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Early Separation and the Development of Impaired Thermoregulation in Rats: A Risk Factor in Gastric Ulcer Susceptibility

by

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Abstract

EARLY SEPARATION AND THE DEVELOPMENT OF IMPAIRED THERMOREGULATION: A RISK FACTOR IN GASTRIC ULCER SUSCEPTIBILITY

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Danielle Greenberg

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Previous work showed that rats prematurely separated from dams at 15 days of age subsequently have heightened vulnerability to restraint induced gastric erosions (RGEs). This vulnerability was highly correlated with a fall in body temperature during restraint at normal room temperatures. The present work examined the nature of the thermoregulatory disturbances of prematurely weaned rats.

As available fat is important in heat production, I investigated differences in fat deposition of early and normally weaned rats. Early weaned rats had significantly less white and brown adipose tissue than normally weaned rats. Early weaned rats also had relatively greater fat depletion during conditions of food deprivation and restraint. A high correlation was found between hypothermia or RGE susceptibility and low body fat content.

The competence of 30 day old rat's behavioral thermoregulation was assessed. Rats were tested on a thermal runway where free choice of ambient temperature was available, and in situations of inescapable heat and cold. Robust differences in temperature selection behaviors of early and normally weaned rats were not found. However, rats of both weaning conditions evidenced the ability to detect and avoid temperature extremes. In inescapable cold early weaned rats responded appropriately, showing significantly greater incidences of heat producing behaviors than normally weaned rats. However, early weaned rats became relatively hypothermic during 20 minute test sessions. Thus 30 day old early weaned rats mobilize behavioral mechanisms for heat production but these behaviors are ineffective in preventing hypothermia.

Oxygen consumption measurement was used to assess physiological thermoregulatory mechanisms of early and normallly weaned rats. During food deprivation or food deprivation and restraint, early weaned rats became hypothermic and used significantly less oxygen than normally weaned rats. When food was available early weaned rats used as much oxygen as normally weaned rats. Food deprived early weaned animals did not increase oxygen consumption in response to exogenous norepinepherine.

I concluded that early weaned rats are more vulnerable than normally weaned rats to hypothermia during food deprivation and restraint because heat production is diminished and fuel stores are more depleted.

Dedicated to

My mother: Ray S. Greenberg M.D.

and

My husband: Victor F. Klebanoff

(in order of appearance)

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GENERAL INTRODUCTION

Ulcer disease in man is considered the classic case of a psychosomatic disease wherein disease susceptibility is linked to both physiological and psychological factors (Weiner, Thaler, Reiser, & Mirsky, 1957). Animal models have been used to investigate the mechanims uderlying the pathogenesis of ulcer disease. Many methods for experimentally inducing ulcers in various animal species have been used.

Among the techniques that have been used to elicit gastric ulcers in experimental animals are drug administration, brain lesions and stimulation, burning or freezing, surgical procedures such as pyloric ligation, inescapable electric shock, approach-avoidance learning paradigms, and immobilization (Brodie, 1968; Ader, 1971). Prior to the 1960's the primary technique for the study of ulcer disease in experimental animals was that of pyloric ligation (Brodie, 1968). However, it has been shown that increased gastric acid secretion is an artifact of the pyloric ligation technique and ulcers produced by this technique are not a result of the animals own physiological processes.

A relatively noninvasive means of producing gastric erosions reliably is that of immobilization or restraint. It is a simple technique and, as it does not involve surgical intervention or drug

administration the technique is particularly useful in the study of the effects of psychological factors in the susceptibility to ulcers. Selye (1936) first used this technique to elicit gastric erosions in rats. Brodie and Hanson (1960) tested rats, mice, guinea pigs and hamsters and determined some of the parameters that were likely to affect lesion susceptibility. The percent of animals who formed gastric lesions was found to increase with the duration of immobilization. Food deprivation was also found to lead to gastric erosions and food deprivation combined with immobilization resulted in the greatest extent of gastric erosions in rats. Brodie and Hanson (1960) also found that there was an inverse relationship between lesion incidence and body weight with smaller animals being more likely to form gastric erosions. It was also found that the smaller the cage volume for restraint the greater the number of RGEs (Bonfils, Liefooghe, Gelle, Dubrasquet & Lambling 1960).

A number of factors in the life history of experimental animals have been shown to affect their susceptibility to RGEs. Individually housed rats and mice are more likely to form gastric lesions when restrained or food deprived for 24 hours than are animals housed in groups (Ader, 1965; Essman & Frisone, 1966). Handling of young rats has also been shown to reduce their susceptibility to RGEs when tested later in life (Weininger, 1956; Winokur, Stern & Taylor, 1959). There have also been numerous studies of how mother-infant interactions

could affect later RGE susceptibility.

Premature separation of infants from their mothers has been shown to produce both immediate and long term alterations of behavioral and physiological processes in numerous mammalian species (Ackerman, Hofer & Weiner, 1978a; Hofer 1975a, 1975b, 1976; Kaufman & Hinde, 1961; Scott, Ross, & Fisher, 1959). In the rat, developmental factors have been shown to affect susceptibility to experimentaly induced gastric erosions later in life (Ackerman, Hofer & Weiner, 1975; Ader, Tatum & Beels 1960). Weaning rats at 15 days of age rather than at the typical 21 days, increases the risk of restraint induced gastric erosions at 30 days of age from approximately 20% to from 70-90% Ackerman, Hofer and Weiner (1975) examined the lesion susceptibility of both early weaned and normally weaned rats at various times during the rat life span. Normally weaned rats typically are unlikely to form gastric erosions early in life and as they age RGE susceptibility increases. Early weaned rats show the opposite pattern, they are extremely susceptible to RGEs early in life with a gradual decline in susceptibility as they age. By day 150 early and normally weaned rats have similar RGE susceptibility.

When young rats are separated from their dams at 15 days of age, they are unlikely to survive on their own. The cause of death is linked to the animals' becoming hypothermic (Hofer, 1975b). Thus Hofer (1975b) has shown rat pup survival is greatly increased if they are

provided with an external heat source. The susceptibility to gastric erosions in prematurely weaned 30 day old rats is also linked to hypothermia (Ackerman et al. 1978a). At 30 days of age, rats weaned at 15 days of age become hypothermic when subjected to food deprivation and restraint at normal room temperature. Rats weaned at 21 days of age are able to maintain body temperature under the same conditions (Ackerman et al. 1978a). The incidence of gastric erosions is highly correlated with the tendency to become hypothermic. Ackerman et al. (1978a) have shown that it is also possible to protect 30 day old rats, weaned at 15 days, both from becoming hypothermic and from forming gastric erosions by food depriving and restraining these animals at the elevated ambient temperature of 30 °C. Additionally, 21 day weaned rats tested at 30 day of age will both become hypothermic and form gastric erosions if food deprived and restrained at the relatively low ambient temperature of 18 °C.

The main focus of the present work will be to investigate what thermoregulatory mechanisms are disrupted by premature weaning and how these disturbances lead to ulcer susceptibility.

Hypothermia and Gastric Erosions

Bartlett and his associates (1953, 1954) have shown that restrained animals have impaired thermoregulatory capabilities. When placed in an environment with an ambient temperature of 6 °C, restrained mice showed significantly greater loss of core body temperature than did unrestrained animals. Bartlett attributed part of this deficiency in thermoregulatory ability to a stress factor. This he based on the fact that familiarizing a group of animals with the restriction cage for a brief time each day and feeding them while they were in the restriction area led to fewer disturbances in thermoregulation than in those animals who were not first familiarized with the restriction area. Additional evidence for a stress factor being involved in causing the hypothermia associated with restraint was shown in a study where animals were first pretested for emotionality. Animals that were found to be more "emotional" (using Hull's open field test to measure emotionality) were significantly more likely to show restraint induced hypothermia than were animals rated low in "emotionality" (Bartlett, Bohr, Helmdach, Foster & Miller 1954).

Body temperature regulation is also a factor involved in the formation of restraint induced gastric lesions. If adult rats are food

deprived for 24 hours and then restrained at various ambient temperatures for 24 hours, the number of gastric erosions formed is inversely related to ambient temperature. In addition, the number of erosions is negatively correlated with body temperature at the end of the restraint period (Martin, Martin, & Lambert 1970). Further investigation as to the influence of body temperature on the production of restraint induced ulcers has shown that significantly fewer animals who are restrained but who have their body temperatures maintained at normal levels show more ulcers when compared to those whose body temperature is allowed to fall (26% as opposed to 100% of those tested) (Antoon & Gregg 1976).

There is evidence as to the mechanism that links hypothermia and gastric ulcers. Witty and Long (1970) and more recently Ackerman (1981) have shown that decreasing ambient temperatures leads to increased gastric acid secretion. Witty and Long (1970) used a pyloric ligation technique for measuring acid output while Ackerman (1981) used continuous perfusion of the gastric lumen. The perfusate is collected and measured for acid content. Using this technique Ackerman was able to measure the effect of changing ambient temperature on acid output in awake as well as anesthetized animals. Even when core temperature was maintained at a constant level, lowering ambient temperature led to a significant increase in acid output.

Both early weaned and normally weaned 30 day old rats are able to

maintain their body temperature when housed with litter-mates under typical laboratory conditions. However, as previously mentioned prematurely weaned animals are unable to maintain body temperature when subjected to food deprivation followed by restraint, while normally weaned rats are able to maintain thermoregulation under these conditions (Ackerman, Hofer, & Weiner 1978a). Thus it seems that premature weaning effects temperature regulation, and that thermoregulatory deficits brought about by early weaning appear to link early separation to an increased susceptibility to gastric lesions.

Development of Homeothermy

Neonatal rats take on the temperature of the external environment and have little control over their body temperature. However, by 14 days of age rat pups are able to maintain their body temperature at a relatively constant level (Antoschkina as cited in Adolph 1957; Taylor 1960; Conklin & Heggeness 1971). The development of this capacity involves a number of systems broadly defined as heat production mechanisms and heat conservation mechanisms. Heat production in mammals is largely aerobic and thus oxygen consumption can be and has been used as a full measure of heat production.

Lavoisier was the first to show that metabolism could be measured

by calorimetry. He also showed that oxygen consumption and carbon dioxide production were involved in the metabolic process. Since Lavoisier's time heat production has typically been measured in one of two ways. One method is by direct calorimetry where the subject is placed in a thermal chamber in which all heat changes can be measured. The other method involves indirect calorimetry where oxygen consumption or carbon dioxide production are measured as indicators of heat production. Haldane (1892) utilized these principles to develop a mechanism for indirect calorimetric measurements for small mammals. His device consisted of an animal chamber connected to jars containing either sulfuric acid used to absorb water or soda lime used to absorb carbonic acid. By pumping air through the system and weighing the jars both before and after the experiment it was possible to calculate the amount of oxygen consumed and carbon dioxide produced.

In 1895 Pembry used a similar device to measure the effects of variations in ambient temperature on both carbon dioxide production and body temperature of young mice and rats. In adult mice he found that a decrease in temperature from 30 °C to 18 °C resulted in a 74% increase of carbon dioxide production and no change in body core temperature. However, when 1 day old mice were exposed to the same external temperatures no increase in carbon dioxide production was found and body temperature steadily declined. Three day old mice were found to have responses that were similar to those of neonatal mice

when exposed to decreased ambient temperatures. Eight day old animals however, showed an initial increas in carbon dioxide production in response to cooling, although this effect was transient. By ten days of age animals showed response patterns similar to those of adults. A similar study was conducted using 1 to 2 day old rats. These animals showed a decrease in carbon dioxide production in response to cooling with a simultaneous decline in body core temperature.

Antoschkina (cited in Adolph, 1957) found that neonatal rats were poikilothermic, developing some ability to maintain body temperature by 14 days of life. Adult thermoregulatory capabilities developed by 28 days of life. Fairfield (1948) extended research in this area by simultaneously measuring body temperature and oxygen consumption while varying ambient temperature. Oxygen consumption was measured by placing the animal in a respiratory chamber where the decrease in the volume of air could be measured. Animals from birth to 17 days of age were studied. Ambient temperature ranged from 30 °C to 5 °C. Intraperitoneal temperature was found to depend on environmental temperature and oxygen consumption was found to decrease as temperature decreased for neonates. Three to 4 day old animals were able to maintain body temperature about 1 °C above ambient temperature, although a steady decline in oxygen consumption was observed when ambient temperatures fell. Seventeen day old animals were able to maintain a body temperature 3 °C above ambient temperatures and showed

an initial increase in oxygen consumption as temperature was lowered followed by a subsequent decline as temperature was further lowered.

Recent research has confirmed that it is somewhere between the second and third week of life that rats temperature becomes relatively independent of ambient temperature. Taylor (1960) measured oxygen consumption of rats aged up to 22 days at various ambient temperatures. The method for measurement of oxygen consumption used a spirometer and soda lime as a carbon dioxide absorbant. Oxygen consumption was considered equal to carbon dioxide production which was determined by increses in the weight of soda lime. Newly born animals were found to use 19.7 ml/100g/min of oxygen with no substantial increase in response to cold. The body temperature of these animals was found to decrease in correspondence to ambient temperature decreases. As animals became older they showed a proportionately higher basal oxygen consumption rate. In addition, with increasing age animals began to show an increase in oxygen consumption in response to cold. Taylor also determined the physiological thermoneutral range for animals from 0 to 22 days of age. Thermoneutrality has two typical definitions. First, physiologically, thermoneutrality is defined as that environmental temperature which elicits minimal oxygen consumption. Second, behaviorally, thermoneutrality is defined as that environmental temperature at which no thermoregulatory behaviors are observed. As very young animals show little increase in oxygen consumption in

response to environmental stimuli they were found to have a wide physiological thermoneutral range. The range for physiological thermoneutrality decreased from 6 °C for 1 day old pups to 2 °C for 21 day old animals (Taylor, 1960). However, behaviorally, even very young animals do exhibit responses to changing ambient temperature (Alberts, 1978; Johanson, 1980). Thus using the behavioral definition of thermoneutrality a narrower range would be found. Conklin and Heggeness (1971) were able to define more precisely the physiological thermoneutral range for young animals by using less severe cold challenges. Utilizing measures of oxygen consumption and colonic temperature these investigators found that the width of the thermoneutral range decreased by 5 °C as animals went from 5 to 12 days of age. A further decrease of 5 °C was found in the width of the thermoneutral range of animals as they aged from day 12 to day 21. Younger animals colonic temperature was found to decrease upon exposure to cold despite a fivefold increase in oxygen consumption, suggesting that heat conservation and not heat production is compromised in very young animals. As animals became older both the speed and intensity of the metabolic response to cold increased.

Body temperature maintainence is also related to food consumption. Brobeck (1948) first documented the relationship between feeding and temperature regulation in rats. Using adult rats Brobeck (1948) placed rats that had been acclimatized to approximately 80 °F in

ambient temperatures from 65 °F to 92 °F. He found that at low temperatures rats increase the amount of food eaten, and show weight gains, while at high temperatures rats eat less and have weight losses. Brobeck (1948) concluded that food intake was controlled as if it were a mechanism of temperature regulation. Kraly and Blass (1976) note that this relationship is valid for many mammalian species. They note that alterations in food intake in respose to changes in ambient temperature have been found in such diverse mammalian orders as primates, ungulates, carnivores, and lagomorphs. Additionally, enhanced feeding occurs even after relatively short exposures to cold ambient temperatures.

Some investigations into the mechanisms that control the relationship between food intake and ambient temperature have focused on the interaction of peripheral and central thermosensitive structures. Hamilton and Ciaccia (1971) found that heating the preoptic anterior hypothalamic area led to decreases in core body temperature and increases in food intake. Heating of other hypothalamic areas such as the ventromedial or posterior hypothalamus did not result in changes in body temperature or food intake. These results suggest that feeding may at least in part be a thermoregulatory mechanism mediated by the preoptic anterior hypothalamus. Kraly and Blass (1976) found that low ambient temperature was a sufficient stimulus to increase feeding behavior, and ambient temperature rather

than energy need was most important in effecting this increase. They also found that peripheral sensation of cold increased the motivation for feeding as measured by the operant rate of rats on a VI 30 schedule, or as measured by rats response to quinine adulterated food. In addition Kraly and Blass (1976) confirmed earlier findings (Sleeth & Van Liere, 1937) that cold ambient temperatures increase the rate of gastric emptying. These results suggest that peripheral sensation of cold is an adequate stimulus for feeding.

Recent work by Johanson and Hall (1980) show that the feeding behavior of young rats at high and low ambient temperatures is quite different from that of adults. In rat pups less than 10 days old it was found that warm ambient temperatures feeding increased while at low ambient temperatures feeding decreased. Johanson and Hall suggest that this parallels the young rats response to the natural stimulus of the mother. They argue that warmth acts as a "comfort" stimulus and that in this "comfort" state, when the mother would normally be present, rats are more likely to be responsive to food stimuli. The developmental course wherein the response to cold ambient temperature changes from one of decreased food consumption to increased food consumption has not been detailed. However if 30 day old rats are responding to ambient temperatures as would adults then cool ambient temperatures should elicit feeding. If early weaned rats normally respond to low ambient temperature with increased food intake, then

food deprivation may impose a challenge to thermoregulation by disrupting this thermoregulatory behavior.

Hofer's (1975b) work shows that 15 day old rats separated from their mothers have abnormalities in a number of systems. That is after separation, body weight, cardiac rate, and respiratory rate declines, although these return to normal levels later in life. Body temperature also declines when rat pups are weaned at this age. And as mentioned earlier survivability of pups is greatly increased by providing an external heat source. An external heat source is not equivalent to heat provided by the mother especially as such a source is generally provided constantly and mothers heat is provided cyclically. Thus weaning a rat pup at 15 days of age has the effect of a thermal challenge as the mother is no longer available to provide warmth. This challenge occurs at the time when the animal's physiological means of thermoregulation are maturing. It is possible that weaning at this time could disrupt the maturation of thermoregulation. It could be this disruption that later makes these animals more likely to respond to the challenge of food deprivation and restraint by becoming hypothermic and developing concomitant gastric erosions.

In addition to maintainance of body temperature by physiological means, young animals also are able to thermoregulate through behavioral strategies such as huddling with littermates or their mother, chosing favorable environmental temperatures, and positioning

their bodies to maximize or minimize contact with littermates and the mother. Historically, temperature selection has been investigated most extensively in adult ectotherms. There is a great deal of evidence that ectothermic animals such as frogs, lizards, and some insects regulate their internal temperature by locomoting direction of preferred temperature (Herter 1934, Workman & Fisher 1941; Grunn & Walshe 1942). Herter (1934) was the first to use a thermal gradient to test the temperature selection behavior of rodents. He used a copper bar heated at one end and cooled at the other to test adult mice. These mice were found to come to rest at 34.6 °C.

The temperature selection behavior of young endothermic mammals has also beeen investigated. Oglive and Stinson (1966) studied the temperature selection behavior of mice aged from 1 to 84 days. Mice were placed in a box with a thermal gradient from 5 to 25 °C. Animals were tested in groups of 6, animals were placed either as a group at the region of 29-31 °C or placed as individuals spaced equally along the gradient. One to 11 day old animals were found to migrate toward the warm part of the gradient whether placed in groups or singly so that at the end of a 2 hour testing period most animals were found at about 37 °C. This was true for animals starting either at relatively hot or relatively cool portions of the gradient. However, 1 day old animals starting at portions of the gradient less than 16 °C showed no movement, apparently being immobilized by the cold. The level of final

selection temperature was nearly constant for 1-11 day old mice. After 11 days of age the final selection temperature was lower, so that adult animals preferred a temperature near 31 °C. For all animals tested group contact seemed less important than environmental temperature since animals place in groups separated and then reformed their groups at a higher temperature. Thus there is evidence that young mice select a particular temperature and this temperature drops as mice age. However, it is not necessarily true that they are accomplishing thermoregulation through the behavior of temperature selection. That is temperature selection is not necessarily equivalent to temperature regulation.

The difference between thermal choice and thermoregulation has been pointed out in a study on young hamsters (Leonard 1974). Hamsters 4 to 5 days old were found to move along a thermal gradient in the direction of a heat source. This was true even though their core temperatures rose to from 40 to 43 °C and these animals evidenced discomfort by squealing and jumping about. This positive "thermotaxis" was seen from birth till 8 days of age. Pups older than 8 days showed more erratic behavior when placed upon the thermal gradient, presumably due to development of competing behavioral responses. Young hamsters locomote toward a heat source even to the detriment of core temperature. Thus heat thermotaxis can override temperature regulation. In their nest environment this positive

"thermotaxis" would act to promote thermoregulation since the warmest object with which the pup is likely to come into contact is the mother; and the mother will be at a temperature that is physiologically appropriate for hamsters.

In a more recent investigation of behavioral thermoregulation in young hamsters (Leonard 1982) pups tested in groups rather than individually showed a variety of behaviors that suggest behavioral thermoregulation is available to these animals. That is, if allowed to show behaviors other than temperature selection alone, pups will thermoregulate even before they are mature enough to be capable of physiological thermoregulation. Four to 14 day old pups tested on either a moderate thermal gradient, a strong thermal gradient or no gradient showed several behaviors which could serve thermoregulatory functions. Pups might locomote toward a region with a particular temperature, or maintain or decrease contact with littermates, or become more or less active within a group of huddling animals. Rectal temperature measurements indicated that the use of these behaviors in numerous combinations led to relatively constant core temperatures. Leonard concluded that falling environmental temperature led to more activity and active social contact, while rising environmental temperature led to quiet and little group contact. As pups matured the behavioral responses became less clearly coupled to external temperature. Maturation of physiological temperature regulation was

thought to be responsible for this effect since behavioral responses would therefore be less essential. Temperature regulation has been described as a model system for the idea that ontogeny recapitulates phylogeny, with behavioral regulation being the more primitive and the later emerging physiological regulation being the more advanced.

Species other than rodents, such as pigs, have been found to use the behavioral response of temperature selection for thermoregulation. Mount (1963) used a thermal gradient with a range from 23-37 °C. Large white breed pigs aged from 1 to 41 days were tested. When tested individually or in groups animals of all ages tended to stay at temperatures of about 30 °C after a 45 minute test period. As Mount points out this was true although as pigs aged their physiological thermoneutral ranges had lower end points. It was found that rectal temperature of animals had changed only minimally (.07 ± .03 °C) suggesting that temperature selection was indeed used for thermoregulation by prephysiologically regulating animals. Thus a variety of young mammals have been shown to use behavioral means of thermoregulation even though these animals have differing levels of precosity at birth, and differing levels of physiological thermoregulatory mechanisms available to them.

Young rats have been shown to exhibit positive "thermotaxis" at least under certain conditions. Johanson (1980) tested 3 and 6 day old pups in a temperature selection situation with a thermal gradient

ranging from 18 °C to 37 °C. Pups were either deprived of maternal contact for 24 hours prior to testing or tested immediately following separation. Rat pup activity is known to increase following maternal deprivation (Moorcroft, Lytle, & Campbell, 1971; Randall & Campbell, 1976), and therefore it was believed that deprivation might lead to increased thermotaxic behavior. Pups were given one minute tests starting at one of three starting positions: the cold, the middle or the warm. Deprived animals did show thermotaxic responses greater than nondeprived animals. Three day old animals moved more from the cold than did six day old pups. They also showed a greater tendency to move toward the warm end from the moderate starting position than did six day animals. Animals starting in the warm end tended to remain at their starting position. The failure of deprived older animals to exhibit the thermotaxic response was explained by the fact that they probably have less heat loss due to increased surface area to body weight ratio, increased skin, thickness, and hair growth. And since they lose less heat they would not find it as necessary to utilize environmental temperature for temperature control.

Kleitman and Satinoff (1980) have reported behavioral thermoregulation by young rats placed on a 17 to 45 °C gradient. However, these animals were given long testing periods since often no movement was observed until after half an hour had passed. They concluded that rats older than 2 days of age were capable of

thermoregulation through temperature selection, although a great deal of time might be necessary to observe the behavior. It was also concluded that there was an interaction between latency for temperature selection and initial gradient temperature. From these results it appears that rats are capable of the sensory detection, central integration, and motor response necessary for voluntary thermoregulatory behavior from about 2 days of age.

Temperature selection by young rats has also been demonstrated in a somewhat different type of apparatus. Rat pups from 1 to 13 days of age were studied in a choice box with regions that had different floor temperatures (Fowler & Kellogg, 1975). Pups were place in the center of three compartments. The central compartment was at approximately 23 °C. One end of the apparatus was at 35-36 °C, while the other end was at approximately 22 °C. Rectal and skin temperatures were measured immediately prior to and following testing. In three minute tests animals less than 6 days of age never left the starting position, while older animals consistantly chose the warmer area, and as rats became older they were progressively better at placing themseleves in the warm environment. L-dopa was administered to younger animals to see if this would improve their thermal choice performance. Fowler and Kellogg (1975) argued that the important effect of L-dopa administration was to increase motor activity. They found that although motor activity was increased the administration of L-dopa did not increase positive

thermotaxic resposes in animals less than 6 days of age. This is in contrast to Johanson's maternal deprivation induced activation which suggests that maternal deprivation causes more than mere activation of the motor system but instead a more general arousal. From these studies it seems likely that young rat pups do indeed respond to environmental temperatures by choosing a warm environment and at least to some extent use this behavior for thermoregulation.

As previously mentioned, thermotaxis is not the only thermoregulatory behavior available to young rats. One extremely important behavior is that of huddling with littermates. Alberts (1978a) has shown that for rat pups from 5 to 20 days of age, huddling reduces oxygen consumption elicited by cool temperatures. Huddling also attenuated the rate of heat loss as measured by rectal temperature when 5 and 10 day old rats were removed from the nest and placed at room temperature. Pups placed singly in this condition lost heat at a significantly greater rate than did groups of rats. Additionally, the number of rats participating in a huddle was inversely related to metabolic expenditure as measured by oxygen consumption. Another significant finding of Alberts (1978b) work was that the total surface area of a huddling group correlates with ambient temperature so that more surface area is found at higher temperatures. Thus it appears that young rat pups use the huddling response to thermoregulate and this response is at least partially determined by sensitivity to

external thermal cues.

In the present study one aspect of temperature regulation that will be examined is that of behavioral thermoregulation. If early weaned animals have disrupted protection against heat loss, then in a temperature selection paradigm they should select warmer temperatures than normally weaned animals. Other mechanisms for behavioral thermoregulation in response to both heat and cold challenges will also be observed in early and normally weaned animals.

Neural Substrates for Thermoregulation

The search for the neural basis for thermoregulation has been actively going on since the mid nineteenth century. In 1885 Aronsohn and Sachs used a "piqure" needle to destroy part of what is now known as the anterior hypothalamus of rabbits. Puncturing this area resulted in animals with "fever like" temperatures from 40-41 °C. These animals also had a concomitant increase of oxygen consumption of from 8-20% per degree of body temperature rise. Electrical stimulation of this brain area caused body temperature increases. These results led Aronsohn and Sachs to conclude that the anterior hypothalamus acted as a "thermostat" controlling body temperature.

In addition to lesioning and stimulating portions of the brain, another strategy for localizing the brain area responsible for body

temperature regulation has been transecting the brain at various levels. Isenschmid and Krehl (1912) made cuts at the level of the telencephalon, mesenchephalon and diencephalon of rabbits. Whenever the diencephalon was destroyed or separated from the mammilary bodies animals became essentially poikilothermic. The area described by Isenschmid and Krehl now known as the posterior hypothalamus, is indeed necessary for normal thermoregulation. Thus at the beginning of the 20th century two different brain "heat centers" had been described. Later work concentrated on the mechanisms by which these areas functioned to control body temperature. Barbour (1912) was the first to use thermal stimulation to investigate the brain area described by Aronsohn and Sachs. Using a double concentric cannula through which he could pass water of varying temperatures, Barbour (1912) found that warming this brain area led to heat loss responses, while cooling led to what he called 'kaltefieber', that is, cold induced heat production.

Magoun and his coworkers (1938) used diathermic stimulation of specific areas of the hypothalamus and confirmed Barbours earlier results. Hemingway, Rasmussen, Wickoff and Rasmussen (1940) used diathermic stimulation in dogs and found that very different physiological effects were produced when the anterior and posterior hypothalamus were the target of the external heating. Heating of the anterior hypothalamus led to suppression of shivering and peripheral vasodilation in the cold, while heating the posterior hypothalamus did

not lead to these responses. Lesion studies had shown that cats with anterior hypothalamic damage could not maintain body temperature in a warm environment, but could maintain body temperature in the cold (Clark, Magoun, & Ranson, 1939). Cats with lesions in the posterior hypothalamus could not maintain their temperatures in either condition. This and other work led to a dual-center temperature control hypothesis with the anterior hypothalamus being responsible for heat loss mechanisms and the posterior hypothalamus being responsible for heat production mechanisms Ranson, 1940).

The dual center hypothesis has been called into question in recent years. Rather particular brain areas seem responsible for particular thermoregulatory functions. Satinoff (1974) argues for a hierarchical organization of brain areas responsible for thermoregulation. She cites evidence that there are temperature sensitive neurons in the spine, at the level of the pons, midbrain, and telencephalon as well as the hypothalamus, and notes that transecting the brain at various levels below the hypothalamus does not lead to a total lack of thermoregulation. An experiment by Chambers, Seigel, Liu, and Liu (1974) supports the concept of a hierarchical organization of the brain with regard to temperature regulation. Using cats, Chambers et al. (1974) first cooled the spinal cord of unanesthetized cats. This led to shivering, vasoconstriction, and piloerection, while core temperature was maintained. Decerebration of these animals at the

level of the colliculi abolished these responses and rectal temperatures fell. Decerebration at the level of the caudal pons reinstated the responses to cooling. As Satinoff (1974) points out this suggests that the spinal cord and medulla facilitate responses to cold. Normally these areas are inhibited by the midbrain, which explains why high level decerebration leads to an abolition of thermoregulatory responses. Lower level decerebration releases the medullary and spinal centers from inhibition and thus thermoregulatory responses can be observed.

Other work has led to the idea that particular brain regions are responsible for specific thermoregulatory responses. Roberts and Mooney (1974) noted that there are a number of heat reducing responses typically seen in the rat. Among these are grooming (saliva spreading) escape, prone extention, and vasodilation. Roberts and Mooney (1974) looked for brain areas sensitive to warming. They used diathermic warming of particular brain areas while ambient temperatures were kept at 21 to 23 $^{\circ}$C. They were able to elicit all of these thermoregulatory responses but responses were selectively elicited by specific placement of diathermic electrodes. Specifically, prone extention was elicited by placement in the anterior preoptic area. Locomotion was elicited by placement in the ventral midbrain and dorsal medulla. Vasodilation was elicited by placement in the anterior preoptic area, the septal area, and the midbrain. Further evidence for the

dissociation of various thermoregulatory responses was demonstrated in an experiment in which rats were warmed either peripherally via a warm cage floor or more centrally by subcutaneous heaters located in the nasal cavity, pharynx and esophagus. Peripheral warming led to grooming, locomotion and vasodilation but not to prone extention. Central warming was able to elicit prone extention, and different threshold temperatures were found for the other behaviors (Roberts & Martin, 1974). This separation of thermoregulatory responses was also found in other laboratory rodents (Roberts, Mooney, & Martin, 1974). Roberts and Martin (1977) provided more evidence for specific brain areas controlling specific thermoregulatory responses by selectively lesioning thermosensitive brain areas. Lesions in the preoptic anterior hypothalamus abolished the response of prone extention to ambient temperatures of 37 $^{\circ}$C, but had no effect on locomotion, grooming or tail vasodilation. Lesioning the medial midbrain led to reduced body extention but left other responses intact. Lesions in the septum, dorsomedial medulla, and posterior hypothalamus had few significant effects on the thermoregulatory behaviors that were measured.

There is also evidence for different neurological substrates for certain behavioral and physiological thermoregulatory responses. Weiss and Laties (1961) demonstrated that cold rats would bar press for the reinforcement of a brief pulse from a heat source. It should be

noted that rats that were either hypothyroid or fed a diet deficient in pantothenic acid, and they were found to bar press for heat at higher rates than normal rats when normothermic. Satinoff and Shan (1971) used the operant paradigm to test the effects of various brain lesions. They found that lesions of the lateral hypothalamus abolished the behavioral response of bar pressing for heat reinforcement, but left physiological thermoregulation intact. Lesions of the preoptic anterior hypothalamus led to a loss of physiological mechanisms for regulating temperature but left the behavioral responses of bar pressing for heat reinforcement and nest building intact.

These experiments suggest that specific thermoregulatory responses are linked to specific brain areas. In the current studies there are no manipulations of the brain per se. However, identification of specific disturbances in thermoregulation that early weaned animals may display should allow one to infer some of the specific CNS regions that might be involved.

Nutrition, Body Weight Ontogeny, and Homeothermy

Rats weaned at 15 days of age weigh significantly less at 30 days of age than do rats weaned at 21 days of age (Ackerman et al. 1978b). This fact has a number of consequences for temperature regulation, especially as early weaning necessitates nutritional restriction. A number of factors in the mother-infant relationship could influence the increase in gastric erosion susceptibility in rats separated early from their mothers. To test whether loss of behavioral interaction with the mother or loss of maternal milk was most important Ackerman et al. (1978b) separated rats at postnatal day 15. Litters were assigned to one of four conditions. In the "normal" condition rats remained with their mothers until day 21. In the "early weaned" condition rats were removed from mothers at day 15 and fed ground and solid rat chow. The "behavioral interaction" group remained with their mothers. However, in this group the mothers teats were ligated on day 15, and young rats had access to only ground or solid rat chow. The "milk fed" group was removed from their mothers on day 15 but fed a high fat milk diet from day 15 to day 21. All groups were fed only rat chow from day 21 until day 30. When all animals were 30 days of age they were food deprived for 24 hours at an ambient temperature of 20 °C, and subsequently restrained in individual wire mesh cones for 24 hours at an ambient temperature of 20 °C. Animals that had been left with non-lactating

mothers showed increased gastric lesion susceptibility while prematurely separated high fat fed animals showed susceptibility equivalent to control animals (Ackerman et al. 1978).

The mechanisms by which nutritional restriction leads to erosion susceptibility remains to be examined. However, a connection between nutritional restriction and impaired thermoregulation has been shown. Blackmore (1972) separated young rats from their mothers for limited periods of time each day during development and then tested the temperature at which animals used minimum oxygen. The age when a given thermoneutral temperature was reached was delayed by maternal separation and concomitant nutritional deprivation. Greater delays in reaching a particular thermoneutral temperature were found for animals who were subjected to the longest periods of maternal separation. Blackmore also found that increased maternal separation led to lower increases in oxygen consumption response to a given deviation from thermoneutrality. Blackmore suggested that the effects of separation from the mother may be a delay in general development rather than specific metabolic deficiencies.

Bignall, Heggeness, and Palmer (1974) have demonstrated an interaction between acute starvation and metabolic response to cold in young rats. This response is altered during the ontogeny of the rat. When pups of 5, 10, 15 or 20 days of age were exposed to temperatures equivalent to thermoneutrality, oxygen consumption was maintained at a

constant level even after 20 hours of starvation. In contrast pups responded to cold exposure with an initial increase in oxygen consumption. Five and 10 day old pups responded to starvation during cold exposure with a decrease in oxygen consumption which was more intense as starvation was prolonged. The metabolic increase to cold that is normally observed was completely abolished by 10 hours of starvation in animals 5 and 10 days old. In 20 day old animals no alteration of oxygen consumption in response to cold exposure was seen even after 20 hours of starvation.

Bignall et al. (1974) considered possible mechanisms for the interaction of starvation and cold in effecting oxygen consumption. It seemed unlikely that starvation for the short periods of time used in testing would reflect differences in heat conservation, thus differences in the capacity for heat production were examined. Exhaustion of food stores was an unsatisfactory explanation as estimates of energy available from fuel stores far exceeded the energy needed to maintain body temperaure in the cold. Rather an unspecified active CNS inhibitory mechanism was proposed. This active suppression of heat production would be advantageous for conserving body stores during times of nutritional deprivation. Analagous mechanisms are thought to exist for adult animals in hibernation, with a slowing of metabolism leading to conservation of body stores. It should be noted that changes in the functioning of brown adipose tissue are thought to

be involved in the initiation and termination of hibernation. Bignall et al. (1974) proposed that during ontogeny the effectiveness of this CNS inhibitory system declines. While during this same time period animals heat conserving capabilities are rapidly increasing. Thus at weaning acute starvation does not lead to a slowing of metabolism in cold environments, and additionally, the animals conserves what heat is produced via additional insulation.

Early weaning could disrupt or delay the normal progress away from active inhibition of the metabolic response to cold. Thus the acute starvation which normally precedes the restraint paradigm typically used by Ackerman et al. (1975, 1978a, 1978b) to induce gastric lesions, could inhibit thermoregulation in these animals. Differences in heat production during starvation and cold would be expected for early weaned animals if delay in maturation of this unknown CNS inhibitory was occuring. Heat production during starvation and restraint at 30 days of age may be disrupted for early weaned animals. This will be tested by measuring oxygen consumption for early and normally weaned animals during food deprivation and restraint at day 30.

As noted previously early weaned animals are smaller than normally weaned animals at 30 days of age. This difference is largely abolished by feeding of a high fat diet (Ackerman et at., 1978b). This suggests that fat deposits may be important in thermoregulation. Fat deposits have two important functions in thermoregulation. One is as

insulation, and the other is in supplying a source of energy for heat production. Heat conservation in the mammal is largely accomplished by subcutaneous fat, skin, and hair. In the rat skin thickness increases rapidly from conception to 16 days of life. After this time a 35 day cycle of thickening and thinning of skin layers occurs, with skin gradually thickening to adult dimensions (Butcher, 1943). Hair first emerges on the second day postnatally, with an increase in length for 18 to 20 days thereafter. Hair follicles show little change from 20 to 56 days postnatally at which time adult hairs are added to those already present (Adolph, 1957). The total skin and hair is 200 times as thick in the adult rat as in newly born animals. Thus heat conservation is drastically reduced in young animals.

It has been shown that adipocyte development in the rat continues from birth till 180 days of age (Greenwood & Hirsch, 1974). The greatest proliferation of adipocytes is in rats less than 35 days of age. After 180 days, adipocyte number becomes stable and any change in quantity of body fat is due to increase in adipocyte size (Greenwood & Hirsch, 1974). Cells that proliferate, such as adipocyte cells have been shown to do so at differing rates depending on several environmental factors. Among these factors are temperature and dietary intake (Cameron, 1971; Faust, Johnson, & Hirsch, 1977). Food restriction before 35 days of age in rats leads to permanent deficiencies in adipocyte number. Specifically reductions of up to 40%

of epididymal fat pads are seen. Conversely weanling rats fed a high fat diet are able to regenerate surgically removed subcutaneous fat pads (Faust et al.,1977). Early weaning is thus likely to effect adipocyte proliferation both directly through nutritional deprivation and indirectly through temperature changes. Feeding of high fat diets to early weaned rats enables them to resist hypothermia and gastric lesions. This supports the idea that differences in the quantity of fat may be of importance in sustaining normal thermoregulation. In the current studies quantitative differences in fat deposits of rats weaned at 15 or 21 days will be examined.

As previously noted early weaned animals are smaller than normally weaned animals, and there is some evidence that body mass per se is important in thermoregulation. Farkas and Donhoffer (1974) report no correlation between body weight and fall in core temperature in response to cold for either neonatal guinea pigs, or rabbits. The cold stimulus was however, a moderate one. Close and Mount (1978) and Close, Mount and Brown (1978), however, found that guinea pigs who weighed more were more resistant to low ambient temperatures. They also found that animals who were fed a diet with a high proportion of fats were more cold resistant than animals fed low fat or normally balanced diets. In human infants studied with a direct calorimetric method, responses at either 28 °C, 32 °C or 37 °C showed no significant differences in heat loss in terms of body surface area for small as

opposed to large neonates. However, differences were found in rates of heat loss relative to body weight. It was suggested that the larger surface area relative to body weight allows for more heat loss due to convection for smaller rather than larger infants (Sulyok, Jequier, & Prod'hom, 1976). The greater loss of heat due to body surface area to body weight ratios is supported in animal research as well (Brody, 1945; Conklin & Heggeness, 1971). Early weaned animals may therefore be compromised in their ability to thermoregulate not only by having less insulation due to inadequate nutrition but also merely by being smaller.

Brown Adipose Tissue and Nonshivering Thermogenesis

Although young rats do not respond to cold by such activities as shivering they are able to produce heat. When young animals are exposed to cold an initial increase in oxygen consumption is observed that is not associated with muscular activity. This response is referred to as nonshivering thermogenesis (NST). Young animals have been shown to increase metabolism with no associated increases in electrical activity of the musculature (Bruck, 1961).

In young rats NST has been shown to be based mainly on the calorigenic effects of norepinepherine (NE) on brown adipose tissue (Moore & Underwood, 1963). NE is released by the sympathetic nervous

system in response to cold (Hsieh & Carlson, 1957), and is known to occur in higher concentrations in young rats compared to adults (Adolph, 1957). Two types of NST have been delineated (Jansky, 1973): first, obligatory or basal NST, which refers to the heat production occurring normally to maintain basal metabolism; second, regulatory NST which occurs at ambient temperatures below thermoneutality. Newborn rats are able to exhibit regulatory NST of approximately 2 to 3 times the basal metabolic rate, but are unable to maintain body temperature due to inadequate heat conservation capabilities (Jansky, 1973).

It has been suggested that the disruption of regulatory NST in young rats that is exhibited as a decreased response to cold is due to a decreased release of endogenous NE (Bignall et al. 1977). Stimulation of endogenous NE by administration of 6-Hydroxydopamine elevated metabolic rates of 5 day old rat pups that were exposed to cold. This response was eliminated by administration of reserpine. Reserpine acts to increase membrane permeability of biological amines. As NE is released it is destroyed by monoamine oxidase, the result is depletion of NE from cells. If heat production is less for early weaned animals than for normally weaned animals exogenous stimulation of heat production via NE might correct this deficiency. If this were the case and heat production deficits were important in gastric erosion production, then exogenous administration of NE might lead to fewer

gastric erosions in early weaned rats. On the other hand if early weaned animals were already responding with maximal metabolic output during food deprivation and restraint then administration of exogenous NE would not increase heat production and would not serve to protect against gastric erosion formation.

For young animals an important way in which NE exerts its calorigenic effect is by the mobilization of brown adipose tissue. Brown adipose tissue has been described as an organ of heat generation (Himms-Hagen & Desautels, 1978). Brown adipose tissue contains specialized mitochondria that release energy as heat rather than storing it as ATP (Himms-Hagen & Desautel, 1978; Nicholls, 1979). Young animals including humans have a substantially higher proportion of brown fat to white fat tissue than do adults (Heaton, 1972; Smith & Horowitz, 1969). There is a great deal of evidence that NST in young mammals mainly depends on the heat production of brown adipose tissue (Smith & Horowitz, 1969; Hull & Segall, 1965a; 1965b). Normal newborn rabbits show increased oxygen consumption when exposed to cold or when injected with NE (Hull & Segall, 1965a). When interscapular brown fat was excised in newborn rabbits the metabolic response to both cold and NE administration was abolished. It was also found that the metabolic response to cold and NE decreased in proportion to the degree of the extirpation of brown adipose tissue.

Other experiments showed that the amount of brown adipose tissue

young rabbits had partially depended on the fat content of the diet, with greater amounts of brown adipose tissue being correlated with higher fat content of the diet (Hull & Segall, 1965b). Heim and Hull (1965) found that in newborn rabbits the amount of oxygen that is extracted from the blood that has passed through the brown adipose tissue accounts for two thirds of all NST. In adult rats brown adipose tissue accounts for only about 8% of total NST (Imai, Horowitz, & Smith, 19[illegible]). Jansky (1973) measured the metabolic capacity of brown fat as exhibited in cytochrome oxidase activity. He found that the heat production capacity of brown fat is adequate to account for total NST in the rat from birth to 21 days of age. After that time the available brown fat cannot account for total NST. Thus, as animals normally mature they depend less on brown adipose tissue for their total thermogenesis.

If early weaned animals show a generalized delay of maturation, they may be slower to lessen their reliance upon brown adipose tissue for thermogenesis. If it is also true that they have less fat and less brown fat due to dietary inadequacy then they may be doubly compromised in there ability to generate heat. The current studies will examine both the amount of brown adipose tissue available to early weaned and normally weaned rats and how they metabolize what fat they do have.

It is possible that along with quantitative deficiencies in brown adipose tissue early weaned animals may be compromised in their ability

to mobilize what brown adipose tissue they have available. It has been shown in rats less than 2 weeks of age that blocking the β-adrenergic receptors with propranalol leads to a 60% fall in oxygen consumption, with an accompanying decrease in colonic temperature for animals exposed to cold. As animals matured colonic temperature was less affected even when oxygen consumption was similarly depressed (Pinkerton, 1976). Presumably this is due to the improved heat conservation capacities of older animals. Other investigators have used α and β-adrenergic agonists and antagonists. Both types of adrenergic receptors are associated with activation of brown adipose tissue, although heat output is thought to be via β-adrenergic receptors alone (Flain, Horowitz & Horowitz, 1977).

In the present study while oxygen consumption is measured, exogenous NE will be administered at various times during food deprivation and restraint of early and normally weaned animals 30 days postnatally. This will allow for assessment of the ability of early and normally weaned animals to activate the heat production capabilities of brown adipose tissue.

To summarize, previous work has shown that premature weaning effects temperature regulation. Specifically the loss of the high fat content of mothers milk leads to this deficit. Temperature regulation is important in altering susceptibility to gastric erosions resulting from restraint. The purpose of the present work will be to investigate

three main issues. First, does the nutritional deprivation associated with early weaning lead to differences in body fat content of 30 day old rats, and how might differences in adiposity and fat utilization affect thermoregulation under conditions that lead to increased RGE susceptibility. Second, does early weaning effect the behavioral thermoregulation of 30 day old rats. Third, how does early weaning affect the oxygen consumption of 30 day old rats.

Thermoregulatory mechanisms can be categorized in the following ways:

1. mechanisms involved in the insulation of the animal
2. behavioral mechanisms for heat production or heat conservation
3. physiological mechanisms of heat production or heat conservation

Differences between early and normally weaned animals will be investigated in these areas. Aspects of thermoregulation will be viewed in relationship to gastric lesion susceptibility. Manipulation of variables found to be important in differentiating early and normally weaned animals thermoregulation should be reflected in differing ulcer susceptibility.

PART I: BODY FAT CONTENT AND GASTRIC ULCER SUSCEPTIBILITY

Introduction

Thirty day old rats weaned prematurely at postnatal day 15 (15w) have increased susceptibility to gastric erosions induced by food deprivation and restraint as compared to rats weaned normally at postnatal day 21 (21w) (Ackerman et al. 1975). Body temperature regulation is important in the rat's susceptibility to restraint induced gastric erosions (RGEs). In 30 day old rats the occurrence of gastric erosions is highly correlated with the tendency to become hypothermic when subjected to food deprivation and restraint at normal room temperatures (Ackerman et al. 1978a). Also early weaned animals can be protected from forming RGEs by raising the ambient temperature at which they are food deprived and restrained. Conversely RGEs can be induced in normally weaned animals by lowering the ambient temperature at the time of food deprivation and restraint (Ackerman, 1981).

In adult rats the incidence of RGEs is inversely correlated with ambient temperature (Martin, Martin & Lambert, 1970). Additionaly adult rats that are restrained at low ambient temperature (4-5 $^{\circ}$C) will form RGEs in 1 or 2 hours (Senay & Levine, 1967). The mechanism by which a failure to regulate body temperature is related to gastric

pathology is unknown. However, Ackerman (1981) has shown that lowering ambient temperatures in awake restrained rats leads to increased gastric acid secretion. This increase in acid secretion may affect gastric pathology when an animal's body temperature falls. In any case maintainence of body temperature seems crucial to protecting against RGEs. The present study will examine some ways in which 15w animals may be compromised in their ability to thermoregulate.

As previously described, Ackerman et al. (1978b) demonstrated that milk loss rather than loss of behavioral interaction with the mother is the critical factor leading to 15w rats greater RGE susceptibility when tested at 30 days of age. Alterations in dietary fat are known to effect alterations in lipogeneis and lipolysis (Greenwood & Hirsch, 1974), and early nutritional status is known to be one of the factors determining the size and number of adipocytes (Knittle & Hirsch, 1968). Decreased adiposity would have a number of consequences in terms of body temperature regulation. In the laboratory 30 day old 15w rats are found to be an average of 14% lighter than 21w rats (Ackerman et al. 1978b) The size of the 15w animals leaves them at a disadvantage in their ability to conserve heat, due to a high surface area to body weight ratio. A small body size is known to reduce an animals ability to conserve heat (Farkas & Donhoffer, 1974).

Decreased adiposity could also lead to hypothermia due to a lack

of insulation. Lack of subcutaneous fat would also decrease heat conservation. In addition, lack of adequate energy stores could compromise 15w animals ability to generate heat during food deprivation and restraint. I hypothesized that the nutritional deprivation caused by early weaning would lead to differences in body composition. Specifically I hypothesized that 15w rats would have less white and brown adipose tissue as compared to 21w rats. I further hypothesized that reduced body fat would lead to a compromised ability to both generate and conserve body temperature.

An important means of generating heat for young rats is through brown adipose tissue (BAT) (Jansky, 1973). If early weaned animals have decreased stores of BAT as well as lessened general adiposity then they would also be compromised in their abilty to produce heat via nonshivering thermogenesis (NST). NST refers to heat produced without involving muscular activity. BAT has been described as an organ of thermogenesis, and supplies up to 65% of heat derived from nonshivering thermogenesis in young rats (Jansky, 1973).

The present study adresses the question of whether body fat content differs in 15w and 21w rats. I will examine whether fat differences are related to the ability to maintain body temperature during food deprivation and restraint. I also investigate whether differences in fat content can account for differential lesion susceptibility.

Part I -- Experiment 1

The first experiment examines quantitative differences in adiposity of 30 day old, 15w and 21w rats subjected to the same conditions of food deprivation and physical restraint that have been found to differentially elicit RGEs in these animals (Ackerman et al. 1978b). Differences in white and brown fat mass of 15w and 21w rats were examined. Differences in the weights of skin punch biopsies were used as an indicator of relative quantity of subcutaneous adipose tissue of 15w and 21w rats. Decreased skin thickness would be especially important in heat conservation ability, while differences in adiposity would be important both for heat conservation and heat production.

Method

Subjects

Albino rats of a Wistar derived strain born in the laboratory served as subjects. One day after birth all litters are culled to 12 animals with equal distribution of sexes when possible. Litters were not used if fewer that 8 pups were present at birth. Thus litter size from birth to day 15 is from 8 to 12 pups. Two types of litter

conditions are used throughout these studies the first condition I will call the split litter condition. In the split litter condition at the time of early separation, which was from day 14 to day 16 one half of the litter was removed and placed in a 24 liter plexiglass tank. In the split litter condition only litters with 12 pups were used. For split litters those animals separated early were matched for sex and body weight with those animals that remained with the mother. The other condition is what I will call the whole litter condition. In this condition an entire litter is either prematurely weaned at day 14-16 or the entire litter is normally weaned at day 21.

Criteria for premature separation were that at least 2 pups in a litter would have open eyes and the average weight of pups was at least 28 g. These criteria were used since it has been found that if average body weight is than this or pups are less developed, as indicated by eye opening, survival rates are drastically reduced, and weaning is accomplished as early as possible to still have high survival rates (Ackerman, personal communication 1981). Although I refer to these rats as separated on day 15, this is the average day of separation. If litters met these criteria on day 14 they were separated at that time and if litters did not meet these criteria until day 16 they were then separated. No animals were separated prior to day 14 nor after day 16.

For either split litter or whole litter conditions, following separation rats that were early weaned were fed ground purina lab chow

mixed with warm water as well as dry lab chow pellets. Early weaned rats were maintained in the plexiglass tanks on heating pads until 21 days of age. Maintainence on heating pads has been shown to greatly reduce their mortality (Hofer, 1975). However, early weaned rats still have increased mortality rates such that 25-40 % more early than normally weaned animals die prior to age 21. It should be noted that as wet mash was placed in open glass containers on the cage floor the wet food mash was also kept warm by the heating pads. At 21 days of age pups were transferred to 18 X 35 X 18 cm standard wire mesh cages, where they remained undisturbed until day 30. Animals separated from their dams at 21 days of age were moved with their littermates to standard wire mesh cages at that time.

For both weaning conditions from 21 to 30 days of age rats were kept with littermates of the same weaning condition and fed only purina lab chow pellets. Water was available freely at all times. Subjects were tested from day 28 to day 30 postnatally. Animals were maintained on a 12:12 day/night cycle with lights on at 6:00 am. The ambient temperature in the animal housing room was relatively constant at 22 °C ± 2 °C.

Procedure

In this experiment 10 early weaned and 10 normally weaned litters from the whole litter condition were used. Littermates of a given weaning condition were matched for weight and sex when animals were 28 days old. Half the 28 day old animals of each litter were sacrificed and autopsied immediately. The other half litter was first food deprived as a group for 26 hours at 20 °C and subsequently restrained in individual wire mesh cones for an additional 26 hours at 20 °C. The size of the wire mesh cone allows the animal to just turn around but not to fully extend hindlimbs or forelimbs. Body weights were determined with an Ohaus balance (model 1600) that allows weight to be determined within ± .5 g at the start of the experiment, after food deprivation and following restraint. Rectal temperatures were determined with a thermal probe at these same times. Following restraint animals were immediately sacrificed and autopsied.

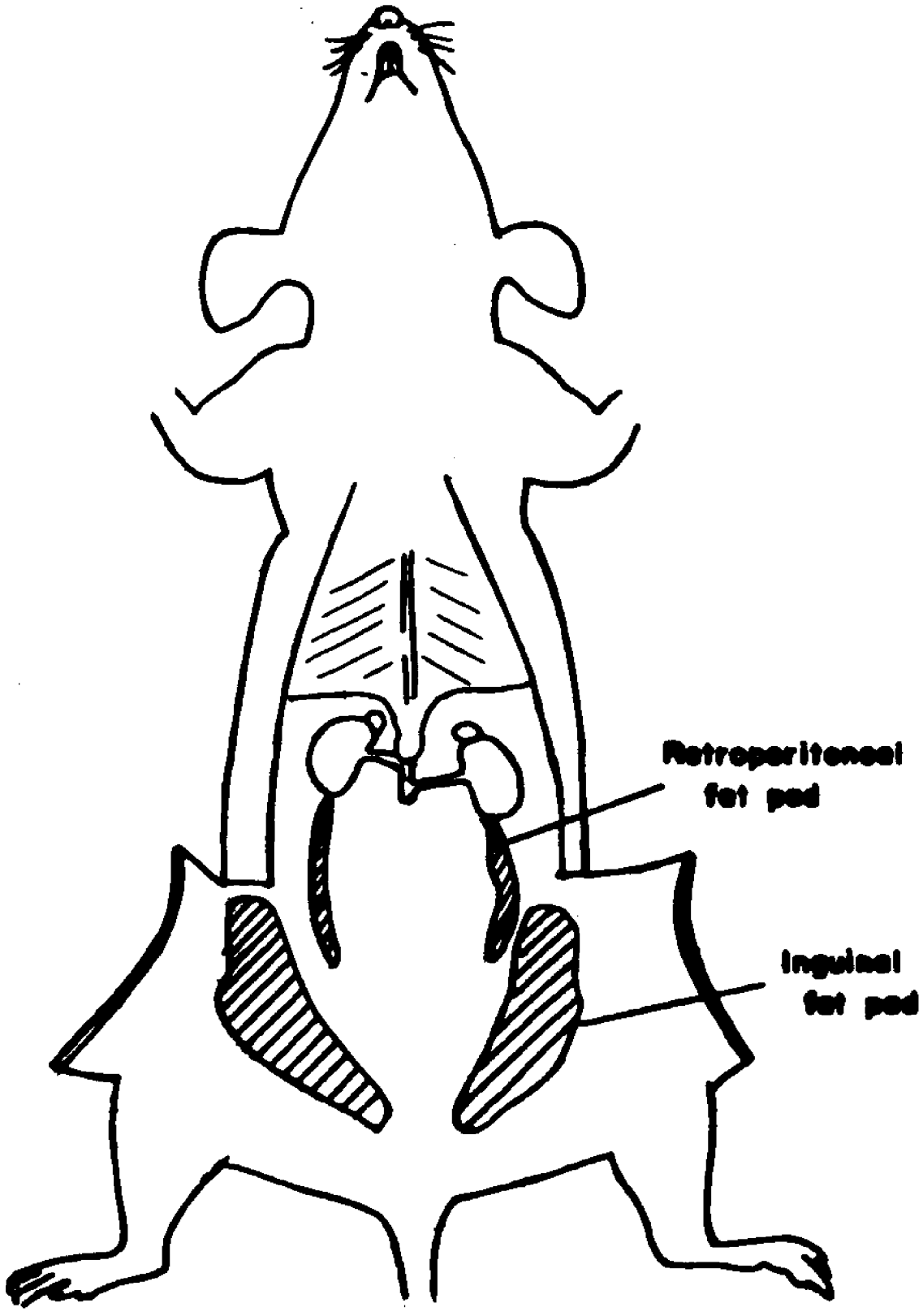
At autopsy stomachs were removed, opened along the greater curvature, pinned flat and examined for gastric erosions. Examination of stomachs was performed by a second investigator blind as to the weaning condition of the animal. A dissection microscope with 30x magnification was used to examine the gastric mucosa. The number of gastric erosions were noted, and their severity was estimated by

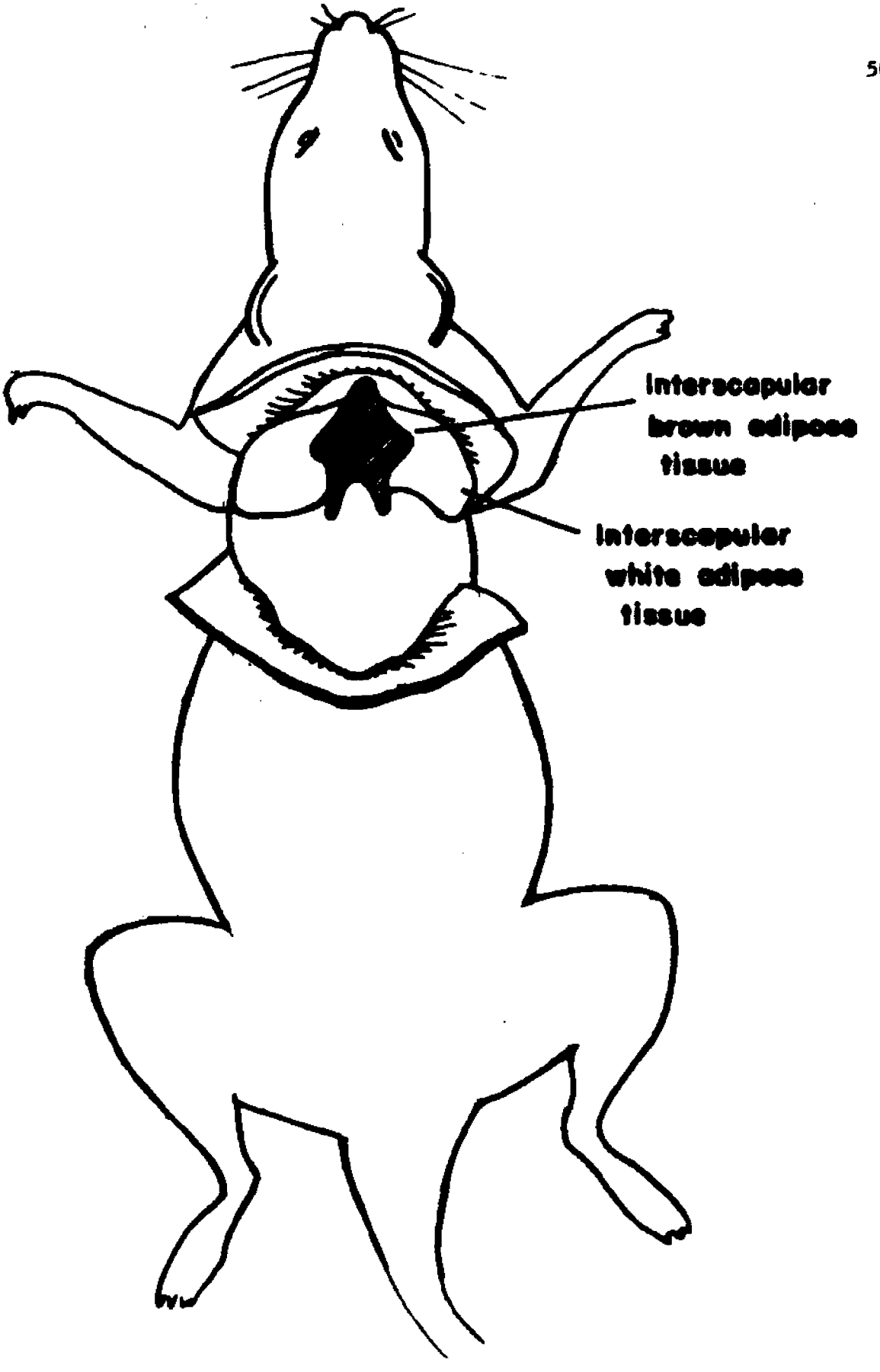
measuring the total length of lesions for each animal.

At autopsy I excised and weighed inguinal, retroperitoneal and interscapular white fat pads, as well as interscapular brown adipose tissue. Excision of fat pads was by dissection using a technique adapted from Faust, Johnson and Hirsch (1976). Figures 1 and 2 show the location of these fat deposits. I also examined skin thickness by weighing .8 cm punch biopsies of skin from four body areas. I took skin biopsies from the interscapular region, the flank, at the sternum and over the inguinal fat depot. Vascular landmarks were used to insure consistency of the areas measured. Fat deposits and skin punch biopsies were weighed using a Mettler analytical balance (model 1-910) which is accurate to within ± 0.0005 g.

Figures 1 and 2

Figures 1 and 2 show the location and approximate extent of the excised adipose deposits. Figure 1 shows the inguinal area at the lower abdomen and leg, and the retroperitoneal behind the kidneys along the spinal region. Figure 2 shows the interscapular white fat immediately below the skin in the interscapular region, and the interscapular BAT, just ventral to the interscapular white fat.


Retroperitoneal
fat pad
Inguinal
fat pad


Interscapular brown adipose tissue
Interscapular white adipose tissue

Results

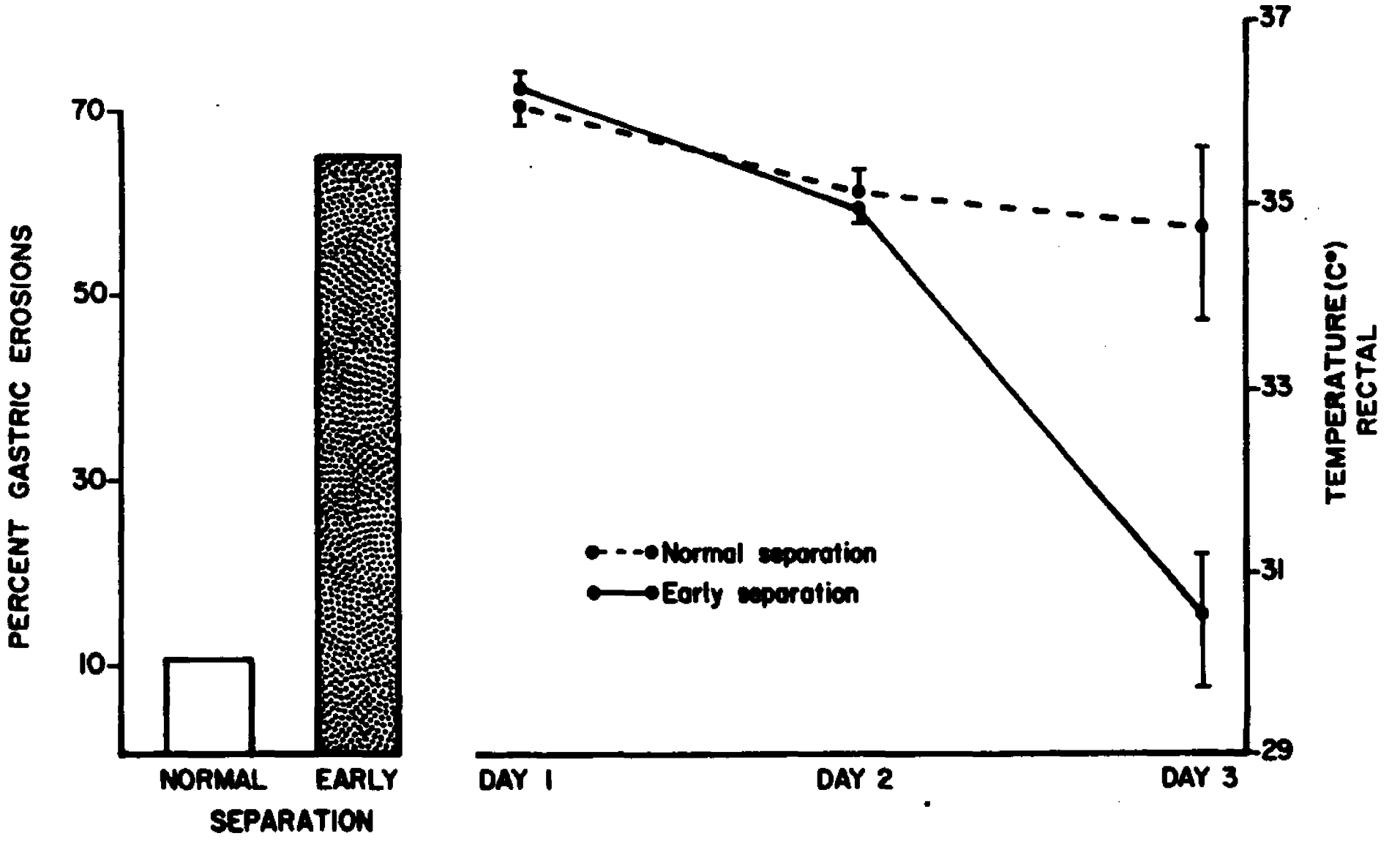
Sixty five percent of 15w animals had RGEs while only 10% of 21w animals had erosions, which replicates previous findings (Ackerman et al. 1975, 1978a, 1978b). This difference was highly significant with 15w rats having greater incidences of gastric erosions than 21w rats $\chi^2(1) = 13.7$, $p<.0001$, (see Figure 3). Early weaned animals also had greater severity of gastric lesions as indicated by the number of lesions each animal had ($M = 5.85$ for 15w rats, $M = 0.18$ for 21w rats) and as indicated by lesion length ($M = 7.54$ mm for 15w rats, $M = 2.1$ mm for 21w rats). For the number of lesions an analysis of variance with litter, sex and weaning condition as factors showed a significant main effect for weaning condition $F(1, 50) = 26.32$, $p<.0001$. Significant main effects were not found for sex ($F(1, 50) = 0.03$, $p>.8$) or litter ($F(18, 50) = 1.39$, $p>.1$), nor were then any significant interactions. for the length of gastric erosions a similar analysis also found main effects for weaning condition ($F(1, 50) = 39.51$ $p<.0001$) and litter ($F(18, 50) = 2.37$, $p<.01$) but not for sex ($F(1, 50) = 1.04$, $p>.3$), and no significant interactions were found.

Animals of different weaning conditions show no differences in body temperature at the start of the experiment ($M = 36.1$ °C for 21w, $M = 36.4$ for 15w). During food deprivation and restraint 15w rats develop significantly lower body temperatures than 21w rats $F(1, 50) =$

42.67, $p<.0001$ (see Figure 3). Body temperatures and number of gastric erosions were highly inversely correlated, (for all animals analyzed together Pearsons $r= -.87$; for 15w rats analyzed alone Pearsons $r= -.88$; for 21w rats analyzed alone Pearsons $r= -.67$. The correlation between lowered body temperature and lesion susceptibility also replicates previous findings (Ackerman, 1981; Ackerman et al. 1978a).

Figure 3

Figure 3 shows the percent of animals who had gastric erosions. This figure also shows the rectal temperatures of early weaned and normally weaned rats. Day 1 is prior to any experimental manipulation. Day 2 is after food deprivation and day 3 is subsequent to restraint.


PERCENT GASTRIC EROSIONS
70
50
30
10
NORMAL
EARLY
SEPARATION
Normal separation
Early separation
DAY 1
DAY 2
DAY 3
37
35
33
31
29
TEMPERATURE(C°)
RECTAL

I found that early weaned animals had significantly less adiposity than did 21w rats. Early weaned animals had smaller fat deposits both prior to and following food deprivation and restraint. This is true both in absolute terms and when fat deposits are expressed as a percentage of total body weight. Figure 4 shows differences in total excised white adipose tissue for 15w and 21w rats prior to and following food deprivation and restraint. Prior to food deprivation and restraint the weight of the total excised white fat of 15w rats is less than 50% of the weight of the total excised white fat of 21w animals. When the amount of fat is expressed as a ratio of fat to body weight 15w animals have significantly less adiposty compaired to 21w animals, $F(1, 50) = 10.4$, $p<.0001$. The means and standard errors of absolute weights for excised brown and white fat are shown in table 1. Means and standard errors of weights of excised fat expressed as a percent of total body weight are shown in table 2.

For fat depletion I inferred that measures determined from littermates sacrificed prior to food deprivation and restraint were comparable to values that would have been measured from animals subjected to food deprivation and restraint. I therefore treated measurements for animals sacrificed prior to food deprivation and restraint as equivalent to measures from a pre restraint condition and measurements for animals sacrificed after food deprivation and restraint as equivalent to measures from a post restraint condtion and

determined change scores as reflecting fat depletion. Although 15w rats used less (in absolute weight) white fat than did 21w animals during food deprivation and restraint (see figure 5) during this time early weaned animals are relatively more depleted of fat following food deprivation and restraint (see figure 6). Early weaned animals have more depleted fat stores for each individual fat pad as well as total excised white fat. However, the effect was most pronounced in the retroperitoneal fat pad. Early weaned animals would often have no visible retroperitoneal fat following food deprivation and restraint. Comparisons of fat used by 15w and 21w animals in absolute terms and of percents of body weight used during food deprivation and restraint are shown in table 3 and 4.

Figure 4

This figure shows the quantities of total excised white fat for early weaned and normally weaned animals. Quantities are for littermates matched for body weight prior to or following food deprivation and restraint. On the left quantities are the absolute weights of adipose tissue, while on the right adipose tissue is expressed in terms of percent body weight.

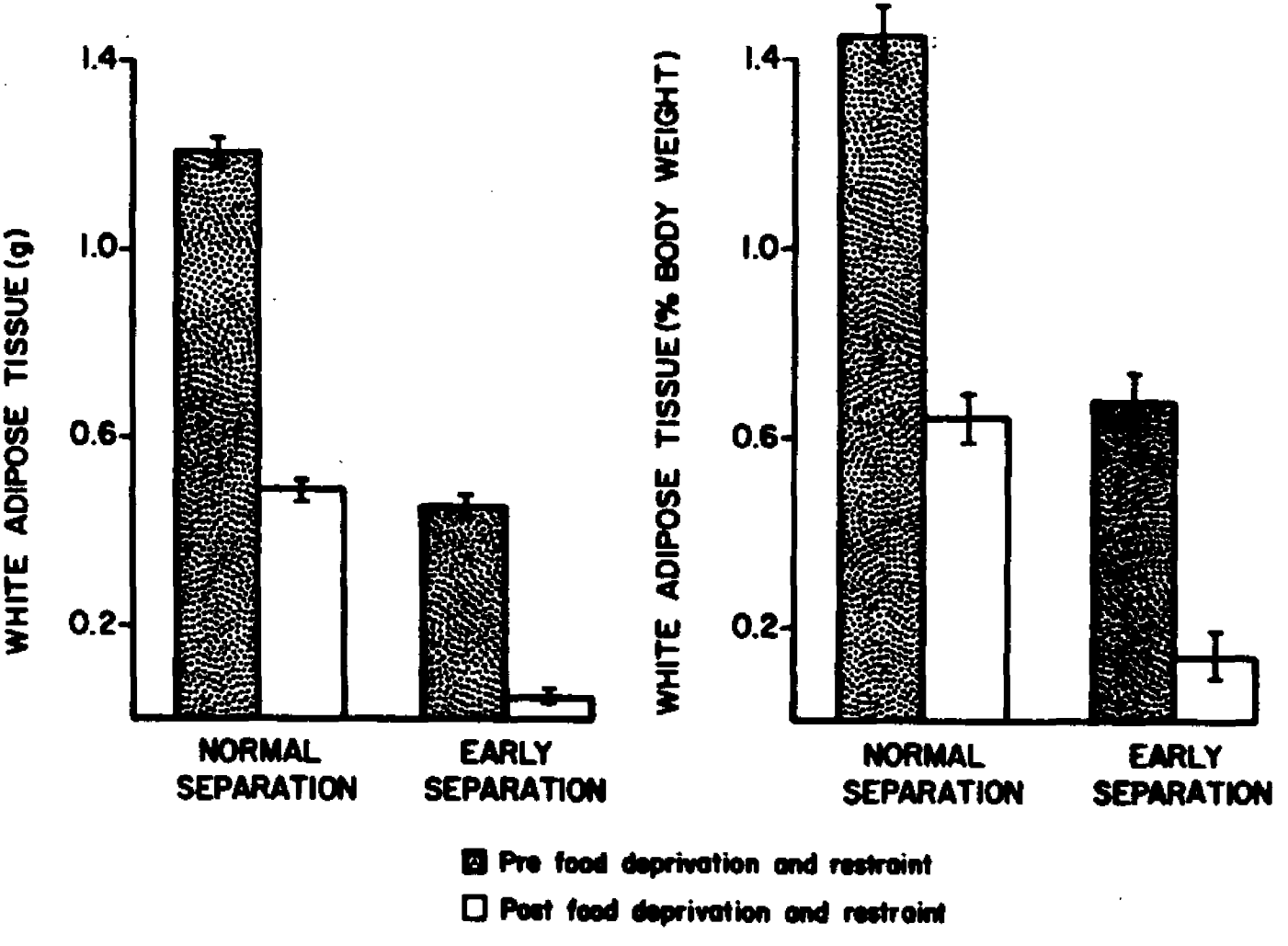
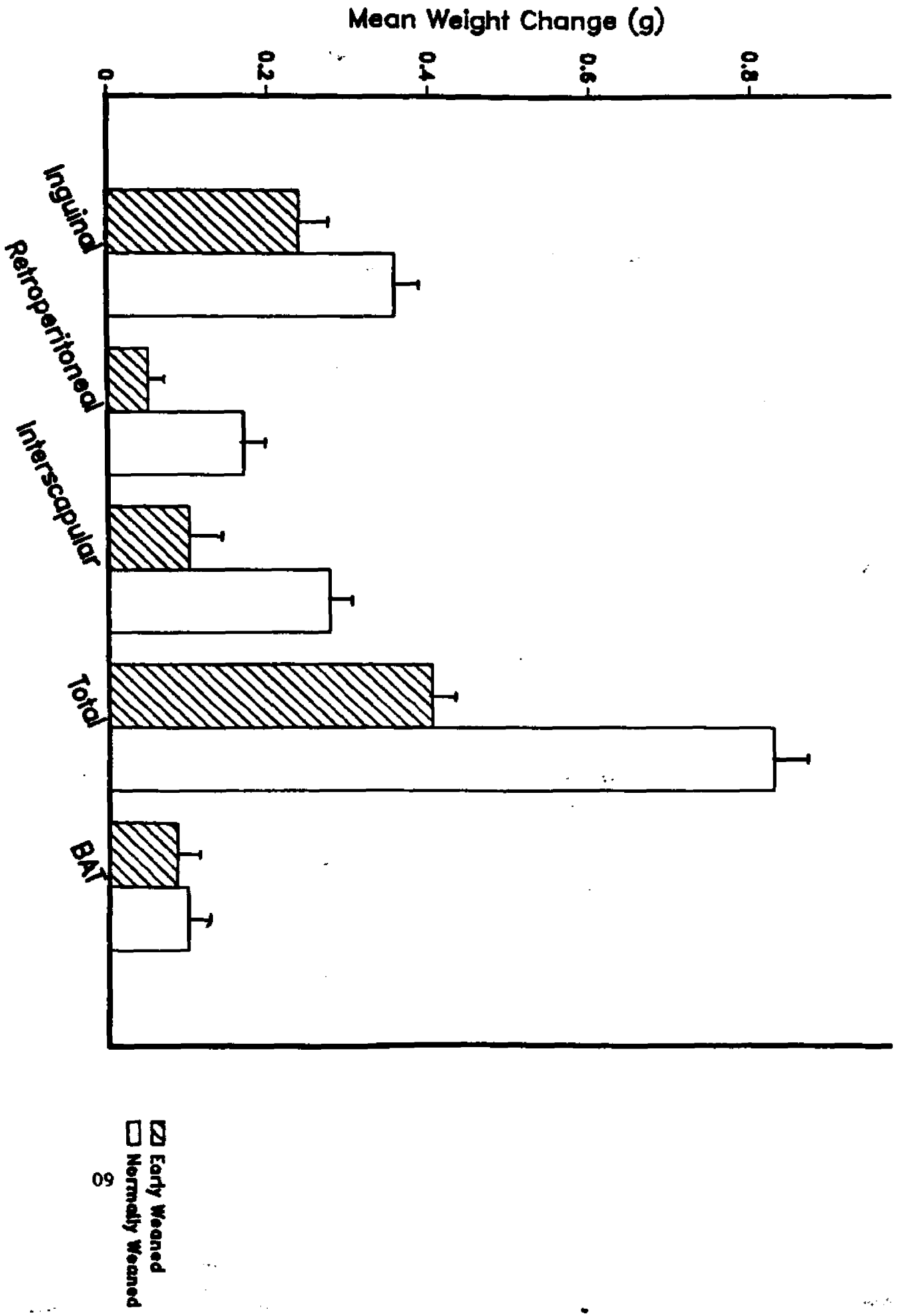

WHITE ADIPOSE TISSUE (g)
1.4
1.0
0.6
0.2
NORMAL SEPARATION
EARLY SEPARATION
WHITE ADIPOSE TISSUE (% BODY WEIGHT)
1.4
1.0
0.6
0.2
NORMAL SEPARATION
EARLY SEPARATION
Pre food deprivation and restraint
Post food deprivation and restraint

Figure 5

This figure shows the average depletion of fat during food deprivation and restraint in absolute terms. Initial weight values for fat pads were determined from animals taken directly from housing cages. Final values were determined from littermates of the "pretreatment" animals, but following food deprivation and restraint.

Figure 6

This figure shows the average depletion of fat during food deprivation and restraint in terms of percent depletion of available fat. Initial weight values for fat pads were determined from "pretreatment" animals, while final value were determined from the "posttreatment" animals subsequent to food deprivation and restraint.


Mean Weight Change (g)
0
0.2
0.4
0.6
0.8
Inguinal
Retroperitoneal
Interscapular
Total
BAT
Early Weaned
Normally Weaned

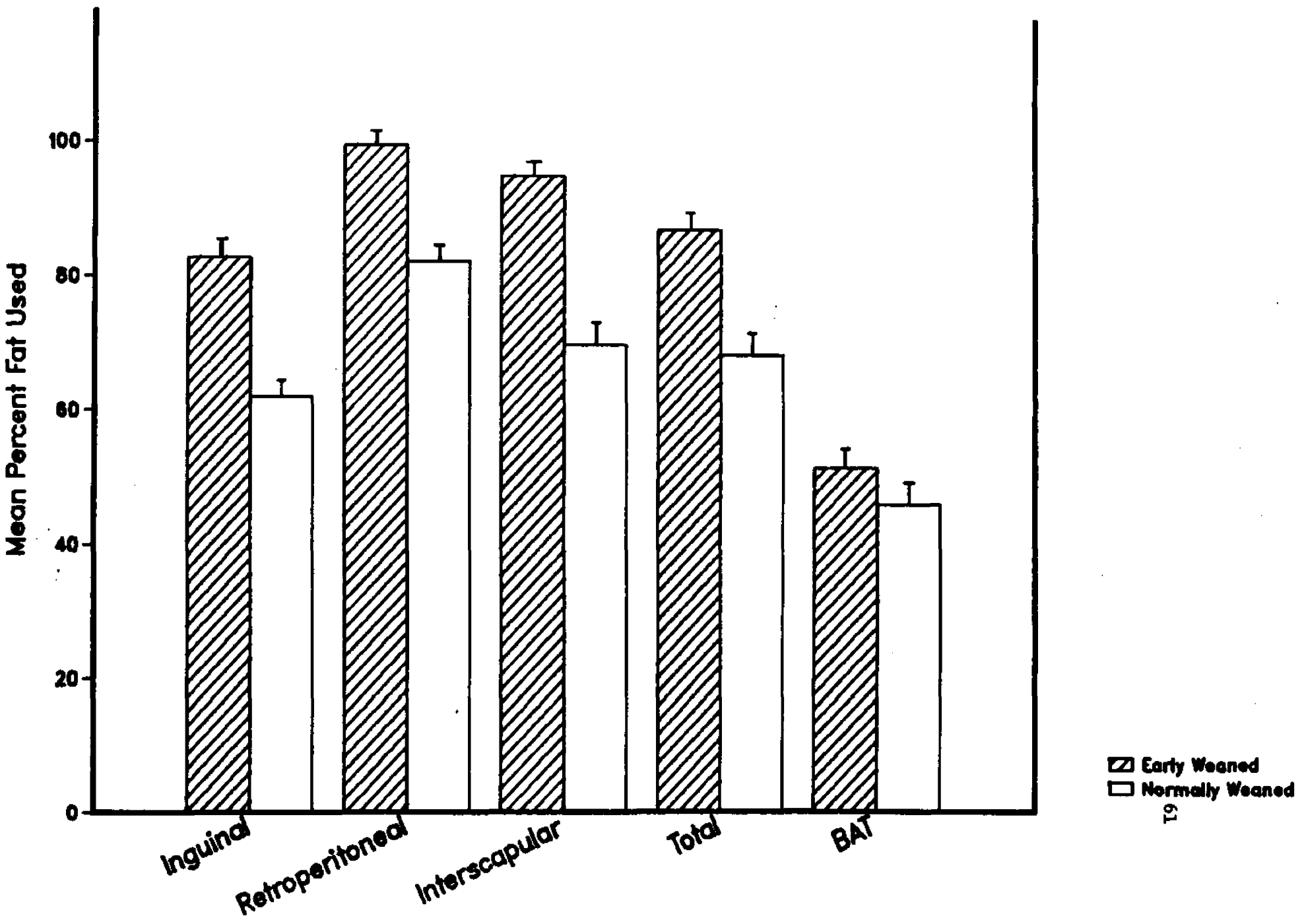

Mean Percent Fat Used
100
80
60
40
20
0
Inguinal
Retroperitoneal
Interscapular
Total
BAT
Early Weaned
Normally Weaned

Table 1--Absolute Weights of Brown and White Adipose Tissue

Fat Pad		Before Restraint				After Restraint			
		15w		21w		15w		21w	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Inguinal	m	.306	.021	.605	.038	.054	.008	.217	.024
	f	.277	.021	.551	.047	.045	.007	.246	.022
R. P.	m	.56	.009	.222	.025	.001	.001	.039	.008
	f	.48	.007	.203	.037	0.0	0.0	.039	.009
I. S. white	m	.018	.013	.407	.051	.005	.002	.107	.020
	f	.109	.012	.395	.052	.006	.002	.123	.015
I. S. BAT	m	.161	.008	.214	.014	.082	.009	.115	.008
	f	.134	.011	.214	.019	.067	.006	.110	.009

Mean weight in grams of excised fat pads. Means are given for both males and females of each group.

R. P. is retroperitoneal
I. S. is interscapular

Table 2-- Weights of Brown and White Adipose Tissue Expressed as a Percent of Total Body Weight

Fat Pad		Before Restraint				After Restraint			
		15w		21w		15w		21w	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Inguinal	m	.443	.026	.704	.033	.119	.016	.349	.031
	f	.442	.024	.692	.050	.109	.015	.432	.035
R. P.	m	.081	.011	.257	.028	.003	.003	.061	.012
	f	.073	.011	.249	.041	0.0	0.0	.067	.015
I. S.	m	.153	.017	.474	.057	.011	.005	.168	.029
white	f	.172	.016	.496	.065	.013	.005	.217	.025
I. S.	m	.236	.012	.250	.016	.185	.021	.192	.013
BAT	f	.218	.018	.270	.021	.165	.014	.196	.015

Mean percents of body weights of excised fat pads. Percents are for males and females of each group.

R. P. is retroperitoneal
I. S. is interscapular

Table 3-- Weights and Percents of Adipose Tissue Utilized During Food Deprivation and Restraint

Fat Pad	Weight in grams				Percent used			
	15w		21w		15w		21w	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Inguinal	.239	.013	.357	.024	82.7	1.66	61.9	2.09
R. P.	.051	.007	.170	.029	99.3	.706	82.0	2.23
I. S.	.102	.010	.276	.046	94.6	1.61	69.6	3.53
Tfat	.402	.035	.824	.091	86.5	1.58	68.1	2.11
BAT	.085	.011	.099	.015	51.2	3.66	45.8	3.21

R. P. is retroperitoneal
I. S. is interscapular white fat
Tfat is total excised white fat

Table 4 t Tests of Fat Depletion
Comparing 15w and 21w R

Fat Pad	t score	DF	prob
inguinal			
weight	4.36	18	<.0004
percent	8.17	18	<.0001
R.P.			
weight	3.87	18	<.0011
percent	7.35	18	<.0001
I.S.			
weight	3.67	18	<.0017
percent	6.47	18	<.0001
Tfat			
weight	4.30	18	<.0004
percent	6.98	18	<.0001
BAT			
weight	.73	18	>.10
percent	1.32	18	>.10

Based on difference scores comparing animals of the same litter in non food deprived conditions or after food deprivation and restraint.

R.P. refers to retroperitoneal fat
I.S. refers to interscapular white fat
Tfat refers to total excised white fat

Early weaned animals also have significantly less brown adipose tissue both in absolute terms and as a percent body weight in non food deprived conditions and following food deprivation and restraint. (see figure 7). However, following food deprivation and restraint no significant difference in BAT depletion (either in absolute terms or as a percentage of body weight) was found $t(18) = 1.3$, $p>.2$ (see figure 7 and table 3).

Thirty day old 15w animals have skin that is significantly thinner than that of 21 w animals, $F(1, 25) = 39.06$, $p<.0001$, (see figure 8 and tables 4 and 5). Both groups lose skin thickness during food deprivation and restraint probably due to loss of subcutaneous fat. However 15w animals show a greater percent reduction of their skin weights. This was true for all skin areas measured. Figure 8 illustrates the data for the skin taken above the interscapular fat deposit.

In order to assess the effects of restraint rather than prolonged food deprivation on body temperature maintainence and RGE susceptibility an additional group of rats were food deprived but not restrained. Four litters of 15w rats and five litters of 21w rats from whole litters and weaned and housed as previously described were used. Procedures were identical to those previously described except that animals were not restrained following the initial 26 hour food deprivation time. Instead animals were food deprived for an additional

26 hours at 20 °C. At autopsy stomachs were examined for gastric erosions and retropeitoneal and interscapular BAT were dissected and weighed.

Following food deprivation, 15w rats food deprived but not restrained, had a lower mean body temperature ($\underline{M}$ = 34.06 °C) than did 21w rats ($\underline{M}$ =35.08 °C) but this difference was not significant. This in contrast to 15w rats both food deprived and restrained whose mean body temperature following restraint is 30.55 °C. In addition neither 15w nor 21w rats food deprived but not restrained were found to form RGEs. However, 15w rats in these additional litters still had less retroperitoneal adipose tissue to begin with ($\underline{M}$ = 0.06 g for 15w rats, $\underline{M}$ = 0.18 g for 21w rats) and inferred utilization again showed 21w rats using a greater amount of fat in absolute terms ($\underline{M}$ = .09 g for 21w rats, $\underline{M}$ = .06 g for 15w rats). This was also true of the weight of excised BAT ($\underline{M}$ = .16 g for 21w rats, $\underline{M}$ = .13 g for 15w rats) determined prior to prolonged food deprivation; and for inferred usage during prolonged food deprivation ($\underline{M}$ = .08 g for 21w rats, $\underline{M}$ = .06 g for 15w rats). It seems as if restraint is important in eliciting low body temperatures and susceptibility to RGEs but food deprivation alone affects the relative depletion of fat stores in 15w rats. These results will be discussed in conjunction with the results of Part I experiment 2.

Figure 7

This figure shows the quantities of interscapular brown adipose tissue for early weaned and normally weaned animals. Quantities are for littermates matched for body weight prior to or following food deprivation and restraint. On the left quantities are the absolute weights of BAT, while on the right BAT is expressed in terms of percent body weight.

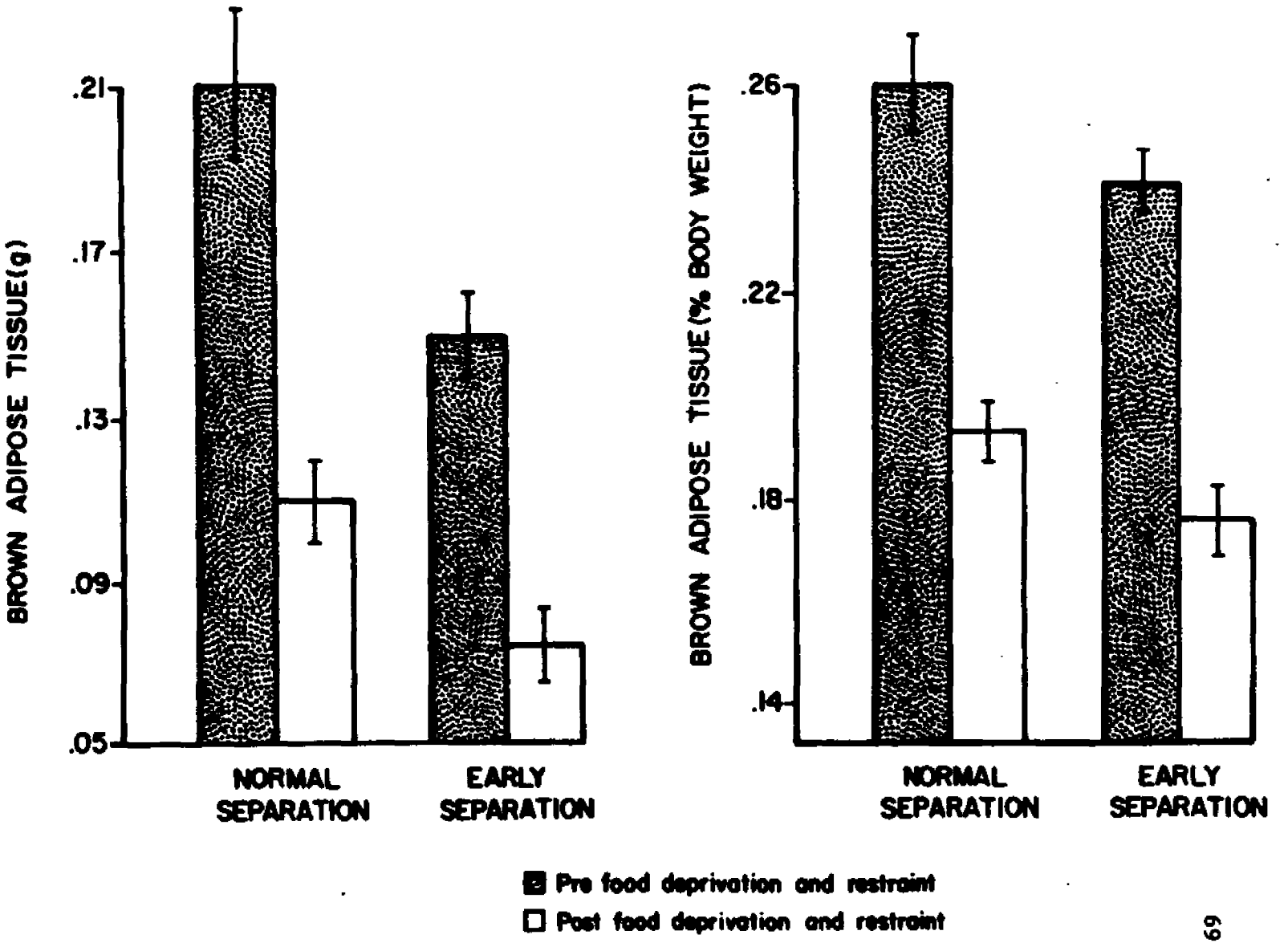

BROWN ADIPOSE TISSUE(g)
.21
.17
.13
.09
.05
NORMAL SEPARATION
EARLY SEPARATION
BROWN ADIPOSE TISSUE(% BODY WEIGHT)
.26
.22
.18
.14
NORMAL SEPARATION
EARLY SEPARATION
Pre food deprivation and restraint
Post food deprivation and restraint

Figure 8

Figure 8 shows the weights of skin biopsies from the interscapular region. Weights are shown for tissues taken from animals matched for body weight either prior to or following food deprivation or restraint.

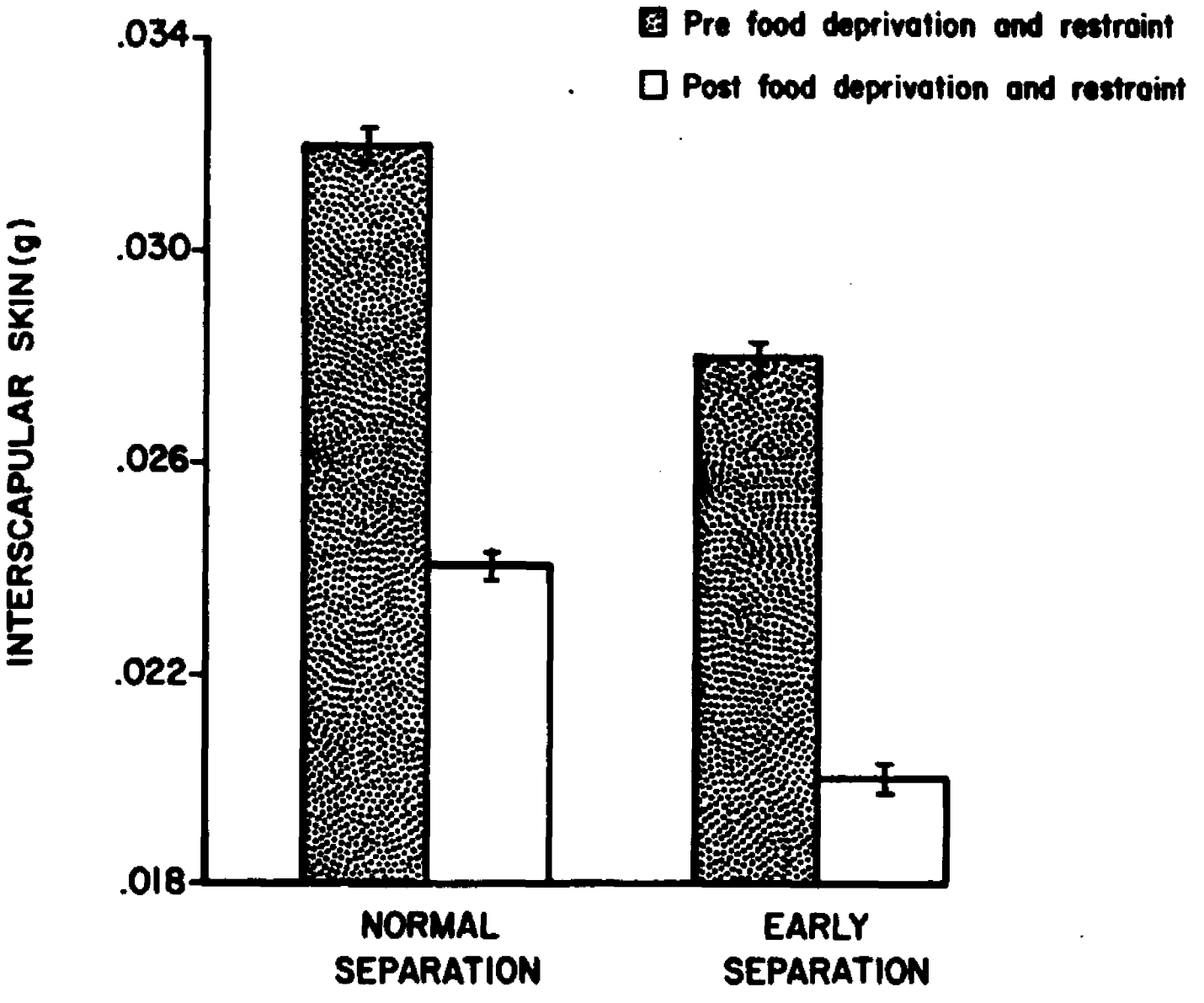

Pre food deprivation and restraint
Post food deprivation and restraint
INTERSCAPULAR SKIN (g)
.034
.030
.026
.022
.018
NORMAL SEPARATION
EARLY SEPARATION

Table 5-- Weights in grams of Skin Biopsies

Skin Area		Before Restraint				After Restraint			
		15w		21w		15w		21w	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Sternum	m	.016	.002	.022	.001	.012	.001	.017	.001
	f	.024	.009	.030	.009	.012	.001	.017	.001
Flank	m	.019	.001	.026	.002	.018	.001	.024	.001
	f	.020	.001	.026	.001	.017	.001	.022	.001
Leg	m	.019	.001	.024	.001	.016	.001	.020	.001
	f	.019	.001	.023	.002	.014	.001	.020	.001
I. S.	m	.028	.001	.033	.001	.019	.001	.026	.001
	f	.027	.002	.030	.002	.021	.001	.023	.001

Weights of .8 cm skin biopsies from various body areas

I. S. is the area above the interscapular fat deposit.

Table 6--Mean Percent Body Weight of Skin Punch Biopsies

Skin Area		Before Restraint				After Restraint			
		15w		21w		15w		21w	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Sternum	m	.024	.002	.026	.001	.029	.002	.027	.001
	f	.046	.009	.041	.013	.039	.002	.030	.001
Flank	m	.029	.002	.031	.002	.049	.002	.041	.002
	f	.033	.002	.034	.002	.043	.002	.041	.002
Leg	m	.028	.001	.030	.001	.037	.002	.034	.002
	f	.032	.002	.031	.001	.037	.003	.037	.002
I. S.	m	.042	.002	.039	.002	.043	.002	.044	.002
	f	.044	.003	.038	.002	.052	.003	.041	.002

I. S. refers to the skin area above the interscapular fat deposit.

Part I -- Experiment 2

As I had determined that there were differences in adiposity for 15w and 21w animals at 30 days of age I wished to further explore the roles of white and brown fat in determining RGE susceptibility. The next experiment addressed the question of whether one could cause 30 day old 21w animals to become more susceptible to hypothermia and concomminant RGEs by reducing either their white or brown fat stores. Presumably, removing white fat would reduce available fuel stores. Thus, if merely having less stored white fat available for conversion into heat was a critical factor in 15w rats' increased RGE susceptibilty, then surgically reducing white fat stores in 21w rats should lead to increased RGE susceptiblity. Removal of brown fat would directly inhibit the ability to produce heat via nonshivering thermogenesis. If direct production of heat via nonshivering thermogenesis was a critical factor in 15w rats' increased RGE susceptibility then BAT removal should increase RGE suscetibilty in 21w rats.

For white fat excision I removed bilateral inguinal fat deposits as this reduced available fat stores by approximately 45% (Faust & Hirsch, 1977). For BAT excision I removed the BAT found in the interscapular region since for young rats the BAT of the interscapular region contains approximately 85% of all BAT (Jansky, 1973).

Method

Subjects

Subjects were 28 day old 21w rats raised and housed as described in Experiment 1. Eight litters of the whole litter condition were used for white fat removal and eight litters of the whole litter condition were used for BAT removal. One male and one female from each litter served as experimental animals and one male and one female from each litter served as sham operated controls. Littermates serving as operated animals and controls were chosen such that they were matched in body weight.

Procedure

On day 28 postnatally animals were weighed and rectal temperatures taken. For white fat removal, operated animals were anesthetized with ether and bilateral inguinal fat pads were surgically removed. Wounds were closed ulizing wound clips. Sham operated controls were anesthetized and bilateral incisions were made at the inguinal region. However, no tissue was removed. Sham operated animals were anesthetized for the same amount of time that the experimental animals were anesthetized. Wound clips were also used for sham operated animals.

For BAT removal, operated animals were anesthetized with ether, an incision was made at the interscapular region, and interscapular BAT was removed. As the interscapular BAT lies beneath a layer of interscapular white fat, removal of BAT involved some disturbance of white fat. The surgical procedure was undertaken in such a way as to minimize any excision of white adipose tissue. Sham operated animals were anesthetized and an incision was made at the interscapular region. A small incision in the white adipose tissue was made to mimic the disturbance of white fat necessitated in the operative procedure.

Following surgery a 24 hour recovery period was allowed for all animals. After recovery weights and rectal temperatures were again recorded. Animals were then food deprived with their littermates for 26 hours at 20 °C. Following food deprivation weights and temperatures were recorded. Animals were then restrained in individual wire mesh cones for an additional 26 hours at 20 °C. Following restraint weights and temperatures were recorded and animals were sacrificed and examined for gastric erosions as described in Experiment 1. In all animals remaining white fat stores in the inguinal retroperitoneal and intescapular regions were excised and weighed, as was remaining BAT.

Results

ANOVAS were performed with surgical condition, litter and sex as factors. For the number of lesions per animal significant main effects were found for all factors ($F(2, 28) = 5.77$, $p<.03$ for surgical condition; $F(11, 28) = 4.90$, $p<.005$ for litter; $F(1, 28) = 4.99$, $p<.03$ for sex). No significant interactions were found. Post hoc analysis with Tukey studentized range tests showed that significant differences existed between animals with BAT excised and sham operated animals, animals with BAT and white fat excised but not between animals with white fat excised and shams.

Analysis of the incidence of lesions showed significant main effects for surgical condition and sex of animal ($F(2, 28) = 5.84$, $p<.01$ for surgical condition, $F(1, 28) = 4.35$, $p<.03$ for sex of animal). No other significant effects were found. Post hoc analysis again showed significant differences between BAT and white fat excised animals and between BAT excised animals and shams but not between white fat excised animals and sham operated controls. For the length of lesions a significant main effect was found only for litter ($F(11, 28) = 3.2$, $p<.01$), and no significant interactions were found between surgical condition, litter or sex of animal.

No significant differences were found between any groups in body temperature measured at any part of the experiment. Significant main

effects in initial body weight were found for sex and litter ($F(1, 28) = 3.45$, $p<.03$ for sex; $F(11, 28) = 4.21$, $p<.01$ for litter). Significant main effects for body weights measured after surgery were found for surgical condition, sex and litter but no significant interactions were found ($F(2, 28) = 3.71$, $p<.03$ for surgical condition; $F(1, 28) = 4.63$, $p<.01$ for sex; $F(11, 28) = 3.83$, $p<.01$ for litter).

Discussion

I have demonstrated that early weaned animals not only are smaller than normally weaned animals but also have less body fat. This is true both in absolute terms and when fat mass is expressed as a percent of total body weight. During food deprivation and restraint 15w animals deplete their adipose tissue to a greater extent than do 21w animals. However, during this time 15w rats use less white fat in absolute terms than do 21w animals. During food deprivation and restraint 15w rats have decreasing body temperature while 21w rats do not and this lowered body temperature is highly correlated with incidences of RGEs. Thus either the total fat that 15w rats have is insufficient for supplying caloric needs during food deprivation and restraint, or they are not utilizing available fat stores in a manner that is effective in maintaining body temperatures at normal levels.

The fact that 15w animals utilize a greater percentage of the fat

stores available to them argues against their having a sensory defect in which they fail to recognize the need for supplying energy while being food deprived and restrained. Early weaned rats have less overall white fat, less brown fat and thinner skin than normally weaned rats. It is possible that they are more likely to become hypothermic when food deprived and restrained due to inabilities to protect against heat loss as well as to deficiencies in heat production.

The results of experiment 2 suggest that mechanisms of heat production via BAT mediated nonshivering thermogenesis are especially important in determining lesion susceptibility. Excision of BAT but not white adipose tissue led to increased RGE susceptibility in 21w rats. Although the 21w rats that had BAT excised were not significantly more hypothermic than sham operated controls or than animals with white fat excised it shoud be noted that body temperatures were depressed relative to body temperatures at the start of the experiment. Excising BAT in 21w rats does not appear to lead to the full complement of thermoregulatory deficits typical of 15w rats, however, it does increase lesion susceptibility. The ob/ob mouse, which has known deficits in BAT mediated thermogenesis (Himms-Hagen & Desautels, 1978) are more susceptible to RGEs than are lean siblings (Greenberg & Ackerman, 1983). And ob/ob mice have excess white fat stores as compared to lean mice. Early weaned animals have less BAT. Early weaned animals may also have qualitative defects in their BAT.

However, qualitative differences in BAT were not examined in the current study.

During food deprivation and restraint early weaned animals use a much larger proportion of their white adipose tissue than do normally weaned animals. Excising the inguinal white fat of normally weaned animals does not result in depletion of remaining white fat stores. Similarly, when the BAT of 30 day old normally weaned rats is excised these animals do not deplete remaining white fat stores. While non operated 15w rats typically deplete white fat stores during food deprvation and restraint. It is likely that the depletion of white fat stores in 15w rats is due to the relative sparseness of white fat in these animals. Excision of inguinal white fat in 21w rats did not lead to increased RGE susceptibility. This argues against the hypothesis that excising 21w rats bilateral inguinal fat pads leaves them with fat stores analagous to those of 15w rats. However, as BAT excision leads to increased RGE vulnerability in 21w rats, it seems that directly decreasing the ability to produce heat via nonshivering thermogenesis is important in RGE susceptibilty. It may also be true that active depletion of brown and white fat stores is especially important in leaving early weaned rats vulnerable to RGEs.

The mechanism by which having decreased fat stores leads to vulnerability to RGEs is speculative. However, increased gastric acid secretion is found when ambient temperatures are lowered in awake rats

(Witty & Long 1970, Ackerman, 1981). And in anesthetized rats, lowered core temperature leads to increased acid secretion (Ackerman, 1980). Both 15w and 21w 30 day old rats are capable of increasing acid secretion in response to cold ambient temperature (Ackerman, 1981). The diminished adiposity of 15w animals compromises their ability both to produce and conserve heat, and 15w animals become hypothermic during food deprivation and restraint. This hypothermia may trigger the normal acid secretory response to cold core temperature which may eventually lead to the formation of RGEs. Normally weaned animals do not become hypothermic and thus the acid secretory response to cold is not elicited. Any cold stimulus should lead to increased acid output, whether due to lowered body core temperature or to external cold stimuli. Indirect evidence for a connection between responsiveness to cold stimuli and the acid secretory response is given by the fact that cold ambient temperature increases the severity of RGE formation in adult rats (Martin, Martin, & Lambert 1970), and restraining rats at low temperatures increases RGE incidence (Senay & Levine, 1967).

In addition to increased acid secretion in response to cold, another factor that may link decreased fat stores and RGE formation is that heat production to maintain body temperature may actively involve the stomach. It has been shown that under normal conditions active thermogenesis takes place in the stomach and other upper gastrointestinal areas. The upper GI tract has a temperature higher

than that of the liver or aortic blood, and it has been estimated that in adult dogs the heat produced by the upper GI tract accounts for between 25% and 40% of total body heat (Durotoye & Grayson, 1971). However, this heat production could be partially derived from perigastric, peripancreatic, or other periorganic BAT, rather than from the stomach and other organs themselves.

It is possible that in the attempt to maintain normal body temperatures heat production capabilities of the upper GI tract are mobilized. In the early weaned rat there would be a greater need to call upon upper GI tract heat production as the early weaned rat has less interscapular BAT for heat production and less subcutaneous fat for heat conservation. BAT excised 21w animals would also be compromised in their ability to produce heat from sources outside the GI tract (although not compromised in their ability for heat conservation), and it is these 21w animals that show increased RGE formation. White fat excised 21w animals do not have increased incidence of RGEs, and they are not directly incapacitated in their ability to produce heat. Thus a requirement for increased heat production is likely to be particularly important in RGE susceptibilty. Mobilizing heat production in the upper GI tract may alter metabolism in the stomach which could lead to a change in mucosal defenses against increased acid secretion. Early weaned animals may have both increased acid secretion in response to lowered core

temperature and a decrease in the effectiveness of mucosal defenses resulting from altered metabolism of the stomach.

I have demonstrated that one of the effects of weaning rats prematurely on day 15 is an alteration in body compostition. Specifically 15w rats have a lower fat to body weight ratio than do 21w rats. As a consequence of decreased adiposity 15w rats are more vulnerable to hypothermia induced by food deprivation and restraint. This is consistent with Ackerman et al. (1978b) finding that 15w animals fed a high fat diet are no more susceptible to RGEs than are 21w animals. This is also consistent with studies showing that postnatal nutrition is an important factor in determining adipocyte number and size (Knittle & Hirsch, 1968). I believe that the alteration in body composition effected by premature weaning is a critical component in 15w animals greater susceptibility to RGEs.

PART II: BEHAVIORAL THERMOREGULATION IN 30 DAY EARLY AND NORMALLY WEANED RATS

Introduction

As previously indicated temperature regulation is an important factor in susceptibility to RGE formation, and weaning rats at day 15 has a number of immediate and long term consequences that may effect their abiltiy to thermoregulate. In addition to some of the direct physiologic effects on metabolism explored in the first section it is possible that 15w rats have disturbances in behaviors that affect thermoregulation. The present studies examine whether there are differences in behavioral thermoregulation between 15w and 21w rats.

Neonatal rats are essentially exothermic, taking on the temperature of their external environment (Adolph, 1957, Taylor, 1960, Conklin & Heggeness, 1971). The second and third week of life are the times at which the normally developing rat begins to be able to maintain body temperature independent of the environment (Taylor, 1960). Early weaning thus occurs during the normal maturation of thermoregulation. And it may be that weaning at this time disrupts the normal maturation of thermoregulatory mechanisms.

Differences in temperature regulation between 15w and 21w rats

have, until now, only been found during food deprivation and restraint. Under normal laboratory conditions, if body temperature is measured from day 25 to day 30 in freely moving rats, no significant differences are found between 15w and 21w rats (Ackerman et al., 1978). It is possible, however, that 15w rats have disturbances in their ability to thermoregulate not elicited by normal laboratory conditions. For example, it is not known whether 15w rats exposed to hot or cold ambient temperatures would exhibit appropriate behavioral responses. When rats are exposed to extremes in ambient temperatures they will attempt to escape if such a response is possible. If heat is unavoidable rats will typically engage in behaviors such as saliva spreading, assuming a prone extended position, slow locomotion, and avoiding contact with littermates (Roberts & Mooney, 1974; Alberts, 1978a, 1978b). If cold is unavoidable rats will engage in behaviors such as increased activity, shivering, piloerection, and huddling with littermates (Satinoff, 1974; Alberts, 1978a, 1978b). These behaviors have been shown to be under control of different regions of the CNS. Early weaned rats may not appropriately display some or all of these responses. The absence of appropriate thermoregulatory responses may contribute to the inabiltiy of 15w rats to maintain normal body temperatures under conditions of food deprivation and restraint. The present studies were carried out to investigate whether 30 day old 15w and 21w rats differ in their behavioral responses to different thermal

stimuli.

Part II -- Experiment 1

One important way in which animals can regulate body temperatures is by placing themselves in a favorable thermal environment. When placed along a temperature gradient many different organisms are found to position themselves in a relatively narrow portion of the available temperature range, and the temperature selected by placement approximates thermoneutrality for these animals (Herter, 1941; Workman & Fisher, 1941; Mount, 1963; Oglive & Stinson, 1966; Leonard, 1974; Johanson, 1980). It has also been shown that as young rodents develop they tend to place themselves at relatively lower temperatures along temperature gradients (Adolph, 1957; Oglive & Stinson, 1966; Fowler & Kellogg, 1975); this tendency corresponds to a developmental decrease in thermoneutrality.

Early weaning might have the effect of disrupting the maturation of homeothermy. Presumably this disruption would be reflected in their temperature selection behavior, and 15w rats would be expected to select temperatures more typical of younger rats, that is a higher temperature. Temperature selection differences could also indicate differences in the ability to sense temperature gradients. If 15w rats select different temperatures than 21w rats it may indicate either

sensory differences or differences in thermoneutrality, and increased variability might indicate a reduced ability to detect differences along a gradient. However, temperature selection behavior will not distinguish between these possibilities. I tested the temperature selection behavior of 15w and 21w animals on a thermal gradient.

Method

Subjects

Rats were raised, weaned and housed as previously described. In order to reduce the variance in observed behaviors in this and all subsequent experiments only male rats were used as subjects. Male rats from sixteen litters of the split litter condition were used as subjects. One early weaned (15w) and one normally weaned (21w) rat from a given litter was used for testing. All animals were 30 days old at the time of testing.

Apparatus

A runway was constructed to present a thermal gradient to 30 day old rats. The runway consisted of a steel pipe bisected along its diameter 127.5 cm long and 13.4 cm in diameter. Hinged along the top portion of the pipe were plexiglass lids that allowed an observer to visually determine the location of the animal inside the runway. A thermal gradient of from 20 °C to 40 °C was produced by wrapping the warm portion of the runway with electrical heat tape and cooling the cold portion of the runway with "blue ice". The density of the heat tape wrapping was adjusted to obtain the heat gradient. The runway was lined with protective padding so that an animal would not have direct contact with the heated metal surface. Measurements of air temperature just above the surface along the runway were determined with the padding in place. The padding was changed after each test session to eliminate any odor cues.

The runway was divided into 5 temperature regions, the temperature at the center of each region was determined both prior to and following each animal's test session. Each of the five temperature regions was delineated by the edge of a 25.5 cm piece of plexaglass, which served as the previously mentioned cover for the runway. The temperature at the center of the five regions were: 40 °C, 35 °C, 29 °C, 24 °C, and 20 °C. These temperatures were maintained within ± 2 °C

during all test sessions.

An observer visually determined where along the runway an animal had placed itself and recorded the position of the animal by using a five position switch. This switch connected to a constant voltage source and current was fed into a Beckman polygraph (Type R Dynograph 12-channel). Each switch position produced a deflection of a polygraph pen, which corresponded to the 5 thermal zones. Use of this system allowed for a permanent record of an animals position along the runway.

Procedure

Thirty day old rats to be used as subjects were removed from their home cages at the time of testing. Each animal's weight and rectal temperature was determined prior to and following testing. Observations of an animal's position along the thermal runway were made during 15 minute test periods. Eight 15w and 21w littermate pairs each from a different litter were used in each of two conditions. In the first condition animals were tested individually with initial placement at the warmest portion of the runway and in the second condition they were tested individually with initial placement at the coolest portion of the runway.

Eight additional 15w and 21w littermate pairs were observed in a control condition. Animals were observed for 15 minute test periods as before. However, for control animals there was no temperature gradient

along the runway. The temperature of the entire runway was normal room temperature of 22 ± 1 °C. Four 15w and 21w littermate pairs were initially placed on the part of the runway that was previously the warm end, while the remaining animals were initally placed on the part of the runway that was previously the cool end.

An observer noted the animals location along the runway continually for the 15 minute test period. A scoring system was used such that an animal was scored at a given temperature region only if it remained within that region for 15 seconds or more. Otherwise the animal was said to be in transition from one region to another. The 15 minute test was divided into 30 second intervals. For each 30 second interval an animal was scored as either located at one of the temperature zones or in transition. In this way I calculated the amount of time an animal spent at any given temperature region and the amount of time spent moving from one region to the next. I also measured the total distance an animal moved and the number of different temperature regions that an animal entered.

Results

Consistent with earlier findings (Ackerman et al. 1975, 1978) I found that 30 day old 15w rats weighed significantly less than 21w rats of the same age ($\underline{M}$ = 71.8 g compared to $\underline{M}$ = 89.9 g), $\underline{t}(30) = 4.18$,

$p<.0002$. I also confirmed that there were no significant body temperature differences between 15w and 21w rats when temperature is measured immediately after rats are removed from the home cage, ncr were significant differences found in body temperatures after runway test periods.

An ANOVA with weaning condition, starting position and time as factors, and place chosen along the runway as the dependent variable was performed. No significant main effects or significant interactions were found. I then did separate analyses on the first, second, and last five minutes of testing. Again no significant effects were found for any factor. Separate analyses were performed on the temperature selection behavior of animals that were started at the cold and warm end of the thermal gradient. A multiple regression analysis was performed to analyze the distribution of placement of the animals over time. For animals starting at the cold portion of the runway a significant difference in the distribution of chosen temperature over time was found between 15w and 21w animals, $F(1, 240) = 9.97$, $p<.02$. No other significant differences in temperature selection behavior (such as total distance moved, latency to move, temperature selected at any given time, preference for any given temperature, or mean temperature at which the most time was spent) were found for animals started at the cold end of the runway. For animals started at the warm end of the runway, no significant differences were found in any of

these measures.

Animals were, however, responding to the thermal gradient. Comparisons between the temperature selection behavior of experimental animals (see figure 9 and 10) and control animals (see figure 11 and 12) showed marked differences. Significant differences were found in the distribution of time spent at various temperatures along the runway (χ^2 (4) = 18.6, $p<.005$).

Figure 9

Figure 9 shows the area along the runway at which 15w and 21w rats started at the cold end of the runway placed themselves in 30 second time intervals during the 15 minute test session while the thermal gradient was in effect.

Figure 10

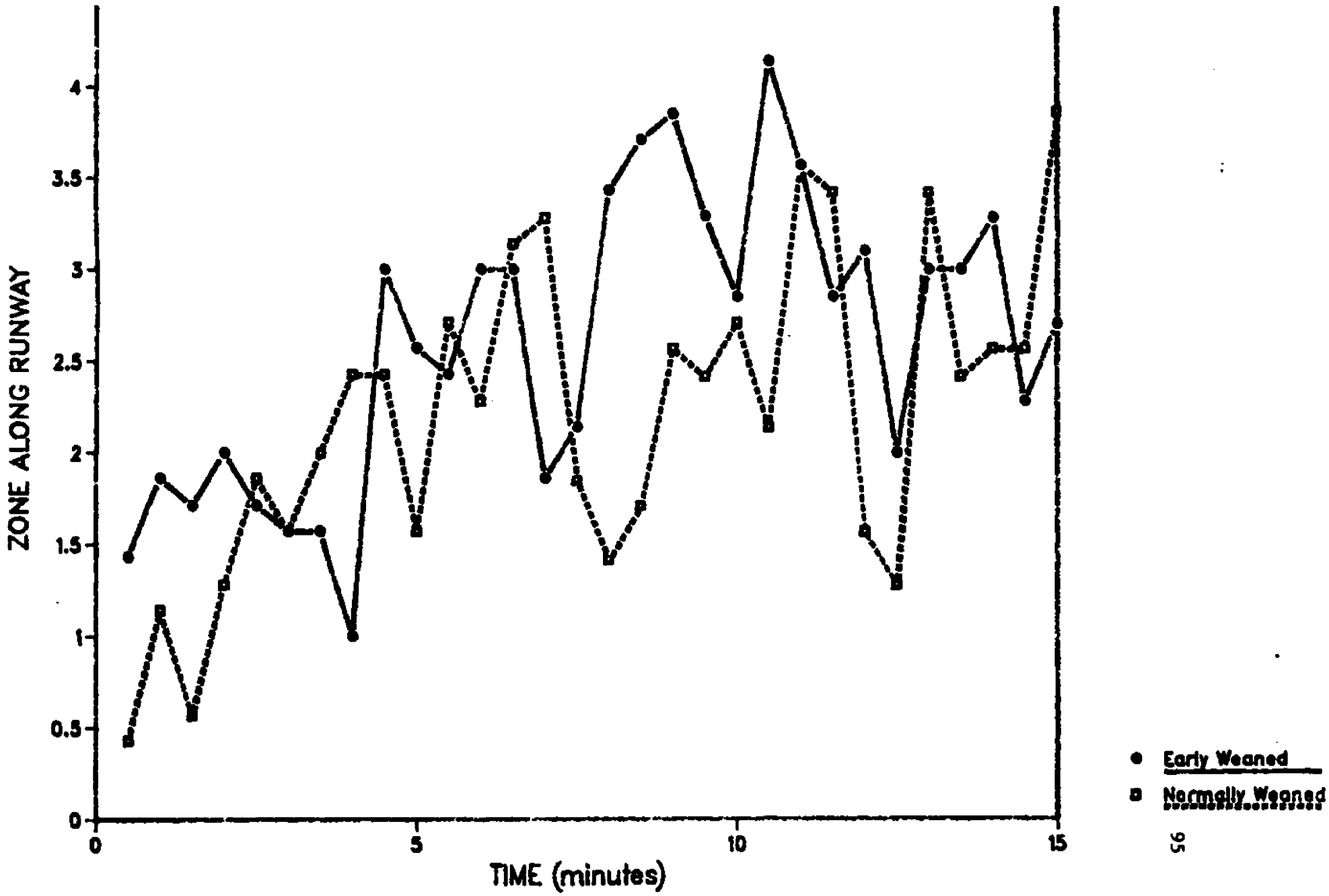
Figure 10 shows the area along the runway at which 15w and 21w rats started at the warm end of the runway placed themselves in 30 second time intervals during the 15 minute test session while the thermal gradient was in effect.

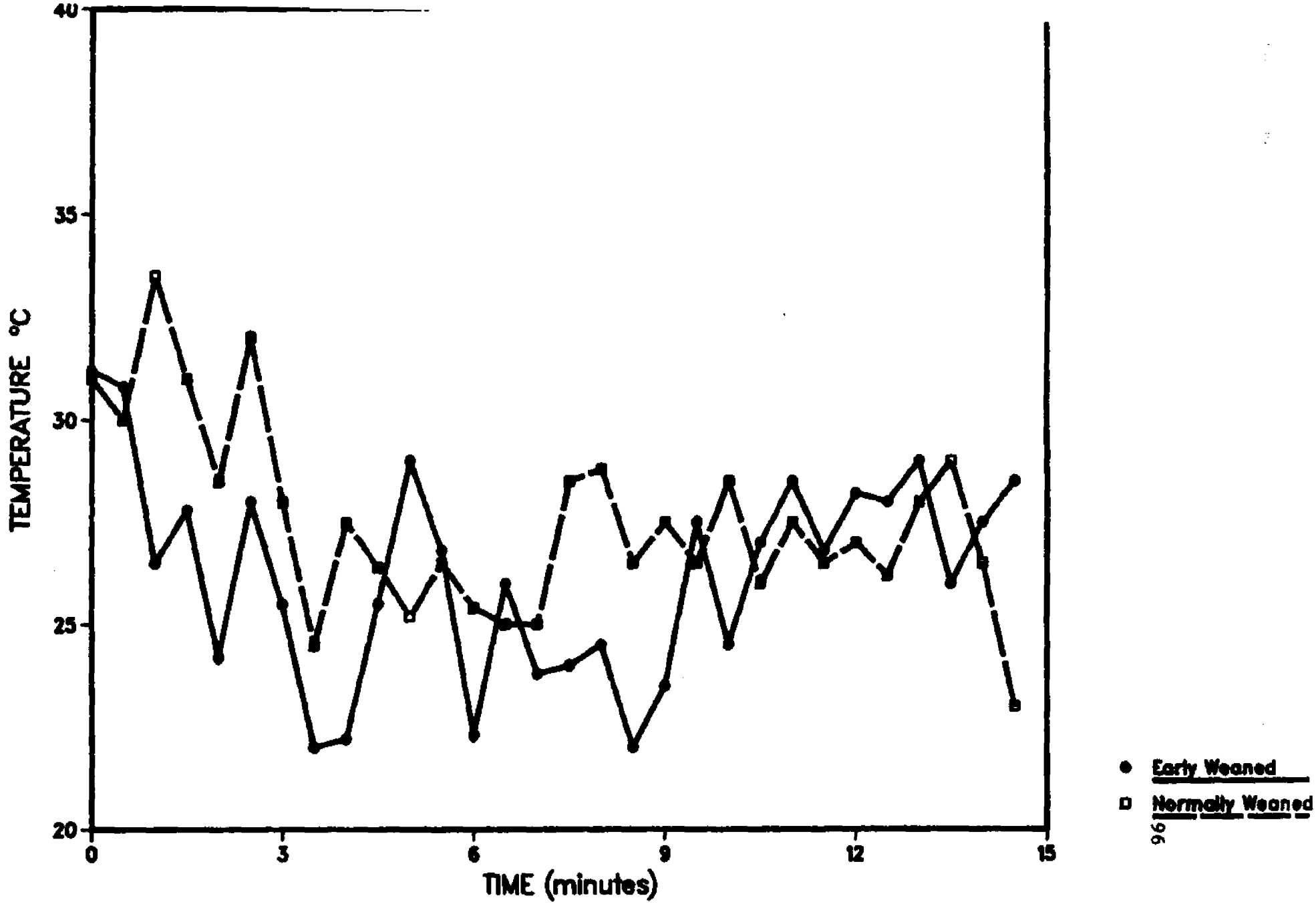
Figure 11

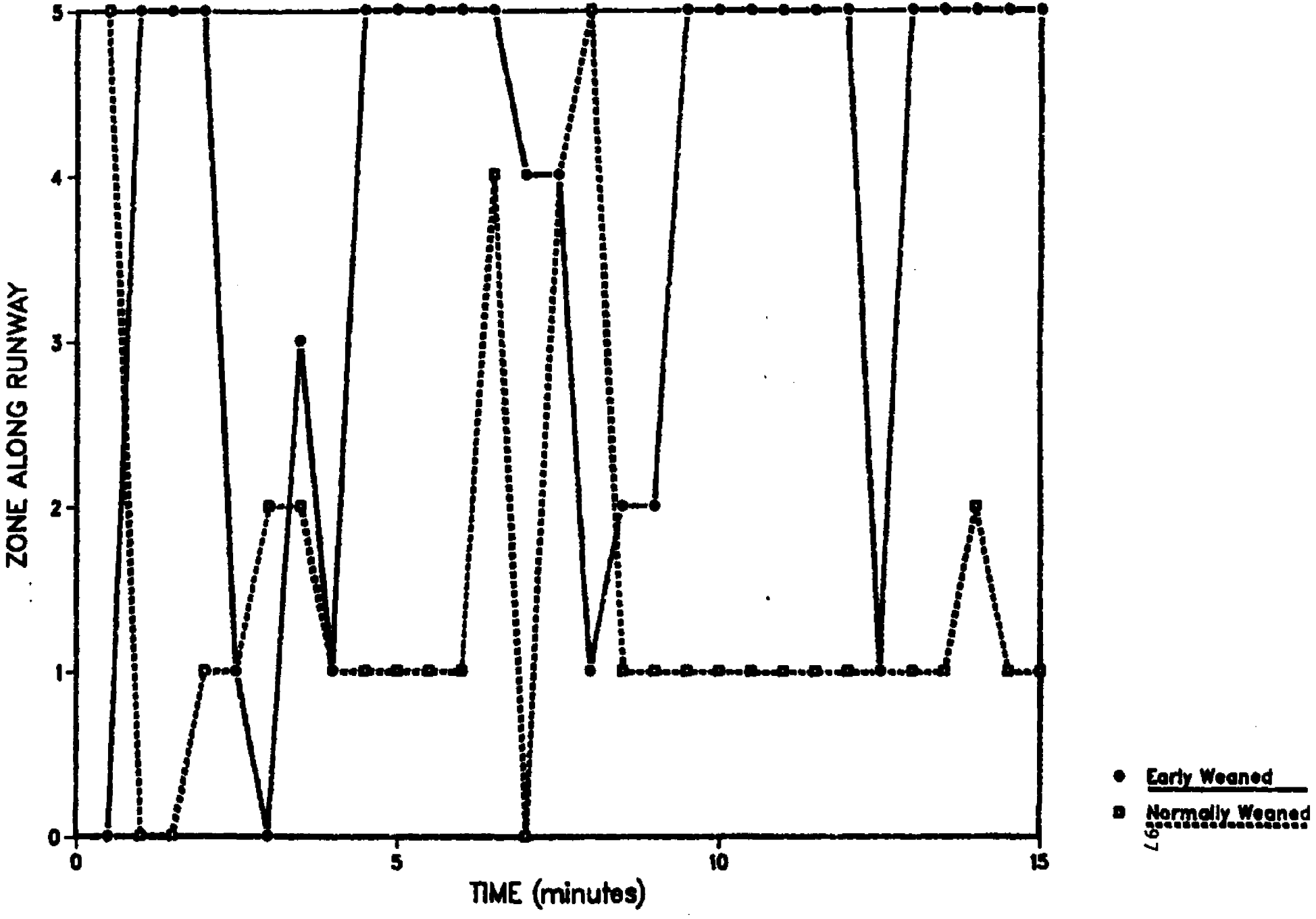
Figure 11 shows the area along the runway at which a representative 15w and 21w rat placed itself for 30 second time intervals during the 15 minute test session. The thermal gradient was not in effect. However, animals were started at what had been the cold end of the runway.

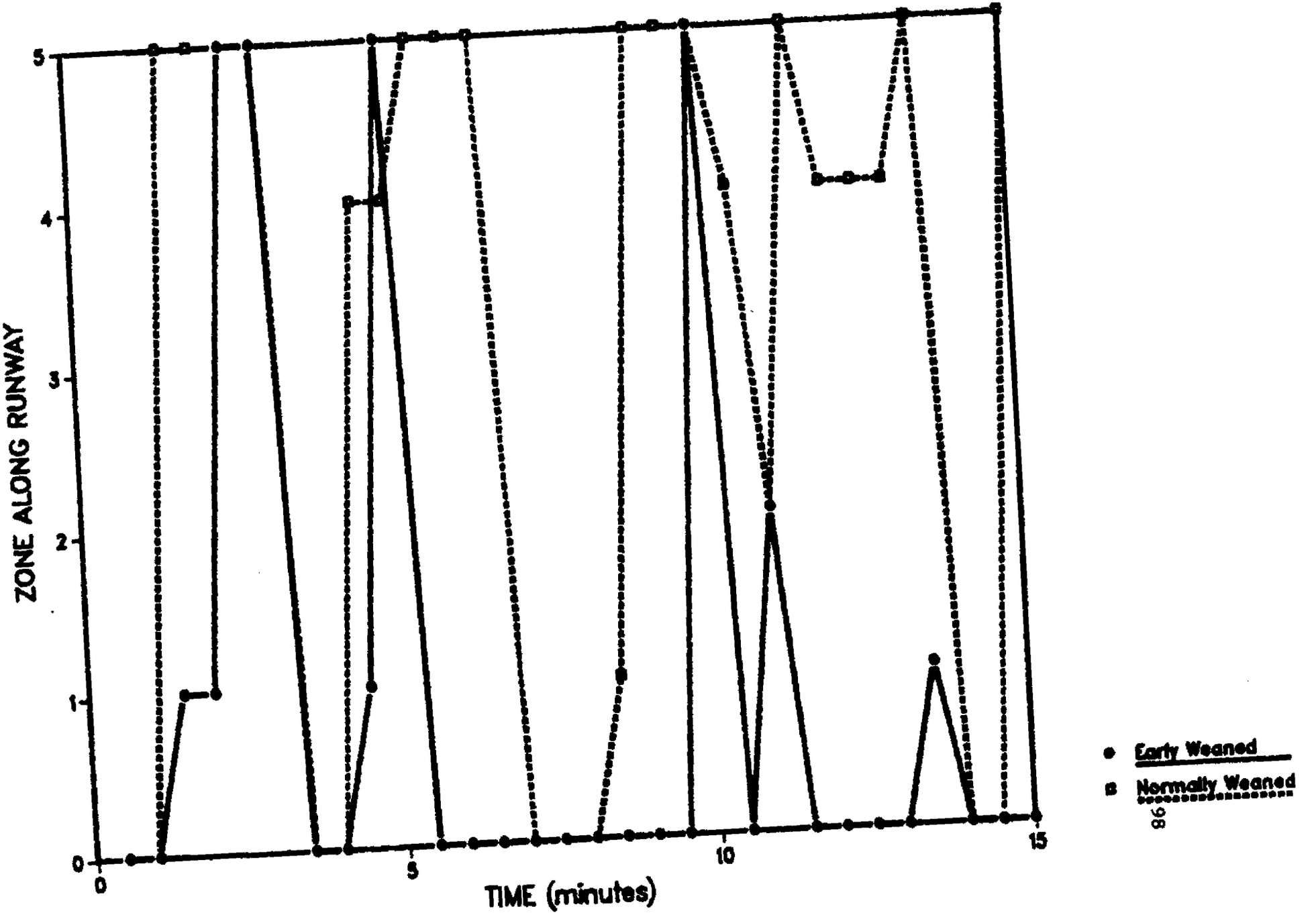
Figure 12

Figure 12 shows the area along the runway at which a representative 15w and 21w rat placed itself for 30 second time intervals during the 15 minute test session. The thermal gradient was not in effect. However, animals were started at what had been the warm end of the runway.


ZONE ALONG RUNWAY
0
0.5
1
1.5
2
2.5
3
3.5
4
0
5
10
15
TIME (minutes)
Early Weaned
Normally Weaned


40
35
30
25
20
TEMPERATURE °C
0
3
6
9
12
15
TIME (minutes)
Early Weaned
Normally Weaned


ZONE ALONG RUNWAY
0
1
2
3
4
5
0
5
10
15
TIME (minutes)
Early Weaned
Normally Weaned


ZONE ALONG RUNWAY
0
1
2
3
4
5
0
5
10
15
TIME (minutes)
Early Weaned
Normally Weaned

Discussion

Robust differences were not found in the temperature selection behavior of 30 day old 15w and 21w rats tested on a thermal gradient with a range of from 20 to 40 °C. Thus neither the hypothesis that 15w rats have a delay in maturation of thermoregulatory response that would be reflected in selecting higher temperatures, nor the hypothesis that 15w rats have severe distubances in thermal sensation were supported.

Both 15w and 21w rats behaved differently when the thermal gradient was in effect and when it was not in effect. When the gradient was not in effect animals of both weaning conditions tended to stay at the ends of the runway (where a corner was formed). When the gradient was in effect animals of both weaning conditions avoided the ends of the runway where more extreme temperatures were in effect. And 15w rats did this to the same extent as 21w rats. This argues against the idea that 15w rats fail to activate thermoregulatory mechanisms due to a sensory defect. At least to the extent of avoiding extremes of temperature 15w rats show the ability to sense and respond to thermal stimuli.

These results do not eliminate the possibility that 15w and 21w rats have differences in their abiltiy to thermoregulate. However, it is possible that these differences are only elicited by a challenge to the animal. A thermal runway does not require an animal to mobilize

heat producing or heat dissipating mechanisms. A thermal gradient does allow the animal to escape severe temperatures, and when escape from extreme temperature is possible 15w and 21w rats do not differ in their temperature selection behavior. The next experiment addressed the question of whether 15w and 21w 30 day old rats differ in their responses to inescapable severe cold or heat, situations which presumably would activate behaviors that produce or dissipate heat.

Part II -- Experiment 2

Thirty day old 15w and 21w rats do not show differences in their temperature selection behavior, nor in their body temperatures under normal laboratory conditions. Early weaned animals do however, become hypothermic when food deprived and restrained while 21w rats do not. Food deprivation and restraint appears to serve as an indirect challenge to 15w animals ability to thermoregulate.

The present experiment examines the responses of 30 day old 15w and 21w rats to direct thermal challenges. The conditions that typically elicit hypothermia in 15w rats involve food deprivation. I therefore tested the responses of rats to direct thermal challenges when they had been food deprived as well as without prior food deprivation. Contact with littermates has been shown to be an important behavioral thermoregulatory mechanism for young rats

(Alberts, 1978a, 1978b). I therefore observed responses to heat or cold stress both in individual animals and in pairs of littermates of the same weaning condition.

Method

Subjects

Male rats from the split litter condition raised, housed and weaned as previously described served as subjects. Six 15w rats and six 21w rats were used for each condition in the experiment. Animals were from 28 to 32 days old at the time of testing. One animal from each litter was the experimental subject, and one littermate of the same weaning condition served as companion.

Procedure

Animals in the non food deprived conditions were removed from their home cages, their body weights and colonic temperatures were recorded and they were placed in the test environment. Twenty six hours prior to testing animals in the food deprivation conditions were removed from their home cages, their weights and temperature recorded, and replaced in the home cage. No food was available subsequently

until testing. The length of food deprivation was the same as has been used prior to restraint to elicit hypothermia and RGEs in 30 day old 15w rats (Ackerman et al. 1975, 1978a, 1978b). The test procedure was identical for food deprivation and non food deprivation conditions. Body weights were determined using a Sartorius model 1212 M P balance with an animal integrator which allows weights to be determined to within ± .01 g.

The test environment consisted of a standard wire mesh cage placed in an incubator (GCA/Precision, Chicago Il). The incubator allowed control of ambient temperature within ±.5 °C. A wide angle lens (typically used to look through entrance doors) was installed in the front of the incubator. The behavior of test animals were easily observed through this "observation lens".

For both the heat and cold challenge conditions animals were initially placed alone in the test environment. Test animals were allowed a 2 minute adaptation period, followed by 8 minutes observation. Observations were made every 15 seconds for 5 seconds. All behaviors observed during that time were recorded, behaviors were not mutally exclusive so that more than one behavior could be noted during an observation period. Following the initial 8 minute observation a companion littermate of the same weaning and food deprivation condition was introduced into the test environment. The companion littermate was marked so as to distinguish it from the test

animal. The companion littermate had been handled exactly as the test animal and had been in the incubator in a separate standard wire mesh cage during the 10 minutes while the test animal had been observed alone.

Following introduction of the companion littermate another 2 minute adaptation period was allowed. The behavior of the test animal was observed for an additional 8 minute period. Observations were made for 5 seconds every 15 seconds for the additional 8 minutes. Following observations the weight and rectal temperature of the test animal was again recorded.

For the heat stress condition the ambient temperature of the test and holding cages was 38 °C. For the solitary condition responses to this temperature were recorded as belonging to one or more of the following categories:

> Prone extension, where the animal was prone or streched out along the cage floor or walls
> Saliva spreading or grooming
> Rising up so that the forepaws were raised off the floor
> Quiet, where there was no visible activity
> Contact with cage walls, where the animal was in contact with more than one surface of the cage, but the animal was not in a prone position
> General activity, or those activities that did not fall into any other category.

In the paired condition the previous categories of responses were used except that the category of contact was now contact with the littermate. Only the responses of the test animal were recorded. The

procedures were identical for animals either food deprivated or not food deprivated.

For the cold stress condition the ambient temperature of the test cage and holding cage was maintained at at 10 °C. In the solitary condition observations were made that placed responses in one or more of the following categories:

Piloerection
Shivering
Hunching up resulting in minimum exposure of the ventrum
Saliva spreading or grooming
Contact with two cage walls
General activity or any activity not falling into the other categories.

When the test animal was accompained by a littermate the above categories were used along with huddling with the littermate. Animals were scorded as huddling when any contact with the littermate was observed. Procedures were again the same for food deprivation and no food deprivation conditions.

Results

Consistent with previous findings (Ackerman et al. 1978a, 1978b) I found that body temperature of 15w and 21 rats was not significantly different when temperature was measured immediately upon removal from the home cage. I also confirmed previous findings that 30 day old 15w rats were smaller than 21w rats of the same age ($\underline{M}$ = 68.9 g, $\underline{M}$ = 89.4 g,

$t(46) = 2.23$, $p<.01$). In all conditions 15w rats had significantly more labile body temperatures over the 20 minute test sessions than 21w rats. In animals tested in the cold 15w rats body temperature decreased significantly more than did that of 21w rats. Thus body temperature change scores which were determined by subtracting body temperature at the end of the test period from body temperature at the start of the test period were greater for 15w as compared to 21w rats. This was true both for animals non food deprived ($M = 1.58$ °C (15w), $M = 0.41$ °C (21w), $t(10) = 2.36$, $p<.05$), and following food deprivation ($M = 0.98$ °C (15w), $M = .43$ °C (21w), $t(10) = 3.07$, $p<.01$). Following food deprivation 15w rats body temperature at the start of the test session was already lowered compared to temperatures determined prior to food deprivation ($M = 36.4$ °C prior to food deprivation, $M = 35.2$ °C following food deprivation, $t(10) = 2.4$, $p<.05$). So that although the magnitude of temperature decrease during the test period was greater for 15w animals that had not been food deprived, 15w animals were more hypothermic following testing after food deprivation.

For animals tested at high ambient temperatures, 15w rats body temperature rose more than did that of 21w rats. When animals had not been food deprived the body temperature change scores were greater for 15w than 21w rats ($M = 2.58$ °C (15w), $M = 2.03$ °C (21w)) although this difference was not significant t (10) = 1.38, $p>.10$. For food deprived animals tested at high temperatures 15w rats body temperature rose

significantly more than did that of 21w rats ($\underline{M}$ = 4.07 °C (15w), $\underline{M}$ = 2.68 (21w), $\underline{t}(10) = 2.71$, $\underline{p}<.025$).

For animals tested at 10 °C a repeated measures multiple ANOVA was performed with weaning condition, food deprivation condition, presense or absense of littermate, and time as factors. A significant main effect of weaning condition was found for shivering, piloerection, activity and grooming ($\underline{F}(1, 255) = 14.06$, $\underline{p}<.005$ (shivering), $\underline{F}(1, 255) = 142.54$, $\underline{p}<.001$ (piloerection), $\underline{F}(1, 255) = 6.61$, $\underline{p}<.01$ (activity), $\underline{F}(1, 255) = 50.98$, $\underline{p}<.001$ (grooming). No significant weaning condition effect was found for hunching up or contact with cage walls (see table 7).

The main effect seen for weaning condition for piloerection was in fact due to differences in latency to piloerect ($\underline{t}(10) = 7.9$, $\underline{p}<.01$), as once a given animal was observed to piloerect that behavior was continuously maintained throughout the test session. The mean latency to piloerect in animals not food deprived was 0.83 min for 15w animals and 2.38 min for 21w animals. For animals that had been food deprived the latency to piloerect was decreased in both weaning conditions. For 21w rats the latency was 1.5 min and for 15w rats the latency was 0.48 min, again latencies were significantly shorter for 15w rats ($\underline{t}(10) = 2.91$, $\underline{p}<.01$).

For food deprivation significant main effects were found for all measures except hunching or huddling with a littermate ($\underline{F}(1, 255) =$

43.5, $p<.001$, for shivering; $F(1, 255) = 54.1$, $p<.001$, for piloerection; $F(1, 255) = 54.0$, $p<.001$, for grooming; $F(1, 255) = 50.1$, $p<.001$, for activity). A significant interaction of weaning condition and food deprivation was found for activity ($F(2, 255) = 7.17$, $p<.005$), but not for other measures, with 15w rats who had not been food deprived showing higher levels of activity than 21w rats but 15w rats that had been food deprived showing lower levels of activity than 21w rats. A significant interaction was also found for food deprivation and time for grooming ($F(63, 255) = 1.57$, $p<.05$) with nondeprived 15w rats showing relatively high grooming levels and deprived 15w rats showing relatively low grooming levels. No other significant interactions of food deprivation and other factors were found.

Presence or absence of littermate was found to significantly effect the observed measures of shivering ($F(1, 255) = 8.61$, $p<.005$), hunching ($F(1, 255) = 8.13$, $p<.005$), and grooming ($F(1, 255) = 58.13$, $p<.005$). It must be noted that the presence of a littermate was always subsequent to testing alone and this factor is therefore confounded with the length of time of the test period.

Time was a significant factor for all measures ($F(63, 255) = 1.42$, $p<.05$ for shivering; $F(63, 255) = 7.97$, $p<.005$ for huddling; $F(63, 255) = 1.34$, $p<.05$ for hunching; $F(63, 255) = 51.45$, $p<.001$ for piloerection; $F(63, 255) = 2.05$, $p<.01$ for grooming; $F(63, 255) = 1.37$, $p<.01$ for activity). With occurrence of behaviors tending to increase

as time went on. See table 7 for a summary of significant differences in the behaviors of 15w and 21w rats.

For animals tested at 38 °C a repeated measures mutiple ANOVA was performed with weaning condition, presence or absence of littermate, food deprivaton and time as factors. A significant main effect for weaning condition was found for all measures except rising up ($F(1, 255) = 55.12$, $p<.001$ for prone extension; $F(1, 255) = 50.46$, $p<.001$ for saliva spreading; $F(1, 255) = 3.89$, $p<.05$ for contact; $F(1, 255) = 56.11$, $p<.001$ for activity; $F(1, 255) = 3.52$, $p<.05$ for quiet.

Significant interactions of weaning condition and time were found for prone extension ($F(63, 255) = 2.13$, $p<.01$) and saliva spreading ($F(63, 255) = 1.6$, $p<.01$), with food deprivation leading to greater reductions in the levels of these behaviors for 15w rats than for 21w rats. A significant interaction of weaning condition and food deprivation was found for saliva spreading ($F(2, 255) = 1.6$, $p<.03$). A significant interaction of weaning condition and the presence or absence of a littermate was found for activity ($F(2, 255) = 3.73$, $p<.05$) with the presence of a littermate leading to increases in behavior for 15w rats and decreases in general activity for 21w rats.

For food deprivation significant main effects were found for all measures ($F(1, 255) = 107.2$, $p<.0001$ for prone extension; $F(1, 255) = 57.7$, $p<.001$ for saliva spreading; $F(1, 255) = 4.97$, $p<.01$ for rising; $F(1, 255) = 8.20$, $p<.01$ for contact; $F(1, 255) = 60.0$, $p<.001$ for

activity; $F(1, 255) = 19.2$, $p<.005$ for quiet). Food deprivation interacted with weaning condition as noted above.

For the presence or absence of a littermate significant main effects were found for prone extension ($F(1, 255) = 10.6$, $p<.01$), rising ($F(1, 255) = 11.7$, $p<.01$), and activity ($F(1, 255) = 13.5$, $p<.01$). The presence of a littermate tended to decrease the level of observed rising or prone extension and increase the level of activity.

For time significant main effects were found for all behaviors ($F(63, 255) = 30.1$, $p<.001$ for prone extension; $F(63, 255) = 4.06$, $p<.03$ for saliva spreading; $F(63, 255) = 3.12$, $p<.04$ for rising; $F(63, 255) = 14.8$, $p<.001$ for contact; $F(63, 255) = 3.89$, $p<.04$ for activity; $F(63, 255) = 4.03$, $p<.03$ for quiet). The incidence of all behaviors tended to increase as time went on. For a summary of the significant differences in behaviors seen in 15w and 21w rats see table 8.

Table 7

Summary of thermoregulatory behaviors for which there were significant differences between early and normally weaned rats tested at 10 °C.

Condition	Behaviors					
	Pilo	Shiv	Hnch	Contct Huddle	Active	Groom
No food deprivation alone 15w	+	+	=	=	+	
21w	-	-	=	=	-	-
No food deprivation with littermate 15w	=	++	=	=	++	=
21w	=	--	=	=	--	=
26 hr food deprived alone 15w	+	++	=	=	+	-
21w	-	--	=	=	-	
26 hr food deprived with littermate 15w	=	++	=	-	=	
21w	=	--	=	+	=	-

The + or - sign indicate significant differences in the number of observed epochs of a given behavior, with the greater number of occurences indicated by the positive sign. A double positive or negative sign indicates a greater difference in the number of observed epochs than in the condition where animals are alone and not food deprived.

Table 8

Summary of thermoregulatory behaviors for which there were significant differences between early and normally weaned rats tested at 38 °C.

Condition	Behaviors					
	Prone ext.	Salva sprd	Rise	Contct	Quiet	Active
No food deprivation alone 15w	-	-	=	=	-	=
21w	+	+	=	=	+	=
No food deprivation with littermate 15w	=	-	=	+	-	=
21w	=	+	=	-	+	=
26 hr food deprived alone 15w	=	--	=	=	++	+
21w	=	++	=	=	--	--
26 hr food deprived with littermate 15w	--	=	=	=	++	+
21w	++	=	=	=	--	--

The + or - sign indicate significant differences in the number of observed epochs of a given behavior, with the greater number of occurences indicated by the positive sign. A double plus or minus sign indicates a greater difference in the number of ovserved epochs than in the condition where animals are alone and not food deprived.

Discussion

Early weaned and normally weaned 30 day old rats exposed to inescapable extremes of ambient temperatures differ in their responses to these stimuli. Early weaned animals are less able to maintain stable body temperatures against thermal challenges. In the cold this is not due to a lack of mobilization of appropriate thermoregulatory mechanisms, but rather the ineffectiveness of these mechanisms. For example, when exposed to cold ambient temperatures 15w rats actually shiver more and respond with piloerection sooner than 21w rats. When with a littermate 15w rats are more active than 21w rats. These responses help 15w rats to maintain normal body temperatures. However, despite these responses 15w rats have relatively large decreases in body temperature when compared to 21w rats. Food deprivaton served to potentiate the differences in the effects of the weaning conditions, and did not attenuate the behavioral thermoregulatory responses of 15w rats.

When animals were exposed to high ambient temperatures, 15w animals had relatively large increases in body temperature compared to 21w animals. In contrast to 15w rats responses to inescapable cold, in the case of inescapable high temperatures 15w rats do not respond with appropriate behavioral thermoregulatory behaviors. Thus it is a lack of response rather than ineffectiveness of thermoregulatory behaviors

that contribute to the inability of 15w rats to defend body temperature against a heat challenge. This lack of response may indicate inappropriate sensation of high temperatures, disturbances in motor responses to high temperatures, or disturbances in the CNS structures responsible for temperature regulation. However, the present study does not differentiate between these possibilities. Early weaned rats showed significantly less saliva spreading, and prone extension, and 15w rats engage in more activity. Without high levels of saliva spreading 15w rats were less competent in defending their body temperature. The reason for 15w rats lower levels of saliva spreading is not known. It is possible that this reflects some CNS disturbance, but it is also possible at least for food deprived rats that 15w rats are more dehydrated than 21w rats. Early weaned rats responded less frequently with prone extension in either food deprived or non food deprived conditions, this may also indicate differences in CNS structures involved in body temperature maintainance.

General Discussion

The results of these experiments suggest that 30 day old 15w rats do have disturbances in behavioral thermoregulation. These disturbances are only elicited by some situations that require mobilization of mechanisms necessarly to defend body temperature

against a challenge. Under normal laboratory conditions there are no apparent differences in body temperature of 15w and 21w rats. And when 30 day old 15w or 21w rats are placed in a thermal gradient no substantial differences in temperature selection behavior are observed. However, when challenged by food deprivation and restraint 15w rats become hypothermic (Ackerman et al. 1978a, 1978b). And if exposed to inescapable extremes of high and low ambient temperatures 15w rats become relatively more hyperthermic or hypothermic than do 21w rats, which suggests that 15w rats ability to regulate temperature is compromised.

The temperature selection response of 15w rats suggest that they are able to avoid extremes of temperature when that opportunity is available. The thermoregulatory disturbance of 15w rats only becomes obvious when they are forced to mobilize heat dissipation or heat production mechanisms. In the situation of unavoidable cold ambient temperature, 15w rats respond appropriately. However, these behaviors are ineffective in maintaining body temperature. In the situation of unavoidable high ambient temperature, 15w rats fail to respond appropriately. This inappropriate response may indicate disturbances in sensation, in motor response or in CNS structures.

Satinoff and Shan (1971) first demonstrated that different brain areas are responsible for specific physiological and behavioral thermoregulatory responses. They found that the lateral hypothalamus

was responsible for certain behavioral responses to cold ambient temperatures while the preoptic anterior hypothalamus was responsible for physiological responses to cold ambient temperatures. In an elegant series of studies Roberts and his coworkers (Roberts & Martin, 1974; Roberts & Martin, 1977; Roberts & Mooney, 1974; Roberts, Mooney, & Martin, 1974) further localized specific brain areas responsible for specific thermoregulatory responses. By stimulating various brain areas with diathermic electrodes it was found that prone extension was elicited by stimulating the anterior preoptic area, locomotion was elicited by stimulating the ventral midbrain and dorsal medulla, and vasodilation was elicited by stimulating the anterior preoptic area or the septal region. While lesioning the preoptic anterior hypothalamus abolished the behavioral response of prone extension in rats subjected to high ambient temperatures, this same lesion had no effect on saliva spreading, locomotion or tail vasodilation.

When exposed to inescapable heat 15w rats show deficits in prone extension and saliva spreading but they show increased locomotion. It is possible to infer that 15w rats have disturbances in the CNS structures involved in the control of prone extension, or saliva spreading but not in those involved with locomotion. Decreased amounts of saliva spreading and prone extension, and increased amounts of locomotion would all contribute to a rise in body temperature at high ambient temperatures. Increased locomotion, although contributing to

a rise in body temperature, may be an appropriate response to high ambient temperatures as increased locomotion may indicate greater attempts to escape aversive stimuli.

When exposed to cold ambient temperatures 15w rats respond in ways such as piloerection and shivering that could help them to maintain normal body temperatures. However these responses are inadequate, as 15w rats become relatively hypothermic during exposure to cold temperatures. This may indicate that 15w rats have a greater need for heat production that is required by smaller body size and less subcutaneous fat than do 21w rats (Greenberg & Ackerman, 1980). It is possible that the small body size and therefore large surface area to body weight ratio is partially responsible for 15w rats inabiltiy to maintain body temperature when exposed to high as well as low ambient temperatures.

Under my test conditions 15w rats were more exothermic than 21w rats. This may reflect a delay in maturation of homeothermy. Or it may be that early weaning effects development so as to alter CNS responses involved in temperature regulation. The existence of disturbances in the normal functioning of the anterior preoptic hypothalamus of 15w rats is speculative, however not inconsistent with what is known of their ability to thermoregulate. Such a disturbance combined with small body size would leave 15w rats vulnerable to any challenge to maintaining normal body temperature.

PART III: OXYGEN CONSUMPTION OF 30 DAY OLD EARLY AND NORMALLY WEANED RATS

Introduction

Thus far I have considered the effects of differing body composition and differing behavioral responses to cold of 15w and 21w rats. The question remains as to why 15w animals are more likely to become hypothermic during food deprivation and restraint. Hypothermia could be the result of inadequate heat conservation mechanisms, inadequate heat production mechanisms, or inadequacies in both heat conservation and heat production. It is possible that 15w rats have relatively high metabolic rates and rapidly deplete available fuel stores. This depletion could subsequently result in hypothermia. Or 15w rats could have relatively low metabolic rates and hypothermia would result from insufficient heat production. Low metabolic rates could also be combined with depletion of fuel stores which would be reflected in even less heat production as food deprivation is prolonged. The current studies measure oxygen consumption of 15w and 21w rats in order to assess their heat production capabilites.

The temperature regulation of 30 day old rats could be affected by

early weaning in a number of ways. Premature weaning occurs at the time in the young rats life when there is a rapid development of thermoregulatory control. By the end of the third week of life the young rat can maintain body temperature within the narrow range typical of the adult, while before that time it cannot (Adolph, 1957; Conklin & Heggeness, 1971). This is in part due to the increasing capability to produce heat via metabolism (Blackmore, 1970). Premature weaning may disrupt the maturation of these systems. However, it is not known if 15w and 21w rats expend different amounts of energy during food deprivation and restraint.

An important way in which young animals maintain body temperature is via non shivering thermogenesis (NST). It has been shown that the primary source of NST in young rats is heat generated by brown adipose tissue (BAT) (Smith & Horowitz, 1969). As 30 day old 15w rats have less BAT that 21w rats it is possible that they have diminished BAT mediated heat producing capacity. It is also possible that 30 day old 15w rats have an inappropriately diminished response to external situations which elicit heat production. Animals such as the ob/ob mouse have mitochondrial defects in their BAT which diminishes the heat generating capacity of this tissue (Himms-Hagen & Desautels 1978). While adult rats exposed to cold show qualitative alterations in BAT which result in enhanced thermogenesis (Smith & Roberts, 1964). Early weaned rats may have qualitatively different BAT from that of 21w rats,

which could affect the ability of 15w rats to produce heat via NST.

BAT mediated NST is normally activated by release of endogenous norepinepherine (NE) which acts on the β-adrenergic receptors of BAT. I wished to determine if administration of exogenous NE would lead to differential oxygen consumption responses in 15w and 21w rats. Such differential responsiveness could allow the inference of qualitative differences in the BAT of 15w and 21w rats.

Part III -- Experiment 1

I first examined the oxygen consumption of 30 day old 15w and 21w rats under conditions that have been shown to lead to hypothermia in 15w but not 21w rats. I measured oxygen consumption during food deprivation followed by physical restraint.

It has been shown that 15w and 21w rats behave differently during the food deprivation and restraint periods associated with RGE formation in 15w rats as 15w rats spend a significantly greater proportion of their time in quiet wakefulness during restraint (Ackerman et al. 1978a). However, measurements of activity of 15w and 21w rats during food deprivation and restraint fail to differentiate these groups (Ackerman et al. 1978a). Differences in metabolism may accompany these behavioral differences, and these differences should be reflected in oxygen consumption.

Method

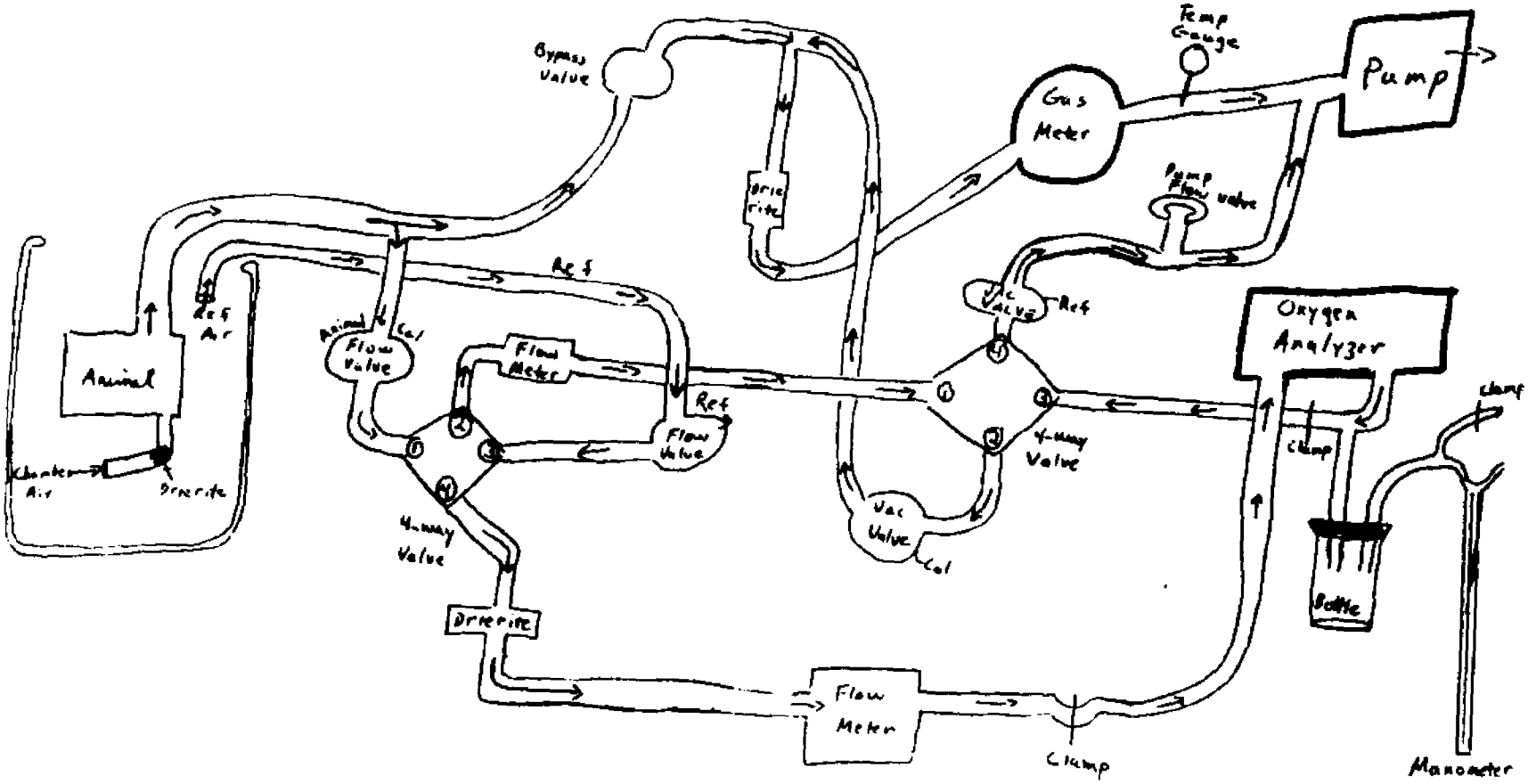
Subjects

Male rats from split litters weaned, raised, and housed as previously described served as subjects. Eight 15w rats each from separate litters and eight 21w rats from separate litters were used. All rats were 28 to 30 days old at the start of the test period.

Apparatus

Oxygen consumption was measured using a S3A/1 oxygen analyzer (Applied Electrochemistry, Sunnyvale, Ca.). Dried air was drawn continuously through a metabolic chamber by use of a vaccum motor. The metabolic chamber was a plexiglass aquarium measuring 10 X 20 X 25 cm in which the animals were able to freely move. Air flow was kept constant by use of air contol valves and flow meters (Roger Gilmont Instruments, Great Neck, N.Y.). The metabolic chamber was placed in an incubator (GCA/Precision, Chicago, Il.) such that air temperature was controlled at all times. Changes in oxygen content of air from the metabolic chamber were recorded on one channel of a strip chart recorder (Houston Instruments, Houston Txs.). Another channel of the recorder was used for measuring temperature. Rectal temperature was

measured during restraint by means of an indwelling rectal probe. By use of a channelizer (Bailey Instruments, Saddle Brook, N.J.) ambient and rectal temperatures were sampled for alternating 1 minute periods and periods and recorded. An illustration of the system used for oxgen consumption measurements is shown in figure 13.



Clamps for O_2 Calibration

Procedure

Rats were food deprived for 26 hours and subsequently restrained in plexiglass adaptations of Bowman cages for 26 hours. A Bowman cage restrains rodents by means of steel rods that run along the length of the animals body. The rods are held in place by the ends of the cage. There is an opening for the animals tail at one end of the cage so that rectal probes are easily kept in place. The adaptation of the cage that I used had plexiglass rather than steel rods so as to reduce thermal conductance. Water was available during food deprivation. Body weights were recorded at the start of the experiment, following food deprivation and after restraint. Rectal temperature was measured once at the start of the experiment and then continuously during restraint. Oxygen consumption was measured continuously while animals were food deprived and restrained. Ambient temperature of the metabolic chamber was maintained at 20 °C throughout the test procedure.

Following restraint, animals were sacrificed and examined for gastric erosions by a second investigator who was blind as to the weaning condition of the test animal.

Results

During both the initial 26 hours of food deprivation and during restraint 30 day old 15w rats used significantly less oxygen than did 30 day old 21w rats (see figure 14). A repeated measures ANOVA with weaning condition and time as factors was performed. Differences between weaning conditions were highly significant $F(1, 106) = 156.35$, $p<.0001$. There was also a significant effect of time $F(51,106) = 3.33$, $p<.02$, and a significant interaction of weaning condition and time $F(51, 106) = 1.48$, $p<.05$. During food deprivation alone, the oxygen consumption of both groups of rats was relatively constant, although 15w rats level of oxygen consumption was consistantly lower than that of 21w rats. Early weaned animals also had smaller standard deviations in their oxygen consumption during the initial food deprivation period than did normally weaned animals (see figure 15). When animals from both weaning conditions were first restrained they showed a sharp rise in oxygen consumption (see figure 14). During the 26 hours of restraint the oxygen consumption of 21w rats declined slightly, while the oxygen consumption of 15w rats decreased precipitously during this time (see figure 14). During restraint the variance of oxygen consumption increased for 15w rats, while that of 21w rats decreased. The increasing variability of oxygen consumption in 15w rats reflects the variation between those animals that form RGEs and become

hypothermic and those that do not.

Trend analyses were performed on the oxygen consumption curves of 30 day old 15w and 21w rats during food deprivation and restraint periods. During food deprivation significant fits were found as linear for both weaning conditions. During restraint there were significant quadratic and cubic components in 15w rats, while the oxygen consumption curve of 21w rats showed only a significant linear component. The fit of these lines reflects the fact that 21w rats show relatively constant levels of oxygen consumption during restraint while 15w rats show declines in oxygen consumption as restraint continues.

Thirty day old 15w and 21w rats did not differ significantly in body temperature measured immediately upon removal from the home cage ($\underline{M}$ = 36.98 for 15w, $\underline{M}$ = 36.44 for 21w, $\underline{t}(14) = .689$, $\underline{p}>.1$). Nor were significant differences in body temperature found after 26 hours of food deprivation ($\underline{M}$ = 37.16 for 21w, $\underline{M}$ = 36.42 for 15w, $\underline{t}(14) = 1.2$, $\underline{p}>.1$). However, during restraint 15w rats became hypothermic while 21w rats maintained body temperature (see figure 16). At the end of the restraint period 15w rats had significantly lower body temperatures than 21w rats ($\underline{M}$=26.32, $\underline{M}$ = 34.46, $\underline{t}(14) = 3.21$, $\underline{p}<.01$).

Early weaned rats had significantly greater incidences of gastric erosions than 21w rats ($\chi^2(1) = 4.2$, $\underline{p}<.05$). The length of lesions was also greater for 15w rats ($\underline{M}$ = 9.8 mm for 15w rats, $\underline{M}$ = .125 mm for 21w

rats, $t(14) = 3.21$, $p<.01$). The incidence of gastric erosions was correlated with occurance of hypothermia ($r = .78$, $p<.005$).

Body weights of 30 day old 15w rats were significantly lower than those of 30 day old 21w rats at the start of the experiment and after food deprivation and restraint. However, during food deprivation and restraint 21w rats lost more weight than did 15w rats (see table 9). The increased weight loss of 21w rats is consistant with their higher metabolic rates.

Table 9

Means and t tests for body weights of 30 day old 15w and 21w rats, before and after food deprivation and restraint.

Condition	Means		t	p
Start	15w	21w		
	69.8	90.5	5.19	<.0001
Post-food deprived	55.9	75.4	5.04	<.0001
Post Restraint	46.6	63.9	4.96	<.0001
Weight loss	23.2	27.6	2.53	<.025

Figure 14

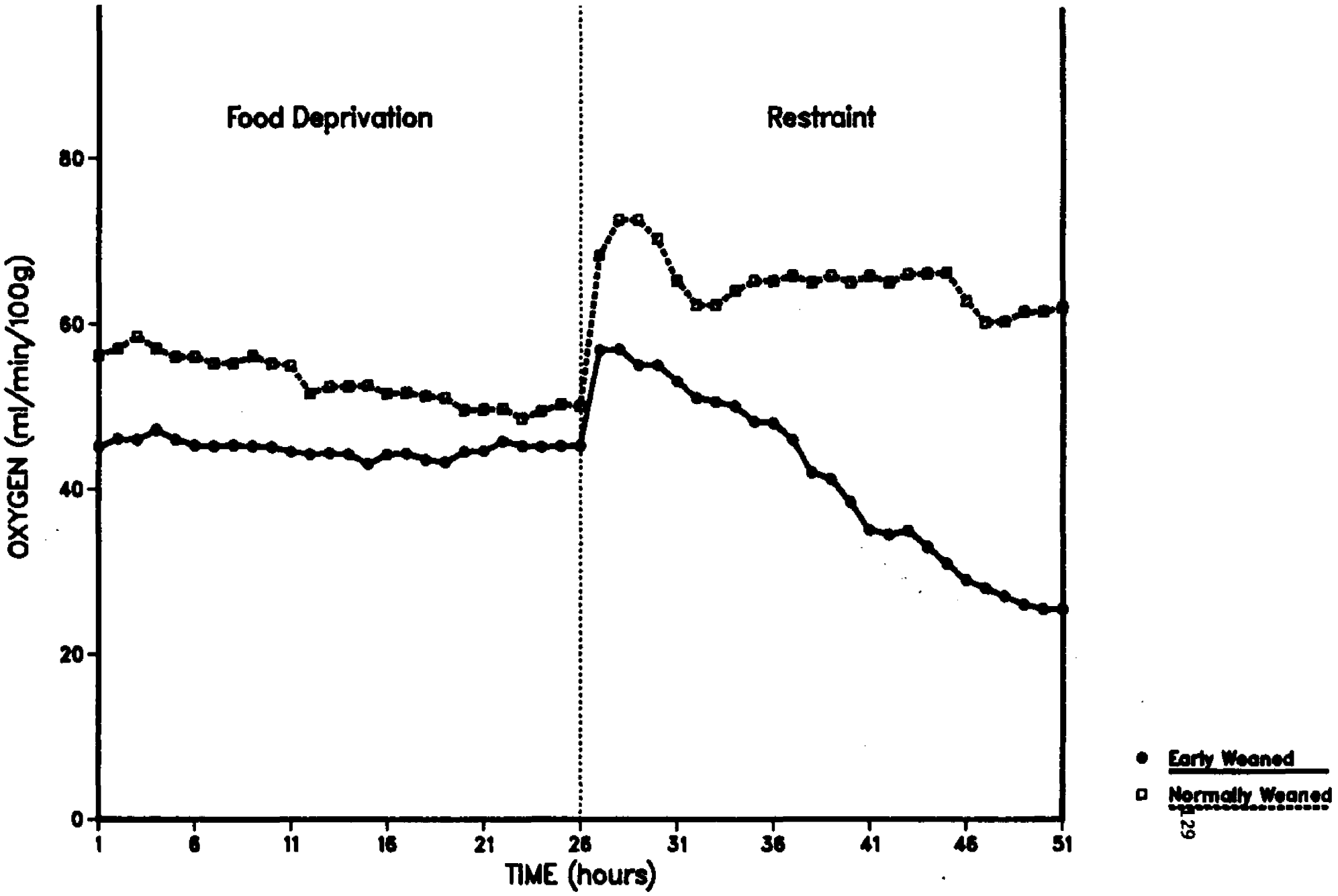
Illustrated is the mean oxygen consumption of 15w and 21w rats during food deprivation followed by physical restraint. Oxygen is expressed in ml/min/100g body weight. Time 1-26 is that of food deprivation and time 27-52 is during restraint.

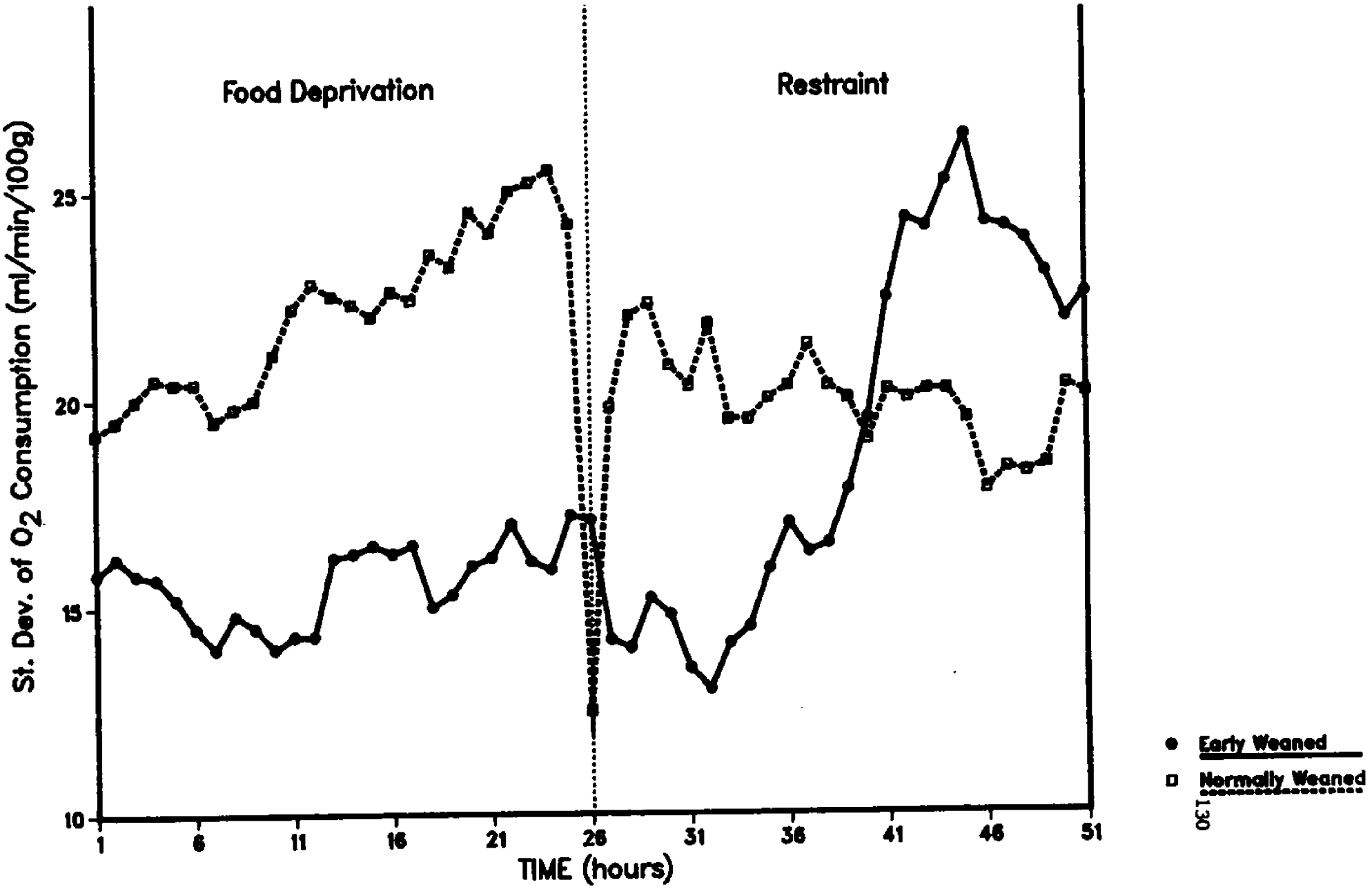
Figure 15

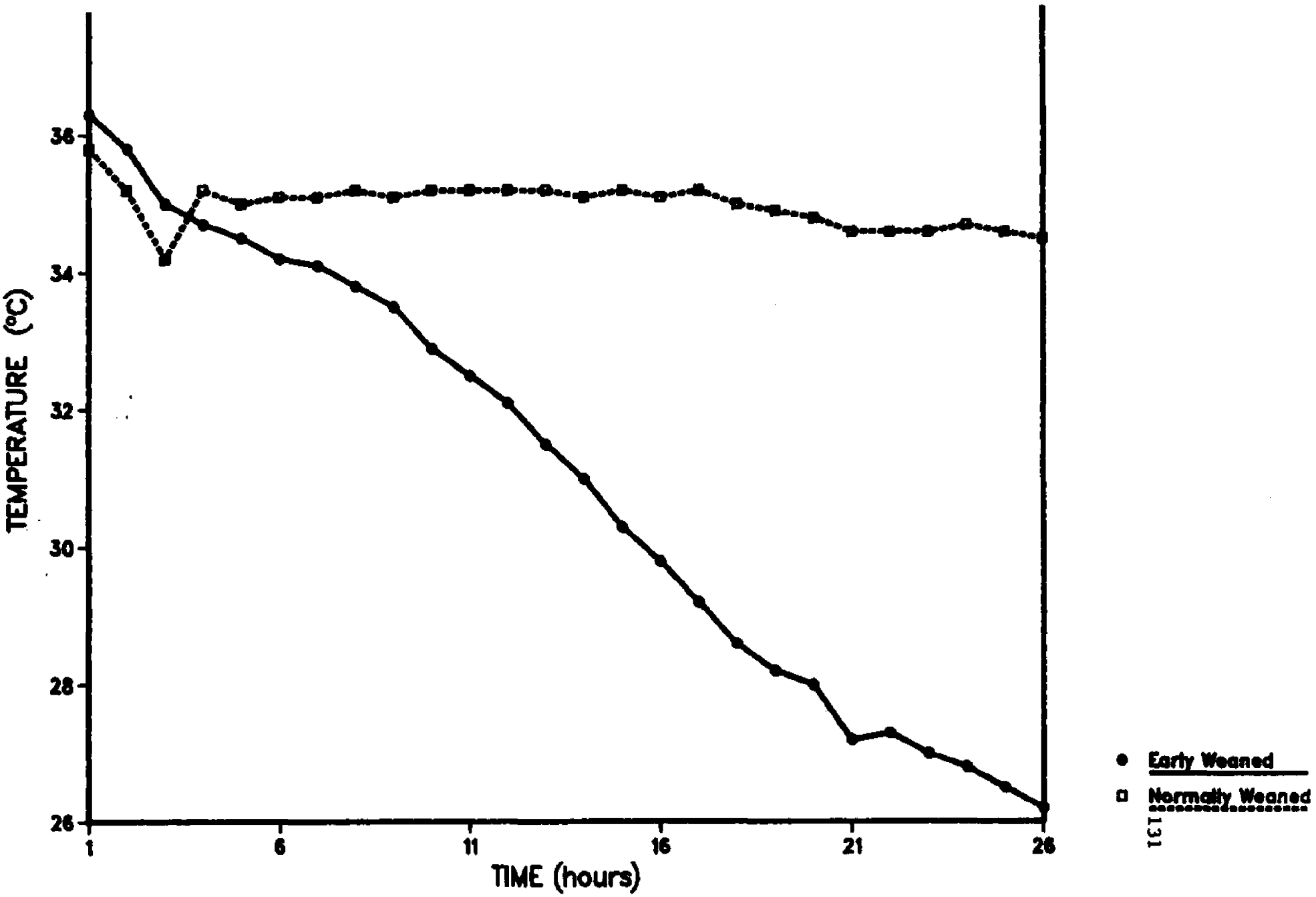
The average standard deviation per hour for oxygen consumption of 15w and 21w rats is shown. Time 1-26 represents the period of food deprivation while time 27-52 represents the period of restraint.

Figure 16

This figure shows the mean rectal temperatures of 15w and 21w during physical restraint.


Food Deprivation
Restraint
OXYGEN (ml/min/100g)
0
20
40
60
80
1
6
11
16
21
26
31
36
41
46
51
TIME (hours)
Early Weaned
Normally Weaned


Food Deprivation
Restraint
St. Dev. of O_2 Consumption (ml/min/100g)
25
20
15
10
1
6
11
16
21
26
31
36
41
46
51
TIME (hours)
Early Weaned
Normally Weaned
L130


TEMPERATURE (°C)
36
34
32
30
28
26
1
6
11
16
21
26
TIME (hours)
Early Weaned
Normally Weaned

Part III -- Experiment 2

In the first experiment I found that 30 day old 15w and 21w rats had oxygen consumption levels that were parallel during food deprivation and at the initial portion of restraint, but diverging levels of oxygen consumption as restraint continued. I wished to determine whether the important factor in the time related decrease in oxygen consumption of 15w animals was physical restraint, or whether this was an effect of prolonged food deprivation. I therefore determined the oxygen consumption of 15w and 21w rats under conditions of prolonged food deprivation but without restraint.

Method

Rats from the split litter condition were used as subjects. Six 15w and six 21w rats each from separate litters were used. Animals were weaned and housed as described in the first experiment. Oxygen consumption measures were determined as previously described. Animals were removed from the home cage, weights and body temperatures were determined and animals were placed in the metabolic chamber without food, but with water available. After 26 hours weights and body temperatures were again recorded. Animals were then returned to the metabolic chamber for an additional 26 hours. After the second 26 hour

period, weights and body temperatures were again recorded. At that time animals were sacrificed and examined for gastric erosions. Ambient temperature was maintained at 20 °C at all times.

Results

A repeated measures ANOVA was performed with weaning condition and time as factors. During prolonged food deprivation significant effects were found for weaning condition ($F(1,500)$, = 37.8, $p<.0001$), and time ($F(49,500)$, = 11.67, $p<.005$). A significant interaction was found for weaning condition and time ($F(51,500)$, = 13.16, $p<.0001$). Early weaned rats used signifcantly less oxygen than did 21w rats (M = 54.87 ml/min/100g 15w rats, M = 72.52 ml/min/100g 21w rats).

Over the first 26 hours 15w and 21w rats maintained oxygen consumption at relatively constant levels (see figure 17). At the start of the second period of food deprivation no substantial increase in oxygen consumption was seen initally as occurred when animals were restrained. As food deprivation continued over the next 26 hours 21w rats had gradual increases in oxygen consumption while 15w rats had progressively decreasing oxygen consumption resulting in the obtained significant interaction between weaning condition and time. Except for the initial rise in oxygen consumption seen in response to restraint in the first experiment, the level of oxygen consumption

during food deprivation alone was similar to that during food deprivation and restraint.

Early weaned animals became hypothermic over the 56 hours of food deprivation while normally weaned animals did not. After 56 hours of food deprivation the mean body temperature of 15w rats was 26.13 °C, while that of 21w rats was 35.08 °C, and this difference was significant ($\underline{t}(10) = 3.91$, $p<.003$). Upon removal from the home cage and after 26 hours of food deprivation no significant differences in body temperature were found. Body temperatures of these 30 day old 15w and 21w rats during prolonged food deprivation alone were similar to those of animals from Part III, experiment 1 who were both food deprived and restrained.

Food deprivation alone produced more gastric erosions in 15w rats than in 21w rats ($\chi^2(1) = 4.33$, $p<.05$; $\underline{t}(10) = 2.3$ $p<.045$). However, the severity of gastric erosions, based on the number of erosions per animal and the length of those erosions, is less in 15w rats that are only food deprived than in 15w rats food deprived and subsequenly restrained (see table 10).

As shown previously (Ackerman et al. 1975, 1978a, 1978b, experiment 1) body weights of 30 day old 15w rats were significantly lower than those of 30 day old 21w rats at all times during the experiment (see table 11). When animals were food deprived but not restrained no significant differences in the amount of weight lost over

the 52 hour time period were found. The body weights of animals food deprived but not restrained were not significanlty different from body weights of animals from the same weaning condition that were both food deprived and restrained. The amount of weight lost during food deprivation alone did not differ significantly from the amount of weight lost when animals were both food deprived and restrained.

Table 10

Extent and length of gastric erosions in rats food deprived or food deprived and restrained

Condition	Mean Lesion Length		Mean Lesion Number	
	15w	21w	15w	21w
Food Deprived	7.2 mm	.991 mm	4.5	.8
Food Deprived and Restrained	9.8 mm	.125 mm	7.5	.125

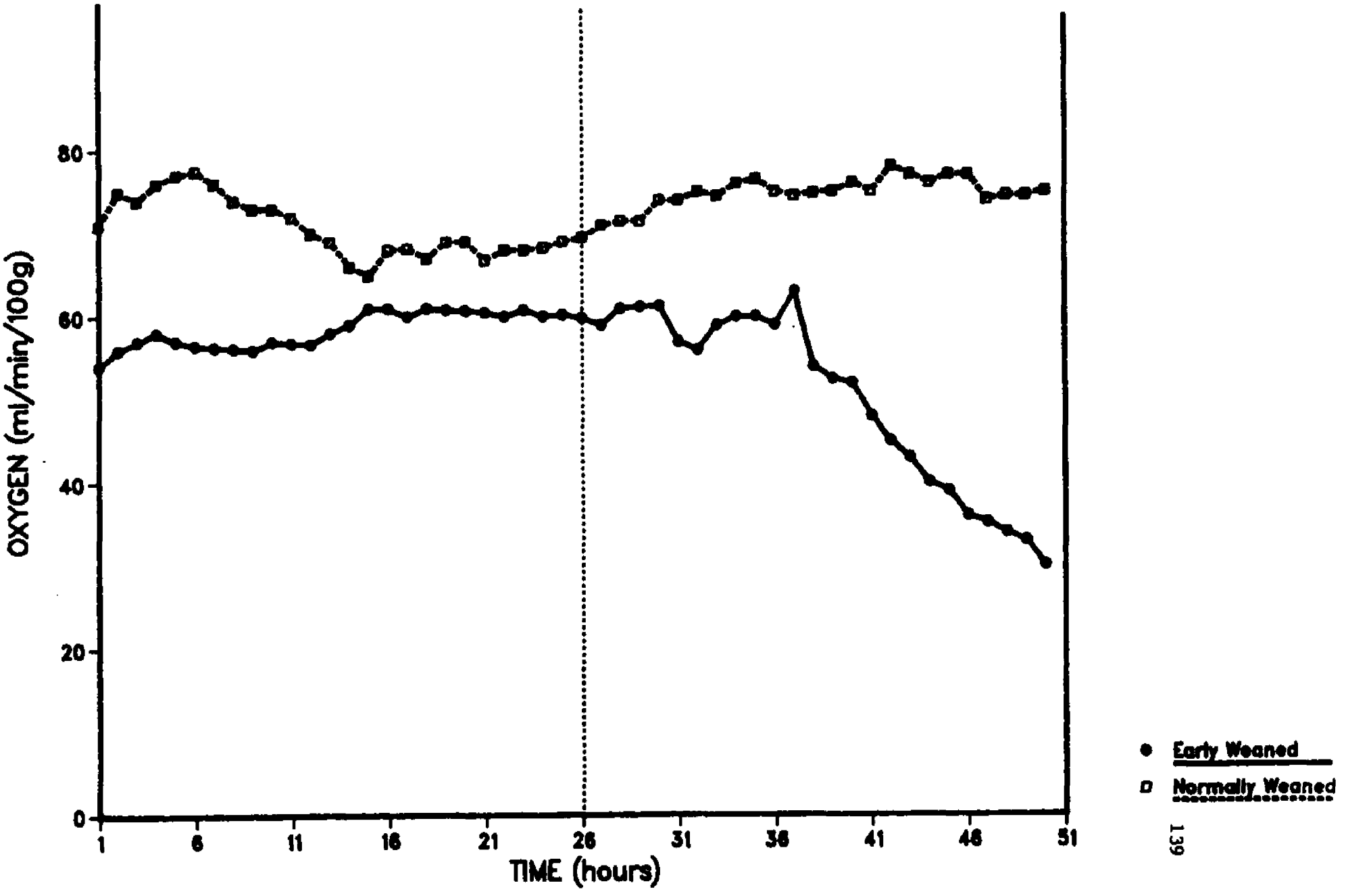
Table 11

Mean Body Weights, and t tests of rats food deprived for 52 hours

Condition	Means		t	p<|t|
Start	EW	NW		
	70.2	93.81	3.34	.0075
26 hours deprived	54.0	78.8	3.78	.0036
52 hours deprived	45.3	66.1	2.86	.016
Weight loss	24.8	27.7	1.61	.272

Figure 17

This figure shows the mean oxygen consumption of 15w and 21w rats during 56 hours of food deprivation. Oxygen consumption is expressed in ml/min/100g body weight.


OXYGEN (ml/min/100g)
80
60
40
20
0
1
6
11
16
21
26
31
36
41
46
51
TIME (hours)
Early Weaned
Normally Weaned

Part III -- Experiment 3

In conditions where animals were food deprived and restrained 30 day old 15w rats used less oxygen than 21w rats. Under these conditions 15w rats also became hypothermic and developed gastric erosions. Under normal laboratory conditions 15w and 21w rats do not exhibit differences in body temperature (Ackerman et al. 1978a, Part 2 this document). To see if 15w rats had generally lowered rates of oxygen consumption or if lowered rates of oxygen consumption was a specific response to food deprivation I measured oxygen consumption of 30 day old 15w and 21w rats under conditions approximating normal laboratory conditions. In this experiment I allowed rats free access to food and water while measuring oxygen consumption.

Method

Individual rats from six litters of 15w and six litters of 21w rats from the split litter were used. Animals were weaned and housed as in the first experiment. Oxygen consumption measures were determined as previously described. Animals were removed from the home cage, weights and body temperatures were determined and animals were placed in the metabolic chamber. Oxygen consumption was measured for 26 hours. During this time food and water were freely available.

After 26 hours weights and body temperatures were again recorded. Rats were then returned to the metabolic chamber for an additional 26 hours. Food and water were again freeely available. After the second 26 hour period, weights and body temperatures were again recorded. At that time animals were sacrificed and examined for gastric erosions. Ambient temperature was maintained at 20 ^{0}C at all times.

Results

Oxygen consumption data was analyzed with a repeated measures ANOVA with weaning condition and time as factors. A significant effect was found for oxygen use across time ($F(51,599)$, $p<.009$), but not for weaning condition. Oxygen consumption for both weaning conditions was much greater than when food was not available (see figure 18). During the first 26 hours with food freely no differences in oxygen consumption of 15w and 21w rats was found. During the second 26 hours while food was available 15w rats actually used more oxygen than did 21w rats although these differences were significant only for hours 30 to 40.

There were no significant differences in body temperatures of 15w and 21w rats at any time (see table 12). Early weaned animals did not become hypothermic over the 56 hours in the metabolic chamber when food was available to them.

Figure 18

Figure 18 shows the mean oxygen consumption of 15w and 21w rats measured over 56 hours. Both groups had food and water freely available at all times.

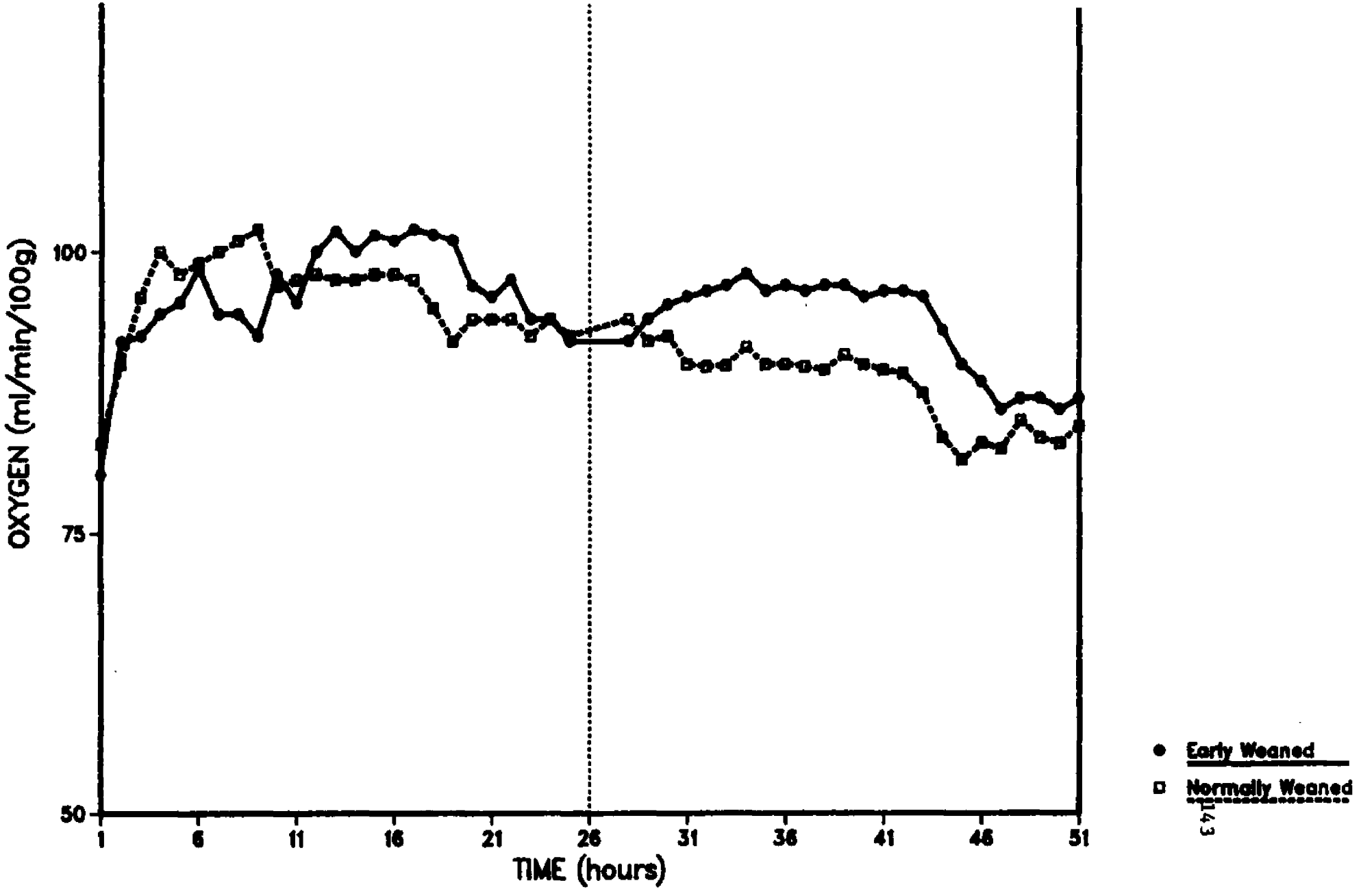

OXYGEN (ml/min/100g)
100
75
50
1
6
11
16
21
26
31
36
41
46
51
TIME (hours)
Early Weaned
Normally Weaned

Table 12

Mean rectal temperatures, and t tests of rats with free access to food and water

Condition	Means		t	p
	EW	NW		
Start	36.6	36.1	1.15	>.1
26 hours	36.2	36.5	.649	>.5
52 hours	36.7	36.2	1.08	>.1

Table 13

Mean body weights and t tests of rats with free access to food and water

Condition	Means		t	p
	EW	NW		
Start	68.7	84.3	-3.48	<.0059
26 hours	74.7	90.1	-3.15	<.0103
52 hours	81.3	97.1	-3.21	<.0093
wt gain	12.6	12.8	.16	>.8

Early weaned animals weighed signifcantly less than 21 rats at all times (see table 13). Animals from both weaning conditions gained weight during both 26 hour periods when oxygen consumption was measured. There was, however, no significant difference between groups in the amount of weight gained during the 52 hour period.

No gastric erosions were found in any animal of either weaning condition.

Discussion

I found that 30 day old 15w rats use less oxygen then 21w rats under conditions of food deprivation at 20 °C. This is true whether animals are only food deprived or if they are both food deprived and physically restrained. Under these conditions 15w rats become hypothermic and develop RGEs while 21w rats are able to maintain body temperature and are relatively RGE resistant. The tendency of 15w rats to become hypothermic and form gastric erosions was associated with insufficient heat production. This does not eliminate the possiblity that 15w rats are less efficient in heat conservation than 21w rats. However, during food deprivation or food deprivation and restraint 15w rats do show reltively low levels of oxygen consumption and are unable to maintain body temperature at normal levels, which suggests that inadquate heat production is at least partially responsible for 15w

rats susceptibility to hypothermia.

When animals were subjected to prolonged food deprivation while oxygen consumption was measured, 15w rats formed lesions and became hypothermic. This is in contrast to Part I, experiment 1 when prolonged food deprivation did not lead to gastric erosions or hypothermia. However, there are several differences in the conditions of prolonged food deprivation in these two studies. In Part I, experiment 1, animals that were subjected to prolonged food deprivation were food deprived with their littermates. The presence of littermates would provide comfort and permit huddling as a thermoregulatory behavior. In addition food deprivation was imposed in the home cage and although the cage was placed in an incubator the environment was somewhat familiar. In the present study animals were isolated for the entire 52 hour food deprivation period and the environment was novel. Presumably isolation combined with a novel environment is an added challenge that 15w rats are unable to meet.

When food is available 30 day old 15w rats use as much or more oxygen than do 21w rats. With food available animals from both weaning conditions are able to maintain normal body temperatures and neither group is susceptible to RGEs. Food deprivation seems to impose a particular challenge to 15w rats wherein they produce too little heat to maintain body temperature. Early weaned rats have previously been described as having a "latent thermoregulatory disturbance" (Ackerman

et al. 1978a) since under normal laboratory conditions 30 day old 15w and 21w do not show differences in body temperatures. However, during food deprivation and restraint 15w rats of this age become hypothermic. In terms of oxygen consumption of 15w and 21w rats a "latent disturbance" is an accurate discription. It is only when they are food deprived or food deprived and restrained that 15w rats shown significantly lower levels of oxygen consumption.

For both weaning conditions physical restraint initially acts as a stimulus to increase metabolic activity. When physical restraint is imposed both 15w and 21w rats show parallel, immediate, sharp increases in oxygen consumption. This suggests that 15w rats are responding to the challenge of restraint as best they can. However, as restraint continues 15w rats are unable to continue producing heat in sufficient quantities to prevent hypothermia.

The first three experiments assessed the oxygen consumption of 15w and 21w rats either under conditions of food deprivation and restraint or under conditions analagous to those of the normal laboratory. I found that food deprivation and restraint is accompanied by low oxygen consumption levels in 15w rats. However, I do not know what mechanisms lead to the failure of 15w rats to produce sufficient heat during food deprivation and physical restraint. Early weaned rats may not adequately sense the need to activate nonshivering thermogenesis, may have deficiencies in heat producing capabilities of

their BAT or may have insufficeint BAT to continue to produce heat during continued food deprivation. A temperature of 20 °C may also present more of a challenge to 30 day old 15w rats than to 30 day old 21w rats, and thus lead to a more rapid depletion of fuel stores and heat generating capacity. The next set of experiments sought to examine these possibilities.

Part III -- Experiment 4

When rats mature normally there is a progressive decrease in the temperature that is considered thermoneutral (Taylor, 1960; Conklin & Heggenes, 1971). Thermoneutrality is defined as that temperature at which minimum oxygen consumption is observed. As mentioned previously, Blackmore (1970, 1972) found that increasing the length of time that young rats were separated from their mothers led to increasing delays in the maturation of homeothermy. This delay in maturation of homeothermy was expressed as a delay of the age at which rats reached a given thermoneutral temperature. Early weaning at day 15 may lead to a delay in the maturation of homeothermy as early weaning necessitates total maternal deprivation. Thus 30 day old 15w rats would presumably have a higher thermoneutral temperature than 21w rats of the same age. If so this would imply that the ambient temperature of 20 °C at which I have food deprived and restrained 30

day old rats would deviate more from thermoneutrality for 15w than for 21w rats. In this case then a temperature of 20 °C would present more of a thermal challenge for 15w than for 21w rats. In this experiment I assessed the temperature at which 30 day old 15w and 21w rats are thermoneutral.

Method

Individual 30 day old rats from the split litter condition were used. Six litters of 15w and six litters of 21w rats served as subjects. Animals were weaned and housed as described in the first experiment. Individual rats were tested to determine the temperature at which there was minimum oxygen consumption. Test animals were removed from the home cage and placed in a metabolic chamber where oxygen consumption was measured. The weight and rectal temperature of test animals were determined at the start and finish of the experiment. Testing was always initiated at 10:00 am. The apparatus was the same as in the first three experiments. No food or water was available during testing.

The initial ambient temperature in the metabolic chamber was 26 °C. That temperature was maintained until oxygen consumption was stable. When stability of oxygen consumption was observed the ambient temperature was increased by 1 °C. That temperature was then

maintained until oxygen consumption was again observed to stabilize. Ambient temperature was then increased by additional 1 °C increments, allowing for stabilization of oxygen consumption between temperature increases, until oxygen consumption was observed to rise. At that time ambient temperature was decreased in 0.5 °C increments until oxygen consumption was again seen to rise. In this way the temperature at which oxygen consumption was at a minimum was determined.

Results

The temperature at which each 30 day old animal used the least amount of oxygen was determined. The mean temperature at which 15w rats used the least oxygen was 30.2 °C, while the mean temperature at which 21w rats used the least oxygen was 29.1 °C and this difference was significant ($t(10) = 2.38$, $p<.038$).

As in previous studies 15w rats were found to weigh significantly less than 21w rats ($M = 70.3$ g for 15w rats, $M = 91.5$ for 21w rats, $t(10) = 2.4$, $p<.032$). However, no significant differences were found in the change in body weight during the experimental period. No significant difference between weaning conditions was found in the rectal temperatures measured at either the start or end of the experiment.

Discussion

The fact that 15w rats have a higher thermoneutral temperature than 21w rats is consistent with the hypothesis that 15w rats have delayed maturation of homeothermy. Taylor (1960) has shown that as rats mature normally they have higher basal rates of oxygen consumption per unit body weight. in addition, as rats mature they show greater increases in oxygen consumption in response to cold ambient temperatures. If 15w rats are delayed in their maturation of temperature regulation they would be expected to have lower basal metabolic rates and respond with less oxygen consumption in response to lowered ambient temperatures. Under conditions of food deprivation or food deprivation and restraint 15w rats do indeed respond with lower levels of oxygen consumption.

The fact that 15w rats use less oxygen under conditions of food deprivation is consistent with a hypothesis of delayed maturation of homeothermy. Bignall, Heggeness and Palmer (1974) have shown that acute starvation leads to a decline in the level of oxygen consumption in response to cold in 5, 10 and 15 day old rats but not in 20 day old rats. During the experimental conditions of food deprivaton and restraint ambient temperatures are at 20 °C. For 15w rats this temperature deviates more from thermoneutrality than it does for 21w rats. Thus 20 °C is more of a cold stimulus for 15w than for 21w rats.

One would predict that rats younger than 30 days would respond to colder ambient temperatures combined with acute starvation with less oxygen consumption than would be observed for 30 day old rats. The predicted response for a younger rat is consistant with the observed response of 15w rats during food deprivation or food deprivation and restraint.

Part III -- Experiment 5

For young rats one important method of producing heat is through non shivering thermogenesis (NST). In young rats NST has been shown to be based mainly on the calorigenic effects of endogenous norepinepherine (NE) on brown adipose tissue (Moore & Underwood, 1963). Endogenous NE is released by the sympathetic nervous system in response to cold (Hsieh & Carlson, 1957), and activates the heat producing capabilities of BAT.

Thirty day old 15w rats have been shown to have less interscapular BAT in absolute terms and less BAT per unit body weight than 21w rats of the same age (Greenberg & Ackerman, 1980; part 1 this document). Early weaned rats may therefore have a decreased capacity for BAT mediated NST. It is also possible that 15w rats may release insufficient endogenous NE to stimulate heat production from the BAT that they have. It has been suggested that the reason young rats are

unable to respond to cold ambient temperatures with increased oxygen consumption is due to insufficient release of endogenous NE (Bignall, Heggeness & Palmer, 1977). If 15w rats were unable to produce adequate heat during food deprivation and restraint due to insufficient release of endogenous NE this would be consistant with a hypothesis of delayed maturation of homeothermy for 15w rats.

It is also possible that 30 day old 15w rats may have qualitatively different BAT from 21w rats of the same age. Qualitative differences in BAT mitochondria that affects the heat producing capacity of this tissue have been found. In ob/ob mice qualitative defects in the mitochondira have been found (Himms-Hagen & Desautels, 1978). These defects have been associated with defective responses to cold and a tendency to become hypothermic (Davis & Meyer, 1954; Trayhurn, Thurlby, & James 1976). Adult rats and lean mice that are acclimated to cold have BAT that is able to increase heat output above normal levels, and these animals show hightened responsiveness to exogenous NE (Smith & Roberts, 1964; Himms-Hagen & Desautels, 1978; Thurlby & Trayhurn, 1980). As 30 day old 15w rats use less oxygen than 21w rats under conditions of food deprivation and restraint it seems more likely that any qualitative differences in BAT would be such that 15w rats would have qualitative defects in heat production capacity but this is not necessarily true.

I hypothesized that if inadequate heat production of 15w rats food

deprived and restrained was due to insufficient release of endogenous NE then administration of exogenous NE should result in an increase in oxygen consumption for these animals. As it is known that 15w rats have quantitative deficts in BAT responses to exogenous NE may allow for the inference of qualitative differences as well. That is if 30 day old 15w and 21w rats respond to exogenous NE with equivalent increases in oxygen consumption it can be inferred that the heat production capacity of 15w rats BAT is relatively enhanced. This same conclusion would be true if 15w rats respond with a greater relative increase in oxygen consumption than do 21w rats. On the other hand if 15w rats fail to show the same degree of increased oxygen consumption in response to exogenous NE this could indicate qualitative defects in BAT or such lack of response could merely be due to quantitative differences in BAT or to lack of sensitivity to NE. In order to evaluate these possibilites I compared the metabolic response of 30 day old 15w and 21w rats to exogenous NE administered at various times during food deprivation and restraint.

Method

Thirty day old rats from eight litters weaned at 15 days and eight litters weaned at 21 days were used as experimental animals. Animals were from the whole litter condition. The whole litter condition was used as three animals of the same sex and weaning condition were desired and this would not have been consistently possible with split litter animals. Additional rats from 8 litters weaned at 15 days served as controls. Animals were weaned and housed as previously described. For animals used in the experimental conditions three rats from a given litter and weaning condition were removed from their home cage at which time body weights and rectal temperatures were recorded. Rats were then assigned to one of the three experimental conditions.

In the first condition (no food deprivation) one animal was immediately placed in the previously described metabolic chamber. The ambient temperature of the metabolic chamber was 20 °C at all time during the expermient. A two hour baseline of oxygen consumption was determined. The animal was then removed from the chamber, physically restrained in a Bowman cage, and replaced in the metabolic chamber. The animal remained in the metabolic chamber until a peak in oxygen consumption was observed. A peak was defined as the first observable decline subsequent to an oxygen consumption increase. When peak oxygen

consumption had been observed the metabolic chamber was opened, the animal was administered 0.1 ml/kg NE (ip) while restrained, and the metabolic chamber was closed. Oxygen consumption was then measured for an additional 24 hours, at which time body weight and rectal temperature was again recorded.

In the second condition (24 hours food deprived) a littermate of the animal used in the first condition was food deprived for 24 hours prior to testing. The animal was then placed in the metabolic chamber. The procedure was the same as in the first condition except that animals had been food deprived prior to testing in the metabolic chamber.

For the third condition the remaining littermate of animals used in the first two conditions was tested. In the third condition (NE prior to restraint) the animal was food deprived for 24 hours, then placed in the metabolic chamber while baseline oxygen consumption was determined for two hours. At that time the metabolic chamber was opened, the animal was administered 0.1 ml/kg NE (ip) and returned to the metabolic chamber. The animal remained undisturbed and oxygen consumption was recorded until a peak in oxygen consumption was observed. At that time the animal was removed from the metabolic chamber, physically restrained in a Bowman cage, and returned to the metabolic chamber until a peak in oxygen consumption was again observed. Subsequently the metabolic chamber was opened, animal was

administered a second dose of 0.1 ml/kg NE (ip) while in restraint, the metabolic chamber was closed, and oxygen consumption was measured for an additional 24 hours. At the end of the experiment the animals weight and rectal temperature was recorded.

In order to see if 30 day old 15w rats who were already responding to prolonged food deprivation with decreased oxygen consumption could be stimulated to increase oxygen consumption, a control condition was analyzed. For this condition individual 30 day old rats from 6 litters that had been weaned at day 15 served as subjects. Test animals were removed from the home cage, body weight and rectal temperatures were recorded and the animal was placed in the metabolic chamber. The ambient temperature of the metabolic chamber was 20 °C. Water but not food was available to the animal.

Oxygen consumption was continuously recorded. The animal remained in the metabolic chamber undisturbed until oxygen consumption was observed to decline consistantly over 1.5 hours. The amount of time that each animal was in the metabolic chamber undisturbed varied from 30 to 38 hours and was determined by the individual animals' metabolic response to food deprivation. When oxygen consumption was observed to be steadily declining the animal was administered 0.1 ml/kg NE (ip). The animal was then returned to the metabolic chamber and oxygen consumption was measured for an additional 4 hours. At that time the animal was removed from the metabolic chamber and body weight

and temperature were again recorded.

In order to insure that the NE that I used was active animals from 2 other 15w and 21w litters animals were administered 0.1 ml/kg NE (ip) when they were not food deprived or restrained. A two hour baseline oxygen consumption level was measured then NE was given.

Results

For both 15w and 21w rats that were neither food deprived and restrained administration of NE led to increases in oxygen consumption.

For animals not food deprived prior to testing, restraint led to an increase in oxygen consumption for both weaning conditions. The administration of NE led to a slight increase in oxygen consumption for restrained 21w rats and a decrease in oxygen consumption for 15w rats (see figure 19). The difference in the change scores from oxygen consumption during restraint to oxygen consumption following administration of NE was not however, significant ($\underline{M}$ = 2.32 ml/min./100g body weight for 21w rats, $\underline{M}$ = -5.55 ml/min/100g body weight for 15w rats; $\underline{t}(14) = 1.36$, $p>.10$). A repeated measures ANOVA with weaning condition and restraint,

r drug condition as factors showed a significant effect for weaning condition ($\underline{F}(1, 84) = 26.89$, $p<.0001$), with 15w rats using less oxygen

at all times.

For animals not food deprived prior to testing, 15w rats weighed significantly less than 21w rats (see table 14). During testing 21w rats lost significantly more weight than did 15w rats ($\underline{M}$ = 13.55 g for 15w rats, $\underline{M}$ = 19.27 g for 21w rats; $\underline{t}(14)$, = 2.87, $\underline{p}<.011$). No significant differences in body temperatures were found (see table 15).

In the second experimental condition animals who were food deprived for 26 hours prior to testing, baseline levels of oxygen consumption were significantly less for 15w rats than for 21w rats ($\underline{M}$ = 56.3 ml/min/100 g body weight, $\underline{M}$ = 84.2 ml/min/100g body weight; $\underline{t}(14)$ = 3.9, $p<.0018$). Both groups increased oxygen consumption in response to restraint however this increase was significantly greater for 21w rats than for 15w rats ($\underline{M}$ = 13.02 ml/min/100 g for 21w rats, $\underline{M}$ = 4.36 ml/min/100 g for 15w rats; $\underline{t}(14)$ = 2.13, $\underline{p}<.05$). For both groups administration of NE while in restraint led to decreased oxygen consumption. The magnitude of this decrease did not differ significantly between weaning groups. A repeated measures ANOVA with weaning condition and restraint and drug condition as factors showed a significant effect only for weaning condition ($\underline{F}(1, 84)$ = 71.96, $\underline{p}<.0001$). Early weaned rats used significantly less oxygen at all times during the experiment (see figure 20).

The body weights of animals food deprived for 26 hours and

administered NE following restraint, differed significantly at all times (see table 13). However, during food deprivation and subsequent restraint 15w and 21w rats did not differ significantly in the amount of weight they lost. Early weaned and normally weaned rats did not differ significantly in body temperatures measured before food deprivation or after 24 hours of food deprivation. However, following restraint and NE administration 15w rats were hypothermic, while 21w rats had maintained body temperatures at normal levels (see table 14).

For animals food deprived for 24 hours and administered NE both prior to and following restraint, baseline oxygen consumption levels were again significantly less for 15w rats than for 21w rats ($\underline{M}$ = 44.3 ml/min/100 g for 15w rats, $\underline{M}$ = 77.5 ml/min/100g for 21w rats; $\underline{t}(14)$ = 3.65, $p<.01$). Initial administration of NE to unrestrained rats led to increases in oxygen consumption for both weaning conditions, however this increase in oxygen consumption was significant only for 21w rats ($\underline{t}(14)$ = 2.14, $p<.05$ (see figure 21). Subsequent restraint led to an increase in oxygen consumption for 21w but not for 15w rats, and the magnitude of this difference was significant. Early weaned rats increased oxygen consumption by an average of $\underline{M}$ = 0.88 ml/min/100 g and 21w rats increased oxygen conumption by an average of $\underline{M}$ = 13.14 ml/min/100 g ($\underline{t}(14)$ = 2.2, $p<.05$). The second administration of NE led to slight decreases in oxygen consumption for both weaning conditions, and the magnitude of this decrease did not differ significantly (see

figure 21). A repeated measures ANOVA with weaning condition and restraint or drug condition as factors showed a significant effect only for weaning condition ($F(1, 84) = 62.86$, $p<.0001$). Early weaned rats again used signifcantly less oxygen than 21w rats.

The body weights of animals food deprived for 26 hours and administered NE prior to and following restraint, differed significantly at all times (see table 13). However, during food deprivation and subsequent restraint 15w and 21w rats did not differ significantly in the amount of weight they lost. Early weaned and normally weaned rats did not differ significantly in body temperatures measured before food deprivation or after 24 hours of food deprivation. However, following restraint and NE administration 15w had somewhat lower body temperatures than 21w rats although this differences was not as great as for animals restrained prior to NE administration (see table 14).

Early weaned animals that were food deprived until oxygen consumption was observed to decline and then administered exogenous NE failed to show an increase in oxygen consumption.

Table 14

Mean body weights (in grams) and t tests of animals given exogenous NE

Experiment Condition	time	Wean cond.	Mean	t	p
Not food deprived	start	EW	71.9	3.8	<.002
		NW	106.4		
	24 hr	EW	58.4	3.3	<.005
		NW	84.5		
24 hours food deprived	start	EW	68.2	4.5	<.0007
		NW	93.3		
	24 hr	EW	57.8	4.1	<.0011
		NW	80.4		
	52 hr	EW	46.9	4.3	<.0006
		NW	69.7		
24 hours food deprived NE prior and after restraint	start	EW	75.6	2.7	<.019
		NW	91.2		
	24 hr	EW	61.9	3.0	<.009
		NW	77.5		
	52 hr	EW	52.6	3.2	<.007
		NW	68.1		

Table 15

Mean rectal temperatures and t tests of animals given exogenous NE

Experiment Condition	time	Wean cond.	Mean	t	p
Not food deprived	start	EW	36.9	.29	>.7
		NW	36.0		
	24 hr	EW	34.8	1.3	>.1
		NW	35.5		
24 hours food deprived	start	EW	36.4	.98	>.1
		NW	36.2		
	24 hr	EW	34.9	1.6	>.1
		NW	35.5		
	52 hr	EW	28.9	3.2	<.005
		NW	35.2		
24 hours food deprived NE prior and after restraint	start	EW	36.3	.09	>.5
		NW	36.4		
	24 hr	EW	35.3	.29	>.5
		NW	36.0		
	52 hr	EW	33.3	1.3	>.1
		NW	35.1		

Figure 19

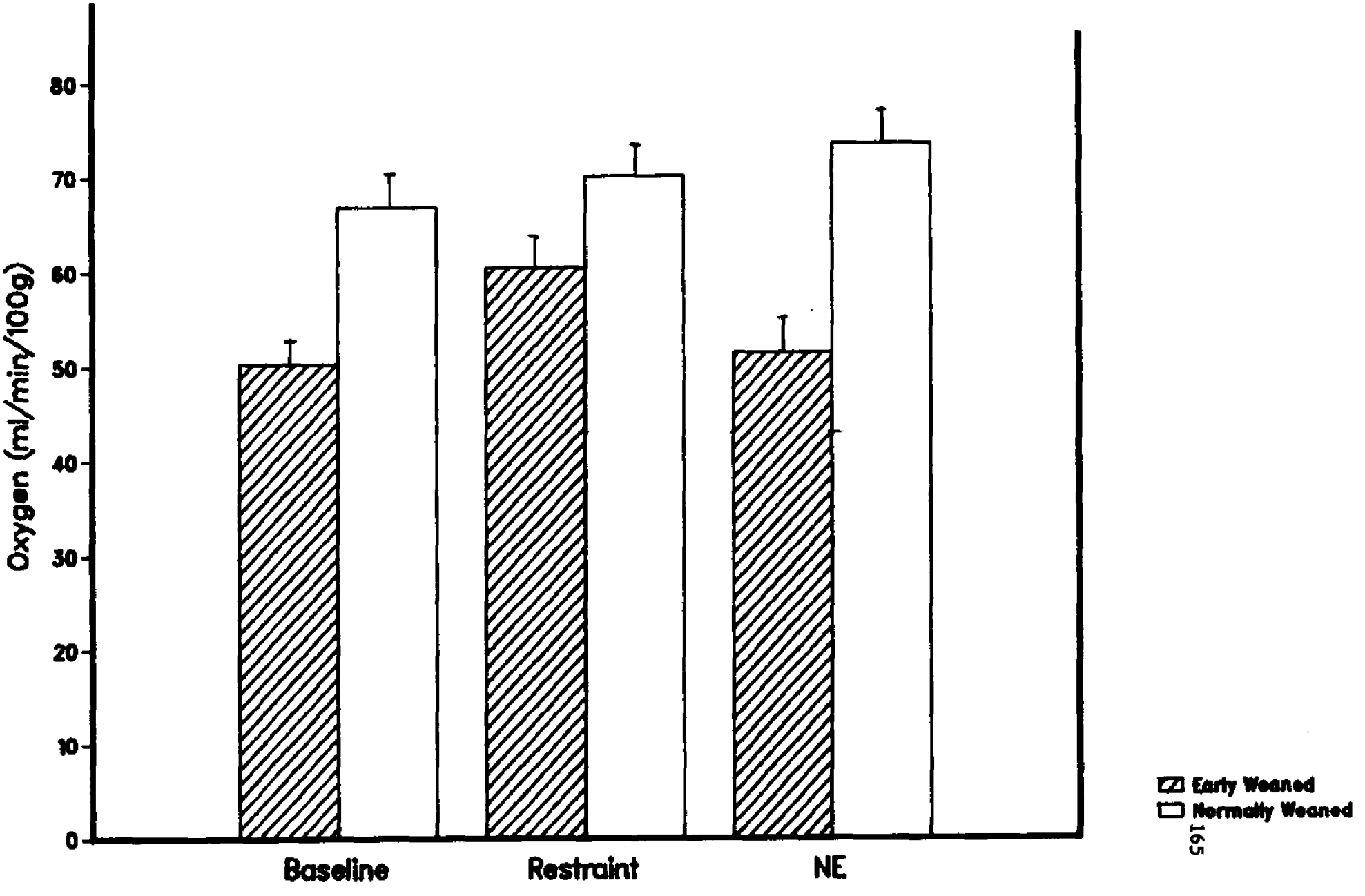
Figure 19 shows the mean oxygen consumption of 15w rats (E) and 21w rats (N). Shown are means for a two hour baseline, response during initial restraint and the the response in the hour after administration of exogenous NE.

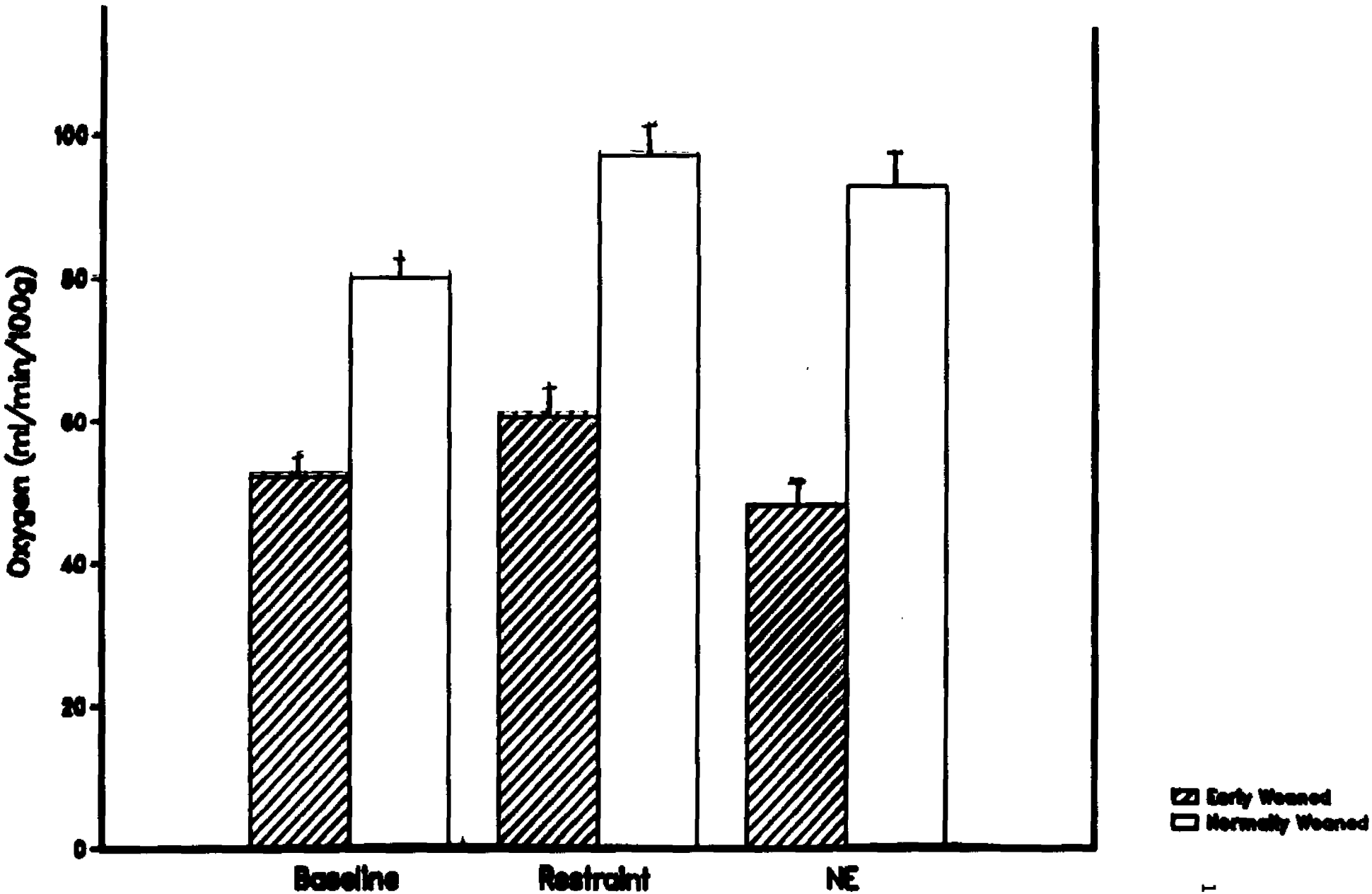
Figure 20

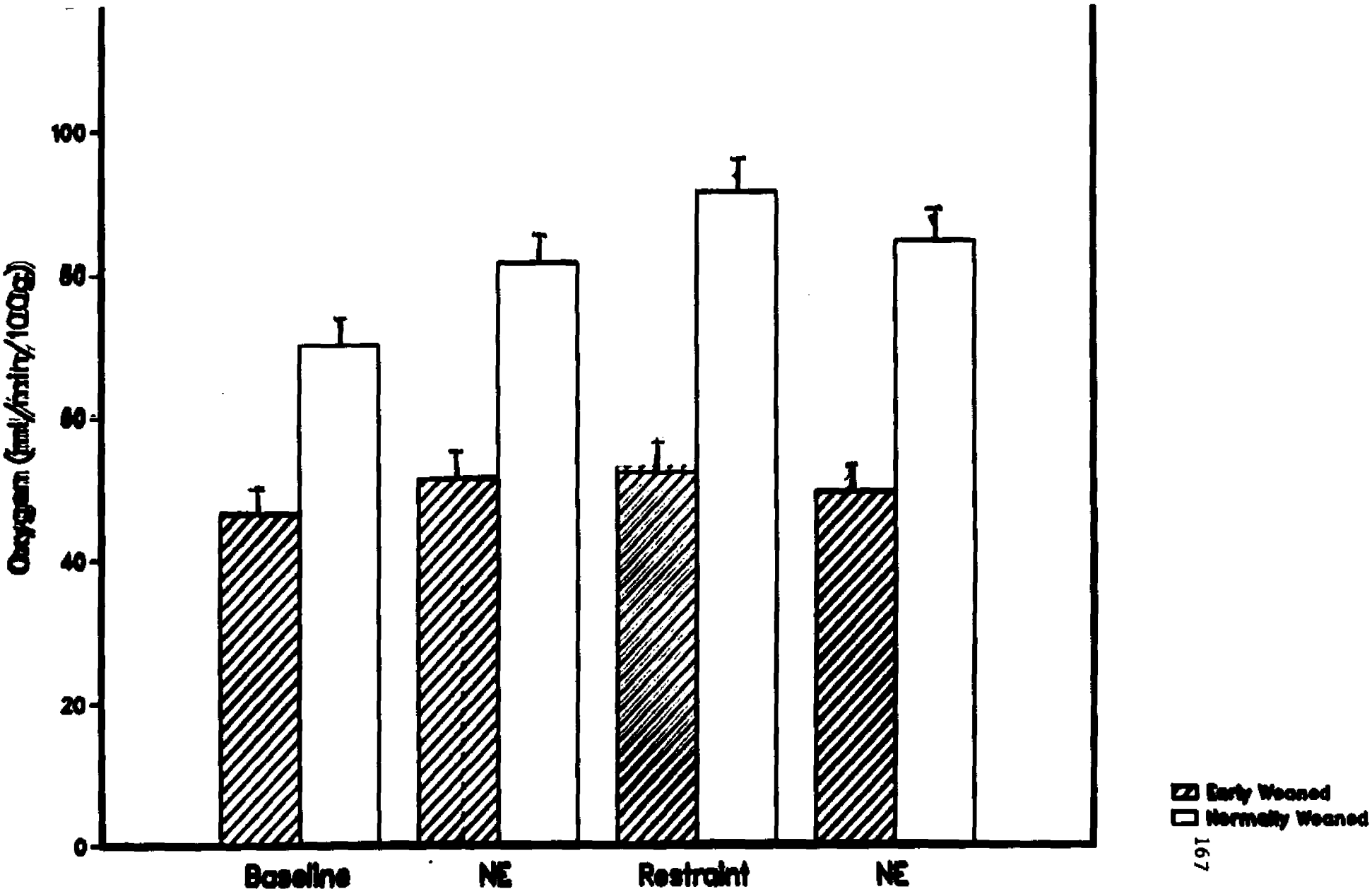
Figure 20 shows the mean oxygen consumption of 15w rats and 21w rats who were food deprived for 24 hours prior to testing. Shown are means for a two hour baseline, response during initial restraint and the response in the hour after administration of exogenous NE.

Figure 21

Figure 21 shows the mean oxygen consumption of 15w rats (E) and 21w rats (N) who were food deprived for 24 hours prior to testing. Shown are means for a two hour baseline, response to an initial injection of NE, response to subsequent restraint, and response to an additional injection of NE.


Oxygen (ml/min/100g)
0
10
20
30
40
50
60
70
80
Baseline
Restraint
NE
Early Weaned
Normally Weaned


Oxygen (ml/min/100g)
100
80
60
40
20
0
Baseline
Restraint
NE
Early Weaned
Normally Weaned


Oxygen (ml/min/100g)
100
80
60
40
20
0
Baseline
NE
Restraint
NE
Early Weaned
Normally Weaned

Discussion

The administration of exogenous NE to restrained 30 day old 15w rats did not lead to increases in oxygen consumption. This was true whether NE was administered prior to or following food deprivation. This suggests that oxygen consumption may be maximally stimulated by restraint in 15w rats, although it is possible that the oxygen consumption of restrained 15w rats might be increased by stimuli other than exogenous NE. The fact that restrained 15w rats do not increase oxygen consumption in response to exogenous NE argues that they are producing what heat they can via NST. If 15w rats are responding maximally to restraint then it is possible that 15w rats do not produce sufficient heat to prevent hypothermia due to insufficient capacity for NST. This could be due to their quantitative deficiencies in BAT or to unspecified qualitative defects in their BAT.

Restrained early weaned rats at no time showed increases in oxygen consumption in response to NE or restraint that were equivalent to or greater than those of normally weaned rats. This relatively low response argues against the hypothesis that 15w rats have qualitatively enhanced heat production capacity of BAT analagous to cold aclimated mice or rats.

The response of both food deprived and non food deprived animals is not consistant with the hypothesis that the decreased oxygen

consumption that is seen during prolonged food deprivation or food deprivation and restraint is due to insufficient release of endogenous NE in 15w rats. Exogenous NE did not bring about increases in oxygen consumption in food deprived 15w rats, nor did it appear to delay the onset of hypothermia in these animals. However, exogenous NE may act quite differently than endogenous NE in response to cold. Presumably endogenous NE released in response to cold would act locally to stimulate the β-receptors of BAT. While exogenous NE acts not only to stimulate these receptors but also to stimulate both α and β receptors elsewhere. To more closely mimic the action of endogenous NE release in response to cold a specific β1-agonist could be used. To clarify whether the lack of response to NE seen in 15w rats is due to saturation of BAT β-adrenergic receptors or due to activation of responses that would otherwise affect oxygen consumption would require further testing with α and β-agonists and antagonists. However, the fact that 21w rats do show increases in oxygen consumption when administered NE following restraint, suggest that these animals have more heat generating capacity than do 15w rats. This may merely be due to the quantitative advantage that 21w rats have in terms of their BAT, as due to having more BAT they have more available adrenergic receptors. It is also possible that 21w rats have qualitatively better heat producing capacity of their BAT as well, however, our results do not directly address this possibility.

General Discussion

I have shown that hypothermia and the formation of gastric erosions in 15w rats is accompanied by low levels of oxygen consumption. Early weaning may also lead to relatively poor conservation of heat. However, inadequate heat production is one factor in the susceptibility of 15w rats to hypothermia. Under the current test conditions food deprivation alone was a sufficient stimulus to induce low levels of oxygen consumption and resulting hypothermia in 15w rats. While physical restraint served to exacerbate the degree of hypothermia and the serverity of gastric erosions in 15w rats.

I also found that 15w rats have higher thermoneutral temperatures than do 21w rats. This is consistent with a hypothesis of delayed maturation of homeothermy in 15w rats. The higher thermoneutral temperature of 15w rats implies that an ambient temperature of 20 °C is more of a thermal challenge for these animals than it is for 21w rats. To maintain a given body temperature at a given ambient temperature below thermoneutrality would presumeably require more heat production in 15w rats than in 21w rats. Instead, 15w rats that are food deprived are not able to produce even as much heat as 21w rats and therefore 15w rats become hypothermic. The lower heat production levels of 15w rats during food deprivation is also consistent with a delayed maturation of

homeothermy (Blackmore 1970, 1972).

I attempted to stimulate increased oxygen consumption in 15w rats with exogenous NE. However, in 15w rats administration of NE did not lead to increases in oxygen consumption above levels induced by physical restraint, whether or not they had been food deprived prior to restraint. It seems likely that for 15w rats physical restraint leads to maximum heat output. Hypothermia in these animals results not from a lack of appropriate heat production responses but rather to quantitative limitations in NST.

From my results I can not state whether this limitation in NST is due to quantitative or qualitative dificiencies of BAT. Early weaned rats have less BAT than 21w rats, and it may be that 15w rats are unable to generate sufficient heat during food deprivation and restraint simply due to their relative lack of BAT. It is also possible that 15w and 21w rats have qualitatively different BAT and that 15w rats are further compromised in heat production capacity due to these differences. Qualitative defects in the BAT mediated NST would be consistent with a lack of responsiveness to exogenous NE. However, the absense of oxygen consumption increases seen in 15w rats could be due to having less BAT rather than any defect in BAT. The overall limitation of heat production in food deprived 15w rats contributes to ensuing hypothermia.

GENERAL DISCUSSION

The work described here leads to the conclusion that premature maternal separation affects the normal development of temperature regulation in rats. Under typical laboratory conditions this disturbance in thermoregulation is not exhibited. However, when early weaned rats are placed in situations where it is necessary to defend body temperature against a challenge they are less able to maintain temperature homeostasis than are normally weaned rats.

It has previously been shown that the relevant component of maternal separation that leaves 15w rats susceptible to hypothermia and RGE's is the loss of maternal milk (Ackerman, Hofer & Weiner, 1978b). One way in which nutritional deprivation affects the competance of 15w rats to maintain temperature homeostasis during prolonged food deprivation and physical restraint is through fat deposition. Hirsch and his coworkers have shown that early nutritional status affects subsequent size and number of adipocytes (Knittle & Hirsch, 1968; Greenwood & Hirsch, 1974). In the present work 15w rats were found to have significantly less white and brown adipose tissue at 30 days of age than were 21w rats. And following food deprivation and restraint 15w rats were found to have depleted the majority of their white and brown fat stores, while 21w rats had not depleted these

stores.

Having less fat has a number of implications for the inability of 15w rats to maintain body temperature during food deprivation and restraint. One factor is that 15w rats have insufficient fuel stores available for heat production. This is supported both by the fact that 15w rats have essentially depleted certain regions of body fat stores during food deprivation and restraint, and the fact that 15w rats show extremely low levels of oxygen consumption towards the end of food deprivation or food deprivation and restraint. At the time when 15w rats are showing these low levels of oxygen consumption they are becoming hypothermic.

Another implication of having less adipose tissue is that 15w rats have less subcutaneous fat and are therefore more susceptible to heat loss from conductance. Early weaned rats were found to have significantly thinner skin than 21w rats; presumably this difference was largely due to less subcutaneous fat. Early weaned animals are also significantly smaller than are 21w rats. Removal of bilateral ingiuinal fat in 21w rats reduced available fuel stores by approximately 45%, however this did not lead to hypothermia in these animals. This suggests that smaller white fat fuel stores alone does not leave rats susceptible to hypothermia. Rather, the smaller body size and reduced subcutaneous fat of 15w rats are important in hypothermia susceptibility. Early weaned rats are in some sense in

"double jeopardy" of becoming hypothermic; they are both less able to protect against heat loss, and during conditions of food deprivation they have less white fat available for conversion to energy.

Along with having less white fat for fuel and insulation 15w rats also have less brown adipose tissue than do 21w rats. This is especially important for heat production via nonshivering thermogenesis. When placed at an ambient temperature of 10 °C 15w rats were observed to shiver more frequently than 21w rats. Thus 15w rats can use shivering to attempt to maintain body temperature in response to temperature stimuli. Although not examining shivering per se Ackerman et al. (1978a) used sensitive activity monitors and EMG recordings and found no differences in activity of 15w and 21w rats during restraint. It seems therefore unlikely that early and normally weaned rats differ in thermogenesis due to shivering during food deprivation and restraint. However, 15w rats use significantly less oxygen when food deprived or food deprived and restrained. Therefore differences in thermogenesis of 15w and 21w rats are likely to be due to differences in nonshivering thermogenesis.

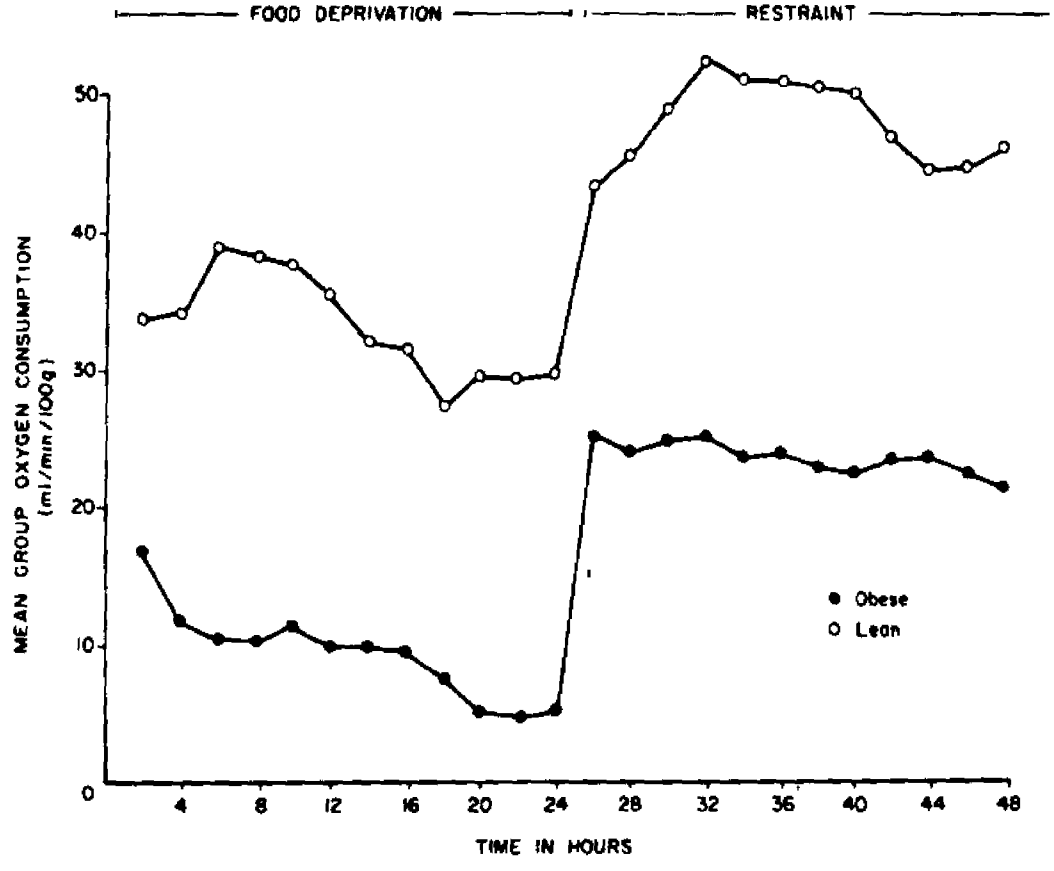
In addition to quantitative differences in BAT, it is also possible that 15w rats have qualitative differences in BAT. Genetically obese mice ob/ob are known to have qualitative defects in their BAT. Studies have shown that the mitochondria of the BAT of ob/ob mice lack specific proteins that normally enable heat production

(Himms-Hagen & Desautels 1978; Nicholls, 1979). Genetically obese mice are known to become hypothermic in response to cold challenges that do not produce hypothermia in lean mice (Davis & Mayer, 1954; Trayhurn, Thurlby & James, 1976). Obese mice also consume less oxygen than lean mice and do not show comparable increases in oxygen in response to exogenous NE. It has been shown that obese mice are significantly more susceptible to RGEs than lean mice and this susceptibility is associated with hypothermia (Greenberg & Ackerman, 1983). In addition, when oxygen consumption of obese and lean mice was measured under the same food deprivation and restraint conditions as those in which I measured oxygen consumption for 15w and 21w rats the responses of obese mice are analagous to those of 15w rats while the responses of lean mice are analogous to those of 21w rats (see figures 16 and 22). Thus although there is no direct evidence that 15w rats have any qualitative defects in their BAT they respond in a similar manner to obese mice in terms of oxygen consumption, hypothermia, and gastric erosion formation.

It is a plausible hypothesis that 15w rats have qualitative as well as quantitative deficiencies in BAT. Future studies could test this hypothesis in several ways. There are in vitro techniques that allow for assessment of the metabolic activity of excised BAT (Correll, 1963; Smith & Roberts, 1964), and it would be possible to compare the activity of BAT from 15w and 21w rats. I would predict that the BAT from food deprived 15w rats would have less capacity for activation than BAT from food deprived 21w rats.

Figure 22

Figure 22 shows the mean oxygen consumption of 3 groups of 5 ob/ob and 3 groups of 5 lean mice, expressed in ml/min/100g body weight. Oxygen consumption for ob/ob mice was significantly below lean animals at all times. Note that the response of ob/ob mice is similar to that of 15w rats while the response of lean mice is similar to that of 21w rats.


FOOD DEPRIVATION
RESTRAINT
MEAN GROUP OXYGEN CONSUMPTION (ml/min/100g)
50
40
30
20
10
0
4
8
12
16
20
24
28
32
36
40
44
48
TIME IN HOURS
Obese
Lean

One way in which 15w rats do not resemble obese mice is that when food is available 15w rats use as much or more oxygen than do 21w rats, while when food is available obese mice use less oxygen than do lean mice. In fact low levels of oxygen consumption has been used as a means of detecting the obese genotype prior to its phenotypic expression in both ob/ob mice (Kaplan, 1974) and fa/fa rats (Bray, 1969; Kaplan, 1979). In addition both ob/ob mice and fa/fa rats have relatively depressed core temperatures under normal laboratory conditions. At normal room temperatures it is only under conditions of food deprivation or food deprivation and restraint that 15w rats show either depressed body temperatures or low levels of oxygen consumption. Food deprivaton may act as a stimulus for 15w rats to conserve what fuel stores they have, so that it is only under conditions of food deprivation that low levels of oxygen consumption would be seen.

Part of an animal's repetoire of heat producing behaviors involves increased searching for and ingestion of food. Food deprivation may be especially important for 15w rats if they normally tend to increase food intake to maintain body tempertures. I did not measure the food intake of 30 day old 15w and 21w rats who had food availab.e to them while oxygen consumption was measured. However, since in all other situations 15w rats had lower oxygen consumption than 21w rats, while during the second day when food was available 15w

rats actually used more oxygen than 21w rats, there must be a connection between the ability of 15w rats to have high levels of oxygen consumption and the availability of food. Had I measured food intake I would have expected that 15w rats would have consumed more during the 52 hours than would 21w rats at least in terms of grams of food eaten per 100 grams body weight.

There is evidence that food deprivation is especially damaging to 30 day old 15w rats. Ackerman et al. (1977) found that 15w rats were significantly less able to withstand starvation than were 21w rats. By day 4 of starvation, early weaned rats had a survival probability of 0.29, while normally weaned rats had survival probabilities of 0.93 on day 4 of starvation.

When ambient temperature is either very low or very high the inability of 15w rats to maintain temperature homeostasis was observed even without food deprivation. When 15w rats were placed in either inescapable heat or inescapable cold they became respectively hyperthermic and hypothermic compared to normally weaned rats. In the situation of inescapable cold 15w rats exhibited appropriate thermoregulatory behaviors such as shivering, piloerection and huddling with a littermate, and in fact were observed to engage in these behavior significantly more frequently than 21w rats. However, despite this 15w rats became more hypothermic than did 21w rats. Food deprivation served to potentiate the hypothermia of 15w rats. Oxygen

consumption was not measured when 15w or 21w rats were exposed to the ambient temperatures that elicited these behaviors, so it is not known whether 15w rats were producing heat comparably with 21w rats. It is likely that even if 15w rats were producing as much heat as 21w rats, they were losing heat at greater rates than were the 21w rats. This would be expected because of the smaller body size and lesser degree of subcutaneous fat of 15w rats. Even activation of behaviors for heat production and the prevention of heat loss may not be sufficient to counter the increased heat loss that small body size and little insulation impose.

Unlike their response to inescapable cold, when 15w rats become hypothermic despite appropriate responses, when 15w rats are exposed to inescapable heat they fail to respond with appropriate behaviors. In the case of inescapable heat 15w rats act in a manner that counters dissipation of heat. For example, 15w rats are more active and fail to exhibit prone extension. Again having a smaller body size would increase the effective challenge upon 15w rats, as conductance of high temperatures would be unfavorable for smaller animals.

As was mentioned in the discussion of the second part of this work, the particular lack of the response of prone extention allows the inference of a possible CNS disturbance. Roberts and his coworkers (Roberts, Mooney, & Martin, 1974; Roberts & Martin, 1974; Roberts & Mooney, 1974; Roberts & Martin, 1977) have shown that specific

thermoregulatory behaviors are controlled by specific CNS structures. That is, prone extention is controlled by the anterior preoptic area of the hypothalamus. From the current work it is not known if there is actually a defect in the anterior preoptic hypothalamus of 15w rats. It is also possible that 15w rats have a sensory defect such that appropriate behaviors are not mobilized. The behavior of early weaned rats in a thermal runway suggests that they can sense extreme temperature at least to the extent that such temperatures are found aversive. However, it is still possible that they do not adequately sense the need to dissipate heat when they are unable simply to avoid an aversive thermal stimulus.

The fact that 15w rats have a higher thermoneutral temperature than 21w rats suggests that early weaning may affect a general disruption of the maturation of homethermy. This is consistant with Blackmore's (1970, 1972) findings that increasing length of daily periods of maternal separation led to delays in such maturation. Since younger rats are relatively more exothermic than older rats, the fact that 15w rats have more labile body temperatures under conditions where ambient temperature deviates from thermoneutrality is also consistent with a delay in maturation of homeothermy.

The question still remains as to why a disturbance in homeothermy should translate into RGE susceptibility. There are a number of factors that appear to link temperature regulation disturbances and

RGE susceptibility. Ackerman (1983) has recently shown that there exists a developmental pattern to basal and maximal gastric acid secretion. Early weaned rats are found to have normally developing gastric acid secretory responses. The pattern of development of these responses are such that 30 day old rats have relatively large secretory responses to cold and various secretogogues when compared either to 15 day old or 100 day old rats.

The prematurely weaned 30 day old rat appears to have a normal development of the gastric acid secretory response to cold. However, these animals have a retarded development of homeothermy. The increased RGE susceptibilty of 15w rats may not be due to any abnormal development but rather to a disturbance in the normal developmental synchrony between two interacting systems. That is the gastric acid secretory system may develop normally while systems involved in maintaining homeothermy are retarded by early weaning.

Food deprivation and restraint at 20 °C imposes a challenge for both 15w and 21w rats. For 15w rats a temperature of 20 °C is more of a deviation from thermoneutrality than it is for 21w rats, and thus poses a relatively greater challege. Early weaned rats are also likely to lose more heat through conductance due both to smaller body size and having less subcutaneous fat than are normally weaned rats. In addition, early weaned rats have less fat available as fuel stores, and they deplete the fat they have to a greater exent than do 21w rats.

Early weaned rats also have less BAT available for producing heat via non-shivering thermogenesis, and they may have qualitative defects in the BAT they do have. During food deprivation and restraint it is consistant with what is known to assume that 15w rats deplete their available fuel, begin to have a fall in core temperature, and have an increase in gastric acid secretion. As food deprivation and restraint continue 15w rats further deplete their ability to produce heat and core temperature continues to fall which potentiates gastric acid output. Eventually the protective mucosal barriers of the stomach are damaged and gastric erosions are formed. In this way susceptibility to hypothermia would be linked to RGE formation.

I have discussed here an animal model where occurrences during early development are shown to affect the subsequent risk of susceptibility to gastric erosions. Early separation leads to a disturbance in thermoregulation that is exhibited during challenges to temperature homeostasis. This animal model may have analogues in some human disorders. In the animal model a developmental occurrence affects the animal's subsequent ability to adapt to certain features of the external environment, specifically ambient temperature. It is possible that some of the regulatory disturbances of persons who are susceptible to ulcer disease may also arise during postnatal development.

It is unlikely that the animal model described here and human ulcer disease have common pathophysiology. However, it is possible that the underlying biological processes are similar. If so then in human development there may also exist processess analagous to the disruption induced by early weaning that disrupt the normal development of systems of homeostasis and lead to later disease susceptibility.

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