THE INFLUENCE OF BREED, SEX AND AGE ON LIPOGENIC ENZYME ACTIVITIES IN SOME OVINE TISSUES

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ABSTRACT

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by Allen Floyd Parr

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SYN activities were significantly ($P < .05$) higher in the adipose tissues than in liver and LM. Tissues of Southdowns, which have a greater propensity to fatten, had greater SYN activities than those of Suffolks. The significant ($P < .05$) decrease in SYN activity observed in SCF from 16 to 32 weeks of age may reflect a trend of decreased rate of fat deposition (lipogenic activity) in lambs between 16 and 32 weeks. CCE activities were significantly ($P < .05$) higher in PRF than in liver and LM. All tissues of Suffolk lambs showed greater CCE activities than those of Southdowns. PRF of neonatal and 24 hour lambs had significantly ($P < .05$) greater CCE activities than those at 8, 16 and 32 weeks.

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CBX activities were highest in SCF followed in order by liver, PRF and LM. Adipose tissues of Southdowns (greater propensity to fatten) exhibited higher CBX activities than those of Suffolks. PRF and SCF of rams and ewes at 32 weeks showed significantly ($P < .05$) greater CBX activities than those at 8 and 16 weeks; whereas, wethers at 32 weeks had lower activities of this enzyme than those at 16 weeks. CBX activities appeared to be closely related to the level of lipogenic activity in ovine adipose tissues.

G-6PDH activities were significantly ($P < .05$) higher in adipose tissues than in liver and LM. There did not appear to be a relationship between G-6PDH activity and the propensity to fatten in any of the tissues studied. The activity of G-6PDH reached its maximum at 16 weeks in PRF and SCF and this observation appeared to correspond to the high level of lipogenic activity generally associated with ovine adipose tissue depots at this age. SCF possessed the greatest 6-PGDH activities followed in order by liver, PRF and LM. The tissues of Southdowns exhibited higher 6-PGDH activities than those of Suffolks and this suggested that 6-PGDH was related to the propensity to fatten. The activities of 6-PGDH in PRF and SCF increased considerably with age and this observation indicated that the activities of this enzyme in lamb adipose tissues were closely associated with the level of lipogenic activity.

Adipose tissue ME activities were significantly ($P < .05$) higher than those in liver and LM. The trend toward increasing ME activities with age in lamb adipose tissues followed the general pattern for lipogenic activity in growing lambs. However, ME contributed only a minor proportion

of the total NADPH generating potential in ovine adipose tissues. Adipose tissues showed greater ICDH activities than LM and liver. ICDH activities did not appear to be closely related to the propensity to fatten. The response of ICDH to different levels of lipogenic activity was generally associated with age between 8 and 32 weeks. However, the presence of significant ICDH activities in ovine adipose tissues indicated that ICDH has the potential to generate NADPH for lipogenesis.

SCF and PRF had significantly ($P < .05$) greater LPL activities than LM. LPL activities in ovine adipose tissue depots did not appear to be related to the propensity to fatten. PRF of lambs at 8 weeks exhibited significantly ($P < .05$) higher LPL activities than those at birth, 24 hours and 32 weeks and this observation seemed to coincide to the high rate of fat deposition usually found in PRF of lambs at 8 weeks of age. Changes in plasma FFA levels usually reflected changes in the energy state and/or the development of rumen function.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Table

Page

LIST OF FIGURES

LIST OF APPENDIX TABLES

INTRODUCTION

In our affluent society where food is plentiful and constantly available, excessive adiposity is common and it represents a definite health hazard to man. In addition, animal fats are frequently incriminated in the problem of cardiovascular diseases and because of the negative attitude toward these fats they are a source of economic concern to the meat animal industry. Consequently, the need exists for minimizing the waste fat depots in livestock because of the low demand for animal fats and because production efficiency decreases during the fattening phase, waste fat results in high production costs. The U.S.D.A. in 1966 reported that about two billion pounds of fat was trimmed from beef carcasses alone. This represented about 500 million dollars in feed costs, but was worth only about 100 million dollars as a by product. However, considerable progress has been made towards the production of leaner animals as evidenced by the decrease in lard production by approximately 30 pounds per pig over the past 20 years. Even so, the average meat animal still contains excessive fat especially in the subcutaneous and perirenal depots. This presents the meat animal industry with the monumental task of increasing efficiency of production coupled with the conversion of a higher proportion of the caloric intake to increased protein and the edible portion. As a consequence of the above problems there is a genuine concern in furthering our understanding of the regulation of adiposity in man and meat animals. The controversy surrounding the relationship between animal fat and cardio-

vascular disease as well as other human health aspects adds even greater impetus to the need for controlling adiposity in meat animals. The information gained from the investigations of the biochemical, physiological, hormonal and metabolic control mechanisms of lipogenesis in domestic animals will not only be of use in the regulation of fat deposition in meat animals, but could play a vital role in the control of adiposity and related diseases in man. While improvement in production efficiency and carcass composition of the meat animals has been made through dietary manipulation, the use of synthetic hormones and genetic selection, there is an urgent need for a thorough understanding of regulating adiposity which would involve a more direct interaction with biochemical events in adipose tissue. This need is even more critical since some growth stimulants such as diethylstilbestrol, are no longer permitted in meat animal feeds. The regulation of fat deposition requires an understanding of the biochemical and physiological sequence of events in lipid biosynthesis and transport. Adipose tissue is a metabolically active tissue with very adaptive and precisely regulated biochemical and physiological functions. A great deal of information has been amassed with regard to the metabolic pathways in adipose tissue and the enzymes catalyzing individual steps of these pathways (Renold and Cahill, 1965; Lehninger, 1970). Virtually all the available information has been collected from investigations involving non-ruminant animals, particularly laboratory animals. Physiological, neural and hormonal mechanisms synchronize the balance between lipogenesis and lipolysis (Adler and Wertheimer, 1967). Many of the metabolic steps are involved in the conversion of glucose or acetate into lipids and

several are considered as possible rate-limiting factors. Research efforts on intracellular metabolic control have been directed towards four major areas: 1) the ability of adipose tissue to synthesize acetyl COA from glucose or acetate; 2) the level of de novo fatty acid synthesis by controlling the incorporation of acetyl COA into long chain fatty acids; 3) the ability to supply the reducing equivalents necessary for fatty acid synthesis and 4) the availability of α-glycerophosphate for triglyceride synthesis.

Thus, this study was undertaken to observe the influence of breed, sex and age on enzymatic activity and lipid biosynthesis in lambs of two strains of sheep differing in their propensity to adiposity with the following specific objectives:

1. To study the influence of breed, sex and age on the ability of various tissues to convert acetate to acetyl COA.

2. To observe the effect of breed, sex and age on the capacity of various tissues that provide the reducing equivalents (NADPH) for lipogenesis.

3. To determine the influence of breed, sex and age on the uptake of lipids by various tissues.

LITERATURE REVIEW

Sites of Lipid Biosynthesis

The study of lipid metabolism has led to concentrated research efforts on the lipogenic capabilities of liver, adipose and mammary tissue. The liver has long been regarded as the primary site of lipogenesis as Pihl and Bloch (1950) in early work, using isotope tracers, found that liver had a high turnover rate of long chain fatty acids. It was concluded from this observation that the liver had a high lipogenic capacity. These investigators also stated that the appearance of labeled triglycerides in extrahepatic tissue must have resulted to a large extent from the uptake of lipids from the bloodstream. However, just prior to this finding Masoro et al. (1949) presented evidence indicating that the conversion of glucose to fatty acids was not limited to a single tissue in the adult rat. This evidence was based on the finding that labeled palmitic acid was isolated in extrahepatic tissues of hepatectomized adult rats. Later, Feller (1954) reported that adipose tissue was capable of active catabolic and anabolic lipid metabolism and that adipose could readily synthesize fatty acids from acetate. Furthermore, when the values obtained for fatty acid synthesis were expressed on a fat free or total nitrogen basis, adipose tissue formed fatty acids at rates equal to or greater than those found for liver slices. The biosynthetic pathways are apparently similar in both liver and adipose tissues, but because of their differences in

response to hormones the extent and control of fatty acid biosynthesis may be markedly affected. Much of the present day research efforts have been directed toward determining the relative contribution made by liver and adipose tissue to total body lipogenesis. The findings of Jansen et al. (1966) indicate that adipose tissue in rats and mice accounts for at least 50 percent of the newly synthesized fatty acids and O'Hea and Leveille (1968; 1969a, b) reported that pig adipose tissue possessed all the attributes of an active lipogenic tissue. Also, the almost complete absence of citrate cleavage enzyme in porcine liver is undoubtedly an important factor contributing to its low capacity for synthesis of fatty acids from glucose and is consistent with the results of O'Hea and Leveille (1969b) indicating that adipose tissue accounted for 99 percent of the newly synthesized fatty acids in the pig. Thus, these results indicate that adipose tissue plays a major role, if not nearly the exclusive role in fatty acid synthesis in pig. Similarly, Ballard et al. (1969) in a review of lipogenesis stated that in ruminants, where acetate is the major precursor for fatty acid synthesis, adipose tissue appears to be the most important site of fatty acid synthesis and recently Hood (1972) reported that in Holstein steers adipose tissue was the only important lipogenic tissue. In contrast, O'Hea and Leveille (1969c) reported that in the chicken the liver is the major site of lipogenesis and Goodridge and Ball (1967) reported similar findings when studying lipogenesis in the pigeon.

In ruminants, the short chain fatty acids, up to a chain length of C_{14} and part of the C_{16} acids, in milk fat originated by de novo fatty acid synthesis from acetate and β-hydroxybutyrate absorbed by the mammary

tissue from the blood (Bickerstaffe, 1971). Earlier Annison et al. (1967) working with goats and Bishop et al. (1969) working with cows reported that the long chain fatty acids, i.e., 18 or more carbon atoms and part of the fatty acids with 16 carbon atoms, in milk fat are derived from plasma lipids. Furthermore, Linzell (1968) showed that even though substantial glucose is absorbed by the mammary gland, it does not contribute to the synthesis of milk fatty acids. In contrast, Linzell et al. (1969) and Spincer et al. (1969) reported that in non-ruminants, milk fatty acids (including a number of short chain fatty acids) are exclusively derived from glucose. Moreover, the latter authors found that fatty acids with chain length up to C_{18} were synthesized from both glucose and acetate and the balance of the fatty acids were contributed by the plasma lipids.

Intracellular Localization of Fatty Acid Synthesis

At least three intracellular sites of fatty acid synthesis are recognized: (1) cytoplasmic, (2) mitochondrial and (3) microsomal. The enzyme systems of the cytoplasmic and mitochondrial sites are similar, yet differences do exist since the end product of the cytoplasmic synthetic system is primarily palmitic acid and that of the mitochondria primarily stearic acid (Masoro, 1968). The extramitochondrial enzyme system is by far the most active and is probably the system chiefly responsible for the de novo synthesis of fatty acids. The system located in the cytosol is responsible for the de novo synthesis of the entire fatty acid molecule. This so-called fatty acid synthetase system requires acetyl COA as a

"sparker" molecule, malonyl COA as substrate and NADPH as hydrogen donor (Lehninger, 1970). Harlan and Wakil (1963) reported that fatty acid synthesis in mitochondria is based on at least two mechanisms. The first mitochondrial system is similar to the cytoplasmic system, while the second system consists of the elongation process and requires acetyl COA as substrate and NADPH as hydrogen donor to elongate acyl-COA esters by two carbon increments (Donaldson et al., 1970).

In addition to the two classical mechanisms (cytoplasmic and mitochondrial) of fatty acid synthesis and elongation, a mechanism of synthesis in microsomes was postulated by Nugteren (1963). According to Masoro (1968), this enzymatic sequence can elongate either saturated or unsaturated fatty acids by reacting malonyl COA with the long chain fatty acyl COA esters to generate a series of COA intermediates with the ultimate formation of fatty acyl COA esters two carbons longer. In recent experiments designed to study the mechanism of microsomal fatty acid synthesis, Rous (1971) found that the reducing equivalents were supplied by NADPH. Moreover, Donaldson et al. (1970) suggested that the microsomes may play a key role in the control of fatty acid synthesis since the elongation and/or desaturation of palmitic acid synthesized de novo would provide a spectrum of fatty acids for subsequent estrification that would confer the proper physical characteristics upon lipids stored in various fat depots.

Anatomical Location and Adipose Tissue Cellularity

Recent advances in the measurement of the cellularity of adipose tissue have made it possible to study the effect of fat cell size and

number upon fat metabolism. Several investigators have confirmed the hypothesis that adipose tissue mass can increase by either cell proliferation (hyperplasia) or by cell enlargement (hypertrophy) or a combination of the two. Hirsch and Han (1969) found that early growth of rat epidymal fat pads and retroperitoneal depots was accompanied by progressive enlargement of adipose cells as well as by increases in number. Moreover, beyond the 15th week, the depot grew exclusively by the process of cellular enlargement, with no further change in cell number. In an experiment designed to study the changes in the total carcass adipose tissue of young pigs, Anderson and Kauffman (1972) found that the changes between 1 and 2 months were primarily due to hyperplasia and between 2 and 5 months the changes were due to a combination of hyperplasia and hypertrophy. Furthermore, the work of Hood (1972) and Anderson and Kauffman (1972) indicated that after 5 to 6 months of age the increase in the total adipose mass of pigs was primarily due to hypertrophy. Hood (1972) also found that during growth of the bovine animal, an increase in adipose mass was accompanied by cellular hypertrophy and hyperplasia. However, by 14 months of age hyperplasia was complete in all but the late developing intramuscular adipose tissue. While working with excessively obese human subjects, Hirsch and Knittle (1970) found that both adipose cell size and number was greater in obese subjects as compared to normal subjects and the degree of obesity was directly and highly correlated to the number of adipose cells. The results of Di Girolamo and Mendlinger (1971) and Di Girolamo et al. (1971) indicate that definite species differences exist

as evidenced by the finding that the guinea pig expands its epididymal adipose tissue by hyperplasia with little hypertrophy; whereas, in the rat, pig and hamster it occurs mainly by hypertrophy. Moreover, the results of Hirsch and Knittle (1970) indicate that childhood obesity is primarily associated with hyperplasia; whereas, the studies of both these authors and Salans et al. (1971) provide evidence that adult onset obesity in humans is mainly due to hypertrophy. The work of Bjorntorp and Sjostrom (1971) showed that moderate enlargement of fat depots in humans is mostly due to cell hypertrophy, whereas, in severe obesity hyperplasia is the dominating factor. However, recent work by Hood (1972) and Lee (1972) indicate that excessive fat accumulation in pigs is caused primarily by cell enlargement and the percent carcass fat was found to be more highly correlated to adipose cell size than with cell number.

In an experiment designed to study the effects of starvation on adipocyte size and number, Hirsch and Han (1969) reported that cell size decreased in the semi-starved rat adipose tissue and observed little change in cell numbers. Hood (1972) reported similar findings when studying the effects of starvation on pig adipose tissue. The results of a study conducted by Knittle and Hirsch (1968) indicated that nutritional restrictions during the suckling period of rats resulted in a permanent decrease in adipose cell number. In contrast, Lee (1972) recently showed that caloric restriction during the suckling period had no effect on adipocyte numbers of adult pigs.

Knittle and Hirsch (1968) demonstrated that in rats the lipogenic capacity of the adipocyte was not related to cell size. However, more

recent work has shown that in human (Smith, 1971a) and porcine (Anderson et al., 1972; Anderson and Kauffman, 1972) adipose tissues, adipocytes of different size, fat depot location and animal age all affect lipogenic rates or enzyme activities. Anderson et al. (1972) also found that pig adipose tissue from areas where fat is readily deposited (perirenal) had higher enzyme activities, larger cells, less connective tissue, greater ether extractable lipid and a lower concentration of adipocytes per gram of tissue than samples from anatomical areas where fat is deposited more slowly (hind limb subcutaneous). Moreover, Hood (1972) reported that the activity of NADP-isocitrate dehydrogenase, NADP-malate dehydrogenase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in bovine adipose tissue was directly related to adipocyte volume.

Recent studies involving various adipose tissue depots reveal that intramuscular fat or marbling varies in many respects from the other fat depots. Studies by Moody and Cassens (1968) and Hood (1972) with bovine intramuscular fat and by Lee and Kauffman (1971) with porcine intramuscular fat show that adipocytes of this depot are smaller than corresponding subcutaneous adipocytes. Also, the findings of Hood (1972) suggest that the increase in intramuscular fat in bovine muscle is due primarily to hyperplasia and the presence of numerous small cells indicates that intramuscular fat is later developing than other adipose tissue depots. Intramuscular fat possesses the same basic lipogenic capabilities as subcutaneous fat, which allows for in situ fatty acid synthesis in the intramuscular depots. However, Lee and Kauffman (1971) and Chakrabarty and

Romans (1972) reported lower lipogenic enzyme activities in intramuscular fat than in subcutaneous fat. Moreover, Lee and Kauffman (1972) reported that the lipogenic enzymes of porcine intramuscular fat showed a different pattern of development than that of subcutaneous adipose tissue. In their study the lipogenic enzyme activities of intramuscular fat was higher at 5 months of age than at younger ages; whereas, the corresponding enzyme activities of subcutaneous fat had already reached its maximum before 5 months of age and was lower at this age than at younger ages. This observation supplies additional evidence to support the findings of Hood (1972) who concluded that intramuscular fat was later developing than other adipose tissue depots.

In an experiment designed to study the lipogenic capabilities of the three layers of the subcutaneous fat depot of the pig, Anderson and Kauffman (1972) found that the magnitude of growth and total enzyme activity was greatest in the inner subcutaneous layer followed in decreasing order by middle subcutaneous and the outer subcutaneous layers. The greatest proportion of the evidence presented indicates that adipose tissue depots of various anatomical locations possess basic differences in cellular and metabolic characteristics. These differences and their interactions have a profound effect on lipid metabolism and much of the recent research effort is being concentrated in this area in order to obtain an explanation for the metabolic interrelationships.

Lipogenesis

Most animals are intermittent eaters and as such their bodies are equipped for storage of much of the energy derived from the ingested food. Fat takes up less volume and weighs less per calorie of stored chemical energy than either carbohydrate or protein. Thus, in animals whose major food nutrient is carbohydrate, the lipogenic process is the primary system and maximum efficiency means by which dietary energy is stored. The major homeostatic function of lipogenesis is to store the energy ingested in excess of immediate energy requirements as fat.

Adipose tissue has long been considered to be a mere fat storage depot in which excess calories are deposited but in the recent years it has captured the interests of many biologists. It is now recognized as an extremely metabolically active tissue with very adaptive and precisely regulated physiological and biochemical functions. According to Adler and Wertheimer (1967), the physiological, neural and hormonal mechanisms synchronize the balance between fat deposition and mobilization with the changing requirements of the animal. The homeostatic regulation of lipogenesis is determined to a large extent by the availability of carbohydrate or appropriate substrates to the tissues involved in lipogenesis and by the enzymatic capacity of these tissues to utilize these substrates. The amount of fat ingested and the state of energy balance also serve in a regulatory capacity (Masoro, 1961). Energy intake in excess of immediate energy requirements results in an increase in adipose tissue mass. This increased adiposity is the result of triglyceride deposition within the individual adipocytes of the various fat depots. The triglycerides

are composed of fatty acids derived from either dietary sources or endogenous sources (de novo fatty acid synthesis).

Substrate for Lipogenesis

Non-ruminant

The one important metabolic difference between ruminant and non-ruminant animals is the relative inability of ruminants to use glucose as a substrate for lipogenesis. This was demonstrated in liver slices and adipose tissue by Hanson and Ballard (1967) and in mammary tissue by Hardwick (1966) when they showed that the rates of lipogenic activity in rat tissues far exceeded the rates found in comparable ovine and bovine tissues when glucose was the in vitro substrate source. Furthermore, O'Hea and Leveille (1969b) reported that when glucose-u-^{14}C was used as the substrate in in vitro and in vivo studies it accounted for 99 percent of the newly synthesized fatty acids in pig adipose tissue and Linzell et al. (1969) obtained similar results when studying porcine mammary tissue.

Considerable interest has been directed toward the volatile fatty acids which are produced in the rumen, but little attention has been given to the VFA production in non-ruminants until Elsden et al. (1946) compared the VFA content of the alimentary tract of several species including the pig. The latter authors found that VFA were produced in the pig but they were appreciably less than in ruminants and these authors as well as Barcroft et al. (1944) reported that the chief sites of VFA production in

the pig were the caecum and colon. More recent work by Friend et al. (1962, 1963a, b) provided additional evidence that considerable quantities of VFA are produced in the digestive tract of the monogastric animal. Barcroft et al. (1944) reported that VFA were absorbed from the caecum and colon and McClymont (1951) found the following percentages of VFA in pig blood: acetic acid, 75.5%; propionic acid, 9.6%; butyric acid, 3.7%; and acids higher than butyric, 11.2%. Later work by Friend et al. (1964) resulted in similar findings and they reported that the VFA were removed from the blood by the liver. In addition, O'Hea and Leveille (1969b) found when using acetate-1-^{14}C as substrate that the liver of the pig accounted for 25 to 30 percent of the newly synthesized fatty acids and Kinsella (1970) demonstrated that dispersed mammary cells from rats could utilize acetate and hydroxybutyrate to synthesize fat. Therefore, it is conceivable that VFA may serve as substrate for lipogenesis in non-ruminants to a limited extent.

Research by Barrick et al. (1953) and Garton and Duncan (1954) showed that the composition of the depot fat of non-ruminants, such as the pig, could be altered by variations in the source of dietary fat. Furthermore, Tove and Smith (1960) found that when elevated depot fat levels of linoleate and oleate were produced by dietary means in mice, specific patterns of fatty acid replacement were observed. Mason and Sewell (1967) and Babatunde et al. (1968) reported that many of the body tissues exhibited a rather sensitive response to changes in the quantity and source of dietary lipids supplied to pigs. Thus, it is evident that the fatty acid composition of dietary fat has a marked effect upon the fatty acid composition of the depot fat of monogastric animals.

Ruminant

Fermentation in the rumen, which precedes gastric digestion in ruminants makes a large proportion of the structural components (cellulose and hemicellulose) of plants available in forms which are directly usable by the tissues of the ruminant. Carbohydrates, proteins and all other fermentable substances are simultaneously converted by microbes to volatile fatty acids, methane, carbon dioxide, ammonia and incorporated into the microbial cells (Leng, 1970). Volatile fatty acids are known to be the major utilizable products derived from ruminant digestion of complex carbohydrates and estimates by Warner (1964) and Bergman et al. (1965) indicate that they account for approximately 70 to 80 percent of the energy requirements. Bergman et al. (1966) reported that over 95 percent of the VFA are accounted for by acetic, propionic and butyric acids. Earlier studies by Shaw and Ensor (1959) had shown that the molar proportions of the rumen VFA, especially acetic and propionic, can be controlled by diet to a remarkable degree. Furthermore, Satter et al. (1964) reported that dietary changes brought about changes in ruminal microorganisms and that both affected the VFA formation from ^{14}C-labeled polysaccharides. The latter authors demonstrated that the lower ruminal acetate to propionate ratios generally observed on high grain diets were due not only to the fermentation of more soluble carbohydrate, but also to an altered fermentation pattern of cellulose and hemicellulose. Thus, it is apparent that ruminal VFA production from any given feed constituent is altered by a number of environmental factors, including changes in the microflora. In addition, the work of

Shaw and Ensor (1959) revealed an altered pattern of VFA production in cows fed unsaturated fatty acids or oils and suggested the possibility of a direct substrate effect on VFA formation. Satter et al. (1967) found that the addition of sodium lactate markedly increased the molar percentage of butyrate and butyrate-^{14}C. The dramatic change in titratable VFA and VFA-^{14}C production caused by the addition of sodium lactate was considered evidence for the direct influence that ration constituents or feed additives exert on the rumen fermentation via altering the metabolic routes used by the existing microbial population. The changes in the molar proportions of VFA and acetate to propionate ratios are mainly due to changes in propionate production in the rumen (Davis, 1967). Church (1969) concluded that the ingestion of immature grass, increasing the amount of carbonaceous or protein supplements, increasing feed intake and pelleted roughages tend to result in increasdd propionate levels; whereas, increased levels of acetate are usually seen with silage and mature pastures.

The influence of the acetate to propionate ratio on lipogenesis was first demonstrated by Armstrong et al. (1957, 1958) and Armstrong and Blaxter (1957) in a series of experiments in which steam VFA were infused into the rumen of sheep fed maintenance rations. They found that when the mixture of steam VFA contained about 70 percent acetic acid on a molar basis, the efficiency with which the absorbed products of digestion were utilized for lipogenesis was 33 percent. Similarly, when the rumen liquor contained about 45 percent acetic acid on a molar basis, the efficiency with which the absorbed products of digestion were used to synthesize body fat was 56 percent. Later work by Blaxter (1966) using roughage

rations (high molar concentration of rumen acetic acid) demonstrated a drastic decrease in the efficiency of body fat synthesis. Moreover, grain rations (lower molar concentration of rumen acetic acid) resulted in a marked increase in the efficiency of body fat synthesis. When given as the sole source of energy, acetic acid is poorly utilized and acid accumulates in the systemic blood causing severe acidosis, decreased blood sugar and a marked increase in protein breakdown (Blaxter, 1962). In experiments with labelled compounds, Davis et al. (1960a, b) demonstrated that acetic acid provided a high efficiency energy source when propionic acid was also present and Blaxter (1962) concluded that acetic acid is not readily utilized unless carbohydrate or a carbohydrate precursor is given at the same time. Thus, it appears that as the proportion of energy derived from acetic acid is increased, the efficiency with which the metabolizable energy of the ration is used for lipogenesis declines.

Substantial amounts of acetic, propionic and butyric acid with smaller amounts of long chain fatty acids, are formed in the rumen and absorbed through the rumen wall. Pennington and Sutherland (1956) reported that the epithelium of the rumen wall is capable of metabolizing butyrate, propionate and to some extent acetate. Cook and Miller (1965) showed that the mixture of fatty acids that is transported to the liver is depleted of its butyrate as compared to the mixture in the rumen fluid. Holter et al. (1963) demonstrated that acetate was not metabolized in perfused goat liver when propionate and butyrate were present and Leng and Annison (1963) concluded that both propionate and butyrate in the portal blood are largely removed during the passage through the liver,

the former giving rise to lactate, glucose and carbon dioxide and the latter producing chiefly β-hydroxybutyrate. Moreover, the work of Cook and Miller (1965) indicated that the propionate in the portal blood was almost quantitatively removed during passage through the liver. They concluded that propionate is mainly metabolized by the liver while acetate metabolism occurs mainly in the extrahepatic tissues. These conclusions agreed with those of Reid (1950) who had earlier concluded that in general, acetate is the only short chain VFA present in significant concentrations in the peripheral blood. Recent results of Smith (1971b) clearly showed that of the VFA produced most abundantly in the rumen, sheep liver mitochondria most effectively metabolized a mixture of propionate and butyrate. Acetate was metabolized only in the absence of the other two, but the presence of acetate did not modify the metabolism of the other two. These results are consistent with the appearance of acetate being the only VFA present in significant quantities in peripherial blood. Thus, it is evident that acetate is essentially the only VFA available to the tissues and is the major substrate for the metabolic processes in extrahepatic tissues in ruminants.

The amount of glucose transported from the ruminant digestive tract to the blood, at least under normal feeding conditions, is usually considered negligible (Roe et al., 1966). One consequence of this difference between monogastric animals and ruminants is the need to produce glucose in the latter group. Glucose synthesized in the liver from propionate, amino acids, lactate and glycerol is used as an energy source in brain and some other tissues, but neither ruminant liver nor adipose

tissues can convert significant amounts of glucose into triglycerides for storage (Ballard et al., 1972). Some enzymes which are necessary for lipogenesis to occur, from glucose as substrate, are absent in ruminants. These enzymes are glucokinase (Ballard and Oliver, 1964), ATP-citrate lyase and NADP-malate dehydrogenase in ruminant liver (Hanson and Ballard, 1967) and ATP-citrate lyase, NADP-malate dehydrogenase and pyruvate carboxylase in adipose tissue (Hanson and Ballard, 1967). Bergman et al. (1970) concluded that it was unlikely that the low activities of hexokinase and glucokinase in sheep liver regulate fatty acid synthesis from glucose; however, the mere presence of these enzymes indicate a physiological role in sheep liver for producing glucose in both the fed and starved states. Baldwin and Ronning (1966), Baldwin et al. (1966) and Young et al. (1969) measured the activities of NADP-malate dehydrogenase and ATP-citrate lyase in liver and adipose tissues of cattle fed high carbohydrate diets. These experiments showed little if any adaption to the diets in either liver or adipose tissue and the activities of both enzymes remained very low compared to the corresponding enzyme activities in rat tissues. Furthermore, Hardwick et al. (1963) and Hardwick (1966) demonstrated that only acetate supplied carbon for milk fatty acid synthesis. The results of a similar study by Bauman et al. (1970) indicated that the lack of glucose utilization for fatty acid synthesis in ruminant mammary gland was due to a nonfunctioning citrate cleavage pathway or more specifically low citrate cleavage enzyme and NADP-malate dehydrogenase. Hood (1972) also concluded that acetate was the only substrate incorporated into fatty acids in bovine adipose tissue, but glucose was quantitatively the more

important substrate for the synthesis of triglyceride glycerol in adipose tissue and intestinal mucosa. However, Ballard et al. (1972) using radioisotope incorporation studies with isolated adipose tissues showed an 18-fold increase in fatty acid synthesis from glucose in sheep with abomasal infusions and a 34-fold increase when glucose was injected intravenously. The glucose infusion caused a different effect in the liver from that observed in adipose tissue; the primary effect in the liver was to stimulate acetate metabolism. Moreover, the enzymes of the citrate cleavage pathway showed much greater adaption in adipose tissues than in liver and they concluded that ATP-citrate lyase and NADP-malate dehydrogenase adapt to dietary stimuli. The low rates of fatty acid synthesis in hepatic or adipose tissues from glucose in these studies emphasize the lesser importance of this lipogenic pathway in the ruminant compared to monogastrics. Nonetheless, glucose plays an important role in supplying reduced coenzyme nicotinamide adenine dinucleotide phosphate via the hexose monophosphate shunt and α-glycerophosphate via the Emden Meyerhof pathway for lipid biosynthesis in the ruminant.

Thus, gluconeogenesis is of obvious importance in ruminant metabolism. Propionate, a major VFA produced from dietary carbohydrate is a major substrate for gluconeogenesis in ruminants (Armstrong, 1965; Ford, 1965). Cook and Miller (1965) concluded that propionate is absorbed from the rumen of sheep and goats largely as such and presented to the liver for metabolism. In contrast, Leng et al. (1967), who infused radioactive propionate in the rumen of sheep, estimated that up to 70% of the propionate was metabolized to lactate before it was converted to glucose and they

suggested the rumen epithelium was the site of this conversion of propionate to lactate. However, Weigand et al. (1972) concluded from propionate infusion studies that the direct conversion of propionate into lactate by bovine rumen reticulum epithelium was small and probably amounts to less than 5 percent. Also, the majority of the propionate reached the liver where it was converted to glucose without being converted to lactate. However, in earlier studies, Bergman et al. (1966) found that propionate accounted for only about 40 percent of the glucose and they suggested that protein probably serves as a substrate for glucose formation in the adult ruminant. The findings of Ford and Reilly (1969) who compared the mean specific activities of plasma amino acids and glucose after the intravenous infusion of a mixture of ^{14}C-amino acids into sheep, substantiated the earlier work of Bergman et al. (1966). They concluded that 11 to 17 percent of the glucose was derived from plasma amino acids. Nonetheless, considerable controversy still exists as to the specific substrates for gluconeogenesis and their physiological importance to lipogenesis in ruminants.

Ruminant depot fats are relatively insensitive to changes in the fatty acid composition of the diet when the usual fats rich in C_{18} fatty acids are fed (Tove, 1965). This unique feature of ruminant metabolism is the result of dietary lipid modification in the rumen before absorption. Hydrolysis effected in the rumen includes the release of fatty acid from ester combination and the release of galactose from galactoglyceride (Garton, 1965). The rumen lipase system responsible for this hydrolysis is due to lipolytic bacteria (Hobson and Summers, 1966). Wood et al. (1963)

and Polan (1964) concluded that dietary fatty acids are not degraded to VFA in the rumen, while McCarthy (1962) reported that only small amounts of the long chain fatty acids are absorbed through the rumen wall. One of the most important fates of dietary long chain unsaturated fatty acids is their hydrogenation in the rumen by both bacteria and protazoa (Wright, 1960). The net effect of hydrolysis and hydrogenation results in long chain saturated free fatty acids which constitute the major lipid class present in digesta and they are passed from the rumen to the lower digestive tract. These studies also indicate that stearic acid is the major free fatty acid present (Keeny, 1970).

Metabolism in the Young Ruminant

Many metabolic parameters in lambs and calves during the first few weeks of age, when the rumen is non-functional and they are dependent primarily on milk, resemble those of adult non-ruminants more than those of adult ruminants. Carbohydrate metabolism in young calves and lambs shows a pattern characteristic of monogastrics. Jarrett et al. (1964) and House and Phillips (1968) observed a gradual decline in plasma glucose concentration in growing lambs and this decline was associated with a progressive decrease in the glucose entry rate into the vascular system. Ballard et al. (1969) suggested that this gradual decline may be partly due to the development of ruminal fermentation which diminishes the absorption of glucose from the gut. The data of Filsell et al. (1963); Goetsch (1966) and Bartley et al. (1966) imply that the activities of the

gluconeogenic enzymes increase with age, while the activities of the glycolytic enzymes decrease with age. More recent work by Leat (1970) indicates that the development of a functional rumen results in a reduced capacity for hepatic glucose oxidation via the pentose and glycolytic pathways and an increase in gluconeogenesis in the liver. Thus, as the ruminant matures it becomes more dependent on gluconeogenesis for its supply of glucose rather than dietary carbohydrates.

The blood concentration of VFA, principally acetic acid, increases gradually in young ruminants, although it is variable and less striking than the change in the blood glucose level (Reid, 1953). It was suggested by Keech and Utter (1963) that the COA esters of these acids may stimulate gluconeogenesis by activating pyruvate carboxylase and Ballard et al. (1969) emphasized that this enzyme plays an important physiological role in gluconeogenesis. Also, Randle et al. (1966) suggested that the increased acetyl COA to COA ratio inhibits some of the glycolytic enzymes.

Ballard et al. (1969) reported substantial gluconeogenic activity in the liver of fetal calves and lambs and this was in contrast to low gluconeogenic activity found in fetal rat livers. The capacity to synthesize glucose allows the fetal ruminant to be less dependent on maternal glucose than fetuses from non-ruminants. In all ruminant species studied by Ballard et al. (1969) the overall activity of the gluconeogenic pathway increased rapidly at birth and was accompanied by increases in the activities of the key gluconeogenic enzymes (phosphoenolpyruvate carboxylase) Pyruvate carboxylase, fructose 1,6-diphosphatase, glucose-6-phosphatase). Furthermore, when lambs were maintained on a diet containing milk, but no

solid food, the progressive increase in the conversion of propionate to glucose characteristic of normal maturing rumen function was delayed. This demonstrates that the biochemical development of a mature ruminant can be delayed experimentally and provides further evidence that many metabolic systems are adaptive to dietary changes.

The enzymes involved in lipogenesis exhibit marked changes during the development of the young ruminant. The fetal calf liver has ATP-citrate lyase and NADP-malate dehydrogenase present as well as a functioning citrate cleavage pathway as measured by the substantial amount of incorporation of the carbon 3 of aspartate and the carbon 5 of glutamate into fatty acids in this tissue (Ballard et al., 1969). However, citrate cleavage enzyme activity in the fetal ruminant diminishes during the first few weeks after birth and continues to decrease to the low level of activity observed in the adult ruminant liver (Hardwick, 1966; Hanson and Ballard, 1967). The fetal ruminant and to a certain degree the young ruminant possess the capacity to utilize glucose carbon for lipogenesis, but this ceases shortly after birth with the acquisition of the rumen microflora which results in the production of VFA to serve as the major source of energy.

Lipogenic Precursors

The de novo synthesis of fatty acids diagrammatically depicted in figure 1 involves the fatty acid synthetase complex which catalyzes the following overall reaction, in which one molecule of acetyl COA and seven molecules of malonyl COA are condensed to form one molecule of palmitic

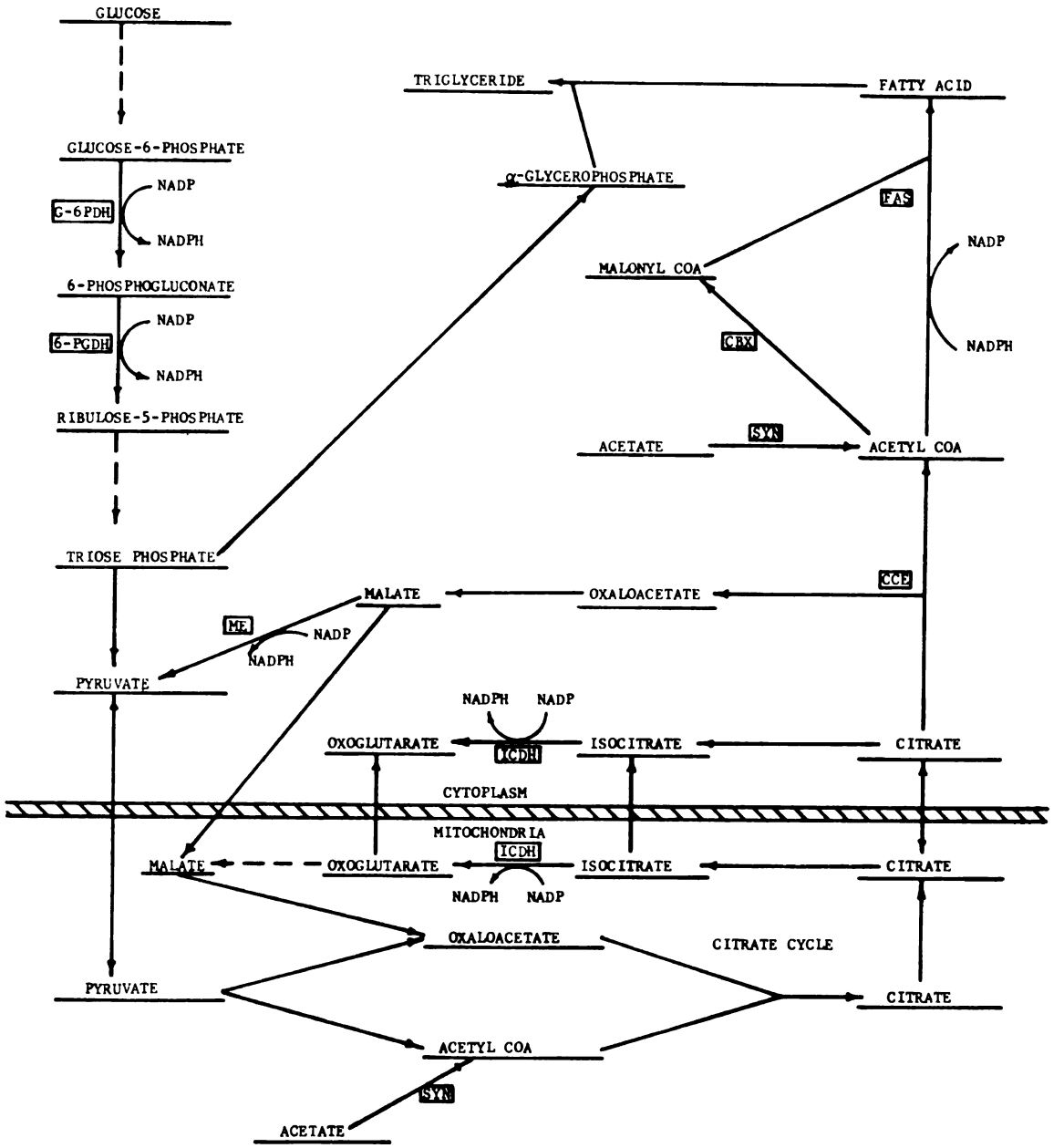


Figure 1. De novo fatty acid synthesis from glucose and acetate. (G-6PDH)-Glucose-6-phosphate dehydrogenase; (6-PGDH)-6-phosphogluconate dehydrogenase; (ME)-NADP-malate dehydrogenase; (ICDH)-NADP-isocitrate dehydrogenase; (Syn)-acetyl COA synthetase; (CBX)-acetyl COA carboxylase; (FAS)-fatty acid synthetase; (CCE)-NADP-citrate lyase.

acid. The coenzyme NADPH supplies the reducing power to drive the reaction to form palmitic acid and one molecule of acetyl COA serves as a primer.

$$\text{Acetyl COA} + 7\ \text{Malonyl COA} + 14\ \text{NADPH} + 14\ H^{+} \longrightarrow$$

$$\text{Palmitic acid} + 7\ CO_2 + 8\ \text{COA} + 14\ \text{NADP} + 6\ H_2O$$

When discussing the research efforts on the precursor supply for lipogenesis in animal tissues it is convenient to divide these studies into four major areas: 1) those concerned with the pathway of carbon flow from glucose or acetate to acetyl COA; 2) studies involving the de novo synthesis of fatty acids from acetyl COA; 3) studies of reducing equivalent (NADPH) supply and 4) studies of the availability of α-glycerophosphate for triglyceride synthesis.

Sources of Acetyl COA

All the carbon atoms for the de novo synthesis of fatty acids are supplied by acetyl COA molecules which are considered to be the building blocks. One important metabolic difference between ruminant and non-ruminant tissues is the source of carbon for lipogenesis (figure 2). In non-ruminant tissues the source of carbon for acetyl COA is glucose, whereas, in ruminant tissues the source of acetyl COA carbon is acetate. Therefore, the metabolic pathways for acetyl formation are somewhat different and will be discussed separately.

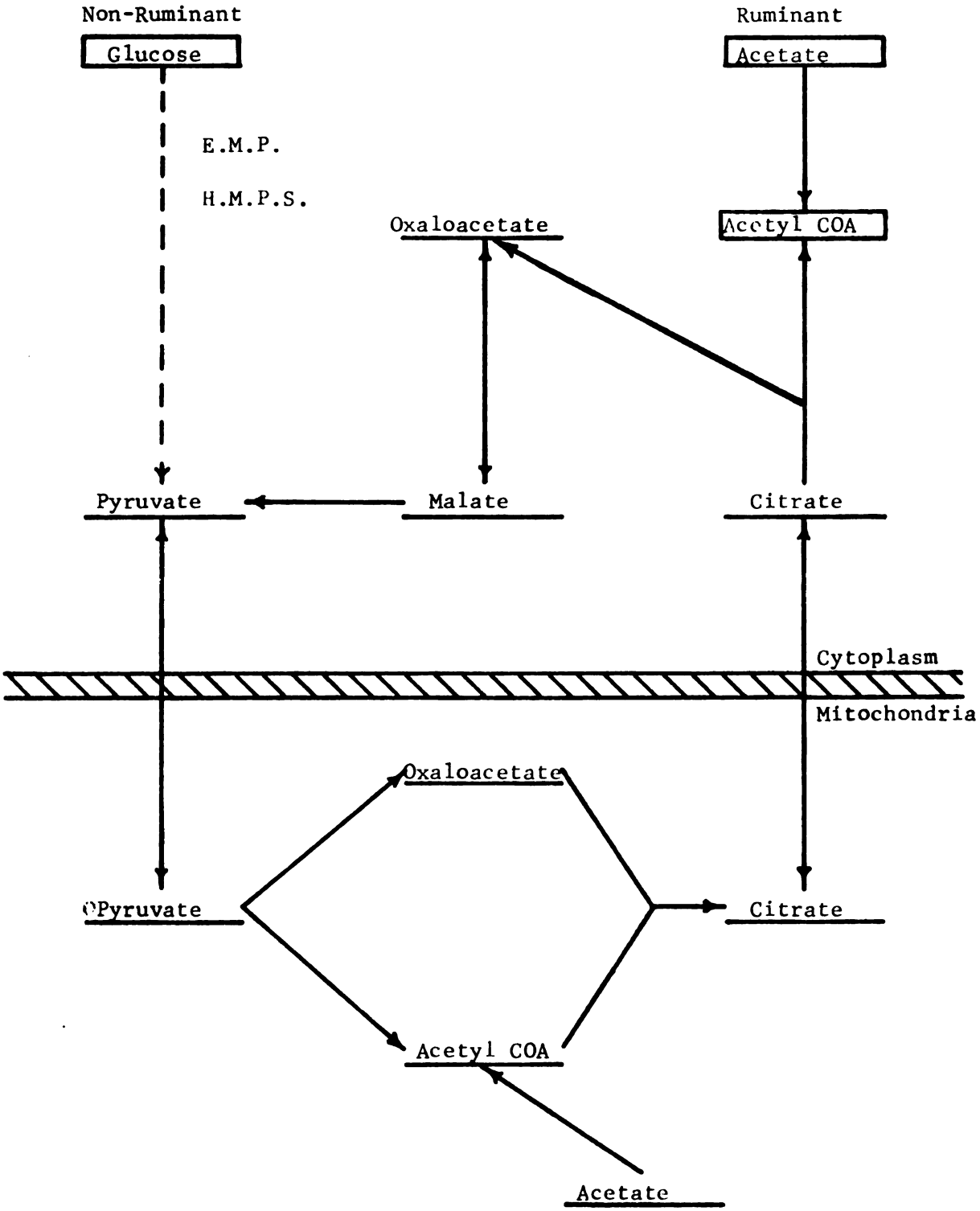


Figure 2. Intracellular location of the reaction sequences involved in the formation of Acetyl COA from glucose and acetate.

Non-ruminant

The conversion of glucose to fatty acids involves a complex series of enzymatic reactions. In the first phase, glucose in metabolized to pyruvate in the cytosol via the Embden-Meyerhof pathway and the hexose monophosphate shunt pathway (figure 2). Pyruvate then enters the mitochondria, where it is oxidatively decarboxylated to acetyl COA. Fatty acid synthesis from acetyl COA occurs within the cytoplasm of the cell, but uses acetyl COA formed in the mitochondria (Srere, 1965). However, Spencer and Lowenstein (1962) reported earlier that the rate of acetyl COA diffusion out of the mitochondria was too slow to meet the demands for fatty acid synthesis. Fatty acid biosynthesis thus requires the production of extramitochondrial acetyl COA from mitochondrial acetyl COA. The observation of Srere (1959) that citrate cleavage enzyme was located in the cytosol of the cell, together with the demonstration that citrate was an effective substrate for fatty acid synthesis (Srere and Bhaduri, 1962; Spencer and Lowenstein, 1962; Formica, 1962) led to the conclusion that citrate played an important role in the generation of acetyl COA in the cytosol (Srere, 1965). The most tenable indirect pathway for the generation of cytoplasmic acetyl COA in mammalian tissues involves the following sequence: formation of citrate within mitochondria from acetyl COA and oxaloacetic acid by citrate synthetase, transfer of citrate to the cytosol and the production of extramitochondrial acetyl COA and oxaloacetic acid from citrate by the action of the citrate cleavage enzyme (Hanson and Ballard, 1968). The above sequence is generally referred to as the citrate

cleavage pathway. Recent work by Watson and Lowenstein (1970) and Martin and Denton (1970a, b) supplied substantial evidence that the citrate cleavage pathway is the major pathway whereby acetyl units for fatty acid synthesis are transferred to the cytoplasm in mammalian tissues. The citrate cleavage pathway and its relationship to lipogenesis from glucose and acetate is diagrammatically depicted in figure 3. Citrate cleavage enzyme has been implicated by numerous researchers as an important control point in the regulation of extramitochondrial acetyl COA for fatty acid synthesis in non-ruminants (Kornacker and Lowenstein, 1964, 1965; Kornacker and Ball, 1965; Ballard et al., 1969). This implication was based on three observations. First, Srere (1959) noted that citrate was localized in the soluble fraction of the cell (cytosol). Secondly, Srere and Bhaduri (1962); Spencer and Lowenstein (1962) and Formica (1962) observed that citrate was an effective precursor for fatty acid synthesis. Thirdly, Brown and McLean (1965); Howanitz and Levy (1965) and Brown et al. (1966) found that under certain physiological conditions, citrate cleavage enzyme and fatty acid synthesis appeared to be altered in a parallel fashion. In contrast to these earlier conclusions, Srere and Foster (1967) provided some preliminary evidence that no necessary relationship existed between citrate enzyme and fatty acid synthesis by showing that during a 24-hour fast the latter decreased to negligible levels without change in citrate cleavage enzyme activity. Moreover, Foster and Srere (1968) demonstrated that in recovery from fasting, fatty acid synthesis increased markedly without change in citrate cleavage enzyme activity and after alloxan administration, fatty acid synthesis decreased prior to any change in citrate cleavage enzyme.

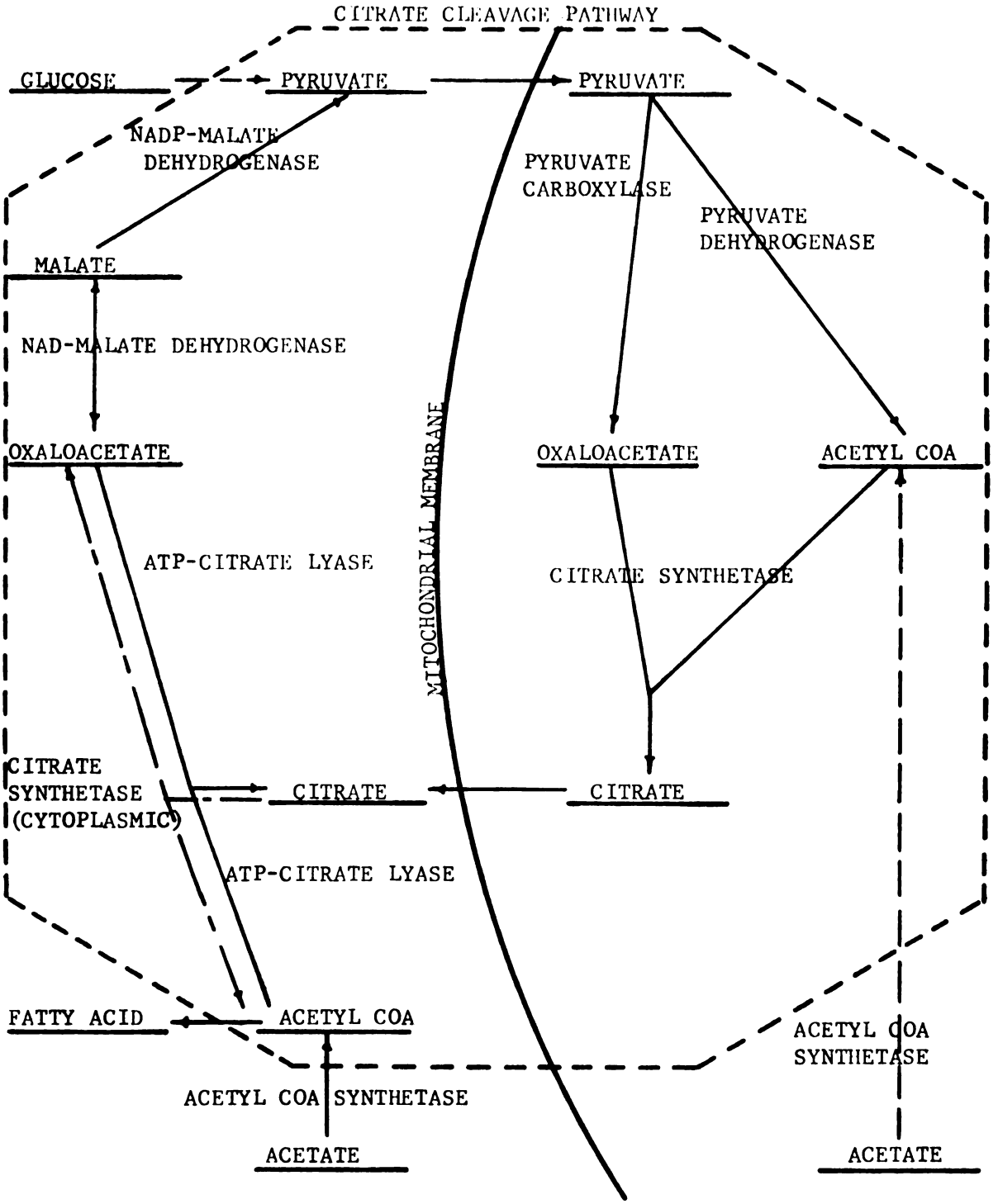


Figure 3. Citrate cleavage pathway and its relationship to lipogenesis from glucose and acetate.

These authors also found that substitution of a high carbohydrate, low fat diet resulted in large increases in citrate cleavage enzyme activity without concomitant change in lipogenesis. Moreover, they showed that the addition of purified citrate cleavage enzyme from rat liver to the fatty acid synthesizing system did not restore fatty acid synthesis. Del Boca and Flatt (1969) found fatty acid synthesis to be higher in the presence of glucose plus acetate than with glucose alone, demonstrating that the acetyl COA transport mechanism is not rate-limiting in adipose tissue.

Fatty acid synthesis via the citrate cleavage pathway requires the continual replenishment of oxaloacetic acid within the mitochondria. Figure 4 diagrammatically depicts the pathways most likely to be involved in the transfer of oxaloacetic acid between the adipocyte cytoplasm and the mitochondria. Regeneration of mitochondrial oxaloacetic acid from oxaloacetic acid formed by citrate cleavage in the cytoplasm may occur in part by the pathway suggested by Martin and Denton (1970a) and Ballard et al. (1972): OAA cyto ---> malate cyto ---> malate mito ---> OAA mito However, Kornacker and Ball (1965) suggested that the most important pathway appeared to be: OAA cyto ---> malate cyto ---> pyruvate cyto ---> pyruvate mito ---> OAA mito. This latter pathway involves the transfer of (H^+) from cytoplasmic NADH to cytoplasmic NADPH which appears to be necessary as Martin and Denton (1970a) demonstrated that the rate of production of NADPH by the hexose monophosphate shunt pathway is not sufficient to account for high rates of lipogenesis. Additional evidence to support the suggestion that the latter pathway is the major means for the

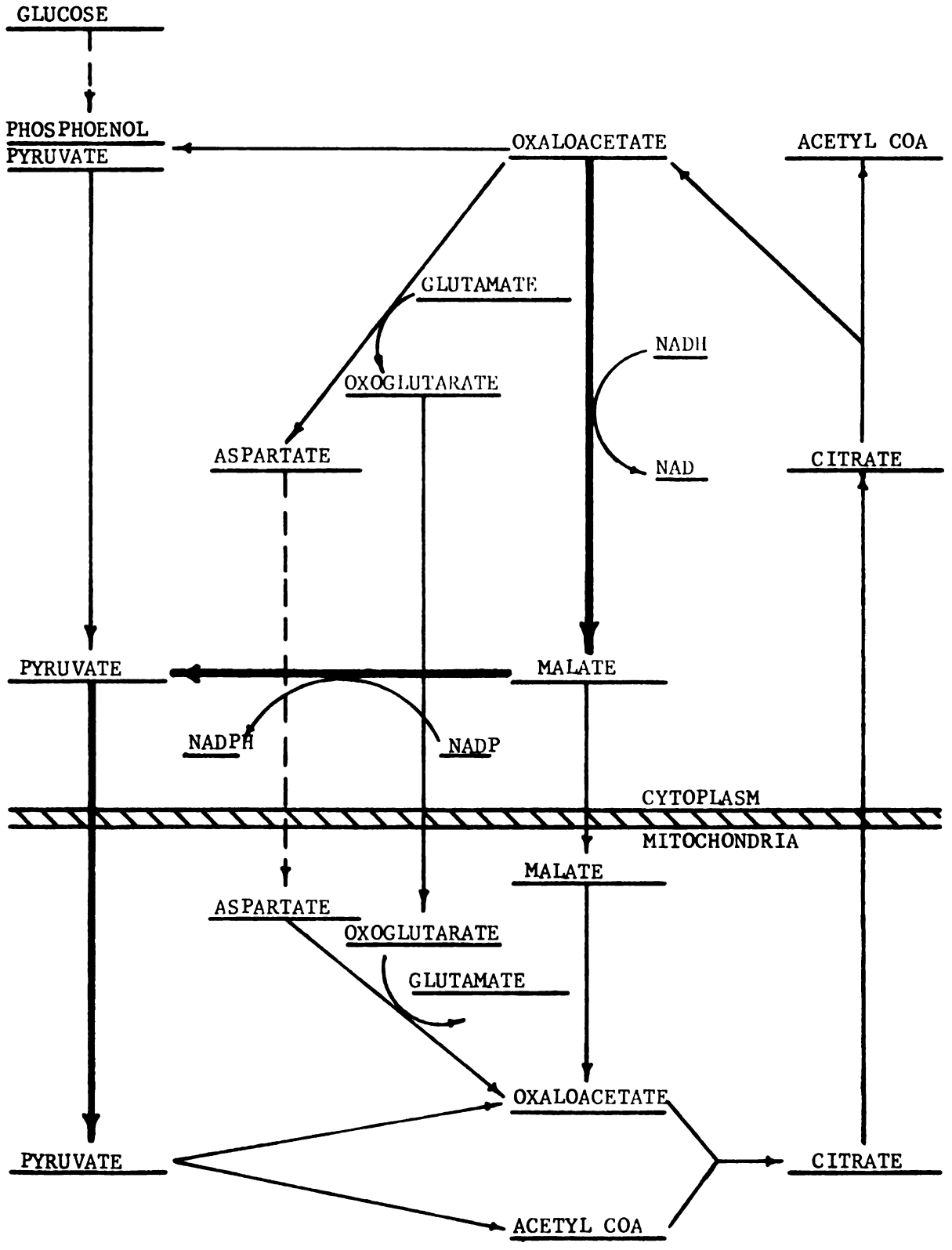


Figure 4. Pathways most likely involved in the transfer of oxaloacetic acid between the fat cell cytoplasm and mitochondria.

replenishment of mitochondrial oxaloacetic acid was supplied by Ballard and Hanson (1967). They reported that pyruvate carboxylase was present in adipose tissue and was active enough to supply the intramitochondrial oxaloacetate to support the citrate cleavage sequence. These authors proposed that pyruvate carboxylase should be considered to be a component of the citrate cleavage pathway in adipose tissue.

The physiological importance of acetyl COA synthetase which converts acetate to acetyl COA has not been totally elucidated in non-ruminant tissues. However, Baldwin et al. (1966) reported the presence of acetyl COA synthetase in the liver of guinea piglets, rats and piglets and Barth et al. (1971) found considerable acetyl COA synthetase in rat liver, kidney, heart and adipose tissue. Although the acetyl COA synthetase mechanism for acetyl COA synthesis has been identified in non-ruminant tissues, it only indicates that the potential of a functional system exists but does not provide unequivocal evidence of its functionality.

Ruminant

In ruminant tissues where acetate is the single most important metabolite from a quantitative standpoint (Davis et al., 1960a; Lee and Williams, 1962; Kronfeld, 1968) the conversion of acetate to acetyl COA for de novo fatty acid synthesis is of profound physiological importance. The initial enzymatic step in the conversion of acetate into fatty acids is the formation of acetyl COA via acetyl COA synthetase reaction (figure 2). Acetyl COA synthesis from acetate can be summarized by the equation:

$$\text{Acetate} + \text{COASH} + \text{ATP} \xrightarrow[\text{Synthetase}]{\text{Acetyl COA}} \text{Acetyl COA} + \text{AMP X PPi}$$

Acetyl COA synthetase allows the ruminant to form acetyl COA directly in the cytoplasm, thus, bypassing the need for citrate to move acetyl units across the mitochondrial membrane. The almost negligible activities of both ATP-citrate lyase and NADP-malate dehydrogenase in both bovine and sheep liver as well as low levels of key enzymes of the citrate cleavage pathway in ruminant adipose tissue reflects the almost total inactivity of this pathway in adult ruminants (Ballard et al., 1969). The occurrence of acetyl COA synthetase activity was first demonstrated by Beinert et al. (1953) in pig heart and Hele (1954) in bovine heart mitochondria. Hanson and Ballard (1967) showed this enzyme to be much more active in both bovine and sheep adipose tissue than in rat adipose tissue. The latter authors and Ballard et al. (1969) found much higher acetyl COA synthetase activities in ruminant liver than in adipose tissue and Bauman et al. (1972) reported that high acetyl COA synthetase activity in rump adipose tissue of wether lambs decreased 7 to 14 fold with fasting. Even though acetyl COA synthetase is present in several ruminant tissues, adipose tissue apparently possesses the most active acetyl COA synthetase system.

There has been considerable discussion as to the intracellular location of acetyl COA synthetase. The work of Aas and Bremer (1968) and Aas (1971) indicated that acetyl COA synthetase was preferentially a mitochondrial enzyme. In contrast, Kornacker and Lowenstein (1965) and Hanson and Ballard (1967) found acetyl COA synthetase activity not only in the mitochondria but also in the cytoplasmic fraction of animal cells. Martin and Denton (1970a) and Barth et al. (1971) used acetate as a substrate and

found 80 percent of the acetyl COA synthetase activity of liver and epididymal fat tissues in the cytoplasmic fraction and only 20 percent in the mitochondria. They concluded that these findings demonstrated a preferential cytoplasmic location in lipogenic tissues. The latter authors reasoned that this disparity could be attributed in part to the use of propionate as substrate which is also activated by butyrl COA synthetase.

Malonyl COA Formation

The malonyl COA required as the immediate precursor of fourteen of the sixteen carbon atoms of palmitic acid is formed from cytoplasmic acetyl COA and carbon dioxide by the action of acetyl COA carboxylase, which catalyzes the reaction shown below:

$$\text{Acetyl COA} + CO_2 + \text{ATP} \xrightarrow{Mn^{++}} \text{malonyl COA} + \text{ADP} + \text{Pi}$$

Acetyl COA carboxylase (figure 1), a biotin dependent enzyme, is the first enzyme involved in the sequence of the cytoplasmic pathway of fatty acid synthesis and it has been implicated by several researchers as the key enzyme in the regulation of fatty acid synthesis in animal tissues (Vagelos, 1964; Numa et al., 1965; Lane and Moss, 1971; Moss and Lane, 1971). The results presented by numerous researchers show a definite relationship between the rate of fatty acid synthesis and acetyl COA carboxylase activity. Korchak and Masoro (1962) studied the effect of fasting on fatty acid synthesis in rat livers and found that a 50 percent reduction in acetyl COA carboxylase activity accompanied a 99 percent depression of fatty acid

synthesis. In a similar study with pigs, O'Hea and Leveille (1969d) reported that following a 4 day fast, the activity of the enzyme was decreased by almost 66 percent, whereas, lipogenesis fell by more than 99 percent. Bauman et al. (1972) studied the lipogenic capacity of adipose tissue in wether lambs and reported that of all the enzymes measured the activity of acetyl COA carboxylase was most closely related to in vitro lipogenic rate. Chang et al. (1967) and Chakrabarty and Leveille (1969) found approximately equal activity of fatty acid synthetase and acetyl COA carboxylase in rat liver and the latter authors reported the response of fatty acid synthetase to meal feeding was similar to that of acetyl COA carboxylase in rat adipose tissue. These latter authors and Dakshinamurti and Desjardins (1969) demonstrated that changes in acetyl COA carboxylase activity in response to dietary alterations were evident to a much greater extent in adipose tissues than in liver of rats. Chang et al. (1967) also reported that fatty acid synthetase and acetyl COA carboxylase activities were both elevated in obese mice compared to nonobese controls. In addition, Chakrabarty and Leveille (1969) reported that acetyl COA carboxylase activity in rat adipose tissues decreased with age and corresponded to the decrease in lipogenic capacity of rat adipose tissue with age as shown by Gellhorn and Benjamin (1965).

The role of acetyl COA carboxylase as a regulatory enzyme has been attributed to its complex regulatory properties. Tubbs and Garland (1963) and Bortz and Lynen (1963a, b) reported that the tissue concentration of long chain fatty acyl COA esters increased with fasting and fat feeding and they suggested that these long chain fatty acyl COA derivatives may

reduce fatty acid synthesis through an inhibitory effect on acetyl COA carboxylase. Cahill et al. (1960) found that the addition of free fatty acids to in vitro systems containing either liver homogenates or epididymal fat pads depressed the fatty acid synthetic activity and Korchak and Masoro (1964) demonstrated that free fatty acids primarily inhibited the acetyl COA carboxylase reaction.

Certain tricarboxylic acids, notably citrate and isocitrate stimulate the acetyl COA carboxylase catalyzed reaction (Martin and Vagelos, 1962; Matsuhashi et al., 1962; Kallen and Lowenstein, 1962; Waite, 1962; Gregolin et al., 1966). The activation of acetyl COA carboxylase involves an equilibrium between an active polymeric form and an inactive polymeric form of the enzyme (Numa et al., 1966; Gregolin et al., 1968a, b). Citrate and isocitrate are capable of shifting this equilibrium in favor of the catalytically active form (Lane et al., 1971) and this has led to the suggestion that citrate activation of acetyl COA carboxylase has a role in the physiological control of fatty acid synthesis. However, Lynen (1967) questioned the physiological significance of citrate activation of the enzyme on the grounds that the intracellular citrate concentration is below that required for the activation of the enzyme in vitro. Moreover, Denton and Halperin (1968) have shown that citrate concentration in adipose tissue is not related to fatty acid synthesis in the manner which is required if fatty acid synthesis were regulated by citrate activation of acetyl COA carboxylase. The results of a study by Iliffe and Myrant (1970) on the sensitivity of rat liver acetyl COA to citrate stimulation indicated that the enzyme is relatively insensitive to citrate in the intact liver

cell. This raises the possibility that citrate may not participate in the physiological control of fatty acid synthesis via regulating the activity of acetyl COA carboxylase despite the undoubted influence of citrate on this enzyme in the purified state. Therefore, it becomes apparent that the cause and effect relationship between the activity of acetyl COA carboxylase and the rate of lipogenesis is currently one of the many subjects of controversy surrounding lipogenesis.

Sources of Reducing Equivalents

The synthesis of long chain fatty acids requires large amounts of NADPH (Langdon, 1957; Brady, 1958; Wakil et al., 1957) and Tepperman and Tepperman (1958) suggested that the availability of this reducing equivalent may be a possible controlling factor in fatty acid synthesis. The fatty acid synthetase complex preferentially oxidizes fourteen moles of NADPH during the formation of one mole of palmitic acid from acetyl COA and malonyl COA.

In non-ruminants, NADPH is generated from glucose via the hexose monophosphate shunt and by the conversion of malate to pyruvate via the citrate cleavage pathway (Ballard and Hanson, 1967; Young et al., 1964; Ball, 1966). The sources of NADPH are diagrammatically depicted in figure 5. A primary source of NADPH for lipogenesis has been considered to be the hexose monophosphate shunt with glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase supplying the cytoplasmic NADPH. The concept that NADPH formed in the oxidative pathway was essentially

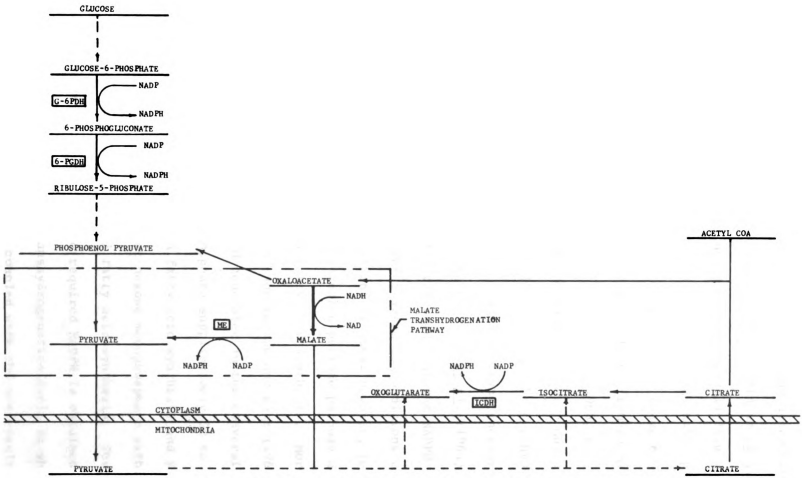


Figure 5. Pathways of reducing power (NADPH) generation for de novo fatty acid synthesis.

and the exclusive source of NADPH for lipogenesis was accepted for a long time (Tepperman and Tepperman, 1958; Wakil, 1961; Abraham et al., 1961). This concept is substantiated by the fact that a parallelism does exist between the observed rates of fatty acid synthesis and NADPH production by the hexose monophosphate shunt pathway for a wide range of incubation conditions for normal tissues and also tissues taken from animals varying in dietary status (Pande et al., 1964; Katz et al., 1966; Saggerson and Greenbaum, 1970). Baldwin and Milligan (1966) and later Gul and Dils (1969) showed that the activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in rat mammary tissue increased markedly with the onset of lactation. Furthermore, Young et al. (1964); Leveille and Hanson (1966) and Leveille (1970) reported that the NADPH generating enzyme involved in the pentose pathway adapted to conditions of decreased and increased lipogenesis in non-ruminant tissues. However, Tepperman and Tepperman (1963) demonstrated that increased pentose pathway dehydrogenase activity was secondary to increased fatty acid synthesis. Moreover, Flatt and Ball (1964), Wise and Ball (1964) and Landau and Katz (1965) showed that intact rat adipose tissue NADPH generated in the conversion of the hexose monophosphate to pentose phosphate supplies only 50 to 60 percent of the reducing equivalents used in fatty acid synthesis and Saggerson and Greenbaum (1970) also found that hexose monophosphate pathway NADPH production does not always parallel fatty acid synthesis. Several authors have suggested that the rest of the required NADPH is obtained from cytoplasmic NADH through the malate transhydrogenation cycle in which the NAD and NADP-malate dehydrogenases are coupled with the net result that NADH

is used to generate NADPH (Pande et al., 1964; Wise and Ball, 1964; Leveille and Hanson, 1966; Rognstad and Katz, 1966). In addition, Young et al. (1964), Saggerson and Greenbaum (1970), Allee et al. (1971) and Hood (1972) reported that alterations in the activity of NADP-malate dehydrogenase in response to different lipogenic levels were similar to those of the NADPH generating enzymes of the hexose monophosphate pathway.

Lowenstein (1961) suggested that cytoplasmic NADP-isocitrate dehydrogenase could provide an alternative pathway for NADPH generation. In contrast, Young et al. (1964) reported that the correlation between isocitrate dehydrogenase and lipogenesis was almost negligible and Pande et al. (1964) and Wise and Ball (1964) found that isocitrate dehydrogenase did not show a close association with lipogenesis. The results of Bauman et al. (1970) and Leveille (1970) indicate that in the non-ruminant, NADP-isocitrate dehydrogenase does not appear to be of major importance in the generation of reducing equivalents because the activity is low and not adaptable to dietary changes that affect lipogenesis.

The source of reducing equivalents in ruminants is less clear. Under physiological conditions enough glucose may be metabolized via the hexose monophosphate pathway to supply adequate amounts of NADPH. This suggestion is supported by the presence of significant activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in bovine and ovine adipose tissue as reported by Raggi et al. (1961); Filsell et al. (1963); Hanson and Ballard (1967); and Opstvedt et al. (1967). Beitz (1972) reported significant levels of glucose-6-phosphate dehydrogenase activity in inner and outer subcutaneous perirenal, omental and intermuscular

adipose tissue depots of Holstein steers. In a similar study, Hood (1972) concluded that hexose monophosphate dehydrogenases particularly glucose-6-phosphate dehydrogenase appear to be important regulators of NADPH production for fatty acid synthesis in bovine adipose tissues. In contrast, Bauman et al. (1970) found that glucose-6-phosphate dehydrogenase activity in bovine mammary gland was only 1/6 of that in rat mammary tissue and they suggested that the ruminant has yet another means of generating reducing equivalents. The almost complete absence of the citrate cleavage pathway in ruminant tissues rules out an active malate transhydrogenation cycle as a major NADPH generator. Bauman et al. (1970) have suggested that an alternate source of NADPH generation in the ruminant is via NADP-isocitrate dehydrogenase. They reported that the ratio of this enzyme to glucose-6-phosphate dehydrogenase was 13 to 1 for the bovine animal and 1 to 9 in the rat. Hood (1972) indicated that NADP-isocitrate dehydrogenase is capable of supplying NADPH for lipogenesis in bovine intermuscular fat. Beitz (1972) found considerable NADP-isocitrate dehydrogenase activity in adipose tissue of fasted and normal steers. Beitz (1972) also found that glucose-6-phosphate dehydrogenase activity was reduced during fasting to 50 percent of normal, but was less than the reduction in lipogenesis; whereas, NADP-isocitrate dehydrogenase activity was not reduced to the same degree as glucose-6-phosphate dehydrogenase activity during fasting. Bauman et al. (1972) studied subcutaneous adipose tissue from wether lambs and reported similar results. They found that fasting reduced the activity of the two pentose pathway enzymes by 50 percent while isocitrate dehydrogenase activity remained unchanged. It

appears that isocitrate dehydrogenase becomes more significant as a source of NADPH and glucose less important as a source of carbon and NADPH for lipogenesis as the availability of acetate is increased. NADP-isocitrate dehydrogenase may be the enzyme system for the generation of reducing power from acetate for lipogenesis in ruminant tissues.

Beitz (1972) suggested that the supply of reducing equivalents does not seem to be the rate limiting factor in fatty acid synthesis in ruminants, rather fatty acid synthesis tends to regulate the amount of NADPH that is generated from glucose oxidation. Nonetheless, there are numerous unanswered questions concerning the NADPH generating system in ruminant tissues.

Transport of Lipids

The transport of lipids by the plasma to the various tissues and the general metabolic routes of fatty acids and triglycerides are diagrammatically depicted in figure 6. Lipid transport in ruminants appears to occur similarly to lipid transport in the nonruminant. In ruminants, as in other mammals, the long chain fatty acids enter the circulatory system via the thoracic duct in the form of chylomicrons. These chylomicrons are composed primarily of triglycerides (Felinski et al., 1964; Wadsworth, 1968). About one-third of the chylomicron triglyceride is absorbed by the liver (Di Luzio, 1960), one-third by adipose tissues (Felinski et al., 1964) and the remaining one-third by other tissues including mammary tissue (Robinson, 1963). The liver hydrolyzes triglycerides taken up as chylomicron triglyceride and these fatty acids along with free fatty acids

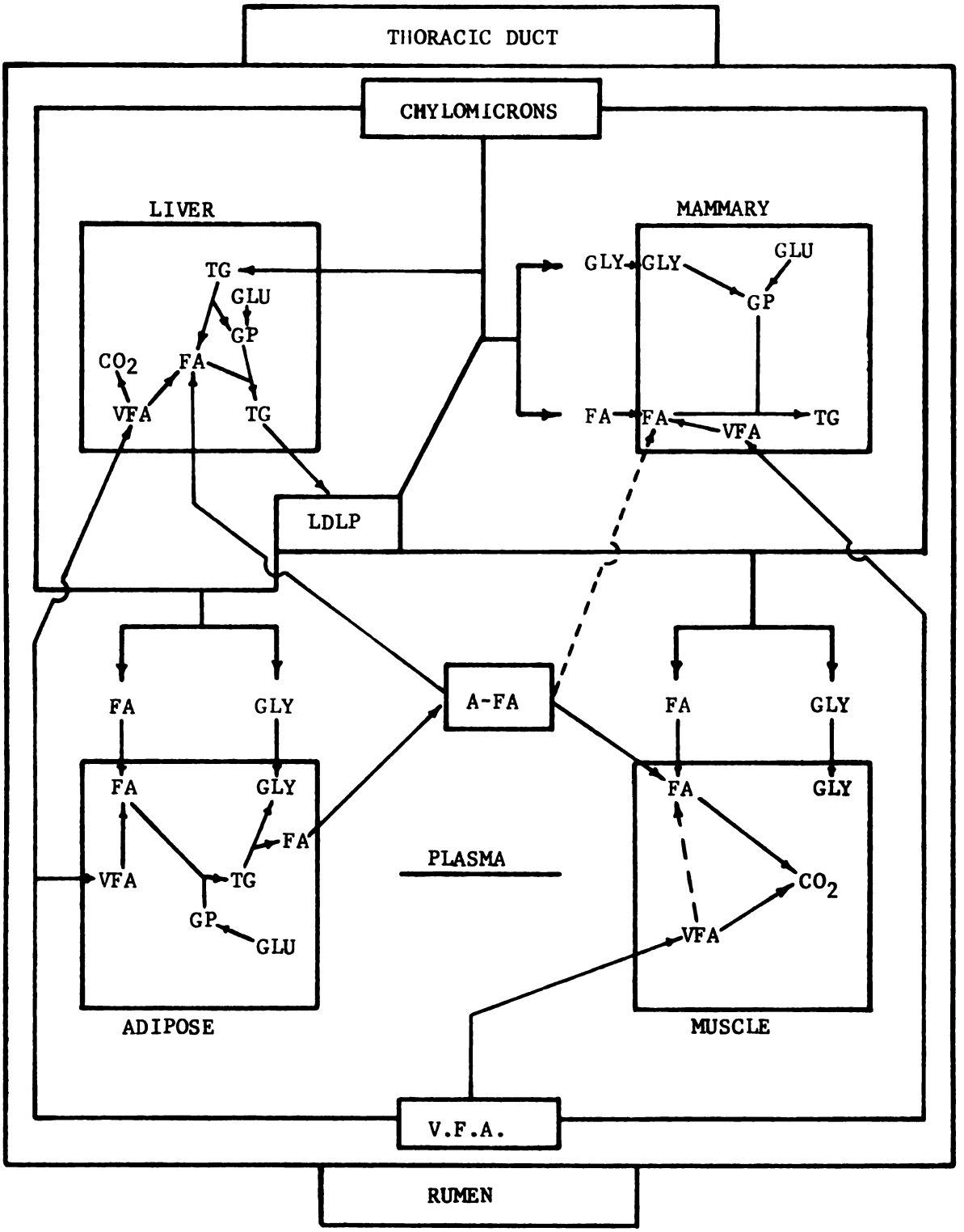


Figure 6. Diagram of the transport and uptake of triglycerides and fatty acids by ruminant plasma and their metabolism in tissues. The following abbreviations are used: VFA, volatile fatty acids; FA, fatty acids; A-FA, free fatty acids in albumin complex; LDLP, low-density lipoproteins; GLY, glycerol; GP, α-glycerophosphate; GLU, glucose.

mobilized from adipose tissues are re-esterified to glycerol to form new triglycerides (Olivecrons, 1962). The majority of the triglycerides synthesized by the liver are combined with protein to form lipoproteins, and they re-enter the plasma and are transported as low-density lipoproteins (Robinson, 1963). Triglycerides of chylomicrons and low density lipoproteins are hydrolyzed for removal from the circulating blood lipids by the action of the enzyme lipoprotein lipase (LPL).

Fatty Acid Uptake and Mobilization

In adipose tissue the processes of circulating triglyceride uptake and of intracellular triglyceride release both require hydrolysis of the triglyceride molecule and these hydrolytic reactions are mediated by two distinct lipase systems.

Lipoprotein lipase or clearing factor lipase first described by Korn (1955a, b) plays an important role in lipoprotein metabolism as it is the enzyme responsible for the hydrolysis of the triglyceride moiety of chylomicrons and low density proteins. It is present in various tissues that utilize plasma triglycerides and several investigators have presented evidence to show that plasma triglycerides are hydrolyzed to free fatty acids before their removal from the blood (Robinson, 1960, 1963; Bezman *et al.*, 1962a). The enzyme has been identified in heart and lung (Anfinsen *et al.*, 1952), adipose tissue (Hollenberg, 1959; Rodbell, 1964; Pokrajac *et al.*, 1967), muscle (Hollenberg, 1960) and in lactating mammary tissue (McBride and Korn, 1963), but not in liver (Olson and Alaupovic, 1966).

Mayes and Felts (1968) reported that LPL was present in liver, but in an inactive state. The activity of lipoprotein lipase in adipose tissue is proportional to the rate of triglyceride uptake, therefore the enzyme may play a significant role in controlling lipid deposition in this tissue (Garfinkel et al., 1967; Bezman et al., 1962b). There is considerable evidence (Pav and Wenkeova, 1960; Salaman and Robinson, 1966; Wing et al., 1966; Reichl, 1970, 1972; Scow et al., 1972; Schotz and Garfinkel, 1972) that LPL activity in adipose tissue is decreased by fasting and diabetes and increased by refeeding. Reichl (1972) found a progressive increase in LPL activity in adipose tissues that coincided with an increase in the plasma insulin concentration during the first 6 hours after feeding. This finding was consistent with the view of Robinson and Wing (1970) who earlier proposed that the increase in LPL activity that occurs during the transition from the starved to the fed state is, at least in part, due to an increased secretion of insulin. Several investigators (Robinson, 1963; Patton and Hollenberg, 1969) have demonstrated that LPL is activated or released by heparin when in both in vitro and in vivo studies. Scow et al. (1972) observed that LPL is rapidly released into the blood stream when heparin is injected intravascularly and suggested that the enzyme is present in or near the vascular wall. This is in agreement with the earlier suggestions that the action of LPL takes place at the luminal surface of the capillary endothelium. These suggestions were based on the findings that the enzyme is released into the circulation of organs involved in triglyceride uptake, such as the perfused rabbit hindlimb (Robinson and French, 1960) and the lactating goat mammary gland (Barry et al., 1963).

Additional evidence to support the suggestions has been revealed by several researchers using the electron microscope. Electron microscopy of adipose tissues by Moskowitz and Moskowitz (1965), mammary gland by Schoefl and French (1968) and of adipose tissue and heart by Blanchette-Markie and Scow (1971) from normal animals have shown that chylomicrons and other lipid particles are attached to the luminal surface of the capillary endothelium, but none was seen inside the cells or in extracellular space. Furthermore, West et al. (1972) concluded that hydrolysis occurs at the luminal surface of the capillary endothelial cells and the liberated glycerol and free fatty acids are then taken up by the tissue. It appears that intact chylomicrons and other lipid particles do not cross the capillary endothelium as intact particles in most tissues.

The mobilization of adipose depot triglycerides as glycerol and free fatty acids requires the action of a lipase that is activated by the catecholamines and a variety of other hormones. The enzyme responsible for initiating the hydrolysis of the triglycerides stored in adipose tissues has been referred to as hormone sensitive lipase (Rizack, 1961). In an experiment designed to determine the intracellular location of hormone sensitive lipase, Khoo et al. (1972) found that a large fraction of this enzyme could be recovered in a large, phospholipid-rich particle present in rat adipose tissue. They suggested that this indicates that the enzyme in the intact cell might be associated with a lipid-rich matrix and their results strongly suggest that hormone sensitive lipase is predominantly a cytoplasmic enzyme. The regulation of this lipase is attributed to intracellular levels of cyclic AMP (CAMP) (Butcher et al., 1965; Butcher and Sutherland, 1967; Meng and Ho, 1967; Davies, 1968; Patton, 1970) and in

this mechanism, cyclic AMP has been likened to a second messenger which carries the message from the hormone to its cellular action site (Robinson et al., 1967; 1968). Butcher (1969) indicated that hormone sensitive lipase regulation is mediated by a cyclic AMP dependent phosphorylation and dephosphorylation mechanism and that cyclic AMP effects the conversion of the enzyme from an inactive to an active form. Recent evidence accumulated by Huttunen et al. (1970a, b), Huttunen and Steinberg (1971), Mayer et al. (1972) and Sidhu et al. (1972) provide confirmation that hormonal activation of hormone sensitive lipase is mediated by cyclic AMP through a protein kinase and involves a phosphorylation activation of the enzyme. Thus, any condition which increases intracellular cyclic AMP would increase the activation of this enzyme. Bauman et al. (1972), Patton (1970) and Thorton et al. (1972) reported that lipolysis due to hormone sensitive lipase was elevated in both fasted lambs, and steers and decreased with refeeding. Beitz (1972) found that as steers matured and fat accumulated the maximum rates of lipolysis generally declined; whereas, Sidhu et al. (1972) concluded that lipolysis seemed to increase with age and fatness in lambs. On the basis of the limited information to date it is difficult to draw conclusions as to the physiological interrelationship of hormone sensitive lipase, lipoprotein lipase and lipid metabolism generally.

Hormonal Influences of Lipid Metabolism

The major portion of the research efforts concerning the mechanisms of adipose tissue regulation has been conducted with non-ruminants, especially the rat. In the ruminant the regulatory mechanisms and metabolic

pathways are somewhat different, although these differences may be quantitative rather than qualitative. Most of the hormones produced in an animal can affect some aspect of fat metabolism either by stimulation and/or inhibition of lipogenesis or lipolysis.

The major lipogenic hormone in the non-ruminant is insulin which is essentially the anabolic hormone of adipose tissue (Renold and Cahill, 1965). Insulin has two major effects on fat cells: 1) it stimulates the entry of glucose into the cell, and this is correlated with an accelerated metabolism of glucose and the synthesis and esterification of fatty acids (Crofford and Renold, 1965a, b; Turner and Bagnara, 1971); and 2) it exerts a potent antilipolytic effect, opposing the lipolytic actions of certain pituitary hormones and the catecholamines (Fain et al., 1966; Rudman and Shank, 1966).

Salans et al. (1968) and Nestel et al. (1969) reported that large adipocytes were less sensitive to the stimulatory effect of insulin on the oxidation of glucose and on the uptake of labeled triglyceride fatty acids than small cells. The former authors also found that biopsy samples of adipose tissues with different mean cell size incorporate glucose at similar rates when calculated on a per cell basis. However, Bjorntorp (1966) reported that on a per cell basis biopsy specimens from obese patients, which had enlarged adipocytes had a greater rate of lipid synthesis than specimens from nonobese patients. Additionally, Zinder et al. (1967) and Smith (1971a) demonstrated that incorporation of glucose into lipids in human adipose tissue is dependent on cell size since the larger cells of a given fat depot had a greater rate of glucose incorporation

than smaller cells of the same fat specimen. Furthermore, the latter investigators observed that the larger adipocytes were less sensitive than smaller adipocytes to the stimulatory effect of insulin on glucose incorporation into lipids.

The manner in which insulin is involved in carbohydrate and fat metabolism is readily apparent, since lipogenesis in adipose tissue is a function of the concentration of circulating insulin, and the circulating insulin concentration depends on blood glucose level (Adler and Wertheimer, 1968). Thus, as long as adipose tissue is responsive to insulin, the plasma level of insulin can regulate the lipogenic rate in adipose tissues. The major quantitative role of insulin in non-ruminants is the conversion of glucose to triglycerides in adipose tissue; whereas, this does not appear to be the case in ruminants because of the relatively low glucose levels and the presence of other substrates (VFA) for lipogenesis. The early work of Reid (1951) indicated that neonatal lambs and calves like non-ruminants are insulin sensitive, however, this insulin sensitivity decreases with increasing age. In more recent investigations, Manns et al. (1967); Horino et al. (1968) and Trenkle (1970) showed an increase in circulating insulin following the administration of butyric or propionic acid and they concluded that these two acids stimulated insulin secretion in ruminants. The actual physiological role of insulin in ruminant lipogenesis has not been thoroughly elucidated.

According to Ball (1970) the vast amount of research work in recent years on insulin has revealed a multiplicity of insulin action on adipose tissues. Perry and Bowen (1962) and Jungas and Ball (1962) reported that

insulin inhibited the customary production of free fatty acids and glycerol from rat adipose tissue following the administration of epinephrine, corticotropin, growth hormone, glucagon or thyroid stimulating hormone. Since the production of glycerol and free fatty acids from triglyceride is inhibited, this action has been termed the antilipolytic action of insulin. Butcher et al. (1965) first showed that the induction of lipolysis in adipose tissue by epinephrine was accompanied by an increase in tissue cyclic AMP. Butcher et al. (1966, 1968) also indicated that the increase in cyclic AMP induced by epinephrine was lowered by the addition of insulin and Jungas (1966) demonstrated that adenyl cyclase activity of adipose tissue was lowered by pretreatment with insulin. Thus, Ball (1970) proposed that the effects of insulin on lipolysis are due to its lowering cyclic AMP levels, which in turn results in diminished activities of lipolytic enzymes. The cellular effects of insulin on lipid metabolism have been studied more intensively than any of the other hormones; nonetheless, the mechanisms of insulin action on adipose tissues is still not completely resolved.

In recent years it has become increasingly apparent that the lipolytic process in adipose tissues plays a key role in the energy metabolism of the animal. By regulating the extent of lipolysis in adipose tissues a variety of hormones exert important influences on the supply of free fatty acids made available as fuel for muscle and other tissues of the body.

The catecholamines, epinephrine and norepinephrine, secreted by the adrenal medulla and the endings of the sympathetic nerve fibers, vigorously

promote hydrolysis of triglycerides to free fatty acids and glycerol in adipose tissues (Rudman, 1965; Steinberg, 1966; Fain, 1968; Corbin and Park, 1969; Exton et al., 1972). The catecholamines apparently do so by activating the rate limiting enzymatic step in adipose tissue lipolysis, i.e., hormone sensitive lipase (Khoo et al., 1972; Schwartz and Jungas, 1971; Corbin et al., 1970). They activate this enzyme by increasing 3',5'-cyclic AMP in adipocytes, which then activates the hormone sensitive lipase (Fain, 1968; Corbin et al., 1970; Exton et al., 1972). Therriault et al. (1969) found that the lipolytic response of isolated adipocytes to norepinephrine was related to cell size since the larger adipocytes were less responsive to norepinephrine than the smaller cells. In an experiment designed to study the influence of cell size on the release of free fatty acids from isolated adipocytes incubated with epinephrine and adrenocorticotropin (ACTH) Zinder and Shapiro (1971) concluded that free fatty acid release stimulated by either epinephrine or ACTH was a function of cell surface area as well as a function of cell number. Hartman et al. (1971) studied the lipolytic response of rat adipocytes to norepinephrine as related to cell size and reported that when the rate of lipolysis was based either on the amount of triglyceride in the incubation medium or on the cell surface area, lipolysis was inversely related to cell size.

The pituitary peptide hormones, adrenocorticotrophin (ACTH), thyroid stimulating hormone (TSH), melanophore stimulating hormone (MSH) and vasopressin, are capable of promoting fat mobilization in a manner identical of the catecholamines. However, the lipolytic activity of each of these peptide hormones is mammalian species specific (Rudman, 1965).

Growth hormone and the adrenal glucocorticoids promote fat mobilization, but their stimulation of lipolysis in isolated adipocytes occurs by a mechanism quite different from that of the other lipolytic hormones (Fain et al., 1965). The lipolytic effect of either growth hormone or the adrenal glucocorticoids, unlike other lipolytic agents, requires a lag period of at least one hour (Fain, 1968; Moskowitz and Fain, 1970). The results of Fain and Saperstein (1970) indicate that the lipolytic action of growth hormone and the glucocorticoids is due to a direct activation of white adipocyte lipolysis. The mechanism by which these hormones stimulate lipolysis is different from the rapid activation of adenyl cyclase seen with other peptide hormones and catecholamines. The lipolytic effect of growth hormone and glucocorticoids is blocked by protein inhibitors and this suggests that the action of these lipolytic agents is to increase the synthesis of a protein or proteins which appears to increase the amount of cyclic AMP that accumulates in white adipose tissue cells (Fain, 1967; Moskowitz and Fain, 1970).

The actions of glucagon in most instances, are antagonistic to those of insulin (Foa, 1964). Glucagon elevates cyclic AMP in liver and adipose tissue and activates hormone sensitive lipase, thereby stimulating lipolysis (Fain, 1968; Manganiello and Vaughan, 1972; Exton et al., 1972). However, the actual physiological role of glucagon in the regulation of fat mobilization has not been fully established.

It is evident that many hormones influence fat mobilization, but the difficult question to answer in regard to these agents is whether their efforts are merely pharmacological or whether these agents function in physiological control mechanisms.

EXPERIMENTAL PROCEDURE

An experiment involving 49 lambs was conducted to study the influence of breed type, sex and age on enzymatic activity and lipid biosynthesis. A commercial flock of 39 Western crossbred ewes was divided into two groups based upon their body length (anterior edge of scapula to tuber ischii) and body weight. The small ewes averaged 76.2 cm in length and weighed 70.9 kg while the larger ewes averaged 79.2 cm in length and 76.6 kg in weight. The smaller bodied ewes were mated to a Southdown ram and the larger bodied ewes were mated to a Suffolk ram in order to obtain two strains of lambs differing in propensity to fattening. Because of the differences in body type of the ewes and ram breed, the term breed type will be used to denote the matings employed in this study. Reference to the lambs resulting from these matings will be identified hereafter by their sire breed. Table 1 shows the design of the experiment and the number of lambs in each breed type, sex and age group.

The ewes were maintained on pasture or hay until approximately one month before the first ewe lambed. The ewes were then moved to dry-lot where they were maintained on hay supplemented with a grain ration. The grain rations for the bred and lactating ewes are presented in tables A and B in Appendix I.

The lambs of the 8, 16 and 32 week age groups were exposed to ad libitum creep feed after 2 weeks of age and green, leafy alfalfa hay was fed twice daily. The composition of the creep feed and grain rations of the subsequent feeding periods are shown in table 2. The lambs were weaned at 2 months of age and remained in dry-lot on full feed until

Table 1. EXPERIMENTAL DESIGN

Age	Sex and breed type						Age group total
	Ram lambs		Ewe lambs		Wether lambs[a]		
	Southdown[b]	Suffolk[b]	Southdown[b]	Suffolk[b]	Southdown[b]	Suffolk[b]	
Birth[c]	1	1	1	1			4
24 hour[d]	2	2	2	3			9
8 weeks	2	2	2	2	2	2	12
16 weeks	2	2	2	2	2	2	12
32 weeks	2	2	2	2	2	2	12
Sub-total	9	9	9	10	6	6	
Total	18		19		12		49

[a]The lambs of this sex group were castrated at 4 weeks of age.
[b]Refers to the breed of the sire.
[c]The lambs of this age group were slaughtered immediately after birth and had not nursed.
[d]The lambs of this age group nursed and were slaughtered at 24 hours of age.

Table 2. COMPOSITION OF CONCENTRATE MIXTURES

Ingredients	Age of lambs			
	2 weeks %	6 weeks %	12 weeks %	16 weeks onwards %
Crimped oats	24.92	14.95	-----	-----
Whole oats	-----	19.94	46.85	56.89
Crimped corn	51.84	23.93	-----	-----
Shelled corn	-----	19.94	34.90	22.95
Wheat bran	7.98	6.98	5.98	9.98
Soybean oil meal (49%)	9.97	9.97	9.97	9.97
Wet molasses	2.99	1.99	----	----
Dicalcium phosphate	1.00	1.00	1.00	----
Trace mineral salt[a]	1.00	1.00	1.00	----
Aurofac[b]-10	0.30	0.30	0.30	0.20
Total	100.00	100.00	100.00	100.00

[a]Guaranteed analysis, %: Co, 0.012; Mn, 0.228; Cu, 0.040; Fe, 0.160; I, 0.007; Zn, 0.011. Product obtained from Michigan Salt Co., St. Louis, Mich.

[b]Chlortetracycline, 22 g per kilogram. Product obtained from American Cyanamid Co., Princeton, New Jersey.

slaughtered. Full feed of grain was defined as the amount of grain consumed in 20 min twice daily.

Slaughter Procedure

Lambs were slaughtered at the designated ages by bleeding without prior stunning. Blood was collected at the time of slaughter in a centrifuge tube coated with heparin to prevent clotting and then centrifuged at 2000 x g for 10 min to obtain the plasma. The plasma was removed via pipette, frozen and stored at -30°C. Immediately following bleeding, samples of liver, longissimus muscle (LM), perirenal (PRF), and subcutaneous fat (SCF) were removed from the carcass. The longissimus muscle was excised from the 12th to 13th thoracic vertebrae region, except in the at birth and 24 hour age groups in which both intact longissimus muscles were excised in order to obtain sufficient sample. Subcutaneous fat samples were removed from the dorsal thoracic and lumbar regions of the carcass except in the at birth and 24 hour age groups where the absence of external fat made it impossible to obtain subcutaneous fat samples.

The following enzymes were assayed on all four tissues: glucose-6-phosphate dehydrogenase (G-6PDH), 6-phosphogluconate dehydrogenase (6-PGDH), NADP-malate dehydrogenase (ME), NADP-isocitrate dehydrogenase (ICDH), acetyl COA synthetase (Syn), acetyl COA carboxylase (CBX) and ATP-citrate lyase (CCE). The adipose tissue and longissimus samples were assayed for lipoprotein lipase (LPL) and free fatty acids (FFA) were determined on blood plasma.

Extractions of acetyl COA synthetase and acetyl COA carboxylase were made on all tissues immediately after sampling and the cytosol and mitochondrial fractions were obtained by centrifugation. These fractions were frozen and stored at -30°C. The remaining portions of the fresh tissue samples were frozen immediately in liquid nitrogen and stored at -30°C until powdered. The frozen tissue samples were powdered in a Waring blendor and the connective tissue particles were removed by sifting. All powdering procedures were carried out in a -30°C freezer and following powdering the samples were stored at -30°C until assayed.

Enzyme Assays

Acetyl COA Synthetase

Acetyl COA synthetase catalyzes the reaction of acetate with COASH to form acetyl COA. The assay measured the disappearance of the (-SH) group of COASH which was accomplished by breaking the S-H bond shown in the following reaction.

$$\text{acetate} + \text{COA - SH} + \text{ATP} \xrightarrow[Mg^{++}]{\text{Syn}} \text{acetyl COA} + H_2O + \text{AMP} + \text{PPi}$$

The procedure followed was that described by Grunert and Phillips (1951).

Enzyme extraction. Approximately 5 g of fresh tissue were homogenized 0° in 20 ml of 0.13M KCl at 2°C with a Virtis homogenizer for 30 seconds. The homogenate was centrifuged in a Sorvall RC2-B refrigerated centrifuge (0°C) for 10 min at 1000 X gravity. The pellet was discarded and the

supernate was centrifuged at 20,000 X g for 20 minutes. The cytosol fraction was decanted into a storage vial and the mitochondrial pellet was resuspended in 10 ml of 0.13M KCl and both fractions were frozen and stored at -30°C until assayed. All preparatory work was carried out in a 2°C coldroom.

Assay procedure. The assay reagents and the assay design are presented in table 3. All reagents, except the enzyme extract, were added to 15 ml conical centrifuge tubes. The reaction was started by the addition of 0.05 ml of enzyme extract diluted with buffer to a protein concentration of 1 to 2 mg protein/milliliter. The tubes were incubated in a gently shaking waterbath at 37°C for 10 minutes. The reaction was stopped by the addition of 2.8 ml of the color reagent (footnote e table 3) and the optical density was read exactly 30 sec after the addition of the color reagent at 520 nm on a Bausch and Lomb Spectronic 20 Colorimeter. The colorimeter was set to zero with the blank.

Calculations.

O.D. (standard) - O.D. (assay) = Δ O.D. (A)

A x 3.243 (conversion factor for 10 min assay) = μmoles M COASH oxidized /tube (B)

$$\frac{B \times \text{extract dilution factor} \times \text{extract aliquot factor}}{\text{mg protein} \quad 1 \text{ ml enzyme extract}} =$$

enzyme units/mg protein = Specific Activity

enzyme unit = 1.0 μmoles M COASH reacting in 60 minutes

Δ O.D. of 0.185 = 0.10 μmoles COASH oxidized

Table 3. REAGENTS AND ASSAY DESIGN FOR ACETYL COA SYNTHETASE

Reagents[a]	Tubes: Blank vol (ml)	Tubes: Standard[b] vol (ml)	Tubes: Assay[c] vol (ml)	Assay conc (μM)
0.25M Mg Cl_2	0.01	0.01	0.01	2.5
0.08M ATP (dipotassium salt)[d]	0.01	0.01	0.01	0.8
0.5M acetate (K)	0.01	--	0.01	5.0
0.05M tris pH 8.6	0.07	0.07	0.06	(35.0)(30.0)
0.017M COASH (tri-lithium salt)	--	0.01	0.01	0.17
Enzyme extract	0.05	0.05	0.05	--
Total	0.15	0.15	0.15	--
	after incubation			
Color reagent[e]	2.8	2.8	2.8	--

[a]Prepared in 0.05M tris pH 8.6.
[b]Duplicated.
[c]Duplicated.
[d]Prepared in 0.05M tris pH 6.8 and diluted 1:3 with 0.05M tris pH 8.6 for each assay.
[e]Mixed fresh for each assay in the following proportions; 1 (2.0M Na_2CO_3 in 0.09M NaCN) : 5 (6.8M NaCl) : 1 (0.09M Na_2 [Fe (NO)$(CN)_5$] $\cdot H_2O$) and stored in an amber bottle.

Acetyl COA Carboxylase

Acetyl COA carboxylase catalyzes the reaction of acetyl COA with ^{14}C-bicarbonate to form radioactive malonyl COA as shown in the following reaction.

$$\text{acetyl COA} + H^{14}CO_3 + \text{ATP} \xrightarrow[mg^{++}]{CBX} {}^{14}\text{C-malonyl COA} + \text{ADP} + \text{Pi}$$

The ^{14}C-malonyl COA was not utilized for fatty acid synthesis as NADPH was not present in the assay medium. The reaction was stopped by acidification and the radioactivity of the ^{14}C-malonyl COA was measured by liquid scintillation counting. The procedure followed was a modified method of Dakshinamurti and Desjardins (1969).

Enzyme extraction. Approximately 5 g of fresh tissue were homogenized in 25 ml of 0.4M glycylglycine in 0.25M sucrose pH 7.0 with a Virtis homogenizer for 30 seconds at 2° centigrade. The homogenate was filtered through a thin layer of pyrex glass wool and centrifuged at 1000 X g for 10 minutes. The pellet was discarded and the supernate centrifuged at 20,000 X g for 20 minutes. The cytosol was decanted into a storage vial and the mitochondral pellet was discarded. All work was carried out in a 2°C cold room and the cytosol fraction was frozen and stored at -30°C until assayed.

Assay procedure. The assay reagents and the assay design are presented in table 4.

All the reagents except acetyl COA (AcCOA) were added to an 12 mm by 75 mm disposable culture tube and incubated at 37°C for 30 min in a gently

Table 4. REAGENTS AND ASSAY DESIGN FOR ACETYL COA CARBOXYLASE

Reagents[a]	Tube: Blank[b] vol (ml)	Tube: Assay[c] vol (ml)	Assay conc µM
0.25M tris pH 7.6	0.32	0.25	(76.8)(60.0)
0.32M $MgCl_2$	0.02	0.02	6.4
0.80M citrate (K^+)	0.02	0.02	16.0
0.12M Glutathione (GSH)	0.02	0.02	2.4
60 mg/ml BSA (fraction V)	0.01	0.01	0.6 mg
0.01M EDTA	0.01	0.01	0.1
H_2O	0.20	0.20	--
0.08M ATP[d]	--	0.05	4.0
0.10M $NaH\ ^{14}CO_3$ (902, 910 CPM/µmole)	0.10	0.10	10.0
0.01M AcCOA	--	0.02	0.2
6N HCl	0.20	0.20	--
Enzyme extract	0.10	0.10	--
Total	1.0	1.0	--
After incubation			
Scintillation solution[e]	10.0	10.0	--

[a]Prepared in 0.05 tris pH 7.6.
[b]Duplicated.
[c]Triplicated.
[d]Prepared in 0.05M tris pH 6.8 and frozen for stability.
[e]Composed of naphthalene (60 g): PPO (4g), POPOP (0.2 g), absolute methanal (100 ml), ethylene glycol (20 ml) and p-dioxane up to 1 liter.

shaking waterbath. The preincubation of the enzyme extract with citrate was necessary to attain maximum CBX activation. The reaction was started by the addition of AcCOA and incubated at 37°C for 2 minutes. After the 2 min incubation, 6N HCl was added to stop the reaction and the protein precipitate was sedimented by centrifugation at 1000 rpm for 3 minutes. A 0.2 ml aliquot was removed via repipet and delivered into a scintillation vial. The vials were placed in a 55°C sandbath to remove excess water and $^{14}CO_2$. After drying, 10 ml of scintillation solution were added and the radioactivity measured with a Chicago-Nuclear scintillation spectrophotometer. The micromoles of $^{14}CO_2$ incorporated were determined from the radioactivity of the malonyl COA and the specific activity of NaH $^{14}CO_3$.

Calculations.

cpm (test) - cpm (blank) = Δcpm (A)

$$\frac{(A) \times \text{efficiency coefficient}}{CC} = \mu\text{moles } ^{14}CO_2 \text{ incorporated/tube (B)}$$

where CC = count coefficient = CPM/μmoles $^{14}CO_2$ = 902, 910 CPM/μmole

$$\frac{B \times \text{assay aliquot factor} \times \text{extract aliquot factor}}{\text{2 minute assay} \times \text{mg protein/ml enzyme extract}} = \mu\text{moles } ^{14}CO_2$$

incorporated/min/mg protein

ATP - Citrate lyase

Citrate cleavage enzyme catalyzes the cleavage of citrate to yield AcCOA and oxaloacetate (OAA). The reaction was measured by coupling it with the NAD-malate dehydrogenase (MDH) reaction. Malate dehydrogenase

catalyzes the conversion of OAA to malate using NADH as the reducing agent. The oxidation of NADH as shown in the reaction below was followed spectrophotometrically at 340 nm.

$$\text{citrate} + \text{ATP} + \text{COA} \xrightarrow[Mg^{++}]{CCE} \text{Ac COA} + \text{ADP} + \text{Pi} + \text{OAA} \xrightarrow[NADH \quad NAD]{MDH} \text{malate}$$

The enzyme was assayed using the procedure described by Kornacker and Ball (1965).

Enzyme extraction. Approximately 2 g of powdered tissue were homogenized in 20 ml of 0.15M KCl in 0.05M tris (CL-) pH 7.4 with a Virtis homogenizer at 2° centigrade. The homogenate was filtered through a thin layer of pyrex glass wool and centrifuged at 1000 x g for 10 minutes. The pellet was discarded and the supernate was centrifuged at 20,000 x g for 20 minutes. The cytosol fraction was decanted over glass wool to remove the fat particles and then frozen and stored at -30°C until assayed.

Assay procedure. The assay reagents and design are presented in table 5.

All reagents except ATP were added to an incubation tube and incubated at 25°C for 5 minutes. The activity of the blank was measured at 340 nm on a Beckman/DU spectrophotometer. The reaction was started by the addition of ATP and the change in optical density was recorded at one minute.

Calculations.

O.D. (blank) - O.D. (test) = Δ O.D.

Table 5. REAGENTS AND ASSAY DESIGN FOR CITRATE CLEAVAGE ENZYME.

Reagents[a]	Tubes: Blank vol (ml)	Tubes: Assay[b] vol (ml)	Assay conc µM
0.05M tris (Cl^-) pH 7.3	1.40	1.30	(70.0)(60.0)
1.0M $MgCl_2$	0.02	0.02	20.0
0.1M Dithiothreitol (DTT)	0.04	0.04	4.0
0.1M KCN	0.02	0.02	2.0
0.4M citrate (K^+)	0.10	0.10	40.0
2.5 mg/ml MDH	0.02	0.02	0.05 mg
1.67 mg/ml NADH	0.10	0.10	0.167 mg
0.008M COA	0.10	0.10	0.8
0.1M ATP	--	0.10	10.0
Enzyme extract	0.20	0.20	--
Total	2.0	2.0	

[a]Prepared in 0.05M tris (Cl^-) pH 7.3.
[b]Duplicated.

$$\frac{\Delta \text{ O.D.}}{\text{E.C. (standard curve)}} = \mu\text{moles NADH oxidized/tube/min.}$$

where E.C. = $\frac{\text{O.D.}}{\text{conc}}$

$$\frac{\mu\text{moles NADH oxidized/tube/min X aliquot factor}}{\text{mg protein/ml enzyme extract}} = \mu\text{moles NADH oxidized/min/mg protein}$$

Glucose-6-phosphate dehydrogenase

and 6-Phosphogluconate dehydrogenase

Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase catalyze the following reactions and their activities were determined collectively by measuring the generation of NADPH at 340 nm upon the addition of both substrates (G-6-P and 6-PG). 6-Phosphogluconate dehydrogenase

$$\text{G-6-P} \xrightarrow[\text{Mg++}]{\text{G-6-PDH, } H_2O} \text{6-PG} \xrightarrow[\text{Mg++}]{\text{6-PGDH}} \text{Ribulose-5-P}$$

NADP → NADPH (G-6-PDH step); NADP → NADPH (6-PGDH step)

was determined by the addition of only 6-PG and G-6-PDH was determined by difference. The enzyme was assayed using the procedure described by Lohr and Waller (1963).

Enzyme extraction. Approximately 2 g of powdered tissue were homogenized in 25 ml of 0.25M sucrose with a Virtis homogenizer at 2° centigrade. The homogenate was filtered through a thin layer of pyrex glass wool and centrifuged at 1000 x g for 10 minutes. The pellet was discarded and the supernate was centrifuged at 20,000 x g for 20 minutes. The cytosol fraction was decanted over glass wool to remove fat particles and frozen

and stored at -30°C until assayed. The mitochondrial pellet was resuspended in 5 ml of 0.25M sucrose and frozen and stored at -30°C until assayed for isocitrate dehydrogenase activity.

Assay procedure. The assay reagents and design are presented in table 6. All reagents, except the substrate(s), were added to an incubation tube and preincubated at 25°C for 5 minutes. The reaction was started by the addition of the substrate(s) and the change in O.D. at 340 nm after 1 min was recorded.

Table 6. REAGENTS AND ASSAY DESIGN FOR GLUCOSE-6-PHOSPHATE DEHYDROGENASE AND 6-PHOSPHOGLUCONATE DEHYDROGENASE.

	Tubes			
		G-6-D[b]		
Reagents[a]	Blank vol (ml)	6-PG vol (ml)	6-PG[c] vol (ml)	Assay conc μM
0.05M glycylglycine pH 7.6	1.6	1.4	1.4	(80.0)(70.0)
0.40 $Mg\ Cl_2$	0.1	0.1	0.1	40.0
0.0015M NADP	-	0.2	0.2	0.3
0.01M G-6-P 0.01M 6-PG	0.2	0.2	-	2.0
0.01M 6-PG	-	-	0.2	2.0
Enzyme extract	0.1	0.1	0.1	-
Total	2.0	2.0	2.0	

[a]Prepared in 0.05M tris (Cl^-) pH 7.6.
[b]Duplicated.
[c]Duplicated.

Calculations.

O.D. (both) - O.D. (blank) = total Δ O.D. (G-6PDH + 6-PGDH)

O.D. (6 - PG) - O.D. (blank) = Δ O.D. (6-PGDH)

Total Δ O.D. - Δ O.D. (6-PGDH) = Δ O.D. (G-6PDH)

$\frac{\Delta \text{ O.D.}}{\text{E.C. (standard curve)}}$ = μmoles NADPH generated/min/tube

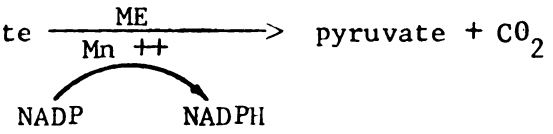
where E.C. = $\frac{\text{O.D.}}{\text{conc}}$

$\frac{\text{μmoles NADPH generated/min/tube X extract aliquot factor}}{\text{mg protein/ml enzyme extract}}$ =

μmoles NADPH generated/min/mg protein

NADP - Malate dehydrogenase

NADP-malate dehydrogenase (ME) catalyzes the decarboxylation of L-malate to pyruvate and the activity was determined by measuring the NADPH generated by the reaction shown below.



The enzyme was assayed using the procedure described by Ochoa (1955) and the generation of NADPH was followed spectrophotometrically at 340 nm.

The enzyme extraction procedure was identical to that previously described for the extraction of G-6PDH and 6-PGDH.

Assay procedure. The reagents and assay design are presented in table 7. All reagents except L-malate were added to an incubation tube and preincubated at 25°C for 5 minutes. The blank contained all reagents except NADP. The reaction was started by the addition of L-malate and the change in optical density at 340 nm was recorded at 1 minute.

Table 7. REAGENTS AND ASSAY DESIGN FOR MALIC ENZYME.

Reagents[c]	Tubes: Blank vol (ml)	Tubes: Assay[b] vol (ml)	Assay conc µM
0.05M tris (Cl^-) pH 7.4	1.6	1.4	(80.0)(70.0)
0.02M Mn Cl_2	0.1	0.1	2.0
0.0015M NADP	-	0.2	0.3
0.015M L-malate	0.2	0.2	3.0
Enzyme extract	0.1	0.1	-
Total	2.0	2.0	

[a]Prepared in 0.05 tris (Cl^-) pH 7.4.
[b]Duplicates.

Calculations.

O.D. (test) - O.D. (blank) = Δ O.D.

$$\frac{\Delta \text{ O.D.}}{\text{E.C. (standard curve)}} = \text{µmoles NADPH generated/min/tube}$$

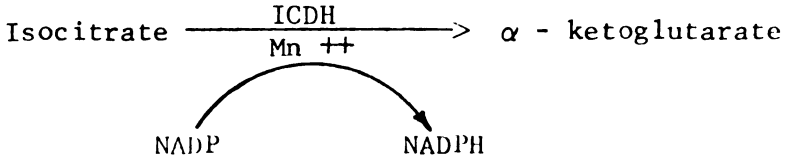
where: $\text{E.C.} = \frac{\text{O.D.}}{\text{conc}}$

$$\frac{\text{µmoles NADPH generated/min/tube} \times \text{extract aliquot factor}}{\text{mg protein/ml enzyme extract}} =$$

µmoles NADPH generated/min/mg protein

NADP - Isocitrate Dehydrogenase

NADP-isocitrate dehydrogenase (ICDH) catalyzes the decarboxylation of isocitrate to α-ketoglutarate both intra- and extra-mitochondrially. The activity of this enzyme was determined by following the generation of NADPH (as shown in the reaction below) spectrophotometrically at 340 nm.



The enzyme was assayed according to the method of Plaut (1962) for both the cytosol and mitochondrial fractions.

The enzyme extraction procedure was identical to that used for the extraction of G-6PDH and 6-PGDH as previously described.

Assay procedure. The reagents and assay design are presented in table 8. In the assay of the cytosol fraction all the reagents except isocitrate were added to an incubation tube and preincubated at 30°C for 5 minutes. The reaction was initiated by the addition of isocitrate and the change in optical density at 340 nm at 1 min was recorded. In the assay of the mitochondrial fraction all the reagents except NADP were added to an incubation tube and preincubated for 5 min at 30° centigrade. The reaction was started by the addition of NADP and the change in optical density at 340 nm was recorded at 1 minute.. The blanks in both cases contained all of the reagents except NADP.

The calculations for ICDH were identical to those for the calculation of malate enzyme already described.

Table 8. REAGENTS AND ASSAY DESIGN FOR ISOCITRATE DEHYDROGENASE.

Reagents[a]	Tubes: Blank vol (ml)	Tubes: Assay vol (ml)	Assay conc µM
0.05M tris (Cl^-) pH 7.4	1.2	1.0	(60.0)(50.0)
0.0034M EDTA	0.2	0.2	0.68
0.03M $MnCl_2$	0.1	0.1	3.0
0.0015M NADP	-	0.2	0.30
0.3% gelatin	0.2	0.2	-
0.015M dl-isocitrate	0.2	0.2	3.0
Enzyme extract	0.1	0.1	
Total	2.0	2.0	

[a]Prepared in 0.05M tris (Cl^-) pH 7.4.
[b]Duplicated.

Lipoprotein Lipase

Lipoprotein lipase is a specific lipase which preferentially hydrolyzes triglycerides when they are components of lipoproteins. The enzyme was assayed by a modification of the method of McBride and Korn (1963).

Enzyme extraction. Approximately 3 g of powdered tissue was homogenized in 12 ml of 0.15M KCl pH 8.5 with a Virtis homogenizer for 30 seconds. The homogenate was centrifuged at 2750 rpm for 10 min and the supernate decanted over a thin layer of pyrex glass wool.

Assay procedure. The reagents and assay design are presented in table 9. The substrate employed in this assay was a mixture of (1:1) fresh

Table 9. REAGENTS AND ASSAY DESIGN FOR LIPOPROTEIN LIPASE.

Reagents	Tubes[a] Boiled extract[b] blank vol (ml)	Assay vol (ml)
10% BSA (fraction V)[c]	1.0	1.0
0.15M KCl pH 8.5	1.0	1.0
1:1 serum:ediol	0.5	0.5
Enzyme extract	0.5	0.5
After 45 minute incubation		
LPL extraction fluid[d]	5.0	5.0
Hexane	3.0	3.0
H_2O	2.0	2.0

[a]Duplicated.
[b]The extract was placed in boiling waterbath for 3 min to inactivate the enzyme.
[c]Prepared in 0.15M KCl pH 8.5.
[d]Composed of isopropanol:heptane:1N H_2SO_4 (40:10:1).

ovine serum and ediol, a coconut oil emulsion (Calbiochem) mixed 1:6 with distilled water. The appropriate amount of serum and ediol were combined prior to assay and incubated for 30 min at 30°C in a gently shaking waterbath. The KCl, BSA and preincubated substrate (serum:ediol) were added to a 25 ml screw cap vial. The specified amounts of the enzyme extracts were added to start the reaction and the reaction mixtures were incubated at 37°C for 45 minutes in a gently shaking water bath. After the 45 min incubation period 5 ml of the LPL extraction solution was added to each vial to stop the reaction and the vials were allowed to stand at room

temperature for 5 minutes. Following the 5 min equilibrium period, 3 ml of heptane and 2 ml of H_2O were added and the mixture was allowed to stand until the layers separated. Two ml of the top layer were transferred to another test tube via repipet and dried in a 55°C sandbath for the subsequent colorimetric determination.

After drying 4 ml of toluene were added to the tubes and the tubes vortexed at low speed. Then 3 ml of a 1:1 mixture of Rhodamine B (1 mg/ml H_2O) and uranyl acetate (1% aqueous solution) were added and the tubes vortexed three times at 10 min intervals. Each time the mixture turns from purple to pink and becomes turbid. The tubes were centrifuged at 2000 rpm for 5 min and the top layer transferred via Pasteur disposable capillary pipette into a spectrophotometer cuvette. The optical density at 545 nm was recorded and compared to that of a palmitic acid standard curve.

Calculations.

O.D. (assay) - O.D. (blank) = Δ O.D.

Δ O.D. compared to the palmitic standard curve to obtain the μmoles FFA released/ml toluene

$$\frac{\mu\text{moles FFA/ml toluene} \times \text{dilution factor} \times \text{aliquot factor} \times \text{extract aliquot}}{\text{mg protein/ml enzyme extract}}$$

$$\frac{\text{factor} \times \text{time factor}}{} = \mu\text{moles FFA released/hr/mg protein}$$

Free Fatty Acids

Assay procedure. A colorimetric method described by Mackenzie et al. (1967) was used to determine the free fatty acid (FFA) concentration in

blood plasma. The reagents and assay design are presented in table 10. One gm of zeolite was added to a 25 ml screw cap test tube followed by 1 ml of the plasma sample and 2 drops of 85% phosphoric acid. With constant mixing 3 ml of methylal:methanol (4:1) were added followed by 4 ml methanol, 1 ml H_2O and 3 ml petroleum ether. The test tubes were capped and vortexed twice at 5 min intervals for 30 sec at low speed. The reaction mixture was centrifuged at 2000 rpm for 5 min and a 1 ml aliquot from the top layer was transferred via capillary pipette to another test tube. The tubes were placed in a 55°C sandbath to evaporate the petroleum ether. After drying the same colorimetric method described for the lipoprotein lipase assay was employed to determine the FFA. The blank was used to set the spectrophotometer to zero.

Table 10. REAGENTS AND ASSAY DESIGN FOR FREE FATTY ACIDS.

Reagents	Tubes	
	Blank vol (ml)	Assay[a] vol (ml)
Zeolite[b]	1 g	1 g
Plasma	-	1.0
85% phosphoric acid	2 drops	2 drops
Methylal:Methanol (4:1)	3.0	3.0
Methanol (reagent grade)	4.0	4.0
Distilled H_2O	2.0	1.0
Petroleum ether (redistilled)	3.0	3.0

[a]Duplicated.
[b]Ground to 100 mesh and dried at 100°C.

Calculations. The test optical density was compared to a palmitic standard curve to obtain the μM FFA/ml toluene.

$$\frac{\mu\text{moles FFA/ml toluene X dilution factor X aliquot factor}}{\text{mg protein/ml plasma}} = \frac{\mu\text{moles FFA/mg}}{\text{protein}}$$

Protein Determination

Protein was determined by the method of Lowry et al. (1951) and expressed on a mg/ml of tissue extract basis.

Statistical Analysis

Data were anlyzed on the C.D.C. 3600 computer at Michigan State University Computer Laboratory. A least squares analysis was used to determine differences between treatment means. The Duncan's Multiple Range Test was employed when significant differences were observed (Snedecor and Cochran, 1969). Simple correlation coefficients were also determined (Snedecor and Cochran, 1969).

RESULTS AND DISCUSSION

The results and discussion of the enzyme analyses will be presented in groups according to the phase of lipogenesis in which they are involved. The first group includes acetyl COA synthetase and ATP-citrate lyase which are involved in the formation of the cytoplasmic acetyl COA necessary for fatty acid synthesis. Acetyl COA carboxylase which is considered to be a key enzyme in de novo fatty acid synthesis from acetyl COA will also be included with the first group. The second group includes glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, NADP-isocitrate dehydrogenase and NADP-malate dehydrogenase which have been regarded as being functional for the generation of the reducing equivalents (NADPH) necessary for lipogenesis. The final group will include the enzyme lipoprotein lipase which plays an important role in lipoprotein metabolism since it is responsible for hydrolysis and uptake of the triglyceride moiety of circulating chylomicrons and low density proteins from plasma.

In the discussion of the results several terms are used which require definition or clarification. In the discussion of the level of lipogenic activity, its use in this dissertation refers to the rate of fatty acid synthesis and it will be used synonymously with the rate of fat deposition. A decrease in lipogenic activity or rate of fat deposition indicates a decreased rate of fatty acid synthesis, but it does not imply that fatty acids are not being synthesized or that fat is not being deposited. An increase in lipogenic activity refers to an increase in the rate of fatty acid synthesis and/or rate of fat deposition. Under normal nutritional regimes, fatty acids are being synthesized and fat is deposited

during the entire growth and development of the lamb and continues, although to a more limited extent, even after maturity has been reached. However, the rate of fatty acid synthesis and fat deposition varies throughout the life of the sheep.

Reference to adipose tissue depots in this dissertation will include the subcutaneous (SCF) and perirenal (PRF) fat. While intramuscular fat (marbling) is generally considered as a fat depot, because of the low quantity of fat observed in the longissimus muscle (LM) of the lambs included in this study, the LM will not be included in the discussion of the adipose tissue depots (SCF AND PRF), but will be discussed separately.

Acetyl COA Generation

Acetyl COA Synthetase

The enzyme acetyl COA synthetase (SYN) allows the ruminant to form acetyl COA directly in the cytoplasm; thus, the conversion of acetate to acetyl COA for de novo fatty acid synthesis is of considerable physiological importance in the ruminant. The means and standard error of the means of acetyl COA synthetase activity are presented in figures 7, 8 and 9. Cytoplasmic SYN (figure 7) was significantly ($P < .05$) higher in perirenal fat (PRF) than in subcutaneous fat (SCF) with both PRF and SCF possessing greater enzyme activities than longissimus muscle (LM) and liver. Mitochondrial SYN activity (figure 8) followed a similar pattern with the adipose tissue depots showing significantly ($P < .05$) higher activity than muscle and liver. A pattern similar to that of both the

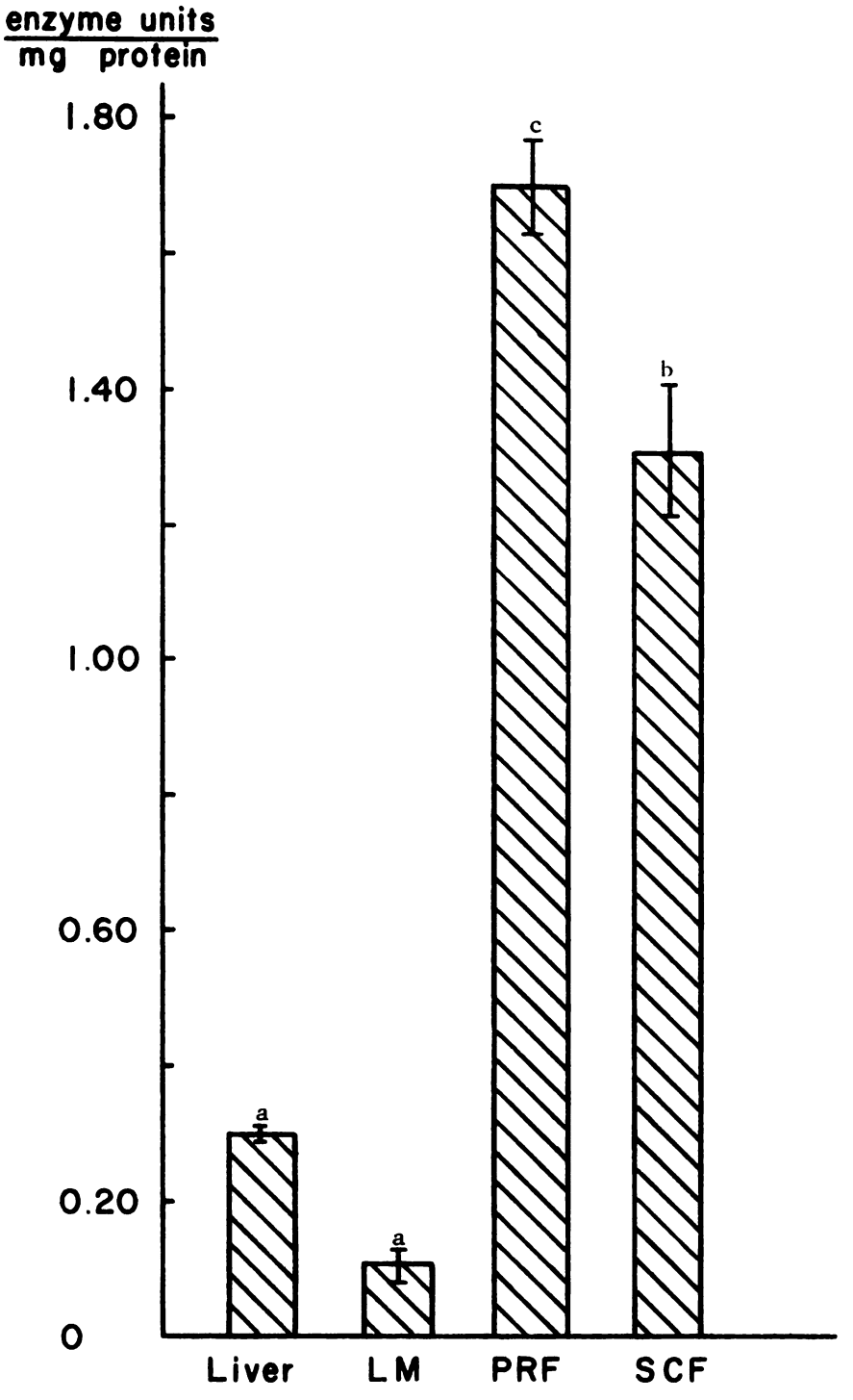


Figure 7. Means and standard error of the means of cytoplasmic acetyl COA synthetase activity in various tissues.
^{a,b,c}Means with different superscripts differ significantly ($P < .05$).

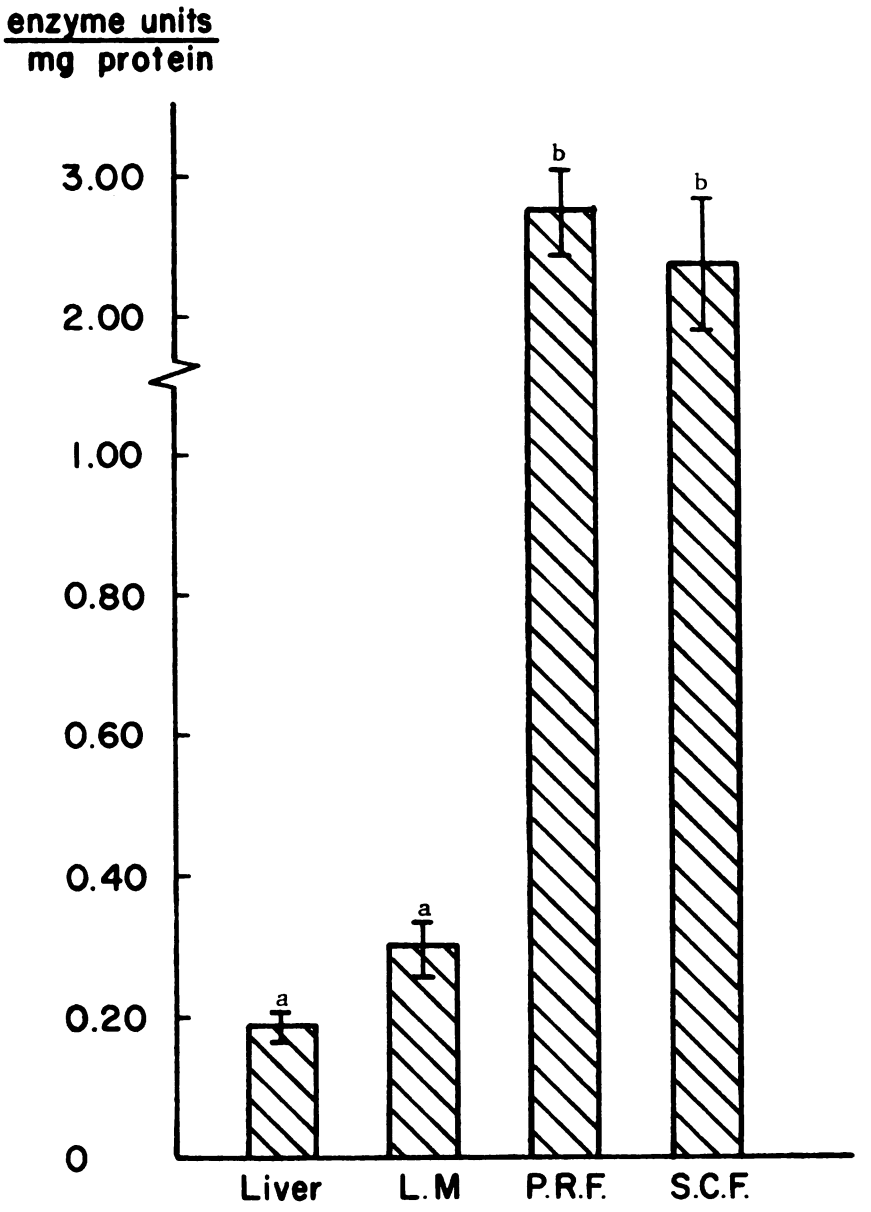


Figure 8. Means and standard error of the means of mitochondrial acetyl COA synthetase activity in various tissues.
[a,b]Means with different superscripts differ significantly ($P < .05$).

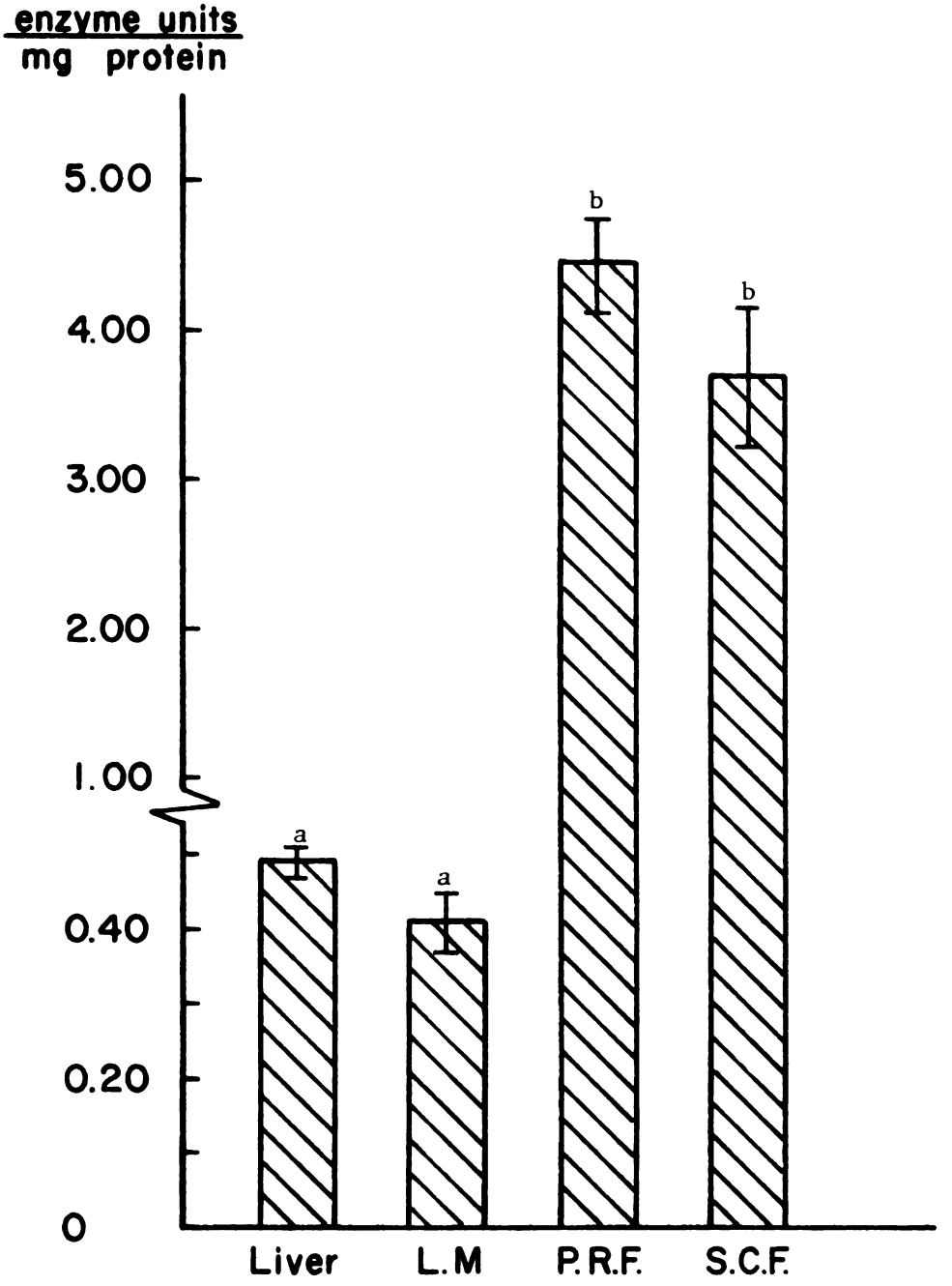


Figure 9. Means and standard error of the means of total acetyl COA synthetase activity in various tissues.
[a,b]Means with different superscripts differ significantly ($P < .05$).

cytoplasmic and mitochondrial SYN activity was observed for total SYN activity in the four tissues. These results are in contrast to the findings of Hanson and Ballard (1967) and Ballard et al. (1969) who reported much higher acetyl COA synthetase activity in liver of adult ruminants than in adipose tissue. However, these results are in agreement with the more recent observations of Cramer (1972) who found very low SYN activity in the liver of steers and an especially higher level of enzyme activity in the adipose tissue depots. The data indicate that SYN is located both in the cytoplasm and mitochondria and agree with the earlier findings of Kornacker and Lowenstein (1965) and Hanson and Ballard (1967). However, these results are in contrast to the findings of Martin and Denton (1970a) and Barth et al. (1971) who concluded that this enzyme was preferentially located in the mitochondria. The results showed that 35 to 40 percent of the SYN activity in ovine adipose tissue was of cytoplasmic origin and 60 to 65 percent of mitochondrial origin.

The means and standard error of the means of cytoplasmic SYN in various tissues are presented by breed, sex and age in table 11. All of the tissues of the Southdown lambs had greater SYN activities than those of Suffolks, but only in PRF were the differences significant ($P = .01$). These data indicate that Southdown lambs which have a greater propensity to fatten, possess a higher potential to utilize acetate for acetyl COA synthesis and possibly for lipogenesis. The influence of sex on cytoplasmic SYN activities in the various tissues was not significant and there was no apparent or consistent pattern of enzyme activity among the various sexes. SYN activity increased with age in the liver. The livers at 32

TABLE 11. CYTOPLASMIC ACETYL COA SYNTHETASE ACTIVITY IN VARIOUS TISSUES BY BREED, SEX AND AGE.

Variable	n[b]	Means and Standard Error of the Means[a] Liver	n	Longissimus muscle	n	Perirenal fat	n	Subcutaneous fat
Breed		(P=.67)[c]		(P=.71)		(P=.01)		(P=.33)
Suffolk	(25)	0.293 ± .020	(25)	0.104 ± .022	(25)	1.462 ± .098[d]	(17)	1.211 + .138
Southdown	(24)	0.313 ± .020	(24)	0.117 ± .022	(24)	1.942 ± .100[e]	(18)	1.406 ± .135
Sex		(P=.44)		(P=.83)		(P=.94)		(P=.52)
Ram	(18)	0.329 ± .023	(18)	0.120 ± .026	(18)	1.711 ± .115	(12)	1.311 ± .165
Ewe	(19)	0.326 ± .022	(19)	0.124 ± .025	(19)	1.664 ± .112	(12)	1.525 ± .165
Wether	(12)	0.255 ± .028	(12)	0.087 ± .032	(12)	1.731 ± .141	(11)	1.089 ± .172
Age		(P=.01)		(P=.45)		(P=.01)		(P=.01)
Birth	(4)	0.197 ± .050[d]	(4)	0.084 ± .054	(4)	3.285 ± .244[f]		
24 hour	(9)	0.263 ± .033[de]	(9)	0.155 ± .036	(9)	2.195 ± .163[e]		
8 week	(12)	0.288 ± .028[de]	(12)	0.119 ± .032	(12)	1.124 ± .141[d]	(11)	1.556 ± .172[e]
16 week	(12)	0.352 ± .028[ef]	(12)	0.061 ± .032	(12)	0.778 ± .141[d]	(12)	1.516 ± .165[e]
32 week	(12)	0.416 ± .028[f]	(12)	0.133 ± .032	(12)	1.128 ± .141[d]	(12)	0.852 ± .165[d]

[a]Values expressed as specific activity = enzyme units/mg protein: where enzyme unit = 1.0 μm COASH reacting in 60 min.

[b]Number of observations.

[c]Level of significance for each variable and tissue shown in parentheses.

[d,e,f]Means with different superscripts within variable and column differ significantly ($P < .05$).

week showed significantly ($P < .05$) greater activities than at birth, 24 hour and 8 weeks and the difference in cytoplasmic SYN activities between the neonate and 16 week lambs was also significant ($P < .05$). As the rumen becomes more functional with age in the young lamb, acetate becomes available in larger quantities for metabolism and the increased SYN activity in liver may reflect the increased molar concentration of acetate in the blood. This does not suggest that there is an accompanying increase of lipogenic activity in liver. The influence of age on cytoplasmic SYN in LM was nonsignificant. The most significant effect of age on cytoplasmic SYN was observed in the adipose tissues. The PRF at birth showed significantly ($P < .05$) greater SYN activities than that at 24 hours and both age groups had significantly ($P < .05$) greater enzyme activities than those at 8, 16 and 32 weeks of age. The presence of high levels of cytoplasmic SYN activity in the PRF of young lambs indicates that the prenatal lamb may be acquiring acetate from the maternal blood. Furthermore, PRF is the most active adipose tissue in young lambs and the high lipogenic activities observed are consistent with the changes in metabolic activity of this depot with age. The observed decrease in SYN activity from the high level at birth to a low level at 16 weeks may reflect a trend of decreased fat deposition in PRF since this is one of the earliest maturing fat depots. These observations indicated that the level of cytoplasmic SYN activity in PRF was closely related to the level of lipogenic activity in perirenal fat.

The significant ($P = .03$) breed X age interaction of cytoplasmic SYN activity in PRF (table 11A) indicates that the influence of age on the

TABLE 11A. BREED X SEX, BREED X AGE AND SEX X AGE INTERACTION OF CYTOPLASMIC ACETYL COA SYNTHETASE ACTIVITY IN VARIOUS TISSUES.[a]

Tissue	Breed X Sex	Breed X Age		Sex X Age
			Suffolk	Southdown
Liver	(P=.07)[b]	(P=.32)		(P=.06)
Longissimus muscle	(P=.66)	(P=.54)		(P=.81)
Perirenal fat	(P=.10)	(P=.03)		(P=.84)
		Birth	2.402 ± .346[d,x]	4.168 ± .346[e,y]
		24 hour	1.893 ± .291[d]	2.498 ± .244[d]
		8 week	1.061 ± .244[c]	1.187 ± .244[c]
		16 week	0.735 ± .244[c]	0.821 ± .244[c]
		32 week	1.217 ± .244[c]	1.038 ± .244[c]
Subcutaneous fat	(P=.19)	(P=.92)		(P=.26)

[a]Means and standard errors of the means are presented only when significant interactions were observed. Values expressed as specific activity = enzyme units/mg protein: where enzyme unit = 1.0 μm COASH reacting in 60 min.

[b]Level of significance for each interaction and tissue shown in parentheses.

[c,d,e]Means with different superscripts within interaction and column differ significantly ($P < .05$).

[x,y,z]Means with different superscripts within interaction and row differ significantly ($P < .05$).

level of activity of this enzyme was not the same between breeds. However, within both breed groups the activities of this enzyme decreased from a high level in the PRF of neonate lambs to a low level at 16 weeks and this was similar to the overall age effect. The significant breed effect on SYN activities in PRF was shown to be primarily due to the neonates where the Southdowns showed significantly ($P < .05$) greater enzyme activities than the Suffolk lambs. Lambs possessing the greatest propensity to fatten (Southdowns) exhibited greater SYN activities at young ages, but as they matured the difference between the breeds disappeared.

At 8 and 16 weeks SCF had significantly ($P < .05$) greater cytoplasmic SYN activities than at 32 weeks of age. At 32 weeks, all lambs were obese and the rate of fat deposition and hence, the rate of lipogenic activity had decreased considerably. The pattern of SYN activity in SCF indicates that at 32 weeks of age the capability of SCF to utilize acetate for lipogenesis had decreased markedly compared to that of younger, more rapidly growing lambs (8 and 16 weeks). The decrease in activity of this enzyme in SCF from 16 to 32 weeks of age reflects a decrease in rate of fat deposition (lipogenic activity) in SCF of lambs between 16 and 32 weeks.

The means and standard error of the means of mitochondrial SYN in various tissues are presented by breed, sex and age in table 12. Mitochondrial SYN activities were higher in all tissues of Southdown lambs except in SCF where the Suffolk lambs showed greater activity. However, none of the tissue differences between the breed means were significant. The influence of sex on the mitochondrial SYN activity in liver, muscle

TABLE 12. MITOCHONDRIAL ACETYL COA SYNTHETASE ACTIVITY IN VARIOUS TISSUES BY BREED, SEX AND AGE.

Variable	n[b]	Means and Standard Error of the Means[a] Liver	n	Longissimus muscle	n	Perirenal fat	n	Subcutaneous fat
Breed		(P=.67)[c]		(P=.88)		(P=.68)		(P=.31)
Suffolk	(25)	0.182 ± .032	(25)	0.300 ± .064	(25)	2.630 ± .418	(18)	2.869 ± .679
Southdown	(24)	0.204 ± .033	(24)	0.316 ± .066	(23)	2.925 ± .436	(18)	1.889 ± .679
Sex		(P=.36)		(P=.98)		(P=.63)		(P=.05)
Ram	(18)	0.237 ± .038	(18)	0.295 ± .076	(18)	3.209 ± .493	(12)	1.602 ± .832[d]
Ewe	(19)	0.238 ± .038	(19)	0.295 ± .074	(19)	2.581 ± .480	(12)	1.544 ± .832[d]
Wether	(12)	0.105 ± .047	(12)	0.333 ± .094	(11)	2.605 ± .630	(12)	3.991 ± .832[e]
Age		(P=.09)		(P=.50)		(P=.15)		(P=.69)
Birth	(4)	0.199 ± .082	(4)	0.368 ± .162	(4)	4.250 ± 1.046		
24 hour	(9)	0.075 ± .054	(9)	0.366 ± .108	(9)	2.134 ± .697		
8 week	(12)	0.155 ± .047	(12)	0.196 ± .094	(12)	1.654 ± .604	(12)	2.209 ± .832
16 week	(12)	0.220 ± .047	(12)	0.207 ± .094	(11)	2.200 ± .630	(12)	1.980 ± .832
32 week	(12)	0.317 ± .047	(12)	0.402 ± .094	(12)	3.649 ± .604	(12)	2.947 ± .832

[a]Values expressed as specific activity = enzyme units/mg protein: where enzyme unit = 1.0 μm COASH reacting in 60 min.
[b]Number of observations.
[c]Level of significance for each variable and tissue shown in parentheses.
[d,e]Means with different superscripts within variable and column differ significantly ($P < .05$).

and PRF was nonsignificant. However, a significant ($P = .04$) breed X sex interaction of mitochondrial SYN (table 12A) was obtained in PRF which showed a significant ($P < .05$) sex effect within the Suffolk lambs and rams had greater enzyme activities than wethers. In addition, the interaction showed a significant ($P < .05$) breed effect within wether lambs as the PRF from Southdown wethers possessed considerably greater mitochondrial SYN activities than those of Suffolk wethers. Subcutaneous fat of wether lambs exhibited significantly ($P < .05$) higher mitochondrial SYN activities than SCF of ewe and ram lambs. When examining the significant ($P = .05$) breed X sex interaction of mitochondrial SYN activities in SCF (table 12A), it can be seen that the sex effect within Suffolks followed a pattern similar to that observed for the overall sex effect; however, sex differences among Suffolks were nonsignificant. In contrast to these findings, the activities of this enzyme in SCF within Southdown lambs was significantly ($P < .05$) higher in wethers than in ewes. Wether and ewe lambs are more predisposed to fattening than rams and the higher enzyme activity in SCF from wether lambs appears to substantiate this observation. The fact that SCF of ewe lambs possessed enzyme activities similar to that of ram lambs is in disagreement with the general pattern of sex influence on predisposition to fattening and cannot be explained from these data. The influence of age on mitochondrial SYN activities in various tissues was not statistically significant.

The means and standard error of the means of total acetyl COA synthetase activity in various tissues are presented by breed, sex and age in table 13. The influence of breed on total SYN activities in the various

TABLE 12A. BREED X SEX, BREED X AGE AND SEX X AGE INTERACTIONS OF MITOCHONDRIAL ACETYL COA SYNTHETASE ACTIVITY IN VARIOUS TISSUES.[a]

Tissue	Breed X Sex		Breed X Age	Sex X Age
Liver	(P=.55)[b]		(P=.29)	(P=.06)
Longissimus muscle	(P=.66)		(P=.84)	(P=.85)
Perirenal fat	(P=.04)		(P=.58)	(P=.54)
	Suffolk	Southdown		
Ram	3.903 ± .697[d]	2.516 ± .697		
Ewe	2.924 ± .662[cd]	2.112 ± .697		
Wether	1.062 ± .854[c,x]	4.147 ± .935[y]		
Subcutaneous fat	(P=.05)		(P=.89)	(P=.88)
	Suffolk	Southdown		
Ram	2.109 ± 1.176	1.094 ± 1.176[cd]		
Ewe	2.840 ± 1.176	0.247 ± 1.176[c]		
Wether	3.657 ± 1.176	4.324 ± 1.176[d]		

[a]Means and standard error of the means are presented only when significant interactions were observed. Values expressed a specific activity = enzyme units/mg protein: where enzyme unit = 1.0 μm COASH reacting in 60 min.

[b]Level of significance for each interaction and tissue shown in parentheses.

[c,d]Means with different superscripts within interaction and column differ significantly (P <.05).

[x,y]Means with different superscripts within interaction and row differ significantly (P <.05).

TABLE 13. TOTAL ACETYL COA SYNTHETASE ACTIVITY IN VARIOUS TISSUES BY BREED, SEX AND AGE.

Variable	n[b]	Means and Standard Error of the Means[a] Liver	n	Longissimus muscle	n	Perirenal fat	n	Subcutaneous fat
Breed		(P=.48)[c]		(P=.79)		(P=.32)		(P=.27)
Suffolk	(25)	0.475 ± .037	(25)	0.403 ± .068	(25)	4.090 ± .444	(17)	4.367 ± .670
Southdown	(24)	0.518 ± .038	(24)	0.433 ± .068	(23)	4.849 ± .463	(18)	3.294 ± .652
Sex		(P=.16)		(P=.99)		(P=.63)		(P=.05)
Ram	(18)	0.566 ± .044	(18)	0.416 ± .080	(18)	4.920 ± .524	(12)	2.912 ± .798[d]
Ewe	(19)	0.564 ± .042	(19)	0.419 ± .078	(19)	4.181 ± .510	(12)	3.069 ± .798[d]
Wether	(12)	0.359 ± .053	(12)	0.419 ± .098	(11)	4.309 ± .670	(11)	5.510 ± .834[e]
Age		(P=.01)		(P=.32)		(P=.04)		(P=.84)
Birth	(4)	0.397 ± .092[de]	(4)	0.451 ± .168	(4)	7.526 ± 1.110[e]		
24 hour	(9)	0.338 ± .062[d]	(9)	0.521 ± .112	(9)	4.320 ± .740[d]		
8 week	(12)	0.443 ± .053[de]	(12)	0.315 ± .098	(12)	2.778 ± .641[d]	(11)	4.196 ± .834
16 week	(12)	0.572 ± .053[ef]	(12)	0.268 ± .098	(11)	2.951 ± .670[d]	(12)	3.497 ± .798
32 week	(12)	0.733 ± .053[f]	(12)	0.535 ± .098	(12)	4.777 ± .670[d]	(12)	3.799 ± .798

[a]Values expressed as specific activity = enzyme units/mg protein: where enzyme unit = 1.0 μm COASH reacting in 60 min.

[b]Number of observations.

[c]Level of significance for each variable and tissue shown in parentheses.

[d,e,f]Means with different superscripts within variable and column differ significantly ($P < .05$).

tissues was not significant. The sex effect on the activity of this enzyme in liver, muscle and PRF was also nonsignificant; however, the SCF of wethers had significantly ($P < .05$) higher total SYN activities than that of ewe and ram lambs. A significant ($P = .02$) breed X sex interaction was observed for total SYN activity in PRF (table 13A) and it showed a significant ($P < .05$) sex effect within Suffolk lambs in that rams had greater enzyme activities than those of wethers. Moreover, the interaction showed a significant ($P < .05$) breed effect within the wethers with the PRF of Southdowns possessing considerably greater total SYN activities than that of Suffolk wethers. The significant ($P = .05$) breed X sex interaction of total SYN activity in SCF (table 13A) showed that the influence of sex on the activities of this enzyme in SCF of Suffolk lambs followed a similar pattern to the overall sex effect. However, within Southdowns SCF of wethers had significantly ($P < .05$) greater total SYN activities than that of ewe lambs.

The pattern of total SYN activity in liver was similar to that of the cytoplasmic SYN activity. At 32 weeks liver possessed significantly ($P < .05$) greater enzyme activities than that at birth, 24 hours and 8 weeks of age. Also, total SYN activities in liver at 16 weeks was significantly ($P < .05$) higher than those at 24 hours. A significant ($P = .01$) sex X age interaction of total SYN activity in liver (table 13A) was obtained and it showed that the age effect within ewe lambs was similar to the overall age effect. Within rams, the major variation from the overall age effect was found in the lower level of total SYN in liver at 32 weeks of age. The effect of age on the activity of this enzyme in liver of wether

TABLE 13A. BREED X SEX, BREED X AGE AND SEX X AGE INTERACTIONS OF TOTAL ACETYL COA SYNTHETASE ACTIVITY IN VARIOUS TISSUES.[a]

Tissue	Breed X Sex	Breed X Age	Sex X Age
Liver	(P=.08)[b]	(P=.09)	(P=.01)
Longissimus muscle	(P=.84)	(P=.85)	(P=.94)
Perirenal fat	(P=.02)	(P=.39)	(P=.66)
Subcutaneous fat	(P=.05)	(P=.77)	(P=.91)

Sex X Age (Liver)

	Ram	Ewe	Wether
Birth	0.773 ± .130^{cd,y}	0.158 ± .130^{c,x}	
24 hour	0.376 ± .092^{c}	0.437 ± .082^{c}	
8 week	0.504 ± .092^{cd}	0.336 ± .092^{c}	0.488 ± .092
16 week	0.687 ± .092^{d}	0.590 ± .092^{c}	0.438 ± .092
32 week	0.490 ± .092^{cd,x}	1.298 ± .092^{d,y}	0.686 ± .092^{x}

Breed X Sex (Perirenal fat)

	Suffolk	Southdown
Ram	5.552 ± .740^{d}	4.288 ± .740
Ewe	4.415 ± .702^{cd}	3.948 ± .740
Wether	2.307 ± .906^{c,x}	6.311 ± .994^{y}

Breed X Sex (Subcutaneous fat)

	Suffolk	Southdown
Ram	3.328 ± 1.128	2.497 ± 1.128^{cd}
Ewe	4.447 ± 1.128	1.691 ± 1.128^{c}
Wether	5.325 ± 1.236	5.695 ± 1.128^{d}

[a]Means and standard error of the means are presented only when significant interactions were observed. Values expresed as specific activity = enzyme units/mg protein: where enzyme unit = 1.0 μm COASH reacting in 60 min.
[b]Level of significance for each interaction and tissue shown in parentheses.
[c,d]Means with different superscripts within interaction and column differ significantly (P <.05).
[x,y]Means with different superscripts within interaction and row differ significantly (P <.05).

lambs was not significant. Furthermore, the interaction showed a significant ($P < .05$) sex effect within the birth and 32 week age groups. Livers of rams at birth exhibited greater total SYN activities than those of ewe lambs; whereas, total SYN activities of ewe lambs at 32 weeks were higher than those of rams and wethers. The effect of age on total SYN activities in LM were nonsignificant. Perirenal fat of neonatal lambs had significantly ($P < .05$) higher total SYN activities than that of all other age groups. The influence of age on this enzyme in SCF was similar to the age effect on cytoplasmic SYN activity.

In summarizing the SYN activity, it was highest in PRF followed in order by SCF, liver and LM. In ovine adipose tissue, 35 to 40 percent of this enzyme activity was of cytoplasmic origin and 60 to 65 percent was located within the mitochondria. There appeared to be a relationship between SYN and the propensity to fatten since the adipose tissue depots of Southdowns (greater predisposition to fatten) exhibited higher enzyme activities than those of Suffolk lambs. The influence of sex on SYN did not show any apparent or consistent pattern. The age effect on the activity of this enzyme in various tissues was significant, but the pattern observed in each instance was tissue specific.

ATP-Citrate Lyase

The enzyme ATP-citrate lyase (CCE) allows the non-ruminant to generate the cytoplasmic acetyl COA necessary for de novo fatty acid synthesis from mitochondrial acetyl COA (Hanson and Ballard, 1968). However, in the adult ruminant where acetyl COA synthetase allows the ruminant to form

acetyl COA directly in the cytoplasm, CCE activity is very low compared to the non-ruminant (Ballard et al., 1969; Bauman et al., 1970). Nonetheless, the young ruminant possesses a limited capacity to utilize glucose for lipogenesis and has an active ATP-citrate lyase enzyme (Ballard et al., 1969; Cramer, 1972).

The means and standard error of means of CCE activity in various tissues are presented in figure 10. SCF could not be obtained from the birth and 24 hour lambs, therefore, a comparison of CCE activities could not be made on this tissue of young lambs. ATP-citrate lyase was significantly ($P < .05$) higher in adipose tissue (PRF) than in liver and LM. These results agree with the findings of Ballard et al. (1969), who reported that adipose tissue from both the adult cow and sheep had much greater CCE activity than liver. Cramer (1972) also found that adipose tissue depots of steers possessed significantly higher levels of CCE activity than liver.

The means and standard error of the means of ATP-citrate lyase activity in various tissues are presented by breed, sex and age in table 14. All tissues of Suffolk lambs showed greater CCE activity than those of Southdowns. The differences between breeds were nonsignificant for the LM, but approached significance ($P = .07$) in liver. However, PRF of Suffolks had significantly ($P < .05$) greater CCE activities than that of Southdowns. PRF of ewes showed greater CCE activities than that of rams with the differences approaching significance ($P = .08$). Since there were no wether lambs in the birth or 24 hour age groups, a comparison of CCE activities could not be made on wethers. CCE activity does not appear to

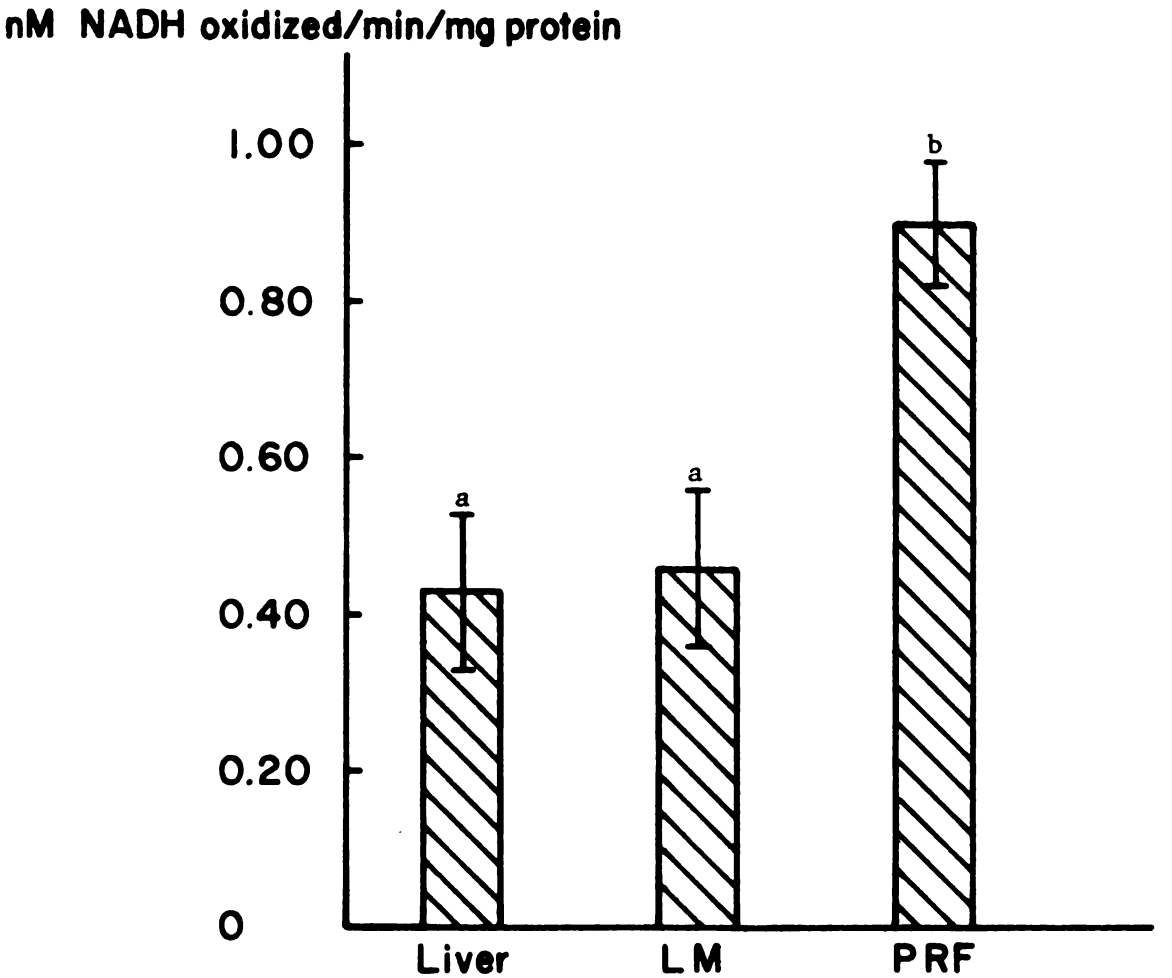


Figure 10. Means and standard error of the means of ATP-citrate lyase activity in various tissues.
[a,b]Means with different superscripts differ significantly ($P < .05$).

TABLE 14. ATP-CITRATE LYASE ACTIVITY IN VARIOUS TISSUES BY BREED, SEX AND AGE.

Variable	n[b]	Means and Standard Error of the Means[a] Liver	n	Longissimus muscle	n	Perirenal fat
Breed		(P=.07)[c]		(P=.86)		(P=.04)
Suffolk	(25)	0.698 ± .164	(25)	0.507 ± .162	(24)	0.961 ± .122[e]
Southdown	(24)	0.180 ± .168	(24)	0.408 ± .165	(21)	0.822 ± .130[d]
Sex		(P=.15)		(P=.90)		(P=.08)
Ram	(18)	0.891 ± .194	(18)	0.380 ± .190	(16)	0.518 ± .149
Ewe	(19)	0.439 ± .189	(19)	0.538 ± .185	(17)	1.256 ± .144
Wether	(12)	0.000 ± ----	(12)	0.000 ± ----	(12)	0.000 ± ----
Age		(P=.04)		(P=.01)		(P=.01)
Birth	(4)	0.724 ± .412[de]	(4)	0.669 ± .404[d]	(3)	2.384 ± .344[e]
24 hour	(9)	1.472 ± .274[e]	(9)	1.743 ± .256[e]	(6)	3.747 ± .244[f]
8 week	(12)	0.000 ± ----[d]	(12)	0.000 ± ----[d]	(12)	0.000 ± ----[d]
16 week	(12)	0.000 ± ----[d]	(12)	0.000 ± ----[d]	(12)	0.000 ± ----[d]
32 week	(12)	0.000 ± ----[d]	(12)	0.000 ± ----[d]	(12)	0.000 ± ----[d]

[a]Values expressed as nanomoles of NADH oxidized/min/mg protein.
[b]Number of observations.
[c]Level of significance for each variable and tissue shown in parentheses.
[d,e,f]Means with different superscripts within variable and column differ significantly ($P < .05$).

be related to the predisposition to fatten. The influence of age on CCE activity was significant in all tissues. At 24 hours, liver exhibited significantly ($P < .05$) higher CCE activity than at 8, 16 and 32 weeks. Muscle and PRF at 24 hour possessed significantly ($P < .05$) greater CCE activity than the corresponding tissues of all other age groups. Even though the liver, muscle and PRF of neonatal lambs showed much greater CCE activity than at 8, 16 and 32 weeks of age, only the difference in CCE activities in PRF were significant ($P < .05$). The significant ($P = .01$) sex X age interaction of CCE in PRF (table 14A) showed that the effect of age within ewe lambs was similar to the overall age effect. However, within rams the PRF of the neonates had significantly ($P < .05$) higher CCE activities than those at 24 hours. Moreover, the interaction showed a significant ($P < .05$) sex effect within the 24 hour age group in that PRF of ewes exhibited greater CCE activities than that of rams. The greater CCE activities at 24 hours than at birth was not entirely surprising, since the 24 hour lambs had nursed and the ingested glucose would be expected to stimulate CCE activity. According to Srere and Foster (1967), CCE readily adapts to dietary changes. The finding of greater CCE activity in young ruminants than in older ruminants is in agreement with results reported by Ballard *et al.* (1969) and Cramer (1972). However, the complete absence of ATP-citrate lyase in the tissues from older lambs (greater than 8 weeks of age) is in contrast to the findings of Ballard *et al.* (1969); Bauman *et al.* (1970) and Cramer (1972), all of whom reported some CCE activity in adult cows and sheep, although the levels were low. This discrepancy in results may have been due to the crude homogenate used

TABLE 14A. BREED X SEX, BREED X AGE, AND SEX X AGE INTERACTIONS OF ATP-CITRATE LYASE ACTIVITY IN VARIOUS TISSUES.[a]

Tissue	Breed X Sex	Breed X Age	Sex X Age					
Liver	(P=.87)[b]	(P=.14)	(P=.65)					
Longissimus muscle	(P=.45)	(P=.99)	(P=.99)					
Perirenal fat	(P=.19)	(P=.08)	(P=.01)					
				Ram		Ewe		Wether
			Birth	3.411 ± .597[e]		1.951 ± .422[d]		
			24 hour	1.626 ± .344[d,x]		5.869 ± .344[e,y]		
			8 week	0.000	----[c]	0.000	----[c]	0.000 ---
			16 week	0.000	----[c]	0.000	----[c]	0.000 ---
			32 week	0.000	----[c]	0.000	----[c]	0.000 ---

[a]Means and standard error of the means are presented only when significant interactions were observed. Values expressed as nanomoles of NADH oxidized/min/mg protein.

[b]Level of significance for each interaction and tissue shown in parentheses.

[c,d,e]Means with different superscripts within interaction and column differ significantly ($P < .05$).

[x,y]Means with different superscripts within interaction and row differ significantly ($P < .05$).

in this study. At low levels of CCE activity, the homogenate must be in a fairly pure state in order to obtain a significant enzyme response.

Although CCE activity was highest in adipose tissues as was SYN activity, it was not closely related to mitochondrial acetyl COA synthetase activity. Simple correlations between CCE and the other enzymes measured are presented in Appendixes IV, V and VI.

In summarizing the results of this study, perirenal fat showed significantly ($P<.05$) greater CCE activities than muscle and liver. There were no significant trends for breed and sex effects on CCE activity in the various tissues studied. Age had the greatest influence with PRF from the neonate and 24 hour age groups possessing significantly ($P <.05$) higher levels of CCE than that at the 8, 16 and 32 weeks. The level of CCE activity was not highly associated with the level of mitochondrial acetyl COA synthetase activity.

Malonyl COA Formation

The immediate precursor of fatty acids, malonyl COA, is formed from cytoplasmic acetyl COA by the action of the enzyme acetyl COA carboxylase (CBX). CBX is the first enzyme involved in the sequence of the cytoplasmic pathway of fatty acid synthesis and has been implicated by many researchers (Vagelos, 1964; Numa *et al.*, 1965; Lane and Moss, 1971) as the key enzyme in the regulation of *de novo* fatty acid synthesis in animal tissues. Bauman *et al.* (1972) reported that in sheep adipose tissue CBX was the most closely related to lipogenesis of all the enzymes measured. However, the specific involvement of CBX in affecting the rate of lipogenesis has not been thoroughly elucidated.

Acetyl COA Carboxylase

The means and standard error of the means of CBX activity in various tissues are presented in figure 11. SCF showed significantly ($P < .05$) greater CBX activity than liver, LM and PRF. PRF and liver possessed significantly ($P < .05$) higher enzyme activity than LM. These results are in partial agreement with the findings of Chakrabarty and Leveille (1969) who also reported greater CBX in rat adipose tissue than in liver; however, the observation that CBX activity in sheep liver was essentially equal to that found in PRF is in disagreement with their results. Lower levels of CBX activity were found in sheep liver, PRF and SCF in this study compared to that reported by the latter authors in rat liver and adipose tissue. These results may be due to the crude homogenate (high protein concentration) used in this study and/or the effect of frozen storage on enzyme activity. However, the possibility of a species difference cannot be excluded since this comparison is made between non-ruminants (rat) and ruminants (sheep).

Means and standard error of the means of CBX activity in various tissues by breed, sex and age are presented in table 15. Except for muscle, the tissues of Southdowns showed greater CBX activity than those of Suffolks, but none of these differences was significant. However, the breed differences in CBX activities in muscle and SCF approached statistical significance ($P = .09$ and $P = .11$, respectively). CBX activities due to sex differences in liver, LM and PRF were nonsignificant, however, those of SCF approached significance ($P = .09$). The SCF and PRF of ram and ewe lambs had considerably greater CBX activities than those from wethers.

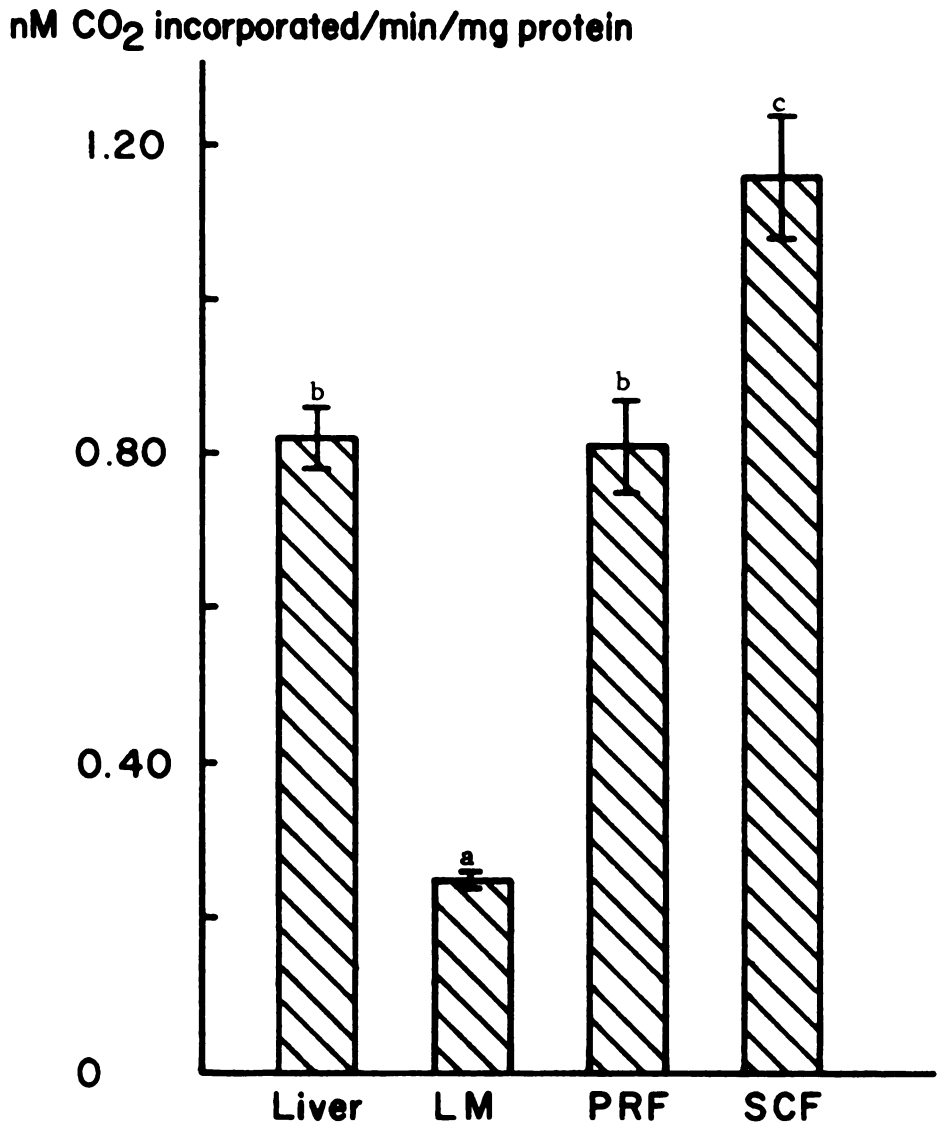


Figure 11. Means and standard error of the means of acetyl COA carboxylase activity in various tissues.
^{a,b,c}Means with different superscripts differ significantly ($P < .05$).

TABLE 15. ACETYL COA CARBOXYLASE ACTIVITY IN VARIOUS TISSUES BY BREED, SEX AND AGE.

Variable	n[b]	Means and Standard Error of the Means[a] Liver	n	Longissimus muscle	n	Perirenal fat	n	Subcutaneous fat
Breed		(P=.84)[c]		(P=.09)		(P=.63)		(P=.11)
Suffolk	(25)	0.815 ± .062	(25)	0.271 ± .014	(25)	0.778 ± .083	(18)	1.018 ± .117
Southdown	(24)	0.837 ± .064	(24)	0.230 ± .014	(24)	0.845 ± .084	(18)	1.292 ± .117
Sex		(P=.23)		(P=.31)		(P=.42)		(P=.09)
Ram	(18)	0.952 ± .074	(18)	0.223 ± .017	(18)	0.961 ± .098	(12)	1.304 ± .144
Ewe	(19)	0.765 ± .072	(19)	0.260 ± .016	(19)	0.810 ± .095	(12)	1.282 ± .144
Wether	(12)	0.760 ± .090	(12)	0.269 ± .021	(12)	0.663 ± .120	(12)	0.878 ± .144
Age		(P=.50)		(P=.01)		(P=.01)		(P=.01)
Birth	(4)	0.823 ± .156	(4)	0.415 ± .036[f]	(4)	0.868 ± .200[de]		
24 hour	(9)	0.652 ± .104	(9)	0.334 ± .024[f]	(9)	0.964 ± .138[de]		
8 week	(12)	0.954 ± .090	(12)	0.235 ± .021[e]	(12)	0.632 ± .120[d]	(12)	0.763 ± .144[d]
16 week	(12)	0.798 ± .090	(12)	0.145 ± .021[d]	(12)	0.784 ± .120[d]	(12)	0.853 ± .144[d]
32 week	(12)	0.902 ± .090	(12)	0.125 ± .021[d]	(12)	1.259 ± .120[e]	(12)	1.849 ± .144[e]

[a]Values expressed as nanomoles of CO_2 incorporated/min/mg protein.
[b]Number of observations.
[c]Level of significance for each variable and tissue shown in parentheses.
[d,e,f]Means with different superscripts within variable and column differ significantly ($P < .05$).

The effect of age on CBX activities in liver was nonsignificant. However, CBX activity in muscle decreased significantly ($P = .01$) between 24 hours and 32 weeks. Longissimus muscles of neonates and lambs at 24 hours had significantly ($P < .05$) greater CBX activities than those at 8 weeks and these three age groups were significantly ($P < .05$) higher than at 16 and 32 weeks. A significant ($P = .03$) breed X age interaction of CBX activity in muscle (table 15A) was observed and showed that the influence of age on enzyme activity within the breeds was not the same. A significant ($P < .05$) difference in CBX activity in LM was observed in Suffolks between neonates and those at 24 hours and lambs at birth exhibited greater CBX activities than at 24 hours. The decrease in CBX activity in LM with age was unexpected. Since intramuscular fat is the latest developing adipose tissue depot, it would seem logical that both lipogenic and CBX activities of older lambs would be higher than in younger lambs. This disparity between CBX activity and lipogenic activity (intramuscular fat deposition) may reflect the increased protein concentration in the crude homogenate of muscle of the older lambs since enzyme activities are expressed on a soluble protein basis.

Perirenal and SCF of lambs at 32 weeks showed significantly ($P < .05$) higher CBX activities than those from lambs at 8 and 16 weeks. The differences in CBX activities in PRF and SCF between lambs at 8 and 16 weeks were nonsignificant. A significant ($P = .01$) sex X age interaction of CBX activity in PRF (table 15A) showed that the influence of age within sex groups differed considerably and deviated from the observed overall age effect. The PRF of neonate rams showed considerably greater CBX

TABLE 15A. BREED X SEX, BREED X AGE AND SEX X AGE INTERACTIONS OF ACETYL COA CARBOXYLASE ACTIVITY IN VARIOUS TISSUES.[a]

Tissue	Breed X Sex	Breed X Age		Sex X Age		
Liver	(P=.80)[b]	(P=.66)		(P=.45)		
Longissimus muscle	(P=.24)	(P=.03)		(P=.59)		
		Suffolk	Southdown			
	Birth	0.538 ± .052[f]	0.291 ± .052[d]			
	24 hour	0.355 ± .032[e]	0.313 ± .036[d]			
	8 week	0.214 ± .030[d]	0.256 ± .030[d]			
	16 week	0.137 ± .030[cd]	0.152 ± .030[c]			
	32 week	0.113 ± .030[c]	0.136 ± .030[c]			
Perirenal fat	(P=.74)	(P=.54)		(P=.01)		
				Ram	Ewe	Wether
				Birth	1.328 ± .294[cd]	0.418 ± .294[c]	
				24 hour	0.794 ± .208[cd]	1.282 ± .186[d]	
				8 week	0.604 ± .208[c]	0.343 ± .208[c]	0.949 ± .208
				16 week	0.659 ± .208[c]	0.723 ± .208[c]	0.970 ± .208
				32 week	1.422 ± .208[d,y]	2.045 ± .208[e,z]	0.715 ± .208[x]

TABLE 15A. BREED X SEX, BREED X AGE AND SEX X AGE INTERACTIONS OF ACETYL COA CARBOXYLASE ACTIVITY IN VARIOUS TISSUES.[a] (continued)

Tissue	Breed X Sex	Breed X Age	Sex X Age		
Subcutaneous fat	(P=.10)	(P=.75)	(P=.01)		
			Ram	Ewe	Wether
		8 week	0.680 ± .203[c]	0.803 ± .203[c]	0.807 ± .203
		16 week	1.130 ± .203[c]	0.494 ± .203[c]	0.935 ± .203
		32 week	2.104 ± .203[d,y]	2.549 ± .203[d,y]	0.894 ± .203[x]

[a]Means and standard error of the means are presented only when significant interactions were observed. Values expressed as nanomoles CO_2 incorporated/min/mg protein.
[b]Level of significance for each interaction and tissue shown in parentheses.
[c,d,e,f]Means with different superscripts within interaction and column differ significantly ($P < .05$).
[x,y,z]Means with different superscripts within interaction and row differ significantly ($P < .05$).

activities than at 24 hours; whereas, the opposite age effect was observed in ewes where the PRF at 24 hour showed significantly ($P < .05$) higher enzyme activities than that at birth, 8 and 16 weeks. Also, ewes deviated from the overall effect of age on CBX activity in PRF since they exhibited significantly ($P < .05$) greater activities at 32 weeks than at birth and 24 hours. PRF of wethers showed lower CBX activity at 32 weeks than at 8 and 16 weeks, but the differences were not significant. The age effect in wethers at 16 to 32 weeks was opposite that observed in PRF of rams and ewes and also the overall age effect. The interaction showed a significant ($P < .05$) sex effect within the 32 week age group since the PRF of ewes showed higher CBX activities than those of rams which in turn had greater CBX activities than wethers.

A significant ($P = .01$) sex X age interaction of CBX in SCF (table 15A) was also obtained and it showed that the age effects within sex groups did not follow similar patterns. The SCF of rams and wethers at 16 weeks showed greater CBX activities than that of rams and wethers at 8 weeks; whereas, the opposite effect was observed in SCF of ewes between 8 and 16 weeks. Moreover, CBX activities in SCF of ram and ewe lambs at 32 weeks were significantly ($P <.05$) greater than those at 8 and 16 weeks. However, the CBX activities in SCF of wethers at 16 weeks were greater than those at 32 weeks, but the influence of age on CBX activities in wethers was not significant. The interaction also showed a significant ($P <.05$) sex effect within the 32 week age group where SCF of ewe and ram lambs had considerably greater CBX activities than that of wethers.

The period of maximum lipogenic activity or maximum rate of fat deposition in lamb adipose tissue probably lies between 16 and 32 weeks

of age. Thus, the adipose tissues at 32 weeks of age while continuing to accumulate fat, are probably doing so at a slower rate than at earlier ages. Lambs at 32 weeks of age are probably beyond the period of maximum lipogenic activity. Thus, the high level of CBX activities observed in the adipose tissue depots of ram and ewe lambs at 32 weeks may not have been as high as at earlier ages since the maximum level of lipogenic activity could have occurred between 16 and 32 weeks. An evaluation of CBX activities in PRF and SCF of wethers revealed that the maximum level of activity occurred at 16 weeks and this observation coincided with the period of maximum lipogenic activity (rate of fat deposition) which is generally observed in adipose tissue of wethers at this age. The lower level of CBX activities in adipose tissue depots of wethers at 32 weeks compared to 16 weeks was expected because at 32 weeks the wethers were beyond the period of maximum lipogenic activity (rate of fat deposition). The observed sex effect on CBX activities in PRF and SCF at 32 weeks may be partially explained by the differences in the propensity to fatten among sexes. The maximum level of lipogenic activity in adipose tissues of wether and ewe lambs usually occurs at earlier ages than in rams. At 32 weeks ewes and wethers are generally further beyond the period of maximum lipogenic activity than rams. Thus, the adipose tissue depots of rams at 32 weeks should show greater CBX activity than those of wethers and ewes. The data in this study actually showed that rams had greater CBX activity than wether lambs in SCF and PRF at 32 weeks. However, the presence of high levels of CBX activity in adipose tissue depots of ewe lambs at 32 weeks of age, which usually have a propensity to fatten

similar to wethers, does not follow the general sex pattern of fattening. The ewes of this study at 32 weeks of age were approaching sexual maturity and this fact coupled with their being slaughtered in October, which coincides with the breeding season could have resulted in physiological (hormonal) changes in these ewes. These events could have produced drastic changes in the lipogenic enzyme activities in the adipose tissue depots of ewe lambs.

The simple correlations for CBX activity with the activity of the other enzymes measured are presented in Appendixes IV, V, VI and VII.

In summarization, acetyl COA carboxylase activity was greatest in the adipose tissue depots and liver and these observations corresponded to those for cytoplasmic acetyl COA synthetase activity which also were greatest in adipose tissue depots.

Although breed effects were not significant, adipose tissue depots of Southdowns (greater propensity to fatten) showed greater CBX activities than those of Suffolks. This trend is similar to that observed for cytoplasmic SYN activity in adipose tissues. There appears to be a relationship between the propensity to fatten (breed effect) and CBX activity in adipose tissue depots. A significant sex effect was observed within the 32 week lambs where PRF and SCF of rams and ewes showed greater CBX activities than those of wethers. The differences in CBX activities observed between rams and wethers were as expected. The high CBX levels of activity in adipose tissues of ewe lambs at 32 weeks might possibly be explained by physiological changes resulting from a combination of approaching sexual maturity and the fact that they were slaughtered during the breeding season.

The CBX activities of PRF and SCF of rams and ewes at 32 weeks were greater than those at all other ages. The adipose tissue depots of wethers at 32 weeks showed lower CBX activities than those at 16 weeks. In older wether and ram lambs the influence of sex on predisposition to fatten tended to be related to CBX activities in adipose tissue depots, however, this relationship did not apply to ewe lambs.

Potential Sources of Reducing Equivalents for Lipogenesis

Lipogenesis requires large quantities of NADPH (Brady, 1958; Wakil et al., 1957) and it was suggested by Tepperman and Tepperman (1958) that the availability of NADPH may serve as a controlling mechanism in de novo fatty acid synthesis. In non-ruminants, NADPH is generated from glucose via the pentose shunt dehydrogenases, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, and by NADP-malate dehydrogenase which is involved in the conversion of malate to pyruvate (Young et al., 1964; Ball, 1966; Ballard and Hanson, 1967). The sources of NADPH in ruminants are less obvious. However, it appears that the pentose shunt dehydrogenases are involved in the generation of NADPH in ruminants (Raggi et al., 1961; Filsell et al., 1963; Hanson and Ballard, 1967; Hood, 1972; Beitz, 1972). The almost complete absence of citrate cleavage enzyme in the adult ruminant negates an active NADP-malate dehydrogenase. Bauman et al. (1970), Beitz (1972) and Hood (1972) have suggested that NADP-isocitrate dehydrogenase may serve as an alternate source of NADPH generation in the ruminant.

Glucose-6-Phosphate Dehydrogenase and 6-Phosphogluconate Dehydrogenase

The hexose monophosphate shunt via the action of glucose-6-phosphate dehydrogenase (G-6PDH) and 6-phosphogluconate dehydrogenase (6-PGDH) is considered a primary source of NADPH for lipogenesis. The means and standard error of the means of G-6PDH activity in various tissues are presented in figure 12. The adipose tissue depots, PRF and SCF, showed significantly greater G-6PDH activities than liver and LM. SCF possessed significantly ($P < .05$) higher G-6PDH activities than PRF and liver had significantly ($P < .05$) greater G-6PDH activities than LM.

Means and standard error of means of 6-phosphogluconate dehydrogenase activity in various tissues are presented in figure 13. Tissue levels of 6-PGDH were considerably higher than those of G-6PDH. Liver and SCF showed significantly ($P < .05$) greater 6-PGDH activities than LM and PRF, and PRF had significantly ($P < .05$) higher 6-PGDH activities than muscle. The finding of significant activities of G-6PDH and 6-PGDH in adipose tissues of lambs agrees with the results of Raggi *et al.* (1961), Filsell *et al.* (1963), Hanson and Ballard (1967), Opstvedt *et al.* (1967), Hood (1972), Beitz (1972) and Cramer (1972) who also reported the presence of significant activities of G-6PDH and 6-PGDH in ovine and bovine adipose tissue depots. The results of this study indicated that 6-PGDH activities were greater than those of G-6PDH in ovine adipose tissues and this observation disagrees with that of Cramer (1972) and Hood (1972) who observed higher levels of G-6PDH than 6-PGDH activity in bovine adipose tissues. This observation suggests that in ovine adipose tissue G-6PDH may be a

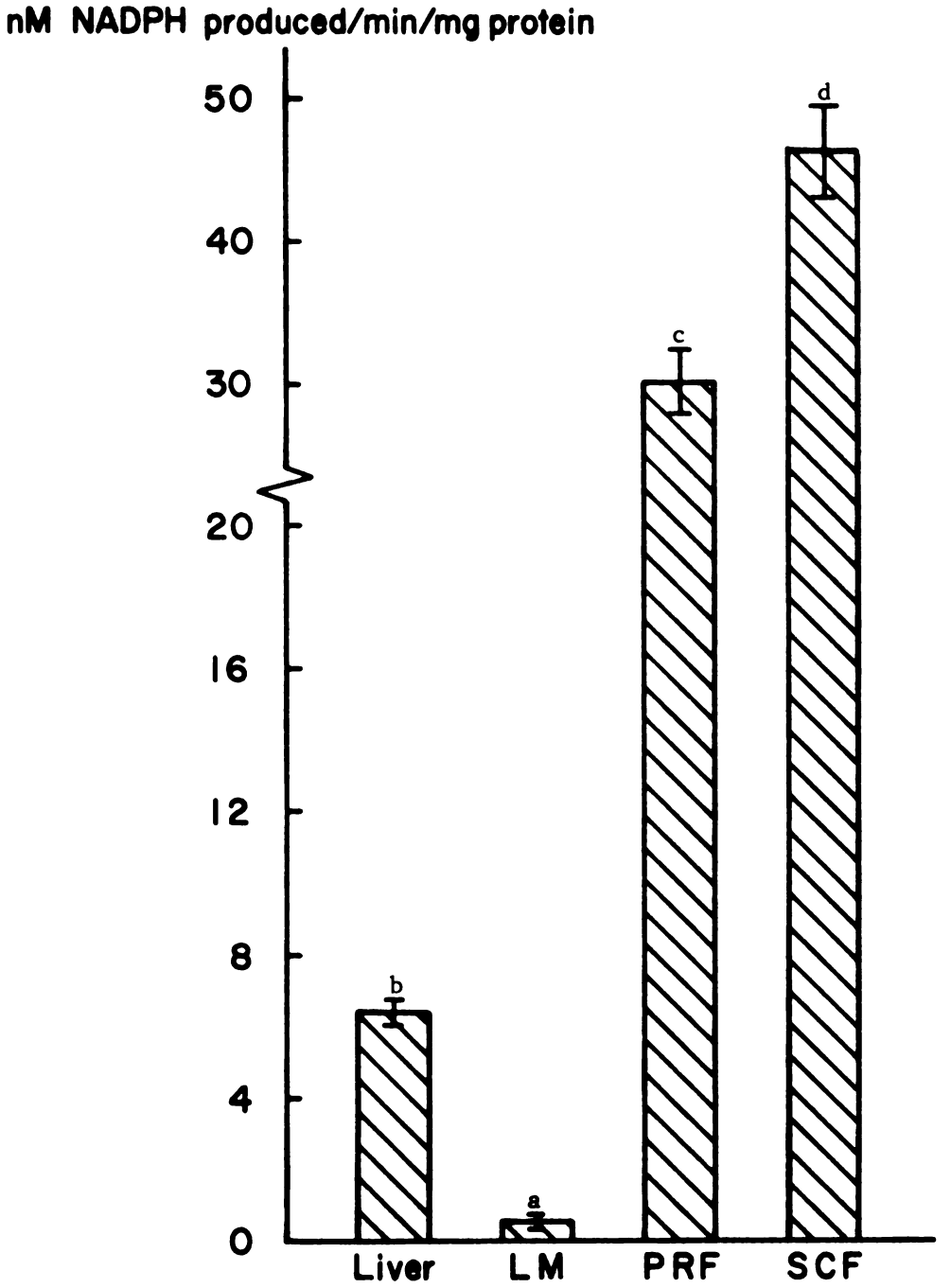


Figure 12. Means and standard error of the means of glucose-6-phosphate dehydrogenase activity in various tissues.
^{a,b,c,d}Means with different superscripts differ significantly ($P < .05$).

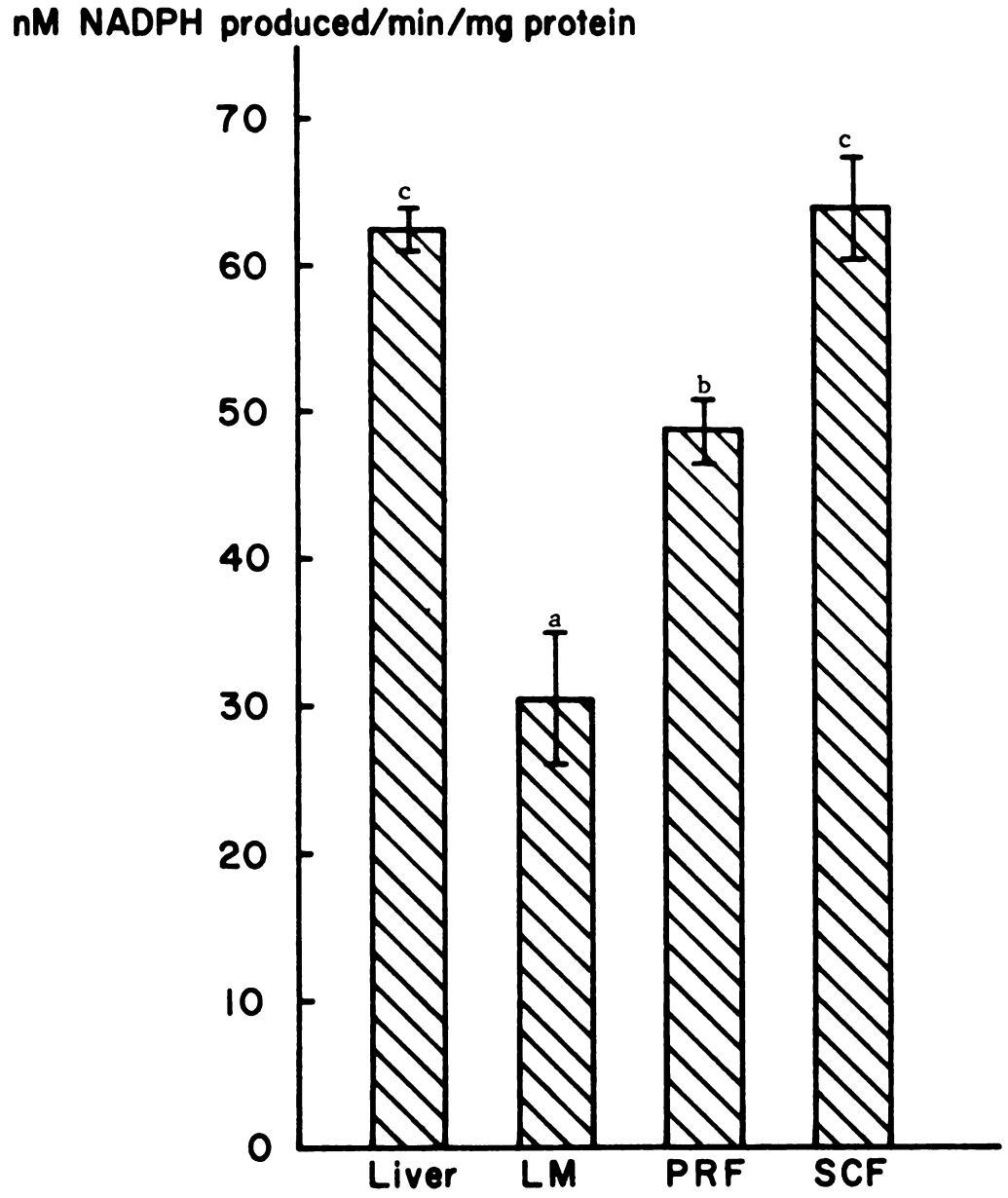


Figure 13. Means and standard error of the means of 6-phosphogluconate dehydrogenase activity in various tissues.
^{a,b,c}Means with different superscripts differ significantly ($P < .05$).

rate limiting enzyme in the pentose shunt and could possibly play a role in the regulation of NADPH generation for lipogenesis.

The means and standard error of the means of G-6PDH in various tissues are presented in table 16 by breed, sex and age. The tissues of Suffolks showed greater G-6PDH activities than those of Southdowns with the differences in liver and SCF approaching significance ($P = .09$). There did not appear to be a relationship between G-6PDH activity and the propensity to fatten in any of the tissues studied. The differences in G-6PDH activities in liver, LM and PRF due to sex were not significant. SCF of rams and wethers possessed significantly ($P < .05$) greater G-6PDH activity than that of ewes. A significant ($P = .03$) breed X sex interaction of G-6PDH in SCF (table 16A) showed different patterns of sex effects within breed groups. Sex effects within Suffolk lambs were not significant; whereas, SCF of Southdown rams showed significantly ($P < .05$) higher G-6PDH activities than that of Southdown ewes. The general pattern of G-6PDH activity among the different sexes does not appear to be related to the predisposition to fatten.

The livers of lambs at 24 hours had significantly ($P < .05$) greater G-6PDH activities than those at birth, 8, 16 and 32 weeks. This high level of G-6PDH at 24 hours probably reflects the increased glucose absorption after the young lambs had nursed. G-6PDH activities in lamb liver decreased to a low level by 8 weeks of age and appeared to stabilize at this level. This might be expected since less glucose would be absorbed and utilized by the liver as rumen function developed; thus, the activity of G-6PDH should decrease. The influence of age on G-6PDH activity in LM

TABLE 16. GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY IN VARIOUS TISSUES BY BREED, SEX AND AGE.

	Means and Standard Error of the Means[a]							
Variable	n[b]	Liver	n	Longissimus muscle	n	Perirenal fat	n	Subcutaneous fat
Breed		(P=.09)[c]		(P=.55)		(P=.46)		(P=.09)
Suffolk	(25)	7.08 ± .52	(25)	0.68 ± .15	(24)	31.98 ± 2.92	(18)	53.22 ± 5.34
Southdown	(24)	5.58 ± .52	(24)	0.53 ± .16	(22)	28.29 ± 3.05	(18)	39.76 ± 5.34
Sex		(P=.81)		(P=.53)		(P=.65)		(P=.03)
Ram	(18)	5.96 ± .60	(18)	0.50 ± .18	(17)	26.26 ± 3.47	(12)	59.64 ± 5.34[e]
Ewe	(19)	6.52 ± .59	(19)	0.78 ± .18	(17)	29.97 ± 3.47	(12)	28.54 ± 6.54[d]
Wether	(12)	6.52 ± .74	(12)	0.53 ± .22	(12)	34.18 ± 4.13	(12)	51.30 ± 6.54[e]
Age		(P=.01)		(P=.35)		(P=.01)		(P=.07)
Birth	(4)	6.46 ± 1.29[d]	(4)	0.26 ± .26	(4)	22.78 ± 7.15[d]		
24 hour	(9)	10.89 ± .86[e]	(9)	0.72 ± .26	(6)	26.46 ± 5.84[d]		
8 week	(12)	4.18 ± .74[d]	(12)	0.96 ± .22	(12)	22.62 ± 4.13[d]	(12)	36.18 ± 6.54
16 week	(12)	5.18 ± .74[d]	(12)	0.76 ± .22	(12)	45.34 ± 4.13[e]	(12)	58.92 ± 6.54
32 week	(12)	4.69 ± .74[d]	(12)	0.54 ± .22	(12)	33.46 ± 4.13[de]	(12)	44.36 ± 6.54

[a]Values expressed as nanomoles NADPH produced/min/mg protein.
[b]Number of observations.
[c]Level of significance for each variable and tissue shown in parentheses.
[d,e]Means with different superscripts within variable and column differ significantly ($P < .05$).

TABLE 16A. BREED X SEX, BREED X AGE AND SEX X AGE INTERACTIONS OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY IN VARIOUS TISSUES[a]

Tissue	Breed X Sex		Breed X Age	Sex X Age
Liver	(P=.38)[b]		(P=.07)	(P=.61)
Longissimus muscle	(P=.58)		(P=.07)	(P=.20)
Perirenal fat	(P=.93)		(P=.89)	(P=.45)
Subcutaneous fat	(P=.03)		(P=.99)	(P=.19)
Ram	59.19 ± 9.26	60.08 ± 9.26[d]		
Ewe	38.04 ± 9.26	19.04 ± 9.26[c]		
Wether	62.42 ± 9.26	40.18 ± 9.27[c,d]		

[a]Means and standard error of the means are presented only when significant interactions were observed. Values expressed as nanomoles of NADPH produced/min/mg protein.

[b]Level of significance for each interaction and tissue shown in parentheses.

[c,d]Means with different superscripts within interaction and column differ significantly ($P < .05$).

was nonsignificant. The G-6PDH activity in PRF of lambs at 16 weeks was significantly ($P < .05$) greater than that of lambs at birth, 24 hours and 8 weeks. The activity of G-6PDH reached its maximum at 16 weeks and this observation appeared to correspond to the high level of lipogenic activity generally associated with perirenal fat at this age. The decrease in G-6PDH activity in PRF by 32 weeks may reflect a decrease in the rate of lipogenic activity between the ages of 16 and 32 weeks. The pattern of G-6PDH activity in SCF was similar to that observed in PRF with the maximum level of activity being attained at 16 weeks. The differences in G-6PDH activity in SCF due to age approached significance ($P = .07$). G-6PDH seemed to be related to lipogenic activity and may possibly play a role in the regulation of NADPH generation for fatty acid synthesis. This conclusion concurs with that of Hood (1972) who stated that G-6PDH appeared to be an important regulator of NADPH production in bovine adipose tissues.

The means and standard error of the means of 6-PGDH activities in various tissues are presented in table 17 by breed, sex and age. The tissues of Southdowns showed greater 6-PGDH activities than those of Suffolks and this observation was opposite to the breed effect observed for G-6PDH activity. The livers and LM of Southdowns had significantly ($P = .03$) higher 6-PGDH activities than those of Suffolks. The activity of 6-PGDH appeared to be related to the propensity to fatten as those lambs which possessed the greatest predisposition to fatten (Southdowns) showed the highest 6-PGDH activities. Sex effects on 6-PGDH were not significant and no consistent or apparent enzyme patterns were observed among

TABLE 17. 6-PHOSPHOGLUCONATE DEHYDROGENASE ACTIVITY IN VARIOUS TISSUES BY BREED, SEX AND AGE.

	Means and Standard Error of the Means[a]							
Variable	n[b]	Liver	n	Longissimus muscle	n	Perirenal fat	n	Subcutaneous fat
Breed		(P=.03)[c]		(P=.03)		(P=.55)		(P=.37)
Suffolk	(25)	58.42 ± 2.30[d]	(25)	24.20 ± 6.26[d]	(24)	47.45 ± 2.92	(18)	60.78 ± 5.15
Southdown	(24)	67.02 ± 2.35[e]	(24)	37.42 ± 6.38[e]	(22)	50.44 ± 3.06	(18)	67.48 ± 5.15
Sex		(P=.99)		(P=.61)		(P=.40)		(P=.41)
Ram	(18)	62.57 ± 2.72	(18)	33.78 ± 7.38	(17)	47.94 ± 3.48	(12)	66.80 ± 6.31
Ewe	(19)	62.65 ± 2.64	(19)	24.08 ± 7.18	(17)	53.46 ± 3.48	(12)	65.47 ± 6.31
Wether	(12)	62.93 ± 3.32	(12)	34.58 ± 9.04	(12)	45.43 ± 4.14	(12)	60.12 ± 6.31
Age		(P=.01)		(P=.61)		(P=.01)		(P=.03)
Birth	(4)	127.82 ± 5.76[f]	(4)	39.49 ± 15.65	(4)	15.87 ± 7.17[d]		
24 hour	(9)	68.77 ± 3.84[e]	(9)	38.82 ± 10.43	(6)	35.36 ± 5.85[e]		
8 week	(12)	39.82 ± 3.32[d]	(12)	34.79 ± 9.04	(12)	53.48 ± 4.14[f]	(12)	50.89 ± 6.31[d]
16 week	(12)	40.54 ± 3.32[d]	(12)	22.60 ± 9.04	(12)	61.62 ± 4.14[f]	(12)	65.48 ± 6.31[de]
32 week	(12)	36.63 ± 3.32[d]	(12)	18.35 ± 9.04	(12)	78.42 ± 4.14[g]	(12)	76.02 ± 6.31[e]

[a]Values expressed as nanomoles NADPH produced/min/mg protein.
[b]Number of observations.
[c]Level of significance for each variable and tissue shown in parentheses.
[d,e,f,g]Means with different superscripts within variable and column differ significantly ($P < .05$).

the different sexes.

6-PGDH decreased with age in liver following a pattern similar to that observed for G-6PDH. The livers of lambs at birth showed significantly ($P < .05$) greater 6-PGDH activities than those at 24 hours and both age groups possessed significantly ($P < .05$) higher 6-PGDH activities than lambs at 8, 16 and 32 weeks. The high levels of 6-PGDH activity observed in the livers of neonate lambs may have resulted from glycogenolysis as these lambs were essentially in a fasted state; whereas, the presence of high 6-PGDH activity in the liver at 24 hours may reflect the increased glucose absorption resulting from milk ingestion. The breed X age interaction of 6-PGDH in liver (table 17A) was significant ($P = .03$). The interaction showed that the age effects within breed groups were different. The significant breed effect observed for 6-PGDH activity in liver was primarily associated with the neonates as Southdowns possessed significantly ($P < .05$) higher 6-PGDH activities than Suffolks. 6-PGDH activities in LM exhibited a gradual, although nonsignificant, decline with age.

The activities of this enzyme in PRF and SCF increased considerably with age. PRF at 32 weeks exhibited significantly ($P < .05$) 6-PGDH activities than that at all other ages. PRF at 8 and 16 weeks showed significantly ($P < .05$) greater 6-PGDH activities than those at birth and 24 hours. The differences in PRF 6-PGDH activities between the 24 hour and neonate lambs were significant ($P < .05$). The low levels of 6-PGDH activity in PRF at birth might be expected as neonate lambs were essentially in a fasted state; thus, available glucose was undoubtedly being utilized for vital functions leaving only a negligible supply for NADPH generation via

TABLE 17A. BREED X SEX, BREED X AGE AND SEX X AGE INTERACTIONS OF 6-PHOSPHOGLUCONATE DEHYDROGENASE ACTIVITY IN VARIOUS TISSUES.[a]

Tissue	Breed X Sex	Breed X Age		Sex X Age
Liver	(P=.21)[b]	(P=.03)		(P=.59)
		Suffolk	Southdown	
	Birth	106.97 ± 8.15[e,x]	148.68 ± 8.15[e,y]	
	24 hr	66.64 ± 5.16[d]	70.90 ± 5.76[d]	
	8 week	38.84 ± 4.70[c]	40.82 ± 4.70[c]	
	16 week	43.52 ± 4.70[c]	37.56 ± 4.70[c]	
	32 week	36.14 ± 4.70[c]	37.12 ± 4.70[c]	
Longissimus muscle	(P=.34)	(P=.61)		(P=.48)
Perirenal fat	(P=.64)	(P=.53)		(P=.41)
Subcutaneous fat	(P=.60)	(P=.45)		(P=.72)

[a]Means and standard error of the means are presented only when significant interactions were observed. Values expressed as nanomoles of NADPH produced /min/mg protein.

[b]Level of significance for each interaction and tissue shown in parentheses.

[c,d,e]Means with different superscripts within interaction and column differ significantly ($P < .05$).

[x,y]Means with different superscripts within interaction and row differ significantly ($P < .05$).

hexose monophosphate shunt dehydrogenases in adipose tissues. The rapid increase in 6-PGDH activities in PRF of lambs at 24 hours reflected increased glucose absorption and availability which resulted from milk ingestion. Since the PRF depot is active in the very young lamb, a rapid increase in NADPH generation would be expected. The gradual increase in 6-PGDH activity with age appeared to coincide with the increased amount of fat accumulation observed in these lambs. The maximum level 6-PGDH activity probably occurred somewhere between 16 and 32 weeks of age; thus, the high level of this enzyme at 32 weeks was probably lower than the maximum that may have been attained earlier. SCF at 32 weeks exhibited significantly ($P < .05$) higher 6-PGDH activities than that at 8 weeks. The pattern of 6-PGDH activities in SCF due to age was similar to that observed in PRF.

These data indicate that the hexose monophosphate shunt dehydrogenases (G-6PDH; 6-PGDH) are capable of generating NADPH for lipogenesis. The latter enzyme appeared to be closely associated with lipogenic activity in lambs and seemed to be an adaptable enzyme. Moreover, 6-PGDH activity was found to be related to the propensity to fatten. This enzyme does not seem to be a rate limiting factor in fatty acid synthesis in ovine adipose tissues; rather the level of lipogenic activity tended to regulate the amount of NADPH generated from glucose oxidation by this enzyme. However, G-6PDH did not seem to be as adaptable as 6-PGDH, although its level of activity was related to lipogenic activity. In addition, the level of G-6PDH activity did not appear to be related to the propensity to fatten. The response of G-6PDH to increased lipogenic

activity was not as marked as the response of 6-PGDH. This suggests that G-6PDH may be a rate limiting factor in NADPH production via the pentose shunt pathway and could possibly play a role in the regulation of lipogenesis in ovine adipose tissues. This conclusion is in agreement with that of Hood (1972); however, Beitz (1972) suggested that the supply of reducing equivalents was not a rate limiting factor in ruminant lipogenesis. It is plausible that reducing equivalent generating enzymes other than the pentose shunt dehydrogenases may provide the NADPH necessary for maximum fatty acid synthesis during peak lipogenic periods.

NADP-Malate Dehydrogenase

Leveille and Hanson (1966), Rognstad and Katz (1966), Saggerson and Greenbaum (1970), Allee et al. (1971) and Hood (1972) reported that in non-ruminants a portion of the required NADPH is obtained from cytoplasmic NADH through the malate transhydrogenation cycle in which the NAD and NADP-malate dehydrogenases are coupled with the net result that NADH is used to generate NADPH. However, in ruminants the functional importance of NADP-malate dehydrogenase (ME) as a NADPH generating enzyme is questionable because of the almost complete absence of the citrate pathway which seems to negate ME as an important NADPH generator.

The means and standard error of the means of ME activity in various tissues are presented in figure 14. Subcutaneous fat possessed significantly ($P < .05$) higher ME activity than PRF and both adipose tissues showed significantly ($P < .05$) greater ME activities than liver and LM. These results are in accord with the findings of Hanson and Ballard (1967)

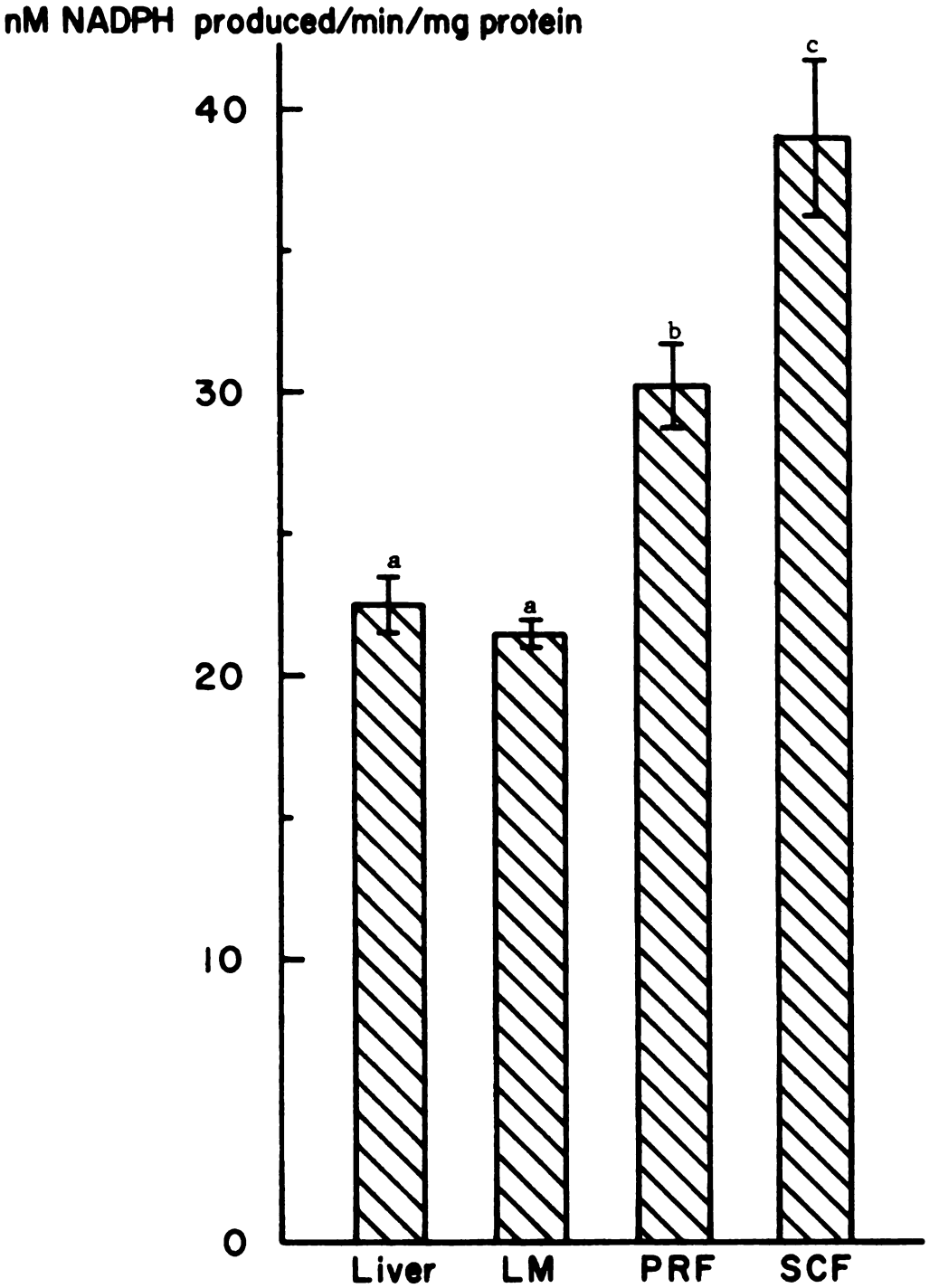


Figure 14. Means and standard error of the means of NADP-malate dehydrogenase activity in various tissues.
[a,b,c]Means with different superscripts differ significantly ($P < .05$).

who reported the presence of considerable ME activity in adipose tissues of the cow and sheep. The ME activities of ovine liver and LM in this study were considerably higher than those previously reported for cows and sheep (Hanson and Ballard, 1967) and in ovine and bovine mammary tissues (Bauman et al., 1970).

The means and standard error of the means of ME activities in various tissues are presented in table 18 by breed, sex and age. Breed differences for ME activity in various tissues were nonsignificant and there was no apparent or consistent pattern of enzyme activity among these two variables. Livers of rams exhibited significantly ($P < .05$) greater ME activities than those of ewe and wether lambs. A similar pattern was observed in LM as rams possessed ($P < .05$) higher ME activities than ewes and both sexes showed significantly ($P < .05$) greater ME activities than wethers. Thus, there was an apparent relationship between sex and ME activity in muscle and liver, but the level of these activities did not appear to be related to the propensity to fatten. The effect of sex on ME activities in SCF and PRF was not significant.

The liver of neonate lambs possessed significantly ($P < .05$) greater ME activities than that at 24 hours and both age groups had significantly ($P < .05$) higher ME activities than at 8, 16 and 32 weeks. LM showed a similar, but slower pattern of decreasing ME activities with age. The LM of lambs at birth and 24 hours had significantly ($P < .05$) greater ME activities than that at 8 and 16 weeks, and all four of these age groups possessed significantly ($P < .05$) higher activities than at 32 weeks. A

TABLE 18. NADP-MALATE DEHYDROGENASE ACTIVITY IN VARIOUS TISSUES BY BREED, SEX AND AGE.

Variable	n[b]	Means and Standard Error of the Means[a] Liver	n	Longissimus muscle	n	Perirenal fat	n	Subcutaneous fat
Breed		(P=.39)		(P=.11)		(P=.71)		(P=.81)
Suffolk	(25)	21.53 ± 1.34	(25)	22.71 ± .87	(24)	29.56 ± 2.04	(18)	38.40 ± 3.99
Southdown	(24)	23.44 ± 1.37	(24)	20.38 ± .89	(22)	30.86 ± 2.13	(18)	39.75 ± 3.99
Sex		(P=.03)		(P=.01)		(P=.92)		(P=.30)
Ram	(18)	27.36 ± 1.58^{e}	(18)	27.78 ± 1.03^{f}	(17)	29.74 ± 2.42	(12)	32.74 ± 4.88
Ewe	(19)	21.71 ± 1.54^{d}	(19)	20.88 ± 1.00^{e}	(17)	31.05 ± 2.42	(12)	42.69 ± 4.88
Wether	(12)	18.39 ± 1.94^{d}	(12)	15.98 ± 1.26^{d}	(12)	29.85 ± 2.88	(12)	41.78 ± 4.88
Age		(P=.01)		(P=.01)		(P=.01)		(P=.27)
Birth	(4)	52.19 ± 3.66^{f}	(4)	34.13 ± 2.18^{f}	(4)	17.33 ± 5.00^{d}		
24 hour	(9)	26.56 ± 2.24^{e}	(9)	29.10 ± 1.46^{f}	(9)	17.14 ± 4.08^{d}		
8 week	(12)	14.41 ± 1.94^{d}	(12)	20.78 ± 1.26^{e}	(12)	29.84 ± 2.88^{e}	(12)	33.92 ± 4.88
16 week	(12)	12.40 ± 1.94^{d}	(12)	19.18 ± 1.26^{e}	(12)	30.56 ± 2.88^{e}	(12)	38.02 ± 4.88
32 week	(12)	14.38 ± 1.94^{d}	(12)	14.84 ± 1.26^{d}	(12)	56.19 ± 2.88^{f}	(12)	45.29 ± 4.88

[a]Values expressed as nanomoles NADPH produced/min/mg protein.
[b]Number of observations.
[c]Level of significance for each variable and tissue shown in parentheses.
[d,e,f]Means with different superscripts within variable and column differ significantly ($P < .05$).

significant (P = .01) sex X age interaction of ME activity in LM (table 18A) showed that the effects of age within sex groups were not the same. Muscles of neonate and 24 hour rams had significantly (P <.05) higher ME activities than those at all other ages. Ewes at 24 hours had significantly (P <.05) greater ME activities in muscle than those at 16 and 32 weeks and neonate and 8 week ewes showed significantly (P <.05) higher enzyme activities than those at 32 weeks. ME activities in muscles of wethers decreased with age, but the differences due to age were not significant. In addition, the interaction showed that the significant sex differences observed were primarily associated with the neonatal and 24 hour lambs.

ME is a key enzyme in the citrate cleavage pathway; thus, it would be expected to decrease with age in young ruminants. The high level of ME activities in the two young age groups reflected an active citrate cleavage pathway which probably resulted from absorption and utilization of considerable quantities of glucose by liver and muscles of lambs on milk diets. The decreased ME activities with age after 24 hours coincided with increased rumen function.

The level of ME activities in PRF increased with age from the neonate and 24 hour age groups to 32 weeks. PRF at 32 weeks exhibited significantly (P <.05) higher ME activities than at 8 and 16 weeks and all three of these age groups had significantly (P <.05) greater activities than those at birth and 24 hours. ME activities in SCF showed a gradual, but nonsignificant, increase with age.

The trend toward increasing ME activities in lamb adipose tissues with age follows the general pattern observed for lipid accumulation in

TABLE 18A. BREED X SEX, BREED X AGE AND SEX X AGE INTEREACTIONS OF NADP-MALATE DEHYDROGENASE ACTIVITY IN VARIOUS TISSUES[a]

Tissue	Breed X Sex	Breed X Age	Sex X Age		
Liver	(P=.19)[b]	(P=.41)	(P=.20)		
Longissimus muscle	(p=.51)	(P=.08)	(P=.01)		
			Ram	Ewe	Wether
		Birth	45.11 ± 3.09^{d,y}	23.16 ± 3.09^{de,x}	
		24 hour	37.54 ± 2.18^{d,y}	26.22 ± 1.96^{e,x}	
		8 week	19.31 ± 2.18^{c}	21.76 ± 2.18^{de}	21.24 ± 2.18
		16 week	20.50 ± 2.18^{c}	18.11 ± 2.18^{cd}	18.94 ± 2.18
		32 week	16.40 ± 2.18^{c}	12.90 ± 2.18^{c}	15.18 ± 2.18
Perirenal fat	(P=.14)	(P=.59)	(P=.30)		
Subcutaneous fat	(P=.62)	(P=.74)	(P=.84)		

[a]Means and standard error of the means are presented only when significant interactions were observed. Values expressed as nanomoles of NADPH produced/min/mg protein.
[b]Level of significance for each interaction and tissue shown in parentheses.
[c,d,e]Means with different superscripts within interaction and column differ significantly (P <.05).
[x,y]Means with different superscripts within interaction and row differ significantly (P <.05).

these depots. It appears that ME is adaptable, but much slower than 6-PGDH, to changes in the level of lipogenic activity. This conclusion agrees with that of Allee et al. (1971) and Hood (1972) who reported that ME activities were similar to those of the hexose monophosphate shunt dehydrogenases in response to different lipogenic activity levels. Even though ME is adaptable to changes in lipogenic activity levels, its slow response indicates that the role of this enzyme in supplying NADPH for lipogenesis in ovine adipose tissues is limited.

NADP-Isocitrate Dehydrogenase

Because of the almost complete absence of the citrate cleavage pathway in ruminants, Bauman et al. (1970) suggested that an alternate source of NADPH generation is via NADP-isocitrate dehydrogenase (ICDH). Hood (1972), Beitz (1972) and Cramer (1972) reported the presence of considerable ICDH activity in bovine adipose tissues.

The means and standard error of the means of cytoplasmic, mitochondrial and total ICDH activities in various tissues are presented in figures 15, 16 and 17. The adipose tissues showed the greatest cytoplasmic ICDH activities and PRF had significantly ($P < .05$) higher activities than all other tissues. Liver and SCF had significantly ($P < .05$) higher levels of cytoplasmic ICDH activities than LM. Muscle exhibited the highest mitochondrial ICDH activity followed in order by SCF, PRF and liver with the differences between tissue means being significant ($P < .05$). PRF and SCF possessed the highest total ICDH activities. PRF had significantly ($P < .05$) greater total ICDH activities than SCF and LM and both showed

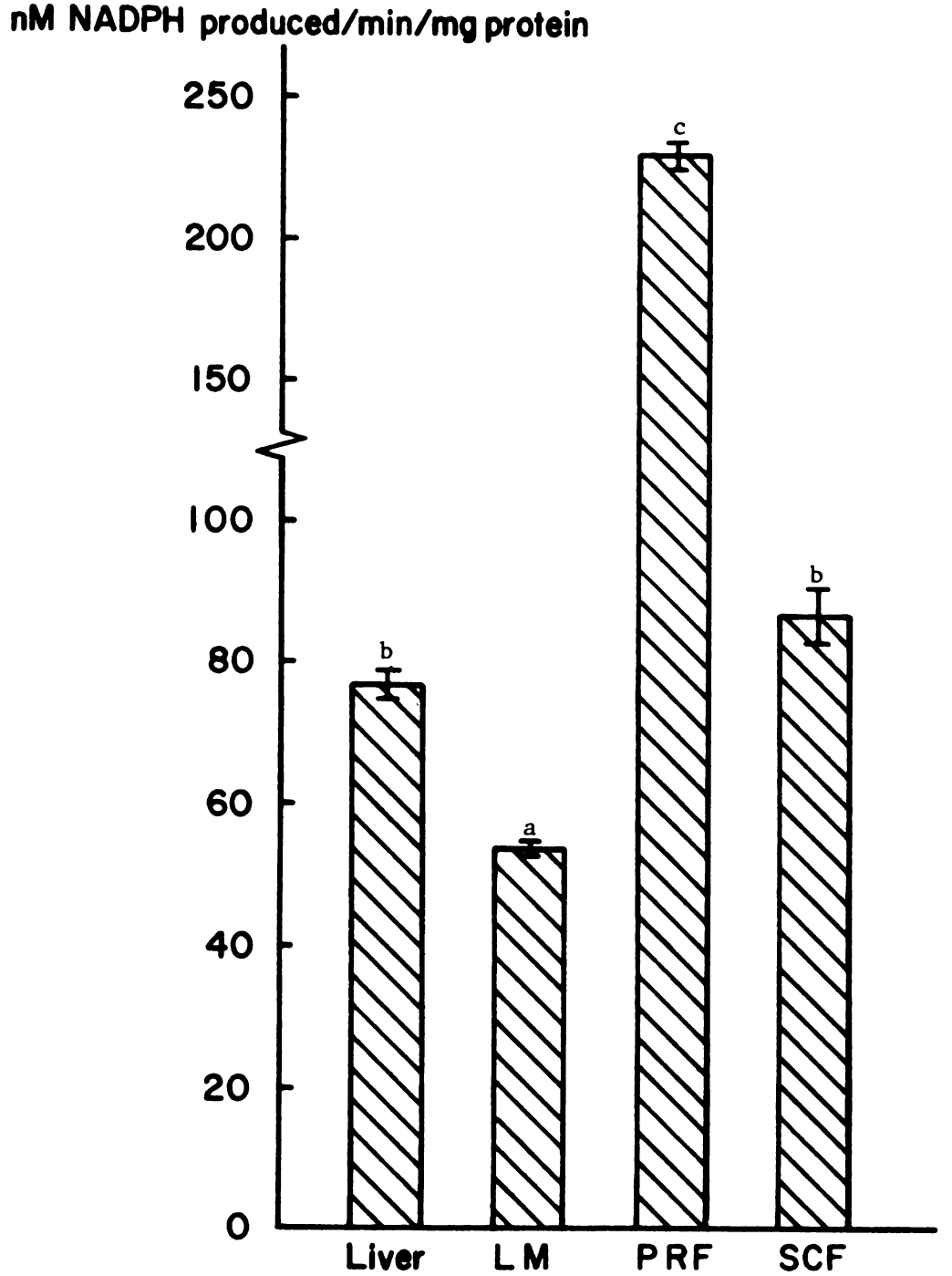


Figure 15. Means and standard error of the means of cytoplasmic NADP-isocitrate dehydrogenase activity in various tissues. ^{a,b,c}Means with different superscripts differ significantly ($P < .05$).

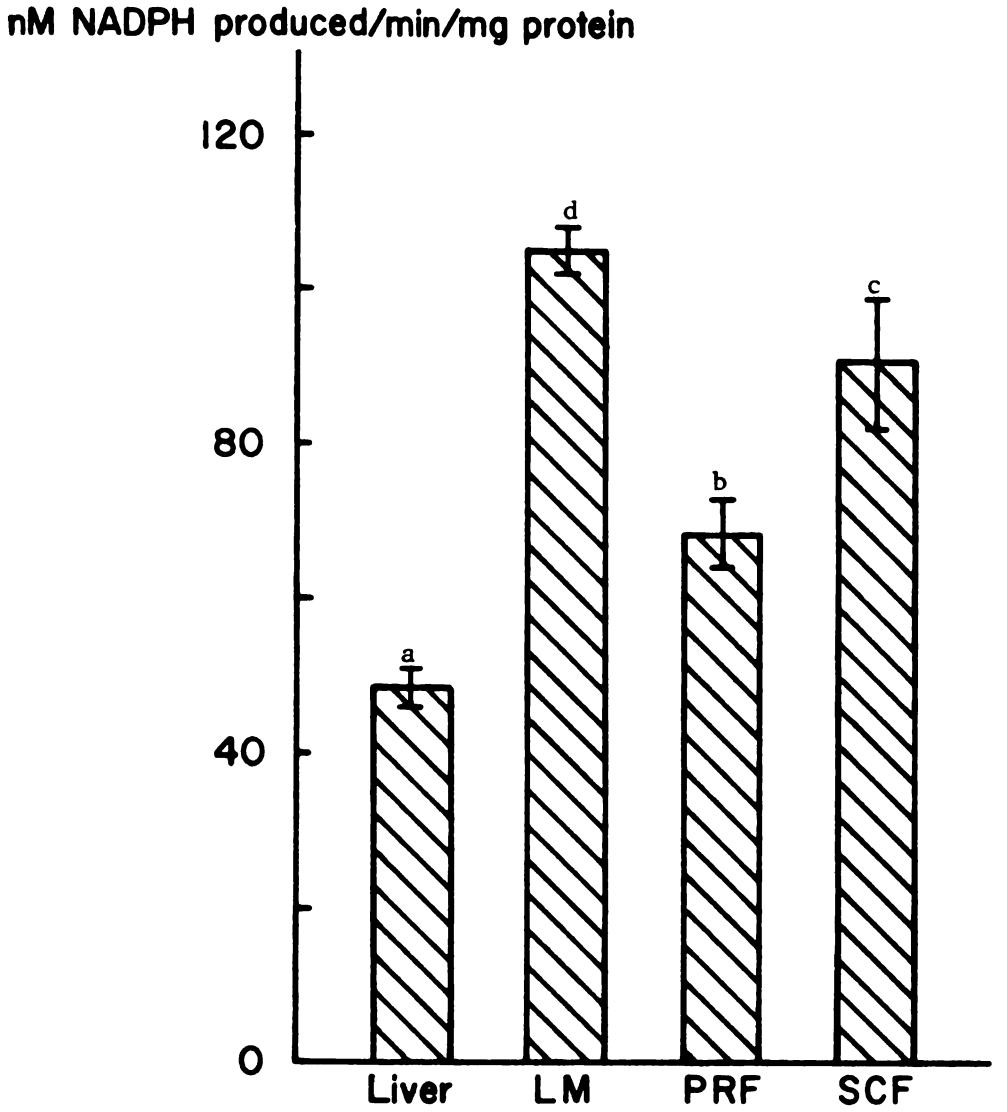


Figure 16. Means and standard error of the means of mitochondrial NADP-isocitrate dehydrogenase activity in various tissues. ^{a,b,c,d}Means with different superscripts differ significantly ($P < .05$).

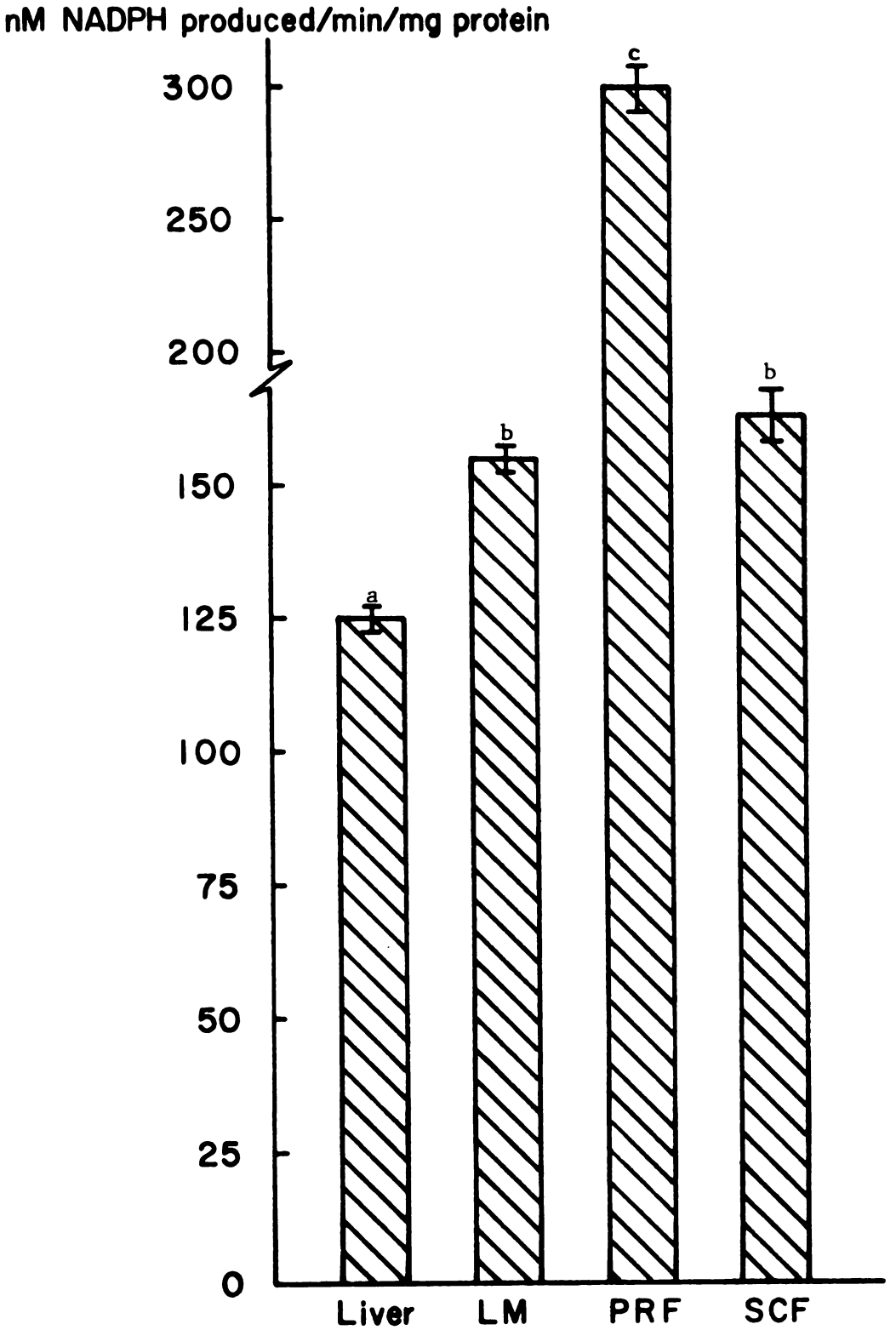


Figure 17. Means and standard error of the means of total NADP-isocitrate dehydrogenase activity in various tissues.
^{a,b,c}Means with different superscripts differ significantly ($P < .05$).

significantly ($P < .05$) higher levels of activity than liver. The finding of significant cytoplasmic ICDH activity in ovine adipose tissues indicates that NADP-isocitrate dehydrogenase may be involved in the generation of NADPH for lipogenesis. This is in accord with the results of Hood (1972), Beitz (1972) and Cramer (1972) who found considerable ICDH activity in bovine adipose tissues.

The means and standard error of the means of cytoplasmic ICDH activity in various tissues by breed, sex and age are presented in table 19. The cytoplasmic ICDH activities in liver and LM of Suffolks were higher than those of Southdowns, but only those in muscle were significant ($P < .01$). However, the differences between breeds in cytoplasmic ICDH activities in liver approached significance ($P < .10$). The effects of breed on adipose tissue cytoplasmic ICDH activities were not significant. Livers of ram lambs showed significantly ($P < .05$) greater cytoplasmic ICDH than those of ewes and wethers. This pattern followed a trend similar to that observed for the effect of breed on cytoplasmic ICDH activities in liver and muscle, in which the lambs with the least propensity to fatten (Suffolks; rams) had the highest enzyme activities. The differences in cytoplasmic ICDH activities in muscle due to sex were not significant. PRF of wethers showed the greatest cytoplasmic ICDH activities followed in order by ewe and ram lambs and the enzyme activities of wethers were significantly ($P < .05$) higher than those of ewes and rams. The SCF of rams exhibited the highest level of cytoplasmic ICDH activities followed in order by wethers and ewes, but these differences were not significant. Cytoplasmic ICDH activities did not appear to be closely related to the propensity to fatten.

TABLE 19. CYTOPLASMIC NADP-ISOCITRATE DEHYDROGENASE ACTIVITY IN VARIOUS TISSUES BY BREED, SEX AND AGE.

Variable	n[b]	Liver	n	Longissimus muscle	n	Perirenal fat	n	Subcutaneous fat
		Means and Standard Error of the Means[a]						
Breed		(P=.10)[c]		(P=.01)		(P=.66)		(P=.91)
Suffolk	(25)	80.04 ± 2.17	(25)	56.82 ± 1.00[e]	(24)	232.69 ± 5.54	(18)	85.32 ± 5.52
Southdown	(24)	74.06 ± 2.22	(24)	50.89 ± 1.02[d]	(22)	228.45 ± 5.78	(18)	86.16 ± 5.52
Sex		(P=.02)		(P=.31)		(P=.02)		(P=.10)
Ram	(18)	83.84 ± 2.56[e]	(18)	54.62 ± 1.18	(17)	206.94 ± 6.58[e]	(12)	96.08 ± 6.75
Ewe	(19)	72.68 ± 2.48[d]	(19)	52.34 ± 1.15	(17)	224.96 ± 6.58[e]	(12)	79.25 ± 6.75
Wether	(12)	74.64 ± 3.13[d]	(12)	54.61 ± 1.45	(12)	259.80 ± 7.84[d]	(12)	81.88 ± 6.75
Age		(P=.01)		(P=.05)		(P=.01)		(P=.09)
Birth	(4)	84.08 ± 5.43[ef]	(4)	78.68 ± 2.52[g]	(4)	406.95 ± 13.57[g]		
24 hour	(9)	65.52 ± 3.62[d]	(9)	80.01 ± 1.68[g]	(6)	334.06 ± 11.08[f]		
8 week	(12)	93.66 ± 3.13[f]	(12)	49.02 ± 1.45[f]	(12)	125.86 ± 7.84[e]	(12)	74.92 ± 6.75
16 week	(12)	70.67 ± 3.13[de]	(12)	35.96 ± 1.45[e]	(12)	92.28 ± 7.84[d]	(12)	85.44 ± 6.75
32 week	(12)	71.34 ± 3.13[de]	(12)	25.62 ± 1.45[d]	(12)	123.76 ± 7.84[e]	(12)	96.86 ± 6.75

[a]Values expressed as nanomoles of NADPH produced/min/mg protein.
[b]Number of observations.
[c]Level of significance for each variable and tissue shown in parentheses.
[d,e,f,g]Means with different superscripts within variable and column differ significantly ($P < .05$).

Liver cytoplasmic ICDH activities of lambs at 8 weeks were significantly ($P < .05$) higher than those at 24 hours, 16 and 32 weeks. Also, the liver of neonates exhibited significantly ($P < .05$) greater enzyme activities than those at 24 hours. Cytoplasmic ICDH activities in LM decreased with age from birth and 24 hours to 32 weeks. The activities of this enzyme at birth and 24 hours were significantly ($P < .05$) higher than that of muscles at 8, 16 and 32 weeks. Moreover, LM of 8 week lambs showed significantly ($P < .05$) greater cytoplasmic ICDH activities than those at 16 weeks and both had significantly ($P < .05$) higher enzyme activities than at 32 weeks. The pattern of cytoplasmic ICDH activities in liver due to age cannot be explained. However, cytoplasmic ICDH activity is highest in muscles of young lambs and seemed to correspond to the more active citrate cycle observed in young lambs. The citrate cycle becomes less active with the development of rumen function and muscle showed a decrease in cytoplasmic ICDH activities with age. Cytoplasmic ICDH in LM does not appear to play an important role in NADPH generation for fatty acid synthesis since the activity of this enzyme decreased while the level of lipogenic activity (intramuscular fat deposition) was increasing.

Cytoplasmic ICDH activities in PRF of neonate lambs were significantly ($P < .05$) higher than those at 24 hours and both age groups had significantly ($P < .05$) greater enzyme activities than those at 8, 16 and 32 weeks. Also, PRF at 8 and 32 weeks showed significantly ($P < .05$) higher enzyme activities than at 16 weeks. A significant ($P = .01$) sex X age interaction of cytoplasmic ICDH activity in PRF (table 19A) was observed and it showed that age effects among sex groups were not the same. The

TABLE 19A. BREED X SEX, BREED X AGE AND SEX X AGE INTERACTIONS OF CYTOPLASMIC NADP-ISOCITRATE DEHYDROGENASE ACTIVITY IN VARIOUS TISSUES[a]

Tissue	Breed X Sex	Breed X Age	Sex X Age		
Liver	(P=.97)[b]	(P=.14)	(P=.57)		
Longissimus muscle	(P=.79)	(P=.43)	(P=.22)		
Perirenal fat	(P=.17)	(P=.44)	(P=.01)		
			Ram	Ewe	Wether
		Birth	388.30 ± 19.20[e]	444.95 ± 19.20[e]	
		24 hour	293.70 ± 15.67[d,x]	372.40 ± 15.67[e,y]	
		8 week	121.12 ± 13.57[c]	142.12 ± 13.57[d]	114.34 ± 13.57[cd]
		16 week	107.64 ± 13.57[c]	84.92 ± 13.57[c]	84.30 ± 13.57[c]
		32 week	124.40 ± 13.57[c]	101.52 ± 13.57[cd]	149.00 ± 13.57[d]
Subcutaneous fat	(P=.40)	(P=.21)	(P=.56)		

[a]Means and standard error of the means are presented only when significant interactions were observed. Values expressed as nanomoles of NADPH produced/min/mg protein.

[b]Level of significance for each interaction and tissue shown in parentheses.

[c,d,e]Means with different superscripts within interaction and column differ significantly (P <.05).

[x,y]Means with different superscripts within interaction and row differ significantly (P <.05).

observed sex effect was primarily associated with the 24 hour age group in which the PRF of ewe lambs possessed significantly ($P < .05$) greater cytoplasmic ICDH activities than that of rams. The high levels of cytoplasmic ICDH activities in PRF at birth and 24 hours probably resulted from a combination of an active citrate cycle and the fact that PRF is the most active fat depot in young lambs. The level of lipogenic activity in PRF of lambs usually increases shortly after birth. Since neonatal lambs had an appreciable quantity of PRF, the late prenatal level of lipogenic activity in this depot would be expected to be substantial and this could possibly explain the ICDH activities observed in neonates.

Cytoplasmic ICDH activity in SCF increased with age and the differences among the means approach significance ($P = .09$). The presence of significant cytoplasmic ICDH activities in adipose tissue depots of sheep indicates that this enzyme has the potential to generate NADPH for fatty acid synthesis. However, the response of this enzyme to different levels of lipogenic activity generally associated with age in lambs from 8 to 32 weeks was not great. Cytoplasmic ICDH activities in response to changes in lipogenic activity were not as great as those observed for the pentose shunt dehydrogenases. These findings are in agreement with those reported by Bauman et al. (1972) and Beitz (1972) who found a greater reduction in G-6PDH and 6-PGDH activities than in ICDH activity in response to fasting.

The means and standard error of the means of mitochondrial ICDH activity in various tissues by breed, sex and age are presented in table 20. The tissues of Suffolks showed slightly higher mitochondrial ICDH activities than those of Southdowns, but the differences were not significant.

TABLE 20. MITOCHONDRIAL NADP-ISOCITRATE DEHYDROGENASE ACTIVITY IN VARIOUS TISSUES BY BREED, SEX AND AGE.

Variable	n[b]	Means and Standard Error of the Means[a] Liver	n	Longissimus muscle	n	Perirenal fat	n	Subcutaneous fat
Breed		(P=.76)[c]		(P=.64)		(P=.77)		(P=.87)
Suffolk	(25)	49.30 ± 3.06	(25)	106.65 ± 4.36	(24)	69.93 ± 6.22	(18)	91.92 ± 12.38
Southdown	(24)	47.74 ± 3.13	(24)	103.25 ± 4.46	(22)	66.78 ± 6.49	(18)	88.94 ± 12.38
Sex		(P=.55)		(P=.92)		(P=.51)		(P=.32)
Ram	(18)	52.06 ± 3.61	(18)	102.96 ± 5.14	(17)	78.45 ± 7.38	(12)	87.04 ± 15.17
Ewe	(19)	51.30 ± 3.52	(19)	103.49 ± 5.01	(17)	70.86 ± 7.38	(12)	87.56 ± 15.17
Wether	(12)	42.20 ± 4.42	(12)	108.40 ± 6.30	(12)	55.62 ± 7.38	(12)	96.70 ± 15.17
Age		(P=.96)		(P=.01)		(P=.03)		(P=.03)
Birth	(4)	50.36 ± 7.66	(4)	96.75 ± 10.92[de]	(4)	83.62 ± 15.22[de]		
24 hour	(9)	43.88 ± 5.11	(9)	146.81 ± 7.28[f]	(6)	45.87 ± 12.43[d]		
8 week	(12)	50.18 ± 4.42	(12)	101.92 ± 6.30[e]	(12)	62.30 ± 8.79[d]	(12)	73.88 ± 15.17[d]
16 week	(12)	49.62 ± 4.42	(12)	99.80 ± 6.30[e]	(12)	55.50 ± 8.79[d]	(12)	71.51 ± 15.17[d]
32 week	(12)	48.57 ± 4.42	(12)	79.47 ± 6.30[d]	(12)	94.50 ± 8.79[e]	(12)	125.92 ± 15.17[e]

[a]Values expressed as nanomoles of NADPH produced/min/mg protein.
[b]Number of observations.
[c]Level of significance for each variable and tissue shown in parentheses.
[d,e,f]Means with different superscripts within variable and column differ significantly ($P < .05$).

Likewise, the effect of sex on the level of mitochondrial ICDH activities was not significant in any of the tissues. The level of mitochondrial ICDH activity in liver did not change significantly with age.

LM of lambs at 24 hours exhibited significantly ($P < .05$) greater mitochondrial ICDH activities than those at birth, 8, 16 and 32 weeks of age. Also, LM at 8 and 16 weeks possessed significantly ($P < .05$) higher enzyme activities than those at 32 weeks. The pattern of decreasing mitochondrial ICDH activity in muscle from 24 hours to 32 weeks of age was similar to the observed pattern of cytoplasmic ICDH activity. A significant ($P = .02$) sex X age interaction of mitochondrial ICDH activities in LM (table 20A) was obtained and indicates that a significant sex effect within the birth and 24 hour age groups was evident. The LM of ram lambs at birth exhibited significantly ($P < .05$) greater enzyme activities than ewes. The opposite effect was observed within the 24 hour age group because muscles of ewes had significantly ($P < .05$) higher mitochondrial ICDH activity than that of rams. The interaction showed that the age effects among sex groups were different.

The influence of age on the level of mitochondrial ICDH activities in PRF was opposite of that observed for cytoplasmic ICDH activity. A gradual increase in mitochondrial ICDH activities was observed in adipose tissue depots between 24 hours and 32 weeks of age. PRF of lambs at 32 weeks showed significantly ($P < .05$) higher mitochondrial ICDH activities than that at 24 hours, 8 and 16 weeks of age. The high level of mitochondrial ICDH activity of the neonatal lambs indicates that PRF of neonates is an active fat depot. Lamb SCF at 32 weeks had significantly

TABLE 20A. BREED X SEX, BREED X AGE AND SEX X AGE INTERACTIONS OF MITOCHONDRIAL NADP-ISOCITRATE DEHYDROGENASE ACTIVITY IN VARIOUS TISSUES[a]

Tissue	Breed X Sex	Breed X Age	Sex X Age		
Liver	(P=.96)	(P=.52)	(P.92)		
Longissimus muscle	(P=.38)	(P=.16)	(P=.02)		
			Ram	Ewe	Wether
		Birth	123.79 ± 15.44^{de,y}	66.25± 15.44^{c,x}	
		24 hour	107.80 ± 10.92^{ce,x}	182.37 ± 9.77^{e,y}	
		8 week	122.04 ± 10.92^{e}	86.72 ± 10.92^{c}	97.00 ± 10.92
		16 week	84.69 ± 10.92^{cd}	114.15 ± 10.92^{d}	100.54 ± 10.92
		32 week	76.47 ± 10.92^{c}	67.94 ± 10.92^{c}	86.02 ± 10.92
Perirenal fat	(P=.79)	(P=.69)	(P=.64)		
Subcutaneous fat	(P=.58)	(P=.41)	(P=.70)		

[a]Means and standard errors of the means are presented only when significant interactions were observed. Values expressed as nanomoles of NADPH produced/min/mg protein.
[b]Level of significance for each interaction and tissue shown in parentheses.
[c,d,e]Means with different superscripts within interaction and column differ significantly ($P < .05$).
[x,y]Means with different superscripts within interaction and row differ significantly ($P < .05$).

($P < .05$) higher mitochondrial ICDH activities than at 8 and 16 weeks of age. The increase in mitochondrial ICDH activity with age in adipose tissue depots indicates that this enzyme may play a role in the generation of reducing equivalents for lipogenesis. However, the relatively stable activity of this enzyme in liver and the decrease in activity in muscle with age suggests that in these tissues, especially muscle (intramuscular fat deposition), mitochondrial ICDH may not be closely related to lipogenic activity. Thus, mitochondrial ICDH may not be an important source of NADPH for lipogenesis in these latter tissues.

The means and standard error of the means of total ICDH in various tissues by breed, sex and age are presented in table 21. All tissues of Suffolk lambs exhibited greater total ICDH activities than those of Southdowns, but the differences were not significant. In addition, the influence of sex on the level of total ICDH activities in the various tissues was not significant. The livers at 8 weeks exhibited significantly ($P < .05$) higher total ICDH activities than those at 24 hours, 16 and 32 weeks. LM at 24 hours showed significantly ($P < .05$) greater total ICDH activities than that from all other age groups. Total ICDH activities in muscle of the neonatal lambs were significantly ($P < .05$) higher than those at 16 and 32 weeks of age. Also, the muscles at 8 and 16 weeks had significantly ($P < .05$) greater total ICDH activities than those at 32 weeks.

A significant ($P = .02$) sex X age interaction of total ICDH activity in LM showed that a significant ($P < .05$) sex effect occurred within the birth and 24 hour age groups (table 21A). Muscles from rams at birth had greater total ICDH activities than those of neonatal lambs. The opposite

TABLE 21. TOTAL NADP-ISOCITRATE DEHYDROGENASE ACTIVITY IN VARIOUS TISSUES BY BREED, SEX AND AGE.

	Means and Standard Error of the Means[a]							
Variable	n[b]	Liver	n	Longissimus muscle	n	Perirenal fat	n	Subcutaneous fat
Breed		(P=.27)[c]		(P=.23)		(P=.65)		(P=.90)
Suffolk	(25)	129.34 ± 4.07	(25)	163.48 ± 4.64	(24)	302.62 ± 9.51	(18)	177.24 ± 12.46
Southdown	(24)	121.80 ± 4.16	(24)	154.14 ± 4.73	(22)	295.24 ± 9.94	(18)	175.10 ± 12.46
Sex		(P=.17)		(P=.85)		(P=.59)		(P=.69)
Ram	(18)	135.89 ± 4.80	(18)	157.58 ± 5.46	(17)	285.40 ± 11.30	(12)	183.12 ± 15.26
Ewe	(19)	123.98 ± 4.67	(19)	155.84 ± 5.32	(17)	295.78 ± 11.30	(12)	166.81 ± 15.26
Wether	(12)	116.86 ± 5.88	(12)	163.02 ± 6.69	(12)	315.62 ± 13.45	(12)	178.59 ± 15.26
Age		(P=.02)		(P=.01)		(P=.01)		(P=.01)
Birth	(4)	134.44 ± 10.18^{de}	(4)	175.44 ± 11.59^{f}	(4)	514.88 ± 23.20^{g}		
24 hour	(9)	109.40 ± 6.79^{d}	(9)	226.82 ± 7.72^{g}	(6)	425.56 ± 19.02^{f}		
8 week	(12)	143.84 ± 5.88^{e}	(12)	150.94 ± 6.69^{ef}	(12)	188.16 ± 13.45^{e}	(12)	148.80 ± 15.26^{d}
16 week	(12)	120.30 ± 5.88^{d}	(12)	135.74 ± 6.69^{e}	(12)	147.79 ± 13.45^{d}	(12)	156.95 ± 15.26^{d}
32 week	(12)	119.91 ± 5.88^{d}	(12)	105.10 ± 6.69^{d}	(12)	218.26 ± 13.45^{e}	(12)	222.78 ± 15.26^{e}

[a]Values expressed as nanomoles of NADP produced/min/mg protein.
[b]Number of observations.
[c]Level of significance for each variable and tissue shown in parentheses.
[d,e,f,g]Means with different superscripts within variable and column differ significantly ($P < .05$).

TABLE 21A. BREED X SEX, BREED X AGE AND SEX X AGE INTERACTIONS OF TOTAL NADP-ISOCITRATE DEHYDROGENASE ACTIVITY IN VARIOUS TISSUES[a].

Tissue	Breed X Sex	Breed X Age	Sex X Age		
Liver	(P=.95)[b]	(P=.38)	(P=.77)		
Longissimus muscle	(P=.41)	(P=.23)	(P=.02)		
			Ram	Ewe	Wether
		Birth	205.10 ± 16.39[d,y]	141.56 ± 16.39[cd,x]	
		24 hour	180.26 ± 11.59[d,x]	269.17 ± 10.36[e,y]	
		8 week	173.12 ± 11.59[d]	135.70 ± 11.59[cd]	144.02 ± 11.59
		16 week	123.15 ± 11.59[c]	147.22 ± 11.59[d]	136.89 ± 11.59
		32 week	106.26 ± 11.59[c]	99.98 ± 11.59[c]	109.08 ± 11.59
Perirenal fat	(P=.75)	(P=.56)	(P=.37)		
Subcutaneous fat	(P=.23)	(P=.14)	(P=.89)		

[a]Means and standard error of the means are presented only when significant interactions were observed. Values expressed as nanomoles of NADPH produced/min/mg protein.
[b]Level of significance for each interaction and tissue shown in parentheses.
[c,d,e]Means with different superscripts within interaction and column differ significantly ($P < .05$).
[x,y]Means with different superscripts within interaction and row differ significantly ($P < .05$).

sex effect was observed within the 24 hour age group. The effects of age on total ICDH activities were different among sex groups.

The patterns of total ICDH activity in liver and muscle suggests that ICDH may not be involved in the generation of NADPH for lipogenesis. PRF from neonatal lambs had significantly ($P < .05$) higher total ICDH activities than those at 24 hours and both age groups showed significantly ($P < .05$) greater enzyme activities than those at 8, 16 and 32 weeks of age. Also, total ICDH activities in PRR at 8 and 32 weeks were significantly ($P < .05$) higher than those at 16 weeks. Total ICDH activities in SCF increased with age and lambs at 32 weeks exhibited significantly ($P < .05$) greater enzyme activities than those at 8 and 16 weeks. In contrast to the observations in liver and muscle, it appeared that ICDH may be related to the level of lipogenic activity in sheep adipose tissues and may be a possible source of NADPH for fatty acid synthesis.

Total NADPH generating potential is defined as the total NADPH generated by G-6PDH, 6-PGDH, ME and ICDH in cytoplasm of adipose tissue cells. The NADPH generating potential was greatest in PRF of lambs at birth and 24 hours of age. The relative proportions of each of the dehydrogenases to the total NADPH generating potential in PRF and SCF are presented in table 22. ICDH was the major contributor to the NADPH generating potential in lamb PRF at birth (89 percent) and 24 hours (83 percent). The contribution of ME was minor at birth (3 percent) and 24 hours (4 percent) and the pentose shunt dehydrogenases, G-6PDH and 6-PGDH, contributed only 8 and 24 percent, respectively, of the NADPH generating potential at these same ages in PRF. The proportion of total NADPH potential provided by

TABLE 22. PROPORTION OF THE TOTAL NADPH GENERATING POTENTIAL OF DEHYDROGENASES IN ADIPOSE TISSUES AT VARIOUS AGES.

	Age				
Dehydrogenase	Birth	24 hr	8 wk	16 wk	32 wk
		Perirenal Fat			
Pentose shunt	8.0[a]	13.0	33.0	47.0	38.0
NADP-ME	3.0	4.0	13.0	13.0	19.0
NADP-ICDH	89.0	83.0	54.0	40.0	43.0
		Subcutaneous Fat			
Pentose shunt			45.0	50.0	46.0
NADP-ME			17.0	15.0	17.0
NADP-ICDH			38.0	35.0	37.0

[a]Percent of total NADPH generating potential.

ICDH in PRF decreased from the high birth and 24 hour percentages to 54, 40 and 43 percent at 8, 16 and 32 weeks, respectively. The importance of pentose shunt dehydrogenases (G-6PDH and 6-PGDH) as supplier of NADPH in PRF increased with age and they accounted for 33, 47 and 38 percent of the total at 8, 16 and 32 weeks, respectively. ME exhibited a comparatively minor role in NADPH generation in PRF of lambs at 8, 16 and 32 weeks, contributing only 13, 13 and 19 percent, respectively.

In subcutaneous fat of lambs at 8, 16 and 32 weeks, the pentose shunt dehydrogenases were the major suppliers of NADPH as they accounted for 45, 50 and 46 percent, respectively, of the total NADPH generating potential. The importance of ICDH in the generation of NADPH in SCF was slightly less than that observed in PRF of lambs at 8, 16 and 32 weeks of age. However, it was a major contributor to the NADPH generating potential in the SCF of 8, 16 and 32 week lambs as it supplied 38, 35 and 37 percent of the total, respectively, to these three age groups. ME played a limited role in the generation of NADPH in SCF as evidenced by its contribution of only 17, 15 and 17 percent, respectively, at 8, 16 and 32 weeks of age.

The pentose shunt dehydrogenases and ICDH appear to be the major sources of NADPH for lipogenesis in ovine adipose tissues. The pentose shunt dehydrogenases, G-6PDH and 6-PGDH, generally accounted for less than 50 percent of the total NADPH generating potential. However, the contribution of G-6PDH and 6-PGDH increased as the level of lipogenic activity increased and this observation indicates that the level of pentose shunt dehydrogenase activities in adipose tissues are closely related to lipogenic activity. The results of this study do not indicate whether the

levels of G-6PDH and 6-PGDH activities are a function of lipogenic activity or whether they are regulators of NADPH production for fatty acid synthesis as suggested by Hood (1972). ICDH appears to play a major role in the generation of NADPH for lipogenesis in ovine adipose tissues as indicated by Bauman et al. (1970), Beitz (1972), Hood (1972) and Cramer (1972), but the results of this study show that ICDH does not appear to respond to changes in the level of lipogenic activity as readily as the pentose shunt dehydrogenases. These findings are consistent with those of Beitz (1972) and Bauman et al. (1972) who found that ICDH activity remained relatively unchanged during fasting, but the activities of the pentose shunt dehydrogenases decreased considerably. The importance of ME to the generation of NADPH for lipogenesis in ovine adipose tissue is minor and this observation probably reflects the almost complete absence of the citrate cleavage pathway in the older lambs following the development of rumen function.

In summarizing the importance of the dehydrogenases to fatty acid synthesis, the adipose tissue depots (SCF and PRF) showed greater G-6PDH activity than liver and LM. SCF and PRF depots possessed greater 6-PGDH activity than muscle. 6-PGDH activities were considerably higher than G-6PDH in adipose tissues. The presence of considerable pentose shunt dehydrogenase activity and the fact that G-6PDH and 6-PGDH contributed a large proportion of the total NADPH generating potential in adipose tissue indicates that these enzymes are capable of generating NADPH for fatty acid synthesis in the ovine animal. 6-PGDH is an adaptable enzyme since the level of lipogenic activity appears to regulate the activity of this

enzyme. Moreover, 6-PGDH activities seems to be related to the propensity to fatten. G-6PDH is not as adaptable to changes in the level of lipogenic activity as 6-PGDH and does not appear to be related to the propensity to fatten. G-6PDH may be a rate limiting enzyme in the pentose shunt pathway; and thus, involved in the regulation of NADPH generation for fatty acid synthesis in ovine adipose tissues.

Subcutaneous and perirenal fat depots exhibited higher ME activities than LM and liver. ME activities appeared to be related to the level of lipogenic activity in ovine adipose tissues, but its response to changes in lipogenic activity was slower than that of the pentose shunt dehydrogenases. Moreover, the minor contribution of ME to total NADPH generating potential indicates that this enzyme is of little importance to lipogenesis in ovine adipose tissues.

Adipose tissue depots showed greater ICDH activities than liver and muscle. The presence of significant ICDH in adipose tissue depots and the fact that this enzyme contributed a large portion of the total NADPH generating potential indicates that it has the potential to supply NADPH for lipogenesis. However, the response of this enzyme to changes in lipogenic activity was not the same as those of the pentose shunt dehydrogenases.

The simple correlations between the activities of the NADPH generating enzymes and the activities of the other lipogenic enzymes measured are presented in Appendixes IV, V, VI and VII.

Circulating Triglyceride Uptake

The process of uptake of circulating triglycerides by tissues requires hydrolysis of the triglyceride molecule and this is mediated by lipoprotein lipase or clearing factor lipase (LPL) (Korn, 1955a, b). LPL is present in various tissues that utilize plasma triglycerides and several investigators have presented evidence to show that the plasma triglyceride moieties of chylomicrons and low density proteins are hydrolyzed to free fatty acids prior to removal from plasma (Robinson, 1960, 1963; Bezman et al., 1962a). The activity of lipoprotein lipase in adipose tissues is proportional to the rate of triglyceride uptake; therefore, the enzyme may play a significant role in controlling lipid deposition (Garfinkel et al., 1967; Bezman et al., 1962b).

Lipoprotein Lipase

The means and standard error of the means of LPL activity in various tissues are presented in figure 18. SCF possessed significantly ($P < .05$) greater lipoprotein lipase (LPL) activities than PRF and both adipose tissue depots showed significantly ($P < .05$) higher LPL activities than LM. These data indicate that adipose tissues are more actively involved in triglyceride uptake than LM and probably reflect the larger quantities of triglycerides being deposited in these depots at the ages studied.

The means and standard error of the means of LPL activities in various tissues by breed, sex and age are presented in table 23. The influence of breed on LPL activities was not significant for any of the

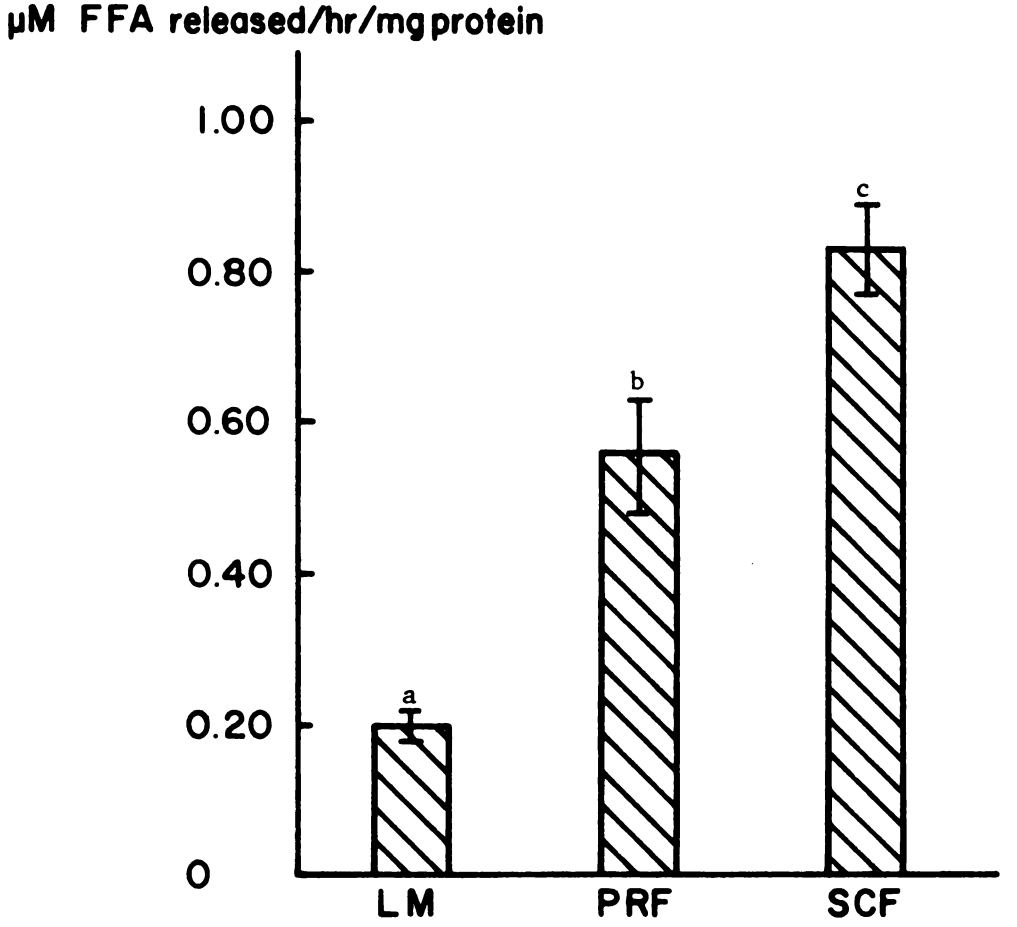


Figure 18. Means and standard error of the means of lipoprotein lipase activity in various tissues.
^{a,b,c}Means with different superscripts differ significantly ($P < .05$).

TABLE 23. LIPOPROTEIN LIPASE ACTIVITY AND FREE FATTY ACIDS IN VARIOUS TISSUES BY BREED, SEX AND AGE.

	Means and Standard Error of the Means							
Variable	n[a]	Longissimus[b] muscle	n	Perirenal[b] fat	n	Subcutaneous[b] fat	n	Blood[c] plasma
Breed		(P=.29)[d]		(P=.99)		(P=.87)		(P=.44)
Suffolk	(25)	0.215 ± .021	(24)	0.569 ± .100	(18)	0.839 ± .079	(25)	17.32 ± 1.06
Southdown	(24)	0.178 ± .022	(23)	0.569 ± .102	(18)	0.820 ± .079	(24)	18.66 ± 1.08
Sex		(P=.01)		(P=.42)		(P=.41)		(P=.67)
Ram	(18)	0.100 ± .024[e]	(18)	0.753 ± .116	(12)	0.824 ± .096	(18)	17.94 ± 1.24
Ewe	(19)	0.194 ± .024[f]	(17)	0.557 ± .120	(12)	0.876 ± .096	(19)	19.10 ± 1.21
Wether	(12)	0.295 ± .030[g]	(12)	0.396 ± .142	(12)	0.788 ± .096	(12)	16.92 ± 1.52
Age		(P=.01)		(P=.01)		(P=.31)		(P=.01)
Birth	(4)	0.214 ± .052[e]	(4)	0.152 ± .147[e]			(4)	38.29 ± 2.64[g]
24 hour	(9)	0.418 ± .035[f]	(7)	0.297 ± .146[e]			(9)	9.70 ± 1.76[e]
8 week	(12)	0.143 ± .030[e]	(12)	1.156 ± .142[f]	(12)	0.837 ± .096	(12)	13.68 ± 1.52[ef]
16 week	(12)	0.133 ± .030[e]	(12)	0.926 ± .142[f]	(12)	0.718 ± .096	(12)	15.64 ± 1.52[f]
32 week	(12)	0.075 ± .030[e]	(12)	0.313 ± .142[e]	(12)	0.934 ± .096	(12)	12.64 ± 1.52[ef]

[a]Number of observations.
[b]Values expressed as μm free fatty acids released/hr/mg protein.
[c]Values expressed as nanomoles free fatty acids/mg protein.
[d]Level of significance for each variable and tissue shown in parentheses.
[e,f,g]Means with different superscripts within variable and column differ significantly ($P < .05$).

tissues studied. LM of wethers exhibited significantly ($P < .05$) greater LPL activities than those of ewes and both sex groups showed significantly ($P < .05$) higher LPL activities than those of rams. These observations indicated that lambs with the greatest propensity to fatten (wethers and ewes) had the highest LPL activities in muscle. However, the opposite sex effects were observed in PRF and SCF where ewes and rams showed greater LPL activities than wethers, but the differences were not significant. Thus, in contrast to the observations for muscle, it appeared that the level of LPL activities in ovine adipose tissue depots was not related to the propensity to fatten.

Muscles of lambs at 24 hours possessed significantly ($P < .05$) higher LPL activities than those at birth, 8, 16 and 32 weeks of age. Muscles of neonate lambs which were essentially in a fasted state, were probably depleted of energy stores. As a result of milk ingestion by 24 hours plasma lipids increased and the increased LPL activities probably reflect the increased fatty acid uptake by muscle to meet energy needs. The significant ($P < .01$) sex X age interaction of LPL activities in LM (table 23A) showed that the significant overall age effect was sex specific as it was limited to the ewe lambs. The interaction showed that the effect of age on LPL activities was not the same in all sex groups. The interaction also showed a significant ($P < .05$) sex effect within the 24 hour age group where muscles of ewes had greater LPL activities than those of rams. There were no apparent or consistent patterns of LPL activities due to breed, sex or age as indicated by the interaction and this suggests that LPL may not be closely related to lipogenic activity in ovine muscle.

TABLE 23A. BREED X SEX, BREED X AGE AND SEX X AGE INTEREACTIONS OF LIPOPROTEIN LIPASE ACTIVITY AND FREE FATTY ACIDS IN VARIOUS TISSUES[a]

Tissue	Breed X Sex	Breed X Age	Sex X Age		
Longissimus muscle	(P=.19)[b]	(P=.22)	(P=.01)		
			Ram	Ewe	Wether
		Birth	0.071 ± .059	0.212 ± .059[c]	
		24 hour	0.069 ± .052[x]	0.423 ± .042[d,y]	
		8 week	0.114 ± .052	0.107 ± .052[c]	0.216 ± .052
		16 week	0.161 ± .052	0.138 ± .052[c]	0.101 ± .052
		32 week	0.084 ± .052	0.099 ± .052[c]	0.040 ± .029
Perirenal fat	(P=.41)	(P=.96)	(P=.82)		
Subcutaneous fat	(P=.34)	(P=.83)	(P=.22)		
Blood plasma	(P=.44)	(P=.77)	(P=.61)		

[a]Means and standard error of the means are presented only when significant interactions were observed. Values expressed as μm free fatty acid released/hr/mg protein.
[b]Level of significance for each interaction and tissue shown in parentheses.
[c,d]Means with different superscripts within interaction and column differ significantly (P <.05).
[x,y]Means with different superscripts within interaction and row differ significantly (P < .05).

PRF of lambs at 8 weeks of age exhibited significantly ($P < .05$) higher LPL activities than that at birth, 24 hours and 32 weeks of age. The high level of LPL activities in PRF appears to coincide with the high level of lipogenic activity usually found in this depot at 8 weeks of age. These observations suggest that during periods of high rates of fat deposition in PRF, deposition of fat may be the result of both lipogenic activity and circulating triglyceride uptake. Also, these results indicate that the maximum rate of fat deposition in PRF probably occurs before the lambs reach 16 weeks of age. The influence of age on LPL activity in SCF was not significant and does not appear to be related to the level of lipogenic activity or rate of fat deposition.

The influence of breed and sex on free fatty acid levels in blood plasma were not significant (table 23). Free fatty acid concentrations in blood plasma at birth were significantly ($P < .05$) higher than those at 24 hours, 8, 16 and 32 weeks of age. These high FFA levels no doubt reflect the fasted state of the neonatal lambs and probably resulted from mobilization of body fat to supply energy for vital functions. After milk ingestion the FFA concentration in blood decreased to a low level as indicated by the plasma FFA at 24 hours. This low level probably resulted from increased blood glucose which stimulated insulin secretion. The effect of insulin is two-fold as it exerts an antilipolytic effect which decreases fatty acid mobilization and it simultaneously stimulates triglyceride uptake. These two effects could account for the low FFA observed in plasma at 24 hours. FFA concentrations in plasma at 16 weeks were significantly ($P < .05$) higher than those at 24 hours. The plasma FFA

increased between 24 hours and 8 weeks, and then essentially stabilized. These results probably reflect increased rumen function which is associated with reduced blood glucose. Reduced blood glucose generally results in decreased insulin secretion which would tend to diminish triglyceride uptake and this coupled with the increased insulin insensitivity of maturing ruminants could possibly account for the higher FFA levels in blood of the older lambs. Moreover, the increase in rumen function generally results in increased plasma FFA. The simple correlations for lipoprotein lipase activity and free fatty acids with the activity of the other enzymes measured are presented in Appendixes IV, V, VI and VII.

In summarization, lipoprotein lipase activities were greater in adipose tissues than in muscle. The level of LPL activity in adipose tissues does not appear to be related to propensity to fatten. LPL activity seems to be related to the level of lipogenic activity only in PRF of lambs. Plasma FFA appeared to be associated with the energy state of lambs as well as rumen development.

SUMMARY

Forty-nine lambs were included in an experiment designed to study the influence of breed, sex and age on lipogenic enzyme activities in some ovine tissues. Thirty-nine western crossbred ewes were divided into two groups based upon body length and body weight. The smaller bodied ewes were mated to a Southdown ram and the larger bodied ewes were mated to a Suffolk ram in order to obtain two strains of lambs differing in propensity to fatten. Lambs were slaughtered at birth, 24 hours, 8, 16 and 32 weeks of age and blood, liver, longissimus muscle (LM), perirenal (PRF) and subcutaneous fat (SCF) samples were collected from the carcass. Enzymes assayed were glucose-6-phosphate dehydrogenase (G-6PDH), 6-phosphogluconate dehydrogenase (6-PGDH), NADP-malate dehydrogenase (ME), NADP-isocitrate dehydrogenase (ICDH), acetyl COA carboxylase (CBX), acetyl COA synthetase (SYN), lipoprotein lipase (LPL) and ATP-citrate lyase (CCE). Plasma free fatty acids (FFA) were also determined.

Acetyl COA synthetase activities were significantly ($P < .05$) higher in adipose tissues than in liver and LM. The results showed that 35 to 40 percent of the SYN activity in adipose tissue was of cytoplasmic origin and 60 to 65 percent was located intramitochondrially. Tissues of Southdown lambs had higher SYN activities than those of Suffolks. These data indicated that Southdowns, which have a greater propensity to fatten, possessed a greater potential to utilize acetate for acetyl COA synthesis and possibly for lipogenesis. There were no consistent or apparent patterns of SYN activities due to sex in the various tissues. The influence of age on the activities of SYN was significant, but the pattern

observed in each instance was tissue specific. The SYN activities appeared to be related to the level of lipogenic activity only in PRF.

PRF showed significantly greater CCE activities than LM and liver. The influence of breed and sex on CCE activities in the various tissues studied were not significant. Neonate and 24 hour lambs possessed greater CCE activities than those at 8, 16 and 32 weeks. CCE did not appear to be related to the predisposition to fatten and the level of lipogenic activity.

SCF had the greatest CBX activities followed in order by liver, PRF and LM. Adipose tissues of Southdowns showed higher CBX activities than those of Suffolks. There appeared to be a relationship between CBX activity in ovine adipose tissues and the propensity to fatten. PRF and SCF of 32 week ewe and ram lambs had greater CBX activities than those of wethers. CBX activities in the adipose tissues of 32 week rams and ewes were greater than those at all other ages; whereas, the 32 week wethers possessed lower CBX activities than at 16 weeks. The influence of sex on predisposition to fatten tended to be related to CBX activities in adipose tissues, but this relationship did not apply to ewes. CBX activities in PRF and SCF appeared to be related to the level of lipogenic activity. CBX appeared to be an important enzyme in fatty acid synthesis in ovine adipose tissues.

PRF and SCF had significantly ($P < .05$) higher G-6PDH activities than liver and LM. 6-PGDH activity was greatest in SCF followed in order by liver, PRF and LM. 6-PGDH activities were greater than those of G-6PDH in ovine adipose tissues. The effect of breed on G-6PDH activities was

not significant and the general pattern of G-6PDH activities among different sexes did not appear to be related to the predisposition to fatten. G-6PDH activity tended to be related to lipogenic activity in lambs as it adapted to changes in the level of lipogenic activity due to age.

Tissues of Southdowns exhibited greater 6-PGDH activities than those of Suffolks which indicated that this enzyme was related to the propensity to fatten. There were no consistent or apparent patterns due to sex on 6-PGDH activities in the various tissues studied. 6-PGDH activities increased considerably with age in PRF and SCF and it appeared that this enzyme was related to the level of lipogenic activity. 6-PGDH activities tended to be regulated by the level of lipogenic activity and this indicated that this enzyme was adaptable as well as not rate limiting. The pentose shunt dehydrogenases, G-6PDH and 6-PGDH, are capable of generating NADPH for lipogenesis as indicated by the fact that they contributed 33 to 50 percent of total NADPH generating potential in SCF and PRF of lambs from 8 to 32 weeks of age.

ME activities in the adipose tissues were greater than those in liver and LM. The effect of breed on ME activities in the various tissues was not significant. Liver and LM of rams possessed the highest ME activities followed in order by ewes and wethers, but the differences due to sex in PRF and SCF were nonsignificant. ME activities decreased with age in liver and LM which appeared to reflect the decreased citrate cycle activity usually associated with maturing lambs. However, ME activities increased with age in PRF and SCF and this trend followed the general pattern observed for lipogenic activity in growing lambs. ME appeared to play a

limited role in NADPH generation in ovine adipose tissues as indicated by its slow adaptation and the fact that its contribution to the total NADPH generating potential was minor (13 to 19 percent) in PRF and SCF.

Cytoplasmic ICDH activities in PRF and SCF were greater than those in liver and LM. Mitochondrial ICDH activities were highest in LM followed in order by SCF, PRF and liver. The effect of breed on cytoplasmic ICDH in the adipose tissue depots was not significant. PRF of wethers possessed greater cytoplasmic ICDH activities than those of ewes and both sexes had greater enzyme activities than rams. However, SCF of rams possessed the highest cytoplasmic ICDH followed in order by wethers and ewes. The effects of breed and sex on mitochondrial ICDH in the various tissues studied were not significant. The response of cytoplasmic ICDH to changes in the level of lipogenic activity was not as great as that observed for the pentose shunt dehydrogenases. Mitochondrial ICDH activities in the adipose tissues gradually increased with age and appeared to be related to changes in the level of lipogenic activity usually associated with maturing lambs. The presence of significant ICDH activities in ovine adipose tissues and the finding that ICDH contributed 35 to 54 percent of the total NADPH generating potential in PRF and SCF indicates that ICDH plays a major role in the generation of NADPH for lipogenesis in the ruminant.

LPL activities were highest in SCF followed in order by PRF and LM which indicated that adipose tissues were more actively involved in triglyceride uptake than LM and this probably reflected the larger quantities of triglycerides being deposited in those depots at the ages studied. The

influence of breed on LPL activities in the various tissues studied was not significant. LPL appeared to be related to the propensity to fatten in LM since lambs with the greatest predisposition to fatten (wethers and ewes) had the greatest activities. LPL activities in PRF appeared to coincide with the changes in rate of fat deposition usually found in lambs between birth and 32 weeks of age. These data suggested that the maximum rate of fat deposition in PRF probably occurred before the lambs reached 16 weeks of age. The results of this study indicated that during periods of high rates of fat deposition in PRF, fat deposition may be the result of both high lipogenic activity and uptake of circulating triglycerides. The influence of age on LPL activities was not significant and this enzyme did not appear to be related to the rate of fatty deposition in SCF.

The influence of breed and sex on plasma FFA levels was nonsignificant. The energy states of the lambs as well as the development of rumen function appeared to be the major factors affecting plasma FFA.

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APPENDIX

APPENDIX I. Ewe Grain Rations

Table A. Composition of Grain Mixture for Bred Ewes

Ingredients	Percent
Oats	55.00
Corn	13.75
Wheat	20.00
Wheat bran	10.00
Wet molasses	1.00
Aurofac	.25
Total	100.00

Table B. Composition of Grain Mixture for Lactating Ewes

Ingredients	Percent
Oats	53.00
Corn	25.00
Wheat bran	15.00
Soybean oil meal	5.00
Wet molasses	2.00
Total	100.00

APPENDIX II. Raw Data[a]

a Values expressed nanomoles NADPH produced/min./mg protein

b 1 = Suffolk breed type, 2 = Southdown breed type

c 1 = Ram; 2 = Ewe; 3 - Wether

d 1 = Birth; 2 = 24 hour; 3 - 8 week; 4 = 16 weeks; 5 = 32 weeks

e 1 = Liver; 2 = Longissimus muscle; 3 = Perirenal fat; 4 = Subcutaneous fat; 5 = Blood plasma

G-6 PDH = glucose-6-phosphate dehydrogenase

6-PGDH = 6-phosphogluconate dehydrogenase

NADP-ME = NADP-malate dehydrogenase

ICDH-CYTO = Cytoplasmic NADP-isocitrate dehydrogenase

ICDH-MITO = mitochondrial NADP=isocitrate dehydrogenase

APPENDIX II. Raw Data (continued)

Animal Number	Breed[b]	Sex[c]	Age[d]	Tissue[e]	G-6 PDH	6-PGDH	NADP-ME	ICDH-CYTO	ICDH-MITO
1	1	2	2	1	16.927	82.904	42.501	82.106	44.652
1	1	2	2	2	2.670	30.726	32.923	80.581	145.931
1	1	2	2	3	19.349	42.112	11.086	409.316	43.727
2	1	2	2	1	9.040	54.821	13.378	76.801	84.549
2	1	2	2	2	1.925	33.660	38.672	77.545	136.516
3	1	2	2	1	5.720	52.915	13.652	64.345	44.733
3	1	2	2	2	0.600	30.656	24.620	82.641	139.878
3	1	2	2	3	46.165	28.211	22.415	333.963	52.104
4	2	2	2	1	16.216	58.712	10.704	37.407	32.928
4	2	2	2	2	0.000	31.442	25.855	77.761	164.857
6	1	2	3	1	3.774	38.583	12.823	91.346	58.122
6	1	2	3	2	1.326	16.802	25.528	53.400	65.568
6	1	2	3	3	30.911	49.803	39.925	192.349	91.603
6	1	2	3	4	42.184	10.123	41.901	71.691	73.286
7	1	3	3	1	3.765	27.613	7.309	85.410	40.008
7	1	3	3	2	0.536	19.344	21.007	47.158	101.237
7	1	3	3	3	27.482	58.393	35.294	106.129	109.295
7	1	3	3	4	55.213	42.741	28.684	47.694	48.089
8	1	1	3	1	4.099	40.999	20.071	110.769	54.050
8	1	1	3	2	0.480	19.235	19.653	49.463	147.467
8	1	1	3	3	14.907	40.264	13.743	135.416	48.094
8	1	1	3	4	46.375	51.518	24.729	64.555	68.696
9	2	2	3	1	1.578	39.291	11.891	95.591	28.333
9	2	2	3	2	2.694	14.234	16.705	52.244	64.111
9	2	2	3	3	41.040	94.893	45.898	121.647	40.500
9	2	2	3	4	25.640	56.427	57.700	69.943	93.429
10	2	2	3	1	4.783	45.440	12.156	89.445	67.625
10	2	2	3	2	1.302	13.894	22.173	43.048	100.762
10	2	2	3	3	14.425	65.725	28.190	98.029	64.111
10	2	2	3	4	6.866	56.679	35.620	46.738	32.971
11	1	1	3	1	4.787	35.540	9.461	87.188	44.816
11	1	1	3	2	0.000	16.431	18.453	52.884	123.308

APPENDIX II. Raw Data (continued)

Animal Number	Breed[b]	Sex[c]	Age[d]	Tissue[e]	G-6 PDH	6-PGDH	NADP-ME	ICDH-CYTO	ICDH-MITO
11	1	1	3	3	34.421	28.347	21.864	144.957	42.756
11	1	1	3	4	40.073	30.052	28.420	89.902	89.767
12	1	3	3	1	6.072	57.001	19.606	92.621	57.368
12	1	3	3	2	2.405	16.350	19.115	54.625	129.850
12	1	3	3	3	8.589	51.518	40.495	123.110	50.174
12	1	3	3	4	46.160	76.933	45.259	77.809	118.385
13	2	1	3	1	2.082	41.962	18.405	111.912	57.418
13	2	1	3	2	0.396	17.847	17.440	48.472	113.436
13	2	1	3	3	12.147	44.547	18.863	112.202	58.878
13	2	1	3	4	6.462	58.193	24.926	82.175	55.839
14	2	3	3	1	2.251	37.510	18.712	88.757	40.390
14	2	3	3	2	0.770	16.158	21.029	42.962	67.175
14	2	3	3	3	18.196	44.189	27.284	82.661	34.357
14	2	3	3	4	21.221	33.941	24.624	58.851	125.909
15	2	1	3	1	6.943	36.450	18.339	107.063	54.648
15	2	1	3	2	0.000	22.120	21.725	53.492	103.973
15	2	1	3	3	11.145	61.333	28.496	91.934	72.966
15	2	1	3	4	42.574	50.451	20.008	52.068	119.260
16	1	1	4	1	5.770	57.706	14.604	74.497	31.803
16	1	1	4	2	1.097	21.434	19.776	40.155	96.167
16	1	1	4	3	46.160	40.016	35.620	75.105	42.320
16	1	1	4	4	81.685	81.685	45.688	69.943	59.844
17	1	2	4	1	6.653	35.928	15.826	73.064	55.180
17	1	2	4	2	1.202	14.427	20.575	35.743	116.421
17	1	2	4	3	44.119	42.349	26.227	85.491	17.884
17	1	2	4	4	32.328	52.344	24.048	77.299	77.857
19	1	2	4	1	5.843	33.827	11.969	63.299	43.283
19	1	2	4	2	0.580	21.502	20.368	38.253	96.188
19	1	2	4	3	64.119	48.849	13.042	99.875	82.914
19	1	2	4	4	34.689	50.450	43.324	65.235	60.737
20	2	1	4	1	5.582	27.765	8.376	62.563	47.704
20	2	1	4	2	1.639	26.776	26.987	37.656	88.769

APPENDIX II. Raw Data (continued)

Animal Number	Breed[b]	Sex[c]	Age[d]	Tissue[e]	G-6 PDH	6-PGDH	NADP-ME	ICDH-CYTO	ICDH-MITO
20	2	1	4	3	45.898	78.693	38.899	115.971	138.917
20	2	1	4	4	61.698	87.101	34.350	106.757	83.846
21	2	2	4	1	4.857	42.098	15.388	62.380	56.223
21	2	2	4	2	0.000	26.036	15.388	30.040	129.630
21	2	2	4	3	52.202	71.617	25.263	83.802	46.000
21	2	2	4	4	14.791	73.978	17.485	65.400	65.683
22	1	3	4	1	5.991	45.660	9.444	59.541	33.856
22	1	3	4	2	2.418	22.984	21.252	33.610	73.559
22	1	3	4	3	64.121	64.121	43.720	82.205	22.192
22	1	3	4	4	96.180	57.700	14.119	62.955	40.500
23	1	3	4	1	4.835	31.204	5.944	75.649	42.349
23	1	3	4	2	0.529	21.727	16.222	46.461	96.175
23	1	3	4	3	68.258	58.944	21.939	85.843	94.044
23	1	3	4	4	86.413	61.333	50.239	78.948	30.780
24	2	1	4	1	8.110	37.032	11.285	66.192	56.301
24	2	1	4	2	0.000	28.957	17.953	32.826	86.241
24	2	1	4	3	46.375	51.518	26.534	104.543	33.457
24	2	1	4	4	126.633	67.325	39.070	124.292	76.943
25	2	3	4	1	4.897	43.289	11.156	67.217	49.577
25	2	3	4	2	1.639	20.219	12.854	29.173	113.659
25	2	3	4	3	41.815	79.446	26.892	91.488	9.625
25	2	3	4	4	19.238	86.562	32.056	85.947	34.977
26	2	3	4	1	4.252	41.391	14.010	79.035	55.180
26	2	3	4	2	0.000	23.540	25.435	36.133	118.794
26	2	3	4	3	35.430	59.052	21.774	77.651	65.400
26	2	3	4	4	59.536	50.381	66.902	96.174	153.867
27	2	2	4	1	3.383	33.827	5.496	62.874	59.887
27	2	2	4	2	0.000	26.257	16.116	28.226	114.378
27	2	2	4	3	17.782	58.184	41.675	70.533	38.480
27	2	2	4	4	28.101	67.000	58.430	96.174	76.933

APPENDIX II. Raw Data (continued)

Animal Number	Breed[b]	Sex[c]	Age[d]	Tissue[e]	G-6 PDH	6-PGDH	NADP-ME	ICDH-CYTO	ICDH-MITO
28	1	1	5	1	1.230	28.906	18.536	75.370	24.553
28	1	1	5	2	0.000	17.793	17.406	25.802	108.739
28	1	1	5	3	43.902	62.728	56.718	130.407	83.367
28	1	1	5	4	45.644	84.763	31.189	108.027	151.143
29	1	2	5	1	3.620	43.429	14.107	75.497	48.090
29	1	2	5	2	0.000	22.520	14.796	26.531	48.083
29	1	2	5	3	37.403	112.208	42.164	91.288	96.176
29	1	2	5	4	51.094	72.125	59.606	87.200	96.167
30	1	2	5	1	4.271	29.211	18.728	70.677	48.523
30	1	2	5	2	0.000	16.029	14.234	21.914	73.974
30	1	2	5	3	11.732	75.061	52.699	81.685	96.200
30	1	2	5	4	43.435	105.484	36.246	97.535	276.500
31	2	1	5	1	5.715	34.074	15.996	83.943	15.387
31	2	1	5	2	0.000	16.449	11.620	28.743	41.431
31	2	1	5	3	34.573	71.326	56.293	97.565	96.188
31	2	1	5	4	90.625	122.067	42.184	166.051	45.794
32	2	1	5	1	5.303	25.459	11.080	60.941	78.860
32	2	1	5	2	0.594	17.864	19.830	28.620	65.943
32	2	1	5	3	38.467	69.253	65.943	100.362	96.167
32	2	1	5	4	32.481	59.948	11.057	80.875	140.577
33	2	2	5	1	7.082	62.441	14.770	58.324	57.189
33	2	2	5	2	1.640	13.123	9.160	24.142	85.489
33	2	2	5	3	45.078	93.172	66.328	99.500	78.370
33	2	2	5	4	32.554	103.569	60.737	73.987	72.125
34	1	3	5	1	4.457	30.046	8.044	73.869	44.624
34	1	3	5	2	0.000	18.070	16.350	20.221	98.914
34	1	3	5	3	37.403	77.472	72.525	130.800	88.769
34	1	3	5	4	32.060	45.797	25.456	94.817	76.933
35	2	3	5	1	4.342	34.019	14.743	59.160	38.138
35	2	3	5	2	0.000	24.690	19.491	20.715	115.400
35	2	3	5	3	38.471	74.186	68.062	123.100	99.483
35	2	3	5	4	46.160	30.773	68.062	70.189	177.538

APPENDIX II. Raw Data (continued)

Animal Number	Breed[b]	Sex[c]	Age[d]	Tissue[e]	G-6 PDH	6-PGDH	NADP-ME	ICDH-CYTO	ICDH-MITO
36	1	1	5	1	0.864	42.515	21.029	86.442	56.191
36	1	1	5	2	1.279	16.615	16.776	35.987	89.767
36	1	1	5	3	55.754	83.623	51.905	169.280	86.871
36	1	1	5	4	75.571	59.536	60.873	112.202	56.588
37	1	3	5	1	8.464	42.702	8.450	77.197	47.651
37	1	3	5	2	0.000	21.857	11.285	27.250	58.303
37	1	3	5	3	20.607	66.405	29.600	153.880	54.952
37	1	3	5	4	58.543	92.000	70.288	116.786	204.375
38	2	2	5	1	8.701	29.832	10.083	69.753	61.038
38	2	2	5	2	0.000	17.173	13.465	23.522	96.184
38	2	2	5	3	22.776	68.342	69.638	119.079	153.900
38	2	2	5	4	6.272	66.902	46.636	82.819	96.179
39	2	3	5	1	5.578	36.932	17.013	64.879	62.628
39	2	3	5	2	0.582	18.070	13.601	24.043	71.457
39	2	3	5	3	15.387	87.200	42.426	188.174	103.577
39	2	3	5	4	17.947	69.253	31.189	71.813	117.087
40	1	1	2	1	10.771	70.786	24.095	95.544	49.106
40	1	1	2	2	1.477	76.938	44.030	80.436	89.321
40	1	1	2	3	11.737	50.573	22.157	293.744	114.000
41	1	1	1	1	10.921	96.511	46.543	77.730	76.933
41	1	1	1	2	0.000	40.669	49.424	86.952	130.800
41	1	1	1	3	13.206	22.394	15.937	420.013	111.677
42	1	1	2	1	10.748	90.431	43.449	82.205	53.853
42	1	1	2	2	0.000	66.646	49.355	73.219	114.000
42	1	1	2	3	22.237	32.243	14.087	302.425	72.784
43	1	2	3	1	5.067	33.295	12.229	77.959	43.324
43	1	2	3	2	1.634	20.162	22.666	47.202	116.459
43	1	2	3	3	21.160	48.090	25.363	156.453	73.100
43	1	2	3	4	24.553	69.574	30.602	117.000	28.850
44	2	1	2	1	5.810	96.391	42.092	58.875	56.097
44	2	1	2	2	0.000	60.457	27.824	55.287	74.000

APPENDIX II. Raw Data (continued)

Animal Number	Breed[b]	Sex[c]	Age[d]	Tissue[e]	G-6 PDH	6-PGDH	NADP-ME	ICDH-CYTO	ICDH-MITO
45	1	1	4	1	2.001	56.780	25.396	101.781	64.120
45	1	1	4	2	0.000	17.449	17.302	43.191	67.595
45	1	1	4	3	16.912	86.659	45.123	134.940	74.806
45	1	1	4	4	65.807	48.930	30.475	96.176	96.167
46	2	2	1	1	3.227	147.600	55.297	80.960	56.097
46	2	2	1	2	0.873	39.345	26.663	72.392	78.370
46	2	2	1	3	25.115	15.926	20.813	462.531	104.909
47	2	1	1	1	5.780	138.716	58.213	78.408	42.164
47	2	1	1	2	1.600	64.117	40.803	75.680	116.786
47	2	1	1	3	14.189	26.803	17.160	356.585	68.611
48	2	2	2	1	8.203	88.998	13.678	55.164	32.700
48	2	2	2	2	0.000	38.831	25.708	76.557	130.536
48	2	2	2	3	16.809	47.932	18.079	373.940	54.952
49	2	1	2	1	6.002	58.216	35.539	99.158	58.066
49	2	1	2	2	0.000	22.966	28.938	80.906	153.867
49	2	1	2	3	20.883	26.380	17.289	290.934	67.325
50	1	2	1	1	10.065	107.919	47.529	78.456	50.174
50	1	2	1	2	0.000	26.160	19.662	81.961	112.208
50	1	2	1	3	20.123	24.858	15.522	388.686	67.325
51	2	3	3	1	4.954	44.245	11.926	85.890	56.029
51	2	3	3	2	0.000	24.906	23.820	43.328	89.767
51	2	3	3	3	37.055	54.706	32.700	145.439	61.821
51	2	3	3	4	76.941	74.089	44.512	120.603	32.067

APPENDIX III. Raw Data

a 1 = Suffolk breed type; 2 = Southdown breed type.

b 1 = Ram; 2 = Ewe; 3 = Wether.

c 1 = Birth; 2 = 24 hour; 3 = 8 weeks; 4 = 16 weeks; 5 = 32 weeks.

d 1 = Liver; 2 = Longissimus muscle; 3 = Perirenal fat; 4 = Subcutaneous fat; 5 = Blood plasma.

e Values expressed as nanomoles of NADH oxidized/min./mg protein.

f Values expressed as μm CO_2 incorporated/min./mg protein.

g Values expressed as specific activity = enzyme units/mg protein, enzyme unit = 1.0 μm COASH reacting in 60 min.

h Values expressed as μm free fatty acids released/hr/mg protein.

i Values expressed as nanomoles free fatty acids/mg protein.

CCE = ATP - citrate lyase.

Ac COA CBX = Acetyl COA carboxylase.

Ac COA syn-cyto = Cytoplasmic acetyl COA synthetase.

Ac COA syn-mito = mitochondrial acetyl COA synthetase.

LPL = Lipoprotein lipase.

FFA-blood = Free fatty acids of blood plasma.

APPENDIX III. Raw Data (continued)

Animal Number	Breed[a]	Sex[b]	Age[c]	Tissue[d]	CCE[e]	Ac COA[f] CBX	Ac COA[g] Syn-Cyto	Ac COA[g] Syn-Mito	LPL-TIS[h]	FFA[i] Blood
1	1	2	2	1	3.578	0.360	0.2318	0.0000		
1	1	2	2	2	1.020	0.133	0.1510	0.4140	0.58870	
1	1	2	2	3	3.675	0.366	2.4416	2.0840	0.59593	
1	1	2	2	5						8.2281
2	1	2	2	1	0.275	0.587	0.3066	0.0566		
2	1	2	2	2	1.112	0.389	0.2287	0.4666	0.34316	
2	1	2	2	3		0.493	1.6682	0.5218		
2	1	2	2	5						9.3590
3	1	2	2	1	1.142	1.045	0.2832	0.1128		
3	1	2	2	2	4.255	0.486	0.1420	0.0000	0.37199	
3	1	2	2	3	5.391	0.878	1.7142	0.9056	0.10315	
3	1	2	2	5						8.3756
4	2	2	2	1	1.664	0.255	0.3093	0.1864		
4	2	2	2	2	1.495	0.412	0.2254	0.7232	0.59986	
4	2	2	2	3		1.836	1.5608	2.0099		
4	2	2	2	5						8.9315
6	1	2	3	1	0.000	0.894	0.2994	0.0733		
6	1	2	3	2	0.000	0.203	0.0126	0.0825	0.21293	
6	1	2	3	3	0.000	0.220	0.2607	0.0000	1.47834	
6	1	2	3	4	0.000	1.077	2.4754	2.8928	0.93122	
6	1	2	3	5						14.9222
7	1	3	3	1	0.000	1.350	0.2010	0.1348		
7	1	3	3	2	0.000	0.209	0.0215	0.0000	0.35176	
7	1	3	3	3	0.000	1.396	0.8648	0.0290	1.33072	
7	1	3	3	4	0.000	0.905		0.1778	1.23345	
7	1	3	3	5						9.0384
8	1	1	3	1	0.000	0.441	0.3322	0.0550		
8	1	1	3	2	0.000	0.200	0.2140	0.3620	0.25377	
8	1	1	3	3	0.000	0.345	1.3046	7.1469	0.93683	
8	1	1	3	4	0.000	0.733	1.8301	3.9828	0.51436	
8	1	1	3	5						3.7959

APPENDIX III. Raw Data (continued)

Animal Number	Breed[a]	Sex[b]	Age[c]	Tissue[d]	CCE[e]	Ac COA[f] CBX	Ac COA[g] Syn-Cyto	Ac COA[g] Syn-Mito	LPL-TIS[h]	FFA[i] Blood
9	2	2	3	1	0.000	0.664	0.1160	0.1288		
9	2	2	3	2	0.000	0.308	0.2874	0.0000	0.00000	
9	2	2	3	3	0.000	0.658	2.2700	1.2960	1.00406	
9	2	2	3	4	0.000	1.116	2.0484	0.0000	0.72122	
9	2	2	3	5						10.4903
10	2	2	3	1	0.000	0.960	0.1924	0.1439		
10	2	2	3	2	0.000	0.277	0.1898	0.2822	0.03475	
10	2	2	3	3	0.000	0.219	0.5071	1.5180	0.41712	
10	2	2	3	4	0.000	0.319	1.9161	0.0000	0.92512	
10	2	2	3	5						17.5053
11	1	1	3	1	0.000	0.974	0.3595	0.2120		
11	1	1	3	2	0.000	0.245	0.1133	0.4771	0.18750	
11	1	1	3	3	0.000	0.725	1.1263	1.5677	0.25000	
11	1	1	3	4	0.000	0.424	1.3376	1.3125	0.46425	
11	1	1	3	5						9.8659
12	1	3	3	1	0.000	1.389	0.1074	0.0657		
12	1	3	3	2	0.000	0.217	0.0154	0.0600	0.10119	
12	1	3	3	3	0.000	0.575	1.0755	0.0000	0.86400	
12	1	3	3	4	0.000	0.200	0.4290	6.9524	0.85751	
12	1	3	3	5						11.1531
13	2	1	3	1	0.000	1.576	0.3225	0.2580		
13	2	1	3	2	0.000	0.288	0.2106	0.2472	0.01599	
13	2	1	3	3	0.000	0.506	0.7318	3.7028	2.46390	
13	2	1	3	4	0.000	0.450	0.3471	2.1257	1.14647	
13	2	1	3	5						11.7916
14	2	3	3	1	0.000	0.852	0.3084	0.4516		
14	2	3	3	2	0.000	0.287	0.1080	0.5054	0.10684	
14	2	3	3	3	0.000	0.360	1.4124	0.1580	0.36338	
14	2	3	3	4	0.000	0.726	1.6375	2.5578	0.66675	
14	2	3	3	5						11.8918

APPENDIX III. Raw Data (continued)

Animal Number	Breed[a]	Sex[b]	Age[c]	Tissue[d]	CCE[e]	Ac COA[f] CBX	Ac COA[g] Syn-Cyto	Ac COA[g] Syn-Mito	LPL-TIS[h]	FFA[i] Blood
15	2	1	3	1	0.000	0.637	0.4108	0.0657		
15	2	1	3	2	0.000	0.123	0.0874	0.0438	0.00000	
15	2	1	3	3	0.000	0.839	0.9014	1.0800	1.07865	
15	2	1	3	4	0.000	1.111	1.9130	0.6246	0.43889	
15	2	1	3	5						22.0138
16	1	1	4	1	0.000	0.442	0.4631	0.8016		
16	1	1	4	2	0.000	0.206	0.0561	0.8541	0.12748	
16	1	1	4	3	0.000	0.901	0.4920	1.2592	1.01304	
16	1	1	4	4	0.000	0.881	1.8270	1.9628	0.54698	
16	1	1	4	5						13.6258
17	1	2	4	1	0.000	0.765	0.3327	0.4598		
17	1	2	4	2	0.000	0.156	0.0708	0.0344	0.19893	
17	1	2	4	3	0.000	0.580	0.7372	1.8514	0.41385	
17	1	2	4	4	0.000	0.220	1.2611	0.8750	1.56165	
17	1	2	4	5						9.2894
19	1	2	4	1	0.000	0.846	0.3670	0.1542		
19	1	2	4	2	0.000	0.127	0.1871	0.0000	0.25000	
19	1	2	4	3	0.000	0.752	1.1688	2.3627	1.18572	
19	1	2	4	4	0.000	0.388	1.2349	3.5610	0.38079	
19	1	2	4	5						11.1996
20	2	1	4	1	0.000	0.573	0.4362	0.2980		
20	2	1	4	2	0.000	0.152	0.0644	0.0582	0.25343	
20	2	1	4	3	0.000	0.675	0.5448	2.8400	1.07975	
20	2	1	4	4	0.000	1.015	2.2024	0.0000	0.70765	
20	2	1	4	5						10.0959
21	2	2	4	1	0.000	0.652	0.4002	0.2584		
21	2	2	4	2	0.000	0.196	0.0928	0.0976	0.09852	
21	2	2	4	3	0.000	0.923	0.7475	1.0088	0.96706	
21	2	2	4	4	0.000	1.000	2.3354	1.4833	0.73573	
21	2	2	4	5						24.2746

APPENDIX III. Raw Data (continued)

Animal Number	Breed[a]	Sex[b]	Age[c]	Tissue[d]	CCE[e]	Ac COA[f] CBX	Ac COA[g] Syn-Cyto	Ac COA[g] Syn-Mito	LPL-TIS[h]	FFA[i] Blood
21	2	2	4	1	0.000	0.652	0.4002	0.2584		
21	2	2	4	2	0.000	0.196	0.0928	0.0976	0.09852	
21	2	2	4	3	0.000	0.923	0.7475	1.0088	0.96706	
21	2	2	4	4	0.000	1.000	2.3354	1.4833	0.73573	
21	2	2	4	5						24.2746
22	1	3	4	1	0.000	0.686	0.4058	0.2305		
22	1	3	4	2	0.000	0.124	0.1670	0.2875	0.04908	
22	1	3	4	3	0.000	1.368	0.6824	3.4644	0.92217	
22	1	3	4	4	0.000	0.724	1.5906	0.0000	0.35216	
22	1	3	4	5						11.8906
23	1	3	4	1	0.000	0.520	0.3086	0.0000		
23	1	3	4	2	0.000	0.073	0.0000	0.0820	0.02375	
23	1	3	4	3	0.000	1.300	0.2722	1.6222	0.93769	
23	1	3	4	4	0.000	1.094	1.4640	4.7722	0.76964	
23	1	3	4	5						26.1936
24	2	1	4	1	0.000	1.112	0.1420	0.0000		
24	2	1	4	2	0.000	0.116	0.0000	0.5192	0.19876	
24	2	1	4	3	0.000	0.236	1.3953	0.0000	0.94945	
24	2	1	4	4	0.000	1.374	2.1673	1.9076	0.78940	
24	2	1	4	5						20.4210
25	2	3	4	1	0.000	0.655	0.2595	0.1522		
25	2	3	4	2	0.000	0.116	0.0070	0.0000	0.27751	
25	2	3	4	3	0.000	0.638	0.8062	3.1137	0.48372	
25	2	3	4	4	0.000	0.995	1.0918	6.3714	0.58666	
25	2	3	4	5						11.7144
26	2	3	4	1	0.000	1.042	0.3946	0.0000		
26	2	3	4	2	0.000	0.199	0.0000	0.0895	0.05219	
26	2	3	4	3	0.000	0.575	1.2345		0.67640	
26	2	3	4	4	0.000	0.927	1.8694	0.8818	0.67855	
26	2	3	4	5						15.5025

APPENDIX III. Raw Data (continued)

Animal Number	Breed[a]	Sex[b]	Age[c]	Tissue[d]	CCE[e]	Ac COA[f] CBX	Ac COA[g] Syn-Cyto	Ac COA[g] Syn-Mito	LPL-TIS[h]	FFA[i] Blood
27	2	2	4	1	0.000	0.770	0.2131	0.1733		
27	2	2	4	2	0.000	0.137	0.0297	0.4118	0.00277	
27	2	2	4	3	0.000	0.636	0.1947	1.2412	0.92363	
27	2	2	4	4	0.000	0.369	0.3262	0.0000	0.83882	
27	2	2	4	5						20.7849
28	1	1	5	1	0.000	0.758	0.4545	0.1134		
28	1	1	5	2	0.000	0.119	0.0794	0.6738	0.21297	
28	1	1	5	3	0.000	1.598	1.5057	4.9846	0.42832	
28	1	1	5	4	0.000	0.570	0.3942	0.0000	1.16264	
28	1	1	5	5						13.7253
29	1	2	5	1	0.000	0.704	0.6128	0.4764		
29	1	2	5	2	0.000	0.123	0.2128	0.0000	0.03722	
29	1	2	5	3	0.000	2.188	0.9529	9.6138	0.33677	
29	1	2	5	4	0.000	2.388	1.5165	0.0000	1.38665	
29	1	2	5	5						9.1762
30	1	2	5	1	0.000	0.880	0.5303	0.6685		
30	1	2	5	2	0.000	0.171	0.1230	0.9816	0.08551	
30	1	2	5	3	0.000	1.625	1.1785	3.3818	0.06540	
30	1	2	5	4	0.000	2.333	1.4210	8.7600	0.76691	
30	1	2	5	5						12.2426
31	2	1	5	1	0.000	1.442	0.3796	0.3020		
31	2	1	5	2	0.000	0.132	0.0558	0.3820	0.03020	
31	2	1	5	3	0.000	1.477	0.8960	2.7000	0.22365	
31	2	1	5	4	0.000	2.747	1.0806	1.9090	0.96917	
31	2	1	5	5						10.9264
32	2	1	5	1	0.000	1.233	0.2316	0.2016		
32	2	1	5	2	0.000	0.157	0.6497	0.0588	0.09258	
32	2	1	5	3	0.000	1.625	0.5192	1.6200	0.21704	
32	2	1	5	4	0.000	2.400	0.7036	0.0000	0.90189	
32	2	1	5	5						12.4977

APPENDIX III. Raw Data (continued)

Animal Number	Breed[a]	Sex[b]	Age[c]	Tissue[d]	CCE[e]	Ac COA[f] CBX	Ac COA[g] Syn-Cyto	Ac COA[g] Syn-Mito	LPL-TIS[h]	FFA[i] Blood
33	2	2	5	1	0.000	1.129	0.7021	0.3132		
33	2	2	5	2	0.000	0.136	0.0849	0.8824	0.15049	
33	2	2	5	3	0.000	1.541	1.0969	2.8174	0.36038	
33	2	2	5	4	0.000	2.398	1.1782	0.0000	0.32753	
33	2	2	5	5						17.8118
34	1	3	5	1	0.000	0.422	0.4134	0.0646		
34	1	3	5	2	0.000	0.065	0.0932	0.1076	0.01861	
34	1	3	5	3	0.000	0.549	1.1320	2.2462	0.49526	
34	1	3	5	4	0.000	1.324	0.2727	0.0000	0.75815	
34	1	3	5	5						13.5858
35	2	3	5	1	0.000	0.511	0.4974	0.3312		
35	2	3	5	2	0.000	0.101	0.0356	0.4813	0.02447	
35	2	3	5	3	0.000	0.787	1.4050	2.7000	0.40815	
35	2	3	5	4	0.000	0.506	0.5037	7.9636	1.04801	
35	2	3	5	5						10.7252
36	1	1	5	1	0.000	1.181	0.2779	0.0000		
36	1	1	5	2	0.000	0.121	0.0568	0.1182	0.00000	
36	1	1	5	3	0.000	0.988	1.9204	3.8118	0.20591	
36	1	1	5	4	0.000	2.697	1.0946	3.4500	1.58158	
36	1	1	5	5						13.4029
37	1	3	5	1	0.000	0.669	0.2438	0.5463		
37	1	3	5	2	0.000	0.081	0.0780	0.4764	0.00827	
37	1	3	5	3	0.000	0.420	0.6147	0.0000	0.04530	
37	1	3	5	4	0.000	0.420	0.0932	10.0400	0.28034	
37	1	3	5	5						14.0412
38	2	2	5	1	0.000	0.861	0.3404	0.4496		
38	2	2	5	2	0.000	0.090	0.0630	0.0000	0.12588	
38	2	2	5	3	0.000	1.067	0.8100	0.0000	0.11473	
38	2	2	5	4	0.000	3.077	0.8582	0.0000	1.05785	
38	2	2	5	5						11.7073

APPENDIX III. Raw Data (continued)

Animal Number	Breed[a]	Sex[b]	Age[c]	Tissue[d]	CCE[e]	Ac COA[f] CBX	Ac COA[g] Syn-Cyto	Ac COA[g] Syn-Mito	LPL-TIS[h]	FFA[i] Blood
39	2	3	5	1	0.000	1.032	0.3114	0.3347		
39	2	3	5	2	0.000	0.200	0.0639	0.6592	0.10973	
39	2	3	5	3	0.000	1.250	1.5000	9.9176	0.86552	
39	2	3	5	4	0.000	1.324	1.1088	3.2375	0.96876	
39	2	3	5	5						11.7968
40	1	1	2	1	0.063	1.077	0.1908	0.0000		
40	1	1	2	2	1.958	0.536	0.1286	0.0000	0.05574	
40	1	1	2	3	2.736	1.187	2.3653	1.8140	0.34240	
40	1	1	2	5						11.3808
41	1	1	1	1	4.828	1.081	0.2949	0.4842		
41	1	1	1	2	0.000	0.394	0.0864	0.1246	0.03596	
41	1	1	1	3	3.411	0.868	2.0533	6.1939	0.22396	
41	1	1	1	5						30.1322
42	1	1	2	1	3.525	0.628	0.2410	0.2534		
42	1	1	2	2	0.000	0.325	0.0935	0.4565	0.13056	
42	1	1	2	3	0.911	1.127	2.4940	3.8630	1.19232	
42	1	1	2	5						16.7536
43	1	2	3	1	0.000	0.767	0.3916	0.0000		
43	1	2	3	2	0.000	0.210	0.0682	0.2972	0.17998	
43	1	2	3	3	0.000	0.275	1.7318	1.8118	2.18109	
43	1	2	3	4	0.000	0.701	1.7306	0.9529	0.88445	
43	1	2	3	5						21.8331
44	2	1	2	1	0.976	0.628	0.3366	0.0000		
44	2	1	2	2	0.000	0.208	0.1970	0.9625	0.00000	
44	2	1	2	3		0.420	2.3839	4.1840	1.23326	
44	2	1	2	5						22.6233
45	1	1	4	1	0.000	1.514	0.4996	0.1099		
45	1	1	4	2	0.000	0.135	0.0624	0.0444	0.06250	
45	1	1	4	3	0.000	0.824	1.0565	3.1578	1.56097	
45	1	1	4	4	0.000	1.250	0.8298	1.9466	0.66922	
45	1	1	4	5						12.7287

APPENDIX III. Raw Data (continued)

Animal Number	Breed[a]	Sex[b]	Age[c]	Tissue[d]	CCE[e]	Ac COA[f] CBX	Ac COA[g] Syn-Cyto	Ac COA[g] Syn-Mito	LPL-TIS[h]	FFA[i] Blood
46	2	2	1	1	0.592	0.926	0.2272	0.2360		
46	2	2	1	2	0.000	0.288	0.1310	0.1421	0.03580	
46	2	2	1	3	2.564	0.362	3.9460	4.4888	0.13942	
46	2	2	1	5						33.2266
47	2	1	1	1	0.200	0.855	0.3564	0.4114		
47	2	1	1	2	0.815	0.136	0.0570	0.1524	0.10524	
47	2	1	1	3		1.788	3.6933	2.7800	0.57274	
47	2	1	1	5						39.7955
48	2	2	2	1	0.698	0.784	0.2845	0.1988		
48	2	2	2	2	0.997	0.313	0.1042	0.0539	0.21332	
48	2	2	2	3	8.541	1.113	3.1654	3.2113	0.30627	
48	2	2	2	5						17.4517
49	2	1	2	1	3.202	0.955	0.1978	0.2846		
49	2	1	2	2	4.017	0.336	0.0482	0.0952	0.08780	
49	2	1	2	3	1.230	0.443	3.0860	2.5100	0.29503	
49	2	1	2	5						13.4046
50	1	2	1	1	0.855	0.600	0.2133	0.2520		
50	1	2	1	2	1.337	0.561	0.0638	0.5690	0.38766	
50	1	2	1	3	1.178	0.473	2.5471	3.4923	0.14199	
50	1	2	1	5						45.5870
51	2	3	3	1	0.000	0.942	0.4143	0.2674		
51	2	3	3	2	0.000	0.252	0.0982	0.0000	0.26611	
51	2	3	3	3	0.000	1.464	1.3022	1.5400	1.50402	
51	2	3	3	4	0.000	1.395	2.0159	4.9333	1.25892	
51	2	3	3	5						19.9150

APPENDIX IV. Simple Correlations - Liver

	G-6 PDH	6-PGDH	NADP-ME	ICDH CYTO	ICDH MITO	CCE	Ac COA CBX	Ac COA Syn-Cyto	Ac COA Syn-Mito	FFA Blood	ICDH Total
6-PGDH	0.392										
NADP-ME	0.301	0.882									
ICDH-CYTO	-0.092	0.143	0.276								
ICDH-MITO	0.173	0.197	0.227	0.376							
CCE	0.594	0.475	0.589	0.026	0.176						
Ac COA-CBX	-0.226	0.032	0.076	0.478	0.373	-0.115					
Ac COA-Syn-Cyto	-0.092	-0.072	-0.074	0.052	0.050	-0.206	-0.044				
Ac COA-Syn-Mito	0.045	0.096	0.102	-0.036	-0.006	0.053	0.005	0.388			
FFA-Blood	0.130	0.666	0.611	0.112	0.242	0.167	0.055	0.012	0.082		
ICDH-Total	0.030	0.201	0.305	0.870	0.784	0.112	0.518	0.062	-0.027	0.204	
Ac COA-Syn-Total	-0.012	0.032	0.036	0.000	0.020	-0.062	0.025	0.760	0.894	0.064	0.010

APPENDIX V. Simple Correlations - Longissimus Muscle.

	G-6 PDH	6-PGDH	NADP-ME	ICDH CYTO	ICDH MITO	CCE	Ac COA CBX	Ac COA Syn-Cyto	Ac COA Syn-Mito	LPL TIS	FFA Blood	ICDH Total
G-6 PDH												
6-PGDH	-0.101											
NADP-ME	0.167	0.392										
ICDH-Cyto	0.180	0.262	0.784									
ICDH-Mito	0.057	0.078	0.468	0.588								
CCE	0.002	0.081	0.321	0.604	0.444							
Ac COA CBX	0.043	0.248	0.566	0.780	0.424	0.598						
Ac COA-Syn-Cyto	0.124	0.009	0.106	0.105	-0.030	0.040	0.194					
Ac COA-Syn-Mito	-0.120	-0.094	-0.064	-0.070	0.028	-0.106	0.048	0.013				
LPL-TIS	0.179	0.132	0.204	0.415	0.520	0.363	0.337	0.064	0.122			
FFA-Blood	-0.121	0.250	0.300	0.362	0.129	-0.018	0.238	0.162	0.080	0.114		
ICDH-Total	0.120	0.170	0.664	0.842	0.931	0.568	0.634	0.027	-0.012	0.534	0.250	
Ac COA-Syn-Total	-0.069	-0.084	-0.023	-0.029	0.016	-0.086	0.112	0.354	0.940	0.136	0.019	-0.002

APPENDIX VI. Simple Correlations - Perirenal Fat.

	G-6 PDH	6-PGDH	NADP-ME	ICDH CYTO	ICDH MITO	CCE	Ac COA CBX	Ac COA Syn-Cyto	Ac COA Syn-Mito	LPL TIS	FFA Blood	ICDH Total
6-PGDH	0.477											
NADP-ME	0.394	0.768										
ICDH-CYTO	-0.098	-0.204	-0.176									
ICDH-MITO	0.190	0.460	0.542	0.327								
CCE	-0.123	-0.244	-0.266	0.703	0.050							
Ac COA CBX	0.206	0.338	0.346	-0.048	0.314	-0.022						
Ac COA-Syn-CYTO	-0.264	-0.360	-0.321	0.728	0.034	0.522	0.024					
Ac COA-Syn-MITO	-0.036	0.206	0.040	0.239	0.228	0.086	0.348	0.266				
LPL-TIS	0.086	0.130	-0.060	-0.156	0.020	-0.250	-0.168	-0.182	-0.004			
FFA-Blood	-0.017	-0.183	-0.148	0.476	0.149	0.110	0.026	0.450	0.077	0.050		
ICDH-Total	-0.035	-0.056	-0.008	0.967	0.557	0.632	0.042	0.649	0.262	-0.131	0.458	
Ac COA-Syn-Total	-0.122	0.052	-0.076	0.454	0.206	0.252	0.305	0.569	0.944	-0.066	0.228	0.454

APPENDIX VII. Simple Correlations - Subcutaneous Fat.

	G-6 PDH	6-PGDH	NADP-ME	ICDH CTYO	ICDH MITO	CCE	Ac COA CBX	Ac COA Syn-Cyto	Ac COA Syn-Mito	LPL TIS	FFA Blood	ICDH Total
6-PGDH	0.630											
NADP-ME	0.614	0.722										
ICDH-CYTO	0.748	0.874	0.776									
ICDH-MITO	0.402	0.610	0.630	0.612								
CCE	0.000	0.000	0.000	0.000	0.000							
Ac COA-CBX	0.498	0.683	0.560	0.672	0.463	0.000						
Ac COA-Syn-CYTO	0.566	0.555	0.518	0.592	0.352	0.000	0.460					
Ac COA-Syn-MITO	0.364	0.434	0.511	0.432	0.581	0.000	0.166	0.180				
LPL-TIS	0.518	0.648	0.663	0.761	0.491	0.000	0.606	0.499	0.270			
FFA-Blood	-0.139	-0.198	-0.193	-0.214	-0.254	0.000	-0.176	-0.094	-0.144	-0.248		
ICDH-Total	0.608	0.800	0.768	0.860	0.929	0.000	0.612	0.503	0.576	0.672	-0.264	
Ac COA-Syn-Total	0.494	0.554	0.613	0.563	0.628	0.000	0.284	0.454	0.958	0.389	-0.158	0.668