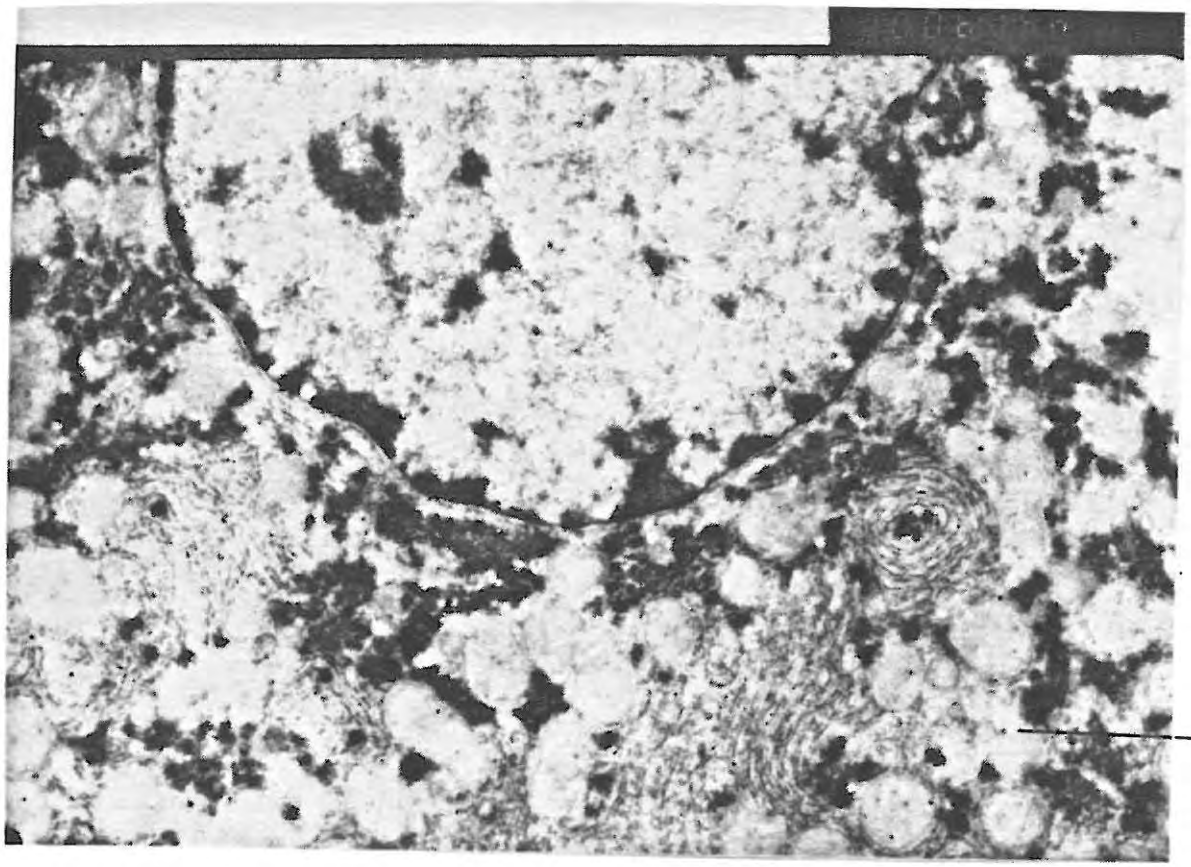
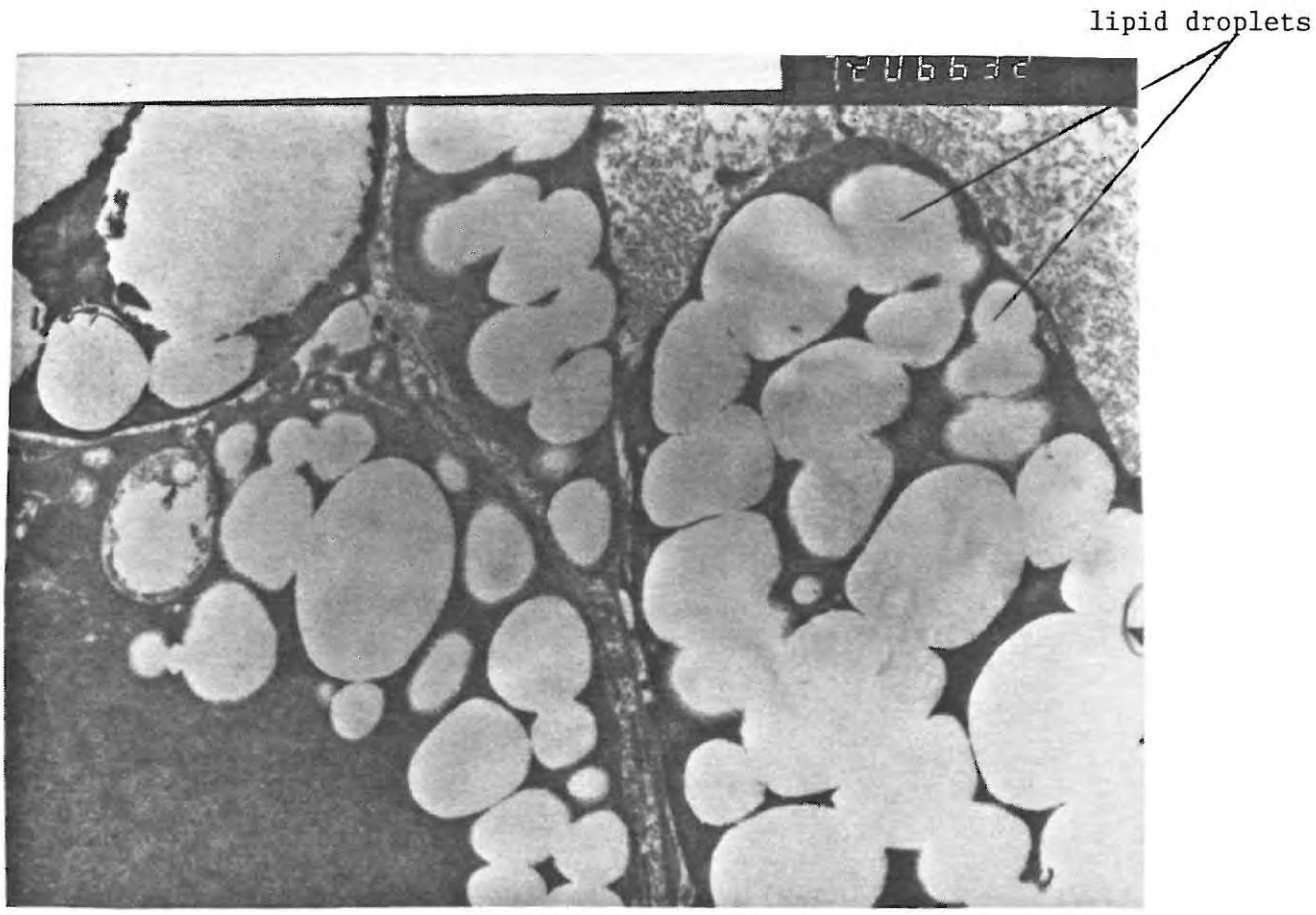


fig V.17 Electron micrograph of fat deposit on liver after 4 weeks of daily IP-OV treatment (mag. x 7200). Note the big lipid droplets, typical for WAT.



SUMMARY & CONCLUSION

CHAPTER I

The BW of most animals seems to be carefully regulated by a variety of physiological and behavioral mechanisms. BW is the result of the energy balance, the balance between energy intake and energy expenditure.
Bray (1989) tries to link the behavioral and physiological factors altering energy balance in his controlled-system approach and in this way to explain the control or adaptation of BW to environmental, physiological, pharmacological, etc. disturbances. This regulated system consists of a controller, located in the brain, a controlled system (which is the energy intake, expenditure and storage), feedback signals informing the controller about the controlled system and finally efferent control mechanisms.

The OH, E and P, are part of those efferent control mechanisms. They are considered as internal signals which animals use in making anticipatory adjustments in behavior and energy metabolism to respond to metabolic challenges such as winter, gestation and lactation.

In adult female rats, withdrawal of OH by ovariectomy induces a hyperphagia and rapid weight gain. The effects of ovariectomy can be reversed by treatment with E alone and P, in the presence of E, is thought to antagonize the effects of E.
OH probably act concurrently in the brain and in peripheral tissues to provoke behavioral (FI and activity) and metabolic (availability of metabolic fuels) alterations, resulting in changes in BW and carcass composition.

CHAPTER II

It can be concluded that the effect of P treatment on the BW of intact female rats depends on the route of administration. Daily subcutaneous P injections provoke an immediate BW gain (which is statistically different from the control level), while the same product, given intraperitoneally, fails to elicit any weight response. It is possible that the weight-increase effect of P depends on a fairly sustained active blood level of the hormone and that an intermittent, fluctuating level, such as may be produced by daily injections at sites giving relatively rapid absorption (IP), is ineffective.
Rats, which have been treated daily IP with the oily P solution or with the OV and which, none the less, do not gain weight, accumulate WAT around and on the liver lobes. Rats injected SC however, do gain weight after P treatment, but do not have this deposit. Again, this shows the importance of the route of administration. It is possible that IP-injected oil is

transported directly to the liver and deposited there while SC OV is metabolized peripherally.

The P-induced BW gain (seen after SC treatment) is not likely to be a serotonin-mediated one, based on an appetite-activating principle. The obesity during SC-P treatment is not accompanied by hyperphagia. Also, those obese rats do not show any change in nutrient selection habits. Thus, there are significant shifts in energy balance and BW, without changes in total caloric- and nutrient intake, indicating the importance of energy expenditure.

CHAPTER III

Energy can be saved by reducing the activity level. Locomotor activity studies show that changes in spontaneous activity make little or no contribution in the P induced energy storage. Consequently, it can be stated that the behaviors, eating and activity are of minor importance in the obesity induced by SC P and the increase in caloric efficiency probably has a metabolic origin.

CHAPTER IV

Measuring the core temperature of both P and OV treated rats could reveal a possible role for thermogenesis in the P induced increase in metabolic efficiency, but no alteration is observed at a depth of 3 cm. This measurement (at a depth of 3 cm) is more likely to

represent surface temperature. Consequently it is concluded that peripherally located BAT thermogenesis is not changed during P treatment. More centrally located thermogenic organs (as the liver) and BAT pads may be more important.

CHAPTER V

Without ruling out possible contributions of other metabolic changes, P induced changes in lipid metabolism seem to be very important in the P induced obesity. Since the liver is a crucial organ in fat metabolism, studies are undertaken to analyse P-induced modifications in hepatic-fatty-acid and cholesterol distribution.

SC P gives a small but significant change in the FA profile, without altering the cholesterol proportion. It is difficult to understand the real meaning of this result, even after comparison with the more obvious change due to phenobarbitone administration, which is known to induce hepatic enzymes some of which are involved in fat metabolism.

Electron microscopy studies examining the ultrastructure of hepatocytes of those obese rats, do not reveal prominent modifications, either in the SER or in the glycogen level.

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