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Influence of feeding frequency on lipid metabolism in adult rats recovering from malnutrition

by

Robert Donald Reeves

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Nutrition

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1971

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INTRODUCTION

An area of investigation which has received much attention during the past few years has been the rate of food ingestion and its regulation of over-all body metabolism. Prior to this, studies related to pathways of carbohydrate, fat, and protein metabolism have focused on energy value and dietary composition as dominant factors in the genesis or accentuation of metabolic abnormalities. Studies of this type have assumed that, as long as food enters the gastrointestinal tract, regardless of whether the calories were consumed as small frequent feedings ("nibbling") or as full spaced meals ("meal-eating") the animal would handle the food in a similar manner. This assumption has been shown to be invalid. The rate of food ingestion appears to play a significant role in the regulation of over-all body metabolism. The consumption of full spaced meals as contrasted with small frequent feedings markedly alters some specific aspects of normal metabolism. The available data which led to the development of this concept have been derived mainly from studies with weanling or young adult animals. A striking dissimilarity may exist between the metabolic responses to limited feeding frequency observed in young animals compared to those observed in mature adult animals.

The study to be presented is part of a series of investigations in this laboratory designed to determine the

influence of kind and amount of dietary fat on lipid metabolism in serum, hepatic and epididymal tissues. The response of tissue lipids and indices of lipid metabolism to variations in dietary energy source and dietary protein have been studied previously in this laboratory (Lee, 1967; Noble, 1967; Stadler, 1969; McAtee, 1970; Mitchell, 1971). The experimental model developed and used for these studies was the mature adult rat in a state of recovery from chronic protein-calorie malnutrition.

The present study, using the previously developed experimental model, was designed to evaluate the influence of altered meal patterns on hepatic, epididymal, and serum lipids and certain lipogenic enzymes in hepatic and epididymal tissue. The possibility exists that states of elevated and reduced lipogenesis characteristic of meal eating and nibbling feeding patterns, respectively, will be modified under conditions of rapid synthesis of body fat, as is the case in the mature adult rat recovering from chronic malnutrition. We also sought an answer to the question whether the response to variations in the dietary energy source persists with time, since previous work in this laboratory (Noble, 1967; Stadler, 1969; Kopec, 1969) had demonstrated dramatic shifts in tissue lipid fatty acid patterns within short periods of refeeding.

REVIEW OF LITERATURE

Part A. Feeding Frequency

Body Composition

Early evidence demonstrating that feeding frequency alters body metabolism was obtained from respiratory quotient studies, performed on rats trained to eat their entire daily ration in a limited period of time. Tepperman *et al*. (1943) reported that rats with hypothalmic obesity showed a significantly higher RQ than intact controls, even when pair-fed *ad libitum* identical amounts of a laboratory chow consisting of 18% protein, 5% fat, and 68% carbohydrate. The only obvious difference between operated and control animals, when pair-feeding was used, was the rate of food ingestion. The operated, pair-fed rats appeared to be voraciously hungry and they consumed a 24-hour ration often within a 1-hour period. In comparison control animals nibbled on the served amount for nearly 24 hours. In an attempt to determine whether the elevated RQ observed in this type of rat was caused by the hypothalmic lesion or by some adaptive change in response to the very unusual eating pattern, unoperated rats were trained to mimic the food habits of pair-fed animals with hypothalmic lesions. These animals, which after a 2 week training period were able to consume the entire 24-hour food allotment within a

3-hour period each day, had RQ values similar to those in the hypothalmic animals. When the time period of feeding was further decreased to 1 hour per day, and the animals were placed on a low-fat high-carbohydrate diet, the RQ values were even higher in the trained group compared with the untrained rats. A mean RQ of 1.22 $\pm$ 0.01 was observed in the trained rats compared with 1.05 $\pm$ 0.01 for the untrained group. As in this case RQ values far in excess of 1.0 suggested rapid conversion of carbohydrate to fatty acids. The possibility that the eating pattern was the cause of the observed changes in respiratory quotient was in keeping with the results of Werthessen (1937), who six years earlier had reported elevated RQ values in rats fed only 1-5 hours per day.

In 1955 two papers on the metabolic effect of feeding frequency appeared independently from two different laboratories. Van Putten et al. (1955) published experiments based on Tepperman's (1943) work. The authors studied the influence of hypothalamic lessions and feeding regimens on carcass composition in the rat. Hyperphagic-hypothalamic rats were pair-fed with controls on which sham operations were performed. The hyperphagic rats consumed all their ration quickly while the controls nibbled their ration throughout the day. After six weeks the hyperphagic rats had a greater percentage of body fat than pair-fed controls

based on carcass analysis. The difference in fat content of the two groups diminished when they were fed in a similar manner, e.g. only 2-hours per day. At approximately the same time, Cohn *et al*. (1955) with an initial interest to determine the reason for the leanness of the adrenalectomized rat studied the effect of food administration on weight gains and body composition. Adrenalectomized rats were placed in two groups, one group being force-fed and the other group having free access to food. Control rats were similarly fed. The force-fed animals were given an amount of food by stomach tube that would allow them to gain ("pair-gain") the same weight as those eating *ad libitum*. After 3 weeks of feeding, all force-fed animals, adrenalectomized or intact, contained almost double the amount of body fat compared with nibbling counterparts which had access to food 24 hours per day (20 vs. 39 gm/rat). An increased fat deposition was observed in spite of a smaller caloric intake for the force-fed group.

Since caloric intakes were not identical between the *ad libitum* fed animals and the "pair-gained" experimental rats, Cohn and Joseph (1959a) further demonstrated in "pair-fed" (identical caloric intakes) rats an increased production of body fat as a result of force-feeding. Growing rats were either fed *ad libitum* or force fed (equal amounts) once daily a carbohydrate diet for 3-4 weeks. The force-fed animals

gained less weight (62 vs. 65 g) during the experimental period, but analyses of body composition showed that water content was lower (55.5 vs. 67.8%), fat content higher (23.6 vs. 7.8%), and protein content lower (17.7 vs. 22.4%) than for those rats fed ad libitum. Further studies (Cohn et al., 1957) have demonstrated that difference in body fat was not due to the presence or absence of adrenals, the sex of the animals, the handling of the animals, the dilation of the stomach in force feeding or the fecal fat excretion. These workers concluded from their studies that the force-fed rats retained more of the digested energy in the form of fat and utilize calories more efficiently than did ad libitum fed controls. This was shown by calculating the ratio of calories retained in newly formed tissue to the calories ingested, which was 11.9% for the rats fed ad libitum compared to 20.6% for the force-fed rats (Cohn and Joseph, 1959a).

Leveille and Hanson (1965a) arrived at a similar conclusion in experiments with rats limited to a single 2-hour meal per day. The meal-fed rat lost weight initially but after the initial adjustment period gained weight at the same rate as ad libitum fed controls. This similar rate of weight gain was achieved while consuming only 75-80% as much food as control rats. The authors concluded that the meal-fed rat becomes more efficient in utilizing the ingested

calories than ad libitum fed controls.

With one exception several laboratories (Beaton et al., 1964; Stevenson et al., 1964; Leveille and Hanson, 1965a) have consistently observed that rats limited to a single 2-hour meal usually gain less body weight than ad libitum fed controls and ingest only 75-80% as much food. In contrast Hollifield and Parson (1962) have reported that their rats trained to eat in a 2-hour period consumed 5-15% more food and were 30% heavier than controls after a 10-week experimental period. However, Stevenson et al. (1964) were unable to reproduce their results in a duplicate experiment.

Subsequent studies (Cohn and Joseph, 1960; Cohn, 1963; Tepperman and Tepperman, 1958a; Tepperman and Tepperman, 1965) have confirmed that the frequency of food intake is a significant factor in regulating intermediary metabolism. A decrease in meal frequency with a proportional increase in meal size causes an increased proportion of the dietary energy to be directed toward fat storage with an alteration of body composition.

Another experiment utilizing a different type of meal pattern had similar implications. Heggeness (1965) fed weanling rats a high-carbohydrate diet (60% glucose) ad libitum for three days followed by restricted feeding for three days during which time only the amount of food necessary for weight maintenance was allowed. Restricted animals

maintained body weight on a lower food intake, had less body protein and greater body fat than controls fed *ad libitum* at 60 and 120 days. After 180 days, the differences in body composition had disappeared. Rats not started on intermittent feeding until 55 days of age had body compositions similar to those observed for *ad libitum* fed controls. This indicates that limiting the feeding frequency may have an influence in young growing animals but not in older animals.

Effect of age and sex

Two studies have recently been published which emphasize the interaction of age with feeding frequency. Friend (1967) conducted three pair-feeding experiments to determine the effect on growth, nitrogen metabolism and body composition of rats fed 5 times per day ("multiple-feeders") or one meal per day ("single-feeders"). In one of 3 experiments, the younger (117 gms body weight) "single-feeders" demonstrated less weight gain, a higher percentage of carcass fat and less body protein than pair-fed controls. Fecal nitrogen loss was similar for both groups, but "single-feeders" had a greater uninary nitrogen loss. In 2 other experiments the rats were older (156 and 184 gms body weight), and the changes noted for the younger rats were not observed. Similar implications were obtained by Wardlaw, Henneyey and Clarke (1969) who investigated the influence of age and

feeding frequency on body composition in mature and immature male and female rats. Ninety-day old male and female rats were force-fed a liquified diet twice daily for a two-week experimental period. The amounts force fed corresponded to *ad libitum* intakes of similar animals fed the diet in a dry state. The manner of feeding had no significant effect on the percentage of fat in the carcass of females while a very slight increase in percentage fat occurred in male rats. Subsequently 35-day old animals were treated similarly. A significant increase in fat deposition occurred only in the young force-fed male rats. In the young female rats there was a trend toward increased fat deposition in the force-fed group, however this was not significantly different from *ad libitum* fed controls. These studies indicate a need to consider the age at which specific feeding frequency patterns are initiated, and possibly the sex of the animal.

Species differences

In addition to the influence of age and sex on the response to limited feeding frequency, consideration must be given to species differences. In contrast to the rat, the chicken appears to utilize food less efficiently when it ingests a 24-hour food allotment in a limited period compared with controls which nibble their food throughout the day. Feigenbaum *et al*. (1962) trained chickens to consume 40 g of

feed twice a day in two 1-hour periods. The meal-eaters lost body weight during the 17-day period, and had a lower body fat content than nibbling controls consuming the same quantity of feed *ad libitum*. A second experiment (Fisher and Griminger, 1963) considered the effect of aging and food restriction in chickens meal-fed 80% of the food consumed by *ad libitum* controls of similar body weight. The two feeding patterns were carried out from 1 day of age until the birds were 38 months old. After this extended period, the full-fed birds were heavier than the restricted ones, but the body composition was essentially the same in both groups.

Similar results were obtained by Leveille and Hanson (1965b) who studied, over a 14 week period, male chickens fed either *ad libitum* or for 2 hours daily. At the end of the experimental period meal-fed chickens, in comparison to *ad libitum* controls, had a lower body weight and lower percent of body fat. But the most interesting finding was that both *in vitro* and *in vivo* studies revealed an enhanced rate of lipogenesis from acetate by liver slices of meal-eaters, and that *in vivo* synthesis of fat from glucose was also enhanced in this group of chickens. Lipogenesis in the chicken appears to be restricted to the liver, with adipose tissue serving primarily as a storage organ (O'Hea and Leveille, 1968; Leveille *et al*., 1968).

Studies conducted with pigs indicate that they too respond differently from the rat to limited feeding periods. Friend and Cunningham (1967) have reported that pigs fed five meals per day, the same quantity as eaten by pigs fed one meal, tended to have slightly greater fat (35.7 vs. 34.2%, $p < 0.05$) and less protein deposits. The authors offer no explanation for the pigs' different response. However, Leveille[1] believes that the different response in the pig versus the rat may be attributed to the slow rate of food passage through the gastro-intestinal tract and the fact that the pig is never in a post-absorptive state during a 24-hour period. Longer periods of fasting in the pig may demonstrate a response to limited feeding frequency similar to that found in rats.

From this point, the review will be confined only to the effects of feeding frequency on the rat, since recent reviews (Fabry, 1967; Tepperman and Tepperman, 1964a) present studies of other species including man.

Energy Metabolism

The possibility that energy expenditure is strikingly altered as a consequence of meal feeding is suggested by

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[1]Leveille, G. A., Department of Animal Science, University of Illinois, Urbana, Illinois. Personal communication. 1969.

two observations: 1) force-fed rats contain a greater concentration of body fat than do *ad libitum* controls receiving the same energy level of ingested food (Cohn *et al.*, 1957, 1959a), and 2) rats fed a single 2-hour meal per day gain weight at the same rate as nibbling control animals but consume significantly less food (Leveille and Hanson, 1965a). Studies have been directed towards both components of total energy expenditure, 1) basal or resting metabolism (measured by O_2 consumption) and 2) activity (recorded).

Cohn *et al.* (1958) observed a decrease in energy expenditure accompanying force-feeding which suggested that a hypothyroid condition might be related to the altered body composition of meal-fed rats. They showed that meal eating was associated with almost a 50% decrease in I^{131} uptake by the thyroid when compared to rats eating *ad libitum*. Further experiments indicated that the decreased activity of the thyroid seemed to be secondary to a decreased formation and/or release of thyroid stimulating hormone (TSH). Consequently Cohn and Joseph (1960) designed a unique experiment in which rats were injected with 10, 20, or 40 ug of thyroxin daily for 14 days, after which total body lipids were determined. Each force-fed rat was pair-fed against one eating *ad libitum*. If hypothyroidsm in the force-fed rat was the cause for the increased body fat, then equilization of body fat content should have occurred provided that the

dose of thyroxin was great enough to inhibit the release of TSH and thyroxin. Force-fed rats still had a greater percentage of body fat, however at higher levels of thyroxin the difference between the two groups became less. The urinary and fecal losses of I^{131} were similar for both groups which negates the possibility that endogenously secreted or exogenously injected thyroxin was lost in the feces and/or urine, and thus not physiologically available. The authors concluded that a decrease in endogenous thyroxin release could not account for the observed differences in body composition.

Fabry et al. (1961) reported an increased basal oxygen consumption in intermittently adapted rats even in the face of reduced thyroid activity. The authors postulated that the higher basal oxygen consumption may be the reflection of a more intense tissue metabolism resulting from the periodic nutrient loads which the animal must metabolize within a relatively short period. These same workers however, later demonstrated that the mean values for oxygen consumption over a 24-hour period was significantly reduced in intermittently adapted rats compared to ad libitum fed controls (Fabry et al., 1963). The basal oxygen consumption during the day, the period of relative rest, was higher in intermittently fasted animals (received food 3 times a week in the a.m.) than controls, but during the night the oxygen

consumption was markedly reduced compared with the controls. The decrease was apparently sufficient to compensate for the higher values at rest and indicated by the lower average value for the 24-hour period.

More recently Heggeness (1969) obtained similar implications using weanling rats adapted to 3 days of *ad libitum* feeding followed by 3 days of restricted (approximately 65% of *ad libitum* intake) food intake. They found that the usual transient rise in resting oxygen consumption of post-weaning animals fed *ad libitum* did not occur in their adapted animals. Resting oxygen consumptions were significantly lower than those of *ad libitum* fed controls. To examine further the role of the thyroid gland in these changes induced by this feeding pattern, thyroxin levels were maintained relatively constant by providing exogenous thyroxine to hemithyroidectomized rats fed continuously or intermittently. In hemithyroidectomized rats, residual thyroid tissue was significantly smaller (4.9 mg) compared with those fed continuously (6.6 mg) after 14 days refeeding. Following replacement of thyroxine in thyroidectomized rats, the differences in metabolic rate as well as body composition (intermittently fed had a greater percentage of body fat) were abolished between the two groups. Results of this study suggested that the differences in body composition associated with intermittent feeding may be mediated, in

part, by a modification of thyroid activity.

Leveille and O'Hea (1967) studied the spontaneous activity of the meal-fed (2 hr/day) and nibbling rats to determine if the energy expenditure of meal-fed rats was reduced. The spontaneous activity of meal-fed and nibbling rats was determined during the day (8:00 a.m. to 4:00 p.m.) and night (4:30 p.m. to 8:00 a.m.) by revolving cages connected to a counter which measured each revolution of the cage. The 24-hour activity of meal-fed rats was decreased by approximately 50%, particularly during the evening hours, as compared with controls. They concluded that the improved food efficiency and potential for obesity in the meal-fed rat was due, in part, to the reduced activity and hence reduced energy expenditure. This conclusion was supported somewhat by a recent study by Nejjar and Heggeness (1969). They found that the greater accumulation of body fat in weanling rats adapted to intermittent feeding (3 days *ad libitum* followed by restricted food intake) did not develop when they were spontaneously active.

Intermediary Metabolism

The frequency with which food is ingested appears to modify over-all metabolism of carbohydrate, fat and protein. The periodic intake of full spaced meals ("meal-eating") results in increased concentrations of utilizable substrate (Leveille and Chakrabarty, 1968). This is probably

the stimulus that leads to adaptive changes in activity of enzymatic pathways of intermediary metabolism (Cohn and Joseph, 1959b; Fabry and Braun, 1967; Leveille, 1970). Such adaptive changes have been termed a "working hypertrophy" of enzyme systems (Tepperman and Tepperman, 1965).

Carbohydrate metabolism

Glucose absorption and utilization   Since glucose may serve as a primary substrate for lipogenesis, Leveille and Chakrabarty (1968) studied glucose absorption and utilization in rats adapted to a daily 2-hour feeding schedule as well as *ad libitum* feeding. Rats were fed a semipurified diet (18% protein, 66.5% glucose, 12% corn oil) for 3 weeks at which time oral glucose tolerance tests were performed. Meal-fed rats absorbed glucose more rapidly than *ad libitum* fed controls (268 vs. 195 mg per 100g bw/hr). This enhanced absorptive rate was explained by an increased absorptive area of the small intestine. The glucose absorptive coefficient and intestinal weight relative to body weight were both increased about 40% in the meal-fed as compared with nibbling animals. These findings are in agreement with the earlier observations of Tepperman and Tepperman (1958a) of enhanced intestinal glucose absorption in rats trained to consume their daily food ration within one hour. Similarly the hypertrophy of the gastrointestinal tract in response to periodic meal-feeding has

been reported (Holeckova and Fabry, 1959; Fabry and Kujalová, 1960). According to Durand _et_ _al_. (1965) the hypertrophy of the intestine in response to food intake occurs primarily during the period of vigorous growth prior to puberty. This may offer a partial explanation for the failure of some investigators (Heggeness, 1965; Wardlaw _et_ _al_., 1969) to produce an increase in carcass fat or caloric efficiency with decreased frequency of feeding in mature rats.

The sudden influx of absorbed nutrients implies that the tissues of meal-fed rats must adapt to handle the increased supply of glucose presented to them. Evidence supporting this concept was obtained by Leveille and Chakrabarty (1968). Blood glucose increased more slowly in meal-fed than in nibbling rats (135 vs. 185% of initial concentration 30 minutes after oral glucose administration) even though glucose was being absorbed more rapidly by meal-fed rats. To determine if the difference in blood glucose level was due to absorption from the gut or to the net removal from the blood or to both, the rats were fasted 22 hours and given 800 mg of glucose intraperitoneally. Fifteen minutes after glucose administration the serum glucose levels of the meal-fed and nibbling rats were 325% and 425% respectively, of the zero time control values. Blood glucose levels of meal-fed rats returned to original

control values much quicker than *ad libitum* fed controls (e.g. 60 vs. 120 minutes). These results illustrate that peripheral tissues of meal-fed rats have a greater capacity to assimilate glucose than those of *ad libitum* fed rats, and are in agreement with those reported by Fabry (1967).

In a similar type study Cohn and Joseph (1970) investigated the relationship between quantitative nutrient intake and periodicity of food ingestion of glucose uptake and insulin sensitivities. Male rats (100 to 120 g) were fed *ad libitum* (controls), pair-force-fed to controls, or force-fed 80% of the nutrient intake of the others. The food intake for the *ad libitum* and pair-force-fed groups was 14.5 g per day over the 30 day period. The group force-fed to 80% received 11.6 g per day. In response to glucose tolerance and insulin sensitivity tests, the blood glucose levels were significantly higher in the force-fed animals, and they were less responsive to the hypoglycemic effect of insulin than those animals eating the same amount *ad libitum*. By contrast the undernourished-force-fed animals were more sensitive to insulin and were capable of disposing of blood glucose faster than *ad libitum* fed controls. The findings in the pair-force-fed rat agree with results reported for humans (Gwinup *et al*., 1963), while glucose tolerance data obtained from the undernourished animals are similar to those reported by Leveille and Chakrabarty (1968) for the meal-fed

rat. These results indicate that the quantity of food consumed as well as the feeding frequency, affects metabolic responses. From an earlier study Cohn and Joseph (1968) had concluded that some "threshold" quantity of caloric intake must be exceeded if feeding frequency is to alter body composition.

Glycogen synthesis During the period of fast between meals, tissues of the meal-fed rat become more efficient than those of *ad libitum* fed controls in converting carbohydrate to energy stores, probably glycogen and lipid. Leveille and Chakrabarty (1967) have estimated that in the meal-fed rat 30% of the ingested energy is lost as energy expenditure while about 70% is stored as glycogen and lipid. Of the stored energy 68% is deposited as lipid and 32% as glycogen. The authors suggest that the capacity for lipid and glycogen synthesis is greatly enhanced in tissues of meal-fed animals compared with *ad libitum* fed controls. A number of studies with meal-fed versus *ad libitum* fed animals have shown that the increased lipogenesis, observed as a result of meal feeding, is accompanied by enhanced liver glycogen deposition (Hollifield and Parson, 1962a; Stevenson *et al.*, 1964; Leveille, 1966; Tepperman and Tepperman, 1958a).

The diurnal variations in tissue glycogen content of meal-eating rats has been investigated by Leveille and Chakrabarty (1967) to gain information on the magnitude of glycogen storage and of its rate of deposition and depletion.

Meal-fed rats (2 hr meal/22 hr fast) were killed at various times after the initiation of the meal, and glycogen content was determined in liver, diaphragm and adipose tissue. Fasting liver glycogen levels were higher (22 vs. 4 mg/g liver) in meal-fed rats than in *ad libitum* fed controls. Meal-fed rats accumulated more glycogen in diaphragm muscle and adipose tissue than nibbling controls. Glycogen increased in these two tissues for up to 8 hours and decreased from 12 to 22 hours, but was not markedly altered during the latter period in tissues of nibbling rats. Between 8 and 10 hours after the start of the meal, the glycogen content in adipose tissue of meal-fed rats fell sharply. The large reduction in tissue glycogen content during this period in meal-fed animals suggests that glycogen was being used as an energy source. Serum free fatty acids (FFA) were determined to provide information on the oxidative fuel available to the meal-fed rat during this period. In the meal-fed rat, the serum FFA were not elevated until 14 hours after the initiation of the meal, implying that carbohydrate was the major source of energy for the first 14 hours. In contrast the serum FFA levels were already elevated 8 hours after the start of the meal in the *ad libitum* fed rats.

Lipid metabolism

Adaptive hyperlipogenesis As previously mentioned the strikingly elevated RQ values in excess of 1.0 as well as the increase in percentage carcass fat suggested an enhanced ability of meal-fed or intermittently adapted rats to synthesize fatty acids and accumulate lipid stores. To confirm the hypothesis that lipogenesis was promoted in animals trained to eat their food allotment in a limited period (e.g. 1 to 2 hrs/day), investigators turned to isotope tracer techniques. Tepperman and Tepperman (1958a) studied liver slices from obese rats trained to eat their 24-hour food allotment in one hour and from rats fed a high carbohydrate diet after a 48-hour fast. These *in vitro* analyses demonstrated an increased incorporation of acetate-1-C^{14} and glucose-U-C^{14} into liver lipids of meal-fed and fasted-refed rats as compared to those eating *ad libitum*. Similarly Hollifield and Parson (1962b) and Stevenson *et al*. (1964) observed enhanced *in vitro* incorporation of labelled acetate into fatty acids of adipose tissue in rats trained to eat their food ration in 2 hours. Leveille (1967c) has recently estimated the relative importance of both liver and adipose tissue as sites of fatty acid synthesis in the rat by observing the rates of glucose-U-C^{14} incorporation in these tissues. Fatty acids synthesis was approximately 200-fold higher in adipose tissue and

9-fold higher in liver of meal-fed as compared to nibbling rats. Calculations based on labelled glucose incorporation suggest that in the nibbling rat between 50-90% of the fatty acids are synthesized in adipose tissue. In comparison when fatty acid synthesis is stimulated by meal-feeding, adipose tissue apparently accounts for about 95% of the total fatty acids synthesized. These data demonstrate that liver plays a very minor role in the total fatty acid synthesis of the meal-fed rat.

Enzyme adaptation To gain further understanding of the factors responsible for the elevated rates of lipogenesis and glucose utilization in the meal-fed rat, the activities of various enzymes in liver, adipose tissue and muscle have been studied. The adaptive enzymes studied can all be related in some way to lipogenesis, and of the tissues studied, adipose tissue shows the greatest adaptability. In general the enzymes studied can be grouped into three broad metabolic categories as suggested by Leveille (1970): 1) those enzymes involved in the phosphorylation of glucose and its conversion to α-glycerophosphate and pyruvate, 2) those enzymes involved in the conversion of glucose to fatty acid and glycerol, and 3) enzymes involved in the generation of NADPH for the support of reductive lipogenesis.

Pyruvate and α-glycerophosphate formation

Chakrabarty and Leveille (1968) recently investigated the activities of hexokinase (E.C.2.7.1.1), pyruvate kinase (E.C.2.7.1.40),

and alpha-glycerophosphate dehydrogenase (E.C.1.1.1.8) in tissues of meal-fed (2-hr/day) and _ad libitum_ fed rats (250-280 g) receiving a diet which supplied approximately 19, 12, and 70% of the calories as casein, corn oil, and glucose, respectively. These enzymes play obvious roles in supporting high rates of lipogenesis: hexokinase, by initiating the utilization of glucose, provides substrate for further metabolism; pyruvate kinase controls the formation of pyruvate and acetyl CoA; and alpha-glycerophosphate dehydrogenase supplies alpha-glycerophosphate, an essential substrate for fatty acid esterification to triglyceride. The activities of these three enzymes were significantly elevated (hexokinase, +283%; pyruvate kinase, +40%; alpha-glycerophosphate dehydrogenase, +69%) in adipose tissue of meal-fed, as compared with nibbling rats. Muscle hexokinase activity was also enhanced by meal-feeding, however the activities of the three enzymes in liver were similar in meal-fed and nibbling rats. The possibility that α-glycerophosphate formation may act as a controlling mechanism in lipogenesis was proposed earlier by Leveille (1967d). Fatty acid synthesis, low in fasting animals, increased as amounts of α-glycerophosphate became available. Thus a feedback mechanism depressed fatty acid synthesis during fasting when tissue levels of FFA were elevated. When

α-glycerophosphate increased as a result of carbohydrate feeding, synthesis and esterification of fatty acid increased.

Fatty acid biosynthesis   Vagelos (1964) and more recently Tepperman and Tepperman (1970) have reviewed current evidence for a possible enzymatic pathway for the *de novo* biosynthesis of fatty acid from carbohydrate. This involves the extramitochondrial formation of pyruvate which is converted to acetyl CoA by oxidative decarboxylation within the mitochondria. Since fatty acid synthesis is thought to occur in the cell cytoplasm, acetyl CoA must be transferred out of the mitochondria. However, the diffusion of acetyl CoA out of the mitochondria is probably too slow to meet demands for rapid lipogenesis. A possible mechanism to circumvent this problem is the intramitochondrial formation of citrate. The citrate passes freely through the mitochondrial membrane and is cleaved to acetyl CoA and oxaloacetate (OAA) by citrate cleavage enzyme. The acetyl CoA is directed toward fatty acid biosynthesis via a sequence of reactions catalyzed by acetyl-CoA carboxylase and the enzyme complex referred to as fatty acid synthetase. Enzymatic pathways for *de novo* synthesis of fatty acid are present also within the mitochondria, however this system appears to be less active than the one outside the mitochondria.

In consideration of the current concept of *de novo* fatty acid biosynthesis Leveille and Hanson(1966a) and Chakrabarty and Leveille (1969) studied the activities of citrate-cleavage enzyme (E.C.4.1.3.8), acetyl-CoA carboxylase (E.C.6.4.1.2) and fatty acid synthetase in liver and adipose tissue of meal-fed and nibbling rats receiving a high carbohydrate (70%) diet. In accord with the contention that adipose tissue is the major site of fatty acid biosynthesis, the activities of acetyl-CoA carboxylase and fatty acid synthetase were significantly greater in adipose tissue than liver of meal-fed as compared to nibbling rats. The activities of citrate cleavage, acetyl-CoA carboxylase and fatty acid synthetase were respectively +1000, +129 and +169% higher in adipose tissue of meal-fed rats compared to *ad libitum* fed controls. In contrast the activities of acetyl-CoA carboxylase and fatty acid synthetase were found to be of the same order of magnitude in liver of meal-fed and nibbling rats. The increase in these three enzymes provide for a rapid conversion of mitochondrially generated acetyl-CoA to fatty acids in the cell cytoplasm.

Generation of NADPH Since reductive fatty acid biosynthesis depends upon an available supply of NADPH, one might assume that the enzymatic steps involved in NADPH generation would show adaptation to limited feeding frequency. Several investigators have observed a marked

increase in the activity of dehydrogenases of the pentose cycle (i.e., glucose-6-phosphate, E.C.1.1.1.49; 6-phosphogluconate dehydrogenase, E.C.1.1.1.44) in liver and adipose tissue of meal-fed rats as compared with nibbling controls (Tepperman and Tepperman, 1964; Hollifield and Parson, 1962a; Cohn and Joseph, 1959b; Leveille and Hanson, 1966a). However, Flatt and Ball (1964) and Rognstad and Katz (1966) demonstrated that only about 50% of the NADPH needed for high rates of lipogenesis (i.e., in response to feeding frequency or recovery from starvation) in rat adipose tissue could be supplied by the hexose monophosphate shunt dehydrogenases (pentose cycle). This lead to a search for alternate enzymatic pathways that could generate NADPH. Experiments by Young _et al_. (1964) and Wise and Ball (1964) suggested that oxaloacetate derived from the cleavage of of citrate could be converted to malate by malic dehydrogenase (E.C.1.1.1.37) and then to pyruvate by malic enzyme (E.C.1.1.1.40), and thus a transfer of hydrogen from NADH to $NADP^+$. Ballard and Hanson (1967) later demonstrated that pyruvate carboxylase was present in the cell cytoplasm of adipose tissue. Thus they proposed a "transhydrogenation cycle" in which NADPH could be generated from NADH with ATP supplying the required energy. The reaction sequence is as follows:

$$\text{oxaloacetate} + \text{NADH} \rightarrow \text{malate} + \text{NAD}^+$$

$$\text{malate} + \text{NADP}^+ \rightarrow \text{pyruvate} + \text{NADPH} + \text{CO}_2$$

$$\text{pyruvate} + \text{CO}_2 + \text{ATP} \rightarrow \text{oxaloacetate} + \text{ADP} + \text{Pi}$$

Since the "transhydrogenation cycle" probably makes an important contribution (50%) in supplying NADPH during enhanced lipogenesis, Leveille and Hanson (1966a) and Chakrabarty and Leveille (1968) studied the effects of limited feeding frequency on enzymes in this cycle. Adipose tissue of meal-fed (2 hours daily) rats demonstrated a significant increase in pyruvate carboxylase (E.C.6.4.1.1) and malic enzyme (E.C.1.1.1.40) activities, +344 and +424% respectively, in comparison with *ad libitum* fed controls. The activity of malic dehydrogenase was not increased significantly, but was high enough not to be rate-limiting. The authors concluded that the "transhydrogenation cycle" was functioning at an increased rate in adipose tissue of the meal-fed rat in support of hyperlipogenesis.

Time sequence of enzymatic adaptations and hyperlipogenesis Although it is apparent that the meal-fed rat is capable of supporting rapid lipogenesis, the actual mechanism(s) responsible for "triggering" these adaptive changes is not known. There is question whether the increased enzyme activity and hence supply of NADPH, stimulates increased lipogenesis or whether the enhanced

lipogenesis stimulates the increased enzyme activity. The possibility exists that the increased enzyme activity of the pentose pathway and "transhydrogenation cycle" is the result of hyperlipogenesis rather than the cause. This is implied by work of Leveille (1966) who studied the *in vitro* incorporation of labeled acetate into fatty acids, and the activities of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenases, and malic enzyme in adipose tissue of meal-fed and nibbling rats. Adipose tissue was obtained from both experimental groups at various times after initiation of their respective feeding patterns. Lipogenesis increased significantly after 5 days of meal-feeding whereas enzyme activity responsible for NADPH production was not elevated above nibbling control levels until the 9th day of refeeding. Similar results were obtained earlier by Tepperman and Tepperman (1961) who showed that lipogenesis in the liver of fasted-refed rats preceded the increase in pentose pathway dehydrogenase activity.

The enhanced activities of the glucose-6-phosphate, 6-phosphogluconate and NADP-malic dehydrogenases, although not essential for the initial increase in fatty acid biosynthesis, without doubt reflect the increased demand for NADPH to support hyperlipogenesis. These findings support the concept that the increased use of an enzymatic pathway

stimulates an increase in enzyme(s) involved in that pathway (Freedland and Harper, 1959; Fitch and Chaikoff, 1960). The mechanism by which an increased flux of substrate stimulates an increase in enzyme activity is not clear. Leveille (1970) has speculated that the increased flow of substrate through the enzymatic pathway may stabilize the protein enzyme and thus reduce catabolism of that enzyme.

Reversibility of metabolic adaptations The reversibility of metabolic adaptations induced by meal-feeding was recently investigated by Muiruri and Leveille (1970). Rats were fed a single daily 2-hour meal for 3 weeks, then changed to *ad libitum* feeding (nibbling) for an additional 3 weeks. The *in vitro* rate of glucose-U-C^{14} incorporation into fatty acids by adipose tissue and the activities of pentose pathway dehydrogenases and malic enzyme remained significantly higher in the meal-fed group returned to a nibbling pattern than control animals maintained on *ad libitum* feeding. These observations indicated that reversal of adaptive changes required a longer period of time than that necessary for their induction.

Composition of adipose tissue Recognition of the prominent role of adipose tissue in hyperlipogenesis of meal-fed rats stimulated interest in the morphological changes and fatty acid composition of adipose tissue. Braun *et al*. (1965) measured the DNA and RNA content of

adipose tissue of rats adapted to eating 2 hours per day and *ad libitum* fed rats. After 7 weeks of experimental feeding, the DNA content of meal-fed rats was increased significantly (by 27%) compared to controls. Histological examination revealed that fat cells were smaller and more numerous than in corresponding tissue of controls. Similarly the RNA content of adipose tissue was significantly elevated (17.4 vs. 23.6 mg/100 g) above *ad libitum* fed controls, indicating protein synthesis. The authors concluded that the greater cellularity, suggested by the increased DNA content and histological examination as well as the increased RNA content, was in agreement with the increased synthesis of fatty acids and the enhanced enzyme activity in adipose tissue of meal-fed rats.

Braun *et al*. (1967) later investigated the effect of frequency of food intake on fatty acid composition of parametrical adipose tissue. After 30 days of experimental feeding a standard laboratory diet the percentage of palmitic acid was higher (31.9 vs. 27.6) and the percentage of linoleic acid significantly lower (15.6 vs. 20.0) in the tissue of the meal-fed rats compared to *ad libitum* fed controls. The authors concluded that the greater percentage of palmitic acid in meal-fed rats as compared with controls reflected the higher biosynthesis of this fatty acid.

Serum and hepatic cholesterol Studies designed to explore the effect of limited feeding frequency on serum and hepatic cholesterol in the rat are limited. Okey *et al.* (1960) determined serum and hepatic cholesterol levels in young male and female rats allowed to eat either *ad libitum* or for two 1-hour periods daily. Basal diets were uniform and adequate except that half the rats in each group were given 1% cholesterol. In the absence of added cholesterol in the diet, no differences in serum or hepatic cholesterol levels were noted, regardless of the rate of food consumption or sex of the animals. When cholesterol was added to the diet, the meal-fed females exhibited increased serum levels of cholesterol, although they were eating less food and hence less cholesterol. No differences were observed in the serum cholesterol content of male rats subjected to the same experimental procedure. When cholesterol was present in the diet, regardless of the sex of the animals, the meal-fed rats had lower hepatic cholesterol levels than controls fed *ad libitum*. Leveille (1967c) did not detect differences in hepatic or serum cholesterol of rats fed a commercial rat chow either *ad libitum* or 2-hours daily for a 2 week period. Similarly Fabry (1967) was unable to observe consistent and reproducible changes in serum cholesterol related to frequency of food intake in rats when the diet had not been supplemented with cholesterol.

Fabry (1967) concluded that in meal-fed rats, the oxidation and removal of cholesterol may be enhanced, which in this species may balance the higher endogenous cholesterol biosynthesis.

Protein metabolism

The manner in which the body handles dietary protein is likewise subject to feeding frequency. A decrease in the number of daily feedings results in the deposition of less protein and enhanced excretion of urinary nitrogen compared to rats fed *ad libitum* (Cohn, 1962). Cohn *et al*. (1964) postulated that these changes occur as a result of enzymatic adaptations. Using male pair-force-fed rats, they measured the urinary excretion of urea N^{15} and the activities of hepatic urea cycle enzymes. The force-fed rats excreted more of the dietary N^{15} as urea and exhibited a greater hepatic arginine synthetase activity than their pair-fed controls with free access to food. They concluded that there is a limit to the magnitude of the load of dietary protein that the animal can utilize in a specific time period for protein synthesis. When this limit is exceeded, there is an increased protein turnover and an increased shunting of amino groups into the Krebs urea cycle. The deaminated remnants of amino acids can then be used as a source of energy or as material for fat synthesis. This concept is supported by Cohn *et al*. (1963) finding that

even on high-protein diets (67%), force-feeding leads to an increase in body fat.

Leveille and Hanson (1966a, 1966b) have demonstrated an increase in the ability of adipose tissue of meal-fed rats to convert aspartic and glutamic acids and more recently leucine (Leveille, 1967a) to fatty acids as compared with nibbling controls. Such a feature of metabolism leads support to the hypothesis that amino acid carbons are incorporated into fat as an adaptive response to meal feeding (Cohn, 1962).

Influence of Diet Composition on Metabolic Adaptations

Numerous studies have established that feeding a high-fat diet will depress lipogenesis in liver (Hill *et al*., 1958; Bortz *et al*., 1963; Kimura and Ashida, 1969) and adipose tissue (Hausberger and Milstein, 1955; Leveille, 1967b) of *ad libitum* fed animals. Much interest has developed therefore regarding the influence of diet composition on metabolic adaptations to altered meal patterns. In early studies of feeding frequency Tepperman *et al*. (1943) observed that the increase in RQ of rats fed for one hour a day was greater on a diet that contained a higher percentage of sucrose than on a standard laboratory diet. Cohn *et al*. (1963) compared changes in body fat content of rats fed two weeks either *ad libitum* or force-fed

a diet ranging in protein content from 0 to 67%. In comparison with controls fed *ad libitum* the relative body fat content of the force-fed animals became increasingly greater as the dietary protein content increased. Similarly Beaton *et al.* (1964) fed a 5% and 20% casein diet to rats either *ad libitum* or for a two-hour period each day. On both levels of protein intake, significantly increased lipogenesis (measured as C^{14}-acetate incorporation) *in vitro* and increased liver glycogen were demonstrated in rats fed 2-hours daily compared with their *ad libitum* fed controls. The authors concluded that meal-fed rats fed a low protein diet do not have impaired lipogenesis in adipose tissue as measured *in vitro*. Fabry (1967) has postulated that the glucogenic amino acids, after deamination, provide material for fat synthesis.

Studies by Leveille (1966, 1967a, 1967b) demonstrated the adaptive enzyme response to meal feeding to be carbohydrate dependent. As the level of dietary fat increased from 10, 20 and 30% the incorporation of glucose-U-C^{14} into epididymal fatty acids decreased in both meal-fed and nibbling rats. Similarly an inverse relationship existed between level of dietary fat and activity of glucose-6-phosphate dehydrogenase and malic enzyme. The depression in lipogenesis and enzyme activity could be related either to an increase in dietary fat or to a decrease in dietary carbohydrate. Thus a second study was conducted in which

the percent of calories derived from fat was kept constant but calories derived from carbohydrate was reduced by increasing the level of dietary protein. Increasing dietary protein level from 9 to 36% decreased carbohydrate calories from 79 to 50% and resulted in significantly less incorporation of labeled glucose and malic enzyme activity in adipose tissue of both meal-fed and nibbling rats. The data suggest that increasing dietary fat or protein reduces the calories from carbohydrate which is a major factor in depressing fat synthesis. However, dietary fat probably has a specific inhibitory effect of lipogenesis since replacement of carbohydrate calories with a calorically equivalent amount of fat is more effective in reducing lipogenesis, particularly in the case of meal-fed rats.

Part B. Dietary Influences on Fatty Acid Composition

Previous theses from this laboratory have extensively reviewed the effects of diet on fatty acid composition of hepatic and epididymal lipids (Noble, 1967; Stadler, 1969; Kopec, 1969). Therefore only a few studies and their conclusions will be presented in this review.

Fatty Acid Composition

Effect of diet on hepatic tissue

Noble (1967) reviewed papers which indicated that the rate of hepatic lipogenesis and the fatty acid profile of the hepatic lipid is greatly influenced by dietary composition. In the absence of dietary fat, endogenous fat synthesis is elevated with relative increases in the four major saturated and monounsaturated fatty acids; palmitic, palmitoleic, stearic and oleic. The polyenes do not appear to be synthesized and their relative concentration decreases. The presence of dietary fat reduced the rate of hepatic lipogenesis with the fatty acid pattern of the tissue lipid reflecting the type of fat fed. An exception is the lack of incorporation of short chain fatty acids into hepatic lipid even when present in the diet. Dietary fatty acids with a chain length less than 14 carbons appear to be converted *in vivo* to palmitic acid. Exogenous sources of polyenoic fatty acids, such as linoleic, are readily incorporated into all major hepatic lipid fractions. The amount of hepatic linoleate deposited increases with increased amounts of dietary linoleic acid and with extended refeeding periods. Evidence was cited which suggested that the primary function of linoleic acid was its *in vivo* conversion to arachidonic acid.

Stadler (1969) has reviewed studies from several laboratories (Morin and Alfin-Slater, 1964; Connellan and Masters, 1965; Glende, Jr. and Cornatzer, 1965) regarding the fatty acid profile of hepatic phospholipids and non-phospholipids and the effect of dietary manipulation of these lipid fractions. Hepatic phospholipids, comprising approximately 72% of the total hepatic lipid, contain a higher proportion of long-chain fatty acids than the non-phospholipid fraction. The C_{20-22} unsaturated fatty acids represent slightly less than 30% of the hepatic phospholipid fatty acids compared to 10 to 15% in the non-phospholipids. Arachidonic acid was the only fatty acid with a chain length greater than 18 carbons reported in hepatic non-phospholipids of adult rats receiving a commercial stock diet. In general hepatic non-phospholipids contain greater concentrations of palmitate, oleate, and linoleate than hepatic phospholipids. Under the influence of various dietary manipulations the hepatic phospholipid concentrations remain relatively constant. In contrast the changes observed in the fatty acid composition of total hepatic lipid are more pronounced in the non-phospholipid. Evidence for this is cited in the numerous reports reviewed by Stadler (1969) and likewise by studies conducted in this laboratory (Stadler, 1969; McAtee, 1970).

<u>Effect of diet on epididymal tissue</u>

Evidence has been reviewed by Noble (1967) which indicates that a characteristic fatty acid pattern develops in rat epididymal tissue (adipose tissue) during *de novo* fat synthesis which utilizes carbohydrate and protein precursors. The percentage of palmitic acid remains fairly constant (25-30%) in adipose tissue of rats receiving low-fat diets. In rats changed from a standard laboratory stock diet to one high in carbohydrate (94% sucrose) or protein (96% casein), the percentage of palmitoleic acid, which is approximately 5%, increased to 20%. Another characteristic of the adipose fatty acid pattern is the relatively low concentration of polyenoic fatty acids when these are absent in the diet. The fatty acid profile resulting from *de novo* fat synthesis is reportedly similar whether carbohydrate or protein serves as the substrate.

The effect of fat-containing diets on rat adipose tissue also has been reviewed by Noble (1967) and Stadler (1969). Body fat of rats maintained on stock rations usually consists of fatty acids with 16 to 18 carbon atoms. However, modifications in the level and kind of dietary fat can result in deposition of fatty acids with chain lengths as short as eight carbon atoms or as long as 20 or more carbons. In a study conducted in this laboratory (Kopec, 1969) medium chain fatty acids (C10 to C14)

comprised 14% of the total fatty acids in rats refed with diets containing butter oil. Stadler (1969) cited evidence that the major portion of dietary fatty acids containing eight or more carbon atoms could be directly esterified by adipose tissue but not by liver. Extremely short-chain fatty acids (C2, C4) do not appear to be incorporated directly. If they are, they probably are elongated to C16 and C18 fatty acids before esterification into adipose tissue.

METHODS AND PROCEDURES

Selection and Treatment of Animals

Experimental protocol

This study was designed to evaluate the influence of altered meal patterns on hepatic, epididymal, and serum lipids and certain lipogenic enzymes in the adult male rat recovering from a state of protein-calorie malnutrition. Table 1 presents the features of the protocol used for this study. The experimental design included three dietary periods, pre-experimental, depletion (period 1), and repletion (period 2). Food intake and body weights were recorded every two days during periods 1 and 2.

Each experimental group contained 10 rats, each being individually housed in one-half inch mesh wire cages in a laboratory maintained at $24 \pm 1^{\circ}C$ with a relative humidity of approximately 40 percent. Throughout the experimental period the laboratory was maintained on a 12-hour photoperiod with the light period extending from 2100 to 0900 and the dark period from 0900 to 2100.

Pre-experimental   Male adult rats of the Wistar strain from the Iowa State University stock colony[1] were fed a stock diet from weaning until they reached a body weight of

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[1]Original breeding stock obtained from Simonsen Laboratories, White Bear Lake, Minnesota.

Table 1. Experimental protocol

Group	Dietary Period	Meal Pattern	Diet		Days of Refeeding
I	Pre-experimental	*Ad Libitum*	stock		0
			Period 1	Period 2	
II	1	8-16[a]	OF,OP[b]	---	0
III	1 + 2	*Ad Libitum*	OF,OP	OF,P[c,d]	10
IV	1 + 2	*Ad Libitum*	OF,OP	OF,P	30
V	1 + 2	8-16	OF,OP	OF,P	10
VI	1 + 2	8-16	OF,OP	OF,P	30
VII	1 + 2	*Ad Libitum*	OF,OP	20F,P[e]	10
VIII	1 + 2	*Ad Libitum*	OF,OP	20F,P	30
IX	1 + 2	8-16	OF,OP	20F,P	10
X	1 + 2	8-16	OF,OP	20F,P	30

[a]8-hour meal, 16-hour fast; dietary restriction.

[b]93% corn starch, 0% protein, 0% fat.

[c]Lactalbumin substituted isocalorically for carbohydrate to provide 1.08 g protein per 100 kilocalories.

[d]87.7% corn starch, 5.3% lactalbumin, 0% fat.

[e]65.1% corn starch, 6.7% lactalbumin, 20% corn oil.

475 to 510 g at 4 1/2 to 6 months of age. When ready for the experiment the animals were fasted 16 hours and either autopsied, Group I, or placed on dietary treatment.

Period 1 Period 1 was a 2-phase period of depletion in which adult male rats were depleted of body fat and nitrogen stores. During the first phase of depletion (Period 1a) animals were fed a protein-free diet for 8 hours a day and fasted 16 hours. The 8-16 meal pattern employed during depletion served to adapt the animals to an 8-hour meal pattern and reduce the total number of days necessary to deplete body weight from 500 to 400 g by the usual ad libitum feeding pattern. The animals began the second phase (Period 1b) of depletion upon reaching a body weight of 400 g or less. During this phase severe caloric restriction was imposed by feeding 5 g of the protein-free diet, divided into two daily portions. Animals remained on the restricted diet until their body weight was reduced to at least 300 g. At this time rats were either sacrificed (Group II), or randomly assigned to Groups III to X for refeeding.

Period 2 This was a period of nutritional rehabilitation during which the animals were fed either ad libitum or a single 8-hour meal followed by a 16-hour fast (8-16 meal pattern). Rats were refed for either 10 or 30 days.

Diets

Stock diet The stock diet consisted of a modified Steenbock XVII ration (Table 2) which was supplemented weekly with 15 g of lean ground beef, 20 g of carrots, 10 g of cabbage and 165 mcg of vitamin A acetate. The stock diet

Table 2. Stock ration for male rats, Steenbock XVII

Dietary component	Percent
Corn meal[a]	48.3
Linseed meal[b]	13.8
Dry skim milk[c]	10.2
Wheat germ[d]	8.6
Yeast, Brewer's USP[a]	8.2
Casein, crude B3F[e]	4.3
Cottonseed oil[f]	3.6
Alfalfa meal[g]	1.7
NaCl[h]	0.4
$CaCO_3$[i]	0.4
Yeast (irradiated)[j]	0.4

[a]General Biochemicals, Inc., Chagrin Falls, Ohio.

[b]Froning and Deppe Elevator, Ames, Iowa.

[c]Des Moines Cooperative Dairy, Des Moines, Iowa.

[d]General Mills, Inc., Chicago, Illinois.

[e]The Borden Company, Chemical Division, New York.

[f]Wesson Oil, Wesson Sales Company, Fullerton, California.

[g]National Alfalfa, Lexington, Nebraska.

[h]Analytical reagent, Mallinckrodt Chemical Works, St. Louis, Missouri.

[i]Matheson Coleman and Bell Division of the Matheson Company, Inc., Norwood, Ohio.

[j]Brewer's yeast irradiated in this laboratory.

Table 3. Fatty acid composition of stock ration, Steenbock XVII, and of corn oil

	% fatty acids						
	14:0	16:0	16:1	18:0	18:1	18:2	18:3
Stock diet	0.6	17.8	2.1	2.7	21.7	49.4	5.7
Corn oil	--	11.0	--	2.4	27.8	57.1	1.8

contained 7.4 percent fat. Stadler (1969) has previously reported the fatty acid composition of the stock diet and of corn oil used in this laboratory (Table 3).

Experimental diets Components of the experimental diets are listed in Table 4. Diet OF,OP was the protein-free diet used during the depletion period. During dietary repletion Groups III through X were fed a diet containing 1.08 g of protein per 100 kilocalories from lactalbumin and either corn starch (Diet OF,P) or corn starch plus 20 percent corn oil (Diet 20F,P) as the source of energy. Ration of calories to mineral mix and non-nutritive fiber were equalized for all diets. The calculated physiological energy value for diets OF,OP and OF,P was 3.72 kilocalories per g and 4.67 kilocalories per g for diet 20F,P. The experimental diets were supplemented daily with 500 mg of a mixture containing crystalline, water soluble vitamins (Table 5) plus

Table 4. Composition of experimental diets

Dietary component	OF,OP	OF,P	20F,P
		% weight	
Corn starch[a]	93.0	87.7	65.1
Corn oil[b]	0	0	20.0
Lactalbumin[c]	0	5.3	6.7
Hawk and Oser salt	4.0	4.0	5.0
Non-nutritive fiber[d]	2.8	2.8	3.0
NaCl[e]	0.2	0.2	0.2
		% kilocalories	
Corn starch	100	94.3	55.7
Corn oil	0	0	38.5
Lactalbumin protein	0	4.5	4.6

[a]Argo, Best Foods Division Corn Products Company, New York, New York.

[b]Mazola, Best Foods Division Corn Products Company, New York, New York.

[c]78.6% protein, Nutritional Biochemical Corporation, Cleveland, Ohio.

[d]General Biochemicals, Inc., Chagrin Falls, Ohio.

[e]Matheson Coleman and Bell Division of the Matheson Company, Inc., Norwood, Ohio.

Table 5. Composition of water soluble vitamin mixture

Vitamin[a]	Allowance per rat per day	Per 1000 doses
Thiamine HCL	40 mcg	50 mg
Riboflavin	60 mcg	60 mg
Pyridoxine HCL	40 mcg	40 mg
Ca-pantothenate	100 mcg	100 mg
Nicotinic acid	500 mcg	500 mg
Folic acid	8 mcg	8 mg
Biotin[b]	1 mcg	100 mg
Vitamin B_{12}[c]	0.75 mcg	750 mg
Ascorbic acid	1 mg	1 gm
Choline chloride	5 mg	5 gm
Inositol	10 mg	10 gm
P-aminobenzoic acid	10 mg	10 gm
Dextrin		to make 500 gm

[a]All vitamins were obtained from General Biochemicals, Inc., Chagrin Falls, Ohio.

[b]Biotin was mixed with dextrin so that 100 mg of the mixture contained 1 mg of biotin.

[c]Vitamin B_{12} in mannitol furnished 0.1 mg of vitamin B_{12} per 100 mg of mixture.

50 mg cod liver oil[1] and 0.75 mg DL-alpha tocopherol[2] in 50 mg of cottonseed oil.[3] The cottonseed oil provided 25 mg linoleic acid per day (Watt and Merrill, 1963). Distilled water was available to the animals *ad libitum*.

Feeding

To cooperate with the rats' normal instincts, food was made available only during dark periods for those animals eating on the 8-16 meal pattern. Meal-fed rats received food from 0900 to 1700 each day to give 8 hours feeding and 16 hours fasting. Animals on the *ad libitum* feeding regimen had food available continuously during both light and dark periods. No attempt was made to determine whether *ad libitum* fed animals consumed more food during the dark period compared to the light period. By shifting the dark period in the animal room to coincide with the lighted period in the laboratory, care and feeding activities could be conducted during the laboratory work day.

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[1] Squibb and Sons, New York, New York, 1700 USP vitamin A, 170 USP vitamin D per g.

[2] General Biochemicals, Inc., Chagrin Falls, Ohio.

[3] Wesson Sales Company, Fullerton, California.

Autopsy

On the last day of the refeeding period animals were fasted 16 ± 1 hours beginning at 1700. Around 0900 the following morning animals were sacrificed by a severe blow on the head. A complete laparotomy was done immediately and blood taken from the abdominal aorta or via heart puncture. One drop of a DL-alpha-tocopherol solution (1.6 mg/ml ethanol) was added per 2 ml blood to retard lipid oxidation. Blood samples were placed under refrigeration ($4^{o}C$) for approximately 30 minutes or until clot retraction was evident. After clotting, blood samples were centrifuged in a clinical centrifuge at $4^{o}C$ for 15 minutes. Serum was decanted, recentrifuged in the same manner and stored in 2-dram vials under nitrogen at $-20^{o}C$ for later cholesterol analysis.

Liver and epididymal fat pads were removed and placed on moist filter paper over ice, where extraneous matter was trimmed away prior to weighing. Approximately 4 g of liver and 2 g of epididymal fat were sampled from the tissues and stored under nitrogen at $-20^{o}C$ for later analysis of lipid and fatty acid content. In addition approximately 1 g of liver and epididymal tissue was homogenized in cold 0.25 M sucrose solution and the 26,500 x g (30 minutes at $4^{o}C$) supernatant stored at $-20^{o}C$ until assayed for enzyme activity.

Chemical Analysis

Lipids and fatty acids

Total lipid extraction The method of Folch, Lees, and Stanley (1957) as modified by Stadler (1969) was used to extract total lipid from liver and epididymal tissue. Tissue samples of known weight which were individually frozen at the time of autopsy were used for lipid analyses. The extraction procedure and subsequent lipid analysis was conducted as much as possible under an atmosphere of nitrogen.

Gravimeteric determinations Gravimetric determinations were made with a one ml aliquot of lipid extract made to a final volume of 5 ml with dry chloroform. The chloroform was evaporated with nitrogen from an aluminum foil weighing cup[1] previously brought to constant weight. The cup was then heated at 100°C for 20 minutes, placed in a desiccator and weighed when cool.

Separation of hepatic lipid fractions Stadler's (1969) modification of Borgstrom's (1952) procedure for silicic acid chromatography was used to separate liver lipid into non-phospholipid and phospholipid fractions. The silicic acid column was approximately 120 mm in length and could

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[1]E. H. Sargent and Company, Chicago, Illinois.

accommodate a maximum load of 120 to 140 mg lipid, although less lipid was often applied. After confirming completeness of separation with thin-layer chromatography, gravimetric determinations were made with 1.0 ml aliquots from each liver fraction.

Esterification To prepare the total lipid extracted from epididymal fat pads and lipid fractions obtained from liver extracts for fatty acid analyses, a modification of the interesterification procedure developed by Stoffel and coworkers (1959) was used to methylate both free and esterified fatty acids. Two percent sulfuric acid in methanol served as the esterification agent and hexane was used to extract the fatty acid methyl esters. The methyl esters in hexane were stored in one-half dram vials, under nitrogen, at -20°C for fatty acid analysis by gas-liquid chromatography.

Gas-liquid chromatography A Varian Aerograph Model 204B Gas Chromatograph,[1] with dual flame-ionization detectors was used for fatty acid analysis of methyl esters. Instrument conditions for fatty acid analysis were as follows: stainless steel columns, 10' x 1/8", packed with 3 percent ethylene glycol succinate on Gas-Chrom G 100/120 mesh HP; column temperature, 180°C; injector temperature, 210°C; detector temperature, 210°C; carrier gas flow, 30 ml/min.

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[1]Varian Aerograph, Walnut Creek, California.

Fatty acid identification and measurement Fatty acid peaks were identified by comparison with commercial standards obtained from Hormel Institute[1] and Applied Science Laboratories.[2] Methyl esters of arachidonic[2] and docosahexaenoic acids[2] were also obtained and incorporated into Hormel GLB Standard 1.[1] From these commercial standards correction factors were obtained by dividing the known weight percent of each fatty acid ester by the area percents obtained by gas-liquid chromatography. These correction factors, determined at intervals, were multiplied by the peak areas of sample fatty acids to obtain corrected peak areas. The corrected peak areas were used to obtain the relative percent of each component fatty acid. Uncorrected peak areas were measured using an Alltek Peakometer[3] which measured peak height and width at half-height. The peak area was the product of these two measurements. Calculation of corrected peak areas and relative percents of each component fatty acid was done by computer programming.[4]

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[1]University of Minnesota, Austin, Minnesota.

[2]Applied Science Laboratories, State College, Pennsylvania.

[3]Altek Associates, Arlington Heights, Illinois.

[4]Iowa State University Computer Programming Service Center.

Cholesterol

Serum   Serum samples prepared at the time of autopsy and stored under refrigeration (-20°C) were analyzed for both free and total cholesterol by a modification of the cholesterol digitonide method of Schoenheimer and Sperry (1934) as described in Hawk's Physiological Chemistry (Oser, 1965). A mixture of acetone-alcohol (1:1) was used to precipitate the serum proteins and extract the cholesterol and cholesterol esters from the serum. The cholesterol was precipitated with an alcoholic digitonin solution, either without saponification (free cholesterol) or after saponification (total cholesterol) with 3 drops of potassium hydroxide (10 g reagent KOH/20 ml distilled HOH). The separated digitonide was purified and subjected to the Liebermann-Burchard color reaction.[1] At 30 to 31 minutes after adding the acetic anhydride-sulfuric acid reagent, the optical density of each sample was determined with a Gilford Recording spectrophotometer[2] at 625 mu. A cholesterol standard was determined with each set of samples. The standard containing 0.06 mg cholesterol per 2 ml was prepared

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[1]The addition of sulfuric acid to cholesterol in the presence of acetic anhydride gives a green (i.e., red-absorbing) chromophore.

[2]Gilford Recording Spectrophotometer, Model 240. Gilford Instrument Laboratories, Inc., Oberlin, Ohio 44074.

from a stock cholesterol solution containing 1 mg per ml. Calculation of cholesterol content was based on optical density of the unknown and standard. Prior to analysis, serum samples were pooled to yield 5 samples per experimental group rather than the usual 10.

Liver Samples of liver lipid extract in chloroform from the previous lipid extraction procedure were used for liver cholesterol analysis. The samples were made to volume in a 5 ml volumetric flask with dry chloroform. One ml aliquots were taken for gravimetric analysis, free cholesterol, and total cholesterol determinations. The 1 ml aliquot for total cholesterol was transferred to a 10 ml volumetric flask, partially evaporated and made to volume with acetone-alcohol (1:1). Triplicate 3 ml samples were analyzed for total liver cholesterol by the same procedure used for serum cholesterol.

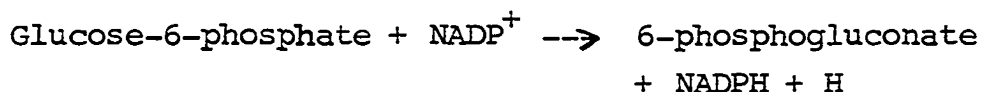
Enzyme studies

Tissue preparation Approximately 1 g samples of liver and epididymal tissue were homogenized in Tri-R Teflon Tissue Homogenizer[1] tubes equipped with a stainless steel pestle having a teflon tip. Samples were homogenized in 3 ml cold 0.25 M sucrose for 1 minute followed by an

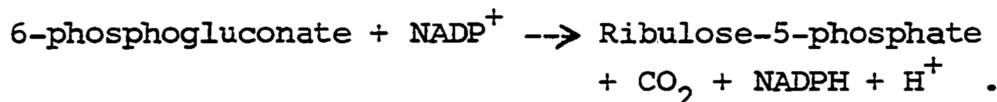
[1]Physicians and Hospitals Supply Co., Scientific and Laboratory Division, 1400 Harmon Place, Minneapolis 3, Minnesota.

additional 2 ml of sucrose and 1 - 1 1/2 minutes of homogenization. The homogenate was made to a final volume of 10 ml and centrifuged for 30 minutes at 4°C at 26,500 x g. The homogenate separated into an upper lipid cake, an intermediate clear liquid layer containing the soluble cell components, and a sediment consisting of fibrous stroma, mitochondria and cell debris. The middle layer was removed by pipette, taking care to avoid contamination with the other two layers. The clear supernatant fraction was divided equally into 3 2-dram vials, one for each enzyme to be assayed, and stored at -20°C until assayed. Young et al. (1969) have reported the combined activity of the hexose monophosphate shunt dehydrogenases (D-glucose-6-phosphate, E.C.1.1.1.49 and 6-phospho-D-gluconate, E.C.1.1.1.44), "malic" enzyme (NADP-malate dehydrogenase, E.C.1.1.1.40) and citrate-cleavage enzyme (ATP-citrate lyase, E.C.4.1.3.8) stable on storage at -15°C for several weeks.

Glucose-6-phosphate and 6-phosphogluconate dehydrogenase (HMPD) The combined activity of the hexose monophosphate shunt dehydrogenases was determined by the procedure of Glock and McLean (1953) as modified by Thorp (1966). Shunt activity was based on the change in optical density at 340 mu produced by the reduction of NADP over a 5-minute interval at 25°C. The reaction sequence was as follows:



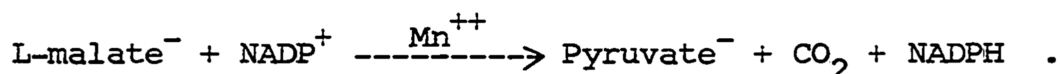
$$\text{Glucose-6-phosphate} + NADP^{+} \longrightarrow \text{6-phosphogluconate} + NADPH + H$$



$$\text{6-phosphogluconate} + NADP^{+} \rightarrow \text{Ribulose-5-phosphate} + CO_2 + NADPH + H^{+}.$$

Concentration and volume of reagents are listed in Table 6. The reaction was initiated by the addition of glucose-6-phosphate. Blank determinations without added NADP were made for each sample.

<u>NADP-malate dehydrogenase</u> A modification of the assay as described by Ochoa (1955) was used with the concentration and volume of reagents as shown in Table 7. The enzyme activity determination were based on the reaction:



$$\text{L-malate}^{-} + NADP^{+} \xrightarrow{Mn^{++}} \text{Pyruvate}^{-} + CO_2 + NADPH.$$

The formation of NADPH was followed for a 5-minute interval with a spectrophotometer[1] at 340 mu at 25°C. Nonsubstrate controls were run for all assays.

<u>Citrate-cleavage enzyme</u> Oxaloacetate formation from citrate, CoA, and ATP was measured as the oxidation of NADH by coupling with NAD-malate dehydrogenase by the method of Srere (1962). The reaction sequence of the citrate-cleavage pathway is as follows:

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[1]All enzyme assays were measured with a Gilford Recording Spectrophotometer, Model 240. Gilford Instrument Laboratories, Inc., Oberlin, Ohio 44074.

Table 6. Concentration and volume of reagents used for a single determination of HMPD shunt activity

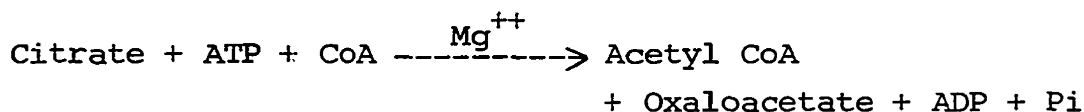
Reagent[a]	Concentration	Volume/cuvette	
		Sample (ml)	Blank (ml)
NADP	0.0015 M	0.2	--
$MgCl_2$	0.1 M	0.5	0.5
Glycyl glycine, pH 7.6	0.25 M	0.5	0.5
Enzyme homogenate	1-10 dilution	0.1	0.1
Distilled water		1.1	1.3
Glucose-6-phosphate	0.05 M	0.1	0.1
	Total Volume	2.5	2.5

[a]Sigma Chemical Co., St. Louis, Missouri.

Table 7. Concentration and volume of reagents used for a single assay of NADP-malic dehydrogenase

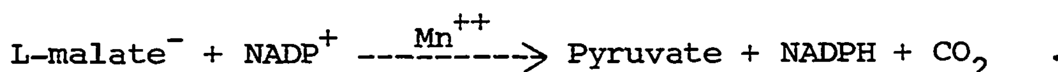
Reagent[a]	Concentration	Volume/cuvette	
		Sample (ml)	Blank (ml)
NADP	0.000675 M	0.24	0.24
$MnCl_2$	0.05 M	0.06	0.06
Glycyl glycine, pH 7.4	0.25 M	0.30	0.30
L-Malate	0.03 M	0.10	--
Enzyme homogenate	1-10 dilution	0.05-1.0	0.05-1.0
Distilled water		2.20	2.30
	Total Volume	3.0	3.0

[a]Sigma Chemical Co., St. Louis, Missouri.



$$\text{Citrate} + \text{ATP} + \text{CoA} \xrightarrow{Mg^{++}} \text{Acetyl CoA} + \text{Oxaloacetate} + \text{ADP} + \text{Pi}$$

$$\text{Oxaloacetate} + \text{NADH} \rightarrow \text{malate} + \text{NAD}^+$$



$$\text{L-malate}^- + \text{NADP}^+ \xrightarrow{Mn^{++}} \text{Pyruvate} + \text{NADPH} + CO_2 \quad .$$

The decrease in optical density at 340 mu produced by the oxidation of NADH over a 5-minute interval was used as an index of enzyme activity. A blank prepared without added ATP, CoA and potassium citrate was determined with each sample. The concentration and volume of components in the assay system are given in Table 8. Since both disodium ATP and potassium citrate are acid in solution, the buffering capacity of the Tris buffer is likely to be exceeded. Therefore both ATP and CoA were neutralized to pH 7.3 prior to being added to the reaction mixture.

Protein determination

Protein in both liver and adipose tissue supernatants was determined by the method of Lowry, Rosebrough, Farr and Randall (1951). The protein assay was based upon the tyrosine and tryptophan content of the protein. The protein was first reacted with 0.5 percent $CuSO_4 \cdot 5H_2O$ in 1 percent potassium tartrate[1] to form a protein-copper complex. The

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[1]Solution A: 1 ml $CuSO_4$ and 1 ml K-tartrate diluted to 100 ml with a solution of 2 percent Na_2CO_3 in 0.1 N NaOH.

Table 8. Concentration and volume of reagents used for a single assay of citrate-cleavage enzyme

Reagent[a]	Concentration	Volume/cuvette	
		Sample (ml)	Blank (ml)
Tris buffer, pH 7.3	0.1 M	0.1	0.1
$MgCl_2$	0.1 M	0.1	0.1
Potassium citrate	0.2 M	0.1	--
CoA	10 mg/ml	0.03	--
Mercaptoethanol	0.1 M	0.1	0.1
NADH	2.5 mg/ml	0.05	0.05
Malic dehydrogenase	10,000 units/ml	0.05	0.05
ATP	0.1 M	0.05	--
Enzyme homogenate		0.1-0.3	0.1-0.2
Distilled water		0.32	0.50
	Total Volume	1.0	1.0

[a]Sigma Chemical Co., St. Louis, Missouri.

complex then reduced the phosphomolybdic-phosphotungstic reagent[1] giving the final color development. The optical density of the unknown samples was determined at 660 mu and the protein content based upon a standard curve.[2] A blank

[1]Solution B: "Folin-Ciocalteu" phenol reagent.

[2]A standard curve was prepared from a stock protein solution containing 24 mg of pure crystalline bovine serum albumin per 100 ml distilled water.

of distilled water was run with each determination series.

This procedure was the method of choice since sucrose buffer has been reported (Young *et al*., 1969) to interfere with the biuret method (Layne, 1957) at low protein concentrations.

Statistical Analysis

A completely randomized design was used consisting of ten experimental groups with ten animals in each group. Two treatment groups, Group I stock animals and Group II depleted animals, served as experimental controls. The remaining eight treatment groups formed a 2 x 2 x 2 factorial experiment with two meal patterns (A), two levels of dietary fat (B), and two time periods (C) during refeeding. The analysis of variance used to evaluate the data is outlined as follows:

Source	Degrees of Freedom	
Treatments		7
A	(1)	
B	(1)	
C	(1)	
AB	(1)	
AC	(1)	
BC	(1)	
ABC	(1)	
Error		72

All treatment factors were considered fixed and tested against the error mean square.

A second analysis of the data was computed involving

all ten treatment groups to allow comparisons between experimental controls I and II and with the factorial combination of treatments.

To aid in making valid statistical comparisons and statements of probability, an Arc Sin^{-1} transformation was made on the percentage fatty acid data. Little difference appeared in the analysis of variance using the Arc Sin^{-1} transformation as compared to using the recorded percentage data. Therefore, the analysis and all comparisons are made using the percentage means.

RESULTS AND DISCUSSION

The present study was conducted with two primary interests: 1) evaluation of altered meal patterns on specific aspects of lipid metabolism in adult rats recovering from chronic malnutrition, and 2) the effect of an extended refeeding period on hepatic and epididymal tissue response to variations in the dietary energy source. Three variables are operant in this study: 1) meal patterns, either *ad libitum* or 8-16 (8-hour meal, 16-hour fast); 2) level of dietary fat, either 0 or 20%; and 3) length of refeeding period, either 10 or 30 days. The main effect of each variable as well as limitations resulting from interaction effects between variables will be discussed in relation to the parameters measured. The effect of these three variables was assessed by measuring a) hepatic and epididymal lipid, b) fatty acid profile of hepatic phospholipid and non-phospholipid fractions and epididymal lipid, c) serum and hepatic cholesterol, and d) hepatic and epididymal enzyme activities (combined hexose monophosphate shunt dehydrogenases, NADP-malate dehydrogenase, and citrate-cleavage enzyme).

Food Consumption and Body Weights

Period 1: Period 1 was a 2-phase period of depletion in which adult male rats were depleted of body fat and nitrogen stores. During Period 1a rats received a fat-free,

protein-free diet (Diet OF,OP) for 8 hours a day followed by a 16-hour fast (8-16 meal pattern) until body weight was reduced from approximately 475 to 400 g. Severe caloric restruction was imposed during Period 1b by feeding only 5 g of diet OF,OP daily until their body weight was reduced to at least 300 g. The mean total length of the depletion period for each experimental group ranged from 33 to 38 days (Table 9). This is approximately 20 days shorter than the length of the depletion period reported in previous studies in this laboratory (Stadler, 1969; McAtee, 1970). This would be expected since the 8-16 meal pattern reduced the food intake during Period 1a to approximately 13 g per day compared to the _ad libitum_ intake of approximately 17-18 g per day (Stadler, 1969; McAtee, 1970). Further food restriction during Period 1b resulted in a 28% increase in the rate of weight loss per day. The mean rate of weight loss for Period 1a and 1b was 4.6 and 5.9 g/day respectively. The length of time each animal remained on the dietary regimen of Period 1 varied with individual rates of weight loss.

Period 2: The meal pattern had a significant effect ($p < .01$) on food intake. _Ad libitum_ fed animals (Groups III, IV, VII, VIII; Table 10) consumed an average of 79 kcal/day compared with an average of 67 kcal/day for the 8-16 fed rats (Groups V, VI, IX, X). For both 10 day and 30 day

Table 9. Mean body weights and mean length of depletion phases during Period 1

Group	Body Weight		Days			Food intake[a]
	Initial gm	Final gm	Phase 1a	Phase 1b	Total	Phase 1a gm/day
II	471±3[b]	296±2	16±0.8	17±1.2	33±2.0	13.8±0.5
III	470±4	299±1	18±2.1	20±1.3	38±2.9	12.8±0.9
IV	477±4	298±1	19±3.0	16±1.9	35±4.4	12.6±1.2
V	473±2	294±3	19±1.5	18±2.0	37±3.3	13.0±0.7
VI	473±2	298±1	19±2.8	17±2.3	37±4.9	13.9±0.9
VII	471±4	299±1	17±2.1	20±1.7	37±3.4	12.6±0.8
VIII	478±3	298±1	19±2.2	15±1.1	34±3.1	14.0±1.1
IX	470±3	298±1	16±1.6	18±1.4	34±2.2	13.5±0.7
X	471±3	298±1	14±1.2	22±1.9	35±2.6	11.9±1.1

[a]Intake for phase 1b was 5 g/day, divided into 2 feedings 8 hours apart.

[b]Mean ± standard error.

Table 10. Weight gain, energy intake and food efficiency for total refeeding period

Group	Diet	Refeeding Period Days	Body Weight: Initial g	Depleted g	Refed g	Gain/Day g	% Recovery g	Food Intake: g/day	kcal/day	Food Efficiency g gain / 100 kcal
I Stock	stock	0		--	--	--	--	--	--	--
II Depleted	OF,OP	0	471	296	--	--	--	--	--	--
III *Ad Libitum*	OF,P	10	470	299	365	6.6	78	20	75	8.9
IV *Ad Libitum*	OF,P	30	477	298	417	4.0	87	20	74	5.4
V 8-16	OF,P	10	473	294	335	4.1	71	15	55	7.4
VI 8-16	OF,P	30	473	298	383	2.8	81	16	61	4.6
VII *Ad Libitum*	20F,P	10	471	299	380	8.1	81	19	87	9.3
VIII *Ad Libitum*	20F,P	30	478	298	434	4.5	91	17	81	5.6
IX 8-16	20F,P	10	470	298	364	6.6	77	17	78	8.5
X 8-16	20F,P	30	471	298	431	4.4	92	16	74	6.0
Factorial standard error			±3	±1	±5	±0.3		±0.7	±3	±0.2

refeeding periods meal-fed animals on the low-fat diet (Groups V, VI) consumed about 75% as much food in 8 hours as did *ad libitum* fed animals in 24 hours (Figure 1). A similar reduction in food intake as a result of meal feeding has been observed in other laboratories (Beaton *et al.*, 1964; Stevenson *et al.*, 1964; Leveille and Hanson, 1965a). However, the inclusion of dietary fat tended to modify ($p < .05$) the difference in food intake between the two meal patterns (Figure 2).

The food efficiency ratio (Figure 3) was influenced significantly by interaction between meal pattern and level of dietary fat ($p < .05$) as well as meal pattern and length of refeeding period ($p < .01$). For those animals eating *ad libitum* the inclusion of dietary fat did not significantly increase the food efficiency (Groups III vs. VII; IV vs. VIII, Table 10). In contrast, the 8-16 fed animals demonstrated a significant increase ($p < .01$) in food efficiency with the inclusion of dietary fat (Groups V vs. IX; VI vs. X, Table 10). With an extended refeeding period the food efficiency ratio decreased as did the difference between meal patterns ($p < .01$, Figure 3). Stadler (1969) likewise reported a decrease in food efficiency with time for *ad libitum* fed animals on both high-fat and low-fat diets. In general the mean food efficiency was greater ($p < .01$) in *ad libitum* fed animals (Groups III, IV, VII, VIII; Table 10,

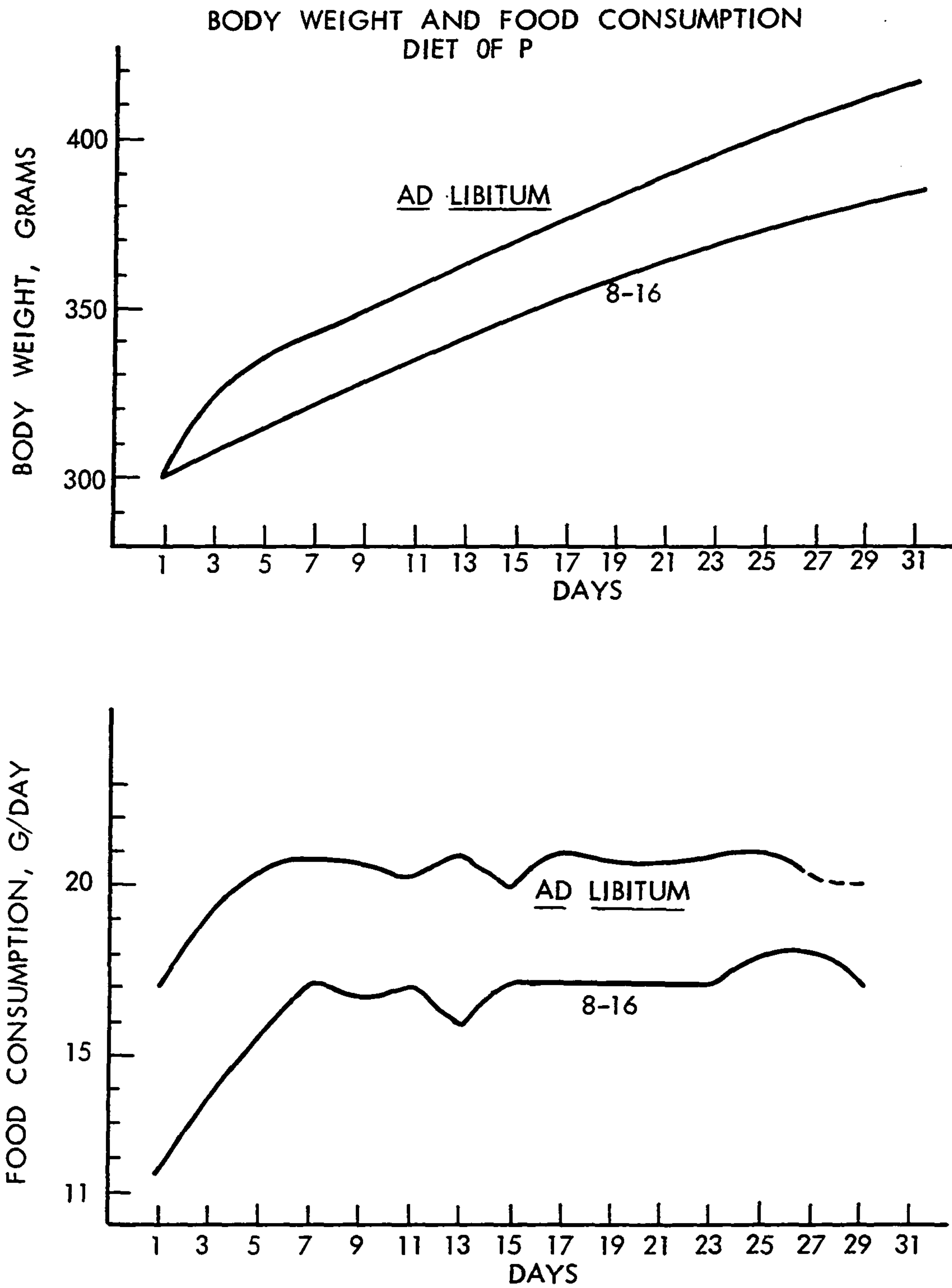
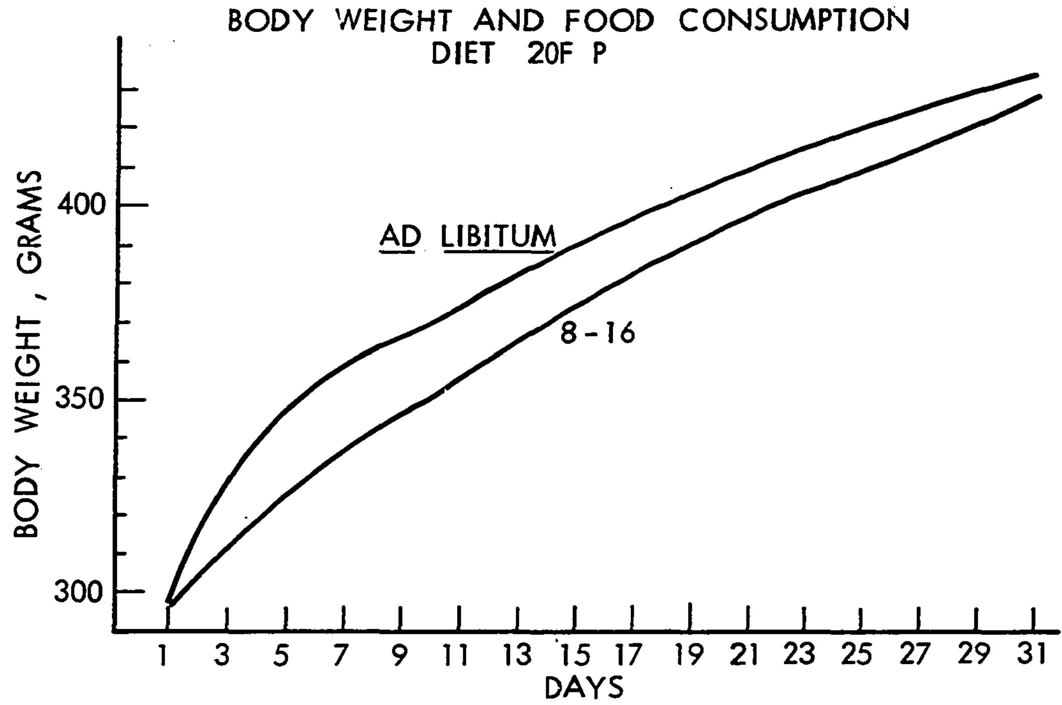


Figure 1. Body weight and food consumption of *ad libitum* (Group IV) and 8-16 (Group VI) fed rats on the low-fat diet during 30 days refeeding.



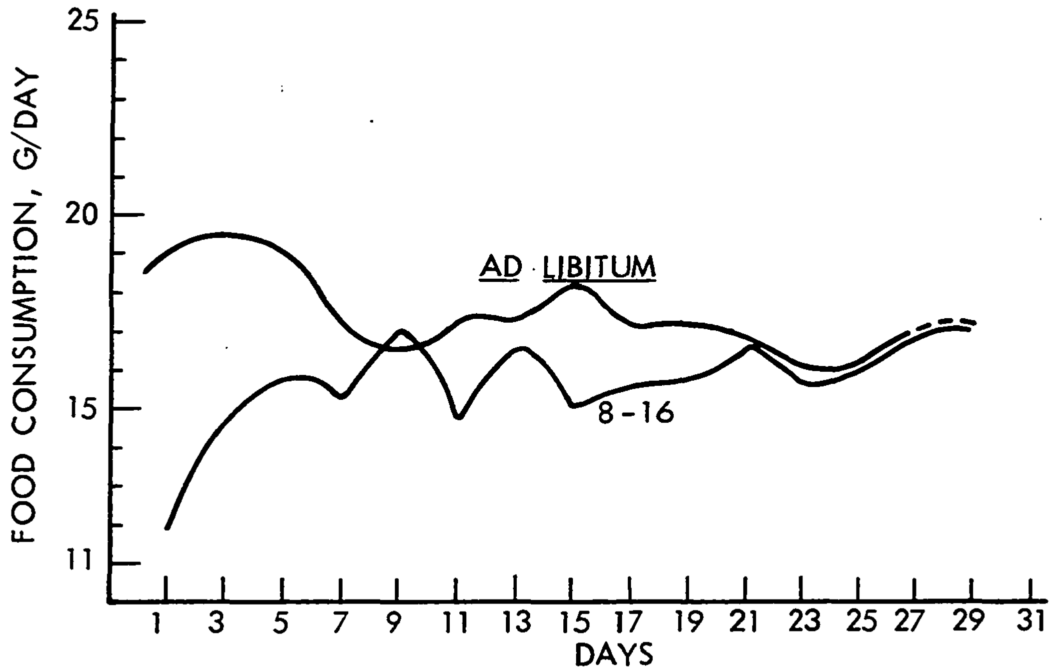


Figure 2. Body weight and food consumption of ad libitum (Group VIII) and 8-16 (Group X) fed rats on the high-fat diet during 30 days refeeding.

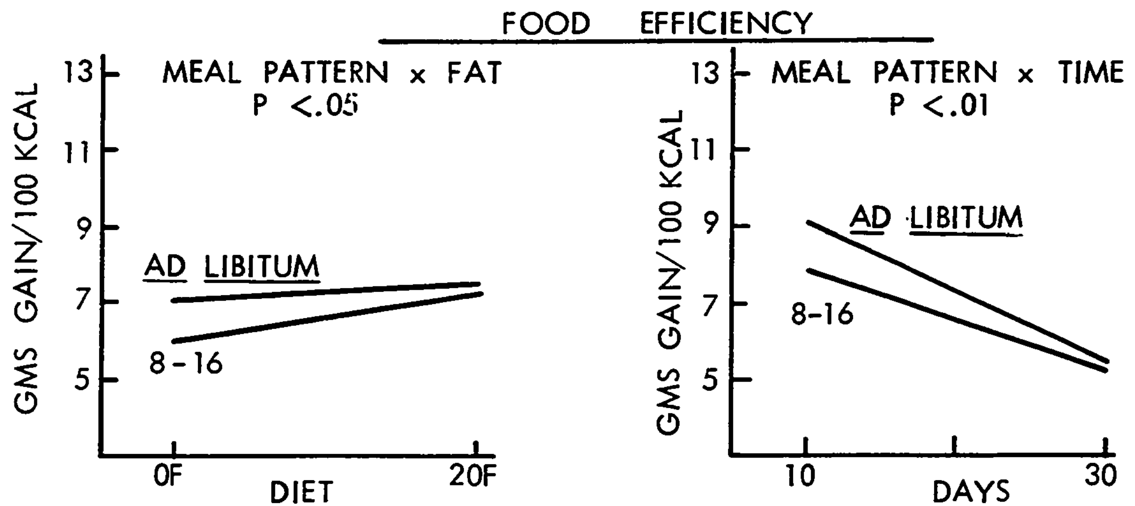


Figure 3. Influence of interactions between meal pattern, fat, and time on food efficiency.

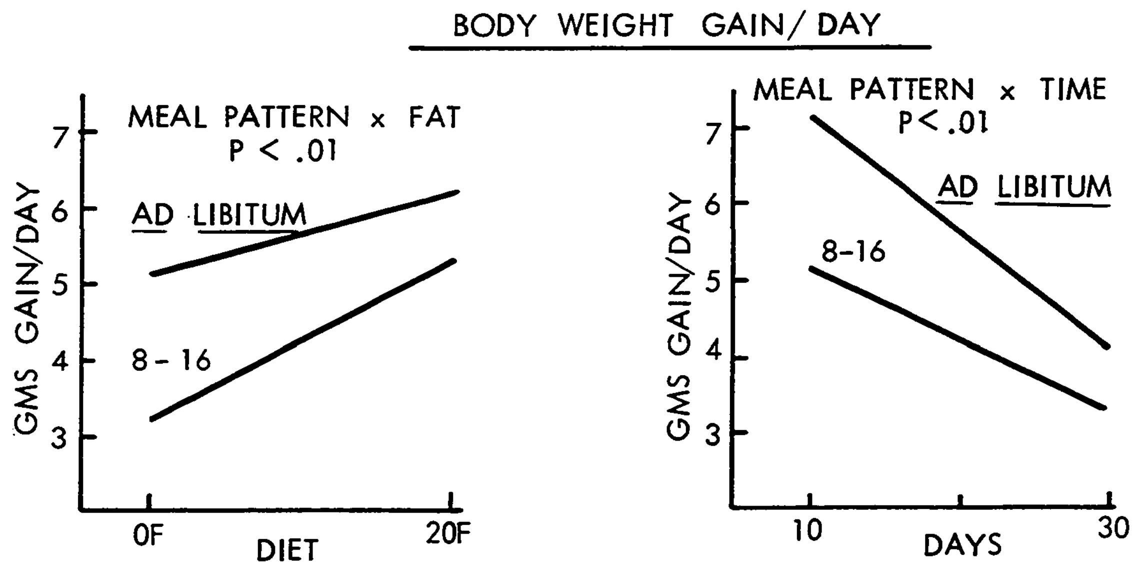


Figure 4. Influence of interactions between meal pattern, fat, and time on body weight gain per day.

Figure 3) than in 8-16 fed rats (Groups V, VI, IX, X; Table 10, Figure 3). An exception however, was the increased food efficiency of the 8-16 compared to *ad libitum* fed rats on the high-fat diet after 30 days of refeeding.

Similarly body weight gain per day was influenced by significant interactions between the variables of dietary fat content, meal pattern, and length of refeeding period (Figure 4). *Ad libitum* fed animals had a greater mean body weight gain per day compared with the 8-16 fed groups on both diets and at both time periods ($p < .01$). The interaction between meal pattern and length of the refeeding period (Figure 4) indicates that the *ad libitum* meal pattern had a greater effect on body weight gain per day during the first 10 days of refeeding than after 30 days refeeding ($p < .01$) compared to the 8-16 meal pattern. Similarly as the length of the refeeding period increased from 10 to 30 days the effect of dietary treatment on body weight gain per day diminished ($p < .05$).

As indicated the difference between *ad libitum* and 8-16 fed rats decreased with the extended refeeding period for both the food efficiency ratio and body weight gain per day (Figures 3 and 4). The rapid rate of body weight gain for *ad libitum* fed rats during the first 5 days refeeding may possibly "mask" the effects of the 8-16 feeding pattern on food efficiency and body weight gain per day.

Table 11. Weight gain, energy intake and food efficiency disregarding the first 5 days of the refeeding period

Group	Diet	Refeeding Period Days	Body Weight			Food Intake		Food Efficiency
			Initial g	Final g	Gain/day g	g/day	kcal/day	g gain / 100 kcal
IV *Ad Libitum*	OF,P	25	336	417	3.2	20	74	4.3
VI 8-16	OF,P	25	317	383	2.6	17	63	4.2
VIII *Ad Libitum*	2OF,P	25	349	434	3.4	17	80	4.2
X 8-16	2OF,P	25	326	431	4.2	16	75	5.6

By considering only the last 25 days of the refeeding period, the rate of body weight gain was not significantly different for *ad libitum* or 8-16 fed rats on either diet (Table 11). The food efficiency ratio was essentially the same for both *ad libitum* and 8-16 fed rats on the low-fat diet. The inclusion of dietary fat stimulated a greater ($p < .01$) increase in food efficiency of 8-16 fed rats compared to those eating *ad libitum*.

In agreement with earlier findings in this laboratory (Stadler, 1969; Kopec, 1969; McAtee, 1970), the inclusion of dietary fat resulted in a greater ($p < .01$) total body weight gain compared to rats receiving the low-fat diet (Table 10). After 30 days of *ad libitum* refeeding the animals on the high-fat diet had recovered 91% of their initial body weight compared to 87% for the low-fat groups (Table 10). In comparison the 8-16 fed animals after 30 days of refeeding with diet 20F,P regained 92% of their initial body weight while only 81% was recovered for those animals on the OF,P diet.

Liver

Tissue weights

During depletion liver mass declined from the average stock control value of 11.7 to 5.8 g for the depleted Group II (Table 12). A decrease of about 50% in liver weight

Table 12. Hepatic tissue weights, total hepatic lipid, non-phospholipid and phospholipid

Group	Diet	Refeeding Period Days	Liver	Liver: Total Lipid		Non-phospholipid		Phospholipid	
			g	mg	% of liver	mg	% of liver	mg	% of liver
I Stock	stock	0	11.7	443	3.8	119	1.0	324	2.8
II Depleted	OF,OP	0	5.8	193	3.3	41	0.7	152	2.6
III *Ad Libitum*	OF,P	10	9.0	504	5.7	275	3.1	229	2.6
IV *Ad Libitum*	OF,P	30	9.9	539	5.5	291	3.0	248	2.5
V 8-16	OF,P	10	8.5	344	4.0	158	1.8	186	2.2
VI 8-16	OF,P	30	9.3	428	4.7	208	2.3	220	2.4
VII *Ad Libitum*	20F,P	10	9.1	494	5.4	252	2.8	242	2.7
VIII *Ad Libitum*	20F,P	30	9.8	731	7.5	474	4.9	258	2.7
IX 8-16	20F,P	10	9.3	384	4.2	154	1.6	232	2.5
X 8-16	20F,P	30	9.8	503	5.2	274	2.8	230	2.4
Factorial standard error			±0.3	±43	±0.5	±39	±0.4	±4	±<.1

has been observed previously in this laboratory under similar conditions (Noble, 1967; Stadler, 1969; McAtee, 1970). Liver weight increased from the depleted weight; 84% of the initial hepatic weight was restored after 30 days refeeding. Earlier work in this laboratory by Stadler (1969) indicated that between 42 and 49% of the original weight was restored during the first 24-hours of realimentation. Hepatic tissue weight was not significantly affected by either the level of dietary fat or the meal pattern but only by the length of the refeeding period ($p < .01$).

Hepatic lipids

Total lipid During dietary restriction the large decrease in lipid weight was accompanied by a concomitant decrease in liver lipid (Table 12) consequently the percentage of liver lipid remained about the same. Refeeding with either diet resulted in a significant ($p < .01$) increase in total hepatic lipid. After 30 days refeeding, the absolute amount of hepatic lipid was greater ($p < .05$) in animals receiving dietary fat compared to those on the low-fat diet. As the length of the refeeding period increased the percent of liver lipid increased for both diets and for both meal patterns ($p < .01$). An exception is the relatively constant concentration of lipid for *ad libitum* fed animals receiving the low-fat diet. *Ad libitum* fed animals on both diets had a

greater percentage of hepatic lipid than the 8-16 fed animals for any given time period ($p < .01$). A tendency toward fatty liver was observed for ad libitum fed animals on both OF,P and 20F,P diets. With similarly treated rats, McAtee (1970) observed that as the level of dietary protein increased from low (4%) to excessive (36%) levels the mild fatty accumulation in the liver disappeared.

Non-phospholipids The non-phospholipid fraction of the liver which consists predominantly of triglycerides, was the lipid fraction most affected by changes in total hepatic lipid (Table 12). During dietary restriction the non-phospholipids decreased 66% from a stock control value of 119 mg compared with a 53% decrease in the phospholipid fraction. After 10 days of refeeding with diet OF,P there was a 570% increase above the depletion value (41 to 275 mg) in the non-phospholipids while the phospholipid fraction increased by only 50% (152 to 229 mg). The inclusion of dietary fat resulted in a significant increase ($p < .05$) in the non-phospholipid weight after 30 days refeeding. This would be expected since the total hepatic lipid, as stated earlier, influenced by dietary fat ($p < .05$). The percentage total liver lipid ($p < .05$) and percentage non-phospholipid ($p < .01$) were affected by interaction between level of dietary fat and length of refeeding period (Figure 5). Compared with rats refed the low-fat diet, the inclusion of

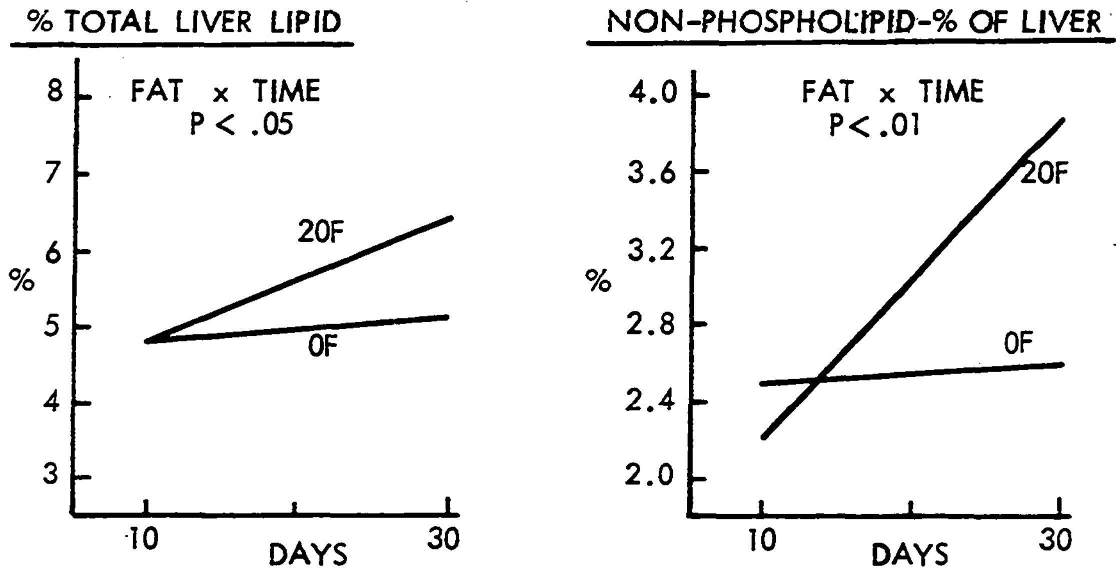


Figure 5. Influence of interactions between fat and time on percentage total liver lipid and non-phospholipid.

dietary fat resulted in a greater increase in the total hepatic lipid and non-phospholipid concentration during the 30-day refeeding period. The non-phospholipid fraction responded to the variables of meal pattern ($p < .01$) and time ($p < .01$) in the same fashion as the total hepatic lipid.

Phospholipid In contrast to non-phospholipids, the concentration of phospholipids remained relatively constant throughout the experiment (Table 12). The stability of this lipid fraction to dietary manipulation has been observed previously in this laboratory (Stadler, 1969; McAtee, 1970) as well as by other investigators (Morin and Alfin-Slater, 1964; Connelan and Masters, 1965). The *ad libitum* fed rats

had a slightly greater ($p < .05$) phospholipid concentration than the 8-16 fed animals. This would be expected since the meal pattern had a similar effect on the absolute amount of lipid deposited in the liver.

Hepatic fatty acid composition

Characteristic fatty acid patterns were observed for both hepatic non-phospholipids and phospholipids (Tables 13 and 14). For example, myristic acid (C14) was completely absent in the phospholipids of all experimental groups but was present in low concentrations (0.7 to 1.6% in non-phospholipids. Palmitic acid (C16) appeared as a major constituent in both lipid fractions; however, it was generally greater in non-phospholipids than in phospholipids (means 35.6 vs. 25.2%). Likewise palmitoleic acid (C16:), oleic acid (C18:), and linoleic acid (C18:2) were present in greater concentrations in non-phospholipids than in phospholipids. However, when an exogenous source of linoleic acid is absent or low as in the case of the low-fat (OF,P) diet, the concentration of linoleic acid (C18:2) can be reversed so that the linoleic acid concentration is greater in the phospholipids (Groups III to VI) than in the non-phospholipids (means 7.9 vs. 5.4%). Of interest was the apparent lack of palmitoleic acid in the phospholipids of rats receiving the high-fat (20F,P) diet. Stearic acid

Table 13. Fatty acid composition of hepatic non-phospholipids

Group	Diet	Days Refed	% Fatty Acids								16 + 16:1 + 18:1	18:2 + 20:4
			14	16	16:1	18	18:1	18:2	18:3	20:4		
I Stock	stock	0	0.7	26.8	2.0	4.2	19.5	36.0	3.8	7.1	48	43
II Depleted	OF,OP	0	1.0	43.5	2.5	8.2	19.0	19.0	0.7	0.7	65	20
III *Ad Libitum*	OF,P	10	1.2	41.0	7.6	3.2	39.1	7.8	trace	trace	88	8
IV *Ad Libitum*	OF,P	30	1.1	41.8	6.8	2.9	43.1	4.2	trace	trace	92	4
V 8-16	OF,P	10	1.6	42.7	7.6	3.6	39.8	4.7	trace	trace	90	5
VI 8-16	OF,P	30	1.3	39.5	6.8	2.7	44.9	4.7	trace	trace	90	5
										mean	90	5
VII *Ad Libitum*	20F,P	10	0.7	30.8	2.0	2.9	20.2	37.8	1.9	3.8	53	42
VIII *Ad Libitum*	20F,P	30	0.5	27.9	0.8	2.9	18.1	43.8	2.1	3.9	47	48
IX 8-16	20F,P	10	0.8	31.9	2.1	3.3	20.1	37.0	1.8	2.9	54	40
X 8-16	20F,P	30	0.6	29.8	1.7	2.9	20.6	39.6	1.5	3.2	52	43
										mean	52	43
Factorial standard error			±<.1	±<.1	±<.1	±<.1	±<.1	±<.2	±<.1	±<.1		

Table 14. Fatty acid composition of hepatic phospholipids

Group	Diet	Days Refed	% Fatty Acids							16 + 16:1 + 18:1	18:2 + 20:4 + >20:4
			16	16:1	18	18:1	18:2	20:4	>20:4		
I Stock	stock	0	18.1	0.5	25.4	8.5	13.0	27.8	6.8	27	48
II Deplt.	OF,OP	0	24.2	0.4	21.7	7.8	14.9	18.0	13.0	32	46
III _Ad Lib_.	OF,P	10	28.3	2.5	21.4	10.5	8.2	15.5	13.6	41	37
IV _Ad Lib_.	OF,P	30	28.5	2.5	20.6	11.3	8.0	13.6	15.5	42	37
V 8-16	OF,P	10	29.3	3.6	18.1	12.4	7.6	13.1	15.7	45	36
VI 8-16	OF,P	30	30.7	2.9	19.8	11.8	8.1	12.6	14.2	45	35
									mean	44	36
VII _Ad Lib_.	20F,P	10	23.1	trace	24.4	5.1	12.2	23.3	11.6	28	47
VIII _Ad Lib_.	20F,P	30	23.1	trace	24.0	4.9	13.2	22.6	12.3	28	48
IX 8-16	20F,P	10	23.6	trace	22.8	5.3	14.2	22.2	12.1	29	48
X 8-16	20F,P	30	23.2	trace	24.4	5.3	13.0	22.4	11.7	28	47
									mean	28	48
Factorial standard error			±<.1	±<.1	±<.1	±<.1	±<.1	±<.1	±<.1		

(C18), arachidonic acid (C20:4) and polyunsaturated fatty acids longer than arachidonic (>C20:4) were present in greater concentration in hepatic phospholipids than in non-phospholipids. In fact, the fatty acid >C20:4 was apparently absent in non-phospholipids. The gross differences observed in the fatty acid profile of both hepatic non-phospholipids and phospholipids in this study are in agreement with those observed by Stadler (1969) in this laboratory and by other investigators (Morin and Alfin-Slater, 1964; Connellan and Masters, 1965; Glende, Jr. and Cornatzer, 1965).

In addition to the characteristic make up of each hepatic lipid fraction, the variables meal pattern, dietary fat level, and time influenced the fatty acid profile. Compared with animals refed *ad libitum*, the 8-16 refed rats had a significant increase in selected saturated and monounsaturated fatty acids. Myristic acid was the only fatty acid in the non-phospholipid fraction which was increased as a result of the 8-16 meal pattern (Table 13), while an increase in palmitic ($p < .05$), palmitoleic ($p < .01$) and oleic ($p < .05$) acids occurred in the phospholipid fraction (Table 14). A decrease occurred in the case of stearic acid ($p < .01$). These slight shifts in fatty acid profile may possibly suggest an enhanced *de novo* fatty acid synthesis in the 8-16 fed rat. Although these changes were statistically significant, they were not of sufficient magnitude

to be considered of physiological importance. The remaining discussion of the effects of fat level and time on the fatty acid profile of both hepatic lipid fractions is presented as if the values for ad libitum and 8-16 fed rats were combined.

Non-phospholipids Dietary restriction resulted in significant shifts in the fatty acid profile of hepatic non-phospholipids. Palmitic and stearic acids were greatly ($p < .01$) increased while the relative concentrations of linoleic and linolenic acids decreased ($p < .01$). Although similar directional shifts in fatty acid profile have been observed in this laboratory (Kopec, 1969; McAtee, 1970), the changes in this study were more evident. Palmitic acid continued to remain higher in all treatment groups compared with comparable treatment groups in previous experiments e.g. 36 vs. 22% (Stadler, 1969; McAtee, 1970). The selective retention of palmitic acid may possibly be related to the method of depletion used in this study. The 8-16 meal pattern used to deplete rats in this study differed from the ad libitum manner of depletion used in previous studies in this laboratory (Stadler, 1969; Kopec, 1969; McAtee, 1970).

Refeeding with diet OF,P greatly elevated the relative concentrations of palmitic (C16), palmitoleic (C16:1), and oleic (C18:1) acids above control values ($p < .01$). The combined value for these fatty acids averaged 89.9% (avg.

of Groups III-VI) compared with 48.3% for stock controls (Table 13, Figure 6). In contrast the percentage of linoleic acid decreased from 36.0% to 7.8% (Group III) with a further decline to 4.2% after 30 days refeeding. The relative concentration of palmitic (C16) remained fairly stable with time, however, palmitoleic (C16:1) acid decreased ($p < .01$) and oleic (C18:1) acid increased ($p < .05$). These alterations in fatty acid pattern are considered indicative of _de novo_ fatty acid biosynthesis via a system of desaturation and elongation enzymes (Wakil, 1961; Vagelos, 1964). Since palmitic (C16) and palmitoleic (C16:1) were elevated in hepatic lipids of rats fed a low-fat diet, it would appear that the _in vivo_ conversion of palmitic (C16) to oleic (C18:) acids may have been accomplished by palmitic acid desaturation and elongation.

Very little difference was observed in the fatty acid profile of rats receiving the 20F,P diet compared to stock controls (Figure 6). This would be expected since the fatty acid composition of the corn oil diet resembled closely that of the stock diet (Table 3). The combined values for palmitic (C16), palmitoleic (C16:1) and oleic (18:1) acids averaged 51.5% compared with 48.3% for stock controls (Group I).

Phospholipids The hepatic phospholipids are predominately structural lipids, and little alteration in

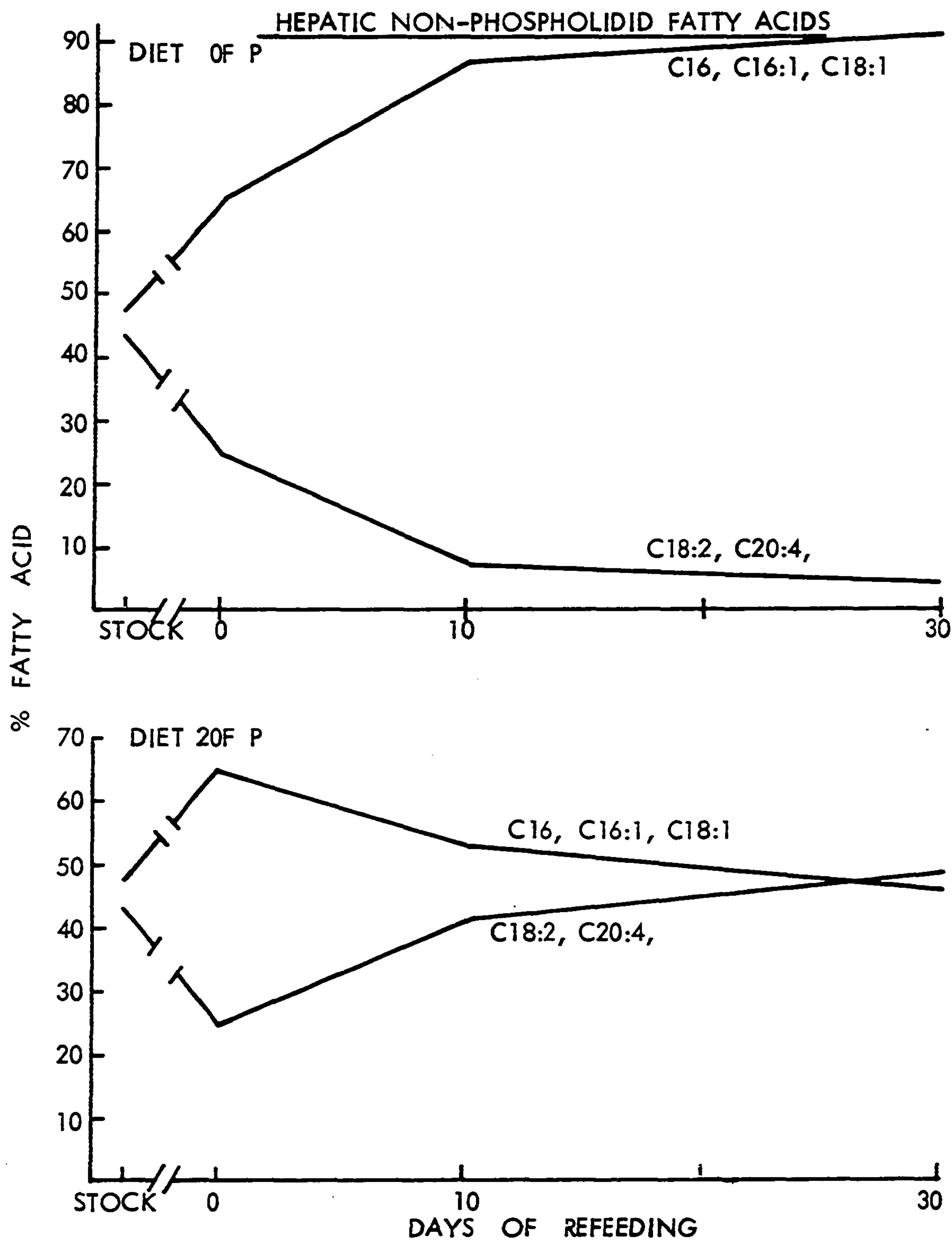


Figure 6. Response of hepatic non-phospholipid fatty acids with time in *ad libitum* fed rats.

fatty acid profile as a result of dietary restriction would be expected (Table 14, Figure 7). A decrease occurred, however, in arachidonic acid (C20:4), but this was apparently off-set by an increase in the C>20:4 fraction. Although shifts in the polyunsaturated fatty acids occurred, the combined percentages of all three polyunsaturated fatty acids (C18:2; C20:4; >C20:4) remained relatively constant at 46 (Group II) to 48% (Group I).

As in the case of the hepatic non-phospholipids the variable of dietary fat had a significant ($p < .01$) effect on the hepatic phospholipid fatty acid pattern. The phospholipid fatty acid profile of rats fed the low-fat diet showed trends similar to those observed in the non-phospholipid fraction, but the changes were much less marked (Figures 6 and 7). The combined percentage of palmitic (C16), palmitoleic (C16:1) and oleic (C18:1) acids was 43.6% for phospholipids compared to 90.2% for non-phospholipids (Table 14). Linoleic (C18:2) and arachidonic (C20:4) declined ($p < .01$) and the fraction >C20:4 increased ($p < .01$) in comparison to stock control (Group I) values. The decrease in the relative concentration of linoleic acid in both hepatic lipid fractions may reflect an absolute decrease or it may indicate a dilution effect due to the rapid synthesis of other fatty acids (e.g. C16, C16:1, C18:1). The inclusion of dietary fat resulted in little change in the overall

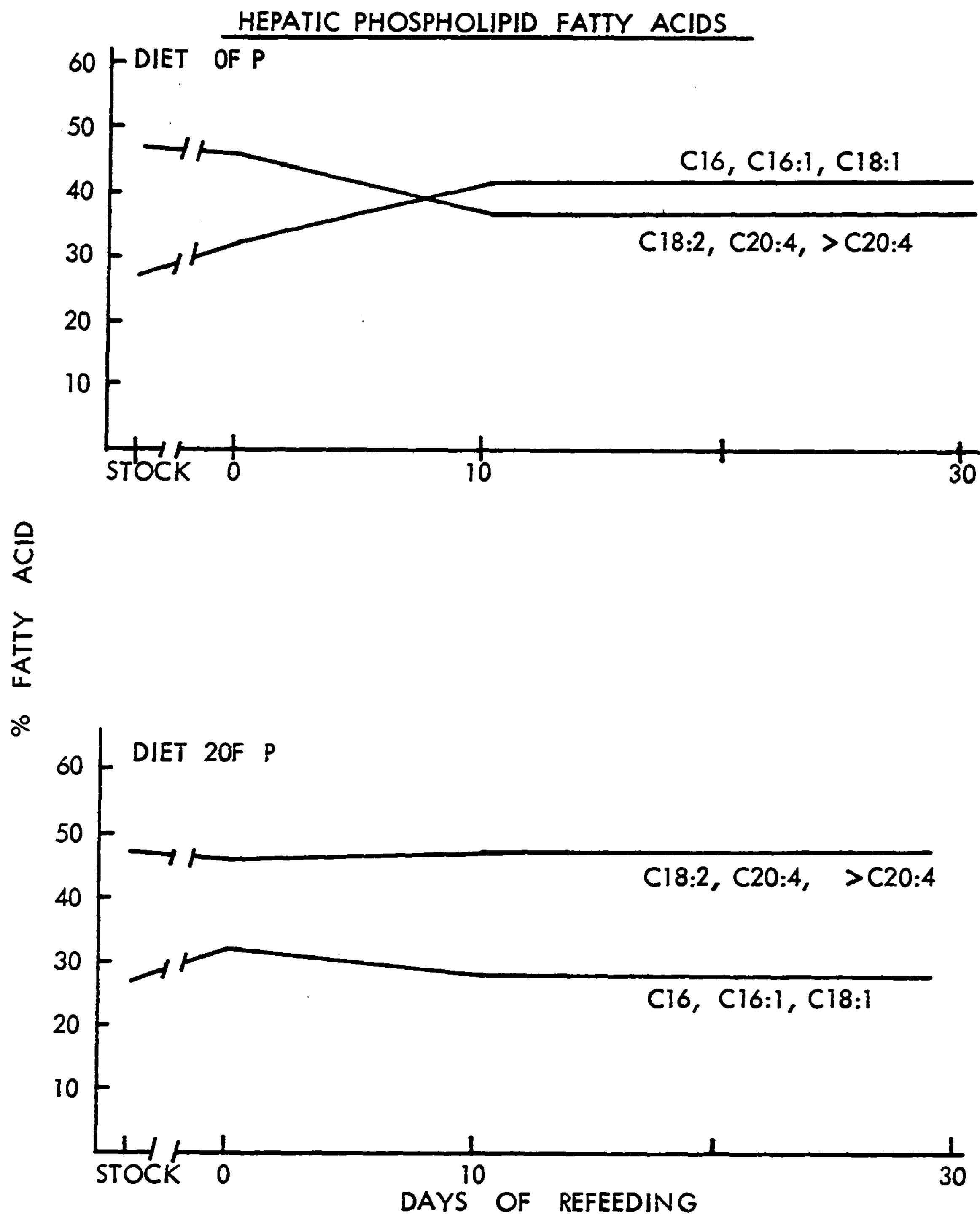


Figure 7. Response of hepatic phospholipid fatty acids with time in ad libitum fed rats.

phospholipid fatty acid pattern compared with the fatty acid pattern of stock controls (Table 14, Figure 7). The combined average percentage of palmitic (C16), palmitoleic (C16:1) and oleic (C18:1) acids was 28.4% compared to 27.1% for stock controls. Similarly the combined average of the polyunsaturated fatty acids (C18:2, C20:4, >C20:4) for Groups VII through X was identical with that for stock controls, 48%.

The length of the refeeding period did not significantly affect the fatty acid pattern of the hepatic phospholipids (Figure 7). In contrast, time ($p < .01$) affected the saturated and monounsaturated fatty acids of the non-phospholipid fractions (Figure 6). In earlier work in this laboratory Stadler (1969) observed that the greatest alteration in fatty acid pattern of both hepatic lipid fractions occurred within 24 hours after refeeding. After the first day only a slight change was seen in the non-phospholipid fatty acids, and the phospholipids remained stable through the 20 day refeeding period. The alterations in the fatty acid profile of both hepatic lipid fractions in this study are in agreement with those reported by Stadler (1969) and McAtee (1970) for similarly treated rats in this laboratory.

Selected hepatic lipogenic enzymes

Several enzymatic adaptations occur in liver and adipose tissue of young meal-fed rats which permit an enhanced rate

of lipogenesis (Leveille and Hanson, 1966a; Chakrabarty and Leveille, 1968, 1969). Key lipogenic enzymes which appear to be readily adapted to the sudden influx of substrate resulting from meal eating are the hexose monophosphate shunt dehydrogenases (glucose-6-phosphate dehydrogenase, E.C. 1.1.1.49; 6-phosphogluconate dehydrogenase, E.C.1.1.1.40), "malic enzyme" (NADP - malic dehydrogenase, E.C.1.1.1.40) and citrate-cleavage enzyme (E.C.4.1.3.8). The combined activities of the hexose monophosphate shunt dehydrogenases (HMPD) and "malic enzyme" are thought to be responsible for supplying the NADPH needed for reductive lipogenesis. The acetate necessary for extramitochondrial fatty acid synthesis may be supplied by the action of citrate-cleavage enzyme. The possibility that these enzymes are altered in the mature meal-fed rat recovering from chronic protein-calorie malnutrition is considered in this study.

Previous work in this laboratory with chronically malnourished rats demonstrated the typical "overshoot" in hepatic HMPD (Thorp, 1966; Lee, 1967) and "malic enzyme" activity (McAtee, 1970) after refeeding for 3 days and 10 days with a low-fat, low-protein diet. Therefore another objective of this study was to ascertain if these changes in enzyme activity persist with an extended refeeding period. The activities of hepatic HMPD, "malic enzyme" and citrate-cleavage enzyme in response to the variables dietary

Table 15. Activities of hepatic HMP shunt dehydrogenases, "malic enzyme", and citrate-cleavage enzyme

Group	Diet	Refeeding Period	HMPD n moles of NADPH / min/mg protein	"Malic enzyme" n moles of NADPH / min/mg progein	Citrate-cleavage enzyme n moles of NAD+ / min/mg protein
I Stock	stock	0	11.2±0.6[a]	2.6±0.3	0.4±0.2
II Depleted	OF,OP	0	10.1±0.7	2.3±0.6	0.2±0.1
III *Ad Libitum*	OF,P	10	59.6±5.6	50.8±4.1	4.5±0.6
IV *Ad Libitum*	OF,P	30	20.1±1.9	29.5±5.7	1.6±0.5
V 8-16	OF,P	10	34.9±5.9	33.1±4.4	3.2±0.5
VI 8-16	OF,P	30	17.4±1.8	33.7±5.0	1.7±0.4
VII *Ad Libitum*	20F,P	10	11.2±0.9	15.6±2.5	0.7±0.2
VIII *Ad Libitum*	20F,P	30	9.6±1.1	15.0±1.7	0.8±0.1
IX 8-16	20F,P	10	10.9±0.7	11.3±1.3	1.0±0.2
X 8-16	20F,P	30	8.5±0.8	14.1±1.9	0.7±0.2
Factorial standard error			±3.1	±3.6	±0.4

[a]Mean ± standard error.

fat, meal pattern and time are presented in Table 15.

Hexose monophosphate shunt dehydrogenases (HMPD) All three variables had a highly significant effect ($p < .01$) on the combined activities of HMPD. *Ad libitum* refeeding with a low-fat diet for 10 days resulted in approximately a 6-fold increase ($p < .01$) in HMPD activity over the stock control value. After 30 days refeeding, HMPD activity had declined ($p < .01$) to about twice that of stock controls. The inclusion of dietary fat reduced HMPD activity to approximately that observed for controls ($p < .01$). Figure 8 illustrates the interaction ($p < .01$) between level of dietary fat and length of refeeding period. In contrast to the decrease in HMPD activity with time for those rats on diet OF,P, time had little influence in enzyme activity of rats receiving dietary fat. Interaction between meal pattern and level of dietary ($p < .01$), and meal pattern and time ($p < .05$) influenced HMPD activity (Figure 8). *Ad libitum* fed rats had greater ($p < .01$) HMPD activity than 8-16 fed animals. However, this difference diminished when fat was present in the diet and the refeeding period extended.

"Malic enzyme" "Malic enzyme" activity was greatly ($p < .01$) elevated over stock controls when depleted rats were refed either the low-fat or high-fat diet. However, rats fed the low-fat diet had a greater "malic enzyme" activity ($p < .01$) than did those receiving the high fat diet.

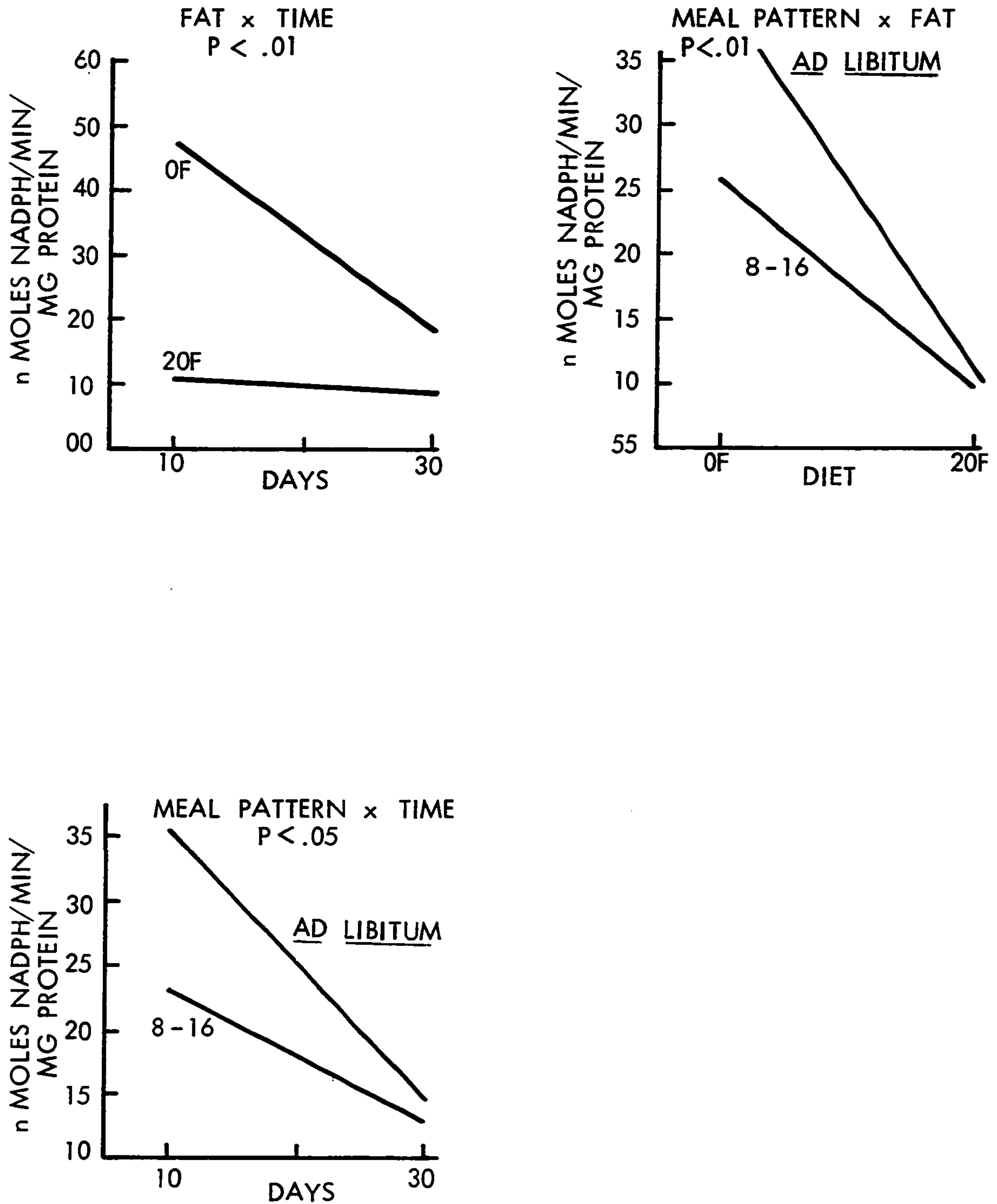


Figure 8. Influence of interactions between dietary fat level, meal pattern, and time on hepatic HMPD activity

After 10 days of ad libitum refeeding, diet OF,P produced approximately a 17-fold increase in enzyme activity above the stock control value. In comparison there was a 5-fold increase in activity for those rats on diet 20F,P.

During the refeeding period the activity of "malic enzyme" was influenced by interaction between fat level and time, and meal pattern and time ($p < .05$; Figure 9). "Malic enzyme" activity was depressed ($p < .05$) as the length of the refeeding period increased for rats receiving the low-fat diet ad libitum (Table 15). Little change occurred

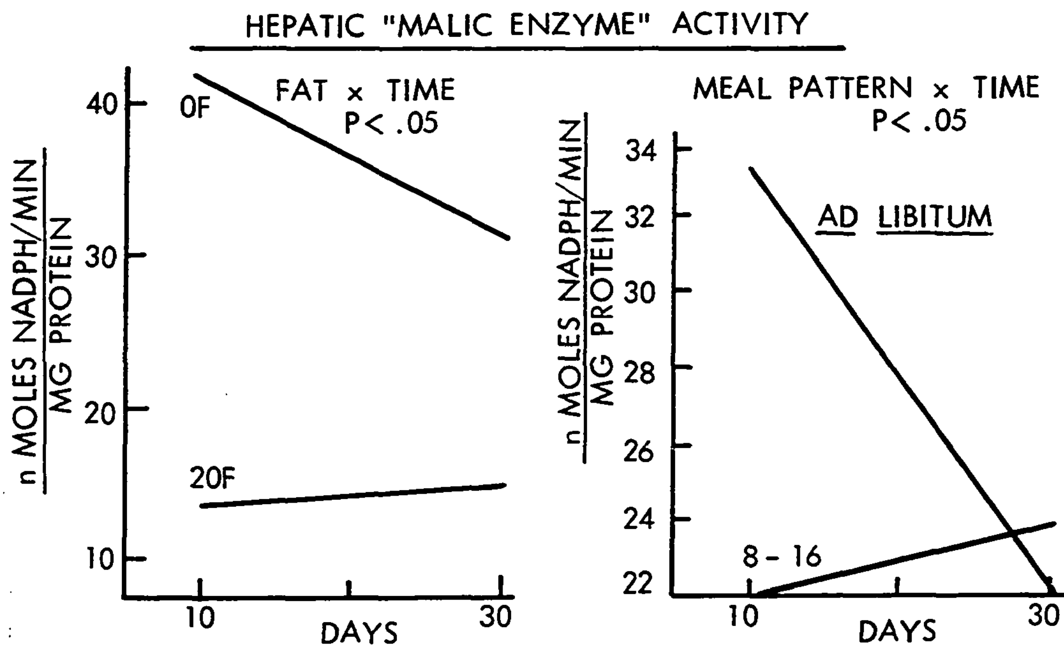


Figure 9. Influence of interactions between dietary fat level, meal pattern, and time on hepatic "malic enzyme" activity.

with time for the 8-16 fed rats. In contrast, meal fed rats receiving the high-fat diet showed a slight increase (27%) in malic activity with time, whereas ad libitum fed rats remained fairly stable. Irrespective of the level of dietary fat or meal pattern, "malic enzyme" remained significantly ($p < .01$) elevated above stock control values after 30 days of refeeding.

Citrate-cleavage enzyme As was the case for HMPD and "malic enzyme", the activity of citrate-cleavage enzyme was greater ($p < .01$) for those animals refed with diet OF,P compared with those fed diet 20F,P. After 10 days of ad libitum feeding with diet OF,P, citrate-cleavage activity was approximately 6 times greater compared to the value for rats receiving dietary fat. Interaction between the level of dietary fat and time significantly ($p < .01$) influenced the activity of citrate-cleavage enzyme (Figure 10). As the length of the refeeding period increased, enzyme activity decreased for those rats on the low-fat diet. However, even after 30 days of refeeding enzyme activity was still elevated above control values by about 4-fold. In contrast, rats receiving dietary fat showed little change in citrate-cleavage activity with time. The inclusion of dietary fat did not significantly increase citrate-cleavage enzyme activity above stock control values. In general enzyme activity was not influenced by meal pattern. An exception

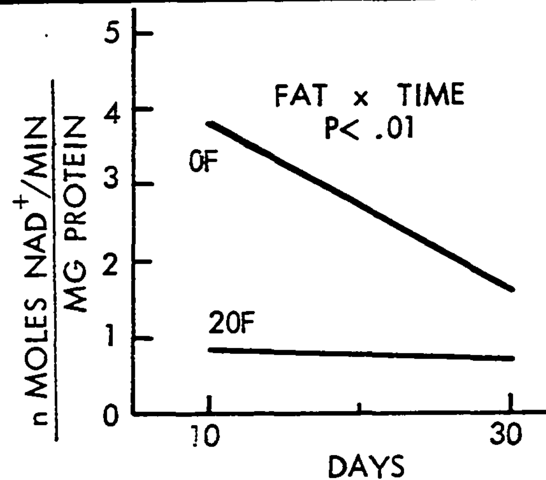


Figure 10. Influence of interaction between dietary fat level and time on hepatic citrate-cleavage enzyme activity.

however, was the increased ($p < .05$) activity of <u>ad libitum</u> fed rats on the low-fat diet after 10 days refeeding compared to 8-16 fed animals.

Epididymal Tissue

<u>Tissue weights</u>

Mean epididymal tissue weights are given in Table 16. The epididymal tissue was reduced by 80% of the initial weight during depletion from 5.4 to 1.1 g. During this period the total body weight had been reduced 37% from the initial weight. The increase in epididymal tissue weight during realimentation was influenced by an interaction between meal pattern and level of dietary fat ($p < .01$),

Table 16. Epididymal tissue weights and lipid

Group	Diet	Refeeding Period Days	Epididymal Fat Pad	Lipid	
			g	g	%
I Stock	stock	0	5.4	3.4	64
II Depleted	OF,OP	0	1.1	0.6	38
III *Ad Libitum*	OF,P	10	3.1	1.9	65
IV *Ad Libitum*	OF,P	30	3.9	2.7	69
V 8-16	OF,P	10	1.9	1.1	57
VI 8-16	OF,P	30	3.6	2.4	65
VII *Ad Libitum*	20F,P	10	3.8	2.5	66
VIII *Ad Libitum*	20F,P	30	5.0	3.4	66
IX 8-16	20F,P	10	3.5	2.1	64
X 8-16	20F,P	30	6.2	4.4	68
Factorial standard error			±0.3	±0.3	±3

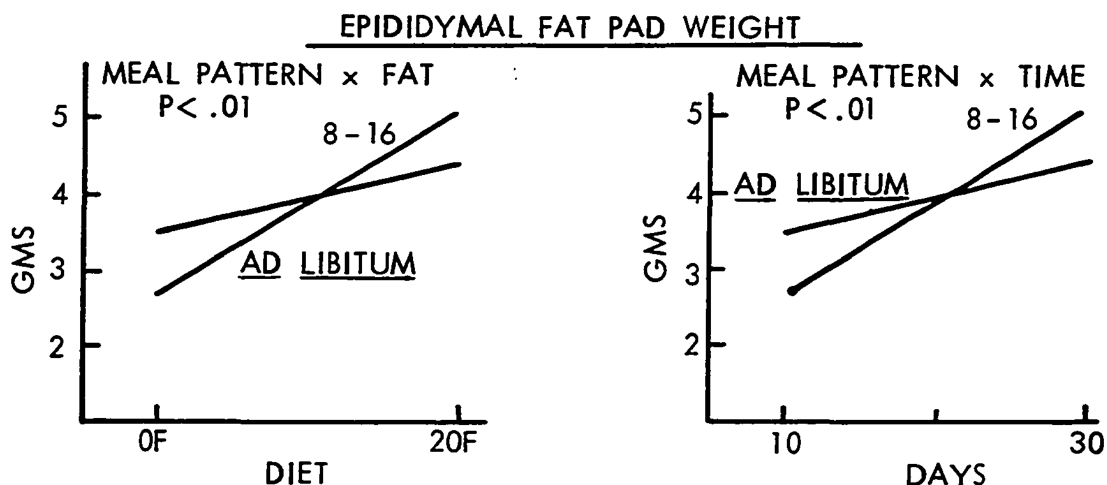


Figure 11. Influence of interactions between level of dietary fat, meal pattern, and time on epididymal fat pad weight.

and meal pattern and length of refeeding period ($p < .01$). The inclusion of dietary fat resulted in heavier epididymal fat pads ($p < .01$) compared with animals receiving the low-fat diets. The mean tissue weights for rats on the high-fat diet were 4.4 and 5.1 g for the *ad libitum* and 8-16 meal patterns respectively, compared with 2.6 and 3.5 g respectively on the low-fat diet. The epididymal tissue weight of the 8-16 fed groups responded more to the high-fat diet than those on the *ad libitum* feeding regimens (Figure 11). The increase in weight of the epididymal fat pads may be related to the greater caloric density of the fat diet as well as the effect of interaction between meal pattern and

level of dietary fat. As expected, the increase in epididymal fat pad weight was significantly ($p < .01$) correlated with the length of the refeeding period; the 8-16 fed animals demonstrating a greater gain ($p < .01$) in epididymal tissue weight than animals eating *ad libitum* (Figure 11). After 30 days of refeeding with the high-fat diet, the *ad libitum* fed animals had regained 93% of the initial weight of the epididymal fat pad compared to 124% with the 8-16 meal pattern. In contrast, the *ad libitum* fed animals of the low-fat diet after 30 days had regained 72% of the original fat pad weight compared to 67% for meal-fed animals.

Epididymal lipid

Changes in epididymal fat pad weight were reflected in the absolute amounts of epididymal lipid present (Table 16). The epididymal fat pads from control stock (Group I) rats contained 3.5 g or 64% lipid. At the end of the depletion period the values had been reduced to 0.6 g or 38% lipid. The recovery of epididymal lipid was influenced by a significant interaction between meal pattern and level of dietary fat ($p < .05$), and by meal pattern and length of refeeding period ($p < .05$). The interaction effects were graphically similar to those for epididymal fat pad weight (Figure 12). With the inclusion of fat in the diet and

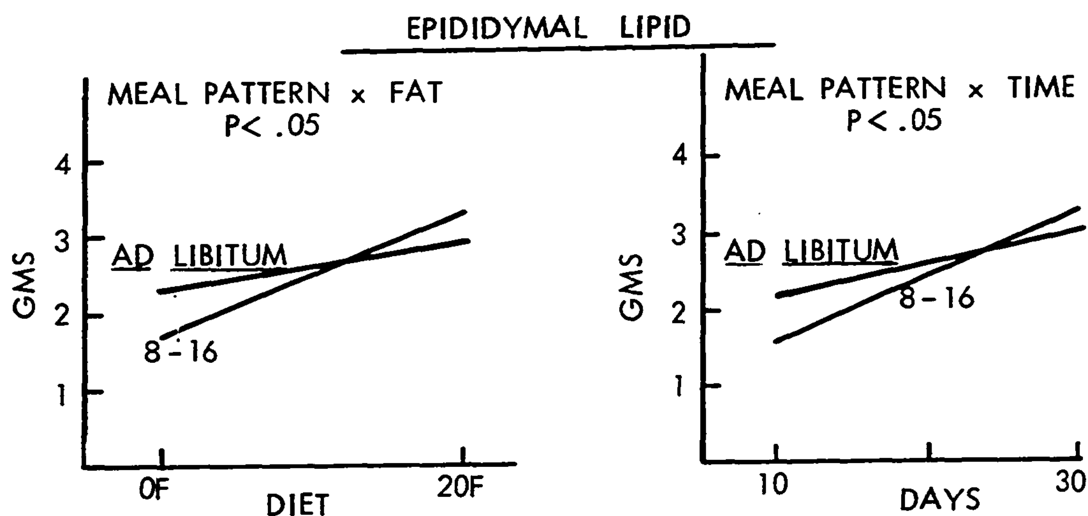


Figure 12. Influence of interactions between level of dietary fat, meal pattern, and time on total epididymal lipid.

with the longer refeeding period, the 8-16 fed animals were able to accumulate a greater amount of lipid in the epididymal tissue than ad libitum fed animals. After 30 days refeeding, the animals on the high-fat diet had essentially regained all of the epididymal lipid by ad libitum feeding while the 8-16 fed animals exceeded the initial lipid level by 26%. In contrast, both the ad libitum and 8-16 fed animals receiving the low-fat diet for 30 days regained approximately 75% of the original epididymal lipid.

Fatty acid composition of epididymal lipid

The relative fatty acid composition of epididymal lipid is presented in Table 17. Dietary restriction prior to

Table 17. Fatty acid composition of epididymal lipid

Group	Diet	Days Refed	% Fatty Acids							16 + 16:1 + 18:1
			14	16	16:1	18	18:1	18:2	18:3	
I Stock	stock	0	0.6	20.0	3.1	3.6	28.3	38.9	5.4	51.4
II Depleted	OF,OP	0	0.9	20.3	3.1	5.1	31.0	36.8	2.8	54.4
III Ad Libitum	OF,P	10	1.8	31.8	9.4	3.4	34.0	18.4	1.2	75.2
IV Ad Libitum	OF,P	30	2.0	37.2	10.1	4.2	36.1	10.5	trace	83.4
V 8-16	OF,P	10	1.7	33.1	9.2	3.6	33.5	18.8	trace	75.8
VI 8-16	OF,P	30	1.7	38.5	9.4	4.4	35.6	10.4	trace	83.5
									mean	79.5
VII Ad Libitum	20F,P	10	0.9	22.3	3.6	3.0	27.8	41.2	1.2	53.7
VIII Ad Libitum	20F,P	30	0.8	20.4	3.1	2.8	27.1	44.5	1.4	50.6
IX 8-16	20F,P	10	0.8	21.4	3.3	2.9	27.0	42.8	1.9	51.7
X 8-16	20F,P	30	0.9	23.7	3.8	2.9	26.9	40.3	1.6	54.4
									mean	52.6
Factorial standard error			±<.1	±<.1	±<.1	±<.1	±<.1	±<.1	±<.1	

refeeding resulted in small shifts in the fatty acid profile of epididymal lipid. The percentages of myristate (C14), stearate (C18) and oleate (C18:1) were increased, while the percentages of linoleate (C18:2) and linolenate (C18:3) decreased. Similar changes in the relative percentages of fatty acids were observed by others in this laboratory (Stadler, 1969; McAtee, 1970) except that they also observed a decrease in palmitate (C16). These shifts in fatty acid profile during depletion were not of sufficient magnitude to be considered of physiological importance.

Refeeding a diet low in fat resulted in large increases in myristic (C14), palmitic (C16), palmitoleic (C16:1) and oleic (C18:1) acids above stock control values and above the values for animals receiving the high-fat diet ($p < .01$). The combined mean value for C16, and its desaturation and elongation products C16:1 and C18:1 was 80% compared with 51% for stock controls. As expected the percentage of linoleic acid decreased ($p < .01$) from the stock control value since it cannot be synthesized by the animal. Palmitic (C16), stearic (C18) and oleic (C18:1) acids increased ($p < .05$) and linoleic acid (C18:2) decreased with time (Table 17, Figure 13). These results confirm previous studies (Stadler, 1969; McAtee, 1970) from this laboratory with similarly treated rats.

With the inclusion of dietary fat, small shifts were observed in individual fatty acids, however, the fatty acid

Table 18. Activities of epididymal HMP shunt dehydrogenases, "malic enzyme", and citrate-cleavage enzyme

Group	Diet	Refeeding Period Days	HMPD n moles of NADPH / min/mg protein	"Malic enzyme" n moles of NADPH / min/mg protein	Citrate-cleavage enzyme n moles of NAD+ / min/mg protein
I Stock	stock	0	25.5±3.2[a]	3.7±1.5	1.8±0.3
II Depleted	OF,OP	0	26.6±3.2	8.3±2.1	0.8±0.2
III *Ad Libitum*	OF,P	10	328.4±34.9	753.6±238	3.9±0.5
IV *Ad Libitum*	OF,P	30	77.5±17.8	95.4±20	2.7±0.5
V 8-16	OF,P	10	193.8±29.3	329.6±43	2.5±0.5
VI 8-16	OF,P	30	122.7±23.3	211.4±42	2.2±0.4
VII *Ad Libitum*	20F,P	10	129.3±18.9	235.1±57	2.0±0.3
VIII *Ad Libitum*	20F,P	30	39.9±5.9	23.3±4	1.4±0.2
IX 8-16	20F,P	10	139.4±27.4	183.1±33	1.9±0.4
X 8-16	20F,P	30	45.4±4.7	44.4±6	1.9±0.6
Factorial standard error			±22.6	±90.3	±.5

[a]Mean ± standard error.

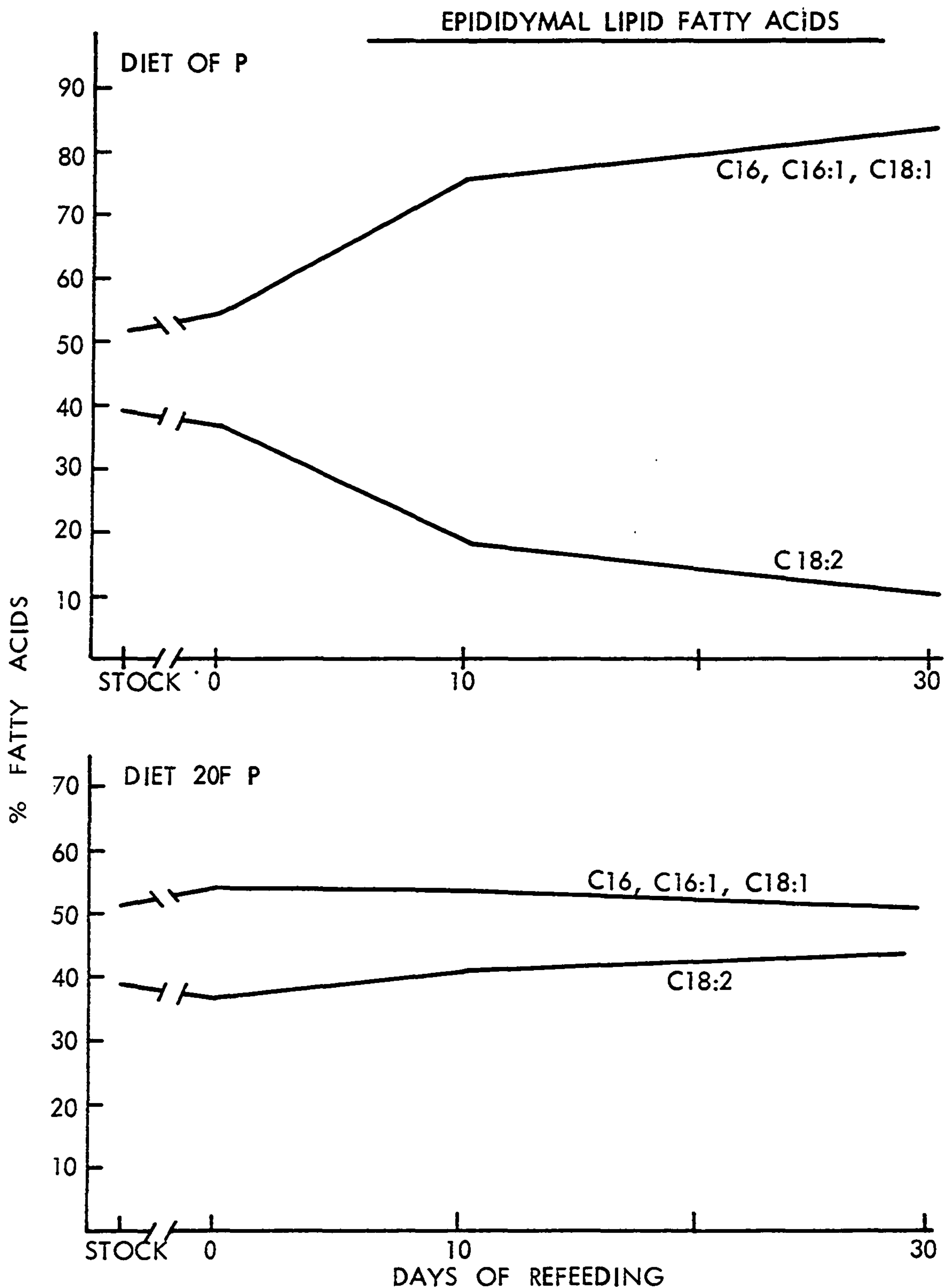


Figure 13. Response of epididymal lipid fatty acids with time in *ad libitum* fed rats.

profile was not different from Groups I and II. The combined mean value for C16, C16:1 and C18:1 was identical (53%) for animals receiving the high-fat diet and the average value of control Groups I and II (Figure 13). Thus even with the great influx of linoleic acid with the high-fat diet, the rat appears to maintain a constant fatty acid profile with time and the tissue does not "get softer".

Selected epididymal lipogenic enzymes

Hexose monophosphate shunt dehydrogenases (HMPD) Depletion appeared to have little effect on the activity of liver (Table 16) or adipose (Table 18) HMPD. This may result from the fact that all animals were fasted 16 hours prior to autopsy thereby causing about the same loss of enzyme activity in both groups I and II. Refeeding either the low-fat or high-fat diet produced an increase ($p < .01$) in epididymal enzyme activity above stock controls (Group I). However, the response was less ($p < .01$) for rats receiving dietary fat. After 10 days of *ad libitum* refeeding, rats receiving diet OF,P had a 13-fold increase above controls (Group I) compared to a 5-fold increase for those receiving diet 20F,P. The depression of HMPD activity with the inclusion of dietary fat is in agreement with observations reported by Tepperman and Tepperman (1964) and Leveille (1967a, 1967b).

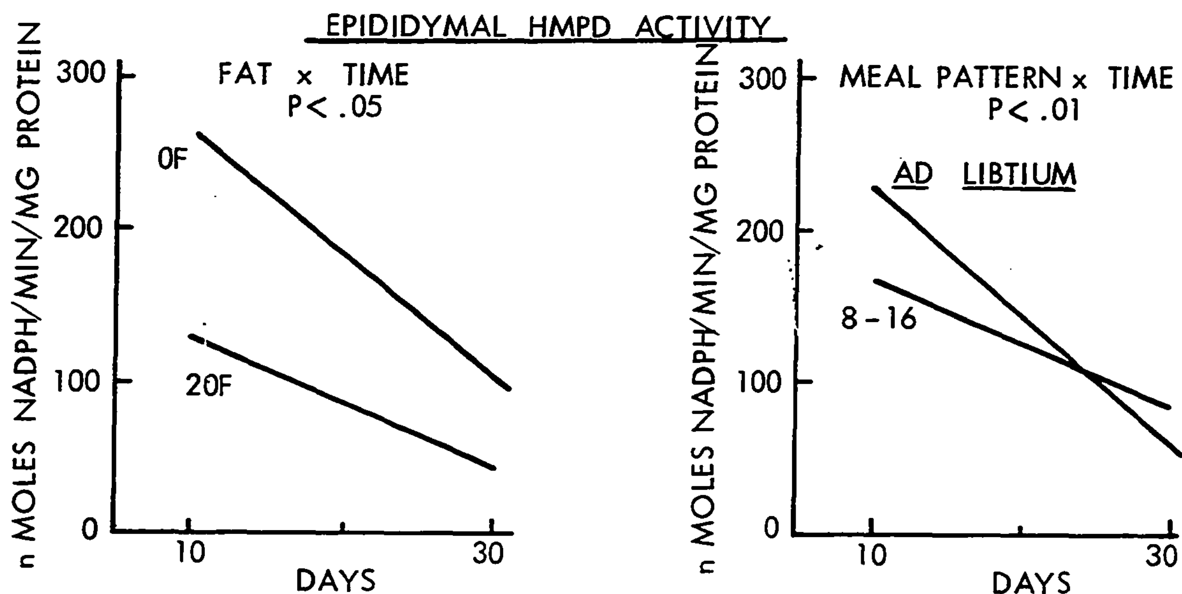


Figure 14. Influence of interactions between level of dietary fat, meal pattern, and time on epididymal HMPD activity.

Figure 14 illustrates the interaction between dietary fat level and time ($p < .05$) and meal pattern and time ($p < .01$) on HMPD activity. As the length of the refeeding period increased to 30 days, HMPD activity decreased ($p < .01$). However, even after 30 days of *ad libitum* feeding the activity was 210 and 52% above the stock control value (Group I) with the low-fat and corn oil diets respectively. *Ad libitum* fed rats had a greater ($p < .05$) HMPD activity than the 8-16 fed rats after 10 days of refeeding, but this relationship was reversed after 30 days refeeding for animals receiving both diets. Significance ($p < .01$) was obtained only for those rats receiving the low-fat diet.

The enhanced HMPD activity as a result of meal feeding a low-fat diet for 30 days is similar to the observations reported in other laboratories (Hollifield and Parson, 1962a; Leveille and Hanson, 1966a).

"Malic enzyme" Depletion did not significantly affect "malic enzyme" activity (Table 18). "Malic enzyme" activity responded to refeeding in the same manner as HMPD activity. Refeeding with either diet OF,P or 20F,P resulted in the typical "overshoot" ($p < .01$) in "malic enzyme" activity above stock control values. The inclusion of dietary fat lowered the "malic enzyme" activity ($p < .01$). As the length of the refeeding period increased "malic enzyme" activity decreased ($p < .01$). A slight interaction ($p < .05$) between the meal pattern and refeeding period influenced "malic enzyme" activity (Figure 15). "Malic enzyme" activity was greater ($p < .05$) for ad libitum fed animals during the first 10 days of refeeding compared with meal-fed rats. As in the case of HMPD activity this was reversed by 30 days refeeding.

Citrate-cleavage enzyme Citrate-cleavage enzyme activity was significantly ($p < .01$) influenced by the fat content of the refeeding diet (Table 18). Refeeding with a low-fat diet produced a two fold increase in activity above the stock control value (Group I). The inclusion of dietary fat reduced citrate-cleavage enzyme activity to values

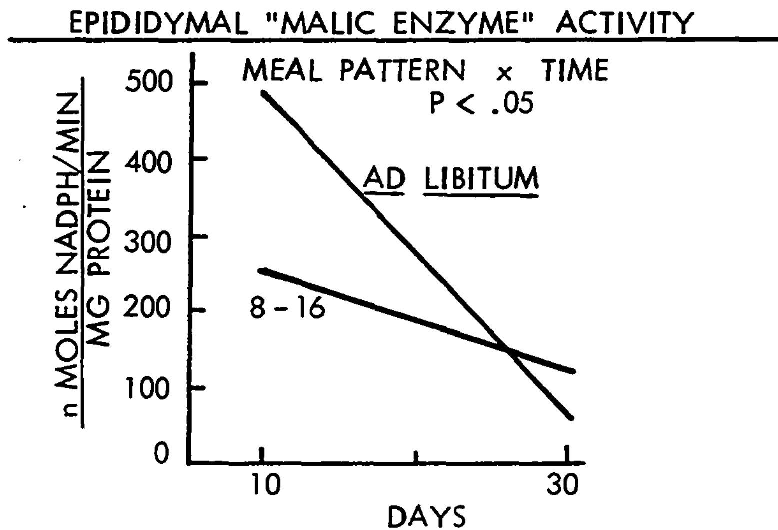


Figure 15. Influence of interaction between meal pattern and time on epididymal "malic enzyme" activity.

observed for controls. The length of the refeeding period or meal pattern during refeeding had no effect on enzyme activity. However, a tendency for a decrease in activity with increasing time was observed for Groups III-VIII. The enhanced citrate-cleavage activity resulting from feeding a diet high in carbohydrate has been observed by Leveille and Hanson (1966a). These same authors reported a +1000 fold increase in citrate-cleavage enzyme activity as a result of meal feeding a 2-hour meal compared to rats fed *ad libitum*. The failure of the 8-16 fed rats in this study to differ in citrate-cleavage enzyme activity from rats fed *ad libitum* was unexpected.

Serum and Hepatic Cholesterol

Serum and hepatic cholesterol concentrations are shown in Table 19. Chronic food restriction during depletion reduced ($p < .01$) the total serum cholesterol from 49 to 33 mg/100 ml. The esterified fraction was similarly reduced ($p < .01$) from 32 to 21 mg/100 ml. Hepatic cholesterol and the circulating free cholesterol of the plasma were little changed from stock control values as a result of depletion and fasting. In contrast Mitchell (1971) using similarly treated rats from this laboratory observed a 42% increase in the circulating free cholesterol while the esters remained fairly stable. Although Mitchell (1971) used *ad libitum* depleted-refed rats, the 8-16 method of depletion for the animals in this study was more severe.

The level of dietary fat during refeeding did not influence the total serum cholesterol nor the free or esterified fractions. Similar observations were made by Mitchell (1971) in this laboratory. However, the length of the refeeding period as well as the meal pattern affected the free, esterified and total serum cholesterol ($p < .01$). Interaction between meal pattern and length of the refeeding period ($p < .05$) was observed for the total serum cholesterol and the esterified fraction (Figure 16). Meal-fed rats had a higher serum cholesterol concentration than

Table 19. Serum and hepatic cholesterol concentrations

Group	Diet	Period Refed Days	Serum[a] Total mg/100 ml	Free mg/100 ml	Esters mg/100 ml	% Free	% Esters	Liver[b] Total chol. mg/g
I Stock	stock	0	49.0	16.8	32.2	34	66	1.91±0.15[c]
II Depleted	OF,OP	0	33.0	14.8	21.2	40	60	2.05±0.21
III *Ad Libitum*	OF,P	10	57.6	24.6	33.0	43	57	--[d]
IV *Ad Libitum*	OF,P	30	33.0	15.8	17.2	49	51	2.67±0.38
V 8-16	OF,P	10	71.0	28.2	42.8	40	60	--[d]
VI 8-16	OF,P	30	56.2	21.0	35.2	38	62	2.29±0.21
VII *Ad Libitum*	20F,P	10	59.6	24.0	35.6	41	59	--[d]
VIII *Ad Libitum*	20F,P	30	40.4	15.0	25.4	38	62	2.44±0.22
IX 8-16	20F,P	10	64.2	24.8	39.4	39	61	--[d]
X 8-16	20F,P	30	59.4	19.6	39.8	32	68	2.10±0.16
Factorial standard error			±2.7	±1.1	±1.9			

[a]Mean of 2 pooled samples, using 2 rats/sample.

[b]Mean of 5 rats.

[c]Mean ± standard error.

[d]No analysis.

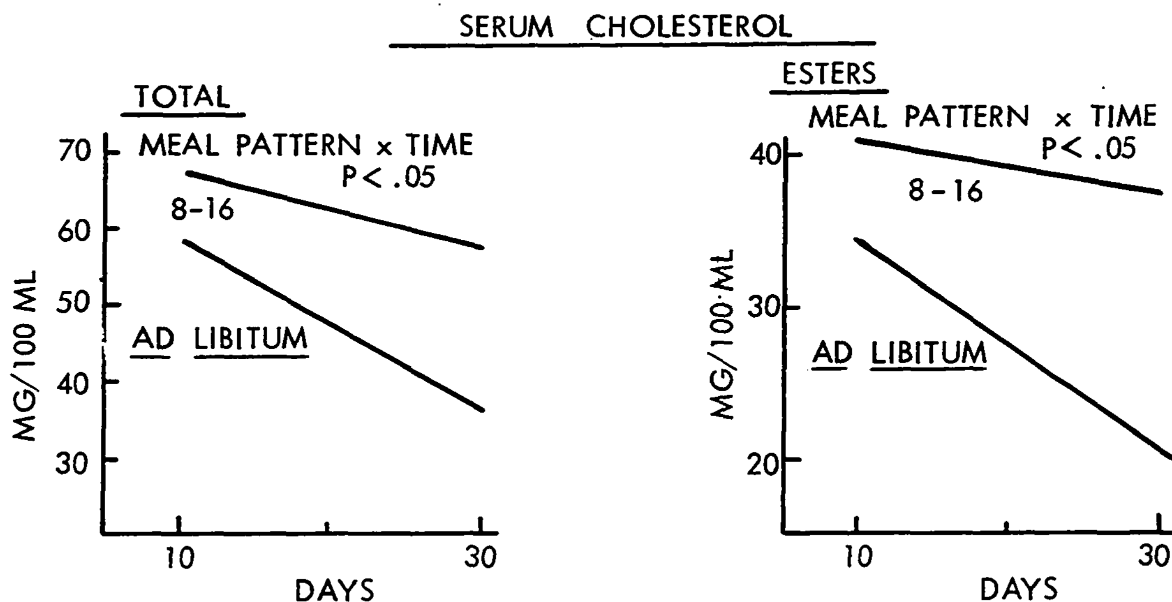


Figure 16. Influence of interaction between meal pattern and time on total serum cholesterol and esterified serum cholesterol.

did ad libitum fed rats. The greatest difference was found after 30 days refeeding. Total serum cholesterol of the 8-16 fed rats was elevated 70% above the value for ad libitum fed animals receiving the low-fat diet for 30 days. Similarly meal-fed rats receiving the high-fat diet for 30 days had a total serum cholesterol 47% above ad libitum fed rats. As the length of the refeeding period increased serum cholesterol concentration decreased with both feeding patterns; but ad libitum fed rats exhibited the greatest decrease with time ($p < .01$). Hepatic cholesterol varied inversely with serum cholesterol.

General Discussion

This study indicates that metabolic adaptations which have been reported consistently for young meal-fed rats appear to be "masked" in older animals during the first few days of recovery from malnutrition. However, after the initial phase of recovery, lasting from 5 to 10 days of refeeding, meal-fed rats developed metabolic characteristics which have appeared in younger animals adapted to a single 2-hour meal daily.

During the initial phase of recovery *ad libitum* fed animals tended to "gorge" food which was reflected in a period of accelerated body weight gain. However, after the first 5 days of the refeeding period, food consumption began to stabilize. With the inclusion of dietary fat, food intake even declined slightly in *ad libitum* fed rats. The rate of body weight gain also decreased; consequently *ad libitum* fed animals maintained a fairly uniform rate of weight gain during the remaining 25 days of refeeding.

As indicated in the review, young meal-fed rats consume voluntarily only 75-80% of the amount of food eaten by nibbling controls, but weight gain often has been similar, resulting in enhanced food efficiency. Meal-fed rats in this study responded in a similar manner after the first 5 days of refeeding. By disregarding the first 5 days, the

period of greatest body weight gain in *ad libitum* fed rats, meal-fed animals gained weight at approximately the same rate as did those fed *ad libitum*. Meal-fed rats on the high-fat diet consumed only 5-10% less food than *ad libitum* fed controls during the remaining 25 day refeeding period and had a greater food efficiency ratio than nibbling controls.

The reasons for the enhanced food efficiency are not clear. The increase in body weight gain per unit of food energy may reflect decreased energy expenditure. This decrease could be represented by a decreased metabolic rate or a reduced activity increment, or both. A decreased metabolic rate as a consequence of meal feeding (Fabry *et al*., 1963; Heggeness, 1969) as well as a depressed thyroid activity (Cohn *et al*., 1958; Fabry *et al*., 1961; Heggeness, 1969) has been documented in other laboratories. In addition, Leveille and O'Hea (1967) observed that meal-fed rats had a lower level of spontaneous activity than nibbling rats. Such factors also may be operating in adult rats in this study.

In contrast, meal-fed rats on the low-fat diet consumed approximately 75-80% of the food eaten by *ad libitum* controls, but food efficiency was essentially the same for both meal patterns. The failure of the meal-fed animals to demonstrate a significantly greater food efficiency ratio compared to *ad libitum* fed animals on the low-fat diet may

be related to the low caloric density of that diet. The possibility exists that meal-fed rats on the low-fat diet are unable to consume enough food of sufficient energy value during the 8-hour meal to exhibit the enhanced food efficiency characteristic of meal feeding. Such may be the case since Cohn and Joseph (1968) have suggested that a "threshold" value of caloric intake must be exceeded if feeding frequency is to alter body metabolsim. In addition, the mature meal-fed rat may not develop the extensive hypertrophy of stomach and small intestine observed in young meal-fed rats which allows for consumption and utilization of large quantities of food in a short period. This possibility is supported by work conducted by Durand et al. (1965) who observed that hypertrophy of the stomach and intestine in response to food intake occurs primarily during the period of vigorous growth prior to puberty. Rats in this study were well past the age of puberty. Another explanation may be that the low (4%) protein level in these diets was not sufficient for maximum recovery from chronic malnutrition. McAtee (1970) reported an increase in food efficiency when protein was increased from low (4%) to adequate (12%) levels. This latter explanation, however, does not seem adequate since the 8-16 fed rats on the low-protein, high-fat diet were able to regain 92% of their original body weight after 30 days refeeding.

Both recovery from malnutrition and meal eating enhanced rates of lipogenesis. Several laboratories have reported studies which illustrate the superior lipogenic capacity of adipose tissue from young meal-fed, compared to nibbling animals (Hollifield and Parson, 1962a; Leveille and Hanson, 1965a; Stevenson _et al_., 1964). Data in the present study indicate a similar response in epididymal tissue from mature adults after the initial phase of recovery from malnutrition. As in the case of the food efficiency ratio, the effect of meal-feeding on epididymal lipid accumulation was more pronounced in those rats receiving dietary fat. During the first 10 days of the refeeding period, as shown below, _ad libitum_ fed rats on the

	Days	Epididymal		
		Total Lipid (g)	Lipid Recovery (g)	Lipid Recovered per Day (g)
Depleted	0	0.6		
Low Fat:				
Ad libitum	10	1.9	1.3	.13
Ad libitum	20	2.7	0.8	.04
8-16	10	1.1	0.5	.05
8-16	20	2.4	1.3	.06
High Fat:				
Ad libitum	10	2.5	1.9	.19
Ad libitum	20	3.4	0.9	.04
8-16	10	2.1	1.5	.15
8-16	20	4.4	2.3	.12

high-fat diet accumulated lipid at the rate of 0.19 g per day compared to 0.15 g per day for meal-fed animals. However, during the remaining 20 days, meal-fed rats accumulated epididymal lipid at a rate 3 times that of _ad libitum_ controls e.g. 0.12 vs. .04 g per day.

The lipogenic response reflected in epididymal tissue was accompanied by increased activity of selected lipogenic enzymes from fasting levels. However, as the length of the refeeding period increased the activity of both enzymes decreased; but the rate of decrease was greater for _ad libitum_ fed rats than for 8-16 fed animals (Figures 14 and 15). After 30 days refeeding on the low-fat diet, HMPD activity decreased 76% for _ad libitum_ fed animals compared with only a 36% drop for meal-fed rats. A similar response was observed for "malic enzyme" with activity of _ad libitum_ animals decreasing 87% compared with a 36% decrease in meal-fed rats. The presence of dietary fat, however, tended to modify the differences in enzyme activity between the two meal patterns. Therefore, meal-fed rats seem to have a slightly greater source of NADPH for fatty acid synthesis than _ad libitum_ fed rats after 30 days. This is particularly evident for animals on the low-fat regimen.

Although hepatic lipids did not reflect any influence of the 8-16 feeding pattern, both hepatic HMPD and "malic enzyme" responded to the _ad libitum_ and 8-16 feeding pattern

in a manner similar to the corresponding epididymal enzymes (Figures 8 and 9). In fact, hepatic "malic enzyme" activity increased slightly with time for meal-fed rats on the high-fat diet. Hepatic HMPD activity in meal-fed rats decreased at a slower rate than in *ad libitum* fed animals. In contrast to epididymal tissue, hepatic HMPD activity was not greater in 8-16 fed rats compared with rats fed *ad libitum* after 30 days refeeding. Refeeding with either dietary meal pattern resulted in fatty livers. This condition was more extensive with the high-fat diet. The low protein level of the diets appears to be a contributing factor in producing fatty liver. McAtee (1970) observed in a similar experiment a decrease in hepatic lipid concentrations as the protein level increased from low to adequate levels.

The failure of the 8-16 meal pattern to influence either hepatic or epididymal citrate-cleavage enzyme during the 30 day refeeding period was unexpected, because Leveille and Hanson (1966a) observed a 1000-fold increase in enzyme activity in rats meal-fed 2-hours daily. The lack of response may be related to the low level of dietary protein. This speculation is supported by work of Jomain and Hanson (1969) with fasted-refed rats. They observed a reduction in carbon flow over the citrate-cleavage enzyme in epididymal fat pads of young rats refed a protein-free diet. Although

the repletion diets in this study were not protein-free, the low level of dietary protein was shown by McAtee (1970) to be insufficient for maximum recovery from chronic malnutrition. The available dietary protein may have been utilized primarily for synthesis of NADPH-generating enzymes. Such a possibility appears reasonable since reducative fatty acid biosynthesis is thought to be dependent upon an available supply of NADPH. The activity of citrate-cleavage enzyme, however, does not appear to limit the rate of fatty acid synthesis of either liver (Foster and Srere, 1968) or adipose tissue (Jomain and Hanson, 1969).

The elevated serum cholesterol in 8-16 fed rats compared with those fed ad libitum was also unexpected. Indices of lipogenesis used in this study e.g. total lipid, fatty acid pattern, and NADPH-generating enzymes, had indicated accelerated rates of fatty acid synthesis. Enhanced cholesterol biosynthesis was not expected since Tepperman and Tepperman (1958b) had shown a significant decrease in acetate incorporation into cholesterol when lipogenesis operated at an enhanced rate. This would indicate that biosynthesis and lipogenesis, which compete for acetate units, are inversely related. Assuming that these relationships did hold in this experiment, one might postulate that the elevated cholesterol of meal-fed rats most likely reflected an enhanced transport of cholesterol from the liver.

This postulation is supported by a lower liver cholesterol concentration in meal-fed rats compared to _ad libitum_ fed animals after 30 days of refeeding.

In contrast to our data, other studies (Okey _et al_., 1960; Leveille, 1967c; Fabry, 1967) failed to demonstrate an increase in serum cholesterol when rats were meal-fed a cholesterol-free diet. These studies differed from the present one in that young adult (220-250 g) or weanling rats were used, and the meal feeding period was less than 21 days. Rats in this study were mature adults and were adapted to meal-feeding during depletion (approximately 30 ± 2 days) and refed by a 8-16 meal pattern for 30 days.

As indicated, the age of the rats in this experiment was an important factor in evaluating the metabolic response to the stress of meal feeding. Rats in this study were 4 1/2 to 6 months of age and weighed approximately 475 g compared to the 180-250 g rats used by Leveille and Hanson (1966a). Although 8-16 fed rats exhibited some characteristics of young meal-fed rats after 30 days refeeding, the response was not as marked as that observed in younger rats. One reason may be that the length of the meal period for rats in this study was much longer (8 hours) than the 2-hour period frequently used for younger animals. We were unsuccessful in a preliminary experiment to adapt adult rats recovering from malnutrition to either a daily

2 or 4-hour meal period. Rats in the preliminary study were unable to gain weight during the refeeding period and in some cases weight loss continued at rates similar to those observed during depletion.

In addition, a decreased rate of body fat formation with age may be a factor in mature rats in this study. A decrease in lipogenesis with aging (Gellhorn and Benjamin, 1965) and the failure of mature rats to demonstrate changes in body composition characteristic of meal-fed rats (Wardlaw *et al*., 1969) has been documented. In contrast, however, we observed that 8-16 fed rats receiving dietary fat were able to accumulate more epididymal lipid after 30 days refeeding than rats fed *ad libitum*. Since epididymal tissue probably reflects changes in body adipose tissue, these animals may have a greater percentage of carcass fat than their nibbling controls. Although Wardlaw *et al*. (1969) failed to detect changes in body composition of mature force-fed rats, the experimental feeding period was only 2 weeks compared to the 30-day period in this study. A longer refeeding period may be essential for mature rats to exhibit metabolic characteristics of meal feeding.

Another finding in this study was the failure of the 8-16 meal pattern to stimulate a major increase in the relative concentration of those fatty acids considered indicative of *de novo* fatty acid synthesis. The slight increase

in palmitic, palmitoleic, and oleic acids in the phospholipid fraction of meal-fed rats was not considered of sufficient magnitude to be of physiological importance. Neither hepatic non-phospholipids nor epididymal lipids responded to the 8-16 feeding pattern. Similarly, Friend and Cunningham (1967) failed to detect shifts in depot fatty acids of pigs fed once or five times daily except for oleic acid, the proportion of which increased slightly from 42.9% in "multiple feeders" to 44.6% in "single feeders". Braun _et_ _al_. (1967) reported an increase only in palmitic acid in parametrial adipose tissue of meal-fed rats compared to _ad_ _libitum_ controls (31.9 vs. 27.6%). These young rats were fed a single 2-hour meal daily compared to the 8-hour meal used in this study. If the older rats in this study could have adapted to a shorter meal period greater differences might have been detected in fatty acid composition.

Previous work in this laboratory has demonstrated dramatic shifts in tissue lipid fatty acid patterns within short periods of _ad_ _libitum_ refeeding (Noble, 1967; Stadler, 1969; Kopec, 1969). Therefore, another aspect of this study was to ascertain whether or not the lipogenic response to variations in the dietary energy source persist with time.

A marked increase in the relative concentration of fatty acids of endogenous origin, palmitic, palmitoleic, and oleic

acids, above stock control levels occurred in both hepatic and epididymal tissue of rats refed the low-fat diet. Epididymal lipids and hepatic non-phospholipids registered the modification in fatty acid composition to a greater extent than did the more stable hepatic phospholipid fraction. Increases in these saturated and mono-unsaturated fatty acids have been correlated with heightened lipogenesis in fasted-refed animals (Allman et al., 1965). The rapid accumulation of epididymal lipid, the elevated activity of HMPD, "malic enzyme", citrate-cleavage enzyme and the increased concentration of saturated and mono-unsaturated fatty acids are all indicative of enhanced de novo fat synthesis.

The greatest change in fatty acid composition occurred during the first 10 days of refeeding. After that only minor shifts in individual fatty acids took place during the remaining 20 days (Figures 6, 7, and 13). Since the relative concentration of palmitic, palmitoleic, and oleic acids in epididymal lipids of rats refed the low-fat diet exceeded that for stock animals, it is uncertain what the final concentration of these fatty acids might be with a long-range refeeding period.

Very little alteration in fatty acid profile occurred in hepatic or epididymal lipids with the inclusion of corn oil in this diet. This would be expected since the fatty

acid composition of corn oil resembles closely that of the stock diet. Both hepatic non-phospholipids and epididymal lipids reflected the continuous influx of linoleic acid. However, the combined mean value for palmitic, palmitoleic and oleic was essentially the same as that for stock controls. Although a high concentration of linoleic acid is present in the corn-oil diet, the rat still appears to maintain a constant fatty acid profile with time.

From the data presented, the activities of both HMPD and "malic enzyme" appear to be well correlated with the indices of lipogenesis used in this study, namely adipose lipid accumulation and fatty acid composition. During the first 10 days of refeeding, particularly with the low-fat diet, the activities of both enzymes were greatly enhanced. During this period total epididymal lipid and fatty acids indicative of _de novo_ lipogenesis (C16, C16:1, C18:1) were significantly increased above the values for depleted controls. As mentioned earlier, epididymal lipid and NADPH-generating enzymes were slightly greater in meal-fed versus _ad libitum_ fed rats after 30 days of refeeding.

Although the 8-16 meal pattern influenced hepatic NADPH-generating enzymes, the response was not as pronounced as in epididymal tissue. Similarly, the activities of HMPD, "malic enzyme" and citrate-cleavage enzyme were greater in adipose tissue than liver. Other investigators have made

similar observations (Young *et al*., 1964; Young, 1969; McAtee, 1970).

From this study one may conclude that mature adult rats, after the early phase of recovery from malnutrition, develop some of the metabolic characteristics observed in young meal-fed rats. This conclusion is based on the evidence of increased food efficiency, accelerated epididymal lipid accumulation, and enhanced NADPH-generating enzyme activity. As is the case in young meal-fed rats, our data supports the conclusion that adipose tissue of adult rats reflects the adaptations to meal feeding to a greater extent than does liver. Since the differences between the two meal patterns were not as marked as those observed in younger rats adapted to a limited feeding period, a longer feeding period may be required. If the rate of change in epididymal lipid accumulation and enzyme activity remains constant, then one might speculate that with a longer feeding period, differences between the two meal patterns might become more pronounced.

In addition, one may conclude that as a consequence of age, the mature adult rat on a low-fat, low-protein diet does not adapt to the 2-hour daily meal frequently used in young meal-fed rats. Consequently the 8-hour meal pattern which had to be used in this study may not have given the high concentration of nutrients required for extensive changes in body metabolism. Since young rats adapted to a 2-hour

meal often develop extensive changes in metabolism after only 3 weeks, the older rat may require as long as 2 or 3 months of meal feeding to develop changes in metabolism and body composition of a magnitude observed in the younger rat. However, major alterations in body metabolism and body composition may not develop in mature rats unless changes in gastrointestinal tract morphology occur to allow an enhanced absorptive capacity.

Since fatty acid composition did not change appreciably after the first 10 days of refeeding with the high-fat diet, one can conclude that the integrity of the tissue fatty acid pattern is maintained with time even under the influx of high concentrations of linoleic acid. In the case of refeeding a diet high in carbohydrate, the lipid component of tissue appears to become more solid at body temperature as indicated by the increased concentration of saturated fatty acids. A conclusion regarding the final physical character of adipose tissue of carbohydrate fed rats is not feasible on the basis of these data since the concentration of palmitic, palmitoleic, and oleic acids had not stabilized at the end of the extended feeding period.

SUMMARY

This study was designed to explore the influence of altered meal patterns on selected parameters of lipogenesis in adult rats recovering from chronic malnutrition and to compare these responses with young meal-fed rats. Also of importance was to determine whether the short term response to variations in dietary energy source would persist with time. Previous work in this laboratory had demonstrated dramatic shifts in tissue lipid patterns within a few days.

Adult male rats were depleted of body fat and protein. Body weight was reduced by 20% of the initial weight by feeding a fat-free, protein-free diet for 8 hours coupled with a 16-hour fast (8-16 meal pattern). A further 20% reduction in body weight was accomplsied by imposing a period of severe caloric restriction with the same diet. Control groups were killed before and after depletion. Remaining rats were refed for 10 or 30 days either _ad libitum_ or by the 8-16 meal pattern with a diet containing approximately 4% of the calories from protein. The remainder of the energy was furnished by corn starch or corn starch plus 20% corn oil. The influence of the variables 1) meal pattern, 2) dietary fat level, and 3) time were considered for the following parameters: 1) body weight and food efficiency, 2) total hepatic and epididymal lipid, 3) fatty acid composition of hepatic phospholipids,

hepatic non-phospholipids and epididymal lipid, 4) hepatic and epididymal HMPD, "malic" and citrate-cleavage enzymes, and 5) hepatic and serum cholesterol concentrations.

During the initial phase of recovery _ad libitum_ fed animals tended to "gorge" food, which was reflected in a period of accelerated weight gain. However, after this first 5 days of refeeding, food consumption began to stabilize and body weight gain decreased from about 5.5 to 3.3 g per day. Depending upon the level of dietary fat, meal-fed (8-16) rats consumed 75-90% of the amount of food eaten by _ad libitum_ controls, but gained weight at approximately the same rate. Consequently the food efficiency ratio of meal-fed rats on the low-fat diet was essentially the same as for _ad libitum_ controls. But meal-fed rats on the high-fat diet had a greater food efficiency ratio than _ad libitum_ fed animals. The reasons for the enhanced food efficiency were not clear from this data, but may be related to alterations in resting energy metabolism or physical activity or both.

Both recovery from malnutrition and meal eating enhanced rates of body fat formation. During the first 10 days of the refeeding period, _ad libitum_ fed rats on the high-fat diet accumulated epididymal lipid at the rate of 0.19 g per day compared to 0.15 g per day for meal-fed rats. However, during the remaining 20 days, meal-fed rats accumulated epididymal lipid at a rate 3 times that of _ad_

libitum controls e.g. 0.12 vs. .04 g per day. Consequently meal-fed rats on the high-fat diet accumulated more epididymal lipid than nibbling controls after 30 days of refeeding. With the low-fat diet, the rate of lipid accumulation during the last 20 days was essentially the same for either meal pattern.

The lipogenic response in epididymal tissue was accompanied by increased activity of HMPD, "malic" and citrate-cleavage enzymes. Citrate-cleavage enzyme activity was not influenced by the 8-16 meal pattern. Both HMPD and "malic enzyme" activities decreased with time, but the rate of decrease was much greater in *ad libitum* compared with meal-fed rats. After 30 days of refeeding on the low-fat regimen the activities of both enzymes were slightly greater in meal-fed rats compared to nibbling controls. The inclusion of dietary fat reduced the activities of all three enzymes.

Neither hepatic lipids nor citrate-cleavage enzyme were influenced by the 8-16 meal pattern. However, both hepatic HMPD and "malic enzyme" activities responded to the *ad libitum* and 8-16 feeding pattern in a manner similar to the epididymal enzymes. After 30 days refeeding on the low-fat diet, enzyme activity was not different for either meal pattern.

Both total and esterified serum cholesterol were elevated in meal-fed rats over *ad libitum* control levels at

either time period. As the length of the refeeding period increased, serum cholesterol concentration decreased. However, the rate of decrease was greater for *ad libitum* compared to meal-fed rats. Hepatic cholesterol varied inversely with serum cholesterol, which suggested an enhanced rate of transport of cholesterol out of the liver in meal-fed rats.

The fatty acid profile of hepatic or epididymal lipid was only slightly affected by feeding patterns. The main influence on fatty acid composition was exerted by the level of dietary fat. Refeeding with the low-fat diet elevated levels of fatty acids characteristic of endogenous synthesis (palmitic, palmitoleic, and oleic acids) above those of depleted controls. This response was observed in both epididymal and hepatic lipids. In the case of hepatic lipid, the non-phospholipid fraction most reflected the shift in fatty acid composition.

The greatest change in fatty acid composition occurred during the first 10 days of refeeding. During the remaining 20 days the combined mean percentage of palmitic, palmitoleic, and oleic acids continued to increase slightly for rats on the low-fat diet. During this period linoleic acid continued to drop. Refeeding with the high-fat diet did not cause a marked shift in fatty acid composition from that of stock controls for either hepatic or epididymal

lipid. Both epididymal and hepatic non-phospholipids reflected the continuous influx of linoleic acid.

The data in this study indicate that metabolic adaptations which have been reported frequently for young meal-fed rats appear to be "masked" in adult rats during the early stages of recovery from chronic malnutrition. After the initial phase of recovery, mature meal-fed rats develop metabolic characteristics observed in young rats adapted to limited feeding patterns e.g. increased food efficiency, accelerated epididymal lipid accumulation, and enhanced NADPH-generating enzyme activity. As is the case in young meal-fed rats our data supports the conclusion that adipose tissue of adult rats reflects the adaptations to meal feeding to a greater extent than does liver tissue.

Since the fatty acid profile did not change appreciably after the first 10 days of refeeding with the high-fat diet, one can also conclude that the integrity of the tissue fatty acid pattern is maintained with time even under the influx of high concentrations of linoleic acid. The relative concentration of palmitic, palmitoleic, and oleic acids in adipose tissue of carbohydrate-fed rats had not stabilized at the end of the extended refeeding period. It is uncertain what the final concentration of these fatty acids might be with long-range refeeding.

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APPENDIX

Table A1. Probability of significance of variables for experimental parameters

Text Table Number	Variables[a]						
	A	B	C	AB	AC	BC	ABC
Table 10							
Initial weight	NS[b]	NS	NS	NS	NS	NS	NS
Depleted weight	NS	NS	NS	NS	NS	NS	NS
Refed weight	.01	.01	.01	.01	NS	NS	NS
Gain	.01	.01	.01	.01	NS	NS	NS
Food intake							
g/day	NS	.01	NS	.05	NS	NS	NS
kcal/day	.01	.01	NS	NS	NS	NS	NS
Food efficiency	.01	.01	.01	.05	NS	.01	NS
Table 12							
Liver weight	NS	NS	.01	NS	NS	NS	NS
Total lipid							
mg	.05	.01	.01	NS	NS	NS	NS
% liver	NS	.01	.01	NS	.05	NS	NS
Non-phospholipid							
mg	.05	.01	.01	NS	.05	NS	NS
% liver	NS	.01	.01	NS	.05	NS	NS
Phospholipid							
mg	NS	.05	NS	NS	NS	NS	NS
% liver	NS	.05	NS	NS	NS	NS	NS

[a] A=% fat, B = meal pattern, C = time.

[b] NS = non-significant.

Table A1 (Continued)

Text Table Number	Variables[a]						
	A	B	C	AB	AC	BC	ABC
Table 13							
Non-phospholipid							
mg/g liver	NS[b]	.01	.01	NS	.05	NS	NS
% total lipid	NS	.01	.01	NS	.05	NS	NS
% Fatty acids							
14:0	.01	.01	.01	NS	NS	NS	NS
16:0	.01	NS	NS	NS	NS	NS	NS
16:1	.01	NS	.01	NS	NS	NS	NS
18:0	NS	NS	.01	NS	NS	NS	NS
18:1	.01	NS	.05	NS	.01	NS	NS
18:2	.01	NS	NS	NS	.05	NS	NS
18:3	.01	NS	NS	NS	NS	NS	NS
20:4	.01	NS	NS	NS	NS	NS	NS
Table 14							
Phospholipid							
mg/g liver	NS	.05	NS	NS	NS	NS	NS
% total lipid	NS	.01	.01	NS	.05	NS	NS
% Fatty acids							
16:0	.01	.05	NS	NS	NS	NS	NS
16:1	.01	.01	NS	.05	.05	NS	NS
18:0	.01	.01	NS	NS	NS	.01	NS
18:1	.01	.05	NS	NS	NS	NS	NS
18:2	.01	NS	NS	NS	NS	NS	.05
20:4	.01	.05	NS	NS	NS	NS	NS
>20:4	.01	NS	NS	NS	NS	.05	NS

Table A1 (Continued)

Text Table Number	Variables[a]						
	A	B	C	AB	AC	BC	ABC
Table 15							
HMPD	.01	.01	.01	.01	.01	.05	.05
"Malic enzyme"	.01	NS[b]	NS	NS	.05	.05	NS
Citrate- cleavage enzyme	.01	NS	.01	NS	.01	NS	.01
Table 16 and 17							
Epididymal fat pad	.01	NS	.01	.01	NS	.01	NS
Lipid							
g	.01	NS	.01	.05	NS	.05	NS
% lipid	NS	NS	.05	NS	NS	NS	NS
% Fatty acids							
14:0	.01	NS	NS	NS	NS	NS	NS
16:0	.01	NS	NS	NS	.05	NS	NS
16:1	.01	NS	NS	NS	NS	NS	NS
18:0	.01	NS	NS	NS	.01	NS	NS
18:1	.01	NS	NS	NS	.05	NS	NS
18:2	.01	NS	NS	NS	.05	NS	NS
18:3	.01	NS	NS	.01	NS	NS	.01
Table 18							
HMPD	.01	NS	.01	NS	.05	.10	.10
"Malic enzyme"	.01	NS	.01	NS	NS	.05	NS
Citrate- cleavage enzyme	.01	NS	NS	NS	NS	NS	NS

Table A1 (Continued)

Text Table Number	Variables[a]						
	A	B	C	AB	AC	BC	ABC
Table 19							
Cholesterol							
Total	NS[b]	.01	.01	NS	NS	.05	NS
Free	NS	.01	.01	NS	NS	NS	NS
Esters	NS	.01	.01	NS	NS	.05	NS

Table A2. Individual body weights, body weight gains and food intake

Rat Number	Body weight				Food intake/day	
	Initial g	Depleted g	Refed g	Gain g	g	kcal
			Group I			
78	461					
79	465					
85	488					
86	453					
90	466					
91	466					
101	476					
102	494					
114	470					
115	474					
Mean	471					

Table A2 (Continued)

Rat Number	Body weight				Food intake/day	
	Initial g	Depleted g	Refed g	Gain g	g	kcal
			Group II			
56	462	298				
51	471	292				
62	480	298				
58	470	291				
95	471	287				
98	462	302				
99	462	298				
104	461	300				
109	482	300				
112	487	299				
Mean	471	296				
			Group III			
9	470	296	343	47	17	63
6	483	300	361	61	18	67
34	497	296	360	64	18	67
17	466	299	364	65	20	74
26	468	300	370	70	18	67
37	466	300	372	72	20	74
52	464	300	371	71	23	86
57	468	296	381	85	27	100
65	469	302	376	68	24	89
81	451	300	358	58	16	60
Mean	470	299	365	66	20	75

Table A2 (Continued)

Rat Number	Body weight				Food intake/day	
	Initial g	Depleted g	Refed g	Gain g	g	kcal
			Group IV			
19	468	300	428	128	19	71
12	479	294	412	118	20	74
5	482	300	447	147	20	74
29	505	290	397	107	18	67
36	490	297	395	98	17	63
46	483	300	431	131	23	86
39	474	296	413	117	20	74
64	461	301	448	147	23	86
76	467	300	424	124	21	78
83	460	299	377	78	17	63
Mean	477	298	417	120	20	74
			Group V			
28	490	299	334	35	12	45
8	477	300	341	41	15	56
13	472	298	341	43	13	48
24	468	300	348	48	15	56
20	471	296	333	37	14	52
41	474	284	319	35	15	56
49	470	275	330	55	20	74
66	468	300	339	39	13	48
73	471	300	335	35	15	56
97	468	291	330	39	16	60
Mean	473	294	335	41	15	55

Table A2 (Continued)

Rat Number	Body weight				Food intake/day	
	Initial g	Depleted g	Refed g	Gain g	g	kcal
			Group VI			
1	470	291	354	63	14	52
11	470	300	383	83	16	60
7	481	300	385	85	14	52
27	490	300	373	73	14	52
38	470	300	366	66	15	56
45	474	304	373	69	18	67
53	471	290	405	115	20	74
74	464	300	392	92	20	74
69	470	300	376	76	17	63
67	472	300	427	127	17	63
Mean	473	298	383	85	16	61
			Group VII			
31	483	295	374	79	18	84
15	461	300	376	76	18	84
10	477	299	380	81	18	84
35	473	299	385	86	18	84
59	456	297	375	78	22	103
60	473	300	381	81	20	93
71	497	300	391	91	20	93
87	469	300	385	85	18	84
96	452	302	382	80	20	93
93	466	300	372	72	15	70
Mean	471	299	380	81	19	87

Table A2 (Continued)

Rat Number	Body weight				Food intake/day	
	Initial g	Depleted g	Refed g	Gain g	g	kcal
			Group VIII			
3	486	300	408	108	16	75
30	485	300	434	134	17	79
22	475	286	422	136	18	84
21	466	300	407	107	14	65
40	490	297	456	159	19	89
48	475	300	421	121	16	75
54	473	298	451	153	18	84
63	481	295	443	148	19	89
72	483	302	437	135	18	84
84	464	300	458	158	18	84
Mean	478	298	434	136	17	81
			Group IX			
33	493	289	332	43	12	56
18	458	300	345	45	13	61
16	476	300	370	70	15	70
23	468	300	362	62	15	70
42	460	300	363	63	17	79
43	475	300	380	80	20	93
68	467	300	390	90	20	93
77	466	298	363	65	17	79
80	473	300	362	62	17	79
92	466	298	378	80	20	93
Mean	470	298	364	66	17	78

Table A2 (Continued)

Rat Number	Body weight				Food intake/day	
	Initial g	Depleted g	Refed g	Gain g	g	kcal
			Group X			
32	491	298	423	125	15	70
14	462	300	406	106	13	61
2	482	298	417	119	15	70
25	468	300	436	136	16	75
44	474	300	450	150	18	84
55	463	300	460	160	17	79
70	474	300	415	115	15	70
75	472	293	428	135	17	79
88	461	290	441	151	17	79
89	466	398	430	132	15	70
Mean	471	298	431	133	16	74

Table A3. Individual epididymal and hepatic tissue weights and respective lipid weights

Rat Number	Epididymal fat pad		Hepatic tissue			
	Weight g	Lipid g	Liver weight g	Hepatic lipid Total mg	NPL mg	PL mg
			Group I			
78	5.5	3.7	10.8	470	125	345
79	4.2	2.4	10.0	427	105	322
85	4.5	2.1	14.0	513	135	378
86	4.3	2.7	11.1	439	149	290
90	4.8	2.8	13.0	478	77	401
91	4.3	2.7	12.3	402	130	272
101	5.6	4.0	12.6	392	91	301
102	4.9	3.6	12.4	436	130	306
114	6.6	4.4	10.9	480	174	306
115	9.0	6.4	10.3	394	73	321
Mean	5.4	3.4	11.7	443	119	324
			Group II			
56	0.3	0.0	4.1	102	35	67
51	1.1	0.3	6.2	243	52	191
62	0.5	0.3	6.0	168	39	129
58	0.6	0.1	6.4	269	82	187
95	2.4	1.7	6.6	215	6	209
98	2.5	1.7	7.1	208	52	156
99	1.5	0.9	5.8	211	41	170
104	1.6	0.7	6.2	230	48	182
109	0.4	0.0	5.3	164	52	112
112	0.3	0.0	4.2	121	7	114
Mean	1.1	0.6	5.8	193	41	152

Table A3 (Continued)

Rat Number	Epididymal fat pad		Hepatic tissue			
	Weight g	Lipid g	Liver weight g	Hepatic lipid		
				Total mg	NPL mg	PL mg
			Group III			
9	2.3	0.8	9.3	801	579	222
6	2.6	1.6	7.6	562	332	230
34	2.1	0.7	9.1	824	630	194
17	3.1	2.9	9.2	417	211	206
26	3.0	1.8	8.4	538	306	232
37	4.2	2.6	9.8	365	141	224
52	2.9	1.5	8.8	429	208	221
57	2.5	1.6	9.6	379	120	259
65	4.8	3.1	10.0	362	149	213
81	3.7	2.7	7.9	365	75	290
Mean	3.1	1.9	9.0	504	275	229
			Group IV			
19	4.7	3.6	10.1	428	230	198
12	3.7	2.3	10.2	490	265	225
5	4.6	2.9	9.4	364	184	184
29	2.5	1.6	9.1	614	394	220
36	3.1	2.0	9.2	986	639	347
46	4.9	3.3	9.9	506	239	267
39	4.4	3.0	9.4	396	159	237
64	3.8	2.9	10.9	403	153	250
76	4.0	2.9	10.9	647	355	292
83	3.1	2.2	9.7	556	295	261
Mean	3.9	2.7	9.9	539	291	248

Table A3 (Continued)

Rat Number	Epididymal fat pad		Hepatic tissue			
	Weight g	Lipid g	Liver weight g	Hepatic lipid Total mg	NPL mg	PL mg
			Group V			
28	2.3	1.5	8.1	235	106	129
8	1.9	1.0	8.5	338	115	223
13	1.7	0.9	8.2	278	122	156
24	2.4	1.5	8.2	288	114	174
20	2.4	1.2	7.0	287	116	171
41	1.3	0.7	9.0	303	133	170
49	1.5	0.7	9.4	569	354	215
66	1.4	0.8	8.5	266	89	177
73	1.9	1.3	8.4	286	117	169
97	1.8	1.1	9.7	586	310	276
Mean	1.8	1.1	8.5	344	158	186
			Group VI			
1	2.2	1.3	9.5	552	364	188
11	3.0	2.0	8.2	347	146	201
7	4.5	2.8	9.9	479	205	274
27	4.1	2.9	7.6	462	240	222
38	2.5	1.6	7.3	365	99	266
45	3.5	2.3	9.3	436	245	191
53	4.4	2.9	10.9	417	248	169
74	2.7	1.6	11.0	377	124	253
69	3.5	2.1	8.6	418	227	191
67	5.2	4.0	10.5	424	177	247
Mean	3.6	2.4	9.3	428	208	220

Table A3 (Continued)

Rat Number	Epididymal fat pad		Hepatic tissue			
	Weight g	Lipid g	Liver weight g	Hepatic lipid Total mg	NPL mg	PL mg
			Group VII			
31	4.0	2.9	8.7	502	292	210
15	3.3	1.7	9.1	602	402	200
10	4.2	3.0	9.1	604	321	283
35	4.0	2.6	8.8	499	282	217
59	3.6	2.3	9.2	522	207	315
60	3.5	2.3	10.1	471	163	308
71	3.6	2.3	10.2	478	227	251
87	3.4	2.5	9.1	537	340	197
96	3.3	2.0	8.9	409	187	222
93	5.2	3.7	7.4	318	97	221
Mean	3.8	2.5	9.07	494	252	242
			Group VIII			
3	2.3	1.2	8.1	818	611	207
30	5.1	3.3	9.3	668	391	277
22	5.4	3.9	9.7	487	326	161
21	4.0	2.8	9.0	539	24	294
40	4.7	2.9	9.7	702	37	333
48	4.2	3.2	9.2	850	570	280
54	5.3	3.4	10.3	589	374	215
63	7.9	6.1	10.3	963	727	236
72	3.0	1.7	11.5	966	641	325
84	7.9	6.0	10.4	730	482	248
Mean	5.0	3.4	9.8	731	474	258

Table A3 (Continued)

Rat Number	Epididymal fat pad		Hepatic tissue			
	Weight g	Lipid g	Liver weight g	Hepatic lipid Total mg	NPL mg	PL mg
			Group IX			
33	1.6	1.0	9.3	250	83	167
18	2.3	1.2	7.5	256	87	169
16	3.9	2.5	8.4	322	120	202
23	3.2	2.1	8.4	407	184	223
42	2.9	0.3	9.9	345	188	157
43	4.5	2.5	11.0	626	220	406
68	5.5	4.0	9.9	536	248	288
77	3.1	2.0	9.1	368	118	250
80	4.5	3.1	9.3	389	143	246
92	3.7	2.4	10.0	366	149	217
Mean	3.5	2.1	9.3	387	154	232
			Group X			
32	10.2	4.6	9.9	337	127	210
14	5.6	4.3	7.9	399	179	220
2	5.2	4.0	8.9	623	424	199
25	6.8	3.8	9.1	548	340	208
44	6.0	4.2	10.9	445	148	297
55	8.9	6.0	9.8	532	261	271
70	5.7	4.7	9.3	584	314	270
75	5.2	3.2	10.1	712	504	208
88	6.1	4.3	10.1	474	255	219
89	7.2	5.3	12.0	382	183	199
Mean	6.7	4.4	9.8	504	274	230

Table A4. Activities of hepatic and epididymal HMP shunt dehydrogenases

Rat Number	HMP shunt dehydrogenases					
	Hepatic			Epididymal		
	ΔOD/min / g tissue	ΔOD/min / 100 mg protein	n moles NADPH / min/mg protein	ΔOD/min / g tissue	ΔOD/min / 100 mg protein	n moles NADPH / min/mg protein
			Group I			
78	4.28	2.9	12	.29	3.1	12
79	2.47	2.2	9	.48	5.1	20
85	2.75	2.0	8	.91	6.8	27
86	3.04	2.3	9	.61	4.1	16
90	4.54	3.0	12	.54	3.9	16
91	3.80	3.0	12	.86	6.4	26
101	3.47	2.9	12	.55	11.1	45
102	4.11	3.6	14	.61	8.5	34
114	2.96	2.4	10	.42	6.0	24
115	3.25	3.4	14	.49	8.8	35
Mean	3.47	2.8	11	.58	6.4	26
			Group II			
56	2.67	2.4	10	1.53	3.4	14
51	2.11	1.7	7	2.39	6.5	26
62	4.20	3.4	14	.67	3.3	13
58	2.42	1.7	7	4.35	7.4	30
95	3.85	3.2	13	3.08	6.8	27
98	3.32	2.6	10	.87	12.3	49
99	1.94	2.0	8	.61	5.6	22
104	3.21	2.6	10	2.68	7.7	31
109	3.24	2.7	11	2.60	7.5	30
112	2.48	2.7	11	.96	6.0	24
Mean	2.94	2.5	10	1.97	6.6	27

Table A4 (Continued)

Rat Number	HMP shunt dehydrogenases					
	Hepatic			Epididymal		
	ΔOD/min / g tissue	ΔOD/min / 100 mg protein	n moles NADPH / min/mg protein	ΔOD/min / g tissue	ΔOD/min / 100 mg protein	n moles NADPH / min/mg protein
	Group III					
9	11.12	12.4	50	19.40	104.0	418
6	24.13	20.4	82	14.76	94.0	378
34	21.64	19.0	76	9.57	61.6	248
17	13.33	12.6	51	7.21	58.7	236
26	15.03	12.9	52	8.06	74.4	299
37	13.02	10.7	43	13.64	57.3	230
52	9.32	7.2	29	12.76	118.1	475
57	23.84	20.4	82	13.19	103.1	414
65	19.85	17.8	72	7.99	109.1	439
81	14.40	14.6	59	4.40	36.6	147
Mean	16.57	14.8	60	11.10	81.7	328
	Group IV					
19	16.52	6.8	27	1.39	18.3	74
12	5.50	5.8	23	.44	4.1	16
5	2.73	2.8	11	1.10	20.3	82
29	3.47	3.4	14	1.58	14.5	58
36	5.28	4.6	18	1.04	11.2	45
46	3.82	2.9	12	.93	8.2	33
39	6.05	5.7	23	1.54	29.0	116
64	5.37	4.9	20	1.04	20.4	82
76	7.68	6.8	27	2.53	53.8	216
83	6.22	6.5	26	1.41	13.1	53
Mean	6.26	5.0	20	1.30	19.3	78

Table A4 (Continued)

Rat Number	HMP shunt dehydrogenases					
	Hepatic			Epididymal		
	ΔOD/min / g tissue	ΔOD/min / 100 mg protein	n moles NADPH / min/mg protein	ΔOD/min / g tissue	ΔOD/min / 100 mg protein	n moles NADPH / min/mg protein
			Group V			
28	3.51	3.8	15	3.79	34.0	137
8	9.35	9.8	39	9.50	54.5	219
13	5.51	5.6	22	7.10	42.2	170
24	8.32	7.6	30	15.76	86.0	346
20	7.03	6.0	24	1.70	17.0	68
41	5.74	5.4	22	2.36	13.3	53
49	17.76	15.6	63	12.40	60.3	242
66	13.08	7.1	28	8.96	51.2	206
73	8.75	8.6	34	8.46	48.6	195
97	17.14	17.9	72	16.58	75.3	302
Mean	9.62	8.7	35	8.66	48.2	194
			Group VI			
1	5.10	5.4	22	.83	11.7	47
11	4.44	4.8	19	1.91	25.1	101
7	2.67	2.5	10	.99	7.5	30
27	2.95	2.7	11	1.14	12.8	51
38	4.85	3.2	13	2.55	19.6	79
45	3.63	3.3	13	2.53	30.2	121
53	5.57	5.2	21	3.14	60.8	244
74	7.18	7.0	28	2.04	40.4	162
69	3.53	3.8	15	3.70	47.5	191
67	5.35	5.6	22	3.81	50.0	201
Mean	4.53	4.4	17	2.26	30.6	123

Table A4 (Continued)

Rat Number	HMP shunt dehydrogenases					
	Hepatic			Epididymal		
	ΔOD/min / g tissue	ΔOD/min / 100 mg protein	n moles NADPH / min/mg protein	ΔOD/min / g tissue	ΔOD/min / 100 mg protein	n moles NADPH / min/mg protein
			Group VII			
31	4.07	3.2	13	2.09	16.4	66
15	2.11	2.0	8	4.27	44.4	178
10	1.95	1.8	7	1.04	20.0	80
35	4.36	3.7	15	2.14	21.2	85
59	2.92	2.4	10	5.33	29.4	118
60	3.14	2.9	12	2.97	20.8	84
71	3.88	3.4	14	4.47	60.5	243
87	4.14	3.6	15	4.30	51.3	206
96	3.13	2.2	9	2.80	31.1	125
93	2.75	2.3	9	2.54	26.8	108
Mean	3.24	2.8	11	3.20	32.2	129
			Group VIII			
3	1.08	.9	4	.70	6.4	26
30	1.34	1.4	6	.66	7.6	31
22	2.18	2.3	9	.43	6.0	24
21	2.40	2.2	9	.62	6.4	26
40	2.33	1.9	8	.82	9.3	37
48	3.84	2.6	10	.82	10.3	41
54	4.98	2.9	12	.79	16.4	66
63	3.28	3.2	13	.79	20.0	80
72	3.36	3.9	16	.66	8.8	35
84	2.36	2.3	9	.56	8.2	33
Mean	2.72	2.4	10	.68	9.9	40

Table A4 (Continued)

Rat Number	HMP shunt dehydrogenases					
	Hepatic			Epididymal		
	ΔOD/min / g tissue	ΔOD/min / 100 mg protein	n moles NADPH / min/mg protein	ΔOD/min / g tissue	ΔOD/min / 100 mg protein	n moles NADPH / min/mg protein
	Group IX					
33	2.56	2.5	10	2.37	18.7	75
18	2.05	2.2	9	2.04	18.8	76
16	2.67	2.1	8	2.35	21.5	86
23	2.74	2.2	9	2.65	18.6	75
42	3.15	2.7	11	8.40	67.0	269
43	2.25	2.1	8	1.27	7.7	31
68	3.78	3.2	13	3.20	37.6	151
77	3.45	3.6	15	6.83	65.8	264
80	3.19	3.3	13	3.85	34.4	138
92	3.55	3.3	13	7.82	57.0	229
Mean	2.94	2.7	11	4.08	34.7	139
	Group X					
32	2.14	2.1	8	.62	8.0	32
14	2.50	2.3	9	.68	11.7	47
2	1.87	1.5	6	.54	4.2	17
25	2.08	1.7	7	.71	11.1	45
44	2.00	1.6	6	.92	10.4	42
55	2.03	2.2	9	.53	9.5	38
70	1.85	1.7	7	.52	10.9	44
75	3.77	3.6	15	.94	16.2	65
88	2.32	2.6	10	1.00	15.3	62
89	1.57	1.9	8	1.14	15.4	62
Mean	2.21	2.1	8	.76	11.3	45

Table A5. Activities of hepatic and epididymal "malic enzyme"

Rat Number	"Malic enzyme"					
	Hepatic			Epididymal		
	ΔOD/min / g tissue	ΔOD/min / 100 mg protein	n moles NADPH / min/mg protein	ΔOD/min / g tissue	ΔOD/min / 100 mg protein	n moles NADPH / min/mg protein
	Group I					
78	.51	.3	1	.04	.5	2
79	.26	.2	1	.06	.6	3
85	.57	.4	2	.14	1.0	5
86	.53	.4	2	.04	.3	1
90	1.06	.7	3	.05	.3	1
91	.81	.6	3	.05	.4	2
101	.43	.4	2	.17	3.5	17
102	1.04	.9	4	.04	.5	2
114	.95	.8	4	.03	.5	2
115	.60	.8	4	.02	.4	2
Mean	.68	.6	3	.06	.8	4
	Group II					
56	.35	.3	1	.15	.6	3
51	.20	.2	1	.17	.4	2
62	.54	.4	2	.50	1.4	7
58	.14	.1	0	.08	.4	2
95	.89	.5	2	.79	1.4	7
98	.63	.5	2	.44	.9	4
99	1.38	1.4	7	.26	3.7	18
104	.37	.3	1	.49	4.4	21
109	.46	.4	2	.60	1.7	8
113	1.00	1.1	5	.76	2.2	11
Mean	.60	.5	2	.42	1.7	8

Table A5 (Continued)

Rat Number	"Malic enzyme"					
	Hepatic			Epididymal		
	ΔOD/min / g tissue	ΔOD/min / 100 mg protein	n moles NADPH / min/mg protein	ΔOD/min / g tissue	ΔOD/min / 100 mg protein	n moles NADPH / min/mg protein
	Group III					
9	11.12	12.4	60	25.15	134.8	650
6	11.35	9.6	46	18.74	119.4	576
34	16.92	14.8	71	16.28	104.8	505
17	13.70	12.9	62	7.82	63.6	306
26	12.10	10.4	50	12.57	116.0	559
37	15.45	12.7	61	10.75	45.1	217
52	9.63	7.5	36	21.08	195.2	941
57	12.57	10.8	52	20.47	160.0	771
65	9.58	8.6	41	4.24	579.5	2793
81	6.25	6.3	30	5.44	45.2	218
Mean	11.87	10.6	51	14.25	156.4	754
	Group IV					
19	10.25	10.6	51	1.78	23.5	113
12	6.49	6.8	33	1.00	9.3	45
5	4.61	4.7	23	2.61	48.0	231
29	3.56	3.5	17	1.97	18.2	88
36	11.11	9.7	47	1.46	15.8	76
46	7.72	5.9	28	1.70	15.0	72
39	10.55	9.9	48	2.00	37.6	181
64	9.54	8.7	42	.56	11.1	54
76	4.59	.4	2	3.29	7.0	34
83	8.05	.8	4	1.34	12.5	60
Mean	7.65	6.1	30	1.77	19.8	95

Table A5 (Continued)

Rat Number	"Malic enzyme" Hepatic ΔOD/min / g tissue	Hepatic ΔOD/min / 100 mg protein	Hepatic n moles NADPH / min/mg protein	Epididymal ΔOD/min / g tissue	Epididymal ΔOD/min / 100 mg protein	Epididymal n moles NADPH / min/mg protein
			Group V			
28	5.59	6.0	29	6.12	55.0	265
8	7.52	7.9	38	6.62	38.0	183
13	2.62	2.7	13	9.86	58.6	282
24	11.35	10.4	50	16.85	91.9	443
20	6.15	5.3	26	2.04	20.4	98
41	6.36	6.0	29	20.51	115.0	554
49	12.59	11.1	54	19.25	93.5	451
66	5.67	3.1	15	12.75	72.8	351
73	6.42	6.3	30	10.40	59.8	288
97	9.15	9.5	46	17.41	79.1	381
Mean	7.34	6.8	33	12.18	68.4	330
			Group VI			
1	8.65	9.2	44	2.14	30.0	145
11	9.88	10.8	52	3.46	45.6	220
7	7.78	7.2	35	1.16	8.8	42
27	3.69	3.4	16	.72	8.1	39
38	11.59	7.6	37	5.60	42.9	207
45	10.52	9.6	46	4.33	51.7	249
53	6.63	6.2	30	5.24	101.4	489
74	10.70	10.5	51	2.96	58.4	281
69	4.02	4.3	21	4.97	63.8	308
67	.98	1.0	5	2.13	27.9	134
Mean	7.44	7.0	34	3.27	43.9	211

Table A5 (Continued)

Rat Number	"Malic enzyme" Hepatic ΔOD/min / g tissue	Hepatic ΔOD/min / 100 mg protein	Hepatic n moles NADPH / min/mg protein	Epididymal ΔOD/min / g tissue	Epididymal ΔOD/min / 100 mg protein	Epididymal n moles NADPH / min/mg protein
			Group VII			
31	3.89	3.0	14	2.87	22.5	108
15	7.59	7.2	35	7.08	73.6	355
10	2.66	2.4	12	1.76	33.6	162
35	4.05	3.4	16	2.14	21.2	102
59	4.89	4.1	20	8.80	48.5	234
60	4.51	4.1	20	4.24	29.6	143
71	2.37	2.1	10	10.64	144.1	695
87	2.16	1.8	9	4.72	56.3	271
96	3.61	2.5	12	3.39	37.7	182
93	1.95	1.6	8	1.96	20.5	99
Mean	3.77	3.2	16	4.76	48.8	235
			Group VIII			
3	4.04	3.3	16	.28	2.6	12
30	3.43	3.5	17	.22	2.6	12
22	4.36	4.7	23	.52	7.3	35
21	4.40	4.1	20	.19	2.0	10
40	4.27	3.5	17	.72	8.1	39
48	5.85	4.0	19	.38	4.8	23
54	5.09	2.9	14	.44	9.1	44
63	1.82	1.8	9	.55	1.4	7
72	1.01	1.2	6	.38	5.1	25
84	2.09	2.0	10	.36	5.3	26
Mean	3.64	3.1	15	.40	4.8	23

Table A5 (Continued)

Rat Number	"Malic enzyme"					
	Hepatic			Epididymal		
	ΔOD/min / g tissue	ΔOD/min / 100 mg protein	n moles NADPH / min/mg protein	ΔOD/min / g tissue	ΔOD/min / 100 mg protein	n moles NADPH / min/mg protein
			Group IX			
33	3.30	3.2	15	4.46	35.2	170
18	1.37	1.4	7	3.45	31.8	153
16	3.82	3.0	14	1.92	17.5	84
23	2.16	1.7	8	4.95	34.7	167
42	4.54	4.0	19	10.89	86.8	418
43	2.93	2.8	13	1.15	7.0	34
68	2.27	1.9	9	4.57	53.7	259
77	2.85	2.9	14	4.99	48.1	232
80	1.50	1.5	7	4.55	40.6	196
92	1.57	1.4	7	3.37	24.5	118
Mean	2.63	2.4	11	4.43	38.0	183
			Group X			
32	4.55	4.4	21	.90	11.6	56
14	1.83	1.7	8	.68	11.7	56
2	2.69	2.2	11	.16	1.2	6
25	2.55	2.0	10	1.10	17.3	83
44	5.32	4.3	21	.86	9.8	47
55	2.10	2.3	11	.43	7.7	37
70	3.32	3.1	15	.79	8.9	43
75	1.07	1.0	5	.26	4.4	21
88	3.48	3.8	18	.74	11.4	55
89	3.85	4.6	22	.62	8.3	40
Mean	3.08	2.9	14	.65	9.2	44

Table A6. Activities of hepatic and epididymal citrate-cleavage enzyme

Rat Number	Citrate-cleavage enzyme					
	Hepatic			Epididymal		
	ΔOD/min / g tissue	ΔOD/min / 100 mg protein	n moles NAD+ / min/mg protein	ΔOD/min / g tissue	ΔOD/min / 100 mg protein	n moles NAD+ / min/mg protein
Group I						
78	.82	.6	1	.17	1.9	3
79	.10	.1	0	.06	.6	1
85	.42	.3	0	.15	1.1	2
86	.33	.2	0	.11	.7	1
90	.34	.2	0	.12	.8	1
91	.45	.4	1	.05	.4	1
101	.19	.2	0	.06	1.1	2
102	.06	.0	0	.04	.5	1
114	.14	.1	0	.07	1.0	2
115	.07	.0	2	.12	2.2	4
Mean	.29	.3	.4	.10	1.0	2
Group II						
56	.72	.6	1	.23	.4	1
51	.03	.0	0	.06	.2	1
62	.05	.0	0	.08	.4	1
58	.28	.2	0	.12	.2	0
95	.04	.0	0	.10	.2	0
98	.07	.1	0	.11	1.5	2
99	.05	.0	0	.00	.0	0
104	.00	.0	0	.15	.4	1
109	.00	.0	0	.20	.6	1
112	.38	.4	1	.10	.6	1
Mean	.16	.1	.2	.12	.4	1

Table A6 (Continued)

Rat Number	Citrate-cleavage enzyme					
	Hepatic			Epididymal		
	ΔOD/min / g tissue	ΔOD/min / 100 mg protein	n moles NAD + / min/mg protein	ΔOD/min / g tissue	ΔOD/min / 100 mg protein	n moles NAD+ / min/mg protein
	Group III					
9	2.99	3.3	5	.64	3.4	5
6	5.04	4.3	7	.44	2.8	4
34	4.81	4.2	7	.25	1.6	3
17	4.53	4.3	7	.36	2.9	5
26	2.29	2.0	3	.13	1.2	2
37	4.08	3.4	5	.52	2.2	4
52	1.55	1.2	2	.20	1.8	3
57	2.53	2.2	4	.49	3.8	6
65	1.44	1.3	2	.29	4.0	6
81	1.62	1.6	3	.10	.8	1
Mean	3.09	2.8	4.5	.34	2.4	4
	Group IV					
19	2.73	2.8	5	.22	2.9	5
12	.88	.9	1	.11	1.0	2
5	1.37	1.4	2	.10	1.8	3
29	.77	.8	1	.10	.9	1
36	.27	.2	0	.08	.9	1
46	.91	.7	1	.08	.7	1
64	.56	.5	1	.05	.9	1
76	.53	.5	1	.14	2.9	5
83	.39	.4	1	.19	1.8	3
Mean	1.01	1.0	1.6	.12	1.7	3

Table A6 (Continued)

Rat Number	Citrate-cleavage enzyme					
	Hepatic			Epididymal		
	ΔOD/min / g tissue	ΔOD/min / 100 mg protein	n moles NAD+ / min/mg protein	ΔOD/min / g tissue	ΔOD/min / 100 mg protein	n moles NAD+ / min/mg protein
			Group V			
28	1.52	1.6	3	.22	2.9	3
8	1.74	1.8	3	.36	2.1	3
13	2.48	2.5	4	.26	1.6	3
24	2.68	2.5	4	.25	1.3	2
20	1.67	1.4	2	.17	1.7	3
41	1.37	1.3	2	.00	0.0	0
49	3.44	3.0	5	.35	1.7	3
66	1.66	.9	2	.09	.5	1
73	.76	.7	1	.14	.8	1
97	3.39	3.5	6	.77	3.5	6
Mean	2.07	1.9	3.2	.26	1.5	2
			Group VI			
1	1.47	1.6	3	.12	1.7	3
11	2.07	2.2	4	.16	2.1	3
7	1.22	1.1	2	.06	.4	1
27	1.16	1.1	2	.10	1.2	2
38	1.90	1.2	2	.21	1.6	3
45	.45	.4	1	.00	0.0	0
53	1.39	1.3	2	.14	2.7	4
74	.38	.4	1	.09	1.8	3
69	.00	.0	0	.00	0.0	0
67	.20	.2	0	.13	1.7	3
Mean	1.02	1.0	1.7	.10	1.3	2

Table A6 (Continued)

Rat Number	Citrate-cleavage enzyme					
	Hepatic			Epididymal		
	ΔOD/min / g tissue	ΔOD/min / 100 mg protein	n moles NAD+ / min/mg protein	ΔOD/min / g tissue	ΔOD/min / 100 mg protein	n moles NAD+ / min/mg protein
			Group VII			
31	1.59	1.2	2	.21	1.6	3
15	.77	.7	1	.18	1.8	3
10	.36	.3	0	.10	1.8	3
35	.62	.5	1	.10	1.0	2
59	.88	.7	1	.14	.7	1
60	.20	.2	0	.06	.4	1
71	.20	.2	0	.10	1.3	2
87	.23	.2	0	.11	1.3	2
96	.64	.5	1	.08	.9	1
93	.43	.4	1	.14	1.2	2
Mean	.59	.5	0.7	.12	1.2	2
			Group VIII			
3	.81	.7	1	.14	1.3	2
30	.59	.6	1	.13	1.5	2
22	.54	.6	1	.03	.4	1
21	.70	.6	1	.10	1.0	2
40	.52	.4	1	.05	.6	1
48	.86	.6	1	.10	1.2	2
54	.10	.1	0	.00	0.0	0
63	.42	.4	1	.04	1.0	2
72	.14	.2	0	.05	.7	1
84	.39	.4	1	.05	.7	1
Mean	.51	.5	0.8	.07	.8	1

Table A6 (Continued)

Rat Number	Citrate-cleavage enzyme					
	Hepatic			Epididymal		
	ΔOD/min / g tissue	ΔOD/min / 100 mg protein	n moles NAD+ / min/mg protein	ΔOD/min / g tissue	ΔOD/min / 100 mg protein	n moles NAD+ / min/mg protein
			Group IX			
33	1.15	1.1	2	.14	1.1	2
18	1.14	1.2	2	.13	1.2	2
16	.53	.4	1	.10	.9	1
23	.59	.5	1	.12	.8	1
42	.84	.7	1	.12	.9	1
43	.30	.3	0	.12	.7	1
68	.60	.5	1	.05	.7	1
77	.67	.7	1	.33	3.2	5
80	.30	.3	0	.18	1.6	3
92	.66	.6	1	.14	1.0	2
Mean	.68	.6	1.0	.14	1.2	2
			Group X			
32	1.25	1.2	2	.07	.9	1
14	.75	.7	1	.10	1.7	3
2	.58	.5	1	.04	.3	0
25	.57	.5	1	.00	.0	0
44	.54	.4	1	.23	2.6	4
55	.68	.7	1	.03	.6	1
70	.07	.1	0	.00	.0	0
75	.21	.2	0	.06	1.0	2
88	.00	.0	0	.23	3.5	6
89	.24	.3	0	.10	1.3	2
Mean	.49	.5	0.7	.09	1.2	2

Table A7. Cytosol protein - mg protein/ml

Rat	I	II	III	IV	V	VI	VII	VIII	IX	X
					Liver					
1	14.30	10.77	8.40	11.70	11.70	12.15	14.55	13.60	15.33	11.60
2	12.03	16.58	13.14	8.62	10.37	12.00	10.90	11.78	8.34	12.92
3	13.05	14.74	12.34	11.57	14.25	8.92	12.25	10.22	14.52	10.46
4	12.34	14.46	17.32	10.46	12.65	10.34	11.32	10.77	12.70	13.14
5	13.10	17.05	14.68	12.55	13.23	14.50	16.37	9.30	12.37	16.12
6	14.18	16.67	13.97	15.78	8.52	12.06	16.03	15.35	14.18	12.34
7	12.90	14.03	12.43	15.10	12.55	16.22	16.77	17.45	15.42	15.54
8	20.50	12.83	14.28	15.48	13.35	13.35	20.15	14.74	12.95	14.80
9	25.75	11.10	16.25	25.54	19.07	13.26	17.72	12.95	16.18	14.00
10	14.28	12.21	13.38	17.30	13.32	14.55	16.34	13.05	13.02	10.65
Mean	15.24	14.04	13.62	14.41	12.90	12.74	15.24	12.92	13.50	13.16
					Epididymal					
1	1.06	2.61	1.75	.68	1.00	.60	1.22	.78	.91	1.12
2	.81	2.49	1.06	.97	1.21	.72	1.08	.99	.85	.60
3	.88	2.10	1.25	.54	1.28	1.14	.55	1.16	1.14	1.60
4	1.39	2.43	1.21	1.10	1.86	.86	1.04	1.02	1.21	.81
5	1.18	3.23	1.25	.57	.88	.92	2.01	.86	1.06	.77
6	1.25	1.48	1.85	1.34	1.13	.86	1.30	.84	1.43	.84
7	.45	.65	.83	.62	1.46	.74	.76	.50	.82	.64
8	.94	.90	1.30	.54	1.02	.57	1.19	.50	.86	.68
9	1.00	1.17	.88	.52	1.25	.80	1.06	.68	1.60	.72
10	.91	1.73	1.86	.84	1.58	.86	1.12	.73	1.51	.78
Mean	.99	1.88	1.32	.77	1.27	.81	1.13	.81	1.14	.86

Table A8. Serum and hepatic cholesterol

Rat Number	Serum			Liver
	Esters mg/100 ml	Free mg/100 ml	Total mg/100 ml	Total mg/g
		Group I		
78-79	33	18	51	2.25
85-86	30	14	44	2.29
90-91	40	23	63	1.56
101-102	24	16	40	1.67
115-116	34	13	47	1.79
Mean	32	17	49	1.91
		Group II		
51-56	20	17	37	2.09
58-62	23	18	41	1.73
95-99	25	11	36	1.44
100-110	15	15	30	2.54
105-106	23	13	36	2.47
Mean	21	15	36	2.05
		Group III		
6-9	25	26	51	
17-26	33	23	56	
34-57	35	21	56	
37-52	33	24	57	
65-81	39	29	68	
Mean	33	25	58	

Table A8 (Continued)

Rat Number	Serum			Liver
	Esters mg/100 ml	Free mg/100 ml	Total mg/100 ml	Total mg/g
		Group IV		
29-39	14	16	30	3.04
19-83	10	11	21	2.48
5-12	13	16	29	1.70
36-46	23	14	37	2.18
64-76	26	22	48	3.94
Mean	17	16	33	2.67
		Group V		
8-28	41	32	73	
13-41	42	28	70	
20-66	46	27	73	
73-94	42	24	66	
82-97	43	30	73	
Mean	43	28	71	
		Group VI		
1-74	32	21	53	2.36
11-69	40	20	60	2.02
7-67	44	24	68	2.44
27-38	24	19	43	1.67
45-53	36	21	57	2.94
Mean	35	21	56	2.29

Table A8 (Continued)

Rat Number	Serum Esters mg/100 ml	Serum Free mg/100 ml	Serum Total mg/100 ml	Liver Total mg/g
		Group VII		
31-96	33	30	63	
15-10	46	24	70	
35-93	32	19	51	
59-60	40	24	64	
71-87	27	23	50	
Mean	36	24	60	
		Group VIII		
3-30	32	17	49	2.87
22-40	18	15	33	1.89
21-72	23	14	37	2.92
48-54	33	17	50	2.56
63-84	21	12	33	1.94
Mean	25	15	40	2.44
		Group IX		
18-33	39	25	64	
16-42	48	29	77	
43-92	42	21	63	
77-80	32	25	57	
23	36	24	60	
Mean	40	20	59	
		Group X		
2-25	35	16	51	2.04
14-98	48	24	72	1.70
44-55	34	14	48	2.53
89-70	44	28	72	2.38
32	38	16	54	1.84
Mean	40	20	59	2.10