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Meal patterns : some ontogenetic, endocrinological, and neurological considerations.

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MEAL PATTERNS: SOME ONTOGENETIC, ENDOCRINOLOGICAL, AND NEUROLOGICAL CONSIDERATIONS

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ABSTRACT

Adult rats ingest their daily quota of nutrients in nine to twelve discrete meals. Each meal is separated from the next by a period of no eating. The pattern in which these meals are distributed in time has been thought to reflect the activity of a basic underlying regulatory mechanism. The amount of food ingested in a meal reliably predicts the length of time which will pass before the next meal is initiated. This correlation is termed the postprandial relationship. The relationship between the length of time passing prior to the initiation of a meal and the size of that meal is termed the preprandial relationship. This relationship has not been observed in adult rats.

Experiment 1 investigated the development of feeding patterns from 16 to 80 days of age. It was found that, with advancing age, there was a rise in the amount of food ingested daily via an increase in the size of each meal, and this in turn resulted from an increase in the rate of ingestion of food. Although, even at 16 days, there was a light-dark difference in the amount ingested, it was not until 35 to 40 days of age that the full adult circadian rhythm emerged. From 16 to 25 days, the weanlings demonstrated a preprandial intake pattern. This subsequently declined, while the postprandial relationship began to emerge, such that, by 30 to 35 days, a fully adult pattern was observed.

The possibility that the early preprandial relationship resulted from premature weaning onto a high carbohydrate diet was investigated in experiment 2 by monitoring the development of meal patterns in rats weaned onto a high fat diet. Diet did not make a difference and the results were similar to those of experiment 1.

Since weanling rats do not respond to glucostatic challenges, experiment 3 attempted to reinstate the preprandial relationship in adults by the induction of diabetes with strepzotocin injections. After the onset of diabetes, the rats became hyperphagic, eating large meals. However, the animals retained their postprandial responsiveness.

In experiment 4, an attempt was made to reinstate the preprandial relationship by ablating the lateral hypothalamus in adult rats. It was found that "recovered lateral" rats, indeed, demonstrated preprandial responsivity.

It was concluded that weanling rats lack adult mechanisms for both meal initiation and circadian feeding rhythmicity. These effects could not be accounted for by either diet, or the lack of glucostatic control. In addition, it was concluded that the adult mechanism for meal initiation appears to emerge as a direct result of a maturation of the lateral hypothalamus. Theoretical implications of the results are discussed.

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DEDICATION

To my mother and father whose sacrifices

have reaped rewards for their children

GENERAL INTRODUCTION

> "An individual developing from egg to adult manifests its most obvious growth in size and shape. Behind the visible changes are many invisible ones, such as the acquisition of new chemical constituents, new capacities for sensation, and new ways of using energy. Least visible of all is the development of controls over one process after another, that is, the development of various means by which processes, instead of conflicting, fit into a pattern of continuous living." (Adolph, 1957, pg. 89.)

The aim of this dissertation is to study and thereby delineate some of the ways in which such controls or regulations of food intake develop in an organism.

The developing organism is faced with two basic problems. First, it must develop into a viable, independent adult entity, and second, it must adapt to its specific real time ecological conditions. These two requirements are not always compatible. However, it is good to keep in mind the precept espoused by Adolph (1957), that each stage of development is, in and of itself, functionally complete. Accepting such a precept will prevent an observer from falling into the trap of viewing the developing animal as an incomplete adult, inferior to the adult in its regulatory capabilities. In fact, in some ways the neonatal organism is superior to the adult (e.g., resistance to hypoxia). It will furthermore tend to eliminate the prevalent preconception that the development of physiological regulations is a smooth transition from zero to an adult pattern.

In order to survive in its ever changing niche during development, the organism must, at times, take a roundabout developmental course. This is particularly evident in the case of feeding behavior where the organism must give up its metabolism based upon a high carbohydrate diet in fetal life to survive on the high fat diet offered during suckling and then to readapt during weaning back to a metabolism based upon a high carbohydrate diet (Hahn and Koldovsky, 1966). Thus, it is best to conceive of the developing organism as uniquely prepared to meet the requirements of survival in the particular circumstances in which it finds itself.

In the context of development, our ability to make interspecies comparisons is severely limited. Since the behavior of the adults of various species differs widely, the goals of development differ between species. It would, thus, not be expected that a rodent would follow the same sequence of development as a bird, a reptile, or a human. Furthermore, the ecology of development differs greatly between species, and thus the developmental adaptations are also expected to be ideosyncratic. For example, the rook, after hatching, responds to the sound "kar-r", with beak opening in anticipation of feeding. Their structural development reflects this ecology. The cochlear elements for the reception of the sound "kar-r" are developed at birth, while other auditory receptor elements are not. In some species of birds which make their nests in hollows, the newborn respond to the darkening of the hollow (mother entering) with beak opening for feeding. In these species, the visual receptor elements for changes in total luminous

flux are particularly well developed at birth (Anokhin, 1964). Thus, these two species of birds, adapting to different niches, show a different structural developmental process.

All of this dictates that, if we are going to study the process of development, we must focus on a single species. We should begin by gaining an understanding of what the behavior of the goal organism (adult) is like and also an understanding of the particular ecology of this species' development. Given such an understanding, the unraveling of structural-functional development should follow a logical course and produce insights into basic regulatory processes.

The species selected for this dissertation is the rat. This choice was dictated by the facts that more is known about the adult feeding behavior of the rat than any other species, the ecological conditions of development are simple and well understood, and there is a great deal of information on the metabolic changes occurring during ontogeny in the rat. Clearly, then, study of this species' development can be fit into a logical framework, and thus should result in a clear understanding of the totality of the behavioral ontogeny.

The ingestive behavior of the rat undergoes a rapid and dramatic transformation during development. From the time of conception until about two and a half weeks postnatally, it is a virtual parasite on the mother. At parturition, ingestive behavior begins abruptly. However, it is simple

and automatic. The neonate simply sucks at the nipple for milk, a fluid of fixed composition, which provides both adequate water and nutrients. In a matter of weeks, this relatively simple mode of ingestion is transformed into a set of complex phenomena. Feeding and drinking become separate activities, and the animal regulates with amazing accuracy while consuming a food supply of heterogeneous composition. During this time, the organism progresses from a parasite to a free foraging adult. "Our ignorance of this transformation is about the equal of its drama. We have only begun to understand how it occurs" (Houpt and Epstein, 1973, pg. 58).

During prenatal development (21 days in the rat), both the behavioral and metabolic regulation of food intake are unnecessary. Nutrients are transferred across the placenta from the mother to the fetus. Glucose and amino acids are taken directly from the mother, and the metabolic end products are also dialyzed by the placenta. Thus, in utero, there is no requirement for feeding behavior. Some of the components of feeding behavior, nevertheless, are "practiced" in the uterus (Gesel, 1945).

The transplacental diet is high in carbohydrate (Lockwood and Bailey, 1971), and the fetal rat's metabolism is well adapted for the efficient utilization of carbohydrates (Lockwood and Bailey, 1970). Since glucose is present in excess of the amount needed to sustain life, gluconeogenesis is absent and some of the supplied glucose is converted into storage forms;

glycogen and fat (Eisen, Goldfine and Glinsmann, 1973; Schaub, Gutman and Lippert, 1972; Walker, 1969). This prenatal nutrient storage is facilitated during the final four days of gestation by an outpouring of insulin from the pancreas. This secretion promotes the development of adipose tissue cells and the shift in fetal metabolism towards the formation and storage of lipids, and plays a permissive role in the synthesis and hepatic storage of glycogen (Chernick, 1960; Eisen, Goldfine and Glinsmann, 1973). This accentuation of nutrient storage is an important safeguard, insuring at least temporary postpartum viability.

Upon parturition, the neonate is suddenly faced with a new dietary situation. It must perform behaviors in order to receive nutrients and its metabolism, adapted to a diet high in carbohydrate, must, now, adapt to a diet high in fat (mother's milk). Rapid and effective adaptations ensue.

After birth, the independent newborn animal rapidly uses up the carbohydrate and fat it stored during the last four days of gestation. It is now completely dependent upon the mother's milk. To deal with this situation, it retains its capacity to utilize glucose, which is also supplied in the milk, and develops the capacity to synthesize glucose (Ballard and Hanson, 1967; Walker, 1969; Schaub, Gutman and Lippert, 1972).

Free fatty acid levels in the blood rise rapidly after birth. This occurs because more fat is supplied and absorbed and more fat is mobilized and utilized (Hahn and Koldovsky, 1966). The ability of the

liver to break down the abundant free fatty acids increases after parturition, as indicated by a rise in plasma ketone bodies (Hahn and Koldovsky, 1966) and a rise in the activities of enzymes associated with lipid degradation (Lockwood and Bailey, 1970; 1971). This metabolic change is reflected in the finding that, in the suckling rat, infusions of casein (protein) do not increase liver glycogen levels, while infusions of olive oil (fat) substantially increases the glycogen content of the liver (Hahn and Koldovsky, 1960). Exactly the opposite pattern is found in adult rats. Thus, the suckling rats' metabolism has adapted to promote glycogen production from fat.

The effects of total starvation on suckling animals reflects the high level of fat utilization. Adults lose more protein than fat and show a rise in the plasma concentrations of free fatty acids, while suckling animals lose more fat than protein and show a fall in plasma-free fatty acid concentrations. During starvation, the suckling uses free fatty acids to the same extent as when fed, as indicated by stable plasma ketone body concentrations, but, since no further substrate is supplied, both plasma and hepatic-free fatty acid levels decline (Hahn and Koldovsky, 1966).

The neonatal rat adapts, as outlined above, efficiently to its new metabolic situation. It must, however, also adapt to its new behavioral situation. It is born at a very immature stage of development relative to other mammalian species (Widdowson, 1971). This prompted an early investigator to comment, "The rats at birth are bright red, ugly and

helpless little creatures" (Small, 1899, pg. 81). However, as far as feeding behavior is concerned, they are far from helpless. Nursing begins a few hours after birth, and even at this early time the rats struggle for position at the mother. Their sucking behavior is fully developed, and they hold onto the nipple tenaciously, even to the extent of supporting their entire body weight when the mother rears up (Boles and Woods, 1964).

From birth to about day 13 or 14, the neonatal rat is highly dependent upon its mother. During this period, the mother cares for the needs of her progeny and she initiates feeding by approaching the young in the nest, licking them, and crouching over them (Rosenblatt, 1969). Thus, at birth, the rat is fully equipped to deal with nutrient acquisition. Feeding is initiated by the mother and the neonate need only respond to the nipple and suck.

During the first week after birth, food consumption, in relation to body weight, is equal to that of adults. However, the rate of growth is higher than during any other period of life. The great efficiency in converting food into new tissue results, in part, from the high protein content of the milk, which makes minimal demands on the suckling's metabolism (Kennedy, 1966). Furthermore, during the first week, the rat is basically poikilothermic, and its body temperature is maintained by contact with the mother. Since calories are not expended for temperature regulation, it

can devote a large proportion of its caloric intake to growth. Later, as the animal acquires the ability to regulate its own body temperature, its caloric requirements for maintenance increase and a smaller proportion of its intake is devoted to growth (Kennedy, 1957; 1966). During the first five weeks, the percentage of caloric intake devoted to growth declines rapidly and are respectively 80%, 28%, 23%, 8%, and 9% (Widdowson, 1971).

It has been claimed that the newborn rat has no means of restraining its food intake until the weaning period. The more milk it can get, the more it will take, and the faster it will grow (Widdowson, 1971). Indeed, with litters smaller than five pups, each suckling rat has access to more milk than it needs. It overeats and grows considerably faster than rats from normal sized litters. The high growth rate does not continue beyond weaning, but the early lead in weight is maintained far into adulthood (Kennedy, 1957; 1966; Widdowson and McCance, 1960; Widdowson, 1971).

Although this litter size phenomenon does tend to support the notion that suckling animals do not have restraints on their feeding behavior, that is, they do not regulate, other data tend to refute this notion. Newborn rats or puppies reduce their subsequent food intake following a stomach load (Satinoff and Stanley, 1963; Houpt and Epstein, 1973). Furthermore, newly born rats and puppies do not ingest as much as they conceivably could, as reflected by the fact that, when food deprived,

they will compensate by eating more than usual (Satinoff and Stanley, 1963; Houpt and Epstein, 1973). Thus, neonatal animals do appear to have a degree of regulatory capacity. However, it only appears to operate under extreme conditions, and incompletely at that.

This could reflect an immature state of development in the physiological mechanisms for intake regulation. Indeed, suckling rats do not respond to many feeding stimuli which are effective in adults. 2-deoxy-d-glucose (2DG), an agent which blocks glucose utilization and increases food intake in adults, is without effect in suckling rats (Houpt and Epstein, 1973). Amphetamine, an anorexic agent which reduces adult food intake, has no effect on the subsequent intake of five or ten-day-old rats (Lytle and Moorcroft, 1971). Finally, thyroidectomy, which depresses intake in adults, or thyroxine, which increases intake in adults, does not affect the intake of rats younger than 12 days (Schapiro, 1966).

By day 14, the young rat has come a long way. It has developed hair and opened its eyes (Rosenblatt, 1969). The external auditory meatus has opened (Rose and Ellingson, 1970). They have begun to smell objects at a distance and can discriminate tastes (Small, 1899; Jacobs, 1964) and have developed most of the adult repertoireof motor responses (Boles and Woods, 1964). They are, thus, ready to undertake the last major metabolic and behavioral step toward complete independence.

Unlike the suckling period, the weaning period is characterized by a gradual shift in behavior and in metabolism, stretching over a 15-day period. The process of weaning begins on day 14 and continues until approximately day 30. Its occurrence results from an interaction between behavioral changes in the pup and behavioral changes in the mother. At 13 to 14 days, the pup begins to manipulate, carry, sniff and chew both edible and inedible objects, such as food pellets, dried boluses from the mother and nesting materials. There appears to be little discrimination between food and non-food objects. By day 16, the young rat shows a definite preference for food objects and eats some of the solid food (Boles and Woods, 1964). It is interesting that this same rapid postingestional pattern of development of the preference for edible over inedible objects during a "critical period" can also be seen in the guinea pig (Reisbick, 1973) and even in the chicken (Hogan, 1973a; 1973b).

Forays for food objects are facilitated, during this period, by a remarkably high activity level which peaks at about day 15 (Moorcroft, Lytle and Campbell, 1971). By day 16, the rat eats food pellets in an adultlike manner, sitting on its haunches and nibbling on a pellet held with the forepaws (Boles and Woods, 1964). From this time forward, solid food makes up an ever increasing proportion of its caloric intake (Hahn and Koldovsky, 1966).

Beginning around day 14 or 15, the mother starts to evade feeding approaches on the part of the pup and by day 16 most suckling occurs as a result of the pup, and not the mother, initiating the feeding. The onset and acquisition of independent feeding behavior is facilitated by social factors. Independent feeding occurs at an earlier age and further from the nest site if the mother is present than if she is absent (Galef, 1971). The weanling appears to learn to eat what the adults of the colony eat by developing a preference for the flavor of the food ingested by the mother (Galef and Sherry, 1973) and by following the adults of the colony to the food supply (Galef and Clark, 1971).

Between the 20th and 22nd day, the amount of nursing drops off radically to the point where its occurrence is fairly rare. However, nursing does occur until the 30th postnatal day (Boles and Woods, 1964; Rosenblatt, 1969). Thus, by postnatal day 30, the rat has achieved complete functional autonomy.

Why is this weaning process so prolonged? It appears that a gradual transition from the high fat to the high carbohydrate diet is necessary so that the animals' metabolism can adjust. Premature weaning can have detrimental effects. Early weaning results in a decrease in brain weight, aspartic acid concentrations, RNA content and threonine concentrations (Plaut and Davis, 1972), autonomic activity (Hofer, 1970), growth, life span (Hahn and Koldovsky, 1966), fertility and spermiogenesis (Kubat,

Flandera, Hahn and Koldovsky, 1961). These physiological deficits are translated into learning deficits in later life (Novakova, 1966). Of those deficits that have been looked at, though, they can be prevented by weaning the rat onto a high fat diet from 15 to 30 days of age (Hahn and Koldovsky, 1966). Thus, the dietary shift would appear to be the precipitating event in premature weaning effects. This indicates that, in contrast to the shift occurring at parturition, the dietary, and hence the metabolic transition during weaning, must be gradual.

The weanling rat makes a slow transition from a metabolism based upon the breakdown and utilization of fat to one based upon utilization of carbohydrate and protein. At 18 days, the rat is unable to utilize protein for gluconeogenesis and does not survive if placed on a diet of protein alone. By 30 days, it responds to a casein infusion with an increase in blood glucose and it can survive on protein alone (Hahn and Koldovsky, 1966).

Fat metabolism is still pronounced during the early weanling period. Plasma and hepatic free fatty acid levels remain high until about 20 days of age. Ketone body concentrations, indicative of free fatty acid breakdown, slowly decline during weaning (Hahn and Koldovsky, 1966; Lockwood and Bailey, 1971). On the other hand, plasma glucose levels slowly increase over this same period (Lavine, Chick, Like and Nakdisi,

1971). This pattern of plasma concentrations is also reflected in enzymatic activities. The enzymes associated with lipid degradation decrease, while those associated with lipogenesis slowly increase during weaning (Walker and Eaton, 1967; Lockwood and Bailey, 1970; 1971).

Hepatic enzymatic processes adapt over the weanling period to the normal dietary regimen, but adapt, only incompletely, to abrupt or artificial dietary changes (Walker and Eaton, 1967; Lockwood, Bailey and Taylor, 1970). Hence, early weaning onto a high carbohydrate diet would be expected to result in inefficient utilization of nutrients. Indeed, feeding a 60% carbohydrate diet to weanling animals results in an elevation in oxygen consumption, reflecting an increase in metabolic rate and a decrease in food utilization (Heggeness, 1961).

The weanling rat responds to the natural depletion-repletion signals. By 21 days of age or earlier, it responds to food deprivation with an increase in intake, to diet dilution, either solid or liquid, with an increase in intake, and to hypertonic saline injections with an increase in drinking (Teitelbaum, Cheng and Rozin, 1969a; 1969b; Satinoff and Stanley, 1963; Houpt and Epstein, 1973). In addition, they show an adultlike circadian cycle of food intake; high at night, low during the day (Zucker, 1971; Bernardis, 1973; Hirsch, 1973) and an adultlike increase in feeding when in a cold environment (Hahn and Koldovsky, 1966; Teitelbaum, Cheng and Rozin, 1969a; 1969b). Thus, the weanling rat progresses in less than one week's time from a suckling to a nearly normal adult in terms of its intake regulation.

This progression is so rapid that, in order to study how it develops, drastic procedures must be employed. One method that has been used is to thyroidectomize rats shortly after birth, to slow down the developmental process. Using this procedure, an interesting analogy has been discovered. The ontogeny of solid food intake regulation proceeds along lines similar to the stages of recovery after lateral hypothalamic lesions in the adult. At 21 days of age, some of the thyroidectomized rats are completely aphagic and adipsic. This is similar to stage I of recovery from lateral hypothalamic destruction. The stunted weanlings nurse reflexively but refuse to eat either wet palatable foods or ordinary dry foods and will die of starvation if not allowed to suckle. Others, who do not show as great a growth impairment at 21 days, are anorexic and adipsic (stage II). They will accept wet palatable foods but cannot maintain their body weight on them alone and they refuse completely to drink. Still others, with even less of a growth impairment, are adipsic (Stage III). They will eat and maintain their body weight on wet palatable foods. If maintained on a liquid diet, they will compensate for caloric dilution with an increase in intake. They still, however, refuse to drink and would perish on dry food and water alone. Finally, some of the thyroidectomized weanlings, who showed the least growth impairment, will both eat dry foods and drink water and will maintain their body weight on such a diet (Stage IV) (Teitelbaum, Cheng and Rozin, 1969a; 1969b). This progression

appears to be much like the normal weaning process, with a decreasing dependence on wet palatable foods (milk) and an increasing dependence on dry food and water.

We should not conclude that the rat at 21 days of age uses adult-like regulatory mechanisms. There are indications that the young rat may use a different mechanism for its depletion-repletion responses than does the adult. Amphetamine, an anorexic agent, does not suppress food intake in rats younger than 15 days (Lytle and Moorcroft, 1971), but is effective in older animals (Wade and Zucker, 1970). Estrogens, which act to suppress food intake, do not appear to affect feeding behavior until around the 40th postnatal day in female rats (Wade and Zucker, 1970; Wade, 1974). In addition, glucostatic regulation of feeding behavior does not develop until around day 30. Insulin injections do not increase the amount of food ingested until around 25 days of age, and, even at that, at only very high doses. An adult response to insulin does not appear until around day 30 (Lytle and Moorcroft, 1971; Teitelbaum, Cheng and Rozin, 1969a; 1969b). The glucoprivic drug 2DG is without noticeable effect in weanlings of less than 28 days of age (Houpt and Epstein, 1973). Furthermore, the weanling rat does not drink water in the absence of food. It is not until 28 days of age that the adult pattern of independent eating and drinking emerges (Kissileff, 1971; Teitelbaum, Cheng and Rozin, 1969a; 1969b).

There are indications that adultlike function is not completely developed in the hypothalamus of weanling rats. Although lesions of the lateral hypothalamus produce aphagia and adipsia in 10, 20, 30 and 50-day-old rats as in adults (Lytle, 1971; Balagura and Raubeson, unpublished), lesions of the ventromedial hypothalamus (VMH), which result in a marked hyperphagia in adults, do not affect either intake or body weight in the weanling (Kennedy, 1957; Han, Lin, Chu, Mu and Liu, 1965; Kurtz, Rozin and Teitelbaum, 1972). No effect on feeding behavior occurs in spite of the fact that the same metabolic defects that are found in adults after VMH lesion are also found in the weanling (Goldman, Bernardis, Frohman and Schnatz, 1968; Frohman and Bernardis, 1968; Frohman, Bernardis, Schnatz and Burek, 1969; Bernardis and Frohman, 1970; Schnatz, Bernardis, Frohman and Goldman, 1971; Frohman, Goldman and Bernardis, 1972; Slaunwhite, Goldman and Bernardis, 1972; Bernardis, 1973). This lack of an overeating effect of VMH destruction in weanlings is probably due to the high circulating growth hormone levels. In fact, lesion of the VMH in a hypophysectomized weanling does result in a marked increase in food consumption (Groome, 1966).

The weanling rat does react appropriately to depletion-repletion signals. However, as indicated by the above mentioned abnormalities, it cannot be concluded that they react in the same way, using the same mechanisms, as do adults. Nevertheless, by the end of the weaning

period (day 30), the young rat has developed to a point nearly equivalent to adults in regulatory capability and is ready to inhabit its niche.

We have reviewed the ecology and the metabolic adjustments involved in the ontogeny of feeding behavior. Now let us turn our attention to the adult and look at its pattern of feeding. The adult rat eats about 9 to 11 discrete meals each day (Balagura and Coscina, 1969; LeMagnen, 1971) and eats more in the dark than in the light. Furthermore, there appears to be a pattern to the way in which the meals are distributed; a relationship between the size of the meal and the time passing between each meal (intermeal interval, IMI). There is a positive correlation between the size of the meal and the following intermeal interval (IMI). This is termed the postprandial relationship and indicates that the more the rat eats in a meal the longer it will wait before beginning a new meal. In contrast, there is no relationship between the length of the preceding IMI and the amount of food ingested in the subsequent meal. This is termed the preprandial relationship and indicates that the length of time prior to a meal does not predict how big a meal will be eaten. Thus the adult rat regulates in a postprandial fashion with the repleting value of the meal determining the length of the subsequent fast (LeMagnen and Tallon, 1963; 1966; LeMagnen, 1969; 1971; LeMagnen and Devos, 1971; Larue and LeMagnen, 1972; Thomas and Mayer, 1968; Snowdon, 1969; Balagura and Coscina, 1968; 1969; Balagura and Devenport, 1970).

Although there has been a great deal of attention paid to this adult pattern, there is little or no information as to how it develops. There are a number of possibilities. The postprandial relationship may be present right from the beginning of independent feeding. Prior to this time, the feeding is mainly initiated by the mother, and thus the regulatory mechanisms of the pup are overshadowed. This possibility is supported by the fact that the basic pattern of higher intake in the dark than in the light is present in the 23-day-old rat (Zucker, 1971). Furthermore, at least in guinea pigs, the number of meals ingested daily is adultlike at 26 days of age (Hirsch, 1973). Another possibility is that the postprandial relationship might develop with age from no relationship to the adult positive relationship. In guinea pigs there is little or no postprandial relationship at 26 to 28 days of age Although a strong postprandial relationship is not found in adult guinea pigs, at least some of the adults do show this pattern (Hirsch, 1973). A final possibility (of course there are many others) is that the young rat may show a completely different relationship (e. g., preprandial) following weaning and subsequently make a transition to the adult postprandial relationship. The facts that the adult glucostatic mechanisms are not yet developed in the weanling and that the animals' metabolism is vastly different from the adult suggest that there may be a different meal patterning mechanism present in the weanling period.

Now let us briefly turn our attention to the metabolic consequences of the pattern of intake. The frequency and size of meals appear to have some rather distinct metabolic consequences. Animals allowed to eat, or force fed, large meals at infrequent intervals show a marked increase in body fat and a decrease in body protein and water (Cohn, Joseph and Shrago, 1957; Cohn, 1963; Cohn, Joseph, Bell and Allweiss, 1966; Fabry, 1967). This increase in fat deposition in animal "gorgers" has an interesting analogy in human obesity. Obese individuals tend to eat fewer, albeit larger, meals than normal weight individuals (Monnello, Seltzer and Mayer, 1965; Fabry and Tepperman, 1970). This "gorging" pattern has been proposed as a possible factor in the etiology of human obesity.

This work with animals, however, requires the imposition of a rather artificial feeding regimen, which upsets the animals' normal metabolic processes. Hence, the increased fat deposition may be more the result of the metabolic insult than of the meal frequency itself. The relationship between meal size and frequency and human obesity yields some important therapeutic conclusions. However, it is impossible to discern the direction of causation. Does the "gorger" become fat, or does the fat individual become a "gorger"? Thus, there is a need to investigate the association between the natural, spontaneous meal size and frequency occurring both prior to and after obesity, and the macronutrient composition of the carcass.

EXPERIMENT 1

Development of Feeding Patterns on a High Carbohydrate Diet and the Relationship between These Patterns and the Composition of the Adult Carcass

The purpose of the first experiment was to investigate the ontogenetic development of feeding behavior in normal rats weaned at different times. It was aimed at determining what, if any, changes occur in the pattern of food intake between 16 and 80 days of age. Furthermore, it is aimed at determining the association between infantile and adult feeding patterns and the protein, fat, and water content of the adult carcass.

Methods

Subjects

Twenty-four male Sprague Dawley albino rats bred and raised in the laboratory served as subjects. Prior to weaning, they were maintained in intact and unmanipulated litters with the mother and ad libitum food and water always present. The colony room was maintained at 72° F $\pm$ 1° with lights on at 8:00 AM (0800) and off at 8:00 PM (2000).

Apparatus

All testing was conducted in individual enclosed chambers described in detail elsewhere (Balagura and Coscina, 1969). In short, the

chambers were 12" long, 8" wide, and 12" high and had a wire mesh floor. Illumination was provided, from 0800 to 2000, by a 6 watt bulb, positioned in the center of the ceiling. A built-in ventilation system supplied a continuous flow of fresh air and simultaneously served as a masking noise. Temperatures were maintained at $73^{\circ} \pm 2^{\circ}$.

Each chamber was equipped with a pellet sensing eatometer, described in detail elsewhere (Kissileff, 1970). In short, a V-shaped trough 3/8" deep, held a 45 mg (Noyes) food pellet. In compartments on opposite sides of the trough, a light and a photoresistor were positioned. A beam of light passing across the trough activated the photoresistor, which in turn activated a relay. The pellet interrupted the light beam. Whenever the rat removed a pellet, the photoresistor was activated, closing the relay, which in turn resulted in the delivery of another food pellet from a pellet dispenser (Lehigh Valley). Hence, every time the rat took a pellet from the trough, it was replaced with another. Water was available ad libitum from a drinking tube positioned 1" to the left of the eatometer and 1" above the floor.

Each pellet delivery was counted by an electromechanical counter and continuously recorded on a 20 pen event recorder (Esterline Angus). In addition, pellet deliveries were monitored by a Mod-Comp I digital minicomputer, which was programmed to recognize a meal and to print out on a teletypewriter the characteristics of that meal, the time of

occurrence of the meal, the number of pellets eaten in the meal (meal size), the number of pellets eaten between meals, the length of time encompassed by the meal (meal duration), and the time from the end of the preceding meal to the beginning of the present meal (intermeal interval, IMI).

Procedure

Behavioral Testing - Four groups of six rats each began testing at 16, 20, 25 and 30 days, respectively. They were weaned and placed in the testing chambers the night prior to the first testing day, and the period from 2000 to 0800 hours was considered an adaptation period. Data collection began at 0800 of the first testing day. Pups were selected from the colony with the restrictions that no more than two pups from a single litter were used and that each group contained only one pup from a single litter. For the 16-day weaning group, to help insure survival, the heaviest male was selected from each litter. For all other groups, selection was essentially random. Four pups, one from each group, were eliminated; one from the 16-day group due to innanition and death; one each from the 20 and 25-day groups due to excessive spillage ($>$ 25% of the total daily intake), and to equalize group sizes one pup from the 30-day group was randomly eliminated. Hence, the final groups consisted of five pups each, weaned the night of their 15th, 19th, 24th, and 29th days, respectively, and first monitored on the morning of their 16th, 20th, 25th, and 30th days, respectively.

Four testing cycles were run, consisting of five days in the testing chambers, wherein feeding was monitored, and ten days of isolation in standard individual cages in the colony room, wherein only daily weights were recorded. Hence, the four groups were in the testing chambers from 16 - 20, 20 - 24, 25 - 29, and 30 - 34 days, respectively, during the first cycle; 31 - 35, 35 - 39, 40 - 44, and 45 - 49 days, respectively, during the second cycle; 46 - 50, 50 - 54, 55 - 59, and 60 - 64 days, respectively, during the third cycle; and 61 - 65, 65 - 69, 70 - 74, and 75 - 79 days, respectively, during the fourth and final cycle.

Carcass Composition Analysis - Ten days after the last testing period, the animals were sacrificed, shaved, and eviscerated. The carcasses were then dried in an oven at 60° C. When the weight of the carcass had stabilized (equal weight on two successive days), the dried carcasses were weighed and ground to a 16-mesh powder in a Waring blender. The water content was calculated by subtracting the weight of the dried carcass from that of the wet eviscerated carcass. The fat content of the carcass was analyzed (0.5 g sample) by a double ether extraction method (Leshner, Litwin and Squibb, 1972). The protein content was analyzed (7 mg sample of fat extracted material) by a colorimetric method (Lowry, Rosebrough, Farr and Randall, 1951).

Data Analysis - A meal was defined as the removal of at least five pellets from the trough with the time between any two pellets not exceeding ten

minutes. All variables were first analyzed with a 1 between, 2 within mixed design analysis of variance, and individual cycle comparisons were performed with a 1 between, 1 within mixed design analysis of variance (Myers, 1971). All correlations were Pearson Product-Moment correlations on raw data. Since r is a nonlinear variable, statistical analysis of group correlational data was performed on r to z transformed data (Dixon and Massey, 1957).

Results and Discussion

Intake Characteristics

The results for a variety of food intake measures are graphically summarized in Figure 1. The animals had a not too surprising increase in their total intake of food as they grew ($F = 50.6$, $df = 3, 48$, $p < .001$). This occurred solely via a marked increase in meal size ($F = 58.6$, $df = 3, 48$, $p < .001$). In fact, the number of meals ingested actually declined with age ($F = 6.0$, $df = 3, 48$, $p < .005$). This marked increase in meal size occurred mainly via a large increase in the rate with which pellets were ingested during the meal, that is a rise in the number of milligrams eaten per second duration of the meal ($F = 37.1$, $df = 3, 48$, $p < .001$). Meal duration declined slightly during the first cycle ($F = 3.6$, $df = 3, 16$, $p < .05$) and thereafter increased slightly over time ($F = 3.6$, $df = 3, 48$, $p < .025$).

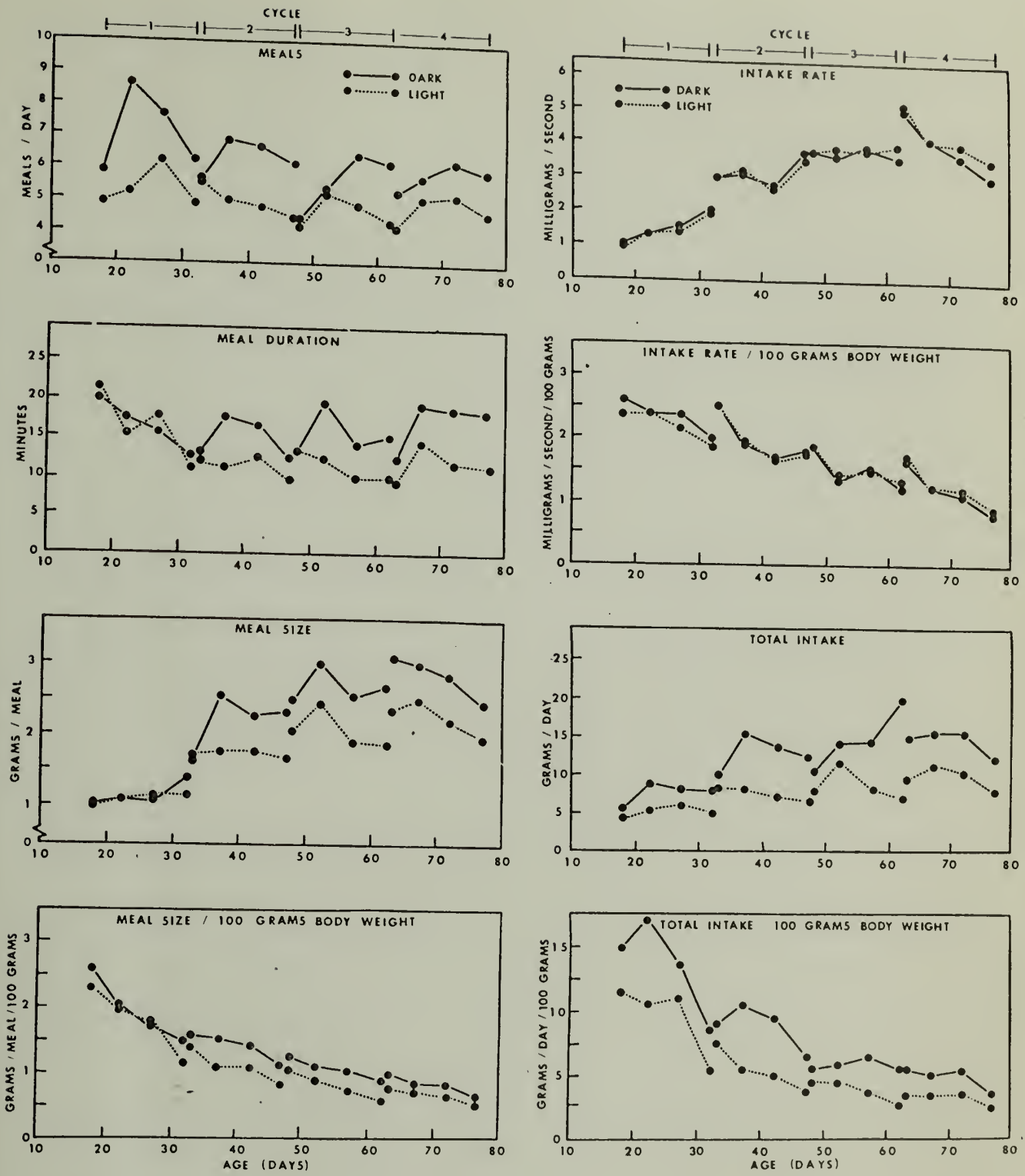

FIGURE 1: Intake characteristics on a high carbohydrate diet as a function of age

If the results are expressed in terms of intake per 100 grams of body weight, a different picture emerges. Relative to body weight, total intake declines over time ($F = 82.1$, $df = 3,48$, $p < .001$) as is also the case with both meal size ($F = 49.9$, $df = 3,48$, $p < .001$) and intake rate ($F = 82.1$, $df = 3,48$, $p < .001$). Thus, although young rats eat smaller meals and eat slower than older animals in absolute amount, relative to their body weights, they ingest more, in larger meals, and at a faster rate than at any other age. These results are similar to those found in the guinea pig (Hirsch, 1973). They also confirm and extend Kennedy's (1969) observation that, just after weaning, intake per gram body weight is higher than during later periods.

An adultlike light-dark difference in feeding behavior was not present during the period of from 16 to 35 days of age. Although a significant difference between total intake in the dark and that in the light was apparent in the first cycle ($F = 18.1$, $df = 1,3$, $p < .025$), the difference was smallest during this period (Light-Dark X Cycle interaction; $F = 5.9$, $df = 3,58$, $p < .005$). Furthermore, there were no significant light-dark differences during the first cycle in either meal size ($F = 2.6$, $df = 1,3$, $p > .05$) or meal duration ($F = 1.1$, $df = 1,3$, $p > .05$), while significant differences were found on both of these measures in the 2nd, 3rd, and 4th cycles ($F = 70.3$; 62.2; 45.4, $df = 1,3$, $p < .01$, for meal size and $F = 21.2$; 20.6; 20.5, $df = 1,3$, $p < .05$, for meal duration on

cycles 2, 3, and 4, respectively). In fact, the light-dark difference in total intake during the first cycle is solely due to a significant difference in the number of meals ($F = 25.6$, $df = 1,3$, $p < .025$). This difference in number of meals eaten is greater during this first cycle than during any other cycle (Light-Dark X Cycle interaction; $F = 3.26$, $df = 3,58$, $p < .05$). Hence, during early development, the difference between food intake in the dark and that eaten in the light, results solely from the number of meals eaten during these portions of the day. Later, at approximately 35 to 40 days of age, an adultlike pattern of light-dark feeding differences emerges, including differences in total intake, the number of meals, meal size, and meal duration.

It is interesting that, in the guinea pig, these later light-dark intake differences never develop. Circadian differences in feeding occur solely due to differences in the number of meals. Differences are not found in meal size or duration far into adulthood (Hirsch, 1973). These results confirm Zucker's (1971) findings of light-dark feeding rhythms in 23-day-old rats. However, his conclusion that light-dark feeding rhythms are essentially adultlike in form in these animals must be modified. True adultlike rhythms do not develop until after 35 days.

At no time during development was a significant difference between the rate of intake in the light and that in the dark found. Balagura and Coscina (1968), measuring free food intake with an operant response

(bar press), also did not find a difference in rate of bar pressing (intake) between dark and light. Furthermore, at no time during the development of the guinea pig was such a difference found (Hirsch, 1973). Thus, feeding behavior during the meal would appear to be rather stereotyped and constant, lacking circadian rhythmicity. Light-dark intake differences occur via circadian controls over meal initiation (frequency) and meal termination (size and duration), and only the former controls are present during development up to 35 days of age.

Meal Pattern Correlations

Correlations, reflecting the pattern of meal taking, were calculated between a number of variables. Those on which significant correlations were found are summarized graphically in Figure 2. No significant correlations were found between the rate of intake and either the preceding or following IMI, between successive satiety ratios (following IMI / Meal size; seconds of non-eating per pellet ingested), or between successive deprivation ratios (Meal size / preceding IMI; pellets ingested per second of fasting). Also, although significant effects were found both between satiety ratios and following deprivation ratios and between deprivation ratios and following satiety ratios, only one, satiety ratio - deprivation ratio, appears in Figure 2.

As can be seen in Figure 2, the most marked and interesting changes in the meal pattern correlations occurred on the pre and post-

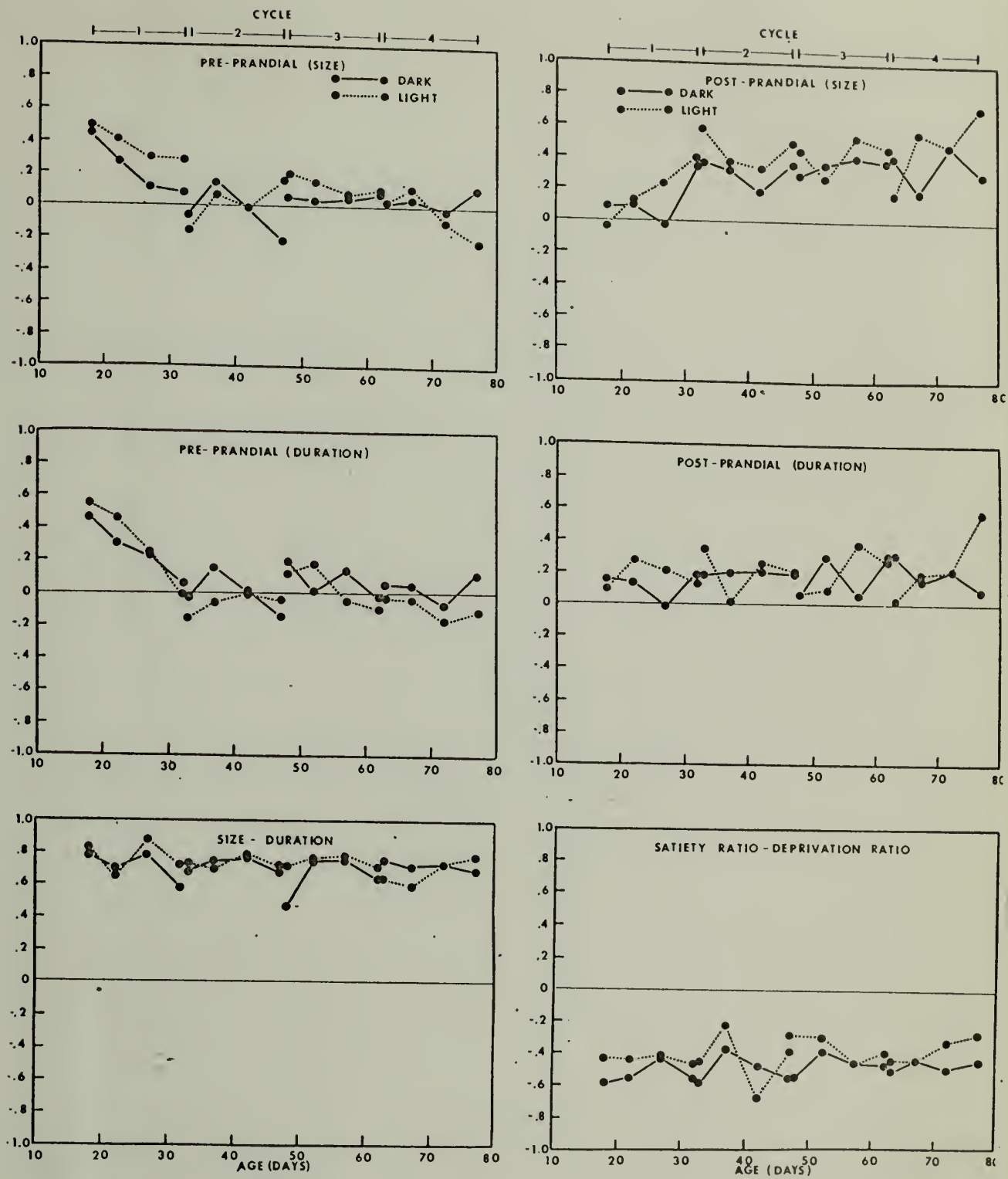

FIGURE 2: Development of meal pattern correlations in animals fed a high carbohydrate diet as a function of age.

prandial correlations. The preprandial relationship (meal size or meal duration and the preceding IMI) began high and sharply declined with age ($F = 10.2; 12.2$, $df = 3,48$, $p < .001$ for size and duration, respectively), with the most marked changes occurring during the first cycle ($F = 5.5; 15.5$, $df = 3,16$, $p < .01$ for size and duration, respectively), wherein the relationship went from highly significant to negligible. The postprandial, size, relationship (meal size and following IMI) followed the opposite course, increasing with age ($F = 9.9$, $df = 3,48$, $p < .001$), especially during the first cycle ($F = 3.2$, $df = 3,16$, $p < .05$), wherein the relationship went from negligible to highly significant. It is interesting to note that, with the postprandial, duration, relationship, although in the appropriate direction, there were no significant effects. Thus, during this early period of 16 to 35 days of age, a marked change in the regulatory behavior occurs with a transition from preprandial to postprandial patterning.

The existence of the postprandial relationship in the adult has been well documented (LeMagnen and Tallon, 1963; 1966; LeMagnen, 1969; 1971; LeMagnen and Devos, 1970; Balagura and Coscina, 1968; 1969; Balagura and Devenport, 1970; Larue and LeMagnen, 1972; Snowdon, 1969; Thomas and Mayer, 1968). However, its emergence at 30 to 35 days has not been previously reported. Hirsch (1973) found only a very slight tendency toward a postprandial relationship in 23-day-old or

adult guinea pigs. However, Hirsch utilized a duration measure of meal size, and, as can be seen in Figure 2, duration is not a reliable measure, resulting in low correlations. In fact, if duration alone was measured in the present study, a conclusion similar to Hirsch's would have been reached. Indeed, many failures to replicate the postprandial relationship probably resulted from the use of a duration measure of meal size (Levitsky and Collier, 1968; Panksepp, 1973; Kenney and Mook, 1974).

The predominance of the preprandial relationship, between 16 and 25 days of age, has not been previously noted. In fact, to this author's knowledge, this is the first report of a significant preprandial relationship under any conditions. This finding suggests that, during this early period, rats regulate in a different manner and probably by a different or more rudimentary mechanism than during later periods or adulthood.

The satiety ratio - following deprivation ratio appears to be most impressive. This correlation is high and stable throughout development. In a recent paper (Panksepp, 1973) it was proposed that this kind of correlation may provide a better description of meal patterning than the pre and postprandial correlations. At first glance, this would appear to be so. However, it is a statistical artifact, signifying nothing. The same term, meal size, appears in the denominator of the satiety ratio and the numerator of the deprivation ratio. This forces a negative cor-

relation. Indeed, when a series of correlations were calculated between these ratios, using random numbers instead of real data and comparable numbers of observations, a mean correlation of -0.45 was produced. A glance at Figure 2 reveals that the correlations calculated from the real data do not differ from the one derived from random numbers. Hence, correlations between such ratio measures appear to have dubious value in feeding research.

Carcass Composition and Intake Characteristics Analysis

Correlations were computed between the composition of the carcass and five measures of feeding behavior for the four cycles. Results for intake in the dark, in the light, and overall were very similar and thus only the correlations with overall intake are reported. There were no significant differences between any of the groups in the percentage or ratio measures of carcass composition. However, since the groups were sacrificed at different ages, their terminal weights differed. Hence, the correlations were calculated using absolute weight measures normalized within groups.

Table 1 contains the correlations between these measures. The correlations between the meal characteristics and the normalized weight measures (whole animal weight, wet carcass weight, dry carcass weight, lean body mass, and the amount of fat, protein, and carbohydrate (not shown)), present a reasonably clear picture. At all ages, heavy animals,

TABLE 1: Pearson product-moment correlation coefficients between carcass composition and meal characteristics, observed during the four cycles of Experiment 1.

					LEAN			FAT		PROTEIN	
		WT.	WET WT.	DRY WT.	BODY MASS	% H_2O	$\frac{H_2O}{LBM}$	% WET WT.	% DRY WT.	% WET WT.	% DRY WT.
CYCLE 1	MEAL SIZE	.59**	.39*	.50*	.41*	-.30	-.41*	-.22	-.33	.15	.14
	MEAL DURATION	-.06	-.09	-.03	-.12	-.35	-.23	.27	.17	.12	.19
	INTAKE RATE	.52	.44	.47	.46	-.05	-.15	-.16	-.19	.12	.09
	TOTAL INTAKE	.44*	.25	.38*	.27	-.35	-.40*	-.06	-.19	.48*	.42*
	MEAL FREQUENCY	-.31	-.23	-.23	-.20	.03	.07	.12	.14	.56**	.49*
CYCLE 2	MEAL SIZE	.64**	.67**	.69**	.64**	-.31	-.19	.24	.13	.12	.12
	MEAL DURATION	-.10	-.02	-.07	-.05	.07	.24	.52**	.54**	.20	.23
	INTAKE RATE	.57**	.53**	.60**	.54**	-.26	-.32	-.15	-.26	.09	-.01
	TOTAL INTAKE	.57**	.44*	.48*	.43*	-.39*	-.24	.37	.25	.41*	.35
	MEAL FREQUENCY	-.38*	-.53**	-.51**	-.52**	-.03	-.04	-.03	-.03	.19	.10
CYCLE 3	MEAL SIZE	.69**	.72**	.74**	.71**	-.36	-.31	.18	.04	.14	.08
	MEAL DURATION	.11	.21	.09	.17	.15	.36	.41*	.48*	-.10	-.11
	INTAKE RATE	.39*	.34	.47*	.37	-.44*	-.57**	-.27	-.43*	.31	.31
	TOTAL INTAKE	.40*	.30	.31	.33	-.20	-.14	.10	.02	.40*	.32
	MEAL FREQUENCY	-.26	-.34	-.35	-.31	.19	.16	-.10	-.09	.23	.24
CYCLE 4	MEAL SIZE	.67**	.72**	.77**	.69**	-.39*	-.25	.38*	.22	.13	.07
	MEAL DURATION	-.07	.04	-.08	.01	.35	.55**	.39*	.54**	.01	.06
	INTAKE RATE	.51**	.41*	.59**	.42*	-.69**	-.76**	-.11	-.38*	.26	.18
	TOTAL INTAKE	.80**	.59**	.74**	.57**	-.69**	-.52**	.41*	.17	.32	.20
	MEAL FREQUENCY	-.09	-.38*	-.25	-.38*	-.24	-.23	-.09	-.16	.14	.05

* $< .05$, ** $< .01$

not very surprisingly, tended to have larger total intakes than light animals. This difference resulted from a tendency on the part of the heavy animals to eat larger meals. This in turn resulted from eating faster rather than from an increase in meal duration. There is also a tendency, albeit not always significant, for heavy animals to eat fewer meals than light animals. Hence, heavy animals can be characterized as "gorgers", eating few but large meals. These results are in keeping with the above findings on meal characteristics during growth. Increases in intake during growth result from a slight reduction in the number of meals and an increase in meal size and intake rate. Similarly, heavy animals have elevated intakes via the same intake pattern of slightly fewer but larger meals, eaten quickly.

The percent of the wet carcass weight devoted to the water compartment and the water - lean body mass ratio had higher correlations with meal characteristics during the final cycle than during earlier cycles. Fourth cycle meal characteristics correlated with both the percent water and the water - lean body mass ratio in a fashion opposite to that of the weight measures. Animals, whose carcasses contain a large amount of water relative to either wet carcass weights or lean body mass, tended to eat less, eat smaller meals, and eat at a slower rate than animals with a low relative water content. This may result from the fact that, although body weight and absolute grams water are

positively correlated ($r = .79$, $p < .01$), body weight and percent water are negatively correlated ($r = -.40$, $p < .05$). Since body weight and percent body water are negatively correlated, the fact that they have an opposite correlational pattern with meal characteristics is not too surprising. It, thus, would seem probable that these two sets of correlations are not independent effects.

The correlations between percent fat and protein and meal characteristics present an interesting and reasonable pattern. Percent carcass protein is significantly related to total intake and the number of meals eaten in the first cycle. These correlations decline progressively in later cycles. During early development, a large proportion of intake is devoted to growth (Widdowson, 1971). It is, thus, reasonable that high levels of intake during this period correlate with carcass protein content. On the other hand, the opposite trend was found with the percent fat content, with stronger correlations with meal characteristics occurring in later cycles. In particular, animals with high percent carcass fat tend to eat more, eat larger meals of longer duration, and eat slower than their lean compatriots.

This relationship between fat content and both meal size and duration appears to partially corroborate previous work, in which only allowing or force feeding a few large meals daily was found to result in an elevated level of fat in the carcass (Cohn, Joseph and Shrago, 1957;

Cohn, 1963; Cohn, Joseph, Bell and Allweiss, 1966; Fabry, 1967). On the other hand, the lack of a relationship between fat content and the number of meals eaten, during any period of life, does not corroborate these studies.

It can be argued, reasonably, that the present study was not looking at "nibbling versus gorging". The fewest number of meals eaten during any period by any animal was 7.2. Hence, the present study could be viewed as comparing various degrees of "nibbling", rather than "nibbling versus gorging". At any rate, the evidence from the present study does not support the notion that natural, spontaneous "gorgers" tend to have a high proportion of fat; rather it indicates that the so-called "gorging" pattern is the normal mode of increasing intake. This pattern occurs both in heavy animals (not necessarily fat animals) and as the basic mode of increasing intake during growth.

It is highly likely that the phenomenon of increased fat deposition in animals forced to eat a few large meals results from the fact that the animal is not himself controlling intake. Elevated fat deposition may be a metabolic adaptation to a disynchronous situation where intake is occurring relatively independent of the metabolic events which normally signal feeding. Furthermore, the present results suggest that the correlation between meal pattern and human obesity results from a tendency for heavy individuals to eat a few large meals, rather than from the meal pattern producing the obesity.

EXPERIMENT 2

Development of Feeding Patterns on a High Fat Diet

The above discussion criticized the experiments on "nibbling versus gorging" in that they impose a pattern of intake on a metabolism adjusted to a different pattern. A similar criticism, however, applies to the meal pattern data of Experiment 1. It was found that, during the period from 16 to 25 days of age, the meal patterning of the young rat was best described by the preprandial relationship. Not until 30 to 35 days of age does the adult postprandial relationship emerge. It is possible that these effects result, not from a basic ontogenetic change in regulatory behavior, but from a sharp change in diet. Particularly with the 16-day weaning group, there is a sudden shift from a high fat diet (mother's milk), to a high carbohydrate diet (Noyes pellets). The metabolism of weanlings at this age is adapted to the high fat diet (Hahn and Koldovsky, 1966; Lockwood and Bailey, 1970; 1971; Walker and Eaton, 1967) and a sudden shift to a high carbohydrate diet results in an increase in metabolic rate and a decrease in food utilization (Heggeness, 1961). Hence, it is possible that the observed preprandial relationship is the product of the ingestion of a diet which is different in composition from the one to which the animal is metabolically adapted.

In those cases in which it has been looked at, weaning onto a high fat diet successfully prevents any of the observed changes from developing (Hahn and Koldovsky, 1966). In addition, adult feeding behavior on high fat diets has not been found to differ from feeding on other diets, except for the effects of caloric content (Levitsky, 1968). Thus, Experiment 2 investigated the development of feeding patterns in rats maintained on a high fat diet.

Methods

Subjects

The subjects consisted of 8 male albino Sprague Dawley rats, bred and raised in the laboratory under the same conditions as described in Experiment 1.

Apparatus and Diet

Testing was conducted in a single room maintained at 72° F $\pm$ 1°. Lighting was provided from 0800 to 2000 by overhead fluorescent lights. Feeding was monitored in six individual adjoining cages, 16" long, 8" wide, and 8" high. The cages had wire mesh floors and ceilings. The sides and the back wall were constructed of 3/8" plywood and the front wall was constructed of plexiglass. Water was available, ad libitum, from a drinking tube, centered on the front plexiglass wall 1" from the floor. The liquid diet was contained in plastic cups, 1 1/2" deep and 2 1/4" in diameter, inserted through a hole in the wire mesh floor in the corner

next to the front wall. The lip of the cup extended 1/2" above the level of the floor. The wire mesh floor and the bottom of the cup were connected through a drinkometer circuit, such that each time the rat licked the contents of the cup the circuit was closed. Each lick was recorded on a 20-pen event recorder (Esterline Angus) and also by a Mod-Comp I digital minicomputer which printed out the same information as in Experiment 1.

A commercial high fat diet (Esbilac, The Borden Co.) was the only nutrient available to the animals throughout the course of testing. This diet has a protein, fat, and carbohydrate composition similar to rat mother's milk of late suckling (Luckey, Mende and Pleasants, 1954).

Procedure

Testing commenced when the pups had attained 17 days of age. The pups were weaned the night of their 16th day and placed in the individual cages with the high fat diet present in the plastic cups. They were allowed overnight to adapt to the environment and the diet. Data recording began at 0800 the next morning.

Animals were selected at random except that only one pup per litter was selected. Two animals failed to eat and were eliminated from the experiment. Intake patterns were monitored continuously over a 25-day period (17 to 42 days of age). The animals were weighed and fresh diet placed in the cups, daily, at the beginning of the light cycle. The amount

of diet consumed each day was estimated by the difference between the combined weight of the cup and its contents when filled and after 24 hours. Evaporation was measured daily by leaving a freshly filled cup in the same room over the same period, and recording the change in its weight. Daily intakes were then adjusted by subtracting the daily amount of evaporation.

Data Analysis

The average lick was estimated for each animal by dividing the total daily adjusted intake by the total number of licks occurring in that day. Meal size and intake rate were then estimated by multiplying the average lick size times the number of licks or the number of licks per second in the meal. This procedure did not in any way change the results. Indeed, analysis of the results in terms of number of licks yielded analogous findings. The definition of a meal and the data analysis procedures were the same as in Experiment 1 except that all analysis of variance designs were of the repeated measures type.

Results and Discussion

Intake Characteristics

The results for a number of measures of feeding behavior are graphically presented in Figure 3. Total daily intake increased over the period of observation ($F = 12.9$, $df = 4,20$, $p < .001$) due to an increase in meal

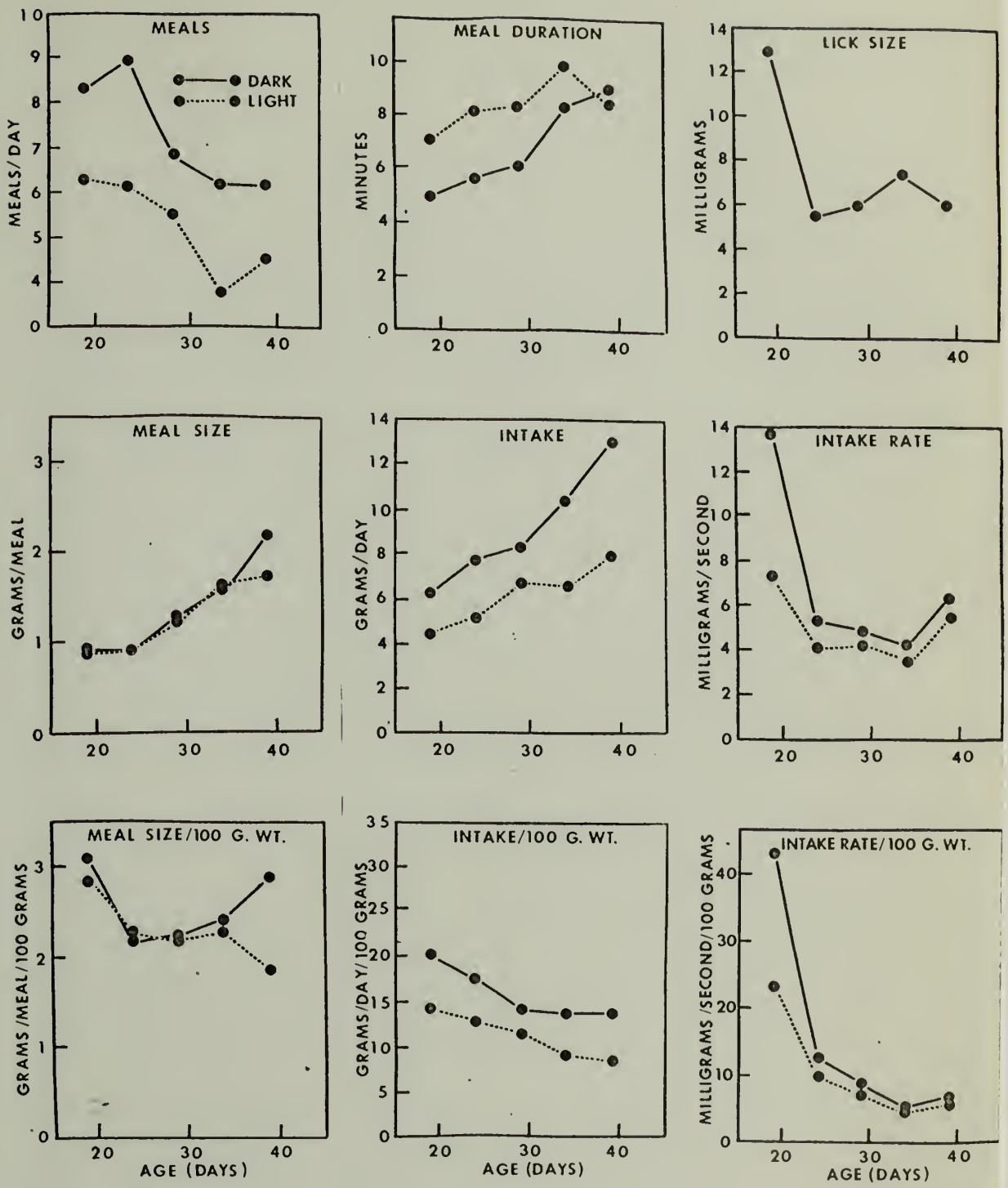

FIGURE 3: Intake characteristics on a high fat diet as a function of age.

size ($F = 13.7$, $df = 4,20$, $p < .001$). The number of meals per day decreased during growth ($F = 5.8$, $df = 4,20$, $p < .005$). These results are similar to those found in Experiment 1. However, the other intake measures diverged from the pattern discerned in Experiment 1. Intake rate did not increase. The number of licks per second occurring within a meal remained relatively constant and there was even a trend, albeit not significant, for a decrease in the rate of intake. The increase in meal size resulted solely from an increase in the duration of the meal ($F = 2.9$, $df = 4,20$, $p < .05$). Thus, while in Experiment 1 meal size increased with age by an increase in the rate of intake, and not in the duration of the meal, in Experiment 2 meal size increases with age by an increase in meal duration and not in intake rate. This might have occurred because of the differing composition of the diets used. However, it is more likely that this resulted from the response system involved. Drinking behavior is a relatively stereotyped behavior, with little if any variation in the lick rate under a variety of conditions (Stellar and Hill, 1952). Hence, only by increasing the duration of the meal can the amount of intake be increased.

When the data are expressed in terms of intake per 100 grams of body weight, relative intake, as in Experiment 1, was found to decrease with age ($F = 8.7$, $df = 4,20$, $p < .001$). Contrary to the findings of Experiment 1, this decline did not result from smaller relative meal sizes,

but rather from a slower relative intake rate ($F = 4.2$, $df = 4,20$, $p < .001$).

Total intake in the dark differed, albeit not significantly, from total intake in the light. This lack of a significant difference is probably due to the experimental procedure. When fresh diet was given at the beginning of the light cycle, a large and prolonged eating bout often occurred. Such behavior tended to mask light-dark differences by increasing the amount of food ingested in the light.

Light-dark differences were not found in either meal size or meal duration. On the other hand, there was a significant difference between the number of meals eaten in the dark and in the light ($F = 6.8$, $df = 1,5$, $p < .05$). Thus, as found in Experiment 1, the adult pattern of light-dark differences is not present during this early period of development. Differences in intake occur solely as a result of differences in meal frequency.

Experiment 2, then, essentially replicated the major findings of Experiment 1 on meal characteristics. Intake increased during growth due to an increase in meal size and an adultlike circadian intake pattern is not fully developed during the period of early postweaning development. Other differences between the results of the two experiments appear to originate in procedural differences.

Meal Pattern Correlations

The same pattern of development of meal pattern correlations observed in Experiment 1 were also found in Experiment 2. The results

are summarized in Figure 4. Initial postweaning food regulatory behavior could be characterized as preprandial. That is, the size of the meal correlated with the preceding and not the postceding IMI. Gradually, over the course of the 25 days of testing, the preprandial relationship declined, while the postprandial relationship increased to the point where, by the end of testing, the animals' regulatory behavior could be characterized as postprandial ($F = 2.9; 4.3$, $df = 4,20$, $p < .05$ for pre and postprandial, size, correlations, respectively). Once again, the same trends could be seen in the meal duration correlations, but these trends were not significant and were not of the same magnitude as the meal size correlations. Duration measures were particularly unstable in this experiment as indicated by the fact that the correlation between meal size and duration was lower in this experiment than in Experiment 1.

The preprandial relationship was, thus, observed in the early postweaning period regardless of whether a high fat (Experiment 2) or a high carbohydrate diet was used. Since the high fat diet had an almost identical composition as mother's milk, it can be concluded that the relationship between meal size and the preceding IMI did not result from the ingestion of a diet which is different in composition from the one to which the organism is metabolically adapted. This relationship, then, would appear to be a true ontogenetic phenomenon, indicating a markedly different mode of intake regulation during early postweaning development than in the adult.

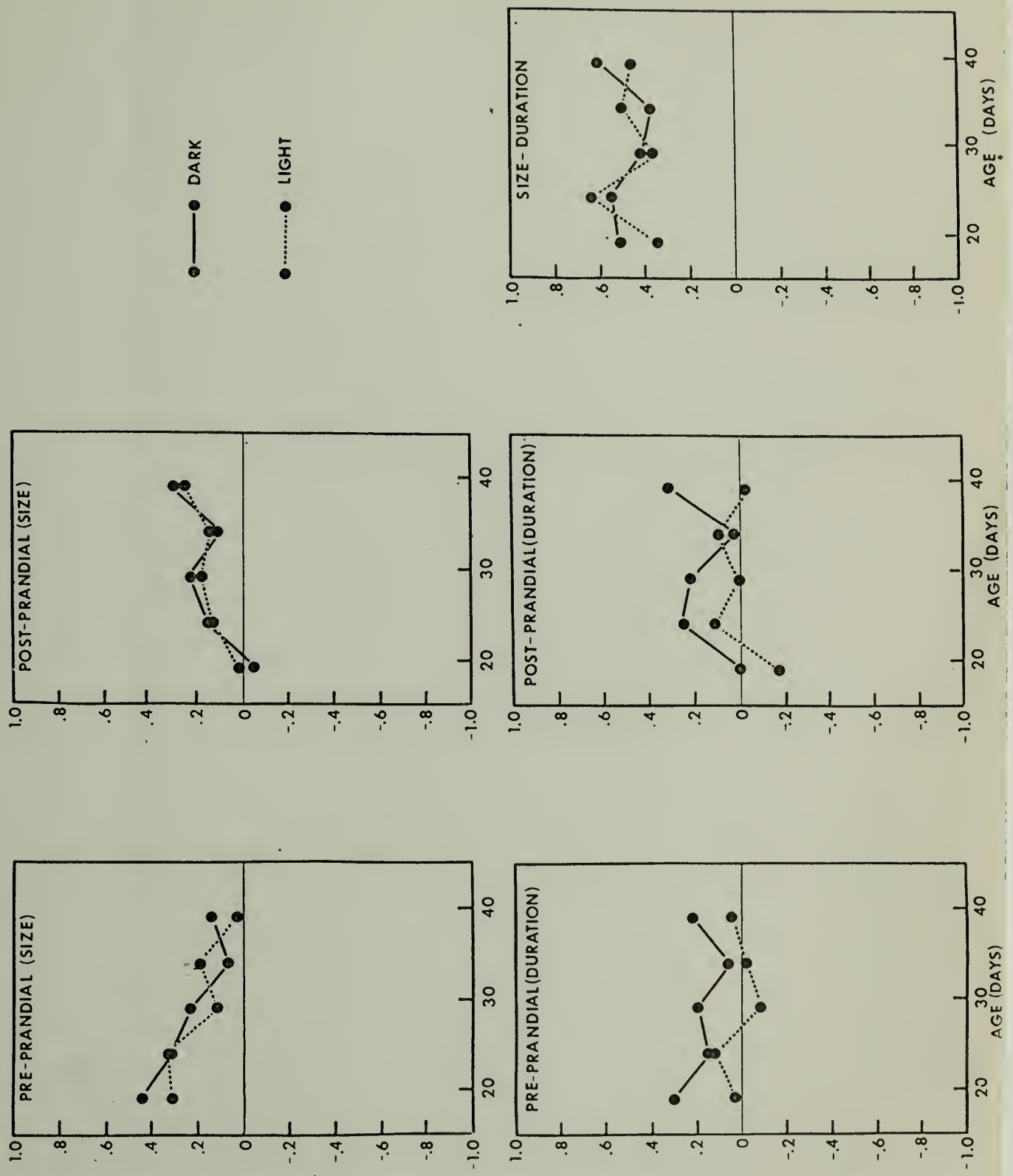

FIGURE 4: Development of meal pattern correlations on a high fat diet as a function of age.

EXPERIMENT 3

Meal Patterning in Adult Diabetic Animals

The young rat (16 to 25 days) does not respond to manipulations of the glucostatic system. Neither insulin nor 2DG affects feeding in the weanling as they do in adults (Lytle and Moorcroft, 1971; Houpt and Epstein, 1973). It is conceivable that this lack of glucostatic modulation of feeding behavior in young rats is responsible for the observed preprandial feeding pattern. A number of lines of evidence tend to support this notion. Hyperinsulinemia, induced either by prolonged insulin injection or by lesion of the ventromedial hypothalamus, tends to accentuate the postprandial relationship (Panksepp, 1973; Thomas and Mayer, 1968). Hence, high levels of insulin are correlated with high postprandial correlations. In addition, plasma insulin concentrations rise rapidly following a meal and decline progressively until the onset of the next meal (Steffens, 1970). This could indicate that insulin plays a role in either or both meal onset and meal offset. Furthermore, diabetes, induced by strepzotocin injection, results in a disappearance of the postprandial relationship (Booth, 1972). All of this evidence strongly suggests that insulin plays a crucial role in meal patterning.

If, in fact, the preprandial relationship in weanlings results from the inability of insulin to affect the feeding pattern, then adult diabetic rats, totally lacking insulin, may show analogous feeding patterns. Indeed, in a number of ways, the feeding patterns of adult diabetics are analogous to weanlings. They are hyperphagic relative to their body weights (Kumaresan and Turner, 1965; Smith, 1972; Panksepp and Nance, 1972; Booth, 1972; Panksepp, 1973). They eat a large number of small meals at a slow intake rate relative to intact controls (Booth, 1972). Although an elimination of the postprandial relationship has been observed following the induction of diabetes, preprandial correlations were not calculated (Booth, 1972). Thus, Experiment 3 attempted to discover whether diabetic adults demonstrate a preprandial intake pattern and, furthermore, whether other observed differences in weanling versus adult feeding behavior also occur after strepzotocin induced diabetes.

Methods

Subjects

The subjects consisted of ten male Sprague Dawley albino rats born and bred in the laboratory under the same conditions as in Experiment 1. Following weaning (28 days of age), the animals were kept in groups of four in the colony room. Testing began when they were 90 to 120 days old, weighing 320 to 450 grams.

Apparatus

The testing apparatus was the same as in Experiment 1.

Procedure

The subjects were placed in the testing chambers and allowed 24 hours to adapt to the environment. Their feeding behavior was subsequently monitored for a five-day period, after which they were anesthetized with sodium pentobarbital (40 mg/kg). Half of the rats (n = 5) were then injected intravenously with 65 mg/kg streptozotocin (kindly supplied by the UpJohn Co.), a dose which reliably produces diabetes (Booth, 1972), and the other half of the subjects (n = 5) received vehicle (saline) injections. The animals were subsequently isolated in individual cages, in the colony room, with food and water ad libitum, for a period of ten days. This insured the appearance of the complete diabetic syndrome. During this period all streptozotocin injected animals developed the symptoms of diabetes; polydipsia, polyuria, and glucosuria (TesTape, UpJohn Co.). After this period, all animals were again placed in the testing chambers and their feeding behavior was monitored for a five-day period.

Data Analysis

Data handling and analysis procedures were the same as in Experiment 1.

Results and Discussion

Meal Characteristics

The results for a variety of food intake measures are presented in Figure 5. The induction of diabetes resulted in hyperphagia. The strepzotocin-treated animals had a marked increase in the amount of food eaten per day (Sessions X Groups interaction, $F = 24.3$, $df = 1,8$, $p < .005$) and the amount eaten per day per 100 grams body weight (Sessions X Groups interaction, $F = 36.5$, $df = 1,8$, $p < .001$), while the saline-treated animals did not. This essentially replicates previous findings.

This increase in total intake did not result from an increase in the number of meals eaten, but rather from a marked increase in meal size ($F = 7.2$, $df = 1,8$, $p < .05$) via an increase in meal duration ($F = 11.1$, $df = 1,8$, $p < .025$). This pattern, except for meal duration, is opposite to that found by Booth (1972). It is difficult to reconcile this difference. However, Booth's animals were considerably smaller (200 to 230 grams) and may have been less well able to withstand the stress of the initial diabetic weight loss. Furthermore, Booth used a tunnel feeding system wherein the rat was required to enter and withdraw repeatedly during a meal, receiving one pellet per entry. If his animals were ill, a fact supported by his own statement, then they may have been either less

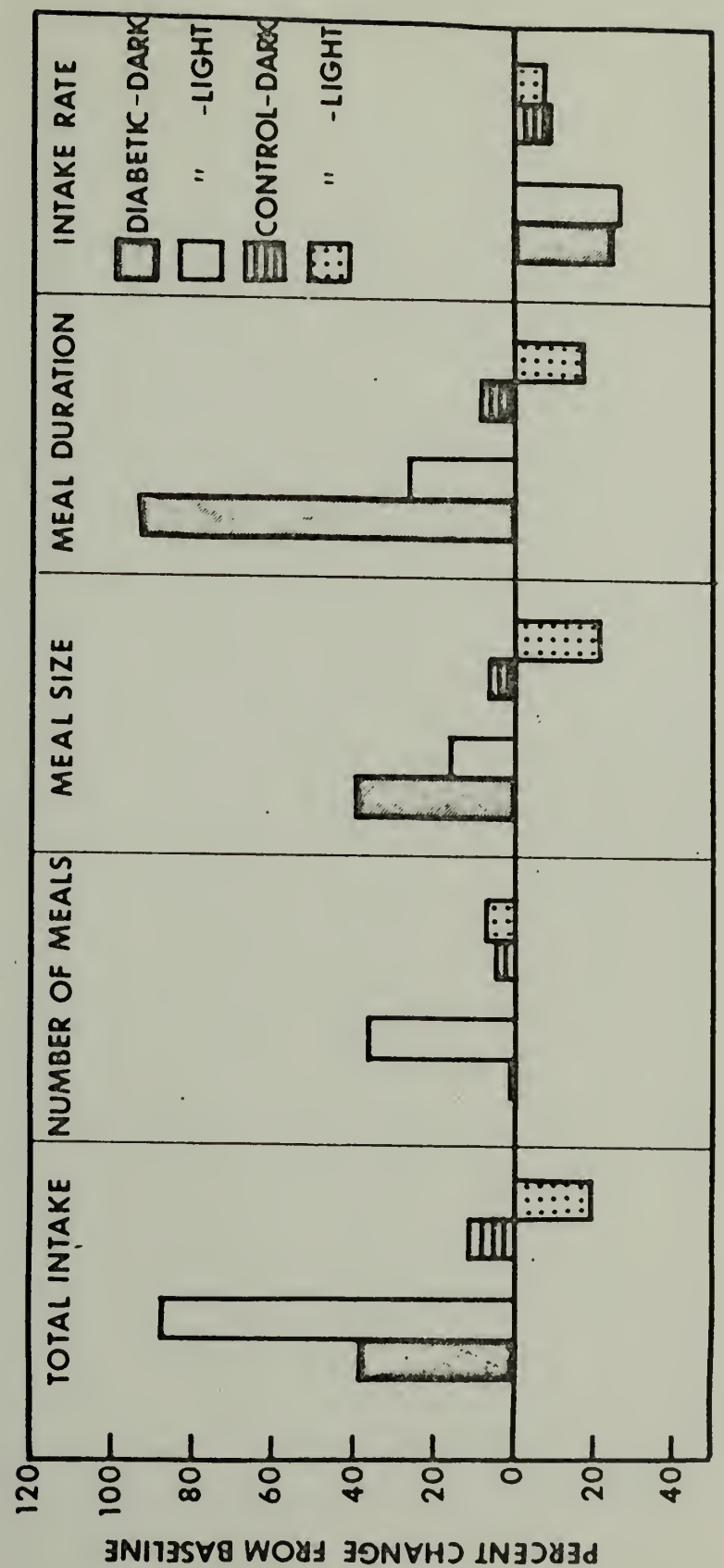

FIGURE 5: Mean percent change in meal characteristics from pre to post-injection sessions for streptozotocin (diabetic) and saline (control) injected animals.

able or less willing to perform the operant response continuously over a prolonged period. This would tend to reduce the size of each meal, and the animals may have compensated by increasing meal frequency. Furthermore, diabetic animals are known to have elevated plasma free fatty acid levels (Spitzer and Gold, 1965) and exercise is known to also elevate free fatty acid concentrations. Hence, the added exertion required to perform the operant response may have further imbalanced the diabetic animals metabolism, making the animals even sicker. Given more easy access to food, as in the present study, increased intake occurs via increased meal size.

The induction of diabetes did not alter light-dark feeding differences. Both groups maintained circadian differences in total intake ($F = 34.9$, $df = 1,8$, $p < .001$), the number of meals ($F = 17.7$, $df = 1,8$, $p < .005$), meal size ($F = 30.6$, $df = 1,8$, $p < .001$), and meal duration ($F = 11.9$, $df = 1,8$, $p < .01$). Also, no light-dark differences and no differences between the groups were found on intake rate. Thus, insulin is not required for the maintenance of circadian feeding rhythm.

Meal Pattern Correlations

The results of the correlational analysis are summarized in Figure c. They both failed to substantiate the experimental hypothesis and also failed to replicate the findings of Booth (1972). There were no significant changes in either the pre or postprandial correlations calculated either

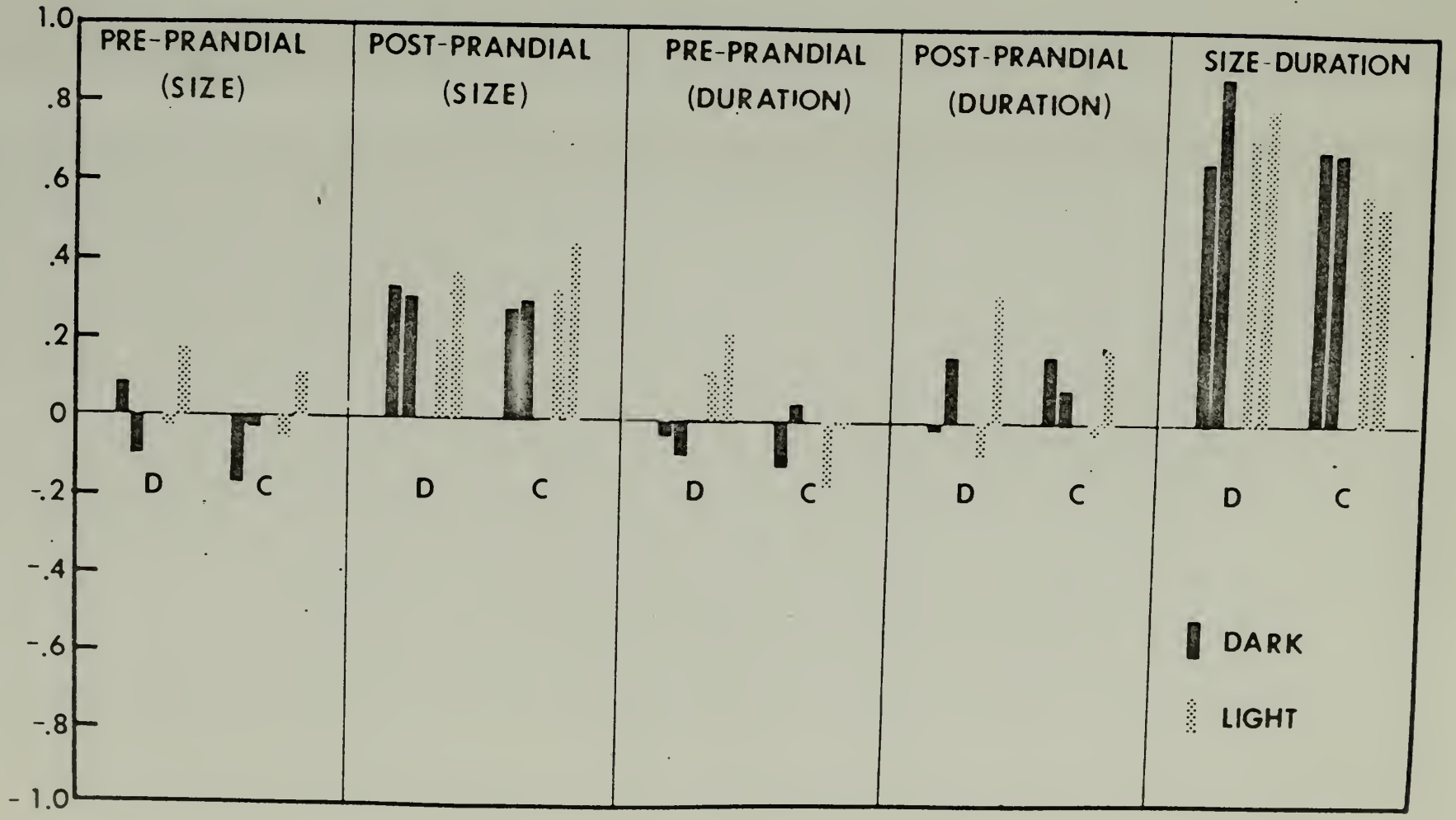

FIGURE 6: Mean Pearson product moment correlation coefficients for streptozocin, diabetic (D), and saline, control (C), injected animals. The first bar of each pair refers to the pre-injection session, the second to the post-injection session.

on the basis of meal size or meal duration following the induction of diabetes. The only significant finding was that the diabetic animals had a closer correspondence between meal size and duration than the controls ($F = 6.0$, $df = 1,8$, $p < .05$).

Once again, it is difficult to explain the discrepancy between the present results and those of Booth (1972). However, it is felt that the same explanation for the difference in meal size (above) also applies here. Indeed, Booth's data indicate very little difference between diabetic and nondiabetic subjects in the postprandial relationship with meal sizes of less than 75 pellets. Since his procedure resulted in very few meals larger than 75 pellets, and since it is with these large meal sizes that the postprandial relationship is the strongest, it is conceivable that the reduction of the sample size within the range of strongest correlation resulted in a lowering of the overall postprandial relationship. It should also be noted that neither actual correlations nor statistical evaluation of the stated effect were reported. Thus, neither the degree nor the statistical significance of his reported effect can be evaluated.

The present experiment found little difference between the feeding patterns of diabetic and nondiabetic animals. Only a hyperphagia, associated with an increased meal size and duration, was observed. Neither the number of meals, the intake rate, circadian rhythm, nor pre and

postprandial correlations were affected. A number of conclusions are warranted. First, the analogy between adult diabetics and neonatal animals does not appear to be valid. Second, since postprandial correlations occur and preprandial correlations do not occur in the absence of insulin, the meal pattern correlation differences between neonates and adults are not related to the lack of glucodynamic feeding control in the weanling. Third, since the number of meals and the postprandial relationship remained stable after the induction of diabetes, insulin does not appear to play a role in the mechanism for meal initiation. Fourth, insulin does not mediate circadian intake differences. Finally, since meal sizes were larger in diabetic animals, insulin may play an important part in the mechanism of meal termination. Of course, the above conclusions must be tempered with the fact that it was impossible to discern whether insulin was totally absent or simply markedly reduced in the present experiment.

EXPERIMENT 4

Meal Patterns in Rats Recovering from Lateral Hypothalamic Lesions

Experiment 3 was inconsistent with a possible analogy between diabetic adult and weanling meal patterning. There is, however, another analogy. Adult rats, after and during recovery from lateral hypothalamic (LH) lesions, show some remarkable similarities to neonatal rats. The development of independent feeding behavior in the weanling rat corresponds closely to that seen in the redevelopment of feeding after LH lesions (Teitelbaum, Cheng and Rozin, 1969a; 1969b). Also, recovered LH lesioned animals are prandial drinkers, with drinking occurring only in combination with food intake, just like weanlings (Kissileff, 1971; Teitelbaum, Cheng and Rozin, 1969a; 1969b). Recovered LH lesioned animals do not respond to either insulin or 2DG injections (Smith and Epstein, 1969; Epstein and Teitelbaum, 1967) as is also the case with weanlings (Lytle and Moorcroft, 1971; Houpt and Epstein, 1973). Furthermore, recovered LH animals, at least applying the meal definition used in the present study, eat a large number of small meals, as do weanlings (Kissileff, 1970).

If this analogy is valid, then recovered LH lesioned adult rats should, at least for a short time, show the preprandial relationship. The present study attempted to test this prediction.

Methods

Subjects

The subjects consisted of 15 male Sprague Dawley albino rats bred and raised in the laboratory under the same conditions as in the preceding experiments. They were 90 to 120 days old and were 350 to 480 grams in weight at the beginning of testing.

Apparatus

The testing apparatus was the same as that used in Experiment 1.

Surgery and Histology

Surgery was performed under sodium pentobarbital anesthesia (40 mg/kg) with atropine methyl nitrate (0.75 mg/rat) given as premedication. Lesion coordinates from the interaural line, with incisor bar at zero were: 7.0 mm anterior to the interaural line, 2.0 mm lateral to the midsaggital sinus, and 7.7 mm below the dorsal surface of the cortex. Lesions were made by passing 1.2 mA anodal current for 25 seconds through a No. 3 stainless steel insect pin (diameter 0.5 mm) insulated with Insulex except for 0.5 mm at the tip.

At the completion of testing, the animals were sacrificed with an overdose of ether, and were perfused through the heart with 10% neutral buffered formalin. The brains were removed and 100μ

sections were cut on a freezing microtome. Every other section through the lesion area was stained for cells and fibers by the Auletta method (Wolf, 1971).

Procedure

The subjects were placed in the individual testing chambers and given a 24-hour adaptation period. Thereafter, for a five-day period, their feeding behavior was continuously monitored. On the morning of the sixth day, surgery was performed. A total of 10 rats received LH lesions. Immediately after surgery, the animals were returned to the testing chambers. Three of the lesioned animals failed to recover from the aphagia and died from inanition. Another two lesioned animals did not become aphagic. Histological examination of the lesions in these animals revealed inaccurate midline symmetry, with the lesion too lateral on one side and too medial on the other. This left a total of five lesioned animals who both became aphagic and spontaneously recovered from the aphagia. Five control animals were then selected from the colony on the basis of similar age and weight. Their feeding behavior was also monitored for a period of five days prior to surgery. Sham operations were performed in the same way as the lesions except that the scalp was sutured immediately after incision. Each sham operated control was then paired with a lesioned animal and the feeder was turned off and the water bottle removed for a period of time equal to the period

of aphagia occurring in its paired counterpart. The subsequent feeding behavior of both the sham operated and the lesioned groups was continuously monitored over a 20-day period, commencing the day after feeding began in the lesioned animals and the day after the pellet dispenser was turned back on and the water bottle returned for the controls.

Data Analysis

Data handling and analysis procedures were the same as in Experiment 1. All data points were calculated on the basis of five-day measurement periods.

Results and Discussion

Histology

There was considerable variation in the lesions. However, the damage was fairly bilaterally symmetrical and centered in the lateral hypothalamus at the level of the ventromedial nucleus. In all cases, the medial edge of the internal capsule was damaged. Reconstructions of the area of maximum damage for the largest and smallest lesions in the series are presented in Figure 7.

Meal Characteristics

The results for a variety of measures of feeding behavior are graphically depicted in Figure 8. Alterations occurred in the feeding behavior of the lesioned group, particularly in terms of their nighttime

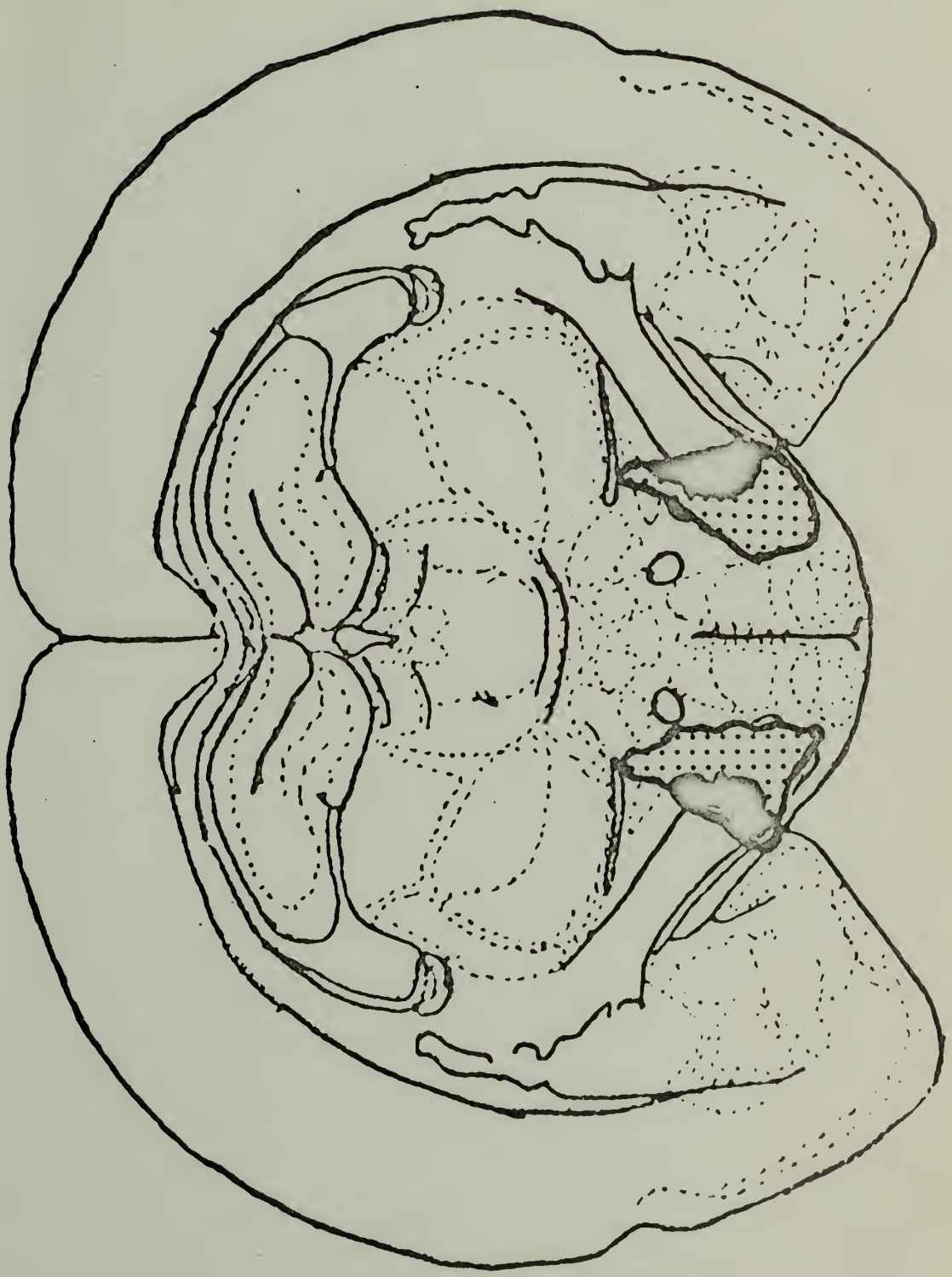

FIGURE 7: Reconstruction of the largest (Stippled) and smallest (solid) lesion damage incurred in Experiment 4, drawn on a coronal section at the level of maximum extent of the damage.

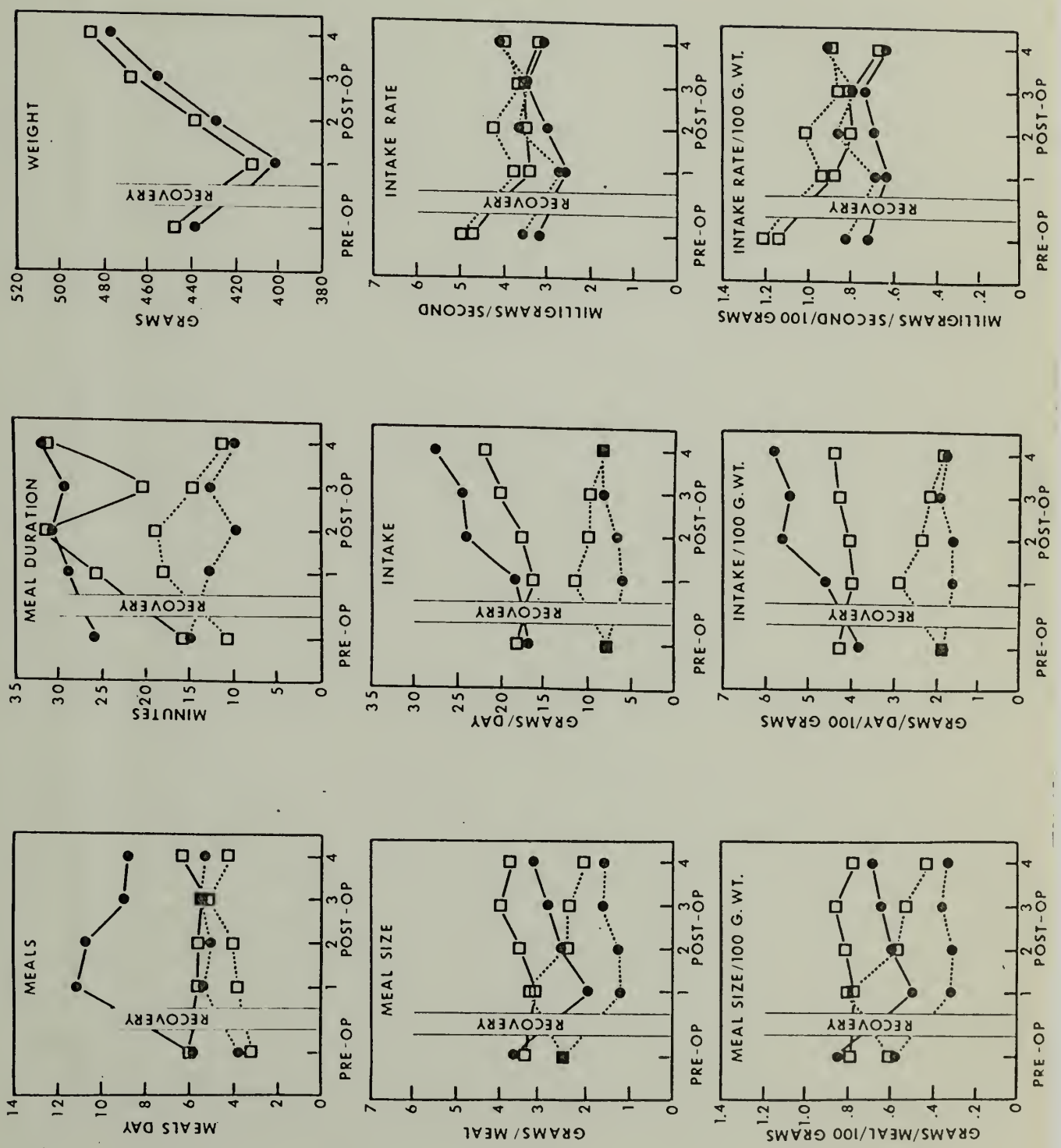

FIGURE 8: Intake characteristics of adult animals both prior to and after recovery from LH lesions (circles) or sham operations (squares).

feeding. Relative to the baseline period and to the sham operated controls, the recovered LH lesioned animals ate more in the dark portion of the cycle and the same amount or slightly less in the light portion (Light-Dark X Cycle X Group interaction, $F = 3.6$, $df = 4, 32$, $p < .025$). This effect was particularly evident during the first two postoperative cycles (Groups X Light-Dark interaction, $F = 6.5; 10.9$, $df = 1, 8$, $p < .05$ for postoperative cycles 1 and 2, respectively), but returned to levels which were not significantly different from controls in later cycles.

Replicating previous findings (Kissileff, 1970), recovered LH lesioned rats had a marked increase in the frequency of meal taking (Cycles X Groups interaction, $F = 3.4$, $df = 4, 32$, $p < .025$). The elevated overall meal frequency resulted mainly from an increase in meal frequency during the night (Light-Dark X Cycles X Groups interaction, $F = 3.2$, $df = 4, 32$, $p < .05$). Even though Figure 8 shows a tendency toward normalization of meal frequency in later cycles, the groups still differed significantly even in the fourth postoperative cycle ($F = 5.77$, $df = 1, 8$, $p < .05$).

Meal sizes decreased following LH lesions (Groups X Cycles interaction, $F = 2.9$, $df = 4, 32$, $p < .05$). However, unlike meal frequency, this effect was uniform across the daytime and nighttime periods. Furthermore, the difference in meal size between lesion and control groups decreased with time ($F = 12.0; 5.7; 2.2; 0.92$, $df = 1, 8$, $p < .01$;

$< .05; < .05; < .05$ for postoperative cycles 1 through 4, respectively). Neither meal duration nor intake rate were affected by the lesions.

These results on meal characteristics are compatible with the hypothesis that recovered LH lesioned animals have feeding characteristics similar to weanlings. The large light-dark difference in meal frequency, with a large number of meals eaten in the dark in the recovered LH lesioned animals, is remarkably reminiscent of the pattern seen in Experiment 1 with weanling animals. On the other hand, the recovered LH rats did not show the same pattern of light-dark feeding differences observed in the weanlings. Recovered LH animals maintained their preoperative light-dark differences in meal size and duration, while weanlings do not show these differences. Although this finding is incompatible with a model based upon a strict correspondence between recovered LH rats and weanlings, the model need not be rejected. It needs to be only slightly modified, to reflect the fact that circadian feeding differences are mediated elsewhere.

Meal Pattern Correlations

The predictions of the LH-weanling analogy were clearly substantiated in the meal pattern correlation analysis as graphically summarized in Figure 9. As in the preceding experiments, the correlations between meal duration and IMI were unreliable. Hence, only the size-IMI

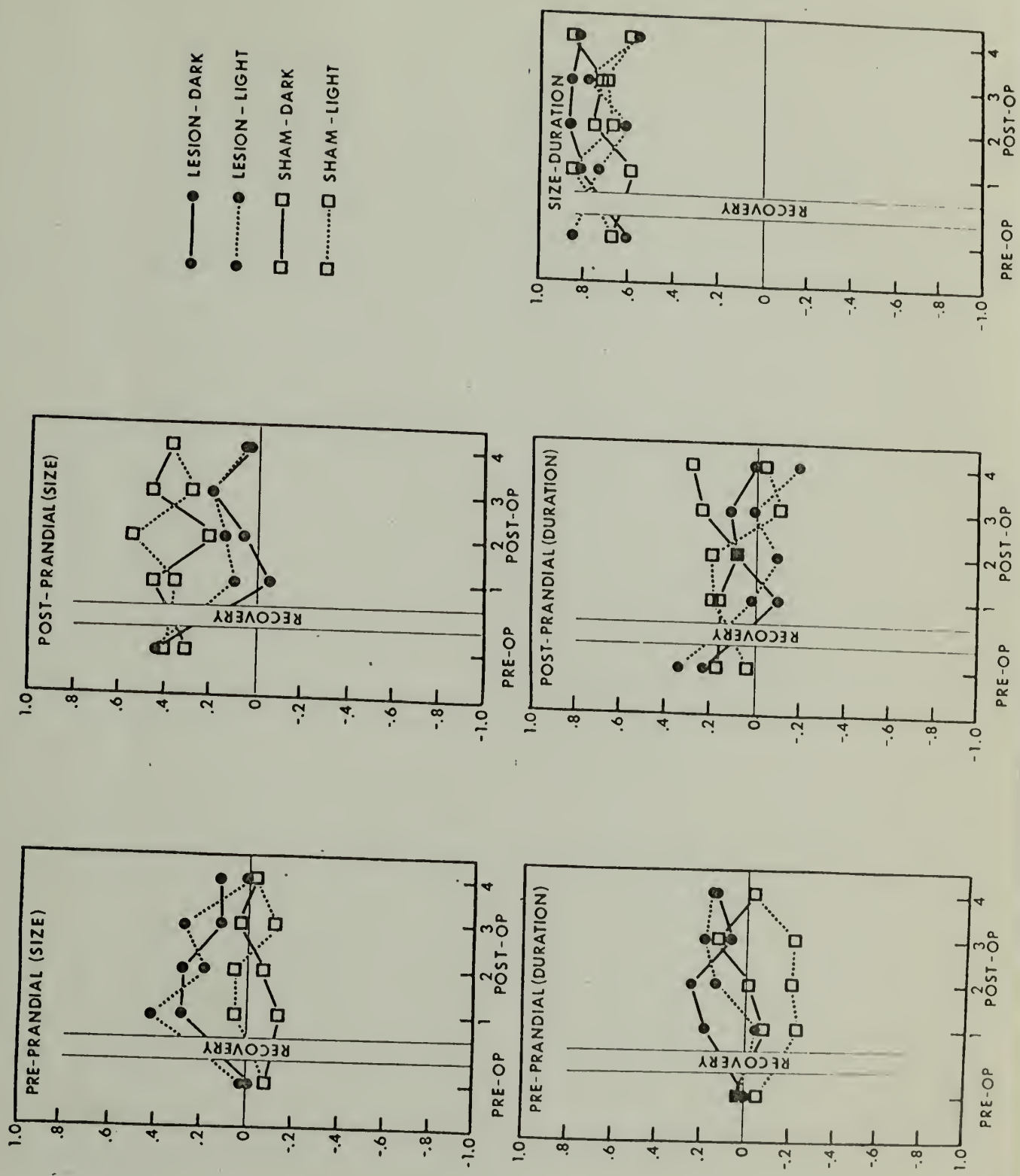

FIGURE 9: Meal pattern correlations of adult animals both prior to and after recovery from LH lesions of sham operations.

correlations are discussed in detail. In addition, the size-duration relationship was not affected by the lesion and will also not be discussed.

Prior to surgery, both groups had negligible preprandial correlations. After surgery, whereas sham operated animals maintained negligible correlations, recovered LH lesioned animals demonstrated a marked rise in the magnitude of the preprandial relationship (Cycle X Group interaction, $F = 5.6$, $df = 1,8$, $p < .05$). The relationship subsequently declined to near zero by the fourth cycle.

The postprandial relationship, present preoperatively, declined postoperatively to negligible for the lesioned animals, but remained stable for the controls (Cycle X Group interaction, $F = 9.9$, $df = 1,8$ $p < .025$). Unlike the preprandial relationship, the postprandial relationship did not return to control levels by the fourth cycle; the groups still differred significantly ($F = 7.3$, $df = 1,8$, $p < .05$). This is not to say that the postprandial relationship never recovers. If the animals were followed longer, possibly the relationship would have reattained control levels. Furthermore, it should be remembered that there was a point in development (25 to 30 days of age) wherein neither pre nor postprandial correlations were significant. Hence, the precept of recovery recapitulates ontogeny (Teitelbaum, Cheng and Rozin, 1969a; 1969b) is not necessarily violated.

The present experiment is consistent with Teitelbaum et al's (1969a; 1969b) analogy between recovered LH rats and weanlings. It further suggests that the LH plays a crucial role in the transition in ontogeny from pre to postprandial intake regulation and that this transition is, probably, the direct result of maturation of the lateral hypothalamus. It should be noted that, within the confines of the present study, it can not be discerned whether LH-medial forebrain bundle damage or damage to the nigro-striatal dipaminergic system (Ungerstedt, 1971) was responsible for the effect.

GENERAL DISCUSSION

The rat ingests its daily food ration in a number of discrete bursts of eating, meals, separated in time by periods of noneating, IMI's. To completely describe the rat's food intake behavior as measured in the laboratory, a theory need only deal with these two phenomena. In addition, a theory need not account for time per se, but only the biological states signalling the initiation of meals and those states signalling the termination of meals. Intervening time may be accounted for by either the presence of a meal termination signal or the absence of a meal initiation signal.

The adult postprandial intake pattern can be described simply as labile nutrient control of meal initiation. That is, in order for a relation-

ship to be explained between meal size and the subsequent IMI, one need only determine how ingested nutrients delay or modulate the meal onset signal, and thus control the length of the fast prior to the next meal. On the other hand, the weanling preprandial intake pattern can be simply described as labile nutrient control of meal termination. That is, in order to explain the relationship between the preceding IMI and the subsequent meal size, one need only determine how ingested nutrients overcome the depleting effects of the period of non-eating, to turn on or modulate the meal termination signal.

It can be readily argued that the metabolic signal for meal termination is glucostatic in nature. Steffens (1969; 1970) has elegantly demonstrated that both blood glucose and insulin levels begin to rise almost immediately after meal onset, reaching peak values near or slightly after the time of meal termination. His data further show that the elevation in insulin secretion is secondary to the elevation of blood glucose levels.

This signal operates independent of the volume of intake. Instead, the critical variable appears to be the caloric content of the meal. This is indicated by the fact that, when intragastric infusion is paired with oral intake, the total caloric content of the meal remains remarkably stable. If water or saline is infused, the animal is unaffected and eats a normal amount orally. There is an increase in the total volume of

diet reaching the stomach, but the caloric content remains stable. If a concentrated diet is infused, the animal compensates and eats less orally. The total volume reaching the stomach is, thereby, reduced, but again the total number of calories remains stable (Thomas and Mayer, 1968). An exactly analogous process occurs when a liquid diet is either diluted or concentrated. Meal size increases in the former case and decreases in the latter case (Levitsky and Collier, 1968; Snowdon, 1969). These effects are compatible with a glucostatic model of meal termination. Glucose and insulin concentrations in the pasma can accurately reflect the caloric content of the ingested nutrients (Steffens, 1969; 1970).

It further appears that this meal termination signal is associated with the rate of glucose utilization, rather than absolute glucose levels, as proposed by Mayer (1955). In the presence of exogenous insulin, which promotes glucose utilization, meal sizes are small (Panksepp, 1973). This could be simply accounted for as a facilitation of the meal termination mechanism. Furthermore, in the absence of insulin, diabetes, there is an elevation of absolute glucose levels, but a decline in glucose utilization. Under these conditions, meal sizes become large (Experiment 3). Hence, there is a suppression of meal termination. Since only glucose utilization rate is down under these conditions, it follows that glucose utilization rate and not absolute glucose levels is the important factor.

Manipulations of the meal terminus signal should not affect the postprandial relationship. Since postprandial correlations are dependent, not on the size of the meal ingested, but on the mechanism controlling the onset of the next meal, meal initiation, it would be expected that such correlations would remain stable in spite of manipulations of the terminus signal. Indeed, diabetes did not alter the relationship (Experiment 3), insulin injections, if anything tend to promote the relationship (Panksepp, 1973), and diet dilution or paired nutrient infusion does not affect the relationship (Snowdon, 1969; Thomas and Mayer, 1968; Levitsky and Collier, 1968).

The presence of the preprandial relationship in weanlings indicates either a dominance of intake regulation by this meal terminus mechanism or a suppression of a meal initiation mechanism. Since the preceding IMI predicts the size of the next meal, it is reasonable to assume that regulation occurs by terminating a meal at a size commensurate with the degree of nutrient depletion resulting from the intervening fast. What factor or factors are controlling meal initiation in the weanling is unknown. However, it can be concluded that the factor is not related to the amount of ingested nutrients in the prior meal.

As noted in the introduction, every point in development is, in and of itself, functionally complete and exquisitely adaptable, given the ecology. The preprandial relationship is no exception. The suckling rat has little

need for a meal initiation system. One is already built into the environment. The mother initiates feeding. In order to regulate, the neonate need only meter his intake and terminate the meal appropriately. Hence, this preprandial relationship is exactly what is needed under these ecological conditions.

The weanling rat is relatively insensitive to glucostatic challenges, neither insulin nor 2DG raise intake levels (Lytle and Moorcroft, 1971; Houpt and Epstein, 1973). If the rat at this age is responding primarily to a glucose utilization signal for meal termination, then why are glucostatic challenges ineffective? The reason appears to reside in the lack of an adult meal initiation mechanism. As pointed out above, insulin injection actually reduces meal size. It facilitates intake strictly by increasing the frequency of meals (Panksepp, 1973). Meal frequency changes are a function of the frequency of meal initiation signals. If the weanling is insensitive to the adult meal initiation signal, then insulin would not be expected to increase meal frequency in these animals. Insulin injection in the weanling, then, should decrease meal size and not affect meal frequency. Thus, insulin should produce a slight hypophagia. Such an effect has been noted both with insulin (Lytle and Moorcroft, 1971) and with tolbutamide (de Castro, unpublished observations).

The results from Experiment 4 suggest that the transition from pre to postprandial responsiveness occurs as a direct result of maturation

of the lateral hypothalamus or systems passing through this area, such as the nigro-striatal dopaminergic system. It is interesting to note that LH lesions did not affect the light-dark difference in meal characteristics. This suggests that it is the maturation of a different structure which is responsible for the adult light-dark intake pattern. This hypothesis is supported by the fact that the transition to post-prandial responsiveness occurred (30 to 35 days of age) prior to the acquisition of adultlike light-dark responsiveness (35 to 40 days of age). A probable structure mediating this function will be discussed below.

The LH, then, would appear to become functionally mature around 30 days of age. Interestingly, this is about the time when myelinization is completed (Buchanan and Hill, 1947; Jacobson, 1973). It would also appear that the maturation of the LH reflects the development of the system for meal initiation. Three lines of evidence suggest that, indeed, meal initiation is a responsibility of the LH system. First, electrical stimulation of the LH results in a stimulus bound initiation of feeding (Larsson, 1955; Miller, 1960). Second, destruction of the LH results, at least for a time, in a complete cessation of meal initiation (Anand and Brobeck, 1951). Finally, the presence of the pre-prandial relationship during recovery from LH lesion, implies the lack of meal initiation regulation. Hence, it would appear that one of the functions of this area is the mediation of adult meal initiation.

Lateral hypothalamic lesions result in aphagia even in ten-day old rats (Lytle, 1971; Balagura and Raubeson, unpublished). This suggests that the Lh is responsible for meal initiation even in the suckling rat. Why, then, is meal patterning not regulated in terms of meal initiation during this period? It is possible that the appropriate metabolic signal for meal initiation regulation is not present or that LH functioning is suppressed by another structure during this period. However, these alternatives appear to be unlikely. Adult animals, who would be expected to have the appropriate metabolic signal and who would have long since had the suppression of the LH removed, show a preprandial pattern of meal taking during recovery from LH lesions. Instead, it would appear that this early form of meal initiation control is rather rudimentary and unsophisticated in reference to adults. Later an elaboration and maturation of this rudimentary system results in adult meal initiation control.

The difference in meal frequency between the light and the dark portions of the cycle in the weanling may be viewed as occurring via a modulation of this rudimentary meal initiation system. The question now arises as to what structure is mediating the adult light-dark feeding pattern. It is unlikely that this is also due to LH maturation. Recovering LH lesioned rats have a pattern of intake differences between light and dark exactly like unlesioned adults. A more likely candidate

is the ventromedial hypothalamus (VMH). It has long been known that destruction of this area results in hyperphagia and obesity (Hetherington and Ranson, 1940). Only relatively recently it has become apparent that the major disruption is in the circadian rhythm. After VMH ablation, total intake, meal size, and the number of daily meals all increase (Teitelbaum and Campbell, 1968). The intake pattern during the night is unaltered. The increase in intake occurs in the daytime. In fact, there is a virtual abolition of circadian feeding differences (Balagura and Devenport, 1970; Becker and Kissileff, 1974; LeMagnen, Devos, Gaudilliere, Louis-Sylvestre and Tallon, 1973). This effect also occurs in weanlings. The weanlings, however, unlike the adults, compensate for the increased intake in the light with a decreased intake in the dark, such that there is no net change in total intake (Bernardis, 1973). Hence, destruction of the VMH in either adults or weanlings abolishes the circadian feeding rhythm.

It is tempting to hypothesize that a direct VMH - LH interaction is responsible for the rhythm. However, it is likely that the interaction occurs indirectly, through alterations in the endocrine system. LeMagnen et al (1973) hypothesized that the VMH normally controls the circadian cyclicity of insulin secretion by the pancreas and this is responsible for the feeding rhythm. Indeed, VMH lesion induces hyperinsulinemia (Frohman and Bernardis, 1968; Frohman, Goldman,

Schnatz and Bernardis, 1971; Frohman, Bernardis, Schnatz and Burek, 1969). This would tend to promote lipogenesis and thus increase the size of the fat stores. In support of this notion, LeMagnen et al (1973) have found that VMH lesion abolishes the circadian cycle of daytime lipolysis and nighttime lipogenesis. However, VMH lesion in diabetic animals still induces hyperphagia (Friedman, 1972). In addition, the circadian rhythm of insulin secretion is in the wrong direction. More insulin is secreted during the day than at night (Gagliardino and Hernandez, 1971). Hence, insulin could not be responsible for the circadian lipogenesis-lipolysis cycle. Furthermore, diabetic animals have a perfectly normal circadian feeding cycle (Experiment 3). Thus, VMH control of the circadian feeding rhythm via modulation of insulin secretion would appear to be unlikely.

A likely alternative choice to insulin is growth hormone. Plasma growth hormone levels are depressed after VMH lesion (Frohman and Bernardis, 1968). Also growth hormone is known to facilitate lipogenesis (Turner and Bagnara, 1971). Further research is required to properly evaluate this hypothesis.

It should be pointed out that the disruption of the circadian cyclicity after VMH lesion is not the primary reason for the hyperphagia. If food intake is prevented during either the night or the day, VMH

lesioned rats still become hyperphagic (Gold, personal communication, 1974). However, the fact that the lesion eliminates day night feeding differences, strongly suggests that a structure in this area, or a system passing through this area, is responsible for the circadian rhythm.

Throughout the course of this discussion, the meal initiation signal has been repeatedly referred to without any attempt to identify what metabolic processes are involved. This was done intentionally. The metabolic signal for feeding is unknown and has long been a point of controversy. No single metabolite appears to be able to survive all of the experimental evidence. If we propose that low blood blucose levels signal meal initiation, we are unable to account for diabetic hyperphagia, wherein blood glucose levels are high. To compensate for this, Mayer (1952) has proposed that a low glucose utilization rate is the feeding signal. However, this notion is contradicted by Experiment 3 of the present study. The constantly low glucose utilization rate of diabetic rats should increase the number of meals eaten. Such an effect was not found. In addition, there is no discernible change in either the glucose or the insulin plasma concentrations prior to the onset of a meal (Steffens, 1969; 1970). Furthermore, i.v. glucose infusions do not always affect intake (Adair, Miller and Booth, 1968). Hence, glucose utilization rate is unlikely.

Kennedy (1953) has proposed a lipostatic feeding signal. Although Kennedy himself did not postulate what the actual signal is, subsequent investigators have suggested that it is plasma-free fatty acid concentrations (Walker and Remley, 1970). Indeed, free fatty acid concentrations increase after a period of food deprivation and tend to rise just prior to meal onset (Walker and Remley, 1970; Steffens, 1969). Hence, it was proposed that high free fatty acid levels signal feeding. A very large problem with this hypothesis is that it cannot explain the circadian feeding pattern. Free fatty acid levels and the rate of lipolysis are high during the day when feeding is low, and are low during the night when feeding is high (Fuller and Diller, 1970; LeMagner and Devos, 1970; LeMagnen, Devos, Gaudilliere, Louis-Sylvestre and Tallon, 1973). Thus, plasma-free fatty acid levels appear to be an unlikely candidate for the meal initiation signal.

What, then, is the feeding signal? In fact, is there a discrete, single metabolite signal? Although no definitive answer can be given at the present time, it would appear that meal initiation probably occurs when a number of metabolic conditions are met simultaneously. Indeed, steps in this direction are currently being undertaken (LeMagnen and Devos, 1970; LeMagnen, Devos, Gaudilliere, Louis-Sylvestre and Tallon, 1973). It is hoped that, in the future, equipped with a fuller

understanding of the interaction between metabolic processes and feeding behavior, the identity of the initiation signal will, at long last, be uncovered.

In conclusion, the present study demonstrated the efficacy of the ontogenetic approach to the understanding of regulatory behaviors. By observing closely the behavioral changes occurring during development, the functioning of an immature feeding system was discovered and described. By then investigating what manipulations in adults would reinstate this pattern, the probable structure overseeing the adult pattern was identified. Furthermore, the role that this structure plays in adult regulatory behavior was considerably clarified.

> "To understand a given machine, one could walk along the assembly line and see the steps by which the parts of the machine are put together. By a similar method, one may hope to understand physiological regulations. The parts in this case are not always visible structures, but they are elementary processes that, when combined in a particular way, do something which they did not do separately. We will watch a function as its elements become available for use in the living fetus or infant. We shall see that the functions at successive stages can operate little or much. We will recognize some of the contributions made to the whole function by individual processes acquired by it. Unable himself to be a creator, the scientist can watch creation going on. He will thus see the accretion of partial processes as they begin to participate." (Adolph, 1957, pg. 90).

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