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We hereby recommend that the dissertation prepared under our supervision by Ellen Clark Leevy entitled THE EFFECTS OF DIET AND FEEDING FREQUENCY ON OXIDATIVE METABOLISM OF RAT TISSUE

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THE EFFECTS OF DIET AND FEEDING FREQUENCY ON OXIDATIVE METABOLISM OF RAT TISSUE

A DISSERTATION

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BY

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CHAPTER

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

At birth man is normally a nibbler. Then, through convenience, customs, and working conditions man is soon made into a meal eater. There is a possibility that a number of diseases associated with abnormalities in carbohydrate, fat, and protein metabolism may be caused or aggravated by eating habits. Whereas the nibbler has a slow continuous absorption of foodstuffs from the gastrointestinal tract, the meal eater must absorb large amounts of foodstuffs in a limited period of time. This change in feeding patterns will involve changes in metabolic pathways as there are rate limiting steps found in most pathways of metabolism. Taking into consideration that these rate limiting steps are present, the degree to which different pathways are used will depend upon varying rates of ingestion.

Enzymes are involved in all areas of intermediary metabolism. The study of enzyme activity in appropriate pathways should reveal significant information as enzymes are involved in all areas of intermediary metabolism. The activity of glucose-6-phosphate dehydrogenase (G-6-PDH) and 6-phosphogluconate dehydrogenase (6-PGDH) of the

hexosemonophosphate oxidative pathway is implicated with the production of reduced nicotinamide adenine dinucleotide phosphate (NADPH) which is necessary for fatty acid synthesis. Thus, these enzymes should demonstrate a reliable picture of fat synthesis.

A question that is of great concern to individuals involved with the study of obesity is whether an eating regimen that has been changed for a prolonged period of time will result in an alteration of metabolism to the point that a return to previous metabolic patterns would be most difficult. Considerable evidence has indicated that changes in dietary regimen and/or dietary composition can produce alterations in metabolism and changes in the concentrations of protein and fatty tissue in the body. Many of these previous studies have been short-term and/or have used the refeeding period after being fasted, and neither of these conditions would be directly applicable to the relationship of dietary patterns of the human population and enzymatic activity. Thus, this present study eliminated the fasting and refeeding procedure.

In view of the above limitations, the purpose of this investigation was to determine the effects of different types of diets fed either continuously or periodically on the following: 1) the food efficiency as measured by weight

gain and food consumption; 2) the enzymatic activity of the hexosemonophosphate dehydrogenases which are presumed to be important in lipogenesis; and 3) the probable relationship of changes in enzymatic activity to adiposity, obesity, and the popular reduction dietary regimens employed today.

BIOCHEMICAL TERMINOLOGY

An explanation of the chemical symbols used in this study is given below:

Carbon dioxide	CO_2
Centimeter	cm
Coenzyme A	CoA
Ethylene-diamine-tetra-acetic acid	EDTA
Glucose-6-phosphate	G-6-P
Glucose-6-phosphate dehydrogenase	G-6-PDH
Grams	g
Hexosemonophosphate	HMP
Kilograms	kg
Kilocalories	kcal
Micromoles	uM
Milligrams	mg
Millimicrons	Mu
Millimoles	mM
Molar	M
Nicotinamide adenine dinucleotide	NAD

Nicotinamide adenine dinucleotide, reduced	NADH
Nicotinamide adenine dinucleotide phosphate	NADP
Nicotinamide adenine dinucleotide phosphate, reduced	NADPH
Optical density	O.D.
6-Phosphogluconate	6-PG
6-Phosphogluconate dehydrogenase	6-PGDH
Probability of statistical significance is less than	P<
Radioisotopic carbon	C^{14}
Standard error	S.E.
Tricarboxylic acid cycle	TCA

STATEMENT OF THE PROBLEM

The laboratory rat is a nibbler by nature, hence this animal appears to be the ideal subject for studying metabolism as influenced by the rate of ingestion of the diet under controlled conditions. One hundred young adult male rats, whose ad libitum feeding patterns had been established, were used for this study. These animals were divided into three main groups and placed on the following diets: 1) Experimental Diet I contained 10 per cent (weight/weight) fat; 2) Experimental Diet II contained 20 per cent (weight/weight) fat; 3) Diet III, Purina Laboratory Chow, contained 4.0 per cent (weight/weight) fat. The protein content was

similar in all three diets and the carbohydrate varied proportionately.

The overall purpose of the present study was to investigate the hexosemonophosphate oxidative enzymes in liver and adipose tissues in young adult rats on different feeding regimens and various types of diets. These oxidative enzymes were chosen because they are considered to be a possible source of reduced nicotinamide adenine dinucleotide phosphate (NADPH), a coenzyme necessary for the synthesis of fatty acids in biological systems. The food consumption and weight gains of the rats were recorded to statistically evaluate the efficiency of the food consumed by the rats.

The specific objectives were to:

1) Analyze the liver and adipose tissues of albino rats for hexosemonophosphate (HMP) oxidative enzyme concentrations.

    a) Compare the glucose-6-phosphate dehydrogenase (G-6-PDH) activities of the meal-fed rats versus the nibblers consuming Diet I;

    b) Compare the G-6-PDH activities of the meal-fed rats versus the nibblers consuming Diet II;

    c) Compare the 6-phosphogluconate dehydrogenase (6-PGDH) activities of the meal-fed rats versus the nibblers consuming Diet I;

    d) Compare the 6-PGDH activities of the meal-fed rats versus the nibblers consuming Diet II;

e) Compare the HMP oxidative enzyme values in the liver of nibbling rats that are on the various diets;

f) Compare the HMP oxidative enzyme values in the epididymal fat pad of nibbling rats that are on the various diets;

g) Compare the HMP oxidative enzyme values in the liver of meal-fed rats that are on the various diets;

h) Compare the HMP oxidative enzyme values of the epididymal fat pad of meal-fed rats that are on the various diets;

2) Determine the food consumption and body weight of the meal-eating and nibbling rats.

3) Determine the efficiency of the food as influenced by the various types of diets.

4) Qualitatively estimate the amount of adiposity observed with the diets considering the meal-fed versus the ad libitum-fed dietary regimens, as well as the composition of the diet.

REVIEW OF LITERATURE

One of the greatest problems seen in today's affluent society is excessive nutrition with consequent acceleration of certain diseases that are aggravated, if not caused by, this enigma. Some of these diseases are diabetes, hypertension, atherosclerosis, gout, and arthritis. MacBryde (41) estimated that in the United States one-fifth of the population over age 30 (approximately 15 million individuals) may be considered overweight. An estimated 5.5 million

persons in the country are pathologically obese, and this constitutes approximately 3.0 per cent of the total population. This situation has pointed to a need for a better understanding of the metabolic factors associated with excessive weight gain.

Essentially, obesity is an excessive accumulation of fat within the adipose cells as well as an increase in the amount of adipose tissue resulting in an abnormally high body weight. Obesity occurs when the upper limits of glycogen storage are exceeded. Quantities of glucose in excess of this upper limit are then converted to fatty acids and stored as triglycerides in the fat depots.

Renold (57) questioned the fact that adiposity and obesity are used interchangeably by some authors. An individual can exhibit adiposity without being obese. Adiposity is a condition in which the proportion of body weight composed of fat is excessive. In the third decade of life the proportion of fat seen in normal persons is: females, 18 to 24 per cent; males, 12 to 18 per cent. Adiposity may be present if the body fat content of a woman exceeds 30 per cent, and the body fat of a man 25 per cent. It is known that the proportion of fat in older men and women tends to be 50 per cent higher than that in younger persons of the same sex, build, and height. Persons of any age who have

poor muscles are apt to exhibit adiposity although the total body weight may be normal or even subnormal.

According to Renold (57) adipose tissue, by definition, is composed of aggregates of cells located throughout the body at specific sites predetermined by embryonic differentiation, the primary role of these cells being the storage of triglyceride as a potential source of energy. Triglyceride has greater caloric density than carbohydrate or protein and does not necessitate accumulation of water and ions as does storage of carbohydrate in the form of glycogen. Thus, this type of energy storage is very economical. If man were to store carbohydrates with the same amount of energy as found in lipids, weight would be doubled.

Renold (57) stated that the deposition of fat within adipose cells is regulated by and localized within the adipose tissue itself and not by the liver or any other organ. Lipogenesis is decreased to a minimum in carbohydrate deficiency and accelerated considerably during carbohydrate availability. In the normal steady state, approximately 30 per cent of ingested carbohydrate is converted to fat. For example, in a 70 kilogram man the total body weight of fat is approximately seven kilograms and the active compartment of the adipose tissue would be approximately 150 grams. If this man consumes 450 grams of carbohydrate per day then

one-third of this, 150 grams, may be transformed daily into fat. This shows quite strikingly that adipose tissue each day may transform its own weight of carbohydrate into fat. Also, if there is excessive caloric intake, the amount of carbohydrate converted can be increased greatly.

Many obese people who want to lose weight seem unable to follow diets which will lead to the achievement and maintenance of the "ideal" weight. The "ideal" weights are described by the National Research Council (51) as the reference man and reference woman. Hollifield (23) and others (44, 45) stated that failure to follow diets has been ascribed to a lack of interest or to psychological factors since no other apparent cause of obesity other than excessive food intake has been recognized.

Stunkard (63, 64) and others (4, 10, 44, 45) noted that unusual eating patterns are often seen in many obese people. A relatively large percentage of today's corpulent population consumes most of their food within a short period of time. These people eat little or no breakfast or lunch but eat voraciously during the evening. They awake in the morning with anorexia but exhibit nocturnal hyperphagia and insomnia. This is one of the reasons studies have been made using ad libitum and meal-fed animals to investigate differences in various biochemical parameters. At birth, man is

normally a nibbler, but because of convenience, customs, and working conditions he soon becomes a meal-eater.

Strang (62) stated that insurance companies have found statistically that even minor degrees of corpulence are accompanied by higher mortality rates. Mayer (44) postulated that overweight increases risk of death from cardiovascular or kidney diseases by 50 per cent. This situation has pointed to a need for a better understanding of the metabolic factors associated with excessive weight gain.

Along with the importance of the recognition of obesity as a medical problem is the equally important investigation of the causes of obesity. The various types of obesity can be characterized with regard to the following possible mechanisms operating at the adipose tissue level: 1) increased de novo synthesis of fatty acids; 2) increased deposition in adipose tissue of fatty acids that may have originated elsewhere; 3) decreased mobilization of fatty acids from adipose tissue; and 4) changes in enzyme and coenzyme concentrations involved with fatty acid synthesis. It is with the latter that this study is involved.

Tepperman and others (72) observed elevated respiratory quotients (R.Q.) in rats restricted to a one meal period. This suggested enhanced lipid synthesis. Dickerson and others (11) investigated the respiratory quotient of liver

in the synthesis of fatty acids from carbohydrate and noted that the same phenomenon could be demonstrated. Recently Vaughn (75) and others (9, 23, 27, 37, 38, 39, 47) have revealed increased fatty acid synthesis in rat liver and epididymal adipose tissue using commercial rat diets, Purina chow and high carbohydrate diets with designated feeding regimens. Antar (2) and others (14, 20, 43, 59) on the effects of various dietary carbohydrates have shown a relationship to serum lipids and oxidative enzyme concentrations in liver and adipose tissue. Simple carbohydrates produce a higher activity of liver and adipose tissue oxidative enzymes involved with fatty acid synthesis than the complex carbohydrates. Fructose and glucose concentrations in the diet also produce a variation in these enzymes.

Numerous reports by Cahill (6) and others (21, 46, 58, 77) have demonstrated an inhibition of lipogenesis by dietary fat in rat liver and adipose tissues. Leveille (34) has shown that feeding a high fat diet completely abolished the adaptive increase in fatty acid synthesis by adipose tissue. A further report by Leveille (36) extended these results by demonstrating that the adaptive changes in glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and malic enzyme in the liver are prevented by the ingestion of a high fat diet.

Kewick (30) and Niemeyer (52) have demonstrated there is a greater weight loss with calories obtained primarily from high fat diets rather than high carbohydrate diets. This suggests that if the diet is sufficiently low in carbohydrate (less than 60 grams), the obese person will lose weight even if the caloric intake considerably exceeds that of the composite diet on which he had previously remained overweight. Miller (49) and others (55, 56) did not support the assumption that energy expenditure is higher on a fatty diet than on a normal food intake.

Specifically, the problem of excessive lipogenesis may be divided into four closely interrelated areas which have been considered in this study. These areas will be discussed in the following order: 1) enzymatic activity; 2) rate of caloric influx; 3) dietary variations in energy nutrients; and 4) types of carbohydrate.

Enzymatic Activity

In 1956 Milstein (50) investigated the in vitro oxidation of glucose-1-C^{14} and glucose-6-C^{14} by liver and adipose tissues of the rat. Oxidation of both compounds occurred in the Embden-Meyerhof and the hexosemonophosphate (HMP) oxidative pathways. High glucose carbon-1 oxidation seemed to indicate extensive extra-glycolytic catabolism of glucose in

adipose tissue. It was much more rapid than that of glucose carbon-6. However, liver slices exhibited significantly higher ratios of C-6/C-1, indicating less C-1 activity than in normal adipose tissue. It was postulated that reduced nicotinamide adenine dinucleotide phosphate (NADPH) may be a critical aspect of lipogenesis in adipose tissue.

In 1957 Langdon (32) presented a paper which indicated that fatty acid synthesis occurs in the soluble cytoplasmic portion of the rat liver cell. In this process, nicotinamide adenine dinucleotide phosphate (NADPH) serves as an electron donor for the reduction of alphs, beta-unsaturated acyl CoA derivatives to their saturated counterparts. The synthesis of fatty acids in the cytoplasm can occur by a sequence of reactions which is essentially the reverse of the oxidative pathway existing in the mitochondria. These results are correlated with a number of independent observations on electron transport and biological fatty acid synthesis. Langdon suggested that abnormal metabolic states which are accompanied by decreased rates of nicotinamide adenine dinucleotide phosphate reduction or increased rates of reduced nicotinamide adenine dinucleotide phosphate oxidation may also be accompanied by decreased rates of fatty acid synthesis. The impairment of lipogenesis which

characteristically accompanies diabetes mellitus may be attributable in part to a decreased availability of reduced nicotinamide adenine dinucleotide phosphate in the extra-mitochondrial portion of the liver cell.

Shaw and others (59), in experimenting with rat liver, demonstrated that washed mitochondria can convert labeled pyruvate to long-chain fatty acids, provided butyryl co-enzyme A is supplied. The formation of butyryl coenzyme A from crotonyl coenzyme A is TPN-dependent (NADP-dependent).

Tepperman and Tepperman (71) demonstrated an increase in glocose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities in liver of meal-fed rats. As a result of these studies these investigators proposed that the availability of NADPH limited fatty acid synthesis and the "supernormal" lipogenesis was dependent upon an increased pentose cycle activity to generate adequate reducing equiva-lents.

By 1961 Foster and Bloom (15) had compared the roles of reduced nicotinamide adenine dinucleotide (NADH) and re-duced nicotinamide adenine dinucleotide phosphate (NADPH) in intracellular reductive reactions of rat liver. In accord with studies at the subcellular level, reduced tritiated nicotinamide adenine dinucleotide phosphate was preferen-tially utilized in fatty acid and cholesterol synthesis,

whereas reduced tritiated nicotinamide adenine dinucleotide was a better source of hydrogen for water formation.

In a study involving the pathway of hydrogen in biosynthesis, Lowenstein (40) investigated the capacity of extramitochondrial enzymes in rat liver to catalyze the conversion of acetate to alpha-ketoglutarate as compared to the normal rate of oxidative metabolism of the whole tissue. Using high speed supernatant fractions of rat liver the rate at which this extramitochondrial portion of the citric acid cycle can occur is equal to or greater than the normal rate of oxidative metabolism of whole liver. Lowenstein proposed that one of the functions of the extramitochondrial reaction sequence was to regenerate reduced nicotinamide adenine dinucleotide from nicotinamide adenine dinucleotide in the extramitochondrial space of the cell.

Tepperman and Tepperman (70) published a study concerning patterns of dietary and hormonal induction of certain nicotinamide adenine dinucleotide phosphate (NADPH) linked liver enzymes. Estrogen and thyroxine induction of hepatic hexosemonophosphate shunt and nicotinamide adenine dinucleotide phosphate-linked malic dehydrogenases was studied in rats on chow and on a high fat and low carbohydrate diet. A different enzyme concentration resulted from treatment with each of the hormones. Estrogen produced a greater effect on

the dehydrogenases of the hexosemonophosphate pathway (pentose shunt) while thyroxine showed a greater influence on the malic enzyme. Both enzymes were effected in animals adapted to a high fat diet, suggesting that accelerated glucose absorption from the gastrointestinal tract is not an essential feature of the mechanism of hormonal induction. However, in the case of the shunt enzymes, availability of dietary carbohydrates resulted in greater increases in enzyme activity than did induction on a high fat diet. Refeeding a carbohydrate diet after a 48-hour fast resulted in a simultaneous increase in both shunt dehydrogenases and malic enzyme, a fact which disproved the hypothesis that the adaptive increase of the former was secondary to a primary increase in the latter.

Young and others (79) conducted a study of the metabolic control of enzymes involved in lipogenesis and gluconeogenesis in 1964. The results indicated that malic enzyme activity in liver and adipose tissue was decreased by fasting and was obtained with a high carbohydrate diet; no significant increase was shown with a low carbohydrate diet. Both liver and adipose tissue are capable of transhydrogenating reduced nicotinamide adenine dinucleotide to reduced nicotinamide adenine dinucleotide phosphate by coupling cytoplasmic malic dehydrogenase to malic enzyme. Thus malic

enzyme may serve a role in lipogenesis either by direct formation of reduced nicotinamide adenine dinucleotide phosphate or by transhydrogenation from reduced nicotinamide adenine dinucleotide. Reduced nicotinamide adenine dinucleotide phosphate generation through the hexosemonophosphate pathway has been claimed not to be sufficiently rapid to account for the reduced nicotinamide adenine dinucleotide phosphate required for lipogenesis in adipose tissue.

Another implication in the above study was that the citrate cleavage enzyme may be important as a regulator of lipogenesis in the liver. The enzyme could form acetyl CoA and oxalacetic acid extramitochondrially. The oxalacetic acid could go to malic acid and pyruvic acid forming reduced nicotinamide adenine dinucleotide phosphate with the help of the malic enzyme.

The early part of 1967 Takeda and others (65) reported the response of various key enzymes related to glucose metabolism in normal and diabetic rat liver. The administration of a high glycerol diet to normal rats strongly induced the various key enzymes concerned with glucose utilization in the liver. These include glucokinase, pyruvate kinase, glucose-6-phosphate dehydrogenase, adenine triphosphate citrate lyase and acetyl-CoA carboxylase. With the induction of diabetes the activities of various key enzymes involved

in glycolysis and lipogenesis in the liver decreased markedly. This also was true for glucose-6-phosphate dehydrogenase. Thus, induction of diabetes produced a great increase in the activities of the key gluconeogenic enzymes in the liver, while it markedly depressed the key glycolytic and lipogenic enzymes. This same condition has been confirmed in fasting.

Rate of Caloric Influx

Tepperman and Tepperman (68) undertook a study in 1958 for the purpose of applying isotope tracer techniques to test the hypothesis that continuous or periodic over-feeding resulted in enhancement of the capacity to form fat from nonfat precursors. Liver slices of aurothio-glucose (ATG) obese mice from the Albino Farms were used. They were fed a high carbohydrate diet after a 48-hour period of fasting.

Using glucose-6-phosphate as the substrate, the NADP-reducing activity of the preparations taken from the 'refed' animals was significantly more active than those preparations from the conventionally 'fed' rats. A 'refed' animal has been fasted for a number of hours and then given a certain diet before sacrifice. The conventionally 'fed' rat has not been fasted. A high positive correlation was found between initial glycogen content and lipogenic performance

of the slices. A high rate of glucose absorption from the gastrointestinal tract was noted in the 'trained' animals. A three to fourfold increase in NADP-linked hexosemonophosphate shunt dehydrogenase activity was found in preparations from the livers of 'refed' rats.

Emerson and Bernards (12) demonstrated that the rate of lipogenesis varied in relation to the time at which food was previously ingested. This research was conducted in 1958 and emphasized the importance of considering the quantity of food ingested as well as controlling the time at which ingestion occurred. It was shown that a proper control of the immediate past diet was necessary if studies involving lipids was to be adequately controlled. Serum lipid concentrations were highest on the two-hour fast animals decreasing to a low at the 12-hour fast.

Cohn and Joseph (9) conducted experiments in 1959 to explore the influence on rats of caloric influx on the usage of alternate enzymatic pathways. Male Holtzman rats, weighing 100 to 120 grams, were either force-fed or fed ad libitum for five to six weeks on a high carbohydrate diet.

By investigating the glucose-6-phosphate and the 6-phosphogluconate dehydrogenase activity as one measure of activity over the shunt, it was found that the dehydrogenase

activity was two to three times greater in both liver and fat homogenates of the tube-fed animals than in similar preparations of animals that had eaten ad libitum. It was concluded that the rate of ingestion of the diet plays a role in the regulation of traffic over specific enzymatic pathways, when multiple alternate pathways are available. Forced feeding probably results in the accumulation of excess body fat by causing the animal to adapt its enzymatic machinery to alternate and perhaps more 'efficient' pathways.

In 1960, Cohn and Joseph (10) stated animal experimentation designed to explore diseases and metabolic abnormalities which afflict man should use the feeding patterns of man. Evidence cited made this especially true for diseases of obesity, atherosclerosis, and diabetes. The rate of ingestion of foodstuffs appeared to play a significant role in regulating the over-all metabolism of fat, carbohydrate and protein, as well as the rate of synthesis (or release) of several protein hormones. Meal eating (spaced, full meals), as contrasted to nibbling (frequent, small feedings), resulted in: 1) a 50 to 100 per cent increase in body fat over pair-fed rats; 2) a three-fold increase in glucose-6-phosphate and 6-phosphogluconate dehydrogenase activity in supernatants of rat liver and epididymal fat pad homogenates; 3) a 10 to 20 per cent decreased I_{131}

uptake by the thyroid and diminished thyroidal I_{131} decay; 4) development of diabetes in partially pancreatectomized rats; 5) enhanced atherogenesis in monkeys and chickens; and 6) evidence that meal eating was associated with a decrease in body protein. These findings were interpreted to indicate: 1) flooding of the organism with calories by the consumption of full meals is associated with a more economical use of calories, leading to obesity; 2) meal eating differentially affects the traffic over alternate metabolic pathways; and 3) the rate of thyrotrophic (thyroid-stimulating) hormone, TSH, release by the pituitary may be decreased by meal-eating. The observed phenomena may be mediated through alterations in the enzymatic machinery of the body secondary to different rates of influx of calories.

Investigating fatty acid components of rat liver in 1961 Okey and others (54) maintained that meal feeding of female rats with diets furnishing either 10 per cent cottonseed or 10 per cent coconut oil with 1.0 per cent cholesterol resulted in higher plasma cholesterol values and lower percentages of arachidonic acid in plasma cholesterol ester than those found either in females fed ad libitum or in males on either regimen.

Variations in the fatty acid components of the liver lipids with the stresses imposed by dietary fat, by

restricted access to food, and by addition of cholesterol to the diet, suggest that there is a tendency toward maintenance of physical properties of each lipid within a characteristic range, rather than maintenance of a relatively constant ratio of saturated to unsaturated acid such as that found in the plasma lipids. Composition of the phospholipids was more nearly independent of diet and methods of feeding than was that of the other lipids. The latter was true of both plasma and liver.

After observing the unusual eating patterns seen in many obese people, Hollifield and Parson (24) examined the liver and adipose tissue glycogen in rats limited to a short daily feeding period. Fifty-six male rats of the Sprague-Dawley strain, weighing 180 to 220 grams were fed Purina Laboratory Chow pellets either ad libitum or for only two hours daily. The experiment lasted up to seven days. These investigators found that adipose tissue from rats limited to a short daily feeding period very quickly developed a markedly enhanced ability to synthesize lipids from acetate in vitro. This increased lipid synthesis is associated with a greatly increased glucose-6-phosphate and 6-phosphogluconate dehydrogenase activity in adipose tissue. The activity of these enzymes in adipose tissue from animals fed for two hours per day for five days increased over 1000 per

cent above the fasting level within two hours after the animals started to eat. Also, the free fatty acid content of adipose tissue decreased as the rate of lipogenesis in adipose tissue increased. Rats fasted for 24 hours after four to five days on a two-hour feeding program had much higher levels of liver glycogen than did control animals fed ad libitum after a similar fast.

Hollifield and Parson (25) reinforced the study mentioned above by investigating rats over a longer period of time, checking their body weights to learn how long these adaptive changes persist when the animals were again allowed food ad libitum. The following results were obtained:

1) After 10 weeks rats allowed access to food for only two hours per day were more than 30 per cent heavier than animals allowed food ad libitum.

2) Rats allowed access to food for only two hours per day ate more than controls fed ad libitum, within a few days after this feeding program was initiated.

3) Acetate-1-C^{14} incorporation into lipids by adipose tissue *in vitro* and liver glycogen remained high in rats allowed food ad libitum for seven days following seven days on a two-hour per day feeding program. These changes were no longer present after similarly treated rats were allowed food ad libitum for 20 to 28 days.

4) In rats allowed access to food for only two hours per day for seven days, a three-day fast reduced the rate of acetate-1-C^{14} incorporation into lipids by adipose tissue *in vitro* after a two-hour feeding period to that of controls fed ad libitum.

By 1964, it has been the observation of Stevenson and others (61) that restriction of feeding time of rats usually resulted in a body weight gain less than that of ad libitum-fed controls. In this experiment the authors duplicated the experiment of Hollifield and Parson (25) in which meal-fed animals were 30 per cent heavier than were the ad libitum-fed after 10 weeks of experimental feeding. The results of this study were:

1) Using young male rats fed chow for only two hours daily, the increased lipogenesis *in vitro* and liver glycogen concentration reported by others was confirmed. The phenomena have been demonstrated also in young male rats fed a purified, high carbohydrate diet for only two hours daily.

2) In contrast to the findings of Hollifield, young rats whose feeding time was restricted to two hours daily had a lower food intake and body weight gain than did ad libitum-fed controls whether provided with chow or the high carbohydrate diet. These "restricted" animals had slightly lower proportion of carcass fat and higher proportion of water and ash.

3) Adult male rats when provided with the high carbohydrate diet for only two hours daily, ate less than ad libitum-fed controls and failed to gain weight.

Tepperman and Tepperman (67) discussed the adaptive changes involved with rate of caloric influx. Rats "trained" to eat their food in three hours contained 20 to 22 ml material in the stomach immediately after a restricted

feeding period, as compared to five to six ml for ad libitum-fed controls. Results of rats starved for two or more days and refed high carbohydrate diets were an extremely high capacity to convert nonfat precursors into fatty acids. The absorbing area increased with intermittently starved animals. The blood of the "trained" rats contained more insulin-like activity after feeding; thus, more glucose was introduced to the interior of lipogenic cells resulting in a type of "hypertrophy" of the enzymatic machinery of the cell.

Leveille and Hanson (37) reported an experiment in 1965 concerning the influence of periodicity of eating on adipose tissue metabolism in the rat. The findings contradicted those of Hollifield and Parson (25) in that meal-eating rats consumed more food and gained more weight than did animals fed ad libitum. However, Leveille and Hanson did note an increased feed efficiency for the meal-eating animals. This finding would support the concept that the increased fat deposition observed by Cohn and Joseph (9) was the result of the pattern of feeding rather than a consequence of force-feeding per se.

This study also showed a greater C^{14} production by adipose tissue from glucose-1-C^{14} in meal-fed rats, with a C-1/C-6 ratio of 9.03 as compared to a ratio of 3.87 for nibbling animals. These results agree with the increased hexosemonophosphate shunt activity in theory.

A study by Leveille and Hanson (34) in 1966 emphasized the changes in metabolic pathways of meal feeding by investigating enzyme activity involved with lipogenesis. Glucose-6-phosphate dehydrogenase and NADP-malic dehydrogenase were more active in adipose tissue from ad libitum-fed rats. The activity in adipose tissue of isocitric dehydrogenase did not increase significantly in response to meal-feeding the high carbohydrate diet. No increase in lipogenesis or enzyme activity could be demonstrated in adipose tissue from rats meal-fed a high fat diet. Lipase activity of adipose tissue was increased by high carbohydrate meal-feeding and decreased by feeding a high fat diet. The in vitro uptake of palmitate-1-C^{14} by adipose tissue was depressed by a high fat diet and enhanced in rats meal-fed a high carbohydrate diet. Diaphragm muscle or slices of liver from high fat-fed rats oxidized palmitate-1-C^{14} more rapidly than did tissue from ad libitum-fed animals.

Leveille's report (35) on glycogen metabolism in meal-fed rats and chicks investigated the time course of the lipogenic and enzymatic adaptations to meal-feeding (access to food for a single daily two-hour period). The incorporation of acetate-1-C^{14} into fatty acid had increased in rat adipose tissue after five to seven days of meal-feeding, and in chick liver after seven days. The activities of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase

and NADP-malic dehydrogenase did not increase in rat adipose tissue until after nine days of meal-feeding and did not increase over control values in liver of meal-fed chicks. The data were interpreted as demonstrating that the hyperlipogenesis induced by meal-feeding was not dependent upon increased activities of the dehydrogenase enzymes studied. These data were discussed in relation to the reduced nicotinamide adenine dinucleotide phosphate requirements for the support of lipogenesis.

In both rats and chicks, the level of glycogen in liver of meal-fed animals was depleted to a lesser extent by 22 hours of fasting than that in liver of nibbling animals. Therefore, it is conceivable that part of the overall adaptive response to meal-eating in the rat involves the increased ability of tissues, including adipose tissue to store abnormally large amounts of glycogen during the meal period to be used judiciously during reactions requiring or preferentially utilizing glucose. Such reactions would include the supply of glucose as an energy source for nervous tissue and alpha-glycerophosphate formation in adipose tissue. Since adipose tissue cannot incorporate glycerol resulting from lypolysis for esterification of fatty acids, alpha-glycerphosphate must be formed from dihydroxyacetone phosphate, and presumably pyruvate or lactate can serve as

precursors for the alpha-glycerophosphate. This report concluded that gluconeogenesis and glycogenesis are greater in tissues of meal-fed animals or those fed a high fat diet.

Leveille (36) reinforced the earlier studies by identifying the influence of dietary fat and protein on metabolic and enzymatic activities in adipose tissue of meal-fed rats. This article was published in 1967 and stated that meal-feeding stimulated the incorporation of pyruvate carbon into fatty acids and the oxidation of pyruvate by isolated adipose tissue. This response to meal-feeding was completely abolished by feeding a high fat diet. The activities of glucose-6-phosphate dehydrogenase and malic enzyme were higher in adipose tissue and liver of meal-fed rats consuming a high carbohydrate diet, but were unchanged in tissue of rats meal-fed a high fat diet. The activities of glucose-6-phosphate dehydrogenase and malic enzyme were depressed in adipose tissue of rats fed the high fat diet, whereas only glucose 6-phosphate dehydrogenase activity was depressed in liver of nibbling rats consuming the high fat diet. Adipose tissue from rats fed the high fat diet was able to convert significantly more pyruvate-2-C^{14} to glyceride-glycerol than tissue from rats fed the high carbohydrate diet. The increased use of this pathway is contended to be the reversal of glycolysis.

Dietary protein did not influence the response of adipose tissue to meal-feeding. Increasing the dietary protein level did increase hepatic glutamic-oxaloacetic (GOT) and glutamic-pyruvate (GPT) transaminase activities and decreased the ability of isolated adipose tissue to oxidize glucose-C^{14} and leucine-C^{14} and to incorporate these substrates into fatty acids. Adipose tissue from rats meal-fed diets containing 9.0, 18 or 36 per cent casein oxidized and incorporated into fatty acids significantly more glucose and leucine than tissue from animals fed the same diets ad libitum.

In 1968 Leveille (38) reported a study on fatty acid synthesis in vivo in which he obtained information on the relative importance of adipose tissue and liver as sites of fatty acid synthesis. The calculations suggested that in the nibbling rat 50 to 90 per cent of the fatty acids are synthesized in adipose tissue whereas when fatty acid synthesis is stimulated by meal-feeding, adipose tissue apparently accounts for about 95 per cent of the total fatty acids synthesized.

Leveille and Chakrabarty (33) performed a study on male rats of the Sprague-Dawley strain weighing 250 to 350 grams. The meal-fed rats ate for only two hours each day. The nibblers were the controls. All the animals were

maintained on these feeding schedules for at least three weeks before analysis, since this period has been shown to be of sufficient duration to induce the lipogenic response to meal-feeding. The change in blood glucose levels following the oral administration of glucose was studied in meal-fed and nibbling rats deprived of food for 22 hours. Food was removed from the nibbling animals on the day preceding the experiment so that both the meal-fed and the nibbling rats had been without food for 22 hours before refeeding. The rats were force-fed five milliliters of a 40 per cent glucose solution. The results showed that meal-fed rats absorbed glucose more rapidly than nibbling animals and this enhanced absorptive rate could be explained by an increase in the weight of the small intestine. Also, the rate of glycogen storage in muscle (abdominal, gastrocnemius and diaphragm) following food ingestion was significantly greater for meal-fed than for nibbling animals.

At this same time, Chakrabarty and Leveille (8) also compared the activities of various enzymes involved in the conversion of glucose to lipid in tissues of meal-fed (access to food limited to a single daily two-hour period) and nibbling (fed ad libitum) rats. They compared some of the key enzymes involved in: 1) the phosphorylation of glucose, 2) the conversion of glucose to fatty acid and

glycerol, and 3) the synthesis of reduced nicotinamide adenine dinucleotide phosphate essential for reductive lipogenesis, in various tissues of meal-fed and nibbling rats. The diet was 70 per cent glucose.

Activities of hexokinase, pyruvate kinase, alpha-glycerophosphate dehydrogenase, acetyl coenzyme A carboxylase and pyruvate carboxylase were significantly elevated in adipose tissue of meal-fed, as compared with nibbling rats. Muscle hexokinase activity, liver pyruvate carboxylase, and liver phosphoenolpyruvate carboxykinase activities were also enhanced by meal-feeding. The activities of liver glucokinase, hexokinase, pyruvate kinase, alpha-glycerophosphate dehydrogenase and acetyl coenzyme A carboxylase, of muscle pyruvate kinase and alpha-glycerophosphate dehydrogenase, and of adipose tissue phosphoenolpyruvate carboxykinase were similar in meal-fed and nibbling animals.

Dietary Variations in Energy Nutrients

One of the early studies concerning glucose conversion to fatty acids was conducted in 1950 by Masaro and others (46). These authors found that liver slices prepared from rats fed a high fat diet for as short a period as three days had almost no ability to convert glucose-carbon^{14} to fatty acids. The conclusion was drawn that dietary carbohydrate

was essential for maintenance of the capacity of hepatic tissue to convert glucose to fatty acids.

In 1955, a study by Whitney and Roberts (77) reported that liver slices from rats fed a high fat diet for two to three months exhibited a depressed capacity to incorporate acetate-2-carbon^{14} into fatty acids and liver glycogen of fed animals. Evidence was presented suggesting reflections of depressed glycogenesis and fatty acid synthesis and accentuated cholesterogenesis in the liver of fat-adapted animals. These adaptations appear to be established by prolonged fat feeding and tend to persist during the early fasting period.

About the same time a study by Hausberger and Milstein (19) reported dietary effects of lipogenesis in adipose tissue. Male Wistar rats weighing 300 grams or more were used, but only the animals showing an average daily weight gain of two to three grams were studied. The diets were fed ad libitum for at least two weeks before sacrifice.

Fasting or feeding of a high fat diet abolishes lipogenesis and greatly diminishes glucose oxidation. Prior treatment with insulin has little stimulating effect on utilization in adipose tissue of rats fed a high fat diet. Glucose utilization increases as the carbohydrate content increases or the fat content decreases. The lipogenic

activity of the adipose tissue was found to be superior to that of the liver of the same animals.

In 1955, MicKelsen and others (47) produced obesity in three strains of normal male rats by the ad libitum feeding of a diet containing 63 per cent fat and adequate amounts of vitamins, minerals and protein. The rats were on three different diets, a low-fat diet, a high-fat diet, and a stock diet. The three strains of rats were Osborne-Mendel, Sprague-Dawley, and NIH black rats.

The weanling rats on the high fat diet gained weight at a higher rate than rats on a low fat or stock diet. Approximately 70 per cent of the weanling rats of the Osborne-Mendel strain from the stock colony attained weights over or close to 1000 grams within 30 to 40 weeks when fed the high fat diet. After over a year, the maximum weight attained on the high fat diet was 1655 grams. These authors believed that the obesity did not result from any genetic or hormonal disturbance.

In 1956, Kekwick and Pawan (28) investigated which factor was most important--caloric restriction or alteration in composition of the diet. In working with obese males and females, these authors concluded:

1) Using 1000 calorie diets, weight loss was most rapid with the high fat diet, less rapid with

a high protein diet, and the weight was maintained for a short period on a high carbohydrate diet.

2) Weight loss appeared to be total body water (30 to 50 per cent) and body fat (50 to 70 per cent).

3) The rate of insensible loss of water rises with high fat and high protein and falls with high carbohydrate.

In 1957, Barboriak and others (3) conducted an experiment on albino rats consuming high-fat diets in which different types of fat were used. The control group had a high carbohydrate diet using corn oil and animal and vegetable shortening. On the high fat diet, 81 per cent of the calories was in the form of fat. The vegetable oils tested (corn oil, coconut oil, and cotton seed oil) did not promote growth in young rats as efficiently as lard, Crisco, margarine, or butter. With the solid fats, over a long period of time, the groups fed lard and Crisco showed the greatest weight gain, followed by butter and margarine groups. Within nine months some of the animals on the Crisco and lard diets surpassed the weight of 900 grams. Over the whole experimental period, the animals fed the Crisco diet showed the highest food efficiency. The fact that some animals on the corn oil diet ultimately reached a weight of over 1000 grams indicated that the differences in obesity-producing properties of fats may be mainly a reflection of differences in transformation of dietary fat to body fat. The results of

this investigation indicated that the solid fats, when fed in very high concentrations, promote the growth of young rats more effectively than vegetable oils.

The sensitivity of hepatic lipogenesis to fat ingestion was established by Hill and others (21) in 1959. With as little as 2.5 per cent fat in the diet, hepatic lipogenesis was measurably slower than when fat-free diets were fed. When the fat content was raised to only 15 per cent, the liver lost about 90 per cent of its ability to convert acetate carbon to fatty acids. This was observed as early as one hour after fat administration. No change was noted in either hepatic fat or glycogen content.

By 1960, Cahill and others (6) showed that free fatty acids (FFA) added to adipose tissue in vitro inhibited lipogenesis. This was shown by triglyceride lipolysis increasing free fatty acid levels in the blood with epinephrine added to the tissue. It would appear that dietary fat does not influence lipogenesis by a simple feedback mechanism involving the concentration of fat; the free fatty acid level in the blood may be the primary determinant.

Pilkington and others (56) conducted a weight reduction program in 1960 to determine which type of isocaloric diet would result in the greatest loss in weight. An earlier study by Kekwick and Pawan (1956) stated that weight loss

was greater on high fat diets than on high carbohydrate diets over short periods of time. They suggested the difference was due to a specific dynamic affect of fat much larger than previously observed. Pilkington and co-workers (56) indicated these experiments should be repeated for longer periods. They found if the study lasted a sufficient length of time to achieve a "steady state," the rate of weight loss on a diet consisting mainly of fat did not differ significantly from the weight loss on an isocaloric diet consisting mainly of carbohydrate.

About the same time, Olesen and Quaade (55) published an article in which they stated that specific dynamic action of foodstuffs shows the action of fat seems to be smaller than that of carbohydrate. In treating eight obese women in a hospital, they came to the following conclusions:

1) These experiments do not support the assumption that energy expenditure is higher on a fatty diet than on a normal food intake.

2) A high-fat/low-carbohydrate diet may reduce the weight of obese people considerably. But, this weight loss ceases after a few days and it may be explained largely--perhaps wholly--by release of water from deposits in the body.

3) Continued intake of a high-fat/low-carbohydrate diet affects body weight in the way expected from the number of calories ingested.

4) A diet containing a relatively high proportion of carbohydrate, but with a calorie intake below the theoretical minimum, may be compatible

> with a stable body weight; at any rate if taken after a period of fatty diet. This is most likely attributable to simultaneous increase of the body's fluid content.

In rebuttal to Pilkington, Olesen and Quaade (55, 56) Kekwick and Pawan (29) were in disagreement with the fact that both groups of authors had fallen back on the old argument that the different short-term effects of these diets must be to increase or decrease total body water. After further experimental observations, Kekwick and Pawan believed that these weight changes from a high-fat diet were brought about in part by changes in metabolism which have resulted in different rates of tissue catabolism. The following points were outlined to substantiate their position:

1) When total body water was measured at the beginning and end of a period of weight change produced by different isocaloric diets, changes in the measurable body water did not account for the whole of the weight change as it does, for example, when the same measurements are made in patients undergoing treatment for cardiac failure.

2) The different diets used produced measurable changes in insensible loss in many subjects.

3) One thousand calorie diets supplied in the form of 90 per cent fat or 90 per cent protein both resulted in subjects producing a substance in the urine which will mobilize fatty acids from adipose tissue in vitro and cause loss of carbass fat when injected into mice. The same subjects on a 1000 calorie diet supplied as 90 per cent carbohydrate for periods up to two weeks do not produce detectable amounts of this substance.

4) The metabolic response of obese subjects to a fixed dextrose or fat load in terms of 24-hour respiratory quotients differed according to the previous dietary intake.

5) High fat feeding in animals inhibited the ability of adipose tissue to synthesize fat from carbohydrate.

Lochaya and others (39) published an article in 1961 involving adipose tissue metabolism of obese mice and their nonobese littermates on standard and high fat diets. In tissue from nonobese mice fed the high fat diets, glucose metabolism to carbon dioxide (CO_2) and to fatty acids was diminished while glucose carbon incorporation to glyceride-glycerol was increased. The metabolism of adipose tissue from obese mice was slightly, if at all, affected by high-fat feedings.

In 1961, Tepperman and Tepperman (69) conducted a study on liver slices of refed rats after feeding a high carbohydrate/low fat diet. The adult male rats were maintained on a chow diet before beginning a 48-hour starvation period. At 24 and 48 hours the ratios of glucose-1-carbon^{14} and glucose-6-carbon^{14} in both carbon dioxide and lipid were consistent with an increase in glucose metabolism via the hexosemonophosphate pathway, sometimes called the pentose shunt.

By 1962, Niemeyer and others (52) had published an article concerning the activities of glucokinase, uridine diphosphoglucose-glycogen transglucosylase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. The activity decreased in the liver of animals fed with a high-fat diet for six days and was maintained at normal levels in rats fed a high carbohydrate/fat free diet.

The enzymes decreased after a 48-hour fasting period and returned to normal values after subsequent feeding with an equilibrated diet. The administration of a high-fat diet did not induce recovery of enzyme activities; only 6-phosphogluconate dehydrogenase recovered partially. In contrast, following administration of a diet consisting solely of carbohydrates, glucokinase recovered to normal levels within one day, while glucose-6-phosphate and 6-phosphogluconate dehydrogenases remained at the same low levels observed in fasted animals. The technique of feeding, following a fasting period, is considered a good tool for the study of the dependence of enzyme activities on the nature of the diet.

Miller and Payne (48) conducted a study on young pigs and rats (28 to 30 days old) in which the dietary protein was always the same and the regimens differed only in the concentration of protein in the diets and the amount fed. The two pairs of pigs in this study were kept at constant

weight for periods of 40 days varying the food intake from low and high calorie regimens, the amount of protein staying the same but the concentration being greater in the low calorie diet. The evidence presented eliminated any conventional reasons for metabolizing energy and the authors concluded that the animals converted some of the food energy directly to heat. Considering these results, it is difficult to understand why the reducing diets normally prescribed contain a high proportion of protein. Finally, the assumption from this experiment is that very high caloric intakes of diets low in protein do not necessarily result in weight gains.

Vaughan and Winders (75) conducted a series of experiments in 1964 involving the effects of diet on hexosemonophospate (HMP) dehydrogenase and malic (NAD) dehydrogenase in the rat. They showed that dietary fat depresses the activity of both of these enzymes. A diet which contained only 15 per cent by weight had this marked effect. At 25 per cent fat by weight, or the level usually eaten by humans in the Western world, activity of these enzymes was depressed to virtually minimal levels. The inference drawn from these results was that fat prevents the flooding of the pathways involved with these enzymes. The rats receiving the high carbohydrate diet on alternate days exhibited elevated

levels of both enzymes. When rats were allowed to eat part of their total carbohydrate intake in the absence of fat, enzyme activities were not depressed to the extent which occurred when fat was always eaten with the carbohydrate.

In another experiment Vaughn and Winders (75) showed that carbohydrate was not necessary for rapid regeneration of maximum levels of hexosemonophosphate dehydrogenase activity. These results imply that on a high protein diet, large amounts of amino acid residues are diverted toward glucose-6-phosphate and thence through the hexosemonophosphate pathway, instead of flowing immediately toward the tricarboxylic acid (TCA) cycle. These authors confirmed the results of Niemeyer and others (52) that the nitrogen-free diet has a very low stimulatory effect on hexosemonophosphate dehydrogenase.

In contrast to the behavior of hexosemonophosphate dehydrogenase, stimulation of malic (NAD) dehydrogenase occurred only on the high-carbohydrate/nitrogen-free diet. Large amounts of dietary protein had no effect.

From an experiment conducted in 1964, Kekwick and Pawan (30) concluded that the rate of weight loss in mice produced by restricting caloric intake below normal requirements was influenced by the type of food prodominant in the diet; fat produced a rapid loss and carbohydrate a

slower one. The amount of weight loss in both instances could be accounted for almost entirely by the extent of the negative carbon balances. The varying rates of weight loss were due to differences in the rate of fat loss and not to changes in total body water. In mice on a normal diet and in carbon equilibrium, about half the carbon was excreted as CO_2 and half in the form of organic molecules with an energy value of their own. With restricted calorie, high carbohydrate diets, the proportion excreted as carbon dioxide rose to over 80 per cent. With restricted calorie, high fat diets, the proportion excreted as carbon dioxide remained normal, about 50 per cent. Therefore, the demands on its own fat stores were consequently greater when fat was the major constituent of such a diet. It was suggested that this view provides a partial explanation of the different rates of weight loss with alteration in dietary composition.

In 1965, Reynolds and others (58) stated nutritional obesity had been produced in rats of the Osborne-Mendel strain by means of a high fat diet. It was suggested that this tendency to obesity may be a genetic predisposition in this strain of rats. Genetic obesity has been studied extensively in mice, and differences in metabolism of carbohydrates and lipids have been observed.

Allmann and others (1) found that refeeding starved rats a fat-free diet over a 48 hour period brought about a marked elevation in the activity of the enzymes in liver cytoplasm which catalyze the synthesis of saturated fatty acids from acetyl CoA and malonyl CoA. Acetate incorporation into palmitoleic and oleic acid is also accelerated during this period. Enhanced capability for the synthesis of these fatty acids is reflected in the net accumulation of saturated and monounsaturated fatty acids, as well as the triglyceride fraction of the liver lipids.

In 1967, Miller, Mumford and Stock (49) extended an earlier study conducted by their laboratory of over-eating a low- or high-protein diet. Sixteen young adult men and women were fed, for periods of four to eight weeks, diets which contained either about 2.8 per cent or 15 per cent of protein calories, and provided an excess of about 1400 kilocalories per day above their normal intake.

The mean weight gain of the low protein groups was 1.1 kilograms compared with a theoretical figure of 5.0 kilograms if the excess calories are calculated as being converted to adipose tissue containing 66 per cent fat; for the high protein groups the mean weight gain was 3.7 kilograms compared with a theoretical figure of 5.4 kilograms. Since none of the indices of body composition showed any real

change during the experimental period, and since activity was both low and unchanged, it was apparent that the excess caloric intake of the subjects was disposed of by an increased heat production. This view was supported by the measurement of oxygen consumption reported in a second paper and had implication in the etiology of obesity.

Types of Carbohydrate

One of the first indications of differences in fatty acid synthesis from various types of carbohydrates was noted by Dickerson and others (11) in 1943. The livers of Sprague-Dawley rats trained to eat food in a one-hour period were studied for evidences of fatty acid synthesis from glucose and fructose. Livers of normal rats were investigated also.

Following fructose administration the respiratory quotient (R.Q.) increased about 40 per cent in both the trained and untrained rats; with glucose administration only the trained rats showed an increased R. Q. Oxygen consumption tended to increase whenever the respiratory quotient increased in response to the sugar medium.

Womack and Marshall (78) studied sugars and related factors affecting liver fat and nitrogen balances in adult rats fed low levels of amino acids. Liver fat was reduced and negative nitrogen balances were decreased in adult

protein-depleted rats fed extra threonine, corn, rice or wheat starch, or corn dextrin, when compared with the levels found for animals fed diets containing sucrose and low levels of amino acids. The results were not influenced by the type of carbohydrate fed during protein depletion. Substitution of potato starch or glucose for sucrose reduced liver fat but did not improve nitrogen balances. The addition of niacin, methionine, cellu flour or sulfasuxidine, or substitution of fructose for sucrose did not change liver fat values or nitrogen balances from those obtained with sucrose. Doubling the essential amino acid intake of animals receiving sucrose, glucose or fructose reduced liver fat to normal or near normal values.

In working with hypophysectomized rats in 1957, Hill, Bauman and Chaikoff (20) felt that some change in the carbohydrate metabolism of the liver occurs after the rat is completely deprived of anterior pituitary hormones. They found that the liver of the hypophysectomized rat fed a diet in which whole ground wheat was the principal carbohydrate source manifested an inability to convert glucose to carbon dioxide and fatty acids at normal rates. Also, this defect was observed in the livers of hypophysectomized rats fed a synthetic diet containing 25 per cent glucose. When the glucose content of the diet was increased to 60 per cent,

the conversion of glucose to carbon dioxide was normal, but the conversion of glucose to fatty acids still remained below normal. The livers of hypophysectomized rats also failed to incorporate the C^{14} of fructose into fatty acids even though the C^{14} recoveries were normal. These findings with glucose and fructose indicated that the conversion of hexose carbons to fatty acids by the liver requires the concurrence of one or more of the anterior pituitary hormones.

In view of the conflicting results with animals and humans, Van Itallie and Shull (74) felt it essential to obtain information about the relative roles of the liver and of the extrahepatic tissues in the disposal of ingested glucose after a period of fructose feeding. From previous reports liver glucokinase activity in man was not decreased by a chronically lowered concentration of glucose in the portal vein blood as seen with rats and dogs in other experiments.

Although over-all glucose tolerance and rate of uptake of glucose by the forearm tissues were markedly diminished following ingestion of a carbohydrate-free diet for three days, the five-day fructose diet did not induce any impairment of net tolerance to glucose or in rate of peripheral glucose uptake. Indeed, the rate of peripheral glucose removal was significantly increased following ingestion of

the fructose diet. It was concluded that the impaired glucose tolerance associated with carbohydrate deprivation is due principally to a decreased uptake of glucose in the extrahepatic tissues, and that the effect of fructose feeding on glucose tolerance is entirely different from that of carbohydrate restriction.

In 1958, Siperstein and Fagan (60) published an article on the influence of glycolysis (Embden-Meyerhof pathway) on the synthesis of cholesterol and fatty acid in normal rat liver. Long-Evans rats weighing 150 to 250 grams were fed Purina Laboratory Chow. Two to three days before sacrifice they were given a 67 per cent glucose diet. The authors concluded that the Embden-Meyerhof pathway seemed to be relatively unimportant in controlling lipid synthesis. Thus, it would follow the cofactors produced during operation of this pathway are not limiting in the synthesis of fatty acids and cholesterol. However, glucose that uses the hexosemonophosphate pathway has a profound influence on the synthesis of both cholesterol and fatty acids.

To further the understanding of the effects of fructose feeding, Fitch, Hill and Chaikoff (13, 14) studied glycolytic enzyme activities of livers of normal rats fed fructose or glucose as the sole carbohydrate in the diet. The activities of phosphoglucomutase, glucose-6-phosphatase, phosphoglucose

isomerase, and the dehydrogenases of 6-phosphogluconate and glucose-6-phosphate were measured in the livers of normal rats fed a 60 per cent fructose diet.

The 60 per cent fructose diet failed to bring about a decrease in the activities of any of the five enzymes studied, yet there was a loss in capacity to utilize glucose. The conclusion drawn was a decrease in the glucokinase activity of the liver or an interference in the mechanism responsible for transporting glucose to the site of glucokinase activity in the cell. Also, in comparing glucose-fed rats against fructose-fed rats, the following enzyme activities were higher in the fructose-fed rats: glucose-6-phosphatase, phosphogluconate dehydrogenase, aldolase, fructokinase, and malate dehydrogenase (nicotinamide adenine diphosphonucleotide phosphate).

In 1964, Vaughn and Winders (75) compared the effect of cornstarch, dextrin, and glucose on the activity of hexosemonophosphate dehydrogenase and malic (NAD) dehydrogenase. The results showed that the more complex carbohydrates, dextrin and starch, depress the activity of hexosemonphosphate (HMP) dehydrogenase and malic (NAD) dehydrogenase when fed ad libitum. This phenomenon may be somewhat similar to that which occurs when dietary fat is increased. The intestinal breakdown of dextrin and starch

may have the effect of spreading out glucose uptake, and the liver does not receive as large intermittent loads. Thus, alternate pathways of disposal become less important.

In 1965, Antar and Ohlson (2) published the results of an experiment conducted on eight young healthy men and women in which the effect of simple and complex carbohydrates upon total lipids in serum was investigated. Serum total lipids, nonphospholipids and phospholipids were found to be significantly reduced with the high cereal diet and increased with the high sugar diet when the calories and fats were held constant for both males and females. An hypothesis was suggested for the mechanism of the lipid-lowering effect of the complex carbohydrates in contrast with the lipemic effect of high sucrose diet.

In 1966, Macdonald (42) noted the influence of fructose and glucose on serum lipid levels in men and pre- and postmenopausal women. The results obtained were compatible with the view that dietary fructose increases serum glycerides in men and postmenopausal women. Dietary glucose, when compared with dietary fructose or starch, seems to be associated with an increase in fasting serum phospholipids in men and a decrease in this fraction in pre- and postmenopausal women.

Macdonald (43) published a further study on ingested fructose and glucose in serum lipids in healthy men and

after myocardial infarction. The specific activity of the serum triglycerides was greater after the ingestion of fructose-C^{14} than after glucose-C^{14} in both groups, suggesting that fructose is preferred to glucose by the lipid synthetic pathways in the fasted state. When glucose was given, the specific activity of the serum triglycerides, when compared with control, was greater after a myocardial infarct.

CHAPTER II

PLAN OF PROCEDURE

One hundred male Sprague-Dawley rats were purchased for this study. When the rats arrived they were four to six weeks of age and weighed 120 to 160 grams each. The rats were housed in aluminum cages equipped with raised wire floors and were kept in a room in which the temperature was thermostatically controlled at 30 degrees centigrade.

DIETARY PROCEDURE

The rats were fed Purina Laboratory Chow in pellet form for about two months, after which time some of the animals were placed on special experimental diets and maintained on these diets until the time of sacrifice.

The rats were divided into three groups:

1) Special Experimental Diet I (40 rats)
2) Special Experimental Diet II (40 rats)
3) Diet III: powdered Purina Laboratory Chow (20 rats)

In this study the author will refer to the groups as Diet I, Diet II and Diet III. Since the special experimental

diets were in powdered form, it was considered advisable to have the Purina Laboratory Chow in powdered form also. All the rats were fed isocaloric diets but the food intake varied with each group.

The special experimental diets contained the following nutrients expressed in grams per cent (weight/weight):

SPECIAL EXPERIMENTAL DIET I

	Grams Per cent (weight/weight)
D (+) Sucrose	5.5
Dextrin (White Tech.)	2.1
Corn Starch	51.75
Crisco	7.2
Corn Oil	2.4
Casein-Purified-High Nitrogen	27.0
Salt Mixture XIV	4.0

Plus complete Vitamin Diet Fortification Mixture.

SPECIAL EXPERIMENTAL DIET II

	Grams Per cent (weight/weight)
D (+) Sucrose	3.7
Dextrin (White Tech.)	2.4
Corn Starch	48.5
Crisco	16.1
Corn Oil	3.25
Casein-Purified-High Nitrogen	22.0
Salt Mixture XIV	4.0

Plus complete Vitamin Diet Fortification Mixture.

The composition of the salt mixture was:

Salt Mixture U.S.P. XIV	Grams /1000 grams
Trace element mixture*	16.2
Calcium carbonate	68.6
Calcium citrate	308.3
Calcium biphosphate	112.8
Magnesium sulfate	38.3
Magnesium carbonate	35.2
Potassium chloride	124.7
Dibasic potassium phosphate	218.8
Sodium chloride	77.1

*The composition of the trace element mixture was:

	Grams /100 grams
Cupric sulfate	0.48
Ferric ammonium citrate	94.33
Manganese sulfate	1.24
Ammonium alum	0.57
Potassium iodide	0.25
Sodium fluoride	3.13

The composition of the Vitamin Diet Fortification Mixture was:

Vitamin Diet Fortification Mixture in Dextrose	Grams /100 lbs. Diet
Vitamin A concentrate (200,000 units per gram)	4.5
Vitamin D concentrate (400,000 units per gram)	0.25
Alpha tocopherol	5.0
Ascorbic acid	45.0
Inositol	5.0
Choline chloride	75.0
Menadione	2.25
p Aminobenzoic acid	5.0
Niacin	4.5
Riboflavin	1.0
Pyridoxine hydrochloride	1.0
Thiamine hydrochloride	1.0
Calcium pantothenate	3.0
Biotin	20.0
Folic acid	90.0
Vitamin B-12	1.35

The above formulation is used in supplementing specific diets where vitamin fortification of a diet mixture is specified. This vitamin mixture is commercially produced using the weights as indicated.

DIET III

(Powdered Purina Laboratory Chow)

The Ralston Company has stated that the Purina chow is a constant formula ration:

		Grams Per cent (weight/weight)
Protein	minimum	23.0
Fat	minimum	4.0
Fiber	maximum	6.0
Ash	maximum	9.0
Carbohydrate	minimum	56.0

As indicated above, Purina Laboratory Chow contains 56 per cent carbohydrates. Of this, approximately 5.0 per cent is composed of simple sugars as found in cane molasses; 5.2 per cent is fiber and presumed to be indigestible by the single-stomach animal. Polysaccharides comprise the remainder which approximates 90 per cent of the total carbohydrates. Plant starches constitute the real bulk of these polysaccharides.

Studies by Macdonald (43) and others (2, 14, 20) demonstrated that simple and complex carbohydrates have different effects on serum lipid concentrations as well as on the concentration of certain oxidative enzymes found in tissues.

Thus, sucrose and dextrin were added to Diets I and II. The remainder of the carbohydrate was macromolecular in the form of corn starch. There was no fiber added to the experimental diets.

It was not considered necessary to increase the casein or vitamins in the moderately high fat diet (Diet II) to compensate for the reduced quantity of food intake which resulted from the higher caloric density of the diet. The author did not consider the increased fat intake excessive in comparison to other experiments cited in this paper. The fat in Diet II was comparable to the percentage of fat observed in most American diets for the human population.

The caloric distribution among the various nutrients given in percentage of total calories for the three diets were:

Diets	Carbohydrate	Protein	Fat	Kcal/g
Experimental Diet I	55.0	25.0	20.0	4.5
Experimental Diet II	45.0	19.0	36.0	5.1
Diet III	68.4	22.3	9.3	4.2

The fat content in the three diets varied in grams percentage approximately as follows:

Diets	Grams Per cent (weight/weight)
Diet I	10.0
Diet II	20.0
Diet III	4.0

The two feeding regimens used in this study were as follows:

1) Ad libitum--food was available all day. These rats were designated as "nibblers" in this study.

2) Meal-fed--food was available for one two-hour period each day. The term "meal eaters" was used when referring to rats fed this type of regimen.

The animals were given isocaloric diets. Each rat in each group had access to 100 kilocalories of food each day. The food left each day was weighed and recorded. After being fed ad libitum on the special experimental diets for two weeks, 20 rats fed Diet I and 20 rats fed Diet II were placed on the meal-fed regimen in which they were allowed only the one two-hour feeding each day. The remainder of the rats in each group were fed ad libitum. Weekly weight gains for each rat were recorded from the time the rats were 11 to 12

weeks of age. After the meal-fed rats had been eating only one two-hour feeding each day for three weeks, the daily food consumption and the weekly weight gain for each rat in all the groups were recorded throughout the remainder of the study. Leveille and Hanson (37) have shown that three to four weeks is a sufficient length of time to induce adaptive changes to meal-feeding.

The rats were classified into their respective groups according to the following number system:

Rats	Diet I
Series 100	Meal-fed
Series 300	Ad libitum-fed

Rats	Diet II
Series 200	Meal-fed
Series 400	Ad libitum-fed

The complete study started in October, 1968, and continued into May, 1969. At the time of sacrifice the rats were 8.5 to 9.5 months of age.

METABOLIC STUDIES

The existence of the metabolic pathway involved with this study was established by Warburg, Dickens, Lipmann, and Dische. This pathway is referred to as the hexosemonophosphate

or the pentose phosphate pathway of glucose metabolism. (See Figure 1.) The reactions in this pathway yield reduced nicotinamide adenine dinucleotide phosphate (NADPH), a co-enzyme which appears to be a reducing agent in the synthesis of fatty acids and cholesterol. These reactions also involve the oxidative enzymes, glucose-6-phosphate dehydrogenase (G-6-PDH) and 6-phosphogluconate dehydrogenase (6-PGDH). By measuring the activity of these oxidative enzyme concentrations in liver and adipose tissue, it is possible to determine whether or not there is increased lipogenesis occurring. The reduced nicotinamide adenine dinucleotide phosphate (NADPH) is produced when glucose-6-phosphate and 6-phosphogluconate are oxidized. Langdon (32) and others (15, 18, 40, 50, 65) have shown that reduced nicotinamide adenine dinucleotide phosphate is a critical aspect for fatty acid synthesis in the cytoplasm as well as the mitochondria.

The meal-fed rats were sacrificed the day after their last meal and the nibbling rats had access to food until the time of sacrifice. The author believed a fasting period would not be directly applicable to this study since the dietary patterns, as related to the human population, were an important consideration. The meal-fed group consumed their last meal the day before sacrifice. From observations

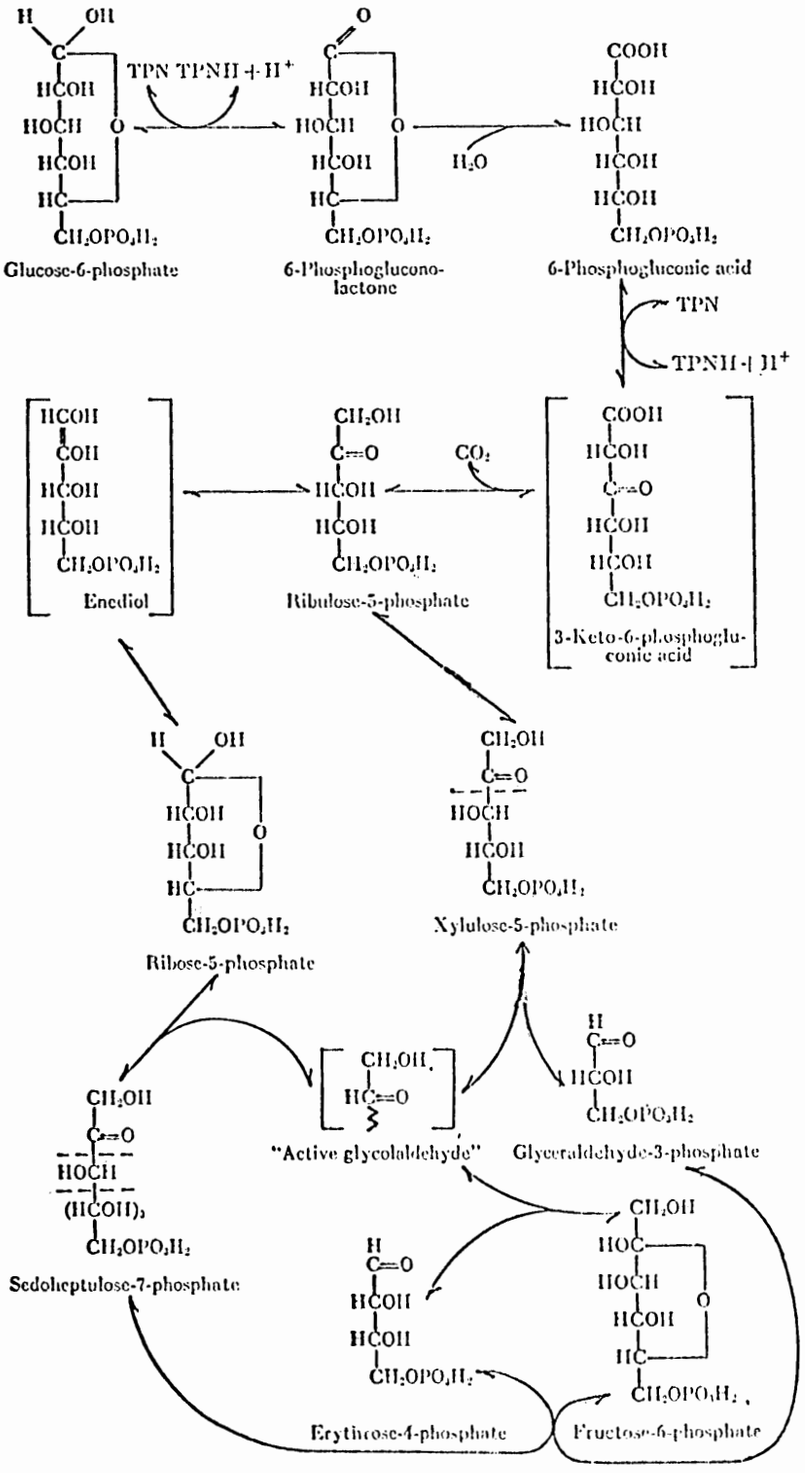

Figure 1

Hexosemonophosphate Oxidative Metabolism According to Cantarow (7)

throughout the study, the nibbling rats ate most of their meal as soon as they were fed. Although the food ration from the day before was available the day of sacrifice, very little, if any, was left for eating on the day of sacrifice. Upon sacrifice some of the rats had digestive juices present in the duodenum but this was not limited to any one group. A few animals in all the groups had traces of digestive juices in the duodenum.

Preparation of Samples

The animals were anesthetized with ethyl ether and exsanguinated by femoral or aortic puncture. The liver was excised quickly and blotted on filter paper. For removal of the epididymal fat pads the testicles were lifted into the abdominal cavity. The fat pads were held by blunt forceps and excised just above the epididymal vessels. A 10 per cent (weight/weight) homogenate of both liver and adipose tissue was prepared by sonifying one gram of tissue in nine milliliters of cold physiological saline (0.9 g per cent) containing 6.6×10^{-4} Moles ethylenediaminetetraacetic acid disodium salt ($EDTA\text{-}Na_2H_2 \cdot 2\ H_2O$). All manipulations were carried out at reduced temperatures. The resulting suspensions were centrifuged in a refrigerated centrifuge, International B-20, for 10 minutes at 5000 x gravity. Aliquots for analysis of glucose-6-phosphate dehydrogenase and

6-phosphogluconate dehydrogenase activities in both liver and adipose tissues were removed and assayed immediately upon completion of the centrifugation. The remaining supernates were frozen and stored for future investigations.

Enzymatic Assays

According to Bergmeyer (5), enzymatic activity may be expressed in various units, but all are related to the rate at which a substrate is converted per unit weight of tissue, tissue protein, or total tissue nitrogen. In view of the fact that the rate of conversion was the parameter being measured, it was imperative that conditions for optimum consistent conversion existed. The factors which influence the rate of an enzymatic reaction vary from enzyme to enzyme. These factors include temperature, pH, concentration of substrate,concentration of enzyme, or ancillary enzymes, and the presence of activators or inhibitors.

Glucose-6-Phosphate Dehydrogenase (G-6-PDH).--This enzyme is responsible for catalyzing the oxidation of glucose-6-phosphate to the corresponding 6-phosphogluconolactone. Its activity may be determined by following the concomitant conversion of oxidized nicotinamide adenine dinucleotide phosphate (NADP) to the reduced form which absorbs ultraviolet light.

The reaction involved is:

$$\text{G-6-P} + \text{G-6-PDH} \rightleftharpoons \text{6-phosphogluconolactone} + \text{NADPH} + H^+$$

Figure 2 shows the effect of substrate concentration on the activity of liver glucose-6-phosphate dehydrogenase. The indication is that a maximum rate of activity is reached at a concentration of 10^{-3} Molar and decreased rather rapidly at concentrations lower than 10^{-4} Molar. Between a pH of 7.4 and 8.6 there is little change in enzymatic activity (Figure 3). However, the activity drops sharply at a pH above or below this range. As can be seen from Figure 4, the rate of reaction at pH 7.6 is quite linear for the first 10 minutes. The Michaelis constant for glucose-6-phosphate dehydrogenase at pH 7.6 is 1×10^{-5} Molar (26). This is a much lower figure than that of the 6-phosphogluconate dehydrogenase enzyme.

6-Phosphogluconate Dehydrogenase (6-PGDH).--This enzyme catalyzes the oxidation of 6-phospho g luconate by the oxidized form of NADP with the resulting formation of NADPH. As mentioned earlier, this can be detected because of its ability to absord ultraviolet light at 340 millimicrons wavelength.

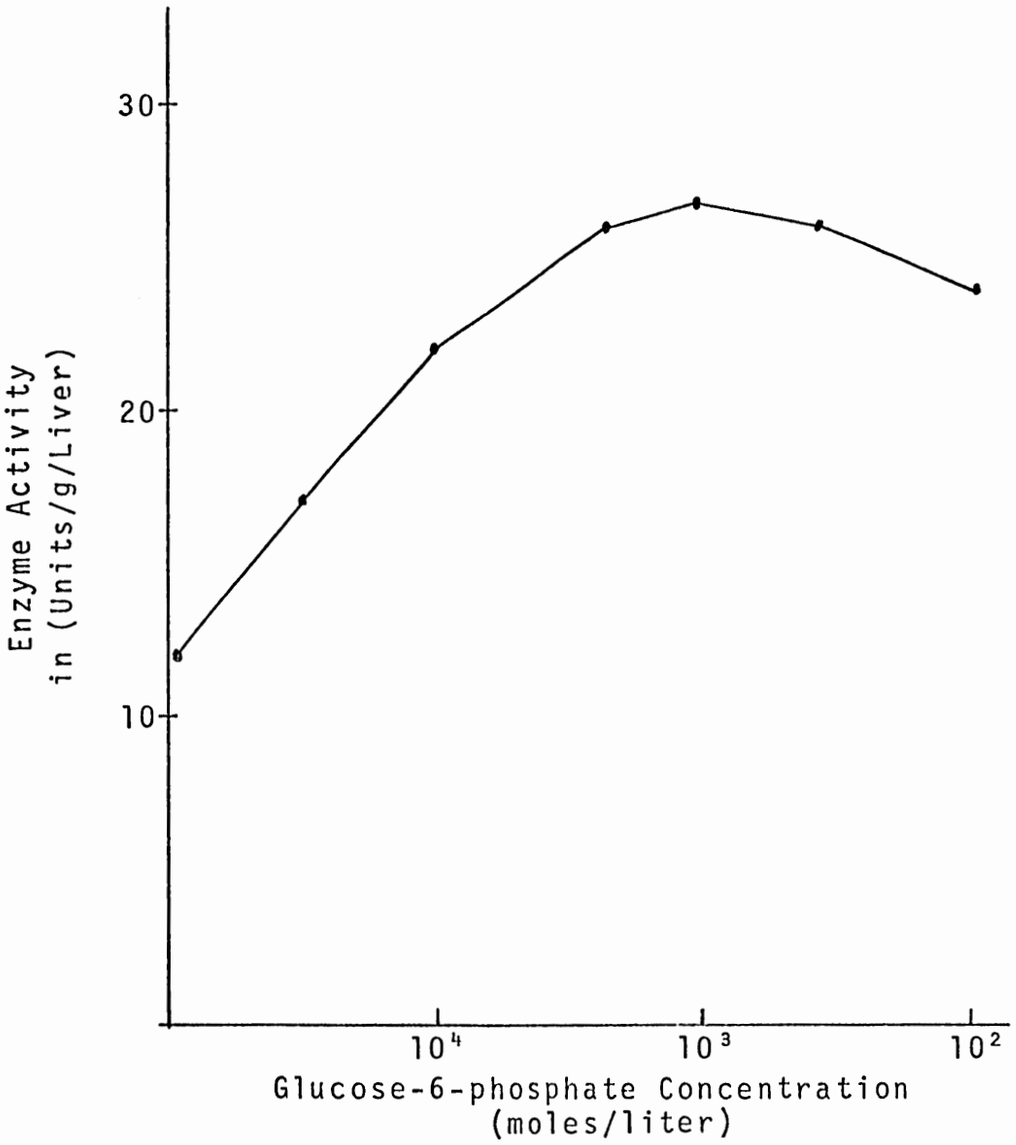

Figure 2

Dependence of G-6-PDH Activity on Substrate Concentration According to Bergmeyer (5)

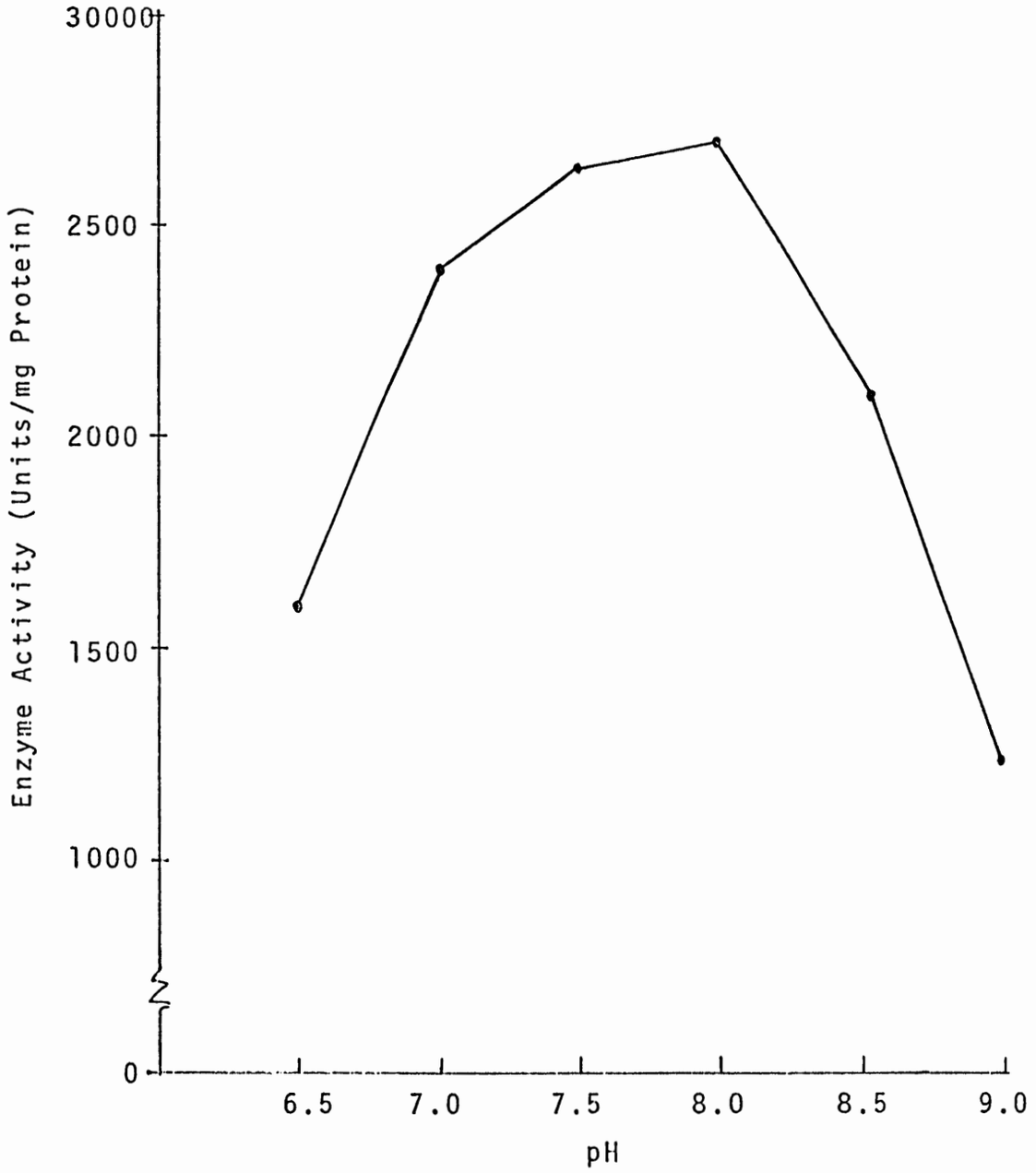

Figure 3

Dependence of G-6-PDH Activity on pH of Media According to Bergmeyer (5)

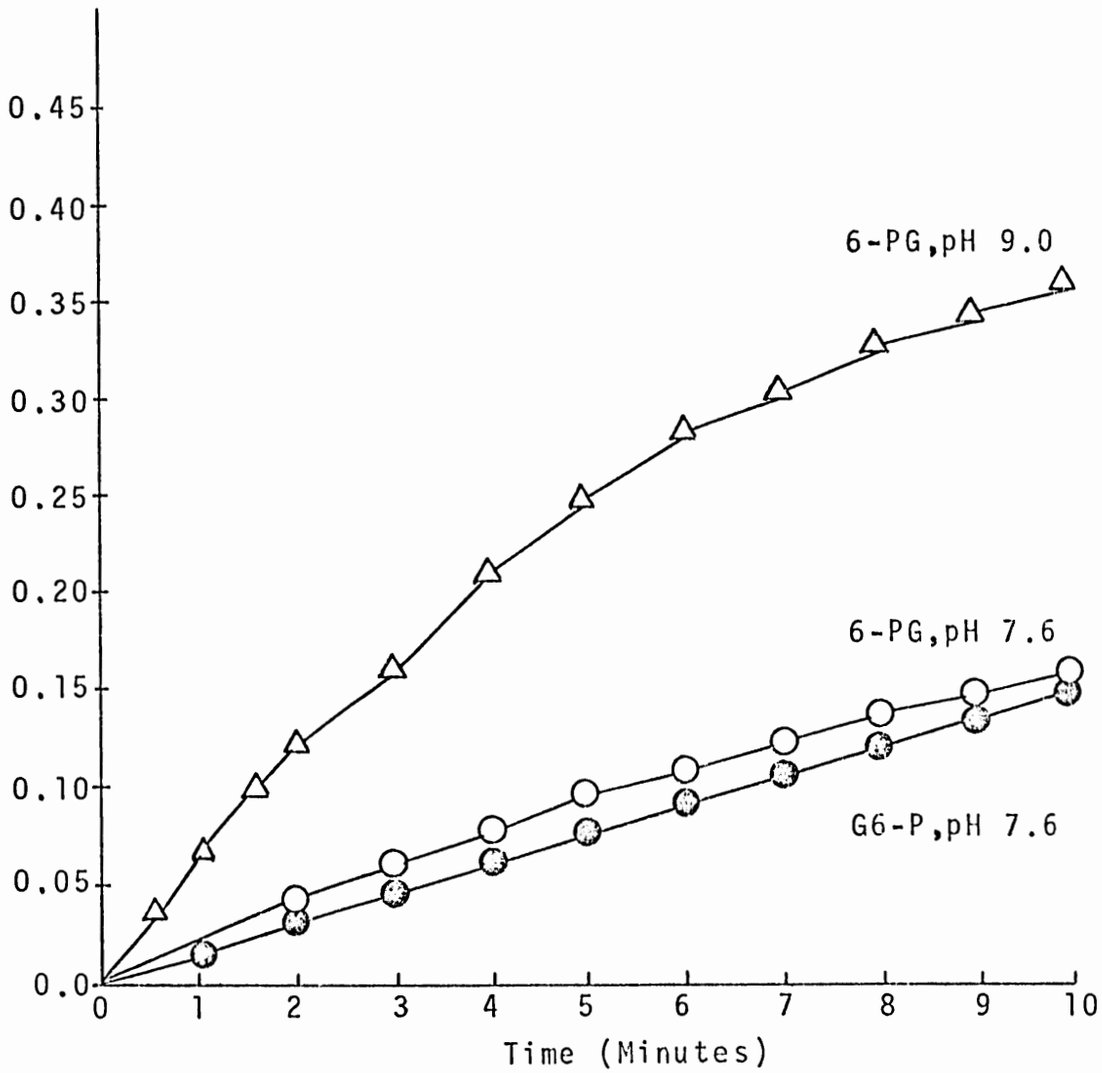

Figure 4

Effect of pH on Rate of Enzymatic Activity

According to Glock and McLean (16)

The reaction involved is:

$$\text{6-PG} + \text{NADP} + \text{6-PGDH} \rightleftharpoons \text{6-phospho-3-ketogluconate} + \text{NADPH} + H^+$$

The equilibrium of this reaction lies far to the right, as shown by the Michaelis constant of 5×10^{-5} Molar. Hohorst (22) found the optimum pH for the above reaction to be 9.0; however, a pH between 7.0 and 8.0 and a slight excess of NADP is sufficient to obtain a quantitative oxidation of 6-phosphogluconate. On the basis of these data, a pH of 7.5 was selected for the dual enzyme assay system.

Experimental Techniques

Reagents.--

1) Triethanolamine buffer, 0.05 Molar, pH 7.5.
   Dissolve 0.93 g triethanolamine hydrochloride and 0.2 g EDTA-$Na_2H_2 \cdot 2H_2O$ in approximately 50 milliliters of water, adjust to pH 7.5 with 0.1 N NaOH and dilute to 100 milliliters with distilled water.

2) Glucose-6-phosphate (4×10^{-2} Molar)
   Dissolve 130 milligrams of G-6-P$\cdot Na_2$ in 10 milliliters distilled water.

3) 6-Phosphogluconate trisodium salt (4×10^{-2} Molar)
Dissolve 137 milligrams of 6-phosphogluconate in 10 milliliters distilled water.

4) Nicotinamide adenine dinucleotide phosphate (approximately 2×10^{-2} Molar beta-NADP)
Dissolve 15 milligrams of the monosodium salt in 1.0 milliliters of 1.0 per cent $NaHCO_3$ solution.

The procedures for evaluation of G-6-PDH and 6-PGDH activities were based on the methods described by Glock and McLean (16, 17) and by Horecker (26).

Assays.--In the assays described below, cuvettes of 1.0 centimeter light path and containing approximately 3.2 milliliters of assay mixture were maintained at 30 degrees centigrade by circulating water from a Haake constant temperature water bath through thermospacers located within the cell compartment of the Hitachi Perkin Elmer Spectrophotometer, Model 139. Changes in optical density measured at 340 millimicrons were recorded at two-minute intervals for a period of 10 minutes or until the rate of change in

optical density was constant. Assay media for each tissue were:

Liver Assay

Cuvette 1 (blank)	Cuvette 2 (6-PGDH)	Cuvette 3 (G-6-PDH + 6-PGDH
3.0 ml buffer	3.0 ml buffer	2.8 ml buffer
0.02 ml supernate	0.05 ml NADP	0.05 ml NADP
	0.05 ml 6-PG	0.10 ml 6-PG
	0.02 ml supernate	0.10 ml G-6-P
		0.02 ml supernate

Adipose Tissue Assay

Cuvette 1 (blank)	Cuvette 2 (6-PGDH)	Cuvette 3 (G-6-PDH + 6-PGDH
2.9 ml buffer	2.8 ml buffer	2.7 ml buffer
0.1 ml supernate	0.05 ml NADP	0.05 ml NADP
	0.05 ml 6-PG	0.10 ml 6-PG
	0.10 ml supernate*	0.10 ml G-6-P
		0.10 ml supernate

*In certain groups of animals it was necessary to reduce the volume to 0.05 ml.

Calculations.--In keeping with the formally accepted recommendation made by Racker to the Enzyme Commission of the International Union of Biochemistry and to the Clinical Chemistry commission of the International Union of Pure and Applied Chemistry (5), the investigator defined a unit of enzyme activity as the amount of enzyme which converts one micromole of substrate per unit time. This activity is usually determined at 25 degrees centigrade, but technical problems prevented the use of a temperature lower than 30

degrees centigrade in this study. The final results have been expressed as units of activity per gram of wet tissue.

As indicated in the equations given on the preceding pages, for every micromole of substrate (either G-6-P or 6-PG) converted, one micromole of NADPH is formed. The NADPH absorbs ultraviolet light at 340 millimicrons and has an extinction coefficient of 6.22×10^6 cm^2/mole, or 6.22×10^3 cm^2/millimole, or 6.22 cm^2/micromole, or 0.622 cm^2/0.1 micromole. To compensate for the 3.0 cm^2 area used in this study, the adjusted value would be 0.207 $3cm^2$/0.1 micromole. Using this figure the activity of the enzyme within the cuvette may be calculated according to the following formula:

$$\text{Activity } (\mu M/hr) = \frac{(0.1\ \mu m)(O.D.\ \text{units/hr.})}{0.207\ O.D.\ \text{units}}$$

The data were evaluated statistically by the Student "t" test. Values were obtained for food efficiency, variations in the group average weight gains, and comparison in enzymatic activity of the rats on three different diets and two dietary regimens.

CHAPTER III

PRESENTATION AND ANALYSIS OF DATA

Experimental data obtained by Leveille (37) and others (13, 20, 23, 46, 54, 68) have indicated that changes in dietary pattern and/or dietary composition can produce alterations in metabolism. These alterations have been demonstrated in a number of systems:

1) Enzyme activity supporting lipogenesis, glycogenesis, and/or gluconeogenesis.

2) Relative concentrations of saturated and unsaturated fatty acids.

3) Concentrations of serum lipids notably cholesterol, lipoproteins, triglycerides, free fatty acids, esterified fatty acids, and phospholipids.

4) Rate of synthesis of fatty acids in the liver and adipose tissue.

5) Use of lesser metabolic pathways.

6) Fatty acids synthesis from different substrates (glucose, acetate, pyruvate, glycogen).

7) Rate of synthesis of coenzymes, particularly the pyridine nucleotides.

In this study the author has investigated the concentrations of the dehydrogenases operating in the hexosemonophosphate pathway, the body weight and food consumption of

96 adult male Sprague-Dawley rats. These young adult rats were placed on various diets and two different dietary regimens. The body weights and food consumption were recorded over a period of 18 weeks. Records were kept on the daily food consumption and the weekly weight gains of each rat. Sixteen rats were fed Diet III (Purina Laboratory Chow) ad libitum. Forty rats were fed a moderately high fat diet (Diet II), either ad libitum or one two-hour meal each day; 40 were fed a moderately low fat diet (Diet I), either ad libitum or as a single two-hour meal each day.

The factors taken into consideration for planning the different dietary regimens were:

1) Percentage carbohydrate, protein and fat intake.
2) Rate of caloric influx.
3) Type of carbohydrate.

Experimental Diets I and II were purchased in four different batches. Sometimes the food varied in uniformity of texture upon arrival; therefore, on two occasions the rats eating only one meal each day did not maintain their usual weight gains as they had to readjust to the new food. Thus, the weeks that new batches of food arrived had to be eliminated from the statistical analysis.

FOOD EFFICIENCY STUDIES

The rats were approximately three and a half months of age at the time they were given the experimental diets. Until that time they were all eating Diet III and were gaining weight at a normal rate. The rats placed on the meal-feeding program had a three-to-four-week period of adjustment and during this time the meal-eaters had an average weight loss of approximately 45 grams.

Overall Study of 96 Adult Rats

The initial effects of the various diets and dietary regimens on average body weight are shown in Table I. This table included a summary of the first four weeks of the 18-week study. The fourth week ended January 17th. At that time the food intake of the meal-eaters in both groups was less than that of the nibblers and continued to be lower throughout the remaining 14 weeks (Figures 5 and 6).

The rats fed Diet I by meal-feeding had an average loss of 16 grams of their initial body weight over a period of two weeks, then proceeded to gain weight. At the end of the 18 weeks the mean weight gains of the rats on the two dietary regimens were nearly identical (Tables II and III). The rats fed Diet II by meal-feeding had an average loss of 11 grams of their initial body weight over a period of two

TABLE I

AVERAGE BODY WEIGHTS OF ALL GROUPS OF MEAL-EATING AND NIBBLING RATS FED DIETS I, II, AND III

Diet	Regimen	Average Initial Weight (g)	Weeks			
			1	2	3	4
I	Meal-Eaters	333	332	317	324	326
	Nibblers	334	338	353	363	372
II	Meal-Eaters	329	327	318	325	331
	Nibblers	332	330	352	369	384
III	Nibblers	328	331	339	349	362

The weights for the fourth week were taken January 17th.

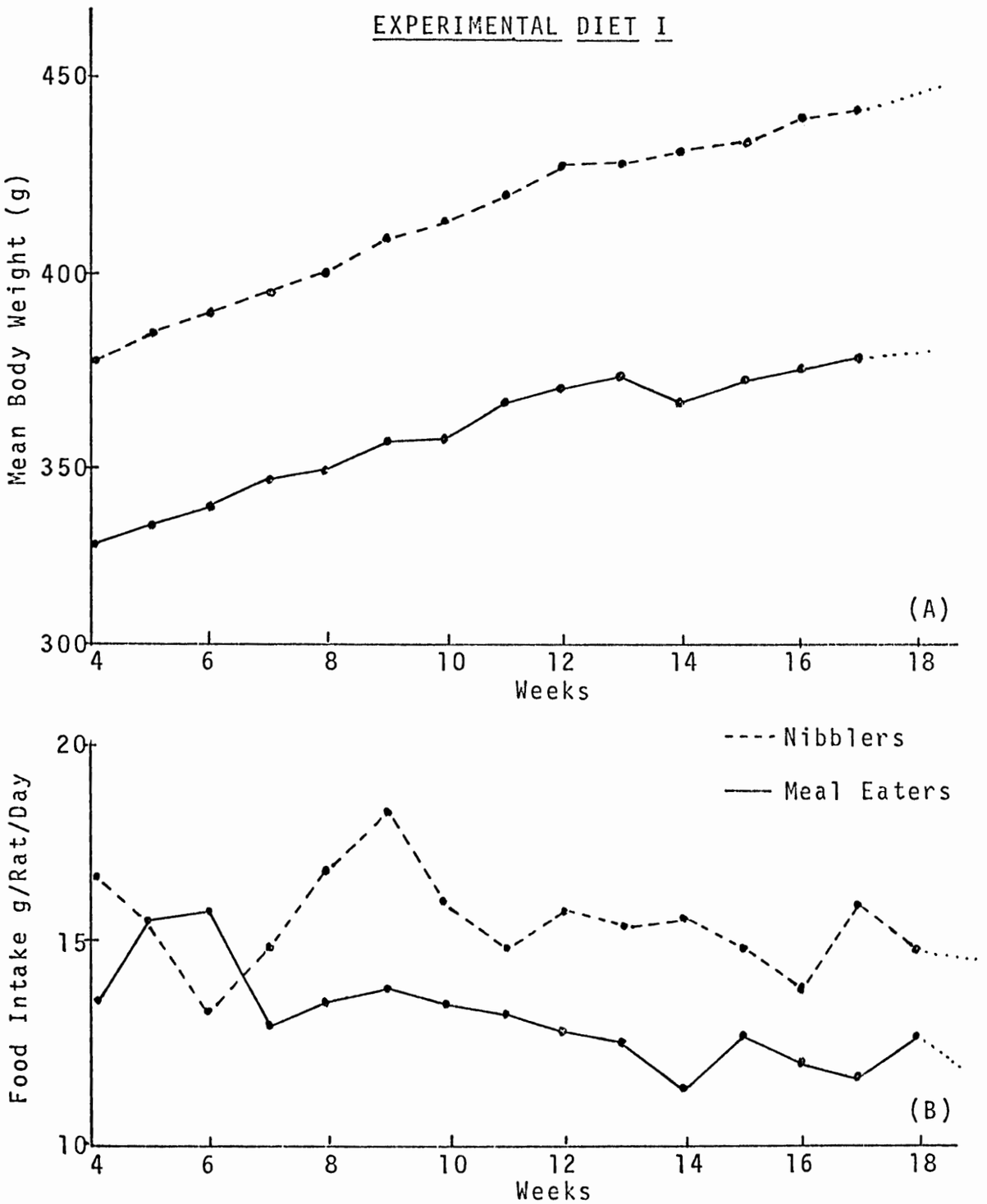

Figure 5

The Mean Body Weights (A) and the Mean Food Intake (B) of 20 Nibbling Versus 20 Meal-Eating Rats

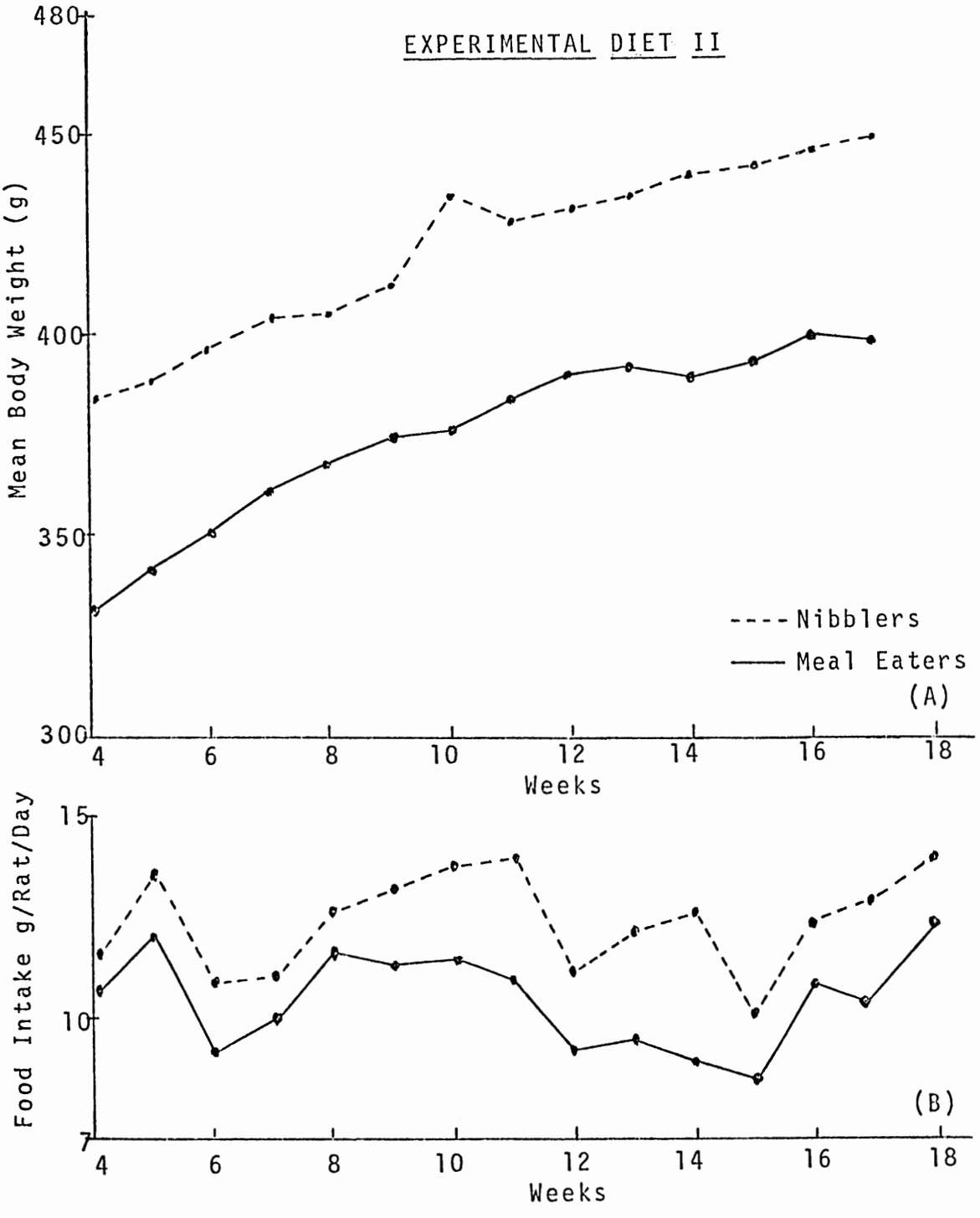

Figure 6

The Mean Body Weights (A) and the Mean Food Intake (B) of 20 Nibbling Versus 20 Meal-Eating Rats

TABLE II

THE AVERAGE DAILY FOOD INTAKE AND WEEKLY WEIGHT GAIN OF 20 ADULT MALE RATS FED DIET I AD LIBITUM

Date	Average Weight (g)	Average Weight Gain (g)	Average Food Intake (g)	Average Food Intake (kcal)	Food Efficiency (Per cent)
January					
17	372				
24	379	7	15.6	70.3	10.0
31	385	6	13.3	60.0	10.0
February					
8	390	5	15.0	67.4	7.4
14	395	5	16.6	75.0	6.7
21	403	8	18.3	82.3	9.7
28	408	5	16.2	72.8	6.9
March					
7	415	7	14.8	66.9	10.5
14	422	7	15.8	71.0	9.8
21	423	1	15.5	69.9	1.4
30	426	3	15.6	70.0	4.3
April					
4	427	1	14.9	67.0	1.5
11	434	7	13.8	61.9	11.3
18	436	2	16.0	72.0	2.8
Average	408	4.9	15.4	69.7	7.1

TABLE III

THE AVERAGE DAILY FOOD INTAKE AND WEEKLY WEIGHT GAIN OF 20 MEAL-EATING ADULT MALE RATS FED DIET I

Date	Average Weight (g)	Average Weight Gain (g)	Average Food Intake (g)	Average Food Intake (kcal)	Food Efficiency (Per cent
January					
17	326				
24	330	4	15.7	70.6	5.7
31	335	5	16.0	71.9	7.0
February					
8	342	7	13.2	59.4	11.8
14	345	3	13.6	61.3	4.9
21	353	8	13.8	62.3	12.8
28	353#	-#	13.6#	61.2#	0.0#
March					
7	362	9	13.3	60.0	15.0
14	366	4	12.9	58.2	6.9
21	369	3	12.6	56.7	5.3
30	361#	8#	11.4#	51.2#	15.6#
April					
4	367	6	12.7	57.1	10.5
11	370	3	12.1	54.8	5.5
18	373	3	11.7	52.6	5.7
Average	354	5.0	13.4	60.4	8.3

#Eliminated from statistical analysis

weeks, then proceeded to gain weight. At the end of the 18 weeks the mean weight gains of the rats on the two dietary regimens were similar (Tables IV and V). The rats consuming Diet II had a slightly higher mean weight gain than the rats eating Diet I, whether meal-fed or fed ad libitum. The rats consuming Diet III had a slightly lower mean weight gain than that of the rats fed Diets I or II (Table VI).

The final weights of all the rats were taken just before sacrifice and recorded in Table VII. As shown below, at the time of sacrifice the Diet II nibblers weighed 19 grams more on the average than the Diet I nibblers. Diet II meal-eaters weighed 10 grams more on the average than Diet I meal-eaters.

Series	Number of Rats	Diet	Regimen		Final Average Weight (g)
			Nibblers	Meal-Eaters	
300	19	I	X		452
100	20	I		X	380
400	17	II	X		471
200	17	II		X	390
500	16	III	X		424

The rats fed Diet III were three weeks older than the other rats at the time they were sacrificed.

Experimental Diet I (40 rats).--At the beginning of the experiment, 20 rats remained on the ad libitum feeding program

TABLE IV

THE AVERAGE DAILY CALORIC INTAKE AND WEEKLY WEIGHT GAIN OF 20 ADULT MALE RATS FED DIET II AD LIBITUM

Date	Average Weight (g)	Average Weight Gain (g)	Average Food Intake (g)	Average Food Intake (kcal)	Food Efficiency (Per cent)
January					
17	384				
24	388	4	13.6	70.2	5.7
31	396	8	11.0	56.5	14.2
February					
8	404	8	11.0	56.8	14.0
14	405	1	12.8	65.5	1.5
21	412	7	10.4	53.1	13.2
28	436	24	14.0	71.8	33.4
March					
7	428	-8	14.0	72.2	-11.1
14	432	4	11.2	57.8	6.9
21	435	3	12.4	63.4	4.8
30	440	5	12.8	65.5	7.6
April					
4	443	3	10.2	52.6	5.6
11	446	3	12.6	64.5	4.6
18	450	4	13.0	67.0	6.0
Average	421	5.1	12.2	62.8	8.2

TABLE V

THE AVERAGE DAILY CALORIC INTAKE AND WEEKLY WEIGHT GAIN OF 20 MEAL-EATING ADULT MALE RATS FED DIET II

Date	Average Weight (g)	Average Weight Gain (g)	Average Food Intake (g)	Average Food Intake (kcal)	Food Efficiency (Per cent)
January					
17	331				
24	341	10	12.1	62.4	16.0
31	350	9	9.2	47.2	19.0
February					
8	361	11	10.2	52.3	21.0
14	368	7	11.7	60.3	11.6
21	375	7	13.3	68.4	10.2
28	377#	2#	11.5#	59.4#	3.4#
March					
7	384	7	11.1	57.1	11.2
14	390	6	9.3	48.1	12.4
21	392	2	9.6	49.4	4.0
28	390#	-2#	9.0#	46.2#	3.2#
April					
4	394	4	8.6	44.1	9.0
11	401	-3	10.9	55.9	-5.4
18	400	-1	10.7	55.0	-1.8
Average	374	5.4	10.6	54.6	9.9

#Eliminated from statistical analysis

TABLE VI

THE AVERAGE DAILY CALORIC INTAKE AND WEEKLY WEIGHT GAIN OF 16 ADULT MALE RATS FED DIET III AD LIBITUM

Date	Average Weight (g)	Average Weight Gain (g)	Average Food Intake (g)	Average Food Intake (kcal)	Food Efficiency (Per cent)
January					
17	362				
24	369	7			
31	378	9	20.1	86.5	10.4
February					
8	375	-3	19.2	82.6	3.6
14	381	6	20.8	89.5	6.7
21	391	10	19.0	81.8	12.2
28	391	0	19.1	82.2	0.0
March					
7	398	7	16.3	70.2	9.1
14	403	5	16.4	70.6	7.1
21	405	2	15.9	68.5	2.9
30					
April					
4	409	2	15.3	65.9	3.0
11	412	3	15.6	67.1	4.5
18	413	1	17.1	73.6	1.4
Average	391	4.1	17.7	76.2	6.3

TABLE VII

FINAL WEIGHTS OF ALL THE RATS BEFORE SACRIFICE

Meal-Fed Rats				Nibbling Rats					
Diet I		Diet II		Diet I		Diet II		Diet III	
Rat Number	Weight (g)	Rat Number	Weight (g)	Rat Number	Weight (g)	Rat Number	Weight (g)	Rat Number	Weight (g)
101*	371	201*	429	301*	475	401*	439	501*	388
102*	401	202	Dead	302	450	402	456	502*	425
103	376	203	406	303*	488	403	555	503	420
104	449	204	367	304*	534	404	467	504	415
105	386	205*	404	305	442	405*	395	505*	481
106*	366	206*	451	306*	479	406*	490	506*	420
107	327	207	Dead	307*	413	407	462	507	Sick
108*	357	208*	397	308*	477	408*	553	508*	403
109*	398	209	441	309	455	409*	491	509*	416
110	361	210	429	310	473	410	495	510*	413
111	353	211*	441	311*	445	411	Dead	511*	384
112	416	212*	445	312	397	412	Sick	512*	411
113*	393	213	427	313*	459	413*	492	513	Dead
114	340	214*	349	314*	428	414	440	514*	473
115*	425	215*	433	315	Dead	415*	454	515	437
116*	377	216*	410	316*	392	416*	516	516	460
117*	356	217	383	317	456	417*	423	517	442
118	364	218*	436	318	Sick	418*	507	518	412
119	352	219	380	319	460	419	392	519	Dead
120*	420	220	Dead	320	413	420	Sick	520	Dead

*The rats selected for the food efficiency statistics involving only 10 rats

and 20 rats were placed on one two-hour meal each day. At the time of sacrifice, there were 19 nibblers and 20 meal-eaters remaining. After the initial weight loss for the meal-eaters, the body weight curves were practically parallel for the 14 weeks (Figure 7). The percentage food efficiency (grams gain per kilocalorie of food consumed) was higher for the meal-fed group. The ad libitum group had a percentage food efficiency of 7.1 ± 3.4 (mean for 20 rats ± S.E.). The meal-eating rats had a percentage food efficiency of 3.8 ± 3.4 (mean for 20 rats ± S.E.). Data analysis revealed this difference was not significant, indicating the meal-eaters were just as efficient in utilizing their food as were the nibblers. Although there was no real difference between the means, the difference was in favor of better utilization of food for the meal-eating rats. The average weight gain over the 14 weeks period for the two groups was almost identical, but the average caloric intake for the nibbling rats was 9.3 kilocalories per day higher than for the meal-eaters.

Experimental Diet II (40 rats).--Twenty rats continued to eat ad libitum and 20 rats were placed on the meal-feeding program. At the time of sacrifice 17 rats were left in each group. After the initial weight loss for the meal-eaters, the weekly weight gain and daily food consumption

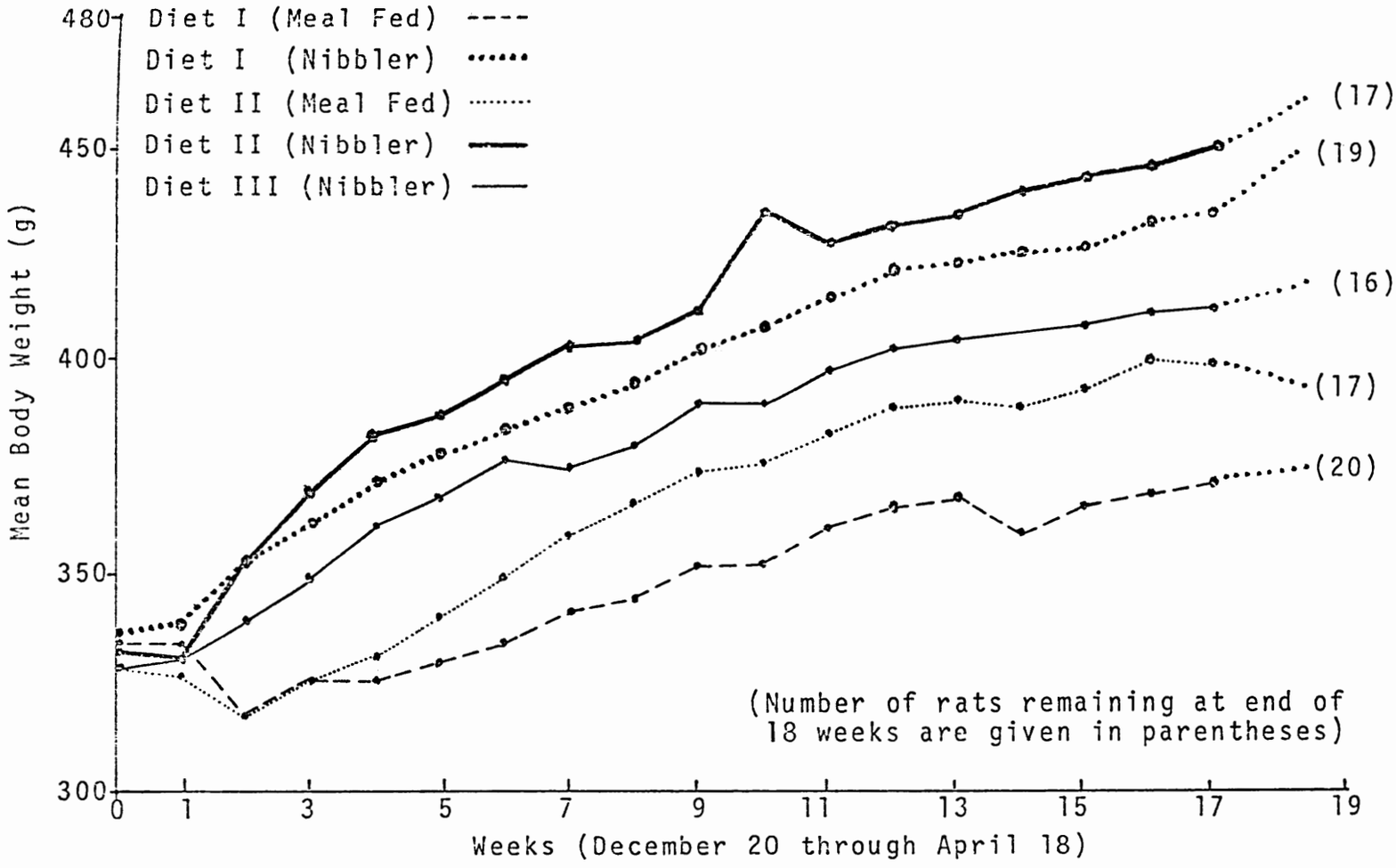

Figure 7

The Mean Body Weights of Each Group of Rats

Over a Period of 18 Weeks

were recorded for 14 weeks. The meal-eating rats exhibited a slightly better weight gain the first few weeks, but the last two weeks they began to lose weight. The food consumption did not follow the pattern of weight loss. Several of the rats in the meal-eating group became sick the last two weeks and two died within the week.

As shown in Table VIII, the ad libitum group had a food efficiency of 8.2 ± 2.8 (mean for 20 rats ± S.E.). The meal-eating group had a food efficiency of 9.9 ± 6.7 (mean for 20 rats ± S.E.). The results indicated that the meal-eaters were slightly more efficient in utilizing their food but the difference was not significant. The mean weight gains for the two groups were similar but the caloric intake for the nibblers averaged 8.2 kilocalories per day higher than for the meal-eaters.

Diet III (16 rats).--The 16 rats in this group were fed ad libitum. The percentage food efficiency was 6.3 ± 4.0 (mean for 16 rats ± S.E.). In comparing the percentage food efficiency with the ad libitum rats eating Diets I and II, this group did not seem to utilize their food as well. A

TABLE VIII

SUMMARY OF MEAN WEIGHT GAINS, CALORIC INTAKE, AND PERCENTAGE FOOD EFFICIENCY OF FIVE GROUPS OF RATS FOR A 14-WEEK PERIOD

Diets	Mean Weight Gain (g)	Mean Food Intake (kcal)	Per cent Food Efficiency (Mean ± S.E.)
Diet I			
Nibblers (N=20)	4.9	69.7	7.1 ± 3.4
Meal-eaters (N=20)	5.0	60.4	8.3 ± 3.4
Diet II			
Nibblers (N=20)	5.1	62.8	8.2 ± 2.8
Meal-eaters (N=20)	5.4	54.6	9.9 ± 6.7
Diet III			
Nibblers (N=16)	4.1	76.2	6.3 ± 4.0

comparison of the average weight gains of the groups eating the three diets ad libitum is given below:

Diets	Mean Weight Gain (g)
I	4.9
II	5.1
III	4.1

Although the differences indicated the rats consuming Diet II utilized their diet more efficiently than the other groups, the differences were not significant.

In comparing the average weight gain of the 40 meal-eaters consuming Diets I and II, the differences were not significant. The 20 meal-eaters consuming Diet I had an average weight gain of 5.0 grams, whereas the 20 consuming Diet II gained 5.4 grams on the average.

Study of 50 Selected Rats

Certain problems are inherent in animal experimentation. Therefore, 10 rats were selected as most representative of each group and their weekly weight gains and daily food consumption were compared (Figures 8 and 9). These data were tabulated in Tables IX, X, XI, XII, and XIII. The animals not used were eliminated for the following reasons:

1) Death.

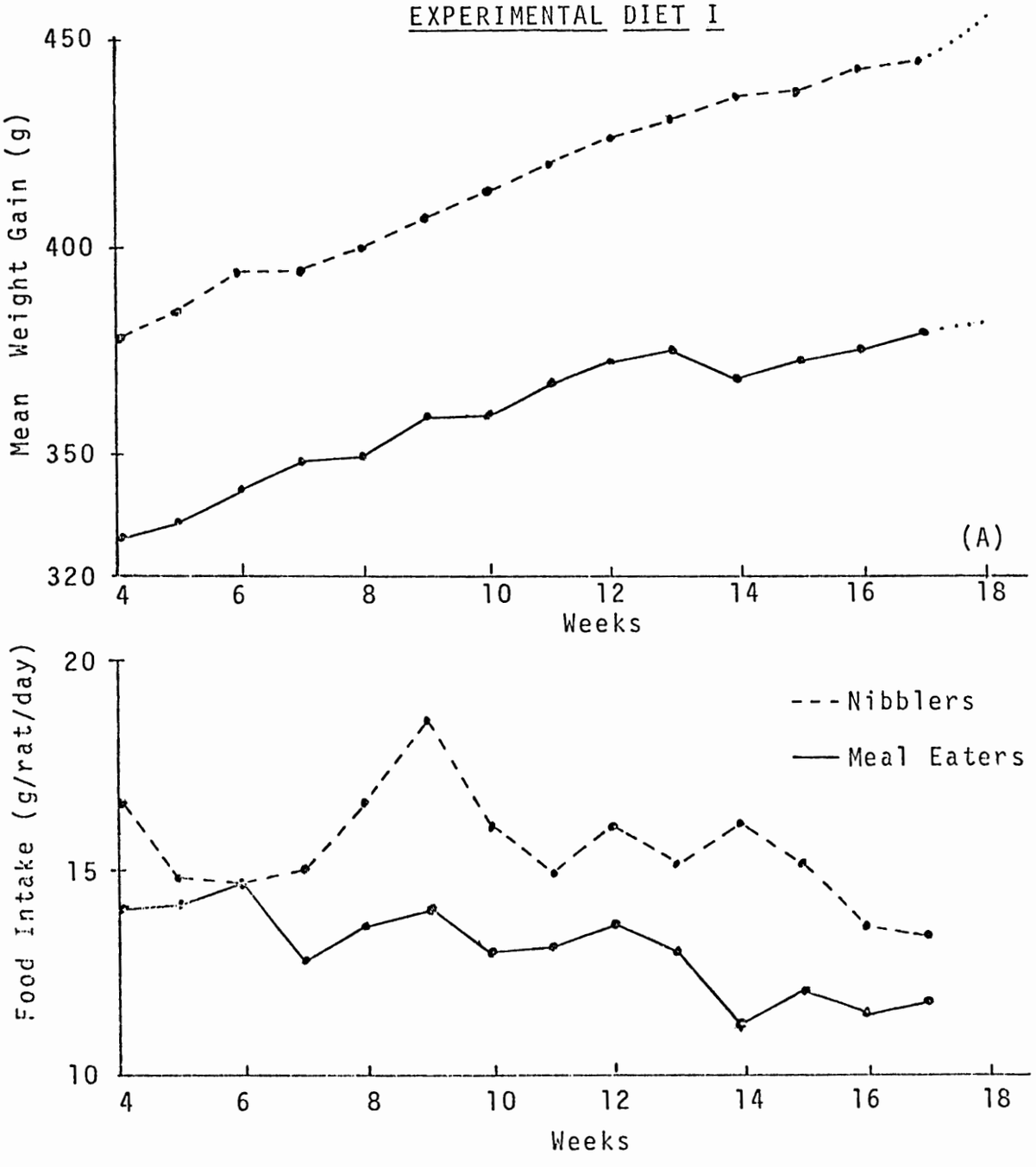

Figure 8

The Mean Body Weights (A) and the Mean Food Intake (B) of 10 Nibbling Versus 10 Meal-Eating Rats

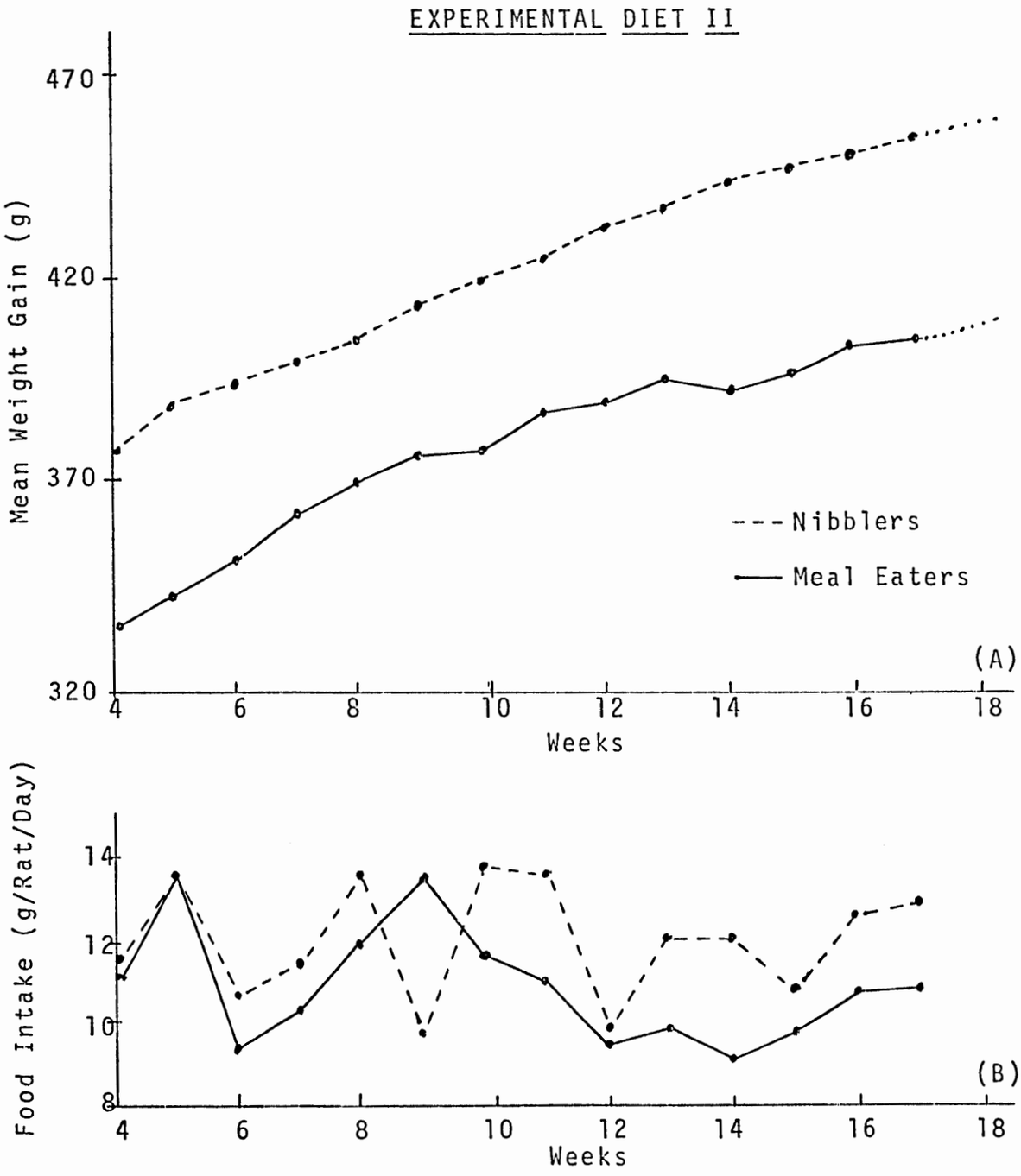

Figure 9

The Mean Body Weights (A) and the Mean Food Intake (B) of 10 Nibbling Versus 10 Meal-Eating Rats

TABLE IX

THE AVERAGE DAILY CALORIC INTAKE AND WEEKLY WEIGHT GAIN OF 10 ADULT MALE RATS FED DIET I AD LIBITUM

Date	Average Weight (g)	Average Weight Gain (g)	Average Food Intake (g)	Average Food Intake (kcal)	Food Efficiency (Per cent)
January					
17	378		16.6	74.7	
24	385	7	14.8	66.7	10.5
31	394	9	14.6	65.8	13.7
February					
8	394	0	15.0	67.6	0.0
14	400	6	16.6	74.8	8.0
21	407	7	18.6	83.8	8.4
28	414	7	16.0	72.0	9.7
March					
8	421	7	14.9	67.0	10.4
14	427	6	16.0	72.0	8.3
21	431	4	15.1	68.0	5.9
28	436	5	16.2	72.9	6.8
April					
4	438	2	15.1	67.9	2.9
11	443	5	13.6	61.2	8.2
18	445	2	13.4	60.3	3.3
Average	417	5.2	16.6	70.0	7.3

TABLE X

THE AVERAGE DAILY CALORIC INTAKE AND WEEKLY WEIGHT GAIN OF 10 MEAL-EATING ADULT MALE RATS FED DIET I

Date	Average Weight (g)	Average Weight Gain (g)	Average Food Intake (g)	Average Food Intake (kcal)	Food Efficiency (Per cent)
January					
17	331		14.1	63.4	
24	334	4	14.2	63.9	6.2
31	342	8	14.7	66.2	12.1
February					
8	348	6	12.8	57.6	10.4
14	349	1	13.6	61.2	1.6
21	359	10	14.0	63.0	15.6
28	359#		13.0#	58.5#	
March					
8	367	8	13.1	60.9	13.1
14	372	5	13.7	61.6	8.1
21	375	3	13.0	58.5	5.1
28	368#	-7#	11.2#	50.4#	
April					
4	372	4	12.1	54.4	7.4
11	375	3	11.5	51.8	5.8
18	379	4	11.8	53.1	7.5
Average	359	5.1	14.4	65.0	8.4

#Eliminated from statistical analysis

TABLE XI

THE AVERAGE DAILY CALORIC INTAKE AND WEEKLY WEIGHT GAIN OF 10 ADULT MALE RATS FED DIET II AD LIBITUM

Date	Average Weight (g)	Average Weight Gain (g)	Average Food Intake (g)	Average Food Intake (kcal)	Food Efficiency (Per cent)
January					
17	379		11.6	59.4	
24	390	11	13.6	69.6	15.8
31	395	5	10.7	54.8	9.1
February					
8	400	5	11.4	58.6	8.5
14	407	7	13.6	70.0	10.0
21	414	7	9.8	50.4	13.9
28	420	6	13.8	70.9	8.5
March					
8	427	7	13.6	69.9	10.0
14	433	6	10.9	56.0	10.7
21	437	4	12.1	62.2	6.4
28	443	6	12.1	62.2	9.6
April					
4	447	4	10.8	55.5	7.2
11	450	3	12.6	64.8	4.6
18	454	4	13.0	66.8	6.0
Average	421	5.1	12.0	62.2	9.2

TABLE XII

THE AVERAGE DAILY CALORIC INTAKE AND WEEKLY WEIGHT GAIN OF 10 MEAL-EATING ADULT MALE RATS FED DIET II

Date	Average Weight (g)	Average Weight Gain (g)	Average Food Intake (g)	Average Food Intake (kcal)	Food Efficiency (Per cent)
January					
17	337		11.2	57.6	
24	343	6	13.6	69.9	8.6
31	352	9	9.4	48.3	18.6
February					
8	364	12	10.4	53.4	22.5
14	371	7	11.9	61.2	11.4
21	378	6	13.5	69.4	8.6
28	379#	1#	11.7#	60.1#	
March					
8	388	9	11.1	57.0	15.8
14	391	3	9.5	48.8	6.1
21	396	5	9.9	50.9	9.8
28	393#	-3#	8.2#	42.1#	
April					
4	397	4	8.8	45.2	8.8
11	404	7	10.8	55.5	12.6
18	405	1	10.9	56.0	1.8
Average	377	5.3	10.9	56.1	12.0

#Eliminated from statistical analysis

TABLE XIII

THE AVERAGE DAILY CALORIC INTAKE AND WEEKLY WEIGHT GAIN OF 10 ADULT MALE RATS FED DIET III AD LIBITUM

Date	Average Weight (g)	Average Weight Gain (g)	Average Food Intake (g)	Average Food Intake (kcal)	Food Efficiency (Per cent
January					
17	363				
24	369	6			
31	378	9	20.1	86.5	10.4
February					
8	372	-6	19.2	82.6	7.3
14	383	11	20.8	89.5	12.3
21	388	5	19.0	81.8	6.1
28	390	2	19.1	82.2	2.4
March					
8	399	9	16.3	70.2	12.8
14	403	4	16.4	70.6	5.7
21	406	3	15.9	68.5	4.4
28					
April					
4	411	5	15.3	65.9	7.6
11	414	3	15.6	67.1	4.5
18	413	-1	17.1	73.6	-1.4
Average	391	4.2	17.1	76.2	6.8

2) Illness at any time during the study.

3) Sudden unexplained weight losses.

4) Large amounts of food waste.

5) Change in temperament.

Experimental Diet I (20 rats).--After eliminating all but 20 rats (10 nibblers and 10 meal-eaters), for various reasons mentioned previously, the weight gains and food consumption were charted again. The percentage food efficiency for the nibbling rats (Table XIV) was 7.3 ± 3.2 (mean for 10 rats ± S.E.). The percentage food efficiency for the meal-eaters was 8.4 ± 1.2 (mean for 10 rats ± S.E.). Again, the mean difference was not significant but the meal-fed group did show a slightly higher food efficiency. This indicated a possibility of a slightly better utilization of the food for the meal-eaters than for the nibblers. The mean weight gains for the two groups were almost identical but the mean caloric intake was five kilocalories per day higher for the nibblers.

Experimental Diet II (20 rats).--After eliminating all but 20 rats (10 nibblers and 10 meal-eaters), the weight gain and food consumption were compared. The percentage food efficiency for the nibblers was 9.2 ± 0.8 (mean for 10 rats ± S.E.). The percentage food efficiency for the meal-

TABLE XIV

SUMMARY OF MEAN WEIGHT GAINS, CALORIC INTAKE, AND PERCENTAGE FOOD EFFICIENCY OF 50 RATS SELECTED FOR STATISTICAL ANALYSIS

Diets	Mean Weight Gain (g)	Mean Food Intake (kcal)	Per cent Food Efficiency (Mean ± S.E.)
Diet I			
Nibblers (N=10)	5.2	70.0	7.3 ± 3.2
Meal-eaters (N=10)	5.1	65.0	8.4 ± 1.2
Diet II			
Nibblers (N=10)	5.1	62.2	9.2 ± 0.2
Meal-eaters (N=10)	5.3	56.1	12.0 ± 1.4
Diet III			
Nibblers (N=10)	4.2	76.2	6.8 ± 1.5

eaters was 12.0 ± 1.4 (mean for 10 rats ± S.E.). According to the Student "t" this difference was significant ($P<0.05$), indicating that the meal-eaters were significantly more efficient in utilizing their food although the nibbling rats weighed more at the end of the 18 weeks.

Diet III (10 rats).--In tabulating data on the 10 rats eating Purina chow, the percentage food efficiency was 6.8 ± 1.5. The average weight gain, the daily caloric intake, and percentage food efficiency were nearly the same for the 10 rats as for the 16 rats fed Diet III.

Adiposity

Nine animals died during the course of the study, two of these at the very beginning. The data for the final weights of each rat at the time of sacrifice were recorded. The final average weights of each group were tabulated for the 89 surviving animals at the time of sacrifice. The results are shown as follows:

Series	Number of Rats	Diet	Regimen		Final Average Weight (g)
			Nibblers	Meal-Eaters	
300	19	I	X		452
100	20	I		X	380
400	17	II	X		471
200	17	II		X	390
500	16	III	X		424

The rats fed Diet III were three weeks older than the other rats at the time they were sacrificed.

The rats exhibiting the most adiposity were the heaviest group of rats. The rats consuming Diet II, the moderately high fat diet, gained the most weight. Assuming that adiposity appeared in all the rats that weighed 450 grams, or more, the majority of the nibbling rats consuming Diets I and II exhibited adiposity. However, several of the meal-eaters appeared to have much more fatty tissue than their weights indicated. The nibbling rats consuming Diet III did not have the fatty tissue apparent in the other groups. This group was sacrificed approximately three weeks later than the other groups, and during this time the average weight gain was 11 grams.

METABOLIC STUDIES

The type of diet and the frequency of feeding has considerable influence on the enzymatic profile of most tissues. In the hexosemonophosphate oxidative pathway, the enzymes G-6-PDH and 6-PGDH are affected by changes in caloric influx

as well as by dietary composition. The diets used in this study had the following composition:

Diet	Per cent (weight/weight)		
	Fat	Protein	Carbohydrate
I	9.6	27.0	59.4
II	19.4	22.0	54.6
III	4.0	23.0	50.0

Diet III, a commercially available laboratory feed recommended for optimum maintenance of rats and certain other laboratory animals, was used as the control diet. It is approximately 4.0 per cent fat by weight. This percentage is considered adequate for the rat but low for the human population. The carbohydrate present was of high molecular weight and, therefore, slowly digested and slowly absorbed

Diet I, a commercially prepared synthetic mixture, was approximately 10 per cent fat by weight. Although this percentage is twice as high as that recommended for the rat, it is considered "acceptable" for the human population. Of the total fat present, approximately 25 per cent was corn oil and 75 per cent a commercially hydrogenated product. Of the carbohydrate present, approximately 87 per cent was of high molecular weight corn starch, 9.0 per cent were disaccharides, and 4.0 per cent oligosaccharides. In this diet fat contributed approximately one-half the kilocalories supplied by

the fat in Diet II, and is referred to as a moderately low fat diet in this study.

Diet II contained 20 per cent fat by weight. This percentage is four times that recommended for the rat and is near the percentage most frequently consumed by the Western world. Of the total fat present, approximately 17 per cent was corn oil and 83 per cent a commercially available hydrogenated fat. Of the carbohydrate present, 89 per cent was in the form of high molecular weight polysaccharides and 11 per cent was composed of di- and oligosaccharides. In this study, Diet II is referred to as a moderately high fat diet.

The results for the data analyses giving the effect of diet and dietary regimen on the hexosemonophosphate oxidative enzymes of adult rat liver are found in Table XV. The results for the data analyses giving the effect of diet and dietary regimen on the hexosemonophosphate oxidative enzymes of adult rat adipose tissue are given in Table XVI.

Glucose-6-Phosphate Dehydrogenase

The data in Tables XVII, XVIII, and XIX show the effects of diet and dietary regimen on G-6-PDH activities of the livers of adult male rats. A summary of the data, including the standard errors of the mean for each group, is given in Figure 10.

TABLE XV

EFFECT OF DIET AND DIETARY REGIMEN ON THE HEXOSEMONOPHOSPHATE OXIDATIVE ENZYMES OF ADULT RAT LIVER

Diet and Dietary Regimen		Glucose-6-Phosphate Dehydrogenase		6-Phosphogluconate Dehydrogenase	
		Mean ± S.E. (μM/hr/g)	Significance of Differences	Mean ± S.E. (μM/hr/g)	Significance of Differences
I	Meal-eaters versus Nibblers	382 ± 48 869 ± 70	P<0.05	1306 ± 106 687 ± 80	P<0.001
II	Meal-eaters versus Nibblers	1431 ± 105 1903 ± 173	P<0.001	949 ± 95 813 ± 82	N.S.
Meal-eaters	Diet I versus Diet II		P<0.001		P<0.02
Nib-blers	Diet I versus Diet II		P<0.001		N.S.
Nib-blers	Diet I versus Diet III	1664 ± 362	P<0.02	1669 ± 206	P<0.001
Nib-blers	Diet II versus Diet III		N.S.		P<0.001

TABLE XVI

EFFECT OF DIET AND DIETARY REGIMEN ON THE HEXOSEMONOPHOSPHATE OXIDATIVE ENZYMES OF ADULT RAT ADIPOSE TISSUE

Diet and Dietary Regimen		Glucose-6-Phosphate Dehydrogenase		6-Phosphogluconate Dehydrogenase	
		Mean ± S.E. (μM/hr/g)	Significance of Differences	Mean ± S.E. (μM/hr/g)	Significance of Differences
I	Meal-eaters versus Nibblers	151 ± 39 38 ± 1	P<0.02	108 ± 10 102 ± 7	N.S.
II	Meal-eaters versus Nibblers	261 ± 40 225 ± 43	N.S.	140 ± 15 142 ± 15	N.S.
Meal-eaters	Diet I versus Diet II		P<0.05		P<0.05
Nib-blers	Diet I versus Diet II		P<0.001		P<0.02
Nib-blers	Diet I versus Diet III	225 ± 35	P<0.001	118 ± 78	N.S.
Nib-blers	Diet II versus Diet III		N.S.		N.S.

TABLE XVII

GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY

IN LIVERS OF RATS FED DIET I

Rat Number	Nibblers (μM/hr/g)	Rat Number	Meal-Fed (μM/hr/g)
301	928	101	174
302	638	103	565
303	798	104	370
304	1410	105	130
305	580	106	406
306	1060	107	189
307	725	108	855
308	914	109	232
309	784	110	435
311	1072	111	318
312	956	112	262
313	769	113	103
314	754	114	494
316	666	115	435
319	1118	116	262
320	725	117	276
		118	507
		119	595
		120	638
Mean ± S.E. = 869 ± 70		Mean ± S.E. = 382 ± 48	

Significance of differences between means $P<0.05$

TABLE XVIII

GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY

IN LIVERS OF RATS FED DIET II

Rat Number	Nibblers (μM/hr/g)	Rat Number	Meal-Fed (μM/hr/g)
401	1960	201	1030
402	319	202	464
403	2335	203	1595
404	1015	204	638
405	1640	205	1203
406	1970	206	1232
407	2440	208	2020
408	2540	210	1100
409	2310	211	1840
410	900	212	1540
413	1380	213	1568
415	1435	214	1362
416	2410	215	1510
417	2975	216	2070
418	2235	217	1595
419	2700	218	1350
420	1782	219	1595
		220	2040
Mean ± S.E. = 1903 ± 173		Mean ± S.E. = 1431 ± 105	

Significance of differences between means $P<0.001$

TABLE XIX

GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY IN LIVERS AND ADIPOSE TISSUES OF RATS FED AD LIBITUM

Rat Number	Liver (μM/hr/g)	Adipose Tissue (μM/hr/g)
501	1220	398
502	2420	158
503		162
504	3420	204
505	812	224
506	2520	178
507	218	26
508	595	455
509	3940	272
510	609	351
511	1855	200
512	203	145
514	2120	154
Mean ± S.E. =	1664 ± 362	225 ± 35

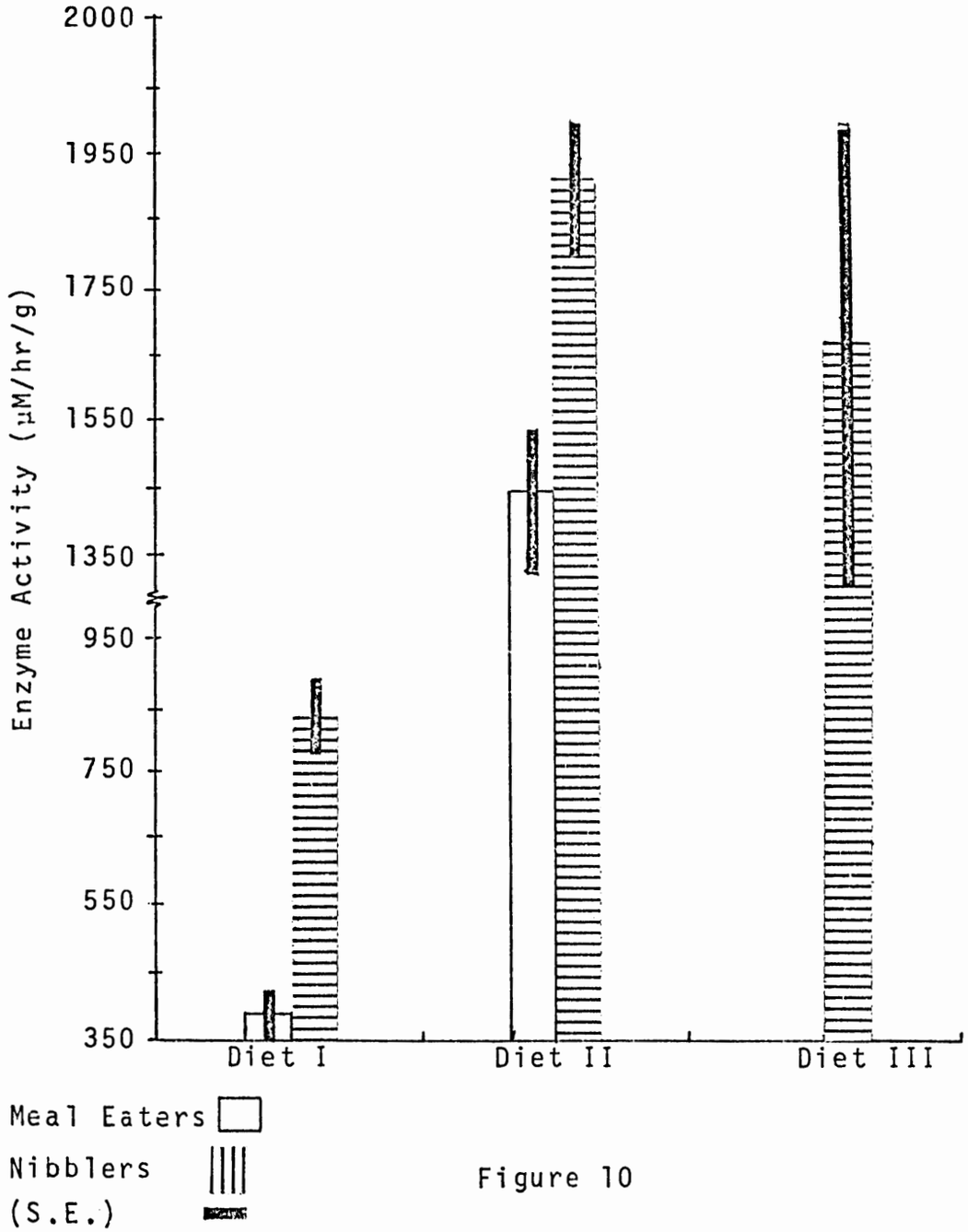

Figure 10

Effect of Diet and Dietary Regimen on G-6-PDH Activity in Rat Liver

A comparison of the effect of Diets I and II on the activity of G-6-PDH in the liver indicate that the consumption of the moderately high fat diet resulted in a significantly higher enzyme level than did the moderately low fat diet. This difference was statistically significant not only for the meal-eaters ($P<0.001$) but for the nibblers ($P<0.001$) as well. The difference in Diets I and III for the nibblers was significant also ($P<0.02$). The wide variability in the data obtained from analyses of the rats in the Diet III group made the difference between the mean G-6-PDH levels of animals on Diets II and III statistically insignificant.

The difference in G-6-PDH activity in the livers of meal-eaters and nibblers eating Diet I was significant at the 0.05 level. The activity was much higher for the nibblers. The difference in liver G-6-PDH activity between the meal-eaters and nibblers eating Diet II was highly significant ($P<0.001$).

On the basis of the information given above, the G-6-PDH activity in the liver was higher for the rats eating ad libitum than for the meal-eaters. In comparing the experimental diets, the diet containing 20 per cent fat by weight (Diet II) had higher G-6-PDH activity than the diet containing 10 per cent fat by weight (Diet I). The difference

cannot be explained on the basis of percentage of dietary fat in view of the fact that the liver G-6-PDH of rats fed the 4.0 per cent fat was not significantly different from that of animals fed the moderately high fat diet (Diet II).

The nibbling rats consuming Diet II had significantly higher G-6-PDH activity in the liver than the nibbling rats consuming Diet I. Also, the meal-eating rats consuming Diet II had significantly higher G-6-PDH activity than the meal-eaters consuming Diet I. The nibbling rats consuming Diet III exhibited nearly as high a G-6-PDH activity as the rats on the moderately high fat diet (Diet II).

The data presented in Tables XIX, XX, and XXI show the G-6-PDH activity in the adipose tissue of adult male rats on the three different diets fed either ad libitum or only one two-hour feeding each day. A summary of these data is given in Figure 11.

The activity of G-6-PDH in adipose tissue was higher in the rats eating Diet II than in the rats eating Diet I. This difference was highly significant in comparing the meal-eaters ($P<0.05$) as well as comparing the nibblers ($P<0.001$). The difference between the Diet I nibblers and the Diet III nibblers was highly significant also ($P<0.001$). However, there was little or no difference between the liver G-6-PDH of Diet II nibblers and the Diet III nibblers.

TABLE XX

GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY

IN ADIPOSE TISSUE OF RATS FED DIET I

Rat Number	Nibblers (μM/hr/g)	Rat Number	Meal-Fed (μM/hr/g)
301	40	101	58
302	29	103	81
303	48	104	46
304	23	105	502
305	86	106	17
306	32	107	128
307	57	108	235
308	42	110	151
309	25	111	90
311	40	112	29
312	17	113	235
313	42	114	214
314	23	115	200
316	29	116	258
319	34	117	84
		119	90
Mean ± S.E. = 38 ± 4		Mean ± S.E. = 151 ± 39	

Significance of differences between means $P<0.02$

TABLE XXI

GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY

IN ADIPOSE TISSUE OF RATS FED DIET II

Rat Number	Nibblers (μM/hr/g)	Rat Number	Meal-Fed (μM/hr/g)
401	113	201	139
402	87	202	104
403	365	203	386
404	125	204	352
405	296	205	246
406	606	206	372
407	197	208	119
408	151	210	309
409	73	211	122
410	3	212	368
413	113	213	81
415	470	214	119
416	324	215	336
417	342	216	650
418	15	217	160
419	458	218	55
420	81	219	481
		220	393
Mean ± S.E. = 225 ± 43		Mean ± S.E. = 261 ± 40	

Significance of differences between means N.S.

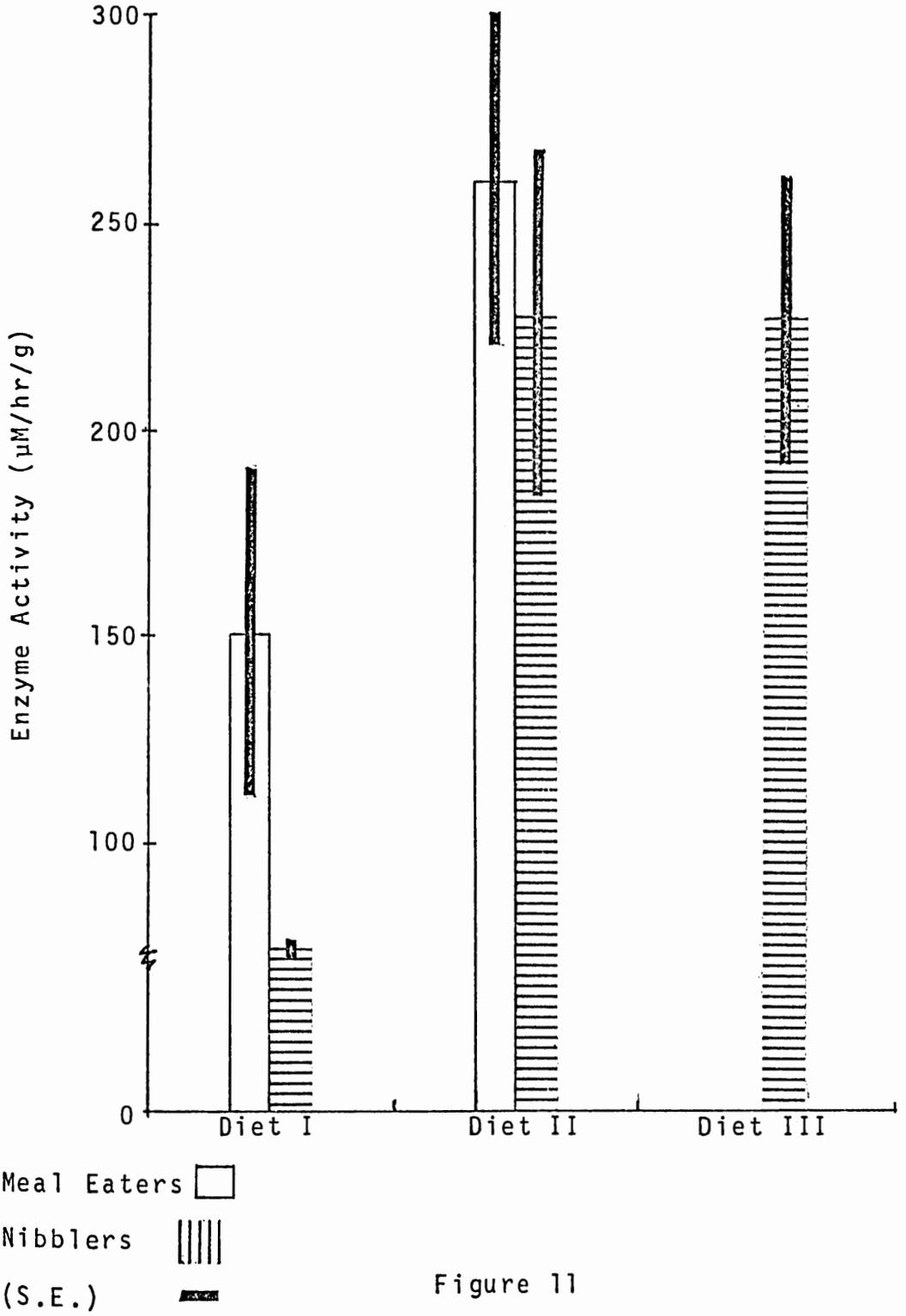

Figure 11

Effect of Diet and Dietary Regimen on G-6-PDH Activity in Adipose Tissue of Rats

The difference between the G-6-PDH activity in adipose tissue of the meal-eaters and nibblers eating Diet I was highly significant ($P<0.02$). The activity was better than three times higher for the meal-eaters in this study. With the rats consuming the Diet II, the difference in the G-6-PDH activity of the meal-eaters was higher than that of the nibblers, but the difference was not statistically significant.

On the basis of this information the rats consuming the moderately high fat diet (Diet II) exhibited higher G-6-PDH activity in adipose tissue than the rats consuming the diet with the lower fat content (Diet I). The differences between the meal-eaters of Diets I and II and the nibblers of Diet I and II were highly significant. The meal-eaters exhibited higher G-6-PDH activity than the nibblers in both diets, but the differences were only significant in the lower fat diet (Diet I). These differences existed in the liver, but the nibblers exhibited higher activity than the meal-eaters.

The nibbling rats eating Diet III exhibited as high a G-6-PDH activity as the nibbling rats eating the moderately high fat diet. Yet, the G-6-PDH was much higher for the rats fed Diet III than for the nibblers eating Diet I.

6-Phosphogluconate Dehydrogenase

The data presented in Tables XXII, XXIII, and XXIV show the 6-PGDH activity in the liver of adult male rats on the three diets fed either ad libitum or only one two-hour feeding each day. A summary of the findings is shown in Figure 12.

The 6-PGDH activity in the liver was significantly higher for the meal-eating rats consuming Diet I than the meal-eaters consuming Diet II ($P<0.02$). The difference between the nibbling rats on the two different diets was not significant. The 6-PGDH activity for the rats consuming Diet III was much higher than that found in the animals fed the synthetic diets. The difference between Diet I and Diet III was significant at the 0.001 level. The same degree of significance was found between Diets II and III. The 6-PGDH activity of the rats fed Diet III was significantly higher than that of the rats on any of the other diets or feeding regimens used in this study.

The difference between the 6-PGDH activity in liver of the meal-eaters and nibblers eating Diet I was highly significant ($P<0.001$). The activity nearly doubled. In comparing the meal-eating rats and nibbling rats consuming Diet II, the 6-PGDH activity was not significantly different.

TABLE XXII

6-PHOSPHOGLUCONATE DEHYDROGENASE ACTIVITY

IN LIVERS OF RATS FED DIET I

Rat Number	Nibblers (μM/hr/g)	Rat Number	Meal-Fed (μM/hr/g)
301	638	101	610
302	478	103	1190
303	770	104	1308
304	1045	105	1729
305	319	106	930
306	655	107	1305
307	855	108	1890
308	478	109	551
309	334	110	1408
311	638	111	957
312	522	112	986
313	1740	113	1361
314	610	114	1625
316	710	115	855
319	435	116	1015
320	770	117	1598
		118	1890
		119	2080
		120	1520
Mean ± S.E. = 687 ± 80		Mean ± S.E. = 1306 ± 106	

Significance of differences between means $P<0.001$

TABLE XXIII

6-PHOSPHOGLUCONATE DEHYDROGENASE ACTIVITY

IN LIVERS OF RATS FED DIET II

Rat Number	Nibblers (μM/hr/g)	Rat Number	Meal-Fed (μM/hr/g)
401	740	201	565
402	929	202	768
403	870	203	696
404	580	204	1030
405	1013	205	870
406	1013	206	841
407	1303	208	595
408	1102	210	1350
409	624	211	1670
410	406	212	1202
413	334	213	900
415	465	214	450
416	653	215	450
417	1630	216	1450
418	944	217	870
419	666	218	450
420	552	219	1640
		220	1302
Mean ± S.E. = 813 ± 82		Mean ± S.E. = 949 ± 95	

Significance of differences between means N.S.

TABLE XXIV

6-PHOSPHOGLUCONATE DEHYDROGENASE ACTIVITY IN LIVERS AND ADIPOSE TISSUES OF RATS FED AD LIBITUM

Rat Number	Liver (μM/hr/g)	Adipose Tissue (μM/hr/g)
501	1522	157
502	1840	113
503		93
504	1043	139
505	1598	104
506	1335	151
507	695	75
508	1450	148
509	3130	122
510	1174	81
511	1900	90
512	2390	145
514	2080	116
Mean ± S.E. =	1669 ± 206	118 ± 78

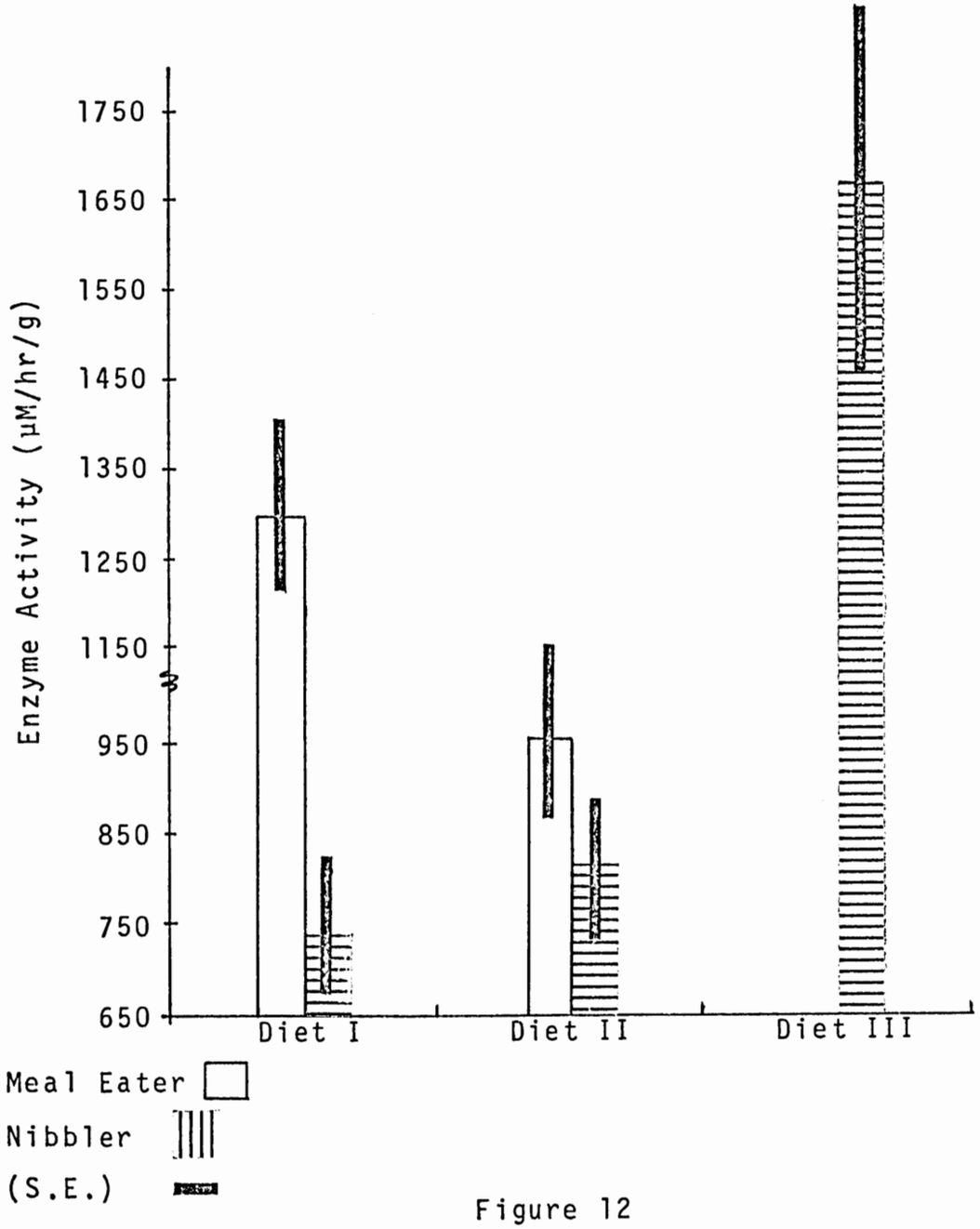

Figure 12

Effect of Diet and Dietary Regimen on 6-PGDH Activity in Rat Liver

As stated above, the meal-eaters consuming the experimental diets exhibited a higher 6-PGDH activity than the nibblers. Also, the rats eating Diet III exhibited significantly higher 6-PGDH activity than the rats on the synthetic diets. If increased carbohydrate content in the diet and meal-feeding were the causes for increased 6-PGDH activity in this study, these results paralleled other studies mentioned in the review of literature.

The data presented in Tables XXIV, XXV, and XXVI show the 6-PGDH activity in adipose tissue of adult male rats on the three diets which were fed either ad libitum or as a single two-hour feeding each day. A summary of this data is given in Figure 13.

The 6-PGDH activity in adipose tissue was significantly higher for the meal-eating rats consuming Diet II than the meal-eaters consuming Diet I ($P<0.05$). The difference between the nibbling rats was significant in favor of the rats consuming Diet II ($P<0.02$). The 6-PGDH activity in adipose tissue of the nibbling rats consuming the Diet III revealed no significant difference in comparing with Diet I or Diet II. Again, there was a wide variability of responses among the animals fed Diet III. There was little or no difference in 6-PGDH activity between the nibblers and the meal-eaters consuming either diet.

TABLE XXV

6-PHOSPHOGLUCONATE DEHYDROGENASE ACTIVITY

IN ADIPOSE TISSUE OF RATS FED DIET I

Rat Number	Nibblers (μM/hr/g)	Rat Number	Meal-Fed (μM/hr/g)
301	119	101	84
302	46	103	81
303	133	104	93
304	119	105	154
305	93	106	70
306	93	107	90
307	99	108	145
308	142	109	99
309	61	110	113
311	73	111	84
312	75	112	46
313	116	113	174
314	110	114	197
316	72	115	160
317	145	116	101
319	119	117	72
320	116	118	99
		119	90
Mean ± S.E. = 102 ± 7		Mean ± S.E. = 108 ± 10	

Significance of differences between means N.S.

TABLE XXVI

6-PHOSPHOGLUCONATE DEHYDROGENASE ACTIVITY

IN ADIPOSE TISSUE OF RATS FED DIET II

Rat Number	Nibblers (μM/hr/g)	Rat Number	Meal-Fed (μM/hr/g)
401	90	201	145
402	58	202	75
403	319	203	139
404	145	204	171
405	168	205	139
406	203	206	168
407	110	208	93
408	139	210	133
409	116	211	99
410	90	212	162
413	129	213	64
415	99	214	145
416	128	215	145
417	139	216	267
418	258	217	81
419	116	218	96
420	110	219	203
		220	197
Mean ± S.E. = 142 ± 15		Mean ± S.E. = 140 ± 15	

Significance of differences between means N.S.

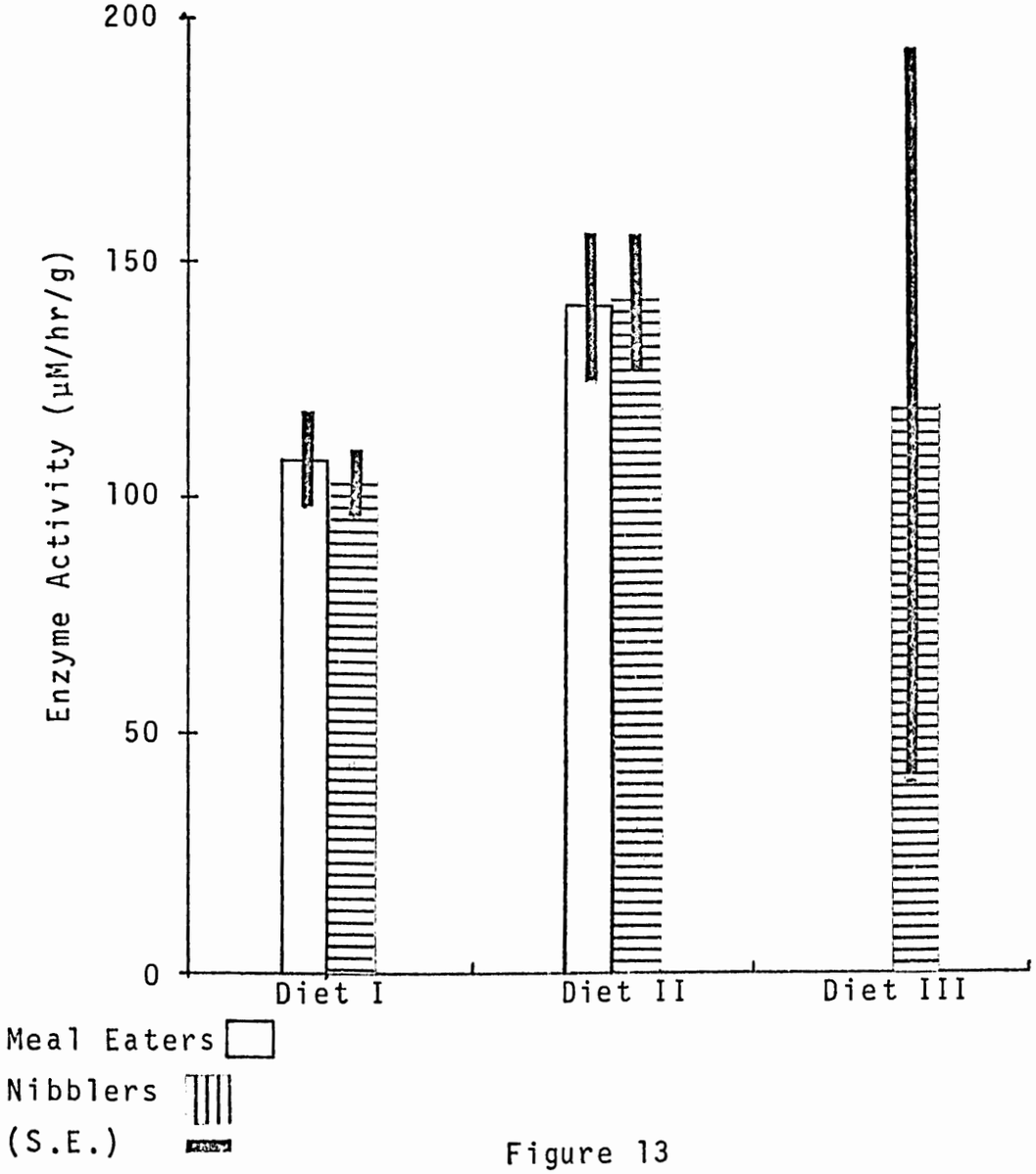

Figure 13

Effect of Diet and Dietary Regimen on 6-PGDH Activity in Adipose Tissue of Rats

When analyzing the data on 6-PGDH activity in adipose tissue, the results did not conform in all instances with other papers cited in the review of literature. In this study the rats that consumed the diet which contained the highest percentage of fat showed the highest 6-PGDH activity which is in disagreement with other studies mentioned previously. The fact that the difference in the activity between the nibblers and meal-eaters was not significant did conform with other statistical analyses.

The differences in some of the results of this study as compared to studies by Leveille (36) and others (9, 12, 34, 38, 69) can be accounted for by the treatment of the rats before sacrifice. In this study the meal-eaters had not eaten since the day before and the nibblers had little, if any, food left from the ration given the previous day. Other studies treated the animals in the following manner:

1) Leveille (36) sacrificed the meal-fed animals immediately after feeding; the nibblers had access to food up until time of sacrifice.

2) Tepperman and Tepperman (69) used a high carbohydrate refeeding program after a 48-hour fast and sacrificed the rats 12 to 48 hours after feeding. These investigators stated dehydrogenase activity in the liver was increased beginning about the 12th hour and became 10 to 20 times greater at the end of 48 hours.

3) Emerson and others (12) demonstrated that the rate of lipogenesis varied in relation to the time at which food was previously ingested. The work demonstrates the importance in designing lipogenic experiments, of considering not only the quantity of food ingested but also the control of the time at which ingestion occurred.

4) In another experiment, Leveille (38) fasted rats from 10:00 a.m. on the day preceding the experiment until 8:00 a.m. on the day of the experiment. At this time both meal-fed and nibbling rats were given 10 grams of food which all animals rapidly consumed. Four rats from each group were killed three, six, and nine hours after the start of the meal period.

5) Leveille and Hanson (34) fasted all their animals for 22 hours and then re-fed them for two hours prior to sacrifice.

6) Cohn (9) force-fed in amounts to pair-gain with the controls. The rats were killed four to five hours after being force-fed three grams of glucose by stomach tube. The liver and epididymal pad were homogenized, contrifuged, and supernatants assayed for dehydrogenase activity after an 18-hour dialysis, essentially as described by Glock and McLean (17).

As indicated by this study and other experiments reviewed, there are variations in carbohydrate metabolism in the whole animal following fasting, alteration in the composition of the diet and changes in dietary regimens. Fitch and Chaikoff (14) concluded that the level of enzyme activity is related to the activity of the metabolic pathway in which it participates and that any changes in enzyme activity

reflects alterations in the "traffic" over the "parent" pathway. These alterations in enzyme activity occur with:

1) Changes in diet
2) Refeeding after a 48-hour fast.
3) Meal-feeding.

Also, recent publications reviewed in this dissertation have indicated that a diet containing a high percentage of fat and little carbohydrate has been suggested for reducing the weight of obese patients. Other reports have indicated that this more obvious weight reduction only occurs until a "steady state" has been reached, eight to 10 days, and then weight reduction is a matter of caloric intake rather than of dietary composition or dietary regimens. Olesen and Quaade (55) and others (47, 58) have produced nutritional obesity by means of a high fat diet. Results of this study indicate there is a larger weight gain and more adiposity among rats eating a moderately high fat diet ad libitum than among rats meal-eating the same diet or rats eating a moderately low fat diet either consumed ad libitum or by meal-eating. Over-nutrition may be involved. In an intact complex multicellular organism it is extremely difficult to attribute a physiological change to one particular system. There are other recognized metabolic systems, and probably some unidentified,that are involved with deposition of

neutral fat in body tissues. Some of the findings obtained in this study concerning the effects of a moderately high fat diet may be explained as follows:

1) More glyceride-glycerol formation occurred by the reversal of glycolysis (Figure 14). In this system there is increased glycerol formation and eventual triglyceride synthesis from dihydroxyacetone phosphate.

2) With increased release of citrate from the mitochondria to the cytoplasm, the reversal mechanism involving the citrate cleavage enzyme could have been increased forming excessive quantities of acetyl CoA and NADPH via the malic enzyme system (Figure 15).

3) Inside the mitochondria there may have been more catabolism of fatty acids with increased release of citrate and acetate to the cytoplasm bringing about increased formation of acetyl CoA (Figure 16).

4) Increased formation of acetyl CoA brings about increased condensation of malonyl CoA.

5) Increased condensation of malonyl CoA and acetyl CoA produces increased butyryl CoA.

6) With the formation of butyryl CoA, the amount of stearyl CoA is increased in the nitochondria and increased palmityl CoA outside the mitochondria.

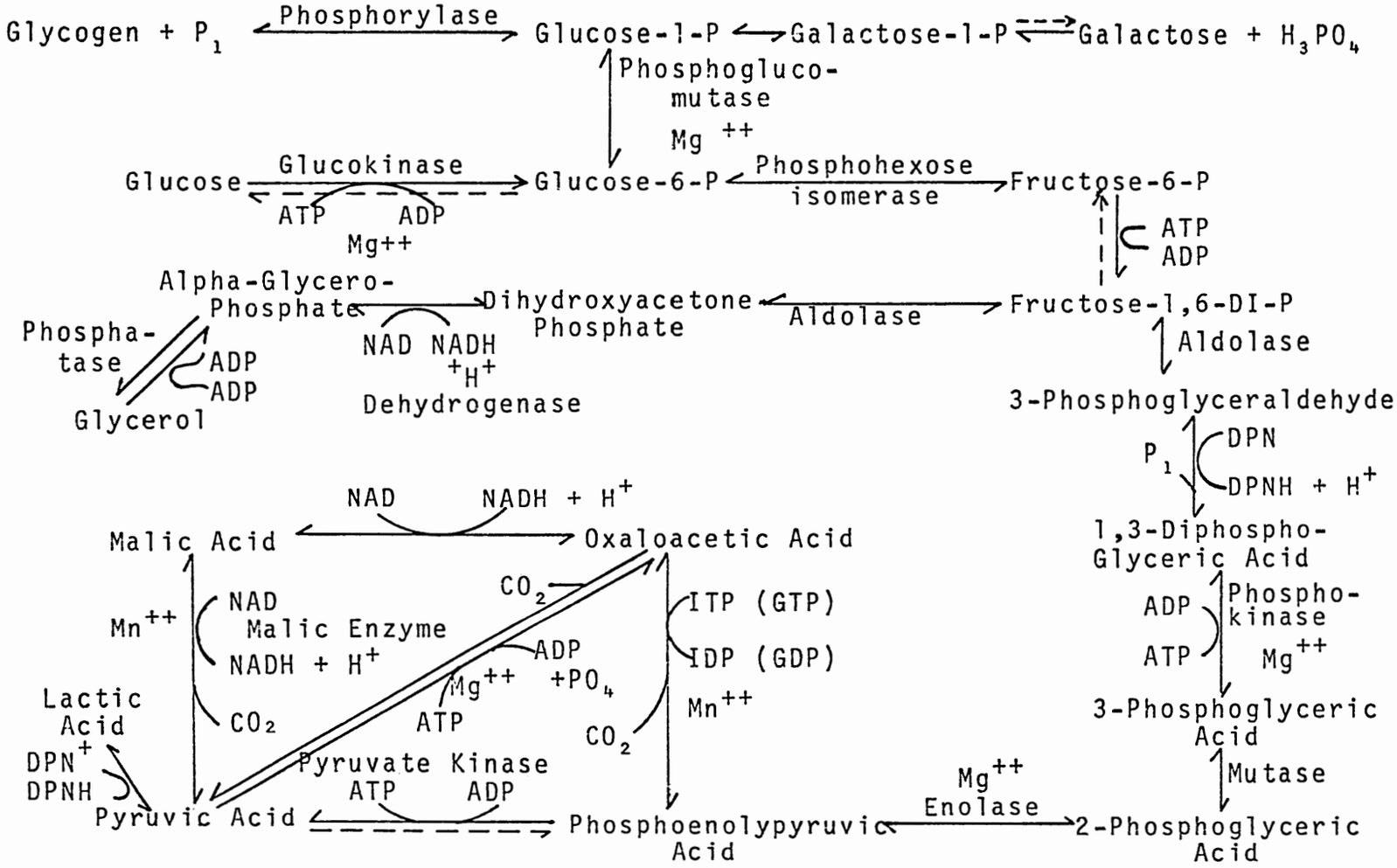

Figure 14

GLYCOLYSIS and REVERSAL OF GLYCOLYSIS*

(*An Adaptation of a Chart Found in West and Others (76))

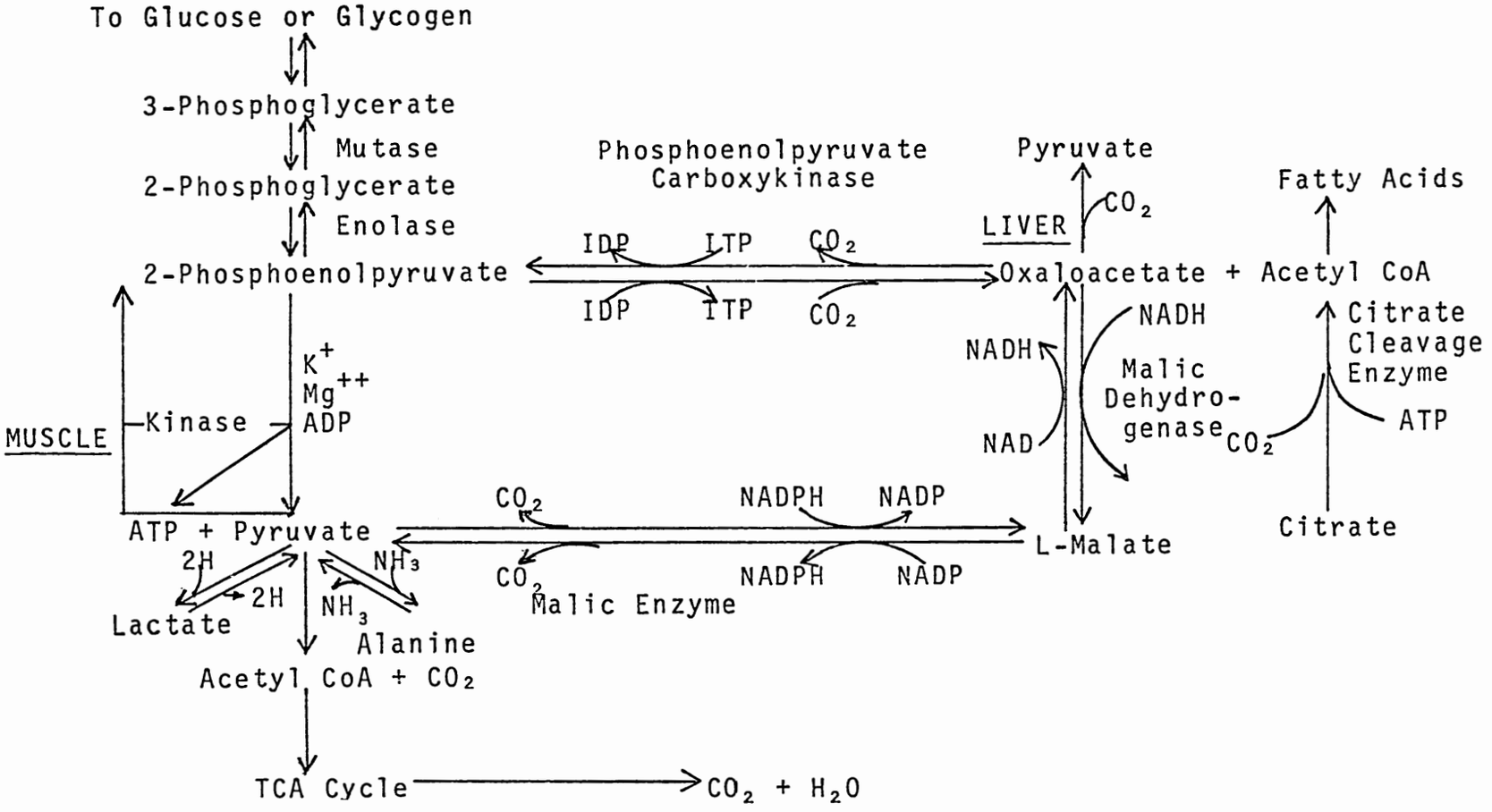

Figure 15

PYRUVATE METABOLISM*

(*An Adaptation of a Chart Found in West and Others (76))

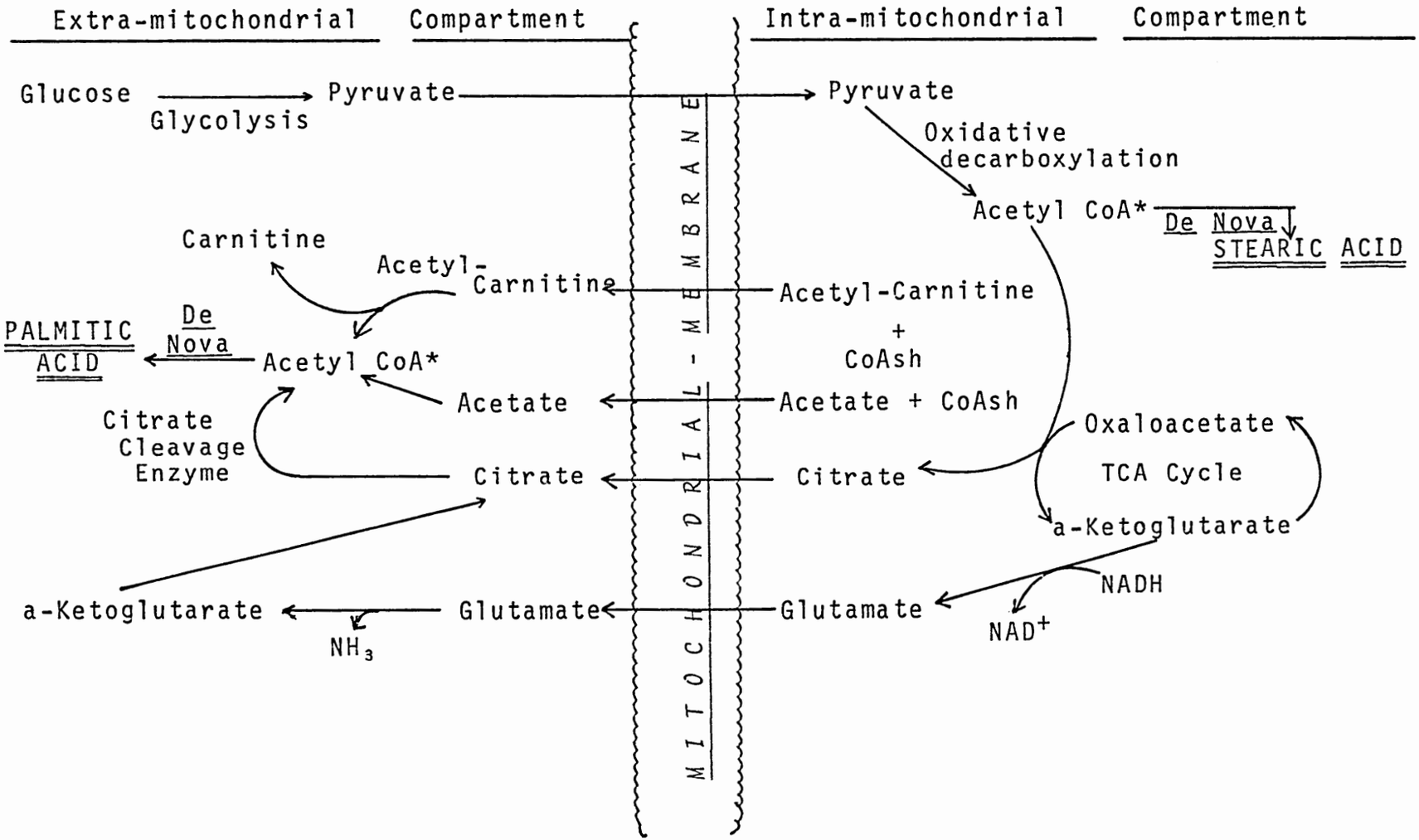

Figure 16

De Novo Synthesis of Fatty Acids

CHAPTER IV

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

SUMMARY

Ninety-six adult male Sprague-Dawley rats were placed on various experimental diets in order to determine the effects of dietary composition and different dietary regimens on the hexosemonophosphate oxidative dehydrogenase activities. The changes observed in the activities of these enzymes are believed to be related to increased lipogenesis.

The three diets used in this study were as follows:

1) Experimental Diet I--approximately 10 per cent by weight fat content (a moderately low fat diet).

2) Experimental Diet II--approximately 20 per cent by weight fat content (a moderately high fat diet).

3) Diet III (Purina Laboratory Chow)--approximately 4.0 per cent by weight fat content.

All three diets contained some sucrose and the protein content in each diet was approximately the same. Half of the rats fed either Experimental Diets I or II were placed on one two-hour feeding per day, and the remainder of the rats

were fed ad libitum. For a period of 18 weeks weight gains and the daily food consumption were recorded. The rats were sacrificed for analyses at 9.5 to 10 months of age.

CONCLUSIONS

The following conclusions were drawn from data obtained in this study:

Food Efficiency Studies

Although the percentage food efficiency was slightly higher for the meal-eaters than the nibblers throughout the study, the differences were not significant except for the 10 selected meal eaters consuming the moderately high fat diet ($P<0.05$).

Adiposity

This parameter was not quantitatively evaluated; however, while dissecting the animals, the author observed a definite difference in the amount of fat surrounding the organs in the peritoneal cavity. The greatest amount of adiposity appeared to be in the nibbling rats consuming Diet II, the moderately high fat diet. This group was closely followed by the nibblers consuming Diet I, the moderately low fat diet. Although a few meal-eaters consuming Diet II appeared to have excessive adiposity in comparison to the

others in this group, the average weight gain did not indicate this.

Metabolic Studies

Glucose-6-Phosphate Dehydrogenase.--The mean G-6-PDH activity of livers taken from rats maintained on Diet II was considerably higher ($P<0.001$) than that taken from rats fed Diet I regardless of the feeding regimen. This did not appear to be due to the total fat content of the diets in view of the fact that the mean enzyme activity of livers from rats placed on Diet III, a low fat diet, more closely paralleled that of the animals on the moderately high fat diet, Diet II. In view of the wide range of values obtained in all analyses of tissues from animals fed Diet III, a commercial feed, it would be premature to draw any definite conclusions without further investigation.

The frequency of feeding had a pronounced effect on the G-6-PDH levels in the rat livers. In comparing the meal-eaters versus the nibblers, the Diet I nibblers had a two-fold increase over the Diet I meal-eaters ($P<0.05$) , and the Diet II nibblers had a 1.5-fold increase over the Diet II meal-eaters ($P<0.001$).

The difference in dietary composition did not have as great an effect on the G-6-PDH activity of adipose tissue as

found in the liver, but the relative change was the same in that there was higher activity in the tissues of rats fed Diet II than in those of rats fed Diet I. The adipose tissue revealed a greater difference among the nibblers ($P<0.001$) than among the meal-eaters ($P<0.05$) fed Diets I and II.

The G-6-PDH activity in adipose tissue was higher for the meal-eaters than for the nibblers, the opposite of the findings in the liver. In Diet I, the enzymatic activity of the meal-eaters was three times that of the nibblers ($P<0.02$) The differences between the meal-eaters and nibblers consuming Diet II was not significant. In referece to Diet III, the adipose tissue and the liver enzyme activity more nearly corresponded to the Diet II animals.

6-Phosphogluconate Dehydrogenase.--The nibblers eating Diets I and II showed no statistically significant difference in the 6-PGDH levels in the liver. However, the 6-PGDH activity of the meal-eaters consuming Diet I was significantly higher than the meal-eaters consuming Diet II ($P<0.02$).

There was a two-fold increase ($P<0.001$) in the hepatic 6-PGDH activity of the meal-eaters on Diet I as opposed to the ad libitum group. This is the reverse of the effect the meal-eating regimen had on the G-6-PDH activity in the same tissue.

The differences in the hepatic enzymatic activity of the Diet III nibblers as compared to Diet I and II nibblers was statistically significant in both instances ($P<0.001$). This was the only instance in which the data obtained from those rats on Diet III was significantly higher than the data from Diet II. Generally, the differences between Diets II and III were not significant. When comparing the meal-eaters, the 6-PGDH enzyme activity in adipose tissue was significantly higher for the Diet II rats than for the Diet I rats ($P<0.05$). The same differences were observed for the nibblers ($P<0.02$).

In comparing the meal-eaters versus the nibblers, the feeding frequency and the dietary composition had no effect on the 6-PGDH activities of adipose tissue.

Except for the 6-PGDH activity in the liver, the dehydrogenase activities for the rats eating the moderately high fat diet (Diet II) was higher than the dehydrogenase activities of the rats eating the moderately low fat diet (Diet I). The only instances where the meal-eaters exhibited significantly higher enzyme activity than the nibblers were:

1) G-6-PDH activity in adipose tissue of rat consuming Diet I ($P<0.02$).
2) 6-PGDH activity in the liver of rats consuming Diet I ($P<0.001$).

The author believes the differences found in this study compared to other studies cited, concerning high fat diets versus high carbohydrate diets, or meal-feeding versus nibbling are as follows:

1) The treatment of the feeding patterns before sacrifice.

2) The extremely high fat diets or the extremely high carbohydrate diets used in other studies cited were not used in this study.

According to the results of this study, the high fat diet, suggested as a reduction dietary regimen by some authors, may not be an acceptable procedure.

RECOMMENDATIONS

There are several modifications of procedure as well as additional analyses that would greatly reinforce this study. The following recommendations are suggested by the author:

1) Explore various feeding patterns prior to sacrifice.

2) Vary the dietary composition, particularly the various sources of complex and simple carbohydrates.

3) Explore the reasons for the variations in Diet III, and incorporate a meal-eating regimen with this group.

4) Employ a total tissue nitrogen standard for enzymatic analysis rather than wet weight of tissue.

5) Investigate carcass fat and carcass nitrogen.

A critical evaluation of the relationship between dietary factors and fat synthesis would be impossible without investigating other pathways involved in the synthesis of fatty acids, glycerophosphate, and NADPH. Although the HMP pathway is considered to be a very significant system to investigate when studying the alterations in metabolic pathways brought about by fat synthesis, the author recommends an investigation of other enzymatic systems implicated in adaptive changes in adipose tissue:

1) Malic enzyme.
2) Alpha-glycerophosphate dehydrogenase
3) Alpha-glycerokinase.
4) Citrate cleavage enzyme.
5) Crotonyl CoA reductase.

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