THE EFFECT OF A HIGH FAT RATION ON BODY WEIGHT, BODY COMPOSITION AND ADIPOSE TISSUE WEIGHTS OF RATS AS INFLUENCED BY AGE, STRAIN AND WEIGHT REDUCTION OF OBESE RATS

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Rachel Schemmel

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ABSTRACT

THE EFFECT OF A HIGH FAT RATION ON BODY WEIGHT, BODY COMPOSITION AND ADIPOSE TISSUE WEIGHTS OF RATS AS INFLUENCED BY AGE, STRAIN AND WEIGHT REDUCTION OF OBESE RATS

by Rachel Schemmel

Rats fed a high fat ration throughout their lives became extremely obese. Up to 150 days, they had similar amounts of protein, moisture and ash in their bodies as the grain-fed "lean" controls of the same ages. At all ages,the increased weight of the high fat-fed rats consisted primarily of fat. However, adult obese rats had approximately a 10% increase in body protein, assumed to be associated largely with proliferation of adipose tissue.

For all ages, adipose tissues of the obese rats were larger than those in the lean controls. In young rats, the subcutaneous adipose tissue was larger than the abdominal adipose tissue. Except for the interscapular depot subcutaneous tissue weights of male rats weighing over 450 g paralleled body weight gains. Perirenal adipose tissue weights and that of the depot surrounding the xiphoid process continued to increase more rapidly with age than body weight. Genital depots showed maximal relative weight increases at the time of sexual development and then the rate of increase declined. The mesenteric and omental depots in the young rats increased more than body weight but this was reversed in older rats.

The high fat ration produced increased body weight gain, percent of body fat and adipose tissue weights in the six strains of rats to

which it was fed. The strains were: Osborne Mendel, Sprague Dawley, Hoppert, Wistar-Lewis, Hooded and MSU Gray rats. Osborne Mendel rats showed a greater propensity to weight gain and deposition of body fat than any of the other strains tested. This was especially true between 13 and 23 weeks of age.

The rats of all strains were more efficient in converting feed energy to body tissue when fed the high fat ration than when fed the grain ration. For the first 10 weeks following weaning, males fed high fat had a 22 to 32% feed efficiency whereas grain-fed males had a 11 to 14% feed efficiency. High fat-fed females had a feed efficiency of 14 to 23% whereas for grain-fed females it was 8 to 12%.

Obese Osborne Mendel rats (males = 1000 g, females = 650 g) were reduced by being fed (1) the high fat ration ad libitum on 2 of 7 days, (2) the high fat ration in daily amounts which in 7 days equalled the weekly intake of (1), (3) the grain ration ad libitum and (4) a semipurified diet approximating (3) in composition. During the first week of the reducing regimen, the rats in all groups and of both sexes lost 7% of their initial body weight. After another 24 weeks, the rats were sacrificed. By then the rats in (1) and (2) lost 40 to 49% of their body weights; those in (3) 30 to 35% and those in (4) 20 to 25%.

Nearly all the weight lost by the rats fed the grain or semipurified rations was fat. A small percentage of body protein was lost besides fat by the rats reduced on the high fat ration.

All adipose tissues decreased in weight during weight reduction. Those showing the largest losses of weight were the subcutaneous, mesenteric and genital depots. (These latter 2 had shown some decrease in relative weights with aging). The perirenal depots and that depot surrounding the xiphoid process showed a lesser decrease in weight with weight reduction.

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In memory of my Father

Mr. Frederic A. Schemmel

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INTRODUCTION

Obesity is invariably more common in countries with continued prosperity and greater mechanization. Accompanying this trend is an increased number of deaths from degenerative diseases to which obesity appears to play a contributory role (Body Build and Blood Pressure Study, 1959; Dublin, Latka and Spiegelman, 1949; Dublin and Marks, 1953). In these studies obesity was judged exclusively on the basis of body weight.

Behnke et al. (1942) and Brožek (1952) suggest that a more reasonable criterion for obesity is based on the percent of body fat rather than on weight alone. The importance of a more specific measure of obesity is stressed in a review by Keys and Brožek (1953). There are numerous methods for measuring of body fat content; these include densitometry, hydrometry, roentgenography, anthropometry, whole body counting for potassium, helium dilution, creatinine excretion (Brožek, 1963) and ultrasonic reflections (Bullen et al., 1965). All have proven useful for determination of body fat or lean body mass, and ultimately, obesity.

A caloric intake beyond expenditure results in weight gain. However, many individuals, men as well as animals, without thought or concern are able to balance caloric intake and expenditure for long periods of time. For others, positive caloric balance is readily maintained. This condition appears to be the result of a number of disturbances, (Anand, 1960; Bruch, 1958; Chirico and Stunkard, 1960; Mayer, 1953; 1961; Young, 1964) thereby causing a complexity of obesities which may behave physiologically and metabolically quite

differently and be further complicated by psychological and emotional factors. The solution of the problems in any one of these types of obesities will greatly contribute to the overall elucidation of obesity.

An ultimate goal is a thorough understanding of the involvement of food in producing a healthy and attractive individual with a long life span. At the present time, social acceptability and life insurance statistics indicate the negative role played by obesity. Due to the long life span of man, his inadaptability to the rigors of strict experimentation and the possibility of adverse effects associated with long-continued studies makes it imperative to find a substitute test animal. The laboratory rat has been chosen for many studies of different facets of the obesity problem. The insufficient criterion of weight alone in man gives way more readily in the rat to more highly controlled food efficiency data, deposition of body fat and chemical analysis for percent of body fat, so that hereditary and environmental factors contributing to obesity can be more clearly evaluated. Likewise, cheating or false insinuations of cheating, are eliminated in weight reduction studies which thereby permit a more thorough and unbiased study of the value of weight reduction than can be done in man. Data on stabilization at the reduced weight are needed since so few men or women who have been reduced maintain their reduced weights.

Since overeating most frequently represents the primary physiological factor involved in human obesity, rats made obese by concentrated sources of calories (Mickelsen et al., 1955) should make a unique contribution to the further understanding of the obesities.

REVIEW OF LITERATURE

FEED EFFICIENCY--ITS RELATION TO OBESITY

Interrelationships of dietary components

The composition and nature of the ration may have a marked influence on the rate of body weight gain of experimental animals. Unfortunately, these studies have involved so many dietary variables (e.g. proportions of cellulose, or fiber, type of carbohydrate, type and amount of fat, etc.) that it is impossible to draw definite conclusions about what the ideal ration ought to be to support greatest weight gain. One observation does stand out, and that is: that many nutritionally adequate semipurified rations fed to rats give better weight gains than do stock or chow diets (Mickelsen et al., 1955; Reed et al., 1930; Barboriak et al., 1958).

Di Giorgio et al. (1962) reported that lipogenic activity of epididymal adipose tissue is less in rats fed stock diets than in animals fed any of the purified diets with which they worked. These rations contained either safflower, corn,olive, hydrogenated cottonseed, butter or cocoanut oil with either glucose or starch as the carbohydrate.

Hajdu (1942) reported that an equal proportion of fat and carbohydrate produced better growth and work performance in rats than did either a high or low intake of carbohydrate or fat. On the other hand, Hoagland and Snider (1940) maintained that when the basis of measurement was efficiency of utilization expressed as gain in weight per 100 calories of food consumed, rats fed the low fat diets were more efficient since the greater growth on the higher fat diets required a greater consumption of food. Data from both Forbes et al. (1946a, 1946b) and Deuel et al. (1947) indicated that diets containing 30 to 60% fat

caused greater gains in body weight, not only because of increased feed consumption, but also because of increased utilization of calories for weight gain as determined by calories wasted for heat production. The least efficient combination of food stuff as indicated by maximum heat increments was a diet containing no fat which was composed of cellulose and beef protein (Forbes and Swift, 1944). All seem to be in agreement, however, that rats consuming a 40 to 60% hydrogenated animal or vegetable fat will grow more rapidly than controls consuming a diet lower in fat content.

Glucose produced greater weight gains in rats when substituted for starch in a semi-purified ration where the percentage of protein in the diet was just adequate for growth (Howe and Gilfillan, 1963). Similarly, weight gains were not as great in baby pigs when the dietary carbohydrate was corn starch or dextrin in comparison to glucose (Sewell and Maxwell, 1966). The greater growth of the glucose-fed pigs was associated with a greater feed consumption rather than any difference in feed efficiency.

Method of feeding

Rats either trained (Hollifield and Parson 1962a; b) or forced to consume a daily portion of food at two feedings (Cohn and Joseph, 1959; Cohn, 1963) gained the same amount of body weight but deposited a larger percentage of it as fat if force-fed. If the rats were fed a high fat ration, this was even more pronounced (Cohn et al., 1965).

After a week of adaptation to a meal eating pattern, Leveille and Hanson (1965) reported that "meal eating" rats had a greater food efficiency. Actually, the meal eaters never ate as much food in a day as the nibbler rats, but their gain in weight was greater.

Increased lipogenesis (Tepperman et al., Dickerson et al., 1943)

in the meal eaters has been considered a contributory factor to the increased percent of body fat. More specifically, Tepperman and Tepperman (1958) reported that lipogenesis from acetate and glucose was greater in liver slices from rats trained to eat their food in a short time, and Hollifield and Parson (1962a) observed increased incorporation of acetate into fatty acids in adipose tissue of meal eating rats.

Breeding

Slight individual differences within the same species and even the same strain allow for some differences in metabolism and utilization of feeds. By selective inbreeding of a male and female of greatest food efficiency and also inbreeding of the least efficient, it is possible to produce strains which show high or low feed efficiency.

Palmer and Kennedy (1931) reported that rats of their stock colony, though inbred for several years, still maintained variations in food efficiency. By the fifth generation of selective breeding, they separated two strains with a difference in feed efficiency of 40% (Calverley et al., 1946). The difference in feed efficiency was also associated with a difference in body composition. Even at weaning time, when the rats of both strains weighed 60 g, the animals of the more efficient strain had a higher percentage of body fat and a smaller percentage of protein. Six weeks later both males and females of the high efficiency strain weighed around 50 g more than the low efficiency strain for both males and females and had 3.0 to 5.0% more body fat (ibid). In this study, and the one reported by Morris et al., (1933) the females were less efficient in food utilization than the males.

Zucker (1960) likewise, after selective inbreeding of successive generations of Norway black and white hooded rats secured two strains which differed in feed efficiency. The high efficiency strain which produced better weight gains and more fat later produced an obese mutant (Zucker and Zucker, 1961) which is discussed in more detail in the section on genetic obesities.

A complicating factor has been introduced by the observation that a reduction in litter size during the suckling period produces a rat which increases its body weight very rapidly but a high proportion of its weight is fat (Widdowson and McCance, 1960).

Activity

The amount of volitional or forced exercise obviously plays an important role in the development of obesity. Increased physical activity, regardless of its nature, especially if it requires a considerable fraction of the daily energy intake, inhibits the development of obesity. At the opposite end of the scale, a decrease in activity, verging on complete inactivity, makes it difficult for the animal to adjust its caloric intake to its requirement. Consequently, obesity develops (Mayer, 1955).

Ingle produced obesity in rats by forced feeding (1946) and claimed that ad libitum feeding with restriction of the animals' cage size (1949) produced the same effect. Actually, Ingle's ad libitum feeding was complicated by the fact that he incorporated water into his ration. When the rats became thirsty, they had to eat the wet ration. However, others suggest that the obesity produced by feeding normal rats a special diet (Mickelsen et al., 1955) or as a result of hypothalamic lesions in mice (Liebelt, 1963) results in curtailed activity so that additional

confinement isn't really necessary. Whether the obesity results in decreased activity or whether decreased activity results in the obesity does not appear to be resolved. However, Bielschowsky and Bielschowsky (1956) claim that in NZO genetically obese mice the inactivity was the result of the obesity.

CHARACTERISTICS OF REGULATORY, GENETIC AND DIETARY OBESITIES

Regulatory obesities

With Clark's modification (1939) of the Horsley-Clarke apparatus, surgical lesions could be placed specifically in the ventromedial nuclei of the hypothalamus of the rat to produce hyperphagia and obesity (Hetherington and Ranson, 1940). These first experiments were no doubt an outgrowth from the controversy over whether or not a faulty pituitary or hypothalamus resulted in a characteristic obesity in man described by Fröhlich (1901; Bruch, 1939).

Hetherington and Ranson (1940) described hyperphagic rats made obese with bilateral surgical lesions of the hypothalamus as showing enlargement of adipose tissues in the abdomen, in the mesenteries and around the kidneys. These rats also had large accumulations of fat in the perineal region and over the neck and upper chest. Both sexes showed enlargement of the subcutaneous fat depots as well.

The weight gain in these obese rats was primarily due to the storage of fat; true growth with synthesis of protein was not apparent in hypothalamic obesity (Hetherington and Weil, 1940). Brecker and Waxler (1949) report similar accumulations of fat in the abdominal cavity of obese mice injected intraperitoneally with gold thioglucose which caused similar lesions in the hypothalamus (Marshall et al., 1957).

Invariably, the nose to anus length of the obese rat with surgical or electrolytic lesions is shorter than its control (Hetherington and Ranson, 1940; Kennedy, 1951; Sétálo, 1965). Tibias (Sétálo, 1965) and femurs (Han et al., 1965; Han and Lin, 1966) also were shorter in the lesioned rats. Brobeck et al., (1943) indicated that the body length of lesioned rats showed no deviation from normal, but these rats were operated on as adults. Bernardis (1963) found that lesions in all sites of the hypothalamus except the ventromedial nuclei resulted in growth impairment.

There is some confusion as to the effect of obesity on the mineralization of the skeleton. Brecker and Waxler (1949) on the basis of radiographic examination of gold thioglucose induced obese and control mice observed no differences in size, shape or density of bones in the two groups. Furthermore, analyses of the whole carcasses indicated no differences in the weights of ash in the two groups. On the other hand, Hetherington and Weil (1940) reported some depletion of calcium, phosphorus and iron in the hypothalamic obese rats.

The hypothalamic lesioned obese rats after gaining a great deal of weight developed sores and scales on their hind feet, their hair became sparse with the skin in these spots appearing scaly and irritated. Sometimes, the very obese animal would begin losing weight and die in a short period of time with necropsy revealing some slight respiratory infection (Hetherington and Ranson, 1942; Kennedy, 1951).

Males usually developed shrunken scrota and the testes lay in the inguinal canal. Necropsy revealed the testes of the male to be small in most cases with atrophy of the reproductive glands (Hetherington and Ranson, 1940). Liebelt (1963) reported abnormal ovarian cycles in mice

made obese by chemical lesions. These obese mice did not breed but if copulation occurred prior to administration of the gold thioglucose, pregnancy proceeded to term. There was some indication in these mice that lactation was impaired. In these obese mice, the adrenals were slightly enlarged while pituitaries and thyroids were smaller (Setälo, 1965). Liebelt (1963) reported hyperfunctioning of the thyroid gland in mice. Alterations have been observed in pancreatic beta cells, (Gepts, 1963; Hausberger et al., 1964) as well as enlargement and alterations in their structure and function (Brobeck et al., 1943). Stomach weights showed no significant differences but gastric secretions did increase in hypothalamic obesity in rats (Ridley and Brooks, 1965). The increased gastric secretion may partially explain the acute gastric ulcers in the chemically lesioned mice observed by Liebelt (1963).

Implantation of electrodes in the ventromedial nuclei which can be stimulated periodically will cause even greater hyperphagia in rats than the electrolytic or chemical destruction of the "satiety" center (Steinbaum and Miller, 1965) and even more excessive weight gains.

Following the surgical lesions, rats became hyperactive for a couple days postoperatively, followed by a large decrease in spontaneous running activity (Hetherington and Ranson, 1942). Liebelt (1963) calls the mice injected with gold thioglucose "active" but doesn't feel that the methods used to study activity have been entirely satisfactory.

Genetic obesities

Four strains of genetically obese mice have been identified, three of which have been associated with a single gene. One of these is dependent on a dominant gene associated with a yellow coat color (Danforth, 1927). Two of these are due to a single recessive gene, one

identified as ob (obese) (Ingalls et al., 1950) and the other as ad (adipose) (Falconer and Isaacson, 1959). The genetics of the New Zealand (NZO) strain of obese mouse has not been determined (Bielschowsky and Bielschowsky, 1956).

The mice displaying the ob gene have been studied extensively by Mayer (1960). These mice with the obese hyperglycemic syndrome display many of the characteristics of the gold thioglucose induced obesity of mice. They have large deposits of body fat with decreased lean tissues, (Vlahakis and Heston, 1959) display decreased activity, enlarged liver, heart, pancreas, and small uteri and ovaries (Mayer, 1960). They are resistant to mating (Runner and Gates, 1954) and are produced from some pairs of non-obese littermates in a 1:3 ratio. They display pancreatic dysfunction with increased insulin and glucagon secretion. Weight gain is at a maximum on a high carbohydrate diet with less weight gain on a high protein or a high fat diet (Mayer, 1960).

Castle (1941) indicated that the obese mouse with the yellow coat color in addition to being obese, had a slightly longer body length. Females became more obese than did males of this strain (Danforth, 1927). Adipose mice (Falconer and Isaacson, 1959) don't develop obesity for 5 to 6 weeks after birth, the main weight gain being body fat. On the other hand, Bielschowsky and Bielschowsky (1956) reported that the NZO strain of obese mice did reproduce, although reproduction was not as favorable as in other strains. Gestation and lactation, however, delayed the obesity. Estrus cycles in the obese NZO females are, more frequently than not, elongated beyond the four or five day normal cycle. The fat

depots of the abdominal cavity, inguinal and retroperitoneal area were enlarged with accumulations of fat which were largely responsible for the weight increase. In conjunction with a high resistance to insulin was a very much altered pancreas with a larger proportion of beta cells. Until reaching extremely marked adiposity, these mice were as active as other strains of mice.

An obese mutation in the rat dependent on the presence of the homozygous recessive gene, fafa, has been described by the Zuckers (1961, 1962, 1963, 1964, 1967). The increased weight of this rat called "fatty", primarily consists of fat, with no increase in skeletal size nor muscle mass. There is a large accumulation of fat in the subcutaneous area surrounding the neck and chest. Heterozygous sibs of "fatty" rats (Genotype Fafa) became fat if fed a high fat diet with a somewhat different proportioning, that is, more fat in the abdominal cavity (Zucker and Zucker, 1963). Fat accretion in this rat was more closely associated with weight gain than with age. Hyperlipemia was a definite characteristic of these rats (Zucker, 1964).

Dietary obesities

That environmental dietary factors can modify genetic predisposition to normal weight is clearly demonstrated by Fenton and Dowling (1953) and Fenton and Chase (1951). In their studies of growth rate and efficiency of food utilization these investigators (ibid) reported that C_3H and A strains of mice gained weight which was roughly proportional to the fat content of the diet. They became obese when fed a 47.5% fat ration. Increasing the dietary fat level above 15% did not cause similar gains in body weight in the C_{57} strain of mice. Fenton (1956) concluded that a 50% hydrogenated fat ration caused an increased percentage of carcass fat,

despite the fact that weight gains were not proportional to percent of dietary fat (Fenton and Dowling, 1953). I strain mice do not show this similar increase in carcass fat on a 50% fat ration. Other strains which exhibited similar obesity when fed a high fat ration were $DBA/_2JN$, STR/N and $C_{57}L/HeN$, (Sokoloff et al., 1960).

By the use of rations containing 40 to 60% hydrogenated fat, Mickelsen et al. (1955) and Barboriak et al. (1958) reported large weight gains of rats thus fed in contrast to stock fed controls or rats fed semi-purified diets containing liquid fats. Although others (Reed et al., 1930; Hoagland and Snider, 1941; Haldi et al., 1942; Forbes et al., 1946 a, b, c, d; Lundbaek and Stevenson, 1947; Hoagland et al., 1952) reported slightly increased weight gains in rats fed high fat diets, none of these maintained their rats sufficiently long for true obesity to develop.

The increased weight in these rats made obese by high fat rations was composed primarily of fat (Mickelsen and Anderson, 1959). There were slight increases in skeletal length (Mickelsen et al., 1955). Those rats maintained on a 50% hydrogenated fat diet have an increased quantity of fat in the skin (Haldi et al., 1942; Page and Babineau, 1953) in contrast to those animals fed a stock ration or even a ration containing a liquid fat.

Histological work on obese 40 week old rats (Barboriak et al., 1958) indicated that these rats are resistant to arterial lesions, that the livers have slight infiltration of fat and that the kidneys show some mild swelling of the tubules. Yamamoto et al. (1959) reported increased proteinuria in these rats consuming a high fat diet.

Metabolism, at least in the young rats fed this high fat diet, did not differ markedly from controls on a 50% fat diet (Reynolds and Pringle, 1965).

OBESITY AND BODY COMPOSITION

Because of the constancy of lean body mass, and the variability in body fat, determination of any one gross component of the body as water, protein or fat can be used to calculate percent of body fat, and ultimately the degree of obesity. Brožek has comprehensively reviewed the analytical methods (1965; 1963; Brožek and Henschel, ed., 1961). The work of Reid et al. (1955; 1956; 1957; 1963) Van Niekerk et al. (1963) and Pearson (1963) have contributed greatly to body composition data on large animals. For the most part the review here will be limited to the rat, and consideration will be given only those data obtained by direct chemical analyses for the major components of the body as fat, protein, minerals and water. Body composition as affected by weight reduction, will be considered in the section devoted to "weight reduction."

Fat is the major variable in the body. However, water, in most instances, makes up the largest percentage of body mass. After reaching puberty, water tends to make up a constant portion of the lean body mass (about 72.3% of the latter) (Reid et al., 1955). Protein will make up 17 to 20% of this total body mass (21-25% of lean body mass) with the remainder being primarily minerals (ibid).

In view of the large amount of work being done in this area, there is very little besides the early work of Rathbun and Pace (1945) which relates the formulae to actual results of bodies that were analyzed for fat, protein, water and ash.

Effect of age

The percentage of body fat in the human subject is lowest during puberty. It is high in the infant, decreases during childhood and adolescence and increases during adulthood to a peak in late maturity. Mayer (1948; 1949) states that passage from protein synthesis in the young to fat synthesis in the adult constitutes a phase of aging.

During growth, lean muscle mass accounts for a large increase in intracellular water (Kerpel-Fronius, 1958; Yannet and Darrow, 1938). In senescence this intracellular compartment decreases leaving a porportionately increased extracellular compartment (Lowry et al., 1942). Associated with this is a reduction in total lean body mass with a compensatory or greater increase in fat.

The adipose tissue cell engorged with fat is composed of only 6-20% water rather than 75% as is muscle tissue. Nevertheless, the percent of water in the fat-free portion of adipose tissue is comparable to other tissues. With aging, however, fat composes a considerably larger proportion of the fat cells (Behnke, 1964).

Effect of sex

There are many reports that among "normal weight" human beings, adult women have a considerably higher percentage of fat in their bodies than men (Wilmer, 1940; Reynolds and Asakawa, 1950; Garn, 1957a; Edwards, 1951; Keys et al., 1950; p. 173).

The values for the body fat content of men range from 10-20% (Brožek, 1952; Mitchell et al., 1945; v. Dobeln, 1956; Pascale et al., 1956; Edelman et al., 1952; Ljunggren et al., 1957) and of women from 20-30% (Brožek et al., 1953; Brožek, 1962; v. Dobeln 1956; Young et al., 1961;

Edelman et al., 1952; Ljunggren et al., 1957). Garn (1957a) claims that the absolute amount of fat in the bodies of normal weight men and women is the same but since women have a smaller lean body mass, the percentage of fat is greater.

The period of life when this differentiation in percentage of body fat first occurs seems to be less clearly established--many claiming it first begins at puberty, while others claim it is evident at birth (Owen et al., 1962; Hanna, 1963) and perhaps even before birth (Forbes, 1962). Where variations in proportion of body fat between the sexes are slight, as in fetuses and new born infants, it becomes difficult to establish whether these differences are due to sex or individual differences. Although the difference is not statistically significant, the trend indicates a greater percentage of fat is already present in the female during the gestation period.

Body composition of the rat

The summary review table (Table 1), indicates the composition of the four major components of the body of the rat as determined by different investigators. Procedures for preparation of the carcasses varied from grinding raw or frozen carcasses with meat grinders to partially hydrolyzing the carcasses and homogenizing. Moisture and fat were analyzed on whole animals or on aliquots while nitrogen and ash were frequently analyzed on dry, fat-free samples.

Table 1

Body composition of rats as determined by various investigators

Sex	Age Days	No.	Wgt. g	Total Body Composition Water %	Prot. %	Fat %	Ash %	Lean Body Mass Water %	Prot. %	Ash %	Investigator
M,F[1]	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.

Use of the soft tissue radiograph for in vivo measurement of body fat

In lieu of direct chemical analysis for body composition indirect methods have been developed for measurement of body fat or lean body compartments. One such measurement is the soft tissue radiograph from which the measurement of skin to muscle shadow can be made. Formulae can probably be developed for converting these measurements to body fat content.

Through the work of Garn (1954; 1957a; 1957b; 1957c) and his associates (Garn and Harper, 1955; Garn et al., 1956) the systematic measurements of the depth of subcutaneous fat layers introduced by Stuart et al., (1940) has become recognized as a valuable tool for prediction of adiposity in man. A comprehensive review of the soft tissue radiographic technique and complications involved in its use for human subjects was presented by Garn (1961) at a symposium on the techniques for measuring body composition.

Animal work is more limited. Hogreve (1938) used radiographic measurements in a study of the process of fat deposition in the hog. Wussow and Weniger (1953) measured fat thickness at four different sites on hogs and believed this method had applicability in studies of the inheritance of fatness. Stouffer (1963) used this method successfully for evaluation of fat thickness over the back of the hog.

Mickelsen (1958) introduced this work in laboratory animals. Crofford and Davis (1965) in the NZO obese mouse have used the radiograph primarily to show the similarity of skeletal size of this genetically obese mouse and a lean control of another strain. Although they have done chemical analyses for body composition no correlation with radiographic measurements was attempted.

Since the depth of subcutaneous fat on a linear surface is being measured on the radiograph, the data obtained from this measurement are only useful insofar as they are able to be mathematically transformed into a value for body fat. Garn (1957a; b) proposed the simple straight line equation of $y = ax+b$ wherein y (total body weight) bears a straight line relationship to the fat thickness (x) with a slope defined by the constant (a). Weight is made up of two components (Behnke 1953), the more variable fat component and the constant lean body mass (b in the above equation). If the lean body mass is subtracted from each side, y' (weight of fat) = ax. The constant (a) is determined by the equation, $a = r \frac{\sigma y}{\sigma x}$ in which r is the correlation between the fat pad thickness and total body weight, σy and σx are the standard deviations of weight and thickness of the fat pad respectively.

Garn (1957a) reported data obtained from this formula on the trochanteric fat pad in men and the iliac crest fat pad in women to be in agreement with body fat determined by densitometry and blood dilution methods of comparable groups of older men, 20-64 year old women and young men. However, Young et al., (1963) applying this formula to women found values for fat calculated from the iliac spine fat pad to be roughly two-thirds of those values determined on the same women by densitometry measurements. Thus far, application of the above formula or similar formulae to animals wherein they can be verified with actual analyses for fat, has not been attempted. In the human, they have been primarily verified with densitometry measurements. In turn, a formula for prediction of body fat from specific gravity has been derived from linear regression equations developed from underwater weighings and chemical analyses of fat in guinea pigs (Rathbun and Pace, 1945; Pitts, 1956).

CHARACTERISTICS OF THE WHITE ADIPOSE TISSUE

Formation of the fat cell

Two theories (Töldt, 1870, Flemming, 1871b) have been proposed for the development of the fat organ, and combinations of these theories have been held by others (Kölliker, 1886; Hammar, 1895). According to Flemming (1871a; 1871b; 1876), adipose tissues were included among the connective tissues. He concluded that they belonged to the mesenchymal cells until such time as they accumulated fat. The fact that the fat cell did not occur randomly in the connective tissue but developed wherever there was a rich vascular supply tended to support the theory of Töldt (1870) that the adipose tissue was clearly separated from the surrounding connective tissue.

Kölliker (1886) and Hammar (1895) identified small localized areas along blood vessels and their capillary networks wherein fat accumulated. Töldt (1870) had called these sites "primitive fat organs." Hammar (1895) showed these "primitive organs," to be the precursors of brown fat and not white fat, so he accepted Flemming's concept that the white fat cell is a modified fibroblast originating from the connective tissue. This idea was supported even as late as 1962 by Bloom and Fawcett (1962). The development of these theories has been reviewed by Wassermann (1964; 1965), Wertheimer and Shapiro (1948) and Wells (1940).

These highly organized formations which were called "primitive organs of the white adipose tissue" were demonstrated to be present in human embryos by Wassermann (1926) and in the bovine embryo by Bell (1909). Many species (man, Tedischi, 1946; rats, Hausberger, 1938; 1955; mice, Liebelt, 1956; and birds, Clara, 1923) exhibit specific anatomical sites wherein adipose tissue is present and able to proliferate and accumulate lipid.

"The first adipose cells appear in the mesenchymatous lobules from which hair follicles and sebaceous glands also develop. The primitive fat cells differentiate from the reticular cells with the penetration of a capillary bud into the lobule which proliferates first along the axis of the lobule, then spreads and sends branches into the periphery" (Wassermann, 1926). "Adipogenesis is connected to this proliferation of the capillary thus resulting in the newest cells appearing at the periphery of the lobule," (Simon, 1965).

"No fat or glycogen is found in other fibroblasts of the connective tissue except in these "primitive organs"," (Wassermann, 1965). "This "primitive organ" is formed both by the growth of the endothelial and adventitial cells. These areas can first be recognized because of the relatively thick layer of adventitial cells in conjunction with the arterioles and venules of that area. By this vascular growth, the primitive organs are built into the connective tissue as separate formations from the connective tissue itself," (Wassermann, 1958). The lobules of the adipose tissue retain the original organization of the primitive body although sometimes it is difficult to identify this organization because of the accumulation of fat in the cells.

Description

The mature white fat cell has a spherical shape and large size. The single large fat droplet causes the cytoplasm to be displaced so that it forms a thin ring around the fat droplet. The nucleus is flattened and pushed to the edge of the cell, so it causes a bulge of the cell membrane in that area. Around the nucleus are observed numerous elongated rod shaped mitochondria (Tedischi, 1960).

Electron microscopic studies (Napolitano and Gagne, 1963) of white adipose tissue from the inguinal or epididymal fat depots of the young white rat or mouse show a very thin rim of cytoplasm around the lipid inclusion. The cytoplasm contains mitochondria and other organelles. There appears to be no membrane separating the lipid droplet and the cytoplasmic membrane. On the cell exterior, the cytoplasm is bound by the plasma membrane which is surrounded by an amorphous area--the basement membrane.

The nucleus is displaced to the periphery of the cell and is flattened. The nucleus contains a distinct nucleolar region. Most of the cytoplasm is also concentrated in the area surrounding the nucleus, and the cell organelles are concentrated in this area. The mitochondria vary in shape from ovals to very fine filaments. They do not contain as many cristae nor are they as large as in other cells (Napolitano and Fawcett, 1958). The cytoplasm contains tiny granules identified as ribonucleo-protein, and occasionally, endoplasmic reticulum, Golgi bodies and in immature white adipose cells, glycogen is present. After the cells attain maturity, the glycogen disappears from the cell. The basement membrane of the signet ring is surrounded by an area of collagen fibers and individual collagen fibers are seen throughout the intercellular area. This fatty area also contains an occasional non-myelinated nerve, other mesenychymal cells and blood vessels.

An electron microscopic study by the same authors (ibid) wherein animals were maintained in good health but gradually depleted of their fat stores, shows that the cells do undergo morphological changes. Fat depleted cells now become ovoid rather than spherical, diminish in size and the cell surface is no longer smooth but shows many indentations. The intercellular space is enlarged and more highly concentrated with collagen fibers in

proximity to the outer fat cell surface. Mitochondria and nuclei appear to be the same as in the spherical lipid-filled cells. The depleted cell contains a large nucleus.

The lipid free component of adipose tissue

Mature fat-free adipose tissue has a gross composition similar to other tissues and organs; approximately 79% water, and 21% residue which is chiefly protein (Allen et al., 1959). An amino acid analysis of this protein (Bowes and Kenton, 1949) shows a distribution of amino acids which is very similar to that of collagen except lower in proline and hydroxyproline.

Sugars primarily found in adipose tissue are the structural sugars, mucopolysaccharides and glycoproteins. The large linear molecules of mucopolysaccharides consist of N-acetylated or sulfated hexosamines in conjunction with a hexuronic acid or hexose repeating disaccharide units (Dorfman,1959). The glycoproteins exist in small heteropolysaccharide units attached to peptide chains (Spiro, 1963). The nature of these sugars suggests they are primarily structural rather than storage depots as is glycogen. There is little or no glycogen found in the adipose tissue, with the mature cell being especially devoid in this component. The entire protoplasm of this cell occupies 2.4% of the entire volume of the fat cell (Gersh & Still, 1945). The intercellular spaces are shown to contain a specific nerve supply (Böecke, 1933) and an abundant vascular supply (Gersh and Still, 1945) equivalent to or greater than other tissues and organs.

During periods of increased deposition of body fat, Liebelt (1956) has shown that the lipid-free component of inguinal and gonadal fat

depots in mice increases in proportion to the increase in body weight.

Pitts (1956) reported that in female guinea pigs the fat-free adipose tissue makes up a larger percentage of the lean body mass than in male guinea pigs. This comprises 7 % of the total lean body mass in the female, while in the male guinea pig it makes up 4 % of the total lean body mass.

The lipid component of the fat cell

Adipose tissue in the newborn rat is fat-free (Hausberger and Gujot,1937). As early as three days after birth the inguinal fat body contained 54% fat and by the end of 2 weeks 79% in the rat (ibid). In the lean adult mammal, adipose tissue had at least 60% or more lipid substance (Behnke, 1964). The accumulation of more lipid enlarged the fat cells so that they contained a larger percentage of fat and at maximum accumulation contained 85% fat. The Hausbergers (Hausberger and Hausberger, 1957) reported that in extremely obese mice the diameters of the fat cells increased by 35% compared with cells from normal mice. This increase in diameter permitted an accumulation of about 150% more fat. However, new cells also developed in the obese mouse and 75% of the increased quantity of fat was deposited in them. Actually, there were four times as many cells in the obese mouse as in the control (Hausberger, 1959). Further evidence for an increase in the number of cells was demonstrated by Bjurulf's (1959) measurements on cadavers which suggested that as the amount of fat under the skin increased the number of fat cells also increased. Pitts (1956) from his studies on accumulation of body fat in guinea pigs concluded that additional body fat was deposited by both mechanisms, that is, filling up the available adipose tissue cells until the total body fat reached about 25%, at which point, the lipid-free cellular component began to increase in quantity.

In reviews concerning adipose tissue development, Wells (1940) and Behnke (1964) stated that, when the fat cell reaches maturity, it only maintained an ability to accumulate more fat and did not divide. Hellman and Hellerström (1961) using autoradiographic methods involving the uptake of the DNA precursor, thymidine, labelled with either tritium or radioactive carbon, studied mitotic division in the subcutaneous gluteal adipose tissue of the white rat from 4 to 154 days of age. They concluded that mitotic division of the fat cells continued but at a slower rate in the older rats. For this age group, renewal of fat cells was greater in the subcutaneous fat depots than in epididymal fat depots.

The amount of fat deposited and the number of cells made available for this deposition is a characteristic of the tissue itself. This was demonstrated by the "boxing glove" effect of an abdominal skin graft to the back of the hand of a 12 year old girl whose hand was severely burned. Evidently Strandberg (1915) in grafting the skin from the abdomen had also grafted some of the underlying abdominal adipose tissue which with advancing age accumulated fat and resulted in the puffiness on the back of the hand (for pictures, see Strandberg, 1915; Hashim & Van Itallie, 1965 and Wells, 1940).

That this property is inherent in the tissue itself and that genetic factors impose specific developmental patterns on the adipose tissues is further demonstrated by the parabiotic transplants of Hausberger (1955). He transplanted abdominal tissue from immature genetically lean mice to immature genetically obese mice and vice versa. There was histological evidence for the original developmental pattern of the host animal with enough change to indicate that there were also hormonal and regulating

mechanisms involved. Liebelt's work (1963) consisting of transplants of obese tissue to the ear of the lean mouse is supporting evidence for this.

Distribution

The adipose tissue's capacity to accumulate or lose fat appears to be the body's defense mechanism available for periods of feast or famine in man as well as in animals (Fredrickson, 1964).

How adaptive processes operate to fill or deplete adipose tissues aren't clearly elucidated. Nevertheless, there is evidence that fat stores are sacrificed to provide energy for the development of lean body tissue and that fat depots are depleted before lean body tissue (Sarett et al., 1966; Young et al., 1963). One exception to this, however, would be the sucking pad or corpus adiposum buccae which exists despite emaciation (Scammon, 1918-1919; Goldzieher, 1946). In emaciated infants, the sucking pads may become extremely prominent (Neff and Billingsley, 1930).

In a study to evaluate the deposition of body fat into certain adipose depots of the white rat including the intermuscular region of the forelimb, the lumbo-dorsal region, the inguinal region, the genital, perirenal and the mesenteric and omental areas, Reed et al., (1930) concluded that neither the nature of the diet nor exercise had an effect on the proportion of fat deposited in each area. The four diets which they used to study this contained 17% of the calories as casein, the other 83% of the calories coming from cornstarch, mutton tallow, "Crisco" and soybean oil respectively. Rats were sacrificed at 50, 150 and 250 g for each feeding regimen. The adipose tissues were approximately twice as heavy in the rats fed the three fat rations as in those fed the constarch ration. For rats of the same body weight, there were no differences in the proportions of fat in each of the six adipose tissues.

In a study designed to investigate the effects of feeding a high fat diet for protection in the cold, Page and Babineau (1953) concluded as did Reed et al.(1930) that the nature of the diet did not affect the distribution of body fat. However, their results showed that in rats kept at room temperature and fed the high fat diet, a higher proportion of the fat was in the adipose tissue depots with lesser amounts in the skin and skeletal area. (The skeletal area included everything except the skin, pelvian and scapular belts of fat, perirenal and retroperitoneal fat, testes and testicular fat, G. I. tract and mesenteric and omental fat, pancreas, and adrenals.)

Liebelt (1959) showed that sex and genetic factors influence the distribution of lipid between the inguinal and gonadal fat organs in mice. Although the fat-free weights of the fat organs followed a pattern similar to body weight in NH and CBA mice the lipid deposition in these two fat organs didn't necessarily do so. In the NH mice, the female deposits 25-30% more lipid in each of the two fat organs when compared to the NH male of the same body weight. The CBA female mice deposited 25-30% more gonadal fat depot but inguinal fat depots were the same size as in the CBA male. The gonadal fat depots of both male and female CBA mice were proportionately heavier (as a % of body wt.) than those in NH mice. This was true until the mice weighed 20 g when the size of the fat depots became comparable in the two strains. Insofar as the inguinal depot is concerned, CBA male and female and male NH mice had the same amount of fat deposited for comparable body weights. After reaching body weights of 12 g, female NH mice deposited considerably more fat in this area. In gold thioglucose induced obesity of CBA/Ki mice, Liebelt et al. (1965) reported a direct relationship between

the amount of lipid in the inguinal or in the gonadal fat depot and body weight. The pattern was similar to that of the non-injected control mice, but both body fat and depot fat were correspondingly greater.

Reed et al. (1930) studied the distribution of fat in rats as affected by undernutrition and exercise. The rats (180 g) receiving the starch diet (83% of calories) showed a greater depletion of adipose tissue in the genital area when exercised. This was not true for the rats consuming a ration which provided 83% of the calories from fat. During fasting (with no forced activity), rats previously fed the high fat diet showed a smaller relative depletion of omental fat than from other areas. This was not true for rats that had been fed the 83% cornstarch diet. No specific conclusions can be drawn on the effect of undernutrition or exercise on distribution of body fat. There were too few rats in this study, and the sex of the animals was not given. The limited data suggests that the relative distribution of fat in the rat's body is changed by exercise or undernutrition.

It appears also that age affects the proportional distribution of body fat. Peckham et al. (1962) indicated that for the epididymal fat depot, the ratio of depot fat to body fat closely paralleled the age of the rat with a decrease in the ratio with advancing age. However, other depots were not studied in this series.

The distribution of fat in human subjects shows considerable individual variation. There are, however, certain types which have been characterized by such terms as "upper extremity pattern", "girdle fat pattern" and "lower extremity pattern" (Garn, 1955).

On the basis of specific gravity determinations, Young et al. (1963) calculated that as women increased in age from 16 to 70 years, their body

fat increased by 55.3%. On the basis of skinfold measurements the increase in body fat content was only 46%. The lower value for body fat content secured by the skinfold technique suggested to the investigators that as women age, more fat is deposited in the areas other than the subcutaneous.

WEIGHT REDUCTION

Although weight reduction has been advocated for the obese subject by both medical personnel and the lay public, there is still not too much factual evidence to indicate the lasting benefits that may accrue from such an ordeal. Most of the earlier reports were based on insurance statistics which indicated that underweight individuals live longer than those who exceed their "normal" weight (Body Build and Blood Pressure Study, 1959). A few reports from the insurance companies suggest that the obese insurees who reduced their weight lived longer than those who didn't (Dublin et al., 1949). The inability of most overweight individuals to "stay reduced" makes the latter statistics of dubious value. It is very likely that a fair proportion of the subjects who reduced and thereby secured a reduction in their insurance premiums may have returned to their obese state shortly after their medical examination.

The psychological advantages of weight reduction have been stressed especially by the writers of articles aimed at the general public. The theory apparently is that if individuals are not likely to do something that might improve their health, they may do the same thing if it improves their physical appearance. There are undoubtedly many psychological advantages that may accrue to the obese individual once he has "slimmed down" (Stunkard and McLaren-Hume, 1959). However, weight reduction may not be an unmixed blessing for the obese individual. This has been stressed

by Bruch (1957) who insists that unless the proper motivation is used, more damage than good may result from attempts to get the overweight patient to lose weight.

Many of the publications aimed for popular consumption suggest that the overweight individual suffers from some metabolic or physiological disturbance which can be circumvented by the use of a special diet. Credence was given to this assumption by the writings of Pennington (1953; 1954), who suggested that metabolism of pyruvic acid may be impaired in obese subjects. Since then much of the popular writing and a fair amount of professional work has been directed toward the use of a diet high in fat as a means of producing weight reduction.

There is ample evidence that a high-fat diet will produce a loss of weight (Yudkin and Carey, 1960; Kekwick and Pawan, 1956, 1965). This occurs even though the subject consumes as much of the diet as he desires. However, the major share of the "lost weight" is body water (Pilkington et al., 1960; Olesen and Quaade, 1960). A diet high in fat produces a loss of body water even though the caloric intake meets or slightly exceeds the caloric requirement. Finally, the loss in body water resulting from the ingestion of a high-fat diet is undoubtedly of limited duration. Although no studies have been extended beyond 10-14 days, the loss of weight resulting from a loss of water appears to have terminated by the end of the second week. Furthermore, most diets sufficiently high in fat to produce such an effect are likely to result in nausea (Pilkington et al., 1960; Taller,1962) and a loss of appetite by the individual who consumes them for more than one day. Kekwick and Pawan (1964) showed similar results in mice but the loss of body water was not as great in mice. These authors felt the additional weight loss on the high fat diet

could be accounted for by an increased quantity of intermediary products containing carbon being lost.

Weight changes which take place in the normal adult man primarily consist of changes in body fat (Behnke et al., 1953). These authors concluded this from determination of the tissue either gained or lost during fluctuations in body weight. This is not true for people undergoing starvation (Keys et al., 1950) wherein protein and water as well as fat are lost. The nitrogen balance study has been used by Passmore et al. (1958) to evaluate the content of the lost tissue during weight reduction. In the first week nitrogen losses were usually large and balances varied from a -1 to -9 g N/day indicating daily losses of from 25-60 g protein. The loss of weight in the first week was always high and associated with large losses of water.

On the other hand, Christian et al. (1964) found that lean body mass remained constant in 51 "clinically normal" obese subjects on a weight reduction regimen over a period of eight weeks. For determination of lean body mass they measured K^{40} which had a 3.5 - 4.0% in men and up to a 5.0% error in women because of the lesser quantity of lean body mass in women. This would indicate the loss of lean body mass was not greater than 3.5 to 5.0% after weight reduction.

Rath and Slabochová (1964) found that in 32 obese women, a 1200 cal. diet which consisted approximately of 90 g protein, 40 g fat, and 120 g CHO caused a weight loss of 1.2 kg per week. The lost weight consisted of 0.67 kg of fat. These values were obtained from densitometry measurements. When this was accompanied by 4 hr. of physical exercise daily, the total body weight lost was made up entirely of fat. The loss of adipose tissue in the group

which exercised was 30% greater than that attained by the low calorie diet alone.

Young et al. (1960) reported that 4 of 9 obese women, 18-21 yr. of age stored nitrogen or were in nitrogen equilibrium while 5 were in negative balance after 6 weeks of weight reduction. This was on a daily intake of 90 g of protein. In only one instance, however, was this loss of N greater than 5.0% of the nitrogen. The mean percentage of body fat dropped from 35.77 to 29.19%. The mean net change in body fat content was 6.58%. These latter data were determined from underwater weighings.

Passmore et al. (1958) calculated the composition of tissue lost by seven patients over a 6 week period of weight reduction when the subjects received each day a 400 cal. diet (Strong et al., 1958). For the seven patients the composition of the lost tissue varied from 73 to 83% fat, from 0.4 to 7.0% protein and from 10 to 23% water. All patients lost large quantities of water during the first 5-7 days but these water losses were not maintained beyond the initial period of weight reduction.

More comprehensive studies of the effect of weight reduction on body composition have been carried out with animals. Sarett et al., (1966) provided adult rats with one half their ad libitum feed intake until the animals lost 25% of their initial body weights. Under these circumstances, "normal" weight male rats lost tissue which consisted of 8 g of protein and 30 g fat. This represented a loss of 12% body protein. On the other hand, rats classified as "obese" weighing 392 g lost 47 g fat and 2 g protein representing a loss of 3% body protein.

Another group of rats weighed 700 g (ibid) and had around 35% fat in their carcasses. They had been fed "Metracal" ad libitum.

Their weights were reduced by 234 g using one of the following regimens: (1) 60% of their previous intake, (2) 37.5% of their previous intake and (3) fasted. In the two groups receiving restricted amounts of food, the lost tissue contained only 4 to 5% protein and about 79% fat, whereas in the fasted animals the lost tissue contained 10% protein and only 64% fat. After the rats lost 234 g, the carcasses of the restricted groups had 13.0 and 15.0% fat whereas the carcasses of the fasted group had 18.7% fat.

Weight loss in vital organs during weight reduction

Some vital organs increase in direct proportion to body weight, others increase in weight according to allometric proportions to body weights, while still others remain the same size after maturation, even though body weight increases. The behavior of these vital organs during the development of obesity tends to be simulated later on, if weight reduction takes place in the adult life span of the rat. During development of obesity due to hypothalamic lesions Brobeck et al., (1943) reported that heart, liver and kidneys increased in size with increases in body weight. On the other hand, the adrenals remained about the same size as those in control animals and ovaries showed a definite decrease in size. More recently, Spencer and Coulombe (1966) indicated that liver has an allometric relationship to body weight.

Allometry was first advanced by Huxley (1932) and many allometric relationships of organ weight to body size were done by Brody (1945; pp. 398-401). The formula used by Spencer and Coulombe (ibid) was $W = AB^x$ or $\log W = x \log B + \log A$. W = organ weight, B = body weight and A is the parameter calculated (that is the predicted organ weight) which increased at a constant "x". For liver the constant "x" is approximately 0.78 to 0.87

of the increase in body weight. When Binder et al. (1966) applied this formula to the livers of obese hyperglycemic mice, the weight of the liver was greatly underestimated. Instead, the obese mouse had a disproportionately large liver for its body weight. Marshall et al. (1957) reported that livers in both young and adult obese hyperglycemic mice weighed twice as much as those in the controls. Also mice made obese by gold thioglucose or hypothalamic lesions showed increased liver size. On the other hand, Pagé and Babineau (1953) found no increase in the absolute weights of livers in rats consuming a high fat diet. However, these "obese" rats were only 30 g heavier than the controls. On reduced food intakes, Peters and Boyd (1966) reported that livers lost weight faster than over-all body weight. In general then, the liver varies with the weight of the rat, but the nature of the diet also causes a variation in liver weight with a high protein diet producing an increase in liver weight (Eaton, 1938; Walter and Addis, 1939) as did hypothalamic or genetic obesity. When comparing rats consuming chow to rats consuming purified diets of sucrose or starch, liver weights showed no differences (Peters and Krijnen, 1966).

With increase in body weight growth of kidneys, likewise, can be expressed by an allometric equation (Stahl, 1965). No doubt, this equation may be disrupted by high protein diets which cause kidney enlargement (Reid, 1963; Osborne, et al., 1923; 1927; Addis et al., 1926) as well as by hypothalamic obesity (Brobeck et al., 1943) and the obese hyperglycemic syndrome in mice (Marshall et al., 1957). On reduced food intakes loss of weight of the kidneys was equivalent to body weight lost (Peters and Boyd, 1966).

Beznák (1954) found that the weight of the heart followed changes in body weight. Thus, the same cardiac weight corresponds to a given body weight irrespective of whether that body weight was achieved through growth or by losses of weight as a result of starvation. Hypothalamic obesity caused an increased heart weight in rats (Brobeck, et al., 1943) and in mice (Marshall et al., 1957). On reduced food intakes Peters and Boyd (1966) and Walter and Addis (1939) likewise reported that heart weights in rats decreased in proportion to body weight. Beznák (ibid) suggested that the reduction in heart weight may be due to a decreased functional demand. In guinea pigs, however, Eaton (1938) found that hearts were practically the same weight regardless of body weight. On the other hand, Peters and Krijnen (1966) reported that rats fed purified diets containing sucrose or starch had slightly heavier hearts than controls of the same body weight consuming a commercial stock ration.

Weight changes in adult rats do not significantly affect the absolute size of the adrenals. Rats with hypothalamic hyperphagia did not have enlarged adrenals (Brobeck et al., 1943). Hyperglycemic obese mice only showed an enlargement of the adrenals in later adulthood (Marshall et al., 1957). Young obese mice when compared with controls did not show any increase in adrenal weights. Likewise, losses in body weight did not cause a corresponding decrease in adrenal weight (Beznák, 1952; Peters and Boyd, 1966).

Consistently, the spleen and lungs of the obese mice weighed the same as those in normal weight controls (Marshall et al., 1957). With decreased food intakes, the spleen showed more papid weight loss than did the body as a whole (Peters and Boyd, 1966).

Reducing by fasting

Subjects undergoing semi-starvation became hyperirritable, restless, sensitive to noise, unable to concentrate and nervous (Keys et al., 1950, p. 836). On the other hand, after the human being has fasted for three days, hunger pangs are mitigated, the desire for food is suppressed, and he can continue the fast without undue distress (ibid, p. 821; Bloom, 1959; Blondheim et al., 1965; Duncan et al., 1962; Drenick et al., 1964; Hashim and Van Itallie, 1965; Silverstone et al., 1966). Zucker's (1967) weight reduction studies indicate that a similar phenomenon exists in rats.

In view of the above observations, clinicians and scientists interested in reducing the obese have investigated numerous parameters associated with fasting both in humans and to a lesser extent in rats.

With fasting immediate weight losses occur in man. This is partially accounted for by naturesis and loss of water (Bloom and Mitchell, 1960). Upon refeeding, readjustment in body water accounts for the rapid initial weight gains observed (Rapoport et al., 1965a; Bloom, 1962).

Other physiological responses to fasting include a decrease in plasma volume (Rapoport et al., 1965a) hypotension (Drenick et al., 1964; Drenick and Smith, 1964) ketonemia (Cubberley, 1965; Duncan et al., 1962; Drenick et al., 1964; Rapoport et al., 1965a) and an increase in non-esterified fatty acids in serum (Castelli et al., 1966). The obese, however, are more resistant to ketoses than lean individuals (Pawan, 1957; Azar and Bloom, 1963). Liver glycogen reserves are decreased (Haro et al., 1965; Shoemaker et al., 1959) but other aspects of liver function are apparently normal (Rapoport et al., 1965b). Serum glucose is also decreased (Haro et al., 1965; Rapoport et al., 1965b)

Fasting has been reported to produce an elevation of serum uric acid levels. This occurs in the human when fat is metabolized whether it be from an exogenous source such as a high fat diet (Harding et al., 1927) or from endogenous fat (Lennox, 1924; Lecocq and McPhaul, 1964; 1965; Rapoport et al., 1965b; Murphy and Shipman, 1963; Drenick and Smith, 1964; Cristofori and Duncan, 1964). According to Rapoport (1965b) the presence of increased serum urates and decreased urine urates during fasting are associated either with an increased reabsorption of filtered urate or a diminished secretion of urate by the renal tubules. In 33 patients who fasted not less than one month and not more than two months "Probenecid"[1] prevented hyperuricemia (Drenick and Smith, 1964).

An evaluation of the composition of the weight loss during weight reduction was done previously. However, the work of Sarett et al. (1966) indicated that slightly larger quantities of lean body mass are lost from fasting rats than from those rats restricted in food intake.

[1]"Probenecid" is the generic name for para dipropyl sulfamyl benzoic acid. This is frequently sold under the trade name of Benamid.

PART I

BODY COMPOSITION AND GROWTH OF ADIPOSE TISSUES IN OBESE AND NORMAL RATS

INTRODUCTION

Throughout the life span, weight gains occur as the result of growth or maturation, deposition of muscle tissue and/or deposition of fat. During maturation, (Tanner, 1963; 1964) and during physical training periods (Behnke et al., 1942; Brožek et al., 1963; Parízková, 1963; 1965) protein synthesis is the primary contributor to this weight gain. However, with maturation, fat synthesis normally takes preference over protein synthesis and under ordinary conditions, weight increments result in additional body fat (Brožek, 1952; Keys and Brožek, 1953; Mayer, 1948; 1949).

Obesity can be produced in rats by feeding them a diet composed of 40 - 60% hydrogenated fat (Mickelsen et al., 1955; Barboriak et al., 1958; Hoagland et al., 1952; Deuel et al., 1944). These rats continue to gain weight at a fairly rapid rate throughout most of their lives. The major portion of this weight gain is due to the accumulation of fat.

The development of obesity is associated with an increase in the size of the adipose tissues. As far as the adipose tissue cells are concerned, Hausberger (1959) suggests that the existing cells are filled with fat after which a growth of new cells takes place (Behnke, 1964; Hausberger, 1959; Bjurulf, 1959). There is still some controversy as to the relative growth of different fat depots. The rate at which the different fat pads increase in size as an animal matures has not been studied very completely. Reed et al. (1930) proposed that in young rats the different fat depots grew at a proportionately equal rate unless influenced by exercise or under-nutrition. Reports contradicting this statement have appeared more recently. Liebelt (1956) stated that inguinal

and genital fat depots in mice develop at quite different rates. In dietary obese rats, the data of Peckham et al. (1962) suggested that epididymal fat depots grow less rapidly with aging. Additional evidence for a differential growth of fat pads comes from studies of rats exposed to a cold environment. Rats so acclimated had epididymal fat depots that were 1/3 as large in proportion to body weight as those in rats acclimated to warmer temperatures (Himms-Hagen, 1965). Based on differences in percent of body fat obtained from densitometry measurements and skinfold measurements in mature adult women, Škerjl (1959), Škerjl et al. (1953) and Young et al. (1963) proposed that with aging, accumulations of fat resulted in a more rapid increase in the size of the abdominal fat depot when compared to the subcutaneous depots.

Most investigators have frequently used only one or two fat depots of animals when studying physiological and biochemical phenomena. Frequently the assumption has been made that what happens in one fat depot is characteristic of what happens in all fat depots in the body. As a start in evaluating this hypothesis, it would appear desirable to determine how the different fat depots change with changes in body weight. Many individuals are presently involved in determining body fat in human beings by skinfold measurements. They depend on measurements of subcutaneous tissues only or radiographic techniques which are limited to subcutaneous depots which can be estimated on soft tissue radiographs.

For these reasons, it becomes important to know whether or not fat depots all grow at the same rate or whether during certain periods of rapid weight accretion or during aging, fat is sequestered in certain depots. To this end, both normal and obese male and female Osborne-Mendel

rats were sacrificed at 150 g intervals to investigate the weights of individual adipose tissues.

EXPERIMENTAL

Weanling male and female Osborne Mendel littermates from NIH stock and bred in our laboratory were fed either (1) a grain ration (M-1), (Campbell et al., 1966) or (2) a ration containing 60% fat (Mickelsen et al., 1955) with slight modifications (M-15, Table 2). The experimental design provided that 5 male and 5 female rats be sacrificed at weaning, and 5 rats of each sex when they reached weights of 150, 300, 450 and 600 g. This was true for rats fed the grain or high fat rations. Female rats consuming the grain ration never reached 450 nor 600 g, so the data reported for the female rats do not include these two groups. The work of Mickelsen et al.(1955) indicated that the rats consuming the high fat ration became obese so a group of 5 rats were included for both males and females at 750 g and for males only at 900 and 1050 g. Since there was some variation in the time required by the rats to attain a given weight, the animals were placed in their specific groups at the time of weaning. This predetermined the ultimate weight the rats should attain before being sacrificed. The rats from one litter were distributed among the different groups. Consequently, no two rats from one litter fed the same ration were sacrificed at the same weight. In addition, data from the four groups of older animals (both male and female rats that had been fed the high fat or grain ration for about 400 days) used as controls in Part II are included here since these data help in extending this experiment time-wise.

At the time of sacrifice, the rats were weighed and then anesthetized until death with ether. Immediately, they were placed on their abdomens, and nose to anus lengths measured. The skin was cut at the base of the rib cage around the entire rat to permit separating the skin from the subcutaneous fat depots by gently removing the skin from the fore and hind parts of the rat. Care was taken to expose only those portions of the rat where the individual fat depot was being removed; the skin was replaced after removing the subcutaneous depot. To further reduce the evaporation of water from the tissue, the working surface was kept moist. Nevertheless, moisture losses through evaporation could account for 0.5 to 1.1% of the body weight as determined by progressive decreases in total carcass weight during the dissecting procedure. In subsequent calculations of moisture, adjustment in the percent of moisture was made for this evaporation loss.

Right and left inguinal fat depots were separated from the body wall by tearing and occasional cutting. Care was taken to exclude the femoral artery and vein. In the larger rats, the inguinal fat depot appeared to merge with subcutaneous fat of other areas. In these cases, the inguinal fat depot was arbitrarily separated from the cephalad half by an incision at the costal border and from the dorsal side by an incision parallel to the backbone and on the ventral side, by the location of the sternum. This inguinal fat depot did not include the intermuscular fat which developed in the thighs of both male and female obese rats. That fat was definitely imbedded below a thin layer of muscle and did not appear to be a part of the depot. On the other hand, where the depot had expanded to incorporate the mammary gland, it was non-distinguishable and thus included in the depot weight. Subcutaneous fat cut from below both

the right and left forelimb extended to the upper esophagus and sternum which made it possible to differentiate it into a right and left side. The interscapular fat depot was separated from the skin and the back of the neck. This depot included the entire dorsal side of the rib cage and extended to the lower costal border.

After the removal of the subcutaneous fat pads, an incision was made into the abdomen. The testes of the male rats were pulled up into the abdomen and the testicular fat depot separated by cutting it away from the testes and epididymis caput and testicular artery. In addition a very small fat pad was removed from the caudal end of the epididymis and called epididymal fat to differentiate it from the larger testicular fat body which frequently has been called epididymal fat. It's weight was negligible, increased in relationship to testicular fat so individual data for it are excluded. The comparable fat depot in female rats was pulled away from the perirenal fat and then cut away from the uterus and follicles and separated into right and left pads at the base by centrally cutting it in a line with the bladder.

An accumulation of fat at the base of the sternum around the cartilage of the xiphoid process was observed with aging and with accretions in body weight. This was easily separated from the cartilage of the xiphoid process and weighed.

The esophagus was cut at its base, and the gastrointestinal tract and the mesenteric and omental fat depots were removed from the abdominal cavity. Any portion of the pancreas adhering to this could be distinguished from the fat by its slightly deeper brown color and thereby readily separated from the fat. The mesenteric fat depot was gently pulled from the

gastrointestinal tract. When resistance was felt, a slight cut facilitated its removal. The capillary plexus supplying the gastrointestinal tract remained as a part of the mesenteric fat.

Lastly, the retroperitoneal and perirenal fat depots were removed as a single mass. By cutting inward from the outer edge of the depot, then removing it from the adrenals and kidneys and, finally, separating it from the backbone and abdominal aorta the process could be completed without loss of blood.

Upon removal, all of these depots were immediately weighed on a Roller-Smith or Torsion balance depending on the size of the depot; and after weighing placed into a previously tarred pint or quart wide mouth jar with a cover always intact to avoid unnecessary loss of moisture. The carcass was placed in this same jar. The gastrointestinal tract and its contents were weighed. The contents were removed from the G. I. tract which was then rinsed with water, gently blotted with absorbant paper and weighed again before being placed in the jar with the carcass. The weight of the gastrointestinal contents was subtracted from the live weight of the rat to secure the "corrected weight". Since special attention was given to prevent any loss of blood during the dissection, the "corrected weight" of the rat compensated for any evaporation losses from the carcass. By actual weighing of the jar before and after adding all the tissues, it was found that evaporative losses amounted to only a few grams for the larger rats.

The jar containing the entire carcass was then autoclaved at 15 lb. pressure for 20 to 25 minutes depending upon the size and age of the rat.

After autoclaving and cooling, the jar and rat were weighed to verify any gain or loss of moisture during the autoclaving procedure. Variations usually were no larger than ± 1 to 2 g of the original weight. The rat was then homogenized using amounts of water equivalent to body weight for the smaller rats and lesser amounts for the larger rats as described by Mickelsen and Anderson (1959). Blenders ranging in size from 1 cup to 2 gal. were used depending on the size of the rat.

After homogenization, aliquots were analyzed for moisture, fat, nitrogen and ash by methods described by Mickelsen and Anderson (ibid). Since ashing was as complete at 12 hours as at the 24 hrs. which they used, samples were ashed 12 hrs.

RESULTS

Although every effort was made to sacrifice the rats at the weights originally chosen, this was not always possible. A variation of ± 15 g of body weight was accepted since some rats gained this much in a week-end when it was difficult to get the animals x-rayed. All rats were sacrificed the morning of the day following the radiograph. Some of the small rats gained as much as 7 g whereas some of the obese rats lost weight during the period intervening between the radiograph and necropsy.

The body weights listed in Table 3 represent the averages for each group just prior to sacrifice and the weight after removing the contents of the gastrointestinal tract. The rats fed the grain ration had about twice as much material (both on a volume and weight basis) in their gastrointestinal tracts as those fed the high fat ration.

Body weights

Rats fed the high fat ration gained weight more rapidly than those fed the grain ration (Fig. 1). This resulted in the high fat-fed rats being younger than the grain-fed rats at the same body weights (Table 3). These differences were significant ($P<0.05$) for the 450 g male and 300 g female rats, and highly significant ($P<0.01$) for the 600 g male rats.

The rate of weight gain for the high fat-fed male rats was rapid and uniform until they attained a weight of 450 g. After this, weight gain occurred at a uniform but less rapid rate. A partial explanation of this decreased rate in the older animals is that some rats lost 20-30 g and then regained this weight. Female rats fed the high fat ration showed a uniform rate of body weight increase up to 855 g.

The grain-fed rats showed a plateau in their body weights at about 600 g for the males and 365 g for the females.

Skeletal size

The skeletons of the obese rats were the same length from snout to anus as those of the lean rats for each of the weight categories (Table 3). When the obese rats began to exceed the maximum weights of the lean controls, differences in skeletal lengths began to appear. The 1050 g male rats measured 29.0 ± 0.4 cm while the 600 g lean rats were 27.7 ± 0.6 cm. Although this difference is significant, its interpretation is open to some question. The large accumulation of fat in the subcutaneous area of the obese rat makes it difficult to evaluate the true skeletal length.

The ash content of the obese rats was for each weight category less than that of the lean rats. A partial explanation for this difference

stems from the fact that the accumulation of body ash is related to the age of the animal (Fig. 2 and 3). Again up through 150 days of age, the absolute weight of ash in the bodies of both obese and lean rats is the same. However, at each age, the rats fed the high fat ration are much heavier than those fed the grain ration. Consequently, when comparisons are made on an equal weight basis, the obese rats are younger and have less ash in their bodies.

Admittedly, the total body ash provides at best only a crude index of skeletal size. For this reason, additional studies are underway to determine the rate of accretion of such specific elements as calcium, phosphorus and magnesium in the skeleton length and weight of specific bones and the ash content of the entire skeleton.

For each weight category through 300 g the female rats have the same skeletal lengths as measured from snout to anus as the males. At each of these weights the females were older than the males which suggests that at comparable ages, the males would have greater snout to anus lengths.

Both the male and female obese rats weighing 450 g had similar body lengths (24.8 cm). However, above this weight, the females showed no appreciable changes in length whereas the males did. For the males, this apparent increase in length continued up to a weight of 1050 g.

Body protein

The increase in body protein was related to age up through 150 days (Fig. 4 and 5). After that age, both male and female rats fed the high fat ration appeared to deposit more protein in their bodies than the grain fed rats. This may have been associated with the marked expansion of the adipose tissue in the obese rats. The cellular material within which

the fat was deposited contains some protein and this with an expansion in the skin may have accounted for the extra protein.

Body fat

For both the males and females, the increase in absolute body fat is linear with time (Fig. 6 and 7) if the rats were fed the high fat ration. This was also true for rats consuming the grain ration as long as there was an increase in body weight. However, when body weights plateaued body fat behaved similarly. The rate and absolute amount of fat accumulated in the body was significantly ($P<0.01$) greater for the high fat-fed rats than for the grain-fed animals. Within each weight group, it appeared that the animals gaining weight most rapidly had a larger amount of fat in their bodies than those reaching the same weight but at a slower rate. The latter may be associated with the observation that the gain in body protein is related to the age of the animal.

Fat depots

Subcutaneous: There was no consistent correlation between the weights of the 3 subcutaneous fat depots studied and body weight of the animals. If there were no loss in weight, the inguinal fat depot more nearly mirrored the changes in body weights especially for the rats fed the high fat ration. Neither the fat depots underlying the forelimb nor the interscapular depot were directly related to body weight changes when fed the high fat ration. On the other hand, they were quite closely related to body weight changes when fed the grain ration.

The inguinal fat depot of the high fat-fed rats was about twice as heavy as the same depot in the grain-fed rats when expressed as percent of body weight (Fig. 8). The inguinal fat pads in the 150 and 300 g male

and female rats fed the high fat ration were the same relative weight (as a percent of body weight). Above that, the weight of this depot in male rats represented a larger but constant proportion of the body weight. For the grain-fed rats, the relative size of the inguinal fat depot was also approximately the same for males and females up to 300 g or 150 days. After that, this depot in males became relatively larger. In the grain-fed male rats, the inguinal fat depot was increased in relative weight more rapidly than the body weight as long as the rats continued to gain weight. It was probably associated with the increased amount of body fat laid down in male rats during this period (Table 3).

Fat accumulated in depots underlying the forelimb at a faster rate in the high fat-fed than in the grain-fed rats (Fig. 9). Especially for the latter and to a certain extent for the former, the accumulation of fat in this depot was proportional to the gain in body weight.

The rats fed the high fat ration showed large accretions of fat in the interscapular area spreading throughout the cephalad portion of the body to the base of the ribs. The females showed a greater propensity in this direction than the males (Fig. 10). As the females increased in weight from 300 to 885 g, this fat depot changed from slightly less than 2.0% of body weight to 10.0%. In comparison to weanling female rats, the obese female rats showed an increase in relative weight of the interscapular depot which was over 300 times greater than the corresponding change in body weight. The interscapular fat deposit in the female obese rat was often so large and loose that it could readily be moved from one shoulder to the other.

In the rats fed the grain ration, the interscapular depot represented only 0.2 to 0.8% of body weight wherefrom it did not deviate as the rats became heavier.

Abdominal: The genital depots in both male and female rats fed the high fat ration were about twice as large as in the animals fed the grain ration (Fig. 11). This difference was highly significant ($P<0.01$). On a relative basis, this depot was larger in the females for both dietary groups. For all but the male rats fed the grain ration, the greatest rate of increase in the relative weight of the genital depots occurred early in life. During this time, these depots increased in relative size more rapidly than the body weight. When the rats became older, these depots grew at a rate comparable to body weight changes or even less rapidly. The exception to this was the group of male rats fed the grain ration. Here a marked increase in relative size of the genital depots occurred as body weight went from 450 to 600 g.

The perirenal and retroperitoneal fat depots increased in weight at a much more rapid rate than body weight (Fig. 12). This was true for both groups of rats and in both cases; the curves for the male and female animals were essentially the same. The rats fed the high fat ration, especially above body weights of 150 g, had heavier perirenal and retroperitoneal fat depots than those fed the grain ration ($P<0.01$). The male rats fed the grain ration showed a marked increase in the weight of these depots after the body weights of these animals exceeded 450 g. The acceleration in the growth of these depots at that stage may be a reflection of the deposition of large amounts of fat in the bodies of the grain-fed rats at that age (Table 3). In the high fat-fed rats, the rapid

deposition of body fat had been occurring from an early age; the steeper slope for the perirenal and retroperitoneal fat in these animals may be a reflection of this phenomenon.

Since the mesenteric and omental depots were both associated with the gastrointestinal tract and occurred in one area, they were weighed together (Fig. 13). The capillary plexus of the mesentery depot was included in this depot. The presence of the capillaries in this depot may partially explain why there was no difference in their weights for the 150 g rats ($P>0.05$). Although the weights of the depots were the same, it was apparent on inspection that more fat was present in this tissue in the high fat-fed animals. For the grain-fed rats, the relative size of the depot was proportional to the body weights of the animals whereas in the high fat-fed rats, the size of this depot increased more rapidly than body weight and showed a relative decrease in weight with aging (Fig. 13).

Young rats, especially those fed the grain ration, showed very little fat around the xiphoid process (Fig. 14). The female rats fed the high fat ration showed a proportionately rapid rate of increase in the weight of this depot. This rate of increase was much greater than that for the male rats fed the same high fat ration. The latter showed an increase in the size of this depot which paralleled that of the grain-fed rats. In the obese rats, the size of this depot and other fat deposits underlying the diaphragm were so prominent as to suggest possible interference with cardiovascular activities.

In the young rat, weight of the subcutaneous fat depots was greater than those in the abdominal area. However, as the animals matured, the situation was reversed. For the female rats, this occurred at a body weight of 300 g and for the males at 600 g (Table 4).

DISCUSSION

The obese rat does not develop a larger skeleton to support its additional body weight. However, since the total ash represented the minerals present in many tissues besides the skeleton, it cannot be considered a true index of size or degree of mineralization of the skeleton. The justification for the use of body ash as a crude index of skeletal size comes from the similarity in size of the lean body mass in the grain and high fat fed rats. Studies are under way to more critically evaluate skeletal size and composition in the lean and obese rats.

For both male and female , the consistently lower quantity of protein in rats eating the high fat ration reflects the difference in age at sacrifice. For the same age, the development of lean body mass was similar whether the rats consumed the grain or the high fat ration. The older obese rats showed a slight increase in total protein. When adipose tissues contain a certain amount of fat, these tissues proliferate with development of more structural material (Hausberger, 1959). The increased fat-free component of the adipose tissues most likely accounts for some of this increase in protein. Future work will be directed to check this assumption. It should be pointed out that in the adult rats, protein represented 22 to 24% of the lean body mass regardless of whether the rats were fed grain or high fat ration. Other investigators have reported similar results (Fig. 15).

Numerous investigators have shown that weight gains in obesity are due to increased body fat (Hetherington and Weil, 1940; Mickelsen and Anderson, 1959; Vlahakis and Heston, 1959; Zucker and Zucker, 1963). This present series of analyses would indicate that this is true for all ages.

In every instance, the consumption of the high fat ration caused large increases in adipose tissue weights. This difference was always highly significant ($P<0.01$) except in the case of the depots underlying the forelimb where the difference was significant ($P<0.05$). The one exception to this was the mesenteric and omental depots in rats weighing 150 $\pm$ 10 g wherein the weight of the capillary plexus obscures the difference in weight of the fat depots (Table 5). Adipose tissues displaying particularly extensive accumulations of weight with the consumption of the high fat ration were the perirenal depot, the adipose tissue surrounding the xiphoid process and the interscapular depot; the latter was especially true for female rats.

With few exceptions, weight gains of the subcutaneous adipose tissues, the mesenteric and omental depots and that depot surrounding the xiphoid process were similar in males and females. However, accretions of weight in the interscapular area of females consuming the high fat ration were particularly pronounced. The inguinal depot and adipose tissue underlying the forelimb appear able to increase markedly in the male. Genital depots were larger in females while perirenal depots were larger in the males. The difference in the subcutaneous adipose tissue weights of the weanling male and female rats reflects the slightly heavier weight of the females (Table 3).

Table 6 summarizes the effect of age upon the weight gains of adipose tissues. From weaning to 99 days, when rats were fed the high fat ration, all depots showed increases in weight which were greater than the increases in body weight. This was particularly pronounced for the genital and perirenal depots. The large percentage of increases of the

latter depots, however, reflect the fact that in weanling rats these depots are relatively smaller than the subcutaneous depots. This would likewise partially account for the extensive percentage increase in these two depots when rats were fed the grain ration. After the first 100 days, subcutaneous depots increased in weight similar to body weight increases. The exception to this was the interscapular fat depot when rats were fed the high fat ration. This depot continued to show increases throughout the lifespan. For this depot, the relative weight increase ranged from 120 to 199% of body weight increase. For each 100 day interval, the increase in weight of this depot for the males was always at the lower range, while that for females was at the upper range.

After the initial large increases in relative weights of the genital depots, they showed no further relative weight gains when rats were fed the high fat ration. With further aging this depot began to decrease in relative size for both males and females. When rats were grain-fed, the same trend occurred but later. At the end of 400 days, the same drop-off in relative weight had not yet occurred in the females. The mesenteric and omental depots showed a similar drop-off in relative weight with aging for the rats fed the high fat ration.

Only the perirenal depot and the depot surrounding the xiphoid process continued to gain in relative weight. However, in the case of the grain-fed female, no changes in relative weight were observed.

Table 2

High fat ration (M-15)

Ingredients	%
Crisco	60.0
Casein	25.0
Mineral mix[1]	5.0
Vitamin mix[2]	2.2
Non-nutritive fiber[3]	2.0
Aureomycin[4]	0.01
Liver powder	2.00
dl-Methionine	0.25
Sucrose	3.54

Calories per g; 6.62

[1] Salt mix, Wesson modified (Osborne-Mendel) General Biochemicals, Chagrin Falls, Ohio.

[2] Vitamin diet fortification mixture from Nutritional Biochemicals Corp., Cleveland, Ohio.

[3] Non-nutritive fiber, cellulose type from General Biochemicals, Chagrin Falls, Ohio.

[4] Generously provided by American Cyanamid Company, Princeton, New Jersey.

Table 3

Body weights, age, length and composition of Osborne Mendel rats fed either a grain or high fat ration

Ration	Body Weight sac. (g)	Body Weight less G.I. content(g)	Age (days)	Body Length (cm)	Body Composition Moisture (g)	Fat (g)	Protein (g)	Ash (g)
				Male				
W.	58.0± 3.6[1]	51.6± 3.1[1]	23.2± 0.4[1]	12.9± 0.4[1]	37.37± 0.79[1]	3.39± 0.56[1]	8.74±0.64[1]	1.50±0.10[1]
M-1[2]	149.6± 6.37	139.8± 6.5[6]	38.4± 4.3[4]	18.5± 0.5[4]	96.34± 1.23[4]	12.32± 2.19[5]	26.03±2.89[4]	4.34±0.43[6]
M-15[3]	158.2± 7.41	153.8± 7.7	38.4± 7.7	18.9± 0.8	95.44± 1.63	28.30± 4.49	25.62±2.84	3.45±0.52
M-1	298.2±11.1	282.0±18.5[4]	61.0± 7.0[4]	23.2± 0.4[4]	179.66± 4.66[4]	33.38± 5.55[5]	57.98±8.16[4]	8.64±1.64[6]
M-14	304.8± 6.4	299.0± 7.6	57.8± 9.0	23.1± 1.0	163.67± 2.30	73.08± 5.75	55.00±4.01	6.70±0.89
M-1	444.8± 9.7	428.0± 9.7[6]	130.2±18.8[6]	25.7± 0.4[4]	265.71± 3.34[5]	51.47± 5.75[5]	95.86±4.96[5]	13.66±1.58[5]
M-15	455.2± 8.3	448.0± 8.4	76.2± 6.0	24.8± 1.0	210.45± 8.51	148.20±20.38	79.04±2.43	10.19±0.35
M-1	589.8± 8.6	571.4±10.8[5]	274.0±57.6[4]	27.7± 0.6[4]	324.00± 8.83[5]	119.30±17.93[5]	105.06±5.43[5]	17.01±2.49[5]
M-15	608.6± 5.6	598.8± 5.4	147.6±30.5	27.4± 1.1	291.89± 6.36	196.65±12.68	92.84±4.70	13.64±1.10
M-15	748.0± 8.8	741.0±11.5	200.8±16.0	27.9± 0.9	335.87± 9.82	277.28±34.94	108.42±3.41	14.17±1.07
M-15	893.0± 6.9	883.4± 9.8	261.6±68.5	28.3± 0.9	353.81± 8.69	389.85±19.06	112.00±6.99	15.45±1.47
M-15	1052.8±12.8	1043.0±13.71	331.2±82.4	29.0± 0.4	375.13± 3.83	524.88±16.82	123.65±8.26	15.39±2.37

1 Means and standard deviation of 5 rats in each group.

2 M-1 (grain ration).

3 M-15 (high fat ration).

4 Not significantly different from grain control.

5 $P<0.01$.

6 $P<0.05$.

Table 3 (continued)

Body weights, age, length and composition of Osborne Mendel rats fed either a grain or high fat ration

Ration	Body Weight sac (g)	Body Weight less G.I. content(g)	Age (days)	Body Length (cm)	Body Composition Moisture (g)	Fat (g)	Protein (g)	
				Female[1]				
W.	61.6± 5.9[1]	57.5± 6.4[1]	23.8± 0.7[1]	13.4± 0.1[1]	39.71± 1.41[1]	4.65± 1.87[1]	10.49±1.91[1]	1.94±0.35[1]
M-1[2]	158.2± 6.6	150.6± 3.0[4]	45.4± 5.4[4]	19.0± 1.0[4]	100.01± 1.99[5]	15.55± 2.54[5]	28.42±2.04[4]	4.75±0.56[4]
M-15[3]	152.2± 7.6	146.0± 7.5	40.2± 6.0	18.1± 1.1	85.94± 2.99	30.32± 6.00	25.66± 1.73	3.89±0.58
M-1	295.4± 6.7	281.4± 7.0[6]	125.0±34.7[6]	23.3± 0.5[4]	168.61± 2.06[5]	45.00± 4.81[5]	56.37± 5.18[6]	9.59±0.91[4]
M-15	298.0± 7.6	294.6± 7.8	83.4±12.6	22.2± 0.6	131.23± 5.33	106.41±13.46	48.43± 4.07	7.61±1.52
M-15	450.4± 7.52	443.6± 8.1	158.2±19.6	24.8± 0.8	172.01±12.21	196.48± 7.06	64.88± 5.65	10.57±1.26
M-15	599.6± 7.14	593.2± 7.6	244.0±26.8	25.3± 0.3	240.54± 5.98	268.61±20.99	69.60± 3.31	10.77±0.66
M-15	744.2± 8.59	734.8±10.3	322.2±49.7	25.6± 0.4	246.82± 6.45	404.95±34.56	75.98± 4.18	11.32±1.05

1 Means and standard deviation of 5 rats in each group.

2 M-1(grain ration).

3 M-15 (high fat ration).

4 Not significantly different from grain control (M-1).

5 $P<0.01$.

6 $P<0.05$.

Table 4

Total weight of subcutaneous fat depots vs. abdominal fat depots

Ration	Weight sac. (g)	Subcutaneous depots[1] (g)	Abdominal depots[1] (g)
		Males	
W.	58.0± 3.6[2]	2.19± 0.20[2]	1.09± 0.16[2]
M-1[3]	149.6± 6.3	2.80± 0.89	1.85± 0.38
"	298.2±11.1	3.42± 0.47	3.17± 0.73
"	444.8± 9.7	3.75± 0.52	3.67± 0.59
"	589.8± 8.6	4.75± 0.91	6.45± 1.06
M-15[4]	158.2± 7.4	6.42± 1.40	3.44± 0.59
"	304.8± 6.4	8.13± 1.57	8.64± 0.62
"	455.2± 8.3	12.68± 1.94	9.93± 0.80
"	608.6± 5.6	11.81± 2.09	12.97± 3.08
"	748.0± 8.8	10.86± 1.67[5]	12.93± 3.32[5]
"	893.0± 6.9	12.84± 3.27	14.24± 3.53
"	1052.8±12.8	14.18± 1.74	18.02± 1.72
		Females	
W.	61.6± 5.9	2.98± 1.03	1.16± 0.40
M-1[3]	158.2± 6.6	3.36± 0.63	2.31± 0.47
"	295.4± 6.7	3.84± 0.40	5.15± 0.62
"	363.3±36.1[6]	2.91± 0.32[6]	6.26± 0.47[6]
M-15[4]	152.2± 7.6	7.53± 1.66	4.58± 0.65
"	298.0± 7.6	9.04± 1.87	10.86± 1.21
"	450.4± 7.5	10.95± 1.75	15.88± 2.43
"	599.6± 7.1	12.43± 1.41	15.01± 2.18
"	744.2± 8.5	15.46± 3.40	19.11± 2.41

[1] Weights expressed as g per 100 g body weight.

[2] Mean and Standard Deviations.

[3] M-1-grain ration.

[4] M-15-high fat ration.

[5] Two rats in the 750 g group lost 20-30 g which weight was regained prior to sacrifice. Subsequent data (Part III) indicate the inguinal, testicular and mesenteric depots immediately reflect this weight loss.

[6] This is the mean weight and Stand. Dev. of 10 grain-fed female rats. These rats were 400 da. and were the lean controls from the weight reduction experiment (Part III).

Table 5

Relative weights of fat depots in rats fed either grain (M-1) or high fat (M-15) rations. The lowest and highest average values for all groups are listed. All weights expressed as g per 100 g body weight. The average weights represent 5 animals in each weight group ranging from 150 to 1162 g.

Fat depot	Sex	W[1]	Range in average depot weights. M-1[2]	M-15[3]
Inguinal	M	1.44	2.1 - 3.9[4]	4.8 - 9.1[5]
	F	2.00	1.8 - 3.0[6]	4.8 - 7.1[7]
Forelimb depots	M	0.48	0.5 - 0.6	1.0 - 2.0
	F	0.62	0.3 - 0.5	0.7 - 1.5
Interscapular	M	0.27	0.3 - 0.8	0.7 - 3.3
	F	0.35	0.4 - 0.8	1.4 -10.3
Genital	M	0.20	0.5 - 2.6	1.0 - 4.0
	F	0.27	1.0 - 2.9	2.3 - 6.1
Perirenal	M	0.23	0.3 - 3.7	1.4 -14.0
	F	0.25	0.5 - 1.7	1.0 - 9.3
Mesenteric and	M	0.62	0.9 - 1.4	1.0 - 3.0
Omental	F	0.61	9.8 - 1.4	1.2 - 3.5
Depot surrounding	M	tr	0.02- 0.15	0.02- 1.00
Xiphoid process	F	tr	0.02- 0.20	0.02- 0.90

1 Weanling rats.
2 Grain ration.
3 High fat ration
4 Range in rat weights; 140 to 571 g.
5 Range in rat weights; 154 to 1162 g.
6 Range in rat weights; 150 to 363 g.
7 Range in rat weights; 146 to 885 g.

Table 6

Summary of weight changes in adipose tissues relative to body weight changes as influenced by age, diet and sex.

Fat depot	Sex	Age in days and diet							
		21 to 99 days		100 to 199 days		200 to 299 days		300 to 425 days	
		M-1	M-15	M-1	M-15	M-1	M-15	M-1	M-15
Inguinal	M	+	++++	+	same	same	same	same	same
	F	+	++	same	same	same	same	-	same
Forelimb depots	M	same	+++	same	same	same	same	same	same
	F	same	++	same	same	same	-	-	-
Interscapular	M	+	++++	+	same	+	+	same	+
	F	same	++++	same	+	same	+	same	+
Genital	M	++++	++++[1]	+	same	+	-	-	-
	F	++++	++++[1]	+	same	same	same	same	-
Perirenal	M	+++	++++[2]	+	+	+	+	3	+
	F	+++	++++[1]	same	+	same	+	same	+
Mesenteric and Omental	M	+	+++	same	same	same	same	same	-
	F	+	+++	+	+	+	same	same	-
Depot surrounding Xiphoid process	M	+	+++	+	++	+	+	+	+
	F	+	++++	same	+++	same	+	same	+

++++ 4.2 times larger (520 increase)

+++ 2.6 to 4.19 times larger (360 to 519% larger).

++ 1.0 to 2.59 times larger (200 to 359% larger).

\+ 0.2 to 0.99 times larger (120 to 199% larger).

same - No change in relative weight -0.19 to +0.19 (80 to 119% of last value).

\- - 0.2 to 0.99 times smaller (120 to 199% smaller).

[1] Over 10 fold increase (1100%).

[2] Over 20 fold increase (2100%).

[3] Slight increase or decrease; possible "no change".

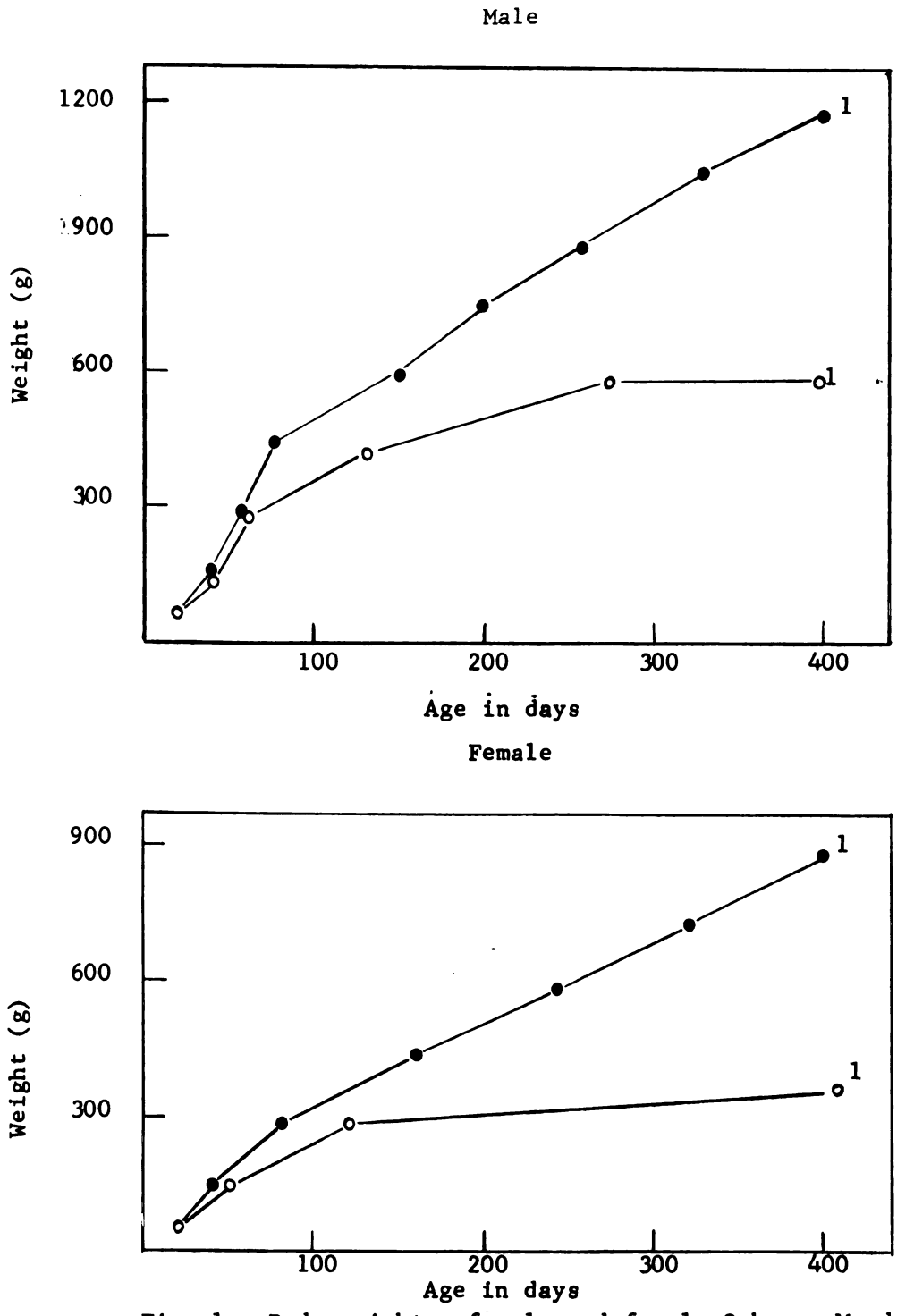


Fig. 1. Body weights of male and female Osborne Mendel rats fed either the high fat or grain ration. Each point represents the mean weight of the five rats analyzed.
1. Mean weight of 400 da. grain-fed and high fat-fed controls (Part III).
●——● High fat ration o——o Grain ration

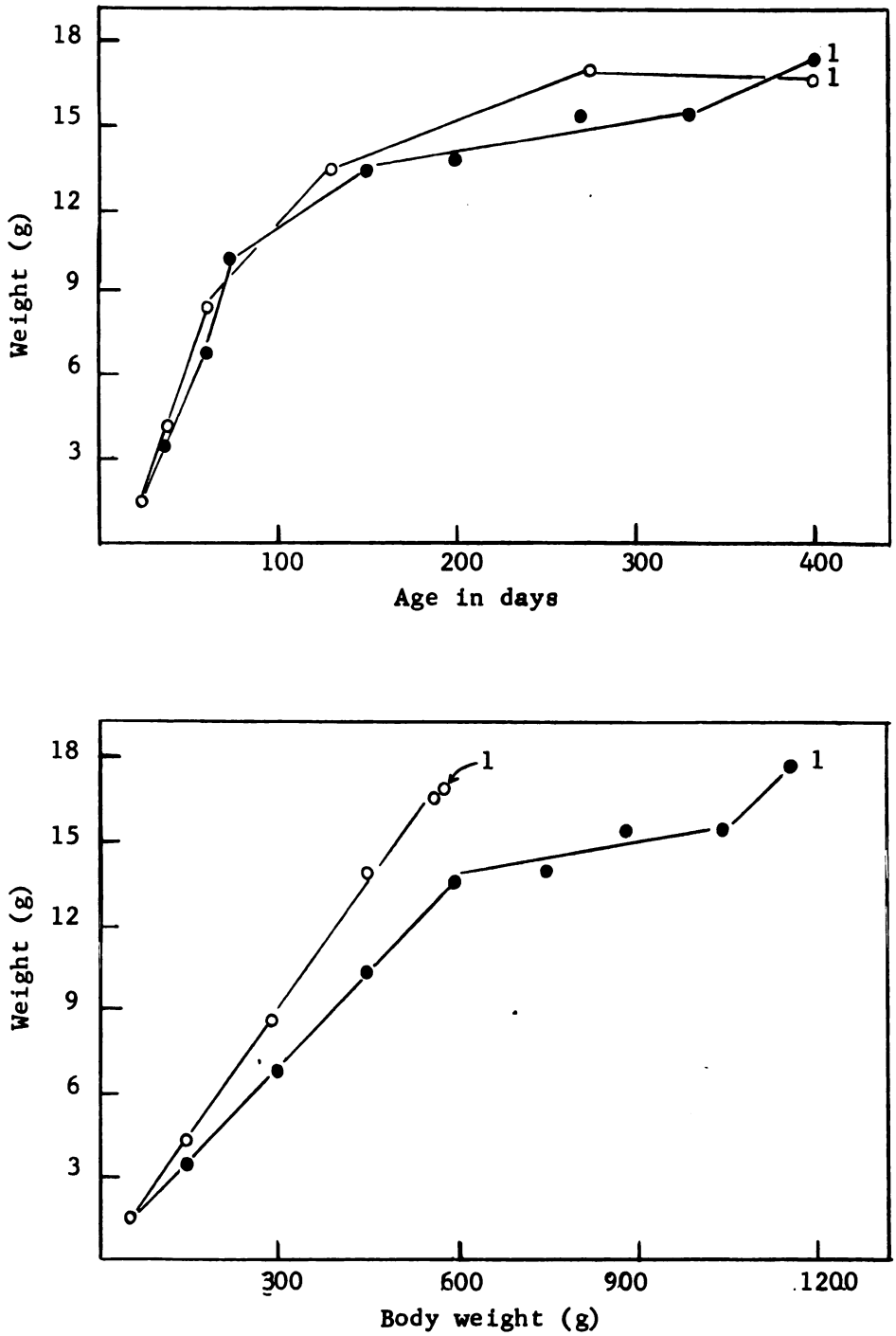
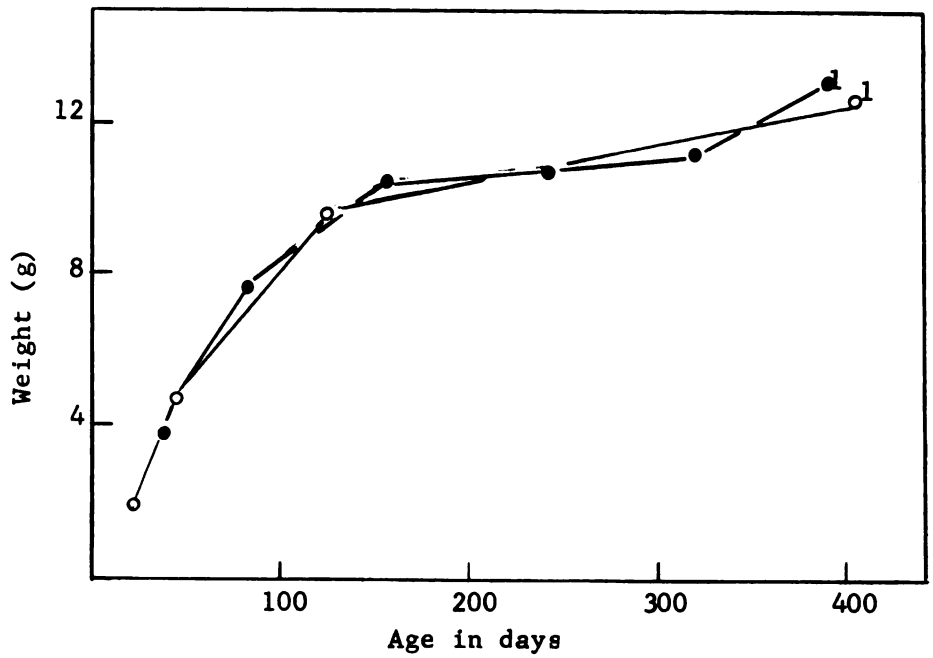


Fig. 2. Increase in body ash content of male Osborne Mendel rats. One group of rats was fed the high fat and the other, the grain ration. Five rats were analyzed for each point on the curves. 1, Mean ash of 400 da. grain-fed and high fat-fed controls (Part III).

●——● High fat ration o——o Grain ration



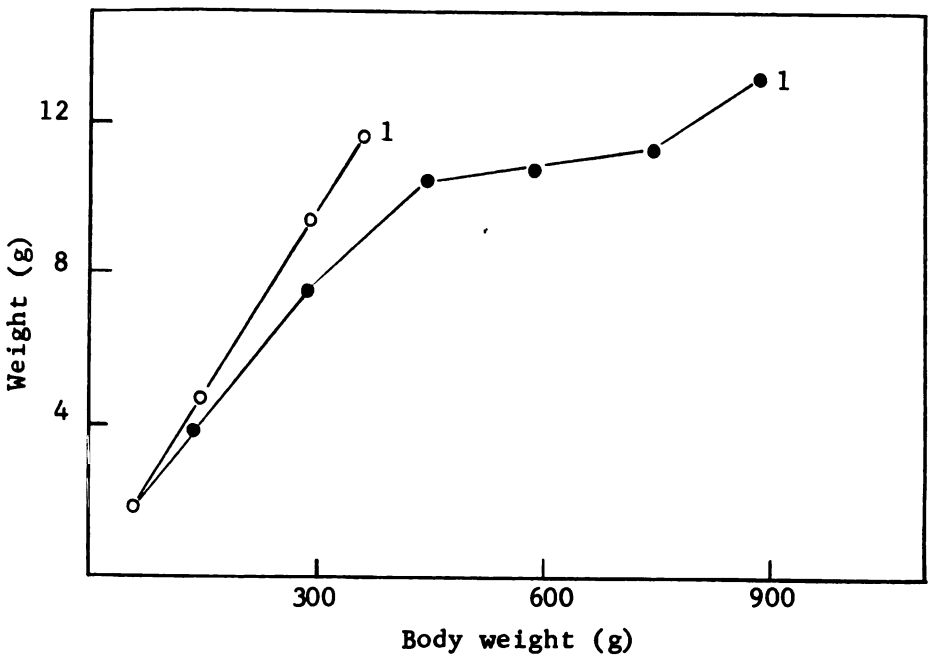
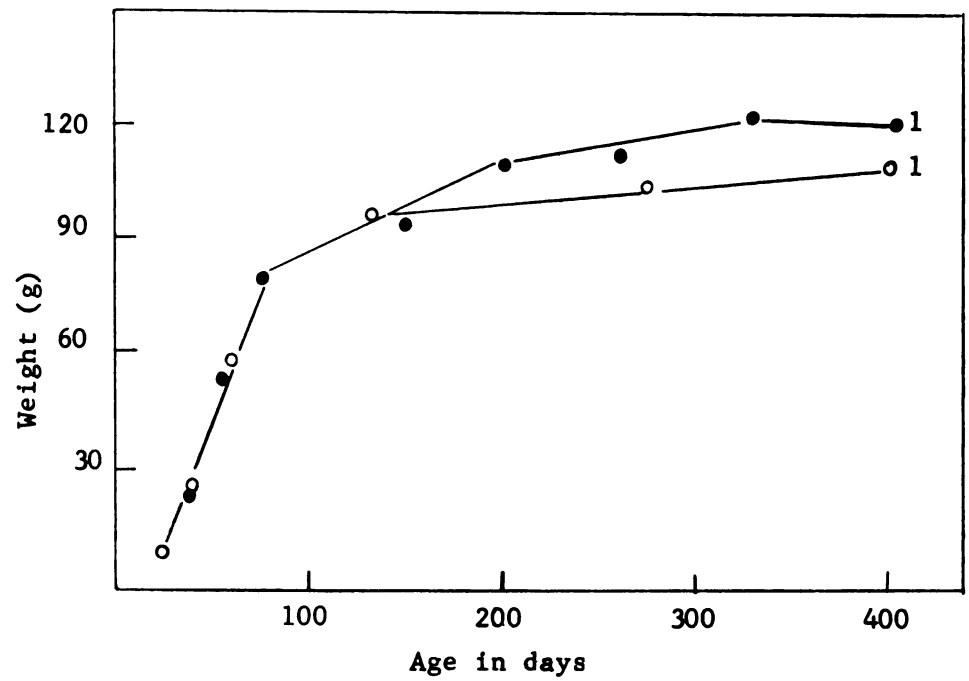


Fig. 3. Increase in body ash content of female Osborne Mendel rats. One group of rats was fed the high fat and the other, the grain ration. Five rats were analyzed for each point on the curves. 1, Mean ash of 400 da. grain-fed and high fat-fed controls (Part III).

●——● High fat ration o——o Grain ration



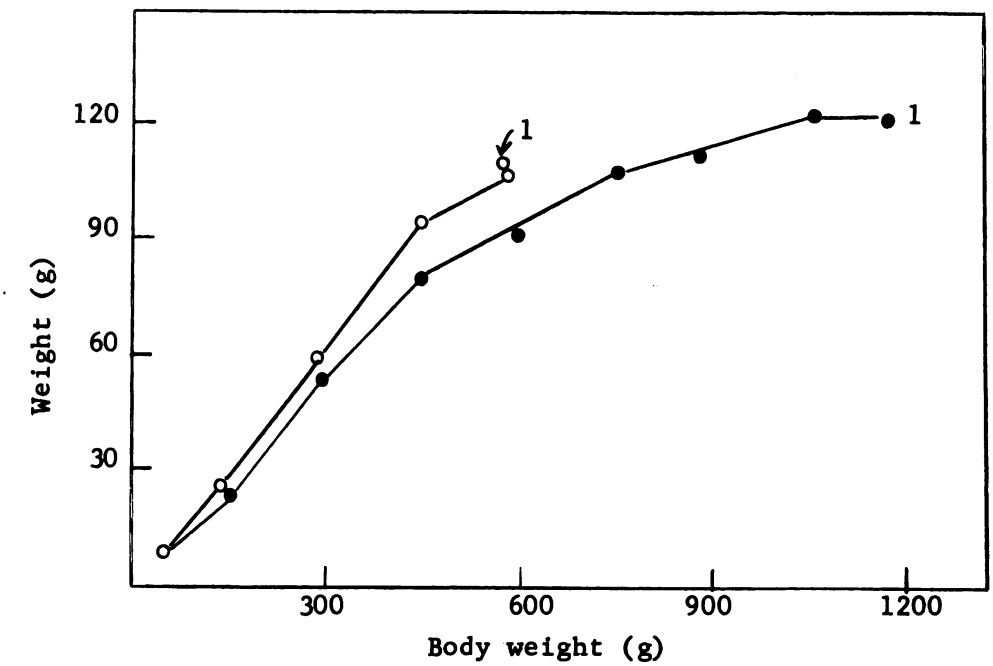
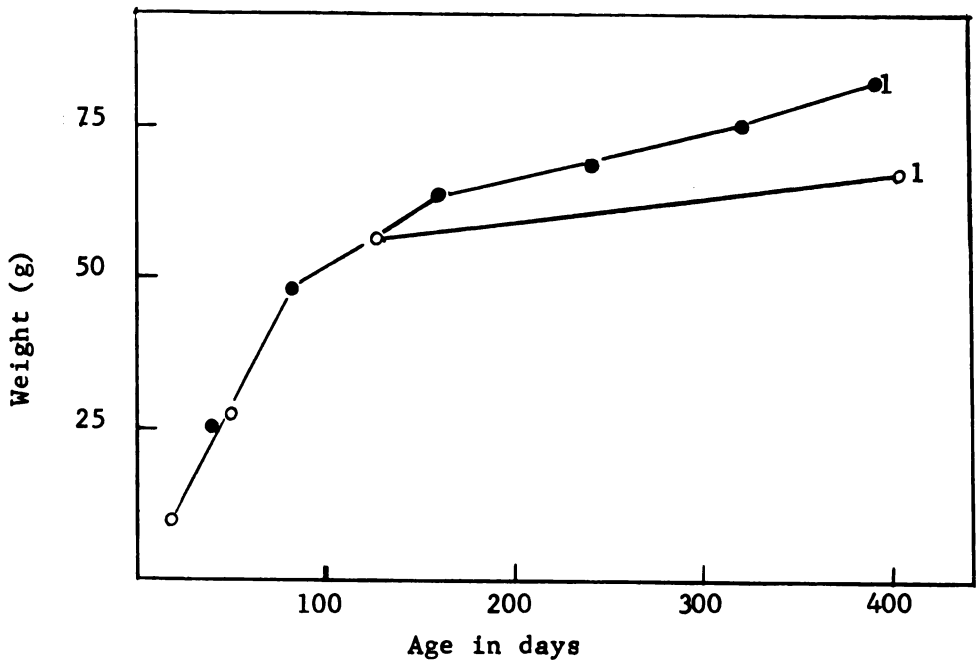


Fig. 4. Increase in body protein content of male Osborne Mendel rats. One group of rats was fed the high fat, and the other, the grain ration. Five rats were analyzed for each point on the curves. 1, Mean protein of 400 da. grain-fed and high fat-fed controls (Part III).

●——● High fat ration o——o Grain ration



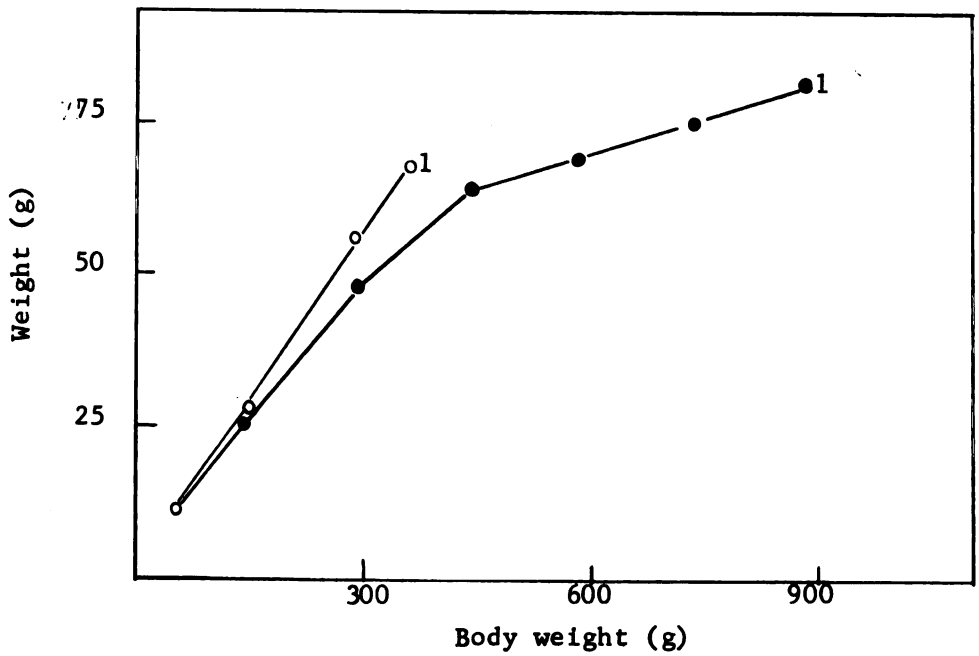
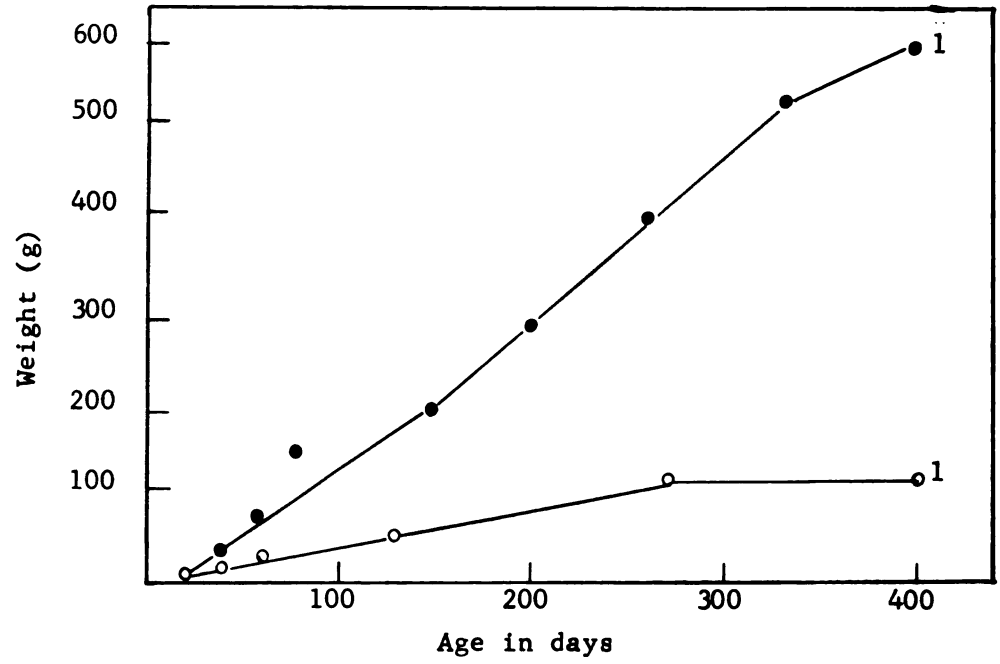


Fig. 5. Increase in body protein content of female Osborne Mendel rats. One group of rats was fed the high fat, and the other, the grain ration. Five rats were analyzed for each point on the curves. 1. Mean protein of 400 da. grain-fed and high fat-fed controls (Part III).

●——● High fat ration ○·····○ Grain ration



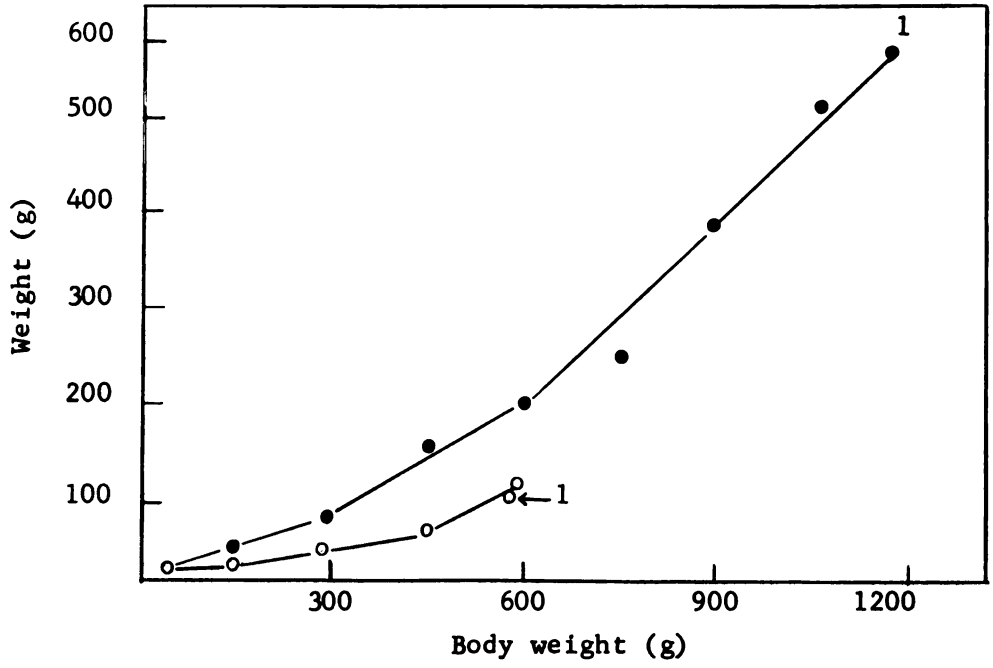
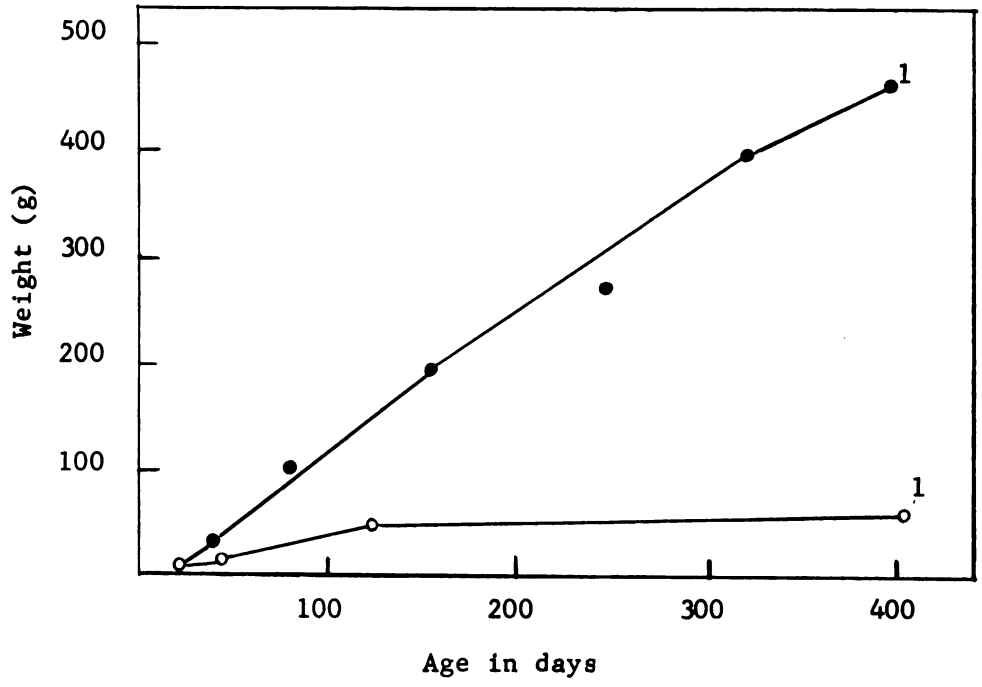


Fig. 6. Increase in body fat content of male Osborne Mendel rats. One group of rats was fed the high fat and the other, the grain ration. Five rats were analyzed for each point on the curves.

1. Mean fat of 400 da. grain-fed and high fat-fed controls (Part III).
●——●High fat diet o——o Grain ration



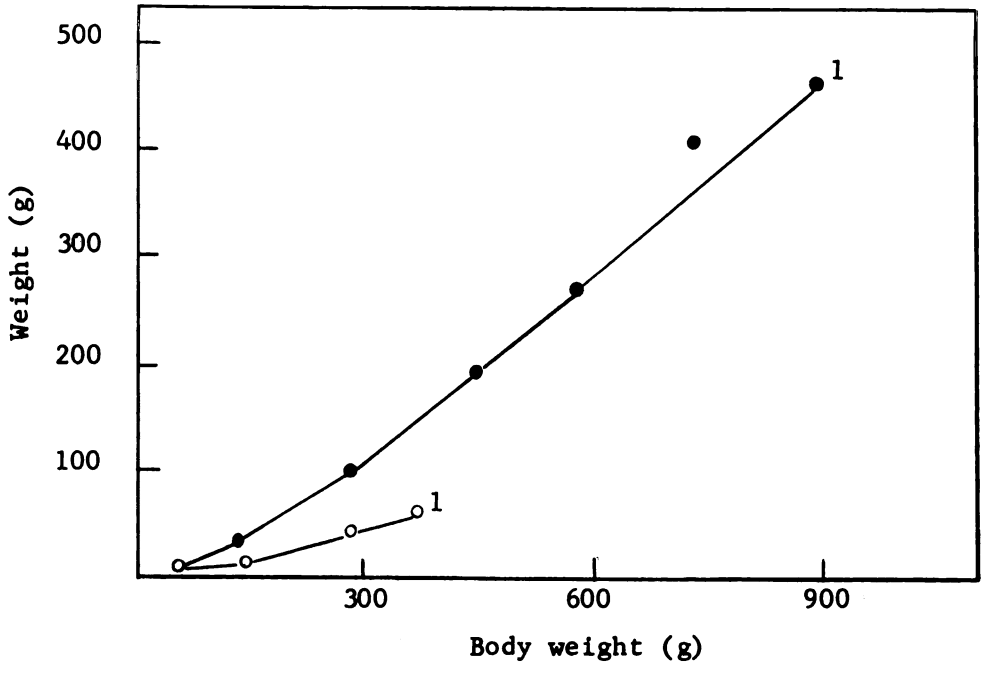


Fig. 7. Increase in body fat content of female Osborne Mendel rats. One group of rats was fed the high fat and the other, the grain ration. Five rats were analyzed for each point on the curves.

1. Mean fat of 400 da. grain-fed and high fat-fed controls (Part III).

●——●High fat diet ○——○ Grain ration

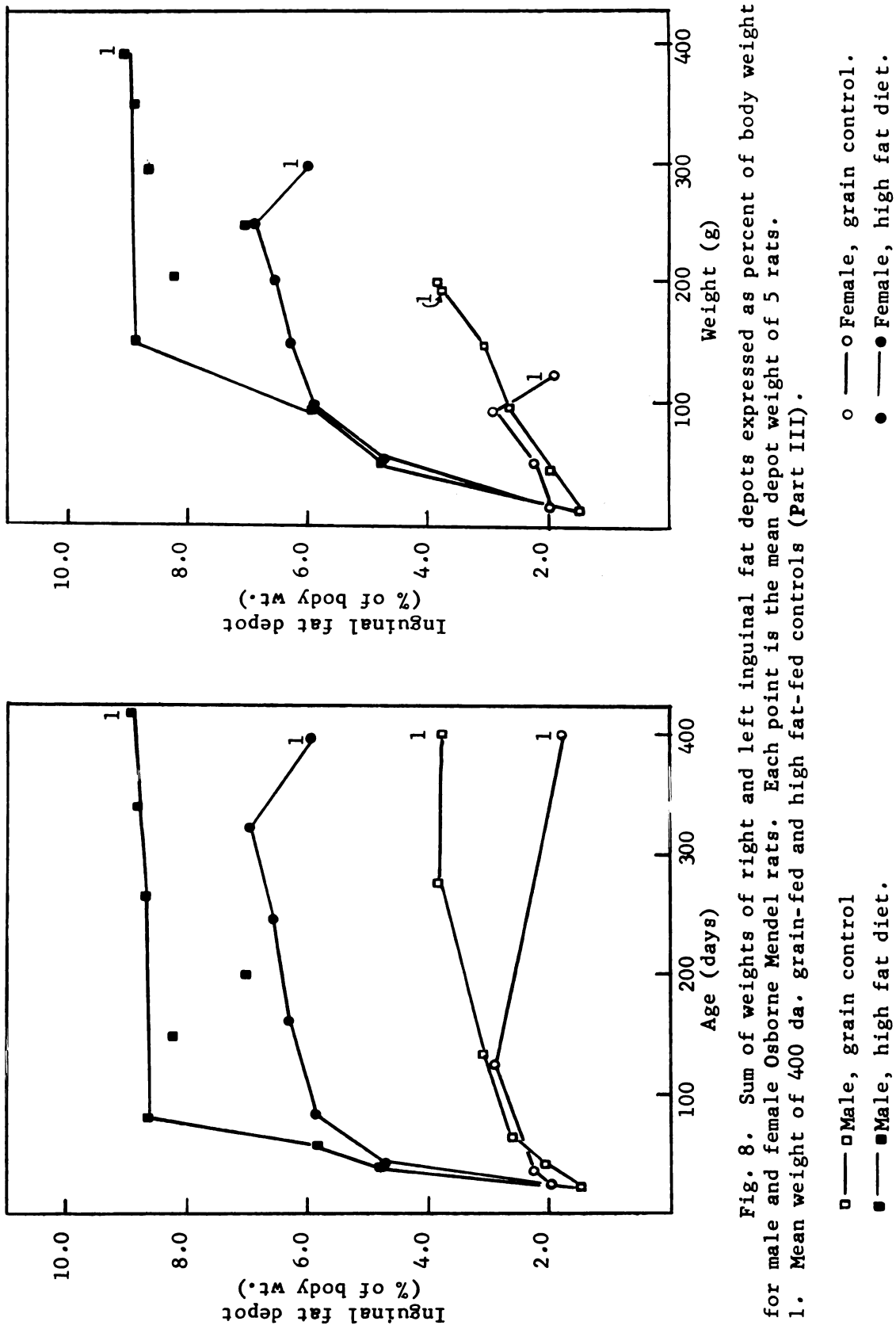


Fig. 8. Sum of weights of right and left inguinal fat depots expressed as percent of body weight for male and female Osborne Mendel rats. Each point is the mean depot weight of 5 rats. 1. Mean weight of 400 da. grain-fed and high fat-fed controls (Part III).

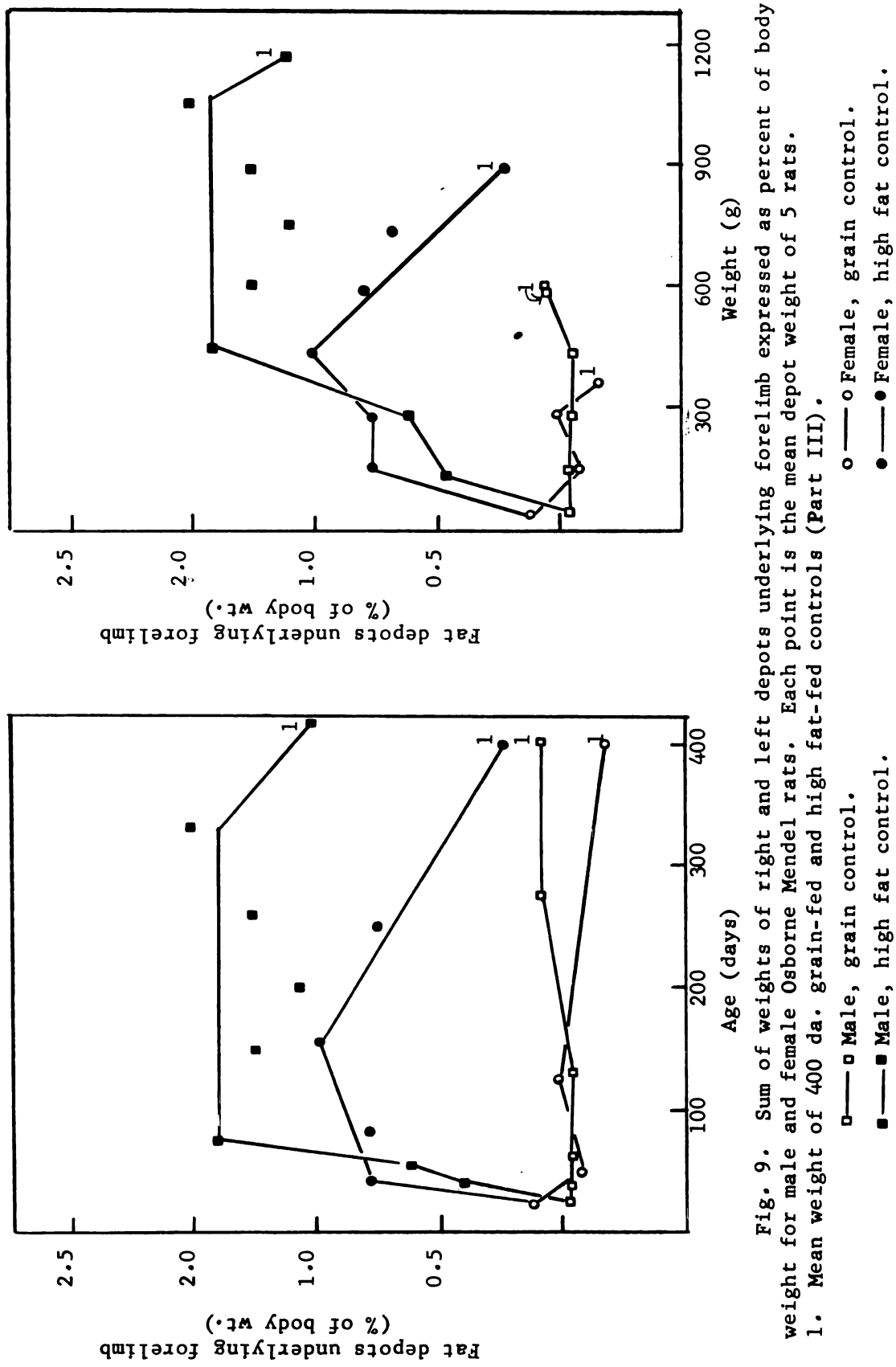


Fig. 9. Sum of weights of right and left depots underlying forelimb expressed as percent of body weight for male and female Osborne Mendel rats. Each point is the mean depot weight of 5 rats. 1. Mean weight of 400 da. grain-fed and high fat-fed controls (Part III).

□——□ Male, grain control.
■——■ Male, high fat control.
o——o Female, grain control.
●——● Female, high fat control.

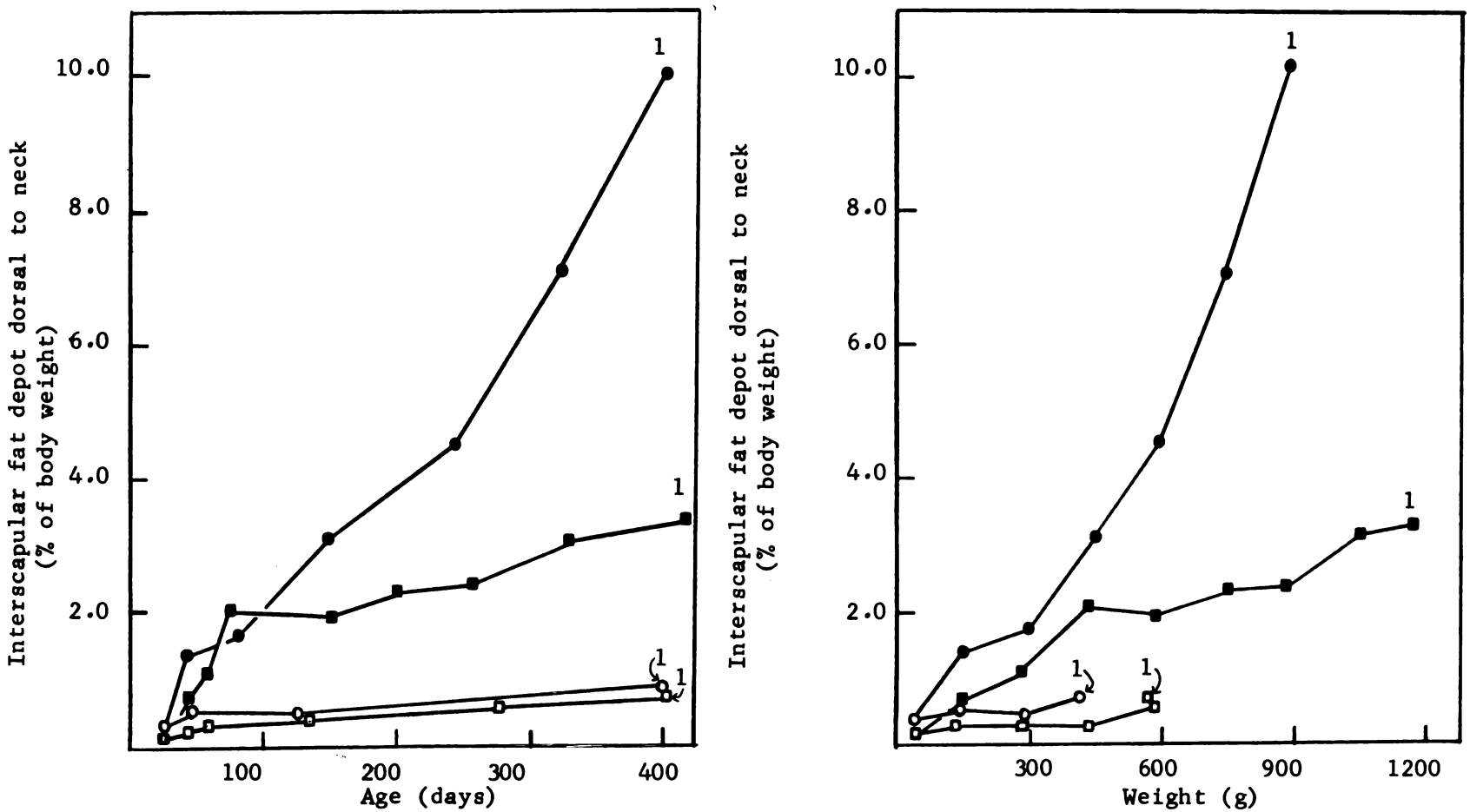
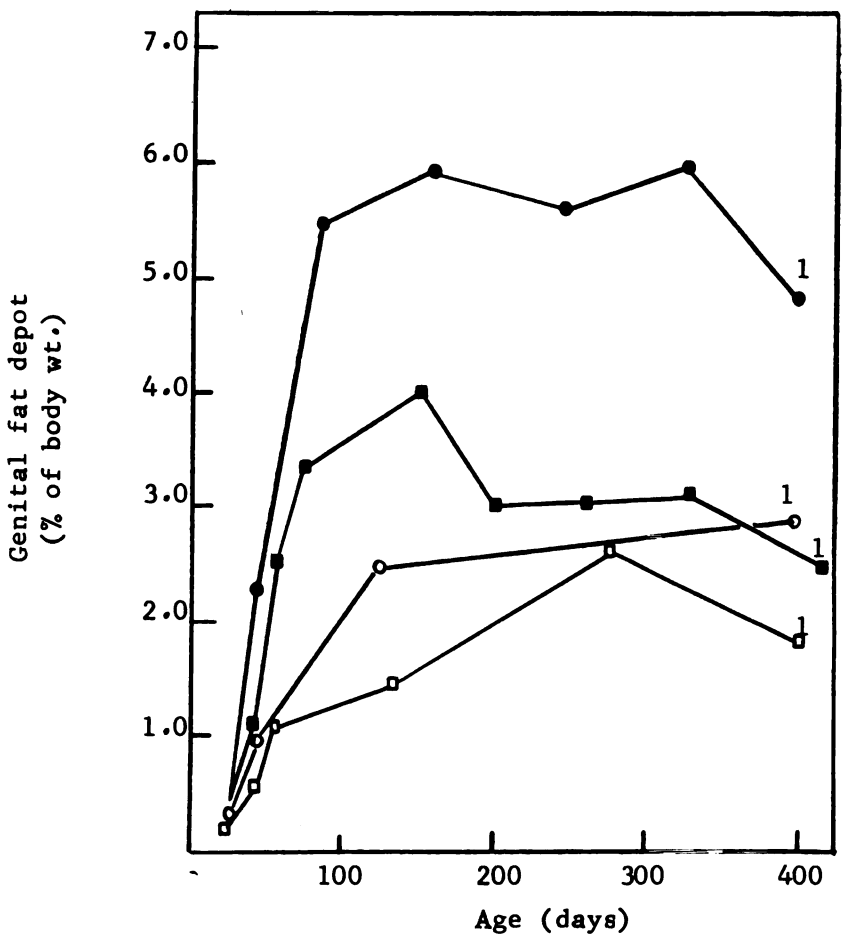


Fig. 10. Weight of interscapular fat dorsal to neck expressed as percent of body weight for male and female Osborne Mendel rats. Each point is the mean depot weight of 5 rats.
1. Mean weight of 400 da. grain-fed and high fat-fed controls (Part III).

□——□ Male, grain ration.
■——■ Male, high fat ration.
○——○ Female, grain ration.
●——● Female, high fat ration.



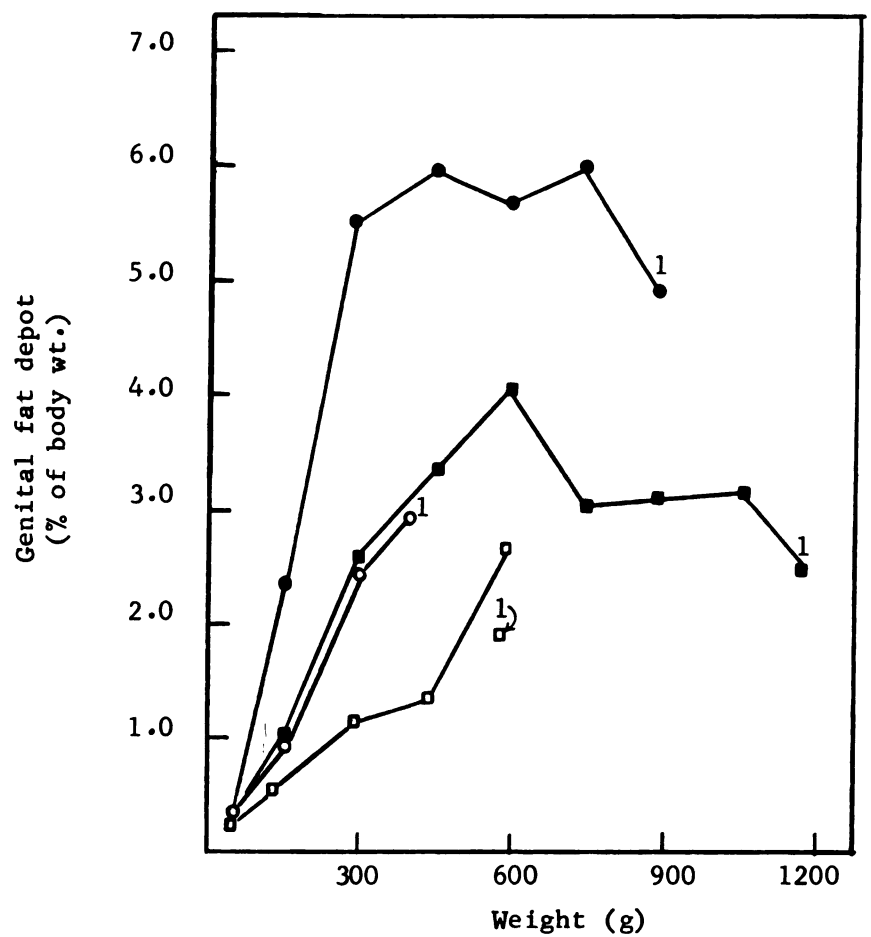
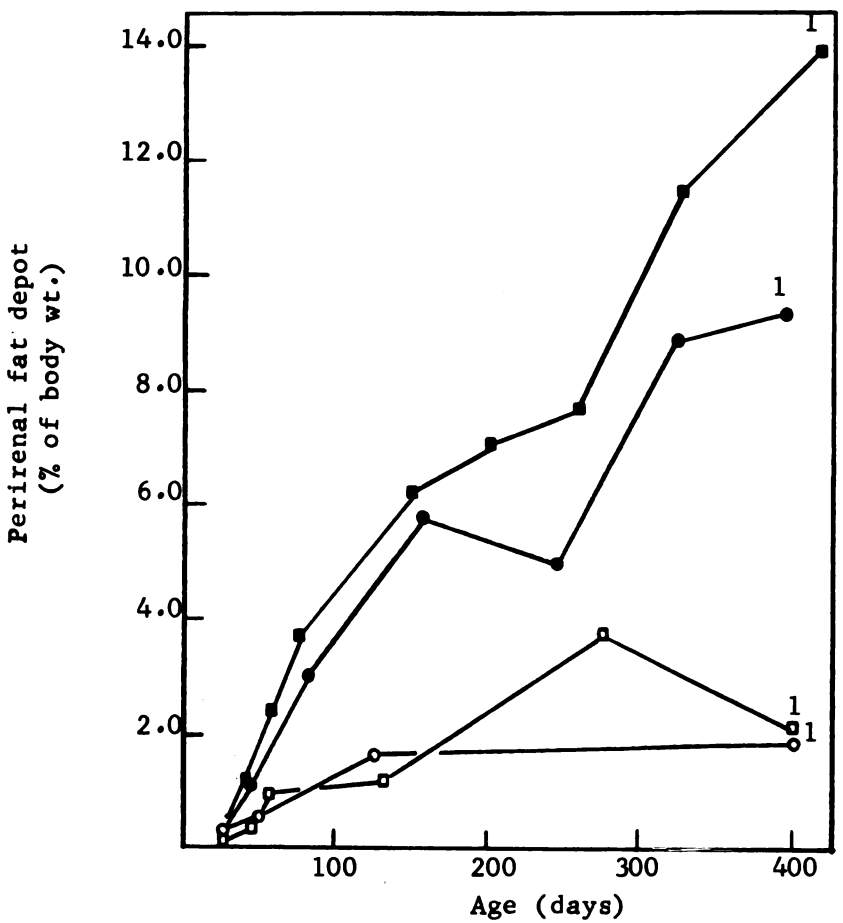


Fig. 11. Sum of weights of right and left genital fat depots expressed as percent of body weight for male and female Osborne Mendel rats. Each point is the mean depot weight of 5 rats.
1. Mean weight of 400 da. grain-fed and high fat-fed controls (Part III).

□——□ Male, grain ration.
○——○ Female, grain ration.
■——■ Male, high fat ration.
●——● Female, high fat ration.



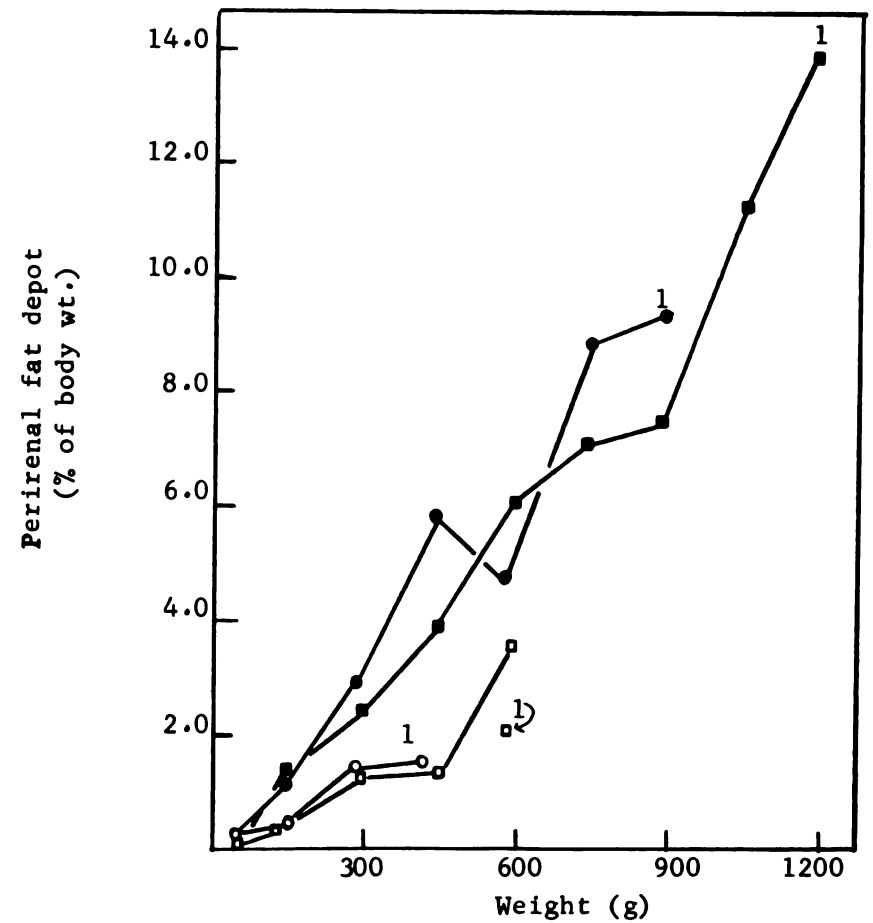


Fig. 12. Sum of weights of right and left perirenal and retroperitoneal fat depots expressed as percent of body weight for male and female Osborne Mendel rats. Each point is the mean depot weight of 5 rats. 1. Mean weight of 400 da. grain-fed and high fat-fed controls (Part III).

□——□ Male, grain ration.

■——■ Male, high fat ration.

○——○ Female, grain ration.

●——● Female, high fat ration.

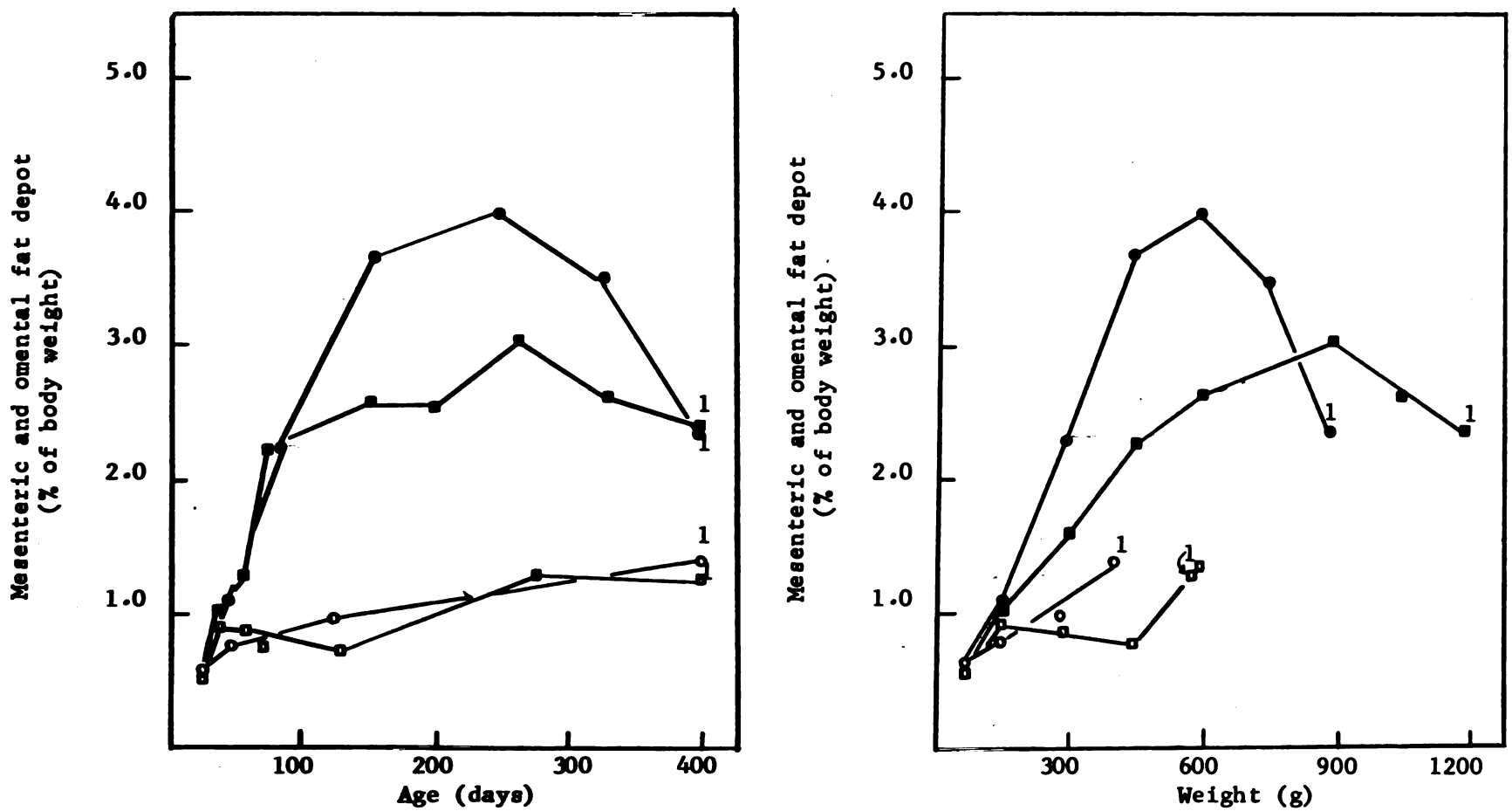


Fig. 13. Weight of mesenteric and omental fat depots expressed as percent of body weight for male and female Osborne Mendel rats. Each point is the mean depot weight of 5 rats.
1. Mean weight of 400 da. grain-fed and high fat-fed controls (Part III).

□——□Male, grain ration.
■——■Male, high fat ration.
○——○ Female, grain ration.
●——● Female, high fat ration.

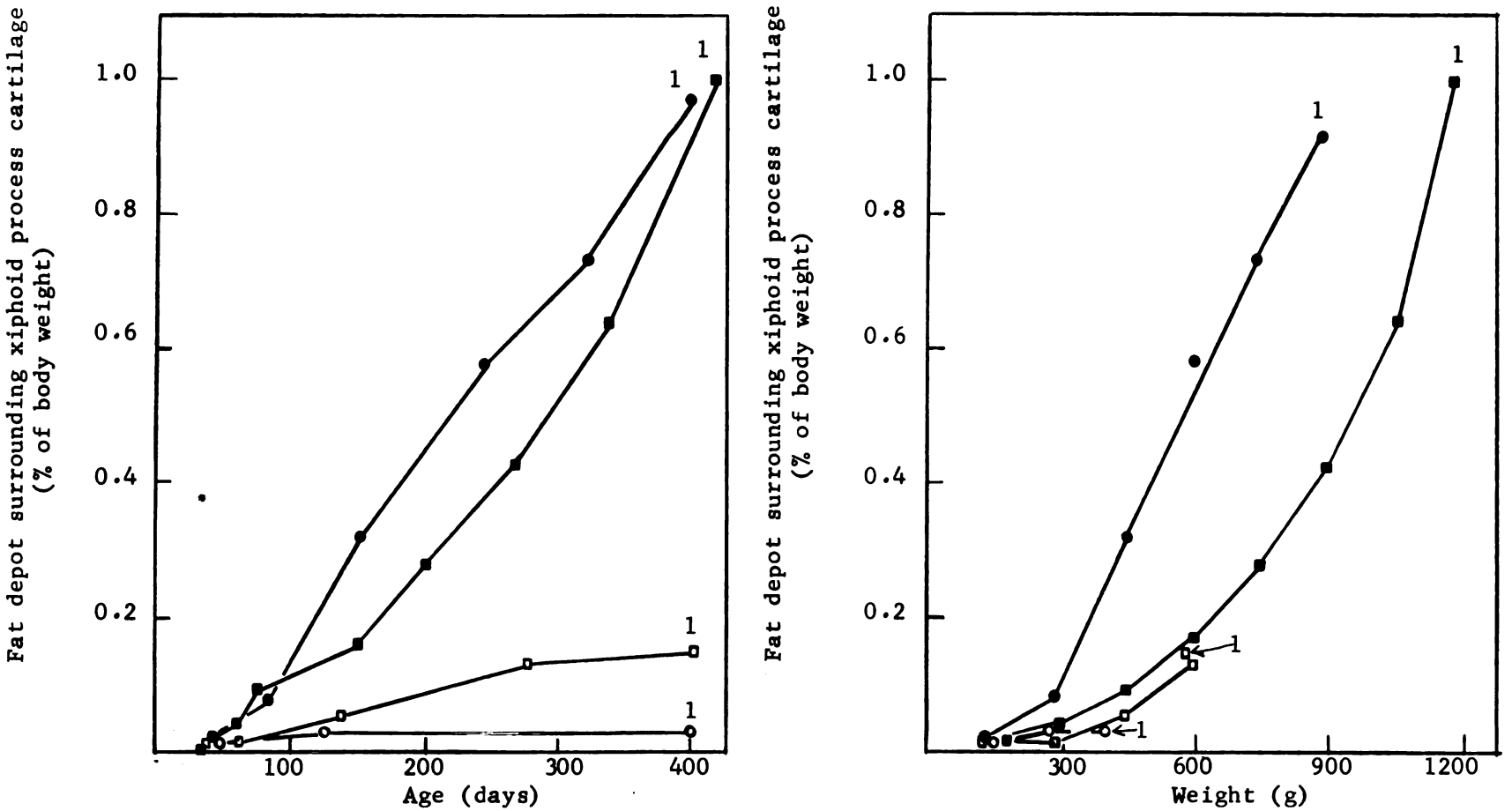


Fig. 14. Weight of fat depot surrounding xiphoid process cartilage expressed as percent of body weight for male and female Osborne Mendel rats. Each point is the mean depot weight of 5 rats.
1. Mean weight of 400 da. grain-fed and high fat-fed controls (Part III).

▫——▫Male, grain ration.
▪——▪Male, high fat ration.
o ——o Female, grain ration
● ——● Female, high fat ration.

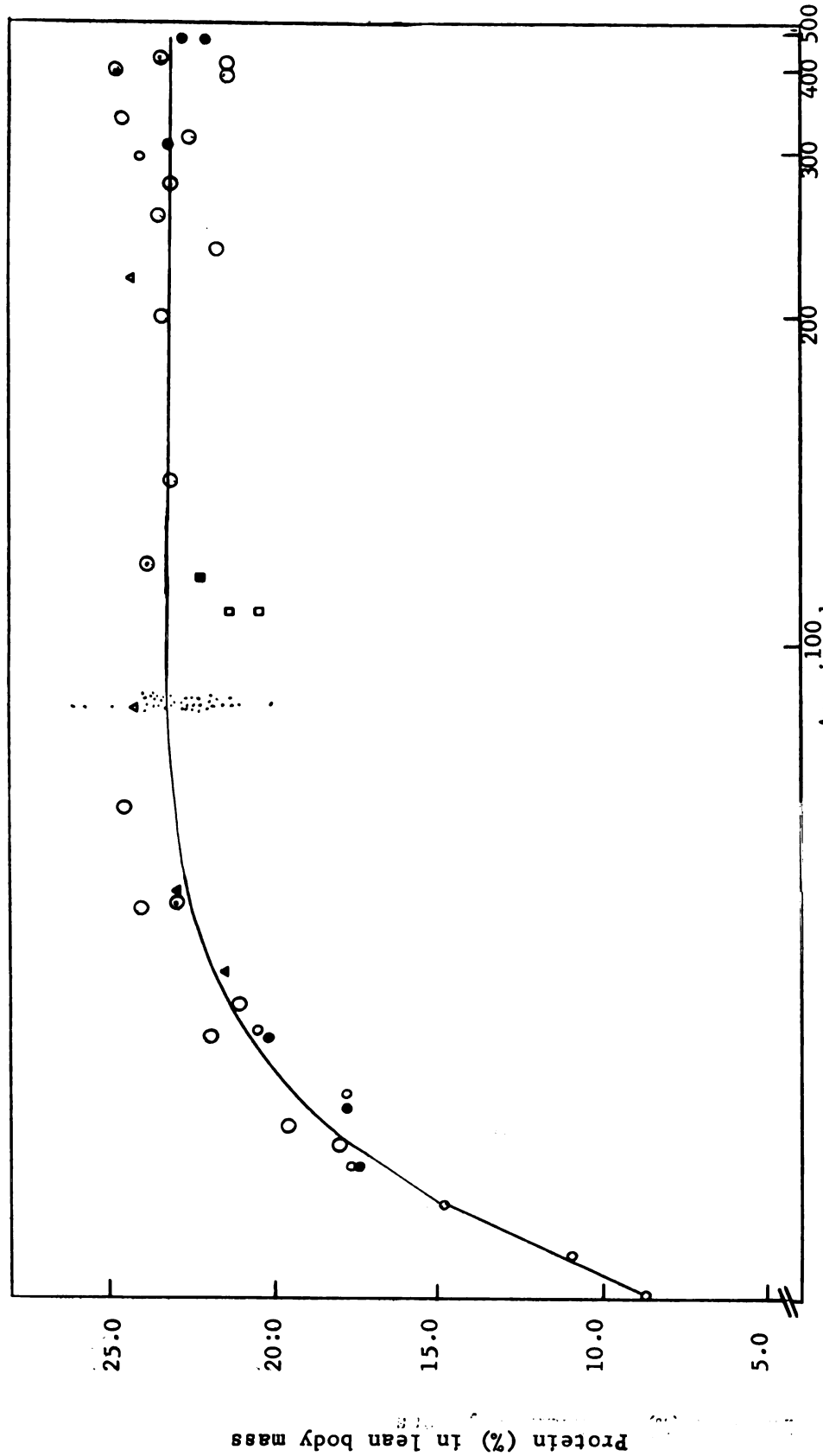


Fig. 15. Percent of protein in rats of various ages expressed as percent of lean body mass as determined by different investigators.

■ - Light et al. (1934) (7 rats).
▫ - Deuel et al. (1944) (102 rats).
▲ - Chanutin (1930,1931) (56 rats).
○ - Hatai, (1917) (29 rats)

●-Mickelsen & Anderson (1959) (15 rats).
O-High fat and ⊙ -grain ration, Mean for 5 rats—this study.
·-Individual data, Part III.

PART II

FEED EFFICIENCY, BODY COMPOSITION AND DEPOSITION OF BODY FAT IN SIX STRAINS OF RATS FED THE GRAIN RATION OR THE HIGH FAT RATION

INTRODUCTION

There are certain inherited metabolic factors which predispose some individuals to obesity and others to leanness (Tepperman, 1957). Mayer has reviewed the evidence for this for man (1965) and for animals (1957). The manner of inheritance, however, continues to elude the investigators.

For purely genetic studies, the choice experimental animal has been the mouse. That gene mutations have resulted in four obese types of mice is well documented (Bielschowsky and Bielschowsky, 1956; Danforth, 1927; Falconer and Isaacson, 1959; Mayer, 1960). These are the exceptional cases of obesity rather than the usual type and it would seem that in man, only the exceptional cases of obesity would parallel these. However, the individuals who are genetically obese probably account for only a small percentage of the obese population as a whole.

However, a genetic predisposition to obesity frequently becomes manifested with the ingestion of a high fat diet. Fenton (Fenton and Dowling, 1953; Fenton, 1956) investigated certain strains of mice which became obese when fed a high fat ration, while other strains did not. The same strains which became obese in the foregoing experiment did not become obese when fed a high protein ration. Still other strains of mice have been known to exhibit similar phenomena (Sokoloff et al., 1960).

Rats, also show a similar characteristic with certain strains becoming obese when fed a high fat ration (Mickelsen et al., 1955). When these same strains were fed grain or chow rations, no obesity

occurred. To genetically characterize this obesity, it was necessary to locate strains of rats wherein such obesity (that is, obesity with the consumption of the high fat diet) was not manifested. No previous investigation of the nature of the obesity induced by the high fat diet has been done. For this reason, information was needed on the propensity of several strains of rats to become obese when fed the high fat ration. This paper reports the results of such a study together with the distribution of fat in the bodies of the different strains of rats as they became obese.

EXPERIMENTAL

Six strains of rats have been investigated for weight gains and deposition of body fat when fed the high fat ration. These are: (1) Osborne Mendel, (2) Sprague Dawley, (3) Hoppert (4) Hooded, (5) M.S.U. Gray and (6) Wistar-Lewis. The parent stock for the Osborne Mendel rats used in this study came from NIH. Weanling Sprague Dawley rats were obtained from a local producer.[1] Weanling Hoppert rats were procured from the Department of Biochemistry, M.S.U. This strain was developed by Drs. Hunt and Hoppert and used extensively in their dental caries work. The Hooded and Gray rats were both descended from the Norway Black and White rats. Parent stock was obtained from the M. S. U. Psychology Department. Breeding stock for the Wistar-Lewis rats was secured through the kind assistance of Mr. Samuel Poiley[2] from a commercial producer.[3]

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[1]Spartan Research, Williamston, Mich.

[2]Mr. Samuel Poiley, National Cancer Institute, NIH, Bethesda, Maryland.

[3]Batelle Memorial Institute, Columbus, Ohio.

For all strains, litters at the time of birth were cut to eight, except for the Gray rats where litters were cut to six. This was done because previous investigators indicated that the dams of this strain could only easily raise six young.

For the rat strains raised in this laboratory, the rats fed the high fat and grain rations were paired according to sex, body weight and litter so that all groups were uniform. Five male and 5 female rats were sacrificed at weaning. Ten male and 10 female rats were fed the high fat ration (Table 2, Part I) and the same number were fed the grain ration (Campbell et al., 1966). Since weanling Hoppert and Sprague Dawley rats were obtained from outside sources, these rats were not littermates. Individual weekly weight records and food intakes were obtained throughout the lifetime of the rats. For each strain, twenty rats or five from each group were sacrificed at 10 weeks and the remainder at 20 weeks.

An estimate of activity was made by observation of the rats. The animals were observed at regular intervals throughout one day (around 9:00 A.M., 4:00 P.M. and 9:00 P.M.) each week. On each of these occasions a record was made of their activity. The following scale was devised to quantitate the observed activity. Value of "0" --rat lying down either asleep or awake, value of "1"--rat sitting either asleep or awake, value of "2" --rat eating, drinking, cleaning self or other comparable motion and value of "3"--rat climbing cage or moving rapidly.

At sacrifice, fat depots were removed as described in Part I and carcass analyses carried out for moisture, fat, nitrogen and total ash (Mickelsen and Anderson, 1959). All data were analyzed by means of the computer at Michigan State University. Means and standard errors were

thus obtained and T tests (Dixon and Massey, 1957) between diets as well as T tests comparing each strain of rat with the Osborne Mendel strain fed the high fat ration.

In 1 g of the grain ration there were 3.24 Calories and in 1 g of the high fat ration there were 6.62 Calories. These values were multiplied by the grams of the ration eaten throughout the 10 week or 20 week interval.

To determine the calories in the body, the grams of fat in the body were multiplied by 9 and the grams of protein by 4. These values were added. The calories present in the body of control weanling rats were subtracted from this to correct for calories already present before the consumption of any food. The calories consumed divided by the calories in the carcass gave a value for calories consumed to make 1 calorie of body energy. To calculate the calories consumed to make 1 calorie of body protein the calories consumed were divided by the g of protein gained in the carcass and were multiplied by 4. To calculate the % of energy retained in the body of the ration consumed, the number of calories gained in the carcass (using 9 calories for fat and 4 for protein) was divided by the calories consumed.

RESULTS

Body weights

In most cases, the Osborne Mendel rats were the heaviest and gained the most weight when fed the high fat ration (Table 7). In male rats of all strains this weight difference between the grain and high fat-fed animals became accentuated during the second 10 week period (Fig. 16 and 17). This was also true of female Osborne Mendel and Lewis rats. This

difference in the latter strain, however, was more dependent on the fact that the grain-fed Lewis rats showed little weight gain during the second ten weeks.

Feed efficiency

The male Hoppert rats were the most efficient in converting energy into body tissue calories (Table 8). This was true for the grain-fed rats and partially so for those fed the high-fat ration. At the 20th week, the Osborne Mendel rats fed the high fat ration were the most efficient. The latter strain (Osborne Mendel) for both males and females were most efficient when fed the high fat ration (Table 9). For the other strains, no consistent pattern in feed efficiency was apparent. The percentage of calories converted to body energy was always greater at ten weeks than at 20 weeks and greater in males than in females. The percentage of calories converted to body energy was highest in the 20 week old Osborne Mendel rats (Table 10) and the 10 week old Hoppert rats. For all strains of rats, the efficiency of depositing calories in the body is about twice as great when the high fat ration is fed in contrast with the grain ration.

When feed efficiency was related to body protein deposition, the differences among the strains and for the two rations became much smaller (Table 11). When feed efficiency is expressed on the basis of calories of protein gained, the only prominent difference is the greater efficiency of the males. This holds true for both rations and age groups. On the basis of increase in total body energy (Table 10), there was no difference between males and females. The efficiencies listed in Table 10 for the 10 week old male rats are essentially the same as those for the same groups in Table 11. However, for the other groups, there is a

pronounced increase in efficiency when based on calories per calorie of body protein gained.

Activity

Rats fed the grain ration consistently showed a greater degree of activity than those fed the high fat ration (Table 12). Exclusive of the Sprague Dawley and the Hoppert rats, grain fed rats increased their activity between the 10th and 20th weeks of the experiment. The activity of these two groups of rats was affected somewhat by an outbreak of murine pneumonia which very likely influenced the results. Rats consuming the high fat ration showed decreases in activity during this same period except for the Gray and Hooded rats. A rank order of activity beginning with the least active strain is given in Table 13. The Gray rats were the most active, and the Osborne Mendel rats were the least active as a whole. However, variations between all the strains were not great.

Body protein and ash

At the end of 10 and 20 weeks, Osborne Mendel and Sprague Dawley rats consistently had more protein (Table 14) and ash (Table 15) in their bodies than did the other strains of rats.

These two strains of rats showed a more rapid formation of body protein even though there were no pronounced differences in the body protein content of the weanling rats.

The Sprague Dawley rats tended to have more ash in their bodies than the other rats; this was especially true for the males and to a lesser extent for the females (Table 15). There was no consistent difference in body ash content between the rats fed the high fat or grain rations. A

plot of the body ash data indicates a rapid increase between the 10th and 20th week of the study.

Fat

All the male rats fed the high fat ration had at least twice as high a percentage of fat in their bodies compared with their littermates fed the grain ration (Table 16). This was also true of female rats for four strains. However, at 10 weeks, both the Hoppert and Gray rats fed the high fat ration did not have twice as much fat in their carcasses as the animals fed the grain ration. For all strains, except the Hoppert rats, the increased percentage of fat in the carcass of those rats fed the high fat ration was highly significant ($P<0.01$). At the 10th week there was no significant difference in carcass fat between the female Hoppert rats fed the grain or high fat rations ($P>0.05$). However, for males of this strain, the difference in carcass fat was significant ($P<0.05$).

The percentages of fat in the male and female rats in each ration group were essentially the same. For some groups, the females had a slightly higher percentage of fat than the males but for others, the reverse was true (Table 16).

The differences in the increases in body weights of the rats fed the high fat or grain ration is practically linear for the 20 weeks of the study. This is especially true of the males of all but the Lewis strain (Fig. 16) and for all but the Sprague Dawley females (Fig. 17).

These differences in the increase in body weight are almost mirrored in the increases in body fat (Figs. 16 and 17). The increase in body fat does not quite account for the increase in body weight. For instance at the 20th week of the study, the male O.M. rats fed the high fat ration weighed 247 g more than comparable rats fed the grain ration while the difference in carcass fat content was 213 g. The increase in body fat was obviously associated with the formation of additional lean body tissue in the obese rats. At the end of the experiment, the obese rats had 97.9 g of protein in their bodies while the grain-fed rats had 95.1 (Table 14). This difference of 2.8 g of protein represents approximately 11 g of lean body tissue. Even this does not explain completely the difference between the increase in body weight and fat content.

Inguinal fat depots

For all strains of rats, within each dietary group, the males had larger inguinal fat depots than the females (Fig. 18). This was true at both the 10th and 20th week of the experiment. Since the weight of the fat pads is expressed on the basis of body weight, the difference in weight of this depot in the two sexes cannot be attributed to the larger weight of the males. When weaned, female rats had inguinal depots that were as large or in some strains (Osborne Mendel, Sprague Dawley, and Lewis) larger than the male (Fig. 18).

T tests indicated that in four strains this depot was significantly larger in all cases ($P < 0.01$) when rats consumed the high fat diet, than when they consumed the grain ration. For Gray and Hoppert rats this significance was decreased ($P < 0.05$) or didn't exist ($P > 0.05$).

In comparing each of the other strains of rats to the Osborne Mendel rats fed the high fat ration only, the Hoppert rats showed no difference in depot weight ($P > 0.05$). The lack of significance for the inguinal fat depot in the Hoppert rats despite the fact that the average value appears to be lower (Fig. 18) is due to the great variability in this parameter. Three of the other strains (Sprague Dawley, Hooded and Lewis) had inguinal fat depots which were significantly smaller than the Osborne Mendel ($P < 0.01$) ranging to no significant difference ($P > 0.05$) (these differences and degree of significance are shown in Fig. 18). When compared to the Osborne Mendel rats, only the Gray rats showed differences in inguinal fat depots based on g per 100 g body weight which were significant in all groups. For Gray males and females the difference was significant ($P < 0.02$) at the 10th week and highly significant at the 20th week ($P < 0.01$).

Interscapular fat depot

For all strains of rats, females had a larger percentage of their body fat just dorsal to the neck than did males. This was true whether rats consumed the grain or high fat ration (Fig. 19). However, differences between males and females were greater when the animals were fed the high fat ration. For weanling rats, this depot was relatively the same size in the two sexes, or in some strains, slightly larger in the female rats.

For the Osborne Mendel and Lewis strains, the accumulation of fat in this area was significantly increased when the rats were fed the high fat ration in contrast to when fed the grain ration ($P < 0.01$). Hooded rats behaved similarly but the level of significance for 20 week old

male rats was decreased ($P < 0.05$). The enlargement of this depot in Gray and Sprague Dawley rats fed the high fat ration varied in degree of significance when compared to the grain fed controls. The Hoppert strain was the only one in which the interscapular fat depot in the rats fed the high fat ration was not significantly larger than in the animals fed the grain ration. This was true except for the males at the 10th week where the difference was significant ($P < 0.02$).

For all strains of rats fed the high fat ration, the size of this depot was compared to that in the comparable Osborne Mendel group. No significant differences existed between the relative size of these fat depots between Hoppert and Osborne Mendel rats ($P > 0.05$). In the Hooded, Lewis and Sprague Dawley strains, this depot varied from being significantly smaller than the Osborne Mendel ($P < 0.05$) to not significantly different ($P > 0.05$). Only the high fat-fed Gray rats in all groups had a significantly smaller depot than the Osborne Mendel rats. For all groups except the twenty week high fat-fed females this depot was significantly smaller than that of the Osborne Mendel rats ($P < 0.01$). The level of significance decreased for 20 week high fat-fed females ($P < 0.05$).

Genital fat depots

Parametrial depots in the female rats for all strains at 10 and 20 weeks were always relatively larger than testicular depots in males (Fig. 20). To compensate for the difference in size of the two sexes, these values are listed on the basis of percent of body weight.

For male rats, the consumption of the high fat ration resulted in a significantly greater size of the genital depot for all strains ($P < 0.01$).

This was also true of four strains of the (Lewis, Osborne Mendel, Gray, and Hooded) female rats. Female Hoppert rats fed the high fat ration showed no significant increase at 10 weeks but a significant increase ($P < 0.05$) at twenty weeks when the relative size of this depot was compared with the grain-fed rats. Female Sprague Dawley rats showed different levels of significance at 10 weeks ($P < 0.05$) and at 20 weeks ($P < 0.01$).

In comparing genital depots in each strain of rats to its size in the Osborne Mendel strain two differences appeared. Male Lewis rats fed the high fat ration had a smaller testicular depot at 10 and 20 weeks. This difference is significant ($P < 0.02$). The other difference is that at ten weeks, both male and female Gray rats have genital depots which are consistently smaller than those of the Osborne Mendel rats. This difference is highly significant ($P < 0.01$).

After the 10th week of the experiment (13th week of life), the genital fat depot grows at almost the same rate as does the body. This is true for all strains except the Gray rats where it grows more rapidly than does the body as a whole. At 20 weeks this depot in the Gray rat is no longer significantly smaller than it was in the Osborne Mendel rat ($P > 0.05$).

The apparently deviant behavior of the parametrial fat in the older female Gray rats may have been associated with a pathological observation. Twenty percent of these rats had some abnormal adipose tissue in this depot. Dr. Vance Sanger of the Pathology Department indicated this to be necrotic adipose tissue apparently resulting from a restricted blood supply.

Mesenteric and omental fat depots

The relative size of the omental and mesenteric fat depots differed in some strains of rats when the effect of diet was evaluated, but for all strains within each dietary group there was no difference between males and females (Fig. 21).

As a general rule, the consumption of the high fat ration caused a significant increase in the size of the mesenteric and omental depot in comparison to grain-fed rats. For four strains this difference was always significant ($P < 0.05$) and frequently highly significant ($P < 0.01$). Only the Gray and Hoppert rats did not show this significant difference. In Gray rats, the difference was not significant at 10 weeks ($P > 0.05$) but significant at 20 weeks ($P < 0.02$). At no time was the difference significant in the Hoppert rats.

Comparing the mesenteric depot of each strain to the Osborne Mendel rats fed the high fat ration, it was found that this depot was significantly smaller ($P < 0.05$) at both ten and twenty weeks in males of all strains. In contrast, the depot was significantly smaller in females of only two strains ($P < 0.05$). These were the Gray females at 10 weeks, and the Hooded females at 20 weeks.

Perirenal fat depots

With two exceptions, for all strains and both diets, the perirenal depot comprised a larger percentage of body weight in male rats than in females (Fig. 22). These two exceptions were the grain-fed Hooded and Gray rats at 10 weeks where the differences were slight.

Both male and female Osborne Mendel, Lewis and Hooded rats fed the high fat ration had much larger perirenal depots than comparable rats

fed the grain ration. This was true for these three strains at the 10th and 20th week and for the Gray rats with the exception of the females at the 10th week. The increase was not significant in the Hoppert rats. For Sprague Dawley rats, the increase was highly significant in females ($P < 0.01$) but significant only for the males at the 10th week ($P < 0.02$) but not at the 20th week ($P > 0.05$).

Lewis and Hoppert rats fed the high fat ration showed no significant differences ($P > 0.05$) in the weight of the perirenal depot when compared to comparable Osborne Mendel rats. This was also true of Sprague Dawley female rats. However, the perirenal depot was smaller in the male Sprague Dawley ($P < 0.02$) and Gray ($P < 0.05$) rat than the same depot in the male Osborne Mendel rat.

The relative size of the perirenal depot was smaller in the Sprague Dawley ($P < 0.01$), Gray ($P < 0.01$) and Hooded ($P < 0.02$) female rats at the 20th week than in the Osborne Mendel rats. In male Hooded rats the perirenal depot weighed significantly less ($P < 0.02$) at ten weeks but was not significantly different in weight from the Osborne Mendel male rats at twenty weeks.

DISCUSSION

This study indicated that Osborne Mendel rats responded to the high fat ration with a larger gain in body weight, a greater deposition of body fat and relatively larger fat depots than did the animals of the other five strains. When fed the high fat ration, the Osborne Mendel rats were least active physically and most efficient in converting feed to body energy. There were instances when this was not the case, but in general, this was true.

The Gray rats were least affected by the high fat ration. They showed the smallest increase in body weight, body fat and relative size of adipose tissue. They were least efficient in converting feed to body tissue and expended more energy on physical activity than any other group of rats. Again, for certain parameters this may not be the case, but it is generally so.

The other four strains were in between these two extremes as far as response to the high fat ration was concerned. For these strains, however, some parameters point to particularly important phenomena. For instance when the Hoppert rats were fed the grain ration they had the largest percentage of body fat and the largest subcutaneous adipose tissues of all the strains investigated. In the Hoppert rats these subcutaneous tissues did not get significantly larger when the rats were fed the high fat ration. The fact that they were so large already is a partial explanation for this as well as the fact that greater individual differences existed in this strain of rats. This would indicate that with selective breeding it should be possible to produce a high efficiency and a low efficiency strain of Hoppert rats.

During the second 10 week interval, the grain-fed female Lewis rats gained very little body weight. Therefore, an accentuated response existed in those rats fed the high fat ration during this period.

The relative distribution of fat in the different depots of rats varied both with strain and sex as well as with ration fed. Since the Gray rats had the smallest accumulation of body fat, it was to be expected that the relative size of their subcutaneous adipose tissue should be the smallest. The females of that strain did not show the

accretion of fat in the interscapular area so characteristic of female rats of other strains.

When mesenteric and genital depots of Osborne Mendel, Lewis, Hoppert and Sprague Dawley rats had already increased to make up a high percentage of body weight and were increasing in weight at the same rate as body weight increases, these depots in the Gray and Hooded rats were comparatively smaller and were still increasing in weight at a faster rate. This was less pronounced in the latter strain. As a whole, fat patterning in the Hooded rats was similar to that in Gray rats but in Hooded rats the depot was always composed of a slightly larger percentage of body weight.

Table 10 showed that rats were twice as efficient in converting a calorie of the high fat ration to body energy when fed the high fat ration as compared to the grain ration. This was true of both males and females of all strains. It appears that the energy of a high fat ration can be readily transferred into body tissues with a minimum of energy expended by the animal.

Investigators have frequently calculated feed efficiency on the basis of grams of weight gain for grams or calories of ration consumed. Mayer and Krehl (1948) reported that 50 day old rats have a feed efficiency of 35%. This was calculated as grams of weight gain per grams of ration consumed. During periods of maximum weight gain, food efficiency for grain-fed rats (male) would be similar in this study. However, when male rats were fed the high fat ration, food efficiency increased to 50%.

Forbes et al. (1946c) recognized an increase in energy retained in rats fed high fat rations. Three experimental rations used by these

investigators consisted of 2, 10 and 30% fat. These all contained 2% corn oil; additional fat was lard. Protein was provided daily at a level of 2.2 g for each rat regardless of the ration fed. To provide this amount, the protein levels in the different rations had to be varied since all rations were isocaloric.

The percent of calories retained in the body were 17.18, 18.43 and 19.76 respectively. Energy lost in the feces was 3.6, 4.5 and 5.3% of the calories respectively for the rats fed the 3 rations. Energy lost in the urine was 4.7, 4.8 and 4.9% respectively. The remainder of the calories were "energy output as heat". This was determined by the increased heat increment when the rats were fed an amount of the ration beyond that sufficient for maintenance. From this was subtracted the heat increment of the maintenance ration. Calories retained were determined from body composition data.

The calories present in a gram of protein or fat in the body will vary. Paladines et al.(1964) reported that various parts of the body of sheep produced a range in calories when combusted. For protein, these values were 5.3 to 5.8 kcal/g; for fat 7.4 to 9.4 kcal/g. Blaxter and Rook (1953) have reported other values. In view of this, the value of 9 kcal/g fat and 4 kcal/g protein was accepted. On the other hand, if the actual caloric value for the protein in the carcass was 5.3, the percent of retained energy would be increased by 16% in the small rats and 9.5% in the larger rats in this study.

Table 7

Body weights of six strains of male, female and weanling rats fed either the high fat or grain ration

Strain	Weanling	10 weeks		20 weeks	
		High fat	Grain	High fat	Grain
Male					
Osborne Mendel	53.18± 1.85[1]	503.56± 22.82[1]	336.14± 12.82[1]	692.60± 27.62[1]	445.32± 17.13[1]
Sprague Dawley	48.90± 1.13	480.18± 16.68	368.22± 6.49	592.86± 19.47	422.98± 22.50
Hoppert	49.98± 1.77	425.08± 27.82[3]	338.15± 21.81	495.90± 69.34[2]	402.53± 12.11
Lewis	55.22± 3.39	339.38± 36.60[3]	302.20± 21.32	515.42± 7.95[2]	363.72± 12.94
Hooded	51.00± 2.14	384.88± 17.35[2]	305.78± 10.60	557.93± 25.83[2]	392.36± 14.38
Gray	45.50± 4.86	376.58± 14.75[2]	298.30± 4.87	493.54± 20.18[2]	378.66± 8.88
Female					
Osborne Mendel	57.36± 2.90	294.98± 21.09	220.34± 9.65	452.28± 35.51	301.04± 6.18
Sprague Dawley	46.68± 1.19	313.18± 5.92	246.14± 8.21	331.00± 10.99	279.32± 10.01
Hoppert	49.54± 1.73	246.80± 15.31	220.38± 15.48	296.23± 38.17[3]	243.80± 11.05
Lewis	47.14± 2.21[3]	235.54± 7.79[3]	188.18± 10.50	309.74± 14.72[3]	204.02± 7.70
Hooded	51.76± 2.57	225.96± 11.64	183.38± 4.10	269.62± 11.90[2]	216.90± 9.70
Gray	48.94± 2.30	215.68± 6.25[2]	188.16± 2.71	248.98± 10.41[2]	203.16± 2.48

1 Body weights in grams ± Std. Error.

2 T tests with Osborne Mendel rats fed high fat are highly significant ($P<0.01$).

3 T tests with Osborne Mendel rats fed high fat are significant ($P<0.05$).

Table 8

Feed efficiency for six strains of male and female rats. Efficiency is expressed as calories consumed from weaning to sacrifice for each calorie deposited in the body during that time.

Strain	Feed Efficiency			
	10 weeks		20 weeks	
	High fat	Grain	High fat	Grain
		Male		
Osborne Mendel	3.55± 0.14[1]	7.86± 0.42[1]	4.59± 0.39[1]	12.28± 0.85[1]
Sprague Dawley	4.35± 0.22	7.04± 0.32	6.56± 0.38	12.92± 1.12
Hoppert	3.21± 0.29	6.68± 1.04	5.98± 0.89	10.81± 1.12
Lewis	4.64± 0.41	8.94± 0.81	5.54± 0.31	12.79± 1.04
Hooded	4.20± 0.23[3]	8.85± 0.26	5.41± 0.29	12.73± 0.96
Gray	4.71± 0.33[3]	8.73± 0.31	6.08± 0.53	11.08± 0.55
		Female		
Osborne Mendel	4.58± 0.20	12.67± 0.74	5.66± 0.70	15.22± 0.92
Sprague Dawley	4.76± 0.22	9.71± 0.55	8.39± 0.41	16.04± 1.35
Hoppert	5.20± 0.73	8.21± 0.54	9.39± 2.21	16.71± 1.75
Lewis	5.02± 0.27	10.88± 0.99	6.82± 0.48	20.81± 2.29
Hooded	5.94± 0.57[2]	11.59± 0.80	8.03± 0.46[2]	19.74± 0.66
Gray	7.18± 0.37[2]	10.94± 0.53	9.28± 0.35[2]	21.17± 2.39

[1] Average for 5 rats in each group ± Std. error.

[2] T tests with Osborne Mendel rats fed high fat are highly significant (P 0.01).

[3] T tests with Osborne Mendel rats fed high fat are significant (P 0.05).

Table 9

Rank order of feed efficiency[1]

Strain	10 weeks		20 weeks	
	High fat	Grain	High fat	Grain
		Male		
Osborne Mendel	2	3	1	3
Sprague Dawley	4	2	6	6
Hoppert	1	1	4	1
Lewis	5	6	3	5
Hooded	3	5	2	4
Gray	6	4	5	6
		Female		
Osborne Mendel	1	6	1	1
Sprague Dawley	4	2	2	2
Hoppert	6	1	4	3
Lewis	2	3	3	5
Hooded	3	5	5	4
Gray	5	4	6	2

[1] Within each ration group, the most efficient rats are ranked as number "1" etc.

Table 10

Percent of calories converted to body energy[1]

Strain	10 weeks		20 weeks	
	High fat	Grain	High fat	Grain
		Male		
Osborne Mendel	28.48	12.92	22.42	8.33
Sprague Dawley	22.82	14.33	15.66	7.99
Hoppert	32.16	14.78	19.03	7.17
Lewis	22.80	11.56	18.41	7.96
Hooded	24.04	11.36	18.68	8.08
Gray	21.92	11.49	17.00	9.15
		Female		
Osborne Mendel	22.03	8.00	18.84	6.63
Sprague Dawley	22.71	10.43	12.05	6.41
Hoppert	21.11	12.38	12.97	6.33
Lewis	20.09	9.46	13.20	5.00
Hooded	14.37	8.78	12.67	4.32
Gray	14.06	9.22	10.86	4.85

1 These values were calculated by dividing calories gained in the body (calories at 10 or 20 weeks minus those in the weanling rats) by the calories in the food consumed throughout the 10 or 20 week period.

Table 11

Calories consumed for calories of protein gained in the body[1]

Strain	10 weeks		20 weeks	
	High fat	Grain	High fat	Grain
		Male		
Osborne Mendel	19.85± 0.68[2]	17.34± 0.24[2]	31.92± 1.90[2]	26.52± 0.55[2]
Sprague Dawley	17.21± 0.65	17.29± 0.17	32.95± 0.42	29.45± 0.93
Hoppert	18.12± 0.92	16.13± 1.09	30.44± 2.11	29.26± 1.78
Lewis	16.93± 0.47	16.62± 0.57	30.85± 1.92	27.98± 1.75
Hooded	17.14± 0.60	16.83± 0.66	29.97± 0.36	26.86± 0.56
Gray	16.15± 0.15	15.63± 0.70	26.40± 1.11	26.81± 0.84
		Female		
Osborne Mendel	23.53± 0.86	22.97± 0.70	41.43± 1.06	36.51± 1.40
Sprague Dawley	24.00± 0.43	21.40± 0.56	42.71± 1.09	38.39± 0.85
Hoppert	20.62± 1.09	21.06± 1.78	39.04± 3.16	36.36± 1.10
Lewis	22.78± 0.21	24.16± 0.99	39.96± 1.18	39.67± 1.91
Hooded	22.82± 0.75	20.88± 0.68	39.46± 0.85	36.46± 0.61
Gray	20.90± 1.07	21.32± 0.57	38.21± 1.30	40.42± 2.26

[1] These values were calculated by dividing calories of protein gained in the body(Calories obtained from protein at 10 or 20 weeks minus those in the weanling rats) divided by the calories consumed throughout the 10 or 20 week period.

[2] Mean ± Standard Error.

Table 12

Summary of activity[1]

	10 weeks		20 weeks	
	High fat	Grain	High fat	Grain
		Male		
Osborne Mendel	1.06	1.22	1.01	1.47
Sprague Dawley	1.41	1.84	1.11	1.40
Hoppert	1.45	1.64	0.88	1.18
Lewis	1.32	1.42	1.14	1.52
Hooded	1.37	1.54	1.61	1.91
Gray	1.60	1.63	1.53	1.79
		Female		
Osborne Mendel	1.30	1.53	1.30	1.91
Sprague Dawley	1.34	1.89	1.16	1.89
Hoppert	1.54	1.56	1.42	1.18
Lewis	1.60	1.57	1.15	1.59
Hooded	1.44	1.42	1.68	1.80
Gray	1.82	1.79	1.86	2.03

[1] The least active group is listed as No. 1. Activity varied for the different groups from 0.88 to 2.03. The activity of the rats was rated on the basis of a scale from 0 to 3 with 0 used to designate no activity at time of observation (see text).

Table 13

Rank order for activity[1]

	10 weeks		20 weeks	
	High fat	Grain	High fat	Grain
		Male		
Osborne Mendel	1	1	2	3
Sprague Dawley	4	6	3	2
Hoppert	5	5	1	1
Lewis	2	2	4	4
Hooded	3	3	6	6
Gray	6	4	5	5
		Female		
Osborne Mendel	1	2	3	5
Sprague Dawley	2	6	2	4
Hoppert	4	3	4	1
Lewis	5	4	1	2
Hooded	3	1	5	3
Gray	6	5	6	6

[1] The least active group is listed as No. 1. Activity varied for the different groups from 0.88 to 2.03. The activity of the rats was rated on the basis of a scale from 0 to 3 with 0 used to designate no activity at time of observation (see text).

Table 14

Protein in the carcasses of six strains of male and female rats fed either a high fat or a grain ration

Strain	Weanling (g)	10 weeks		20 weeks	
		High fat (g)	Grain (g)	High fat (g)	Grain (g)
Male					
Osborne Mendel	9.03± 0.43[1]	80.16± 3.27[1]	72.09± 2.54[1]	97.86± 3.71[1]	95.13± 4.77[1]
Sprague Dawley	8.18± 0.16	83.89± 2.58	73.47± 0.60	95.16± 3.06	86.83± 3.08
Hoppert	8.34± 0.37	62.72± 2.67[3]	63.45± 4.11	73.33± 2.57[2]	74.78± 3.25
Lewis	9.69± 0.50	72.25± 7.32	65.73± 5.00	81.63± 3.50[3]	79.00± 5.43
Hooded	8.83± 0.34	67.90± 4.15	61.07± 2.52	85.96± 3.52	81.44± 3.45
Gray	7.80± 1.04	63.82± 2.05[3]	63.82± 2.05	85.03± 4.10	75.81± 1.67
Female					
Osborne Mendel	10.49± 0.87	48.38± 1.68	42.67± 1.63	58.68± 2.26	56.69± 0.36
Sprague Dawley	7.45± 0.20[3]	49.32± 0.97	48.97± 1.68	51.32± 1.96	53.86± 2.03
Hoppert	8.07± 0.46	39.59± 1.05[2]	38.05± 2.33	45.68± 3.13[3]	46.03± 1.38
Lewis	8.25± 0.29	38.93± 0.09[2]	36.65± 2.33	43.21± 0.07[2]	42.96± 1.81
Hooded	9.42± 0.43	36.82± 1.11[2]	37.69± 0.65	43.71± 2.18[2]	42.75± 1.38
Gray	8.99± 0.70	36.16± 0.92[2]	38.53± 2.21	39.83± 1.86[2]	41.32± 2.68

[1] Body protein in grams ± Std. Error.

[2] T tests with Osborne Mendel rats fed high fat are highly significant ($P<0.01$).

[3] T tests with Osborne Mendel rats fed high fat are significant ($P<0.05$).

Table 15

Ash content in grams of carcasses of male and female rats of six strains

Strain	Weanling	10 weeks		20 weeks	
		High fat	Grain	High fat	Grain
			Male		
Osborne Mendel	1.49± 0.09[1]	9.77± 0.38[1]	9.77± 0.27[1]	12.88± 0.76[1]	13.74± 0.62[1]
Sprague Dawley	1.42± 0.04	11.90± 0.38	11.99± 0.12	13.50± 0.36	14.59± 0.50
Hoppert	1.52± 0.09	8.62± 0.25	9.73± 0.65	10.32± 0.35	11.13± 0.52
Lewis	1.72± 0.10	9.60± 1.02	9.57± 0.82	11.87± 0.58	12.42± 0.72
Hooded	1.62± 0.06	9.52± 0.41	8.69± 0.38	11.73± 0.74	11.91± 0.57
Gray	1.26± 0.22	9.68± 0.59	9.95± 0.29	12.41± 0.45	12.61± 0.40
			Female		
Osborne Mendel	1.71± 0.06	6.85± 0.31	6.39± 0.38	9.02± 0.52	8.39± 0.68
Sprague Dawley	1.31± 0.04	7.98± 0.33	8.73± 0.51	7.75± 0.53	9.04± 0.52
Hoppert	1.47± 0.13	6.65± 0.30	6.50± 0.73	7.72± 0.26	8.32± 0.38
Lewis	1.49± 0.07	6.87± 0.40	6.57± 0.40	7.45± 0.23	8.39± 0.38
Hooded	1.75± 0.10	5.91± 0.58	5.50± 0.78	7.12± 0.49[2]	7.45± 0.24
Gray	1.43± 0.15	5.82± 0.72	6.37± 0.75	7.73± 0.45	8.26± 0.41

[1] Body ash in grams ± Std. Error.

[2] T tests with Osborne Mendel rats fed high fat are significant ($P<0.05$).

Table 16

Percent of body fat in six strains of rats

Strain	Weanling	10 weeks		20 weeks	
		High fat	Grain	High fat	Grain
Male					
Osborne Mendel	6.54± 0.31[1]	34.13± 1.35[1]	12.77± 0.86[1]	38.98± 3.06[1]	12.96± 1.23[1]
Sprague Dawley	9.85± 0.20[2]	24.28± 1.29[2]	15.37± 0.93	30.38± 1.86	14.12± 1.11
Hoppert	8.12± 0.28[3]	33.00± 3.13[3]	14.88± 1.55	31.12± 5.37	16.44± 1.73
Lewis	6.01± 0.59[3]	24.47± 2.10[3]	11.20± 1.12	33.86± 3.02	13.64± 1.12
Hooded	5.25± 0.21[3]	26.15± 2.14[3]	10.17± 0.71	32.57± 1.55[3]	12.27± 1.13
Gray	6.23± 0.73	22.15± 2.00[2]	9.62± 0.79	27.51± 2.34[3]	14.56± 1.51
Female					
Osborne Mendel	7.91± 0.31[3]	31.92± 1.07	11.40± 0.68	40.43± 3.06[3]	14.94± 0.81
Sprague Dawley	11.15± 0.34[3]	30.76± 1.65	13.57± 0.98	30.38± 0.86[3]	14.83± 1.46
Hoppert	8.00± 0.40	26.12± 3.79	16.33± 1.97	28.24± 5.11	13.74± 1.82
Lewis	6.98± 0.41	29.21± 1.08	14.66± 1.07	35.31± 2.24[3]	12.48± 1.52
Hooded	6.28± 0.58	25.46± 2.90[3]	11.38± 0.61	30.70± 1.42[3]	11.08± 0.35
Gray	7.44± 0.34	18.70± 1.17[2]	12.25± 0.54	26.05± 1.18[2]	11.98± 1.13

[1] Percent body fat ± Std. Error.

[2] T tests with Osborne Mendel rats fed high fat are highly significant ($P<0.01$).

[3] T tests with Osborne Mendel rats fed high fat are significant ($P<0.05$).

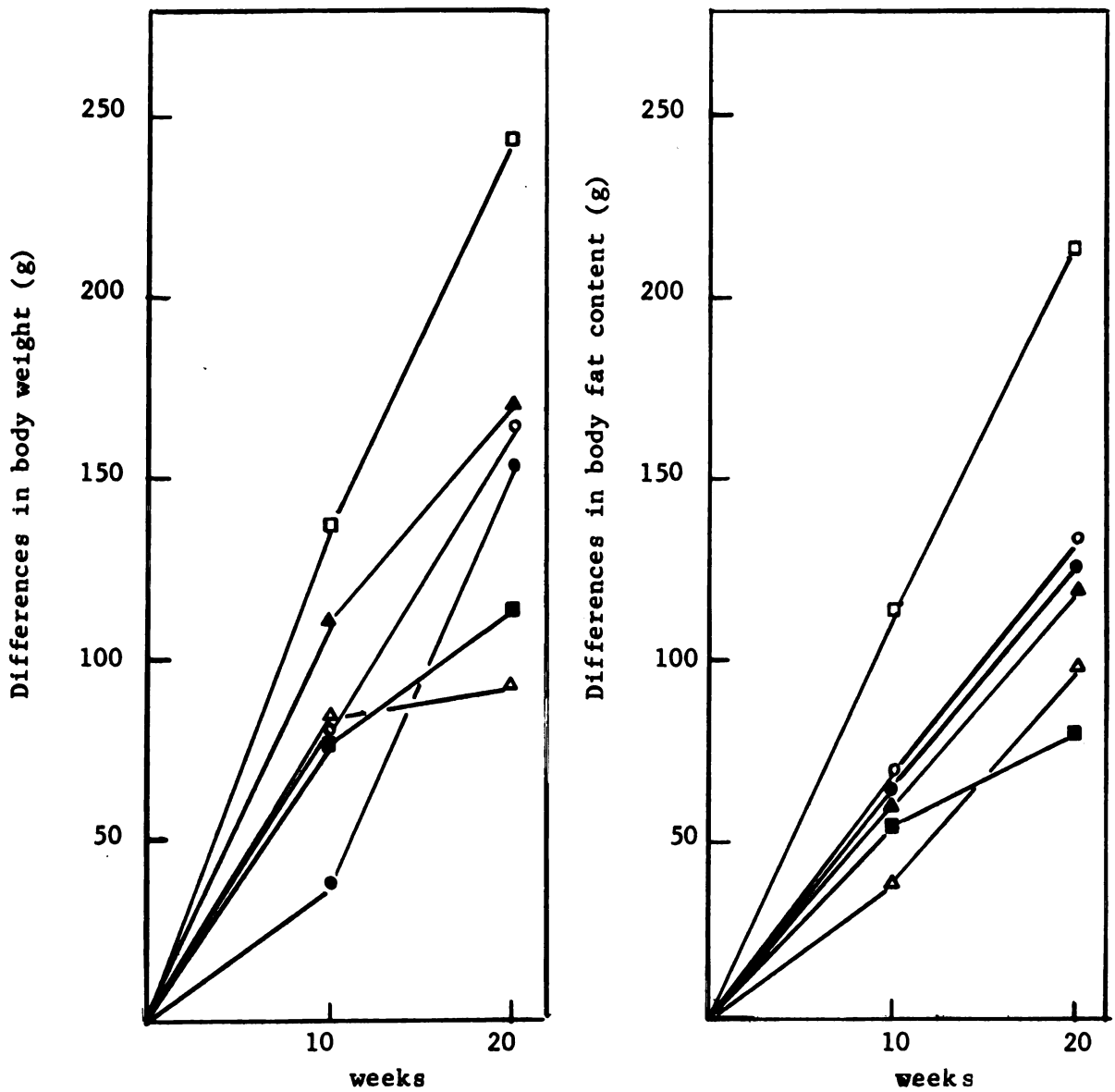

Fig. 16 Differences in body weights and body fat contents of male rats of each strain fed the high fat and grain rations. Each point represents the difference in the average values for 5 rats fed each diet.

□ Osborne Mendel
▲ Sprague Dawley
△ Hoppert
● Lewis
o Hooded
■ Gray

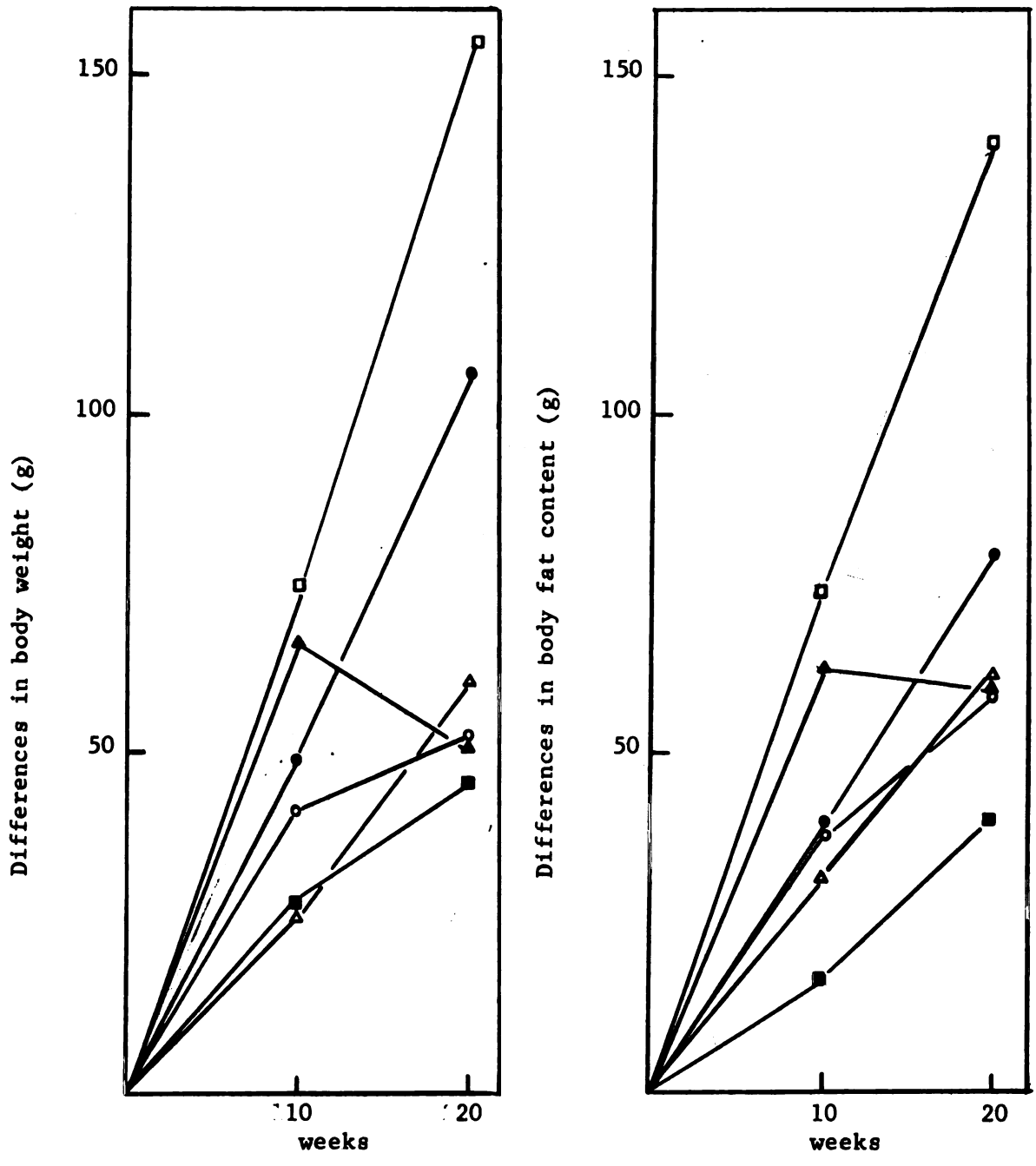

Fig. 17. Differences in body weights and body fat contents of female rats of each strain fed the high fat and grain rations. Each point represents the difference in the average values for 5 rats fed each diet.

□ Osborne Mendel
▲ Sprague Dawley
△ Hoppert
● Lewis
○ Hooded
■ Gray

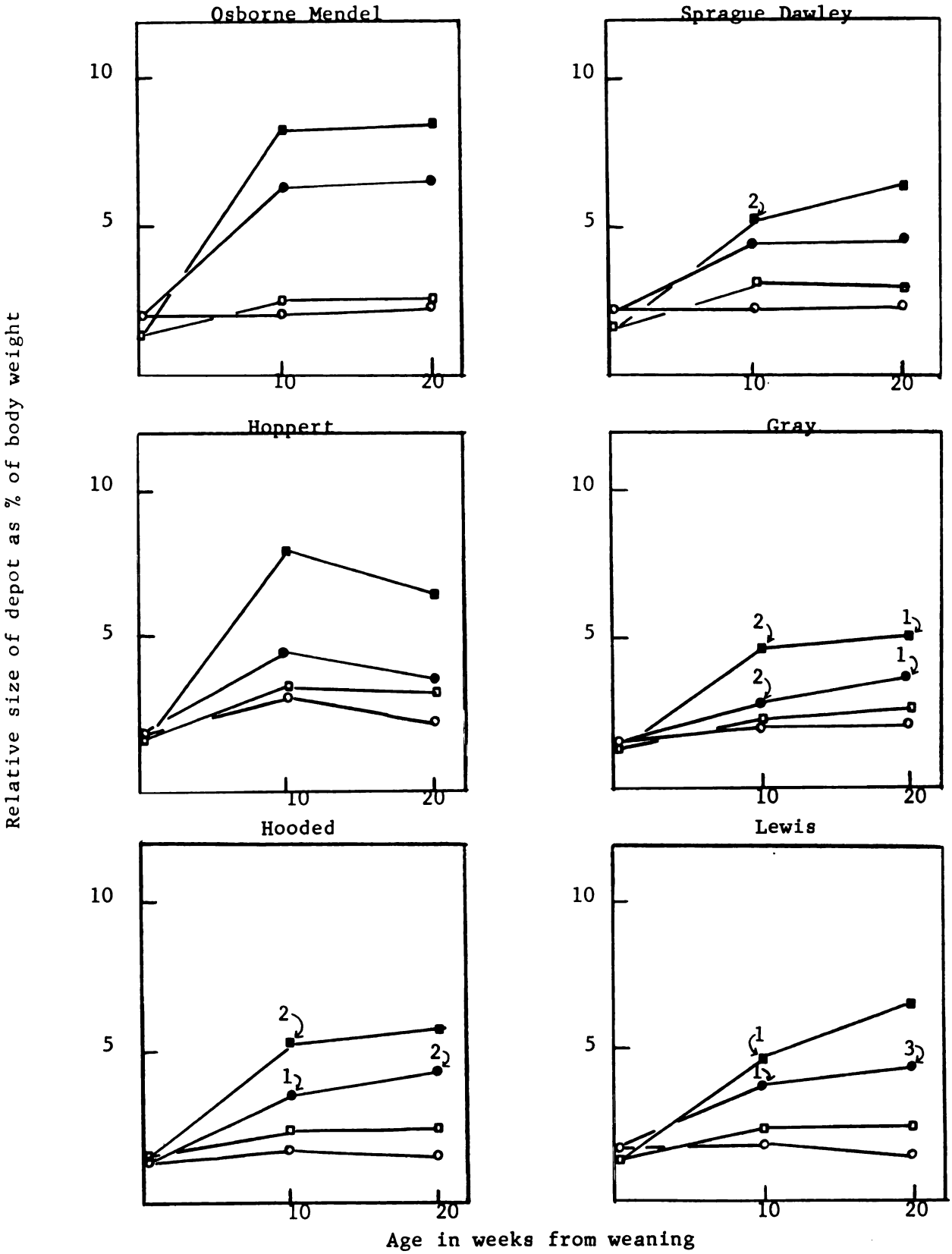

Fig. 18. Sum of the weights of the inguinal fat depots expressed as g per 100 g of body weight. The significance of the difference when referred to the comparable group of Osborne-Mendel rats is listed where applicable. The time intervals indicate the number of weeks the rats were on the experiment. Five rats in each group were sacrificed at the designated time intervals. (1. $P<0.01$; 2. $P<0.02$; 3. $P<0.05$).

■ , Male, high fat; □ , Male, grain.
● , Female, high fat; ○ , Female, grain.

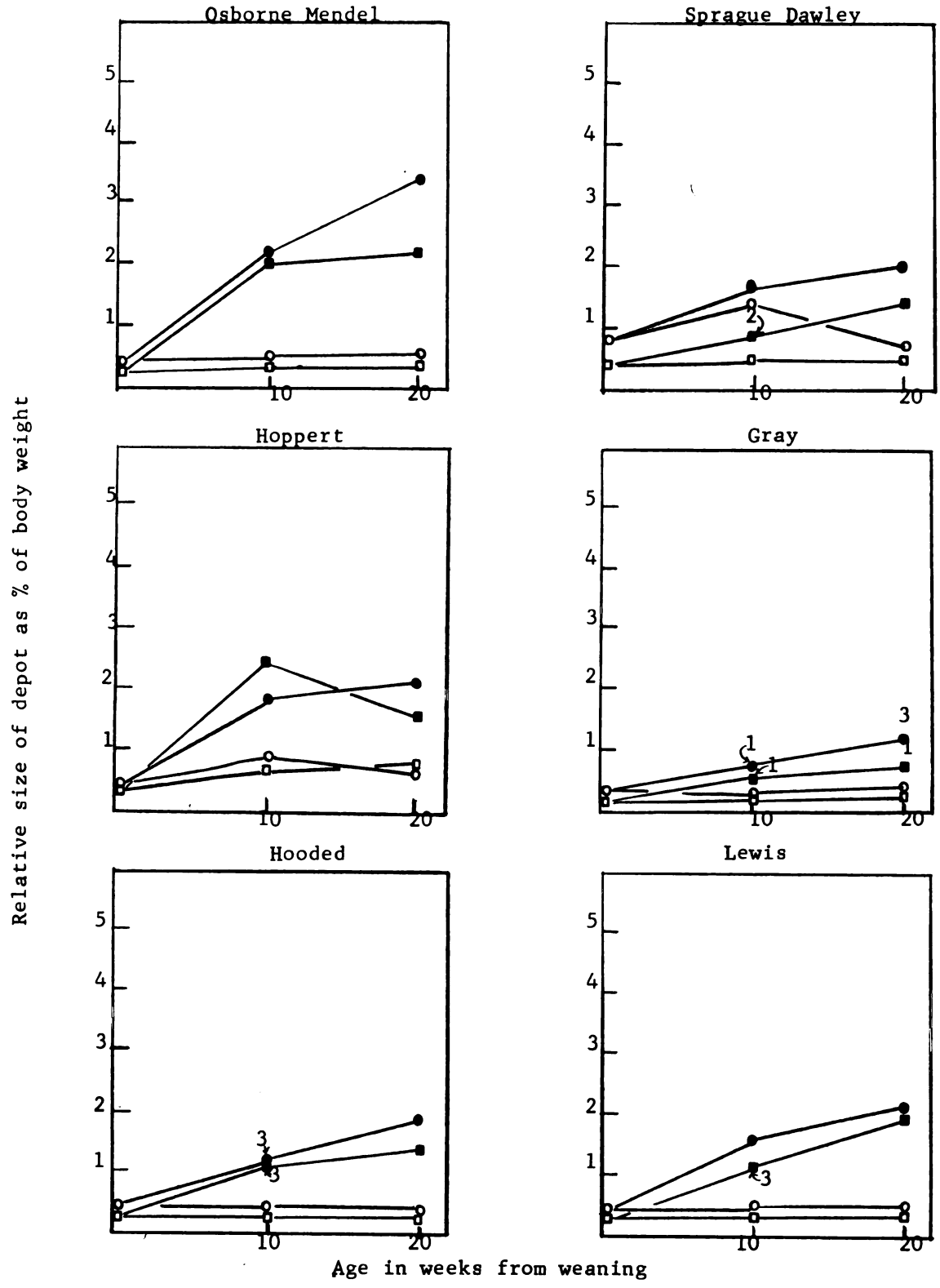

Fig. 19. Weight of the interscapular depot expressed as g per 100 g of body weight. The significance of the difference when referred to the comparable group of Osborne Mendel rats is listed where applicable. The time intervals indicate the number of weeks the rats were on the experiment. Five rats in each group were sacrificed at the designated time intervals.
(1. $P<0.01$; 2. $P<0.02$; 3. $P<0.05$).

■, Male, high fat; □, Male, grain.
●, Female, high fat; ○, Female, grain.

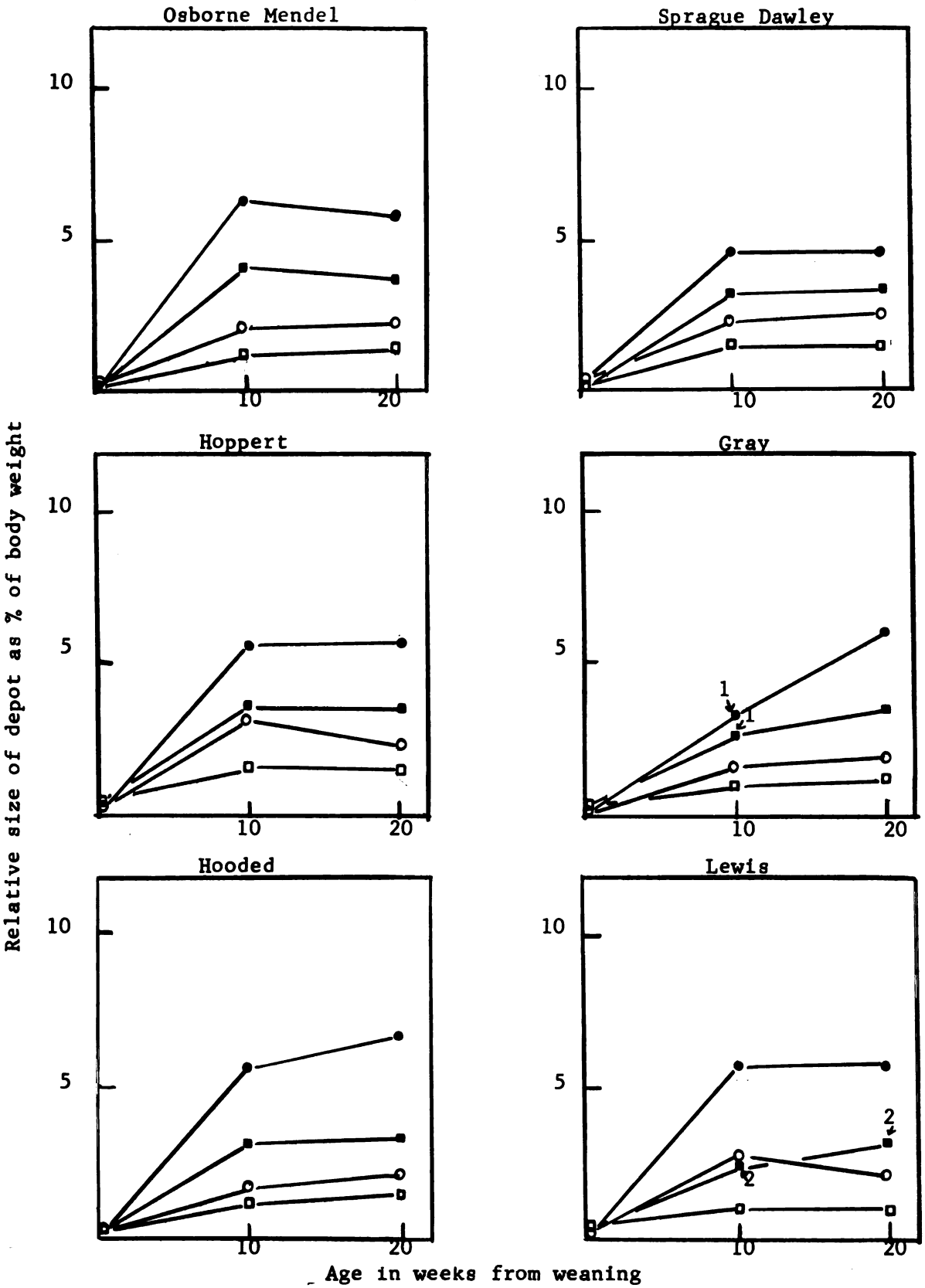

Fig. 20. Sum of the weights of the genital fat depots expressed as g/100 g of body weight. The significance of the difference when referred to the comparable group of Osborne Mendel rats is listed where applicable. The time intervals indicate the number of weeks the rats were on the experiment. Five rats in each group were sacrificed at the weeks designated time intervals. (1. $P<0.01$; 2. $P<0.02$)

■, Male, high fat; □, Male, grain
●, Female, high fat; ○, Female, grain

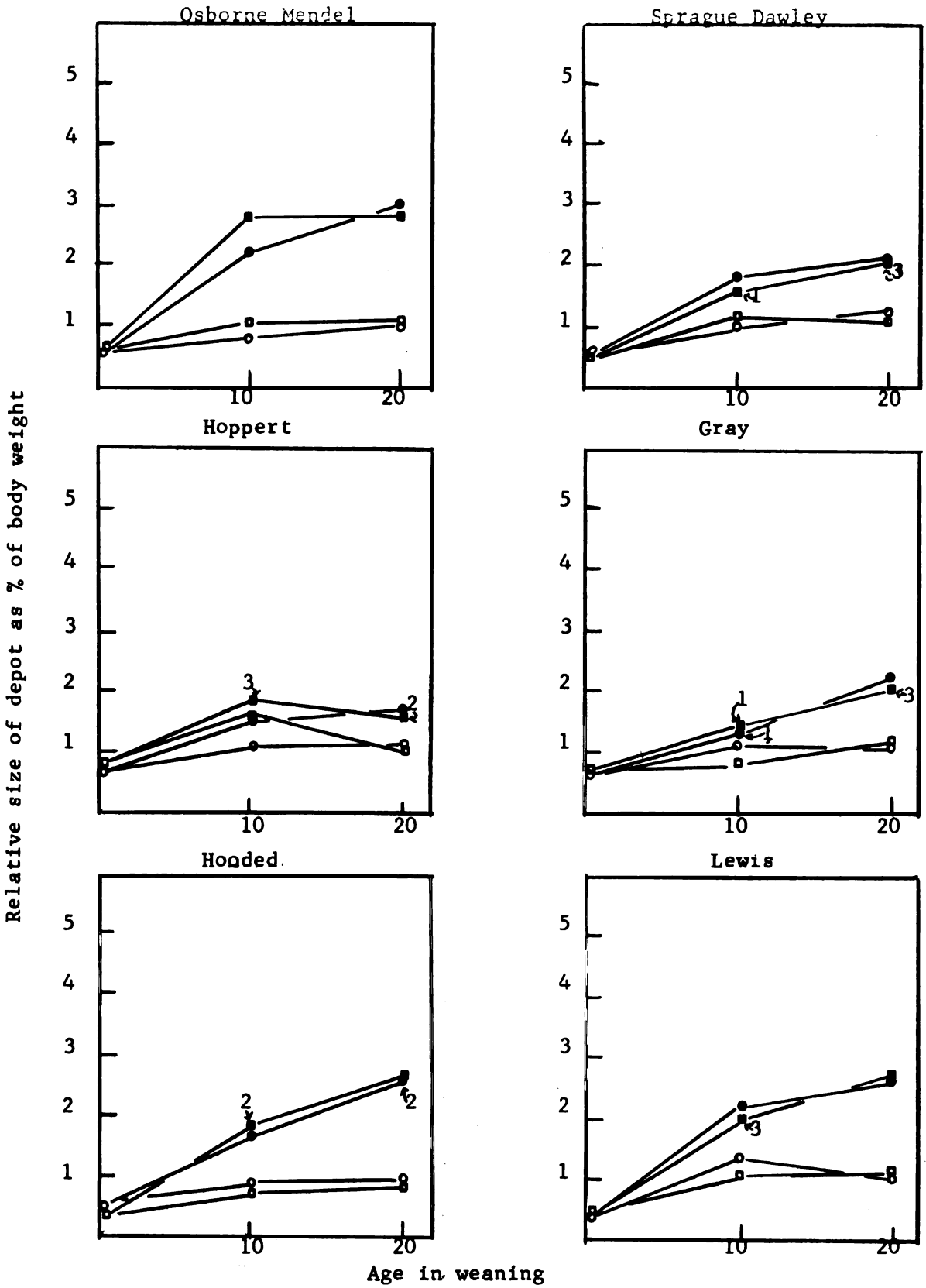

Fig. 21. Sum of the weights of the mesenteric and omental depots expressed as g/100 g of body weight. The significance of the difference when referred to the comparable group of Osborne Mendel rats is listed where applicable. The time intervals indicate the number of weeks the rats were on the experiment. Five rats in each group were sacrificed at the designated time intervals. (1. $P<0.01$; 2. $P<0.02$, 3. $P<0.05$). ● , Female, high fat.
■ , Male, high fat; □ , Male, grain. ○ , Female, grain.

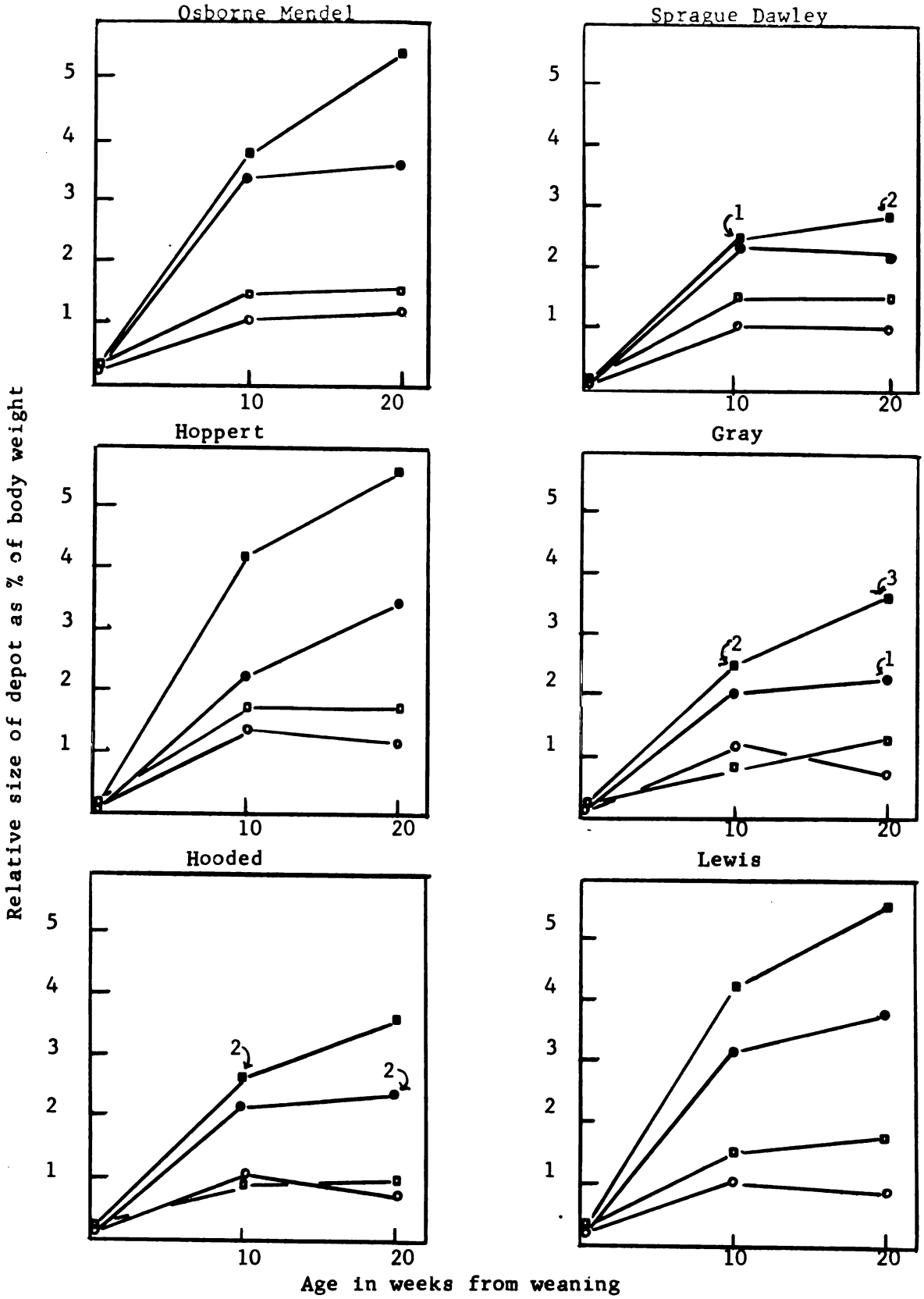

Fig. 22. Sum of the weights of the perirenal depots expressed as g/100 g of body weight. The significance of the difference when referred to comparable group of Osborne Mendel rats is listed where applicable. The time intervals indicate the number of weeks the rats were on the experiment. Five rats in each group were sacrificed at the weeks designated time intervals. (1. $P<0.01$; 2. $P<0.02$, 3. $P<0.05$).

■, Male, high fat; □, Male, grain
●, Female, high fat; ○, Female, grain

PART III

WEIGHT REDUCTION OF OSBORNE MENDEL RATS: EFFECT ON BODY COMPOSITION, WEIGHT OF ORGANS AND ADIPOSE TISSUES

INTRODUCTION

An affluent society, whether that of the Greeks, Romans, or the U. S. today has an easy access to food. Economic factors which lead to more luxurious living make it easier to become obese. In the past, obesity was considered a perogative only of the wealthy. Today an obese individual is looked upon with disdain and a premium is placed on leanness. Part of the latter stems from reports of improved health and increased longevity among individuals who are underweight. Additional advantages of the lean state come from life insurance statistics which suggest a prolongation of life for the obese individual who has lost some of his excess weight (Body Build and Blood Pressure Study, 1959).

To help the obese individual achieve a loss of weight, a number of business operations have sprung up which concentrate their activities in this area. Simultaneously, an endless parade of diets has appeared which claim to be very effective in removing "fat" from the obese. Most of these dietary programs, at best, result in extremely short-term effects with the individual losing weight for only a week or so. Failure to adhere to the diet thereafter is the usual explanation for the long-term failure. In many cases, even the initial weight loss is not entirely fat but largely water (Olesen and Quaade, 1960; Pilkington, 1960). Few individuals reduce and stay reduced.

However, the real merits of weight reduction have not been evaluated. This applies to both groups--those few who stay reduced as well as those whose body weights fluctuate in concert with alternating periods of feast or famine. An evaluation of either type of weight reduction can be done

with rats. The obesity developed in rats by the use of a ration high in hydrogenated fat (Mickelsen et al., 1955) makes this a useful experimental animal for such evaluations. On the other hand, most rats fed a grain ration never become obese.

This study was designed to determine whether or not a chow or grain ration was just as effective in producing weight reduction as it was in maintaining normal weight in rats that had never been obese.

A great deal of research effort has been expended in attempting to determine the nature of the tissue lost during a weight reduction regimen. For human beings, nitrogen balance (Passmore et al., 1960) K^{40} analyses as an index of lean body mass (Christian et al., 1964) and densitometry (Behnke et al., 1953; Rath and Slabochová, 1964) have been used in such studies.

The rat made obese by simple dietary means provides a unique opportunity to evaluate the nature of the tissue lost during weight reduction. Actual carcass analyses of representative rats before and after the weight reduction can provide information about the nature of the tissue lost.

Sarett et al., (1966) reported that this tissue was primarily fat with fasted rats losing somewhat more protein when compared to rats reduced by restricted feed intakes. Initial weight losses frequently involved mobilization of lean body tissue which tended to become less as the weight reduction program was prolonged. In view of this, we became interested in evaluating the gross body composition of obese rats after being reduced to and maintained at their ideal body weights long enough so that equilibrium was established. In addition, we wished to determine the contribution of each adipose tissue to the weight loss since this had not been investigated

previously. Whether fat was mobilized more quickly from certain areas than from others was one of the primary factors in this investigation.

EXPERIMENTAL

Both male and female Osborne Mendel rats bred in our laboratory, were fed a high fat ration from weaning. When male rats reached 1000 g and female rats reached 650 g, they were put into their respective experimental groups and subjected to weight reduction. Littermate rats were assigned to an experimental group according to a predesigned rotation plan to equalize the number of days required to reach the above weights. This allowed no more than one rat from each litter to be placed in each experimental group. The experimental group of 10 male rats was composed of two rats of 2 different litters which reached 1000 g in the shortest number of days, two rats which were the second to reach 1000 g, two rats which were third to reach 1000 g, two rats which were the fourth to reach 1000 g and two rats which were the last to reach 1000 g. The female rats were similarly divided except the division into experimental groups was done at 650 g. The groups consisted of (1) the rats permitted to eat the high fat ration ad libitum; they served as the obese controls, (2) the rats which were fed the grain ration ad libitum, (3) the rats fed a semipurified ration (M-16) (Table 17) which was designed to be similar in proximate composition to the grain ration (Table 18) and Campbell et al. (1966), (4) the rats fed the high fat ration ad libitum every Tuesday and Friday; the twenty-four span beginning and ending at 9:00 A.M., on the other days, the rats received only water, (5) the rats fed restricted quantities of the high fat ration daily between 9:00 and 10:00 A.M. For this group the total amount of high fat ration consumed

per week was similar to that consumed by the group described under "4", except that the weekly feed consumption of the latter group was divided into 7 equal portions one of which was served each day to the rats in group 5 and (6) eight male and 10 female rats were maintained throughout life on the grain ration. They were fed ad libitum and never became obese.

After achieving the respective weights, and after being assigned to an experimental group, records of weekly food intakes were kept for each rat. The high fat ration was of such a consistency that no food spillage occurred. Food cups which contained the grain ration or semipurified ration were fastened in an upright position to the side of the cage. This curtailed spillage except for a few rats that persisted in spilling food throughout the experiment. Feed consumption values were corrected for this spillage.

The rats were maintained at their reduced weight until the latter stabilized. For those animals which showed no weight change thereafter, they were continued on the reducing regimen for an additional 8 to 10 weeks when they showed only a slight weight change. This was done to make sure that their physiological and biochemical parameters had achieved a state of equilibrium. Some groups of rats, especially those fed the semipurified ration (M-16) never did show a weight plateau; they started to increase in weight shortly after an initial weight loss. These animals were sacrificed after being on the reducing regimen for 25 weeks which was the average time for the other rats.

At the time of sacrifice, the rats were weighed and then anaesthesized until death with ethyl ether. Nose to anus lengths were measured. Fat

depots were removed as previously described (Part I). From each animal, the liver, kidneys, adrenals, spleen and heart were removed and weighed. The entire carcass was then placed into a wide mouth jar and prepared by a modification of the carcass analysis procedure previously described (Part I and Mickelsen and Anderson, 1959).

RESULTS

Reduction in weight

All dietary regimens produced reductions in body weights but there was a difference in their effectiveness (Fig. 23 and 24). For all rats, the first week produced the largest decrements of weight loss. This loss in body weight ranged from 5.6 to 8.4% for all groups, irrespective of diet or sex. More pronounced differences between the groups began to appear in cumulative weight losses by the fifth week for both males and females. Male rats consuming the semipurified ration (M-16) showed consistently less weight ($P<0.05$) loss than rats in the other groups. Female rats consuming the semipurified ration behaved similarly but the differences were not significant until the 20th week. This was due to the fact that there were large individual differences in weight losses in the females consuming the semipurified ration. This group had two rats which lost unusually large quantities of weight in comparison to the other rats fed this ration, and had three rats with abnormal tumor growths which contributed to the weight gains. Body weights for both male and female rats consuming the semipurified (M-16) or grain (M-1) ration began plateauing or showed very slight losses in weight at the end of the tenth week.

This represented for both males and females, a 25 and 20% decrease in weight respectively for the rats consuming the semipurified ration, and a 39 and 33% decrease in weight respectively for the rats consuming the grain ration. At the end of ten weeks, males fed the grain ration weighed significantly less ($P < 0.05$) than those males fed the semipurified ration, but weighed approximately the same as the rats fed restricted quantities of the high fat diet (M-15) either daily or 2 days out of 7. On the other hand weight losses of the females fed grain (M-1) were not significantly different ($P < 0.05$) from those fed the semipurified ration until the 20th week.

There were no significant differences in accumulated losses of weight in the two groups of rats consuming restricted quantities of the high fat ration except at the 5th week ($P < 0.05$) for males and at the 15th ($P < 0.01$) and 20th ($P < 0.05$) week for females. These differences in the rats consuming the high fat ration ad libitum two days per week and those fed a restricted quantity resulted from a variation in food intake. It was impossible to pair-feed the rats and still use the rotation plan described in the experimental procedure. This was because animals were being added to the different groups at varying times depending on when the rats reached their obese weights. Both male and female rats fed the high fat ration in restricted quantities each day reflected the constancy of their intakes by showing consistent weight losses between the 5th and 10th, and 10th and 15th weeks of the reducing regimen (Fig. 23 and Fig. 24). This is in contrast to the three groups of rats reduced by other regimens wherein animals adjusted to their new diets and consistently increased their food intakes. After the 15th week, rats fed restricted

quantities of high fat diet daily, were given more food in order to equalize their final body weight with those rats fed the high fat ration two days out of seven.

Food intakes

Immediately after changing to the weight reduction regimens, all rats except those fed a restricted quantity of the high fat diet daily went through a period wherein food intakes were greatly decreased. More specifically, during the 1st week male rats fed grain ration ate 6 g per day. By contrast, rats of the same age fed grain ration throughout their lives consumed about 18 g per day (Table 19). By the end of the fifteenth week, the feed intake of the rats being reduced on the grain ration had increased to equal that of the lean controls (Table 19). This was true of both male and female rats.

Rats fed the semipurified ration consumed only 9 and 7 g daily for males and females respectively during the first week of weight reduction. By the 10th week this had increased to 20 g and 17 g daily for males and females, respectively.

On a dry weight basis, the proximate composition of these two low fat rations was the same (Table 18). They differed, however, in moisture content; the grain ration had 6 - 8% while the semipurified had 2.5%. When feed intakes were corrected for moisture content, the rats consuming the semipurified ration received slightly more calories than those fed the grain ration. This may have accounted for the smaller weight losses of the rats fed the semipurified ration (Fig. 23 and 24).

Male rats fed the high fat ration two days out of seven ate 14 g each day they were fed during the first week, and later increased this to

30 g per day (Table 19). Comparable values for the female rats in this group were 11 g per day in the first week with an increase to 28 g by the 15th week. After the tenth week, male and female rats fed two days out of seven consumed about 60% as much food in a week as the control obese rats of comparable age which had consumed the high fat ration ad libitum from weaning. When food was first put into their cages these rats immediately ate large quantities.

Both male and female rats fed restricted amounts of the high fat ration each day, ate all the food given them in less than five minutes except one female which generally had some food for a couple hours after feeding. In the beginning, male rats received 7 g daily. This amount was increased to 9 g at approximately the fifteenth week for most male rats. Females were fed 4 g in the beginning; later, between the 10th and 15th week this was increased to 6 or 7 g daily. These adjustments in intake allowed for a weight loss similar to those fed two days out of seven.

Following weight loss, all groups of rats appeared to be more active than previously. This conclusion was based only on casual observation of the rats. The rats fed the high fat ration daily in restricted amounts started to vigorously chew on their cages as soon as they heard the noise associated with the feeding operation. Rats fed the high fat ration two days per week did not respond in this manner.

Morbidity and mortality

After the weight reduction program started, one male rat died (Table 21). This was in the group fed the semipurified ration which, on an average, lost less weight than any other group. All other male rats

subjected to weight reduction survived that phase of the study in apparent good health. At the time of sacrifice, two rats in the semi-purified group had kidneys which were the same size or slightly larger than those in the obese control rats.

All of the rats fed the high fat ration on an ad libitum basis two days each week, had markedly enlarged stomachs. The latter very likely stemmed from the fact that in one day they were consuming an average of 30 g of ration whereas the obese controls consumed only half that amount (Table 19).

Of the 13 obese male control rats that were started on this program, 5 died before the study ended. The high mortality rate explains why this group initially was larger than any of the others. Prior to death male rats lost considerable quantities of weight. Fluctuations in weight gains and losses frequently occurred in the last 3 to 4 weeks of life.

All of the lean control male rats survived the study in apparent good health. When sacrificed, one rat had a testicular fat depot that was twice the size of the other; the smaller of these two depots weighed 2.3 g in contrast to 5.7 g for the other testicular depot. In other rats on this study, right and left testicular depots weighed the same.

The information in Table 21 suggests that the obese females did not respond to the weight reduction program as well as the males. This is in contrast to the mortality among the obese control rats. Among the males in that group 38% died during the weight reduction program whereas only 20% of the females died (Table 21). During the development of obesity and prior to weight reduction, 10% of the males had died and only 2% of the females had died.

During the weight reduction program, four of the female rats that were being reduced died. These were distributed among three of the four reducing groups -- the only one in which there was no mortality was the group fed the semipurified ration. The mortality rate among the obese female rats that were being reduced was 10% which is higher than the 2.5% among the comparable male group. The female rats during this period showed a high incidence of tumors. It is impossible to say whether these tumors were initiated by the weight reduction program or whether their presence became more apparent as a result of the loss of weight. One rat in the group receiving the semipurified ration had a large subcutaneous lymphoma type tumor located in the interscapular area. This was removed[1] and weighed 36 g. At the time of surgery, this rat weighed 577 g. One female rat fed the semipurified ration had enlarged and spongy kidneys. One other female reduced on restricted quantities of high fat ration and fed daily had enlarged kidneys and adrenals, abnormally shaped liver and dislocated gastrointestinal tract and genital organs.

Ketosis

Although it is recognized that rats are resistant to ketosis under most conditions, it appeared desirable to check this situation among the animals that were being reduced by alternate periods of feasting on the high fat ration followed by two or three days of starvation. To test this, 24 hour urine samples were collected from 10 male and 10 female rats. Length of time that rats were on weight reduction at the time of this collection varied. These animals were last fed 2 days prior to the

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[1] We wish to thank Dr. Robert Schirmer of the Veterinary Surgery Clinic for performing the operation on this rat.

urine collection. All samples gave negative results when tested for ketone bodies using Acetest tablets[1]. On this basis, it would appear that in the rat ketosis did not occur even when the animals were fed a high fat ration two days out of seven.

Body composition of reduced rats

The primary purpose of this phase of the study was to determine the more effective means of producing weight reduction in obese rats. This has been accomplished as is evident from the results presented in Figs. 23 and 24. However, since there were differences in the average final weights attained by the different groups of reduced rats, it is impossible to compare their body compositions directly (Table 22).

It is possible to compare the body composition of the reduced rats with that of the lean controls by means of a graph (Fig. 25 and 26). This type of presentation indicates that weight reduction for all groups resulted in a proportionately greater loss of fat for each unit of body weight lost. Consequently, the formerly obese rats after weight reduction approached the lean rats of the same body weight insofar as body composition was concerned. This was only partially true of the rats that were reduced by twice-a-week feeding of the high fat ration. These rats more closely resembled the "obese" rats of comparable weight fed the high fat ration from weaning. The other group of rats fed the high fat ration in restricted amounts each day was closer to the "lean" rats in body fat content.

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[1] Manufactured by Ames Company, Elkhart, Indiana. These tablets contain sodium nitroprusside and will detect "small" amounts of acetone according to the literature accompanying them.

The rats that were maintained in the obese state throughout the study increased their body fat content so that the value for the latter fell on the same line as that for the obese rats that had been studied earlier (Figs. 6 and 7, Part I).

The body protein content of the obese rats that were reduced in weight approached that of the animals that had always been lean (Fig. 26). Again, the rats that were fed the high fat ration two days out of seven more nearly resembled the "obese" rats of comparable weight as far as body protein was concerned.

Ash

Although there were variations in the grams of ash in the carcasses of the rats in the different groups, none of the differences were significant ($P>0.05$, Table 22). Even though the differences are non-significant, it should be pointed out that for both the males and females, there was a slight increase in total ash when the obese rats were reduced by being fed the grain (M-1) ration. In both cases, the ash contents exceeded the values for the lean rats which had been fed the grain ration throughout the study. The significance of this observation can only be assessed in a repetition of this work which is planned as a more definitive evaluation of obesity on skeletal size.

Composition of tissue lost

The absence of an effective and accurate method of determining body composition in the intact animals explains some of the apparent anomalies that occur when calculations are attempted to evaluate the nature of the weight lost during the reduction regimen. The composition of the rats used as representative of the reduced animals at the time

the weight reduction regimen started may have differed from the actual composition. When the amount of any component such as protein represents only a very small fraction of the total, it is difficult to secure accurate values when they are obtained by subtraction. For these reasons, the ratios between water and protein as secured from the data in Table 23 are not what they theoretically should be. Data such as these provide an indication of the relative proportions of fat and protein that were lost by the rats on the different reducing regimens.

For all groups, fat made up, by far, the largest fraction of the weight lost. For both males and females, the semipurified ration resulted in weight losses which appeared to be associated with only minor losses of protein. Unfortunately, this apparently ideal situation was marred by the fact that the rats fed this ration lost the least amount of weight. The male and female rats fed the grain ration appeared to lose different proportions of fat. The apparently greater loss of the fat by the females cannot be attributed to an initially higher percentage of fat since there is practically the same relative amount of fat in the bodies of both sexes (Fig. 6 and 7, Part I). The high fat ration in both cases (ad libitum and 2/7 day feeding) resulted in the lowest proportion of fat and highest percentage of protein lost. Whether the greater weight loss of the latter two groups of rats is responsible for their greater loss of protein cannot be determined from these data.

Changes in organ size with weight reduction

Kidneys: The kidneys in the obese rats were larger on an absolute basis than in the lean controls. However, the increase in size was not proportional to the increase in body size (Table 26). The two kidneys

in the male rats that remained obese throughout the experiment weighed 5.97 g compared to 4.19 g for the males that had always been lean (Table 26). In proportion to body weights these values became 0.51 g and 0.74 g per 100 g body weight respectively. A similar situation existed in the female rats.

Weight reduction was associated with a decrease in the size of the kidneys. The exception was the group of rats fed the semipurified diet on an ad libitum basis. In both the male and female rats fed this ration, the weight of the kidneys was essentially the same as that in the obese rats. For the other groups of rats, the size of kidneys decreased to that of the lean controls.

Adrenals: Obesity and weight reduction had no effect on the weight of the adrenal glands (Table 26). This was true for both male and female rats. Although there appeared to be differences between the obese and lean male rats (147 vs 103 mg) these differences were non-significant ($P > 0.05$). In other studies carried out in this laboratory, the adrenal weights of the obese rats were larger than those of the lean animals.

When the adrenal weights were calculated on the basis of body weight, the females showed a range from 17 to 34 mg per 100 g while the range for the males was from 11 to 18 mg. The relatively larger size of the adrenals in the females was seen in all groups. Since there were no differences in the absolute weights of the adrenals for any of the groups, representation of these weights on a body weight basis showed relatively smaller adrenals in the obese rats with no differences for any of the other groups.

Heart: Weight reduction produced a significant decrease in the size of the hearts of all rats except those fed the semipurified ration (Table 26). The weights of the hearts in both the male and female reduced rats approached the weight of the hearts in the lean controls. Although there were no statistical differences between the heart weights of the reduced rats and the lean controls, in no reduced group did the weight equal that of the lean control.

When expressed on a relative size, the hearts for all groups other than the obese controls were essentially the same. For the obese rats, the heart weight for both males and females was 210 mg per 100 g body weight. For males in all other groups of males, the relative heart size ranged from 290 to 310 mg and in the females from 290 to 350 mg.

Liver: Weight reduction produced a significant decrease in the size of the liver for both male and female rats in all groups except the ones fed the semipurified ration (Table 26). When expressed on the basis of relative body weight, there were only minor differences in the size of the liver for all groups.

Spleen: During weight reduction, the spleens of the rat were reduced in weight to those of control rats which were never obese (Table 26). When the weight of the spleens was expressed as percent of body weight, they were significantly smaller ($P < 0.05$) only in the obese control rats. Females (except obese controls) had a larger spleen than did males when the weight of the organ was related to body weight.

Summary: In all rats, hearts and spleens followed most nearly body weight losses, liver and kidney showed a lesser degree of weight loss and adrenals showed little change in weight with weight reduction.

Reduction in fat depot weights

All fat depots were reduced in weight when rats were subjected to any one of the four reducing regimens (Table 27). However, each fat depot had a pattern of weight loss which emphasized dissimilarities in the reduction of the various depots.

Inguinal depots: For all rats subjected to weight reduction, the inguinal depot lost sufficient weight to make this depot significantly different ($P<0.01$) in size from that of the obese rats even when its weight was expressed as a percent of body weight (Table 26). Both male and female rats fed the semipurified ration showed lesser total body weight losses than the rats in the other groups; the inguinal depot reflected this difference -- it was significantly different ($P<0.01$) from that of all other groups of reduced rats whether male or female when expressed as a percent of body weight. Male rats fed any one of the three other reducing rations had final weights of inguinal depots which were equivalent to that of the grain controls ($P>0.05$). Obese female rats fed the grain ration ad libitum still maintained a weight about 30% greater than the lean controls fed the same ration throughout their lives. The weights of the inguinal depots in the reduced rats were almost twice as heavy as in the lean controls. However, when expressed as percent of body weight, the difference in weight of this depot was not significantly different ($P>0.05$) from that of the lean controls. Inguinal depot weights for the two groups of female rats reduced by limiting the quantity of high fat diet were likewise not significantly different from the lean controls.

Genital depots: Male rats reduced on the grain ration showed a larger proportionate weight loss for the testicular depot (Table 27) than the rats reduced by any other means ($P<0.05$).

In proportion to body weight, the testicular depot was largest when male rats were 600 g or 148 days of age. At that time, this depot was 4% of body weight. This depot in control obese rats (about 400 days of age) represented only 2.5% of body weight. This ratio was maintained in the obese rats that were reduced by any of the regimens except the grain ration. The rats reduced by feeding the grain ration had testicular depots which represented only 1.46% of body weight; the same as the lean controls.

The parametrial fat depots in the reduced female rats represented the same proportion of body weight as in the lean controls. In all reduced groups, this depot had not only decreased in size but it represented a smaller percentage of body weight than in the obese rats ($P<0.01$ except for the group fed semipurified ration where $P<0.05$, Table 27).

Lean control male and female rats of this age showed a slight reduction in genital depot weights when compared to younger rats (Part I, Fig. 11).

Perirenal and retroperitoneal depots: None of the weight reduction regimens for either sex resulted in reducing the relative weights of the perirenal depots to those of the lean controls (Table 27). All remained significantly larger ($P<0.01$) although all showed a loss of weight when compared to the obese controls. For both males and females the semipurified ration resulted in the least weight loss of this depot.

This was probably related to the smaller losses in body weight for these rats. However, male and female rats fed the high fat diet ad libitum 2 days of 7 had slightly more fat in this area than those rats restricted to a limited amount of high fat ration daily or those reduced on the grain ration.

Mesenteric and omental fat: Weight reduction was highly effective in decreasing the mesenteric and omental fat depots to weights equal to or below those of the lean controls except for the female rats consuming the semipurified ration.

For male rats reduced by any of the weight reduction regimens other than the purified ration, the relative weights of the mesenteric and omental fat depots were significantly lower than those in the lean controls ($P<0.05$).

Fat surrounding xiphoid process: Compared to the lean controls, this depot remained relatively larger ($P<0.05$) in the rats of all reduced groups except the females fed restricted amounts of the high fat ration. For those females, the relative weight of this depot was the same as that of the lean controls. Although the differences are not significant, it is noteworthy that for both male and female rats, the high fat ration had a differential effect during the reducing program depending on how it was fed. When this ration was fed 2 days out of 7, the fat in the xiphoid process was 1.5 times larger than when the ration was fed each day in restricted amounts.

Recapitulation

During weight reduction of obese rats, the loss of weight by the different depots was influenced by (1) depot, (2) type of reducing regimen, (3) age of animal and to a lesser extent by (4) sex of the animal.

Depot: The rank order in which the fat depots lost weight depended upon whether the obese or the lean controls were used as the reference (Table 28). In both cases, the subcutaneous fat under the forelimb appeared to lose weight most readily. The perirenal fat depots were at the end of the list in both cases. Since the interscapular fat depot of females begins to increase very rapidly in size only after the animal's weight begins to exceed that of the lean controls, it shows a marked reduction compared to the obese rats and much less when compared with the lean controls.

The genital and mesenteric fat depots made up a relatively smaller percentage of the body weight in the older than in the younger animals (see Part I). If an adjustment were made for this, their place in the relative order might be changed.

Type of reducing regimen: The ration fed during the reducing program produced some differences in the percentages of weight lost from individual fat depots. The feeding program had a pronounced effect on the final body weights of the reduced rats. This, in turn, affected the final weights of the depots and thereby, their relative weight losses. The different final weights attained by the reduced rats makes it difficult to adequately evaluate the effect of the reducing regimen on the weight lost by the different depots. The weight of the fat depots in males reduced by feeding the grain were the same weight or lighter than those rats reduced on the high fat regimens. This was true even though male rats reduced on the grain ration were 30 g heavier. For other depots e.g. the interscapular and perirenal depots, there was a suggestion that when rats were fed restricted amounts of the high fat

ration, there was a difference in final depot weights depending on whether restricted amounts of the ration were fed every day or the rats were permitted to eat ad libitum two days out of seven (Table 27).

Age: Weight reduction of two depots (the genital and mesenteric) was enhanced with aging. Weight reduction of the perirenal depot and that depot surrounding the xiphoid process was curtailed by aging.

Sex: When the final weights of the fat depots in the male and female rats are compared (Table 29), it becomes apparent that the male rats during the reducing program lost relatively less fat than the females from the inguinal, forelimb and perirenal depots. The females lost relatively less fat from the interscapular, the genital, mesenteric and for the rats fed the grain and semipurified rations, the xiphoid depots. To a certain extent, the relative weight losses of each sex reflected the sex differences in the size of these depots that existed in the obese rats (Table 29).

DISCUSSION

Initial weight loss

The primary factor in determining the initial rate of weight loss during a reducing program is the deficiency of calories to which the animals are exposed. The nature of the ration is of only minor consequence as evidenced by the similar weight losses experienced by all groups of obese rats during the first few weeks of the reducing programs (Fig. 23 and 24). This is in contrast to the report by Kekwick and Pawan (1964) who claimed that weight reduction in mice was greater when most of the calories in the reducing diet came from fat than when they came from carbohydrate. They maintained that the differences in the

composition of the reducing diets were reflected in metabolic shifts in the mice brought about by the diets.

The primary difference between this work and that of Kekwick and Pawan is the relative amount of fat in our animals. In these studies, the animals that were reduced were grossly obese. This condition, plus the fact that they had been fed a high fat ration for a long time prior to weight reduction, meant that they were adapted to a metabolic scheme involving primarily fat. When they were put on the reducing regimens, they still secured a fairly high percentage of their energy from the metabolism of fat part of which was of endogenous origin.

The animals used by Kekwick and Pawan (1964) were "normal" mice that undoubtedly had relatively small stores of body fat--they had been fed a high carbohydrate ration throughout their lives. Although a number of critical comments could be made about the interpretations Kekwick and Pawan made from their results, suffice it to point out that they ignored the dehydrating effect that the high fat diet has when it is first consumed.

Grain vs. semipurified rations

Work of Mickelsen et al. (1955) indicated that when weanling rats were fed a grain ration from weaning, they did not become obese. When, however, they were fed a ration of purified ingredients in such amounts that the proximate analysis of the semipurified and grain rations were the same, about 80% of the rats eventually became obese. In view of this differential effect of the two rations on the initiation of obesity, they were compared as a means of producing body weight losses in obese rats.

The low-fat semipurified ration was not as effective as the grain ration in lowering the weight of obese rats. Actually, some of the female rats fed this semipurified ration started to regain their body weights after an initial loss; some of them weighed slightly more at the end of the reducing regimen than they did at its start. It is difficult to decide what is responsible for this difference in effectiveness of the two rations. There is a slight difference in moisture content of the two rations. Since the two groups of rats consumed essentially the same weight of feed, the slightly smaller moisture content of the semipurified (2.5%) compared to the grain ration (7%) resulted in a 5% difference in caloric intake. This small difference hardly appears adequate to explain a difference of 100 g of body weight which existed between the rats in these two groups.

The rats more readily accepted the semipurified ration than the grain ration when they were changed to it from the high fat ration. After the 10th week these initial differences in consumption disappeared and more nearly the same quantities were consumed by the two groups. The difference in this early acceptability could easily be related to the sweetness of the sucrose ration.

Sewell and Maxwell (1966) suggest this as the reason why baby pigs consumed more of a sucrose than of a grain ration. Other work in this laboratory (Taylor, unpublished data) indicates that rats have a great avidity for sucrose solutions and when offered this, they will drink more than half their body weight in a 24-hour period.

There was an apparent lower mortality among the rats that were reduced in weight than among the obese controls. For females, however, the evidence is not nearly as clear. A number of the obese females that were subjected

to weight reduction manifested lymphomas in their subcutaneous adipose tissue. Whether they developed as a result of the weight reduction or became more apparent with the loss of weight could not be determined. Furthermore, a number of female rats that were being reduced on the high fat regimen died before the study was completed. The validity of these observations can be established only by further work.

Organ weights

Obesity, per se, is associated with enlargement of the kidneys. The work of Sokoloff (unpublished data) has indicated that disturbances of the kidneys are one of the primary causes of death among the obese rats. Whether the enlargement seen in these obese rats stems from any pathological alterations will have to await the report of the pathologist. The present data do, however, indicate that body weight reduction of the obese rats is associated with a reduction in the size of the kidneys. All but one group of rats showed a decrease in kidney weights until they were, proportionately the same size as in the lean controls. In those rats fed the semipurified ration, the weights of the kidneys remained essentially the same size as when the animals were obese.

Preliminary work indicates that during the development of obesity, the kidneys show a reduction in their ability to clear the blood of phenolsulfonphthalein (PSP). The impairment sets in at a fairly early stage of obesity as evidenced by a reduction in PSP in the 450 g rats fed the high fat ration from weaning. As these rats increase in weight, their ability to clear the blood of PSP decreases even more (Taylor, unpublished data).

These observations indicate that another dietary factor apparently can influence the absolute size of the kidney. This is in addition to the effect of a high protein diet. For years it has been recognized that a

high protein diet produces hypertrophy of the kidneys (Addis, 1926). The hypertrophy per se according to Smith (1951, p. 473) has not been associated with any abnormality of this organ.

It is likely that the hypertrophy of the kidney in the simple dietary type of obesity observed in this study may be similar to that occurring in the rat made obese by hypothalamic lesions (Kennedy, 1957). Within the first week or so after the operation while the rats were eating very large amounts of feed (25% of body weight), the kidney, heart and liver rapidly increased in size. For the kidney, the increase in size was associated with a 1.5 fold increase in deoxyribonucleic acid phosphorus suggesting a considerable increase in the number of kidney cells.

The heart showed an increase in size with the development of obesity. As in the case of the kidneys, the increase in weight of the heart of the obese rats was not proportional to the increase in body weight. Again, Kennedy (ibid) reported a rapid increase in the size of the heart during the first few weeks after hypothalamic lesions in his rats that became obese. Unfortunately no data accompanied that statement. At this time, it cannot be stated whether the hypertrophy of the heart is the result of an increase in the vascular bed. Although no studies of blood volume have been made in these obese rats there is some suggestion for an increase in that parameter. This is based on the volume of blood that can be removed by a syringe inserted into the renal artery. Under such circumstances more blood can be removed from the obese than from the lean rat.

The results of the present study indicated that the weight of the heart returns toward that of the lean control during a weight reduction program. Since the rat is relatively immune to any cardiovascular abnormalities associated with the obese state (Bragdon and Mickelsen, 1955), it becomes difficult to assess the role of weight reduction on that function.

The liver also increased in absolute weight during the development of obesity. However, this increase is closely related to changes in body weight (Table 26). The enlargement of the liver in the rats fed the high fat ration from weaning is not associated with any abnormality either in composition or histological structure (Sokoloff, unpublished data).

Weight reduction is accompanied by a decrease in the size of the liver. This decrease again being proportional to body weight changes.

Protein

The larger loss of total protein on the two weight reduction diets wherein fat was restricted could be associated with a lesser intake of protein on these high fat rations. Although protein made up roughly 25% of the original high fat ration, the reduction in intake of the rats fed reduced amounts of the high fat ration was such that the intake of protein ranged from 6 to 15 g per week. On the other hand, rats reduced on the semipurified and grain rations ate from 8 to 30 g of protein per week. It is doubtful whether even this difference in protein intake can explain the observed differences since the presence of 3.5% protein in the tissue lost by weight reduction agrees with that reported by Sarett et al. (1966).

Fat depots

Here, four behavioral patterns of fat depots seem worthy of discussion. Females appear to favor deposition of fat behind the neck and in the mesenteric depots. Yet, in lean female controls, the relative amount of fat deposited behind the neck is no greater than that present in males. In weight reduction, the grain ration appeared more effective in the removal of fat from this interscapular area than other diets. The rats fed restricted amounts of the high fat ration every day were more effective in

removal of fat from this area than the rats fed the high fat ration ad libitum two days per week.

The mesenteric depots in the obese rats were reduced so that their final weights were nearly the same or less than the weights of lean controls. Could fat be more readily removed from this area because of its proximity to the gastrointestinal tract? Males showed greater evidence of weight reduction here. This appears to counterbalance the lesser reduction of the perirenal fat depot in males. Škerjl (1959) and Young et al. (1963) suggest a reproportioning of fat with a larger quantity of abdominal fat appearing in women with aging. It would appear that the perirenal fat depot and fat deposits in the abdominal cavity under the ribs and around the xiphoid process are sequestering this fat while mesenteric and genital fat depots continue to reduce.

Both of the high fat reducing regimens produced less reduction in testicular fat depot size than either the grain or semipurified rations. Additional work will be required to determine whether this differential effect was due to the differences in the composition of the diets or the method of feeding (i. e., ad libitum versus restricted intake).

Table 17

Composition of Semipurified ration
(M-16)

Ingredients	%
Casein	22.50
Corn oil	2.67
Minerals[1]	6.28
Vitamin mix[2]	2.20
Liver powder	2.00
dl-Methionine	0.25
Aureomycin[3]	0.01
Non-nutritive bulk[4]	3.80
Sucrose	60.29

1 Salt Mixture, Wesson Modified (Osborne-Mendel) General Biochemicals, Chagrin Falls, Ohio.

2 Vitamin Diet Fortification Mixture, Nutritional Biochemicals, Cleveland, Ohio.

3 Generously provided by American Cyanamid Company, Princeton, New Jersey.

4 Non-nutritive Bulk, cellulose type, General Biochemicals, Chagrin Falls, Ohio.

Table 18

Proximate analysis of Grain (M-1) and Semipurified (M-16) Rations

Component	Grain (M-1) %	Semipurified (M-16) %
Protein	23.4	22.5
Fat	3.0	2.7
Fiber	3.8	3.8
Ash	6.3	6.3

The grain ration has 3.24 calories per g; the semipurified ration has 3.48 calories per g of ration. This difference was due to moisture content. The grain ration contained 8.0% moisture; the semipurified ration, 2.5%.

Table 19

Food intakes of rats during weight reduction period

	Week 1 g	Week 2 g	Week 5 g	Week 10 g	Week 15 g	Week 20 g	Week 25 g
				Male			
High fat, control[1]	98.1± 5.4[2]	112.0± 4.3[2]	107.6± 7.3[2]	115.6± 7.4[2]	111.0±10.5[2]	98.5±16.9[2]	109.0±29.5[2]
Grain, control[1]	128.4± 1.4	161.0± 4.1	140.2± 3.3	158.1± 3.7	168.5± 3.6	164.3± 6.3	161.1± 6.0
Grain, reduced	39.9± 3.9	73.2± 5.2	105.9± 5.4	139.3±11.6	158.4± 8.6	163.8± 7.3	166.4±11.1
Semipurified, red.	68.6±10.7	80.9± 7.9	114.8± 9.4	139.8± 9.7	153.1± 9.4	159.1±11.8	162.0±12.2
High fat, 2 da. of 7	25.2± 4.5	37.7± 3.1	41.0± 2.0	52.5± 2.0	60.9± 1.7	61.6± 2.6	60.4± 7.4
High fat, restricted	49.0	49.0	49.0	49.0	49.0	63.0	63.0
				Female			
High fat, control[1]	79.9± 2.0	75.2± 4.7	94.9± 4.6	107.6± 9.9	98.9± 6.3	110.6± 5.3	98.0± 6.5
Grain, control[1]	135.9± 5.2	121.2± 2.7	122.2± 6.5	145.0± 6.3	151.6± 7.6	129.7± 4.0	137.6± 4.5
Grain, red.	41.1± 7.4	65.2± 7.1	95.2± 8.3	125.1±11.3	149.3± 7.6	135.1± 7.6	143.3± 8.4
Semipurified, red.	49.6± 3.8	56.1± 8.0	107.3±12.7	116.6±11.2	138.3± 9.6	138.6± 8.2	135.7±13.9
High fat, 2 da. of 7	22.1± 2.2	26.3± 1.5	30.3± 1.8	43.0±11.8	55.7± 3.7	53.2± 5.7	47.4± 3.5
High fat, restricted	28.0	28.0	28.0	28.0	42.0	42.0	49.0

[1] These rats were never reduced.

[2] Mean food intake for one week in g ± Std. Error.

Table 20

Cumulative average caloric intake per rat during twenty weeks of the reduction regimen

Ration	Week 1 cal.	Week 2 cal.	Week 5 cal.	Week 10 cal.	Week 20 cal.
			Male		
High fat, control[1]	642± 48[2]	1357± 31[2]	3549± 54[2]	7284± 110[2]	14629± 389[2]
Grain, control[1]	416± 6	937± 17	2343± 47	4806± 79	10166± 195
Grain, reduced	129± 11	366± 24	1301± 58	3406± 159	8697± 304
Semipurified, reduced	235± 36	515± 55	1616± 99	4015± 149	9217± 232
High fat, 2 da.of 7	187± 10	436± 24	1205± 45	2749± 66	6615± 117
High fat, restricted	320± 5	644± 5	1618± 5	3251± 6	6716± 5
			Female		
High fat, control[1]	525± 11	1020± 45	2875± 116	6132± 248	12758± 499
Grain, control[1]	440± 17	832± 17	2033± 70	4178± 171	8667± 339
Grain, reduced	133± 24	344± 44	1125± 116	2983± 191	7488± 282
Semipurified, reduced	173± 13	368± 35	1361± 121	3216± 269	7957± 379
High fat, 2 da.of 7	146± 17	327± 22	933± 55	2232± 111	5482± 113
High fat, restricted	191± 6	382± 12	939± 12	1866± 12	4183± 101

1
Mean cumulative caloric intakes ± Std. Error.

2
These rats were never reduced.

Table 21

Some characteristics of rats at termination of wt. reduction regimen (WRR)

Ration	No. of rats	Age(days) start of WRR	Wt(g) at sacrifiee	Number of animals Analyzed	Died	With tumors
			Male			
High fat, control[1]	13	283.3± 12.9[2]	1161.7± 39.2[2]	7[3]	5	0
Grain, control[1]	8	260.8± 28.6	565.6± 15.5	8	0	0
Grain, reduced	10	300.4± 14.8	637.8± 14.2	10	0	0
Semipurified, reduced	10	315.8± 21.8	754.0± 26.7	9	1	0
High fat, 2 days of 7	10	284.0± 17.0	606.5± 10.0	10	0	0
High fat, restricted	10	306.7± 19.6	599.8± 4.7	10	0	0
			Female			
High fat, control[1]	10	249.5± 25.2	885.0± 35.8	7	2	1
Grain, control[1]	10	266.4± 18.3	363.3± 12.1	10	0	1
Grain, reduced	10	258.9± 15.4	447.2± 9.5	9	1	1
Semipurified, reduced	10	263.4± 15.0	575.2± 45.0	10	0	3[4]
High fat, 2 days of 7	11	286.3± 21.3	363.3± 9.0	9	2	2
High fat, restricted	10	269.4± 22.8	358.5± 8.5	8	1	0

[1] These rats were never reduced.

[2] Standard Error of the mean

[3] One rat lost over 300 g and is excluded from the analysis.

[4] One of these rats had a tumor surgically removed. At time of sacrifice, rat had 3 lymphoma-type tumors.

Table 22

Percent body composition of obese and lean controls and reduced Osborne Mendel male and female rats

Diet	Wgt.[1]	Fat %	Protein %	Ash %	Water %
			Male		
High fat, control	1142.0 ± 45.8[2]	51.24 ± 0.86[2]	10.40 ± 0.15[2]	1.52 ± 0.07[2]	36.27 ± 0.92[2]
Grain, control	539.7 ± 21.2	18.21 ± 1.37	20.31 ± 0.44	3.03 ± 0.04	58.24 ± 1.27
Grain, reduced	618.2 ± 15.5	23.69 ± 1.61	18.65 ± 0.54	3.20 ± 0.05	53.81 ± 1.30
Semipurified, reduced	742.2 ± 39.3	31.59 ± 2.56	16.10 ± 0.60	2.53 ± 0.11	49.09 ± 1.96
High fat, 2 da. of 7	590.2 ± 8.8	27.36 ± 1.11	16.95 ± 0.17	2.59 ± 0.07	52.53 ± 0.91
High fat, restricted	585.0 ± 7.2	22.80 ± 1.57	18.55 ± 0.88	3.08 ± 0.14	55.69 ± 1.20
			Female		
High fat, control	856.3 ± 50.2	54.34 ± 1.49	9.69 ± 0.26	1.56 ± 0.11	34.13 ± 1.05
Grain, control	341.4 ± 12.8	17.44 ± 0.88	19.35 ± 0.24	3.40 ± 0.09	59.55 ± 0.66
Grain, reduced	422.8 ± 14.0	23.62 ± 2.50	18.21 ± 1.04	3.30 ± 0.18	55.12 ± 1.92
Semipurified, reduced	510.6 ± 53.1	36.08 ± 6.02	13.91 ± 1.34	2.34 ± 0.23	46.88 ± 4.28
High fat, 2 da. of 7	354.0 ± 11.0	22.57 ± 2.24	17.52 ± 0.73	2.98 ± 0.18	55.89 ± 1.51
High fat, restricted	327.6 ± 6.2	19.70 ± 1.75	17.75 ± 0.21	3.13 ± 0.08	58.58 ± 1.72

[1] This is the weight of the rats exclusive of G. I. contents.

[2] Mean and Std. Error for 5 rats.

Table 23

Absolute body composition of obese and lean controls and reduced Osborne Mendel male and female rats

Diet	Fat g	Protein g	Ash g	Water g
		Male		
High fat, control	585.74 ± 45.67[1]	118.60 ± 3.82[1]	17.21 ± 0.32[1]	414.12 ± 8.24[1]
Grain, control	103.68 ± 4.30	109.91 ± 6.41	16.36 ± 0.78	314.20 ±13.75
Grain, reduced	147.12 ± 12.50	115.13 ± 3.14	19.81 ± 0.74	332.27 ± 8.59
Semipurified, reduced	238.32 ± 31.40	118.59 ± 2.13	18.65 ± 0.47	361.45 ± 2.06
High fat, 2 da. of 7	161.34 ± 6.26	100.10 ± 2.32	15.29 ± 0.38	310.17 ± 8.67
High fat, restricted	133.20 ± 10.59	107.86 ± 4.02	17.92 ± 0.69	324.79 ± 2.85
		Female		
High fat, control	466.39 ± 35.09	83.13 ± 3.21	13.20 ± 3.53	291.75 ±17.57
Grain, control	60.51 ± 3.89	67.17 ± 2.73	11.62 ± 0.59	203.26 ± 7.78
Grain, reduced	101.08 ± 14.09	76.82 ± 4.51	13.86 ± 1.02	232.01 ± 2.66
Semipurified, reduced	190.37 ± 48.56	71.92 ± 2.66	11.45 ± 0.18	235.70 ±22.86
High fat, 2 da. of 7	84.02 ± 9.69	64.56 ± 1.30	10.55 ± 0.83	196.65 ± 2.42
High fat, restricted	67.59 ± 6.46	60.70 ± 1.38	10.25 ± 0.41	191.65 ± 4.53

[1] Mean and Std. Error for 5 rats.

Table 24

Composition of weight lost by obese Osborne Mendel rats as a result of reduction of body weight[1]

Ration fed during wt. reduction	Weight lost	Fat		Protein		Ash		Water	
	g	g	%	g	%	g	%	g	%
				Male					
Grain	384[2]	332.9	86.7	4.9	1.3	4.4[3]	1.1[3]	42.7	11.1
Semipurified	253	241.7	95.5	1.4	0.1	3.2[3]	1.3[3]	13.6	5.4
High fat, 2 da. of 7	404	318.7	78.9	19.9	4.9	0.1	0.0	64.8	16.0
High fat, restricted	405	346.8	85.6	12.1	3.0	2.5[3]	0.6[3]	45.2	11.2
				Female					
Grain	217	213.9	98.6	4.8	2.2[3]	2.8[3]	1.3[3]	10.0	4.6
Semipurified	134	124.6	93.0	0.1	0.1	0.4[3]	0.3[3]	6.3	4.7
High fat, 2 da. of 7	287	231.0	80.5	7.4	2.6	0.5	0.2	45.3	15.8
High fat, restricted	317	247.4	78.0	11.3	3.6	0.8	0.3	50.4	15.9

[1] Weight loss was calculated by subtracting the weight of the body components of the reduced rats when sacrificed from the values for a 1000 g male or a 650 g female rat. The latter were secured by graphing the data for obese rats of different body weights. This plot of the data indicated that a male rat minus its G.I. tract contents would weigh 985 g composed of: 480 g fat, 120 g protein, 15.4 g ash and 370 g water while the female would weigh 640 g composed of: 315 g fat; 72 g protein, 11.0 g ash and 242 g moisture.

[2] These are the mean g of weight lost by the 5 rats in each group analyzed for body composition only. They do not necessarily agree with the weight losses in Fig. 23 and 24 wherein 10 rats for each group are included.

[3] These mean values increased slightly during weight reduction.

Table 25

Percentage of gross body components lost during weight reduction[1]

Ration fed during weight reduction	Body weight %	Fat %	Protein %	Ash %	Water %
		Male			
Grain	39.18	69.35	4.06	28.63[2]	11.54
Semipurified	25.81	50.35	1.18	21.10[2]	36.62
High fat, 2 days of 7	41.22	66.39	16.58	0.71	17.52
High fat, restricted	41.32	72.25	10.12	16.36	12.22
		Female			
Grain	33.90	67.90	6.66[2]	25.18[2]	4.13
Semipurified	20.93	39.56	0.11	3.27[2]	2.60
High fat, 2 days of 7	44.84	73.33	10.33	4.91	18.74
High fat, restricted	49.53	78.54	15.69	7.64	20.81

1 Weight loss was calculated by subtracting the weight of the body components of the reduced rats when sacrificed from the values for a 1000 g male or a 650 g female rat. The latter were secured by graphing the data for obese rats of different body weights. This plot of the data indicated that a male rat minus its G.I. tract contents would weigh 985 g composed of: 480 g fat, 120 g protein, 15.4 g ash and 370 g water while the female would weigh 640 g composed of: 315 g fat, 72 g protein, 11.0 g ash and 242 g moisture.

2 These showed weight gains during weight reduction.

Table 26

Organ weights of obese and lean controls and reduced Osborne Mendel male and female rats

Ration	Kidneys g	Adrenals mg	Heart g	Liver g	Spleen g
		Male			
High fat, control[1]	5.97 ± 0.67[2]	147 ± 32[2]	2.49 ± 0.19[2]	39.07 ± 1.54[2]	1.47 ± 0.09[2]
Grain, control[1]	4.19 ± 0.31	103 ± 9	1.65 ± 0.08	21.70 ± 1.51	0.85 ± 0.13
Grain, reduced	4.37 ± 0.12	113 ± 7	1.86 ± 0.05	24.14 ± 1.25	1.15 ± 0.06
Semipurified, reduced	5.49 ± 0.35	110 ± 7	2.09 ± 0.07	28.46 ± 2.26	1.33 ± 0.07
High fat, 2 da. of 7	3.91 ± 0.16	107 ± 6	1.75 ± 0.05	20.42 ± 1.44	0.89 ± 0.03
High fat, restricted	3.80 ± 0.22	114 ± 6	1.87 ± 0.05	17.71 ± 1.11	0.94 ± 0.06
		Female			
High fat, control[1]	4.47 ± 0.58	142 ± 11	1.88 ± 0.08	24.04 ± 2.41	1.12 ± 0.03
Grain, control[1]	2.58 ± 0.10	136 ± 8	1.19 ± 0.04	12.34 ± 0.83	0.83 ± 0.06
Grain, reduced	2.90 ± 0.12	115 ± 4	1.36 ± 0.04	15.32 ± 0.76	1.00 ± 0.10
Semipurified, reduced	4.39 ± 0.66	123 ± 11	1.57 ± 0.06	22.38 ± 1.15	1.14 ± 0.12
High fat, 2 da. of 7	2.74 ± 0.11	109 ± 4	1.28 ± 0.08	13.68 ± 1.21	0.84 ± 0.06
High fat, restricted	2.52 ± 0.16	122 ± 19	1.21 ± 0.03	12.14 ± 0.80	0.69 ± 0.02

[1] These rats were never reduced.

[2] Mean ± Std. Error.

Table 27
Relative weights of adipose tissues

Fat depot	Control rats		Weight reduction regimen			
	Obese	Lean	Grain	Semipurified	Hi fat 2/7 da.	Hi fat red.
	g	g	g	g	g	g
Male						
Inguinal	9.07 ±0.63[1]	3.83 ± 0.26[1]	4.21 ± 0.34[1]	6.28 ± 0.44[1]	4.08 ± 0.21[1]	4.12 ±0.28[1]
Sub. fat under forelimb	1.54 ±0.15	0.62 ± 0.07	0.44 ± 0.06	0.72 ± 0.05	0.50 ± 0.08	0.50 ±0.06
Interscapular	3.33 ±0.33	0.76 ± 0.08	9.97 ± 0.14	1.79 ± 0.24	1.17 ± 0.14	0.99 ±0.21
Testicular	2.54 ±0.19	1.82 ± 0.11	1.46 ± 0.11	2.15 ± 0.21	2.06 ± 0.19	2.04 ±0.16
Epididymal	0.055±0.005	0.100± 0.004	0.074± 0.004	0.082± 0.03	0.073 0.004	0. 75±0.003
Perirenal	13.95 ±1.25	1.90 ± 0.14	6.53 ± 0.70	8.97 ± 0.76	8.86 ± 0.79	8.09 ±0.59
Mesenteric and omental	2.38 ±0.21	1.27 ± 0.11	0.86 ± 0.11	1.26 ± 0.16	0.85 ± 0.11	0.74 ±0.11
Xiphoid process[2]	1.00 ±0.09	0.15 ± 0.01	0.29 ± 0.03	0.44 ± 0.05	0.44 ± 0.05	0.32 ±0.04
Female						
Inguinal	6.00 ±0.49	1.79 ± 0.20	2.70 ± 0.41	3.80 ± 0.84	1.94 ± 0.19	2.11 ±0.30
Sub. fat under forelimb	0.68 ±0.10	0.34 ± 0.05	0.19 ± 0.05	0.41 ± 0.11	0.12 ± 0.03	0.19 ±0.05
Interscapular	10.26 ±1.07	0.78 ± 0.11	3.01 ± 0.31	5.55 ± 1.03	2.92 ± 0.46	2.15 ±0.55
Genital	4.85 ±0.48	2.94 ± 0.20	2.44 ± 0.33	3.10 ± 0.46	2.31 ± 0.29	2.46 ±0.16
Perirenal	9.31 ±0.45	1.73 ± 0.19	4.65 ± 0.54	6.16 ± 0.95	4.38 ± 0.49	3.69 ±0.40
Mesenteric and omental	2.38 ±0.39	1.39 ± 0.13	1.39 ± 0.14	1.84 ± 0.17	1.29 ± 0.12	1.18 ±0.11
Xiphoid process[2]	0.92 ±0.16	0.20 ± 0.03	0.46 ± 0.04	0.68 ± 0.09	0.40 ± 0.05	0.30 ±0.04

[1] Mean weights of adipose tissues per 100 g body weight ± Std. Error.

[2] This fat was deposited in an area surrounding the cartilage of the xiphoid process.

Table 28

Rank order of weight reduction of individual adipose tissues

	Compared to obese controls[1]			Compared to lean controls[2]	
	Males	Females		Males	Females
Forelimb	1	1	Forelimb	2[3]	1
Interscapular	2	2	Mesenteric	1[3]	3
Xiphoid[4]	3	4	Genital[5]	3	2
Inguinal	5	3	Inguinal	4	4
Mesenteric	4	7	Xiphoid	6	5
Perirenal	6	5	Interscapular	5	7
Genital[5]	7	5	Perirenal	7	6

1 These depots are arranged so that the depot losing the largest percent of weight (compared to obese control weight) is No. 1, etc. (The four rations were averaged).

2 These depots are arranged in the order in which they more nearly approached or were less than the same depot in the lean control - the depot in the reduced rats that had lost the greatest relative amount of weight was listed as 1.

3 These depots in reduced rats weighed less than those in lean controls.

4 This fat was deposited in an area surrounding the cartilage of the xiphoid process.

5 Genital depot means the testicular depot of the male and the parametrial depot of the female.

Table 29

Influence of sex on final weights of fat depots in the reduced obese control and lean control rats. All values are expressed as a ratio of male/female weights.

	Controls		Reducing regimen			
	Obese	Lean	Grain	Semipurified	Hi fat 2/7 da	Restricted
Inguinal	151.17[1]	213.97[1]	155.93[1]	165.26[1]	210.31[1]	195.26[1]
Forelimb	226.47	182.35	231.58	175.61	416.67	294.11
Interscapular	32.45	97.44	32.23	32.25	40.07	46.05
Genital t	52.37	61.90	59.84	69.35	89.18	82.93
Perirenal	149.84	109.83	140.43	145.62	202.28	219.24
Mesenteric	100.00	91.37	61.87	68.48	65.89	62.71
Xiphoid	108.70	75.00	63.04	64.71	110.00	106.67

[1] All values were obtained by dividing the weight of fat depot per 100 g body weight of the male rats by that same value for the females and multiplying by 100.

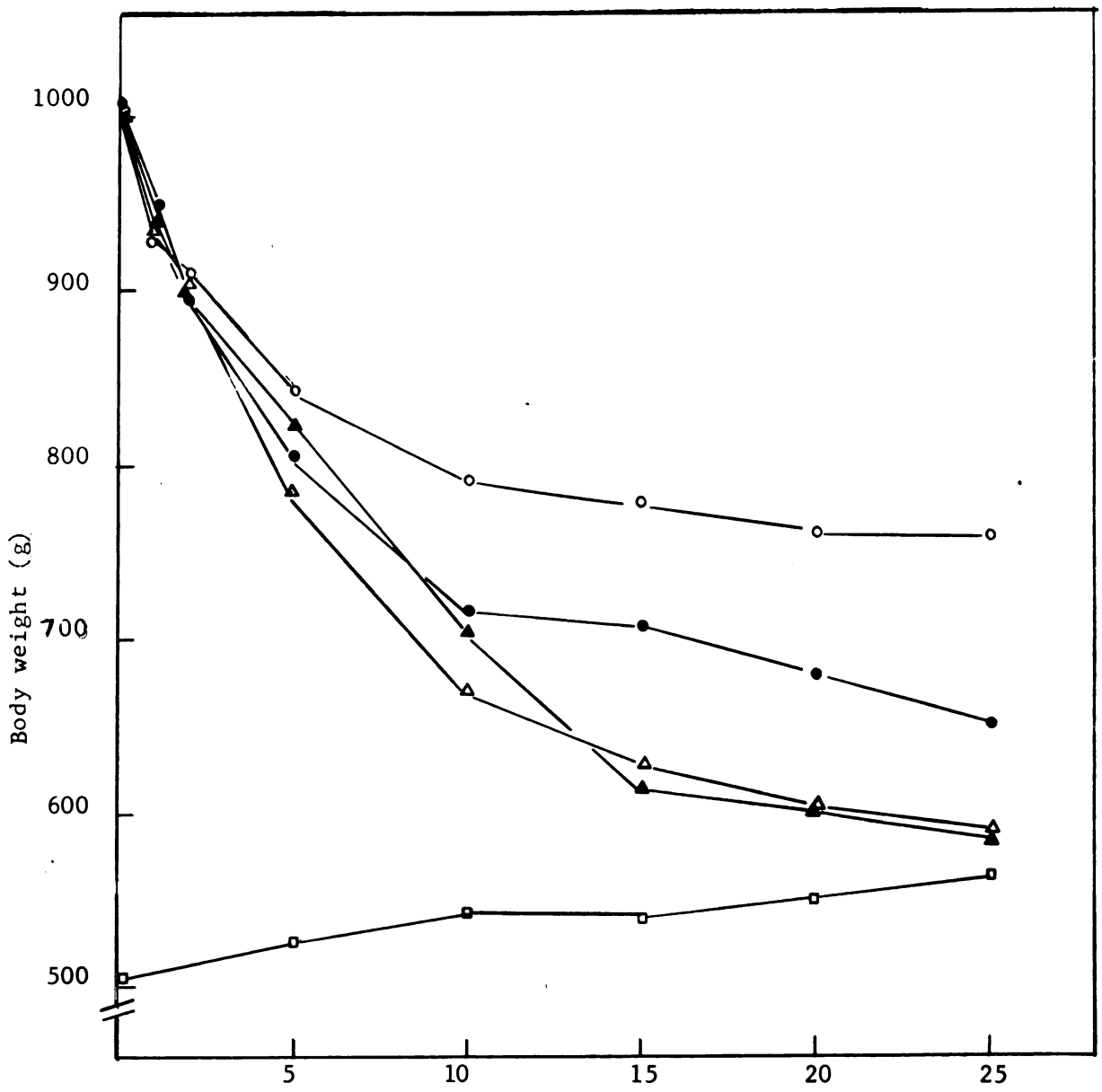


Fig. 23. Decreases in male Osborne Mendel rats subjected to one of the following reducing regimens: o—o semipurified ration, ad lib.; ●—●, grain ration, ad lib.; △—△, high fat ration 2 days each week; ▲—▲, high fat ration in restricted amounts every day and □—□, grain-fed control rats.

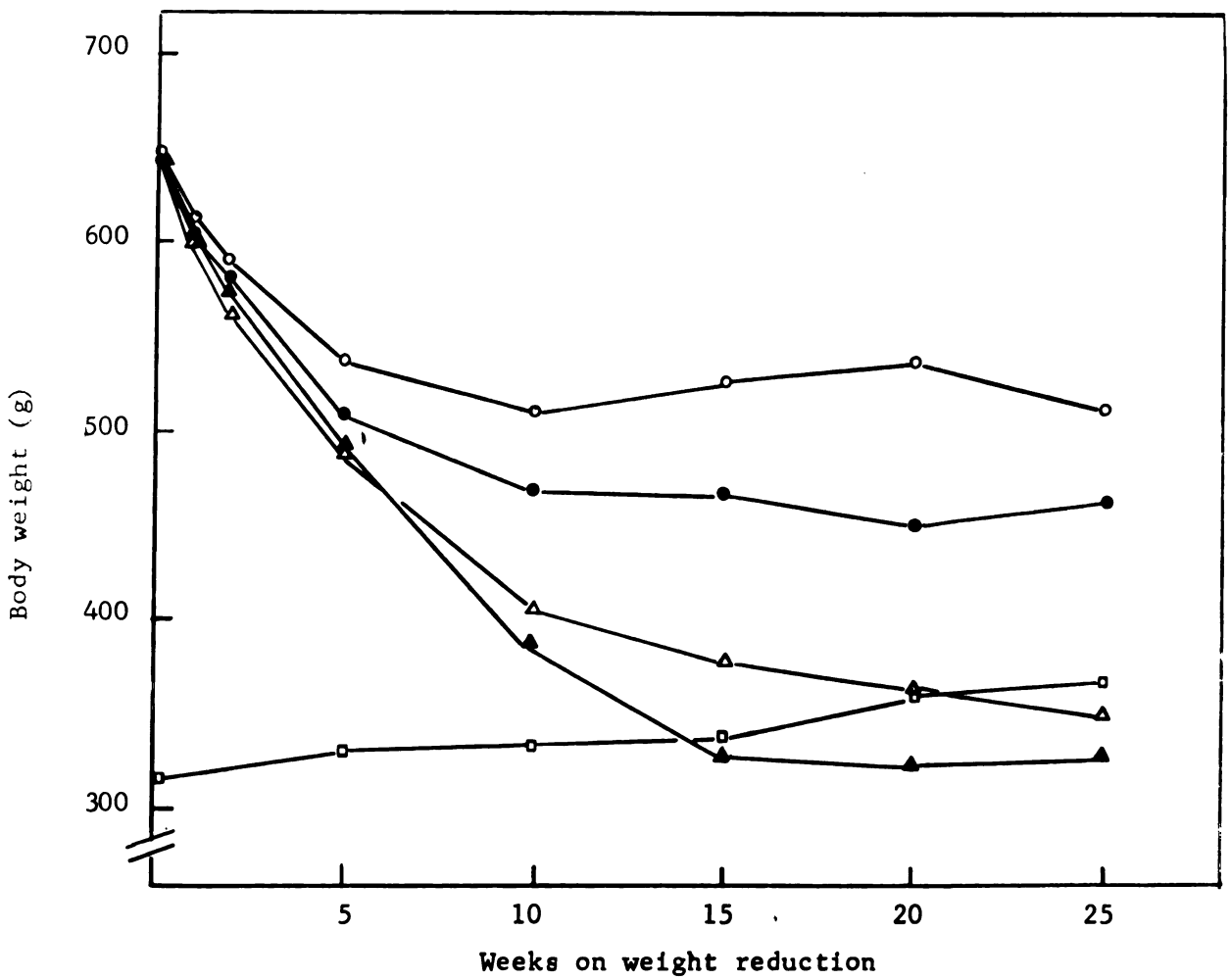
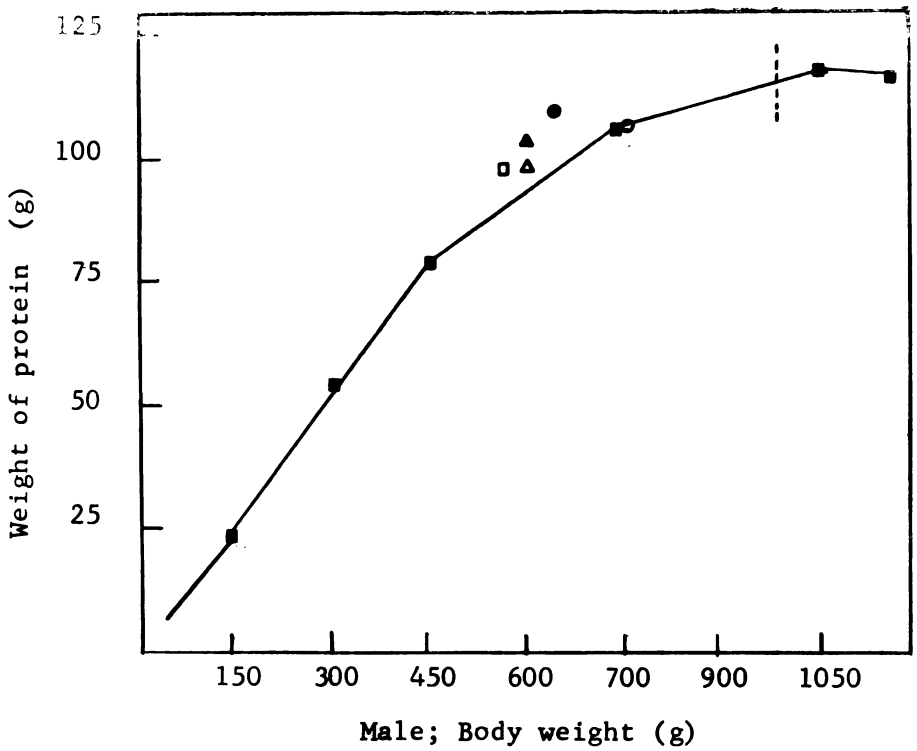


Fig. 24. Decreases in female Osborne Mendel rats subjected to one of the following reducing regimens: o—o semipurified ration ad lib.; ●—●, grain ration, ad lib.; △—△, high fat ration 2 days each week; and ▲—▲, high fat ration in restricted amounts every day and □—□, grain-fed control rats.



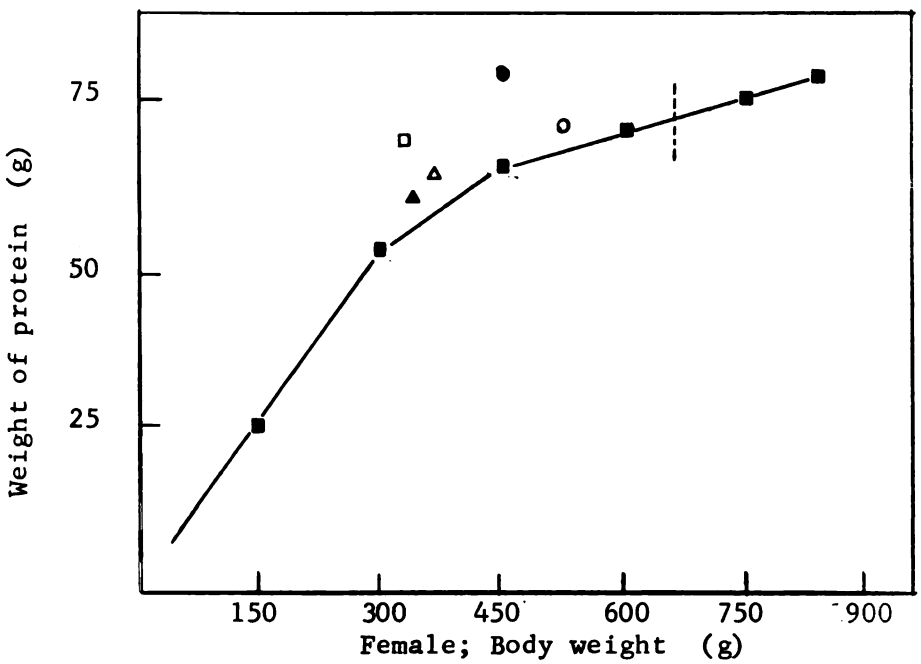


Fig. 25. Weight of protein in reduced Osborne Mendel rats in comparison to weight of protein in younger high fat-fed rats (Part I). The intersect indicates the g of protein in the rats when rats were first fed reducing rations. This figure also shows comparison with lean controls of the same age. ■—■, weight of protein in g during development of obesity; □, grain-fed control; ●, grain-fed, reduced; o, semipurified, reduced; △, high fat, 2 da. of 7; ▲, high fat, restricted.

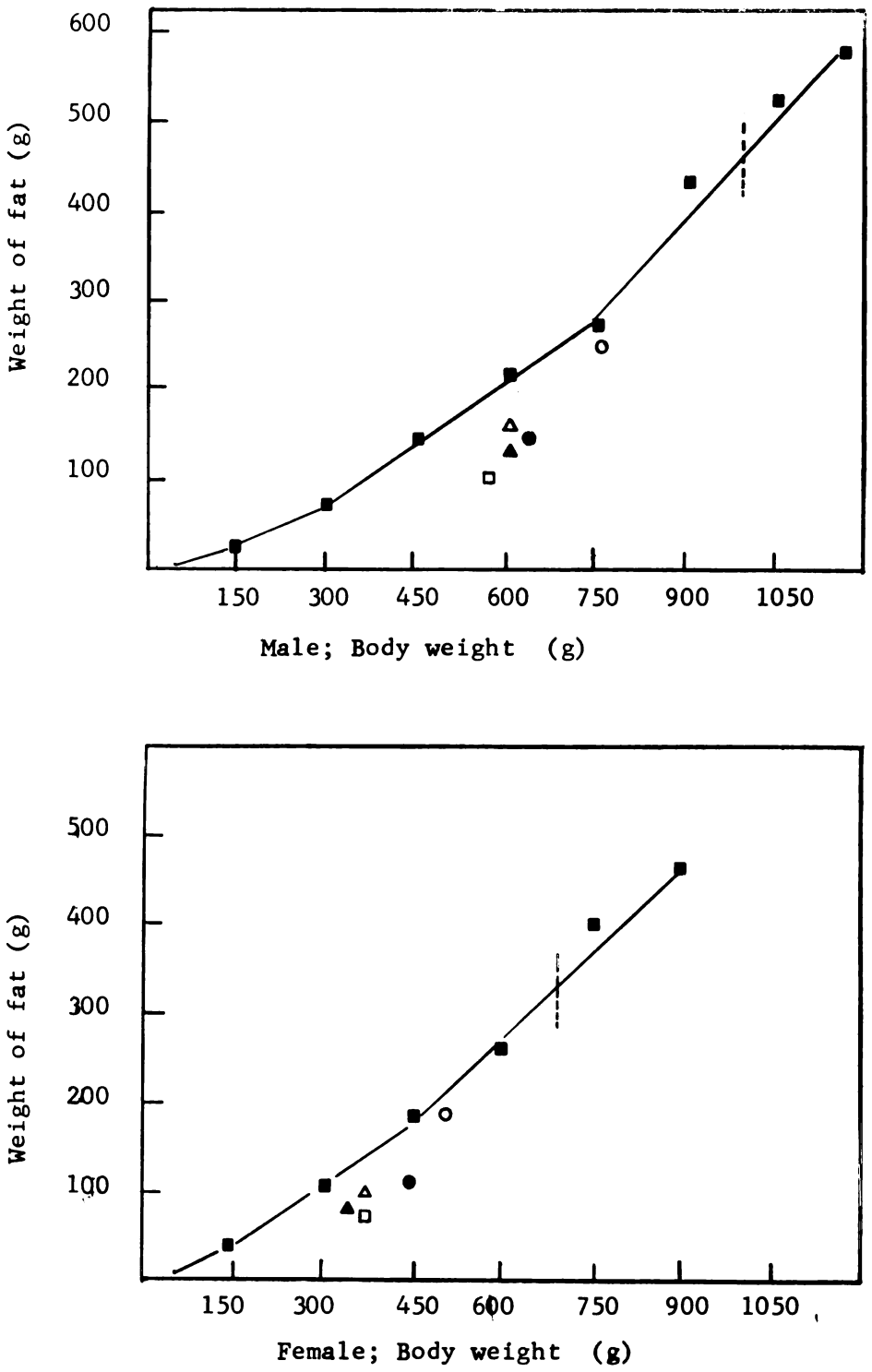


Fig. 26. Weight of fat in reduced Osborne Mendel rats in comparison to weight of fat in younger high fat-fed rats (Part I). The intersect indicates the g of fat in the rats at the time when rats were first fed reducing rations. This figure also shows comparison with lean controls of the same age. ■—■ wgt. of fat in g during development of obesity; □, grain-fed control; ● , grain-fed, reduced; o , semipurified, reduced; △ , high fat, 2 da. of 7; ▲ , high fat, restricted.

SUMMARY AND CONCLUSIONS

To evaluate the effect of excessive weight on body composition and development of fat depots male and female Osborne Mendel rats were fed either a high fat ration or a grain ration. Five male and five female rats fed each ration were sacrificed when they were weaned at 24 days and at weights of 150, 300, 450 and 600 g. In addition, male and female rats fed the high fat ration were sacrificed at 750 g and males at 900 and 1050 g. Analyses for total body fat, protein, moisture and ash were done on the carcasses. Individual fat depots which were dissected from the animals and weighed included the right and left inguinal tissues, right and left subcutaneous tissues underlying the forelimb, interscapular depot, right and left genital depots, right and left perirenal depots, mesenteric and omental fat depots and a small depot surrounding the xiphoid process.

Male rats fed the grain ration never exceeded 600 g in body weight and served as the lean controls. The rats fed the high fat ration gained weight more rapidly than the grain-fed animals and eventually became grossly obese as represented by weights well over 1000 g at the end of 260 days. At all ages, the rats fed the high fat ration had more fat in their bodies both on an absolute and relative basis. Body protein content increased in relation to age and this rate was the same for both the lean and obese rats. In other words, rats of the same age had the same amount of protein in their bodies regardless of their weight. This was true until the high fat rats became very obese. In the latter case, they had about 10% more protein in their bodies than their lean controls.

Adipose tissues doubled in weight when rats were fed the high fat ration. In the young rats so fed, the adipose tissues all increased in weight more rapidly than did body weight. In male rats fed the high fat ration and weighing 600 g or more and in comparable females weighing 300 g or more, inguinal fat depots and subcutaneous tissues underlying the forelimb increased in weight more rapidly than the body. The interscapular fat depot in high fat-fed rats showed a continual linear increase in relative weight. This was much greater for females than for males.

For grain-fed rats all subcutaneous adipose tissues increased in weight relative to increases in body weight.

In high fat-fed rats, the genital and mesenteric depots decreased in relative weight with greater gains in body weight as age increased. Perirenal depots showed a linear increase in relative weight when rats were fed the high fat ration. Also, the perirenal depot showed large increases in weight in the 600 g grain-fed male rats. Adipose tissue deposited around the xiphoid process showed linear increase after male high fat-fed rats reached 450 g, and females, 300 g.

The susceptibility of six strains of rats (Osborne Mendel, Sprague Dawley, Hoppert, Lewis, Hooded and Gray) to become obese and the pattern of changes in both body composition and weight of adipose tissues was studied. Weanling rats of each strain were fed the grain and high fat ration. At 10 and 20 weeks after the start of feeding, 5 males and 5 females from each strain and each dietary group were sacrificed.

Feed consumption records indicated that the rats of all strains and both sexes were more efficient in converting the energy of the high fat ration to body tissue than that in the grain ration. The differences

among the strains in this respect were not very great. During the first 10 weeks, the Hoppert rats were most efficient as far as both rations were concerned. At the end of 20 weeks, both male and female Osborne Mendel rats fed the high fat ration were more efficient than any other strains. The Gray rats were least efficient in converting feed energy to body tissue.

Final absolute values for protein and ash were similar for Sprague Dawley and Osborne Mendel rats. Other strains had less. The increase in body fat in the high fat-fed rats was greatest in the Osborne Mendel rats, intermediary in Sprague Dawley, Hoppert, Lewis and Hooded rats and least in the Gray rats. In general, adipose tissues of high fat-fed rats were heaviest in Osborne Mendel rats, intermediary in the other 4 strains and least in the Gray rats. All strains of rats responded to the high fat ration by some increase in body fat and some increase in adipose tissue weights.

In another study, 40 male and 40 female obese Osborne Mendel rats (1000 g males; 650 g females and around 260 days of age) were reduced. Ten lean and 10 obese rats of each sex and of similar age served as controls and were maintained on the high fat ration throughout the study. Four reducing regimens were used for weight reduction. They were (1) Grain and, (2) Semipurified rations fed ad libitum every day, (3) High fat ration fed ad libitum 2 days of 7 and, (4) Restricted quantities of high fat ration fed daily — the total amount per week to equal that in (3). Rats were reduced and maintained their reduced weights for at least 10 weeks prior to sacrifice. Rats consuming grain reduced their

weights by 40% (males) and 34% (females). Rats fed the semipurified ration reduced their weights by 25% (males) and 21% (females). These rations were similar in composition as to total calories, fat, protein, ash, fiber and carbohydrate. The high fat ration fed in restricted quantities or fed 2 days of 7 resulted in a 41 to 49% weight decrease for males and females.

On all reducing regimens, the lost weight was primarily composed of fat. From 10 to 16% of the original protein was lost when the high fat ration was fed by either method; that is, restricted or fed 2 days of 7.

All adipose tissues decreased in weight during the weight reduction period. Subcutaneous adipose tissues, genital and mesenteric and omental depots lost more weight than did the perirenal depots or the depot surrounding the cartilage of the xiphoid process.

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