GONADAL HORMONES AND FEEDING BEHAVIOR IN THE MOUSE

by

STEWART ALAN PETERSEN

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SUMMARY

This thesis sets out to examine the interaction between feeding and reproduction in mice. It is suggested that reproductive constraints on feeding may arise from two sources, those involved in the maintenance of tonic sexual dimorphisms in body size, and those arising from the need to integrate sexual and other behaviours during the female's period of sexual receptivity. From a consideration of the literature on rats it is argued that the sexual dimorphism is dually maintained by testosterone, which irreversibly facilitates weight gain in the male, and a reversible effect of oestrogen which 'sets' a lower weight in the female. Various criteria allow a provisional separation of this 'regulatory' effect of oestrogens from its disturbing effects at prooestrus. It is suggested that the observation of feeding patterns will aid in the separation and characterisation of reproductive constraints on feeding, and the processes underlying the generation of feeding patterns are discussed in general terms.

In the experimental sections, the existence of a sexual dimorphism in body size in mice is examined, together with the effects of pre- and post-puberal gonadectomy in both sexes on food intake and body weight. Oestrogen is identified as the active gonadal agent in females by replacement therapy. Having established a basic similarity between the mouse and the more commonly studied rat, the nature of the presumed long and short term oestrogenic effects on feeding is examined by following the intake of two foods, one bland, and one sweet, over the oestrus cycle and after ovariectomy. At the time of sexual receptivity the intake of bland food is reduced, whereas that of the sweet food increases. Towards the end of the oestrus cycle, and after ovariectomy, the intake of /

of both foods rises. It is suggested that the changes at prooestrus represent the operation of a biological mechanism producing changes in feeding akin to those that appear in obese human beings.

The two mechanisms are examined further by automatically following feeding patterns over the oestrus cycle, after ovariectomy, and in males and castrate males. Mice consume their daily intake in 25-30 meals, concentrated mainly in the night, during which time they are organised into feeding 'sessions', in which meals are taken at very short intervals. The changes in the intake of the two foods at prooestrus are a result of alterations of meal size, whereas the changes towards the end of the cycle and after ovariectomy are a consequence of changes in meal frequency. The alterations in meal size are produced by changes in mean bout length within meals, themselves a product of changes in the duration of the initial bouts of the meal, which are a consequence of changes in the rate of initial facilitation of bout length. Males show a reduced meal size on bland food as a consequence of similar changes, which are reversed by castration.

Within feeding sessions meal size is correlated with following inter-meal interval. This correlation is absent at prooestrus. Towards the end of the cycle, and after ovariectomy a given meal size staves off feeding for less time than at the beginning of the cycle, so that feeding sessions contain a greater number of meals.

The changes in meal size, it is argued, are a consequence of the integration of feeding with other behaviours, and together with the loss of correlation between meal size and intermeal interval, allow feeding to be /

be a very flexible activity. This accords very closely with the observed changes in obese humans, in which feeding is often determined by factors apart from metabolic needs, and may reflect the operation of a basic biological mechanism for releasing feeding from such control. The changes after ovariectomy, however are characteristic of what would be expected from a regulatory change in feeding.

It is further hypothesised that the regulatory effects of oestrogens are produced via the ventromedial nucleus of the hypothalamus, whereas the prooestrus changes depend on some other structure. Experiments are performed on mice following goldthioglucose injections to investigate this hypothesis, which is to some extent supported.

In conclusion it is argued that the hypothesis of separable constraints of reproduction on feeding may be supported, and that the long term 'regulatory' influences produce the sexual dimorphism in body size by a direct or indirect action on the ventromedial hypothalamus, whereas the short term 'control' effects may reflect the allocation of varying behavioural priorities, by an action on some other neural structure and a consequent release of feeding from strict metabolic control.

TABLE OF CONTENTS

			Page
CHAPTER ONE:	Introduction		1
	1.1	Why?	1
	1.2	A priori	3
	1.3	Reality	4
	1.4	Mechanism	16
	1.5	Short term changes	20
	1.6	Pregnancy	22
	1.7	Real animals?	22
	1.8	Factors influencing the pattern of food intake	24
	1.9	Regulation	26
	1.10	Control	36
	1.11	Feeding patterns and reproduction ..	41
	1.12	Summary	44
CHAPTER TWO:	Outline of Experiments		47
CHAPTER THREE:	Food intake and gonadal hormones in the mouse		
	3.1	Subjects and Maintenance	52
	3.2	General methods for measuring food intake	53
	3.3	Methods for following the oestrus cycle	54
	3.4	Validation of vaginal smear criteria	57
	3.5	The daily intake of laboratory chow over the oestrus cycle	59
	3.6	Sexual dimorphisms in food intake and body weight for mice	61
	3.7	The intake of two foods over the oestrus cycle of mice	68
	3.8	Ovariectomy and golden nuggets ..	71
	3.9	General Discussion	74

			Page
CHAPTER FOUR:	The automated recording of feeding behaviour		
	4.1	Introduction	77
	4.2	Recording	78
	4.3	The feeding Monitor	81
	4.4	Validation	82
	4.5	The organisation of the observations on feeding patterns	84
	4.6	Instrument reliability and the vetting of records	87
CHAPTER FIVE:	General characteristics of the temporal Pattern of feeding in the mouse		
	5.1	Temporal Distribution	91
	5.2	Survivorship analyses	92
	5.3	Selection of variables	94
	5.4	Individual Differences	97
	5.5	Discussion	97
CHAPTER SIX:	The circadian pattern of feeding in the mouse		
	6.1	Introduction	101
	6.2	Total time spent feeding in light and dark	101
	6.3	Variations in some parameters of feeding over the days of the cycle ..	106
	6.4	Summary	113
CHAPTER SEVEN:	Changes in meal pattern over the nights of the cycle.		
	7.1	Introduction	115
	7.2	Changes in meal size over the nights of the cycle	115
	7.3	Changes in meal frequency over the nights of the cycle	116
	7.4	Discussion	117
CHAPTER EIGHT:	Details of the meal size changes		
	8.1	Introduction	119
	8.2	The control of feeding bouts and interbout intervals	119
	8.3	The control of meal taking	122
	8.4	Meal /	

			Page
	8.4	Meal maintenance over the oestrus cycle	128
	8.5	Summary	134
CHAPTER NINE:		Details of the changes in meal frequency	
	9.1	Introduction	136
	9.2	Nature of the changes in IMI	136
	9.3	Correlational analysis between meal size and IMI	139
	9.4	Changes at Prooestrus	141
	9.5	Discussion	142
CHAPTER TEN:		The feeding patterns of males and castrates of both sexes	
	10.1	Males	146
	10.2	Ovariectomised females	150
	10.3	The castrate male	152
	10.4	Discussion: sex hormones and feeding patterns	153
CHAPTER ELEVEN:		Oestrogens and the Ventromedial hypothalamus	
	11.1	Why the Ventromedial Hypothalamus? ..	161
	11.2	Sex and the VMH	162
	11.3	The feeding of males and females after VMH lesions	163
	11.4	Sex and GTG	170
	11.5	Goldthioglucose	171
CHAPTER TWELVE:		Aspects of the feeding behaviour of males and females after GTG	
	12.1	Introduction	177
	12.2	Daily food intake of males and females after GTG	177
	12.3	The endocrine status of animals after GTG	181
	12.4	Combined ovariectomy and GTG	185
	12.5	The effect of oestrogens on food intake in GTG mice	188
	12.6	Intermittant oestrogens and food intake in GTG females	189
	12.7	Intake /	

Page

12.7 Intake of sweet foods after GTG and oestrogens 192

12.8 Feeding patterns of males and females after GTG 194

12.9 Summary 199

CHAPTER THIRTEEN: Food intake and the oestrus cycle in hamsters

13.1 Introduction 201

13.2 Methods 202

13.3 Results 203

13.4 Discussion 204

CHAPTER FOURTEEN: General Summary 206

REFERENCES 219

ACKNOWLEDGEMENTS 232

CHAPTER ONE

I N T R O D U C T I O N

Life is order, and Mother Nature an anarchist. Individual living beings depend for their survival upon the integrity of a large number of interacting, highly complex, systems and sub-systems, each of which is continuously exposed to destructive influences, and a fault in any one of which may destroy the whole. The ultimately inevitable collapse may be postponed for short lifespans by a continuous infusion of energy and raw materials, but no individuals are immortal, so that if species are to survive, individuals must reproduce new members to replace them when they have gone.

Species and self preservation, which are not always fully compatible, must be finely balanced in successful organisms, and it is a particular facet of that balance which will be examined in this thesis.

Feeding is the primary activity by which animals obtain the energy they need to survive, and in the following chapters the potential and actual modulations of feeding by the processes of reproduction will be examined in a common laboratory rodent, the mouse.

1.2 A priori:

Nature discourages absolute uniformity, and in order to survive in a changing world species must retain the capacity for evolution. Chance variations in traits occur relatively infrequently, and the probability of favourable combinations occuring in a single individual is low. Accordingly, in each generation, in many species the process of sexual reproduction produces a mixing of the genetic characteristics of two

individuals /

individuals in varying combinations to produce genetically novel young.

In species where the new embryos require a minimum of parental care they may be produced in very large numbers, and left to the rigours of selection during development. In the more common case, however, to stand any chance of survival at all, they must receive a relatively long period of nurturance and care post conception, and at least one member of the procreating pair must be adapted for this purpose. In mammals this is exclusively the female pre-natally, and most frequently after birth as well.

Females therefore produce, relatively infrequently, relatively few ova (only as many as they can potentially support, allowing for wastage.) Ova are precious, so that in a successful species there will be adaptions to ensure that the maximum proportion are fertilised at the optimum time for their development. This requires the female to show changes indicating that the best time for copulation is at hand, and both sexes to show behavioural adaptations ensuring its rapid completion.

Females place an upper limit upon the breeding capacity of a population by producing the less frequent gamete. This has two consequences, one, the maximum proportion of females should breed, and the other that the production of maladapted young is a very expensive mistake. At least a part of the process of selection should therefore take place before conception. Selection amongst females will reduce total reproductive capacity, whereas selection of males will not, as each can potentially sire many females. The selection

process/

process is therefore well served if males compete, directly or indirectly for the opportunity to procreate, and it might be expected that they will show physiological and behavioural adaptations to that end. (Trivers (1968))

At least two interfaces where reproduction may interact with other behavioural and physiological systems may therefore be identified. On the one hand, the female must ensure the fertilisation of her ovae at the right time, and on the other males will be adapted to compete for the opportunity to perform that service.

Both may potentially interact with feeding, and not necessarily in the same way.

1.3 Reality

It might be predicted, therefore that feeding and reproduction will interact in certain ways, so now we need to determine the details of that interaction in the species under consideration.

As will be noted below, the mouse will be used as subject in the experimental sections of the thesis. There is, however a dearth of information concerning feeding over different reproductive states in the mouse, so that in this introduction hopefully analogous data from rats will be discussed. (The validity of the analogy will be experimentally established in the following chapters)

There is also, however, a shortage of information upon aspects of feeding other than simple measures of intake over relatively long (usually 24 hour) periods of time. For this reason the following

sections /

sections will be concerned exclusively with food intake, rather than feeding behaviour.

Thereafter, an attempt will be made to discuss in very general terms the basics of the mechanisms underlying feeding patterns in rats, as a prelude to the use of feeding pattern data to assist in the characterisation of reproductive constraints upon feeding.

Adult male rats are larger than females and they eat more in both absolute terms and in relation to their body weight. (see later refs.)

In addition to eating chronically less than males females show predictable, relatively short term variations in food intake which males do not. At the time of sexual receptivity, food intake is depressed (Slonaker (1925), Brobeck et al (1946), Kennedy (1963), Tartellin and Gorski (1971), Ter Haar (1972), Drewitt (1973)). If, however, the animal becomes pregnant there is a rapid rise in food intake, which is independent of the presence of a foetus, as it also occurs in pseudopregnant animals (Tartellin and Gorski (1971)) and which is maintained throughout pregnancy and lactation.

We therefore have to find mechanism(s) to account for both the chronic standing differences between males and females, and the periodic changes within females.

1.4 Mechanism

Gonadal hormones are ultimately responsible for the majority of sex-specific morphological, physiological and behavioural changes,

so /

so that it is to direct or indirect hormonal influences that we will look to determine the mechanism of modulation of feeding associated with reproductive phenomena.

Soon after the differentiation of the foetal gonads secretions from the testis begin to influence the course of development. By the time of birth the roots of the majority of sex-specific anatomical characteristics are laid. The same hormones acting around birth further establish the bases of masculinity in adulthood by acting, amongst others, upon the systems that will eventually control the secretion of pituitary trophic hormones ('organisational' effects). At puberty the gonadal hormones begin to be secreted in appreciable amounts in both sexes, and their 'activational' effects lead to the full appearance of sex-specific characteristics, and allow the processes of reproduction to proceed.

It is necessary, when considering the influences of hormones upon feeding therefore, to also divide these into potential 'organisational' and 'activational' effects. (Young (1964))

These questions may be examined by considering data from Bell and Zucker (1971). The authors report data for both food intake and body weight of male and female rats gonadectomised just after birth, as compared to their intact controls. The food intake and body weights of males, females, castrate males, and ovariectomised females are identical at all ages before puberty. After puberty the food intake and body weight of intact males rises more rapidly than any other group for 30 - 40 days, whereas that of the intact females rises most slowly. The neonatally castrate animals of both sexes show identical

rises /

rises of food intake and body weight intermediate between these two extremes.

This data would indicate that influences of gonadal hormones acting after birth are entirely responsible for the observed sexual dimorphism in body size in rats. Furthermore, the hormones of both gonads are involved, as intact females weigh less than their castrate counterparts, whereas intact males weigh more.

Bell and Zucker also injected neonatally gonadectomised animals with various hormones on the fifth day of life. Neither testosterone nor oestrogen had any effect on body weight at 100 days in either sex, so that neonatal hormones do not have any direct effect on adult weight.

If, however, the same hormones are given to the castrates after the expected time of 'puberty', both have marked effects on food intake and body weight. Testosterone facilitates weight gain, and an increase in food intake, whereas oestrogens produce a marked fall in both measures. The perinatal administration of neither testosterone or oestrogen has any influence upon the activational effects of either hormone on food intake, but testosterone given on the fifth day of life does facilitate the later weight promoting effects of the same hormone in adulthood.

From this single set of results, therefore, it is possible to identify which hormones are responsible for the effects of reproduction upon feeding over the long term, and to give some characteristics of their effects.

In /

In the female there are no effects of gonadal hormones on feeding or body weight prior to puberty. The presence of oestrogens after that time leads to a lower rate of gain of food intake and body weight. In the male, the organisational effects of testosterone are confined to an influence, presumably metabolic, upon later weight gain. Activational influence lead to a pronounced stimulation of both food intake and body weight gain after puberty.

The adult sexual dimorphism in body size is therefore made up of two components, one acting in either sex. Males are larger than their early castrate counterparts by an action of testosterone, and females are smaller by an action of oestrogen.

The system may be further examined by considering the effects of castration and ovariectomy in adulthood, after these changes have had time to appear. The castration of adult male rats produces a slight fall in food intake, and a reduction in the rate of body weight gain. The bulk of weight gained prior to the operation is, however, retained. (Kakowleski, Cox, Valenstein (1968)) If, however, females are ovariectomised in adulthood, there is a marked rise in both food intake and body weight gain for some time after the operation (Kakowleski et al (1968), Bell and Zucker (1971), Tartellin and Gorski (1971, 1973), Drewitt (1973), Dubuc (1974)). Dubuc (1974) reports that animals ovariectomised in adulthood show a change in body weight so as to bring them into line with the body weight of animals ovariectomised earlier in their life.

There /

There seems, therefore to be a distinction between the effects of oestrogen and testosterone on body weight in the rat. The male hormone, if present promotes weight gain and food intake. If it is removed, the weight is more or less retained. Oestrogen, on the other hand appears to have a reversible effect, if present food intake and body weight fall, if absent they rise.

It is the comparison between these effects of oestrogens and the changes in feeding over the oestrus cycle which will be the major concern of the rest of this thesis.

Several authors have suggested that oestrogens affect feeding and body weight by an action upon a body weight 'set point' (Kennedy (1969) and others see below). At any given level, body weight, it is argued, must be defended against fluctations associated with mismatches between supply and demand for nutrients. If this control is by some sort of feedback process then a set point must be defined as the optimal weight from which the control system produces an error signal to modulate food intake. (Milsum (1966)) Oestrogens rather than influencing food intake and body weight directly act so as to lower this set point.

The hypothesis has been supported by a number of observations. Firstly, the effects of ovariectomy after puberty. Soon after the operation food intake rises, and continues to rise for some time (e.g. Bell and Zucker (1971), Tartellin and Gorski (1971, 1973), Drewitt (1973)). Thereafter, food consumption falls, and the body weight, which rises following the increased food intake, begins to more or less stabilise at a new, elevated level. This is a response

very /

very characteristic of a feedback control system whose set point has been suddenly increased (McFarland (1972), Milsum (1966)).

Oestrogen replacement therapy given to adults gonadectomised after puberty will reduce food intake and body weight, but the fall in these two measures is not sustained, after an initial rapid phase it tails off, and the animals stabilise at a new, lower, body weight, (Wade (1975) Dubuc (1974)). Dubuc (1974), using oestrogen replacement over long periods from an implanted slow release capsule, observed that animals ovariectomised, and simultaneously implanted kept their body weights similar to intact controls for up to 12 weeks, but as soon as the implant was removed there was a period of rapid weight gain (greater than that in unimplanted animals immediately after ovariectomy), until the animals reached the weight of the unimplanted ovariectomised group.

Implants releasing the same dose produced different rates of fall of intake depending on whether they were placed immediately after ovariectomy (no change), or some weeks later. The rate of fall was proportional to the discrepancy between the body weights of the ovariectomised, unimplanted animals and the oestrogen treated group. These changes are exactly what would be expected if some sort of 'set point' change is produced.

Furthermore, it has been shown (Tartellin and Gorski (1971), Zucker (1972), Mook et al (1972)) that oestrogen treatment of ov-x animals, even at very high doses, reduces post-operative weight only to a level comparable with that of intact controls, and never lower. Also, if ovariectomy is coupled with a technique that raises body weight, such as a high fat diet, or reduced litter size (Tartellin and Gorski(1973),

Zucker /

Zucker (1972), Redick et al (1973), Kennedy (1969)), the effectiveness of oestrogen in reducing body weight is potentiated, and the effects of ovariectomy reduced.

If ovariectomy is coupled with some other technique tending to depress body weight (e.g adrenalectomy - Redick (1973)), there is no gain in weight.

These data taken together, it has been argued provide strong evidence that oestrogens in the long term do not influence food intake directly, but via an action upon the regulatory processes.

There are, however, one or two points to be taken into consideration. Firstly 'set point' changes, even in animals subjected to no external manipulation, for reasons other than the effects of gonadal hormones, such as age. The body weights of both intact and neonatally castrate females increase steadily after puberty for at least 40 days, but there is no simple constant relationship between them, The absolute and relative magnitude of the discrepancy in body weight between the groups increases steadily from 50 to 90 days of age (Bell and Zucker (1971) Swanson (1963)). There is no evidence, so far as one knows to suggest that oestrogen titres change systematically in this way after puberty, so that a mechanism has to be found for a constant hormonal stimulus to produce a changing effect. This is implicit in the data reported by Wade (1975) and Dubuc (1974). Both authors illustrate that, when oestrogen replacement therapy is given to ovariectomised animals some time after the operation, they return, not to their pre-operation weight, but to the weight of intact controls which have been allowed to gain weight over the period during which the group was ovariectomised.

Similarily,/

Similarily, when a longer term picture is considered it can be noted that the apparently stable body weight following ovariectomy is in fact rising at a rate comparable to that of the neonatally ovariectomised animal of the same age. It would appear that the effects of ovariectomy and replacement are, rather than a simple change of 'set point', a 'change of gear', from a growth pattern typical of the intact female, to one typical of the castrate, and vice versa. In this case, it is not simply body weight that is set, but a programmed series of changes over weeks and months.

This brings up another essential question, just what is 'set'?, once it is noted that the effect of oestrogens in the blood stream is to produce a change from one pattern of growth to another, then we must question the assertion that body fat is the only relevant parameter of body weight involved, a point implicit in most previous discussions (e.g. Wade (1972)).

This may be examined by a consideration of the effects of ovariectomy, at various ages, upon the body composition of female rats.

Stotsenberg (1913) was one of the first to record that female animals gain weight after ovariectomy, which he attributed to fat deposition. Soon after this time, however, Mottram (1920) asserted "There is no evidence of a diminution of respiratory exchange, or of a tendency to the deposition of fat in ovariectomised animals" Slonaker (1930) noted an increase in weight, and without supporting evidence, asserted that this was due to fat.

Evans /

Evans (1931), however, found that animals ovariectomised at various ages, from just prepuberally to 100 days of age, did not get fat, they grew. Reed et al (1934) looked particularily at changes in body fat after post puberal ovariectomy, and found that operated animals had no more fat in their bodies (percentage wet weight) than controls. These authors state quite unequivocally that the greater weight was due to a co-ordinated increase in all bodily constituents, although there is a degree of reorganisation of fat. Intact females store relatively more fat in the genital fat depot than males, and after ovariectomy the female adopts the male pattern of storage.

Freudenberger and Billeter, Freudenberger and Hashimoto, Freudenberger and Howard (1935, 1937, 1938) present a series of studies with animals ovariectomised just pre-puberally, and sacrificed at various later times. They report increases in both body weight and body stature, with an increase in 'integument' weight that may well be due to the reorganisation of fat along the lines of Reed (1934).

Holt (1936), using Wistar rats, found increased growth in his animals ovariectomised at 33, 59, or 73 days of age. Operated females had longer bony structures such as the tibia. The author states repeatedly and emphatically that there are no increases in body fat in any of the groups. Tang (1944) reported further quantitative data on ovariectomy, finding again no increase in body fat. Bogart (1944) ovariectomised animals just before puberty, and found no changes in body fat at sacrifice fifty days later. Similar results were obtained by Nyda (1948). Grunt (1964) noted that, in his neonatally gonadectomised animals the increase in body mass was due to increased growth, including

in /

in this case skeletal development. More recently Beatty, Powley and Keesey (1970), using animals ovariectomised just post puberally, found again some redistribution of fat, but no increases. In one of the latest publications in this field (Leshner and Collier (1973)) Sprague Dawley rats were ovariectomised just after puberty and either fed a prepared diet or allowed to self select from protein and carbohydrate fractions. Ovariectomised females eating the prepared diet did indeed show an increase of body fat up to the level of intact males, but if they were allowed to self select they confined their increased consumption almost exclusively to the protein fraction, and showed no increases in body fat content, merely increases in total mass. The proportion of protein selected in the diet was the same as intact males.

On balance, therefore, it would appear that, in animals ovariectomised soon after puberty at least, the increases in body mass that are observed are a product of a co-ordinated change of all bodily constituents rather than a simple increase in body fat. If indeed a set point argument applies, then the entity which is set is rather more complex than body weight, or even a pattern of weight gain, rather more it is a co-ordinated pattern of growth.

There is therefore, for both sexes a co-ordinated, intermediate, 'castrate' pattern of growth, which in the presence of oestrogen is changed to a slower, but still co-ordinated pattern typical of the intact cycling female. This change, in the early postpuberal life at least, is reversible. Testosterone produces an essentially irreversible increase in growth if present after puberty.

It /

It would be of considerable interest to discover whether, in chronically ovariectomised animals treated with oestrogen there is a similarily co-ordinated fall in body weight.

In the normally growing female, however, a mechanism must be found to account for the co-ordinated effect of oestrogens upon growth. There are well known effects of oestrogens upon 'growth', which is unfortunately often taken as increase in body mass. Zondec (1935) noted that oestrogens stunt growth markedly. Meites (1949) observed, that doses of 3 micro-gram per day reduced growth in his rats. The relationship of this dose to physiological levels of the hormone is not examined. A similar point applies to Sullivan and Smith (1957) who demonstrated that male rats given oestradiol at 1, 10, or 100 micro-gram daily showed a lower body weight than controls, and that this fall in body weight could be duplicated in intact animals by pair feeding to the injected group. The implication from this finding, which has been accepted by many later authors is that oestrogens produce their effects on body weight by a direct action upon food intake. (This is equally implicit in the 'set point' hypothesis, as it is assumed that the hormone acts upon the neural mechanism of regulation).

It is of course equally possible that the hormone directly reduce the metabolic demand for nutrient, so that intake falls into line with demand in the usual way.

It is possible that oestrogens may directly inhibit both the secretion and action of pituitary growth hormone. Ovariectomy increases pituitary growth hormone (Jones (1965)), which is reduced by oestrogen (Birge(1966)) /

(Birge (1966)). In mice at least growth hormone titres are higher in males than females (Sinha et al (1973)). Schwartz (1969) has demonstrated changes in calcium and nitrogen balance in human acromegalics after oestrogen therapy consistent with a hypothesis of reduced growth hormone activity. Josimovich (1968) has also demonstrated that oestrogen can directly antagonise tibeal epiphyseal growth induced in rats by exogenous growth hormone. This antagonism is not produced by food restriction alone.

The only point one would like to make from this discussion is that oestrogens could potentially exert a co-ordinated effect on growth, both by a direct action on the neural 'set point', and by influencing the animal's metabolism.

The pattern of growth also, however, influences the effects of oestrogens. Prior to puberty neither food intake nor body weight gain may be affected by injections of oestrogens (Zucker (1972)). At this time lesions of the ventromedial nucleus of the hypothalamus are similarily without affect on body weight and food intake, although they do influence bodily stature, with a stunting of growth (Han (1967), Bernardis and Skelton (1965)). Before puberty, therefore it is argued that the VMH is inactivated, possibly by an inhibition from growth hormone, and that this inactivation is responsible for the ineffectiveness of oestrogens in reducing body weight (Zucker (1972)). The onset of puberty appears to be tied to the achievement of a criterial body weight (Donovan (1969)), and Zucker (1972) has shown that the ability of oestrogens to reduce food intake (although not body weight) does indeed appear when the animal achieves a particular weight, rather than chronological age. Data from Zondec (1935), however, would

suggest /

suggest that oestrogens prior to puberty may, whilst not decreasing body weight gain, stunt the linear growth of the animal, so that it may be in this case that the co-ordination of oestrogenic action is disrupted.

Either way, whilst the evidence is not conclusive for oestrogens exerting a metabolic effect by any particular route, the fact that in the normal animal such a co-ordinated change appears seems reasonably certain. In the long term, therefore the presence of oestrogens in the female specifies a typically female pattern of growth, which is replaced reversibly by one typical of the castrate if they are removed.

1.5

So far only the long term effects of oestrogens have been considered. Unlike the male, however, the endocrine state of the female is by no means constant over time, and shows regular fluctuations associated with the oestrus cycle.

Oestrogen titres may be measuredin intact cycling rats (Brown Grant et al (1970)) "It is found that plasma oestradiol levels are raised by a factor of 10 or so, from 4 to 40 pgm/ml, for only a few hours, beginning during the night of dioestrus preceding the day of pro-oestrus, and ending on the evening of pro-oestrus. Titres remain at or near the lower level for the rest of the cycle."

The effects of oestrogens must therefore be accounted by this pattern of secretion. A few hours after the oestrogen peak the animal becomes sexually receptive, and shows a reduction of food intake (Ter Haar (1972), Drewitt (1973)). Both authors agreed that the oestrus depression of feeding is first manifest as an amelioration of the peak of food intake associated with the onset of darkness, and remains for twelve hours or so. Thereafter, according to Drewitt there is a 'rebound' /

'rebound' hyperphagia, daytime intake being increased by some 150% for the first few hours.

The oestrus depression of feeding can therefore be characterised as rapid in onset and offset, and followed immediately by a compensatory overeating. This must be compared to the very much slower changes following ovariectomy and replacement therapy. From Tartellin and Gorski (1971) it can be seen that the postoperative depression of food intake associated presumably with operative trauma lasts at most two days, thereafter, food intake increases slowly over the next fifteen days or so. If the relationship between food intake and body weight is calculated it can be seen that the degree of hyperphagia, that is to say the amount by which the animal's intake exceeds that of an equivalent weight intact animal increases up until the 12th day. The correcting response of the control system is therefore relatively slow to appear after ovariectomy. A similar pattern is observed with oestrogen replacement. The effects of daily doses of oestrogen cumulate over the first few days, and take time to disappear once the injections have stopped. (Zucker (1972))

A more natural replacement therapy for the female is injections of oestrogen every fourth day. This regime was used by Tartellin and Gorski (1973). They gave 3 micro-gram once every fourth day for a total of 25 days. The day following the injection there is a rapid fall in food intake, which recovers gradually over the next three days. It never, however, regains control levels, and falls again at the next injection, followed by a similar partial recovery. In subsequent cycles, however, recovery to the new, lower level is complete within three days, so that the animal simply oscillates about

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a mean level lower than that of the untreated animal. Exactly the same series of changes is apparent in the first few cycles following the end of a pseudopregnancy (Tartellin and Gorski (1971)), in many ways a more natural situation (see below).

The point is that the rapid oscillations of feeding which follow the oestrogen peaks closely in time are superimposed upon a slower change in the mean level of intake. This is of course exactly the case in the female for the first twenty or so days after puberty, when she is cycling, but the mean level of intake is increasing along the typical female growth curve.

Perhaps, therefore, the set point changes described in the previous section impose a second order modulation upon the basic cyclicity of the female's food intake, so that it 'sets' the point about which the oscillations occur.

The data of Tartellin and Gorski (1973), discussed above suggests that the longer term effects may be produced by the persistence of the effects of an oestrogen peak over several days. Drewitt (1973), however challenges this hypothesis by demonstrating that very low doses of oestrogen, below the minimum dose for vaginal cornification, or the induction of sexual receptivity, can reduce food intake in ovariectomised animals. From this he argues that the tonic, dioestrus component of oestrogenic effect is mediated directly by the low levels of hormone circulating at that time.

It remains clear, however, that the effects of a single injection can persist, so that in the intact cycling animal, Drewitt's effect

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can only account for a part of the dioestrus restraint on eating. It would seem, therefore, that, in the long term the effects of oestrogens may be effectively cumulated, so that the female growth pattern is specified by a 'smoothed' version of the hormonal changes.

If this is the case, then the short term changes in feeding over the oestrus cycle must operate by a different mechanism, as they follow closely the oestrogen peak, and appear if any thing to be a disturbing influence upon the set point, as they are followed immediately by a compensatory rise.

There is another problem. As noted above, one of the supports for the argument that oestrogens influence set point is that oestrogens can never depress set point weight below that of the intact female. Now, during the oestrus cycle, body weight does not deviate by more than 3% (Tartelling and Gorski (1971)), so that the intact, cycling female could never be more than 3% above the minimum intact set weight. Now the sort of fall of intake at pro-oestrus is typical of a massive lowering of set point, which, by one of the basic tenets of the hypothesis cannot occur. If the theory is correct, therefore, it must be that the lowering of food intake at pro-oestrus reflects a direct action upon food intake, rather than an indirect influence through a body weight set point.

It could be suggested, therefore, that the depression of feeding at the time of sexual receptivity appears to be of a different type to the changes in the long term.

1.6 Pregnancy /

1.6 Pregnancy

The intact, regularly cycling female is a very rare bird indeed in the wild. A very high proportion of oestrus cycles will lead to pregnancy in a normal population of wild females.

Vaginal stimulation as a result of copulation induces the female to form corpora lutea and begin secreting progesterone in appreciable quantities. (Hashimoto et al (1968)). Oestrogen titres remain low (apart from a small peak on the fourth day) until towards the end of pregnancy (Shaikh and Abraham (1969), Horikoshi and Weist (1971)), whereas progesterone titres steadily rise.

Tartellin and Gorski (1971) measured the food intake of animals made spontaneously pseudopregnant by vaginal smearing. In this case there are no confounding factors arising from the nutrient demand of a foetus. Food intake and body weight increase steadily over the first fifteen days or so, and thereafter, as the pseudopregnancy ends food intake falls again, and for the following two oestrus cycles is depressed below the pre-pregnant mean. (see previous section) Similar data are reported by Kennedy and Mitra (1963). Superficially, the changes in food intake during a pseudopregnancy are similar to those after ovariectomy, but Beaton et al (1954) have demonstrated that females accumulate fat at this time. Some weight is, however, permanently retained following a pseudopregnancy, so that the animal remains a little heavier than a control with no breeding experience. The amount that is gained is almost exactly the same as that which would appear in an equivalent time of ovariectomy. Leshner, Siegel and Collier (1972) also report that pseudopregnant animals, like those after ovariectomy, select an increased proportion of protein in their diet.

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The effects of progesterone in these changes has been the subject of some debate. Progesterone treatment of intact female rats (Roberts, Kenny and Mook (1972), Galletti and Klopper (1964), Hervey and Hervey (1961)) will elevate food intake, whereas the same treatment in ovariectomised animals has no effect (Galetti and Klopper (1964), Hervey and Hervey (1966), Zucker (1969), Roberts, Kenny and Mook (1972)). This has led some authors to suggest that progesterone exerts its effects in intact animals by blocking the actions of oestrogen, thus producing the superficial similarity with the effects of ovariectomy. (Wade and Zucker (1970), Wade (1975), Ross and Zucker (1974)).

Recently, however, Roberts et al (1972), and Stern Porterfield and Krupa (1974) have challenged this interpretation. Stern et al noted that if rats are simultaneously ovariectomised and adrenalectomised, then given 5.0 mg per day progesterone, they show increased food intake and weight gain in a pattern consistent with an alteration of body weight set point. Roberts noted that treatments which eliminate the effects of ovariectomy (see previous section) restore responsiveness to progesterone, so that maybe, it was argued, progesterone can only elevate body weight set point if it is not already elevated by some other treatment.

Wade (1975) has countered these arguments. He points out that the 5.0 mg dose used in the studies is very large and unphysiological, being 20 times the dose needed to facilitate lordosis in the oestrogen primed animal. In his experiments it is apparent that lower doses were without effect on ov-x/ad-x rats. He also demonstrated, in ovariectomised animals an inhibiting effect of progesterone upon weight loss induced by exogenous oestrogens, at much lower doses, nearer the physiological. From this he argues that the ov-x/ad-x effect is pharmacological, and that physiologically progesterone produces its effects by an inhibition of oestrogen, a process not uncommon in other systems.

1.7 Real/

1.7 Real Animals

Perhaps now we can consider in general terms how the various changes in feeding across the sexes fit into the pattern described in the first section.

Consider first the male. He must compete, and may do this by obtaining and holding territory. This process of competition is probably facilitated by his size, together with other behavioural changes primarily the product of testosterone.

Now the female. In the wild her life will consist of short periods of sexual activity, followed by relatively very long periods of pregnancy and lactation. Only for a very short proportion of her life will she approximate to the intact cycling female in the laboratory, so that her physiology will be oestrogen dominated for only a few days every couple of months.

It has already been noted that the pregnant female grows as if she were ovariectomised, so that a wild animal should grow almost as a castrate for most of her life if she continues to breed. This partially resolves the question as to why a sexual dimorphism should be maintained dually by influences on both male and female, as in the normal case, the female influence may not operate so that the male component will be the major factor. This, however, raises a further question as to the function of an oestrogenic effect which does not often normally appear.

Unreproductive females are useless to the population, and will prove nothing but a drag on useful resources. It may be, therefore, that the oestrogenic mechanism evolved to effectively hold unmated animals

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'in reserve'. Hence the reversibility of the effect. The young, unmated female, will grow very slowly, using minimal resources until she starts to breed. Alternatively, if females continue to cycle during the winter, when most male rodents are non-reproductive, then their growth rate will be lower, and their demands on resources less.

What about the short term changes in feeding? It has been argued above that they operate by a different mechanism to the longer term changes, and if this is the case it is likely that they have a different function. They may be considered together with other behavioural changes at the time of sexual receptivity. Intact, cycling females are much more active in a variety of situations than males. Activity shows a marked peak at the time of sexual receptivity. (Finger (1969)). Wade (1972) considers running an 'energy expending' activity, classing it with nest building as an opposite, energy conserving activity. When, the set ppint is lowered, it is argued, the animal voluntarily exercises to expend energy. The idea of a rat doing physical jerks in order to slim does not, I am afraid, appeal. For one thing it is enormously wasteful of resources.

Look again at an active animal with a low tendency to establish a nest, surely this is the laboratory expression of what would be in the wild an animal on the move. The female if she is on heat, must find a male, even if she attracts males to her by scent, she must still go out to meet them. It may be that the female rat travels away from her home range when she is on heat, in search of new genes. At this time feeding is of low priority, if food is available, then it

may /

may be eaten, but the animal cannot afford to waste a great deal of time eating, not so much because she spends a great deal of time copulating, but because she is engaged on the serious business of selecting a good strong mate.

If this is the case, then it may be possible to distinguish the characteristics of the long and short term influences of oestrogens on feeding by a consideration of the changes in feeding patterns at various times. The following sections therefore are an attempt to determine some of the factors influencing the pattern of food intake, so that those due to reproductive modulation may be identified and characterised.

1.8 Factors affecting the pattern of food intake

All animals must eat, and the earth provides a massive variety of potential foods, which vary considerably in the amount of nutrient they provide for a given bulk. Foods such as grass require a great deal of ingestion and digestion before they supply the nutritional requirements of an animal, whereas relatively small quantities of meat will provide the same nourishment.

The type of food which the animal is adapted to eat will largely define the temporal pattern of its consumption, by virtue of specifying both the total bulk necessary, and the properties of the digestive apparatus. Once this basic pattern is defined, then it may be further modulated by other influences. One of the major such influences is that in both the long and short term nutrient supply and demand should be optimally balanced. Other modulations will arise from the animal's need to be other than a simple eating machine, it must /

must protect itself against attack, sleep, and reproduce, all of which will place constraints on one another and feeding.

The basic influence of the type of food selected may be briefly examined by considering animals at either end of the spectrum of possible foods. Consider grass, animals need to eat a lot of it to keep alive, and once eaten it requires a complex process of digestion, Many grazing animals must spend a very high proportion of their time feeding or ruminating (e.g. Bost (1972) for sheep) The potential for modulation in this pattern is rather small, the animals cannot afford not to eat for very long, and cannot increase the time spent eating much above its usual high level.

At the other extreme is a large predator, such as lion. Lion food comes in large, highly nutritious packets, each of which supplies, once ingested the animal's needs for days. Lion therefore kill only infrequently (Ewer (1968)). The potential for the modulation of feeding in this pattern is great, and the animal may adjust both the frequency and size of its kills to suit its nutrient requirements, and co-ordinate feeding with other activities.

Here we are interested in rats. Rat food comes in reasonably small, moderately nutritious packets, fairly abundant in a good environment. Rats need not, therefore spend a very high proportion of their time feeding. In this case they could either adopt a little and often pattern, or choose to package their daily intake into a number of 'meals'. The little and often paradigm, would require the animal to be engaged in feeding activity, and that is to say away from its nest, and especially vulnerable to predation, for a considerable proportion of its time. For this reason, and possibly also to facilitate

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the efficient operation of its gut, it would be more sense to package feeding into meals. This is in fact the case. There is a wealth of evidence to suggest that normal rats consume their daily ration in a relatively small number of meals (8-10) (LeMagnen and Devos (1970), and many other refs).

This pattern of relatively frequent meals is especially subject to modulation by outside influences, and the first to be considered is the need to optimally match supply and demand.

1.9 Regulation

The problems of regulation will be specified by the temporal pattern of demand. Demand for nutrient may be conveniently divided into three categories. In the first case there is the background continuous demand arising from the normal operation of metabolism, the basal metabolic rate. In the second there are sudden exercise. Thirdly there are those maintainence and growth functions which can conveniently be switched on when nutrient is readily available, and off when it is not. There is an unavoidable lag between the ingestion of food and its availability for metabolic use, so therefore, to cater for the second category, and as an insurance against interruption of supply animals must carry buffer stores of energy providing substances. Very short term needs may be supplied by mobilising muscular and hepatic glycogen but any other disturbances must involve the use of longer term, high capacity stores in adipose tissue.

When we consider regulation as a problem of optimising supply and demand, then there are at least two potential classes of regulation.

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If the animal is free to feed at any time, then so long as it eats in meals the precise timing or size of meals may be used to either compensate or anticipate slight mismatches in supply and demand. As soon, however, as other restrictions apply to when the animal may feed, then the demands upon the buffer stores become much greater, and some sort of regulatory process, acting over several meals which will not necessarily operate by the same route as the short term system, will come into effect.

Firstly, the ideal case of the animal free feeding in a perfect environment, needing only to maintain a short term current balance of supply and demand.

It is first necessary to discover just what happens in the period following the ingestion of a meal. During the ninety minutes of absorption the stomach releases the semi-digested food slowly into the absorptive section of gut, so that nutrient enters the hepatic portal blood stream at a relatively slow rate over a considerable period following the meal. (Steffens (1969))

This food must be used both for feeding metabolism, and replenishing supplies. Its distribution and use are under the control of a number of endocrine systems. Pancreatic islet tissue secretes insulin, which acts upon the membranes of many cells, increasing the availability of glucose for oxidation, and by virtue of the endogenous regulatory mechanisms within the cell effectively producing a swop from the insulin absent mode of primarily metabolising fatty acids, to a virtually exclusive use of glucose. At the same time adipose tissue stores energy from the meal.

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As soon as absorption is complete, insulin levels fall (Steffens (1969) for the rat). Under these conditions the majority of tissues metabolise fatty acids mobilised from the adipose tissue stores, leaving the relatively small stocks of glucose for those tissues, such as the nervous system, which can use no other fuel. The intracellular mechanism controlling this switch is probably essentially a flip-flop.

There is therefore, at the end of absorption of a meal a crucial decision point. The gut is empty, and the general metabolic picture has changed from one using glucose, to the use of fatty acids. The contents of a meal will therefore support the animals total metabolism (including storage) for a time after a meal which is dependent upon its rate of release from the alimentary canal. If more energy is absorped than immediately used, then the buffer stores will be incremented, and this addition may offer further support to metabolism either immediately, or at some later date.

Modulation may be imposed upon this process by altering meal size, meal frequency or both. Maximum limits are imposed on the size and frequency of meals by the operation of the gut. It can only take a certain amount of food at one go and requires time to deal with it before the animal may feed again. These limits appear, however, to be well outside the normal, free-feeding operating conditions. It is well known that the rat can if necessary take the whole of its daily diet in a single meal, or two meals in a very short space of time. (Wiepkema (1972)) Maximum permissible conditions may not, however, be optimum.

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In real animals both meal size and frequency are subject to small, essentially random fluctuations, and these represent the major problem for the mechanisms of hour to hour regulation. The essential question is which is compensatory ? Does meal size compensate variations in meal frequency or vice versa?

The problem has been investigated by a number of authors, principally by the calculation of correlations between the sizes of meals and intermeal intervals juxtaposed in time. If meal size compensates IMI (intermeal interval), assuming that the variations are essentially random, and unpredictable, then after a long or short IMI meal size should be altered to suit the deficit or surplus of energy so produced. If, however, IMI compensates meal size, then the animal if it takes a large meal will follow with a long IMI.

A priori the latter would seem the simpler system. The first requires that the animal be able to accurately monitor in the short term very small deficits and surpluses in energy supply, and use that information to alter the control processes determining meal size. The latter requires only that the animal be able to detect the point at which the products of the meal are no longer able to supply demands.

Le Magnen and Tallon (1966) reported the presence of a strong correlation between meal size and following inter-meal interval, but no correlation between meal size and preceding IMI in their rats. Balagura and Coscina (1968) obtained no correlations between preceding IMI and meal size, but could find significant post-prandial (meal size and following IMI correlations in three out of seven rats. Thomas and Mayer (1968) were able to find strong post-prandial correlations in

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their rats fed on a liquid diet. Le Magnen and Devos (1970) report further data for animals feeding on powdered food. In this case meals were separated into day and night blocks, in both cases a strong post-prandial correlation was observed in the raw data, although the slope of regression varies between night and day. Snowdon (1969) was also able to find a post-prandial correlation with liquid diets. Panksepp (1973) challenges the existence of the correlation by pointing to a statistical artefact in the original Le Magnen and Tallon paper, which arises from the use of mean pooled data. This argument cannot, however, apply to Le Magnen and Devos (1970), Balagura and Coscina (1968), or any of the studies using liquid diets, all of which used raw data for the calculation of correlations. Panksepp was unable to find post prandial correlations in all but one of his control animals, although they did appear after chronic treatment with insulin. He goes on the calculate ratios between meal sizes and IMIs in various combinations, and shows that these vary systematically over the day. If only a limited portion of the night is used strong post-prandial correlations are, however, observed. This is of course exactly the point of the Le Magnen and Devos (1970) separation of night and day records.

Perhaps, just for the moment it may be concluded that within a relative steady state of feeding, the post-prandial correlation between meal size and IMI holds, that is to say following IMI compensates variations in meal size. This fundamental relationship is clearly, however modulated by other factors.

There are two candidates for the signal to resume feeding in this case. Either the animal eats when its gut reaches some criterial level of depletion, or alternatively it eats when some product of the meal /

meal is used up. If total usage (i.e. including movement to stores) is considered then these two signals will occur at more or less the same time, as once the absorption of a meal is complete the nutrient is always either used or stored.

Snowdon (1970) argues that, during the night at least the feeding of the rat is largely peripherally controlled by the operation of its alimentary canal, so that it eats again when the contents of the stomach reach a criterial minimum. The interpretation, he argues, is suggested by the discovery (Snowdon and Epstein (1970)) that vagotomised animals eat small frequent meals, rather the opposite pattern to that which might be expected if the vagus was providing information affecting the termination of meals, suggesting that possibly it was involved in their initiation. A number of experiments were undertaken to determine the role of the stomach in determining meal taking. Firstly the stomach contents of intact animals were removed soon after the ingestion of a meal of liquid diet, and the subsequent time of resumption of feeding noted. Secondly, the rate of gastric emptying was measured and correlated with feeding behaviour, and thirdly animals were given gastric loads. It was discovered that the animal eats much sooner after the removal of its gastric contents than it would if left alone, and that if gastric emptying is measured the animal eats at a time very well correlated with 90% clearance of the previous meal. Gastric loading with water suppressed feeding for a while, but a greater suppression was associated with gastric loading of liquid diet, and in both cases the suppression of feeding correlated well with the measure gastric emptying time (which varies with the energy load of the meal).

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From this data it is argued that the hour to hour control of feeding is by a purely peripheral mechanism, modulated via alterations in meal size by other mechanisms ensuring regulation in the long term.

Provided, however, that the animals rate of usage of energy is less than the rate of supply from absorption, then a portion of the meal will enter storage, and if the IMI is terminated at the end of absorption a surplus will have accumulated. The rate of supply is dependent on the calorie load of the meal, that is to say the meal size, and according to Toates and Booth (1974) (quoting from Hopkins (1966)) bears a square root relationship to the calorie load. Provided, therefore that the animal's meal size exceeds a minimum necessary to provide a rate of supply able to maintain metabolism, if feeding continues according to this paradigm a progressive surplus will be run up. Booth (1972), showed,however that gastric loading with glucose after a meal will prolong 'satiety' following it, so that over the hours after the intubation there is an accurate reduction in calorie intake to allow for the gastric load. This is produced by both reducing meal size and prolonging inter-meal interval over more than one meal. There are, therefore regulatory influences which may act over the relatively long term.

The observed circadian rhythm in feeding activity is an example and will be the first major modulating influence upon feeding to be considered here. Rodents are vulnerable when feeding, and are consequently primarily nocturnal feeders, when the risk is lowest. Some 85% of the rats daily intake is consumed in the hours of darkness (Le Magnen and Devos (1970)). Rather more than 15% of the animal's need for energy appears during the day, so that at this time it must

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be partially living upon reserves. Le Magnen and Devos show that the day deficit is made up by a net lipolysis, and that during the night there is a net lipogenesis. The short term mechanisms discussed above are never, therefore, without modulation from at least circadian influences.

Associated with the circadian rhythmicity there are characteristic changes in feeding pattern, which was the point of Panksepp's (1973) objections. Night time feeding is composed of relatively large meals separated by relatively short intervals, whereas during the day relatively small meals are separated by long intervals. External, long term modulating influences affect both meal size and IMI systematically. (Le Magnen and Devos (1970))

During the day the observed IMI is longer than the presumed gastric emptying time for the small meals. (Although gastric emptying time may vary over the day (Toates and Booth (1974))). If an anti-lipolytic drug is given (propanolol-Le Magnen and Devos (1970), insulin-Panksepp (1973)), the daytime intake is increased by a fall in IMI, implying that daytime lipolysis is responsible for the alteration of the slope of regression between meal size and IMI which is observed. During the night 24% of the ingested food is converted into fat, at this time it would appear that the animal is eating again as soon as absorption from the meal is complete, the excess absorped material being stored. The long term, highly predictable, alterations in energy balance over the day are therefore dealt with rather differently to the hour to hour maintenance of a running balance, and indeed interfere with that process in such a way that the animal is always running a controlled energy surplus or deficit.

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Consider inter-meal interval over these conditions. A given meal will provide the metabolic systems with an input of energy at a rate related to its size. Immediately after a meal blood insulin levels are high (Steffens (1969)), and most tissues will metabolise glucose for their basic needs and the increase in metabolic rate consequent upon the meal. The remainder will enter storage. So long as insulin levels remain high fat mobilisation will be at a minimum. At the end of absorption the first decision point is reached. The animal must decide whether to eat or to support metabolism for some length of time upon reserves. During the day this occurs, and it is of interest to discover the reason for the precise time of the subsequent meal. The post absorptive animal is in an effectively steady state. Le Magnen and Devos demonstrate that meal size and following IMI are still correlated under these conditions, so that some correlate of the preceding meal must be involved, despite the fact that the animal uses more energy in that time than the meal has provided. This can be explained in two ways. Either the time of resumption of feeding is determined by some correlate of the meal other than simply its total energy load, or alternatively lipolysis is controlled to effectively 'top up' the supplies from the meal to a given extent.

Le Magnen and Devos argue that the essential cue involved is the usage of the glucose load of the meal, so that, metabolism is supported by lipolysis until the animal has exhausted the increment to its glycogen stores presumably by supplying its nervous system. The second decision point in this case reflects the point at which the endocrine system would have to initiate gluconeogenesis in earnest.

Toates /

Toates and Booth (1974)present a computer model using the other alternative. They suppose that during the day a net lipolysis tops up the energy supply rate from the meal, and thus effectively prolongs the period during which it can supply metabolism.

In the former case there are effectively two decision points. Either the animal eats when the meal has been absorped, or at some later time possibly specified by glucose availability. If the first decision point is used, then so long as the meal size exceeds a minimum, there will be a net lipogenesis whose magnitude will be determined by the meal size (for a constant other metabolic rate). If the latter is used there will be a net lipolysis, whose magnitude again depends upon the meal size (and possibly glucose load).

In the latter case lipogenesis is turned on during the night, and presumably extracts a fixed proportion of the absorbed meal, leaving the rest to support metabolism (exactly how it is ensured that this will be sufficient is uncertain). During the day lipolysis is turned on. There is here a slight problem, however. It is uncertain that in the insulin dominated animal soon after a meal whether lipolysis could occur. Provided that at least some point after the meal the rate of energy supply from the meal exceeds the metabolic needs, i.e. unless metabolism is carefully controlled to mop up any excess then lipogenesis must occur, and in any case the controlled lipolysis, if it is to bolster the energy supply cannot simply occur at a constant rate, it must change according to the time elapsed since the meal. This requires a complex control process. Similarily it is apparent that after night meals the rate of lipogenesis will vary with time, falling to zero at the end of absorption of the meal, that is to say the /

the time of resumption of feeding. Simply to effectively add a fixed amount of energy availability to the general pool by transfer to and from fat would therefore be a very complex process.

After periods of deprivation meal size is increased (e.g. Levitsky (1970)) with little change in IMI. In this case also, therefore, a relatively long term energy demand (that is to say spanning at least several meals) is dealt with by a co-ordinated change in meal size and IMI (by the normal correlational relationship IMI should fully compensate the increase in meal size, but it does not, so that despite some change in IMI, the mechanisms controlling the time of resumption of feeding must have changed).

In summary, therefore, short term, unpredictable fluctuations in meal size appear to be compensated by changes in following IMI, so that the animal eats again when some product of a meal has dispersed. Predictable, long term changes in energy balance, possibly reflecting a significant departure from a 'set' weight, are corrected by co-ordinated changes of both meal size and IMI, as is the case, for example with the circadian changes.

Feeding may possibly be considered, therefore, as the product of the interaction of nested systems, so that the basic short term mechanism, which maintains a current balance is further modulated by long term influences.

1.10 Control

So far it has been assumed that once some sort of system produces the /

the command 'eat', then the execution of that command is trivially simple. This may not be the case. It is at this level that feeding will be integrated with a great variety of modulating influences. An observed change in feeding may be the product of alterations in these 'control' functions, and indeed in studies of human obesity an inability or unwillingness to perform them correctly and appropriately is thought by some to be of primary importance (Schacter (1972), Bruch (1973)).

The problem in animal terms is to discover by which mechanisms behavioural transitions are produced, and in this case transitions to and from feeding.

Feeding in the rodent is a reasonably well defined activity, which for purposes of argument will be taken as unitary, so that the question reduces to the investigations of transitions to and from a single behavioural category. Feeding could therefore be regarded as an output state of a multistable control system some way up the hierarchy of systems and sub-systems that produce the particular sequence of muscle movements involved in any particular occurence of the activity concerned.

If such a system is to function effectively then it must have some sort of stability. If it is assumed that transitions are the product of influences arising from internal and external stimuli in variable combination, then the system is continuously exposed to stimuli tending it towards one or more activities other than the one in which it is engaged, and unless these are resisted, the animal's behaviour will bounce about like a ball on a pin table.

The majority of activities are bouted, that is to say once they have started, they tend to continue for some time, and feeding is no exception. There must be a mechanism, therefore, by which a behaviour, once started increases its own probability of maintenance. A simple analogy is the bistable circuit, which will, when given an input of sufficient intensity transit rapidly, by a process of self facilitation, from oneoutput state to another. Once there, small, subcriterial inputs will not effect the reverse transition.

There are, however, very few behaviours which will occur independently of a context of other behaviours and other occurences of the same behaviour, so there is a problem not only of considering the very short term interactions of behaviour with itself, but also relatively short term interactions between bouts of similar or different behaviours. The bunching of feeding bouts into meals is a case in point.

Both mice (Wiepkema (1968)), and rats (Le Magnen and Devos (1970)) eat their food in meals. Meals are collections of feeding bouts closely spaced in time. Each feeding bout must therefore affect the probability of occurence of the bouts following in a systematic manner. Hinde and Stevenson (1969) have presented a concept of behaviour being the result of a complex set of interacting incremental and decremental effects that follow any particular response. This may be examined in the meal taking of rodents.

In the rat Le Magnen (1971) illustrates that eating speed varies systematically during a meal. From an initially low level it increases systematically for a while, and thereafter declines steadily towards the end of the meal. Early feeding, or its consequences, therefore facilitates /

facilitates slightly later feeding, but an inhibition builds up towards the end of the meal.

The end product of this process, a meal size, will be multiply determined by these incremental and decremental processes. Le Magnen (1971) demonstrates that the initial rate of facilitation is enhanced by previous deprivation, and that it is also modulated by the sensory properties of the diet, being reduced after quinine adulteration, and enhanced by the addition of corn oil. He argues that these two factors interact to produce the observed rate of facilitation in any particular case.

Towards the end of the meal feeding is progressively inhibited. Whatever the nature of this satiety process it can be modulated by outside factors. Booth (1973) has demonstrated that rats adapt to diets of differing caloric density over a period by gradual changes in meal size. He also shows, by adapting animals to calorically different diets of characteristic flavour that this process is learned and associated with flavour, and if the animals are placed on diets of intermediate caloric density, the meal size continues to be determined by flavour. Furthermore the changes occur in the terminating stages of a meal, supporting the concept of 'conditioned satiety'. (Le Magnen (1971))

Meal size can therefore be altered via effects upon the way in which behaviours or their consequences, influence one another. In the long term animals learn the calorific value of foods, so that the cumulative inhibitory effects of feeding upon feeding are adjusted to produce a meal size containing the necessary caloric load. Deprivation and palatability interact to alter the initial stages of the meal, and modulate meal size in that way.

Wiepkema /

Wiepkema (1968, 1971) has investigated a similar phenomenon in mice. Mice eat their daily intake in a much larger number of meals than rats (18-25 Wiepkema (1968)), but within meals the structure is similar. Meals consist of series of bouts of feeding interspersed with other activities. In the initial stages of a meal De Ruiter and Wiepkema (1969) and Wiepkema (1971) noted a progressive increase in bout length over the first five bouts, with no change in interbout interval. The rate of this facilitation varied with deprivation and palatability in a way similar to the data of Le Magnen (1971). The implication to be drawn from this is that the first feeding bout maredly affects the probability of a second occuring soon after it, and this facilitates the next, and so on till the end of the meal. Wiepkema stresses the importance of behavioural transitions, and notes that both deprivation, and the occurance of a previous bout, increase the probability of feeding beginning. The progressive rise in bout length over the first few bouts, however, reflects changes in the probability of feeding ending, so that the probability of feeding beginning and ending may be dissociated.

De Ruiter and Wiepkema (1969) and Wiepkema (1971b) propose a model, in which feeding is started once 'satiety' falls to some criterial level, and ceases again once that criterion is passed as satiety increases with feeding, which is functionally similar to that of Toates and Booth (1974). The positive feedback mechanisms "insure the organism, once it has given priority to feeding, against interference by other activities", Wiepkema (1971a) p 272. Towards the end of the meal, the interbout interval begins to increase markedly (Wiepkema (1971b)), so that in this case, as would be expected, the probability of feeding beginning is falling.

If /

If the mouse is placed in a strange cage, then the initial facilitation process fails to appear, unless a considerable period of deprivation has preceded the test. The interaction of feeding with other activities is therefore also of vital importance.

Mice therefore appear to show a meal structure controlled by interacting incremental and decremental effects in much the same way as rats, although the ethological approach, as used by Wiepkema et al would seem to allow a more precise specification of these processes.

The approach also suggests differing potential mechanisms for, on the one hand the modulation of the regulatory processes in feeding, and on the other the integration of feeding with other activities, a point which will be taken up in some detail in the experimental chapters to follow.

Firstly, however, we need to consider changes of feeding pattern over various reproductive states in the rats.

1.11 Feeding patterns and reproduction

In the previous sections an attempt has been made to describe in very general terms the processes determining the temporal patterning of feeding in rodents, in the hope that observed changes in pattern over various reproductive states may be interpreted as direct or indirect influences of gonadal hormones on one or more components of the system.

The /

The first sections of the chapter characterised the effects of oestrogens upon food intake as accurately as possible. It was concluded that chronic effects of oestrogens lower body weight by a co-ordinated action upon both the regulatory processes and the growth pattern, producing a characteristically female growth curve in a fashion typical of a regulatory system whose set point has been altered.

It was argued that the changes in feeding at pro-oestrus represent an effect at least partially different to the chronic influences of oestrogen.

There is limited evidence concerning the effects of reproductive phenomena on feeding pattern in rats. Kenney and Mook (1974) show that the increase in food intake following ovariectomy is similar to an exaggeration of the normal, lipogenic, night time feeding pattern of the rat. Meal size is increased, in both night and day, although in this case IMI in both night and day is also to some extent increased. The increase is insufficient to compensate for the change in meal size, so that total intake is increased. This is what would be expected of an animal with a raised body weight set point, which would be extracting a high proportion of the products of the meal during its absorption in order to increment its body weight. Drewitt (1972) indicates that the oestrus depression of feeding is a consequence of a depression of meal size, but in this case there is an increase in meal frequency, although it is insufficient to compensate for the decrease in meal size.

Superficially these effects appear to be simple opposites, but they are not. The essential point is that the ovariectomised animal shows a co-ordinated change of both meal size and frequency. The animal on heat /

heat, however, whilst it shows a reduction in meal size, shows a partially compensatory rise in meal frequency. The implication from this is that the prooestrus influence does not act in a co-ordinated fashion, but rather constitutes a primarily disturbing influence on the short term regulatory process.

The picture is not what would be expected from an animal whose set point is lowered, in which case IMI would be lengthened, as indeed it is during the analogous situation of day feeding.

The evidence, such as it is, regarding feeding patterns would therefore tend to support the hypothesis propounded in section 1.4. The changes after ovariectomy are typical of an animal actively incrementing its stores (just like during the night or after deprivation), exactly as would be predicted if 'set weight' were increased. The processes at prooestrus are not a simple opposite, like the daytime pattern, and seem to reflect a direct effect on meal size.

The hypothesis to be tested for the case of the mouse in the chapters to follow may therefore be made explicit:

> "It is suggested that the effects of oestrogen upon feeding may be divided into at least two classes, those acting in the long term, producing a co-ordinated change in body size, which probably act upon the normal regulatory mechanisms, and those acting in the short term, which are a primarily disturbing, direct influence upon the control mechanisms of meal size, and may be related to the integration of feeding with other activities."

CHAPTER ONE

S U M M A R Y

1. The thesis aims to examine interactions between feeding and reproduction in rodents.

2. The differential parental investment in the care of young by rodents would suggest that:
   (a) sexual selection is practised on the male
   (b) Females are adapted to maximise breeding efficiency

3. The male and female will show a sexual dimorphism in body size which will affect feeding.

4. The female's period of sexual receptivity would be expected to influence feeding, but not necessarily in the same way as the sexual dimorphism.

5. Males are larger and eat more than females. Females on heat eat less than females at any other time.

6. Gonadal hormones are probably responsible for these changes. The sexual dimorphism is dually maintained by testosterone facilitating body weight, and oestrogen reducing it.

7. The testosterone effects are irreversible, whereas those of oestrogen are reversible.

8. Oestrogen acting in the long term probably influences body weight set point by setting a typical 'intact female' growth curve, as opposed to the 'castrate' growth curve.

9. In /

9. In the short term the changes in feeding at prooestrus operate over a different time scale, and may appear as essentially disturbing oscillations upon longer term trends in food intake and body weight.

10. Progesterone appears to inhibit the effects of oestrogen.

11. The longer term effects of oestrogens will only appear in an animal that is not mated, and may represent a holding mechanism, so that non-breeding females grow slowly, using minimal resources, until they are able to breed.

12. Feeding patterns may provide more information as to the nature of the endocrine effects.

13. Feeding pattern is basically determined by the type of food the animal is adapted to eat, and the subject to modulation by a number of factors.

14. The need to balance nutrient supply and demand in the long and short term represents such a major modulating influence.

15. In the short term fluctuations in meal size are compensated by changes in following IMI.

16. Over the relatively long term changes in energy stores developing over several meals appear by virtue of a co-ordinated change in both meal size and meal frequency. The animal in a state of deprivation increases meal size whilst keeping IMI short.

17. Meals are a result of the operation of a behavioural control system which by means of interacting incremental and decremental effects produces a set of feeding bouts arranged together in time.

The /

The system may be modulated by regulatory influences, previous experience with food and other non food related activities in which the animal is indulging.

18. After ovariectomy meal pattern changes in a way consistent with the animal being effectively deprived, that is to say below its set weight.

19. The changes in feeding at prooestrus are produced by a reduction of meal size only, with no co-ordinated change in IMI, rather IMI partially compensates the meal size change.

20. The hypothesis suggested, therefore, is that the alteration of feeding at prooestrus reflects a direct or indirect influence of oestrogens upon the meal control system, whereas the longer term effects act by reversibly, directly or indirectly, affecting the regulatory process, with only secondary influences on meal size.

21. This hypothesis will now be examined in mice.

CHAPTER TWO

OUTLINE OF EXPERIMENTS

In the previous chapter the background to the hypothesis under test in the remaining sections of this thesis was discussed. In this chapter the experiments used to test that hypothesis are briefly outlined, and their aims made explicit.

So far, it has been suggested that in females oestrogens may constrain the operation of the feeding systems in more than one way. In the long term they may set a female pattern of growth, whereas the changes in feeding at prooestrus, which could of course be not solely the product of oestrogens, may reflect the operation of a different mechanism.

The problem is therefore to find experimental manipulations, and ways of analysing feeding which will allow the separation and identification of these two kinds of constraints, thereby verifying, or refuting the hypothesis under test.

The animal subjects chosen for this task were mice. The mouse was chosen primarily because it was readily available in the department, and certain techniques had already been developed for it. A rather less pragmatic aspect of the choice, however, stems from the need to accurately analyse feeding patterns in such a way as to potentially identify the operation of different systems. Wiepkema (1968, 1971a, 1971b), whose work was discussed at the end of the last chapter, has evolved an ethological analysis of feeding patterns in mice, which it was believed could be profitably extended into the present situation if mice were used as subjects.

A major problem produced by choosing to use a species not commonly associated with work in this field was the lack of background material upon which to base experiments. Before, therefore, experiments to test the hypothesis under consideration could begin, some background material had to be collected for the species and strain to be used. The task is to verify, in the mouse, that if it is exposed to the same experimental procedures as used by other workers with rats, behaves in the same, or a similar way.

The next chapter (3) therefore reports attempts to systematically replicate the rat work with mice as subjects. Having evolved methods for following the oestrus cycle and measuring food intake, the existence of a prooestrus depression of intake must be verified. This done, the nature of the sexual dimorphism in body size in mice must be examined, and the effects of gonadectomy at various ages, before and after puberty, discussed. In the rat, oestrogens were identified as the major hormone affecting food intake, so it is necessary to examine the effects of oestrogen replacement therapy, in various regimes, with the mouse.

It is to be hoped that the results of these studies will allow points of agreement and disagreement between the rat and mouse work to be identified, so that the stage may be set for a detailed examination of the changes in feeding over the long and short term.

There are basically two sides to the examination of this hypothesis, on the one hand it is necessary to characterise, in as much detail as possible, the changes in feeding over the long and short term, and on the /

the other to discover manipulations which may differentially affect the operation of the two presumed systems. Both approaches are used in the following chapters.

The female animal on heat, it has been noted, shows a complex of behavioural changes which taken together form a well known image of the sexually receptive animal. Such animals are casually described (usually in species more directly associated with man) as 'temperamental' or 'flighty'. This observation led to the first attempt to separate effects on feeding in the long and short term. It was hypothesised that the receptive animal would show a strong reaction to the attractive/aversive stimulus properties of food, as opposed to its calorific value, so animals were therefore fed one of two foods of similar calorific value, but differing markedly in stimulus characteristics, and the intake followed over the oestrus cycle, and after ovariectomy.

Simple measures of food intake are, however, unlikely to reveal the operations of the feeding systems, in the sort of detail that is required and so the next problem is to evolve a system of recording the pattern of feeding over long periods, so that it may be followed over the oestrus cycle in intact females, and also in ovariectomised females, males and castrates. (Chapter 4)

The output from such a recorder is, however, useless unless condensed into a set of variables, whose changes may be followed over the reproductive states under consideration. The feeding patterns of the mice used here will therefore be examined in general terms (bearing in mind the results of Wiepkema), and a set of variables designated for further study. (chapter 5)

Once /

Once this is done changes in these variables may be followed over the oestrus cycle on either of the foods used in the previous experiment. The inclusion of records from males and gonadectomised animals of either sex will allow any observed changes to be set in context. (chapters 6,7,10)

From the results of Wiepkema (1971b) it might be hypothesised in advance that sexual receptivity would be expected to influence the relationships of feeding to other activities, which may be revealed by an examination of bout structure within meals, whereas changes towards the end of the cycle, and after ovariectomy might be expected to have a more co-ordinated possibly distinct, 'regulatory' influence on feeding.

For this reason, in chapter 8 intra-meal structure is examined in detail, and in chapter 9 the relationships between meals and inter meal intervals are examined.

Even if a detailed study of feeding patterns reveals clear differences between different constraints on feeding, it would be useful to find other operations which dissociate them. The final chapters of the thesis describe an attempt to use a neurological manipulation to this end. It has been suggested that oestrogens exert at least a part of their effects on feeding by action upon the Ventromedial Nucleus of the Hypothalamus. The background to this assertion is discussed in chapter 11, together with a consideration of the use of Goldthioglucose (GTG) to induce lesions of that area in mice. It is hypothesised that lesions of the VMH will abolish one oestrogenic constraint upon feeding (possibly that acting in the long term), whilst leaving the other intact.

In /

In chapter 12 the food intake and feeding behaviour of male and female mice after GTG is compared, together with an examination of the effects of concomitant ovariectomy and GTG, and the injections of oestrogens under various regimes into female mice after GTG. The changes produced are considered in the light of the results of previous chapters.

In the final experimental chapter, a completely new approach is considered. Hamsters show no sexual dimorphism in body size, but do show changes in behaviour over the oestrus cycle (Zucker et al (1972), Richards (1966)). If they also show changes in food intake over the cycle, then this would possibly reflect the operation of a short term mechanism in a species which shows no long term effects of oestrogen on body size. Accordingly, food intake was followed over the oestrus cycle in female hamsters.

In summary, therefore, the experimental sections to follow have a number of aims, firstly to verify that the mouse behaves more or less like the rat when tested in the same way, secondly to characterise, using ethological techniques the parameters of operation of the feeding systems over various reproductive states, and thirdly to attempt to discover experimental manipulations which differentially affect the operation of the two hypothesised mechanisms, which include changing the food, lesions of the hypothalamus, and the examination of other species.

It is hoped at the end of this process that the validity of the basic hypothesis will be either established or refuted.

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CHAPTER THREE

FOOD INTAKE AND GONADAL HORMONES IN THE MOUSE

This chapter describes the methods used for monitoring the food intake and oestrus cycle of the mouse, together with the ways in which they have been used to replicate previously published studies in the rat. In the last section the effect of different foods on the pattern of intake over the oestrus cycle is discussed.

3.1 Subjects and maintenance

For all the experiments described in this thesis, excepting those of chapter 13, the subjects were mice of an inbred C57 strain obtained from the Bush Laboratory Animals centre, University of Edinburgh. The animals arrived usually in batches of 100, of very similar age (within two days), which was usually between 70 and 80 days. Immediately upon arrival they were transferred to stock cages, females at ten to a cage, and males caged individually. Before being used in experiments the animals lived in a stock room for one to eight weeks.

The stock room was kept heated to 25 ± 2 deg C., and maintained on a fully reversed light cycle, lights going off at 6.00 a.m. and on at 6.00 p.m. daily. Stock animals received water and standard mouse chow (of the type used at the laboratory animals centre (Spillers laboratory diet No. 1) ad libitum.

All experimental animals were housed singly in cages 12" x 5" x $4\frac{1}{2}$" in another room, which was fully air conditioned, temperature controlled to $25 \pm \frac{1}{2}$ deg C. The light cycle in this room was phase shifted relative to /

to the stock room, lights going off at 11.00 a.m., and on at 11.00 p.m. This allowed all measurements to be taken both at a convenient time of day, and at the beginning of the animal's dark period. Experimental cages were cleaned once a fortnight during experiments lasting longer than three weeks. Apart from this, and the daily measurements the animals received the minimum of disturbance. Before any experiment subjects lived in the experimental room for at least two weeks, in order to adapt to the new light cycle.

3.2 General methods used in the measurement of food intake

The food consumption of experimental animals was monitored by regularly weighing a feeder insert in the cages. The cages were of a commercial design (NKP Cages Ltd.) with a formed polypropylene base into the top of which fits a galvanised grill with depressions at one end suitable for a water bottle on one side and food on the other. The depressions were constructed of a gently sloping bottom with a near vertical face ([redacted]

The feeder inserts were perspex cubes 5cm along each edge with one face open (the top), and an adjacent face composed for most of its height of a wire mesh, at the bottom of this face there was an angled lip. The wire front of the feeder fits flush to the vertical face of the feeding depression, so that the animal in the cage may chew the food through the two layers of mesh in the same way as they have always fed in the stock cages. Animals feed from these feeders immediately with no difficulty.

Spillage was measured in a proportion of all the animals used in the experiments by mounting a $\frac{1}{4}$" mesh floor $\frac{1}{2}$" above the cage floor, which /

which was covered with a sheet of paper. When faeces have been removed any spilled food may be collected and weighed. In no case did spillage exceed 0.1 gm/day, and in most cases, whilst sometimes visible there was insufficient material to be sensibly measured. Coprophagia was not prevented in any of the experiments. Spillage figures were probably as low as they were because of the moderate difficulty of feeding from the feeders. As a consequence any spilled food will be preferentially consumed.

Unless stated otherwise the feeders were weighed once each day at the beginning of the dark period, on a 'Torbal' torsion balance, accurate to 0.1 gm. They were then refilled to the same level, reweighed and replaced.

It is important to realise that all 24 hour periods described in this thesis are a'night' followed by a 'day', as the feeders were weighed at the beginning of the dark period. It is conventional to label the nights of the oestrus cycle, for example, according to the day that they follow, but as has been reported before (Wiepkema (1968)), and will be shown below, mice show a marked circadian rhythm of food intake some 80-90% being consumed during the hours of darkness. The 24 hour period, where necessary, therefore, is labelled according to the conventional name of the night that it contains, rather than the day.

3.3 Methods used for following the oestrus cycle

During the oestrus cycle the cells present in the vaginal lumen change in type and relative frequency. These variations may be used to monitor the oestrus cycle. (Thung (1956))

Once /

Once each cycle the epithelial lining of the vagina undergoes a process of cornification, in which the cells of the outer layers become keratinsied and die. This cornification is maximum about the time of ovulation, and in rats at least is dependent upon the preovulatory oestrogen surge, (Davidson (1968)) After ovulation the dead cornified cells slough off into the vaginal lumen, and leucocytes migrate through the epithelium to float freely in the lumen, consuming the remains of the cornified cells and presumably any bacteria that may be present. Thereafter the number of leucocytes declines rapidly, and the vagina contains only a few nucleated epithelial cells until the next oestrogen surge.

It is possible to predict from this dynamic picture which cell types will be present on any particular day of the cycle, but the unequivocal definition of cycle stage from a single smear is very difficult. The stage of cycle is best defined by an examination of the changes in the smear picture before and after the day under consideration.

The cycles used in this thesis were all defined relative to one day of the cycle, namely prooestrus. Prooestrus was defined (from Thung (1956)) as that day bounded by the virtual disappearance of leucocytes from the day before, and the appearance of a significant number of cornified cells in the smear of the day after. The day before was labelled 'dioestrus', that following 'oestrus', and the day after that 'metoestrus', irrespective of the vaginal smear picture.

Vaginal smears were taken daily at the beginning of the dark period using a flamed platinum loop cooled in saline. It was inserted into the vagina /

vagina, agitated slightly, removed and the contents smeared onto a microscope slide which was placed immediately into 96% ethanol. All smears were fixed for at least 12 hours before staining, which was undertaken in batches once or twice weekly. Smears were stained with Leishman's stain (see appendix).

The smears were scored for the relative proportion of the three cell types possibly present. At least two fields of view were sampled at 200x magnification, and the abundance of each cell type (nucleated epithelial cells, cornified epithelial cells and leucocytes) assessed on a three point scale. The subjective judgement of frequency was occasionally checked by actually counting a fixed number of cells.

Cycle stages were designated only after several days records were scored by the criteria described above. If any correlation with other data was intended (e.g. food intake), all readings of smears were undertaken blind, before daily intake figures were calculated.

The use of this method of designating cycle days avoids difficulties produced by the irregularity of the oestrus cycle of the mouse. Mice are notoriously irregular in their cycles, but the event of behavioural and physiological importance is ovulation, and the time of this is indicated by the day of prooestrus, the central point in the present analysis. The females were caged in the same room as males, whose small probably helped to maintain regular cycles (e.g. Whitten (1956)).

The cycle length of all cycles (that is to say the number of days between successive prooestruses) monitored in both pilot and main studies has been analysed in Table 3.3(i).

It /

TABLE 3.3(i)

Length of oestrus cycles in Virgin C57 mice

	4 day	5 day	≥5 day
No:	111	65	22
Percentage:	60	38	12

It can be seen that a majority of the cycles lasted only four days, and that the bulk of the remainder lasted five. In the 40% of cycles with 5 or more days the days in excess of four were not considered in the analysis.

3.4 Validation of the Vaginal Smear Criteria

3.4.1 Introduction

Before the vaginal smear criteria may be used to indicate the days of the oestrus cycle their accuracy in predicting events of behavioural importance in the cycle must be determined. This was examined in the following experiment.

The most characteristic and important behavioural event in the oestrus cycle is the period of sexual receptivity. In the rat, at least this occurs during the night following the day of prooestrus. If the vaginal smear can accurately predict the time of sexual receptivity, then this may be used as a reference point for the rest of the cycle. The sexual receptivity of females was therefore monitored over the vaginal oestrus cycle. Sexual receptivity is best monitored by observing the sexual behaviour of the female when presented with an experienced male. There are two possible test regimes, either the female may be tested only once, or she may be tested each day. The latter strategy involves the risk of pseudo-pregnancy developing as a consequence of copulation (or pregnancy if ejaculation is allowed to occur). Accordingly, in the following study, both methods were used.

The index of receptivity chosen was the lordosis to mount ratio which has been used by several previous authors who considered it the best indicator of female sexual activity (e.g. Edwards (1970)).

3.4.2. /

3.4.2. Methods

The experiment was performed in two parts. In the first part eight females were caged in a temperature controlled room on a fully reversed light cycle (lights on 8.00 p.m. off 8.00 a.m.). Vaginal smears were taken each day before 9.30 a.m. (i.e. just after the beginning of the dark period). The animals had been living under this light cycle for four weeks before the experiment began.

Once each day at 4.00 p.m. (equivalent of 2.00 a.m. mouse time) the sexual receptivity of the females was measured by presenting each to a stud male of proven capacity, in the male's home cage. Each male received the same female each day. The animals were tested in two batches of four by a time sampling procedure. The four cages were observed in succession at five second intervals, so that each cage was considered once every 20 seconds. At each time mark the behaviour of the relevant pair was recorded on a sheet. Activities monitored were: Ano-genital sniffing, mounting, intromission and lordosis (the lordosis position is pronounced and easily recognisable in the C57). The testing continued for five minutes with each batch of cages.

A lordosis to mount ratio was calculated for each female on each day of testing and the experiment was continued for 25 days.

In the second experiment 20 females were used. Each was tested only once with a male, its vaginal smear picture having been followed for some days previously, and then four days after, so that the designated cycle stage of the night of testing may be determined. Animals were tested in the same way, by time sampling the behaviour of several pairs tested simultaneously.

After /

After the behavioural testing was completed the smears were read, blind of the behavioural records. (The slides containing the smears were marked with a code by an independent worker, so that individual animals and days could not be distinguished until the records were decoded).

A table of lordosis/mount ratios was constructed for each day of the cycle in each animal.

3.4.3. Results

The results from the two parts of the experiment are identical, so they will be considered together. The lordosis mount ratio is illustrated as a function of the vaginal oestrus cycle in fig 3.4(a). It can be seen that the mean ratio is very much higher on the night following prooestrus than any other night. These results are statistically highly significant. (Friedman's chi = 14.6 $P<0.01$)

3.4.4. Discussion

It would seem clear that, under both conditions of testing, the vaginal smear criteria described in the previous section are clear indicators of the time of sexual receptivity. It can be assumed, therefore, that they provide a valid set of criteria for use in subsequent experiments.

3.5 The daily intake of laboratory chow over the oestrus cycle

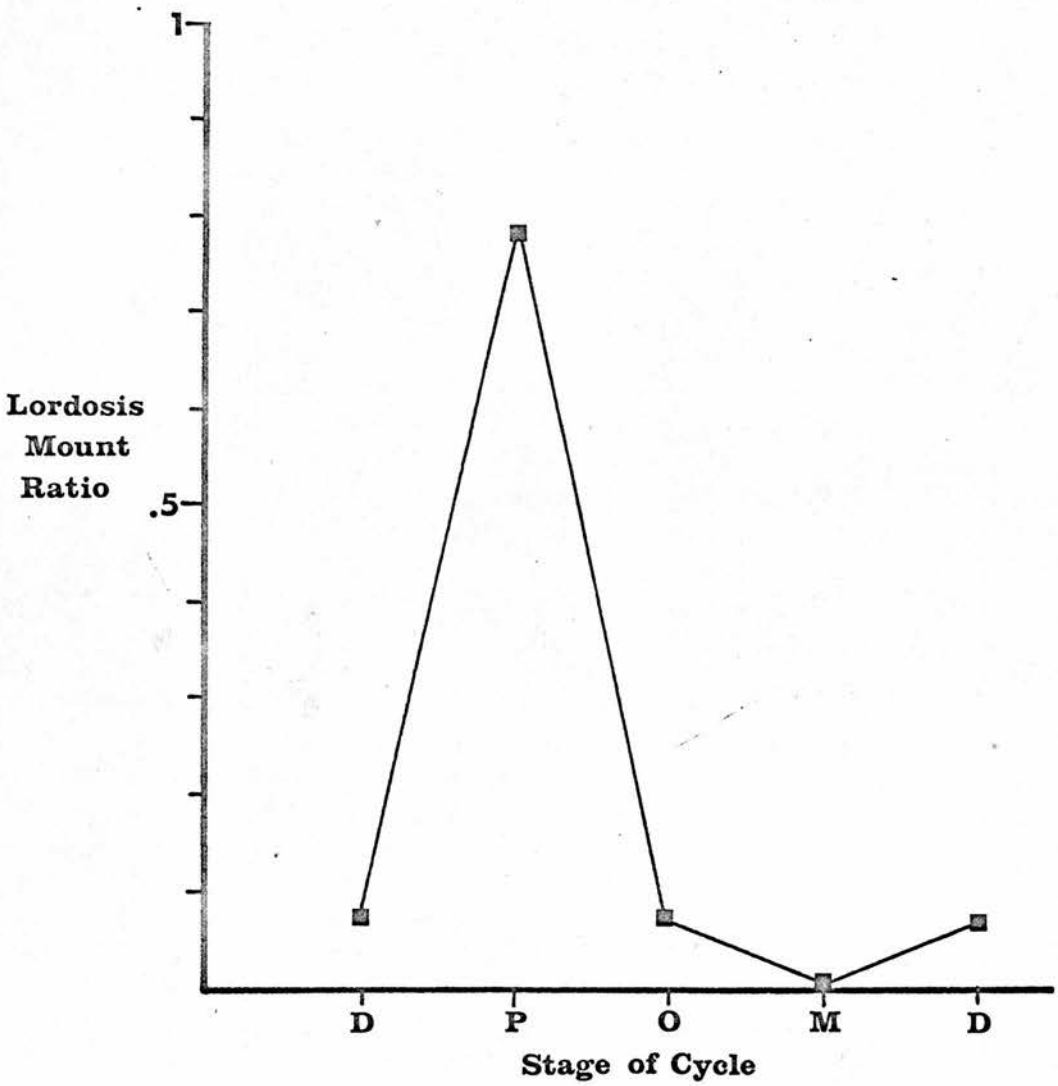
3.5.1 Introduction

Work on rats has indicated that the intake of laboratory food is depressed at the time of sexual receptivity (see introduction). It is of importance to discover whether the same effect may be observed in mice. Accordingly the daily food intake of a number of female mice was followed over several cycles each.

3.5.2 Methods /

Fig. 3.4(a)

Sexual Receptivity of female C57 mice as a function of the vaginal oestrus cycle


1
.5
Lordosis
Mount
Ratio
D
P
O
M
D
Stage of Cycle

3.5.2. Methods

Eight females were caged individually in the temperature controlled experimental room. They were 90 days old at the start of the experiment. The daily intake of standard mouse chow (Spillers Laboratory Diet No. 1) was monitored by the methods described above. Vaginal smears were taken daily at the beginning of the dark period. The smears were scored without knowledge of the daily food intake figures which were not calculated from the raw data until the end of the experiment. At that time the two sets of results were collated and the mean intake on each day of the cycle for each animal was calculated. The data was analysed using Friedman's test.

3.5.3. Results

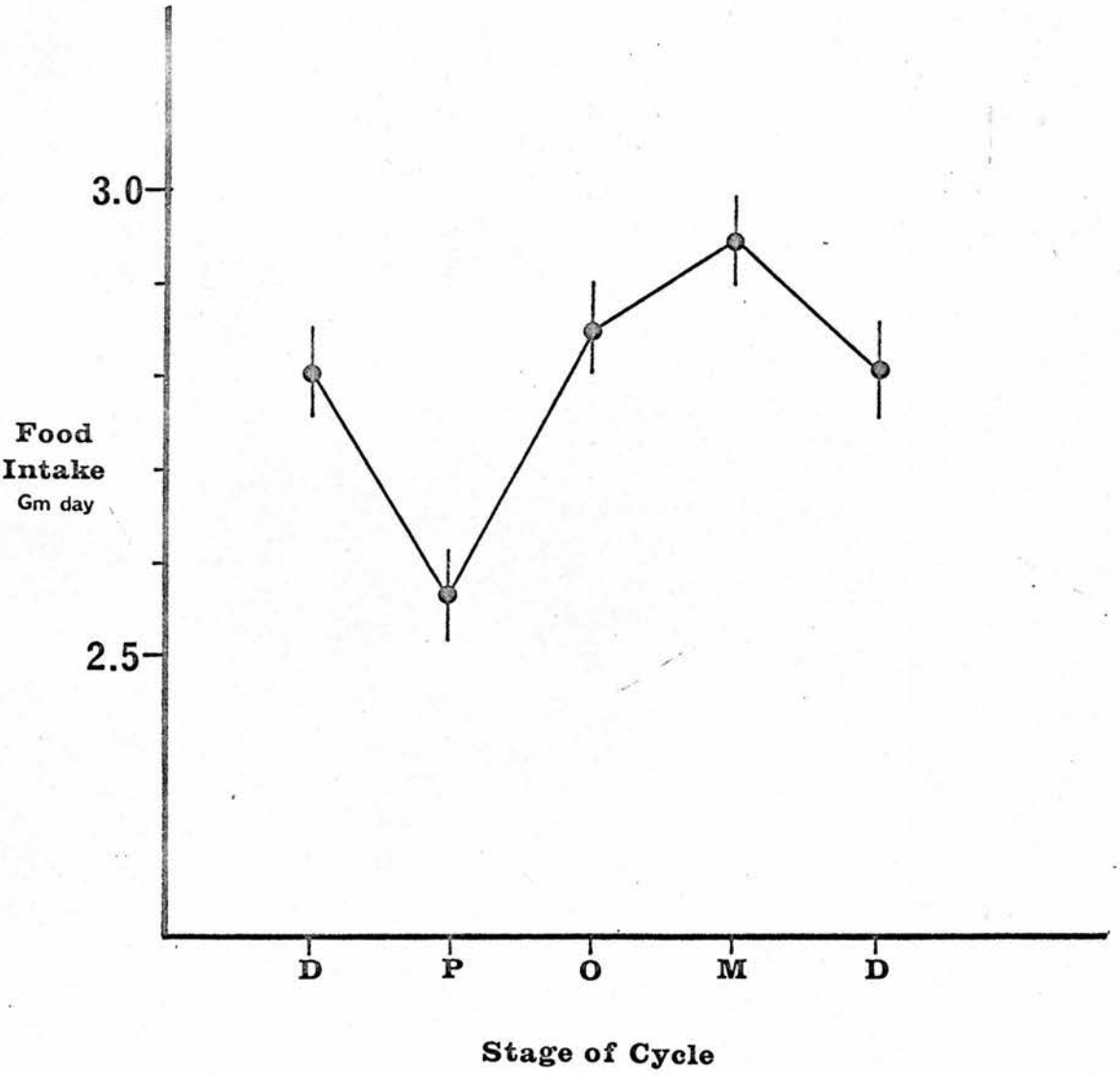
The grand mean intakes and their standard errors are illustrated in Fig 3.5 (a). The individual means are presented in table 3.7 (i). There is a highly significant effect of cycle stage upon food intake (Friedman's chi = 18.3 P 0.001). This is composed of a significant depression in the first part of the cycle, at prooestrus, and a significant elevation later at met-oestrus.

3.5.4. Discussion

The food intake of mice given laboratory chow to eat varies over the oestrus cycle in exactly the same way as that of the rat under similar circumstances, falling in the early stages and rising in the later. This result does not accord exactly with that of Dewar (1957), who found a slight rise in body weight of his mice at 'oestrus' (he defines no pro-oestrus). Body weight changes were only followed in detail for a few of the mice in the present study, and the variability of body weight, even when taken at the same time each day obscured any differences /

Fig. 3.5(a)

The intake of laboratory chow over the oestrus cycle of the female mouse


3.0
2.5
Food
Intake
Gm day
D
P
O
M
D
Stage of Cycle

differences that might have been present. Having demonstrated short term changes of feeding with reproductive state, it is therefore now necessary to examine longer term influences of sex upon feeding.

3.6 Sexual dimorphisms in food intake and body weight in mice

3.6.1. Introduction

Adult rats show a pronounced sexual dimorphism in body weight, which develops in the period following puberty as a consequence of a facilitatory effect of testosterone on male weight gain, and an inhibitory effect of oestrogen acting in the female. Prepuberal gonadectomy prevents the development of these effects and the oestrogen effect at least is reversible by gonadectomy in adulthood, and possibly also by an active breeding life.

In the following sections similar phenomena are examined in mice. The food intake and body weight of animals of both sexes was followed from soon after weaning into adulthood. The effects of pre- and post-puberal gonadectomy were examined, and also the consequences of a long breeding life.

3.6.2 The existence of the sexual dimorphism, and the effects of gonadectomy

(i) Methods: Adult female C57 mice from stock were mated with stock males. The resulting litters were culled to six, or if they contained less than six excluded from the study. At 21 days after birth young males and females were removed from the litters. Sixteen animals of either sex were arranged in two groups of eight animals, caged individually in the experimental room. Daily food intake of each animal was followed in the usual way /

way for seven weeks. Body weight was measured every three days.

At 28 days of age one group of eight young females was ovariectomised, and a group of eight young males castrated (see below for procedure), both under light methoxyflurane anaesthesia. Half of the remaining eight males and females were sham operated.

In a second experiment eight adult males and eight adult females at 90 days of age, were gonadectomised under equithesin anaesthesia. Adult males were anaesthetised with 2.35 ml/kg Equithesin ("Jen-sal" inc.), given intraperitonsally, and prepared for surgery. The testes were exposed in turn through a small incision in the scrotum, clamped off, and excised. The tissue around the point of excision was cauterised with a hot metal rod. The scrotum was not sewn up. Animals recover from this procedure with remarkable ease, and are apparently suffering no ill effects just one day later. Young males received the same surgical procedures under methoxyflurane anaesthesia, which, as it is more controllable, produces far fewer anaesthetic fatalities than barbitutates in the youngsters.

Adult females were anaesthetised using equithesin in the same way as males. The fur in the general region of the ovaries was clipped, and a skin incision made in one flank. After dissecting the underlying fascia a small incision was made in the abdominal wall over the ovary, which could usually be seen. The ovary was withdrawn, ligated and excised. The deep and superficial wounds were repaired with separate sutures. The whole procedure was then /

then repeated, under the same anaesthetic, on the opposite flank. As with the males, young females received the same operation under the inhalation anaesthetic.

The sham operations consisted of making the normal incisions, whilst leaving the gonads undisturbed.

The food intake and body weight of the adult gonadectomy groups were followed for four weeks after the operation. The food intake and body weight of half of each prepuberally gonadectomised group were also followed from 90 to 130 days of age.

(ii) Results: The body weight changes under all of these conditions are show in Fig 3.7(a), and the changes in food intake in 3.7(b).

Considering firstly body weight. Prior to puberty all groups show equal body weights and rates of weight gain (they are therefore shown as one line on the figure to reduce confusion). The temporary weight loss following surgery in the operated groups does not persist for more than three days.

Immediately after the presumed age of puberty (30 - 35 days), the male, female, and gonadectomised groups diverge. Males show a very rapid weight gain at this time, which persists for a relatively short time before their growth curve settles to a more shallow rate of development. A fair proportion of males weaken and die during this period of rapid growth, although by good fortune this did not occur to any of the experimental groups.

The /

Fig. 3.7(a)

Body weight as a function of age in intact males and females and animals gonadectomised before or after puberty

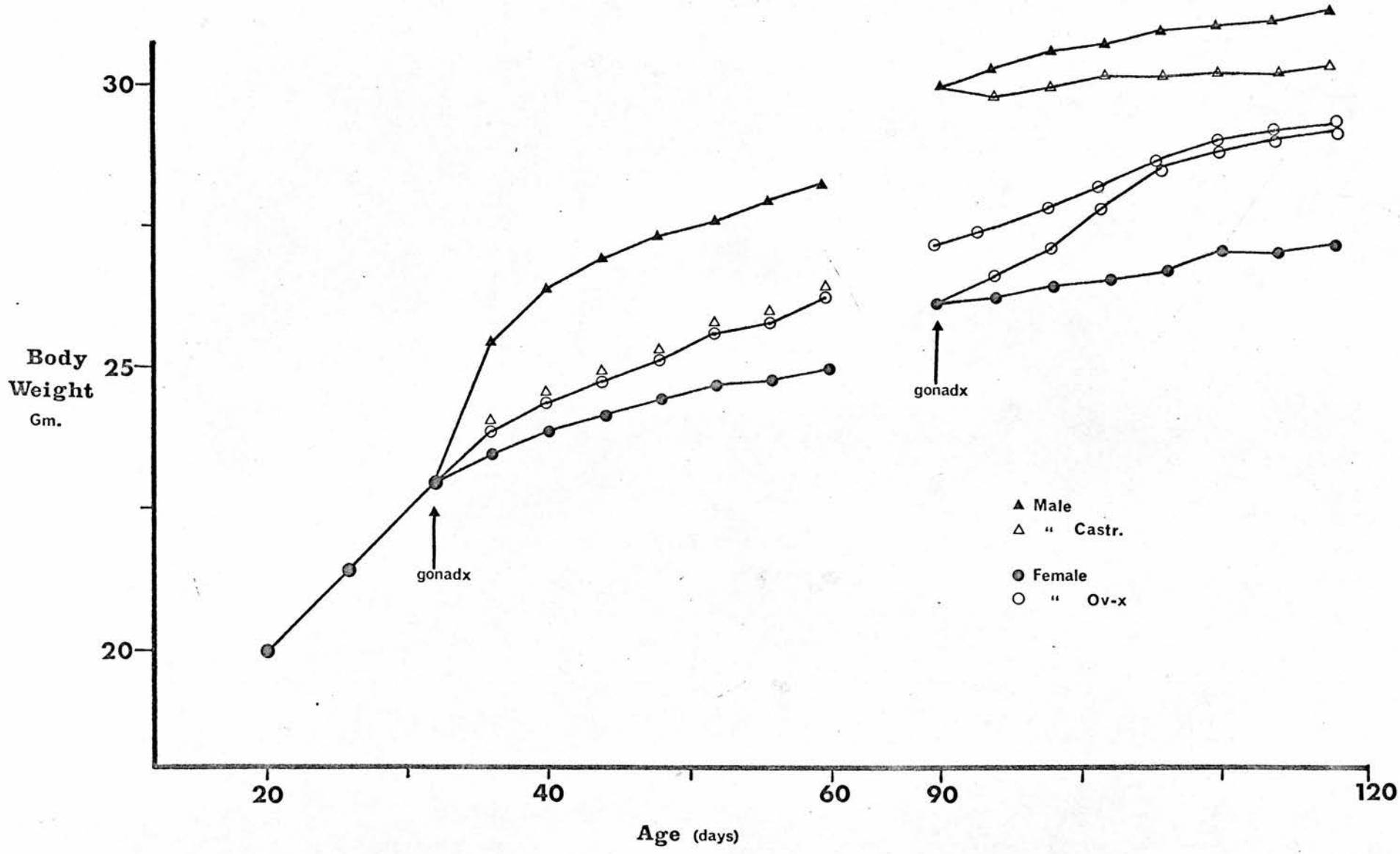

30
25
20
Body
Weight
Gm.
gonadx
gonadx
Male
" Castr.
Female
" Ov-x
20
40
60
90
120
Age (days)

Fig. 3.7(b)

Food intake as a function of age in intact males and females and animals gonadectomised before or after puberty. Symbols same as in Fig. 3

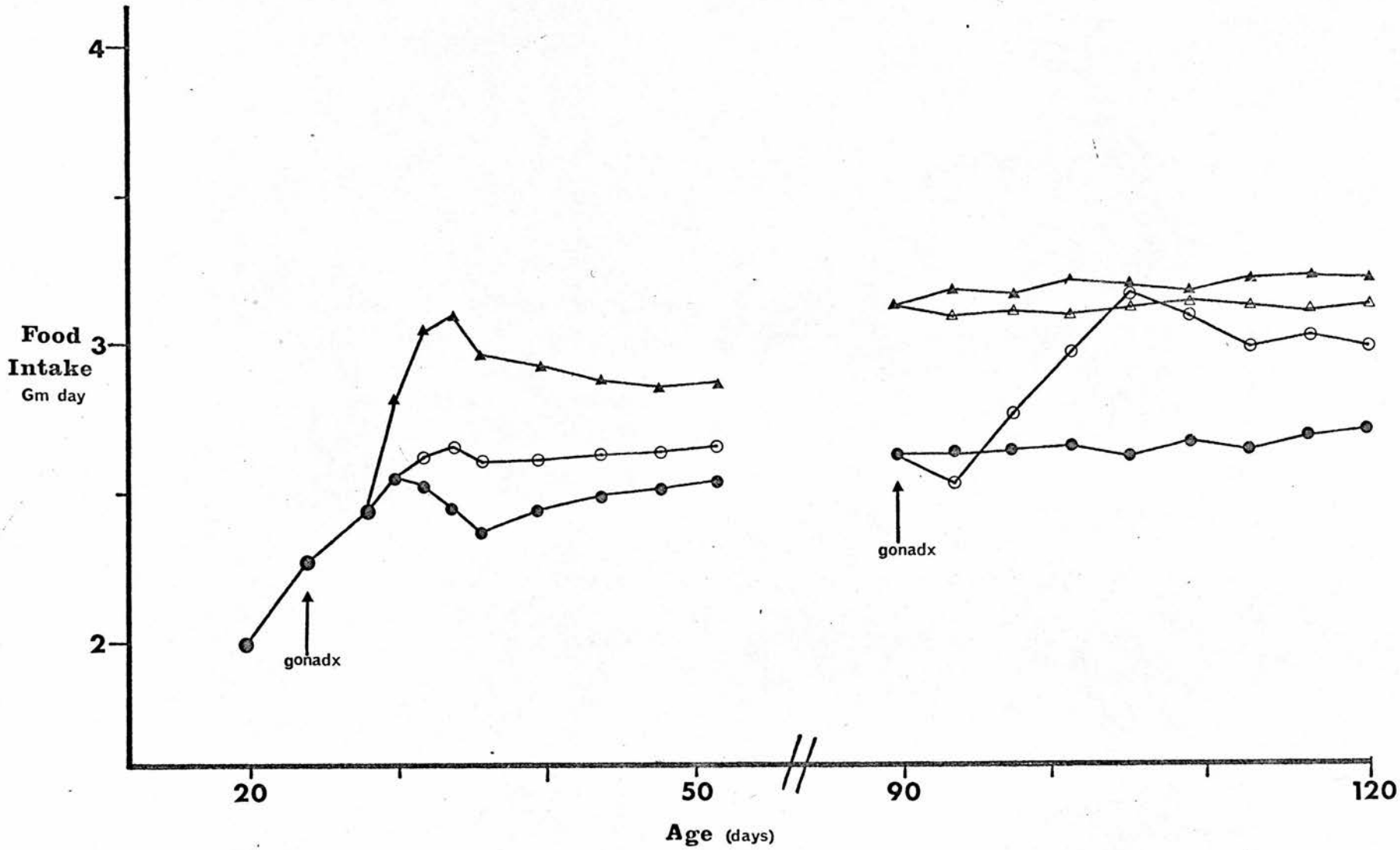
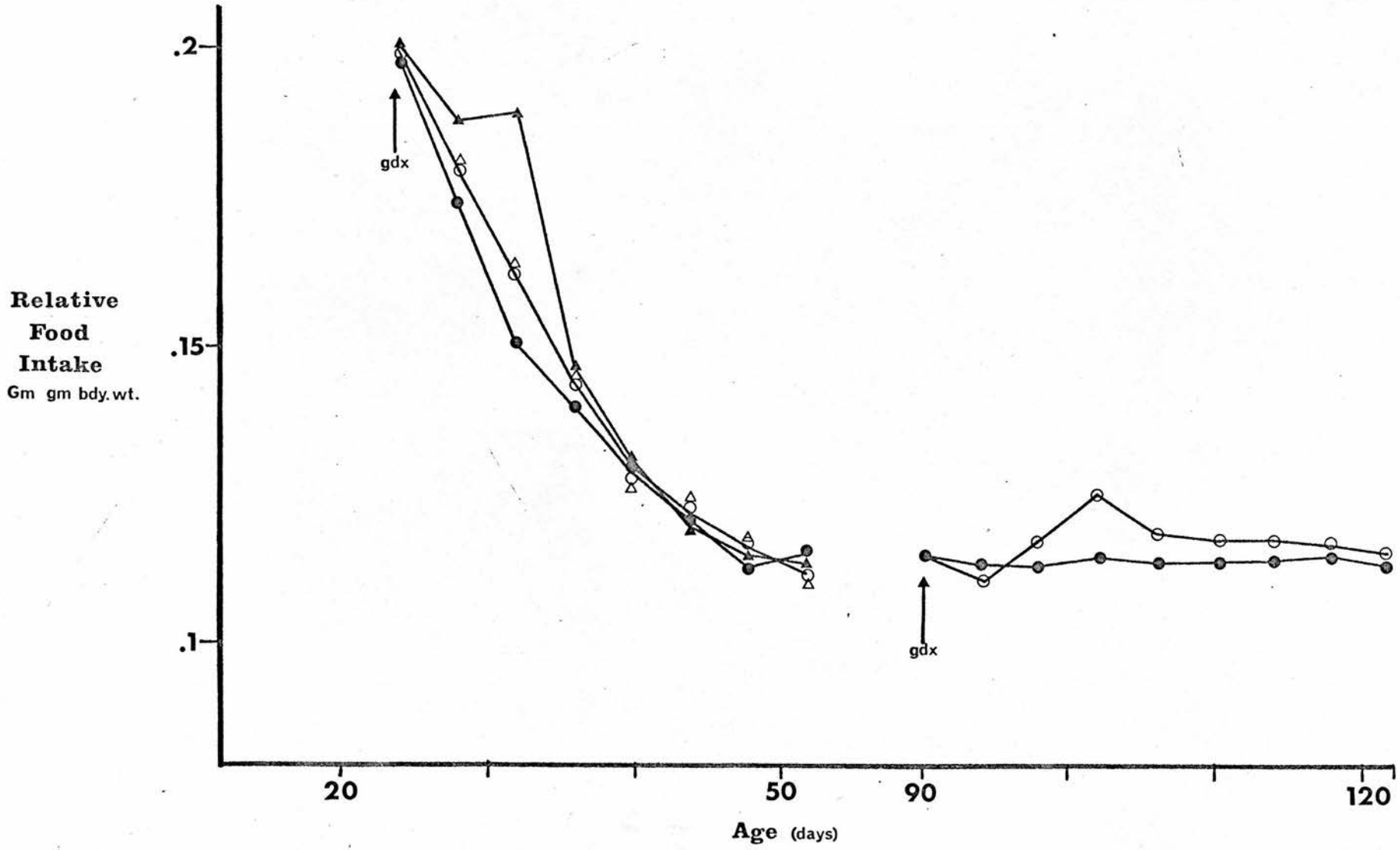

4
3
2
Food Intake
Gm day
gonadx
gonadx
20
50
90
120
Age (days)

Fig. 3.7(c)

Food intake relative to body weight as a function of age in males and females and animals gonadectomised before or after puberty.


Relative
Food
Intake
Gm gm bdy. wt.
.2
.15
.1
gdx
gdx
20
50
90
120
Age (days)

The gonadectomised males and females show identical weight curves, increasing at a lower rate than the male just after puberty. The differences are significant after 40 days ($P<0.01$ Mann Whitney 'U' test). Females show a rate of weight gain post puberty that is lower than the gonadectomised groups. These differences are significant from 44 days on.

From 50 days on the rates of weight gain of all three groups are identical.

Gonadectomy in adulthood produces a levelling off of the weight curve in males, but a considerable (although temporary) elevation of the rate of weight gain in females, until they come to parallel the growth curve of the prepuberally gonadectomised animals. Ovariectomised animals weight significantly more than intact controls from 10 days post injection ($P<0.01$ Mann Whitney 'U'Test).

Food intake changes, as is apparent from fig 3.7(b) parallel these changes in body weight. Fig 3.7(c) illustrates the changes in food consumption per unit body weight with age and after gonadectomy. It can be seen that the difference between the groups are produced entirely in the period before 45 days of age, although late ovariectomy does produce a temporary, and significant ($P<0.01$ Wilcoxon test) elevation of relative intake.

Discussion: Several conclusions may be drawn from these data. Firstly, the effects of prepuberal gonadectomy illustrate that hormones acting in adulthood are necessary for the expression of the normal sexual dimorphism in body size. Furthermore the sexual dimorphism is the consequence of a dual action of male and /

and female gonadal secretions, as the castrates are intermediate in body weight and food intake between the male and female animals. This is very similar to the situation in rats (see Chapter 1). Mice differ from rats, however, in some respects. For one thing the sexual dimorphism in body size is not correlated with differential rates of weight gain after 60 days of age (as was also noted by Wright and Turner (1973)). The sex difference is produced in its entirety in the couple of weeks following puberty, when males gain weight extremely rapidly, whereas females grow relatively slowly. Once this process has occured the growth curves of males, females and castrates are identical in slope.

Adult castration produces a fall in food intake, and a cessation of weight gain. Adult ovariectomy, however, produces a rapid change of gear from the intact female to the young castrate growth curve. The female effects are therefore definately reversible, whereas those of the male are not.

It may therefore be concluded that the mouse behaves more or less like the rat, but with certain relatively minor discrepancies. The next question is to decide whether the same hormones are involved and to investigate the effects of prolonged breeding.

3.6.3. Effects of a long breeding life on body weight

By chance a large number of old female C57's arrived in the laboratory for use in another purpose. These animals had been used by the Bush animal centre as breeding stock, and had each produced several litters. /

litters. The animals were weighed, and their body weights compared to those of intact females of the same age, and females of similar age who had been ovariectomised in early post puberal life (as a part of pilot experiments). The breeders were significantly heavier than the intact females (34 ± 1 g as opposed to 31 ± 1 g, n = 10 in each case, $P<0.01$ T test). The ovariectomised group weighed about the same as the breeders (35 ± 1 g).

A long breeding history, therefore apparently tends to eliminate the female component of the sexual dimorphism, although, of course it may be a different bodily component responsible for the change in weight.

3.6.4. Oestrogen replacement after gonadectomy

Introduction: Experiments in rats have demonstrated that the effects of ovariectomy upon food intake and body weight may be reversed by the administration of oestrogen. It was also suggested that the regime of injections for replacement may reveal details of the oestrogenic action. Accordingly, previously ovariectomised mice were treated with oestrogen either daily, or once every fourth day, and food intake monitored.

Methods: Sixteen adult female mice were ovariectomised by the method described in the previous section. Food intake and body weight were followed for two weeks after the operation. Eight of the animals then received either 100 or 200 ng oestradiol benzoate (Organon Ltd) daily by intramuscular injection in 0.1ml arachis oil. Treatment continued for five days. Food intake was followed for these and a further week. The animals were then given the opposite dose in a similar regime. Four animals received the higher, and four the lower dose first time round, and the groups were reversed on the second set.

The remaining eight animals received intermittent injections of oestrogen. Each animal received, once every fourth day a single intramuscular injection of 500ng oestradiol benzoate in 0.1ml arachis oil. Three such injections were given. Food intake was followed for the period of the injections and a week afterwards.

Two other groups of four ovariectomised animals received oil injections according to these two regimes acting, therefore as control groups. Food intake was followed.

Results: The results of these experiments are show in Fig 3.6(d). Considering firstly the effects of continuous oestrogen, Oestrogen at both daily doses significantly reduced food intake ($P<0.01$ Mann Whitney 'U' Test on the five day means). The intermittent oestrogen injections also reduced food intake, which was significantly depressed throughout the period of injections. There was a considerable fall following the first injection ($P<0.01$ Mann Whitney 'U' test), followed by a partial recovery towards the next. The following two injections produced falls in intake on the second day, but recovery was complete to the low level persisting after the previous injection. The second and third injections therefore essentially produced an oscillation about a maintained low level of intake, which recovered only slowly after the injections ceased.

Discussion: These results demonstrate that in the mouse, as with the rat, oestrogen is a major hormone affecting food intake and body weight. Oestrogens reduce the food intake and body weight of animals after ovariectomy, both when given daily, and every fourth day. The observation that the effects of a single injection may persist over days /

Fig. 3.6(d)

The effect of daily oestrogen injections at two close levels upon the food intake of the ovariectomised, adult female C57 mouse

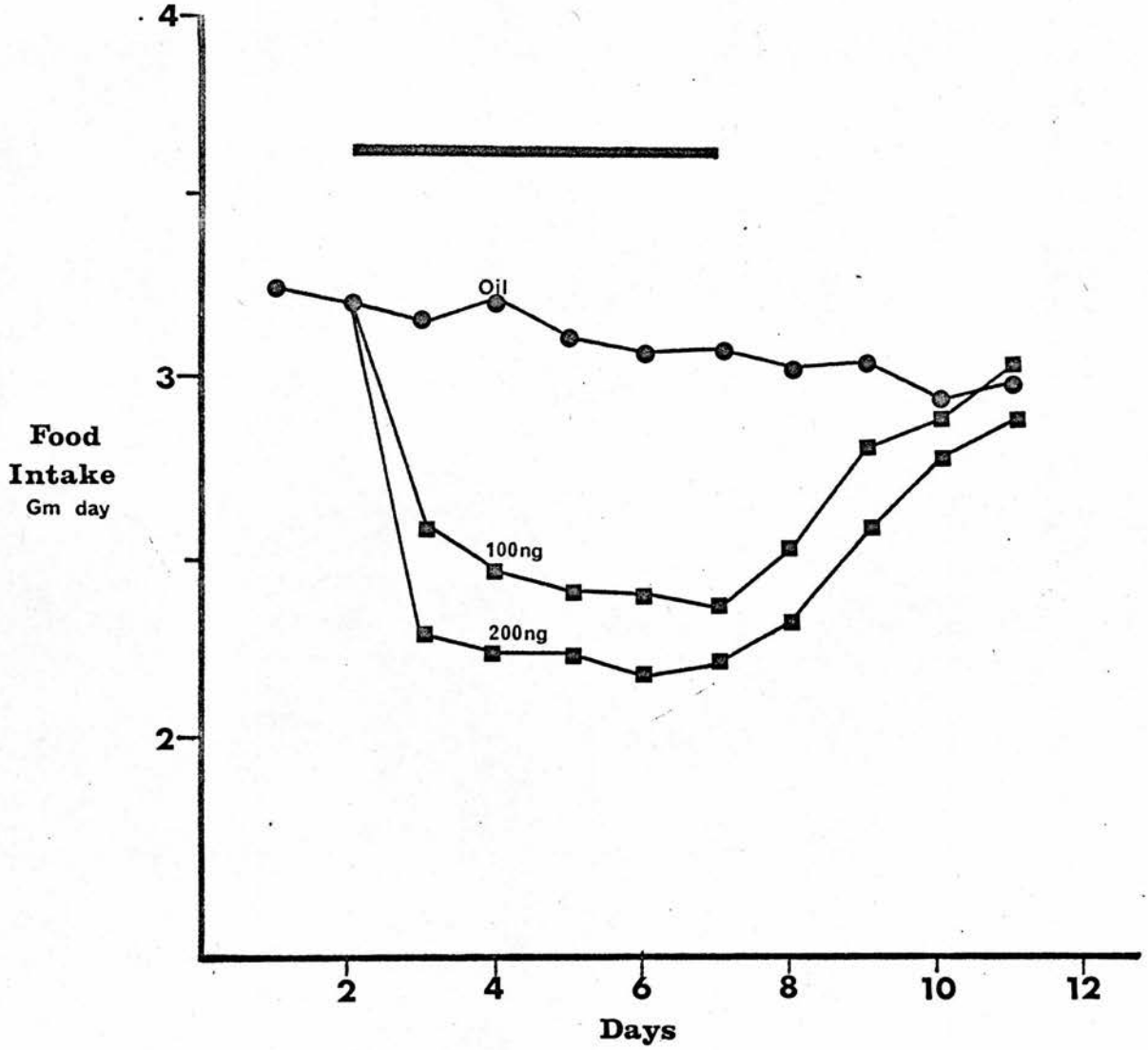
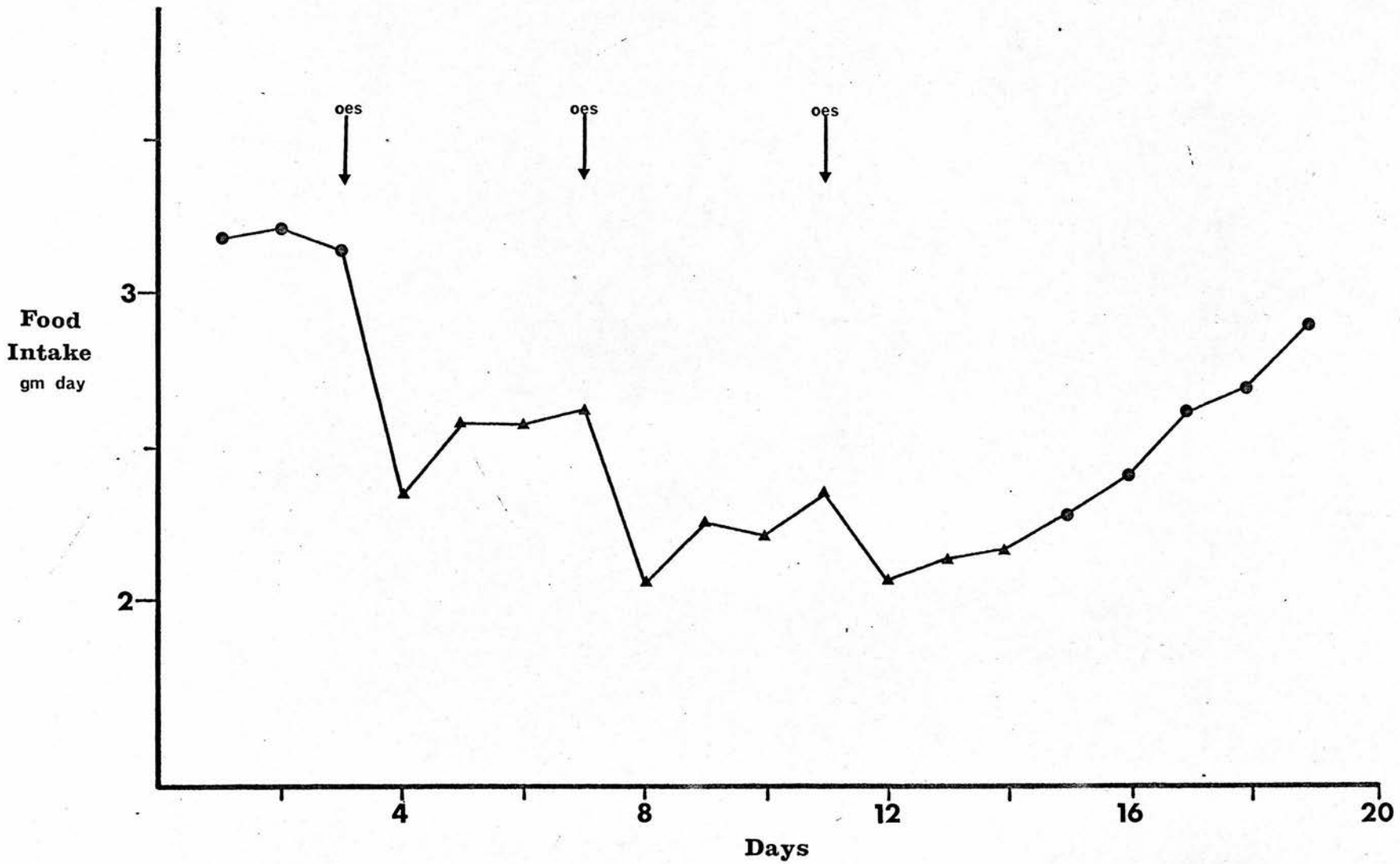

4
3
2
Food
Intake
Gm day
Oil
100ng
200ng
2
4
6
8
10
12
Days

Fig. 3.6(e)

Effect of a single injection of 500ng oestrogen every fourth day upon the food intake of ovariectomised female C57 mice.


oes
oes
oes
Food
Intake
gm day
3
2
4
8
12
16
20
Days

days and lower the general level of food intake is also consistent with the data from rats.

Overall therefore, this section has demonstrated that the C57 mouse, behaves like the rat under a number of conditions, with only minor differences between the two species. The sexual dimorphism in body weight is produced by a dual action of oestrogen in the female and (presumably) testosterone in the male. The effects of oestrogen are reversible and may be produced by summation over doses separated by several days. Food intake is also depressed about the time of sexual receptivity.

The next requirement, therefore, is to try and find means of dissociating the various effects of oestrogen, according to the hypothesis under test. The following section describes the first such attempt.

3.7 The intake of two foods over the oestrus cycle of the mouse

3.7.1. Introduction

There is in folklore outside the normal domains of psychology a common view of the female animal on heat. She is considered to become flightly and temperamental, and to show apparently general changes of mood. Many people experienced with animals can recognise heat by such changes. Although never formally reported many dog breeders, for example, notice that bitches on heat change their feeding habits, becoming apparently more fussy.

With these informal observations in mind, the intake of foods differing in stimulus characteristics, but similar in calorific value, was followed over the oestrus cycle of female mice.

3.7.2. Methods /

3.7.2. Methods

Subjects were C57 female mice, caged individually in the experimental room. Groups of animals were presented with one of two foods to eat. The first was their conventional diet, Spillers Laboratory Diet No. 1, and the second a sweet, roughly isocaloric alternative - 'Klondyke Pete Golden Nuggets' - a childrens breakfast cereal (Nabisco Foods Ltd.). The product is a cereal derivitive, being made from wheat and honey, and produced in lumps of similar size and consistency to lab chow, although it is not quite so dense. It could be used in the feeders with no modification. The calorific value of the two foods is similar, chow being some 6% more calorie dense than nuggets (manufacturer's figures in both cases).

The food intake of twelve animals was followed over five cycles each on the two foods in turn (in counterbalanced order). Animals were allowed a week to adapt to the new feeders, and if necessary food, and then food intake and daily vaginal smears were taken for four weeks. The groups were then swopped, animals previously eating chow, now consuming nuggets and vice versa, allowed another week to adapt, and followed for a further four weeks.

As usual vaginal smears were read blind of the food intake figures which were calculated later. A table of food intake on each day of the cycle for each animal on each food was constructed (Table 3.7 (i)). No vaginal smears were reconsidered in the light of the food intake data, and all complete oestrus cycles included. Data was analysed using Friedman's test.

3.7.3. Results /

3.7.3. Results

Twelve animals received each food for a minimum of three (occasional spontaneous pseudopregnancy), and a maximum of six oestrus cycles. Any results obtained could potentially be confounded by adverse effects of the novel diet, so it is first necessary to discuss the general health of the animals over the period of analysis. Two indices of general health will be used apart from subjective judgement, the animal's body weight, and the persistence of oestrus cycles. Body weight was maintained on all animals given golden nuggets for four weeks, and the rate of weight gain, although slow was not different to that of the control animals eating laboratory chow. The regularity of oestrus cycles may be assessed by considering table 3.7 (ii).

Table 3.7 (ii)

Length of cycle:	4 day	5 day	≥5 day
Chow	60%	38%	12%
Nuggets	58%	36%	6%

It can be seen that the same proportions of cycles of any given length are observed in animals feeding upon nuggets as chow. These two observations, together with subjective impressions, would strongly suggest that animals do not suffer from a prolonged period of eating only the sweet food.

The grand means and standard errors of the intake of each food on each day of the cycle are show in Fig 3.7(a). There is a significant effect of cycle upon food intake in both cases (Chow Friedman's chi

18.3 /

Fig. 1 7.(a)

The intake of two foods over the oestrus cycle of the mouse.

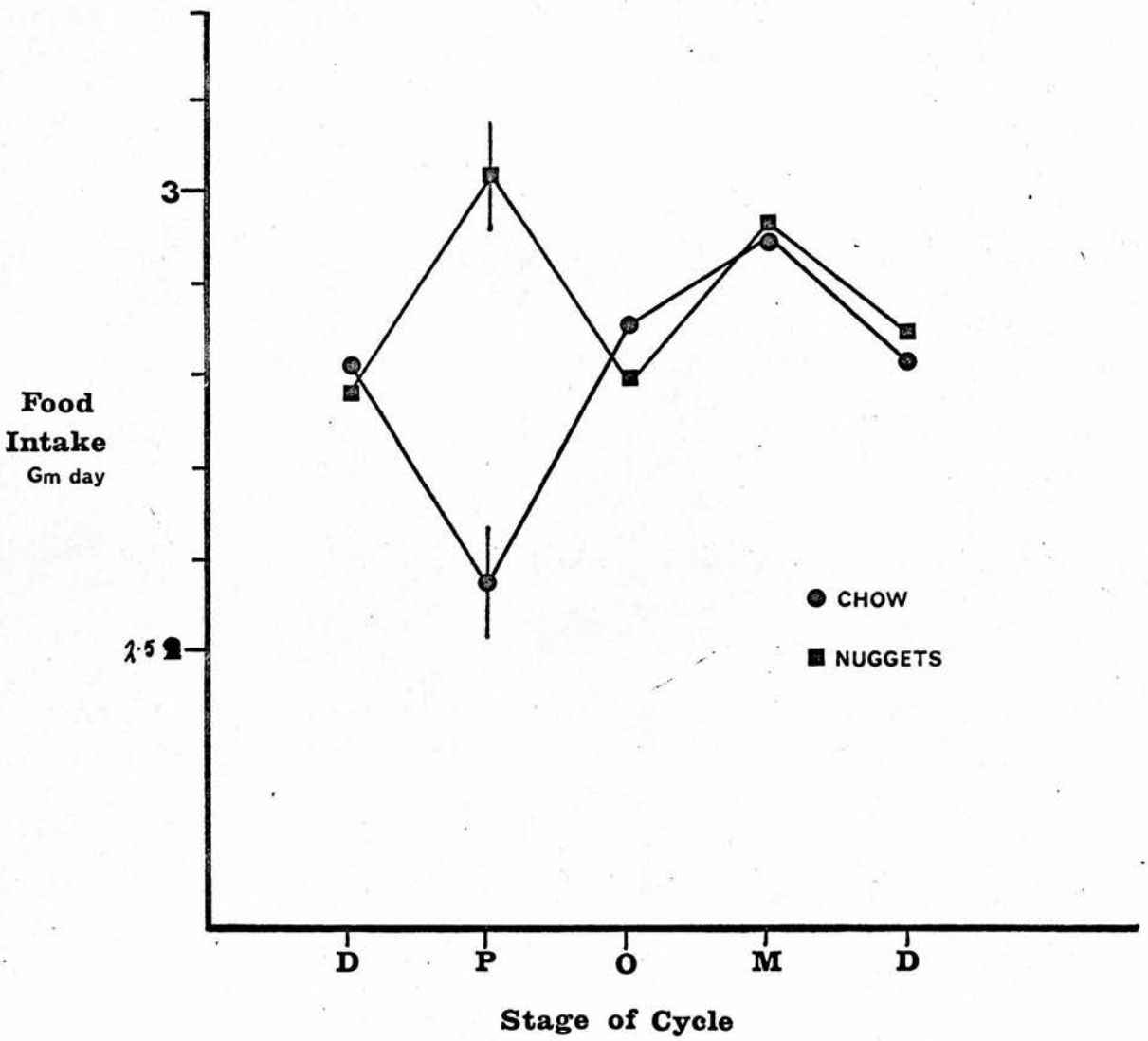

Food
Intake
Gm day
3
2·5
CHOW
NUGGETS
D
P
O
M
D
Stage of Cycle

TABLE 3.7(i)

The intake of two foods over the oestrus cycle in the mouse

(i) LABORATORY CHOW

D	P	O	M
2.82	2.54	3.06	3.10
2.77	2.58	2.53	2.95
2.87	2.65	2.70	2.97
2.85	2.50	3.0	3.02
2.85	2.65	2.93	2.58
2.94	2.70	2.98	3.36
2.86	2.50	3.04	2.72
3.30	2.53	3.35	3.36
2.62	2.36	2.46	2.50
2.52	2.20	2.75	2.97
2.60	2.47	3.0	2.95
3.02	2.58	2.74	2.70
2.83	2.53	2.87	2.94

g/day mean of 5 cycles each

Friedman X = 18.3 P<0.001

(ii) GOLDEN NUGGETS

D	P	O	M
2.6	2.66	2.38	2.45
2.47	2.85	2.62	2.61
2.90	3.40	2.74	3.31
3.0	3.0	3.24	3.27
2.84	2.98	2.98	3.13
2.88	2.98	3.08	3.0
2.92	3.06	2.88	2.93
2.78	3.27	3.14	3.10
2.92	3.68	3.20	3.30
2.34	2.64	2.24	2.80
2.63	2.48	2.33	3.11
2.98	3.42	2.81	3.14
2.77	3.03	2.79	3.01

g/day mean of 5 cycles each

Friedmans X = 15.1 P<0.005

18.3 P<0.001; Nuggets Friedman's chi 15.1 P<0.005). In the case of laboratory chow, this is exactly the same picture as was seen in section 3.5, with a fall in intake at the beginning of the cycle, and a rise towards the end. The picture for animals eating Golden Nuggets is, however, quite different, there being a rise in the early part of the cycle, followed by a fall, and then a second rise. The two patterns of intake cannot be superimposed by any movement of one or the other across days.

3.7.4. Discussion

The nature of the variation of food intake across the oestrus cycle is therefore dependent, in this strain of mice at least, on the stimulus properties of the food presented, the oestrus disturbances being a rise for the sweet food, and a fall for ordinary laboratory chow. The changes in feeding towards the end of the cycle are not, however, affected by these two foods, being the same that is to say a rise, in both cases, (depending on what food was used this could explain the results of Dewar (1957)). This provides evidence for the assertion that the short term changes in feeding associated with sexual receptivity at the beginning of the cycle are quite different to the longer term influences acting towards the end. It is necessary, therefore to determine the effects of ovariectomy upon the intake of Golden Nuggets.

3.8 Ovariectomy and Golden Nuggets

3.8.1. Methods

Eight adult female C57 mice were ovariectomised in the usual way under equithesin anaesthesia. They were caged individually in the experimental /

experimental room for a week before and three to four weeks after the operation. The intake of Golden Nuggets was followed for a pre-operative week. Immediately before and after the operation the animals received laboratory chow for four days in each case (to avoid Garcia conditioning effects - see Chapter 12 for discussion of these). Thereafter the intake of Golden Nuggets was monitored for three weeks in the usual way.

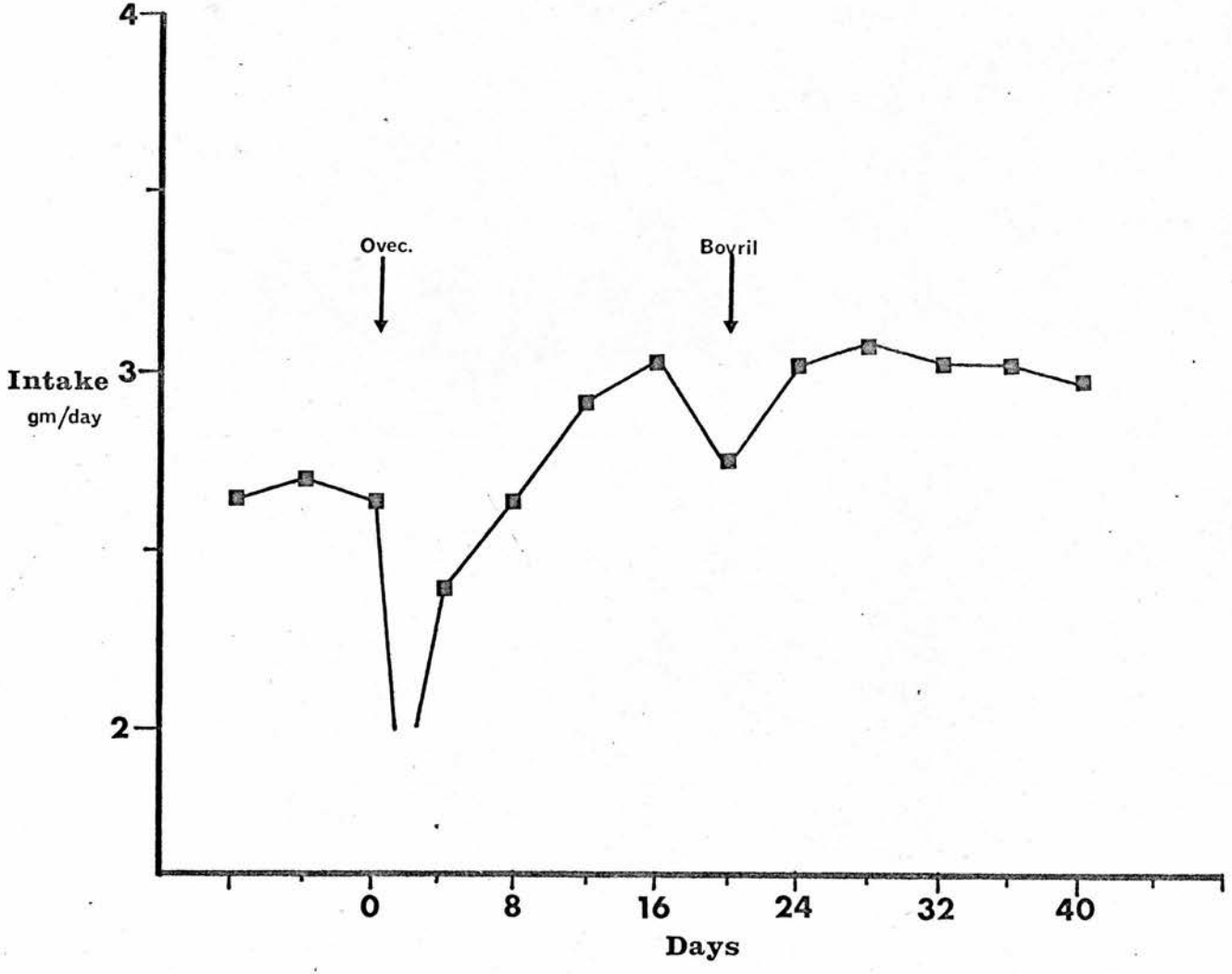
3.8.2. Results

The ovariectomised animals' intake of Golden Nuggets is shown in fig 3.8(a). Soon after the operation the intake begins to rise, and continues to rise for some two weeks. At this time, however, there is a precipitous fall in food intake to levels considerably less than those of intact animals, and the subjects become obviously ill and lethargic. If, however, they are given 'bovril' to drink this process can be prevented, and food intake remains elevated above control levels. The animals were given a solution of 'Bovril' to drink for four hours each day. The solution was made up by mixing four teaspoonfuls of extract to a litre of boiling water. After cooling the solution was placed in water bottles in the cages. All animals were observed to drink almost immediately. (Intake increase immediately post op. $P<0.01$ - Wilcoxon test, two and a half weeks post op. without bovril significant fall $P<0.05$ Wilcoxon, with bovril, increase above pre-op $P<0.01$ Wilcoxon). The animals gained little weight during the initial hyperphagia (ns Wilcoxon) and if not treated with bovril subsequently lost weight. The bovril supplemented animals gained some weight, and after the twelve days were just heavier than pre-operatively (and age control unoperated animals from the same batch) ($P<0.05$ Wilcoxon and Mann Whitney 'U' test against controls).

3.8.3. Discussion /

Fig. 3.9(a)

The intake of golden nuggets after ovariectomy in the female C57 mouse


4
3
2
Intake
gm/day
Ovec.
Bovril
0
8
16
24
32
40
Days

3.8.3. Discussion

The initial hyperphagia following ovariectomy is still apparent when the animals are fed the sweet food. After a while, however, the animals become ill, a process which may be prevented by a dietary supplement of protein rich bovril.

The calorific value of golden nuggets is trivially less than that of lab chow. If the animals were simply showing an increase in body weight set point, then the ingestion of golden nugget calories to build fat should be as good as those from chow. In fact the animal overeats, but still becomes ill, which the intact animal on the same food does not (see previous section). The implication from this is that a diet, sufficient for the intact animal becomes insufficient after ovariectomy. In the light of the observation by Leshner and Collier (1973) in rats, showing that ovariectomised animals increased their intake of protein, it could be that golden nuggets, which are relatively short on protein (most of their calories being contributed by the sugar that makes them sweet) are insufficient to fill the protein demands of the animal after ovariectomy. These insufficiendes will not occur if extra protein is added via the drinking solution, which was the purpose of giving 'Bovril' to drink. This experiment could therefore offer support to the hypothesis that ovariectomised animals grow rather than get fat, although a great many more studies would need to be performed before this could be certain.

With qualifications, however, it can be asserted that, after ovariectomy, the intake of both laboratory chow (see previous section) and golden nuggets is elevated, which is a picture qualitatively different to the situation at prooestrus.

3.9 /

3.9 General Discussion

This chapter has covered a lot of ground in no great detail. The aim was not to provide an exhaustive survey of the effects of gonadal hormones on food intake in the mouse, but to establish a basic framework against which to compare the results of the following chapters.

In the long term mice behave very much like rats, males are larger than females, and this dimorphism is produced by a dual action of testosterone in the male and oestrogen in the female. The oestrogen effects may be reversed by ovariectomy in adulthood, and replaced by both continuous and intermittant oestrogen therapy.

Food intake also varies over the oestrus cycle, there being a depression of the intake of laboratory chow at prooestrus.

The long and short term effects may possibly be dissociated by altering the stimulus properties of the food independently of its calorific value. There is an elevation of the intake of sweet food at prooestrus. At metoestrus, and after ovariectomy the intake of both sweet food and chow rises, implying the operation of a different mechanism in this case.

The short term effects could possibly be characterised by a strong reaction to the stimulus characteristics of foods in isolation from its calorific value. The animals show feeding behaviour that has in humans and dogs been called 'externally controlled' (Schacter (1972)), Jacobs and Sharma (1969)). The same type of feeding is associated with other changes in obese humans (Schacter (1972)).

One /

One such change also appears over the menstrual cycle in normal weight women. Cabanac and Ducleux (1968) have shown that normal weight subjects develop an aversion to sweet tasting solutions for some time after the ingestion of a glucose load. This aversion is attenuated in obese subjects. Wright and Crow (1974) have similarily shown that it is attenuated in normal weight women at the middle of the menstrual cycle, which is the period analogous to the period of sexual receptivity in mice. Furthermore, women gain weight slightly at this time (Russell (1972)), a change quite the opposite to that which might be expected if their intake of food was depressed as was thought to be the case with rodents. It is, however, exactly what would be expected if their feeding came to temporarily resemble that of obese subjects, so that they ate independently of their metabolic needs.

The sexually receptive mouse therefore potentially represents a model of both the changes over the menstrual cycle, and possibly those in obesity, which may illustrate some biological change which could underly some aspects of the development of obesity.

There is, however, one disparate publication. Jennings (1973) reports a fall in the intake of a sugar solution at prooestrus in rats. He, however, presented his rats with sugar for only one hour at a time when they would not be expected to be sexually receptive (referring to Drewitt (1973)). I shall present evidence in Chapter 5 to suggest that the changes described above are quite exclusive to the period of sexual receptivity so that in fact the two findings are not irreconcilable.

It would be of interest to examine in detail the mechanisms of these changes, /

changes, and this may be attacked by considering the details of the temporal pattern of feeding, which will be the subject of the following chapters.

CHAPTER FOUR

THE AUTOMATED RECORDING OF FEEDING BEHAVIOUR

4.1 Introduction

Simple measurement of the amount of food that the animal consumes in any given interval is a very gross indicator of the operations of the feeding system. If the constraints affecting feeding are to be studied in any real detail, then the normal operating characteristics of the systems controlling and regulating feeding must be determined in as much detail as possible.

One of the prime requirements for such a definition is an accurate record of the occurance of feeding behaviour over time, and this chapter describes automated methods used for monitoring the details of the temporal organisation of feeding in the mouse.

The occurance of any given activity may be specified in terms of periods of presence, that is to say 'bouts', and periods of absence 'inter-bout intervals'. The event of theoretical and practical importance are transitions between behaviours which define the boundaries of bouts and interbout intervals. Simple classification of all other behaviours as periods when the behaviour of interest is absent is a considerable simplification, as the behaviour preceding or following a transition will affect its occurance. It is impossible to record for any useful length of time however, the precise nature of the transitions involved, so that in this case only feeding and 'non-feeding' will be considered.

The /

The automated recording of behaviour has two prime requirements. Firstly some method must be obtained for telling when a particular activity is occuring, and secondly, some economic means of recording this information must be derived.

Considering the latter problem first.

4.2 Recording

We will assume that the feeding monitor (described below) yields an output such that so long as, and only so long as, the animal is feeding a switch, normally closed, is open. It is therefore necessary to design a system which will produce a record of hours and days of feeding in a form sufficiently concentrated not to require an impossible number of man hours for scoring. Furthermore, the technical specification of the system must be such that it can withstand recording 24 hours a day seven days a week for several weeks without failure leading to significant loss of data.

The use of a simple event recorder is clearly impossible because of the unbearably large number of hours which would be required to reduce the record to the behaviourally interesting set of bout lengths and interbout intervals. The ideal output would be a digital record of successive bouts and intervals.

This may be achieved in two ways. Either the output from the feeding monitor may be connected to a conventional tape punch with timer, and the resulting tape analysed by computer, or some means be derived whereby the data may be reduced automatically during collection into bout lengths and intervals.

The /

The tape punch available in the department was notoriously unreliable, one being unable to trust it to work for two hours, let alone the minimum of twelve necessary to study feeding over the oestrus cycle. The computer analysis of larger number of twelve hour records would also prove a considerable strain on the available computing resources, so for these reasons a unit was designed which would provide a reduced digital output, without the need for the intervention of computers.

Logically, both in the recording, and the behaviour sequence itself the events of importance are the transitions, and their specification in time identifies the sequence absolutely. The first operation to be performed on the input, therefore, is to reduce the switch openings and closings to a series of pulses, one specific to each transition (i.e. open/close, close/open).

In an ideal world each transition would lead to the establishment of a record of the transition and its time of occurence relative to the previous opposite transition (that is to say either a bout length or interbout interval). The only output devices available, however, take time to act. In the event of a second transition occuring within the operation time of the device, the data would be lost without the intervention of some buffer store. The complexity of the buffer store multiplies inversely with the minimum time between transitions. Transitions separated by very short intervals may arise from two sources, either instability in the behaviour of the animal or in the operation of the feeding monitor.

Preliminary considerations led to the conclusion that the latter

of /

of these two effects was the more damaging, and that 'bounce' in behavioural transitions operated over a relatively long time scale comparable with the operating time of the printer, (that is to say bouts had a minimum duration round about one to two seconds). The artefactual recording bounce, arising largely from true contact bounce, and other artefacts produced by the movement of the animal whilst feeding, was therefore designed out of the system, so that it could not be recorded.

This was achieved by a form of time sampling procedure.

The operation of the recorder was crucially dependent upon a central timer which produces a time mark every two or three seconds depending on the output device. This time mark, a 50 msec pulse can have one of two effects. Either it causes a 'Sodeco' print out counter to increment by one, or to increment, print out, and reset to zero. Which of these alternatives is the case depends on the occurence of behavioural transitions in the interval since the previous time mark. Depending upon the nature of the previously recorded transition pulses from the feeder monitor operate a bistable unit. If the previous recorded transition is off/on, for example, then the opposite transition namely on/off will elevate the bistable to its activated state. Subsequent off/on transitions during the interval will reset the bistable.

At the time mark, if the bistable is reset, then the counter simply counts. If however, the bistable is activated, that is to say there has been a net change in the status of the feeder, then the counter counts, prints and resets. The bistable is also reset, and the nature of the transitions producing activation and resetting reversed.

In /

In this way the logic 'looks' for a net on/off in each time interval. When one is found, it prints out the time since the previous off/On, as a number of whole units, and then proceeds to 'look' for an off/on, which, when found will lead to the printing of another interval. The result of this process is a record of successive bout lengths and inter-bout intervals, each to the nearest number of whole units.

It is important to realise, however, the probabalistic nature of the recording process. Two transitions separated by a very short interval have a low, but not negligible probability of being recorded as a bout of one unit, depending on how they are situated in time relative to the time mark. Similarily, for example, a bout of duration 2.1 seconds is very likely to be recorded as a single two second unit. but it does have a very low probability of being recorded as four seconds (two units) long.

Given these qualifications, the recorder produces from an appropriate input an easily digestible summary of the temporal pattern of feeding in the mouse. Its operation is reliable (see later sections), so that some data can be virtually guaranteed from each days recording.

4.3 The Feeding Monitor

Having devised a suitably economic recording method it is necessary to devise a means of accurately monitoring feeding. The method chosen here was to place the food in a feeder, such that, in order to feed from it, the animal must push open a light door, which operates a switch connected to the recorder.

The /

The feeder was a perspex box 10 x 8 x 6 cm, with a hole 8 x 4 cm 3 cm up one of the larger faces. A light inward opening door was fitted over this hole, and by pushing on the door the animal could gain access to food inside the base of the feeder. The door contained a magnet which held an opposing reed switch closed except when the door was open. The whole feeder was suspended from the wire top of a standard laboratory cage. The spout of a water bottle projected in close proximity to the door of the feeder (see Diag. 4.3(a) for details of feeder).

The feeder base contained ground food of a type appropriate to the experiment concerned. The food was ground so that the animals could not remove sufficient from the feeder to sustain a feeding bout elsewhere. This forced all animals tested into a stereotyped mode of feeding. They would jump onto the feeder, perching with their hind legs on a small lip provided at the base of the door, and lean in, holding the door open with their backs, feeding on the ground food with their mouths and front paws. This strategy was common to all animals observed feeding in the apparatus. Insulated leads connected the feeders to the recorder, which was in most cases in the same room as the cages, although the animals were also tested occasionally in acoustic isolation from the recorder.

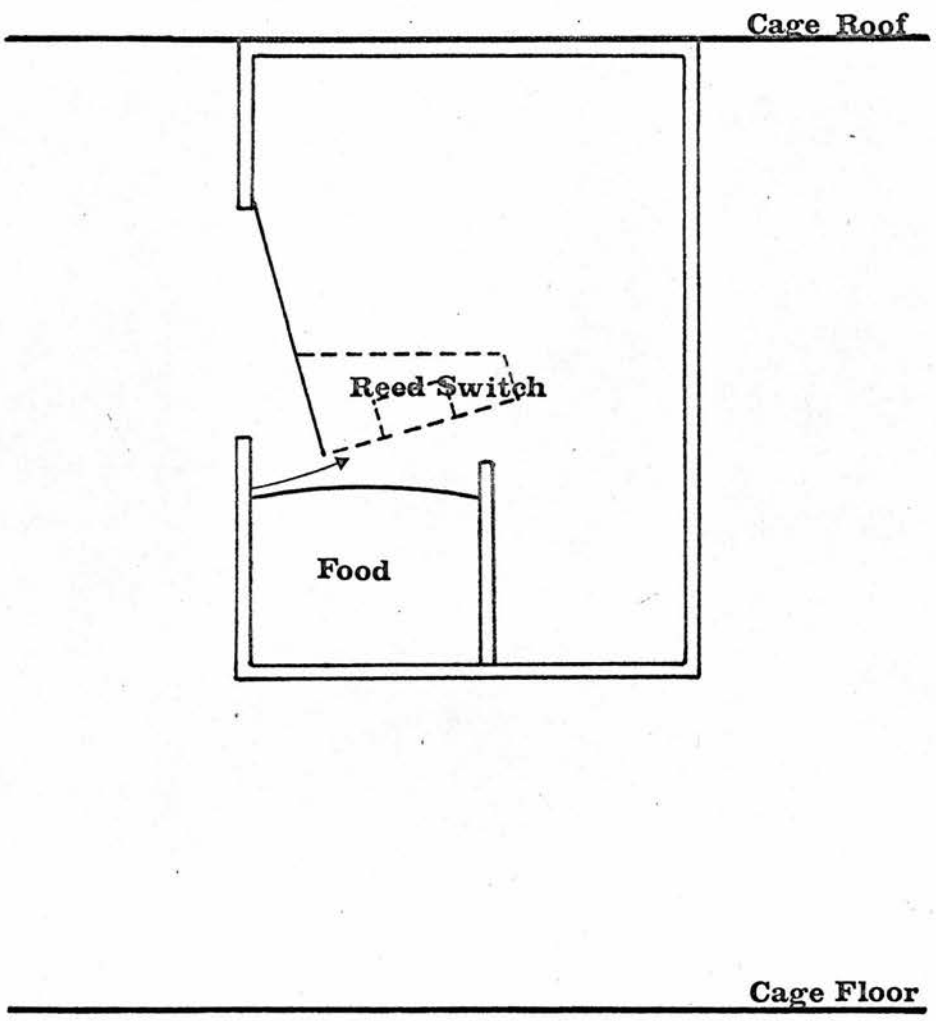
4.4 Validation

Before this apparatus may be used to monitor feeding behaviour over long periods its efficiency at producing an accurate, valid record must be established. Validation has two aspects, on the one hand it must be ensured that the machine records accurately when the animal is feeding, and on the other it must be checked that a record of time spent feeding accurately reflects the amount of food consumed.

4.4.1 /

Fig. 4.3(a)

The feeder monitor (vertical cross section)


Cage Roof
Reed Switch
Food
Cage Floor

4.4.1. Correlation with observer records

In this study the feeding behaviour of mice was monitored automatically, and observed directly at the same time.

Subjects were eight female C57 mice. All were habituated to the feeders and cages, for one week, during which time all food was obtained from the feeder. Following this period, they were food deprived for 24 hours, and then allowed to feed from the feeders, with the recorder switched on.

The feeding behaviour was automatically recorded for half an hour after introduction, being simultaneously observed, and recorded directly on a twelve channel event recorder.

For each animal the total time spent recorded feeding, the mean bout length, and the number of feeding bouts, was scored from each record. Observer recorded feeding was defined as contact of the mouth with food for at least one second.

Correlations were calculated for the pairs of readings of each measure. The figures are presented in Table 4.4(i).

It can be seen that there is a close and significant correlation between the automatic and observer records of total time spent feeding and number of bouts. There is a slight (non-significant - Wilcoxon test) trend for the automatic system to over-estimate the number of bouts, but the total time spent feeding shows a close agreement.

4.4.2. Correlation between time spent /

TABLE 4.4(i)

Comparison between the variables of feeding recorded automatically and those obtained by direct observation

Total Time Feeding (sec)	
obs.	rec.
82	88
97	92
86	94
109	100
124	136
131	128
94	96
108	101
103	104

$r_s = 0.88$ $P<0.01$

No Bouts	
obs.	rec.
11	12
15	15
12	11
14	16
17	19
18	18
16	15
15	17
14.7	15.4

$r_s = 0.79$ $P<0.05$

Mean Bout Length (sec)	
obs.	rec.
7.4	7.3
6.5	6.1
7.2	8.5
7.8	6.3
7.3	7.2
7.3	7.1
5.9	6.3
7.2	5.9
7.1	6.9

$r_s = 0.35$ n.s.

4.4.2. Correlation between time spent feeding and amount consumed

In this case animals were put on a reversed light cycle, and the feeders filled and weighed at the beginning of the dark period. The total time spent feeding in various periods was correlated with the weight lost from the feeder.

Subjects were again eight C57 female mice. The room containing the feeders was placed on a reversed light cycle, lights off at 9.00 a.m. and on at 9.00 p.m. On the days of test the feeders were filled and weighed (to 0.1 gm) at 9.30 a.m. The weight of the feeder, together with the cumulative total time spent feeding was recorded for each animal after 2, 6, and 9 hours. Intakes were calculated for each period, and correlated with the time spent feeding.

Results are shown in 4.4(a) which illustrates the regression between time spent feeding and food intake. The least squares fit regression line yields a value of 1.8 mg of food consumed per second feeding time. This agrees well with the figure of 1.6 - 2.0 mg/sec reported by Wiepkema (1968) for his mice.

4.4.3. Discussion

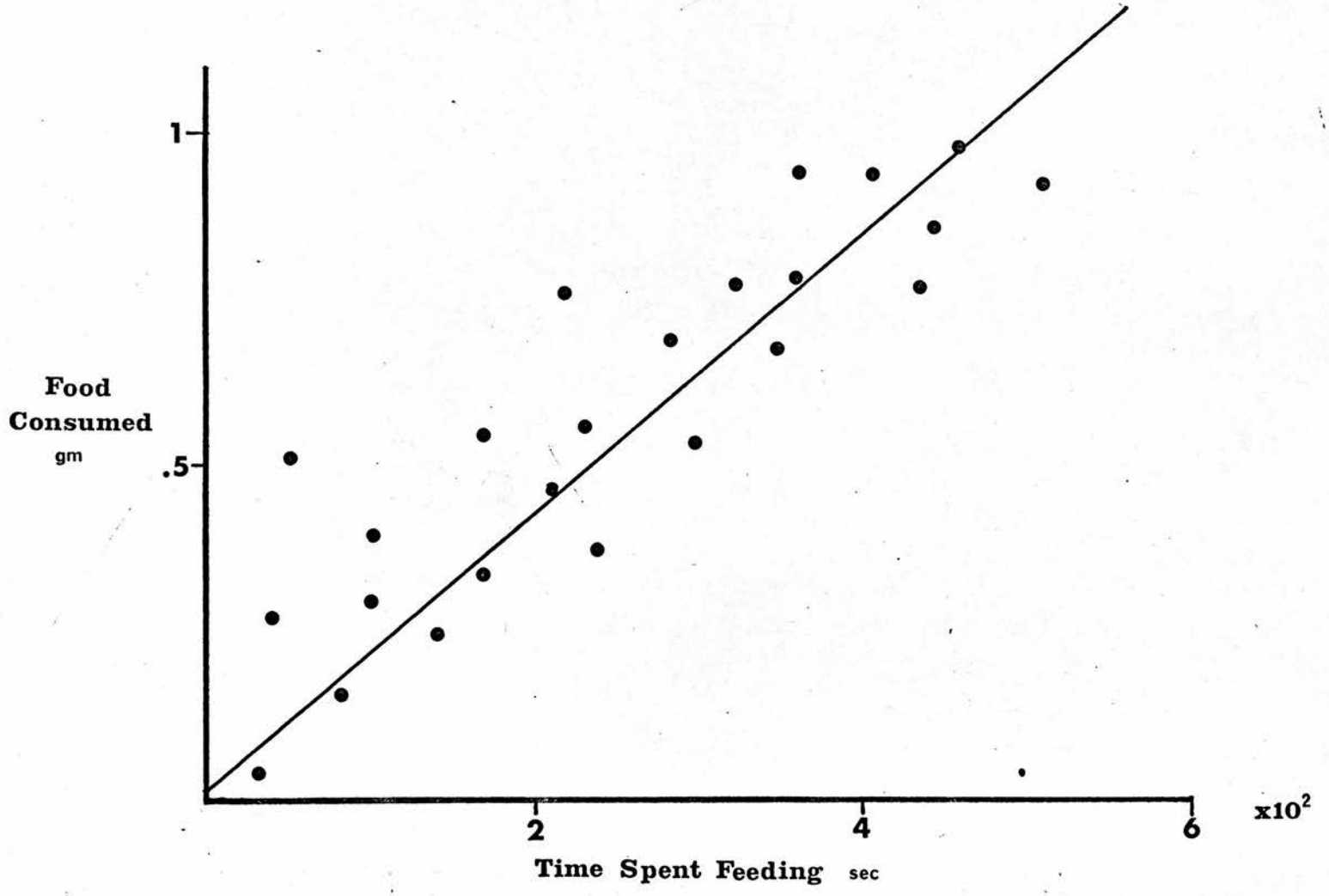
It would appear that the method of recording adopted here yields an accurate picture of the temporal pattern of feeding in the mouse, in that it records most feeding bouts and maintains a faithful record of both total time spent feeding, and indirectly the amount of food consumed.

4.5. The organisation of the observations of feeding patterns

4.5.1. Facilities available /

Fig. 4.4(a)

Correlation between total time recorded as spent feeding and the amount of food consumed


1
.5
Food
Consumed
gm
2
4
6
x10²
Time Spent Feeding sec

4.5.1. Facilities available

The recording circuits described above could in principle be constructed from either electronic or electromechanical logic. Both methods were used. Solid state logic was assembled into a recorder having two channels each outputting through a 'sodeco' printout counter with separate decade units. Another four channel unit was constructed from electromechanical logic. Two channels outputted through 'sodeco' counters of the same types as those used for the electronic logic. The remaining two channels utilised a more rapidly operating sodeco unit. The two counter types differed in the time required for the complete output operation, The four separate decade counters were arranged with a maximum sensitivity of two seconds, whereas the others operated at a one second timing interval. The fact that the two types yielded identical records is another powerful technique of validation. The failure modes of the two systems, a point of vital importance in a study of this type, are discussed below.

In all, therefore, for most of the period of study there were six recording channels continuously available, excepting, that, because of the need to integrate with other members of the department, the solid state logic could often only be used during the night.

4.5.2. Subjects and Organisation

The following sections of the thesis describe the results of experiments in which the feeding behaviour of normal cycling females, males, castrate males, and ovariectomised females is examined. For all of the experiments subjects were C57 mice of appropriate sex, at least 100 days old at the start.

The /

The feeding behaviour of intact females was recorded over the oestrus cycle with either of the two foods described in Chapter 3. Males, castrate males and ovariectomised females were recorded eating only laboratory chow.

In the oestrus cycle experiments the feeding behaviour of seven females was followed on laboratory chow, and five females feeding on golden nuggets. The oestrus cycle experiments took place between March and July 1974. Initially two animals were placed in standard cages with feeder monitors, and put in a sound attenuating compartment on a standard 6.00 a.m. to 6.00 p.m. light cycle. The feeders were connected to solid state logic recorder in another room. Once each day, in the early afternoon, the feeders were filled, and vaginal smears taken from the animals in the usual way. At the same time the record was removed from the printers, labelled and stored. One animal was fed laboratory chow and the other Golden Nuggets, in both cases ground. The behaviour of the animals was recorded for ten days, and then the experiment began in earnest.

Each animal was followed for five weeks. Vaginal smears were stained and read, and the cycle days designated. Then, and only then the stored records were collated into groups for each day of the cycle. Scoring is described in following chapters.

During this experiment the relay logic was assembled, so that more animals could be followed. Thereafter animals for study over the oestrus cycle were recorded using the electomechanical setup, and other groups on the electronic recorder.

The /

The relay logic was in a separate room, with the lights on the same 12 hour normal light cycle. For most of the time the animals lived in the same room as the logic, and its noise, which did not appear to affect them. Of the four animals recorded at any one time two were given laboratory chow, and two golden nuggets. Each food was allocated one two second and one one second channel. Four animals were recorded on each food in two batches of five weeks exactly as above. A further two animals were recorded on laboratory chow during another (abortive)experiment, whose results were incorporated into the following chapters.

In addition the relay logic was equipped so that a record of the total time spent feeding in the night and day was taken (see Chapter 6). Four intact males were recorded on the solid state logic, and four on the relay logic according to a paradigm used for all animals where records were only required over a few days. The animals were brought into the experimental room two weeks before the start of the experiment (to adapt to the new light cycle). After this time they were put onto the feeders for a week, and the final three days data taken for analysis.

The relay logic was also used to record the feeding of six castrate males and seven ovariectomised females according to the same paradigm. Animals were also recorded after injections of gold thioglucose (see below).

4.6 Instrument reliability and the vetting of records

4.6.1. Modes of instrument failure

Before data was allowed to enter the general data pool it and the apparatus were scanned for evidence of machine failure, and whole or part of the record discarded should any suggestion of breakdown occur.

Overall /

Overall the probability of failure was relatively low (see below) but it was essential to remove artefactual data to ensure that it could not distort the results.

Machine failure falls naturally into three classes, those associated with failure of the output devices, those with failure of the logic, and those where the feeder monitor is at fault. 500 hours of preliminary record were checked for machine failure before the experiments began, and strategies devised for at least detecting, and often eliminating such failure.

The printers were by far the most failure prone. Failure here fell into two classes. Firstly, those associated with the mechanism of printing, usually a failure of paper advance, which was detectable but unavoidable, and in fact contributed by far the greatest loss of data. Secondly, in the printers with separate decades there were often failures of carry between decades, so that the number recorded was not the same as that entered. There were also occasional failures of reset.

Both these latter faults could be detected by the fact that, as they always tended to elevate numbers rather than reduce them, they produced an elevation of the total apparent lapsed time. This was checked in the solid state logic by providing an extra counter to count the true total elapsed time directly from the master timer. Comparison of the apparent and total lapsed time shows up any failures of this kind. On the relay logic this was allowed for by having a fifth 'check' counter connected alternatively to the two separate decade units to monitor their operation, and ensure that they were functioning correctly.

The /

The electronic logic was prone to failure in many ways, most of which were obvious. The commonest problem was a failure to alter the sought transition, whose outcome was a concatenation of successive feeding bouts and interbout intervals. This was checked by using one of the decades available on the separate counters to simultaneously record only feeding time (by a separate mechanism). Discrepancies between the two recorded figures indicate logic failures.

Logic failure, whilst it was guarded against in the same way, for the electromechanical logic, was so rare that it caused the loss of very little data. Each channel tended to fail in some small way only once every four or five weeks.

The principal reasons for failure of the feeding monitors were the attentions of mice who proved astoundingly inventive in finding ways of disrupting the experiment. All such disturbances, however, led to easily recognisable changes in the records, so that they could be disregarded.

Overall, given the high probability of failure of the printers, some data at least was lost on a fair proportion of days (30% or so), but a complete days record was lost on only a small number of occasions. The use of a very harsh criterion of data acceptibility, backed up by a number of automated internal checks does however ensure that the record stands a very high chance of avoiding distortion as a result of purely mechanical faults.

4.6.2. Discussion

Over all the experiments the two recording set ups managed about nine /

nine thousand channel hours of recording with no obvious faults, including periods of continuous operation of up to seven weeks.

It can, therefore be concluded that the machine produces to reasonable limits of accuracy, and with reasonable consistency, reliable data as to the temporal organisation of feeding in the mouse. The following chapters will describe the analysis of data so collected.

CHAPTER FIVE

GENERAL CHARACTERISTICS OF THE TEMPORAL PATTERN OF FEEDING IN THE MOUSE

The output from the apparatus described in the previous section comes in the form of an ordered sequence of feeding bout lengths and interbout intervals. In order to form any coherent analysis of changes in feeding patterns some general characteristics of mouse feeding must be recognised, so that variations in these characteristics may be followed over the various test conditions. This chapter aims therefore to develop ways of specifying meaningful variables of feeding behaviour.

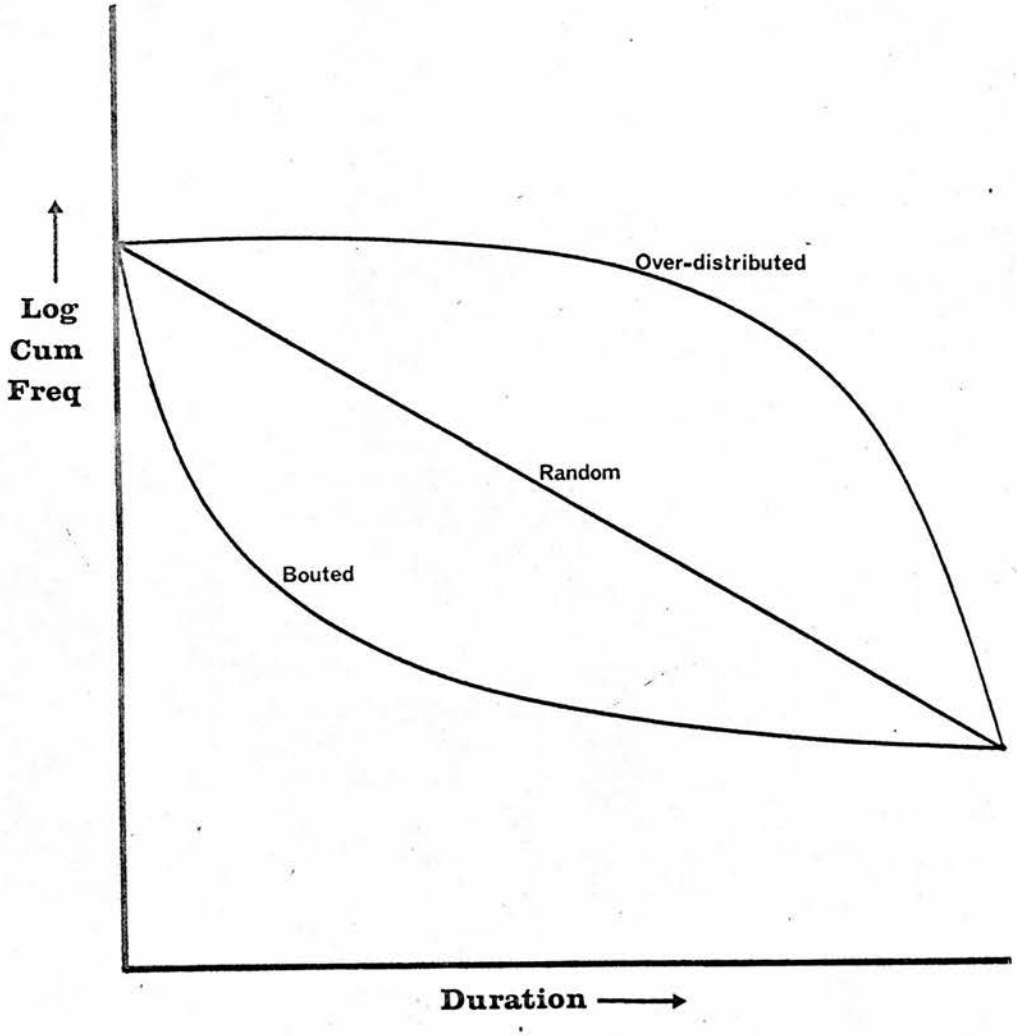
5.1 Temporal distribution

One of the first questions to ask is how the feeding bouts are distributed with respect to one another in time. Given that a feeding bout has occured, then there are three possible conditions governing the occurence of a second. Firstly it may be that the occurence of the second bout is completely independent of the first, secondly it may be that there is a certain time after the occurence of a bout during which another bout cannot occur, so that the bouts are separated by at least some constant time. The third possibility is that the occurence of a feeding bout predisposes the animal to return to feeding soon afterwards, so that the bouts themselves are organised into groups.

These alternatives may be investigated by a consideration of the distribution of the interbout intervals, which is itself best represented for this purpose as a logarithmic survivorship curve (Nelson (1964)). In this analysis the range of potential interbout intervals /

Fig. 5.1(a)

Examples of the shapes of logarithmic survivor plots arising from different distributions of feeding bouts. (adapted from Slater (1975))


Log
Cum
Freq
Over-distributed
Random
Bouted
Duration

intervals (IBI's) is divided into equal or unequal parts, and the number of observations falling in each interval scored. The scores are cumulated from the longest to the shortest interval. In this way the proportion of any starting number of bouts remaining after each interval, that is to say the probability of an interbout interval ending in any particular interval, may be measured.

Considering the three alternatives above, if the first applies then this probability will be constant over time, so that a constant proportion of the IBI's will end in any given interval. As the survivorship curve is plotted on semi-logarithmic axes, (logarithm of frequency), such a situation would produce a straight line plot.

If the second alternative is the case, then soon after a bout the probability of another will be very low for a while, until the criterial period is passed, and will then increase. The survivorship curve should therefore be initially shallow, and then steep. If the third possibility applies, then the probability of a bout occuring a short time after a previous one is very high, so that the curve will be initially steep, and subsequently shallow. The three alternative survivorship curves are illustrated in Fig. 5.1.(a). (see Slater (1975)).

This analysis may be applied to the records obtained from the mouse.

5.2 Survivorship analyses

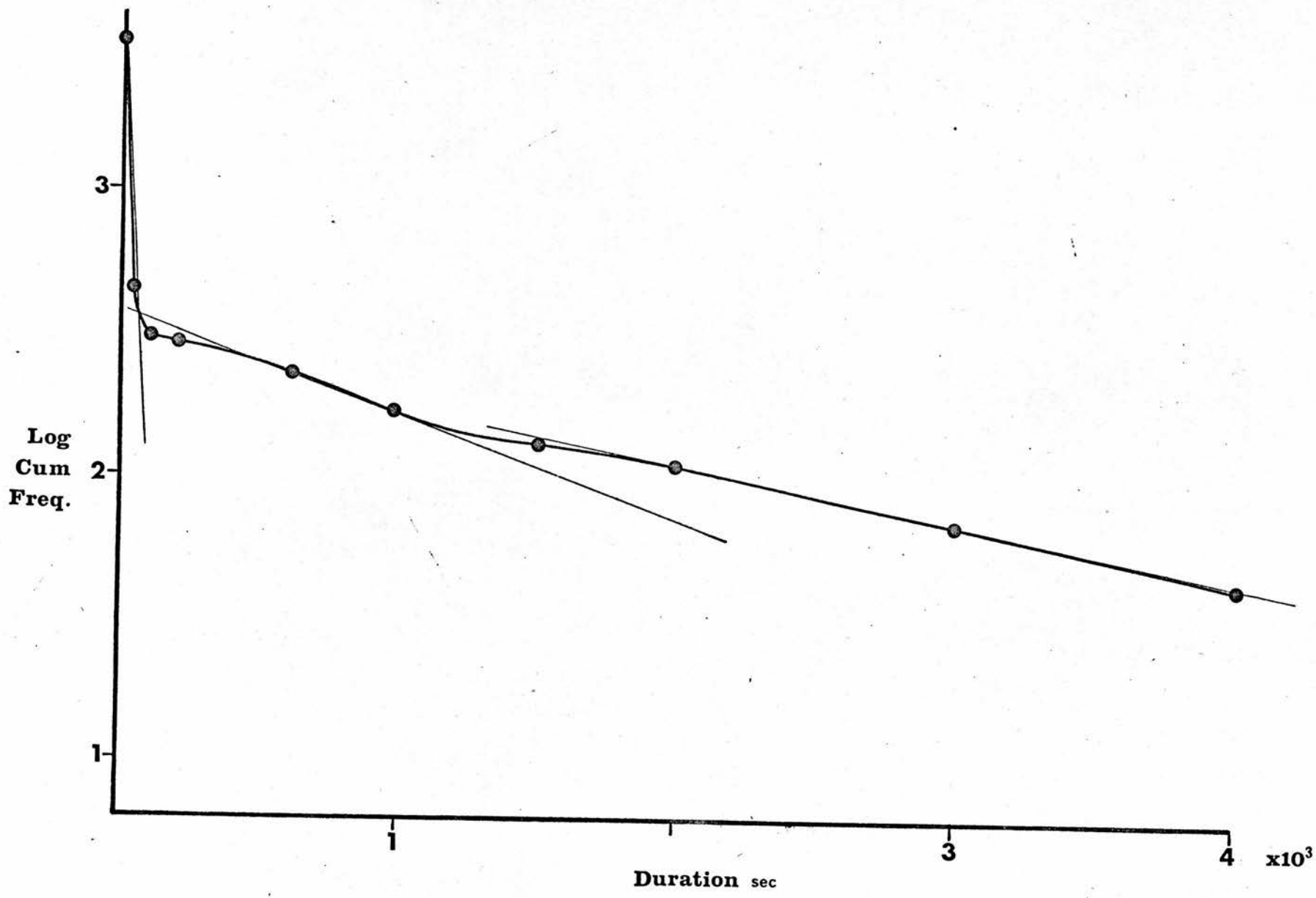
Survivorship data was collected from all feeding records taken on the female C57 mouse over all stages of the oestrus cycle. The product of this analysis is shown in Fig. 5.2.(a). The span of the intervals chosen for analysis varies with the time axis for reasons that are apparent from the shape of the curve.

It /

Fig. 5.2(a)

Logarithmic survivor plot of all interbout intervals

for feeding in the female C57 mouse


3
2
1
Log
Cum
Freq.
1
3
4
x10³
Duration sec

It can be seen that the curve is initially very steep, followed by a relative plateau, then a decline at a more shallow slope than before. The early part of this curve, it will be recalled, conforms to the criteria for a significant bouting of feeding bouts, so that they are organised into groups occuring closely together in time. These groups of feeding bouts may be designated 'meals'. Nearly 90% of all IBI's end before they get to 100 seconds, indicated that feeding bouts are organised into groups of ten or so, separated by intervals very short relative to the longer 'inter meal intervals'.

The second part of the curve, the relative plateau between 75 and 200 seconds conforms to the second paradigm. In this case it would appear that the meals are over separated, and there is a minimum time after a meal is ended before another can begin. Following the plateau the curve again shows a downturn. The rate of decay, however, is still not uniform relative to the time axis, there being an early component from 200 to 1200 seconds which is steeper than the later curve.

In all therefore, the typical semilogarithmic survivorship curve of the female mouse may be divided into four components, representing presumably four characteristics of the temporal pattern of feeding.

The initial steep component reflects the preponderance of very short IBI's within highly associated groups of feeding bouts which may be called meals. The second, practically horizontal section would indicate that after the end of a meal the animal must wait a certain time before another will begin. Once this refractory period is passed then the animal again has a significant probability of returning to feeding. This probability is initially high, but then falls.

This /

This analysis may be further illustrated by actually calculating the probability of an IBI ending in any given interval after it has begun. This is shown in Fig. 5.2.(b). This curve again reflects a division across time into the components discussed above.

The organisation of feeding into meals is not unexpected, and the meal variables have been commonly used in analysis. The changes in probability over longer IBI's is however more novel. The initial relatively steep component, followed by one more shallow is typical of a bouted activity, thus implying that the meals themselves are bouted, or organised into what might be called feeding 'sessions'. The statistical significance of this observation may be examined by comparing the probability of an IBI ending in any given interval over the range 200 to 1200 seconds with that over the ranges 1200 to 2200 and 2200 to 3200. This data is illustrated in table 5.2.(i), yielding a statistic significant at $P<0.02$.

In summary therefore, the temporal pattern of feeding in the mouse is characterised by the organisation of feeding bouts into meals, and the further organisation of these meals into feeding sessions.

5.3. Selection of variables

The pattern established in the previous section allows the specification of certain variables of feeding. Of these meal size and inter meal interval are most important (MS and IMI).

In order to designate a meal some criterial IBI must be chosen, so that groups of feeding bouts bounded on either side by an IBI greater than criterion will be designated a 'meal'. The supra-criterial IBI /

Fig. 5.2(b)

The probability of feeding beginning in any 200 second interval as a function of time since the last feeding bout

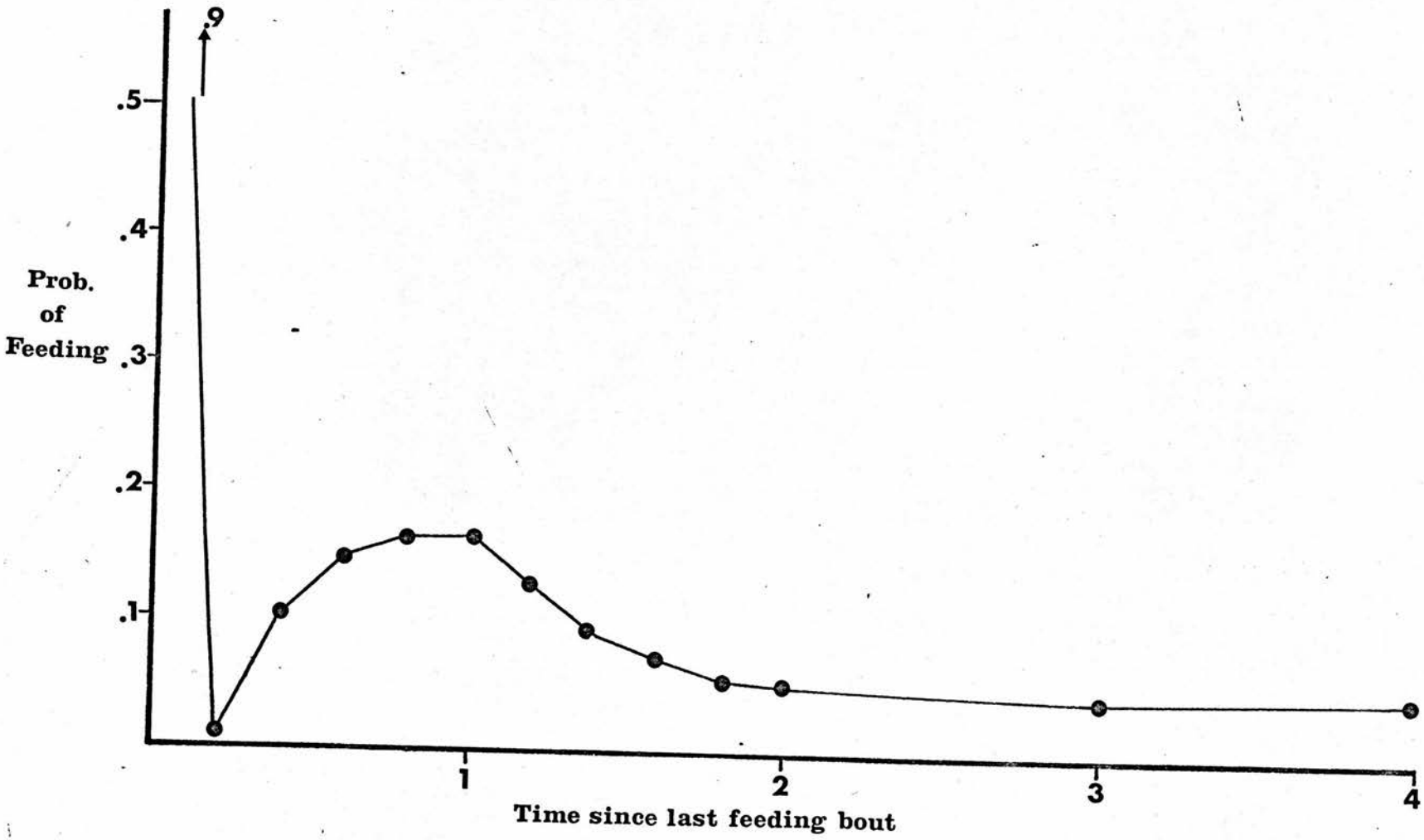

.9
.5
.4
Prob.
of
Feeding
.3
.2
.1
1
2
3
4
Time since last feeding bout

TABLE 5.2(1)

PROB OF ENDING IN RANGE

		Units		
		1200-1600	1200-2200	2200-3200
	1	0.41	0.31	0.34
	2	0.37	0.31	0.20
	3	0.52	0.20	0.26
	4	0.47	0.32	0.27
Animal	5	0.53	0.43	0.30
	6	0.26	0.17	0.28
	7	0.29	0.24	0.36
	8	0.46	0.33	0.34
	9	0.32	0.31	0.38
	10	0.64	0.48	0.26

Friedman's X = 8.6 P<0.02

Table includes only animals in which complete 24 hour records are available

IBI before the meal then becomes the preceding IMI, and that after the following IMI. IBI's within meals may be called intra-meal intervals.

The choice of criterion in this case is relatively simple. It will be recalled that the probability of feeding in the range 75 - 200 seconds is paractically zero. The criterion may be placed anywhere on this plateau, and will not affect the number of meals scored so long as it avoids the ascending portions of the curve.

Accordingly a criterion of 200 seconds (100 recording units) was taken as the most convenient for analysis, and all IBI's greater than 100 units were labelled IMI's.

The investigation of the higher order structuring of the meals into sessions is not quite so easy. The probability changes (see Fig. 5.2.(b)) are merely relative, taking the form of a hump followed by a plateau. The criterial IMI distinguishing and intra from extra session meal was therefore chosen as 1200 seconds, the effective end of the hump.

Consideration of the survivorship curves reported by Wiepkema (1968) for male mice would indicate exactly the same sort of pattern of organisation into meals and sessions, and the same criteria would apply (Wiepkema himself uses a criterion of 300 seconds for IMI's).

The occurrence of feeding sessions may be examined by considering the raw data records. It would appear that the mouse feeds in two or three night time sessions. It is well known that rats and mice show a pronounced /

pronounced circadian rhythm in feeding (Ter Haar (1973), for rats Wiepkema (1968) for mice), and as will be shown in the following chapter C57 females are no exception, eating some 80 to 85% of their daily intake during the night. Consideration of the survivorship curves for day feeding alone (see next chapter) indicates the usual organisation of feeding into meals, but no feeding sessions (with certain exceptions see next chapter). Sessions are therefore confined exclusively to the night.

As darkness falls the mouse begins to eat (as with the rat Ter Haar (1972), Drewitt (1973)), and at this time meals are closely spaced in a pattern typical of a feeding session. This peak of intake is followed by a period when meals are taken relatively less frequently, being separated by IMI's greater than 1200 seconds. Towards the end of the night the typical session pattern reappears at least once.

This may be illustrated by plotting the mean IMI following successive meals into the night over all the animals. This is the closest one can get, given the method of recording to a measure of the rate of intake as a function of time of day, without a very great deal of supplementary analysis. (The inverse of IMI - meal frequency is shown, as this correlates directly with intake - Fig. 5.3.(a).)

The pattern of feeding in sessions towards the beginning and end of the night shows up very well in this analysis, as reflected by the greater mean meal frequencies at these times.

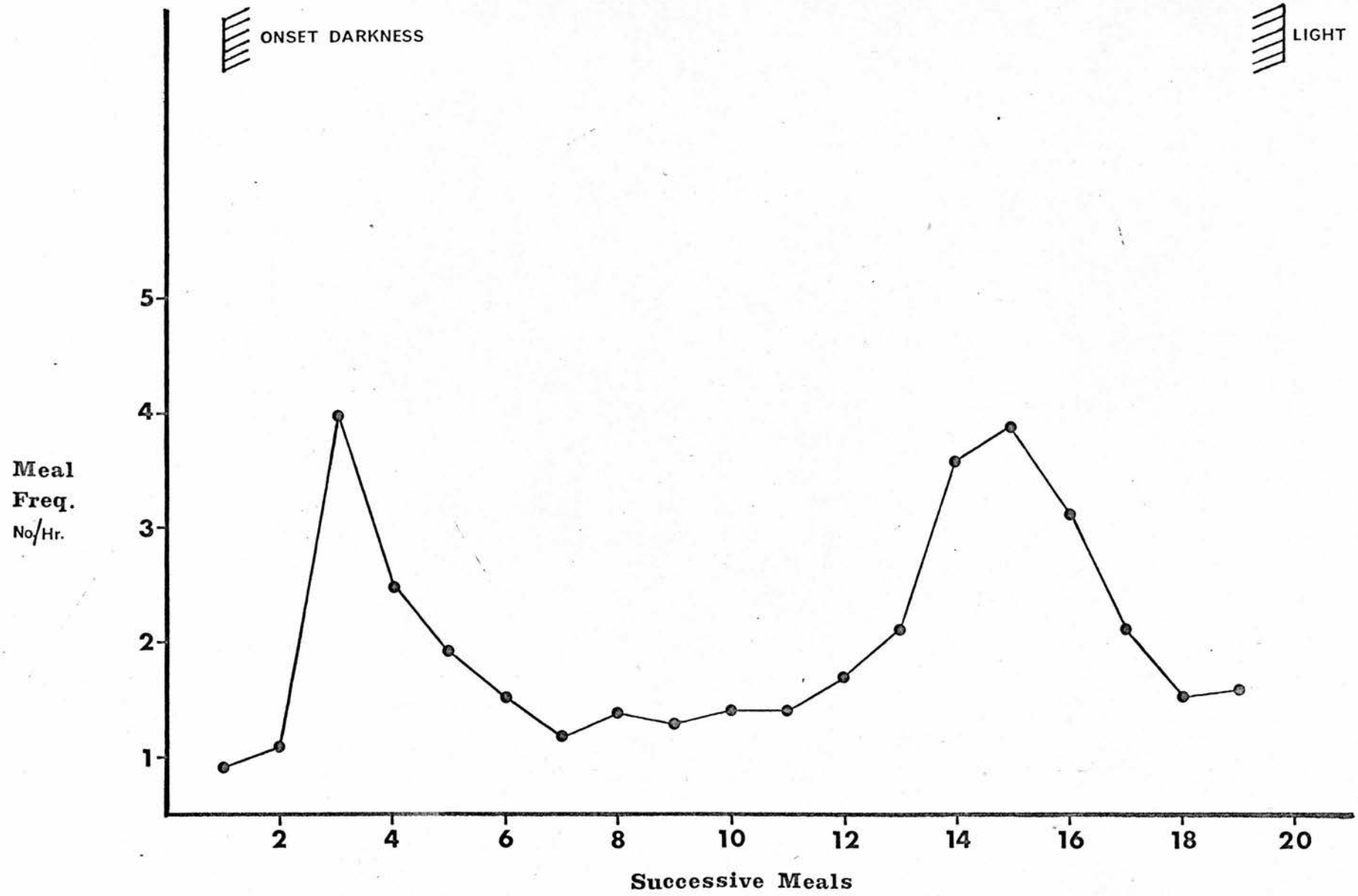
A list of variables characterising the feeding of mice under various conditions may therefore be prepared:

The /

Fig. 5.3(a)

Meal frequency as a function of successive meals into the night.

(Female mouse eating laboratory chow)


ONSET DARKNESS
LIGHT
Meal
Freq.
No/Hr.
5
4
3
2
1
2
4
6
8
10
12
14
16
18
20
Successive Meals

The two most important variables are meal size and inter meal interval. Inter meal intervals may themselves be separated into those falling within sessions, and those without. Within meals, the number of bouts and their lengths are also important and potentially useful variables.

5.4 Individual Differences

Slater (1974) emphasises the importance of considering individual differences in the temporal patterning of activities. Before, therefore the importance of changes in the variables so far designated may be determined, the degree of individual variation in the basic pattern must be assessed.

Unlike Slater's birds, the inbred C57 mice at least are very uniform in their pattern of feeding, which perhaps reflects their basic genetic homogeneity, and standard developmental history. This uniformity is illustrated in Fig. 5.4.(a). It can be seen that the curves derived from the individual animals, of which the most extreme types are illustrated, are sufficiently similar to allow the description produced above general applicability.

5.5. Discussion

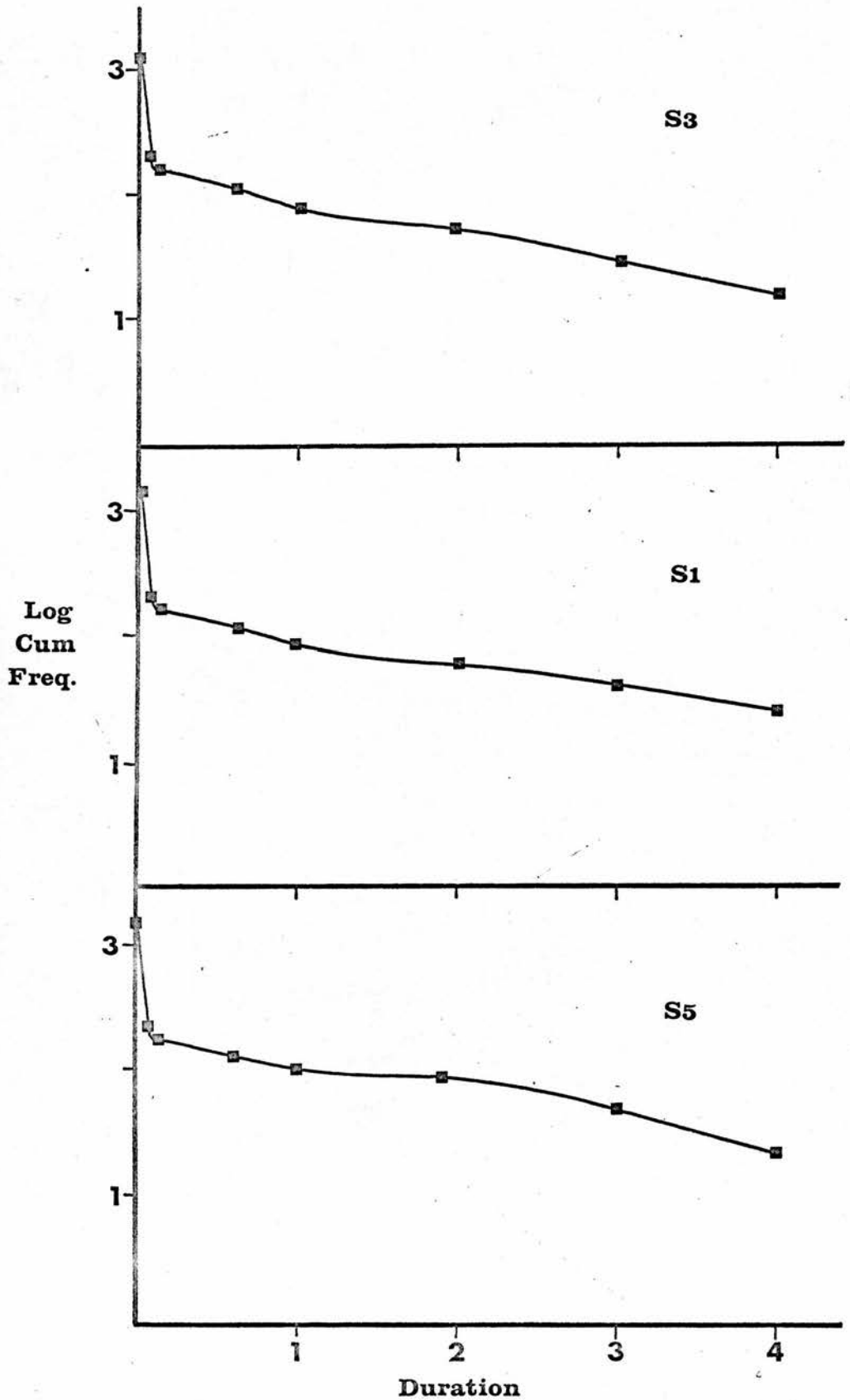
The basic pattern outlined here for the mouse accords quite closely with that described by Weipkema (1968).

The mouse consumes its daily ration in 25-30 meals, each consisting of roughly 100 mg ground diet. The meals are unevenly spaced over the day. Most feeding occurs in the hours of darkness, and is concentrated into feeding sessions.

This /

Fig. 5.4(a)

Extreme examples of the variation between logarithmic survivor plots of IBI's obtained from different animals


3
1
S3
3
1
S1
3
1
S5
Log
Cum
Freq.
1
2
3
4
Duration

This pattern may reflect the operation of constraints arising from the digestive apparatus and lifestyle of the mouse. Observation of the bulk of 100 mg mouse chow (bearing in mind that it swells when wet) would suggest that it compares reasonably with the observed volume of a mouse stomach (noted at autopsy). The animals certainly can eat more, but this may represent a more of less optimum load. Such a limitation upon meal size necessitates a very high frequency of meals, at on average one an hour during the day and night.

Hourly trips out of the nest are very risky even during the night, and during the day may well prove fatal. During the night the risk may be minimised by concentrating feeding into a small number of 'excursions', during which meals are taken at a maximum possible frequency, thus supplying more than immediate needs, and allowing the collection of reserves to supply metabolism at other times. In the wild, therefore the feeding 'session' may well represent the basic pattern of feeding, with extra session meals appearing only in the relative safety of a cage. (Alternatively, it is not impossible that the mouse would consume hoarded food in its nest between excursions.)

Extra session feeding at night (about once an hour) should just support metabolism, but during the day the animal eats much less than its presumed usage. During the night time feeding sessions, food is being consumed at something like four times the rate at which it would be expected to be used. Feeding sessions are therefore a lipogenic activity, and as such their size will determine the increment to stores produced. They would therefore be expected to either anticipate or compensate the daytime depletion of resources. Furthermore, should the animal become chronically deprived for some reason it might be expected /

expected that session size would be increased. Perhaps, therefore the 'session size' of mice would represent a closer analogy than 'meal size' to the meal size parameter of rat feeding described in the first chapter.

The considerable frequency of meals in the mouse also greatly increases the potential for the interaction of meal taking with other activities, and both the timing and size of meals may be affected. In the following chapters changes of these variables over different reproductive states will be discussed.

5.5. Summary

To summarise the main features of the temporal patterning of feeding in the mouse:

1. Consideration of the survivorship curves for interbout intervals indicates a number of separate components.

2. Feeding bouts are organised into groups-meals-within which they are separated by relatively short intervals (less than 200 seconds).

3. Meals are separated by a certain minimum time, during which the probability of feeding is low.

4. Meals are themselves grouped into sessions within which they are separated by 200 - 1200 seconds.

5. These /

5. These sessions contain about half of the night time meals, and there are usually two or three, one towards the beginning of the night, and the others towards the end.

6. Between sessions meals occur relatively infrequently, and have a constant probability of occurence over time.

7. The temporal organisation shows very little variation over different individuals.

CHAPTER SIX

THE CIRCADIAN PATTERN OF FEEDING IN THE MOUSE

6.1 Introduction

In addition to designating certain variables in feeding behaviour the automated method of recording allows a more precise analysis of the daily pattern of food intake. Whilst it is very difficult to partition 24 hours into small time periods, a gross division into day and night is very easy using the recorder. Accordingly, the first sections of the chapter describe how the total time spent feeding in the night and day separately vary over the oestrus cycle in the C57 mouse. The latter sections go on to analyse in detail the variables of feeding described in the previous chapter as they change over the days of the cycle, consideration of night-time changes being left until following chapters.

6.2 Total time spent feeding in dark and light

6.2.1. Methods

In addition to the print-out counters the recording apparatus included a number of counters whose function it was to simply total the number of time marks occuring whilst the animal was feeding. For animals whose behaviour was recorded on the relay equipment only, there were two such counters for each animal. Switches were arranged from timing clocks so that one counter operated during the day, and the other during the night. Preliminary examination of the detailed behaviour records suggested that the circadian rhythm in feeding divides reasonably accurately according to the light and dark phases, so the counters were set to record during the 12 hours of light and dark respectively.

Five /

Five animals on each food were recorded for several oestrus cycles each, on the relay logic recorder, as described in the previous chapter. Daily vaginal smears were taken, together with a record of the total time recorded as spent feeding in the day and night periods. Mean figures were determined for each animal on each day of the cycle, and a table constructed which could be subject to Friedman's test.

6.2.2. Results

The individual data is presented for each food in Tables 6.2(i) and 6.2(ii) respectively. The levels of significance are indicated, together with the value of Friedman's chi statistic. Group data is presented in the form of histograms in Figs. 6.2.(a) and 6.2.(b).

To consider first the daytime data. The pattern of total intake over the days of the oestrus cycle is broadly similar in the two foods. With laboratory chow, intake is high at dioestrus, falls very slightly at prooestrus, and is then markedly raised again at oestrus, only to fall back slightly at metoestrus. With the sweet Golden Nuggetts, intake is again relatively high at dioestrus, with, in this case a very slight rise into prooestrus, followed by a marked rise at oestrus, similar to that for lab chow. There is also a fall to an intermediate value at metoestrus. The essential point however, is that the pattern for the two foods is very similar, differing only quantitatively, not qualitatively.

When we come to look at the night-time intake, the picture is quite different for the two foods, and closely reflects the patterns of food consumption seen in Chapter 5. To consider firstly chow. The intake is /

Fig. 6.2(a)

Total time recorded spent feeding during the days of the oestrus cycle for animals consuming either laboratory chow or golden nuggets

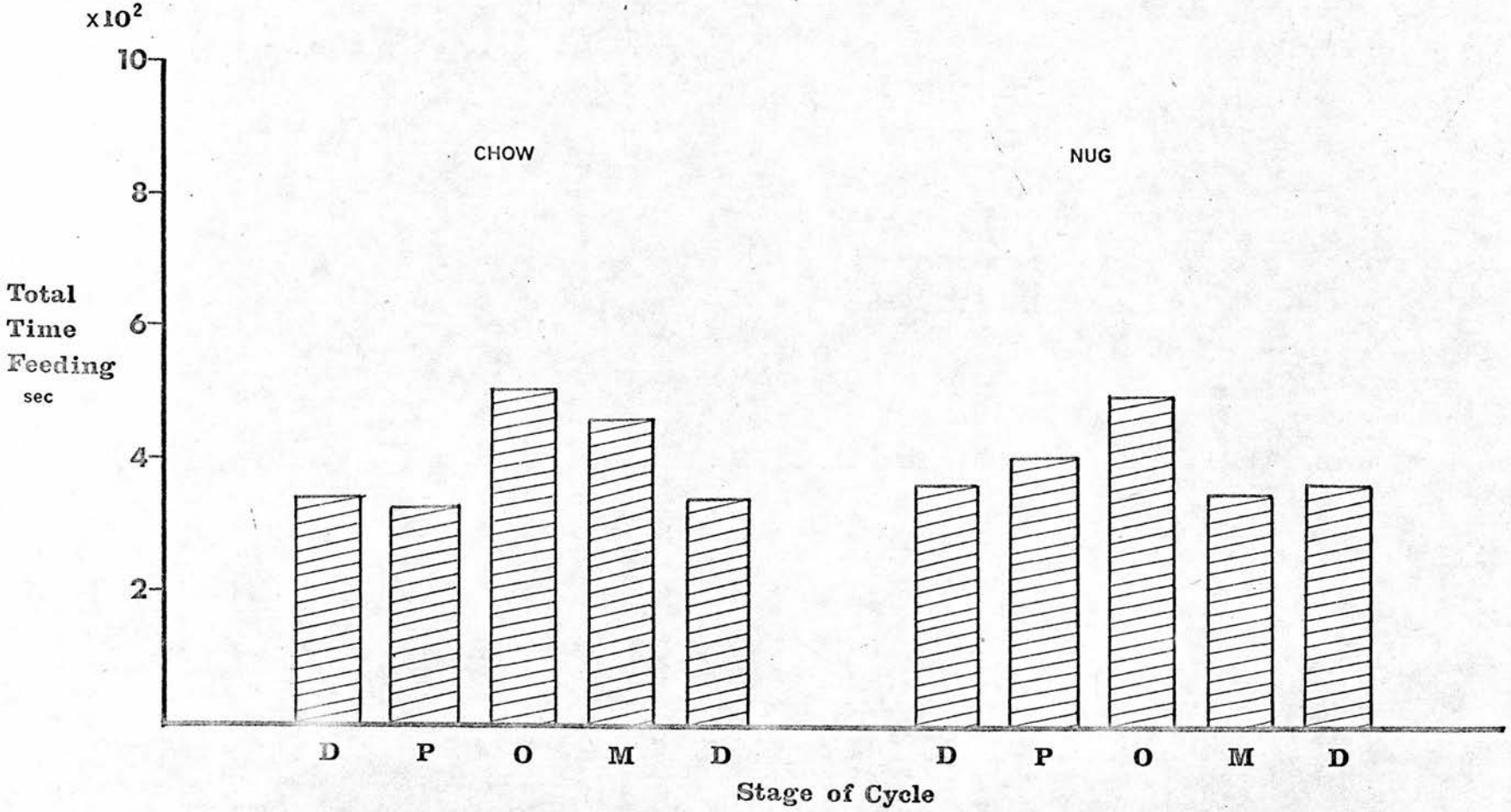

x10²
10
8
6
4
2
Total Time Feeding sec
CHOW
NUG
D P O M D
D P O M D
Stage of Cycle

Fig. 6.2(b)

Total time recorded as spent feeding during the nights of the oestrus cycle for animals consuming either laboratory chow or golden nuggets

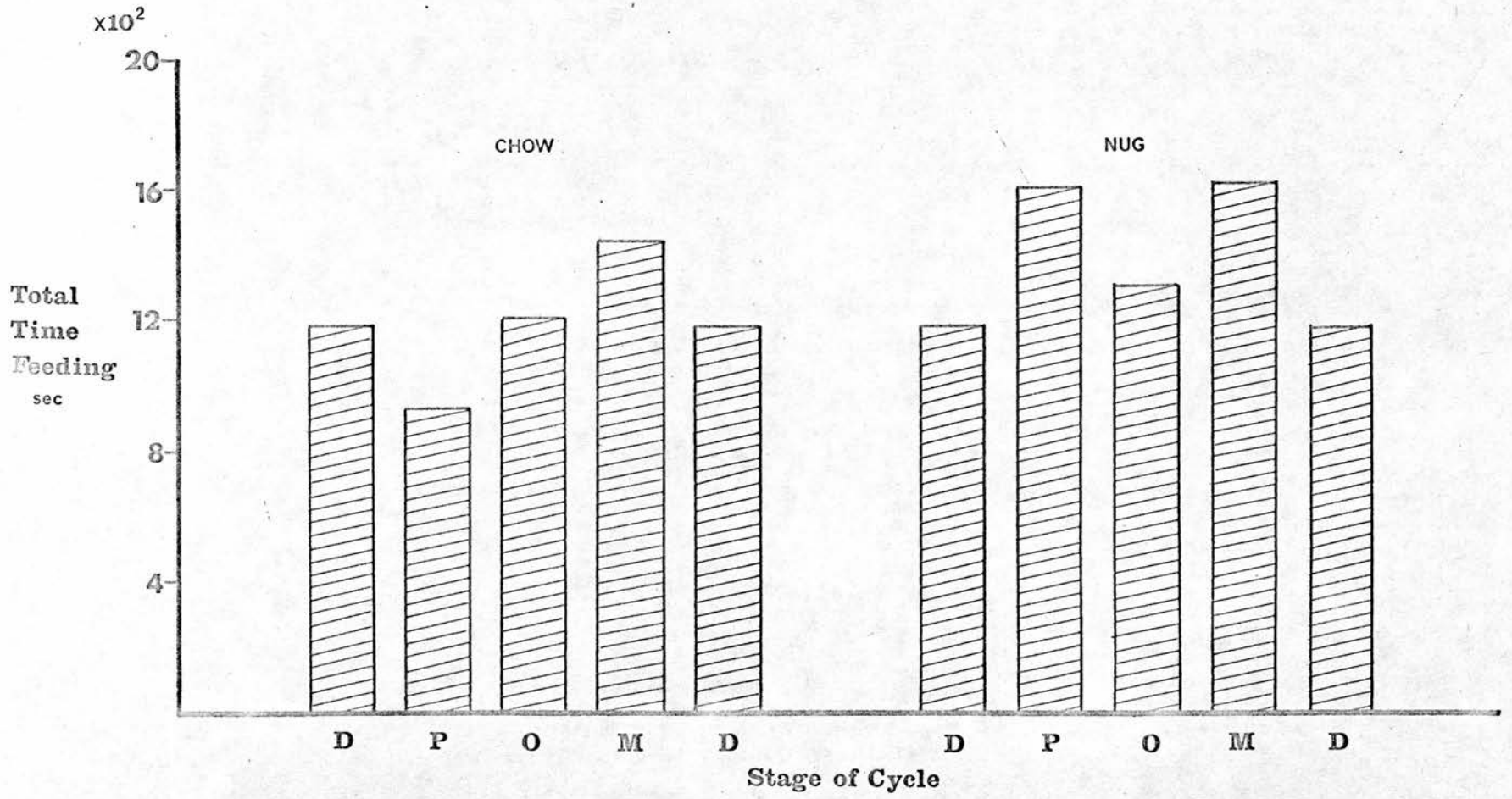

x10²
20
16
12
8
4
Total
Time
Feeding
sec
CHOW
NUG
D
P
O
M
D
D
P
O
M
D
Stage of Cycle

TABLE 6.2(i)

Total time spent feeding on Laboratory chow over the oestrus cycle

All in sec
Means for each animal

DAY

	D	P	O	M
	274	446	524	356
	458	298	470	466
	320	252	464	530
	336	360	536	432
	346	314	528	532
Mean	346	330	504	462

Friedman's X = 10.2 P<0.02

NIGHT

	D	P	O	M
	998	598	746	1396
	1060	912	1090	1156
	918	786	806	1308
	1398	976	1800	1744
	1622	1364	1616	1600
Mean	1198	926	1210	1440

Friedman's X = 9.7 P<0.02

TOTAL

	D	P	O	M
	1444	1132	1102	1670
	1318	1382	1556	1614
	1170	1250	1336	1636
	1778	1512	2232	2080
	1936	1892	2242	1946
Mean	1528	1432	1694	1788

Friedman's X = 8.3 P<0.05

CONVERTED TO GRAMS

D	P	O	M
2.7	2.5	2.95	3.1

TABLE 6.2(ii)

Total time spent feeding on Golden Nuggets over the oestrus cycle

All in sec
Means for each animal

DAY

D	P	O	M
280	232	340	288
338	590	604	318
442	372	574	380
385	388	480	362
398	472	480	406
370	410	494	350

Friedman's X = 9.72 P<0.02

NIGHT

D	P	O	M
1268	1938	1244	1778
1082	1470	1502	1854
1202	1484	1358	1480
824	1222	1134	1406
1562	1928	1330	1618
1186	1608	1312	1626

Friedman's chi X = 9.96 P<0.02

TOTAL

D	P	O	M
1500	2278	1532	2058
1672	2074	1820	2192
1574	1656	1738	1922
1212	1702	1496	1792
2034	2408	1736	2016
1598	2022	1666	1996

Friedman's X = 8.28 P<0.05

CONVERTED TO GRAMS

D	P	O	M
2.79	3.3	2.8	3.3

is close to the cycle mean during the night of dioestrus. During the following night of prooestrus, however, there is a marked, and significant fall in total time spent feeding. This confined exclusively to one night, the next - oestrus - showing a consumption again near the cycle mean. The effect of cycle on total time spent feeding is statistically highly significant.

When the animals are given sweet food the pattern changes. Dioestrus consumption is again near the cycle mean, but there is a marked elevation of consumption during the following night of sexual receptivity, which is not apparent on the third night of oestrus. There is a second elevation of intake during the next night of metoestrus. Again the effect of cycle upon intake is highly significant ($P < 0.01$).

The total time spent feeding in a day corresponds to a mean ingestion rate of about 1.7 mg per second. This is in accordance with the verification data discussed above, and also agrees with a figure of 1.7 - 2.0 mg per second quoted by Wiepkema (1968) for his male mice.

The records of total time spent feeding may be compared more accurately with the quantity of food ingested by summing the night and day pairs in appropriate combinations, that is to say night of pro-oestrus plus day of oestrus, and so on, and converting to an apparent consumption usingthe figure of 1.7 mg per second.

The results of such a calculation are shown, compared with the measured intakes, in Fig. 6.2(c). It is apparent that the total time spent /

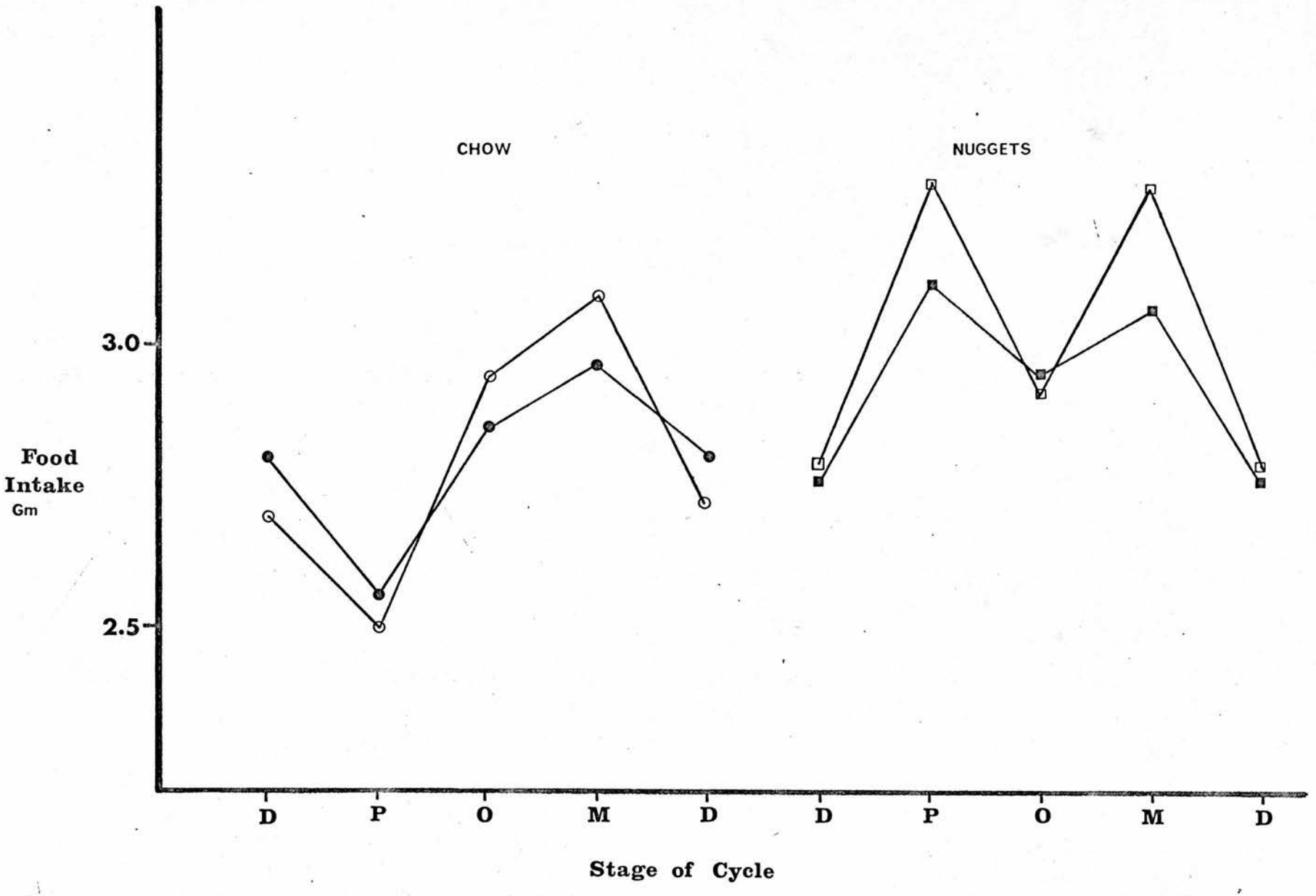

CHOW
NUGGETS
3.0
2.5
Food Intake
Gm
D
P
O
M
D
D
P
O
M
D
Stage of Cycle

Fig. 6.2(c)

Apparent daily intake derived from the record of time spent feeding (open symbols) compared to measured intake (closed symbols), for animals consuming either laboratory chow or golden nuggets

spent feeding correlates reasonably accurately with the total consumption, as would be expected from the validation data.

6.2.3. Discussion

The separation of the day and night components of feeding shows several points of interest. First and foremost, there is a pronounced circadian rhythmicity in the feeding habits of the mouse. 80% of the daily food intake is consumed during the hours of darkness (as in Wiepkema (1968)'s mice). It will also be remembered from the previous chapter that feeding is not evenly distributed during the dark period, being condensed into two or three feeding 'sessions', usually concentrated at the beginning and towards the end of the night.

It is of greater interest to note that the two circadian compponents of intake are differentially affected by the stages of the oestrus cycle. The night-time chow intakes reflect the classical picture of a depression at prooestrus followed by an elevation a couple of days later, and that for the sweet food mimics exactly the pattern of total consumption noted in the previous chapter.

Day time pattern, however, is apparently affected hardly at all by the cycle, except for an elevation in consumption on the day following sexual receptivity irrespective of the food given.

Data from rats, it will be recalled, indicates that the oestrus depression of food intake is sharply defined, with a sudden onset and offset (e.g. Drewitt (1973) Ter Haar (1972)). The data presented here would suggest that a similar pattern is present in mice. There is no depression in the intake of lab chow, on the day preceding sexual receptivity, /

receptivity, and a marked elevation during the day following it. On the day of oestrus, therefore the female would appear to be compensating for eating too little the night before.

This strongly implies that whatever agent is responsible for the depression acts over a limited time scale, and that furthermore it tends to disturb the animal's energy balance so markedly that it will eat more during the day, when it is normally inactive.

The strange thing, however, is that animals which have been given sweet food also increase their eating on the day following sexual receptivity, despite the fact that they have overeaten during the night before. This would suggest that these animals were also in a state of energy deficit. An explanation of this phenomenon would be easy to find if it could be asserted that the animals were more active when on heat (as is the case with rats) so that they may become energy deficient, even if they are eating more than normal, because they were also using more energy than normal. Unfortunately the evidence for an oestrus rhythm of activity in mice is equivocal and it remains to be seen whether it may be demonstrated in the C57 (Barnett and McEwan (1973)).

One thing, however, is clear, the changes in food intake usually described as occuring over the oestrus cycle are typical only of the night, the day time pattern being quite different, and apparently, at least, a consequence of the night-time pattern.

So far we have shown only that the total intake of foods follows this pattern during the days of the cycle, it is of importance to ascertain how the temporal organisation of feeding varies in this period.

6.3 Variations in some parameters of feeding over the days of the oestrus cycle

6.3.1. Methods

Each day, each animal produces a strip of paper tape containing a large number of figures. The analyses performed in the remaining behavioural sections of this thesis depend upon the variables which were designated in the previous chapter most importantly meal size and intermeal interval. Some means had to be devised, therefore, whereby the output from the recorder could be converted into a form most amenable to this type of analysis.

Accordingly the raw data was transcribed onto data sheets for further analysis. These sheets took the form illustrated in Appendix A so that eight designated 'meals' could be recorded on each, together with essential information regarding the animal under test. When scoring raw data the essential operation was to pick out interbout intervals longer than 200 seconds, and they were entered on the lower boxes at the right hand side of the sheet in order. Feeding bouts and interbout intervals preceding each supra-criterial IBI were entered in order to the top and bottom boxes of the record to the left of it. In this way each row represents a meal, which is a set of feeding bouts and interbout intervals bounded on each side by supra-criterial IBI's. Pairs of supra-criterial IBI's separated by a single 'feeding' bout of 2 seconds or less (which is usually not feeding at all, merely a disturbance of the feeder) were run together as a single IMI. Meals consisting of a single long feeding bout, which occasionally occured when the feeders accidentally become practically empty of food were ignored, together with their following IMI. All recorded meals therefore contain 2 or more bouts.

The /

The data from each 24 hour period was separately scored. Knowing the time at which the feeders were filled, and recording re-started, it is possible to cumulate the number of time marks in the record until the recorded time elapsed equals the time between the start of recording and the onset of darkness. In this way data recorded during the day may be scored on to separate sheets to that produced during the night. In practice simply cumulating IMI's (which during the day are very long relatively to the total time involved in a meal) will indicate sufficiently accurately the point at which to divide the raw data into night and day records. All meals and intermeal intervals on the night and day records were then transferred to sheets, except in cases of known machine failure or anomalies in the data suggesting machine failure (see Chapter 4).

In this way, on average, each night record yielded 16-17 meals, and each day record 3-4. The number of meals recorded duringthe day is always less than the number which occured, because of the disturbance of refilling the feeders and taking vaginal smears. This may well lead to a slight distortion of the data which is recorded in the sections to follow, as, in the few cases where day records have not been interrupted, there is a very long (4 - 5 hour) period in the middle of the day during which feeding does not occur. The feeders were unknowingly always refilled right in the middle, with the usual result that the stimulus of new food leads the animals to take a meal very soon after, when they perhaps would not normally eat. For this reason these meals were not entered onto the data sheets, and one very long IMI, which might influence the mean IMI calculated, could not be included in the analysis.

From the observed frequency the mouse would be expected to take approx. 20 meals during the night. The average number of meals recorded is /

is, however, somewhat reduced by meals omitted from the analysis for technical reasons associated with machine failure as described in Chapter 4.

Once the records are transcribed the cumulative total of bout lengths within each meal so designated may be taken as the meal size, and entered in the large box above the IMI.

In this way all relevant variables are recorded in a form which is easily accessible, so that they may be subject to further analysis.

The remainder of this chapter is concerned with the characteristics of day-time feeding. There has already been some discussion of survivorship analyses in chapter 5 with an indication that day and night patterns are different with respect to organisation of feeding into sessions. The day time data was therefore first analysed for the construction of survivorship curves, from which may be derived details of the probability of returning to feeding as a function of the time since the last meal.

This done, mean meal size for each animal on each day of the cycle for each food was calculated, and the table so constructed subjected to Freidman's test. A similar analysis was performed for the mean inter-meal intervals.

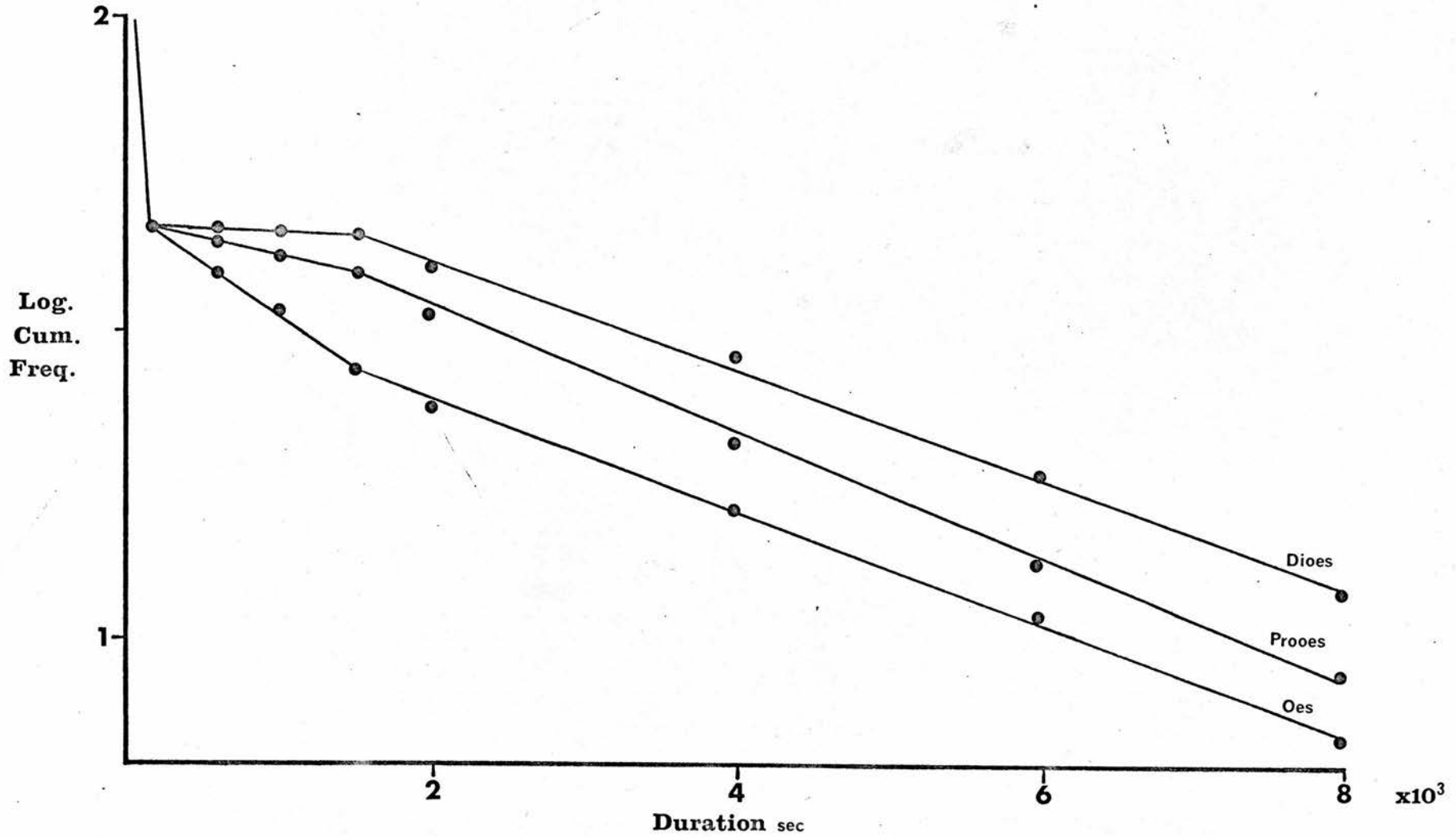
6.3.2. Results

To consider first the survivorship curves for interbout intervals during the day. Fig. 6.3(a) illustrates the survivorship curve obtained from data of the days of dioestrus, prooestrus and metoestrus. It is clear that they are a different form to these for the night-time data. Like /

Fig. 6.3(a)

Logarithmic survivor plot for all IBI's in the feeding of the female mouse over the days of the oestrus cycle (metoestrus and dioestrus data combined).

The curves are deliberately stylised.


2
1
Log.
Cum.
Freq.
Dioes
Prooes
Oes
2
4
6
8
x10³
Duration sec

Like the night-time data, though they do consist of an initial steep component, followed by a later shallow curve, indicating a strong bouting of day time feeding into meals. There is a second component of the curve with a very shallow slope followed by a linear fall. The precise nature of the changes in the components of the curves over the cycle will be discussed below.

The changes in mean meal size, and mean meal frequency over the days of the cycle are indicated in Fig. 6.3(b).

To take firstly meal size: In the animals feeding on laboratory chow, meal size is relatively high on the day of dioestrus, and as will be noted in the following chapter, similar to the night-time meal size. On the day of prooestrus, the day preceding sexual receptivity, meal size falls dramatically, and remains low on the day of oestrus which follows sexual receptivity.

During the following day of metoestrus, however, meal size rises back to the dioestrus level. The effect of cycle on meal size is statistically significant (Freidman's chi = 8.3 $P<0.05$).

It will be remembered from the previous sections, however, that the curve for total time spent feeding during the day looks nothing like this pattern. Total consumption is constant except for a significant elevation at oestrus, when meal size is depressed.

This effect can only be produced if meal frequency alters so as to counteract the changes in meal size and that this is the case may be seen from Fig. 6.3.(b). Meal frequency (the inverse of mean inter-meal interval /

Fig. 6.3(b)

Mean meal size and frequency over the days of the oestrus cycle for animals consuming either laboratory chow (filled circles) or Golden Nuggets (filled squares).

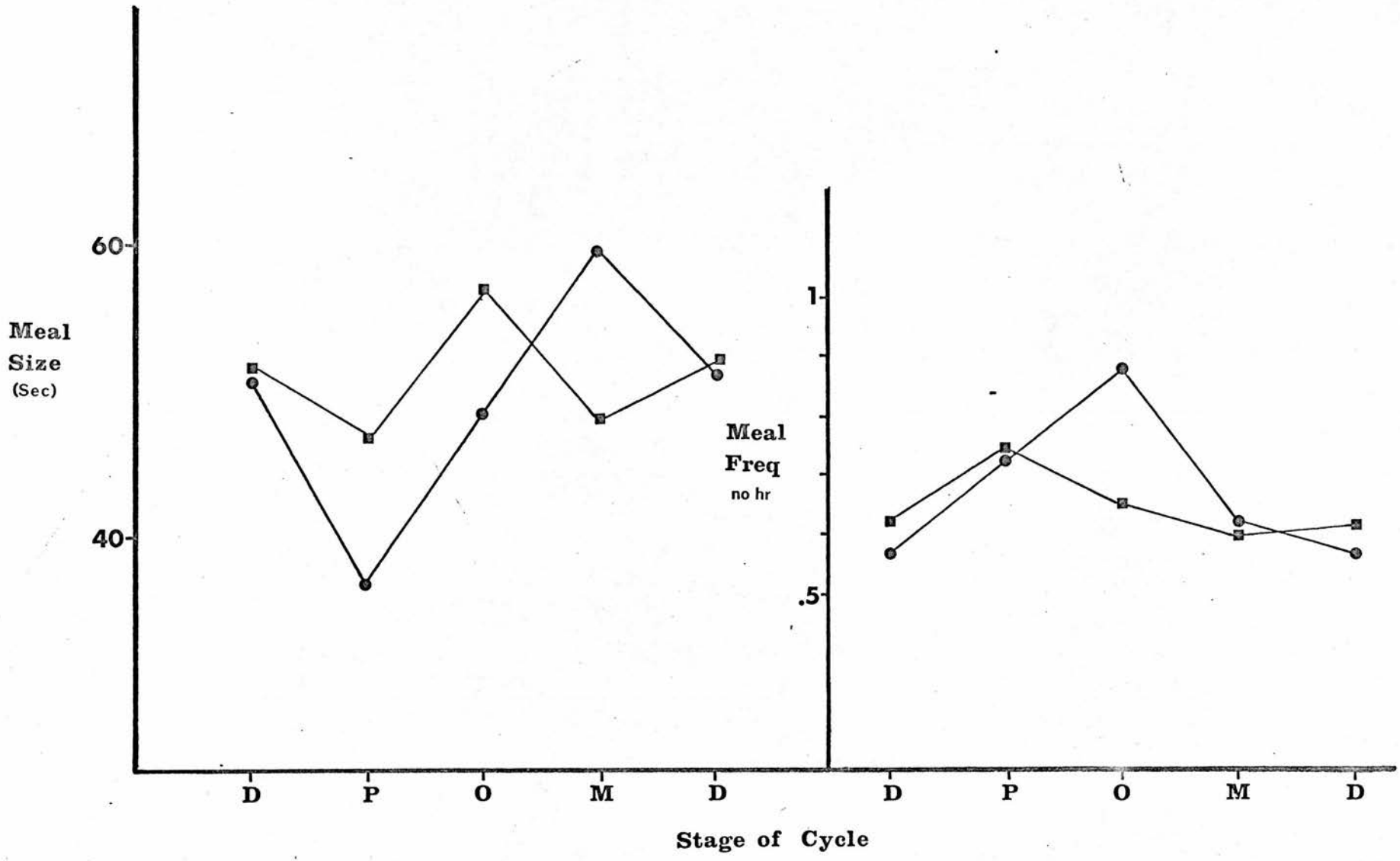

60
40
Meal
Size
(Sec)
1
.5
Meal
Freq
no hr
D
P
O
M
D
D
P
O
M
D
Stage of Cycle

TABLE 6.3(i)

Meal size and IMI over the days of the oestrus cycle

All in sec
Means for each animal

CHOW

Meal Size

D	P	O	M
32.8	40.8	69.2	68.0
63.4	30.6	48.4	55.4
39.6	31.0	33.0	55.4
53.2	42.2	50.0	51.4
65.2	38.8	41.6	73.2
50.9	36.7	48.4	49.6

Friedman's X = 8.3 P<0.05

IMI

D	P	O	M
5160	3940	5580	8328
5972	5120	4440	5128
5338	5292	3060	4500
6816	4790	4018	5120
8120	5320	3400	6418
6281	4892	4099	5898

Friedman's X = 8.8 P<0.05

NUGGETS

Meal Size

D	P	O	M
52.8	56.4	73	62.6
64.4	39.8	57.2	36.0
55.6	41.2	51.0	48.6
33.6	40.6	44.4	36.2
50.0	55.6	64.2	56.6
51.3	46.7	57.9	48.0

Friedman's X = 4.9 n.s.

IMI

D	P	O	M
8214	10484	9254	9266
5360	2914	4090	4698
5414	4768	3828	5524
3748	4518	4000	4318
5414	5084	5714	5994
5630	5553	5377	5960

Friedman's X = 2.04 n.s.

interval in hours), increases from the day of dioestrus to prooestrus, and the magnitude of this increase is sufficient to offset the reduction in meal size at that time, producing the observed constant total time spent feeding. The oestrus rise in food intake is produced by a further increase in meal frequency, which then falls back to a lower level at metoestrus, with the increasing meal size.

Meal size and meal frequency show therefore an inverse relationship during the days of the cycle, if meal size falls, meal frequency rise to compensate, and if meal size rises, meal frequency falls.

The variance in the mean meal frequency is very high but despite this, these changes in frequency apparent in the grand mean, are statistically significant. (Freidman's chi 8.8 $P<0.05$). The changes underlying these trends (which must account for the equivalence of total consumption over days), may be properly analysed by looking at the survivorship curves for long (supra-criterial) interbout intervals. The variation of the survivorship over the days of the cycle curve is represented in Fig. 6.3.(a). The first point to make is that the curves for metoestrus and dioestrus are so similar that they have been shown as a single line. Secondly the curves have been constructed from data lumped across animals, as this is the only way to guarantee sufficient observations.

The curves have been represented in a deliberately stylised fashion as two components, an initial one in the range 0 - 30 mins.(approx), and a second for the remaining period. The slopes of the latter curves do not vary over the oestrus cycle days, whereas the slope of initial portion /

Fig. 6.3(c)

The probability of feeding in any 200 second period as a function of time since the last meal over the days of the oestrus cycle

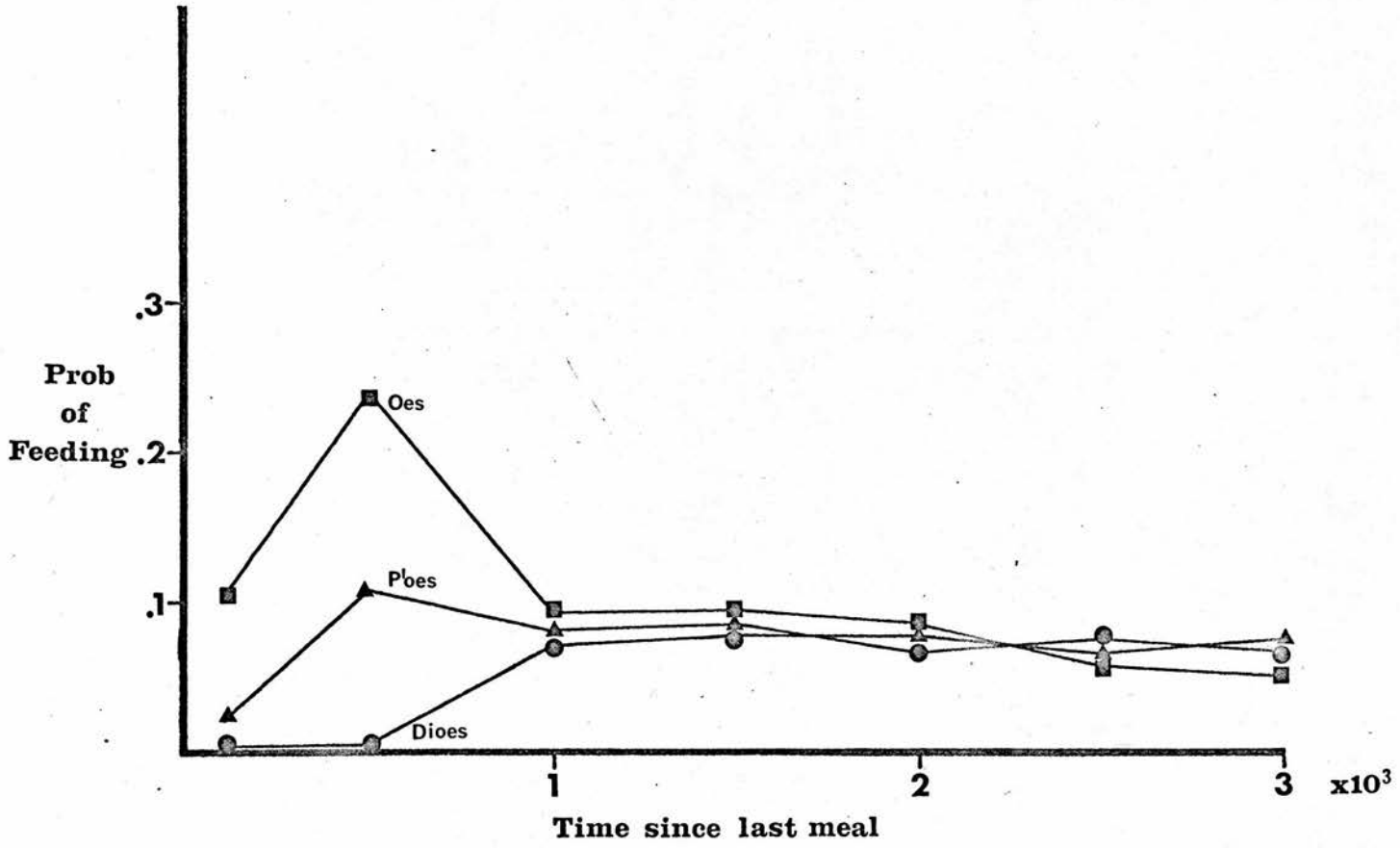

Prob
of
Feeding
.3
.2
.1
Oes
P'oes
Dioes
1
2
3
x10³
Time since last meal

TABLE 6.3.2.

Proportion of short IMI's over the oestrus cycle days

D	P	O	M
0.2	0.4	0.2	0
0.22	0.38	0.33	0
0	0.1	0.63	0.17
0.33	0.38	0.8	0.25
0.15	0.55	0.46	0.13

Friedman's $X = 9.06$ $P<0.03$

portion does. The implication of this finding is that the days of the cycle influence primarily the numbers of relatively short intermeal intervals that occur during the day. This may be illustrated by considering a curve of the probability of returning to feeding as a function of the time since the last meal, which is shown in Fig.6.3(c).

The probability of returning to feeding in any 1000 second interval (16.6 minutes), is quite constant after about 30 minutes, and does not vary during the cycle. The proportion of intermeal intervals falling into this category, does, however, alter, as the probability of an IMI ending in the range 200 - 2000 seconds changes dramatically over the cycle, rising from zero at dioestrus and metoestrus to a maximum of 0.3 at oestrus.

The statistical significance of this effect may be examined by determining for each animal, at each stage of the cycle, the proportion of IMI's falling within feeding sessions, and subjecting the resulting table to a Freidman's test. The results of such a process are shown in Table 6.3(ii). There is a significant change in the proportion of short IMI's over the days of the cycle.

The implication from this finding is that the animal increases meal frequency during the day, not by shortening the relatively long intervals normally seen between meals, but by adding further meals closely following the originals. This pattern is seen most clearly in the oestrus animals, which interpolate a night like feeding 'session' during the day, leaving meals outside of this session to fall into the typical daytime pattern.

The changes in meal frequency over the days of the oestrus cycle are therefore accounted almost exclusively by an increase in the proportion of /

of relatively very short IMI's, which reflects a tendency of the animal to carry over a night time pattern of feeding.

This tendency is a consequence of two factors, on the one hand a lowering of meal size on the two middle days of the cycle, and on the other a presumed energy deficit after the night of prooestrus.

Either way the changes appear to be essentially compensatory, and serve to correct for changes in either meal size or energy demand, and keep total intake in line. Suggestions as to the possible mechanism of these regulatory mechanisms will be made in following chapters.

The pattern of meal size and frequency changes over the days of the cycle are quite similar for animals feeding on Golden Nuggets. Meal size is again relatively high at dioestrus, with a fall into prooestrus. At oestrus however, meal size does not remain depressed, but is somewhat elevated, remaining that way at metoestrus. Meal frequency varies in a way very similar to that of animals eating chow, rising at prooestrus, although there is a fall at oestrus, (presumably due to the meal size changes) and another at metoestrus. The greater variability of the nuggets data, however, means that neither the changes in meal size nor those in IMI are statistically significant. (Friedman's chi = 4.18 for meal size, = 6.32 for IMI both ns).

All that can be said of the golden nuggets data, therefore, is that there is a significant variation in total consumption over the days of the cycle, which is composed of trends to changes in meal size and frequency of the same type as those observed in animals eating lab.chow.

6.3.3. Discussion

There are several points arising from this data. Firstly the daytime pattern of intake is quite different to that prevailing during the night over the oestrus cycle. When, however, the meal size alone is considered, it can be seen to reflect at least the early changes in night time consumption at prooestrus.

The difference between the night and daytime patterns is the consequence of compensatory changes in meal frequency, which override the meal size changes, and which at oestrus, lead to a rise in total intake despite a fall in meal size (in the case of chow). These compensatory changes are brought about not by a shortening of the usual long daytime IMI's, but by the inclusion, during the day, of a night time pattern of feeding in sessions.

It would appear from this analysis that (a) The daytime changes in meal size are primarily disturbing on daytime energy balance, and have to be corrected by meal frequency changes; and (b) The changes in energy balance over the early nights of the cycle are immediately compensated by changes in daytime meal frequency.

In the following chapter the changes in meal size and frequency over the nights of the cycle will be discussed.

6.4 Summary

A. Day and night time changes in total consumption:

1. Night time changes mirror the changes in 24 hour intake described in Chapter Three, the intake of chow being depressed, and tht of nuggets increased at prooestrus, whereas the intake of both is elevated at metoestrus.

2. Daytime /

2. Daytime changes are the same for both foods, intake being relatively constant over the days of the cycle, except for an elevation of the intake of both foods at oestrus.

B. Daytime meal pattern changes:

1. Only the changes for chow are significant.

2. Meal size is depressed at prooestrus and oestrus.

3. These changes are compensated by changes in meal frequency, producing the observed pattern of total intake.

4. Meal frequency changes are the product of introducing a typically night time feeding session into the day.

CHAPTER SEVEN

CHANGES IN MEAL PATTERN OVER THE NIGHTS OF THE CYCLE

7.1 Introduction

The previous chapter has described variations in meal size and frequency over the days of the oestrus cycle, and noted that they combine in different ways to produce changes in the observed total time spent feeding. It was hypothesised that changes in meal size were primarily disturbing influences which were in all cases compensated by alterations of intermeal interval.

In this chapter variations in meal size and frequency are followed over the nights of the oestrus cycle, including the period of sexual receptivity.

7.2 Changes in meal size over the nights of the cycle

Night records of the feeding behaviour of female animals on both foods were collected over the nights of the oestrus cycle as described in Chapter Four. The records were scored onto sheets as described in the previous chapter, and meal size (that is to say the total of all bout lengths) computed for each meal. The mean meal size for each night of the cycle for each animal on either food were calculated, and are presented as a table in Table 7.2(i), and graphically in Fig.7.2(a).

There is a significant effect of cycle stage on meal size for animals consuming both foods. Animals given laboratory chow show a significant reduction of meal size at prooestrus, with no change at any other stage. Nuggets animals, on the other hand, show a significant rise in meal size at prooestrus, with again, no change at any other stage (see tables for statistics).

Meal /

Fig. 7.2(a)

Mean meal size and frequency over the nights of the oestrus cycle for animals consuming either laboratory chow(filled circles)or golden nuggets(filled squares)

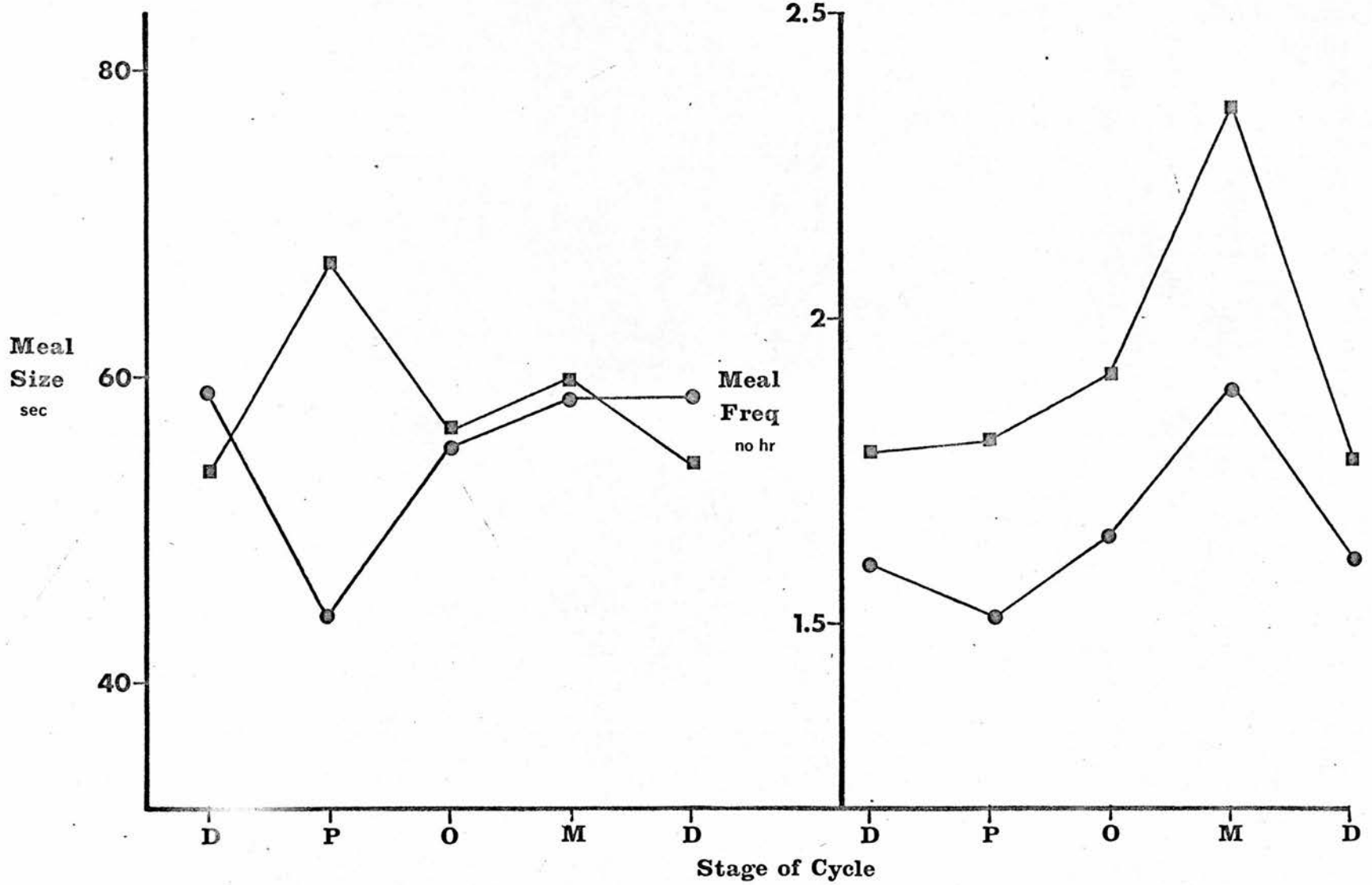

80
60
40
Meal
Size
sec
2.5
2
1.5
Meal
Freq
no hr
D
P
O
M
D
D
P
O
M
D
Stage of Cycle

TABLE 7.2(1)

Meal size over the nights of the oestrus cycle

All in sec
Means for each animal

CHOW

	D	P	O	M
	61	38.8	39.8	63.6
	53.6	47.0	60.8	53.8
	42.6	28.6	30.4	45.6
	62.2	46.6	68.6	64.8
	72.2	53.8	67.4	68.2
	76.4	55.0	69.8	60.8
	48.2	39.0	53.4	57.6
Mean	59.4	44.1	55.8	59.2

Friedman's X = 13.11 P 0.01

NUGGETS

	D	P	O	M
	75.2	101.2	77.4	81
	55.0	81.0	67.8	58.8
	53.0	62.0	51.6	60.8
	40.0	45.6	45.2	48.8
	45.6	50.2	41.0	49.6
Mean	53.7	68	56.6	59.8

Friedman's X = 10.92 P<0.02

Meal size changes can therefore only account for the variations in food intake at prooestrus, and some other mechanism must be proposed for the changes at metoestrus. Meal frequency must therefore be considered.

7.3 Changes in meal frequency over the cycle

Mean IMI (defined as in previous chapters) was calculated for each animal on each night of the oestrus cycle on either food. The data are presented as a table in Table 7.3(i), and graphically in Fig. 7.2(a) as the inverse of IMI, mean meal frequency (in number of meals per hour).

The main point to note is that there are no differences in the pattern of meal frequency changes over the cycle between the two foods. Both show a gradual rise as the cycle proceeds, which is markedly accentuated on the night of metoestrus. The changes over the cycle are not, however, significant in either food taken alone. (see tables for statistics).

If, however, the data from the two foods are combined, then there is a significant effect of cycle upon IMI (Friedmans chi = 18.6 $P<0.05$).

The magnitude of the changes in meal frequency are sufficient to account for the metoestrus peak in consumption of the two foods.

The product of meal size and mean meal frequency yields essentially the same total consumption figures over the cycle as were obtained directly in the previous chapter.

7.4 /

TABLE 7.3(i)

Intermeal Interval over the nights of the oestrus cycle

All in seconds
Means for each animal

CHOW

D	P	O	M
2640	2798	2300	1948
2182	2224	2408	1816
2004	1568	2160	1504
1920	2062	1200	1604
1922	1702	1800	1840
2580	5140	3220	2996
2092	1440	2094	1556
2190	2418	2170	1894

Friedman's X = 5.1 n.s.

NUGGETS

D	P	O	M
2558	2044	2686	1966
2196	2378	1948	1370
1904	1804	1640	1424
2096	1612	1720	1498
1260	1070	1330	1324
2002	1781	1864	1516

Friedman's X = 5.88 n.s.

Overall Friedman's X = 8.6 $P<0.05$

7.4 Discussion

The changes in food consumption over the nights of the oestrus cycle are therefore produced by a complex of changes of both meal size and meal frequency acting relatively independently. The changes in intake at the time of sexual receptivity are the product of changes in meal size, which is altered in different ways depending upon the type of food offered, being increased for sweet food and decreased for laboratory chow.

The changes in intake at metoestrus, however, arise in alterations of meal frequency with no changes in meal size. These changes are the same on the two foods.

It is clear from these data that the changes in feeding at the beginning and end of the cycle may be further dissociated. In addition to being differentially affected by the stimulus properties of the food, receptive animals manifest their changes as alterations of meal size. Meal size variations were also noted during the days of the cycle in the previous chapter. During the day, however, meal size variations were compensated by changes in IMI. During the night this does not occur, and it is this relative independence of meal size and IMI which allows changes in total consumption to appear.

This independence is a problem for investigation in the following chapters. It is also necessary to investigate the details of the changes underlying the variations in meal size, in the hope that they might suggest how sexual receptivity may directly or indirectly interact with the control systems involved in meal taking.

The /

The change in meal frequency at the end of the cycle, however, appears with no change in meal size, and details of changes of this type must also be examined in following chapters.

Overall, there is now strong evidence to suggest that, in the C57 mouse at least, the period of sexual receptivity disturbs feeding in ways quite distinct to the ways in which it varies at the end of the cycle (and after ovariectomy), suggesting that these two effects are possibly mediated by different mechanisms.

CHAPTER EIGHT

DETAILS OF MEAL SIZE CHANGES

8.1 Introduction

The previous chapters have shown that the changes in total consumption of different foods at prooestrus are solely the product of alterations in meal size, whereas those occuring later in the cycle (and after ovariectomy - see below) are produced by modification of meal frequency.

This chapter aims to examine the nature of the mechanism underlying those meal size changes. In the introduction the meal was considered as the product of a set of interacting incremental and decremental effects, which once initiated will lead to the production of a co-ordinated set of feeding bouts associated in time. These effects, it was argued would be expected to be most affected by the need to integrate feeding with other behaviours.

In the following sections the existence and nature of these influences is examined in mouse feeding, and the changes underlying variations in meal size over the oestrus cycle discussed.

8.2 The Control of feeding bouts and interbout intervals

So long as feeding is considered as a unitary output of some hypothetical central control mechanism, then the factors of importance in any situation, which will define it completely, are the probabilities of transition to feeding from any other activity, and the probability of the reverse transition once feeding begins. These probabilities may be stationary or non-stationary over time.

If /

If they are stationary in the long term, that is to say, even though the probability of feeding, for example, may vary during inter-bout intervals, the nature of the variation is the same for all IBI's, then the suvivorship curves may be used to obtain data upon probability changes within bouts and inter bout intervals.

Accordingly such curves have been constructed for all bouts, and intra meal intervals, see Fig 8.2 (a).

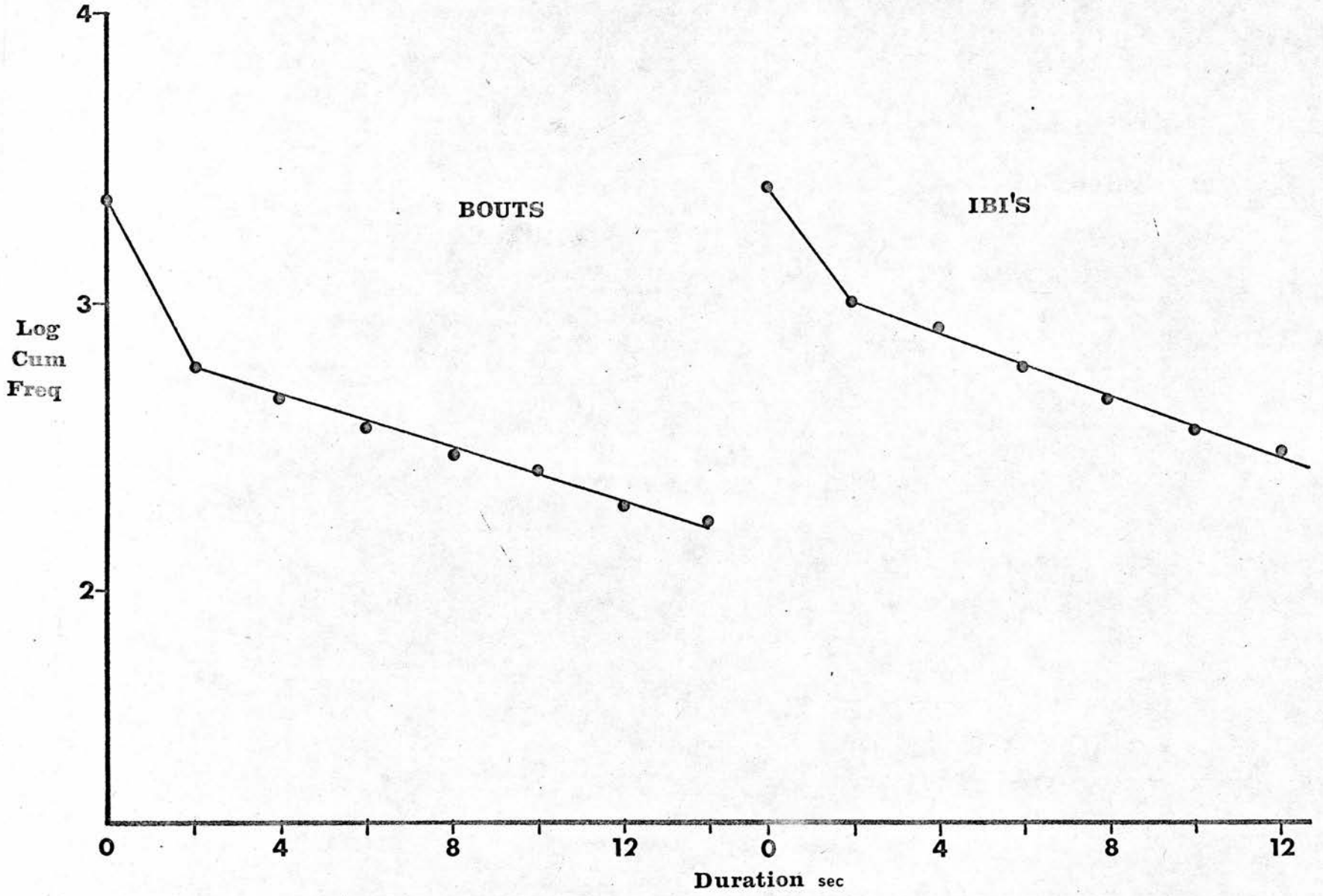
It can be seen that both curves are characterised by an initial very steep component, followed by a shallower straight line. This indicates that from four seconds to twenty seconds at least for both bouts and interbout intervals, the probability of their ending in any given interval is constant over time. The data after 30 seconds is so scarce as to make reasonable interpretation of the changes impossible.

There is, however, a considerable preponderance of very short (less than two seconds) bout lengths and interbout intervals, so that the probability of both a feeding and non-feeding bout ending in its first second or so is very high.

It would appear therefore that animals need a certain time to 'settle in' to a new activity, during which their probability of distraction is very high. This is exactly the sort of effect that would be expected in the form of polystable control system which is hypothesised. It would be expected that any behavioural transition would require a certain amount of 'activation energy' to ensure its completion, so that in a situation where stimuli exist tending towards several alternatives at once, then a fair amount of 'bounce' between alternatives /

Fig. 8.2(a)

Logarithmic survivor plots for bouts and intra-meal interbout intervals in the night time feeding of animals consuming laboratory chow


4
3
2
Log
Cum
Freq
BOUTS
IBI'S
0
4
8
12
0
4
8
12
Duration sec

alternatives would be anticipated before the system finally settles into a stable output. Simple observation of a mouse in its cage illustrates this point very well, the animals oscillate rapidly between behavioural alternatives before settling into one or another.

The recorded 'bounce' could of course arise from two sources, either the behavioural bounce of the animal, or artefactual bounce in the recording apparatus. This point has already been discussed in Chapter Four, and consideration of the validation data obtained by the methods described in that chapter would illustrate that the vast proportion of bounce recorded arises from true behavioural bounce, as that arising in the recording apparatus is eliminated from the recording by the time sampling technique of the recorder.

Furthermore, the time taken for the animal to start an activity, stop it, and leave the feeder is usually sufficient to ensure that at least one time mark occurs during the behaviour. The small proportion of short bouts that are lost are probably almost equally offset (from the validation data) by the inclusion of an equally small proportion of the artefactual recorder bounce. This point is also illustrated by the observation that the proportion of bouts in the range 0-2 secs. remains the same in records obtained from the units operating at both one second and two second sampling intervals. It may be safely concluded that events of behavioural importance in feeding therefore last at least two seconds.

Once, however, a feeding bout has got going, that is to say passed the two second barrier, the survivorship curve is a straight line over the range of durations for which sufficient data is available. The implication /

implication from this is that the probability of both entry to feeding (from the IBI curves), and exit from it (from the bout curves), is constant over time.

These probabilities, which may be calculated from the curves may therefore be used, in addition to mean bout lengths (which are distorted by the preponderance of short bouts and IBI's), as indicators of changes both within and between meals.

8.3 The control of meal taking

Meals are groups of feeding bouts associated in time. Mechanisms must exist therefore to ensure that such a grouping occurs. This can only be produced by an alteration of the probability of a feeding bout beginning. It is necessary therefore to examine changes in the probability of initiation of feeding before, during and after meals. As was noted in the introduction Wiepkema (1971) has reported that bout length also increases over the initial stages of a meal. This can only come about by an alteration of the probability of a feeding bout ending, so that these probabilities should also be observed.

Accordingly in the following sections several variables of bout length and intra meal IBI are calculated over the course of meals in an attempt to characterise the operation of the control systems underlying meal structure such that variations in that structure may be followed over the oestrus cycle.

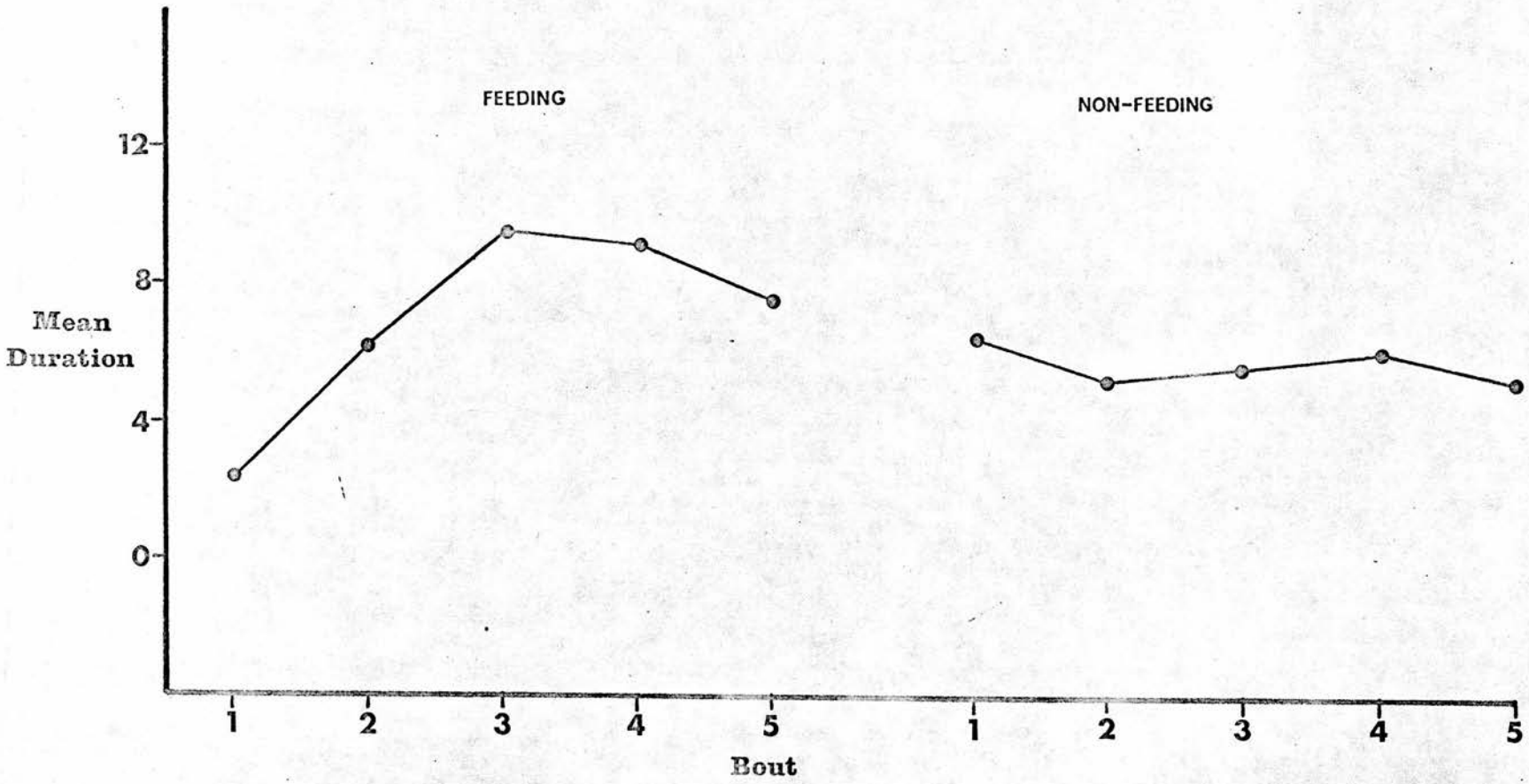
8.3.1. Methods

Data from the recordings of dioestrus, oestrus, and metoestrus animals feeding on laboratory chow were used. (Procestrus data was eliminated for reasons that will become apparent below.) Chow data

is /

Fig. 8.3(a)

The mean duration of the first five feeding and non-feeding bouts in night-time meals of the non-receptive female mouse feeding upon laboratory chow


FEEDING
NON-FEEDING
12
8
4
0
Mean
Duration
1
2
3
4
5
1
2
3
4
5
Bout

is used as it bears a greater similarity to Wiepkema's results, although the basic structure of the nuggets data is very much the same.

A number of variables was computed. The first and most obvious task is to compute the mean feeding bout length as a function of the position of the bout in a meal. A similar computation was applied to intra meal IBI's. This done the nature and complexity of the underlying control systems was examined by constructing survivorship curves for the first, second, third, fourth, penulitimate and last feeding bout and intra meal IBI. From these the probability of feeding beginning and ending at any point in the meal can be calculated.

8.3.2. Results

A. Mean Figures:

Fig 8.3.(a) illustrates the mean duration of the first five bouts of meals in the combined data from dioestrus, oestrus and metoestrus animals. The increase in bout length is significant (Friedman's chi = 35.73 $P<0.001$). Fig. 8.3.(a) also illustrates similar data for the first five non-feeding intervals. It can be seen that there is an initial (non significant) fall in mean IBI from the first to second, but thereafter the value is relatively constant over successive bouts. There is no significant change over the five IBI's (Friedman's chi = $\frac{3.1}{8.3}$ ns).

The penultimate and last feeding bouts are of similar duration to the fifth, so that there is no tailing off of bout length towards the end of the meal. The last IBI is, however, longer than those earlier in the meal. ($P<0.05$ Wilcoxon test)

B. Survivorship /

TABLE 8.3(i)

Initial Facilitation of Bout length

All in seconds
Means for each animal

FEEDING

Bout 1	2	3	4	5
3.2	4.6	5.2	5.4	7.0
2.6	4.2	4.6	4.6	4.8
2.4	4.4	6.2	4.8	6.8
2.0	6.6	16.4	10.6	8.8
2.8	16.6	16.2	18.6	11.8
4.0	5.6	6.8	10.6	9.4
Mean 2.8	7.0	9.2	9.1	8.2

Friedman's X = 16.7 P<0.001

NON-FEEDING

Bout 1	2	3	4	5
7.6	4.8	7.8	7.0	3.8
4.2	5.8	3.8	4.8	3.6
7.6	8.8	8.8	7.4	9.2
5.2	2.6	1.6	5.2	6.6
7.0	4.0	6.4	6.6	6.0
6.0	5.4	5.8	6.2	6.4
Mean 6.2	5.2	5.8	6.2	6.0

Friedman's X = 3.1 n.s.

B. Survivorship analyses

(i) IBI's: The log survivor plots for first, second, third, penultimate and last intrameal IBI's are shown in Fig.8.3(b). Data from other IBI's sampled is not included, as the third and subsequent IBI's yield curves identical to the second. All the curves show the expected initial steep component, (which is not illustrated in the figure for the sake of simplicity) reflecting again a high proportion of the intervals not achieving their fourth second. This is followed by a linear decline, up till 20 seconds, after which data is so scarce, that the slope of the curve becomes almost indeterminate. There is a suggestion, however, that it approaches the horizontal after 30 seconds or so.

Following each feeding bout, therefore, there is a period when the probability of feeding is very high, after which, however, there is a suggestion that the probability returns to a low level.

Only three IBI's in a meal may be isolated as markedly different to the rest, that is the first, penultimate, and last. The first IBI shows a steeper survivor plot in the range 4-30 seconds than the second and subsequent, as does the last. The penultimate IBI also shows a slightly steeper slope, corresponding to that of the first. The probability figures derived from these curves are shown in Fig. 8.3(d). The probability of feeding is somewhat lower after the first bout than the second, and falls again after the third from last bout, and yet again before the last bout. There is insufficient data to calculate probability /

Fig. 8.3(b)

Logarithmic survivor plots for first, second, penultimate and last intra meal IBI's, in the meals of non-receptive female mice feeding upon laboratory chow

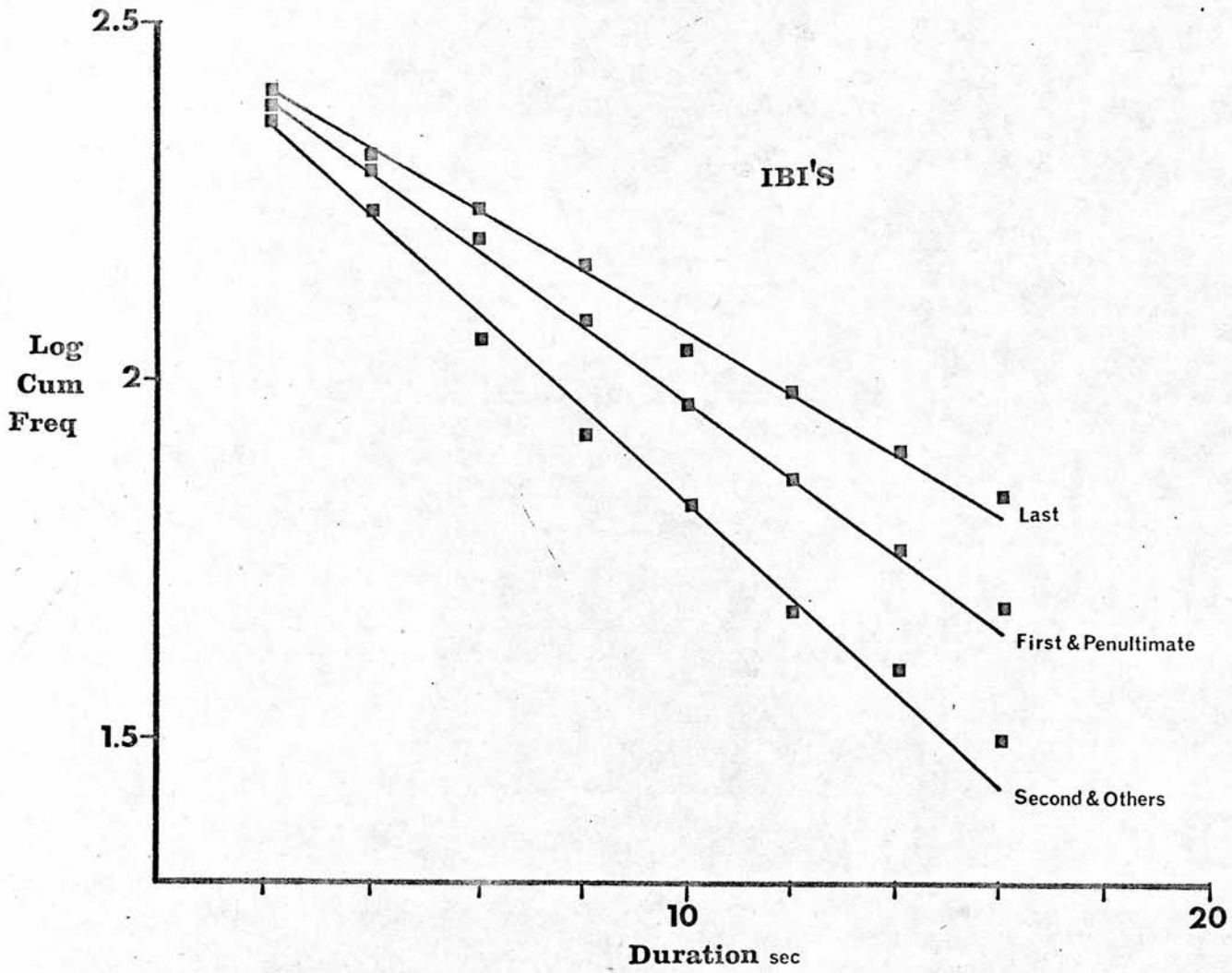
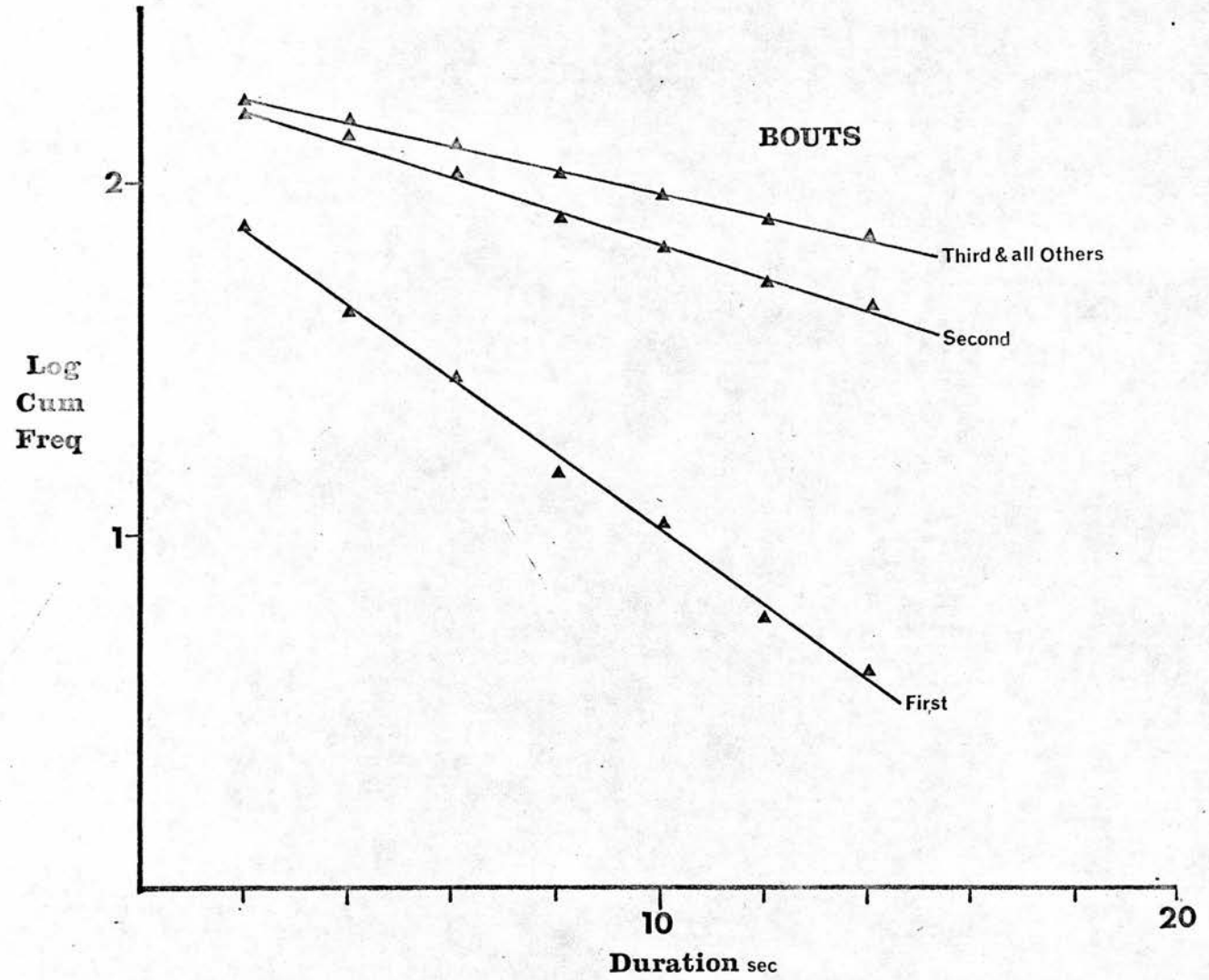

IBI'S
2.5
2
1.5
Log
Cum
Freq
Last
First & Penultimate
Second & Others
10
20
Duration sec

Fig. 8.3(c)

Logarithmic survivor plots for the first, second and third feeding bouts in meals of non-receptive female mice feeding upon laboratory chow


BOUTS
Third & all Others
Second
First
2
1
Log
Cum
Freq
10
20
Duration sec

probability figures of this type for individual animals, so the statistical significance of these probability changes cannot be determined. The fact, however, that the mean IBI figures show significant changes would suggest that the probability changes are of some importance, as the proportion of IBI's ending in the first two seconds is about constant over the various bouts of the meal.

(ii) Bouts: Logarithmic survivor plots for the first, second, third, and subsequent bouts are shown in Fig. 8.3(c). These curves again all show the initial steep component, followed by a linear decline.

The proportion of bouts ending in the first seconds changes from the first to the third bout of the meal, declining from 87% at the first to 72% at the second, and 70% at the third and subsequent. At the same time the slope of the linear portion of the curve varies markedly. The probabilities calculated from these lines are illustrated in Fig. 8.3(d). The probability of a feeding bout ending in any given two second interval falls steadily over the first four bouts of the meal to reach a steady value of c. 0.13, at which it remains until the end of the meal.

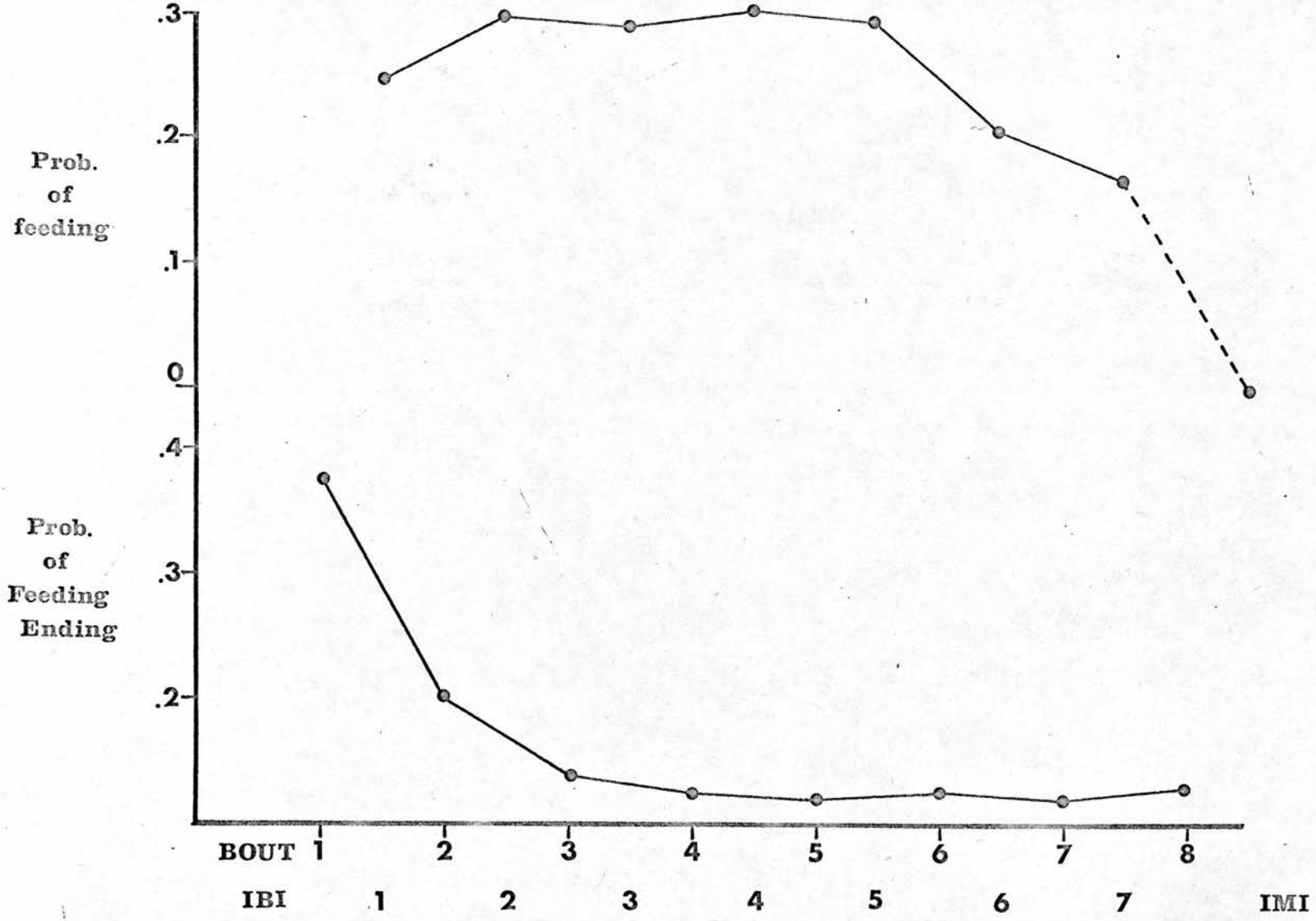
8.3.3. Discussion

The meal structure of the female mouse may therefore also be described as a result of a set of interacting incremental and decremental effects. The initiation of the first bout of a meal brings about a number of changes /

Fig. 8.3(d)

Changes in the probability of feeding beginning in any 2 second period. (in the range 4-15 seconds since the last feeding bout), and ending (in the 4th-15th second of a bout) over the bouts of a typical meal of laboratory chow in the non-receptive female mouse.

(Data for intermediate bouts estimated)


.3
.2
.1
0
.4
.3
.2
Prob.
of
feeding
Prob.
of
Feeding
Ending
BOUT 1 2 3 4 5 6 7 8
IBI 1 2 3 4 5 6 7
IMI

changes. Immediately after the bout the probability of feeding is raised from the very low level prevailing before the meal began to a relatively very high value, such that another bout is virtually certain to follow within a few seconds. This second bout will be longer than the first, by virtue of two changes, on the one hand its probability of being very short is much reduced (to a value that remains more or less constant for the rest of the meal), and on the other its probability of ending in any given period after four seconds also falls. The IBI following this bout will be a little shorter than the first, by virtue of a small increase in the probability of feeding. The third bout will be longer than the second, and the fourth longer than the third as a result of the steady decline in the probability of a feeding bout ending as the meal proceeds. This probability reaches a constant low level at the fourth bout which is maintained for the rest of the meal.

In the initial stages, therefore, the pattern of bouts described by Wiepkema (1971a) is replicated. The rate of consumption of food rises as the meal goes on, as a result of successive bouts facilitating one another by a gradual reduction in the probability of a bout ending.

The end of the meal is preceded by changes of a different kind. The penultimate and last IBI are longer than the rest, this difference being produced by a fall in the probability of a feeding bout beginning. The terminating function of a meal, therefore influences the initiation of feeding, but not its termination, whereas, the changes in feeding at the beginning of the meal affect the termination of feeding independently of its initiation.

It is important to note that the measured probability of feeding does not correspond to any hypothetical 'tendency' to feed, but to the interaction of such a hypothetical entity with the stimulus circumstances of the animal. Considering the probability of feeding beginning, for example, the tremendous rise in this probability between the IMI and first intra meal IBI, is as much a product of the fact that the animal's stimulus circumstances have changed (it has come into the presence of food), as any hypothesised internal changes.

The fall in this probability towards the end of the meal does, however, probably accord more closely with some change in the control system itself, as the stimulus (external that is) conditions are relatively constant. Similarily the changes in the probability of a bout ending as the meal proceeds probably represent a change which is primarily in the control system itself, rather than in the external circumstances of its operation.

Changes in the probability of any given activity ending reflect alterations in the capacity of some or all other stimuli to interrupt that behaviour, that is to say in the general or specific distractibility of the animal. As the meal proceeds, therefore, and the animal gets well and truly into feeding, its distractibility decreases.

Distractibility is a function of two components, the effectiveness of the feeding stimuli at maintaining feeding, and the power of other stimuli to intervene. Changes in either or both will result in alterations of the probability of feeding ending. It is at this level, therefore that one might expect phenomena related to the integration of different behaviours to appear.

Towards /

Towards the end of the meal, changes occur which are a simple opposite of the changes in probability occuring during the long intermeal interval which were noted in Chapter Five. The probability of a feeding bout beginning falls, only to rise again after fifteen minutes or so. These changes accord most closely with the classical concepts of 'satiety', which accumulates during the meal, until feeding is inhibited, and then disperses over the following minutes, allowing feeding to begin again. (e.g Wiepkema (1971b)

It has been observed that meal size changes over the oestrus cycle. It is of importance therefore, to determine which of these two systems is affected during the period of sexual receptivity.

8.4 Meal maintenance over the oestrus cycle

Changes in meal size have been observed, and the question which must be asked is whether these are the product of alterations in the mechanisms underlying the initiation of meals, termination of meals, or both. If only terminating mechanisms are involved then we would expect changes in the number of bouts with no change in bout length. If mechanisms governing the onset and course of the meal are affected then changes in bout length would be expected, which may or may not be associated with changes in the number of bouts, depending on the nature of the mechanisms terminating the meal.

The first problem is therefore to consider whether the number of bouts per meal changes over the oestrus cycle.

8.4.1. Number of bouts per meal over the cycle

The mean number of bouts per meal for each animal on each day of the oestrus cycle on each food were calculated, and are shown in Table /

Fig. 8.4(a)

Mean number of bouts per meal, and mean bout length over the nights of the oestrus cycle in female mice feeding on either laboratory chow(filled circles)or golden nuggets(filled squares)

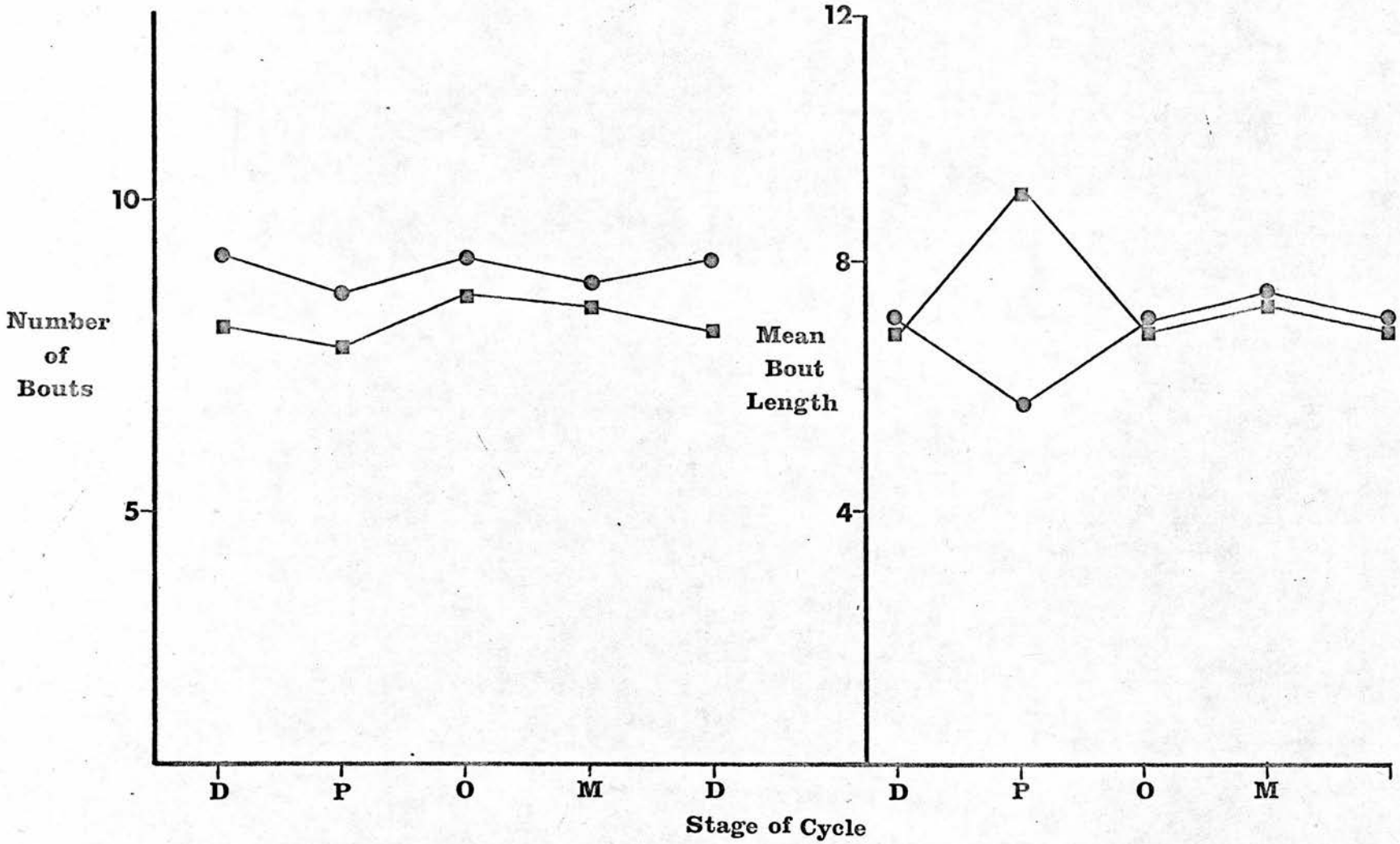

Number of Bouts
10
5
Mean Bout Length
12
8
4
D
P
O
M
D
D
P
O
M
Stage of Cycle

TABLE 8.4(i)

Number of bouts per meal and mean bout length over the cycle

CHOW

No. Bouts				Mean bout length (sec)			
D	P	O	M	D	P	O	M
10.2	10.2	11.3	10.7	5.98	3.8	3.52	5.94
13.2	14.5	14.8	13.9	4.06	3.24	4.04	3.86
9.8	8.9	9.3	7.6	4.34	3.2	3.26	6.0
8.4	6.9	7.9	6.8	7.4	6.74	8.68	9.52
6.2	5.4	5.2	5.6	11.64	9.8	12.96	12.16
6.6	6.2	6.1	6.7	11.56	8.8	11.44	9.06
9.1	7.6	9.3	8.3	5.28	5.12	5.74	6.92
9.1	8.5	9.1	8.5	7.18	5.8	7.08	7.62

Friedman's X = 2.9 n.s.

Friedman's X = 11.91 P<0.005

NUGGETS

No. Bouts				Mean bout length (sec)			
D	P	O	M	D	P	O	M
8.4	5.9	7.6	7.9	8.94	17.14	10.18	10.74
6.4	9.1	7.8	7.3	8.58	8.9	8.68	8.04
7.5	8.0	9.0	9.2	7.06	7.76	5.72	6.6
10.7	9.2	12.9	11.1	3.72	4.94	3.5	4.38
7.0	6.3	6.2	6.6	6.50	7.96	6.6	7.5
8.0	7.7	8.7	8.4	6.96	9.34	6.94	7.44

Friedman's X = 1.56 n.s.

Friedman's X = 9.72 P<0.02

Means for each animal

Table 8.4.(i). The figure does not vary with either food. The changes in meal size are therefore entirely the product of variations in mean bout length, which does vary significantly over the cycle (Table 8.4.(i), Friedman's chi 11.91 $P<0.01$ for chow, chi 9.72 $P<0.02$ for nuggets) (Fig. 8.4.(a)).

The implication from these data is that sexual receptivity affects meal size by an action upon mean bout length, with no effect upon the number of bouts, At least one component of the effects must therefore lie with the mechanisms controlling the termination of feeding bouts. As, however, there is no change in the number of bouts, despite the fact that the mean bout length is altered, then so long as it is assumed that the terminating functions of the meal are somehow produced by an accumulation of the products of feeding, it must also be assumed that there is some compensatory change in these mechanisms to keep the number of bouts constant.

The first problem, however, is to examine the changes in the mean bout length. These could arise by two ways, on the one hand it may be that the bout length is generally altered throughout the meal, or alternatively some changes may appear in the initial facilitation of bout length.

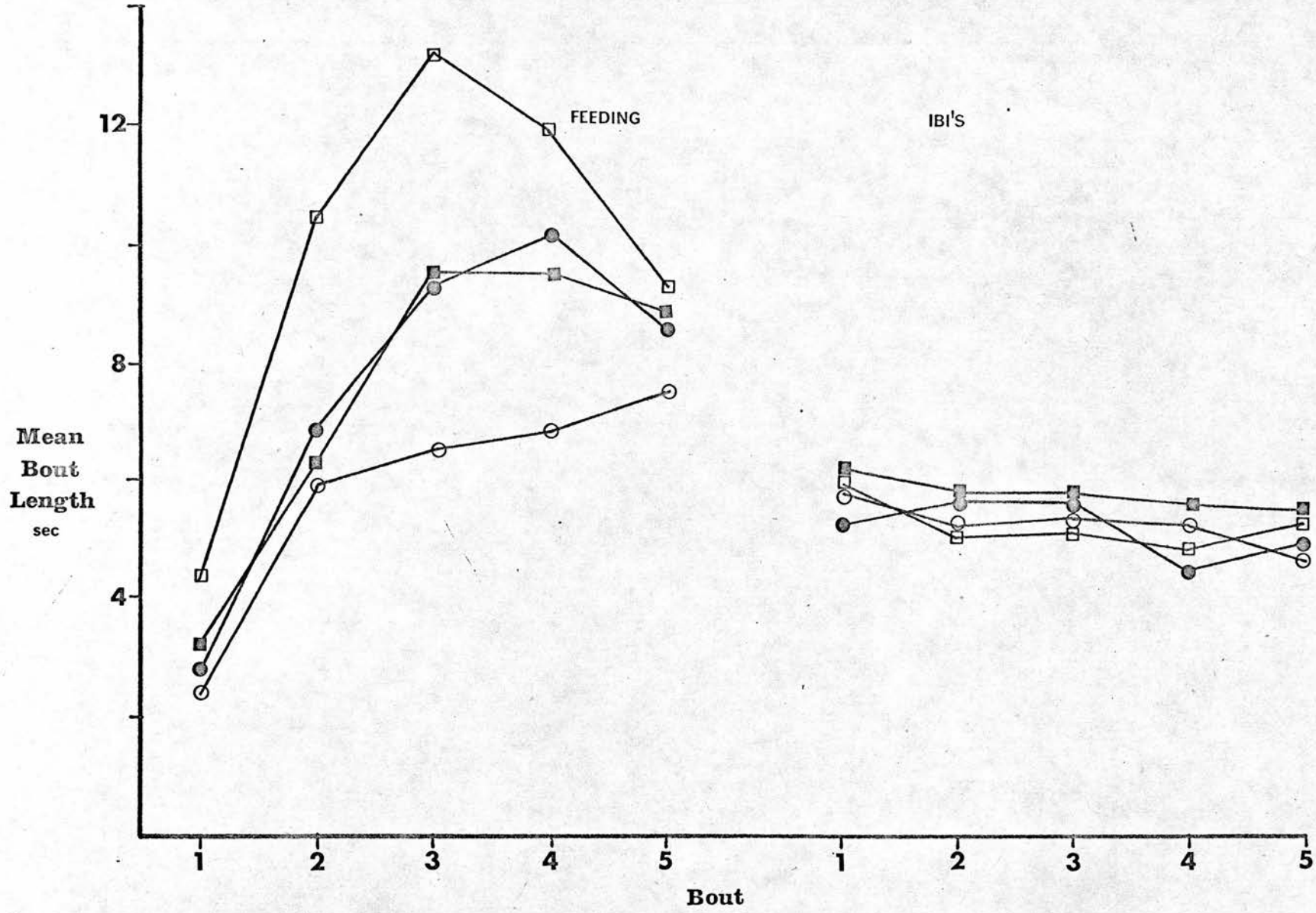
8.4.2. Initial facilitation over the cycle

Mean bout length was calculated as a function of position in the meal for each day of the cycle on each food over the first five bouts.

The results are shown in Fig. 8.4(b). Considering firstly non-receptive animals. The data from the three non-receptive nights of the cycle /

Fig. 8.4(b)

The mean bout length of the first five bouts and IBI's in meals of either laboratory chow (circles) or golden nuggets (squares), taken by receptive (open symbols) and non-receptive (filled symbols) female mice.


FEEDING
IBI'S
12
8
4
Mean
Bout
Length
sec
1
2
3
4
5
1
2
3
4
5
Bout

cycle are so similar that they can and have been combined to produce a single line for each food. The curve for non-receptive animals feeding on chow is exactly the same as that described in the previous sections. Non-receptive animals eating golden nuggets also show a very similar curve, with an equally significant rate of facilitation (Friedman's chi = 18.4 $P<0.005$).

On the night of sexual receptivity this similarity is abolished. If sexually receptive animals are eating laboratory chow, then the rate of initial facilitation of bout length is reduced. The effect of this is that the animals take a greater number of bouts to reach the steady bout length of about eight seconds than they do when non receptive, thus depressing the mean bout length over the whole meal.

If however, the receptive animals are given golden nuggets to eat the rate of facilitation is changed in the opposite direction, being very considerably increased. For animals eating laboratory chow, the third and fourth bouts are significantly shorter than during the rest of the cycle (Friedman's chi respectively 10.33 ($P<0.02$), 12.16($P<0.01$). The first and second bouts are not significantly shorter, and neither are the fifth and subsequent.

If, however, Golden nuggets is being eaten, the reverse is the case, the second, third and fourth bouts are significantly longer than over the rest of the cycle (Friedman's chi respectively 8.2 ($P<0.05$), 8.7 ($P<0.05$), and 9.8 ($P<0.02$), and the remaining bouts the same duration.

Fig. 8.4(a) also shows data for changes in intra-meal interval as

a /

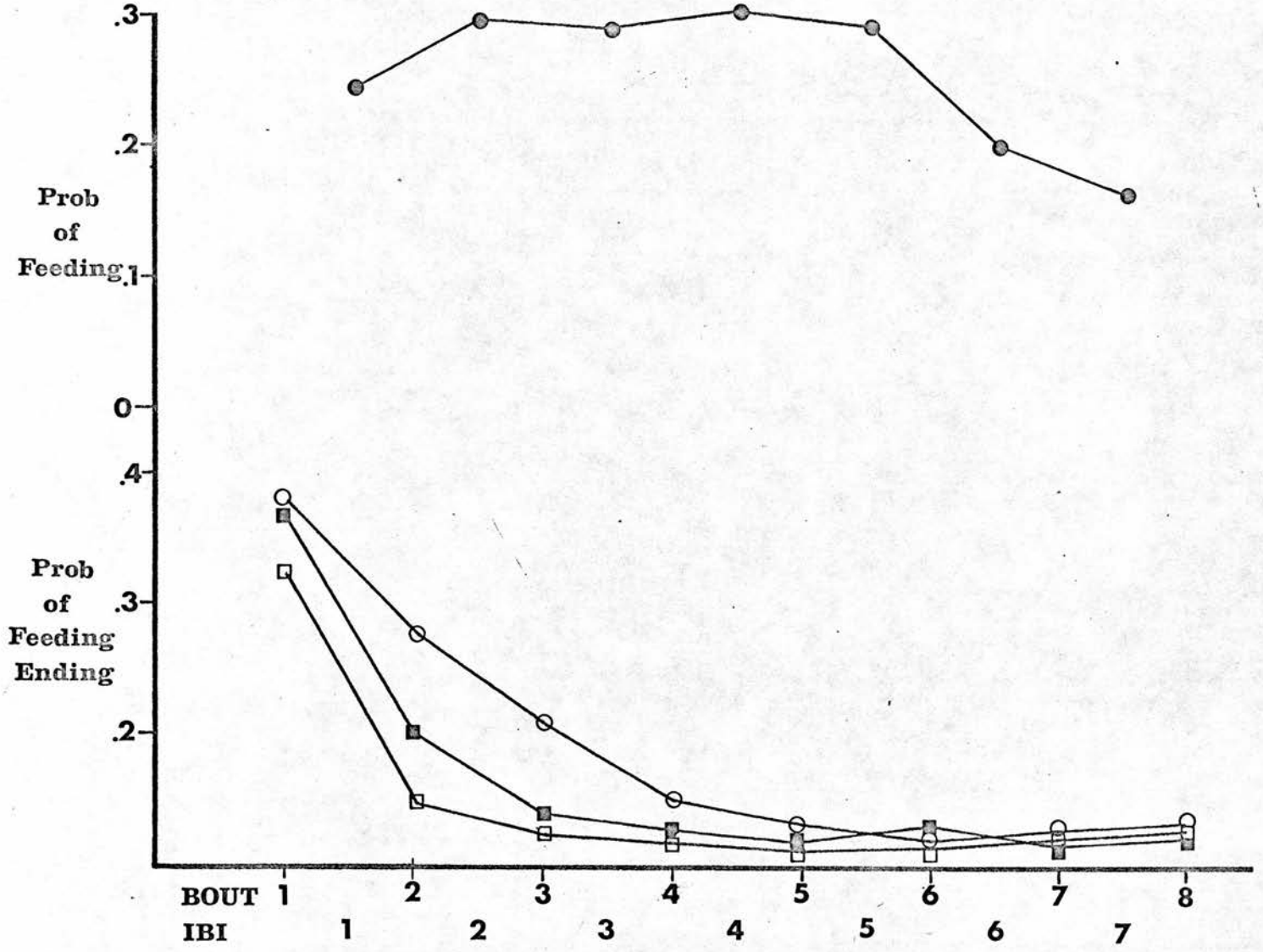
a function of position in the meal and stage of the cycle. There are no effects of either variable upon intra meal IBI.

The changes in facilitation may be further illuminated by an examination of the logarithmic survivor plots of bout lengths. Such plots for the first to fourth bouts of non receptive animals on chow have already been discussed, and a gradual decrease in the probability of feeding ending has been noted. The rate of this decrease is itself affected by sexual receptivity. Survivor plots of bout length for sexually receptive animals feeding on chow and nuggets all show the typical initially steep component, followed by a linear decline, whose slope is dependent upon the position of the bout in the meal. The nature of this dependence is itself affected by sexual receptivity and the type of food offered. This is illustrated in Fig. 8.4(c) in which the probability derived from the slope of the survivor curve is plotted as a function of position in the meal. It will be noted from this figure that the probability changes are very similar to those in mean bout length. Under all conditions the probability eventually reaches a constant value of c. 0.13, the effect of sexual receptivity being to determine at which bout this asymptote is reached. Receptive animals given chow to eat for example do not reach it till the sixth bout, whereas if they are fed golden nuggets it is reached by the third.

There is however, one problem, The mean length of the second, third and fourth bouts of meals taken by sexually receptive animals eating golden nuggets are longer than would be expected from the survivor probabilities. This disparity can only be accounted by changes in the distribution of very long bouts. The number of bouts exceeding the measured linear portion of the survivor plot is the same in both receptive /

Fig. 8.4(c)

The probability of feeding beginning (in the period 4-15 seconds since the last feeding bout), and ending (in the 4th-15th second of a bout),as a function of position in a meal of either golden nuggets or laboratory chow taken by receptive and non-receptive female mice.(To minimise confusion similar curves have been shown as one - all non-receptive animals are represented by filled symbols - receptive females eating chow,open circles - receptive females eating nuggets, open squares. The changes in the probability of feeding do not alter with either food or sexual receptivity, and are shown as a single line(filled circles).


.3
.2
Prob
of
Feeding
.1
0
.4
Prob
of
Feeding
Ending
.3
.2
BOUT 1 2 3 4 5 6 7 8
IBI 1 2 3 4 5 6 7

receptive and non-receptive animals eating golden nuggets, but their positions on the time axis are different, so that there are some bouts which are extremely long (up to 160 seconds), a phenomenon quite unknown in animals under any other condition considered here.

Sexually receptive animals given golden nuggets to eat therefore occasionally emit unexpectedly long bouts, and as these tend to occur at the beginning of the meal, they tend to exaggerate the measured mean bout length at this stage. The fact remains, however, that there are still changes in the probability of bouts ending which are independent of these extra long bouts, so that even if they did not occur the increased rate of facilitation would still be apparent.

The changes in mean bout length at prooestrus are therefore exclusively the product of alterations in the rate of initial facilitation of bout length, which is increased for sweet food, and decreased for laboratory chow. These alter the mean length of the first few bouts and by this route only change the measured mean bout length over the whole meal.

8.4.3. Discussion

Changes in meal size over the oestrus cycle can therefore be shown to be a product of changes in bout length within a meal, with no change in interbout intervals. These changes in bout length are themselves the consequence of an alteration in the mechanism which produces a steady increase in bout length over the first few bouts of the meal. This increase is primarily the product of a reduction in the probability of a bout ending, which may be taken as a measure of the distractibility of the animal whilst feeding. The probability is /

is stationary over bout lengths encompassing at least 95% of all bouts greater than four seconds, and is observed to decrease steadily towards an asymptotic value with successive bouts into the meal. The rate of this decrease is the primary variable affected by sexual receptivity, it being increased for sweet food and decreased for chow at prooestrus.

Meals appear to be terminated after a certain number of bouts irrespective of the stage of cycle. This would imply that either:

(a) The mechanisms terminating a meal are independent of the amount consumed in the meal over the range considered here (the alterations in mean bout length therefore produce changes in meal size), or

(b) The oestrus cycle, in addition to altering mean bout lengths in different directions for different foods has in each case an opposing, compensatory effect on the mechanisms of meal termination which ensures that these changes in bout length are not automatically compensated by changes in the number of bouts.

We have seen that the end of the meal is preceded by a fall in the probability of initiation of a feeding bout, which would suggest some sort of inhibition of the tendency to feed. This fall is no greater in sexually receptive animals feeding upon golden nuggets as would be expected if this inhibition depended upon meal size in this case. The two alternatives described above cannot therefore be discriminated with the data recorded here. The former is, however, more plausible, as the latter would require a very complex system of control to ensure that the differential changes in meal size on the two foods were both compensated accurately. It may be, therefore, that /

that at procestrus at least, the mechanisms terminating meals are to some extent independent of the meal size.

Meals are the product, therefore, of a complex of control processes which may be affected in different ways by the period of sexual receptivity.

8.5 Summary

(i) Meals in the mouse may be considered as the product of interacting incremental and decremental effects.

(ii) Bouts and intra meal IBI's are both characterised by a high proportion of very short members, which may reflect a form of behavioural 'bounce'.

(iii) Once four seconds have passed, however, the probability of both feeding and non-feeding bouts ending is constant over time up to 30 seconds. This probability may be used as a parameter of feeding.

(iv) The results of Wiepkema (1971) have been replicated in the free feeding female mouse. Bout length increases systematically over the first few bouts of the meal. This is a product of a fall in the probability of a bout ending, the 'distractibility' of the animal.

(v) The end of a meal is preceded by a predictive fall in the probability of a bout beginning, with no change in its probability of ending. This may reflect the operation of conventional 'satiety'.

(vi) Changes /

(vi) Changes in meal size over the oestrus cycle are solely the product of changes in mean bout length within meals.

(vii) The changes in mean bout length are themselves the product of alterations in the rate of initial facilitation of bout length.

(viii) The lack of changes in the number of bouts within meals with alteration in meal size would suggest that there is also at prooestrus some interference with the terminating functions of the meal, which could take the form of a removal of the normal 'satiety' influences.

(ix) Sexual receptivity exerts its effects on meal size, therefore primarily by altering the distractibility of the animal in the initial stages of the meal.

CHAPTER NINE

DETAILS OF THE CHANGES IN MEAL FREQUENCY

9.1 Introduction

When discussing changes in meal frequency there are two problems revealed in the preceding chapters which need to be discussed. On the one hand, the gradual change in meal frequency over the oestrus cycle, and on the other, the failure of meal frequency changes to compensate for the alterations of meal size on the night of prooestrus.

In Chapter One possible mechanisms for the short term regulation of energy balance were discussed, and it was concluded that, in the rat at least there is a correlation between meal size and following inter meal interval, which may form the basis of a system whereby temporary mismatches in supply and demand are corrected by alteration of IMI.

The gradual changes in IMI over the cycle may therefore represent a modulation of a mechanism of this type. The sudden dissociation between meal size and IMI at prooestrus will need to be explained by another action on this or a similar mechanism.

9.2 Nature of the changes in IMI

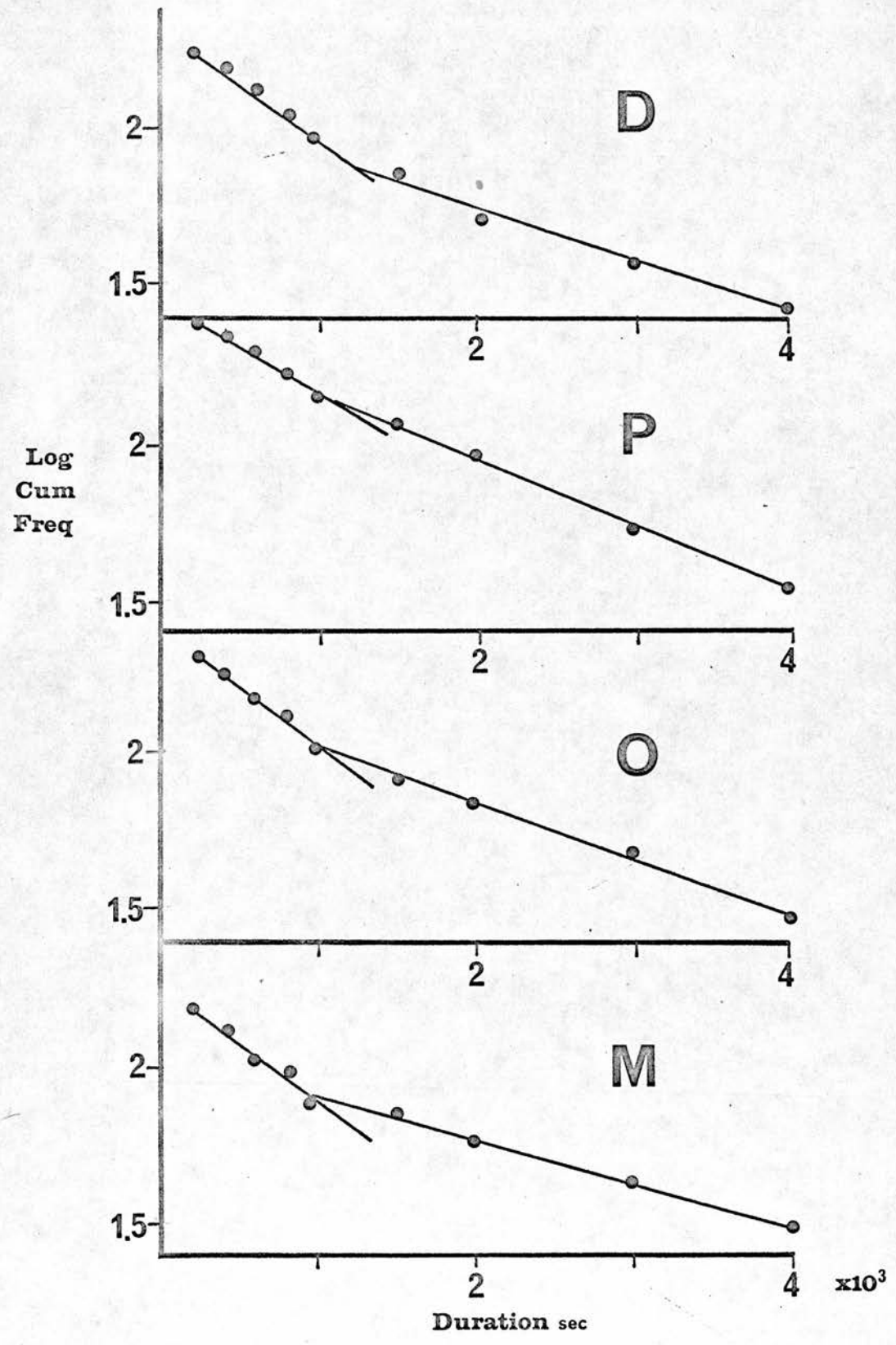
In Chapter Five a case was made for the division of the night time meal pattern into feeding 'sessions', during which meals are separated by relatively short intervals. The sessions are themselves separated by periods in which meals are relatively infrequent.

Observing a change in IMI, it is reasonable to ask whether the change /

Fig. 9.2(a)

The changes in logarithmic survivor plot

of IMI's over the nights of the oestrus cycle


D
P
O
M
2
1.5
2
4
Log
Cum
Freq
x10³
Duration sec

change is due to alterations of intra- session IMI, extra-session IMI, or both. These two possibilities may be examined by considering the survivor curves, and derived probability functions. Fig.9.2(a) shows survivorship curves of IMI (that is, ignoring very short IBI's) for each night of the cycle. It is immediately obvious in each case that the curve divides into two parts, reflecting intra- and extra-session meals. The slopes of the two components of the curve may be estimated, and converted to probabilities of feeding in any 200 second interval. The probability of an IMI, once it has reached 1200 seconds ending in any given 200 second interval does not vary over the oestrus cycle. Outside of sessions, therefore, the spacing of meals remains the same whatever the cycle stage.

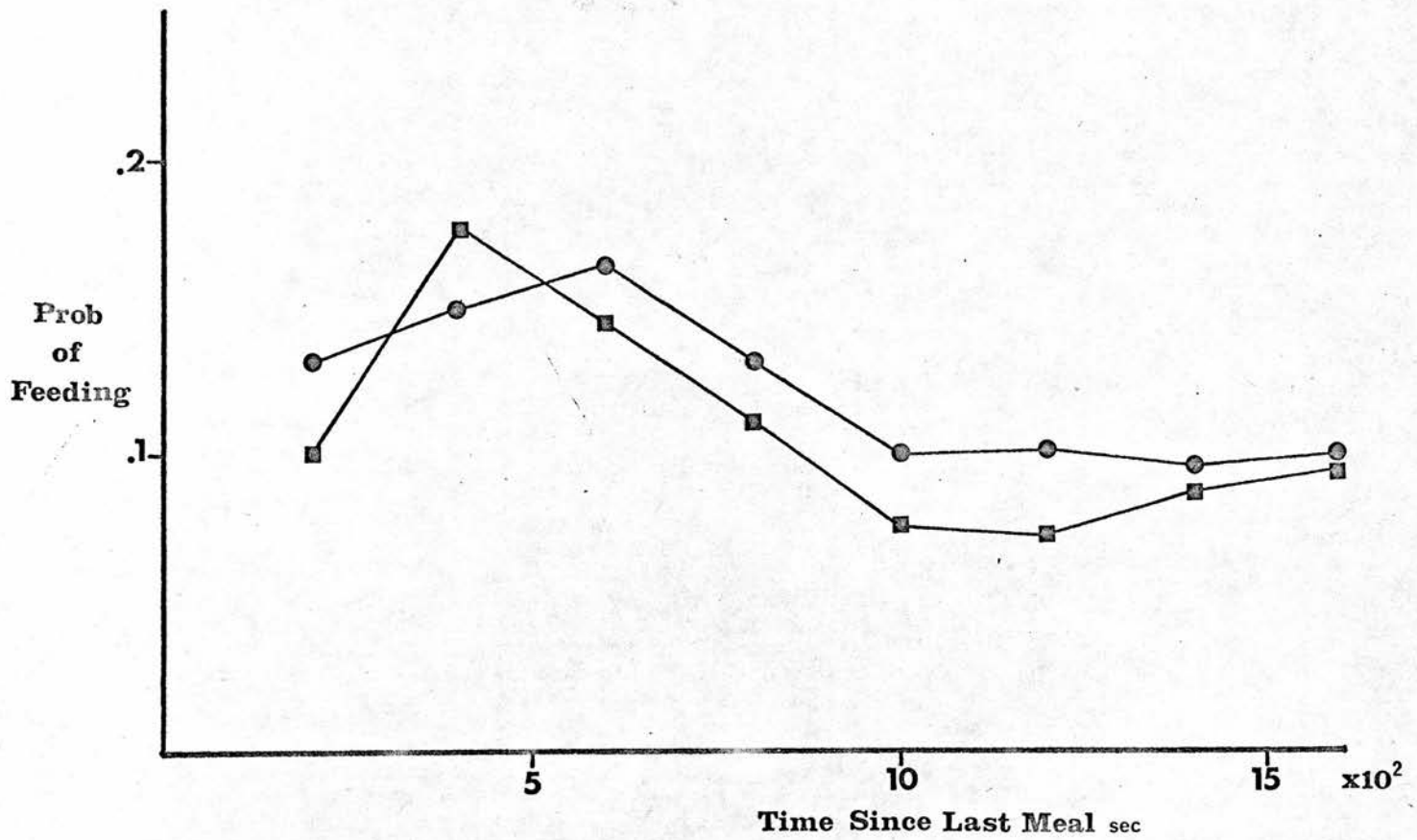
When the initial relatively steep component of the curve is considered, however, there are changes over the cycle. At metoestrus, when the IMI is smallest, the probability of feeding in any given 200 second interval in the range 200-1200 seconds is higher than the rest of the cycle.

Whilst it is possible to fit a good straight line to this initial portion of the curve, it is not perfectly straight, and its non-linearity points to a very important property of intra-session IMI's which distinguishes them from inter session IMI's. This may be illustrated by constructing curves of the probability of feeding as a function of the time since the last meal for animals at dioestrus and metoestrus respectively. Fig. 9.2(b) records the results of such a calculation. The probability curves take the form of a hump, peaking in the range 500-1000 seconds, followed by a straight line. The peak of the

maximum /

Fig. 9.2(b)

Changes in the probability of feeding (in any 200 second interval) as a function of the time since the last meal over two nights of the oestrus cycle. Dioestrus (filled circles) and metoestrus (filled squares).


.2
.1
Prob
of
Feeding
5
10
15
x10²
Time Since Last Meal sec

maximum probability of feeding occurs at a different point in time over the two days of the oestrus cycle which are illustrated, being moved relatively towards the origin of the time axis at metoestrus.

This may be examined in another way. The mean of the intra- and extra- session IMI's may be separately calculated for each day of the cycle. These are shown in Fig. 9.2(c), and tables 9.2(i) and [illegible].

The long inter session IMI's remain practically constant over the nights of the cycle, declining by just 5% from dioestrus to metoestrus (a non-significant change), whereas the short intra-session IMI's show a steady decline typical of the tdal IMI, falling by 19% from dioestrus to metoestrus. This change is significant (Friedman's chi = 8.3 P<0.025). At the same time as the fall in mean short IMI, there is an increase of the proportion of meals that fall within sessions, from 49% to 53%.

The change in mean total IMI during the night from dioestrus to metoestrus would predict the appearance of two extra meals. These are clearly fitted in by increasing the frequency of meals within sessions, which presumably remain roughly the same length. If there are two such sessions during the night, each consisting of roughly five meals(which would be the case as they account for 50% of the night time meals), then the change in short IMI would predict, so long as the total duration of each session remains constant, the addition of one meal to each, which is just right to explain the observed c.10% rise in intake at the end of the cycle.

In summary therefore, with animals feeding on laboratory chow (and also nuggets, although the data are not discussed in detail here), the /

Fig. 9.2(c)

Changes in intra session (less than 1200 sec) and extra session (greater than 1200 sec) IMI over the nights of the oestrus cycle in the female mouse eating laboratory chow.

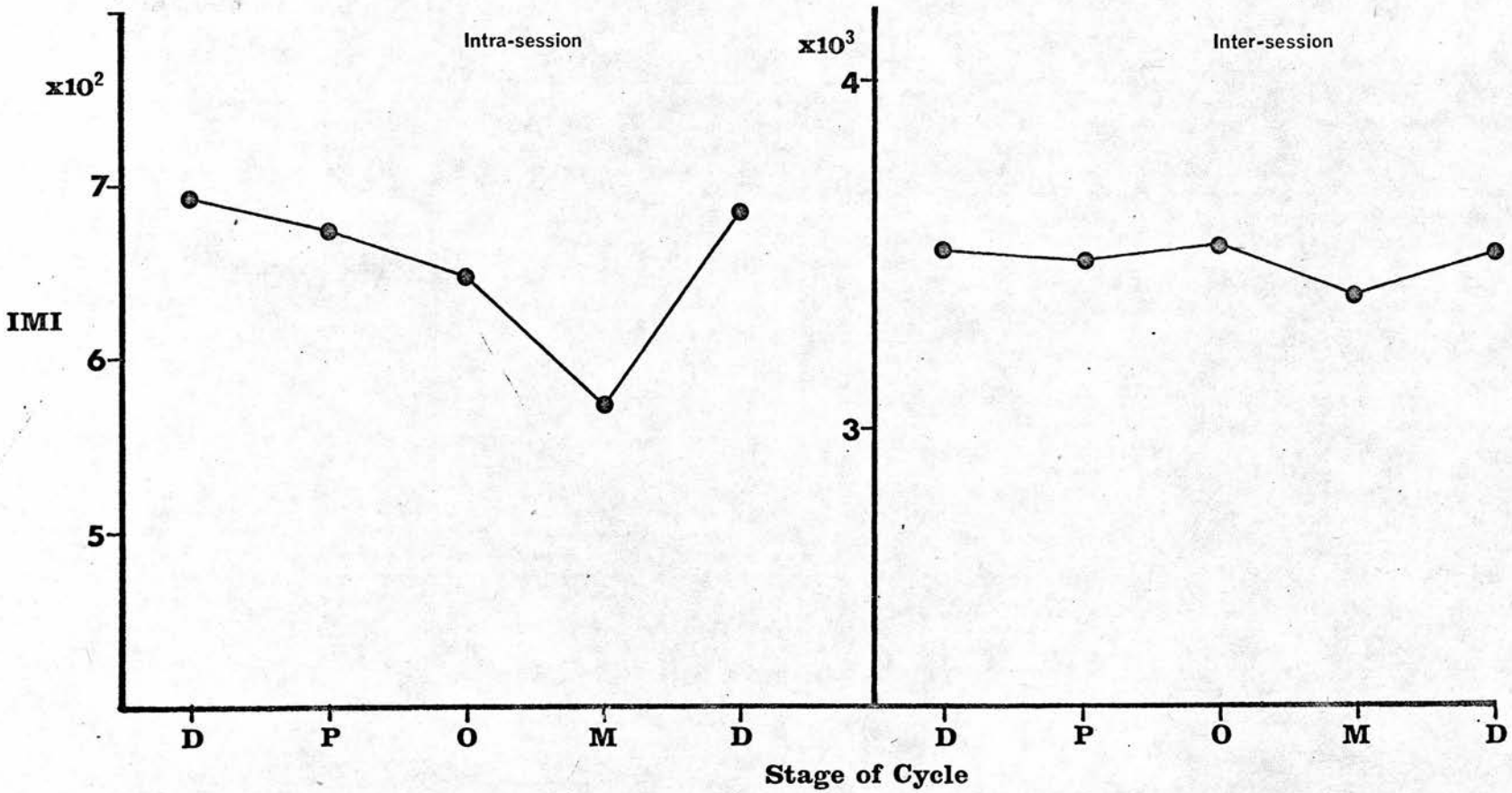

Intra-session
x10²
7
6
5
IMI
D
P
O
M
D
x10³
4
3
Inter-session
D
P
O
M
D
Stage of Cycle

TABLE 9.2(i)

Inter and Intra-session IMI's

All in seconds
Means for each animal

DIOESTRUS

IMI	Intra-session	Proportion	Extra session
2640	674	44	4184
2182	660	52	3830
2004	760	46	3062
1920	724	52	3214
1922	660	54	3402
2580	750	38	3700
2092	720	47	3308
~~1078~~ 2190	706	47.6%	3528

PRO-OESTRUS

IMI	Intra-session	Proportion	Extra session
2798	666	34	3896
2224	676	40	3256
1568	684	69	3534
2062	650	33	2756
1702	662	47	2624
5140	752	17	6038
1440	630	50	2250
2418	674	41%	3478

OESTRUS

IMI	Intra-session	Proportion	Extra session
2300	530	33	3170
2408	648	44	3790
2160	696	52	3746
1200	678	60	1982
1800	656	64	3832
3220	690	38	4770
2094	640	49	3490
2170	648	48%	3540

MET-OESTRUS

IMI	Intra-session	Proportion	Extra session
1948	606	46	3090
2032	640	50	3424
1504	694	44	2140
1604	444	52	2860
1840	536	63	4060
2996	574	46	5058
1556	528	55	2812
1894	574	51%	3348

the observed metoestrus rise in food intake is apparently solely the product of changes in night time meal frequency. These changes are themselves due to an increase in the number of meals included in each feeding session, with no change in the frequency of meals outside of sessions. It is necessary to determine to nature of the mechanisms underlying these changes.

9.3 Correlational analysis between meal size and IMI

Several authors have examined the existence of a relationship between meal size and following IMI in rats, and most agree that there is a significant correlation. Wiepkema (1968) was, however, unable to find such a correlation in male mice. Accordingly correlations between meal size and IMI under various conditions were computed for the mice in this study.

If all meals and intermeal intervals are used, there is no significant correlation between either meal size and following IMI, or preceding IMI. It will be remembered from the previous section, however, that only the short intra-session IMI's are altered to produce changes in meal frequency over the cycle, so it may be that a similar distinction can be made in the case of correlations. Correlations between meal size and following and preceding IMI were therefore separately calculated over each day of the cycle for meals followed by long inter-session and short intra-session IMI's.

Data from each of the days of dioestrus, oestrus and metoestrus is shown for the case of intra-session IMI's following meals, as scatter diagrams in fig. 9.3(a). On each of these days there is a significant correlation between meal size and following intra-session IMI /

IMI, for animals eating both laboratory chow and golden nuggets. Only the data for chow are, however, illustrated in the figure. For dioestrus the correlation coefficient is 0.54 (.01) for chow and 0.39 (.01) for nuggets, at oestrus the figures are 0.64 (.01) and 0.53 (.01), and at metoestrus 0.39 (.01) and 0.54 (.01)

The correlations are calculated lumping raw data across animals, as only a small proportion of the animals yield sufficient data on a single day of the cycle for the reasonable calculation of correlation coefficients. If, however, data is lumped across days for each individual animal, then for six of the seven females eating chow, the correlation is individually significant, (despite variations in the relationship - see below).

No matter how the data are organised, however, there are no correlations between meal size and preceding IMI, nor between meal size and following IMI if the IMI exceeds 1200 seconds.

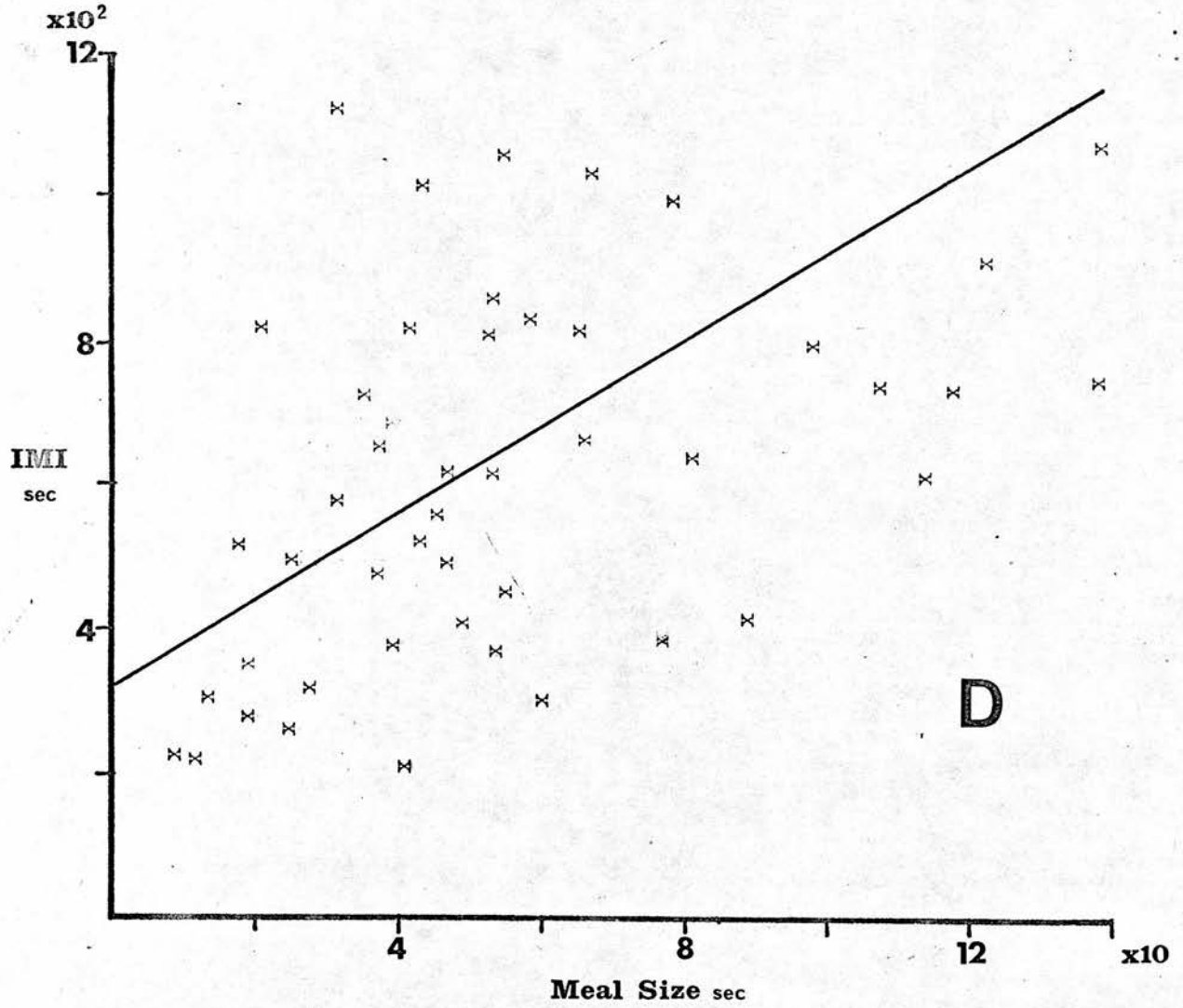
The scatter diagrams also reveal changes in the nature of the relationship between meal size and following IMI over the three non-receptive stages of the oestrus cycle.

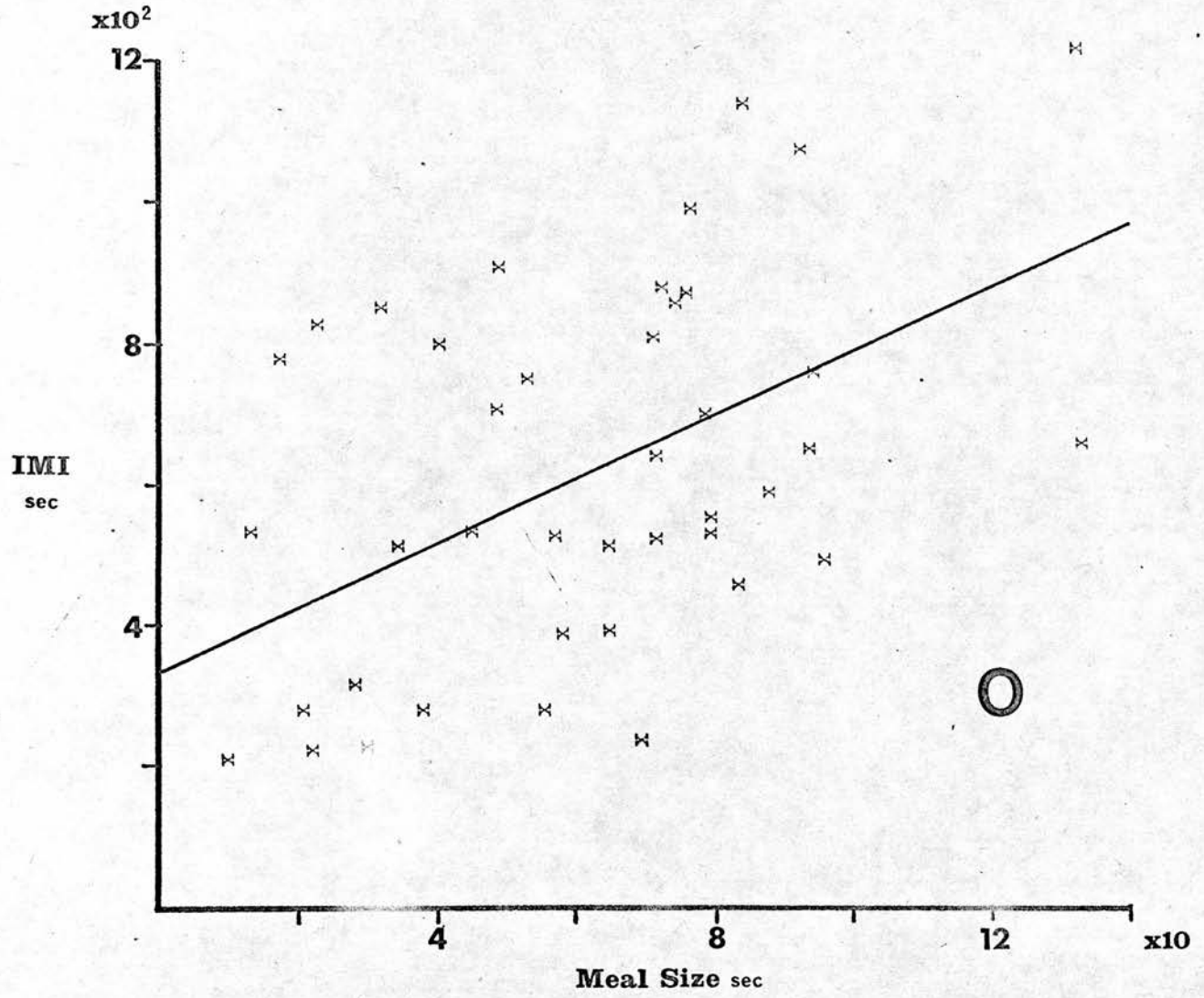
As the cycle proceeds, the slope of the least squares fit regression line between meal size and following IMI decreases. This change in slope is sufficient, to account for the variations in the intra-session IMI. Reading from the regression line, and using the observed mean meal sizes for dioestrus, oestrus and metoestrus, we obtain expected mean short IMI's of 696, 630, and 590 seconds respectively, as compared to the measured values of 706, 648, and 594.

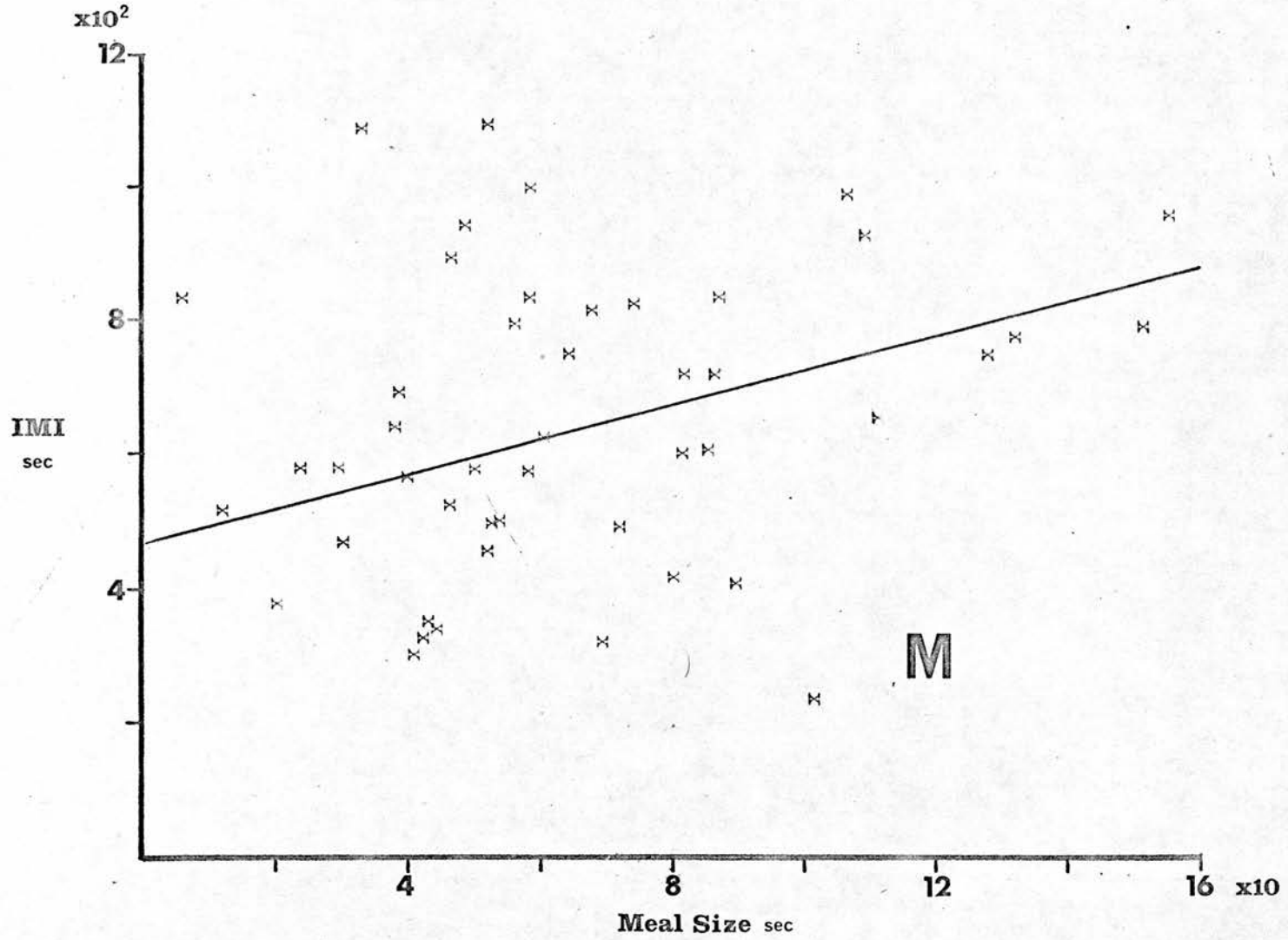
Within /

Fig. 9.3(a)

The following 3 figures show the correlation between meal size and following IMI in the intra-session feeding of female mice on laboratory chow over the three non-receptive nights of the oestrus cycle.


x10²
12
8
4
IMI
sec
D
4
8
12
x10
Meal Size sec


x10²
12
8
4
IMI
sec
4
8
12
x10
Meal Size sec


x10²
12
8
4
IMI
sec
M
4
8
12
16 x10
Meal Size sec

Within sessions, therefore, the power of a given meal size to inhibit feeding varies over the latter stages of the oestrus cycle in a way sufficient to account for the changes in total intake at that time. The statistical significance of the changes in slope of regression was calculated by computing for each animal, at each stage of the cycle a separate regression coefficient, and subjecting the resulting table to a Friedman's test. The result was just significant (Friedman's chi 8.4 $P<0.05$).

The changes in meal frequency responsible for the increase in the intake of both foods at metoestrus may therefore be accounted for by an influenee of the cycle, and presumably, its direct or indirect hormonal effects, on the essentially regulatory mechanism which ensures, within feeding sessions, that chance variations in meal size are compensated by changes in IMI.

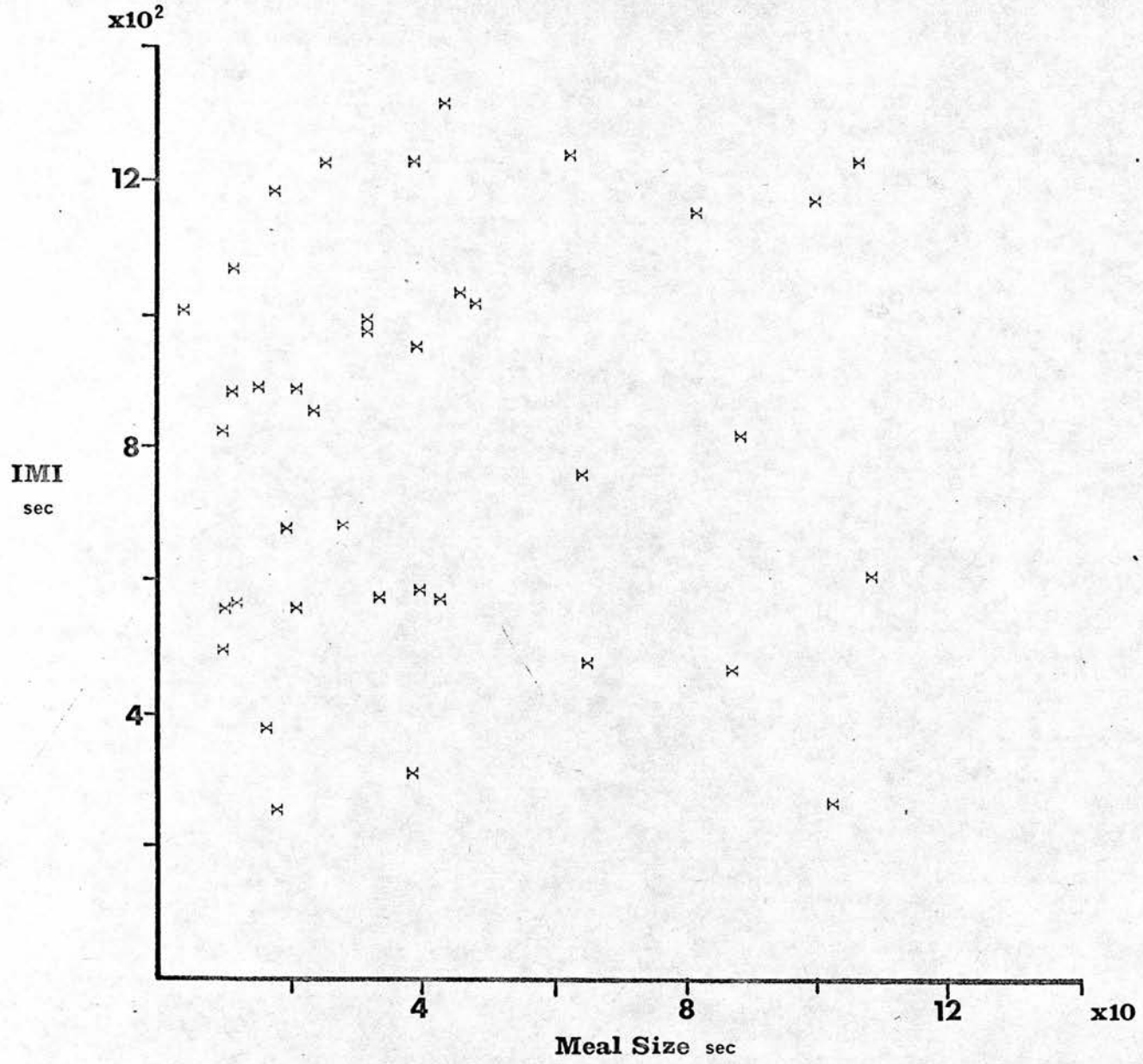
9.4 Changes at Prooestrus

At the time of sexual receptivity meal size is markedly altered. If the mechanism described in the previous section were operating at this time, then equally marked changes in meal frequency should occur. They clearly do not.

The correlation between meal size and following intra-session IMI was therefore examined in sexually receptive animals. The scatter diagram for animals eating chow is shown in Fig. 9.4(a). It is quite obvious that the normal relationship present on the other days of the cycle is absent at this time. There is absolutely no correlation, between meal size and following IMI, the coefficient of correlation being /

Fig. 9.4(a)

Scatter diagram of the relationship between intra-session meal size and IMI in the feeding of sexually receptive female mice upon laboratory chow.


x10²
12
8
4
IMI
sec
4
8
12
x10
Meal Size sec

being in this case fortuitously zero. A similar picture is apparent in the data from animals eating golden nuggets.

At the time of sexual receptivity, the feeding behaviour of the female mouse appears therefore to be released from controls which normally operate, so that meal size may vary independently of IMI. This is the primary change which allows the effects of food on meal size, discussed in the previous chapter, to appear. It will be recalled that they do not appear during the days of the cycle. Correlations were therefore calculated for animals on the day of oestrus, the only time when sufficient intra-session data is available. Even with the small amount of data available, a significant correlation is noted. (although the fact that the correlation appeared with so little data is somewhat fortuitous) The absence of correlation is therefore, unlike the changes in meal size, also confined exclusively to the night of sexual receptivity.

9.5 Discussion

The changes over the oestrus cycle therefore clearly influence the mechanisms underlying the generation of intermeal intervals. These influences may be divided into two categories, influencing what is presumably the same basic mechanism in two different ways.

Like the rat, the mouse shows a correlation between meal size and following IMI provided that certain conditions are met. This correlation is only apparent in the within session meals, separated by relatively short intervals. Outside of the sessions the relatively infrequent meals are spaced independently of their size.

Feeding sessions, by varying the parameters of this correlational relationship may be used to vary the food intake of the animal by an alteration of meal frequency.

This perhaps reflects the use of 'session size' to change the degree of increment to stores produced during feeding. If as would be expected from the discussion of the rat literature in Chapter One, and the experiments of Chapter Three, the animal towards the end of the cycle is behaving as though it were in the first stages after ovariectomy, then it would be expected to be below its set weight, and effectively food deprived. Under such circumstance a pattern of feeding producing an increment to stores would be expected. The observed change is an increase in session size, which will produce a rate of energy input during sessions which exceeds demands by a greater margin than during a smaller session.

The implication from the change in the relationship between meal size and IMI is that the 'maximum' frequency at which it was presumed meals were taken during sessions is in fact capable of variation. The time of resumption of feeding is clearly related to meal size, and unless gastric clearance varies over the oestrus cycle, it cannot provide the limiting, meal size related constraint upon the time of resumption of feeding. It is likely, therefore, in this case, that the usage of some component of the meal determines the IMI. In this context it is of interest to note that oestrogens, in humans at least have effects upon glucose tolerance. At the middle of the menstrual cycle, glucose is cleared less quickly than at other times, by virtue of a reduced hepatic and muscular uptake. (Jarret et al (1968) and Javier (1968)). If a /

If a similar effect is present in mice, then the changes observed above would be predicted, as the rate of utilisation of the meal would be less at the beginning of the oestrus cycle than the end.

The change at the time of sexual receptivity is, however, qualitatively distinct to these changes. Such a change is typical of an animal which is releasing feeding from its dependence upon metabolic factors, and is exactly analogous to the changes observed in obese humans by Cabanac and Ducleux (1968) (particularily in the case of sugar rich golden nuggets), and in women at the middle of the menstrual cycle (Wright and Crow (1974).

Nisbett (1972) and Cabanac (1974) have suggested that such changes in feeding occur in humans below their 'set weight', so that their appearance in obesity is a consequence of a primary pathological elevation of body weight set point.

The data presented here cannot support this assertion in animals, as an operation tending to increase set weight produces changes in feeding which are quite distinct. Rather it would appear that the changes in meal size and the relationship between meal size and IMI may reflect the operation of some biologically definable system for the release of feeding from constraints which may lead to its interference with other temporarily more important activities. If such a system exists then its inappropriate use may well lead to excessive weight gain in animals or humans exposed to palatable diets.

9.6 Summary /

9.6 Summary

1. The changes in IMI over the oestrus cycle are produced by alteration of intra session IMI only.

2. There is on the nights of dioestrus, oestrus and metoestrus a correlation between intra-session meal size and following IMI, which does not appear outside of sessions.

3. The slope of regression between intra-session meal size and following IMI varies over the cycle, so that a given meal size staves off feeding for less time towards the end of the cycle than at the beginning.

4. On the night of sexual receptivity this correlational relationship fails to appear.

CHAPTER TEN

THE FEEDING PATTERNS OF MALES AND CASTRATES OF BOTH SEXES

In this chapter data concerning the details of feeding pattern in male animals will be described, followed by data from ovariectomised females, and castrate males. An attempt will then be made to relate the feeding of these groups of animals to those of the female at various stages of the cycle. In all cases in this chapter animals were consuming only laboratory chow.

10.1 Males

As noted in Chapter Six, eight adult male mice were each recorded for three days each after a period of acclimatisation in the feeder cages. Data was then scored onto sheets in the usual way.

It is first necessary to determine whether the males show the same general temporal pattern of intake as the females, that is to say feeding bouts organised into meals, and meals into sessions. The fact that they do is demonstrated by a log. survivor plot of IBI's in Fig. 10.1(a).

Accordingly meal size and intermeal interval could be calculated for the day andnight records, together with measures of total intake. The data is presented in the form of a table in table 10.1(i). The figure for each animal is obtained from three nights, and up to three days of recording. In the case of animals recorded using solid state logic (1-4), data was collected on only a portion of the days, due to the necessity of integrating recording with the activities of other workers.

Considering /

Fig. 10.1(a)

Logarithmic survivor plot of all IBI's in the night-time feeding of male mice upon laboratory chow.

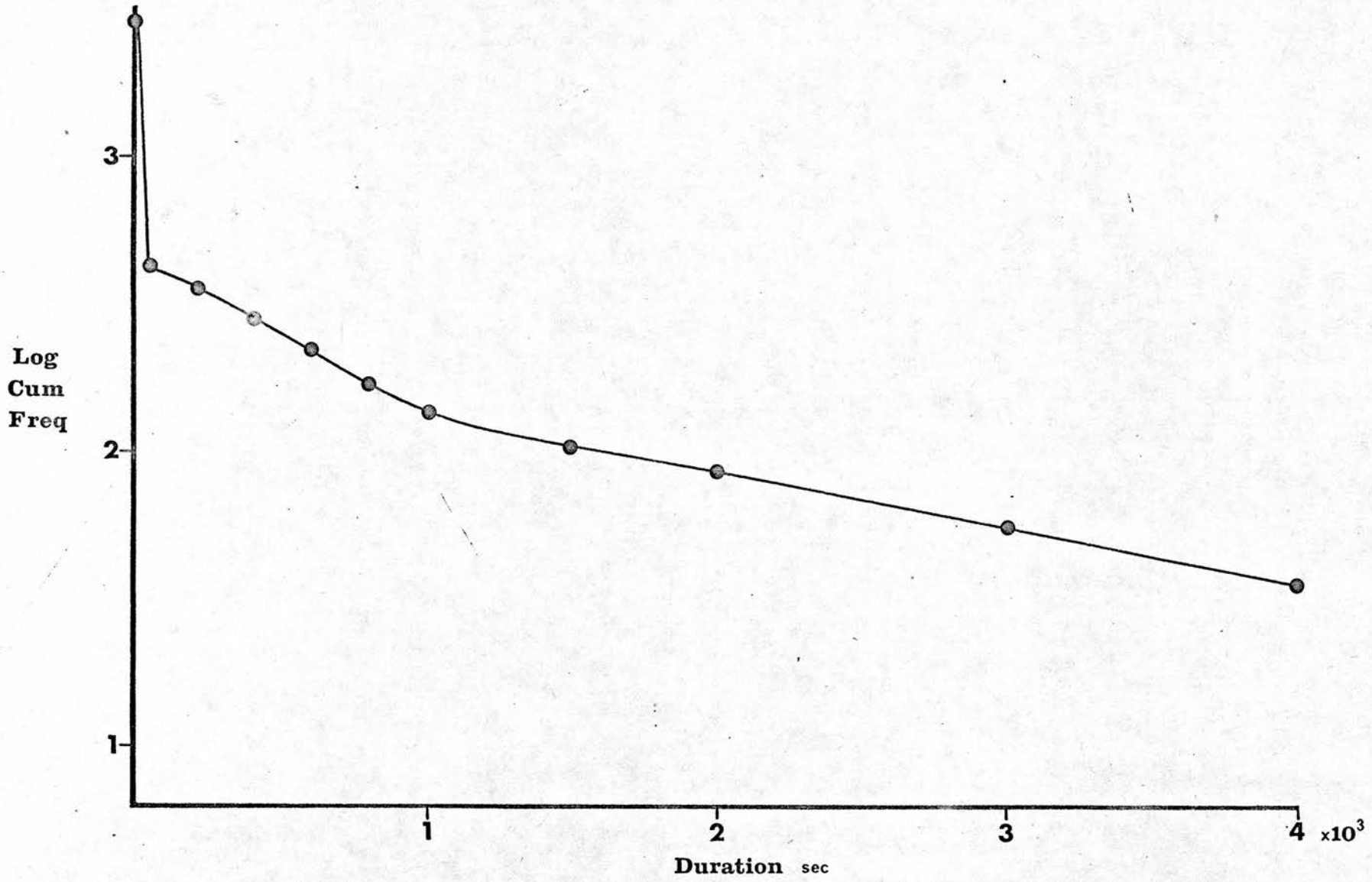

Log
Cum
Freq
3
2
1
1
2
3
4 x10³
Duration sec

TABLE 10.1(i)

Night and day feeding in intact males

All in seconds

Means for each animal

DAY

Meal Size	IMI	Total time feeding
33.4	6706	216
29.0	5968	210
27.0	6034	194
30.8	6008	222
23.8	5758	178
17.8	6444	120
41.2	8764	204
22.4	5780	168
28.2	6432	190

NIGHT

Meal Size	IMI	Total time feeding
38.6	1676	1160
34.4	1156	1498
49.0	1852	1332
56.0	1168	2416
52.8	1768	1504
33.8	1130	1506
43.8	1272	1608
67.8	2142	1594
47.0	1532	1576

Total time spent feeding in 24 hours ≡ 3.1g

Considering firstly the circadian rhythm of feeding in the male mouse eating laboratory chow. In this case the night and day totals are calculated from the observed meal size and frequency, as the counters used to record this directly for the females were not available at this time.

Males consume 85-90% of their food in the hours of darkness. Daytime consumption is relatively less than that of females, and night time consumption considerably greater. The 24 hour totals, when converted to apparent weight consumed show a close agreement to the measured daily intake of adult males.

The daytime feeding of males is characterised by relatively small meals, consumed very infrequently (one every two hours or so). The mean figures for daytime IMI in fact obscure a characteristic daytime pattern. Most males eat little, if at all during the morning, showing often a single long IMI, up to five hours, and then during the afternoon the frequency of meals gradually increases until darkness when the night time pattern begins. (This pattern has been observed only in cases where the feeders were not refilled in the middle of the day, so that there is insufficient data for a more complete analysis.)

The night time pattern of feeding contrasts markedly with that of the day. Meal size is much increased, although not to the level of the intact non-receptive female, and IMI is very much shorter than either the male daytime, or female night time figures.

During the night meals are organised into typical feeding sessions, one at the onset of darkness, another towards light, and a strong likelihood /

TABLE 10.1(ii)

Inter and Intra session IMI males

All in seconds
Means for each animal

IMI	Intra-session	Proportion	Extra-session
1676	514	62	3624
1156	620	71	2468
1852	676	32	2404
1168	622	61	3046
1768	562	64	3912
1130	418	78	3654
1372	426	75	4210
2142	526	51	3822
1532	544	62	3392

likelihood of another in between. On average over 60% of the night meals fall within sessions. Meals outside of sessions are separated by about 55 minutes, figure similar to that observed in the female. Within sessions meals are separated by, on average about nine minutes (see Table 10.1(ii)).

During the night males consume on average 90 mg of laboratory chow in each meal during roughly 50 seconds feeding time. The meal consists of 6-7 bouts, each lasting on average 7.5 seconds.

This may be compared to the meals of female mice. In this case there are two comparisons, between males and non-receptive females, and between males and females on heat.

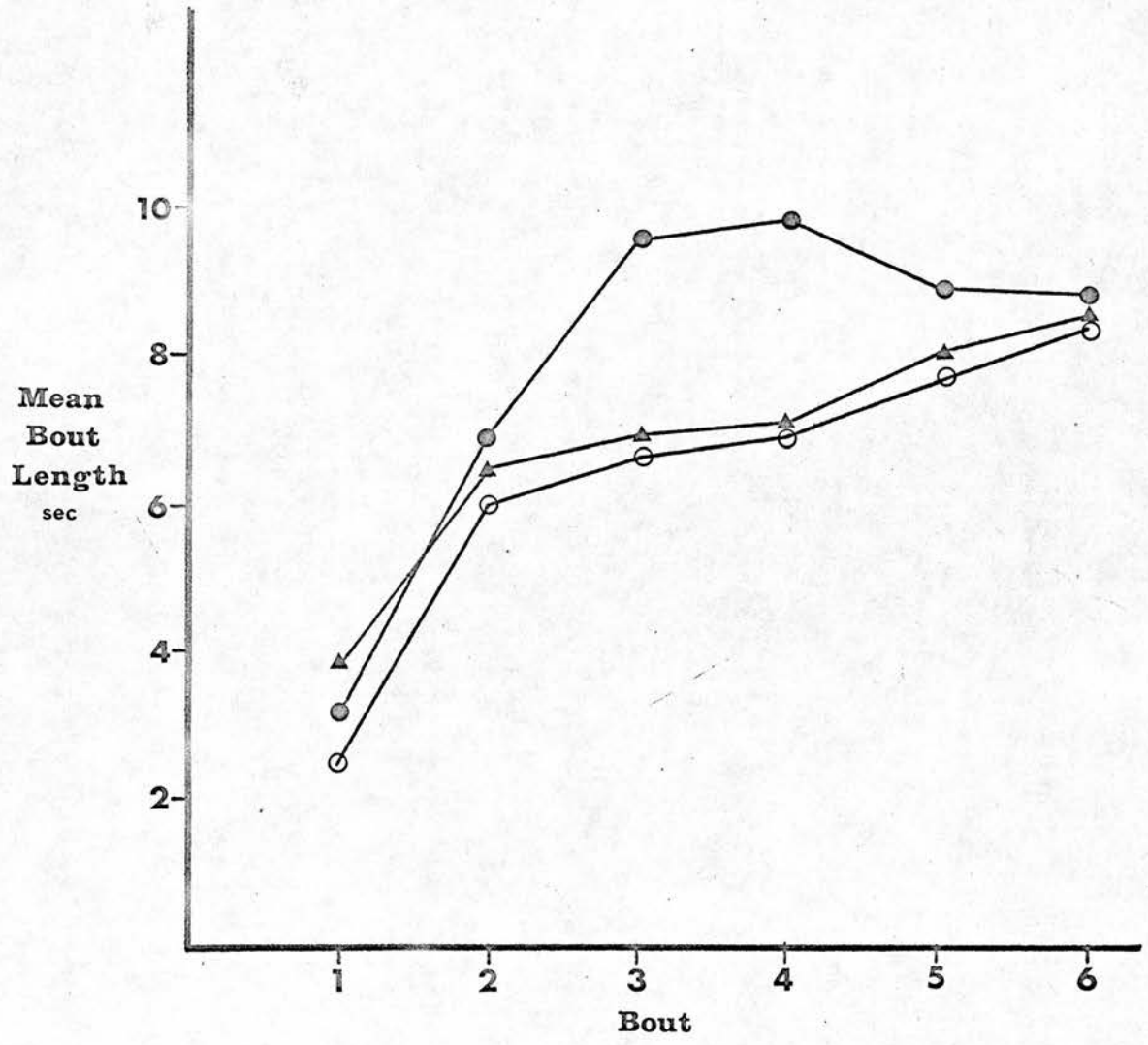
The night time meal size of males feeding on laboratory chow is very similar to that of females on the night of prooestrus, and is significantly smaller than that of the non-receptive females. ($P<0.02$ Mann Whitney 'U' Test).

This difference is produced partly by a reduction in the number of bouts (from 7.5 to 6.5), and partly by a fall in mean bout length (male 7.5 secs, female 8.0 secs). It is of interest to determine the factors producing the differences. Firstly, the difference in mean bout length. It was observed that the changes in meal size over the oestrus cycle were brought about by a reduction in bout length, itself the product of a reduced rate of facilitation of bout length.

Bout length over the first few bouts of a meal was therefore calculated for males, and is shown compared with receptive and non-receptive /

Fig. 10.1(b)

Mean bout length over the first five bouts of meals of non-receptive females (filled circles), receptive females (open circles), and males (filled triangles) eating laboratory chow.


Mean
Bout
Length
sec
10
8
6
4
2
1
2
3
4
5
6
Bout

receptive females in Fig. 10.1(b). The males behaviour can be seen to be very similar to that of the sexually receptive female, but quite different to that of the non-receptive animal.

In terms of meal size, and initial facilitation of bout length, therefore, males are very similar to females on heat. They are dissimilar in the fact that there are a smaller number of bouts in each meal.

When it comes to night time intermeal interval, however, males are quite different to females at any stage of the cycle. It has already been noted that the mean IMI is shorter, and that a higher proportion of meals are eaten within feeding sessions. The within session IMI is significantly shorter than for females at any stage of the cycle except metoestrus.(P 0.01 for dioestrus, oestrus and prooestrus. Mann Whitney 'U' test). The between session IMI shows no significant changes either over the cycle or between the sexes. The differences in total intake between males and females are therefore produced by changes within feeding sessions. Meal frequency within sessions, and the number of meals in each is increased both to offset the lower meal size in males, and to produce the observed sex difference in total consumption in favour of the male.

Over the oestrus cycle it has been seen that females alter IMI by varying the relationship between meal size and IMI. The nature of this relationship was therefore examined in males. Six out of the eight males tested showed a significant postprandial correlation for meals within feeding sessions, whereas none showed either pre-prandial correlation within sessions or any correlations outside of session.(see Table 10.1(iii)).

The slope of regression between intra session meal size and following IMI is indicated in Fig. 10.1 (c). The value of the regression coefficient (least /

TABLE 10.1(iii)

Correlations Males Product Moment Correlations

(i) Post prandial

ms v All IMI	ms v long IMI	ms v short IMI
+0.13 ns	+0.10 ns	+0.54 (P<0.02)
-0.02 ns	+0.01 ns	+0.58 (P<0.01)
+0.36 ns	+0.22 ns	+0.60 (P<0.05)
+0.03 ns	-0.05 ns	+0.38 (P<0.05)
-0.05 ns	+0.33 ns	+0.41 (P<0.05)
-0.18 ns	-0.1 ns	+0.69 (P<0.01)
+0.13 ns	+0.11 ns	+0.22 ns
+0.15 ns	+0.29 ns	+0.27 ns

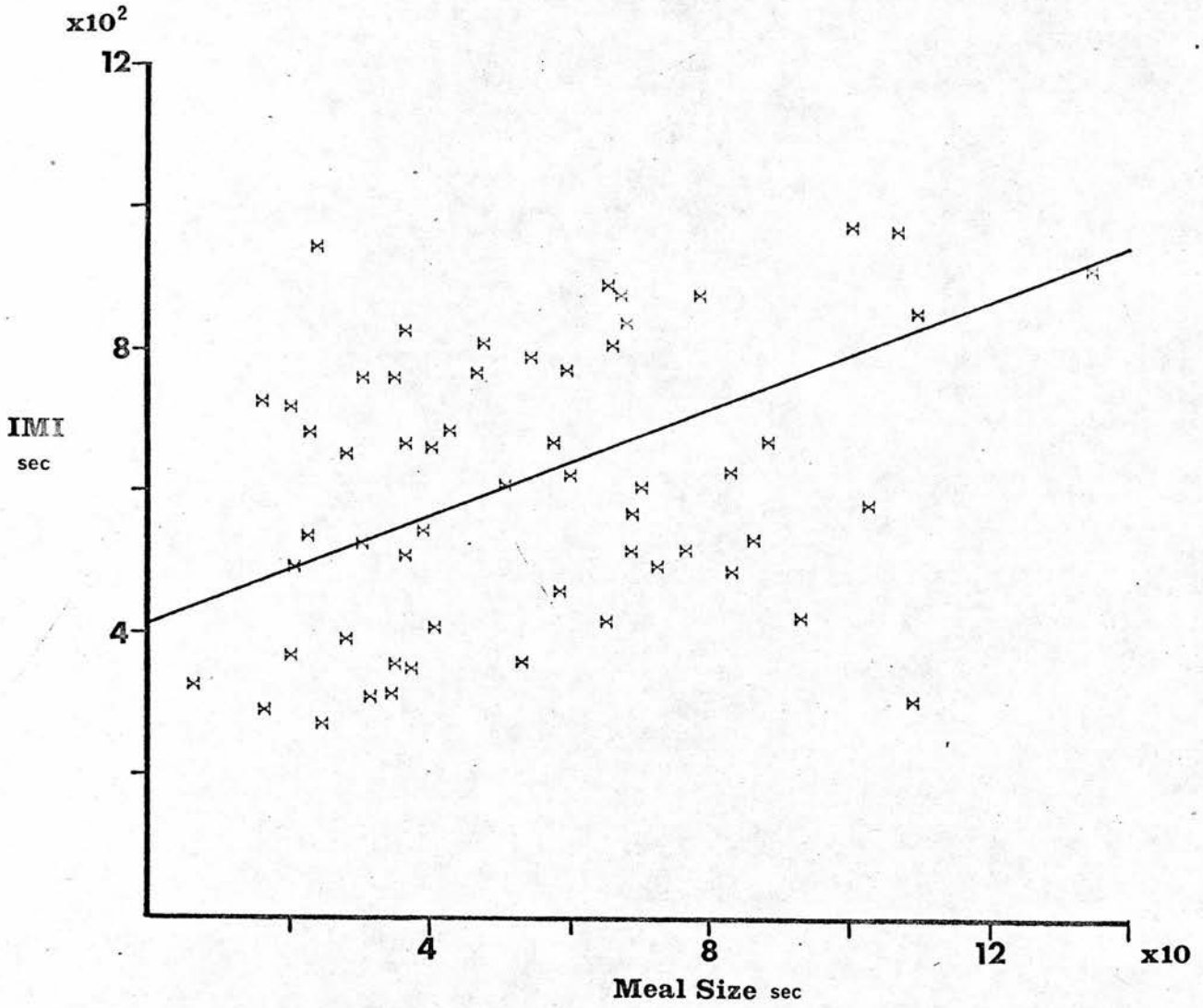
(ii) Pre-prandial

ms v All IMI	ms v long IMI	ms v short IMI
-0.11	+0.00 ns	+0.03 ns
-0.02	+0.05 ns	+0.21 ns
+0.09	-0.02 ns	-0.08 ns
-0.13	-0.08 ns	+0.15 ns
+0.26	-0.12 ns	+0.26 ns
+0.15	+0.13 ns	+0.38 ns
-0.01	+0.05 ns	-0.01 ns
+0.06	-0.11 ns	+0.21 ns

Fig. 10.1(a)

Correlation between intra-session meal size and IMI

in the night-time feeding of male mice


x10²
12
8
4
IMI
sec
4
8
12
x10
Meal Size sec

(least mean squares fit) is intermediate between the values applying over the oestrus cycle in females, so that it would appear that males do not shorten intra-session IMI beyond a value dictated by the smaller meal size, so that the increased consumption is produced by increasing the length of feeding sessions.

Summary of the Male

The feeding pattern of the male mouse is basically similar to the female. There is a pronounced circadian rhythm, the bulk of food being consumed during the night. Daytime feeding is composed of relatively small meals separated by relatively long intervals, unrelated to meal size. At night feeding is organised into more frequent meals, themselves grouped together into feeding sessions. Night meal size is smaller in males than non-receptive females, and as males eat more than females overall this difference is more than offset by alterations in meal frequency.

The principal alteration is in within session feeding, meals are more frequent, and the sessions more prolonged than those of the female. Feeding outside of sessions is similar as far as frequency is concerned in males and females at all stages of the cycle.

In structure the male meal partially resembles that of the sexually receptive female, bout length is reduced by a lowering of the rate of initial facilitation. There is, however, a small reduction in the number of bouts which does not appear in the female.

10.2 Ovariectomised /

10.2 Ovariectomised Females

The feeding patterns of seven females were recorded in the usual way roughly 20 days post operative. It will be recalled that at this stage the animals are still gaining weight more rapidly than their ultimate rate of increase, and will not therefore have reached their stable 'set-point'. Data is not available for animals recorded in the later static phase, so consideration will be restricted to the early dynamic phase of weight gain following ovariectomy.

10.2.1. Basic Pattern

The basic pattern of feeding over the night and day, with meals, intermeal intervals, and feeding sessions is present in the female mouse after ovariectomy.

Considering first the daytime pattern. Day meals are, as with the male, relatively small (Fig. 10.2(a)), and very infrequent, so that the total daytime consumption represents only a small fraction of the total. The daytime pattern is quite typical of the intact female on the days of dioestrus and metoestrus.

10.2.2. Night time pattern

During the hours of darkness the meals of ovariectomised animals are relatively large, but no larger than those of the intact metoestrus, oestrus or dioestrus female. The meal structure is apparently similar, with a similar average number of bouts, and mean bout length. In the dynamic phase after ovariectomy, therefore, there are no changes in either meal size or structure.

The increase in food consumption produced by the operation is therefore exclusively the product of changes in meal frequency(Fig 10.A).

The /

Fig. 10.2(a)

Day and night time meal size, meal frequency and total consumption of dynamic phase ovariectomised females eating laboratory chow.

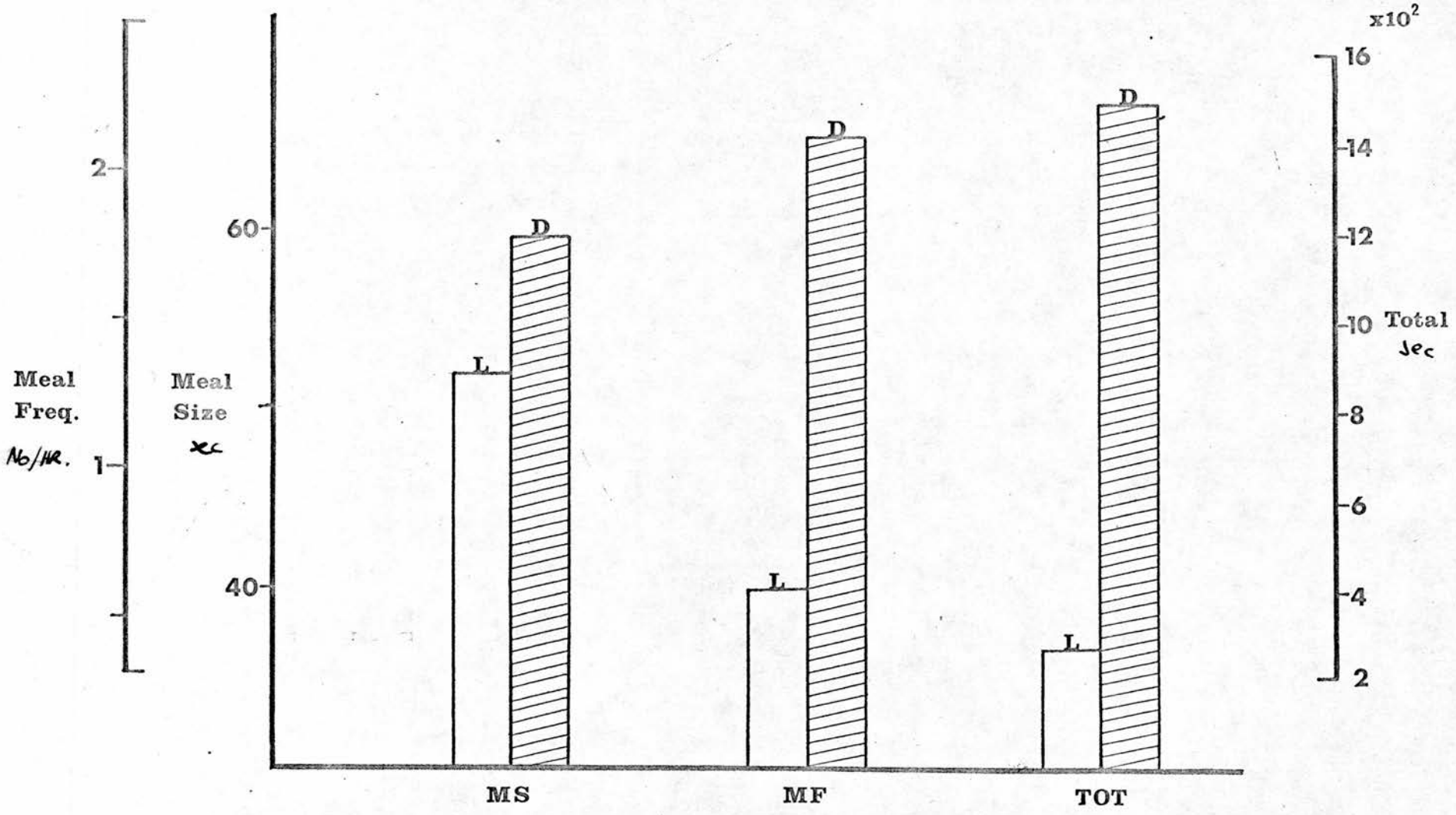

Meal Freq. No/HR.
2
1
Meal Size sec
60
40
L
D
MS
MF
TOT
x10²
16
14
12
10
8
6
4
2
Total Sec

TABLE 10.2(i)

Ovariectomised Females

All in seconds
Means for each animal.

DAY

Meal Size	IMI
42.4	6738
39.6	6228
29.2	6012
54.6	7368
32.0	6386
61.0	9764
45.6	6934
43.6	7060

NIGHT

Meal Size	IMI	Intra-Session	Proportion	Extra-Session
41.6	1254	370	41	1806
40.9	1640	392	54	3104
83.2	1750	620	55	3130
33.0	1628	436	38	2812
61.6	1404	512	52	2836
97.0	2238	562	40	3354
61.6	1866	518	48	3110
59.8	1682	486	58%	2916

The proportion of meals included in the night time feeding sessions is not increased over that of the intact female, remaining constant at about 50%. The intra-session meal frequency is, however, much increased (IMI down from 10 mins to 8). IMI outside of feeding sessions shows a slight, insignificant fall, from the mean over the oestrus cycle. (Although it is just significantly reduced over the dioestrus level $P<0.05$ Mann Whitney 'U' test). (see Table 10.2(i))

The correlation between meal size and following intra-session IMI is retained in the ovariectomised animals, with a regression coefficient rather less than the intact metoestrus animal.

The acute ovariectomised animal therefore tends in most respects towards an exaggeration of the normal metoestrus pattern of feeding, no change in meal size, but a considerable reduction in night time IMI, produced primarily by an alteration of the relationship between meal size and following IMI within feeding sessions.

10.3 The Castrate Male

Six animals were recorded for three days each in the usual way at least 21 days after bilateral castration in adulthood (at 95 days of age).

10.3.1. General picture

Again, these animals showed a basic pattern very similar to the intact males and females. A pronounced circadian rhythm, with relatively small, infrequent day meals, and relatively large frequent night meals. (see Table 10.3(i))

Daytime /

TABLE 10.3(i)

Castrate Males

All in seconds
Means for each animal

DAY

Meal Size	IMI
53.0	8076
56.6	7658
33.2	6464
41.8	5952
46.4	6096
58.8	6794
48.3	6895

NIGHT

Meal Size	IMI	Intra-Session	Proportion	Extra-Session
95.2	3164	854	20	3740
72.8	2054	720	46	3190
61.0	2596	634	40	3904
53.4	2122	640	52	3726
50.8	1972	654	43	2966
63.6	2184	718	41	3202
66.2	2348	702	40%	3444

Night time total Feeding = 1420 sec.

24 hour total = 1672 sec. = 3.01g

Daytime meals are small compared to castrate night meals, but of roughly the same size as the night time meals of intact males (c 90mg). They were slightly less frequent than in the intact males, but not sufficiently to account for the increased meal size, so that castrate males eat a relatively higher proportion of their food during the day than intact animals, a figure closer to the circadian distribution of feeding in the intact female.

The most interesting characteristic of the feeding of castrate males is a considerable rise in night time meal size following the operation. Night meal size is increased by 80% after castration. (Highly significant $P<0.01$ Mann Whitney 'U' test). The rise in meal size is produced, like the difference between males and females, by a rise in mean bout length (similar to that of the non-receptive female), and a slightly greater number of bouts.

Total night time consumption is, however, slightly less in the castrate than the intact male, so that the change in meal size is more than compensated by an alteration in meal frequency, which falls dramatically. This fall is due (Table 10.3(i)), exclusively to changes in the short within session intervals, their forming a lower proportion of the total, and being significantly increased in length. ($P<0.01$ Mann Whitney 'U' test).

10.3.2. Summary

The male castrate shows therefore almost the same total consumption as the intact animal, but in a completely different pattern, far more typical of the non-receptive female than the male. A slightly higher /

higher proportion of the food is consumed during the day, but the major change is one of both night and daytime meal size, which are considerable elevated to a level slightly greater than that of the non-receptive female. Meal frequency shows a correlated reduction due to the elongation of within session IMI's. The relationship between meal size and following intra session IMI remains intact after castration, so that intra session IMI falls naturally into line with the increased meal size.

10.4 Discussion: Sex hormones and feeding patterns

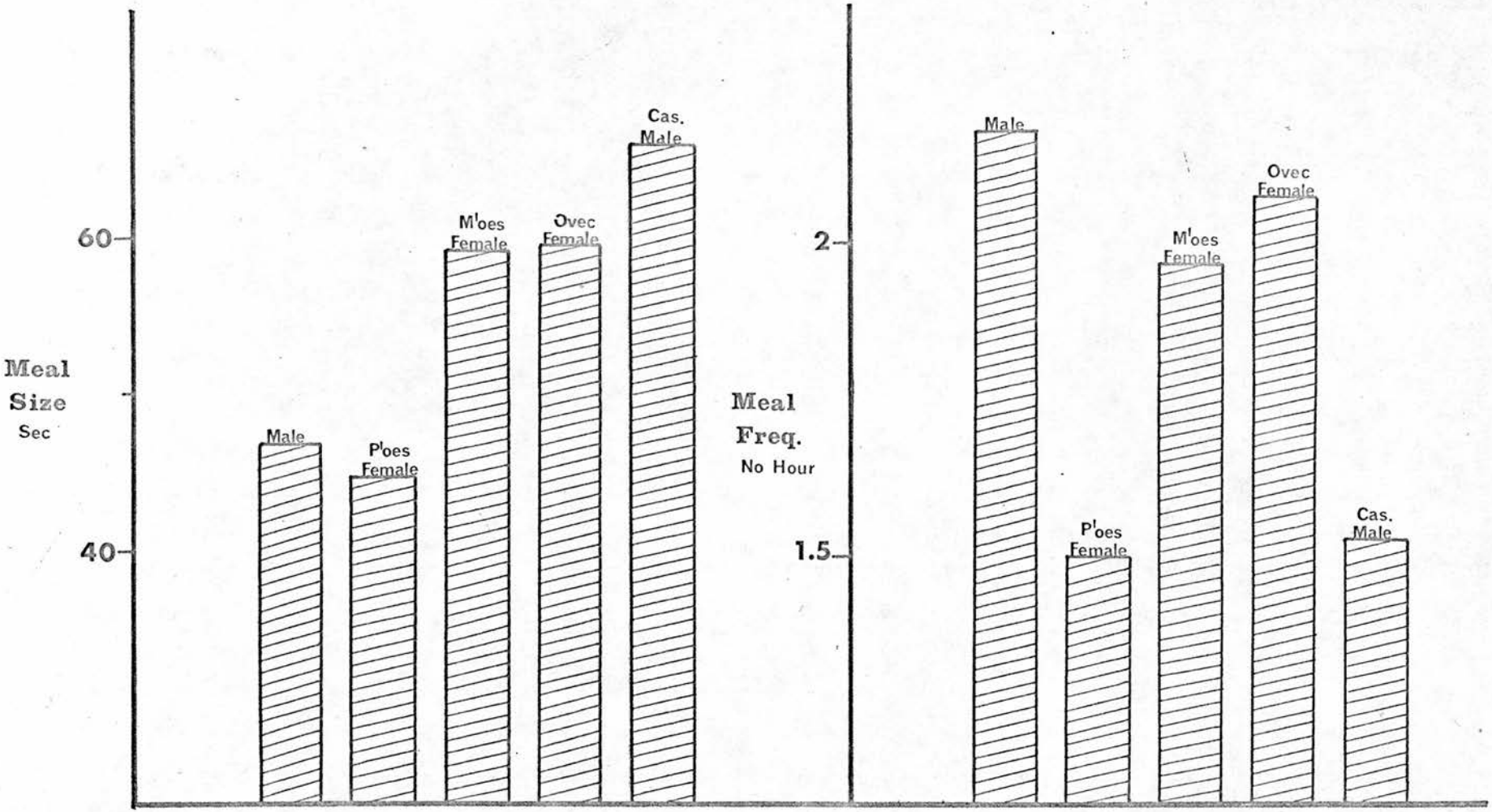
Feeding patterns in the mouse are therefore markedly affected by changes in reproductive state. To summarise, as far as meal frequency is concerned, for a given meal size males eat more frequent meals than females, and females at metoestrus and after ovariectomy eat more frequent meals than at the beginning of the cycle.

Meal size is changed under a different set of circumstances, so that only animals which are potentially sexually active show altered meal size, which, when feeding on laboratory chow is lowered in males and sexually receptive females. Meal size is increased in receptive females eating sweet food.

The dissociation of meal size and IMI changes is greater than would be expected, for example in the rat. A possible reason for this is the far greater meal frequency of mice, which leads to their feeding in sessions. It was suggested in Chapter Five that the feeding session is the major lipogenic activity in mice, and that it is likely that changes in the movement of material to and from stores may well be reflected as alterations of 'session size', rather than meal size, which does /

Fig. 10.A

Night-time meal size and frequency as a function of reproductive state in the mouse


Meal
Size
Sec
60
40
Male
P'oes
Female
M'oes
Female
Ovec
Female
Cas.
Male
Meal
Freq.
No Hour
2
1.5
Male
P'oes
Female
M'oes
Female
Ovec
Female
Cas.
Male

does not appear to be modulated by long term energy changes. The high frequency of meals also considerably increases to potential interference with other activities resulting from the need to feed.

The contol of feeding may be considered to be the product of the operation of a sub-system within the system controlling all behaviour, which of course contains that controlling sexual and related behaviours. Any such sub-system is exposed to two sorts of constraints, on the one hand it must operate as efficiently as possible in its own right, which in the case of a homeostatic system such as feeding means maintaining homeostatsis. On the other hand it must integrate its output with the demands of other systems. Such integration will affect the second to second decision making in the control of behaviour both directly (the sudden appearance of important other stimuli), and as a result of longer term changes of 'balance' - the relative priorities allocated to different possible outputs. Consider as an example the diurnal variation of meal size in the rat, or mouse. During the day meals are small and infrequent and whilst it is true in the normal case that the probability of interruption of feeding is greater during the day, the above experiments have demonstrated that even if animals are kept in partial acoustic, visual and olfactory isolation from the world, the pattern remains as strong as ever. It would appear that the animal is adapted, during the day to allocate a low priority to feeding, so that the balance of behavioural control is switched relatively towards other activities.

Reproductive variations will affect feeding by imposing direct constraints upon the internal operations of feeding, by directly interfering with the second to second decision making of the animal, and /

and also by possibly altering the balance on control in the system, so changing the relative priorities allocated to different outputs.

When titres of gonadal hormones are high in either sex there is a high probability of reproductive activity, or activity related to reproduction. In the female such a raised probability is associated with a discrete period of sexual receptivity, which produces, in rats at least a range of behavioural changes. In the laboratory situation females are more active (Wang (1923) to Finger (1969)). They show alterations of olfactory sensitivity (reviewed by Pietras and Moulton (1974)), as well as alterations of sensitivity to touch (Pfaff et al (1973)), and more bizarre changes such as alteration of bar press duration (Moss (1968)). The point to make is that, in addition to changes in sexual receptivity and feeding females show a complex of other behaviours.

Such a complex of changes has been known for centuries in animals as a change of reproductive 'mood'. The sexually receptive animal presents a characteristic picture to an observer, and individuals experienced with animals can often recognise 'heat' from these signs.

Sexually receptive female rats for example "use a darting form of locomotion . . . involving numerous sudden starts, fast dashes, and sudden stops." Pfaff et al(1973) p 279. On either the day of sexual receptivity or the day before the wild female rat tours a large area (up to ½ acre) dragging her hind quarters on the ground, presumably to lay a scent (Calhoun (1963)).

Such /

Such a complex of changes of behaviour require a complicated alteration of the sub-systems controlling individual behaviours, so as to integrate the whole into a system with a different 'balance', inclined presumably to facilitate the processes of reproduction. Such a complex of changes may come about either by a set of discrete actions of hormones on a number of different sub-systems, or a single action upon a superordinate system exercising modulation upon all the others. (e.g. 'arousal', or 'fear' (Gray and Buffery (1971)))

The system controlling behaviour must, however, consist of interacting sub-systems, and a change in any one will affect not only its output, but that of others integrated with it. The sum total of a small number of relatively specific changes will therefore be a ramnification throughout most behaviours, having the same characteristics of 'mood' or change of 'balance'.

The decision between these alternative depends critically upon the nature of the neural structures underlying the control processes (e.g. Morgane (1969)). Such a change, however caused, would be expected to influence feeding by altering the relationship between it and other activities.

The reduction in meal size which results is a product of an alteration of the initial rate of facilitation of bout length. This facilitation appears by virtue of a steady decline in the probability of feeding ending. As a meal proceeds the changes of the animal being distracted from feeding decreases. 'Distractibility' in this case is a direct measure of the relationship between feeding and other behaviours.

Such /

Such a change of distractibility is just what would be expected, from common sense, of an animal with 'other things on its mind', a way of describing the sort of 'mood' or 'balance' changes discussed above. It is likely that before second to second decisions are made, some mechanism will act so as to allocate priorities to behaviours, and the relative values of those priorities will influence the transitions to and from all behaviours. Under such conditions the changes of feeding on sweet food also make some sense. The receptive female must accord sexual behaviour, and its associated changes a very high priority. If she meets ordinary, mundane food, then the logical strategy is to eat a little, just to keep her going, before carrying on with the task in hand. Routine eating will therefore be allocated a low priority, becoming relatively interruptible. The consumption of choice foods, with the potential for staving off needs for long periods should not, however, be missed, and the way to reduce interference in this case is to eat as much as quickly as possible, as is the case. The allocation of a low priority to feeding therefore need not always imply a reduction in the behaviour.

The second fundamental change at prooestrus also may be considered as the product of a similar process. The removal of the normal relationship between meal size and IMI allows the female not only to vary her feeding according to the foodstuff available, but also to feed when she likes, irrespective of the last meal. She can eat, therefore, whenever circumstances favour the activity, a very flexible system for the integration of feeding and other important behaviours.

It may be, therefore, that there exists a biologically based mechanism for the release of feeding from metabolic necessity, which is activated by the appearance of a reproductive 'mood'.

The /

The male shows a similar change in meal size. He is more distractible during the opening phases of the meal. In this case it would also be expected that sexual and related behaviours would be allocated a relatively high priority. The male, however, is continually sexually active for a long breeding season, and therefore cannot afford the complete flexibility of feeding allowable during the temporary receptivity of the female, as he must regulate his body weight. In the male, therefore, reduced meal size is compensated by changes in meal frequency, and the relationship between meal size and IMI remains intact.

The changes after castration show that some produce of the testis is responsible for the increase in distractibility of the male. Andrew (1972) has show, however, that chicks injected with considerable doses of testosterone show an increase in 'persistence' in the feeding situation, a pattern quite the opposite to that observed here. The effects appear, however, only after injection of the hormones, and are absent in adults, so that their relationship to the natural situation used here is not clear.

A tendency to indulge in sexual or reproductive related activity therefore interacts with feeding in mice influencing primarily meal size by changes of distractibility within meals, which coupled with other changes in receptive females may allow feeding to be a flexible activity which can be easilty fitted in around other activities having a higher priority.

The changes following ovariectomy in the female are quite different. In this case session size is increased by altering the relationship between /

between meal size and following IMI, a simple exaggeration of the metoestrus changes described in the previous chapter. This is a pattern which it has been argued above is one typical of an animal actively incrementing its stores (or alternatively growing). These are constraints arising within the feeding system, affecting its operation by a change in set point, and which may be clearly distinguished from the constraints on the external relations of feeding described above.

In conclusion it would appear that the study of feeding patterns in the two sexes and over the oestrus cycle reveals more than one reproductive constraint upon feeding, and that the hypothesis stated in Chapter One may be supported in the case of mice.

CHAPTER ELEVEN

OESTROGENS AND THE VENTROMEDIAL HYPOTHALAMUS

The control systems underlying the organisation of behaviour as outlined in the previous chapters must be associated with some neural structures, and to produce effects upon behaviour hormones must act directly or indirectly upon these structures. If relatively independent mechanisms exist for the control of behaviourally distinct influences of hormones on feeding then it should be possible, by some neurological manipulation, to dissociate the operations of those mechanisms in such a way to illuminate further the hormonal effects. The following chapter describes the background to the use of lesions of the ventromedial nucleus of the hypothalamus as a tool to study the influences of gonadal hormones on feeding.

11.1 Why the Ventromedial hypothalamus?

The neural structures of the hypothalamus and limbic system in rats have long been associated with the high level control of behaviour, and oestrogens are known to be actively taken up by a number of structures in these areas. Stumpf (1970) reports that oestrogens accumulate in a number of hypothalamic structures, including the medial preoptic area, arcuate nucleus, and ventromedial nucleus, suggesting a variety of potential sites at which the hormone could possibly act to influence food intake.

Implants of oestrogen into the preoptic are (Colvin and Sawyer (1971), Wade and Zucker (1970)) promote an increase of 'running' - general activity typical of behavioural oestrus, and oestrogen acts in this area to facilitate the surge of L.H. release which initiates ovulation. (Donovan (1969))

Wade /

Wade and Zucker (1970) further showed that implants of oestrogen into the Ventromedial Nucleus (VMH) reduced food intake with a latency of twelve hours or so. Implants in other areas, or of other steroids such as cholesterol had no effect upon feeding. Whilst it is true that oestrogens could have diffused away from the implantation site (e.g. Palka et al (1966)), there was no systemic effect, as measured by uterine changes and so as a starting point, the VMH may be taken as at least associated directly or indirectly with some effects of oestrogens upon feeding.

The same nucleus has of course been associated with the general control of feeding for many years since the demonstration by Hetherington and Ranson (1940) that lesions of the area produce animals which overeat markedly for a period - 'dynamic' hyperphagia, until their body weight stabilises at a new elevated level in the 'static' phase of obesity.

The precise nature of the changes underlying these phenomena is still today in considerable dispute, and this is why in the following chapter destruction of the VMH is used only as a tool, to hopefully illuminate some aspects of the influences of oestrogens upon feeding.

11.2 Sex and the VMH

If oestrogens affect food intake directly or indirectly via the VMH, then there should be an interaction of some sort between the effects of the destruction of the nucleus and the endocrine state of the animal involved.

Two general hypotheses have been put forward. On the one hand it may be that destruction of the VMH eliminates directly or indirectly the route /

route by which hormones influence feeding (e.g. Zucker (1972) and others). If this is the case, then, for example, male and female animals should be the same after the operation, despite being different before.

On the other hand, the destruction of the VMH may change the system in such a way that the sex of the animal influences the feeding behaviour after the operation, but possibly in a different way to before. So long as the lesion does not substantially affect the endocrine status of the animal directly, then these two hypotheses may be resolved by comparing the feeding of males and females after the operation. This is the crucial comparison to make, clearly for example, whichever hypothesis is the case the animals will show a change <u>within</u> each sex which is dependent upon the sex, simply because the two sexes are different to start with.

Two sorts of data need to be examined. Firstly a comparison of the feeding of male and female animals after the destruction of the VMH, and secondly as a check the endocrine status of females particularily after the operation, as this crucially affects the interpretation of the comparison of the sexes.

11.3 The feeding of males and females after VMH lesions

Attention was first drawn to the possibility of a sex dependence of the effects of VMH lesions by the controversy, several years ago, about the possible irritative effects of VMH lesions. Reynolds (1963) was unable to demonstrate hyperphagia after radio-frequency lesions of the VMH in male rats, and claimed that the normal VMH effect was a consequency of the irritative effects of deposits remaining after electrolytic /

electrolytic lesions on the lateral hypothalamus. Opponents claimed that the failure to produce hyperphagia was partly due to using male rats, which led Valenstein et al (1968), to survey the literature to date, and conclude that it was indeed easier to produce hyperphagia in female rats, and that the degree of hyperphagia so produced was larger than in males. The same authors conducted a controlled comparison of the effects of identical VMH lesions in male and female rats, and did indeed observe that the change in food intake and body weight following the operation was greater in females than males.

When, however, the more relevant comparison between the sexes after the operation is made, they are found to be consuming equal amounts of food and gaining weights at similar rates (as pointed out by Gold (1970)). Control males eat some 30% more than intact females, so that the effect of the lesion appears to be not the creation of a sex difference, but the abolition of a previous one.

Rehorsky and Wampler (1972) report more data concerning electrolytic lesions in males and females. In their study there were no effects of sex upon either the absolute levels of food intake or body weight gain after the operation, in animals given both high fat, and ground laboratory chow, or upon the absolute weight gain of males and females.

Singh (1970), again using electrolytic lesions of the VMH observed that, when animals are allowed to bar press on a CRF schedule for food, the number of bar presses per day per gram body weight increases more in females than males, and is significantly greater than males after the operation. The absolute number of bar presses in each sex after the operation, which may be calculated from his data, is however, the same in male and female animals. This raises a further point about the way /

way in which the sexes ought to be compared after the lesion. Is it more valid to compare intake, relative to body weight, or absolute intake? The decision between these alternatives unfortunately depends upon the precise nature of the deficit in feeding following VMH lesions. If as has been argued, (Panksepp (1971)), the effect of the lesion is to abolish the relationship between food intake and the long term needs of the animal, then the appropriate comparison should be absolute intakes. Even if this is not the case, however, the observation of equal absolute intakes, is in itself very interesting, as it either implies some very complex sex dependent relationship between intake and body weight which fortuitously produces similar absolute intakes in males and females, or some dissociation of feeding from both body weight and sex dependence. Smith and Britt (1972) incidentally observed the effects of VMH electrolytic lesions in males and females, finding again no significant differences in absolute intake.

In the studies which have used electrolytic lesions, therefore it is apparent, that whilst, in most of them females show both a significantly greater change in food intake, and a significantly greater intake relative to their body weight, than males, the absolute levels of intake of the two sexes are the same.

The least complex interpretation of this data, provided the endocrine status of the female is relatively normal, is that the lesion has, in the dynamic phase at least, released the absolute feeding level from control by both the body weight and sex of the animal.

Gold (1970) used a different technique to produce hyperphagia, namely a knife cut between the medial and lateral hypothalamus, and in this case males /

males and females gained weight at similar weights, although males ate slightly more than females. Smith and Britt (1972) used yet another technique namely the implanting of goldthioglucose into VMH, (see below for discussion of GTG) and they were unable to discover any significant differences in absolute intake of the two sexes after the operation.

Balgura and Davenport (1970) compared the feeding patterns of male and female rats in the dynamic phase of hyperphagia after electrolytic lesions to the VMH. These authors noted that VMH lesions abolish the usual sex difference in meal frequency, so that males and females eat meals at the same rate after the lesion. Meal size, on the other hand showed a partial reversal of the normal sex difference following the lesion, as females increased meal size, whereas males did not.

Apart from the possibly anomalous results of Gold (1970), therefore the consensus remains that males and females, which were different before the lesion do not differ after it.

The abolition of this difference could come about by two means, either the removal of the hormones producing it, or by some interference with their action. Before the latter may be asserted, therefore, the presence of the relevant gonadal hormones must be demonstrated in rats after lesions of the VMH.

Recently Wampler (1975), has reported a detailed examination of the endocrine status of VMH lesioned male and female rats. He observed, in the female the maintenance of oestrus cycles, even when the animals were kept under continuous light, a situation which normally produces persistent oestrus in control animals. Ovarian weight was slightly reduced /

reduced in some animals, but uterine weight was slightly increased. All in all the endocrine status appeared to be more or less normal.

La Vaque and Rodgers (1975) were also able to observe relatively normal oestrus cycles in VMH females, although there was a slight tendency to elongate the period of vaginal cornification in their animals. These authors,unlike others before them, e.g. Kennedy(1963), were also able to observe some sexual behaviour in their animals at the time of vaginal prooestrus, although ejaculation was never achieved by the male.

Such recent systematic studies are well supported by incidental observations of some of the original workers with VMH lesions. Hetherington and Ranson (1940) noted that the ovaries of lesioned animals appeared normal. Hetherington and Ranson (1943), suggest that unless the rostral part of the VMH, bordering upon the anterior hypothalamic region, is involved in the lesion normal oestrus cycles are maintained, whereas if anterior hypothalamic damage appears a state of persistent vaginal cornification is produced. Brooks and Lambert (1946) report a continuation of oestrus cycles in a high proportion of their VMH lesioned females.

Given these observations it is perhaps reasonable to suppose that whatever the endocrine status of the female, it does not approximate to that of a castrate, the animals showing either reproductive cycles which appear normal, or persistent vaginal cornification. In either state the oestrogen dependent reproductive organs such as the uterus maintain their weight. During the normal cycles some components at least of sexual receptivity appear.

Vaginal /

Vaginal cornification is oestrogen dependent (Davidson (1968)), so that the only abnormality that is observed would suggest that the hormone of interest here is still present in significant quantities.

Oestrogens are probably therefore, present in females after electrolytic lesions of the VMH, yet males and females eat very similar absolute amounts after the operation, and in terms of body weight females eat more than males. The implication is that, after the lesion the hormones are still present, but their route of action is directly or indirectly blocked.

This hypothesis may be examined further by the administration of oestrogens to females after VMH lesions, as if it is wholly correct they should have no effect upon food intake.

Kennedy (1969) reports that oestrogens given continuously to VMH females reduce both food intake and body weight, and, if anything, the lesioned animal appears to be rather more sensitive to the weight reducing properties of such 'secondary appetite depressants' as Kennedy calls them.

King and Cox (1973) examined the effects of both oestrogen injections and ovariectomy in VMH females. They similarily found that oestrogens, at two dose levels reduced food intake in the lesioned animals. They also found that ovariectomy increased the food intake of lesioned animals.

These results present a double paradox. In all of the studies described above comparing the food intake of males and females after VMH /

VMH lesions the two sexes were observed to consume insignificantly different amounts of food. Oestrogens are present in the female, and yet they do not lead to her eating less, either in absolute or relative terms, than the male. If, however, exogenous hormones are given, the food intake and body weight are reduced. The effect of ovariectomy, noted by King and Cox, is even more peculiar. These authors report no data for males, but if their animals behave similarily to those of other authors the implication is that their ovariectomised, lesioned females should eat more than males in absolute as well as relative terms.

It is clear that oestrogens can have some effect after VMH lesions, but the question is just what. A clue to the resolution of the paradox can perhaps be gleaned from Fig. 5 of Kennedy and Mitra (1963). In this diagram the food intake of lesioned females is shown in relation to the vaginal oestrus cycle, and at each vaginal oestrus there is a slight down turn of the graph. The presumed oestrogen peak at this time appears therefore to be <u>temporarily</u> reducing food intake, with little overall effect upon the degree of hyperphagia. It could be the case, therefore, that after the lesion the short term effects of oestrogen over the oestrus cycle remain to provide a disturbance to the general hyperphagia, whereas the longer term effects of the hormone on body weight set point (see Chapter One) are abolished or attenuated. This is perhaps not unreasonable in view of the long held view that VMH lesions themselves interfere with body weight set point (Kennedy (1963)).

If this were the case, then the administration of oestrogen doses, sufficient in the intact animal to initiate sexual receptivity, in the unphysiological continuous regime might well depress food intake by a continuous /

continuous activation of the usually short term mechanism. Similarily, the abnormal persistent oestrus female may be subject to a chronic inhibition, and if this is the case it could explain the results of King and Cox as far as ovariectomy is concerned, although there is no strong evidence to support or refute this speculation.

There is however, here a potential situation which could prove useful in the further examination of the various effects of oestrogens upon feeding, and in the following sections the background to the use of GTG induced VMH lesions in the mouse for a similar examination is discussed.

11.4 Sex and GTG

As described in the following section a single intraperitoneal injection of Goldthioglucose (GTG) will produce hyperphagia and obesity in the mouse, which are probably the consequence of a selective damage to the VMH.

It is of interest therefore to compare the effects of identical doses of GTG in the two sexes, to see how they compare with the data obtained from experiments with rats. Sanders and Singh (1973) injected male and female swiss mice with identical doses of GTG, and measured food intake and weight gain on a variety of foods. On normal food, cellulose diluted food, and food with saccharin added male and female mice ate very similar quantities after GTG. The rate of weight gain was similar, and there were no significant differences in the terminal weights achieved by the two sexes.

Wright and Turner (1973) compared the absolute and relative weight gains of female and male C57 mice after GTG, and found in their case that females gained weight significantly more rapidly (in absolute terms) than males, and often achieved higher terminal weights. They did note, however, that GTG given after ovariectomy led to a total weight gain no greater than that which would be expected after GTG alone, suggesting that the effects of these lesion subsumed those of ovariectomy, exactly as would be expected if either the hormones responsible for the effect were removed by the lesion, or their route of action abolished.

Montemurro (1971), however, tested the effects of stilboestrol upon the food intake of male mice bearing electrolytic VMH lesions, and found a significant reduction.

It would appear, therefore, that a similar paradox arises in mice, provided the hormones are present, then it would seem that natural hormones have no effect upon the general level of hyperphagia, but exogenous hormones at high doses reduce it. Experiments aimed at testing in mice, the hypothesis suggested above for rats will be described in the next chapter. The following section describes in general terms the background to the use of Goldthioglucose as a tool to produce selective intracranial lesions.

11.5 Goldthioglucose

In the course of an investigation into the toxicity of certain gold compounds Brecher and Waxler (1949) injected mice intraperitoneally with goldthioglucose (GTG). They observed that animals surviving the injection gained weight and became obese. The drug has subsequently been extensively used as an experimental tool for the easy production of obesity in mice.

There /

There are a wide variety of somatic and neural effects of GTG, the most important of which is its capacity to induce the selective destruction of certain neural structure, particularily the ventromedial nucleus of the hypothalamus, and it is this destruction which is believed to lead to the development of obesity.

Histological examination of the brains of animals given GTG, in those strains for which the drug is not immediately lethal indicates damage in a number of areas, in each case being accompanied by the selective uptake of gold (Debons et al (1962), Schwartz (1960)).

Damage is usually reported in the VMH, the pre-mammiliary area, some parts of the arcuate nucleus, the hippocampus, and medulla. Apart from the VMH, damage is usually slight, producing only small lesions (Brecher (1965), Marshall et al (1955), Perry and Liebelt (1961)). In some strains damage is observed in the preoptic area. In the C57 the damage is greater in animals pretreated with glucose, but may be eliminated by using a relatively low dose of GTG, which restricts damage almost exclusively to the VMH (Perry and Liebelt (1961)).

Perry and Liebelt observe that damage is concentrated in areas where the local blood brain barrier is most permeable to the influx of other substances, such as tryptan blue, silver nitrate, and various radioactively labelled compounds, suggesting that GTG maybe damages cells wherever the permeability of the blood brain barrier allows it to accumulate in sufficient quantities. The uptake of GTG by the hypothalamus only is affected by blood glucose levels (Edelman (1965)), suggesting that in this area some factor in addition to simple diffusion is involved. In addition, gold thio compounds with non-metabolisable sugars /

sugars do not produce lesions (Mayer (1956)). If GTG is given systemically to the rat it proves fatal. However, it has been shown (Smith and Britt (1972)) that implants of GTG into the VMH will induce hyperphagia. Furthermore Smith (1972) has shown that GTG implants lesion the VMH only in the presence of insulin, whereas implants in the lateral hypothalamus produce lesions independently of insulin, but which may be blocked by 2 deoxy glucose. The suggestion is that active insulin dependent, uptake by the VMH is important in the formation of a lesion. Such a blocking of the lesion by the absence of insulin was not, however, observed in alloxanised mice by Perry et al (1964), although Debons et al (1968), have shown that there is some inhibition of the hypothalamic damage in diabetic animals. This inhibition is relieved by adrenalectomy (Debons et al (1974).

The changes in body weight following GTG are accompanied by an increase in food intake, and both the degree of hyperphagia, and the eventual weight gain are directly related to both the GTG dose and the degree of damage to the VMH (McBurney et al (1966)).

Soon after the injection, following a short period of depressed food intake, the hyperphagia appears and is maintained for a variable period (Waxler and Brecher (1950), Gray and Liebelt (1961)), during which time the animals gain weight rapidly. After some weeks food intake falls and body weight more or less stabilises at a new, elevated level. These changes are typical of those observed in rats with lesions of the VMH (see previous section), and mice with electrolytic lesions of the same area (Mayer et al (1955)). The mice also show changes in their reaction to food, so that the degree of hyperphagia is influenced by the nature of the food offered (Larsson et al (1957), Marshall and Mayer (1954)), indicating a 'finickyness' similar to that sometimes reported in VMH rats.

Hyperphagic /

Hyperphagic mice show a normal circadian pattern of feeding (Liebelt et al (1964), Wiepkema (1968)), and their feeding is confined into meals in the usual way (Wiepkema (1968)). Wiepkema was also able to show that, in male animals at least, the increase in food intake following GTG is produced by an increase in meal size, with little or no change in meal frequency.

GTG has a number of other effects influencing feeding only indirectly or not at all, and in this discussion those of primary importance involve the reproductive system of the female. The structures of the hypothalamus are intimately involved in the control of the secretion of gonadotrophins which themselves influence the secretion of gonadal hormones (Everett (1969)), so that damage to these structures might be expected to influence gonadal activity. There are a number of indices of gonadal activity which may be considered, the most important of which are the existence of reproductive cycles, as evidenced by the vaginal oestrus cycle, and the maintenance of both the gonads and other reproductive structures.

Liebelt and Liebelt (1966) followed the oestrus cycles of female BALB/c mice after GTG. A proportion of the animals tested (c.50%) developed persistent vaginal cornification 8-15 days post injection which remained for long periods. The remainder of the animals showed relatively normal oestrus cycles. The animals in which persistent oestrus developed were inevitably destined to show the greatest degree of hyperphagia, and sustained the greatest amount of hypothalamic damage.

As noted above, a continuously cornified smear is indicative of the continuous secretion of oestrogen at a relatively high level, and does not /

not appear after ovariectomy. It is unlikely, therefore, even in those animals whose gonadal function is altered, that oestrogens are not present.

The hypothesis of gonadal dysfunction associated with hyposecretion of oestrogen also cannot be supported by observation of uterine weight . The uterus decreases markedly inweight after ovariectomy, and yet in GTG animals it is, if anything, increased in weight. (Marshall et al (1957), Waxler and Enger (1954)). Ovarian weight has been reported as both increased and decreased following GTG (Deter and Liebelt (1964)). Inmales, normal testicular histology has been described (Liebelt et al (1966)).

Whatever the final outcome of the investigation into the endocrine status of GTG animals, it would seem clear that the same conclusion is justified as in the case of rats namely that it is extremely unlikely that they approximate in any sense to a castrate, and oestrogens are probably present in significant quantities.

Browning and Kwan (1964) report an apparent increase in a hormone stimulating mammary development in ovariectomised GTG mice, which may be prolactin, although Liebelt (1967) refers to experiments which failed to replicate this finding.

Throughout the study of GTG induced changes in mice, strain is always a potential confounding factor. As noted by Liebelt (1967) different strains vary enormously in their susceptibility to GTG, and comparisons between effects produced with different dose levels in different strains proves very difficult. It is essential at every stage therefore, to establish the similarity of various factors when making comparisons across strains.

This /

This is to some extent illustrated by the experiments discussed in the previous section. Sanders and Singh, and Wright and Turner performed the same treatment on different strains, and obtained slightly different results. In the following chapter, the effects of GTG upon male and female C57 mice will be examined in detail in an attempt to test the hypothesis suggested in the previous section.

CHAPTER TWELVE

ASPECTS OF THE FEEDING BEHAVIOUR OF MALES AND FEMALES AFTER GTG

12.1 Introduction

The previous chapter described in general terms the background to the use of goldthioglucose induced lesions of the ventromedial nucleus of the hypothalamus as a tool to investigate the effects of gonadal hormones upon feeding.

Several types of data are required for this purpose, and they are described in the following sections. Firstly we need to examine, in the C57 strain under consideration, the food intake of male and female animals at various times after injection of GTG. The data from this study can only be interpreted in the light of at least a cursory examination of the endocrine status of this strain after GTG.

Thereafter any remaining influences of gonadal hormones upon feeding after GTG can be examined by various hormonal manipulations, including concomitant ovariectomy, and the injection of gonadal hormones under various regimes.

12.2. The daily food intake of males and females after GTG

In the rat and mouse it would appear from the discussion of the previous chapter that males and females eat identical amounts, in absolute terms, after lesions of the VMH, or its isolation from direct connection with the lateral hypothalamus. Sanders and Singh (1974) published data comparing the food intake of male and female swiss mice after GTG, and Wright and Turner (1973), compared weight gains of male and /

and female C57 mice similarily after GTG. In the following experiment an attempt was made to replicate the experiment of Sanders and Singh using the subjects available in the department. This was necessary in the light of the marked strain differences in both the quantitative and qualitative effects of GTG (Liebelt (1967)).

12.2.1. Methods

Adult male and female C57 mice (c.90 days old) were caged individually in the experimental room, on the usual partially reversed light cycle for three weeks. During the last weeks the daily intake of standard laboratory chow (Spillers Laboratory Diet No. 1) was measured in the usual way. Body weights of the animals were taken (accurate to 0.5 gm) every three days for the duration of the experiment.

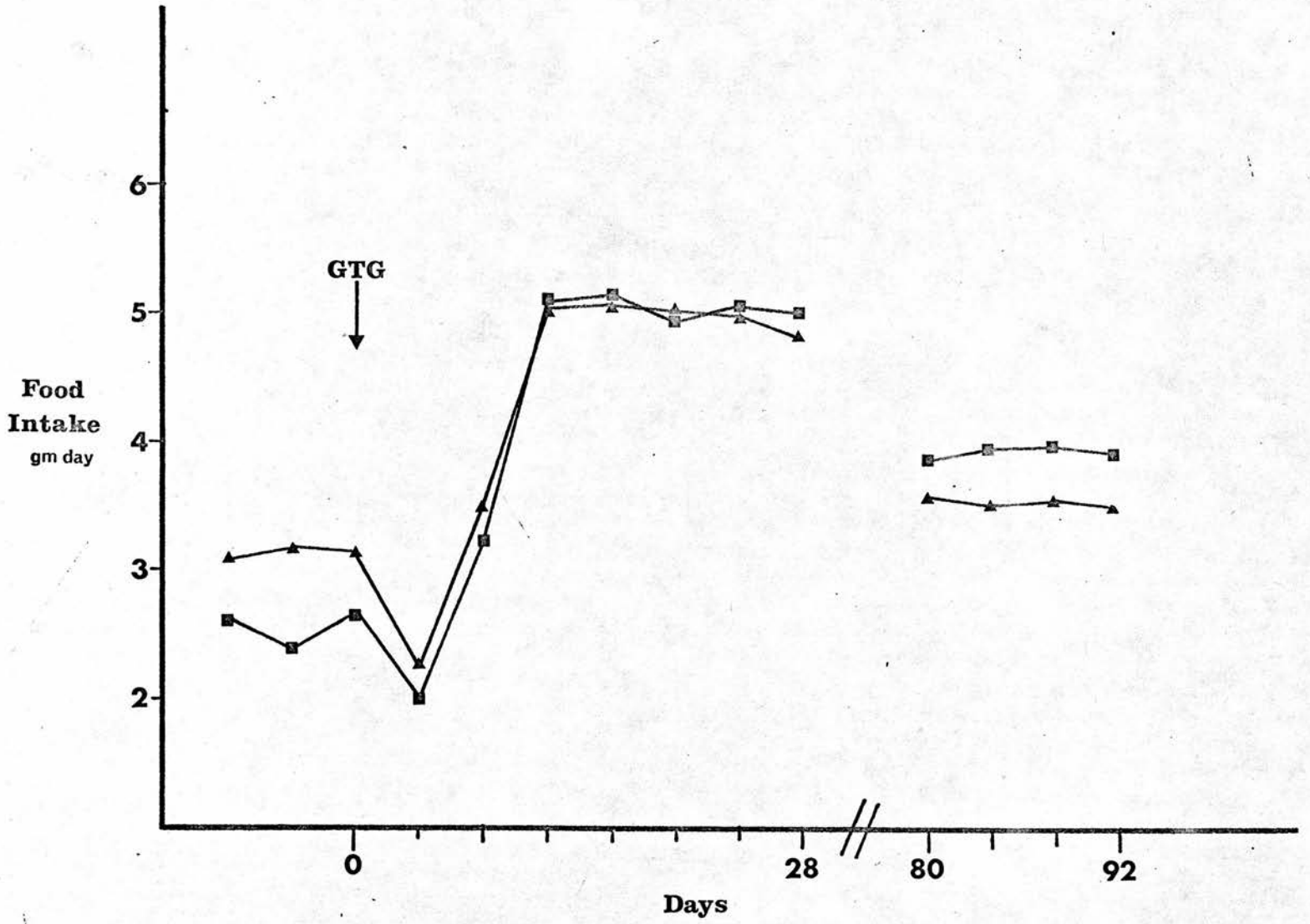
On a single day 16 males and 16 females were injected, intraperitoneally, with 0.6 mg/gm Goldthioglucose (Schering Corporation), taken from a single batch. The solid GTG was dissolved in a minimum of deionised water, and the resulting solution dispersed in an appropriate volume of arachis oil. The suspension was always prepared immediately before injections began, and never used more than two hours after preparation (GTG slowly hydrolyses in solution).

Male and female animals were injected alternately, starting the injections just before the onset of darkness in the experimental room (about 9.00 a.m.). (This was necessary in the light of observed circadian variations in the effectiveness of GTG Wiepkema (1966)). Animals were lightly etherised to facilitate accurate injection.

The /

Fig. 12.2(a)

The food intake (laboratory chow) of male and female mice following the injection of GTC. Males filled triangles, females filled squares.


GTG
Food
Intake
gm day
6
5
4
3
2
0
28
80
92
Days

The food intake of eight randomly selected individuals of either sex was followed for four weeks after the injection, and then for a further two weeks after a break of six weeks (hopefully in the dynamic and static phases respectively). The food intake of the remaining animals, plus several others was measured for one week in the mid dynamic phase. The animals were then sacrificed by cervical dislocation, and various organs weighed and prepared for histological examination as described in the following section.

12.2.2. Results

The results of the study are shown in Fig. 12.2(a). The food intake of intact males and females shows the usual sexual dimorphism. Following the injection of GTG the food intake of both sexes falls, initially markedly, but with a rapid recovery after a few days. During this time on average 20% of the animals of both sexes die. Mortality figures in this and various other pilot experiments of mine show no marked sex difference, although within each fresh batch of animals there are often pronounced sex differences in mortality, favouring each sex in an equal number of cases.

Subsequently food intake rises markedly in both sexes, and body weight follows. Food intake is significantly elevated over preoperative levels from ten days on ($P<0.01$ Wilcoxon Test). In this early 'dynamic' phase of hyperphagia, there are no significant differences in food intake between the sexes ($P<0.4$ Mann Whitney 'U' test on all data, including the spot measurements of intake). Food intake remains elevated in both sexes over the first period observation with a tendency to fall towards the end.

In /

In the later 'static' phase food intake has fallen back towards control levels, although it is still significantly elevated above control levels ($P<0.01$ Mann Whitney 'U' test versus an oil injected control group). At this time, however, there is also a significant difference in food intake between the sexes, in the opposite direction to that of intact animals, with females eating significantly more than males ($P<0.001$ Mann Whitney 'U' test).

The food intake changes are mirrored in changes of body weight, which rises initially very rapidly, and then tails off. In this study, like that of Wright and Turner, the mice, in the case of the females at least, do not appear to reach a true static phase, and continue to gain weight for very long periods, coming eventually to exceed the body weight of males.

12.2.3. Discussion

The results here replicate in general terms those of Sanders and Singh (1974), and extend those of Wright and Turner (1973) in the C57 strain.

There are therefore a number of factors to be considered. Firstly, in the dynamic phase there are no sex specific influences on the absolute level of food intake. This is the same situation as in rats, and therefore raises the same questions, namely are the gonadal hormones still present after GTG, and if they are, then why do they not influence food intake?

In the static phase following the stage of rapid weight gain there are, /

are, in C57's at least, sex differences in food intake, but these are in the opposite direction to those in intact animals, so in this case if the gonadal hormones are present, it is necessary to explain not only why they are not acting, but also why their influence is apparently reversed.

It is clear that the endocrine status of the animals is of vital importance in unravelling the complex of effects of GTG on food intake. This is examined in the following sections.

12.3 The endocrine status of animals after GTG

In the previous chapter various published reports concerning the endocrine status of animals after GTG were discussed. It was concluded that it is difficult to support the assertion that animals of either sex are rendered effectively castrate by the injection. As with the food intake measures, however, different strains vary considerably in their reaction to GTG, and so it remains necessary to examine the situation in the strain used in the other experiments here. The advantage of the supporting studies in this and other strains, however, is that a relatively simple examination should suffice to confirm the situation in C57's.

Accordingly, the following data is not aimed in any way to be at all comprehensive, merely an examination of some of the factors concerned. A number of indices of gonadal action were therefore chosen and examined in GTG mice. It was not feasible to measure directly hormone levels in female mice so that only indirect measures could be taken. The first and most obvious to choose is the vaginal oestrus cycle. The examination of /

of vaginal smears was used for this purpose. A second possibility is the measurement of weights of the various target organs for gonadal hormonal action. Thirdly, the histology of the gonads and their target organs may be examined.

(a) Vaginal smears: In total the vaginal smears of 40 females were followed for up to three weeks after the injection of GTG at 0.6 mg/gm. Each day the vaginal smear was taken in the usual way, stained, and subsequently scored.

Persistent oestrus was defined as at least five consecutive days with a cornified vaginal smear. By this criterion some 25% of the animals (11 out of 40) showed persistent oestrus at some time post injection. These were invariably the animals destined to show the greatest degree of hyperphagia, and to eventually become the heaviest (just as in Liebelt et al (1966)). The remainder of the animals showed normal, mouse-like oestrus cycles, ranging from four to seven days in length, with a normal distribution of cycle lengths (see Table 12.3(i)).

(b) Organ weights and histology: Sixteen static phase, obese female mice were sacrified by cervical dislocation, and the ovaries and uteri immediately removed and weighed, accurate to 2 mg. The organs were fixed in 10% buffered formalin, processed in the usual way for the preparation of paraffin wax blocks, and sectioned at 7μ. The sections were stained with Haematoxylin and Eosin in the standard way. The organs of 12 intact, similar age control females were subjected to the same procedure.

Table /

TABLE 12.3(i)

Oestrus cycles in GTG mice

Length:	4	5	>5 days
No:	51	46	15
Proportion:	46%	41%	13%

Figures do not include persistent oestrus animals

TABLE 12.3(ii)

Organ weights after GTG

(i) Ovaries

GTG	Controls
22	27
18	24
25	21
22	19
19	24
33	21
23	20
21	19
30	26
30	23
27	28
19	21
27	
24	22.8
27	
21	
24.2	

Difference n.s.

(ii) Uteri

GTG	Controls
205	138
276	120
128	88
200	141
84	100
124	133
182	63
155	71
221	138
84	152
120	101
234	106
200	
191	112
242	
297	
145	

All weights in mg.

Difference sig. $P<0.01$ Mann Whitney 'U' test

Table 12.3 (ii) shows the weights of the ovaries and uteri of both GTG and control females. There are no significant differences in the weights of the ovaries in the two groups (Mann Whitney 'U' test). Uterine weight is, however, significantly increased in the GTG group ($P<0.0$ Mann Whitney 'U' test). As with the incidence of persistent oestrus, there is a tendency for the heaviest uteri to appear in the heaviest animals. In fact the uterine weights fall into a bimodal distribution, with a group inside the normal range, and a second group comprising some 50% of the total without it. Only a small proportion of the animals from which organs were extracted received vaginal smears after injection, so that it is unfortunately impossible to say whether the heavy uteri are normally also associated with persistent oestrus.

The ovaries and uteri were examined histologically, and to a relatively inexperienced eye the ovaries appeared quite normal, with ovae in all stages of development. Similarily, in the normal weight uteri there were no obvious differences between the GTG and control groups. The heavy uteri, on the other hand were quite different, showing a marked hypertrophy of the endometrium, and some hypertrophy of the myometrium.

12.3.3. Discussion

The aim of this section was not to characterise in detail the endocrine status of the female GTG mouse, but merely to determine whether they may be considered as effectively castrate. It would appear from both the data presented here, and other published material that /

that at least one conclusion is justified. Whatever the actual hormonal state of GTG females, none of the animals tested shows any signs of gonadal atrophy, or of changes in the gonads or accessory organs suggestive of a reduction in the secretion of gonadal hormones. Rather, such evidence of abnormality as there is would suggest a hypersecretion of some component.

Vaginal cornification is a product of high circulating oestrogen titres (Davidson (1968)), so that the persistent 'oestrus' observed above may be the result of a continuous, elevated secretion of oestrogen. Such a pattern does indeed occur in old mice (Thung et al (1956)), and in rats kept in continuous light. (Wurtman (1967)).

The majority of animals, however, are apparently quite normal, showing the usual vaginal cyclicity and organ weights. There is a possibility (Browning and Kwan (1964)) that GTG females show hypersecretion of some hormone affecting mammary development, which may be prolactin. Sinha and Vander Laan (1971) have also demonstrated an increase in pituitary prolactin content after GTG. Indeed in the few animals used here whose mammary glands were examined histologically, there did appear to be some hypertrophy of the alveoli, with no increase in their numbers.

In the light of the observation by Pfaff (1969) that prolactin injections increase food intake in female rats, but not males, it is possible that the continuing weight gain of females during the 'static' phase may reflect some action of that hormone.

Either way, the fact of importance is that in the long term, after GTG oestrogens appear to be present in either normal or elevated amounts, and /

and yet the food intake of females is not depressed relative to the male. A way to investigate the effect of the gonadal hormones is to combine the operations of ovariectomy and the injection of GTG, and this is discussed in the following section.

12.4. Combined ovariectomy and GTG

12.4.1. Introduction

The induction of lesions by GTG is subject to a great number of interfering influences (Blood sugar levels, strain, time of day etc. Liebelt (1967), Wiepkema (1966)). One such influence is the administration of a barbiturate anaesthetic. Furthermore, animals which have been treated with GTG, and have presumably therefore sustained some hepatic and renal damage are abnormally sensitive to the lethal side effects of anaesthesia. The converse is also true, so that animals previously treated with anaesthetics are abnormally sensitive to the lethal side effects of GTG. Overall, therefore the combined operation of ovariectomy and GTG proves technically very difficult.

In the animals surviving the combined operation, Wright and Turner (1973) noted that the total weight gain was no greater than would be expected from the GTG alone. In the following experiment this study was replicated including measurements of food intake.

12.4.2. Methods

An attempt was made to overcome the problem of high mortality by performing the injection of GTG and ovariectomy simultaneously under a light inhalation anaesthetic (methoxy-flourane). The animals were anaesthetised, ovariectomised by the methods described in Chapter Three, and then given an intraperitoneal injection of 0.6 mg/gm GTG in arachis oil prepared as described above. Food intake andbody weight weight were followed for five weeks after the operation.

Eight /

Eight animals received the combined operation, and a further six received a sham ovariectomy, in which the abdominal incisions were made, but the ovaries left undisturbed, before being injected with GTG in the usual way.

In a later series of experiments, for reasons that will become apparent below, the ovariectomy was delayed for five hours after the injection of GTG. Similar sham operation procedures were used in this case, so that there were eight experimental animals and six controls.

12.4.3. Results

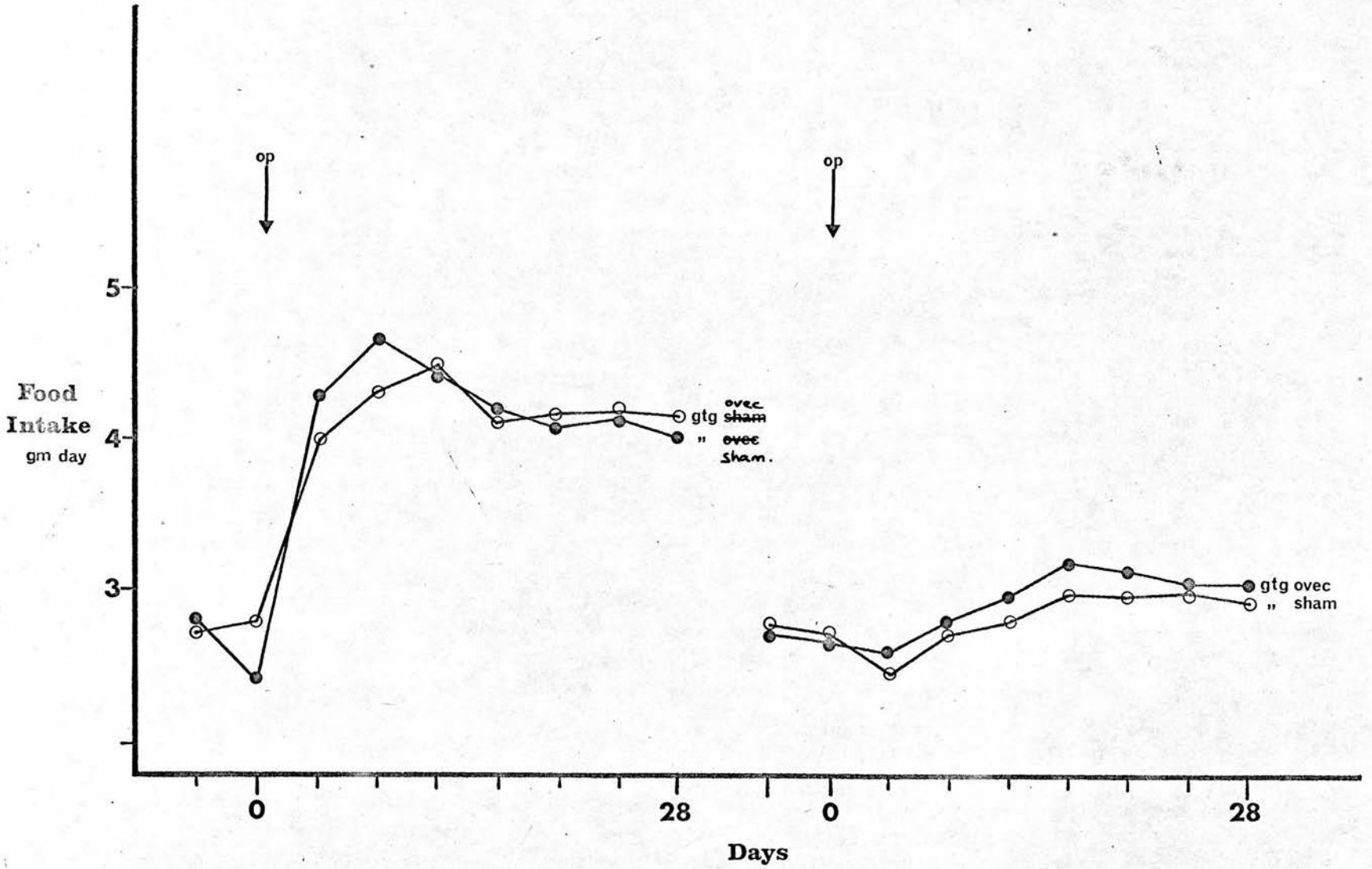
The first method of combining ovariectomy and GTG did indeed minimise mortality, which was much lower in both the sham op and ov-x groups than control GTG injections. There was, however, a further unfortunate side effect, namely a severe reduction in the effects of the GTG. Both sham operated and ovariectomised animals showed some slight increases in food intake (see Fig. 12.4.(a)), and body weight, the ovariectomised animals increasing rather more than the sham op's (but not significantly so). The ovariectomised animals, however, ate no more than ovariectomised animals given no GTG. The rise in food intake is significant over pre-op levels in both groups (Wilcoxon test on intakes two weeks post-op $P<0.05$ for shams, and $P<0.01$ for ov-x's).

It is apparent from these results that some component of the operation (not specifically ovariectomy) was interfering with the action of GTG. The brains of some of the animals from this experiment were processed into paraffin wax blocks in the usual way, and sectioned at 30μ through the hypothalamus. The VMH was clearly present in all animals so checked.

The /

Fig. 12.4(a)

Food intake of mice after combined ovariectomy and GTG injection. Left graph ovariectomy 5 hours after GTG, right simultaneous ovariectomy and GTG.


op
op
5
4
3
Food
Intake
gm day
gtg ovec
" ovec
sham.
gtg ovec
" sham
0
28
0
28
Days

The second surgical procedure, adopted as a consequence of these results proved more successful. (see Fig. 12.4(a)). In this case both sham operated and ovariectomised GTG animals showed a marked, and significant rise in food intake over controls.($P<0.01$ in both cases Mann Whitney 'U' test against age controls). The more important finding, however, is that there were no differences between the sham and ovariectomised groups in either food intake or weight gain over the three weeks of observation. The weights of the two groups at sacrifice four months later were also not significantly different.

Cursory histological examination of the brains of these animals indicated very little of the VMH remaining. No attempt was made, however, to establish whether there were different degrees of hypothalamic damage in the sham operated and ovariectomised groups.

12.4.4. Discussion

These results clarify to some extent the nature of the interaction between ovariectomy and GTG. It may be presumed that the effects of anaesthesia (or possibly surgical trauma it is not important which) is to reduce the effective lesioning power of GTG. (Possibly by some action upon renal clearance?) If the degree of destruction of the VMH is sufficient only to produce a dynamic hyperphagia less than that produced by ovariectomy alone, then the animals in which ovariectomy and GTG are combined will eat more than shams. (There is evidence McBurney, Liebelt et al (1966), that the degree of hyperphagia is related to the degree of hypothalamic damage in GTG mice). If, however, the effect of the GTG is greater than that of ovariectomy alone, then simultaneous ovariectomy has no effect on food intake and body weight, as was observed by Wright and Turner (1973).

Animals /

Animals bearing lesions induced by GTG therefore apparently have oestrogens in their bloodstream which have no effect when present, and there is no change when they are removed, provided that the degree of hypothalamic damage exceeds a certain level.

Montemurro (1971), working with male mice bearing electrolytic VMH lesions, noted that the administration of stilboestrol reduced food intake. If this is the case for the mice used here, with lesions from GTG, then a paradox appears. The normally present hormones apparently have no effect, yet exogenous hormones apparently do, and indeed in rats (Kennedy (1969)) VMH animals appear to be more sensitive to the weight depressing actions of oestrogens. Two problems have therefore to be resolved, on the one hand the effects of oestrogens upon GTG animals of this strain must be examined, and on the other some attempt must be made to resolve the paradox.

12.5 The effects of oestrogens on food intake in GTG mice

12.5.1. Methods

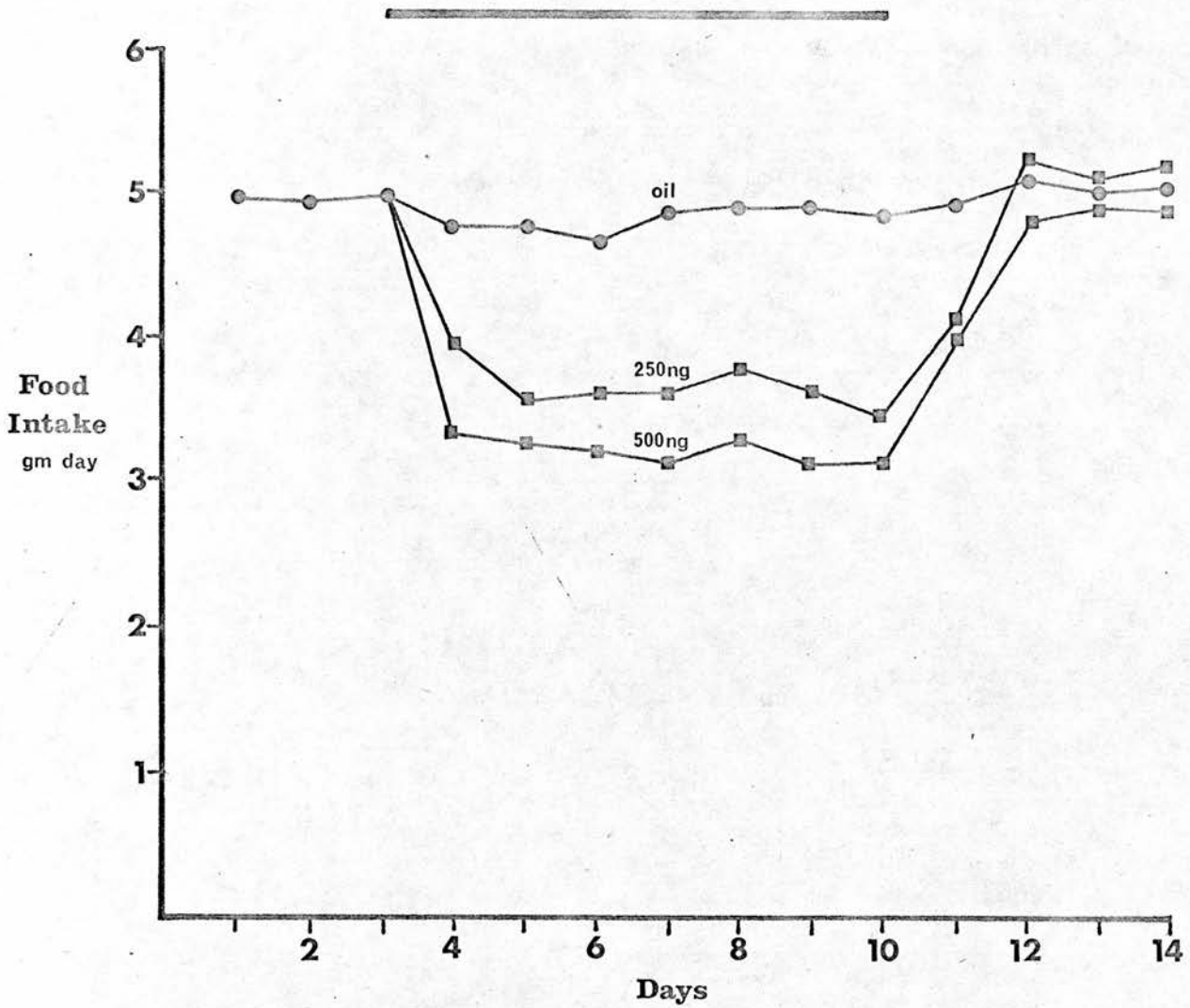
Eighteen dynamic phase GTG female mice, three weeks after injection (in the usual way at 90 days of age) were kept individually in the experimental room. The animals were not ovariectomised. The food intake of three groups of six was measured daily for one week. For the following seven days individuals of each group received daily an injection of either 500, 250 ng oestradiol benzoate in arachis oil (0.1ml total volume in each case), or 0.1 ml oil. Food intake was followed for this and one subsequent week.

12.5.2. Results

Both doses of oestradiol reduced food intake below that of the oil injected /

Fig. 12.5(a)

The food intake of CTG female mice receiving daily injections of either oil, 250ng or 500ng oestradiol benzoate in 0.1ml oil intra-muscularily.


6
5
4
3
2
1
Food
Intake
gm day
oil
250ng
500ng
2
4
6
8
10
12
14
Days

injected control ($P<0.05$ for 250 ng, $P<0.01$ for 500 ng, Mann Whitney 'U' test). (see Fig. 12.5(a)).

Food intake returned to control levels within seven days after the injections ceased.

12.5.3. Discussion

These results, therefore replicate those of Montemurro (1971) in the strain, and with the lesioning technique used here. The paradox therefore remains, if oestrogens are removed, then no change appears, but if they are added it does. One of the possible differences is that in this study, and those on rats, the oestrogen is injected in relatively high doses every day, whereas, in the majority of GTG females at least, the natural oestrus rhythm of intermittant secretion is apparently maintained. Accordingly, the effect of intermittant injections upon the food intake of GTG females was examined.

12.6 Intermittant oestrogens and food intake in GTG females

The best way to consider this problem would be to look at food intake over natural oestrus cycles in GTG females. The period of maximal hyperphagia following GTG is not, however, that long (three weeks or so), so that considering the potential irregularity of the cycles, a large number of animals would have to be followed to obtain sufficient data. Accordingly, in this case it was decided to use a semi-natural situation, in which the oestrus cycles are driven by an external hormonal stimulus. Mann (personal communication) has developed a technique for producing regular, predictable behavioural oestrus cycles in female mice, by the administration of an oestrogen dose every third day. This apparently entrains the animals' natural oestrus cycles.

This /

This procedure was used to produce a predictable cyclical input of hormones to intact GTG mice, having first verified that it produces the expected variations in food intake if given to non-GTG females.

12.6.1. Methods

The experiment was divided into two parts the first using intact, control females, and the second females two weeks after the injection of 0.6 mg/g GTG in the usual way.(at 90 days of age).

Each part used sixteen animals divided into two groups of eight. Within each group each animal received a single injection of either 500 ng oestradiol benzoate in arachis oil (0.1 ml), or oil, intramuscularily, every fourth day, two hours before the beginning of the dark period. Each animal received three such injections, all at the same dose level.

Daily food intake was followed in the usual way. The animals of both groups in each part received Spillers Laboratory Diet No. 1 to eat.

12.6.2. Results

The effects of intermittant injections upon food intake in GTG and intact females are shown in Fig. 12.6(a) and 12.6.(b). In the intact females there is a depression of food intake following each injection, with a subsequent recovery, so that food intake varies cyclically in the usual way. The changes are significant (Friedman's chi = 9.8 $P<0.02$).

The GTG results are somewhat different. In the 24 hours following each injection there is a sharp fall in intake, which is significant (P 0.01 /

Fig. 12.6(a)

The effects of 500ng oestradiol benzoate given once every fourth day upon the food intake of intact female mice.

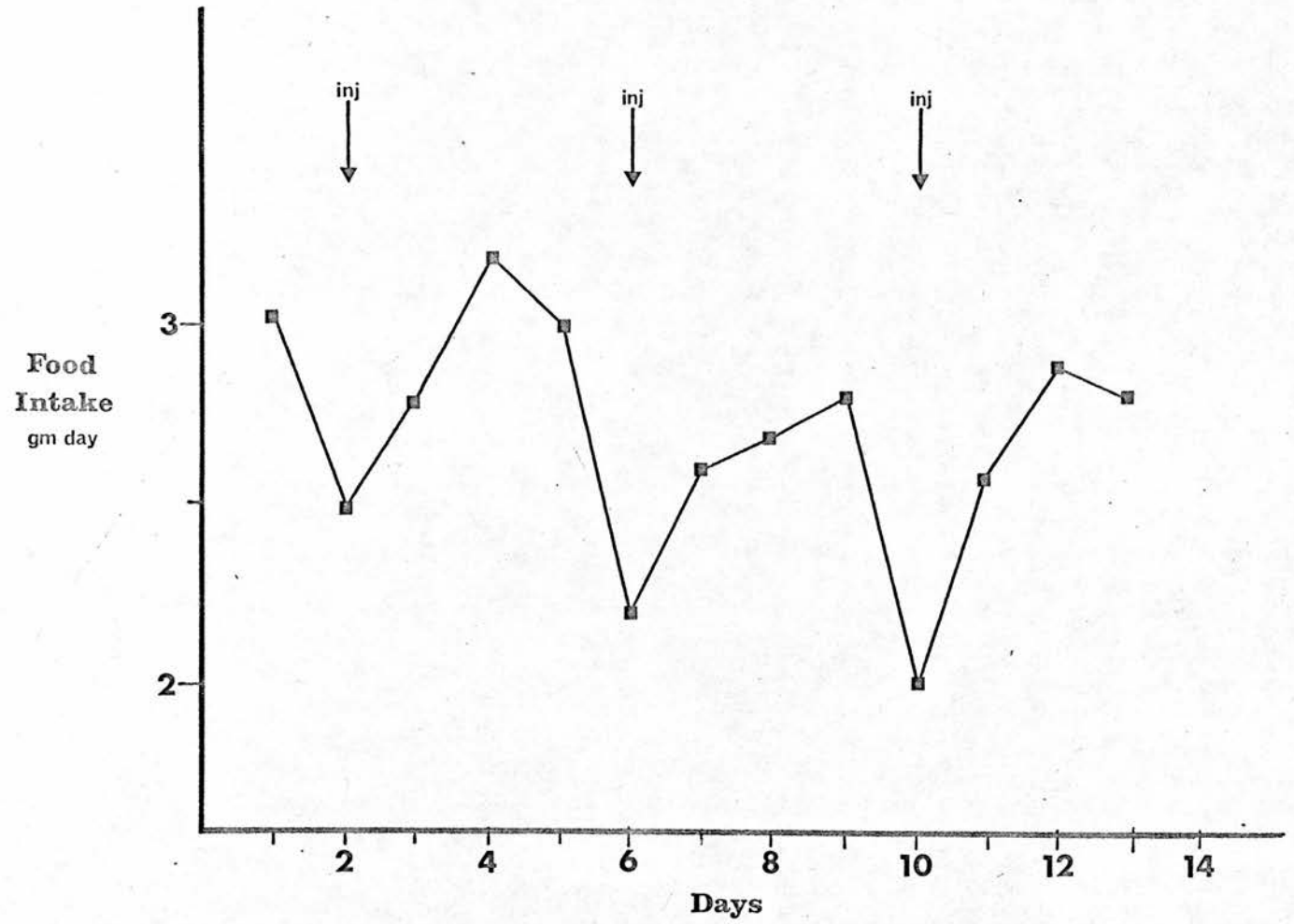
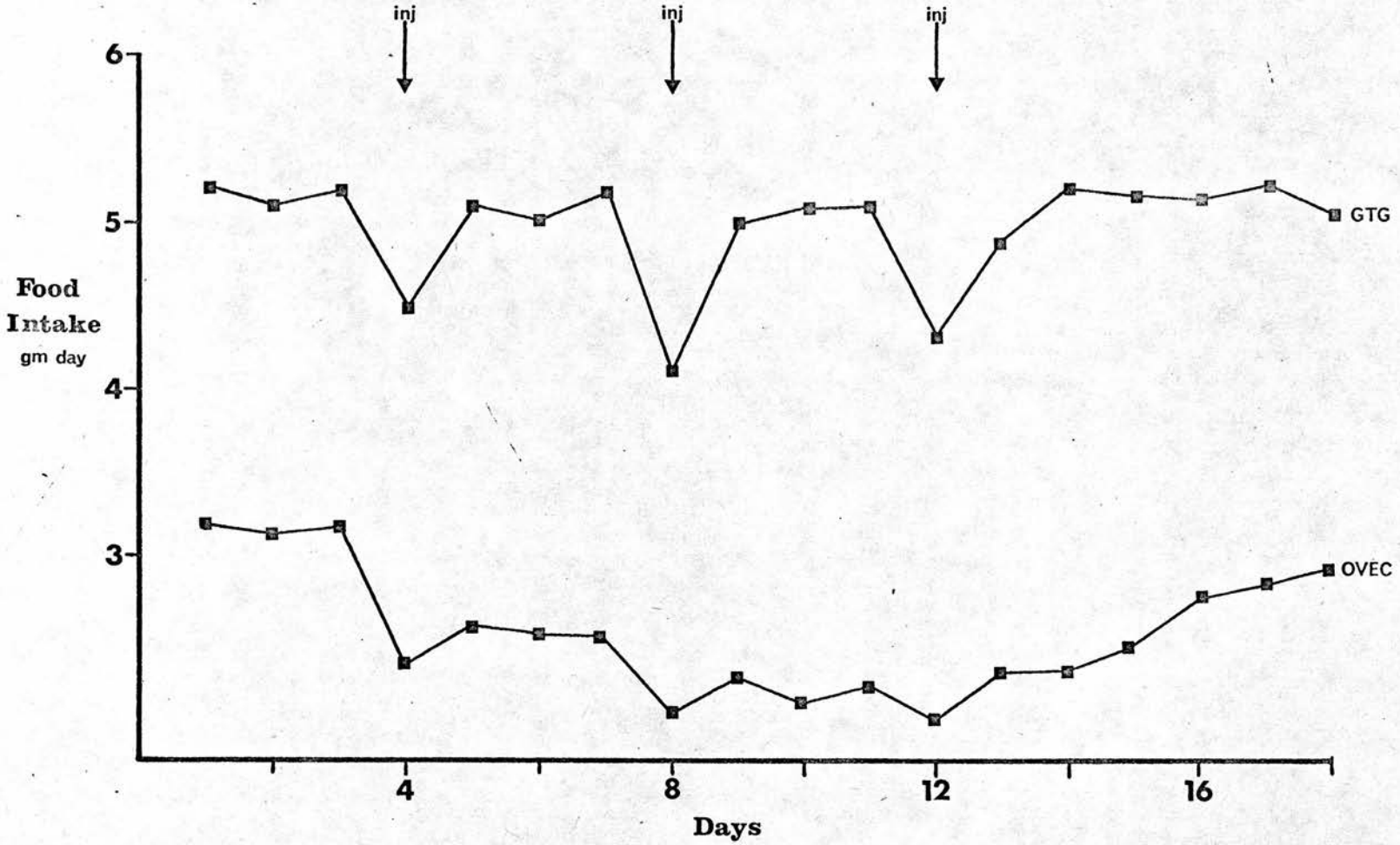

inj
inj
inj
Food
Intake
gm day
3
2
2
4
6
8
10
12
14
Days

Fig. 12.6(b)

The effect of 500ng oestradiol benzoate given once every fourth day upon the food intake of GTG treated female mice (in the dynamic phase). The effect of a similar regime on ovariectomised animals (see Chapter Three) is shown for comparison.


inj
inj
inj
6
5
4
3
Food
Intake
gm day
GTG
OVEC
4
8
12
16
Days

($P<0.01$ Wilcoxon test), the food intake during the following days is, however, insignificantly different to the intact controls (Mann Whitney 'U' test). In both GTG and control groups the effects of the oestrogen injection during the day following it were significantly greater than thos of the oil. ($P<0.01$ in each case Mann Whitney 'U' test).

In the GTG group the mean intake over the four days of each nominal 'cycle' is insignificantly different in the oil and oestrogen injected groups, a situation quite unlike that observed, for example in ovariectomised animals (see Chapter Three).

12.6.3. Discussion

The results first show that the hormonally driven cycles in intact mice ressemble, in terms of the pattern of food intake changes, the natural oestrus cycle. It is not unreasonable, therefore, to assume that the oestrus cycles of GTG animals may be entrained in the same way. If this is done, then a somewhat different pattern of food intake changes appears. Food intake is depressed after each injection, but fails to rise towards the end of the 'cycle' as is the case in the intact animal. This possibly allows a resolution of the paradox presented in the previous sections. In at least the majority of animals showing regular oestrus cycle after GTG oestrogen will be present in significant quantities only once every fourth day. At this time food intake will be altered. In the long term, however, the hormone will have no effect, so that the animal continues in a degree of hyperphagia unaffected by gonadal hormones, as is observed.

When, however, exogenous oestrogens are given in high doses each day, the mechanism presumably activated every fourth day remains active, and /

and depresses intake over the relatively long term. In this case, therefore the pattern and dosage of the hormone is of vital importance. A similar effect may appear in the proportion of animals which show persistent oestrus, and have presumable chronically raised oestrogen levels. These individuals are, however, those destined to eat the most anyway, so that inhibition would tend only to lower them towards the mean GTG intake, rather than below it, so that the depression, if it exists may go unnoticed.

The data presented so far therefore suggest that the lesions induced by GTG dissociate different influences of oestrogens upon feeding. It would seem that the long term effects are absent after injection, whereas the short term effects remain. This assertion may be further examined by observing the effects of oestrogens on the consumption of sweet food by GTG females.

12.7 Intake of sweet foods after GTG and oestrogens

In Chapter Three it was shown that at the time of sexual receptivity during the oestrus cycle of the intact female mouse the intake of sweet food is increased. If, therefore the short term mechanisms remain after GTG then analogous changes should occur in GTG animals.

12.7.1. Methods

Twenty-four adult female mice were fed golden nuggets for two weeks. They were then swopped back to laboratory chow for five days, prior to the injection of 0.6 mg/gm GTG in the usual way. After a further week of feeding upon laboratory chow, they were swopped back to golden nuggets, and food intake monitored in the usual way for two weeks in 16 of the animals, and five weeks in the remaining eight. Twelve adult male /

male mice were subjected to an identical regime. The sixteen females whose intake was not followed for a long period were given intermittant oestrogen of oil injections in a regime absolutely identical to that in the previous section.

12.7.2. Results

Surprisingly, animals of either sex given GTG show no hyperphagia if fed golden nuggets. The mean intake is insignificantly different to controls. (Mann Whitney 'U' test for each sex against oil controls) (Fig. 12.7(a)). If, however, they are swopped onto laboratory chow, the usual dynamic hyperphagia rapidly appears.

There are no significant differences in the consumption of male and female animals after GTG when eating golden nuggets.

Intermittant oestrogens have an influence on the intake of golden nuggets after GTG. During the day following the injection the intake is significantly increased over the oil controls ($P<0.02$ Mann Whitney 'U' test), although the oil injections do have a much greater effect upon the intake of sweet food than chow. At other times the two groups are the same. (see Fig. 12.7(b)).

12.7.3. Discussion

The golden nuggets results are quite peculiar. The failure to show hyperphagia after GTG was characteristic not only of the formal results discussed here, but of many animals used in pilot studies and abortive experiments. At some time at least 80% of the animals were subsequently transferred to chow, and showed hyperphagia immediately.

A /

Fig. 12.7(a)

The intake of golden nuggets by male and female mice after GTG. Males - filled triangles, females - filled squares.

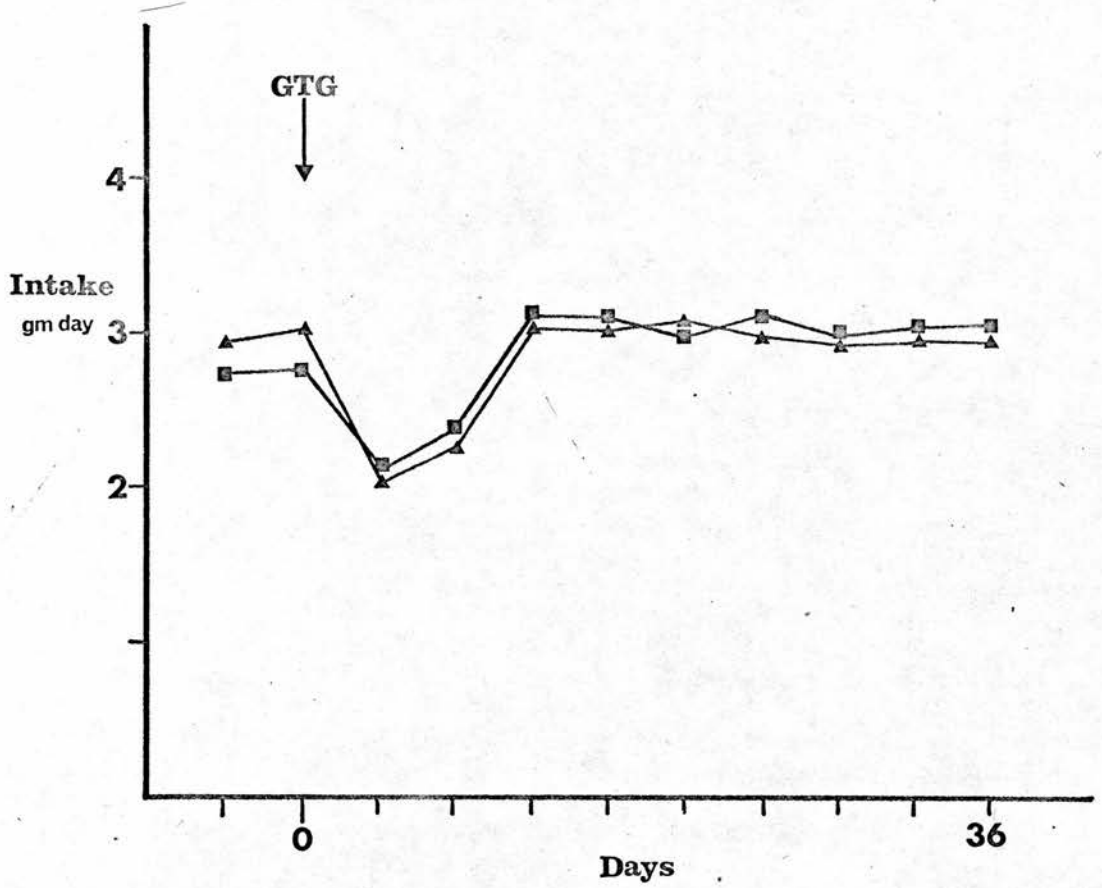
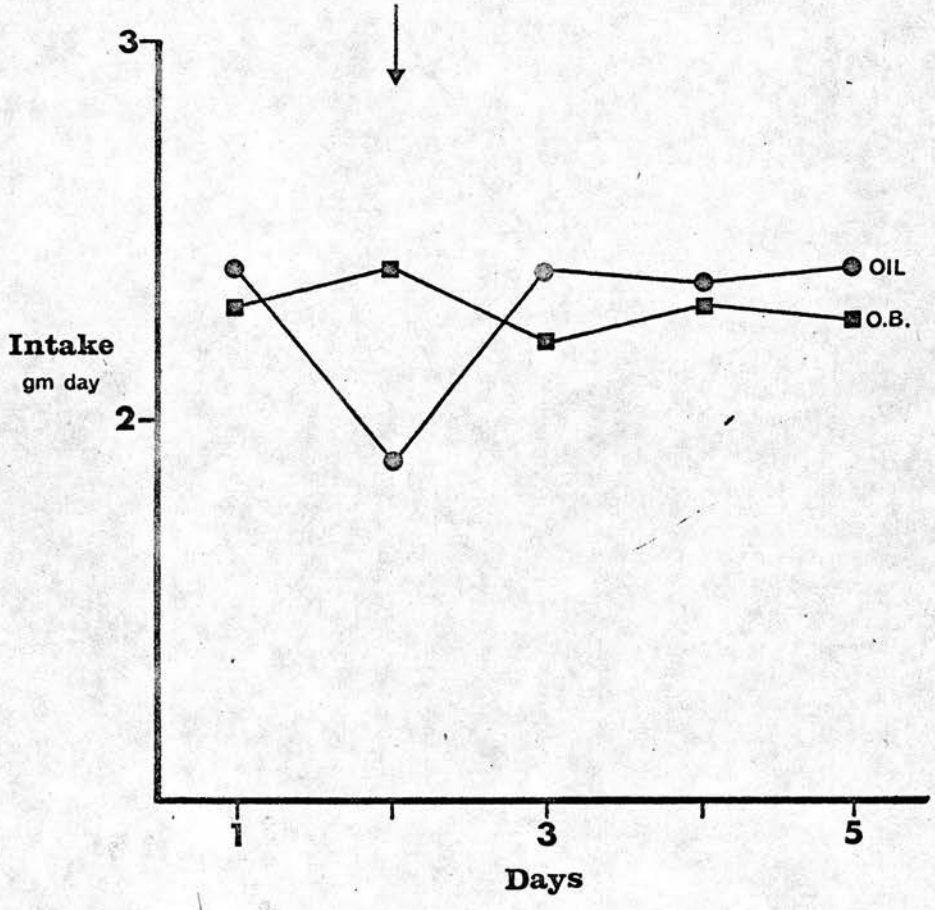

GTG
4
Intake
gm day
3
2
0
36
Days

Fig. 12.7(b)

The effects of externally driven hormonal cycles upon the intake of golden nuggets in GTG female mice


3
2
Intake
gm day
OIL
O.B.
1
3
5
Days

A possible explanation to be examined is that the changes represent a type of conditioning, such as that described by Garcia (1966). Golden nuggets are a novel food, and GTG certainly makes the animals ill. The animals were deliberately left off nuggets for a period before and after injection in the above experiment (to allow the side effects to subside) thus avoiding that possibility.

Singh (1970) showed that VMH animals have a much attenuated hyperphagia when fed sugar, so it may be that VMH lesioned rodents just develop some sort of aversion to sugar, which is of course a major component of golden nuggets.

Whatever the reason the finding is something of a peculiarity, which cannot be readily explained at the moment.

Despite the lack of hyperphagia, males and females eat the same amount after GTG, whilst males eat more than females before. Similarily the effects of oestrogens are an exact opposite of those when the animal is eating laboratory chow, for the day after each injection food intake is raised a direct parallel with the changes over the first part of the oestrus cycle in the intact female.

The results of this study, whilst not as conclusive as they might be, would therefore tend to support the assertion that the short term effects of oestrogens over the oestrus cycle remain intact after GTG, despite the absence of a sexual dimorphism in food intake.

12.8 Details of the feeding patterns of males and females after GTG

Hyperphagic animals must show considerable alterations of feeding patterns /

patterns to accommodate their increased food intake. Wiepkema (1968) described the feeding patterns of GTG male mice, concluding that the bulk of the increase in food intake was accounted in a change in meal size. In the following section the feeding patterns of male and female dynamic phase GTG mice are compared for the existence of sexual dimorphisms.

12.8.1. Methods

The electromechanical recording unit was used to record the detailed feeding pattern of six male and six female mice eating laboratory chow two weeks after the injection of 0.6 mg/gm GTG in the usual way. The animals were kept on a normal twelve hour cycle of lighting.

Three batches of eight, four males and four females in each were injected at one weekly intervals. The food intake of all eight was followed for ten days post injection, and then two clearly hyperphagic animals of either sex transferred to the recorders. They were allowed four days for adaptation to the feeders, and three 24 hour records of feeding taken in the usual way.

The next four animals were selected, and the process repeated, and so on. In this way six animals of either sex were tested at exactly the same time after injection. The records were examined for the existence of meals in the usual way, using the survivorship curve, and then scored again in the usual way onto data sheets, ready for analysis. Only night records were collected.

12.8.2. Results

The general pattern of meals, intermeal intervals, and feeding sessions, described in Chapter Five, is preserved in the GTG animals of /

of both sexes. Furthermore, the circadian rhythm typical of the intact animal is also present. Fig. 12.8(a), shows a summary of the data for meal size, IMI and total consumption over the night for intact and GTG animals of each sex. Night time consumption is increased over control figures in animals of both sex.

The night figures are summarised in Tables 12.8 (i) and (ii). Considering firstly meal size. Males show a highly significant rise in meal size ($P<0.001$ Mann Whitney 'U' test), whereas females show no such rise, provided comparison is made with the non-receptive part of the cycle. When GTG males and females are compared there are no significant differences in meal size.

Males after GTG show no change in meal frequency, but females increase their meal frequency dramatically to the level of the Male ($P<0.01$, Mann Whitney 'U' test).

Both sexes show an increase in the proportion of meals taken within feeding sessions, so that they account for about 65% of the total, irrespective of sex.

The mean within session IMI is unchanged in both males and females, despite the increase in male meal size. The female figure approximates to that at metoestrus in the intact animal. The long between session IMI's remain unchanged after GTG in either sex.

In GTG animals of both sexes the usual postprandial correlation between intra-session meal size and following IMI is retained.

Overall /

Fig. 12.8(a)

The pattern of night-time feeding in GTG male and female mice compared to controls

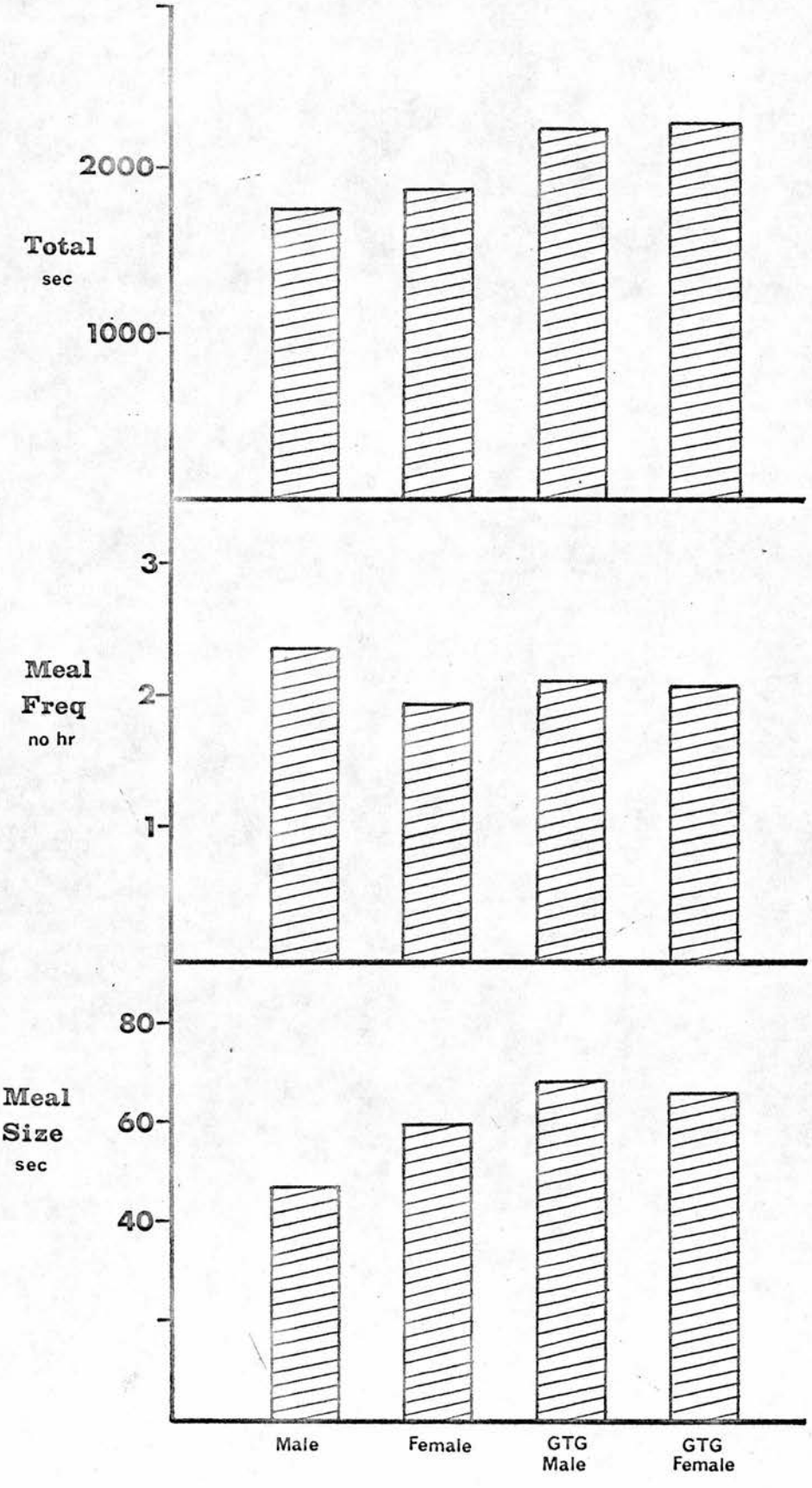

2000
Total
sec
1000
3
Meal
Freq
no hr
2
1
80
Meal
Size
sec
60
40
Male
Female
GTG
Male
GTG
Female

TABLE 12.8(1)

Feeding in GTG Animals

All in seconds
Means for each animal

MALE

Meal Size	IMI	Intra-Session	Proportion	Extra-Session
94.2	890	346	76	2612
92.6	2538	646	54	4758
77.2	1282	534	76	3650
53.2	1874	636	56	3448
41.2	1594	678	64	3744
53.2	1782	480	63	3688
68.2	1660	552	65%	3646

FEMALE

Meal Size	IMI	Intra-Session	Proportion	Extra-Session
38.8	1550	456	60	3190
84.6	1850	554	49	3094
54.8	1560	698	71	3670
45.0	1666	420	69	4438
86.2	1816	746	60	3420
77.2	1514	620	70	3600
64.4	1658	582	63%	3568

Overall the pattern is quite simple. Male and female animals after GTG are identical in all respects as far as feeding is concerned. The changes required to attain the equality are quite different. Males increase meal size, and females meal frequency,

12.8.3. Discussion

These results confirm in part the results of Wiepkema (1968) for male mice, in that the increase in food intake in this sex is produced by changes in meal size. There is, however, one point of disagreement, in that Wiepkema described a fall in the proportion of short IMI's, whereas in this case there is a small rise. The reason for this discrepancy is unclear.

The changes in females are quite different, meal frequency increases so as to abolish the standing differences between the sexes. All chronic sex differences in feeding are therefore abolished by the lesions of GTG. The changes in the male ressemble those following castration as far as meal size is concerned, and this conceals the more fundamental change, which is in the relationship between meal size and intra-session IMI. In the castrate male the meal size changes are compensated, in the GTG male they are not. This lack of compensation is not the product of a removal of the relationship between meal size and IMI, but of its alteration, to a value the same as that of the GTG female. Both the GTG male and female therefore show an alteration of the regression between meal size and IMI, but with the male the increase in meal size compensates so that the meal frequency does not change. It is of interest to note that if the data provided by Wiepkema (1968) is analysed for regression between meal size and IMI this is present in the meals followed by short IMI's in GTG males, thus supporting the data reported here.

The /

The change in meal size in males is typical of that after castration. There is evidence (Wiepkema (1968), and observations of the author not reported in this thesis which will appear elsewhere), that GTG males show much reduced sexual activity and aggression, which is in many ways typical of castration (despite the fact that they have apparently normal testosterone levels). If as was suggested in the previous chapter the smaller male meal size is a consequence of the need to integrate sexual activity and feeding, then the removal of the former should allow meal size to increase as it does after castration. If this is true, then the primary change following GTG is the same qualitatively at least in males and females, that is to say an alteration in the relationship between meal size and IMI. This, it will be recalled is what happens after ovariectomy, and is typical of an animal whose body weight 'set point' has been disturbed, which is of course often assumed to be the result of damage to the VMH.

It is possible to argue, from the limited evidence provided here, therefore, that the change in meal size in males is possibly a secondary effect of GTG, and that the major change is the same as that following ovariectomy, typical of a change in body weight set point.

In conclusion, therefore, examination of the feeding patterns of males and females after GTG reveals that they are identical, and produced by basically the same change, affecting primarily meal frequency within sessions, although in males this is confounded by a change in meal size. This offers further support to the hypothesis that the lesions of GTG (hopefully the VMH) affect the same mechanism as the presumed long term influences of oestrogens on feeding.

12.9 Summary /

12.9 Summary

1. The food intake and rate of weight gain of male and female C57 mice is the same after GTG, during the dynamic phase, but females eat more than males in the static phase.

2. GTG females are not effective castrates. They show in a high proportion of cases normal oestrus cycles and organ weights.

3. If ovariectomy is combined with GTG, then, provided the hyperphagia due to GTG exceeds that expected from ovariectomy alone, ovariectomy has no effect on food intake or weight gain.

4. Daily oestrogen injections will, however, reduce intake in the dynamic phase female.

5. Intermittant oestrogen injections which tend to drive oestrus cycles produce a temporary depression of intake, which is not followed by a rise. There is no long term effects of intermittant injections, unlike the case in ovariectomised animals.

6. GTG animals are not hyperphagic on sweet food, and this is not due to a Garcia type conditioning. Intermittant oestrogenn produce transient rises in the intake of sweet food.

7. The feeding patterns of males and females in the dynamic stage are identical in all respects, although the changes to produce the equality are superficially different between the sexes. The primary change in both sexes appears to be an alteration of the relationship between intra-session meal size and frequency.

8. It /

8. It may be, therefore, that the lesions of GTG abolish the long term effects of oestrogens on feeding, whilst leaving the short term effects intact.

CHAPTER THIRTEEN

FOOD INTAKE AND THE OESTRUS CYCLE IN HAMSTERS

13.1 Introduction

Unlike the rat and mouse, the hamster shows no sexual dimorphism in body size. Females are as large, and sometimes larger than males (Swanson (1970), Swanson (1967), Kowalewski (1969)).

It would appear, therefore, that in this species long term effects of gonadal hormones on body size are absent, and this suspicion is confirmed by results reported by Zucker, Wade and Ziegler (1972): Gonadectomy has no effect upon either food intake or body weight in either sex. Similarily, there are no effects of either oestrogen or testosterone upon food intake and body weight in gonadectomised female hamsters, although testosterone at high doses does reduce food intake in the male. Progesterone markedly stimulates food intake and body weight in hamsters of both sexes both before and after gonadectomy, unlike the situation in rats. (see Chapter One) Female hamsters do, however, show an oestrogen dependent strong saccharin preference.

Female hamsters show oestrus cycles, and their 'general activity', that is to say running in a wheel, varies cyclically in phase with the oestrus cycle, being maximum at prooestrus, as is the case with the rat (Richards (1966)).

Some, at least, of the incidental characteristics of sexual receptivity therefore appear at prooestrus in the hamster. Despite the fact that oestrogen administered continuously at a dose sufficient to induce lordosis does not appear to have any effects upon feeding, it

is /

is of interest to examine directly food intake over the oestrus cycle, as whilst oestrogens clearly have no effect in the long term, cyclical sexual receptivity may be associated with changes in feeding.

13.2 Methods

The measurement of food intake in hamsters is not easy, because of their tendency to store food away from the feeder. A means was therefore devised for presenting the animals with a soggy mash food in a dispenser. Pilot experiments indicated that the hamsters to be used in the experiments would not store a wet mashed food, so that in this case the weight removed from the feeder was an accurate measure of the amount eaten.

Six adult female hamsters (of a pink strain obtained from the Bush Laboratory Animals Centre, Edinburgh), were kept in individual cages in a room with a thermostatically controlled heater set to 21 deg. C. Lights came on at 6.00 a.m. and off at 6.00 p.m. each day, Each cage (15" x 9" x 8") was equipped with a water bottle and a food dispenser, which was similar to a conventional bird drinker, with a vertical reservoir supplying a small trough. Once each day the feeders were weighed, accurate to 0.1g, washed, sterilised with boiling water, refilled, reweighed, and replaced in the cages. Animals received water ad libitum.

The food was a mash prepared from laboratory chow (Spillers Laboratory Diet No. 1). Pellets of the diet were steeped in warm water for about one hour, during which they swell and absorb an apparently fixed amount of water, and them mashed to a fine pulp.

Each /

Each day an accurately weighed sample (to nearest 10mg) of both freshly prepared food and that remaining from the previous day was dried in an oven at 105 deg.C. for three hours, and the percentage volatile matter (presumed as water) calculated. In this way the daily intake of the wet mash could be converted to an equivalent mass of unsoaked chow, using the mean moisture figures from the beginning and end of the 24 hour period the food was in the cage. As noted above, the chow appears to absorb a fixed proportion of moisture, and no more, which it fortuitously holds onto for at least 24 hours, so that the moisture content was similar each day, and also did not alter much over the 24 hours.

Once each day, when the feeders were refilled, vaginal smears were taken from each animal using a flamed platinum loop containing a drop of saline. Smears were stained with leishman's stain (same as for mice see appendix), and read according to the criteria established for mice. No attempt to verify the behavioural predictions from the smears was made. Each animal was followed for at least five cycles, and mean equivalent intakes calculated for each day of the nominal oestrus cycle. The data were analysed with Friedmans test.

13.3 Results

The results are quite clear. Food intake, in this strain, eating this food, varies significantly over the oestrus cycle, being depressed at prooestrus (Friedman's chi = 12.2 $< P$ 0.01). The variation is composed of a sharp depression at prooestrus, with no elevation at metoestrus, a picture unlike that of the rat or mouse (see Fig. 13.3(a) and Table 13.3(i)).

13.4 Discussion /

Fig. 13.3(a)

The daily intake of a wet mash food (converted to equivalent dry weight) over the vaginal oestrus cycle in the female pink hamster.

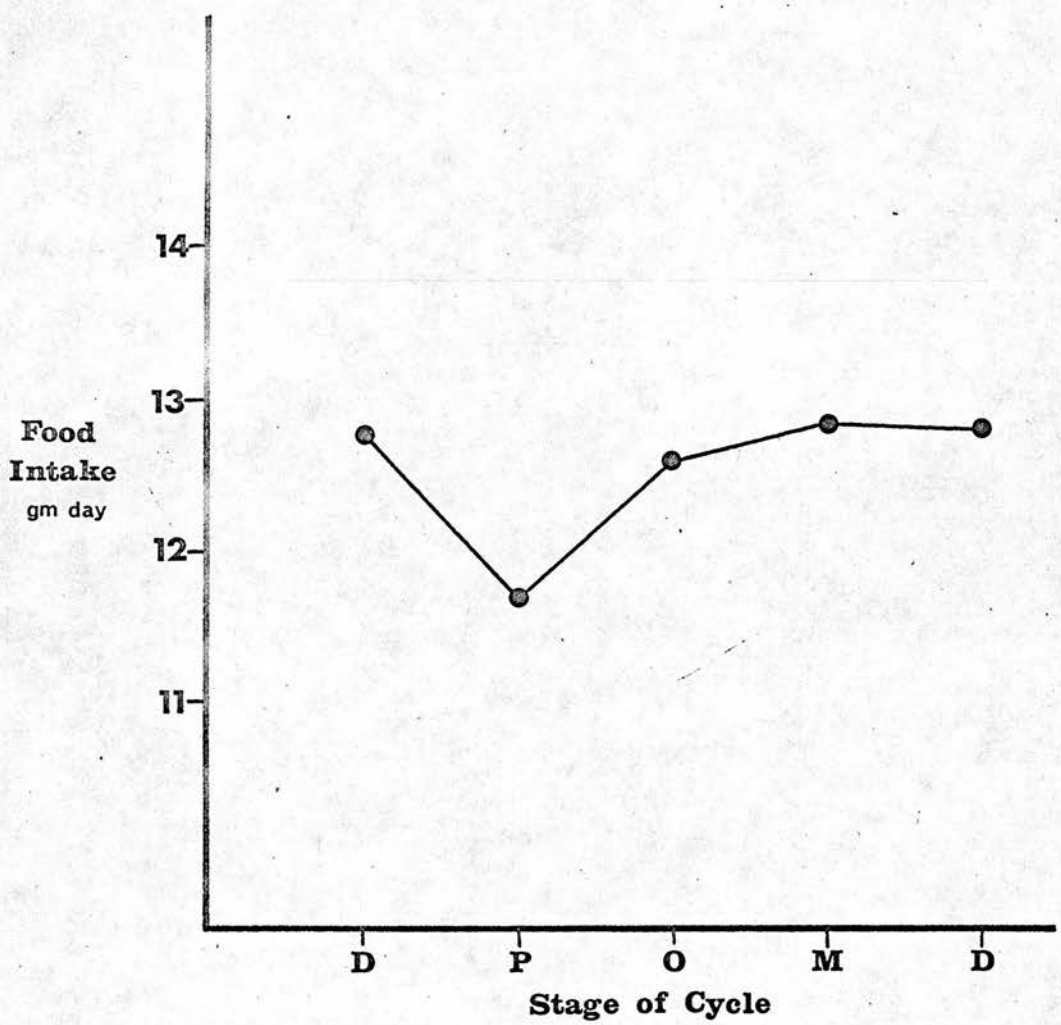

Food
Intake
gm day
14
13
12
11
D
P
O
M
D
Stage of Cycle

TABLE 13.3(i)

Food intake of female hamsters, over the oestrus cycle

Dioestrus	Prooestrus	Oestrus	Metoestrus
38.8	35.9	38.1	36.9
39.1	34.7	35.3	38.8
35.4	34.0	36.7	39.6
39.7	36.2	40.7	41.8
45.0	40.9	43.5	43.8
38.5	35.1	36.5	37.0
39.4	36.1	38.4	39.7

Friedman's X = 12.2 3 d.f. P<0.01

Mean intake of wet food (g/24 hr.) over at least three cycles for each animal.

Equivalent dry chow intake shown fig. 13.3(a)

13.4 Discussion

In the pink hamster, at least, therefore, food intake does vary over the oestrus cycle in a way superficially similar to the changes in the rat (see Chapter One), and the mouse (see Chapter Three). There is, however, one clear difference in the pattern of changes, The depression in food intake at prooestrus, whilst followed by a rise into oestrus, is not accompanied by a further rise at metoestrus, where the intake is only marginally greater than oestrus.

This, it will be recalled, is a pattern quite typical of the GTG female mouse given intermittant oestrogen injections, and was interpreted in that case as the presence of a short term effect of oestrogens in the absence of a long term influence.

The hamster case is, however, slightly different. Unlike the GTG mouse, continuous oestrogens at high doses, sufficient to induce sexual receptivity, do not reduce intake in the ovariectomised female hamster.

The implication from this is that either:

(a) the above results are in some way artefactual,

(b) the effects of oestrogens in the hamster depend on a cyclical release, or

(c) other hormones are involved.

The experiment was designed to eliminate, as far as possible artefactual changes in feeding, particularily those associated with the storage of food. This did, however, require the use of a moist food, which by necessity also supplied a fair proportion of the animal's fluid requirements, so that it could be argued that changes in fluid balance /

balance over the cycle underlie the observed variations in intake. Additional water was observed to be drunk by animals at all stages of the cycle, however, and this reduces the possibility of interference from changes in fluid balance, as any variations couldbe obtained by changing the amount of water drank, rather than food eaten.

The other two possibilities cannot be resolved with the data presented here. Bearing in mind the observation (Zucker et al (1972)) that progesterone stimulates food intake in female hamsters independently of oestrogen, it is just possible that the prooestrus 'depression'of feeding is a product of a temporary absence of progesterone, rather than the presence of oestrogen. Progesterone presumably present for most of the rest of the cycle might keep food intake elevated.

Only further experiments can therefore resolve the origin of the cyclical variations in food intake that have been observed, but either way it points to short term change in feeding which is not present in the long term. This raises a general point of some considerable importance to the data discussed above. So long as separate effects of reproduction upon feeding may be isolated, then it is no longer necessary to assume that all reproduction based changes in feeding are the consequence of oestrogenic action. It may well be that some other hormonal change is involved. It should be noted at this point that Yanai and Nagasawa (1974) have observed a peak of prolactin secretion early in the oestrus cycle of mice which may well influence food intake.

Perhaps, therefore, 'short term effects of oestrogens', should be replaced by 'short term effects over the oestrus cycle'.

CHAPTER FOURTEEN

GENERAL SUMMARY

The survival of mammalian species depends both upon individuals staying alive, and pairs of individuals procreating. These two requirements may interact with one another in complex ways. It was the aim of this thesis to identify, in as much detail as possible, a small component of that interaction, namely the influences of reproductive hormones, particularily oestrogens, upon the food intake and feeding behaviour of mice.

The system controlling and regulating feeding behaviour and food intake is a sub-system of behavioural control in general, and as such is subject to two types of constraints, those specifying its own optimum operation, that is to say in the case of feeding the maintenance of homeostasis, and those arising from the need to integrate feeding with other activities. These latter changes fall into two types, the second to second decision making which determines the minutiae of behaviour, and more general changes of balance in the control systems, which provide the conditions for appropriate integration to occur.

The hypothesis under test is that gonadal hormones, particularily oestrogens have separate constraints serving different functions in the process of maximising the efficiency of reproduction, one acting on the internal operation of the feeding system, and the other on its relations with other systems.

It was argued in the first chapter that there are two aspects to reproductive efficiency, on the one hand the fertilisation and development /

development of ovae must take place as efficiently as possible, and on the other only the fittest animals should breed. In such species as rodents where there is a considerable parental investment, by the female, in the care of young, the two aspects of efficiency are incompatible in the female, whose numbers place a limit on the breeding capacity of the population. In this case the latter, evolutionary efficiency will be best served by direct or indirect competition between numbers of males for the opportunity to procreate.

Provided that the males are virtually always ready to copulate, then the timing of that event, of vital importance to the reproductive process is best controlled by the female. It may also be that the female is responsible for some aspects of the choice of male.

The female is adapted to these pressures by virtue of a period of sexual receptivity, which is relatively circumscribed in duration, and during which a number of behavioural changes appear. The important change, from the point of the present discussion is a disturbance of feeding closely associated with sexual receptivity.

Adult males are larger than adult females, possibly reflecting an adaptation to allow them to compete. Prepuberally castrated males and females have identical body weights in adulthood intermediate between the adult intact male and female. The sexual dimorphism in body size in rats is therefore composed of two components, one a product of the testis, and facilitating weight gain, and the other the product of the ovary, which inhibits it. Males castrated in adulthood do not lose a great deal of weight, whereas females ovariectomised in adulthood show a rapid, co-ordinate, increase in body weight, until their growth curve comes /

comes to be identical to animals ovariectomised before puberty. The ovarian influence is therefore reversible, and a number of lines of evidence suggest that the presence of oestrogens affects the 'set point' of the body weight control system.

Oestrogens are secreted in appreciable quantities for only a few hours every four days in the female rodent, and although titres of the hormone are not undetectable over the rest of the cycle they are very low. The depression of food intake during sexual receptivity is circumscribed in duration, and followed by a temporary hyperphagia, whereas the changes in food intake following ovariectomy, and towards the end of the oestrus cycle are relatively slow to appear. If ovariectomised animals are given intermittant injections of oestrogen once every four days, food intake falls after each injection, but for the first few cycles fails to recover to preinjection levels. Eventually, however, the downward slope of the curve, which carries over successive cycles, disappears, and the injections produce nothing more than an oscillation of intake about a mean level. This suggests that in the long term oestrogens 'set' a body weight, but in the short term they induce an oscillation about that set weight.

The hypothesis therefore reduces to identifying the separate influences of oestrogens (or at least the hormones producing sexual receptivity) on feeding behaviour in the short term, as opposed to the regulation of food intake in the long term.

For various reasons the experimental animal chosen was the mouse. Before, therefore, any new experiments could be undertaken it was first necessary to verify that the mouse behaves in most ways like the rat. /

rat. Adult mice show a strong sexual dimorphism in food intake and body weight. This is absent prior to puberty. Immediately after puberty the sex difference appears as a result of a sudden massive rise in the body weight of males. After a couple of weeks the growth rate of males and females come to be equal and remains so, just preserving the standing difference in body weight previous obtained.

The sex difference is maintained, as in the rat, by a dual action of oestrogen in the female, and some product of the testis in the male. The mouse is quite similar to the rat, therefore, excepting two points, that is males and females gain weight at similar rates in adulthood, and the growth of mice never really reaches a plateau. Oestrogen may be identified as the active agent in the female by replacement therapy.

Mice also show a reduction of the intake of laboratory food during the night of prooestrus which is very similar to the rat.

Intermittant oestrogen injections have the same effect in the mouse as the rat. It would appear, therefore that the basis for the hypothesis under test is as valid in the rat as the mouse, and in the experiments which followed this conclusion attempts were made to differentiate and characterise the effects of oestrogen upon feeding in the C57 mouse.

The first such attempt was an experiment in which the stimulus properties of food were varied independently of its calorific value, and the intake of two foods, one sweet, and one bland was followed over the oestrus cycle. At the time of sexual receptivity the intake of the sweet food is increased, whereas that of the bland food falls. Towards the end of the cycle, and after ovariectomy (with certain reservations)the intake of both foods rises.

At the time of sexual receptivity the disturbance of feeding is different to that following the presumed change in 'set point' after ovariectomy. In the latter case it would appear that any calories will do, whereas in the former, it would seem that the nature of the food determines the amount consumed, rather than the needs of the animal.

Such a move is a sensible strategy for an animal whose greatest priority lies in a behaviour which must be completed in a relatively short time, namely copulation, as it allows feeding to be a very flexible activity which may be fitted around others.

The changes following ovariectomy, however, do not show this flexibility, rather they tend to make feeding a more important activity.

The temporal pattern of an activity will give considerable indication of influences acting upon it, and so data on the temporal pattern of feeding under various hormonal conditions was collected.

The temporal pattern of feeding is multiply determined by a number of influences. The most important factor, dictating the basic pattern modulated by the others is the type of foodstuff and life style for which the animal is adapted. In rats this produces a typical pattern of meals separated by intermeal intervals. The basic pattern is modulated in a number of ways, which fall into the two classes discussed above, namely the efficient maintenance of homeostatsis, and the integration of feeding with other activities.

The problem of maintaining homeostasis - regulation - may be considered at two levels, the relatively short term maintenance of a current balance from meal to meal, and longer term changes acting over several meals.

In /

In the short term variations in meal size are compensated by changes in following IMI, so that the animal eats at a time after a meal dictated by its size. This time could be signalled by a number of events, including clearance of the gut and the end of metabolic usage of the energy load, or some other component of the meal.

Changes over the relatively long term, such as circadian variations in feeding affect both meal size and frequency systematically, so that an increase in body weight set point for example leads to increased meal size with little increase in IMI, so that a higher proportion of the products of the meal enter storage.

Variations in meal size over the short term will arise for reasons not associated with energy balance, which are the product of the control processes determining meal structure. Meals are co-ordinated collections of feeding bouts produced by a set of interacting incremental and decremental effects. The rate of food consumption increases in the opening phases of a meal, and the rate of this increase is dependent upon the stimulus properties of the food.

In the wild rat these short term changes, coupled with the short term regulatory system discussed above, will possibly allow a certain flexibility of feeding. When the time comes to feed the animal may choose to eat a variable amount of the food available at that time. If for example, it is not particularily attractive, or there are other strong stimuli in competition with feeding then meal size may be reduced, and the deficit made up by feeding sooner, when better food may be available in less distracting circumstances.

The /

The basic pattern of the mouse, as discussed by Wiepkema, and from the data presented in the previous chapters, is somewhat different. Mice consume their daily ration in 25-30 meals, each consisting of roughly 100mg standard diet. The meals are unevenly spread over the day. In addition to being concentrated primarily in the hours of darkness meals are organised into sessions, during which a number of meals are taken in quick succession. There are two or three such sessions in a typical night. The survivorship curve reported by Wiepkema (1968) also contains evidence of a higher order structuring of meals, although this is not mentioned explicitly.

The feeding pattern of the mouse may represent the operation of constraints arising from its digestive apparatus and life style. Casual observation of the bulk of 100mg mouse chow (bearing in mind that it swells when wet) would suggest that such a load could very adequately fill the estimated volume of a mouse stomach. Whilst the animals certainly can eat more, it may represent a more or less optimum load. The collection of meals into sessions may be a consequence of the considerable frequency of meals required by the limitations upon meal size. It would be a very risky proposition indeed for the mouse to leave its nest to feed once every hour throughout the day and night, and this risk may be minimised by feeding 'excursions' made on a single trip out of the nest at a reasonably safe time of day, during which meals are taken at a maximum possible frequency, thus supplying more than immediate needs, and allowing the animals energy stores to be incremented.

From the observed daily intake of a normal weight mouse it can be estimated that the normal meal will support metabolism for about one hour, /

hour, so that during sessions energy is being supplied at something like four times the rate required. (although this figure may be reduced by the energy required for the activity of a feeding excursion)

Within sessions, and only within sessions meal size is correlated with following IMI in a way typical of the rat. It would appear that the mouse therefore resumes feeding either when the products of the meal have been absorbed or used in a way similar to the rat. Outside of sessions at night meals occur at a frequency which is just about right (by rough calculation) to maintain a current balance of energy.

During the day, however, meals are eaten very infrequently, so that energy stores must be mobilised to maintain metabolism. Outside of sessions at night, the animal eats just to maintain itself, by some mechanism which is not revealed as a correlation between meal size and IMI. The feeding session therefore appears to be a primarily lipogenic activity. Lipogenesis will be best increased, should the need arise by increasing 'session' size, which may be more or less equivalent to meal size in the rat, which takes far less frequent meals. The night time feeding sessions therefore either anticipate daytime depletion of resources or compensate for them.

Meals in the mouse are produced by interacting incremental and decremental effects in a way similar to the rat. The occurance of a feeding bout markedly facilitates the occurance of another soon after, and so on, such that the bouts become organised into meals. As also noted by Wiepkema bout length increases systematically over the first few meals with no change in inter bout interval. The end of the meal is preceded by /

by a steady increase in successive IBI's. The processes initiating the first bout maintaining the bout sequence, and terminating the meal, therefore affect primarily the probability of feeding beginning, whereas those processes leading to the progressive increase in bout length in the opening phases affect the probability of feeding ending.

It would appear that the influences upon the probability of feeding beginning and ending may be to some extent dissociated by their differential changes over the meal, and that they maybe reflect the operation of slightly different systems.

The basic feeding system is subject to modulation in a number of ways by reproductive changes. After ovariectomy, and towards the end of the oestrus cycle the evidence from rats (see introduction), and mice (Chapter Three) would suggest that body weight set point is raised. Under these circumstances the female is effectively deprived, so that a lipogenic pattern of feeding would be expected (or at least one producing more than immediate needs). The ovariectomised female mouse, and the intact animal at the end of the oestrus cycle increase food consumption by an elevation of intra-session meal frequency, with no change in meal size, although of course 'session size' is increased. The increase is a product of a change in the relationship between meal size and following IMI, such that a given meal size staves off feeding for a shorter period than at the beginning of the cycle. This would suggest that the products of the meal are being used more quickly (either for incrementing stores or growth), and furthermore goes against the hypothesis that gastric clearance is the controlling factor in meal frequency, unless of course that varies after ovariectomy, which is not impossible.

Perhaps, /

Perhaps, then the analogy between session size in the mouse and meal size in the rat is strengthened by these observations. During the day of oestrus, when the female might be expected to be in energy deficit, she also shows a night like feeding session.

Meal size changes in a completely different set of reproductive circumstances, which would not, a priori, be expected to be associated with changes in long term energy balance.

Meals size varies from the standard of 100 mg in only two cases, the intact male mouse, and the sexually receptive female.

The meal size of the intact male mouse eating laboratory chow is less than that of both the female and castrate male. This reduction is primarily the product of a reduced mean bout length, itself produced by a change in the length of the initial bouts of the meal. This reduction appears as a consequence of an attenuation of the rate of facilitation of bout length over the first few bouts.

It would appear that some products of the testis, probably testosterone, directly or indirectly alters the rate of decrease of distractibility during the meal. In the male, however, the reduced meal size has no effect upon the amount of food consumed, as meal frequency increases to compensate. A_s would be expected, only intra-session IMI changes although the sessions are longer in males than females, so that male session size is greater than that of the female, as is the case with meal size in the rat.

Analogous /

Analogous changes occur in the female animal on heat. When feeding on laboratory chow meal size is decreased, again by changes in the rate of facilitation of bout length. In the receptive animal, however, meal size is increased with sweet food.

Unlike the male these changes are not compensated by variations in IMI, so that they are reflected in total intake. This is the fundamental change at prooestrus. In the wild, non-receptive animal meal size would be expected to vary according to the food available at the time feeding becomes necessary. Such meal to meal variations would allow a certain amount of flexibility, although the normal correlative relationship would make sure that they are compensated.

If, however, a single activity is so important that the animal can afford to release feeding from such strict regulation, then a much greater degree of flexibility will result. This may be achieved by freeing IMI's from their correlative relationship with meal size, so that, under these conditions theanimal may decide not only how much to eat of a given food, but also just when to eat. It has been suggested that this allows the female to use a feeding strategy such that choice foods, which it would be a shame to miss, are eaten quickly, whereas mundane foods may be relatively ignored.

It may be, therefore, that there exists a biologically definable mechanism for the release of feeding from certain constraints in times of necessity, and that this mechanism is activated directly or indirectly by the hormonal conditions at prooestrus.

If /

If such a mechanism exists, then it should be possible to dissociate it from the operation of internal constraints on feeding. Lesions of the VMH in rats produce males and females eating about the same in absolute terms, yet oestrogens are present in females after VMH lesions, and exogenous oestrogens will reduce food intake. It was suggested that the short term effects of oestrogens remain after the operation, whereas long term, regulatory ones are absent.

This hypothesis was examined in GTG mice. Males and females eat the same, in the dynamic phase at least, after GTG, and oestrogens are probably present in the female. Ovariectomy has no effect on food intake after GTG, but continuous administration of oestrogens reduces it. Intermittant injections, on the other hand, produce only a temporary fall in intake, with no cumulative effect in the long term. Similarily, intermittant oestrogens produce a temporary elevation of the intake of sweet food. GTG changes feeding patterns in both sexes, and if was argued that the primary change affects the relationship between the meal size and following IMI in way typical of the changes after ovariectomy, once a confusing change in male meal size is ignored. This is what would be expected of an animal with an elevated body weight set point.

It may be, then that oestrogens act directly or indirectly via the VMH to 'set' body weight, in the way that has been suggested for the rat (e.g. Zucker (1972)), or more precisely to set a female pattern of growth. The changes at prooestrus are by a number of criteria different to these effects, however, and presumably reflect the influence of the hormones of sexual receptivity upon some other neural structure, which is maybe associated with the integration of feeding and other activities.

The /

The hypothesis stated at the end of Chapter One is therefore supported by this data from mice. There are probably separate constraints of reproduction upon feeding which operate by distinct neural routes, and may reflect the operation of different selective pressures.

The characteristics of the prooestrus changes illustrate the important principle that the general context in which a behaviour occurs may well greatly influence its appearance, and in higher species, such as man where feeding is embedded in an extremely complex nexus of social constraints the importance of these, and their interaction with the biological constraints described here should not be underestimated.

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I declare that this thesis was composed by myself, and that the work described herein was conceived and conducted entirely by myself.



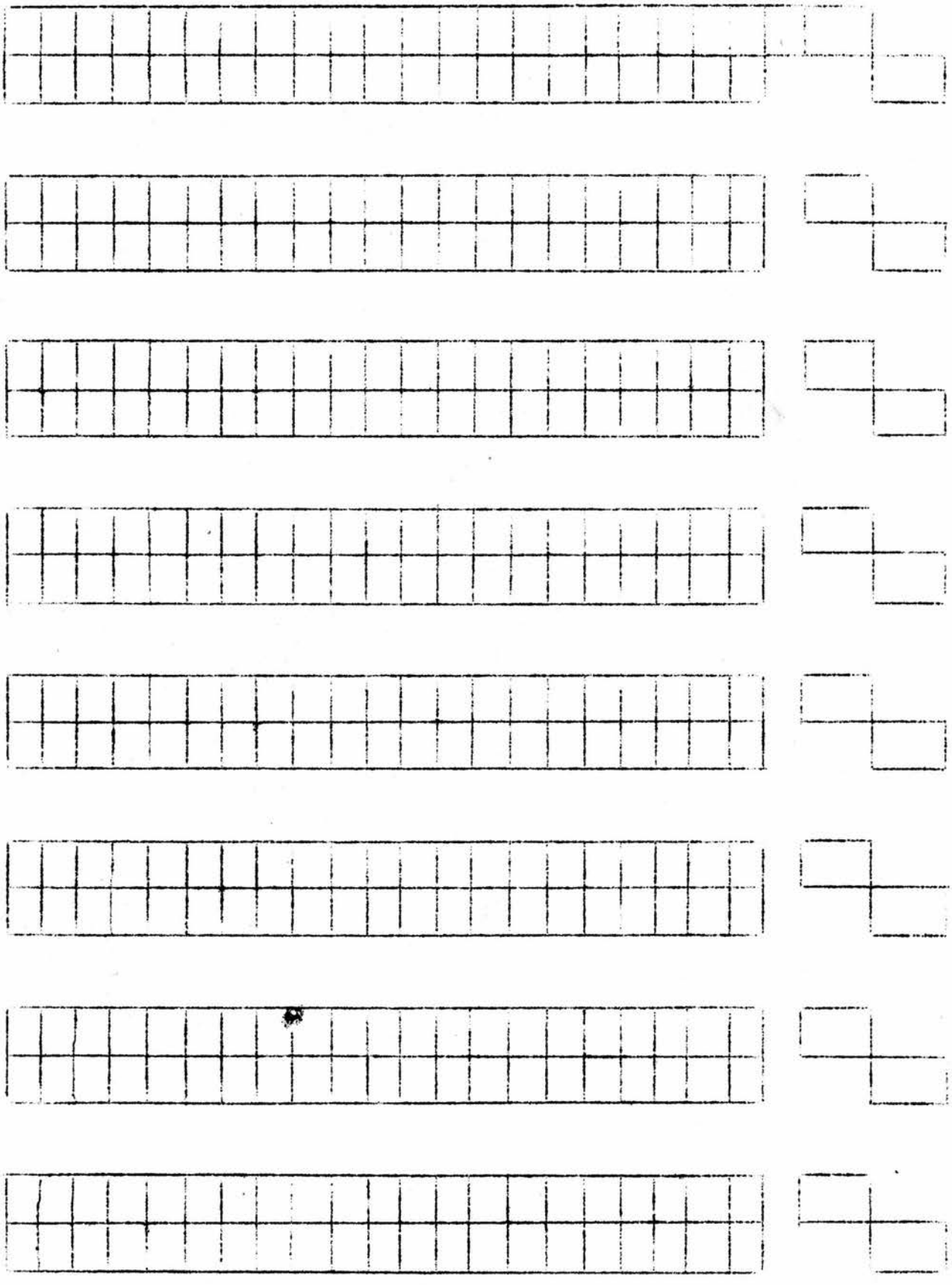
Stewart A. Petersen.

APPENDIX 'A'

ANIMAL _____ DATE _/_/_

SEX_____ STAGE OF CYCLE _____ RESOLUTION_____

SHEET NO._____



CONTINUED ON ANOTHER SHEET? YES/NO

APPENDIX B

Method for staining vaginal smears

1. Smears fixed in 96% ethanol for at least 12 hours.
2. Wash with deionised water.
3. Flood slide with Leishman's stain (George T. Garr Ltd.), 2 minutes.
4. Dilute x 2 with deionised water, leave 20 minutes.
5. Blot, differentiate in 96% ethanol containing 0.025% w/v Colophony resin.
6. Dehydrate, clear mount.

Epithelial cells - blue nuclei

Cornified cells - red, a nucleate

Leucocytes - large, blue, lobulated nuclei

APPENDIX C

Backward cumulative distribution of interboul intervals as a function of position in the meal - non-receptive animals eating chow

Duration	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18
FIRST IBI	613	267	195	157	121	95	74	67	61
2ND IBI	613	242	171	122	89	70	50	41	24
3RD IBI	613	250	180	126	94	71	52	44	28
PEN.IBI	613	260	200	163	130	100	80	70	60
LAST IBI	613	252	203	174	135	110	96	84	72

Backward cumulative distribution of interbout intervals as a function of position in meal - receptive animals eating chow

Duration	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18
FIRST IBI	262	117	74	60	44	38	36	33	27
2ND IBI	262	105	78	54	43	32	28	23	16
PEN. IBI	262	112	83	69	56	44	35	30	26
LAST IBI	262	107	90	75	58	49	43	37	33

Backward cumulative distribution of interbout intervalas a function of position in the meal - receptive animals eating Golden Nuggets

Duration	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18
FIRST IBI	272	119	77	62	47	39	36	33	27
2ND IBI	271	110	84	58	49	33	28	27	17
PEN. IBI	271	116	88	74	62	49	38	31	29
LAST IBI	271	110	90	74	60	51	44	38	34

APPENDIX D

Backward cumulative distributions of bout lengths as a function of position in the meal in Non receptive animals feeding on laboratory chow.

Duration	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18 seconds
FIRST BOUT	613	75	43	27	15	11	6	0	4
2ND BOUT	613	159	132	104	80	69	55	48	41
3RD BOUT	613	179	146	123	103	88	81	74	64
4TH BOUT	613	181	150	108	97	85	80	74	63
LAST BOUT	613	197	160	133	110	97	85	74	65

Backward cumulative distribution of bout lengths as a function of position in the meal - Receptive animals feeding on laboratory chow.

Duration	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18 seconds
FIRST BOUT	262	34	18	14	9	6	4	2	1
2ND BOUT	262	54	34	24	18	16	15	13	12
3RD BOUT	262	60	46	35	30	26	19	15	13
4TH BOUT	262	74	57	49	45	37	27	25	21
LAST BOUT	262	80	62	51	46	40	25	20	19

Backward cumulative distribution of bout lengths as a function of position in the meal - Receptive animals feeding upon Golden Nuggets.

Duration	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18 seconds
FIRST BOUT	271	50	35	29	19	18	17	16	15
2ND B BOUT	271	88	71	64	58	53	43	39	37
3RD BOUT	271	117	88	74	62	55	49	44	43
4TH BOUT	271	112	91	74	65	59	47	43	38
LAST BOUT	271	108	90	75	64	54	48	41	42

APPENDIX E

Individual data, effect of GTG upon food intake in males and females
(Laboratory chow)

MALES

Food intake g/24HR

DYNAMIC							Mean intake over 4 day blocks STATIC		
2.5	2.9	4.5	4.7	4.5	4.6	4.4	3.1	2.5	3.1
2.5	4.6	6.3	5.5	5.7	4.8	4.6	3.1	2.5	3.4
3.0	3.6	4.9	4.2	4.3	4.1	4.0	3.4	3.4	4.1
3.4	2.4	4.1	6.1	6.6	6.5	6.3	3.5	3.4	2.9
3.4	2.5	3.8	3.3	4.5	3.7	3.8	4.0	4.5	4.4
2.6	4.0	6.3	5.1	5.4	5.3	5.3	3.0	3.2	3.5
2.3	4.0	6.3	6.6	6.4	6.0	5.8	3.6	3.9	3.6
2.3	3.9	...	...	DEAD	...	...	...	DEAD	...
	3.5	5.1	5.1	5.3	5.0	4.8	3.4	3.4	3.6

FEMALES

DYNAMIC							Mean intake over 4 day blocks STATIC		
2.5	2.9	5.1	5.1	4.4	4.4	4.3	4.0	4.1	4.3
2.3	3.4	5.2	4.8	4.3	4.8	4.7	3.8	4.1	3.7
2.6	3.8	6.4	6.4	6.0	5.4	5.5	4.3	4.6	4.1
2.9	3.6	5.8	6.7	6.1	5.3	5.4	3.5	4.4	3.9
2.7	3.3	4.8	4.0	3.8	4.0	3.9	3.9	3.9	4.0
2.0	4.0	4.7	5.4	4.9	5.3	5.3	3.5	3.3	4.0
2.6	3.3	4.7	6.4	6.5	6.9	5.9	4.1	4.5	4.1
2.2	3.5	5.4	5.0	5.4	5.1	5.0	3.9	4.1	4.0
	3.4	5.3	5.5	5.2	5.2	5.0	3.9	4.1	4.0

No significant differences between dynamic phase males and females ($P<0.4$ Mann Whitney 'U' test).

Females eat significantly more than males in the static phase ($P<0.02$ Mann Whitney 'U' test on 12 day means).

APPENDIX F

Combined ovariectomy and GTG, Individual data

Food Intake g/24 hr

SHAM OP./GTG

DYNAMIC
Successive 4 day blocks

3.8	4.1	4.0	3.8	3.8	3.7
4.2	4.7	4.6	4.4	4.2	4.0
4.6	5.1	5.2	5.2	5.0	5.1
3.3	3.8	3.4	3.6	3.3	3.3
4.0	4.6	4.5	4.1	4.0	3.9
4.4	5.0	4.4	4.3	4.0	3.8
3.6	4.6	4.6	4.0	3.9	3.9
4.8	5.4	5.2	4.7	4.4	4.2
4.1	4.7	4.4	4.3	4.0	3.95

OVARIEC./GTG

DYNAMIC
Successive 4 day blocks

3.6	3.9	4.4	4.2	4.3	4.4
3.7	4.2	4.6	4.2	4.2	4.3
4.2	4.4	4.5	4.3	4.3	4.4
3.1	3.8	3.9	3.9	4.1	4.0
3.6	4.4	4.3	4.3	4.4	4.5
4.5	4.6	4.7	4.1	4.2	4.3
3.8	4.2	4.45	4.2	4.25	4.3

APPENDIX G

The effects of intermittant injections of oestrogens on food intake in female mice following GTG

Food intake g/24 HR.

		inj *				inj *				inj *				
DAYS	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	6.0	5.6	5.0	5.0	5.2	5.0	5.2	4.7	4.8	4.5	5.4	5.5	5.5	5.0
	5.2	4.3	4.3	5.6	5.6	3.6	5.1	5.8	5.8	5.2	4.9	5.8	5.7	5.9
	5.2	4.9	4.9	4.9	5.0	4.0	4.3	4.3	4.6	4.5	4.2	4.6	4.8	4.7
	5.5	4.1	4.1	5.0	5.0	3.5	5.0	4.7	4.7	4.7	4.4	5.6	5.6	5.4
	5.6	4.6	5.0	5.0	4.9	4.4	4.5	5.3	5.1	4.0	4.6	5.0	5.4	5.8
	5.1	3.0	4.6	4.4	4.6	4.1	5.5	5.4	5.5	3.3	4.4	4.0	4.8	4.9
	4.8	4.7	4.6	4.6	4.7	4.1	4.9	5.5	5.4	4.2	4.9	5.4	5.3	5.4
	5.3	4.9	5.9	5.5	5.5	3.7	5.9	5.0	5.1	4.4	4.6	5.1	5.4	5.5
	5.3	4.5	4.9	5.0	5.1	4.05	5.1	5.15	5.15	4.35	4.7	5.15	5.2	5.2

APPENDIX H

The effects of intermittant injections of oestrogens on food intake in ovariectomised mice

g/24hr

		inj *				inj *				inj *				
DAYS	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	3.2	2.6	2.7	2.4	2.8	2.1	2.2	2.2	2.5	2.0	2.0	2.0	2.3	2.4
	3.0	1.8	2.1	2.3	2.2	0.8	2.0	2.3	2.4	2.1	2.4	2.5	2.4	2.6
	3.1	1.7	2.2	2.0	2.5	1.9	2.1	2.1	2.4	1.7	2.0	2.4	2.5	2.6
	3.2	1.9	2.4	2.1	2.1	2.4	2.4	1.9	2.0	2.0	2.0	2.5	3.0	3.1
	3.0	2.5	2.5	2.9	2.7	1.9	2.3	1.9	2.3	2.1	3.6	2.5	2.9	3.0
	3.6	3.1	3.6	2.9	2.8	3.1	2.6	2.3	2.6	1.6	3.0	2.3	3.1	3.0
	3.0	2.9	2.6	2.9	3.1	1.4	2.0	1.9	1.9	2.1	3.0	2.6	2.8	2.9
	2.7	1.9	2.5	2.6	2.6	2.1	2.1	2.0	2.2	1.9	2.4	2.3	2.8	2.4
	3.1	2.3	2.6	2.5	2.7	2.0	2.2	2.1	2.3	1.95	2.3	2.4	2.7	2.4

APPENDIX I

The effects of intermittant injections of oestrogen on food intake in intact female mice

g/24HR

DAYS	1	2*	3	4	5	6*	7	8	9	10*	11	12	13	14
	2.4	2.1	2.1	2.2	2.9	2.2	2.5	2.6	2.8	2.0	2.3	2.4	2.4	2.1
	2.4	2.1	3.1	3.8	3.4	2.6	2.9	2.9	3.1	1.1	2.4	2.8	2.7	2.6
	3.7	2.7	2.9	2.9	3.1	2.4	2.6	2.7	2.7	2.8	2.9	3.2	3.1	3.0
	3.2	1.9	2.1	3.7	3.2	2.1	2.3	2.6	2.8	2.3	2.5	3.1	3.1	2.8
	4.0	2.8	3.2	3.8	3.6	1.1	2.6	2.7	2.9	0.8	2.8	3.2	3.3	3.0
	3.5	2.8	2.7	3.0	2.9	2.1	2.8	2.8	3.1	2.5	2.5	3.4	3.1	2.8
	3.9	3.2	3.4	4.0	2.8	2.8	2.9	2.9	3.7	2.2	3.0	2.6	2.7	2.8
	2.4	2.2	2.8	2.9	3.0	2.2	2.4	2.6	2.5	2.0	2.3	2.7	2.8	2.6
	3.1	2.5	2.8	3.2	3.1	2.2	2.6	2.7	2.9	2.0	2.6	2.9	2.8	2.7