ADAPTIVE RESPONSES TO SEMISTARVATION AND REFEEDING IN RATS

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ABSTRACT

ADAPTIVE RESPONSES TO SEMISTARVATION AND REFEEDING IN RATS

By

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Rats subjected to prolonged semistarvation showed physiologically desirable adaptive responses. Gonadal hormone activity increased during the latter phase of food restriction after the initial marked decrease. Rate of change in organ weights (as a percent of body weight) due to semistarvation declined during the second period of semistarvation. Semistarved animals were better able to prevent the severe manifestations of infectious disease than animals allowed to feed freely. Rate of decline in weight of fat depots slowed down after an initial rapid fall.

At age 10 weeks, previously ad libitum fed male Sprague-Dawley rats were randomly divided into an unrestricted control and a 50% restricted group. Ten rats from the unrestricted control and 10 from the semistarved groups were killed at 18 weeks and also at 33 weeks of age.

Serum testosterone, as determined by gas-liquid chromatography decreased to 31% of control values after 8 weeks of semistarvation, but were comparable at 23 weeks of semistarvation. Seminal vesicle and coagulating gland weights (% body weight) and their respective contents of citric acid and fructose showed significant ($P < .05$) increases during the latter phase of semistarvation after initial sharp decline. Refeeding for 3 weeks after a 20-week restricted period resulted in values of 3 ng. testosterone per ml. of serum compared to 1.5 ng./ml. for ad libitum controls and 1.2 ng./ml. for semistarved rats of the same age. Complete deprivation of food in 32-week-old freely-fed rats for 6 days lowered the hormone to undetectable levels. Concentration of sperm per gram of testicular and epididymal tissue and weights of these organs were well maintained despite food restriction and refeeding.

Weights of kidneys and heart expressed as a percent of body weight closely followed changes in total body weight during starvation and refeeding. Percent of liver and spleen weights decreased sharply during food restriction. Short-term complete starvation appeared to be a greater stress than prolonged semistarvation as shown by rate of increase in adrenal gland weights. During refeeding the adrenals decreased rapidly relative to total body weight. As body weight increased with age in freely fed control rats, the percent of organ weights declined.

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Increase in the total body weights of ad libitum fed adult rats was associated with marked proliferation of gonadal and mesenteric adipose tissue. Caloric deprivation had the most pronounced effect on subcutaneous, genital and mesenteric fat depots. Weight of the mesenteric depot increased most rapidly when the 50% restricted animal was refed. In the 21 day refed rats, percentage of mesenteric adipose tissue was greater and gonadal fat depots were lighter than in the completely deprived rat (starved for 6 days) of the same body weight and age.

Mortality, incidence of signs and symptoms of chronic respiratory disease, evidence of pulmonary lesions and serum complement levels indicated that the freely fed animal with relatively large accretions of body fat is less able to prevent the age-associated progression of infectious disease than the semistarved animal.

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INTRODUCTION

Shortage of food poses a real problem for a majority of the world's population. A great deal of effort is currently being focused upon the equable distribution of the present food supply. Should the level of food intake increase with the expected increase in available food, millions of people will be suddenly faced with an abundance previously unknown to them. The physiological implications of refeeding the chronically undernourished are not well documented.

In developed countries where semi-starvation is now largely of academic interest alone, relatively excessive food intakes have made obesity the primary concern. As the physiologic and aesthetic hazards of overweight are highlighted, more and more people are voluntarily changing from ad libitum to restricted patterns of food intake. Total starvation has been recommended as a feasible method of achieving "ideal" body weights. The comparative effects of prolonged partial caloric restriction and total deprivation need to be established.

While overall figures for availability of food may fail to reveal any immediate cause for alarm, great

disparities exist in regional distributions of population, cultivable land, and the resources to develop this land. Over half the world's population in the Far East lives on only about a quarter of the world's food supplies (FAO, 1963). Despite a chronic deficiency of calories in their diets, people in many areas of the world are able to function apparently normally. If birth rates can be considered an index of reproductive ability, fertility seems to be unhampered by the caloric insufficiency. Therefore the organism appears to have "adapted" successfully to the constantly lowered levels of food intake. Reports on starvation have shown that undernutrition can impair reproduction.

The word "adaptation" has numerous meanings and applications in physiology. The process generally implied is an active change in an animal initiated by contact with its environment (Bajusz, 1969).

It was Charles Darwin who proposed that the survival of species under changed conditions of external influences is best explained by the existence of adaptive mechanisms. He developed the thesis that, in the final analysis, the external environment determines the nature and behavior of the organism. Based more on intuition than empirical data, Claude Bernard introduced into biology the concept that the purpose of most physiological processes is to maintain the constancy of the "milieu interieur" consisting of blood plasma, interstitial fluids, and

extracellular water. This determines the survival ability of the organism by minimizing the pressures of the external environment. Somewhat later, Walter B. Cannon evolved the term "homeostasis" and proposed that adaptation is initiated by a force that produces a displacement from the constant state, and the displacement, in turn, triggers certain reactions to return the organism to its original internal state. More than two decades ago, with Hans Selye's description of the "General Adaptation Syndrome," the word adaptation in much of the medical literature came to denote phenomena associated with disease. While the biologist, the physician, the sociologist, and the layman have continued to use the word, each in his own way, to denote a multiplicity of genetic, physiologic, psychic, and social phenomena mostly unrelated in their fundamental mechanisms, even today the most consistent characteristic of "adaptation" appears to be its ambiguity.

Studies in ecological genetics suggest that living organisms are the product of evolution controlled not by mutation alone, but largely by selection pressures (Ford, 1964). However, Rene Dubos (1965) has pointed out that Darwinian fitness achieved through genetic mechanism accounts for only a small part of the adaptive responses made by man and his societies. According to him the great adaptability of human beings enables them to become adjusted to conditions and habits that may eventually destroy the values most characteristic of human life.

In biology, adaptive responses of an organism have centered around endocrine phenomena. Interest in the pituitary-adreno-cortical system as a segment of general adaptation reactions developed from the concept of the "adaptation syndrome" of Selye. Studies of interrelationships between the neural and endocrine integrative mechanisms have shown that most living organisms, ranging from invertebrates to mammals, possess a complex adaptive apparatus that serves to integrate extrinsic and intrinsic information necessary for the control and adjustment of autonomic functions. The component parts of this defense system include afferent, somatic and visceral pathways, hormonal feedback actions upon the CNS, central neural circuits, efferent sympathetic and parasympathetic nerves, neurosecretory nerve cells, organs of internal secretion and humoral links acting directly on target organs (Scharrer and Scharrer, 1963). There exist channels, at every level, through which various modifying influences enter the main neuroendocrine control system allowing the appropriate reactions to adapt physiological functions to the changed requirements.

To assess the degree to which a previously well-fed adult animal has successfully adapted to inadequate food intake, it becomes necessary to study the response from various aspects. Adaptation in terms of reproductive ability is of prime importance for the perpetuation of the

species under adverse conditions. Adjustments in metabolism engendered by a stressful environment are reflected in changes in the anatomical and chemical components of the body. To sustain life for any period of time, the immune responses of animal must remain intact.

REVIEW OF LITERATURE

ADAPTIVE RESPONSES TO REDUCED CALORIC INTAKES

Insofar as deviation from an accepted "optimum" level of nutrient intake evokes an adaptive response from the organism, semi-starvation can be regarded as a "stress." Various physiological consequences of caloric deficiency are well documented. Some of the earliest studies on man were done on the professional fasters (Keys et al., 1950). Investigations have been made of civilians and of prisoners of war (Burger et al., 1948). The best-known experiments on man are those of Benedict (1907), Benedict et al. (1919) and the comprehensive study of Keys and associates (1950). They also reviewed some of the literature on under-nutrition and famine.

A state of inadequate food intake may justifiably be considered a response provoking stimuli and therefore a "stress." A state of apparent well-being, however, can be achieved by people on many different diets. Several experimental studies including those during the food rationing associated with World War II (Keys et al., 1950)

have shown that man can adapt to a slightly reduced plane of nutrition rapidly and without visible impairment.

Neumann (1902) at Heidelberg showed that his own body weight was constant for periods of one year at daily caloric intakes of either 1766, 2199, or 2403 Cal. He proposed that the excess, or "Luxuskonsumption" was directly oxidized, and dissipated as heat. The American physiologist, Gulik (1922) studied the apparent discrepancies in energy balance in experiments on himself. These were similar to those of Neumann except that the range of food intakes was greater (1974 to 4113 Cal./day) and he recorded details of his physical activity. Widdowson (1947) found that at every age between one and 18 years, it was possible to find one child in a group of 20 of the same sex who was eating nearly twice as much as another. Rose and Williams (1961), in studies with 6 pairs of large and small eaters whose food intakes ranged from 1600 to 7400 Cal./day showed that the weights of all subjects varied little over a period of some weeks despite similar levels of activity. Grafe and Graham (1911) fed a 20-kg. dog a diet containing about 20% of protein at caloric intakes of 1120 and 2580 Cal./day, and observed weight maintenance on each of these regimens.

Several workers have shown that when the caloric consumption of young rats is restricted to such a level that body weight remains constant, the amount of food required per day decreases gradually. Quimby (1948) observed that

the caloric requirement for weight maintenance of 50-gm. rats fed a synthetic stock ration decreased to about one-half of the original value in 5 weeks. Similar results were obtained by Kaunitz et al. (1956), who measured the caloric requirements for weight maintenance of young rats fed diets high in protein, carbohydrates, or fat. Although the rate and the degree of decrease of caloric requirements depended on the particular diet fed, the general pattern reflected a progressive decline during the first 5 weeks.

Various metabolic alterations involved in this adaptation to caloric restriction have been suggested. It was predicted that basal metabolic rate is more closely related to the "active mass" of the body (the total body mass less the stored fat and the weight of the skeleton), than to the total body weight (Keys and Brozek, 1953). Lee and Lucia (1961) measured the body composition in ad libitum and restricted rats to determine whether changes in energy requirements were reflected in the metabolically active fat-free body mass. Although the proportion of total body fat increased with weight on the ad libitum diets it did not increase with age when total body weight was held constant. The relative constancy of the water and protein components of the fat-free mass indicated that adaptation to caloric restriction did not operate through changes in the composition of the lean-body mass. Data on organ weights showed that the "caloric adaptation" did not make

such metabolic demands that the heart and kidneys would hypertrophy. Measurements of liver total lipid, total cholesterol, and total phospholipid did not indicate that changes in metabolism and transport of lipids were involved.

In an attempt to explain the ability of animals to maintain their weight on different caloric intakes, Miller and Payne (1962) suggest that food energy may be converted directly into heat, and that protein as well as caloric components of the diet are important considerations in assessing energy metabolism. They showed that weight maintenance could be achieved in rats over a wide range of caloric intakes if caloric intake/day/kg.$^{0.73}$ times NDpCals % equalled 624 $\pm$ 12. Two pigs were maintained by them at constant weight with diets providing 243 and 1,180 Cal./day. After 40 days the regimens were reversed and body weight was maintained for a similar period. The difference in caloric intakes could not be accounted for by differences in digestibility, losses in the urine, storage of depot fat, or physical activity. Differences in expired carbon dioxide showed that all the ingested food was being metabolized.

Lee and Lucia (1963) have studied fecal excretion of fat, nitrogen, energy, and ash as a possible mechanism of the adaptive response of calorically restricted rats. Caloric restriction was accompanied by a decrease in caloric requirements approaching 40%. The differences in the fecal loss of nitrogen and fat by ad libitum fed rats as opposed

to that by caloric-restricted rats was relatively negligible. Their data preclude fecal excretion or apparent absorption (intake minus fecal excretion) of nutrients as a primary cause of adaptation in caloric requirements.

Keys and coworkers (1950) have shown that the basal metabolic rate declines during complete starvation. McCance and Mount (1960), found no significant change in oxygen consumption of pigs subjected to caloric restriction without weight loss. In studies on obese patients Apfelbaum and associates (1969), however, concluded that the energy cost of maintenance per unit of the cellular mass is adapted to food intake since by reducing the food intake the basal expenditure decreased in human subjects even when the cellular mass remained constant and nitrogen balance was maintained. In accord with previous studies (Grande et al., 1957), Bray (1969) found that during caloric restriction, basal energy expenditure in six out of 14 obese patients declined by 15%. Activity of enzymes of the glycerophosphate pathway decreased with caloric restriction indicating an enhanced efficiency of energy production as food intake was curtailed. It is well established that the basal energy cost of metabolism decreases with low caloric intake initially. Factors directly responsible for this adaptive response are not well defined.

That active transport by the intestine can adapt to periods of restriction is shown by studies of Hindmarsh and

coworkers (1967). Active transport of D-glucose and L-histidine was measured by the everted gut-sac technique in rats, hamsters and guinea-pigs. Data suggest that the enhanced active transport is not merely a reflection of the thinning of the intestinal wall and that it occurs during complete as well as in partial starvation.

Stekel and Smith (1969) suggest that anemia found after prolonged caloric deprivation is not caused by a primary lack of any essential nutrients, but represents an adaptive response to the decreased metabolic needs of the chronically undernourished organism. Young pigs, anemic after many months of caloric deprivation, responded to phlebotomy with a prompt increase in excretion of erythropoietin and in the rate of erythropoiesis while maintained on a severely restricted caloric intake. The prephlebotomy level of total red cell mass was rapidly re-established.

It appears that maximal growth rate may be incompatible with desirable skeletal characteristics (Saville and Lieber, 1969). Effects of undernutrition on skeletal mass and density were studied in rats from weaning to maturity. Animals restricted to 2/3 ad libitum food intakes had more total body calcium at any given body weight than the controls. The long bones were of the same volume as the controls but had thicker cortices and appeared to sustain a greater load in compression.

EFFECT OF STARVATION AND REFEEDING UPON REPRODUCTION

Inadequate food intake has long been known to exert a deleterious influence upon the reproductive system. In man a close relationship exists between sub-optimal nutrition and impaired reproduction. Some of the first clinical descriptions of hunger and war edema coming from Europe during the First World War mentioned the prevalence of amenorhea among women and impotence among men (Keys et al., 1950). Several workers (Dietrich, 1917; Stekel, 1920) have attributed various endocrine malfunctions related to reproduction to mental disturbances which accompanied the starvation. Reports indicate that amenorrhea was common during World War II (Keys et al., 1950), especially in the Netherlands and in Greece during German occupation. Observations in the Dutch East Indies by Sydenham (1946) suggest that emaciated Chinese women had adapted to chronic starvation better than European women. These Chinese women experienced no cessation of menstruation whereas the European women showed prolonged intermenstrual periods associated with sudden transition to an inferior plane of nutrition. Anorexia nervosa is often accompanied by amenorrhea (Bliss and Migeon, 1957). However, the cessation of menstruation is said frequently to precede any severe loss of weight (Kay and Leigh, 1954). It has been pointed out (Keys et al., 1950) that in periods

of food shortages a real decline in birth rate often occurs though nutritional status may not be the only or primary factor involved. Several studies have shown that atrophy of the reproductive tract and impairment of spermatogenic and endocrine function occur during caloric insufficiency (Jackson, 1925; Klatskin et al., 1947; Keys et al., 1950).

In nearly all animal species the attainment of sexual maturity approximately coincides with attainment of about 60% adult body weight and dimensions (Donovan and Vander Weff Bosch, 1965). It is widely acknowledged that sexual development, as evaluated by the appearance of the secondary sex characteristics, is delayed in children who are generally underdeveloped (Schonfeld, 1943; Talbot and Sobel, 1947). Bruch (1941) found that obese girls are younger at menarche than girls of average body weight. Other investigators have likewise noted that fat children tend to be taller, more advanced in skeletal maturity and reach sexual maturity at an earlier age than children of average body weight (Fry, 1953; Garn and Haskell, 1960). Tanner (1962) has summarized data which suggest that advanced physical development from early childhood on is related to comparatively early menarche. In a study by Van Wagenen (1952), 2 macaques treated from the age of 5 months with testosterone propionate underwent menarche at the age of 12 months, when accelerated growth had resulted in a body weight of 3.5 Kg, the weight at which normally growing macaques also reach menarche (Van Wagenen, 1949).

In farm animals, undernutrition delays the onset of puberty and leads to impaired fertility in mature animals. Reid et al. (1951) fed calves and heifers at varying levels of intake. The underfed animals developed "heat" symptons at later stages, had a subnormal conception rate and underdeveloped udders. In mature cows, underfeeding impaired estrous symptoms and reduced fertility (Moustgaard, 1969). Several investigators demonstrated that caloric insufficiency impaired fertility in sheep (Foote et al., 1959; Bellows, 1963; Casida, 1964) and swine (McKenzie, 1928). In males, gross undernutrition for long periods of time reduced the size of the testes in swine; spermatogenesis ceased, and the interstitial cells atrophied. Upon refeeding, tubules developed rapidly and spermatogenesis recurred (Dickerson et al., 1964). In young bulls, reduced energy intake impaired growth of the endocrine glands and reproductive organs (Van Demark et al., 1969). When restricted to 70% of the recommended allowances bull calves showed a delay in the onset of sexual maturity; sperm concentrations were lower and sperm motility subnormal (Bratton, 1953).

It is well established that caloric undernutrition delays puberty in the rat (Reid, 1960; Allen, 1961). On the basis of changes in reproductive tract weights and their absolute as well as relative nucleic acid contents on a time continuum of 20 to 100 days of age, Desjardins et al. (1968)

have established that pubertal growth occurs between the ages of 40 to 70 days in male and 33 to 60 days of age in female rats. Several studies suggest that time of onset of puberty appears to be related to body weight, rather than to age. McCay et al. (1943) found that when food consumption was restricted so that body weight increased about 1 g per 10 days from weaning, opening of the vagina was delayed in 30% of the animals. Rats suckled in small litters grow faster and mature earlier than when suckled in large litters (Engle et al., 1937; Seitz, 1954; Widdowson and McCance, 1960; Kennedy and Mitra, 1963). It is also reported that premature weaning in rats causes an impairment of spermatogenesis which only becomes manifest after 6 months of age (Hahn and Koldovsky, 1966).

There are many reports on the effects of starvation and undernutrition on the male organs of laboratory animals (Siperstein, 1921; Moore and Samuels, 1931; Mulinos and Pomerantz, 1941; Keys et al., 1950). The effect appears to be age-dependent. When undernourished from birth onward, weights of the testes varied in proportion to body weight at various ages (Widdowson and McCance, 1960), whereas undernutrition of 3-week-old rats had a greater effect on body weight than on testicular growth, so that the testes became relatively larger than normal (Widdowson et al., 1964).

Undernutrition also results in functional changes of the male sex organs. This feature has been clearly illustrated by Mann and his associates (Lutwak-Mann and Mann, 1950; Mann and Lutwak-Mann, 1951; Mann, 1964). In rats, a low energy intake resulted in a depression of the secretory function of accessory sex glands to the same degree as castration. Administration of androgens and gonadotropin restored the secretory function. After 20 weeks of underfeeding, citric acid in bull semen was reduced to 60% and fructose to 30% of initial values (Mann and Walton, 1953). Spermatogenic function of the testes seemed to remain normal.

Several workers have attributed the regression of sexual characteristics to inadequacy in gonadotropin production during malnutrition (Leathem, 1966; Scott and Scott, 1964; Pazos and Higgins, 1945). The primary derangement centers in the pituitary gland resulting in secondary atrophy of the gonads (Stephens and Allen, 1944; Mann and Rowson, 1957). Regressive changes in reproductive function were corrected by injection of chorionic gonadotropin. Administration of estrogen and progesterone enables protein-deficient rats to maintain pregnancy thus suggesting that protein depletion results in impairment of gonadal hormone secretion (Lyons, 1966). Protein deficient rats responded to follicle-stimulating hormone administration with ovarian follicle growth and to interstitial cell-

stimulating hormone by proliferation of interstitial tissue (Kinzey and Srebnick, 1963). Berg (1965) studied the effect of varying levels of intake of a stock diet in female rats on reproductive performance, maternal and fetal weights, onset of resorptions and critical stages of implantation in the rat. Results were similar to rats fed a purified diet containing different amounts of protein (Nelson and Evans, 1953). With a 50%-restricted stock diet, 77% of rats littered as compared with 70% for animals receiving 5% protein in a purified diet. At the 75% level of restriction, 8% to 36% of stock-fed rats littered as compared with zero to 11% for rats receiving 2.5% protein or a protein-free diet. Berg's (1965) data obtained on day 13 of gestation showed that the number of implants in 75% restricted rats was the same as in animals fed ad libitum. Fetation was an all or none phenomenon in that full-sized litters developed or all implants resorbed. Transitory unrestricted feedings on day 8 prevented resorptions in rats fed 75% restricted diets, but delays to days 10 or 12 resulted in an increasing number of resorptions. Level of nutrition during days 8 to 11 of gestation appears to be critical for resorptions.

McClure (1966) studied the effect of fasting on mating behavior and fertility in rats. In the male, short time fasting for a 30 hour period had no effect on these functions. If the starvation period was extended to 36 or 48 hours, the proportion of mated females decreased but

spermatogenesis remained unaffected. Starvation for 36 hours in the female, inhibited estrous behavior. Van Tienhoven (1968) reviewed the literature and concluded that the most pronounced effect of subnutrition occurs at or close to the time of implantation in rats and mice.

ORGAN WEIGHTS DURING GROWTH AND STARVATION

Variations in growth in different organs tend to be adjusted on a time continuum so as to give the animal mechanical stability and metabolic or physico-chemical homeostasis. In order to analyse interrelationships between size, form and function, several researchers have related a part to the whole during the period of increase in size. Based on the rationale that the percentage change in one variable tends to vary directly with the percentage change in a related variable, Brody (1945) analysed organ weights and body size parameters in several species of animals. From these studies, he developed the equation $Y = aX^b$ which acquires linearity in the logarithmic form, $\log Y = \log a + b \log X$ where Y represents organ weight, X the body weight and log a and b the intercept and slope respectively. This equation has been used extensively to delineate patterns of growth. Julian Huxley observed (1932) that many gross body measurements when plotted against body weights yielded straight lines on log-log paper. While some components vary logarithmically with the total body weight,

some organs vary in direct proportion and still others remain constant in the face of changing body size. Brody (1945) found that with the exception of heart, thyroid and spleen, the relative increase in weight of the visceral organs is less than that of the body as a whole. For kidney the slope (b) is .846, for liver .867 and for lung .986 in mature mammals. The value of the exponent b is known to vary with age (MacKay and MacKay, 1927). For both man and the rat, a log-log plot of testis weight as a function of body weight yielded two distinct slopes with a change in the slope at puberty (Spencer, 1968). While the latter part of the slope was less steep in the rat, the reverse held true in man. This was substantiated by Burr et al. (1970) who observed that testicular growth in boys accelerated after 12 years of age.

Interspecie comparisons reveal wide differences in absolute and relative weights of several organs (Schmidt and Ivy, 1937). Donaldson (1924) pointed out that closely related strains in the rat may differ significantly in growth characteristics insofar as body weight and individual organs are concerned. Webster et al. (1947) found no sex differences in relative weights of organs in albino rats. However, their data demonstrate the variation in relative weights of organs with the procedure used in sacrificing the animals. For instance, the weight of the livers of 400-gram rats represented 4.04% of the body weight when the

animals were etherized and 3.66% when bled. The kidneys weighed 0.74% and 0.72% respectively.

Sperling (1953) found considerable variation in the weight ratio of lungs. Latimer (1943) had pointed out that lungs with various lesions have higher weight ratios than normal lungs. Cameron (1925) found that in albino rats ranging in weight from 50 to 200 grams, the relative weights of liver, kidneys and spleen generally decreased with increasing body weight. Webster et al. (1947) found this trend toward lower ratios with increasing body weight to be more pronounced for animals in the lower weight groups.

McLennan and Jackson (1933) reported that in starved adult rats, loss of weight of organs with respect to decrease in body weight, varied considerably, the spleen and liver losing at a relatively greater rate and the kidneys at a relatively smaller rate than the total body. In 1915, Jackson studied the effect of an average of 9 days of acute starvation on some organ weights of 15 albino rats. Relative resistance of the nervous system and the skeleton compared to the adipose and lymphoid tissues which rapidly undergo marked atrophy was pointed out by Jackson in 1925. Peters and Boyd (1968) found that adrenals, brain, testes and stomach lost weight at a much slower rate than the total body in albino rats subjected to acute starvation, thirst and stress by electric shock. Organs which lost weight at a rate greater than total body were liver, spleen and

thymus. The kidneys and heart lost weight at a rate approximately that of the total body. Beznak (1954) reported that relative weight of the heart stays constant during starvation. In a study on the effects of refeeding following complete starvation in albino rats, Peters (1967) observed that refeeding produced an immediate rise in liver weight to values above normal, followed by a gradual return to control levels at 3 weeks. This "overshoot" phenomenon had been noted previously by Čabak et al. (1963). Recovery upon refeeding after inanition depends upon the degree of cell injury which has been sustained. Jackson (1937) and Quimby (1948) reported that in starved young rats, the majority of the weight loss during starvation is attributable to fat. Most organs appear to lose fat somewhat in proportion to their initial fat content, with the exception of the nervous system. Nitrogen loss in albino rats during inanition were measured by Addis et al. (1936). The liver appeared to lose more nitrogen per unit of body weight than other organs.

DISTRIBUTION OF BODY FAT DURING STARVATION AND REFEEDING IN THE RAT

Measurement of Body Fat

Whole-body chemical analyses have provided data on the lipid content of the bodies of a few infants and adults (Forbes, 1962). Due to the difficulties and the limitations

involved in a direct analytical approach, several indirect methods have been proposed to ascertain the fat component of the body. Although adipose tissue undoubtedly accounts for a relatively large percentage of body weight, as early as 1942, Behnke and others challenged the traditional height-weight-age tables as a valid measure of leanness-fatness among men. Since adipose tissue contains a large proportion of body fat (Allen et al., 1959) it should be possible to estimate the lipid content of the body from quantitative measurements of fat depots. The skin-fold measure has been refined and has come to be used as a standard method of assessing obesity (Corbin, 1969). Garn (1961) has developed the soft tissue radiographic technique for prediction of adiposity in man, based on measurements of the depth of subcutaneous fat layers, originally introduced by Stuart and his associates in 1940.

Body Composition of Rats

Several studies have been conducted to determine the major body components of the rat (Table 1). Procedures involved grinding of raw or frozen carcasses, or partial hydrolysis prior to homogenization, after which chemical analysis for moisture, nitrogen, fat and ash were carried out.

TABLE 1.--Body composition of rats as determined by various investigators.

Sex	Age Days	No.	Wgt. g	Total Body Composition				Lean Body Mass			Investigator
				Water %	Prot. %	Fat %	Ash %	Water %	Prot. %	Ash %	
M,F	Birth	43[2]	4.3	87.2	8.7	2.5	1.6	89.2	8.9	1.6	Hatai (1917)
M,F	7	7	10.2	79.8	10.1	8.0	2.0	86.7	10.8	2.2	"
M,F	15	9	13.5	72.9	13.1	11.8	2.2	82.7	14.9	2.5	"
M	21	6	39.9	73.1	16.3	6.8	2.8	78.4	17.5	3.0	Mickelsen & Anderson (1959)
M,F	22	5	24.9	70.6	15.7	10.8	2.9	79.4	17.6	3.3	Hatai (1917)
M	28	2	78.7[3]	73.3	16.7	6.3	3.0	78.2	17.8	3.2	Mickelsen & Anderson (1959)
M,F	32	3	47.6	70.1	15.7	11.1	3.2	78.9	17.7	3.6	Hatai (1917)
M,F	42	3	65.8	69.4	18.8	8.3	3.5	75.7	20.5	3.7	"
M,F	50	7	91.0	68.2[4]	19.5	9.0	3.4	74.9	21.4	3.7	Chanutin (1931)
M,F	62	6	114.4	66.4[4]	20.7	9.2	3.7	73.1	22.8	4.1	"
M,F	90	4	126.2	61.1[4]	20.9	13.9	4.1	71.0	24.2	4.8	"
M	109	51	254.8[3]	58.2	17.3	18.1	3.0	71.1	21.1	3.6	Deuel et al. (1944)
F	109	51	171.2[3]	56.8	16.4	20.1	3.3	71.1	20.5	4.1	"
M	127	7	274.6[3]	61.5	18.8	15.3	3.4	72.6	22.2	4.0	Light et al. (1934)
M,F	150	39	224	61.6	21.1	13.4	3.8	71.3	24.4	4.4	Chanutin (1930)
M,F	294	2	277.5	65.3	22.1	9.0	3.7	71.8	24.3	4.0	Hatai (1917)
M	308	1	1235.0[3]	26.7	8.68	62.3	1.2	70.8	23.1	3.2	Mickelsen & Anderson (1959)
F	497	4	318.7	57.8	18.0	20.9	3.1	73.0	22.8	3.9	
F	497	2	702.6[3]	36.0	10.7	51.4	1.9	74.1	22.0	3.9	"

[1]Data include male and female rats.

[2]Analyses done on pooled samples.

[3]Rats were fed high fat rations.

[4]Percentage of water calculated by difference.

Effects of Starvation and Refeeding on Body Fat in Rats

Carcass analyses on rats before and after weight reduction showed that tissue lost due to complete starvation initially contained somewhat more lipid and nitrogen when compared with partially restricted rats (Sarett et al., 1966).

Schemmel (1969) studied patterns of reduction of individual fat depots in Osborne-Mendel rats made experimentally obese by the method of Mickelsen et al. (1955). While all adipose tissues were affected by food restriction, the subcutaneous, genital and mesenteric deposits appeared more susceptible than did the perirenal and xiphoidal depots. In a study on normal and hypothalamic-obese rats, the nature of the diet did not appear to alter rate, amount or composition of body weight lost when fed at the level of 9 g. per rat per day for 12 days (Karch and Beaton, 1968). Dible (1932) showed the steeper slope of declining body fat versus that of total body weight in starving rats. Cremer (1939) found the gonadal fat depot to be a discrete and sensitive index of body fat changes occurring during food deprivation. He found that when starvation produced losses of 35%, the fat depots had lost 92%; these loses were in relation to the weights of ad libitum animals. The reverse also appears to hold true. During dietary experimental obesity, fat depots increased in weight more rapidly than overall body weight (Schemmel et al., 1970).

The fatty acid synthetic process in an active lipogenic tissue is generally highly adaptive to dietary manipulations. The replacement of dietary carbohydrate by protein or fat depresses fatty acid synthesis in liver and adipose tissue of the laboratory rat (Masoro et al., 1950; Whitney and Roberts, 1955; Hill et al., 1958; Cohen and Teitelbaum, 1966; O'hea and Leveille, 1969). On the other hand, lipogenesis is markedly enhanced in periodically-fed as contrasted to ad libitum-fed animals (Tepperman and Tepperman, 1958; Fabry, 1967). Food deprivation for varying periods of time has repeatedly been shown to depress lipogenesis (Boxer and Stetten, 1944; Jansen et al., 1966; Leveille, 1969), whereas refeeding following a fast rapidly restores lipid synthesis to the level seen in ad libitum-fed animals and frequently induces an "overshoot" (Tepperman and Tepperman, 1958). The decrease in lipogenesis caused by starvation has been ascribed variously to: (a) alteration in carbohydrate metabolism, particularly the activity of the hexose monophosphate shunt (Masri, et al., 1952), (b) inhibitory factors (Korchak and Masoro, 1962), (c) lack of stimulators (Catravas, 1963), (d) lack of α-glycerophosphate receptor (Tzur et al., 1964), (e) loss of activity of citrate cleavage enzyme (Abraham et al., 1964) and (f) loss of activity of acetyl Co-A carboxylase caused by high concentrations of fatty acyl-CoA derivatives (Bortz and Lynen, 1963).

Distribution of Adipose Tissue

It was in 1948 (Wertheimer and Shapiro) that attention was first called to the high metabolic activity of adipose tissue and it was revealed that such tissue is not merely an inert storage depot for fat synthesized elsewhere, but itself actively engages in lipogenesis. Fat is a concentrated reserve of energy which is used preferentially as fuel by animals deprived of all food. The ability to store both fat and carbohydrate (as fat) in the adipose tissue postprandially confers a degree of metabolic resiliency and adaptability advantageous to the animal. Wertheimer and coworkers (1960) demonstrated that the various individual fat depots of the body are not metabolically identical either in their biosynthetic activities or in their responses to hormonal stimulation. In man, the distribution of fat shows considerable individual variation. Skinfold measurements for fat show an increase with age in women (Young et al., 1963). However, these increases are too small to account for the total body fat increases estimated by specific gravity. These observations suggest fat deposition at areas other than subcutaneous.

One of the earliest studies on differential accumulation of body fat in rats was carried out by Reed and associates (1930). Regardless of diet composition and energy expenditure, the relative distribution of fat among inguinal, interscapular, genital, perirenal and mesenteric

regions remained constant. While the nature of the diet appears to be ineffective in displacing the normal pattern of distribution (Pagè and Babineau, 1953), genetic and endocrine factors can influence the relative depositions at inguinal and gonadal regions in mice (Liebelt, 1959). Peckham et al. (1962) found that the ratio of epididymal fat to total body fat closely paralleled the age of the rat. In gold thioglucose induced obesity, Lielbelt and coworkers (1965) reported a direct relationship between the amount of lipid in the inguinal or in the gonadal fat depot and body weight of mice. Though the total body weight and amount of depot fat in the obese rats were larger than in the non-injected controls, the patterns of distribution were similar.

UNDERNUTRITION AND DISEASE

Human Studies

Early attempts were made to learn about nutrition from human experience (McCollum, 1964). Luigi Cornaro (1467-1566), within a few days on a restricted diet, observed an improvement in his constantly indisposed condition and in less than a year found himself entirely freed from all his complaints. Cornaro, a Venetian, lived to be over one hundred years old.

The earliest advocate of vegetarianism, Sylvester Graham (1794-1851) had always suffered from poor health.

Drawing his conclusions from accounts given by travellers, of dietary practices in different parts of the world, and from his personal experience with different dietary regimens, he began using moderation in eating and strongly urged the use of a vegetarian diet for promoting health, strength and longevity.

Using himself as subject, Siven in 1900 published a study of the protein requirements of man which showed that nitrogen equilibrium could be maintained on 28 to 31 grams daily. These figures were in marked contrast to those of German and American investigators who concluded that adequate diets should contain daily allowances greater than 100 grams protein. It was Russel H. Chittenden (1856-1943), a professor at Yale University who presented experimental evidence that a diet which provided much less protein than the accepted standards was not only safe, but had the merit of being decidedly beneficial to health. On the basis of his personal experiences on an abstemious dietary regimen, he was convinced of the superiority from the standpoint of physiological well-being of the low protein, low calorie diet. He demonstrated the effect of restricted protein intake in human volunteers. Personal testimony, athletic record, reaction time, and photographs all supported the claim that the abstemious system of eating was sound, and could to advantage be adopted generally. Chittenden presented these unconventional principles in his book,

Physiological Economy in Nutrition. This elicited a response by Sir James Crichton-Browne, a London physician, who under the title Parcimony in Nutrition strongly opposed the idea that a low-protein diet is favorable to physiological well being.

For many years, insurance companies have been studying the relationship of various factors to the principle causes of death. The statistics clearly show an association between obesity and early mortality though causation is not definitely implicated. One of the first of these studies suggested that with increasing body weight there is increased mortality from cancer. That study by a joint committee of the Association of Life Insurance Medical Directors and the Actuarial Society of America appeared in 1913. It was a comprehensive analysis of principle causes of death. The report dealt with 774,672 policies (1885-1908) on men whose ages were from 20 to 62 years and whose heights ranged from 5'3" to 6'2". The ages, weights, and heights were those of the policy holder at the time his first policy was issued. The policy holders were divided into three groups--overweight, those 50 pounds or more above the average weight; standard, those less than 50 pounds overweight, and not more than 25 pounds underweight; and underweight, those 25 pounds or more under the average weight. The mortality rate is given as "deaths per 100,000 exposed to risk." Data which suggested that with increasing body weight, there is an increased cancer mortality.

A number of life insurance companies have examined their own records to determine whether any risk was associated with overweight. One of these by Dublin (1932) was of men who started a policy at 45 years of age or over and subsequently died of cancer. He utilized approximately 192,000 records (1887-1921) of the Union Central Life Insurance Company. Also, the policy holders were classified according to weights at entry. The analysis resulted in the following distribution of cancer mortality with regard to weight.

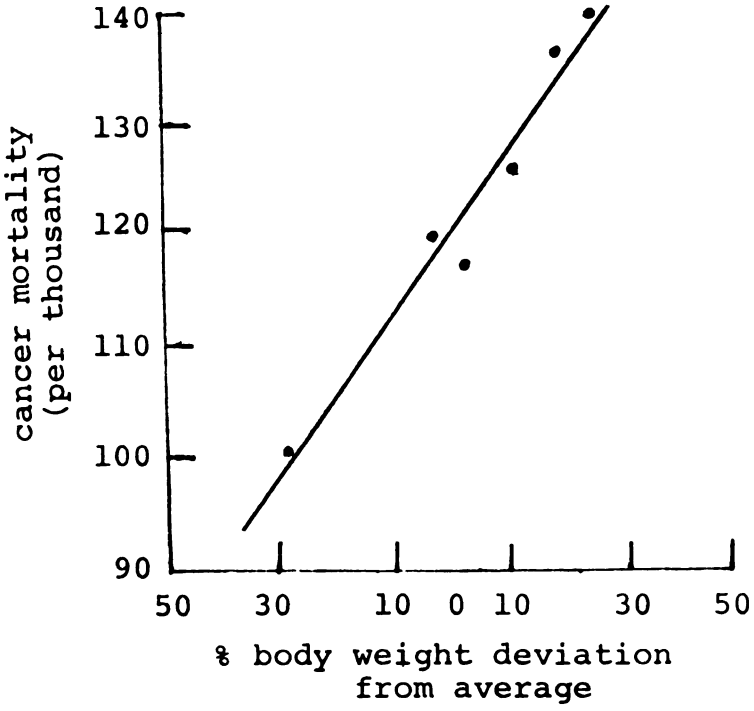

Figure 1.--Correlation between adult body weights and deaths from cancer (Dublin, 1932).

This study reveals a graduation in mortality rates sufficiently consistent to indicate that cancer incidence increases with increasing weight.

Dublin (1929) classified policyholders of Life Insurance Company dying from cancer, on the one hand, and from accidents, on the other, according to their weight when their policies were issued. He considered the accidental death groups comparable in weight distribution to the general population. For policy holders above 45 years, the relative number excessively overweight among persons dying of cancer was nearly 25 percent greater than among those dying of accidents.

Among infants, it appears that insofar as immunological responses are concerned, the undernourished are not at a disadvantage compared to well-fed infants. Keet and Thom (1969) determined concentrations of the immunoglobulins (Ig) G, M and A in the sera of 11 children suffering from kwashiorkor and a variety of infections, and 11 well-fed children with similar infections. There were no differences between the IgG and IgM values of the 2 groups, most of the results being higher than those reported for normal children. The IgA values of the kwashiorkor cases were much higher than those of the controls and normal children. In a study of marasmic infants, Najjar and coworkers (1969) found that in 3-6 month old subjects IgM, IgA and IgG values in serum were significantly higher than in healthy infants. At 13 to 30 months, IgM and IgG were the same, while IgA was significantly higher.

Animal Studies

The relation of longevity to physical growth and particularly to nutritionally retarded growth has been widely studied in experimental animals (McCay et al., 1939; McCay et al., 1943; Silberberg and Silberberg, 1955; Ross, 1961; Berg, 1960; Ross et al., 1970). Rats subjected to caloric restriction after weaning lived a longer time than normally fed animals.

Pioneering studies of McCay and coworkers at Cornell University (1935) showed that restricting food intake of rats resulted in longer life spans due to retardation of onset of disease. Weanling albino rats of both sexes were divided into 3 groups: (1) freely fed, (2) a group fed a normal diet in restricted amounts from weaning, and (3) the same ration in restricted amounts from two weeks post-weaning. Intakes were reduced to allow a 10 gram increment in body weight every 2-3 months. According to survival data, the favorable response due to retarded growth rates was more pronounced in males than in females. In a later experiment, McCay, Sperling and Barnes examined growth, aging, chronic diseases and life span in rats allowed to grow at the rate of 1 gram per 10 days. Autopsies were made at 100-day intervals. Only 5% of the ad libitum fed group exceeded the age of 1000 days. Fifty percent of the restricted rats retarded for over 1 year and then refed exceeded the age of 1000 days. Histological findings

indicated that rats retarded in growth exhibited a much greater resistance to chronic disease than those that grew normally. The diseases appeared ultimately, especially in those groups allowed to feed ad libitum after 300 or 900 days of age. However, at a given age the incidence of chronic disease was much lower in retarded animals than in the unrestricted controls. Inflammatory, neoplastic and degenerative disease are implicated from the pathological data. Retardation of growth tended to equalize the life span of both sexes, as in the earlier experiment.

To distinguish the effects of slow growth and a small body size from the effects of the chronic undernutrition which caused it, Widdowson and Kennedy (1962) employed differential litter-size during suckling as a means of producing variations in size of the animal. Apart from the suckling period, the rats were fed a good ration ad libitum throughout life. Differences in growth rate were maintained despite the free access to food. During the first year mortality among the slow growing animals was greater. On the average, slower growing rats lived about the same length of time as the larger rats. The authors concluded that the longevity which McCay et al. (1939) observed in their undernourished rats was due to the long period of undernutrition they experienced rather than to their smaller body size.

The effect of periodic fasting on life spans of rats was studied by Carlson and Hoelzel (1945). Animals

of both sexes were fed ad libitum on a 30% protein diet until 42 days of age and then divided into groups intermittently fasted 1 in 4 days, 1 in 3 days, and 1 in 2 days. Average life spans of all the fasted rats exceeded that of the controls. Prolongation of life by fasting was proportional to the amount of fasting. Life spans of the males were on the average, increased more than the life spans of females. In each group the life span was found to be correlated with the weight prior to the experiment at 42 days of age. However, prolongation of life due to fasting 1 day in 3 or 2 tended to outweigh the apparent handicap of a poorer nutritional start in life, as indicated by a lower than average pre-experimental weight.

Berg (1960) at Columbia University studied the relationship of nutrition and longevity in rats. Restriction of food intake of weanling Sprague-Dawley rats to levels 33 and 46% below ad libitum levels until 800 days of age resulted in 25 and 40% reduction in body weights respectively. Differences in body weight was largely due to absence of excess body fat which developed in unrestricted animals who apparently consumed more than they needed to meet energy output. Female fertility and health were reportedly better in rats kept on dietary restriction than in unrestricted rats attaining maximum size.

Nolen (1970) studied growth, organ weights, femur lengths and longevity in male and female Simonson rats as

affected by various restricted dietary regimens. The groups restricted throughout life or fed freely till 12 weeks of age and then restricted for life had significantly higher survival rates than the ad libitum fed animals. Forty-three percent of ad lib fed rats were alive at 2 years and none at 3 years of age. Rats restricted initially and then allowed to feed freely throughout life had a mortality similar to the controls.

Undernutrition and Degenerative Disease

In rats on a restricted level of food intake there was a delay in the onset as well as reduced severity of lesions of degenerative diseases associated with aging. Berg and Simms (1960) observed the occurrence of glomerulonephritis, periarteritis, myocardial degeneration and tumors predominant in well fed animals. Care was taken at the beginning of the experiment that none of the rats had lung or middle ear infections. Only 48% of males on the ad libitum regimen were alive at 800 days as compared with approximately 85% survival in restricted animals. Comparative incidence of diseases occurring separately or in combination in rats surviving showed significant differences between unrestricted and restricted animals. At all levels of food intake, incidence of lesions in males was much higher than in females. In both sexes there was a graded relation between the incidence of disease and level of food intake.

In an extension of the study to 1100 days, Berg (1960) observed the development of muscular degeneration characterized by weakness and paralysis of the hind legs--an additional dimension of the age-associated degenerative disease complex. Sprague-Dawley rats of both sexes were fed either ad libitum or 54% of the ad libitum intake. Combined incidence of renal vascular or myocardial lesions in restricted males increased with advancing age from 24% at 800 days to 60% at 900 days and to 75% of 1100 days, frequency being 100% in unrestricted males at 800 and 900 days of age. For females, the incidence of these lesions in restricted rats was zero at 800 days, and 24% at 1100 days as compared with 60 and 90% in unrestricted rats at corresponding ages. Berg concluded that under the limitations imposed on spontaneous activity by prolonged cage confinement, the lean and slightly smaller rat maintained with a restricted food intake adapted to its environment better than the animal fed ad libitum.

Undernutrition and Neoplastic Disease

Long term restriction of food intake effectively delays the time of occurrence and reduces the overall incidence of spontaneous tumors in both rats and mice.

Pioneering experiments to test the effect of simple underfeeding on incidence of tumors (Tannenbaum, 1940) were initiated when it was observed that in experiments in which

mice were being fed ad libitum, smaller animals in the group appeared to develop fewer tumors. Tannenbaum (1947) investigated the effects of calorie intake on different types of tumors in mice. By 100 weeks of age, 26 animals in the ad libitum fed control group of virgin female mice had developed spontaneous mammary tumors while none appeared in the restricted group. When a carcinogenic hydrocarbon was applied to the skin, only 11 tumors arose in the calorically restricted group in comparison with 32 in the control group fed ad libitum. Inhibition of tumors was found to be characterized by both a decrease in the total number of tumors and a delay in the average time of appearance of the tumors. Results of investigations by Visscher and associates (1942), Saxton et al. (1944), White et al. (1944), Rusch and associates (1945) and Larsen and Heston (1945), indicated a generalized effect since other tumor types and spontaneous and induced leukemia were also found to be retarded due to undernutrition.

When a regimen of restriction is imposed at weaning age and maintained for life, the extent of the reduction in overall tumor risk is, within limits, a function of the degree of caloric underfeeding (Tannenbaum, 1947; Ross and Bras, 1965). In the nutrition-longevity studies of Berg (1960) a wide variety of spontaneous tumors developed with greater frequency in well-fed rats. Neoplasms were chiefly of the benign type, the more common being

fibroadenoma of the female breast, adenoma of the thyroid, chromophobe adenoma of the pituitary, pheochromocytoma of the adrenal, and islet adenoma of the pancreas. Malignant tumors included unclassified neoplasms of the central nervous system, carcinomas, sarcomas and lymphomas.

The incidence of adenomas of pituitary gland varies widely according to the strain. In the Wistar rat, Saxton et al. (1948) and Berg and Simms (1960) demonstrated the prevalence of these adenomas was markedly reduced by caloric restriction. In studies where the amount of each of the dietary constituents consumed was controlled throughout postweaning life, Ross and Bras (1965) showed that, although total tumor risk was sensitive to the level of caloric intake, the incidence profile as regards tumor type differed according to the amount of protein consumed. In inbred mice predisposed to a specific tumor type, the incidence of spontaneous hepatomas was reported to relate directly to the protein content of the diet, but the risk of mammary tumors was unchanged. When the basal diet of rats was supplemented with protein, Saxton et al. (1948) found that there was an increase in the number of lymphosarcomas but a decrease in the number of tumors of the pituitary gland. This reduction in frequency of pituitary tumors was ascribed to the life shortening effects of other debilitating diseases which were more prevalent among rats provided the additional protein so that relatively fewer animals were at risk at

later stages. Gilbert et al. (1958) on the other hand reported that when rats were fed a carbohydrate-free, high-protein diet on an ad libitum basis, fewer tumors developed; the incidence of pituitary adenomas was unaffected by reduction in total amount of food intake. Recently, Ross et al. (1970) studied the influence of uniform life long dietary regimens upon incidence of spontaneous tumors of the pituitary gland. Male rats of the Charles River strain were fed purified diets either ad libitum or restricted to 2 grams initially with subsequent increments until a maximum allotment of 6 grams was reached. Dietary regimens were begun at 21 days of age and maintained for life. Body weights of the restricted but isocalorically fed rats stabolized at a level lower than maximum and the average weights remained constant throughout the remainder of the animal's lifetime.

Results indicate that both the caloric intake and the protein-caloric ratio of the diet influenced the prevalence of chromophobe adenomas of the anterior pituitary gland. A change in the level of caloric intake alone had, by far, the greater modifying influence than a change in the relative proportion of dietary protein. The variation in response indicated caloric restriction would more likely influence the incidence of those tumors most common to that strain.

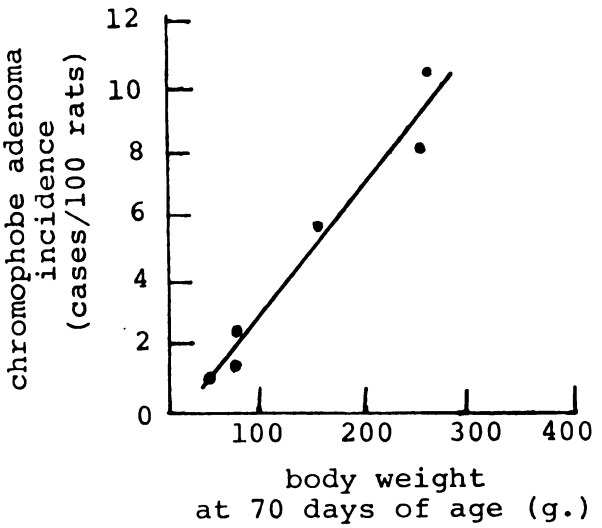

Figure 2.--Correlation between body weight of rats early in life and incidence of chromophobe adenomas of later life (Ross et al., 1970).

Ross and associates suggest that hormonal imbalance is a predisposing condition to the formation of spontaneous pituitary gland tumors. Dietary regimens which promote rapid growth, higher body weights at maturity and obesity may result in higher tumor incidences because such regimens may be conducive to high levels of endocrine activity.

Undernutrition and Infectious Disease

A lowered plane of nutrition can protect animals from some infectious diseases. In deficiencies of the B-complex vitamins replication of the infectious agent in infections of viral or systemic protozoal origin, appears to be impaired and the disease is less severe (Becker and

Smith, 1941; Rasmussen, et al., 1944). Wooley and Sebrell (1942) reported that deficiencies of riboflavin and thiamine increased the severity of type I pneumococcal infection in the mouse whereas very high doses of either of these vitamins increased mortality. Increased susceptibility and mortality in chickens fed high levels of protein and infected with Salmonella gallinarum have been observed by Smith and Chubb (1957) and Hill and Garren (1961). Squibb (1964) found that the severity of Newcastle disease in chickens was increased by both a deficiency and high levels of protein. Hedgecock (1955) reported that mice had greater resistance to tuberculosis when fed diets containing 20% protein and that susceptibility increased when the diets contained 10 or 40% protein.

In order to test the influence of caloric intake on resistance to infection, Newberne (1966) fed a balanced diet at three levels to dogs. The high caloric intake, 90-100 kcal/kg. of body weight per day, resulted in obesity after 5-6 weeks on the diet. When these dogs were inoculated with distemper virus, they demonstrated greater susceptibility and shorter survival times. The moderate intake of 70-75 kcals/kg. body weight per day produced dogs that responded to the virus inoculation in a manner somewhat similar to that observed in dogs fed the high levels of kcals. Bresnahan and Newberne (1968) showed that obese dogs are more susceptible to infection with canine distemper virus

than are their slightly underfed counterparts. Based on studies on lipid metabolism they postulated that the low-fed dog is able to develop a tolerance to the viral stress whereas the obese dog is unable to do this and thus responds more acutely and less effectively to the infection.

Recently, Berger (1969) at the University of France in Paris reported that during the course of a respiratory tract infection, mortality in the ad libitum fed group of 420 rats was 15%. During this same period, only one of a group of 180 chronically malnourished rats died.

Lung Disease in Rats

A spontaneous chronic respiratory disease (CRD) of rats was observed by Newberne, Salmon and Hare (1961) occurring among three strains of albino rats. Approximately 100% of the 400 Sprague-Dawley rats fed freely for 6 months or longer, developed lesions of the disease. Of a group of 360 Charles River rats, 14% showed lesions. Twenty-seven percent of the Alabama Experiment Station stock colony were similarly affected.

Gross and microscopic pathological findings were reported on the pleural surfaces. In earlier stages disseminated foci of reddish-brown color were present. Later, focal areas were pearly-gray, irregularly rounded, and elevated above the surface of the lobe. The cut surface exuded a mucoid or mucopurulent fluid. The authors suggested that the initial lesion of lymphoid infiltration

occurs in response to a viral agent. Subsequent bronchiectasis may be caused by destruction of bronchial musculature or accumulation of mucus and products of bacterial growth.

Nelson (1963) of the Rockefeller Institute characterized the etiological factors in CRD. Prior to 1931, the bronchopneumonia, otitis media, and rhinitis of naturally infected animals were thought of as different manifestations of a single disease entity caused by a Streptococcus moniliformis. However, in an attempt to establish a CRD-free rat colony, Nelson and Gowen (1931) successfully eliminated Streptococcus moniliformis and transmissible otitis media but the pulmonary lesions still persisted. Bacteriological examination of lung suspensions and exudates from naturally infected rats and experimentally infected mice failed to demonstrate a cultivable microorganism capable of reproducing the disease. The etiological factor was finally identified as a virus. The virus was commonly recovered from the lung whether or not frank lesions were present. Experiments indicated that it was not present in new-born rats.

The rate of infection approached 100% as the rats matured. Once acquired, the virus apparently continued to reside in the respiratory tract throughout the life of the animal. The maintenance of infection within a rat population could be largely atrributed to nasal transfer of the

virus from infected mothers to their nursing young. Nelson's work has established that CRD of the rat comprises two separate entities: namely, infectious catarrh caused by PPLO and enzootic bronchioctasis caused by a virus. They may occur independently or co-exist in the same animal. PPLO tend to localize in the upper respiratory tract and middle ears while the lung is the principle locus of the virus. Both infections are transmissible by nasal installation and contact in rats and also in mice.

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PART I

EFFECT OF STARVATION AND REFEEDING UPON TESTICULAR FUNCTION

EFFECT OF STARVATION AND REFEEDING UPON TESTICULAR FUNCTION

ABSTRACT

Serum testosterone as determined by gas-liquid chromatography decreased during 8 weeks of 50% food restriction to 31% of control values in 18 week-old Sprague-Dawley rats. Following another 15 weeks of semistarvation these hormone levels were similar to control values. Weights of accessory reproductive organs and their secretory activities showed increases during the latter phase of food restriction. When allowed to refeed to 21 days after 20 days of semistarvation serum testosterone rose to 3 ng./ml. as compared to 1.5 ng./ml. for ad libitum controls and 1.2 ng./ml. for chronically semistarved rats of the same age. Complete deprivation of food lowered hormone levels to undetectable quantities. Absolute weights of testes and eqididymides and their concentration of sperm (per gram of tissue) were affected less during caloric restriction and refeeding than endocrine criteria.

INTRODUCTION

It is well established that undernutrition adversely affects the reproductive system in rats (Keys et al., 1950;

Berg, 1965; Hahn and Koldovsky, 1966). While spermatogenesis appears relatively resistant to the influence of caloric restriction (Widdowson et al., 1964), the endocrine function of the testes appears to be highly susceptible, as determined by decreased accessory organ weights and their secretory activity (Pazos and Higgins, 1945; Scott and Scott, 1964). That the primary derangement centers in the pituitary gland, has been shown by studies in which regressive changes were corrected by injected gonadotropins (Mulinos and Pomerantz, 1941; Stephens and Allen, 1944; Mann and Rowson, 1957). However, the level of circulating testosterone during starvation has not been explored. The absence of such data stems from the problems associated with determination of testosterone. Another unexplored aspect is on reproductive performance produced by refeeding after prolonged undernutrition. Most previous work has been limited to the short-term effects produced by the complete deprivation of food. The purpose of the present experiment was to study the effect of semistarvation, total starvation and refeeding on testicular functions.

METHODS

The experimental plan is shown in Fig. 3. Male weanling Sprague-Dawley rats were obtained from the Spartan Research Center, Williamston, Michigan. They were allowed to feed ad libitum on a grain ration (Campbell et al., 1966) until puberty at 10 weeks (Desjardins et al., 1968).

Five animals were autopsied at this time to obtain organ weights. Mean daily intakes of unrestricted rats were calculated from weekly food consumption. Amount of ration spilled by the animals was taken into account. Water was available to all animals throughout the study. They were weighed once a week.

The rats were individually caged in wire-screened cages. Temperature was kept constant at 25-27°C. throughout the year. Lighting was regulated to provide 12 hours of illumination and 12 hours of darkness each day.

The animals were randomly divided between the unrestricted control and the 50% restricted groups. Half of the ad libitum level of food was fed once a day between 7 and 8 p.m. to the restricted group.

Ten rats were sacrificed when the weight loss of the restricted group plateaued, after 8 weeks of semi-starvation. A group of 10 unrestricted controls was also sacrificed at this time. The remainder of the 20 rats in the unrestricted and 20 in the restricted group were maintained on their respective levels of food intake until 33 weeks of age, when 10 animals each from the unrestricted and 50% restricted groups were sacrificed. By this time several of the ad libitum fed rats were afflicted with chronic respiratory disease. Three weeks prior to the termination of the experiment, 10 rats from the restricted group were permitted free access to the ration (20 weeks

semistarvation followed by 3 weeks of refeeding). To impose total starvation, a group of 6 previously unrestricted rats was deprived of all food, 6 days prior to being killed. At the second autopsy, the freely fed, 50% restricted, refed and totally starved rats were all 33 weeks of age. The rats were killed by etherization and bleeding from the abdominal aorta. Blood was allowed to clot for 4 hours at room temperature and overnight at 15°C., then centrifuged, and the serum stored at -4°C.

Testosterone analyses were carried out by the electron capture detection of heptafluorobutyrate derivatives following chromatographic purification (Rawlings, 1970).

Seminal vesicles with coagulating glands were carefully excised and stored in saline at 0°C. Prior to fructose analysis (Mann, 1964), coagulating glands were dissected out and homogenized. Citric acid in seminal vesicles was determined spectrophotometrically (Mann, 1964). Weights of adrenals, anterior pituitary and liver were secured at autopsy. To minimize evaporation losses, the working surface was kept moist. For testicular endocrine function, changes occurring in various groups were tabulated as nanograms testosterone per ml. of serum, total citric acid in seminal vesicles and total fructose in coagulating glands. Organ weights were plotted on a time continuum as percent change (expressed on a % of body weight basis) from the initiation of semi-starvation at age 10 weeks.

Correlation coefficients were calculated to determine relationships of serum testosterone for each rat with its accessory productive organ data, weights of liver, adrenals, pituitary, epididymides and testes. Students t test was used to assess the significance of differences between group means.

RESULTS

Changes in body weight are shown in Fig. 4.

To more readily detect any changes that were associated with starvation, the condition of the animals at the end of the preliminary period was used as the standard. Any changes therefrom during the subsequent periods are listed as % alterations.

Semi-starvation for 8 weeks produced a significant decrease ($P < .05$) in the absolute weights of the testes and epididymides. However, since the rate of body weight loss exceeded that of these organs, there was an apparent increase in the weights of the testes and epididymis when expressed as a fraction of the body weight. During refeeding, body weights increased more rapidly than the weights of the sex organs. (See Figs. 3 to 6). At 18 weeks of age, significant ($P < .005$) decreases due to semistarvation in seminal vesicles, coagulating glands, total citric acid and total fructose, were apparent (Table

2). When caloric restriction was continued for another 15 weeks, statistically significant ($P < .05$) increases occurred in relative weights of seminal vesicles, coagulating glands, total citric acid and total fructose. Refeeding for 21 days caused increases in relative weights of seminal vesicles, coagulating glands and in absolute testicular and epididymal weights. When completely deprived of food for 6 days, serum testosterone levels declined to undetectable levels (Table 2). Relative weights of accessory organs and testicular weights were not affected ($P > .05$). However, amount of citric acid and fructose in seminal vesicles and coagulating glands respectively, were markedly reduced ($P < .05$). Concentration of sperm in testes and epididymides (per g. tissue) did not decrease significantly.

Correlation coefficients for serum testosterone and total citric acid, total fructose, accessory reproductive organ weights (% body weight), weights of liver, adrenals and anterior pituitary (% body weight), total weights of testes and epididymides were determined (Table 3).

DISCUSSION

Ability of the testes to produce sperm is unimpaired by food restriction in the adult rat. Although food intakes were maintained at one-half the level of freely fed control animals, spermatozoa continued to be produced even after 23 weeks of semi-starvation when body weights were

less than half of the freely fed controls. Gonadal weights were less susceptible to semistarvation than body weights.

Previous work suggests that undernutrition initiated early in the life of animals caused impairment of spermatogenesis (Widdowson et al., 1964; Hahn and Koldovsky, 1966). However, once sexual maturity has been achieved, gametogenic function of the testes is well maintained in spite of undernutrition. The present data substantiate the work of Moore and Samuels (1931) who found similar results in Vitamin B deficient rats, and of Mann and Walton (1953) on semistarved adult bulls.

Early physiological approaches to study the ability of testes to produce testosterone were based on the effect of this hormone on the growth and secretory activity of accessory sex organs. Relative weights of seminal vesicles, coagulating glands, the prostate and their content of citric acid, fructose and zinc respectively, have been used as indicators of testicular endocrine function (Mann, 1964).

Serum testosterone assays have allowed definitive quantification of gonadal endocrine function in several species of animals (Rawlings, 1970). Resko et al. (1968) reported values ranging from 1.10 to 2.04 ng./ml. plasma in normal adult rats for testosterone as determined by gas liquid chromatography. Values of Bardin (1969) are in the range of 41.0-50.4 ng./ml., while Frick et al. (1969) found 5.04 ng./ml. in rats (using a similar technique). In the

present study, serum testosterone values for adult rats varied from 3.90 at 18 weeks to 1.48 n.g/ml. at 33 weeks of age.

Based on accessory organ data and on the efficacy of gonadotropic hormones in reversing starvation-atrophy of the reproductive tract (Mulinos and Pomerantz, 1941; Lutwak-Mann and Mann, 1950), it was suggested previously that under-nutrition impairs hormone secretion. Although wide differences within groups preclude statistical significance, group means for serum testosterone show that semi-starvation (1.22 ng./ml.) and total starvation (no detectable amounts) cause lowered levels of circulating hormone.

When allowed free access to food for 3 weeks after a 20-week semistarvation period, gonadal endocrine function was greatly stimulated. Dramatic changes occurred during refeeding with hormone levels approaching 3.0 ng./ml.--a two-fold elevation over control values at the same age. Using mating tests as criteria, Jackson in 1915 reported that in restricted-refed rats, reproductive performance actually surpassed that of the controls. It appears that animals "adapt" to prolonged caloric restriction and having arrived at a new set point in the feedback system, are capable of enhanced functional ability insofar as hormone secretion is concerned, when presented with a relative "surplus" input. The adaptive phenomenon is supported by

accessory organ data which show an increasing trend at the latter stage of semistarvation, and significant increments after refeeding.

Serum testosterone levels diminished to undetectable levels during total inanition. The complete absence of food markedly reduced citric acid of seminal vesicles and fructose of coagulating glands.

Correlation coefficients showed that, in general, weights of seminal vesicles and coagulating glands (expressed as % body weight) parallel serum testosterone levels more closely than other reproduction parameters studied. From data on fully fed rats, it appears that male hormone in blood varies directly with accessory sex organ anterior pituitary and epididymal weights ($P < .01$). During semistarvation the total weights of testes and epididymides varied inversely with serum testosterone ($r = -1.0$ and $r = -.96$ respectively). However, serum testosterone was highly correlated with body weight ($r = 0.98$).

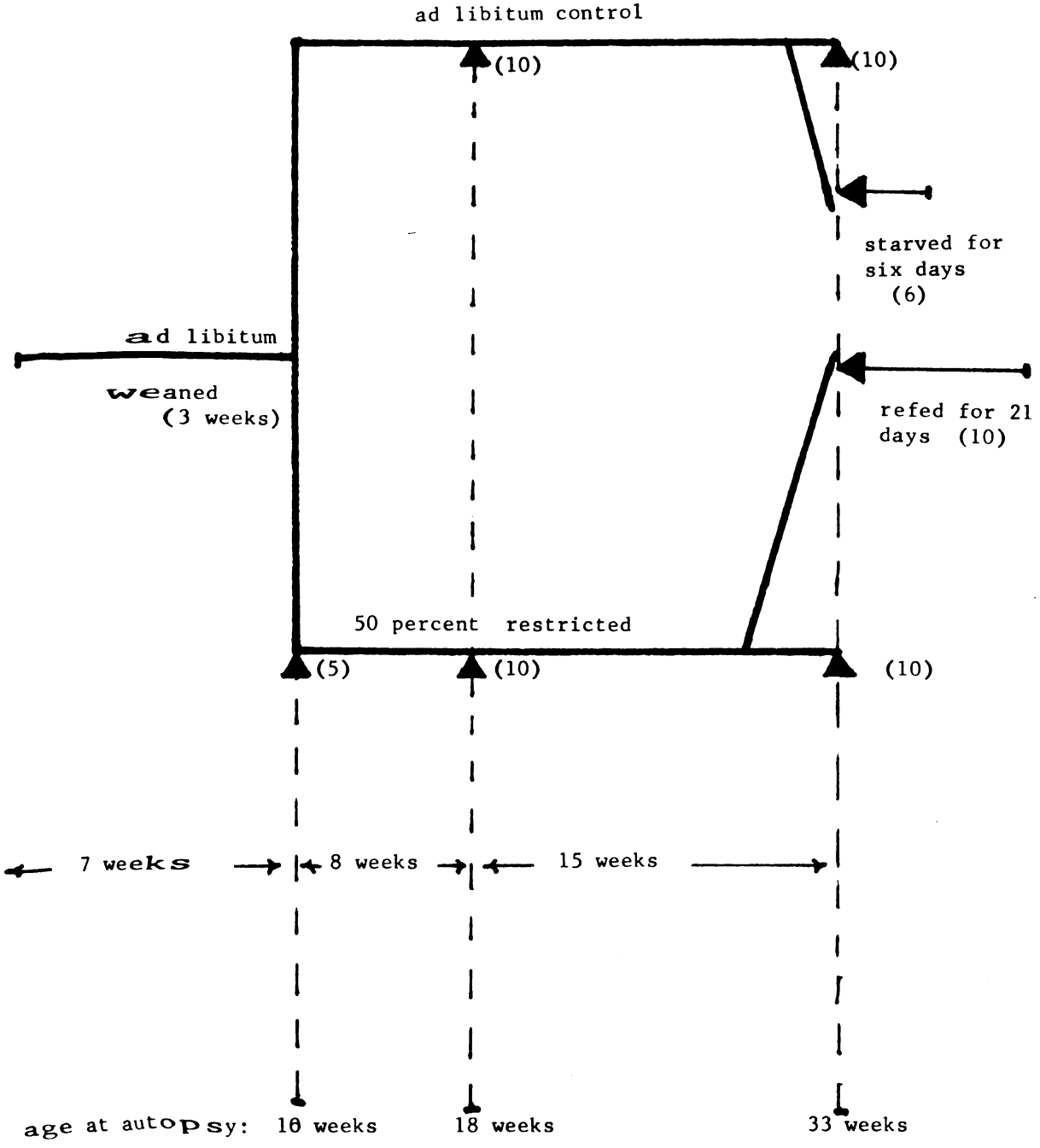


Figure 3.--Experimental design. [Number of animals per group are in parenthesis.]

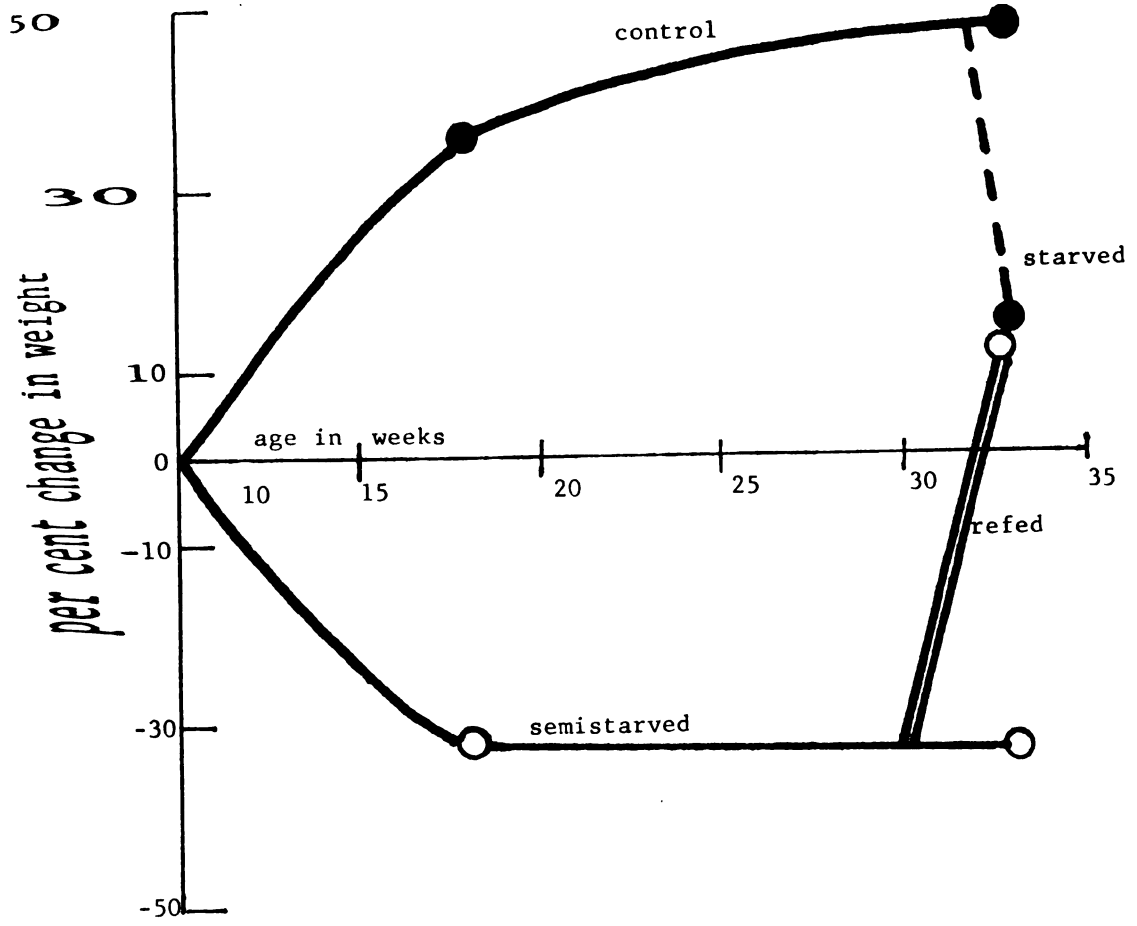


Figure 4.--Influence of semistarvation and refeeding upon total body weights in rats.

Percent deviation from control values at 10 weeks of age during:

50% food restriction
refeeding
complete starvation
unrestricted feeding

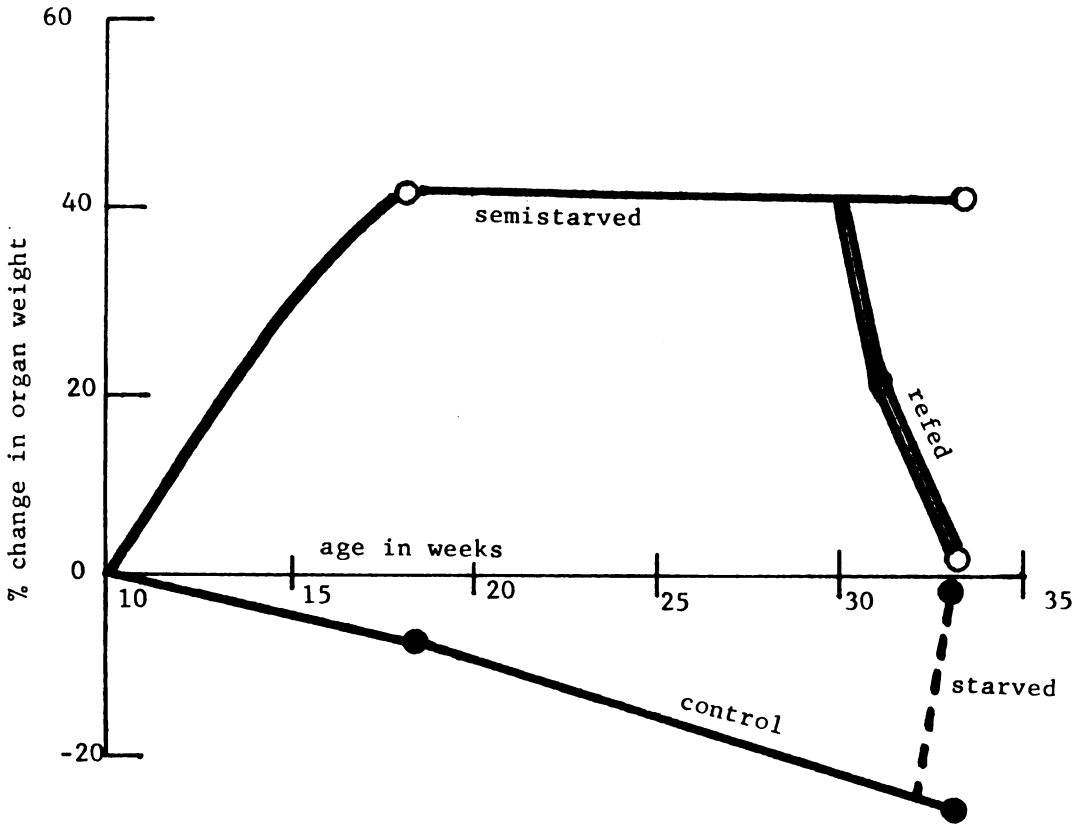
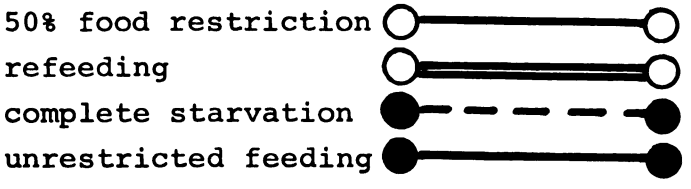


Figure 5.--Influence of semistarvation and refeeding upon testes weights (% body weight) in rats.

Percent deviation from control values at 10 weeks of age during:



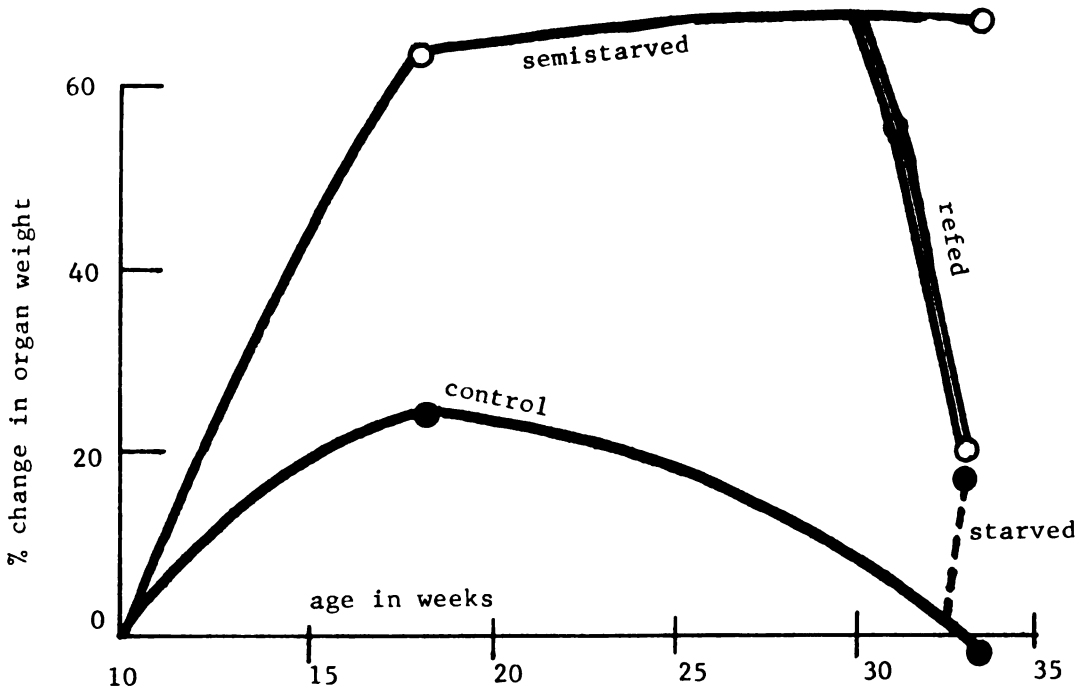


Figure 6.--Influence of semistarvation and refeeding upon epididymides weights (% body weight) in rats.

Percent deviation from control values at 10 weeks of age during:

50% food restriction
refeeding
complete starvation
unrestricted feeding

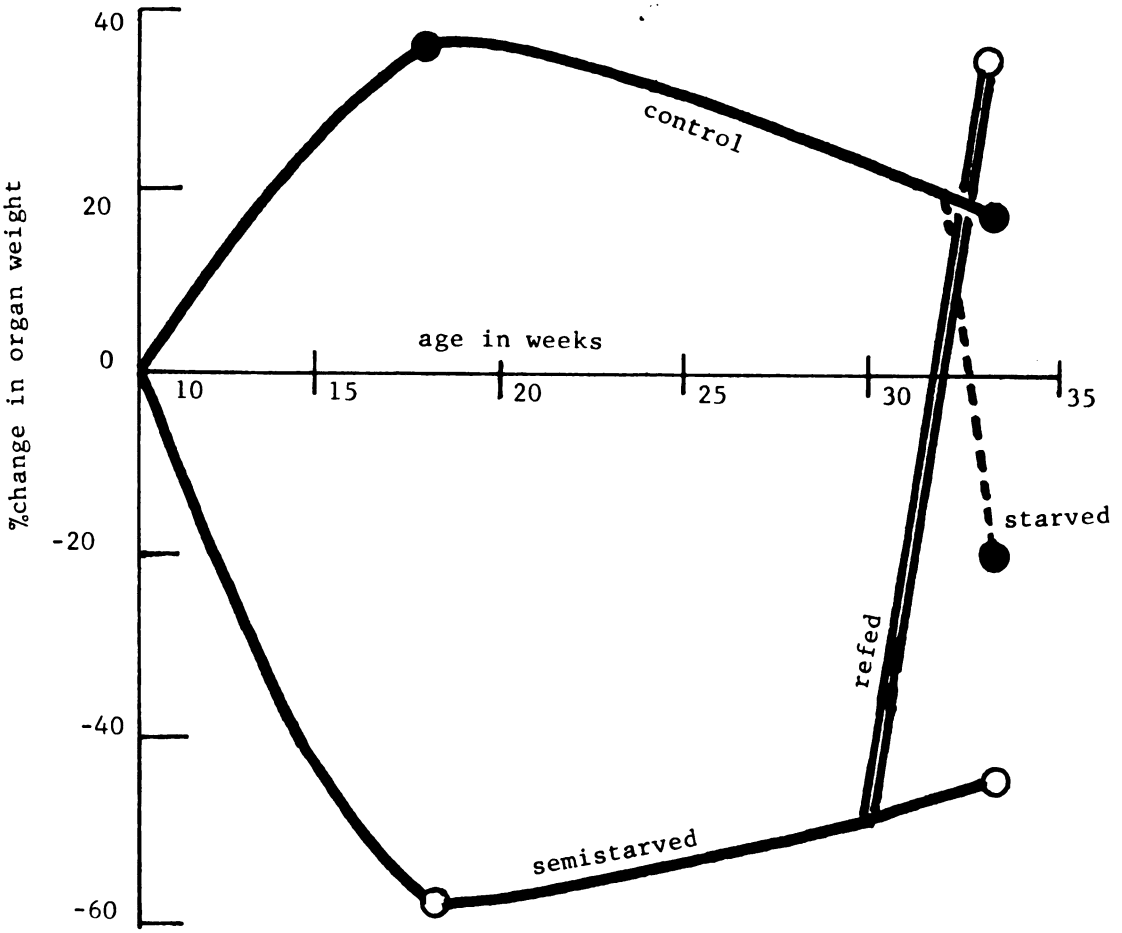


Figure 7.--Influence of semistarvation and refeeding upon seminal vesicle weights (% body weight) in rats.

Percent deviation from control values at 10 weeks of age during:

50% food restriction
refeeding
complete starvation
unrestricted feeding

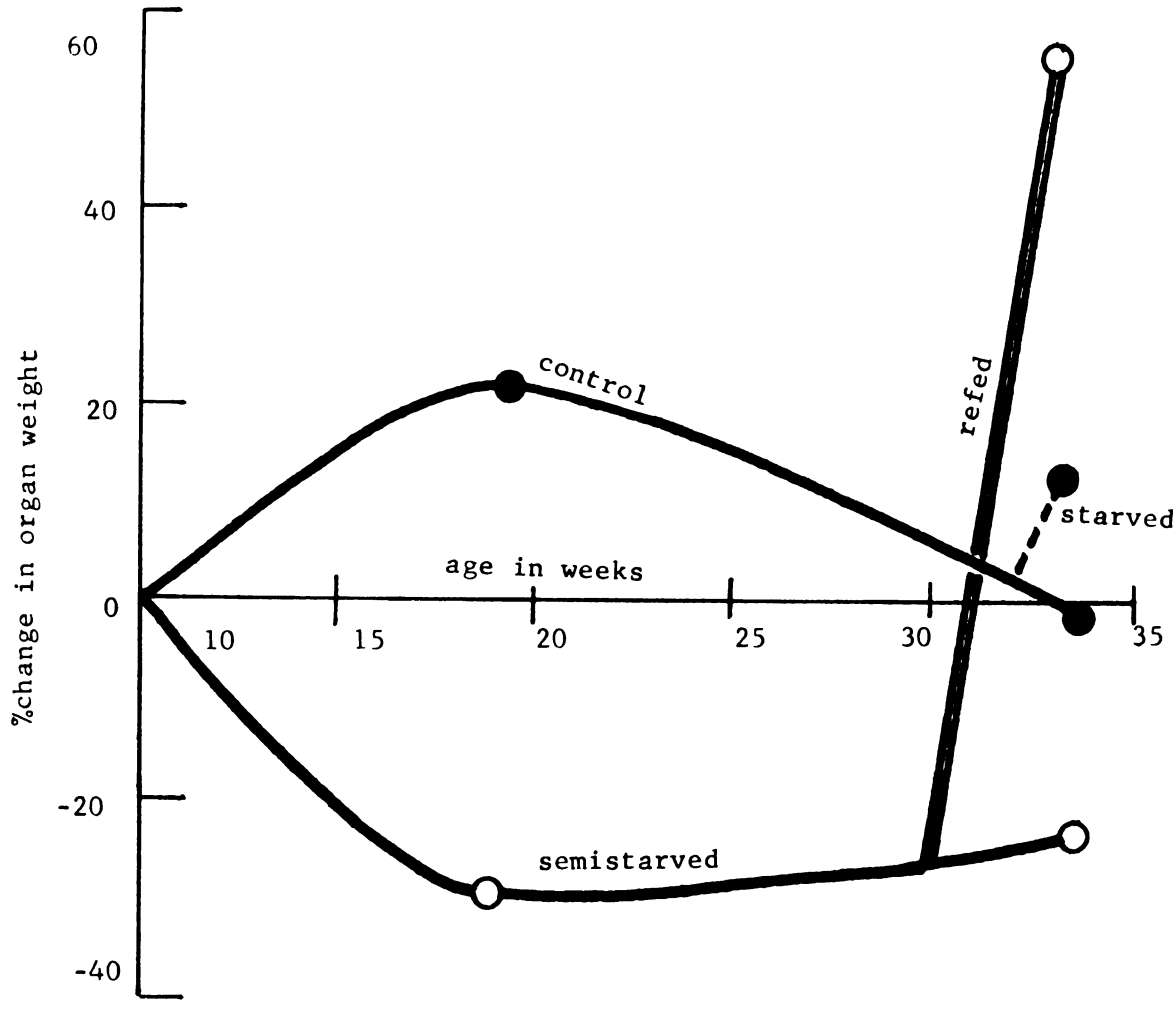


Figure 8.--Influence of semistarvation and refeeding upon coagulating gland weights (% body weight) in rats.

Percent deviation from control values at 10 weeks of age during:

50% food restriction
refeeding
complete starvation
unrestricted feeding

TABLE 2.--Parameters of reproductive function in male rats during starvation and refeeding. (Mean ± Std. Dev.)

Group Age (weeks) No. of Rat	Control 18 wks. (10)	Semi- starved 18 wks. (10)	Control 33 wks. (10)	Semi- starved 33 wks. (10)	Semi- starved Refed (10)	Control- starved (6)
Testosterone (ng./ml. serum)	3.90 ±3.45	1.22 ±2.01	1.48 ±1.08	1.22 ±0.74	3.01 ±3.05	0 0
Seminal Vesicles (% BW)	0.26 ±0.02	0.07* ±0.01	0.22 ±0.03	0.11*[1] ±0.02	0.26[2] ±0.04	0.15* ±0.07
Coagulating Glands (% BW)	0.03 ±0.00	0.01* ±0.00	0.02 ±0.00	0.02[1] ±0.00	0.04[2] ±0.02	0.02 ±0.01
Total Citric Acid (g.)	0.31 ±0.07	0.02* ±0.01	0.33 ±0.05	0.05*[1] ±0.02	0.30[2] ±0.11	0.02* ±0.00
Total Fructose (g.)	0.12 ±0.06	0.05* ±0.03	0.12 ±0.08	0.01*[1] ±0.01	0.08[2] ±0.03	0.02* ±0.00
Testes (g.)	3.77 ± .15	2.82* ± .19	3.32 ± .35	2.82* ± .24	3.01[2] ± .17	3.21 ± .43
Epididymides (g.)	1.31 ± .13	.85* ± .12	1.14 ± .02	0.88* ± .07	1.02[2] ± .19	1.06* ± .13
Sperm Counts (Million per g.)						
Testis	46±12	40±18	39±16	37±9	41±18	41±16
Epididymis						
Head & Body	248±49	283±55	259±58	231±81	291±85	254±48
Tail	342±63	262±31	295±75	314±68	298±91	259±62

*Values significantly different from control group of same age ($P < .05$).

[1]Values significantly different from semistarved group at 18 weeks of age ($P < .05$).

[2]Values significantly different from semistarved group at 33 weeks of age ($P < .05$).

TABLE 3.--Correlation coefficients of serum testosterone with some endocrine and metabolic criteria.

	Control 18 wks.	Semi-starved 18 wks.	Control 33 wks.	Semi-starved 33 wks.	Refed
Total Citric Acid	-.42	-.64	.52	.54	.59
Total Fructose	-.38	.97*	.65	-.52	-.82*
Seminal Vesicles per 100 g. of body wt.	.73*	.97*	.62	-.39	-.24
Coagulating Glands per 100 g. of body wt.	.42	.91*	.63	.18	-.14
Liver per 100 g. of body wt.	-.02	-.94*	-.12	-.74	-.08
Adrenals per 100 g. of body wt.	-.48	-.66	.29	-.09	-.82*
Anterior Pituitary per 100 g. of body wt.	.84*	-.21	.63	.52	-.14
Testes Total Weight	.35	-1.0*	-.32	-.41	-.69
Epididymides Total Weight	.35	.96*	.73	-.12	-.58

*Values significantly different from zero ($P < .01$).

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PART II

INFLUENCE OF VARYING LEVELS OF FOOD INTAKE UPON ORGAN SIZE

INFLUENCE OF VARYING LEVELS OF FOOD INTAKE UPON ORGAN SIZE

ABSTRACT

Male, Sprague-Dawley rats were allocated to unrestricted control and 50% restircted groups at random, after being fed ad libitum until 10 weeks of age. Organ weights of 10 rats from each of these two groups were taken at 18 weeks and also 33 weeks of age. Six rats from each of these two groups were completely starved for 6 days. Sixteen partially restircted animals were permitted free access to food after 20 weeks of semistarvation. Six of these were killed at 6 days following refeeding and the other 10 at 21 days after refeeding.

All changes are expressed as organ weight per 100 g. body weight. Heart and kidneys closely paralleled body weight changes in all groups. Liver and spleen were most susceptible to caloric deprivation. As total body weights increased with age in control rats the relative weights of organ decreased.

INTRODUCTION

Peters and coworkers (1967; 1968) have reported the effects of inanition and refeeding on organ weights of

albino rats. These authors did not study the changes occurring during prolonged semistarvation as compared with complete starvation and when followed by refeeding.

On 50% restricted food intakes, body weights of rats plateau when approximately one-third weight loss has occurred (Mulinos and Pomerantz, 1941). If organ size relative to body size varies linearly, and functionality is reflected in relative size of organs, then a diminishing rate of change with time in organ weights during semistarvation would imply physiological adaptation to a lower plane of nutrition.

In this study adaptive changes in weights of organs relative to body weight are reported during prolonged semistarvation, total food deprivation and restriction followed by refeeding.

METHODS

The experimental plan is shown in Fig. 9. Male weaning Sprague-Dawley rats were obtained from the Spartan Research Center, Williamston, Michigan. They were allowed to feed ad libitum on a grain ration (Campbell et al., 1966) until puberty at 10 weeks (Desjardins et al., 1968). Five animals were autopsied at this time to obtain organ weights. Mean daily intakes of unrestricted rats were calculated from weekly food consumption. Amount of ration spilled by the animals was taken into account. Water was available to

all animals throughout the study. They were weighed once a week.

The rats were individually caged in wire-screened cages. Temperature was kept constant at 25-27°C. throughout the year. Lighting was regulated to provide 12 hours of illumination and 12 hours of darkness each day.

The animals were randomly divided between the unrestricted control and the 50% restricted groups. Half of ad libitum level of food was fed once a day between 7 and 8 p.m. to the restricted group.

Ten rats were sacrificed when the weight loss of the restricted group plateaued, after 8 weeks of semi-starvation. A group of 10 unrestricted controls was also sacrificed at this time. The remainder of the 20 rats in the unrestricted and 20 in the restricted group were maintained on their respective levels of food intake until 33 weeks of age, when 10 animals each from the unrestricted and 50% restricted groups were sacrificed. By this time several of the ad libitum fed rats were afflicted with chronic respiratory disease. Three weeks prior to the termination of the experiment (after 20 weeks of semistarvation), 16 rats from the restricted group were permitted free access to the ration. Six of these rats were sacrificed after 6 days of refeeding and ten animals after 3 weeks of refeeding. To impose total starvation, a group of 6 previously unrestricted rats was deprived of all food, 6

days prior to being killed. At the final autopsy, the freely fed, 50% restricted, refed and totally starved rats were all 33 weeks of age.

Following 6 days of complete starvation, 6 rats each from the unrestricted-starved and semistarved-starved groups were sacrificed. A group of 5 10-week old rats was taken to obtain base line values. All animals were killed by etherization and subsequent bleeding from the abdominal aorta. Organs were removed and weighed. The gastrointestinal tract was slit open, washed and blotted before weighing. To minimize evaporation losses during autopsy, the working surface was kept moist.

Changes which occurred in the various groups were plotted as percent change in organ weights (expressed as % of body weight) from the initiation of semi-starvation at age 10 weeks.

RESULTS

Differential effects of semi-starvation, total starvation, and refeeding, during semistarvation, on various organ weights (% body weight) are shown in Figs. 11-16 at the end of the initial period of ad libitum food intake (age 10 weeks). Changes in heart and kidney weights when expressed as % of body weight closely paralleled body weight (Fig. 11). Liver and spleen weights decreased more rapidly than body weight (Figs. 12 and 13). Weight of the

gastrointestinal tract was larger relative to body weight (Fig. 14). Both adrenals showed a marked increase in weight due to semistarvation initially (Fig. 15). Complete starvation caused more rapid hypertrophy. When refed, there was a sharp decrease in adrenal weight (per 100 g. body weight). The anterior pituitary increased relative to body weight and later decreased during semistarvation (Fig. 16). When completely deprived of food, there was a rapid increase in anterior pituitary weights.

Food restriction caused a greater rate of change in body weight, and weights of epididymides, adrenals, testes, liver, spleen and anterior pituitary during the initial phase of semistarvation (0 to 8 weeks of restricted intakes) as compared to the latter phase (8-23 weeks), (Table 4). When allowed to feed freely after 20 weeks of semistarvation, the rate of change in organ weights (per 100 g. body weight) was greater during the first 6 days than during the next 15 days of refeeding.

During food restriction and refeeding heart and kidneys were highly correlated with total body weight (Table 5). This was true for a mean body weight range of 187 to 520 grams.

DISCUSSION

As ad libitum fed rats increased in body weight, the relative weights of organs decrease. The data

substantiate the work of Cameron (1925) and Webster et al. (1947). Over a range of 187 to 520 grams body weight due to caloric deprivation or refeeding after restriction, heart and kidneys closely parallel fluctuation in total body weight. Similar results were found by Beznak (1954). Liver and spleen decrease at relatively rapid rates compared to total body weight and other organ. This was previously reported by McLennan and Jackson (1933) and Peters and Boyd (1968). When ad libitum feeding is allowed after prolonged semistarvation, weights of liver and spleen (as a percent of body weight) initially increase above those of control rats. This "overshoot" phenomenon was also found by Cabak et al. (1963) and Peters (1967). During chronic caloric deficiency rate of change in organ weights decreases after rapid initial changes.

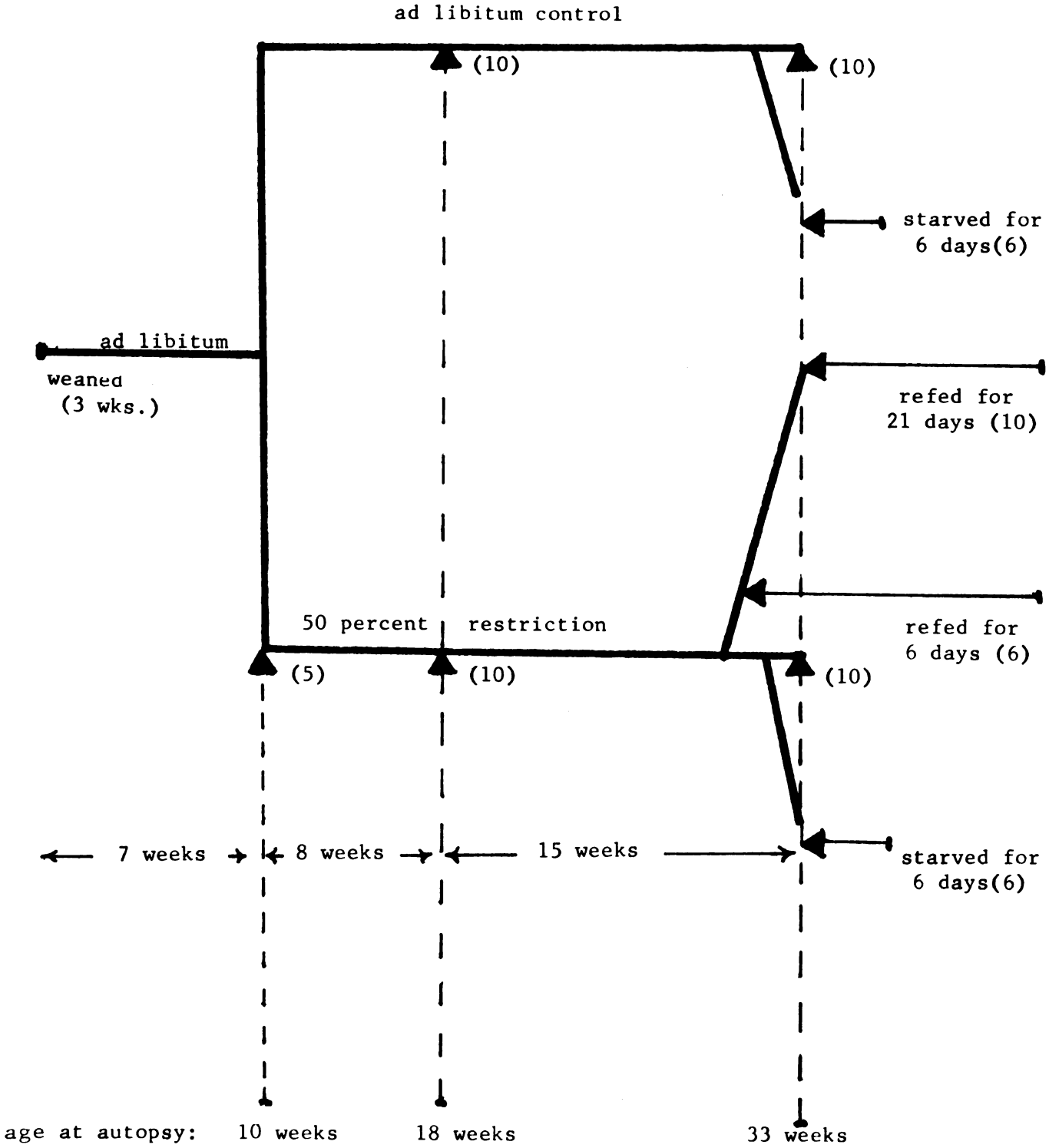


Figure 9.--Experimental design. [Number of animals per group are given in parenthesis.]

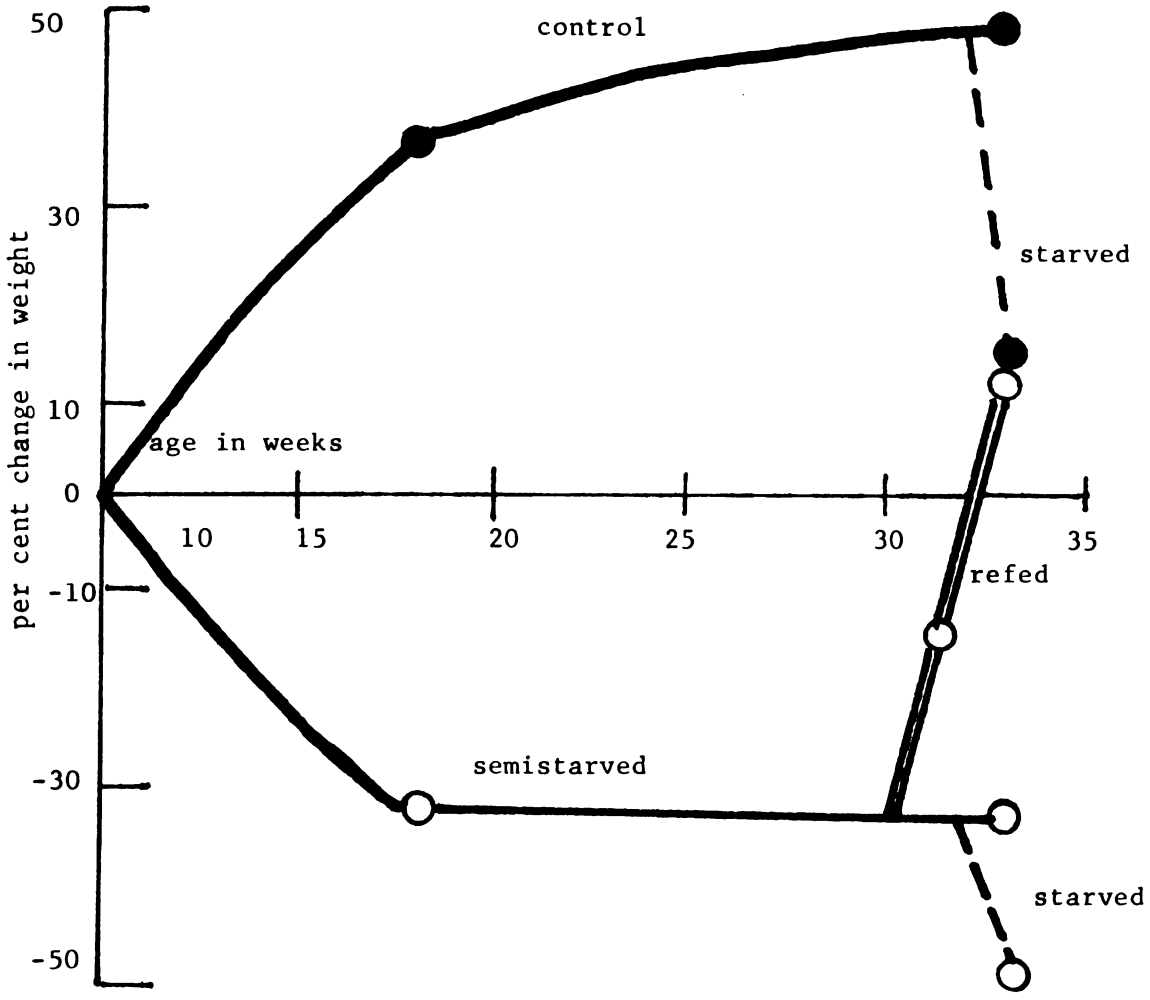


Figure 10.--Influence of semistarvation and refeeding upon total body weights in rats.

Percent deviation from control values at 10 weeks of age during:

50% food restriction
refeeding
complete starvation
unrestricted feeding

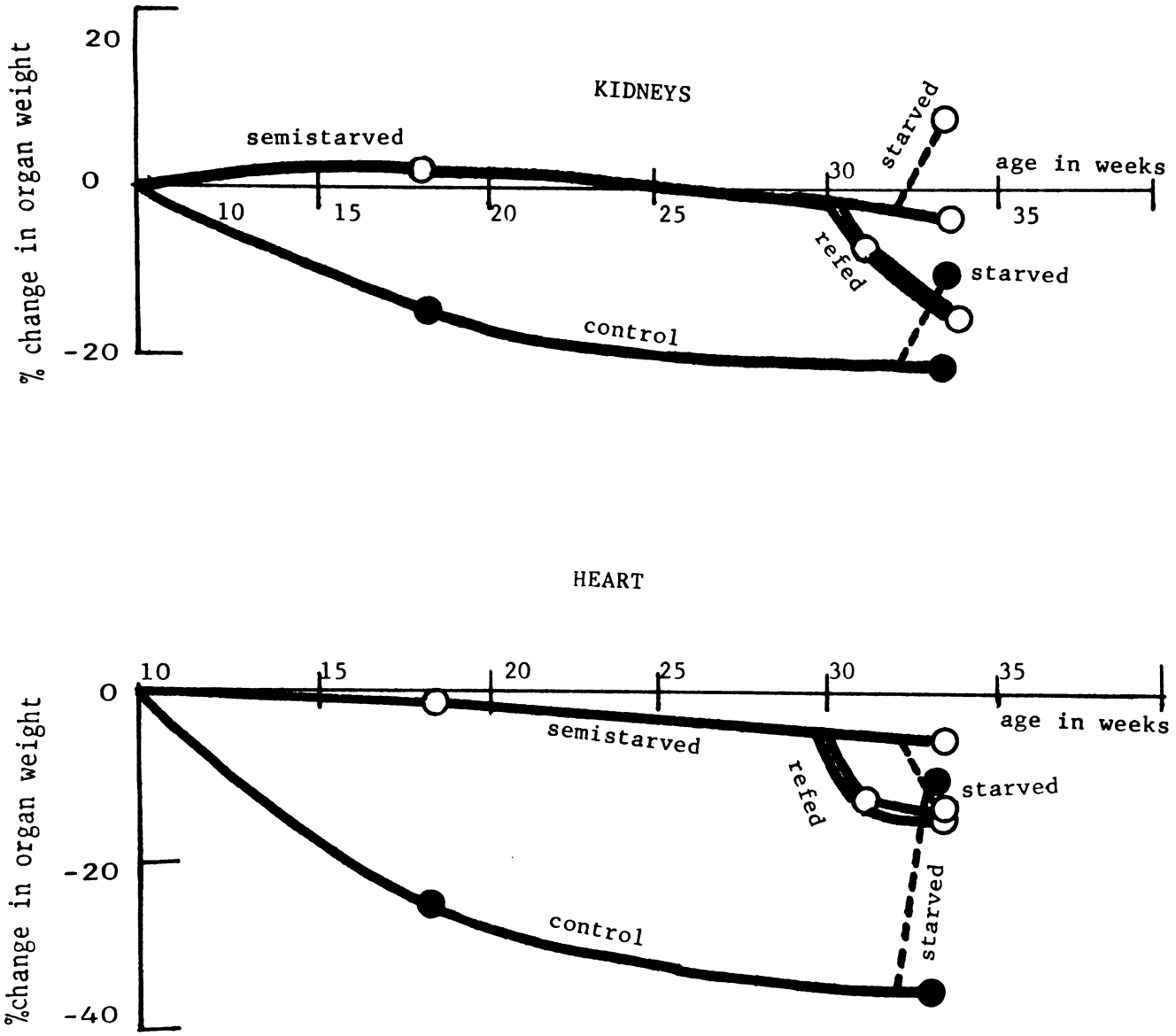


Figure 11.--Influence of semistarvation and refeeding upon kidney and heart weights (% body weights) in rats.

Percent deviation from control values at 10 weeks of age during:

50% food restriction
refeeding
complete starvation
unrestricted feeding

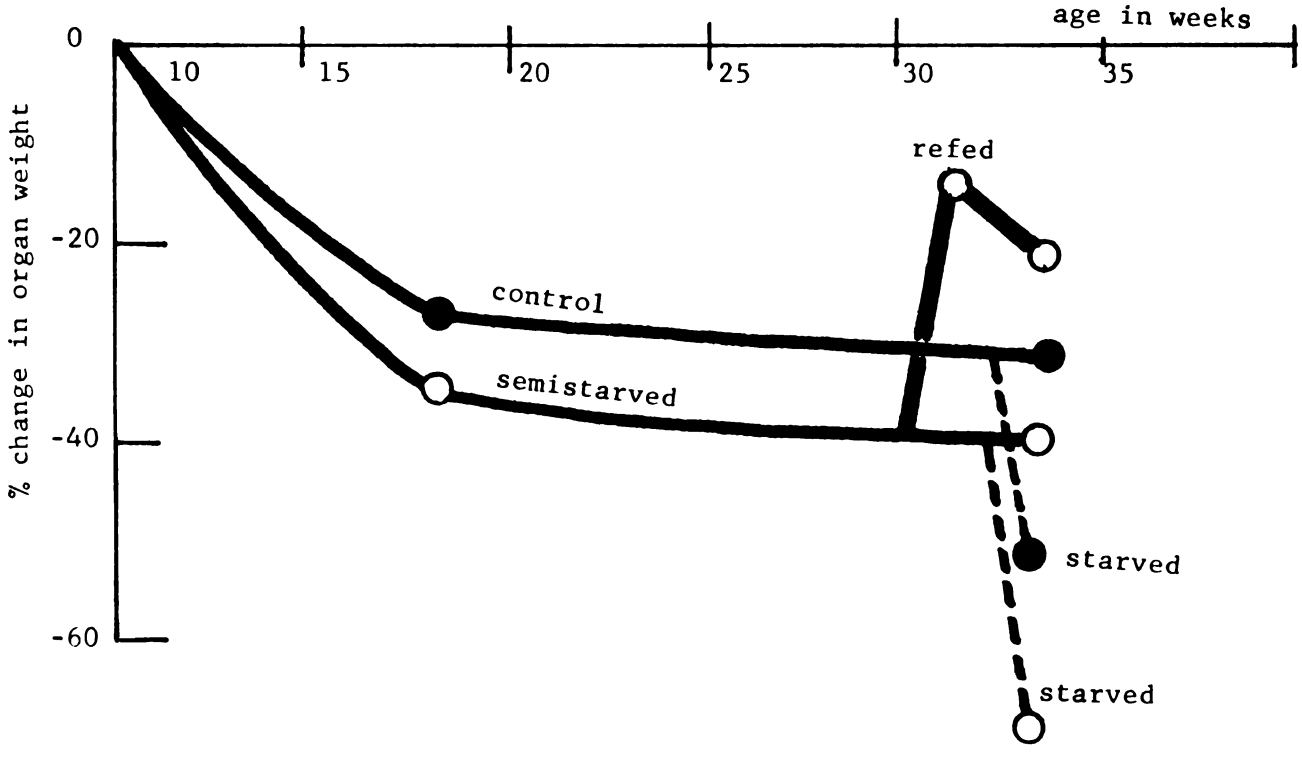
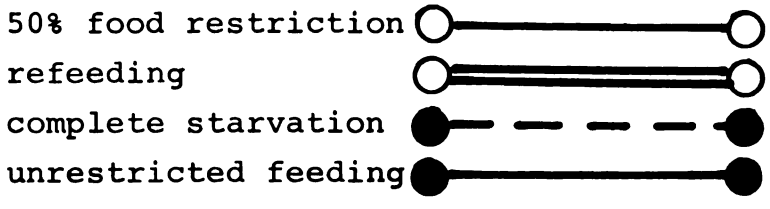


Figure 12.--Influence of semistarvation and refeeding upon liver weights (% body weight) in rats.

Percent deviation from control values at 10 weeks of age during:



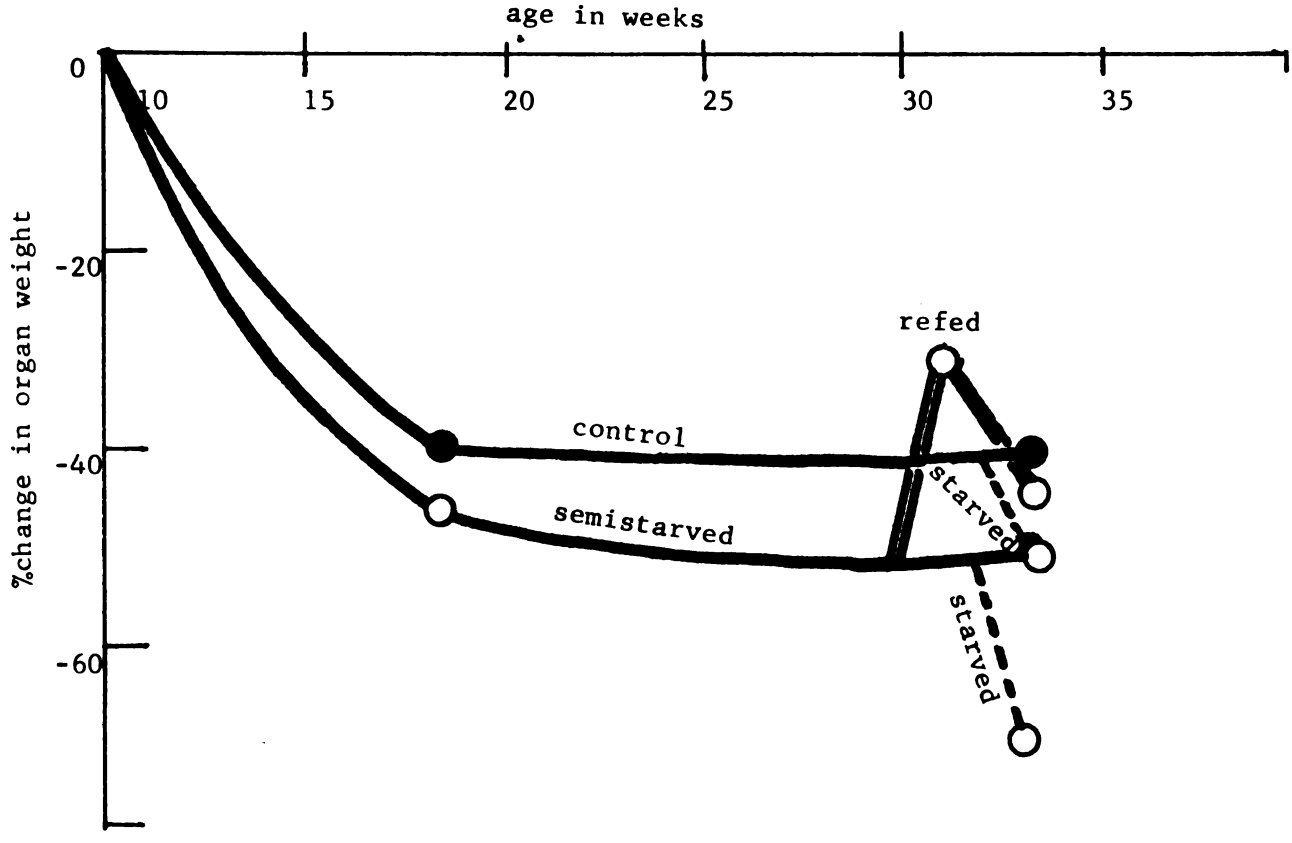
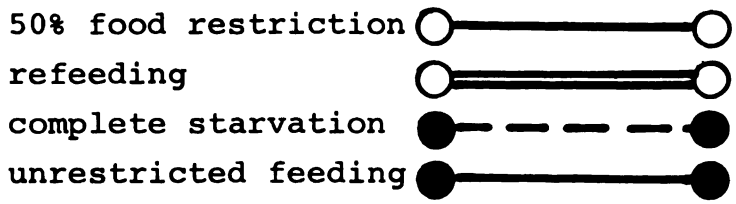


Figure 13.--Influence of semistarvation and refeeding upon spleen weights (% body weight) in rats.

Percent deviation from control values at 10 weeks of age during:



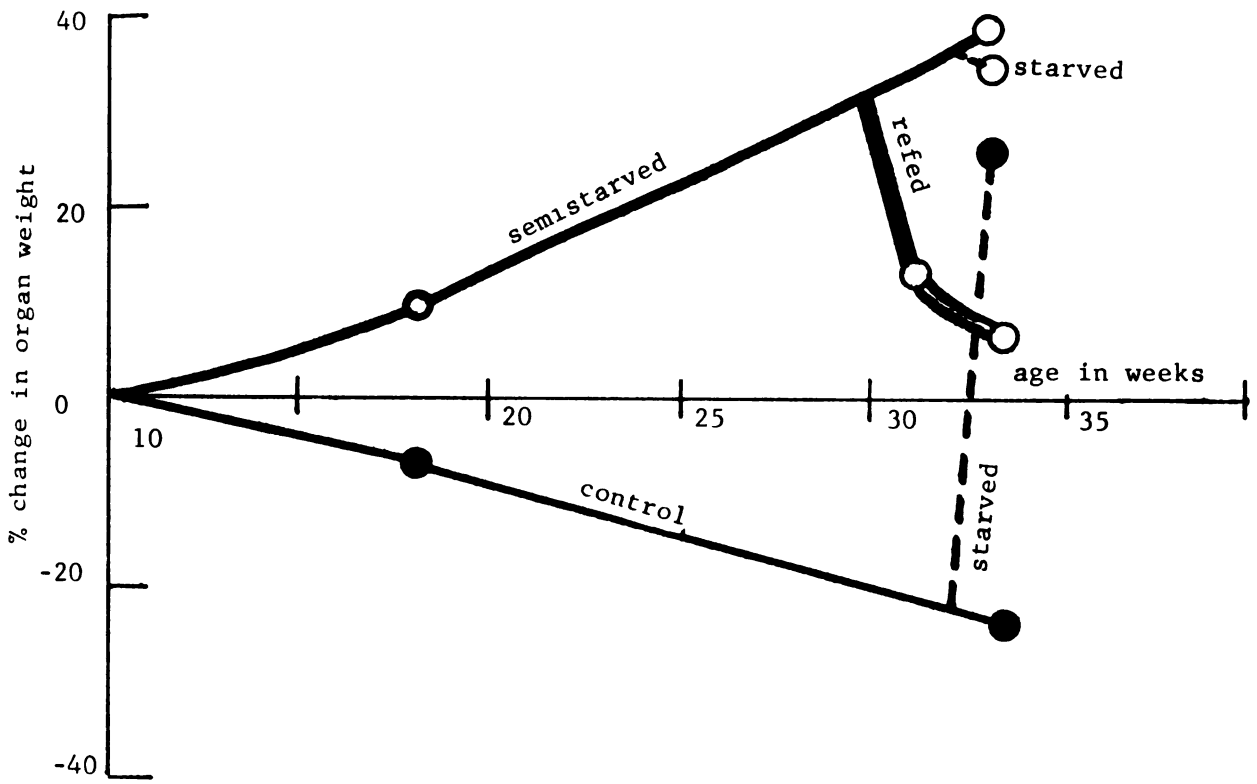


Figure 14.--Influence of semistarvation and refeeding upon gastrointestinal tract weights (% body weight) in rats.

Percent deviation from control values at 10 weeks of age during:

50% food restriction
refeeding
complete starvation
unrestricted feeding

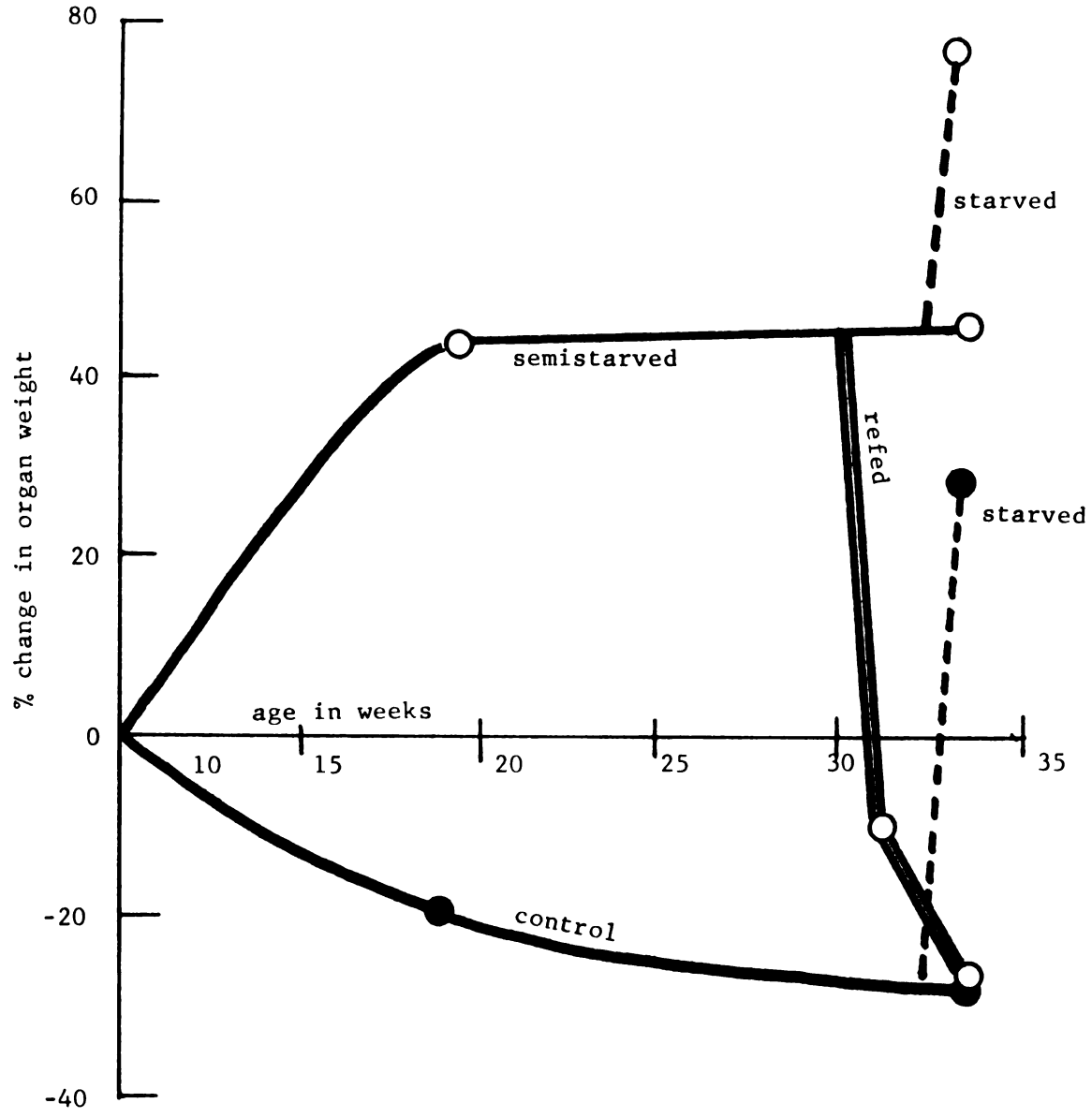
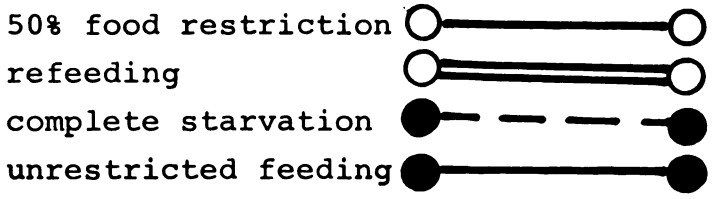


Figure 15.--Influence of semistarvation and refeeding upon adrenal weights (% body weight) in rats.

Percent deviation from control values at 10 weeks of age during:



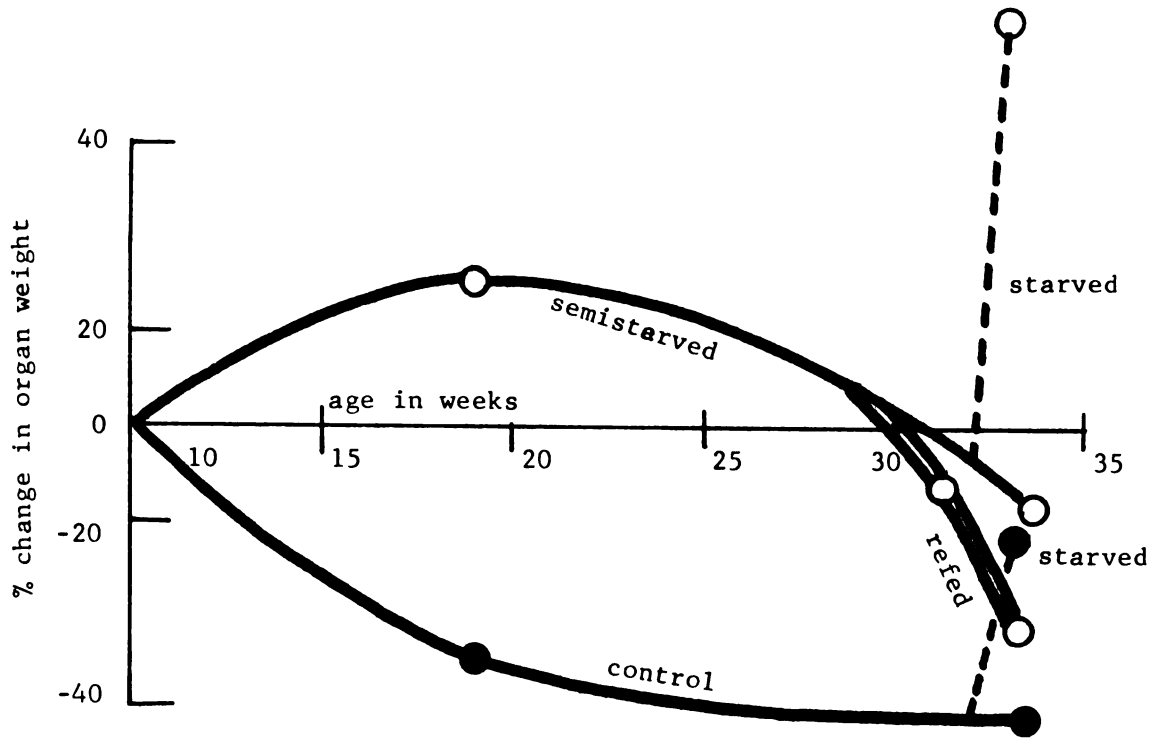


Figure 16.--Influence of semistarvation and refeeding upon anterior pituitary weights (% body weight) in rats.

Percent deviation from control values at 10 weeks of age during:

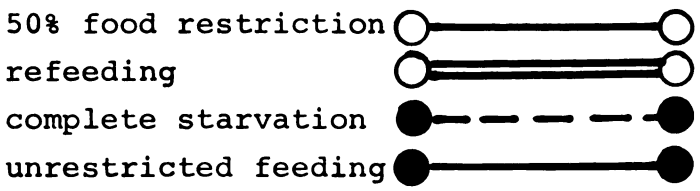


TABLE 4.--Ratio of rate of percent change (β) during initial and later stages of semistarvation.

Organs	Semistarvation Ratios
Body Weight	74.0
Kidneys	.40
Heart	.93
Adrenals	23.24
Epididymides	33.20
Testes	39.75
Liver	13.80
Spleen	13.60
G.I.T.	.67
Anterior Pituitary	1.24

TABLE 5.--Intergroup correlations of total organ weight and body weight means during starvation and refeeding in rats.

Organ	Correlation Coefficient (r)
Heart	.98*
Kidneys	.99*
G.I.T.	.83*
Liver	.92*
Spleen	.94*
Testes	.41
Epididymis	.91*
Adrenals	.42
Anterior Pituitary	.91*
Seminal Vesicles	.88*
Coagulating Glands	.97*

*Significantly different from zero ($P < .01$).

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PART III

DISTRIBUTION OF BODY FAT DURING STARVATION AND REFEEDING

DISTRIBUTION OF BODY FAT DURING STARVATION AND REFEEDING

ABSTRACT

At 10 weeks of age ad libitum fed male Sprague-Dawley rats were randomly divided into an unrestricted control and a 50% restricted group. Ten rats from each of the two groups were sacrificed after 8 weeks and again after 23 weeks of the semistarvation period. Six rats from each of the same two groups were deprived of all food at 32 weeks of age. Six rats were refed after 20 weeks of partial restriction for 6 days and 10 rats were refed for 21 days. At autopsy, fat depots from perirenal, mesenteric, testicular, epididymal, inguinal and interscapular areas were excised and weighed. The mesenteric, gonadal and subcutaneous fat depots were most markedly affected by semistarvation. Refeeding caused the greatest increase in mesenteric adipose tissue. When compared with totally deprived previously ad libitum fed rats, the 21-day refed rats of similar body weight and age had smaller gonadal fat depots but larger mesenteric fat depots. Rate of change in fat depot weights expressed as a percent of total body weight declined with time during semistarvation as well as during refeeding.

INTRODUCTION

Weight reduction can distort the normal pattern of fat distribution in the body. Schemmel (1969) found that while all adipose tissues were affected by food restriction, the subcutaneous, genital and the mesenteric and omental depots lost more weight than the perirenal and xiphoidal depots in Osborne-Mendel rats. The nature of the diet per se appears to be relatively ineffective in causing major changes in the regional distribution of fat (Pagè and Babineau, 1953); however, genetic and endocrine factors can influence relative depositions at inguinal and gonadal regions (Liebelt, 1959).

Trends in fat distribution during different levels of food restriction and refeeding have not been studied in male rats of the Sprague-Dawley strain.

METHODS AND MATERIALS

Experimental design is shown in Fig. 17. Ninety-five male Sprague-Dawley rats were allowed to feed ad libitum on a grain ration (Campbell et al., 1966) from weaning until 10 weeks of age. Thereafter 40 animals were allowed to continue unrestricted feeding while 50 rats were daily given half the food of the ad libitum fed animals. After 20 weeks of semistarvation, 16 rats were allowed free access to food. The experimental period continued until the animals were 33 weeks old. Six days prior to the

termination of the study, 6 unrestricted controls and 6 semistarved animals were deprived of all food. Water was allowed ad libitum in all groups. Throughout the study, all rats were housed in individual screen-bottomed cages in a room maintained at 25 to 27°C. Ten rats from the unrestricted controls and 10 from the 50% restricted group were sacrificed at 18 weeks and the same number at 33 weeks of age. A group of 6 animals were killed after 6 days of refeeding and 10 animals after 21 days of refeeding. Following inanition for 6 days, 6 rats each from the previously unrestricted and previously semistarved groups were sacrificed. Five animals at age 10 weeks were taken to obtain baseline values. All animals were killed by etherization and subsequent bleeding from the abdominal aorta. At necropsy, the inguinal, interscapular, genital, omental and mesenteric and the perirenal depots were removed from the carcass by the method of Schemmel and associates and weighed (1970). Correlation coefficients were calculated for fat depot weights and total body weight within each group and among various group means. Differences in group means were evaluated by applying the t-test.

RESULTS

Figs. 19, 20 and 21 give results of changes in the distribution pattern of fat (depot weights per 100 g. body weight) during semistarvation, refeeding and total

inanition. The genital, subcutaneous, mesenteric and omental depots lost most weight during caloric deprivation. Refeeding caused greater weight gains of depots in non-genital regions. Product-moment correlation coefficients of body weight and depot weights of individual animals within each group (Table 6) indicated that mesenteric and perirenal fat parallels body weight during the first 6 days of refeeding ($P < .05$). Intergroup correlations were uniformly high for all fat depots suggesting that changes in body weight over a range of 187 to 519 g. in rats due to varying food intakes were closely paralleled by changes in weights of adipose tissue. During the period of semi-starvation while mesenteric and omental fat decreased ($P < .05$) subcutaneous and epididymal adipose tissue remained unaffected. However, the testicular fat depot showed a significant ($P < .05$) increase during 15 weeks of semi-starvation despite constant body weight and food intake. When semistarved for 12 weeks and refed for 3 weeks or when previously ad libitum fed and then completely starved for 6 days at age 32 weeks, rats attained similar body weights. Despite the similarity in body weights, fat depot showed marked differences ($P < .005$) for the two sets of animals in mesenteric and omental and in genital areas.

DISCUSSION

Marked differences occur in the rate at which different fat depots change in weight when rats are starved or refed following a period of starvation (either acute, i.e., the complete absence of food, or semi-starvation, i.e., caloric intake inadequate for normal requirements). Furthermore, rats of the same body weight may have fat depots of markedly different weights. For instance, rats that are refed after prolonged periods of semistarvation have lighter genital fat depots but markedly heavier mesenteric depots than the well-fed rats which were subjected to acute starvation until they weighed the same as the refed animals.

When 50% of food restriction was imposed the mesenteric and omental fat showed a relatively gradual decline compared to other fat depots.

The present data indicates that subcutaneous, genital and mesenteric fat are more sensitive to caloric deprivation during semistarvation than the perirenal depot when compared with age matched controls. Results are in accord with Schemmel's work (1969) on weight reduction in high fat-fed Osborne-Mendel rats.

It appears that endocrine factors greatly influence accretion of fat tissue around the genital region in fully fed as well as 50% restricted rats. It is of interest that genital adipose tissue shows an increasing trend in weight during the 15 weeks of semistarvation reminiscent of the

increments in weights and secretory activities of accessory reproductive organs in these rats (unpublished data). Wertheimer and coworkers (1960) demonstrated that various individual fat depots are dissimilar in their response to hormonal stimulation. Relative depositions of fat at the gonadal regions in mice of some strains is known to vary with sex (Liebelt, 1959). Adipose tissue adhering to the testes show a marked proliferation at the time of puberty (Schemmel, 1969).

Interscapular adipose tissue is known to be involved in regulating body temperature (Tepperman and Tepperman, 1964). It is therefore not surprising that when complete starvation was superimposed on semistarvation, resulting in a disappearance of all visible fat tissue, the body temperature of the rats was markedly reduced ($P < .05$).

It is evident that lipogenic activity of adipose tissue is highly adaptive to fluctuations in caloric input. All fat depots show dramatic increases when refeeding follows semistarvation. Previous investigators have referred to these changes as "adaptive" phenomena (Tepperman and Tepperman, 1958; Jansen et al., 1966; Fabry, 1967; Leveille, 1969). The refeeding "overshoot" is reflected predominantly in the mesenteric and omental accretions of fat.

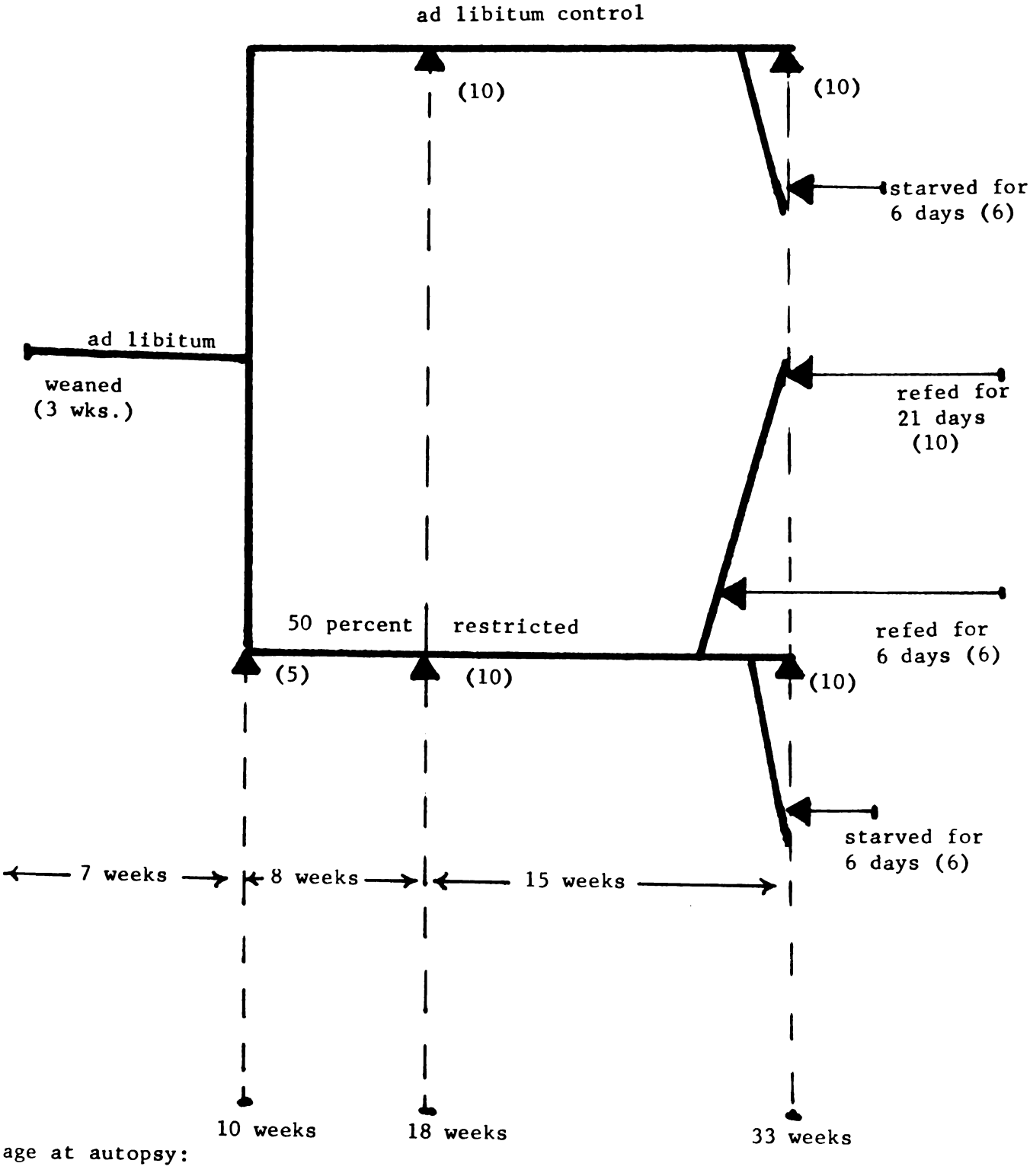

Figure 17.--Experimental design. [Number of animals per group are in parenthesis.]

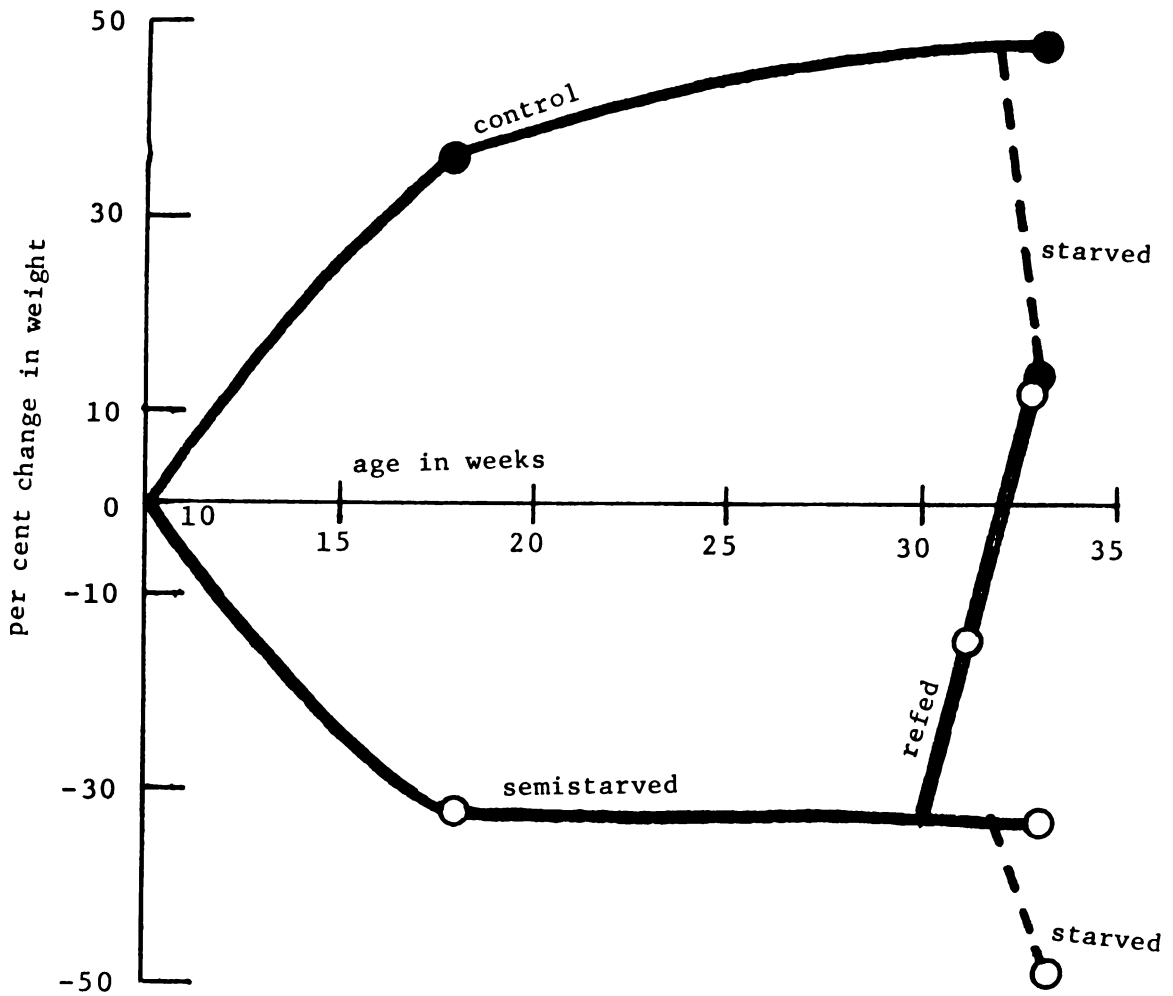

Figure 18.--Influence of semistarvation and refeeding upon body weight in rats.

Percent deviation from control values at 10 weeks of age during:

50% food restriction
refeeding
complete starvation
unrestricted feeding

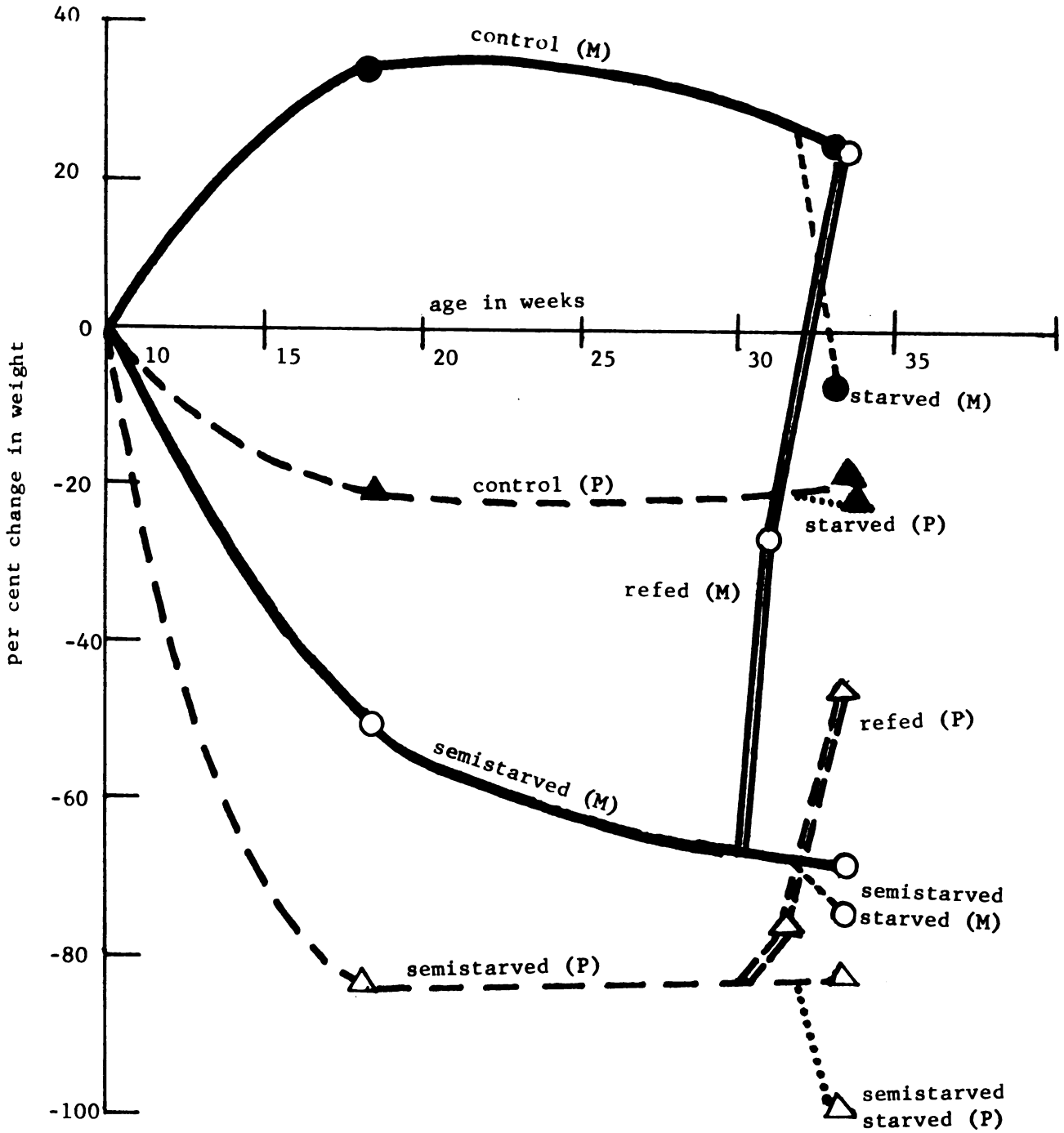

Figure 19.--Influence of semistarvation and refeeding upon mesenteric and omental (M) and perirenal (P) fat depot weights (% body weight) in rats.

Percent deviation from control values at 10 weeks of age during:

50% food restriction
refeeding
complete starvation
unrestricted feeding

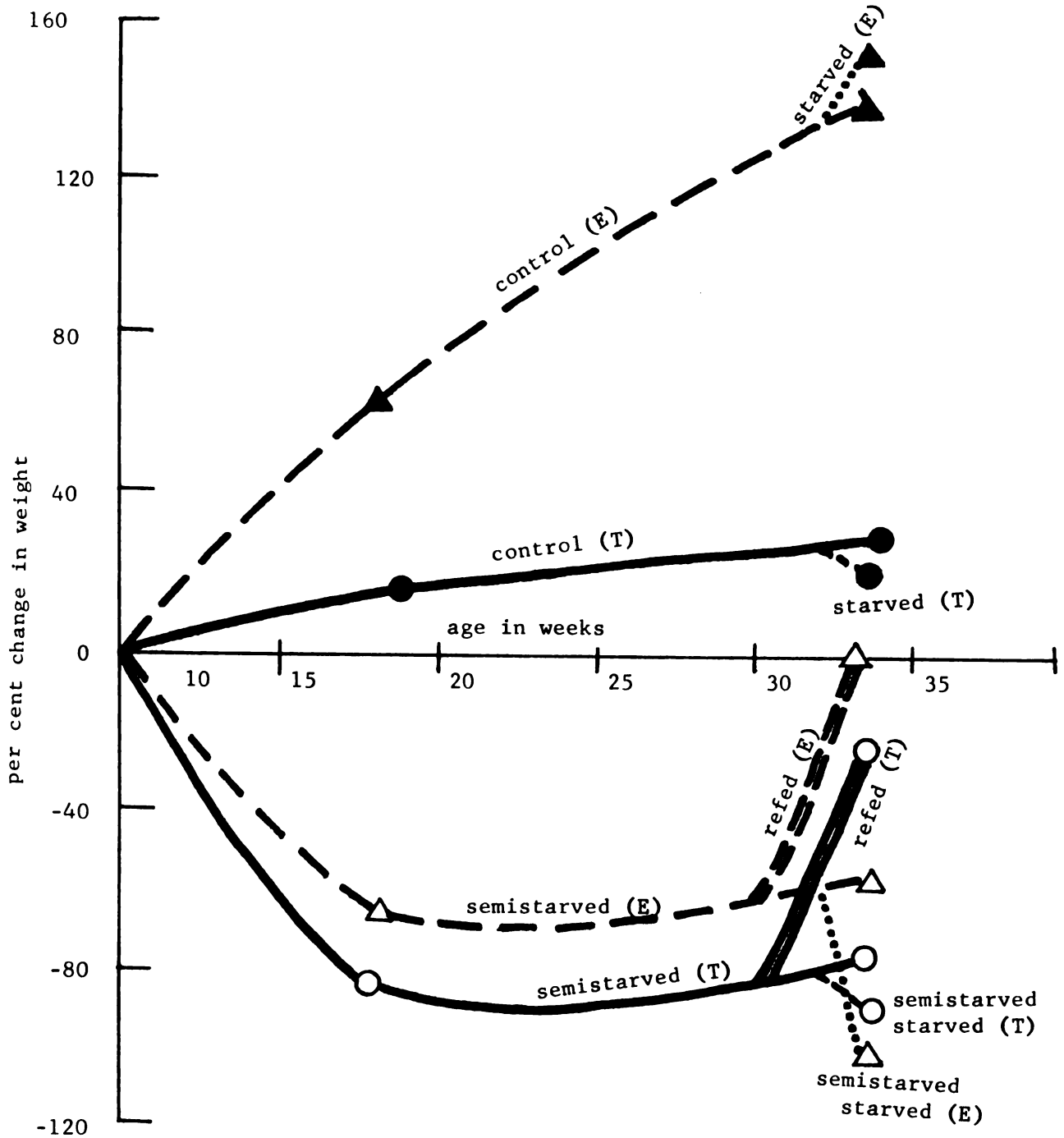

Figure 20.--Influence of semistarvation and refeeding upon testicular (T) and epididymal (E) fat depot weights (% body weight) in rats.

Percent deviation from control values at 10 weeks of age during:

50% food restriction
refeeding
complete starvation
unrestricted feeding

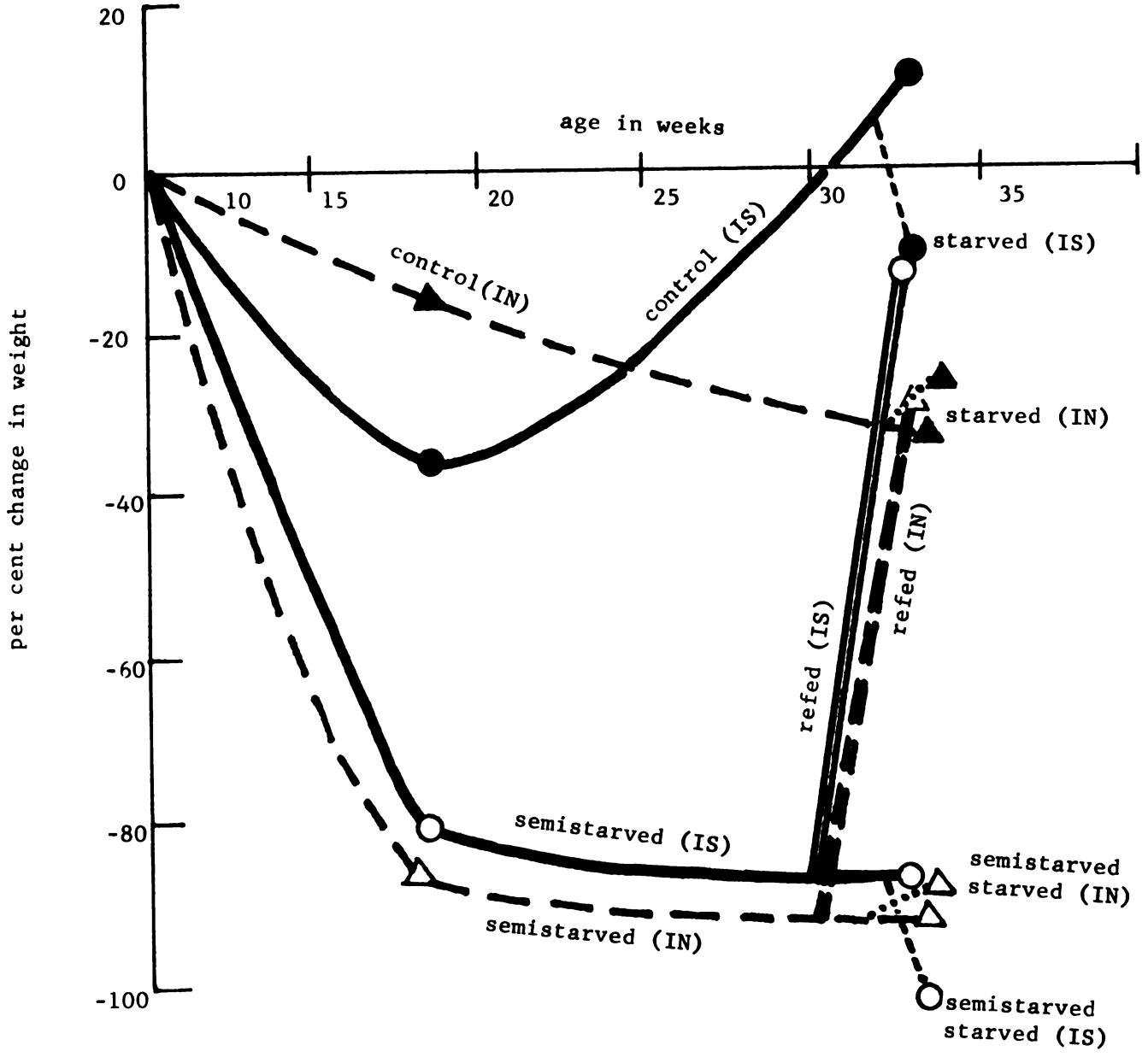

Figure 21.--Influence of semistarvation and refeeding upon inguinal (IN) and interscapular (IS) fat depot weights (% body weight) in rats.

Percent deviation from control values at 10 weeks of age during:

50% food restriction
refeeding
complete starvation
unrestricted feeding

TABLE 6.--Intergroup correlation coefficients for fat depot weights and total body weights during starvation and refeeding.

Fat Depots	Correlation
Mesenteric	.98*
Perirenal	.78
Testicular	.98
Epididymal	.90
Inguinal	.87
Interscapular	.87

*All values are significant at $P < .01$.

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PART IV

FOOD RESTRICTION AND RESISTANCE TO CHRONIC RESPIRATORY DISEASE

FOOD RESTRICTION AND RESISTANCE TO CHRONIC RESPIRATORY DISEASE

ABSTRACT

Male Sprague-Dawley rats allowed free access to food throughout life or refed after a 20-week period of semi-starvation were less able to prevent the age-associated progression of chronic respiratory disease than chronically semistarved animals. At 33 weeks of age, mortality and overt manifestations of the disease, as well as serum complement levels were markedly lower in the rats restricted to 50% of the ad libitum levels of food intake. When permitted ad libitum feeding following prolonged restriction, lesions of the disease which were latent in the region of the bronchus advanced to peripheral areas of the lung.

INTRODUCTION

It is widely acknowledged that infectious diseases often adversely affect malnourished animals through impaired host resistance (Hodges, 1964; Scrimshaw et al., 1969). There is considerable evidence that animals with lower body weights than "normal" controls not only live longer but are less subject to infection (McCay et al., 1939; Berg, 1960; Hill and Garren, 1961; Squibb, 1963).

Experimental work by McCay and associates (1939; 1943), Berg (1960), Newberne and Bresnahan (1968) and Ross et al. (1970) indicates that animals on a lower plane of nutrition live longer than those fed ad libitum. The prolongation of life appeared due to retardation of infectious and age-associated degenerative diseases. Recently, Berger (1969) reported that while respiratory infection caused only a single death among 180 chronically malnourished rats, 15 percent of 420 ad libitum fed animals succumbed to the disease.

Chronic respiratory disease or murine pneumonia is endemic in laboratory animals commonly used in nutritional studies. This report presents evidence suggesting that rats restricted to half the ad libitum food intake of normal controls appeared more resistant to progression of chronic respiratory disease.

METHODS

The experimental plan is shown in Fig. 22. Male weanling Sprague-Dawley rats were obtained from the Spartan Research Center, Williamston, Michigan. They were allowed to feed ad libitum on a grain ration (Campbell et al., 1966) until puberty at 10 weeks (Desjardins et al., 1968). Five animals were autopsied at this time to obtain organ weights. Mean daily intakes of unrestricted rats were calculated from weekly food consumption. Amount of ration spilled by the

animals was taken into account. Water was available to all animals throughout the study. They were weighed once a week.

The rats were individually caged in wire-screened cages. Temperature was kept constant at 25-27°C. throughout the year. Lighting was regulated to provide 12 hours of illumination and 12 hours of darkness each day.

The animals were randomly divided between the unrestricted control and the 50% restricted groups. Half of the ad libitum level of food was fed once a day between 7 and 8 p.m. to the restricted group.

Ten rats were sacrificed when the weight loss of the restricted group plateaued, after 8 weeks of semi-starvation. A group of 10 unrestricted controls was also sacrificed at this time. The remainder of the 20 rats in the unrestricted and 20 in the restricted group were maintained on their respective levels of food intake until 33 weeks of age, when 10 animals each from the unrestricted and 50% restricted groups were sacrificed. By this time several of the ad libitum fed rats were afflicted with chronic respiratory disease. Three weeks prior to the termination of the experiment (after 20 weeks of semi-starvation), 16 rats from the restricted group were permitted free access to the ration. Six of these rats were sacrificed after 6 days of refeeding and ten animals after 3 weeks of refeeding. To impose total starvation, a group

of 6 previously unrestricted rats was deprived of all food, 6 days prior to being killed. At the final autopsy, the freely fed, 50% restricted, refed and totally starved rats were all 33 weeks of age.

The rats were singly caged and arranged on racks so that both groups were equally exposed to nonspecific environmental pathogens. They were in sufficiently close proximity for cross infection to occur. At each weekly weighing, the presence of dyspnea, encrusted pigment around eyes and nose, discolored hair coat and weight loss were noted.

Autopsy

Animals were killed by etherization. Thoracic cavities were exposed, and the lungs examined. Observations on appearance of all lobes of the lungs and evidence of lesions were recorded. After fixation in 10% formalin, pieces of pulmonary tissue were embedded in paraffin, sectioned and stained with hematoxylin-eosin for microscopic study.

Serum Complement

Blood was drawn from the abdominal aorta at necropsy. Serum was stored at 4°C. The 50% hemolytic unit using sheep erythrocytes was determined spectrophotometrically (Mayer, 1961).

A chi-square test was used for estimating the difference in frequency of mortality and occurrence of symptoms between groups. For serum complement data, the student's t-test was employed.

RESULTS

Growth Curves

Unrestricted rats.--Up to 100 days, body weights increased rapidly. Subsequent increments were smaller, plateauing at a weight of 500 grams which was attained at 180 days. The downward trend in the curve beginning at 210 days reflects the increased severity of respiratory disease which caused several rats to lose 10 to 30% of their body weights.

Rats on 50% restriction.--The body weight gains was the same as that of the unrestricted group until 70 days of age since they both received restricted amounts of the same ration. At age 70 days, the rats in this group were restricted 50% of the feed intake of the unrestricted group. When that occurred, body weights declined rapidly from 343 g. until 100 days and thereafter gradually levelled off reaching a plateau of 235 g. at 150 days. Body weights were maintained between 230 and 240 grams until termination of the experiment at 231 days.

Refed rats.--When one group of the previously restricted rats were fed at libitum at 210 days (Fig. 23) they

rapidly increased in body weight. The slope of the body weight curve decreased slightly at 218 days and further at 225 days. Animals reached a final mean body weight of 403 grams, having gained an average of 8.0 grams per day for 21 days of refeeding.

Appearance and Activity of the Animals

Rats fed only 50% of the food eaten by the normal controls consumed their daily food within 30 minutes. At feeding time they became extremely active in anticipation of getting more ration. Especially during the initial period of semi-starvation, the rats appeared to be hypersensitive to movement and sound. Some exhibited aggressive behaviour. In general, environmental stimuli elicited fast responses; animals were able to maneouvre themselves quickly within their cages. Not only was energy intake restricted, but energy expenditure as evidenced by activity within the cages was much greater than in the ad libitum fed rats.

The coats of the control animals fed ad libitum became coarse and discolored. A dark red pigment was often deposited around the eyes and nose. As they grew older and heavier, the rats became passive, sleeping most of the time and were not affected by sound or movement in the vicinity, even at feeding time.

There was a marked transition in activity and appearance in the previously restricted-fed rats when they

were permitted to eat after 20 weeks of restriction. Within 2-3 days the animals became less active, more docile and unresponsive to stimuli. However, a high level of activity at feeding time was retained, the animals relapsing into a passive state after eating to satisfaction. The fur was smooth and clean, and body contours intermediate between those of small semi-starved animals and the larger well fed group.

Body Fat

At autopsy, there was little or no evidence of body fat in the restricted rats. The small accretions around gonadal and mesenteric regions were highly vascular. In contrast, the group permitted to eat ad libitum showed extensive accumulation of adipose tissue distributed in the perirenal, mesenteric and testicular regions within the abdominal cavity and subcutaneously in the interscapular and inguinal areas.

Six days of refeeding following 20 weeks of semi-starvation, increases the total amount of fat and in particular the mesenteric depositions. After 3 weeks of refeeding, marked increases in both visceral and subcutaneous adipose tissue occurred; the small vascular deposits being replaced by well-defined, white fat pads.

Lung Pathology

Three out of 10 lungs in the 18 week old well fed group had extensive lesions distributed over the surface of

the lobes. At 33 weeks, 7 out of 10 animals were similarly affected. Lesions were discrete, grey to red and indurated. In some cases, focal areas became nodular or elevated above the surface of the lobe. Cut surfaces revealed cystic areas filled with mucoid material distributed throughout the lung including bronchi and peripheral areas.

Macroscopic.--Smaller lungs of the restricted animals had pale, clear surfaces. The 10 restricted animals autopsied at 18 weeks of age were apparently free of disease. At the level of the mainstem bronchi, cut surfaces revealed the presence of typical lesions of murine pneumonia in all semi-starved rats at 33 weeks of age. However, only one case showed indurated foci on peripheral pulmonary surfaces. When refed the 3 weeks after restriction, 3 of the 10 animals sacrificed showed lesions identical to those of the affected ad libitum group. Rather than being confined to the area of the bronchus, they had advanced to the periphery of the pulmonary lobes.

Microscopic.--Although there was only slight mesenteric evidence of lung lesions in semi-starved rats, histopathological changes were manifested by the presence of occluding necrotic debris in the bronchus. The epithelial lining was damaged. The foci of inflammation contained mostly lymphocytes and also a few macrophages. Adjacent lung tissue was compressed and air spaces were reduced. Alveolar walls were thickened. Bronchioles contained some

exudate. In 3 animals allowed to feed freely for 3 weeks following restriction, major inflammatory reactions progressed from the mainstem bronchi to bronchioles and peripheral parts of the lobe. The lumens were filled with pus and the bronchioles were surrounded by cuffs of lymphocytic tissue. Atelectasis of lung tissue was extensive. Alveolar walls were thickened and alveoli were collapsed. A non-cellular basophilic staining exudate covered the lung surface. Similar changes were observed in animals fed freely from weaning.

Mortality

One rat out of a total of 90 died during the initial period of ad libitum feeding from 3 to 10 weeks of age. There were no subsequent deaths in the 35 semi-starved or 10 refed animals until the termination of the experiment at 33 weeks of age. For animals fed ad libitum throughout the experiment, cumulative mortality progressed from 1/30 at 20 weeks to 2/30 at 25 weeks, 3/30 at 30 weeks ($P < .10$) and 5/30 at 33 weeks ($P < .05$). Histological examinations confirmed murine pneumonia as the cause of death. One to 3 weeks prior to death all of these animals showed signs of torpor, dyspnea, sniffles, rough hair coat and weight loss.

Serum Complement Levels

Semi-starvation caused a significant reduction in complement activity ($P < .05$). Refeeding appeared to raise

the level of activity although not to a significant extent (Fig. 24).

DISCUSSION

This study indicates that the murine pneumonia so commonly seen in laboratory rats was more severe and mortality was higher in normal weight animals than in those that were underweight. These findings agree with the studies of McCay and associates (1943; 1945), Tannenbaum (1940), Berg (1960), and Ross et al. (1970) which indicated that normal body weight increases susceptibility to degenerative diseases and mortality.

Several reports suggest that healthy animals are more susceptible to experimental viral infections than are their malnourished counterparts (Sprunt, 1948; Squibb et al., 1965; Beveridge, 1967). The antagonistic effect of malnutrition on viral infection has been presumed to be due to a "starvation" at the cellular level with restriction of viral replication. Nelson's studies (1963) implicate a virus as the primary etiological agent in pulmonary lesions of murine pneumonia. Secondary invasion by bacteria cause necrosis of the lung tissue.

Chronic respiratory disease or murine pneumonia is endemic in laboratory rodents. Relative susceptibility to the proliferative form of the disease is species and strain dependent (Newberne et al., 1961). Sprague-Dawley rats are

highly prone to this infection. The present study clearly shows that food restriction causing a significant loss of body weight mostly in the form of fat, enables the animals to effectively retard the onset of the acute phase of respiratory infection.

In the natural state, hunger is a primary instinct in the rat. Under laboratory conditions of cage confinement and constant presence of food, the animals are unable to monitor energy balance and may become obese.

When allowed unlimited access to food, animals grow at a rapid rate and achieve high body weights due largely to the excessive deposition of fat (Mayer, 1968; Newberne, 1963; Berg, 1960). Benedict and coworkers (1932) had one rat that reached a weight of 820 grams when fed primarily a purified diet. An experimental model of obesity has been developed by allowing weanling animals to feed ad libitum on a diet of high caloric density (Mickelsen et al., 1955).

The resistance to the gross lesions associated with semi-starved animals was not permanent; when presented with an unlimited supply of food, the previously restricted animal rapidly developed gross lesions which resembled those in the well-fed controls. By 33 weeks of age, the infection was in the acute phase in the control animals. Elevated antigen levels and the presence of necrotic tissue at the foci of infection appeared to have increased serum complement activity. Although the functions of the

complement system are not well defined at present (Yachnin, 1966), it can be triggered by pathologic processes and may have an important role as a final common pathway of tissue injury. Weimer and coworkers (1963) found increased complement activity in rats semi-starved for 27 days. They did not study the incidence of infectious disease. It is possible that initially food restriction induces some host resistance factor which may be deficient in fully fed animals. Rapid proliferation of the pathogen then produces more severe diseases than those in the semi-starved animals. Kuna and coworkers (1951) found the phagocytosing ability of rats starved for 36 hours greater than in ad libitum fed controls.

McCay (1943) attributed the extended life span of rats which were severely restricted from weaning, to immaturity. In the present study, rats were allowed to grow maximally from weaning until puberty. Preliminary results indicate that reproductive organ function was markedly reduced as a result of food deprivation. That metabolic and endocrine factors may be responsible for the longer life spans of restricted rats, has been pointed out by Berg (1960). However, the precise relationship between storage of energy in the body, endocrine factors and immune response, is not known.

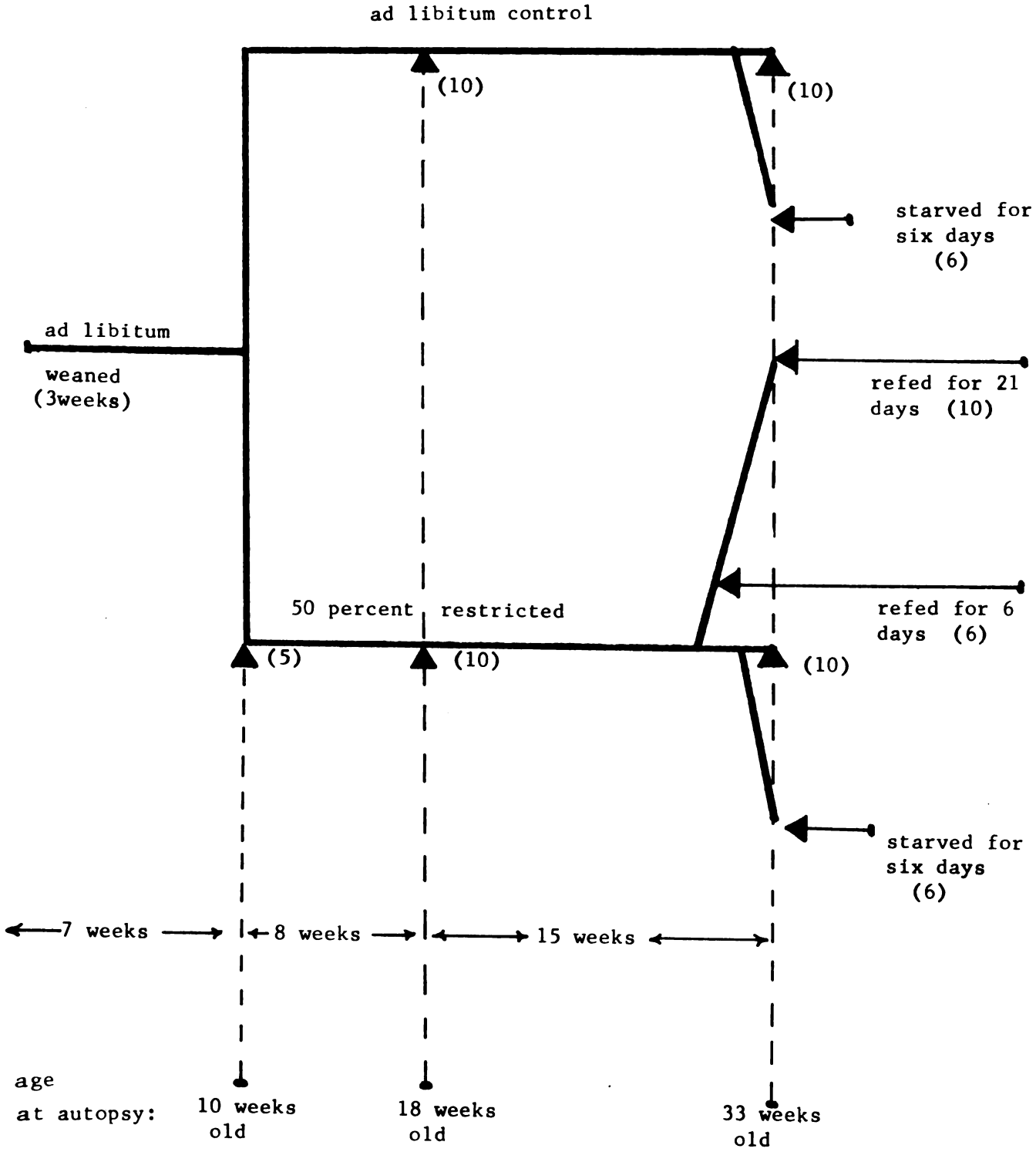


Figure 22.--Experimental design. [Number of animals per group are in parenthesis.]

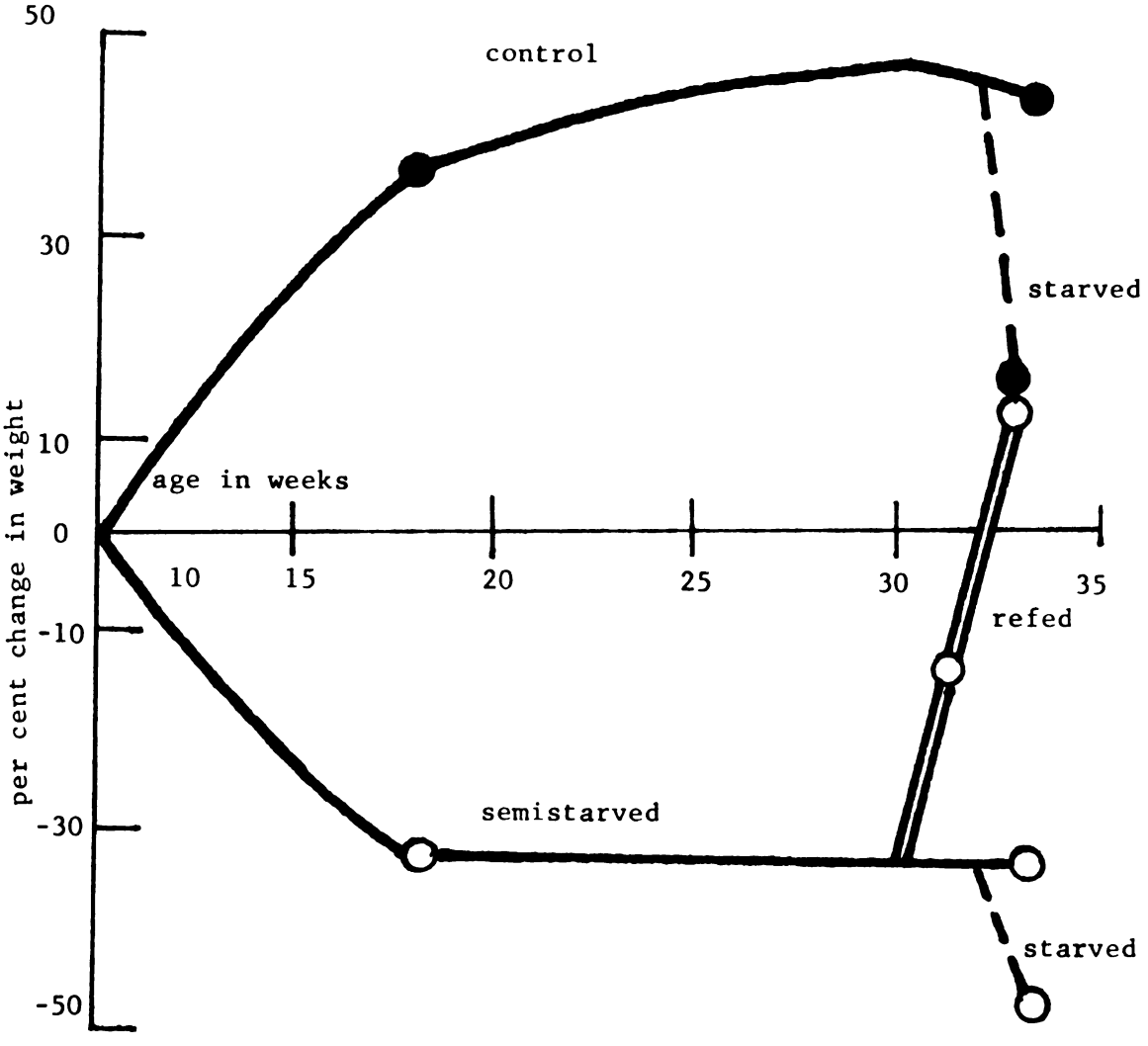
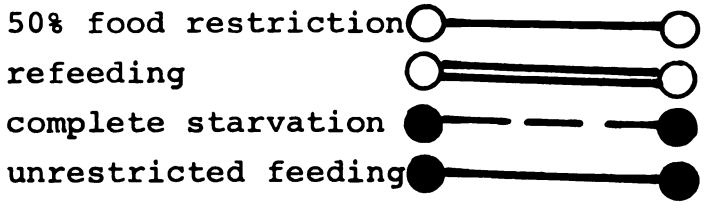


Figure 23.--Influence of semistarvation and refeeding upon total body weight in rats.

Percent deviation from control values at 10 weeks of age during:



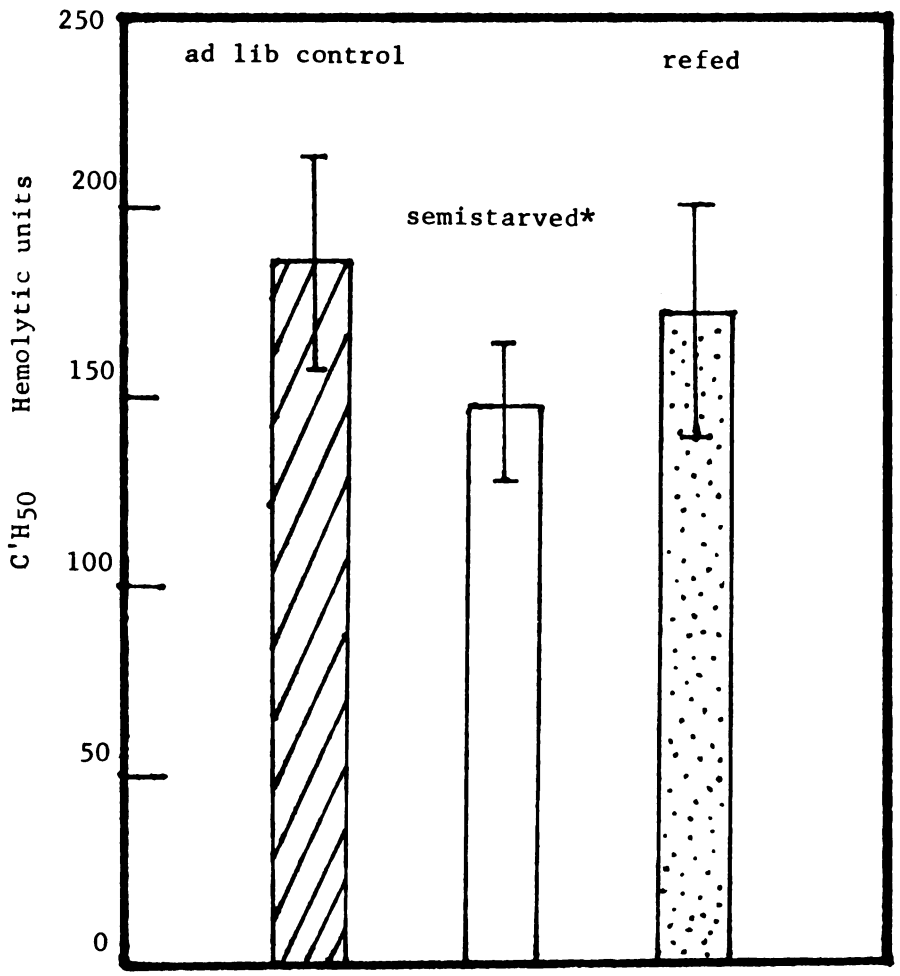


Figure 24.--Serum complement activity during semistarvation and refeeding in rats. [*Significantly different from ad libitum control values P < .05.]

TABLE 7.--Occurrence of pulmonary lesions of murine pneumonia in unrestricted, semi-starved, and refed rats as evidenced by necropsy findings.

Groups	18 Weeks of Age		33 Weeks of Age	
Control	3/10[a]	P < .10[b]	7/10	P < .005
Semi-starved	0/10		1/10	P > .10
Refed	-		3/10	

[a]Number of animals per group showing dark red inflamed regions or discrete, indurated foci on peripheral surfaces of the lung.

[b]Significance of difference in frequency of occurrence of the lesions as estimated by chi-square test.

TABLE 8.--Incidence of chronic respiratory disease in freely fed and 50% restricted rats as evidenced by clinical symptoms.

Age Weeks	Symptoms[a]			Mortality[c]		
	Control	Semi-Starved		Control	Semi-Starved	
10	0/90			1/90		
15	2/40	0/49				
20	1/30	0/39				
25	3/30	0/39	$P < .10$[b]	2/30	0/39	
30	5/30	0/39	$P < .05$	3/30	0/39	$P < .10$
33	6/30	0/39	$P < .01$	5/30	0/39	$P < .05$

[a]Cumulative number of animals per group showing dyspnea, torpor, periocular and perinasal encrustations, sniffles, discolored hair coat, and weight loss.

[b]Significance of difference in frequency of occurrence as estimated by chi-square test.

[c]Cumulative frequency of deaths occurring within each group since the previous autopsy.

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SUMMARY AND CONCLUSIONS

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The effects of semistarvation, semistarvation followed by refeeding and total deprivation of food on testicular function, organ weights, distribution of body fat and incidence of disease were studied in male Sprague-Dawley rats. Weanling rats were allowed to feed ad libitum on a grain ration from 3 to 10 weeks of age when they were randomly divided between an unrestricted control and a 50% restricted group. Semistarvation was continued for a period of 23 weeks. At this time several ad libitum fed rats showed signs of chronic respiratory disease; the animals were 33 weeks old.

Five ad libitum fed rats were sacrificed at 10 weeks of age. When weight loss plateaued in the restricted animals, 10 animals from the unrestricted control and 10 from the 50% restricted groups were killed (18 weeks of age). Following another 15-week period of semistarvation, 10 animals from the restricted and 10 from the freely fed control group were sacrificed (age 33 weeks). Six rats each from the unrestricted and the 50% restricted groups were deprived of all food at 32 weeks of age for 6 days and killed at 33 weeks of age. To study the influence of unrestricted food

intake upon previously semistarved animals, 16 rats from the 50% restricted group (30 weeks old, semistarved for 20 weeks) were allowed free access to the ration. Six of these rats were sacrificed after 6 days (age 31 weeks) of refeeding and the remaining 10 were killed after another 15 days of refeeding (33 weeks of age).

Reproductive function was determined from the number of testicular and epididymal sperm, and levels of testosterone produced. There was a reduction in the total weights of testes and epididymides, and total number of sperm in these organs during semistarvation. However, when expressed as a percentage of body weight, testicular and epididymal weights were higher in the restricted than in freely fed controls. Concentration of sperm per gram of testicular and epididymal tissue were similar in control and restricted rats.

Following 8 weeks of food restriction, serum testosterone was markedly lower in semistarved rats (1.22 ng./ml.) than in ad libitum fed controls (3.90 ng./ml.). At 33 weeks of age, when the animals had been semistarved for 23 weeks, the difference in serum testosterone levels between control and restricted groups was much less (1.48 ng./ml. in restricted vs. 1.23 ng./ml. in restricted rats). Weights of seminal vesicles as percent of body weight and their content of citric acid as well as weight of coagulating glands (as a percent of body weight) and their content of

fructose were markedly lower in the semistarved rats than controls at both 18 and 33 weeks of age. The restricted rats appeared to be successfully adapting to chronic undernutrition since weights of accessory reproductive organs and their secretory products were significantly higher at 23 weeks of semistarvation compared to the values at 8 weeks of semistarvation. When the animals were permitted free access to the ration after being restricted for 20 weeks serum testosterone levels reached 3.01 ng./ml. as compared with 1.48 ng./ml. for ad libitum fed controls and 1.23 ng./ml. for 50% restricted animals at the same age (33 weeks).

At the end of 3 weeks of refeeding, accessory organ weights and their secretory activity was similar to that of unrestricted controls.

Weights of testes and epididymides did not change despite marked reduction in body weight due to complete starvation for 6 days. Concentration of sperm per gram of tissue was similar to pre-inanition values. Serum testosterone decreased to undetectable levels. Testicular and epididymal weights increased less rapidly than total body weight. Number of sperm in these organs were unaffected. Citric acid and fructose in seminal vesicles and coagulating glands respectively, decreased.

At 33 weeks of age the mortality from and signs of chronic respiratory disease (CRD) were markedly greater

among the rats permitted free access to food, than in animals fed only half the ad libitum ration. Observations at autopsy showed that peripheral surfaces of the lungs had lesions of CRD in freely fed rats while these areas were clear in lungs of semistarved rats. However, histological studies revealed lesions at areas adjacent to the bronchus, even in semistarved animals. When these rats were refed for 3 weeks, the lesions appeared to have progressed to the peripheral surfaces of the lungs. Elevated serum complement levels showed that the ad libitum fed and refed rats were more severely infected than the restricted rats.

Weights of heart, kidneys, liver, spleen, gastrointestinal tract, adrenals and anterior pituitary (g. per 100 g. body weight) decreased in ad libitum fed rats as their body weights increased with age. On a percent of body weight basis, heart and kidneys were most resistant to starvation and refeeding, over a body weight range of 187 to 520 grams. The liver and spleen were most severely affected by food restriction. Gastrointestinal tract weights increased during semistarvation relative to body weight. Adrenal glands showed stress hypertrophy due to restricted food intakes. This was more marked in the totally deprived than in the 50% restricted animal. The adrenal weights sharply decreased when restricted rats were allowed free access to food.

Fluctuations in organ weights tended to plateau with time when rats were partially starved for 23 weeks.

Weights of fat depots from 6 different regions of the body showed changes in distribution of adipose tissue during semistarvation, refeeding and total inanition. Weights of subcutaneous, genital and mesenteric fat depots decreased most rapidly during food restriction. While fat depots increased rapidly when the restricted animals were permitted ad libitum food intakes, adipose tissue proliferation at the mesenteric region was most pronounced. Total body weights were similar of rats totally starved for 6 days after 32 weeks of ad libitum feeding, and of rats refed for 3 weeks following 20 weeks of semistarvation. However, in the refed rats mesenteric fat depots were heavier and genital fat depots lighter than in the completely starved rats.

From the evidence presented it can be inferred that when semistarvation is continued over prolonged periods of time, animals show changes in reproductive parameters, weights of organs, fat depot weights and resistance to disease:

1. Ability of testes to produce sperm is more resistant to food restriction than testosterone production.
2. Heart and kidney weights closely parallel body weight during food restriction and refeeding

while liver and spleen weights decrease more rapidly than body weight during starvation.

3. Subcutaneous, genital and mesenteric fat depots are more sensitive to caloric deprivation than the perirenal fat depot and refeeding causes greatest adipose tissue proliferation in the mesenteric region.
4. Animals in the freely fed state are more susceptible to the progress of latent chronic respiratory disease with time, than the partially starved animals.

APPENDIX

TABLE A-1.--Normal adult levels of testosterone and androstenedione from plasma and testicles of different species as estimated by gas liquid chromatography.

Androgen	Species	Plasma	Testis	Reference
		(ng/ml)	(µg/g)	
Testosterone	Rat	41.00	19.50	Bardin (1969)
	Rat	50.40	--	Bardin (1969)
	Rat	1.14	0.11	Resko et al. (1968)
	Rat	5.04	--	Frick et al. (1969)
	Rabbit	3.76	--	Haltmeyer et al. (1969)
	Rabbit	2.10±0.40	--	Seiki et al. (1968)
	Ram	--	17.24±7.57	Skinner et al. (1968)
Androstenedione	Rat	9.20	0.02	Resko et al. (1968)
	Ram	--	1.24±0.62	Skinner et al. (1968)

TABLE A-2.--Variation of plasma and testicular testosterone and androstenedione with age in the rat.[a]

Age (days)	Testosterone		Androstenedione	
	Plasma (ng/ml)	Testicle (µg/g)	Plasma (ng/ml)	Testicle (µg/g)
1	0.27[b]	0.194	ND[de]	0.096
5	0.21	0.134	ND[d]	0.043
10	0.09	0.122	ND	ND
15	0.10	Trace[c]	ND	ND
30	0.15	Trace	0.13	0.008
40	0.64	0.006	0.18	0.004
60	1.10	0.043	0.15	0.003
90	2.04	0.111	0.12	0.015
120	1.14	--	0.57	--[e]

[a]Rawlings (1970).

[b]Resko et al. (1968).

[c]Response not great enough to quantify.

[d]None detected.

[e]Sample lost.

TABLE A-3.--Body weight and total organ weights during starvation and refeeding in rats.

	Body Wt. (g.)	Heart (g.)	Kidneys (g.)	G.I. Tract (g.)	Liver (g.)	Spleen (g.)	Testis (g.)	Epididymis (mg.)	Adrenals (mg.)	Anterior Pituitary (mg.)
C-18 (10)	474.8 ±19.43	1.28 ±.08	3.05 ±.19	11.57 ±.78	15.52 ±1.81	.94 ±.14	1.88 ±.07	.65 ±.06	56.89 ±7.43	10.20 ±1.13
SS 18 (10)	231.3 ±9.75	.80 ±.09	1.74 ±.09	6.68 ±.69	6.71 ±1.00	.41 ±.09	1.41 ±.09	.42 ±.05	48.70 ±8.60	7.4 ±1.26
C 33 (10)	519.2 ±26.57	1.37 ±.01	3.03 ±.18	10.37 ±.59	16.03 ±1.86	1.01 ±.24	1.66 ±.17	.57 ±.01	50.50 ±5.27	9.9 ±1.59
S 33 (10)	234.4 ±5.73	0.80 ±.01	1.67 ±.04	7.25 ±.31	6.30 ±.16	.37 ±.02	1.40 ±.12	.43 ±.03	50.50 ±6.85	6.0 ±1.15
SS-R 6 (6)	291.66 ±29.80	0.92 ±.01	2.03 ±.11	8.53 ±.57	11.13 ±1.30	.65 ±.06	1.50 ±.08	.51 ±.09	39.00 ±2.36	7.83 ±.25
SS-R 21 (10)	402.2 ±17.86	1.24 ±.02	2.56 ±.08	11.22 ±.50	14.04 ±.86	.75 ±.06	1.73 ±.09	.56 ±.02	44.50 ±7.24	8.9 ±1.44
C-S (6)	403.5 ±35.18	1.26 ±.07	2.59 ±.13	13.35 ±1.24	9.08 ±.66	.66 ±.08	1.60 ±.21	.53 ±.06	76.33 ±16.84	9.67 ±.51
SS-S (6)	183.58 ±6.26	0.58 ±.01	1.44 ±.05	6.49 ±.40	2.72 ±.06	.19 ±.02	1.32 ±.10	.33 ±.08	47.67 ±8.95	7.33 ±.75

TABLE A-4.--Absolute weights of adipose tissue during starvation and refeeding in rats.

Groups Age No. Animals	Control 10 wks. (5)	Control 18 wks. (10)	Semi-starved 18 wks. (10)	Control 33 wks. (10)	Semi-starved 33 wks. (10)	Semi-starved Refed for 21 Days (10)	Semi-starved Refed for 6 Days (6)	Control Starved for 6 Days (6)	Semi-starved for 6 Days (6)
Mesenteric (g.)	4.57 ±.23	8.29 ±1.35	1.50 ±.24	8.45 ±1.66	.96 ±.08	6.48 ±.55	3.03 ±.15	.503 ±.10	.65 ±.09
Perirenal (g.)	3.24 ±.13	3.49 ± .58	0.05 0	3.84 ± .85	.05 0	1.91 ±.07	.61 ±.13	2.95 ±.51	0 0
Testicular (g.)	3.02 ±.19	4.40 ± .55	.30 ±.14	5.64 ±1.34	.46 ±.05	2.33 ±.16	.87 ±.19	4.29 ±.10	.20 ±.18
Epididymal (g.)	0.07 0	.16 ±.04	.01 ±.07	.26 ± .02	.02 ±.01	.08 ±.02	.03 ±.01	.21 ±.00	0 0
Inguinal (g.)	4.37 ±.53	5.01 ±.77	.38 ±.14	4.33 ± .47	.36 ±.09	3.47 ±.60	.79 ±.02	3.54 ±.21	0 0
Interscapular (g.)	2.61 ±.68	2.26 ±.85	0 0	4.30 ±..82	0 0	2.59 ±.72	.79 ±.04	2.71	0