LIPID METABOLISM IN THE NEONATAL PIG

By

RONALD GENE WOLFE

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Oklahoma State University

Stillwater, Oklahoma

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Thesis Approved:

Charles V. Maxwell
Thesis Advisor

Eldon C Nelson

Richard C Seubert

Rollin F. Thayer

F. N. Owens

M M Durham
Dean of the Graduate College

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CHAPTER I

INTRODUCTORY CHAPTER

Excessive prenatal and early postnatal mortality continue to plague the swine industry in spite of advances in husbandry and nutrition (1-3). Currently, there is a 20-30% mortality between birth and weaning under normal husbandry practices (2). In a high percentage of instances, death or poor postnatal performance can be attributed to "weakness" which contributes to starvation or inadequate nutrition, chilling, crushing or trampling by the sow, and infection (4). Prior to the mid-1960's, attempts to avoid death loss due to these stresses by the early weaning of pigs were often unsuccessful and associated with diarrhea, dehydration, and death (5-10). Within the past decade, however, techniques have been devised which allow for the rearing of early weaned and colostrum-deprived neonatal pigs (11-13). The success of a program for rearing early weaned or colostrum-deprived pigs depends in part on the degree to which the pig is provided with adequate nutrition. In turn, the problem of formulating a proper diet can be simplified by gaining an understanding of the metabolic capabilities of the baby pig.

In a recent publication on metabolic patterns observed in the neonatal swine, Mersmann (14) has discussed the major metabolic defects in the newborn pig as being: 1.) a low level of glycogen phosphorylase potentially decreasing the rate of production of glucose from glycogen

stores; 2.) a defective gluconeogenic capacity which limits the supply of glucose available for those animals exposed to stressful situations such as starvation, weakness, or cold; 3.) a deficient hepatic mitochondrial number which limits the use of carbohydrate as well as fatty acid for energy production; 4.) a small amount of body fat which impairs both thermoinsulation and the quantitative contribution of a major energy store. Several types of physiological immaturity, with respect to the mobilization of free fatty acids, are also observed in the newborn pig and will be discussed in Chapter III.

While observed metabolic patterns and subnormal physiological responses suggest that the baby pig might be incapable of efficiently utilizing dietary fat as an energy source, the suckling pig thrives on sow's milk which has been shown to contain greater than 30% fat on a dry matter basis (15). In addition, preliminary studies (unpublished observation) show that both corn oil and butterfat will support growth of the colostrum-deprived neonatal pig when the fat level in the diet is 24% (dry matter basis). These observations indicate that the baby pig is quite capable of utilizing certain sources of dietary fat as an energy substrate for growth. While fatty acid synthesis and fatty acid oxidation are important biochemical processes related to lipid metabolism in mammals, little information related to the activity of these pathways and their response to fat feeding in the baby pig is available.

Fatty acid synthesis de novo in a variety of biological systems is catalyzed by two enzyme systems which function sequentially: Acetyl-CoA carboxylase and fatty acid synthetase. The carbon atoms of synthesized fatty acids are derived primarily from acetyl-CoA. In the monogastric animal, acetyl-CoA is derived primarily from the catabolism

of carbohydrate and certain amino acids. Acetyl-CoA carboxylase catalyzes the biotin-dependent carboxylation of acetyl-CoA to form malonyl CoA. Equation 1.1 indicates the stoichiometry for the synthesis of palmitate from acetyl-CoA:

$$8CH_3CO\text{-}S\text{-}CoA + 7ATP + 14NADPH + 14H^+ \longrightarrow CH_3(CH_2)_{14}COOH + 8CoA\text{-}SH + 7ADP + 7Pi + 14NADP^+ + 6H_2O \quad (1.1)$$

The mechanism of action of the cytoplasmic enzyme is similar to that elucidated for biotin enzymes by Lynen (16). Synthesis of saturated fatty acids from malonyl CoA is catalyzed by fatty acid synthetase. Requirements of the reaction include acetyl-CoA and NADPH, as indicated in the following equation:

$$CH_3CO\text{-}S\text{-}CoA + 7HOOCCH_2CO\text{-}S\text{-}CoA + 14NADPH + 14H^+ \longrightarrow CH_3CH_2(CH_2CH_2)_6CH_2COOH + 7CO_2 + 14NADP^+ + 8CoA\text{-}SH + 6H_2O \quad (1.2)$$

The enzyme system was discovered in yeast by Lynen (17) where it exists as a multienzyme complex. Complexes comparable to the yeast system have been isolated and purified from pigeon liver (18), chicken liver (19), rat liver (20), rat (21) and rabbit (22) mammary gland, and Mycobacterium phlei (23).

Since the oxidative decarboxylation of pyruvate to yield acetyl-CoA occurs in the intramitochondrial space, acetyl-CoA must be transferred out of the mitochondria to the cytosol if it is to be incorporated into fatty acids. The acetyl group can be incorporated into citrate followed by translocation and the ATP-dependent cleavage of citrate by citrate cleavage enzyme to form oxaloacetate and acetyl-CoA (24-26). This is the generally accepted pathway for generating

extramitochondrial acetyl-CoA (27-31).

The principle sources of NADPH available for fatty acid synthesis in the cytosol are the hexose monophosphate shunt dehydrogenases (32-36) and malic enzyme (30, 37, 38). Together, the hexose monophosphate shunt dehydrogenases and malic enzyme can produce all of the reducing equivalents necessary for fatty acid biosynthesis in the monogastric animal.

Feeding a high fat diet has been shown to depress fatty acid synthesis in liver and adipose tissue of the laboratory rat (39-44), in adipose tissue of the pig (45-47) and in hepatic tissue of the chicken (48-50). The changes in levels of acetyl-CoA carboxylase, fatty acid synthetase, glucose-6-P and 6-phosphogluconate dehydrogenases, citrate cleavage enzyme, and malic enzyme are closely correlated to variations in the rate of fatty acid synthesis. The strongest evidence for the coordinate changes in activities has been obtained from fasting-refeeding experiments. As a result of fasting, there is a decline in fatty acid synthesis (51, 52) as well as in the levels of the carboxylase (53) and synthetase (53, 54) and the enzymes thought to generate carbon precursors and reducing equivalents (55-62). After refeeding a high-carbohydrate diet, there is a stimulation of fatty acid synthesis (52, 63) and a marked and coordinate rise in the level of all the key enzymes (54, 56-60, 62-66). The regulation of enzymes involved in fatty acid synthesis has been extensively investigated and a number of excellent reviews have been published (67-70).

The complete oxidation of a long-chain fatty acid involves the repetition of the B-oxidation cycle and a 2-carbon fragment of the fatty acid is formed in the final step of each cycle. The overall

equation for seven turns of the fatty acid oxidation sequence acting on palmitoyl-CoA is:

$$\text{Palmitoyl-CoA} + 7\text{CoA} + 7\text{FAD} + 7\text{NAD}^+ + 7\text{H}_2\text{O} \longrightarrow 8\ \text{acetyl-CoA} + 7\text{FADH}_2 + 7\text{NADH} + 7\text{H}^+ \quad (1.3)$$

The quest for an understanding of the mechanism of fatty acid oxidation began with the studies of Knoop (71) in 1904 on hippuric acid formation from phenyl fatty acids in experiments on whole animals. By 1954, the isolation and identification of the five component enzymes of the fatty acid oxidation cycle in liver mitochondria was completed (72-74). The oxidation of fatty acids is an energy yielding process and occurs in the mitochondria of most tissues. After their uptake by cells, and subsequent to their uptake by mitochondria, long-chain fatty acids are activated to their CoA esters by enzymes located primarily outside the mitochondria (75, 76). The extramitochondrial acyl-CoA is not directly available for oxidation by intact mitochondria since the mitochondrial membranes form a barrier which limits the passage of acyl-CoA into the matrix (77) where the enzymes of fatty acid oxidation and the tricarboxylic acid cycle are located (78). Experiments using mitochondria from rat liver (79) and pig kidney (80) indicate the inner membrane as the site of the permeability barrier. Carnitine palmitoyltransferase (EC 2.3.1.23), whose activity is largely mitochondrial (81) and has been shown to be associated with the inner membrane (82), catalyzes the formation of acylcarnitine, which appears to be necessary for the translocation of fatty acyl groups from fatty acyl-CoA derivatives across the barrier to the site of oxidation (77, 83).

Any one of the enzyme-catalyzed reactions mentioned above could be

regulatory for fatty acid oxidation. In 1964, Bode and Klingeberg (84) showed that isolated mitochondria oxidize palmitoyl-carnitine faster than palmitate, in the presence of ATP and CoA. They concluded that the formation of acyl-CoA may limit fatty acid oxidation in the liver. A major objection to this theory is that the site of regulation arises before the metabolic branch point between fatty acid esterification and oxidation and thus would not provide independent control of either process (85). In 1965, Bunyan and Greenbaum (86) assayed the enzymes of the B-oxidation pathway and showed that the activity of the first enzyme (acyl-CoA dehydrogenase) was at least 10-fold less than those of the subsequent enzymes in the pathway. They concluded that this reaction was rate-limiting for fatty acid oxidation. In 1966, Shepherd et al. (87) measured the activities of acyl-CoA synthetase and carnitine palmitoyltransferase in mitochondria isolated from rat liver. The activity of carnitine palmitoyltransferase was about half that of the synthetase, and the rate of oxygen uptake with palmitoylcarnitine was twice that with palmitoyl-CoA plus carnitine (or palmitate, plus ATP, CoA and carnitine). It was concluded that the rate-limiting step in fatty acid oxidation by the mitochondria was the activity of carnitine acyltransferase. Undoubtedly the rate of oxidation can be regulated by the concentration of intracellular fatty acids, which is assumed to vary in the same direction as the concentration of plasma fatty acids (88-91), since it is generally accepted that an increased uptake of fatty acids results in an increased rate of oxidation (92, 93). A number of reviews on fatty acid metabolism have been published. For detailed discussions of many aspects of this subject, the reader is referred to these references (94-98).

Observed changes in the rate of fatty acid oxidation and the activities of enzymes associated with this oxidative process during development of the neonatal pig will be discussed in Chapter III.

CHAPTER II

EFFECT OF DIETARY FAT LEVEL ON PERFORMANCE AND LIPOGENESIS IN THE NEONATAL PIG

Introduction

The effect of different levels of dietary fat on weight gain and feed efficiency (45-47, 99-102) and on adipose tissue lipogenesis and enzymatic activity (45-47, 102) have been reported for the pig older than 14 days. With increasing emphasis on the early weaning of pigs and with development of techniques for rearing colostrum-deprived pigs (11, 13, 103), knowledge related to the effect of varying levels of dietary fat on weight gain, feed efficiency and lipid metabolism in the neonatal pig would be beneficial.

Body fat is approximately 1% of the live weight of the newly farrowed pig and may increase to as much as 15% by 2 weeks of age (104). Sow's milk contains a high level of fat (greater than 30% on a dry matter basis)(15) and the liver (105) and adipose tissue (105, 106) of the suckling pig have a low level of lipogenic activity *in vitro*. This suggests that ingested fat, as opposed to fatty acid synthesis, is the primary source of the fatty acids deposited. The extent to which level of fat in sow's milk influences the rate of fat deposition in the suckling pig has not been well studied. The effect of decreasing the fat to carbohydrate ratio, from that observed in sow's milk, on lipo-

genic activity, body fat deposition, weight gain, and efficiency of gain is also unknown.

The objective of this research was to investigate the effect of dietary fat level on weight gain, percent body fat, fatty acid composition of backfat, and the activity of enzymes associated with fatty acid synthesis when measured *in vitro* in liver and adipose tissue in the 14 day old pig.

Materials and Methods

Materials

Acetyl-coenzyme A (sodium salt), Coenzyme A (lithium salt, grade 1-L), L-cysteine hydrochloride hydrate, ATP (disodium salt, sigma grade), bovine serum albumin (essentially fatty acid-free, fraction V), dithiothreitol, glycylglycine, malate dehydrogenase (beef heart), malonyl-coenzyme A (trilithium salt, grade II), β-NADH (disodium salt, grade III), NADP (monosodium salt, type I), and Trizma base (reagent grade) were purchased from Sigma Chemical Company (Saint Louis, Missouri). Diethylene glycol succinate and Anakrom (40/50 mesh, type ABS) were purchased from Analabs, Incorporated (North Haven, Connecticut). All other materials were of reagent grade.

Methods

Experimental Animals and Diets. Colostrum-deprived male pigs, obtained by cesarean section from either Yorkshire or Hampshire sows, were weighed, placed in individual sterile isolators and randomly allotted from littermate groups to three dietary treatments. Surgical

technique, isolator construction, feeding procedure, and general animal care were as described by Coalson et al. (13). Pigs were fed according to appetite at 4 hr. intervals between 6:00 am and ending at 10:00 pm. The pigs were fed semipurified, isocaloric liquid diets containing either 2, 17, or 32% butterfat on a dry matter basis (Table I). The diets were made isocaloric by substituting glucose for butterfat. Vitamin and mineral supplementation represented approximately 2 times the N.R.C. requirements for the 5-10 Kg pig (107). Energy, protein, calcium, and phosphorous content of the diets is shown in Table II.

After 14 days on one of the three experimental diets, the pigs were weighed, anesthetized with diethyl ether, and sacrificed by exsanguination. Samples of liver and backfat were obtained and chilled in 0.15 M KCl at 0°C. Contents of the thoracic, abdominal, and pelvic cavity were removed, the head was removed at the atlas joint, and the front and hind legs removed at the carpus and tarsus joint, respectively. The remaining carcass was weighed, ground, and samples were obtained for determining total body fat and protein.

Enzyme Assays. Twenty-five percent homogenates (w/v) of liver and adipose tissue were prepared in 0.1 M phosphate-bicarbonate buffer (108), pH 8.0, containing 1 mM dithiothreitol and 1 mM NA_2-EDTA, using a model PT 10-35 Polytron homogenizer (Brinkmann Instruments, Inc., Westbury, New York) at maximum speed for 1 minute. The supernatant fraction resulting from centrifugation of these homogenates at 100,000 x g for 30 min. was used in determining fatty acid synthetase activity according to the spectrophotometric method of Lynen (109) with minor modification. The assay mixture contained potassium phosphate buffer

TABLE I

COMPOSITION OF LIQUID DIETS[a]

	2% Fat	17% Fat	32% Fat
Water	869	815	779
Butterfat	5.4	45.0	86.5
Glucose	182.4	91.2	0.0
Nutritek-900[b]	60.0	60.0	60.0
Calcium Caseinate	80.0	80.0	80.0
Citric Acid	1.3	1.3	1.3
Vitamin Premix[c]	2.0	2.0	2.0
Choline Chloride	0.2	0.2	0.2
Mineral Premix[d]	12.5	12.5	12.5
Lecithin	0.27	0.27	0.27

[a]All values expressed as g/liter.

[b]Foremost Foods Company.

[c]Vitamin premix supplied the following per liter of diet: retinyl acetate, 2230 I.U.; cholecalciferol, 456 I.U.; thiamin, 1.27 mg; riboflavin, 3.03 mg; niacin, 11.15 mg; calcium pantothenate, 6.59 mg; pyridoxine hydrochloride, 1.27 mg; vitamin B-12, 11.15 µg; p-aminobenzoic acid, 4.56 mg; ascorbic acid, 15.17 mg; inositol, 40.48 mg; folic acid, 75.94 µg; biotin, 25.31 µg; α-tocopherol, 22.3 mg; menadione, 1.27 mg.

[d]Mineral premix supplied the following per liter of diet: $NaHCO_3$, 2.5 g; KH_2PO_4, 5.0 g; $MgSO_4 \cdot H_2O$, 630 mg; $MnSO_4 \cdot H_2O$, 25 mg; $ZnSO_4 \cdot H_2O$, 62.5 mg; $CuSO_4 \cdot H_2O$, 12.5 mg; $FeSO_4 \cdot H_2O$, 125 mg; KI, 388 µg; $CaCl_2$, 2.6 g.

TABLE II

ENERGY, PROTEIN, CALCIUM, AND PHOSPHORUS CONTENT OF LIQUID DIETS

	2% Fat	17% Fat	32% Fat
Energy, Kcal/L.[a]	1185	1240	1210
Protein, g/L.[b]	73.54	73.54	73.54
Calcium, g/L.[b]	3.52	3.52	3.52
Phosphorus, g/L.[b]	3.60	3.60	3.60

[a]Determined by bomb calorimetry (Parr Oxygen Bomb).

[b]Diets were formulated to contain these levels of protein, calcium, and phosphorus.

(pH 6.5), 100 mM; Na_2-EDTA, 2.5 mM; cysteine, 10 mM, bovine serum albumin, 0.9 mg; acetyl-CoA, 0.06 mM, TPNH, 1.5 mM; and enzyme in a final volume Of 3.0 ml in the reference cuvette and 2.9 ml in the sample cuvette. The reaction was started by the addition of 0.1 ml of a 2.1 mM solution of malonyl-CoA to the sample cuvette. Twenty-five percent homogenates (w/v) of liver and adipose tissue samples were also prepared in 0.15 M KCl containing 5.0 mM mercaptoethanol. Supernatant fractions resulting from centrifugation of these liver homogenates at 100,000 x g for 30 min. and adipose tissue homogenates at 20,000 x g for 30 min. were used in the citrate cleavage and malic enzyme assay. Citrate cleavage enzyme activity was determined by the method of Cottam and Srere (110). The assay mixture contained tris buffer (pH 7.4), 100 mM; Na-citrate, 20 mM; $MgCl_2$, 10 mM; dithiothreitol, 10 mM; Coenzyme-A, 0.33 mM; NADH, 0.14 mM; malate dehydrogenase, 1.5 μmolar units; and enzyme in a final volume of 3.0 ml in the reference cuvette and 2.9 ml in the sample cuvette. The reaction was started by adding 0.1 ml of 0.15 M ATP to the sample cuvette. Malic enzyme (EC 1.1.1.40) activity was determined essentially by the method of Ochoa (111). The assay mixture contained glycylglycine (pH 7.4), 54 mM; $MnCl_2$, 0.83 mM; TPN, 0.045 mM; and enzyme in a final volume of 3.0 ml in the reference cuvette and 2.9 ml in the sample cuvette. The reaction was started by the addition of 0.1 ml of 11 mM malate to the sample cuvette.

All assays were conducted at room temperature (25°C) using a model 635 Varian Techtron double beam spectrophotometer connected to a Varian A-25 recorder (Varian Techtron, Palo Alto, California). The protein content of all supernatant fractions was determined by the method of Lowry et al. (112) and enzyme activities are expressed as nanomoles

substrate utilized per minute per milligram supernatant protein.

Body Fat Determination. Lipid was extracted from samples of ground carcass by the method of Folch et al. (113). Duplicate samples were homogenized in 20 volumes of chloroform-methanol (2:1, v/v) and allowed to stand for 20 hr. The extract was then drawn through Whatman No. 41 filter paper to remove particulate matter and a portion of the filtrate was washed with 0.2 volume of H_2O to remove non-lipid substances. An aliquot of the washed extract was diluted and analyzed for total lipid by the colorimetric method of Bragdon (114). Percent body fat is expressed on a dry matter basis.

Body Protein Determination. Duplicate samples of ground carcass were subjected to Kjeldahl nitrogen analysis (115) and a factor of 6.25 was used to estimate crude protein content. Percent body protein is expressed on a dry matter basis.

Determination of Fatty Acid Pattern in Backfat. Lipid was extracted from backfat by homogenizing samples in 20 volumes of chloroform-methanol (2:1, v/v). The extract was decanted into a round bottom flask and the solvent removed using a Buchi Rotavapor-R flash evaporator (Rinco Instrument Company, Inc., Greenville, Illinois) with the water bath temperature maintained at 40°C. Methyl esters of the composite fatty acids were prepared according to the procedure of Young and Waller (116) and analyzed on a Barber-Colman 5000 gas chromatograph (Barber-Colman Company, Rockford, Illinois) using a 9.5 ft. glass column packed with 14.0% DEGS on Anakrom support (40/50 mesh, type ABS). The injection port temperature was 190°C with a column temperature of

185°C, a detector temperature of 240°C, and a helium flow rate of 42 ml per min.

Statistical Analysis. Data were analyzed by analysis of variance and calculations were made of standard error of treatment means as outlined by Snedecor and Cochran (117). Treatment sum of squares was partitioned into the sum of squares due to linear and quadratic effects.

Results and Discussion

Weight Gain, Diet Consumption, and Feed Efficiency.

The effect of dietary fat level on weight gain, diet consumption and feed efficiency is shown in Table III. During the 14 day experimental period, an increase in the level of dietary fat from 2 to 32% resulted in an increase in weight gain (significant linear effect, $P < 0.05$) and diet consumption (significant quadratic effect, $P < 0.01$). Allee et al. (45-47) demonstrated that the rate of gain in young pigs (initial wt. $\leq$ 12 Kg) fed for a period of 3 to 5 weeks was improved by adding tallow and corn oil to the diet at levels up to 10 and 32%, respectively. Allee et al. (46, 47, 102) have also reported an improved rate of gain in older pigs (initial weight > 19 Kg) when corn oil, coconut oil, tallow and lard were added to the diet at levels up to 10 or 13%.

Increased weight gain and the tendency for an increased feed efficiency with increasing levels of dietary fat (Table III and references 45-47, 102) have not always been observed in the young pig (99-101, 118). Eusebio et al. (99) and Frobish et al. (101) observed

TABLE III

EFFECT OF DIETARY FAT LEVEL ON WEIGHT GAIN, DIET CONSUMPTION AND FEED EFFICIENCY[a]

	2% Fat	17% Fat	32% Fat
Average 14 Day Gain, g.[b]	1032 ± 140	1420 ± 175	1638 ± 148
Average 14 Day Consumption, ml.[c]	6009 ± 217	7419 ± 295	7076 ± 215
Feed Efficiency (g. liveweight gain/Kcal energy consumed)	0.15 ± 0.02	0.16 ± 0.02	0.19 ± 0.02

[a]Values are mean ± SEM.

[b]Significant Linear Effect ($P < 0.05$).

[c]Significant Quadratic Effect ($P < 0.01$).

no improvement in rate of gain and feed efficiency in 4.4-5.5 Kg pigs by increasing the level of dietary fat. A wide range of fat sources including lard, tallow and butterfat were used, suggesting that the efficiency of utilization of fat by the young pig may not depend on the source of fat.

The reason for the varied results concerning the efficiency of utilization of dietary fat by the young pig is unclear. The physical characteristics of fat utilized in diets would appear to be important, although the decreased rate of gain observed by Frobish et al. (100) in 15 day old pigs upon addition of 10% lard to the diet was not improved by the addition of emulsifying agents. Allee et al. (45) have suggested that if the addition of fat in the diet of the pig decreases the concentration of each nutrient in the diet in relation to metabolizable energy, decreased intake of amino acids and other essential nutrients may limit performance. In support of this theory is the observation that in the present experiment and experiments reported by Allee et al. (45-47, 102) the diets were isocaloric whereas, in reports indicating poor utilization of dietary fat by the young pig (99-101, 118), fat was substituted for an equal amount of carbohydrate resulting in an increased caloric density.

Results from this laboratory not only support the concept that at least certain fats (butterfat) can be utilized very effectively when fed at high levels to the young pig, but also suggest that baby pig performance is better when high levels of fat (32%) are the primary dietary energy source.

Effect of Dietary Fat Level on Enzyme Activity.

An increase in the level of dietary fat from 2 to 32% resulted in a significant decrease in the activity of fatty acid synthetase and citrate cleavage enzyme in both adipose tissue (Table IV) and liver (Table V) and a significant decrease in malic enzyme activity in adipose tissue (Table IV). This response to increasing levels of dietary fat has previously been demonstrated in both suckling and growing pigs. In trials using pigs initially weighing 12 Kg or heavier, Allee et al. (45-47, 102) have demonstrated that an increase in dietary fat level is associated with a decreased rate of incorporation of [U-^{14}C]glucose into fatty acids and a decreased activity of malic enzyme and citrate cleavage enzyme in adipose tissue. Mersmann et al. (105) examined the in vitro synthesis of lipid and the activity of malic enzyme and citrate cleavage enzyme in adipose tissue and liver preparations from pigs 2-60 days of age. In adipose tissue, the incorporation of [U-^{14}C]-glucose into lipid was low until weaning (21 days) and thereafter markedly increased whereas the rate of synthesis of lipid from glucose by liver preparations remained low throughout the experiment. The activities of malic enzyme and citrate cleavage enzyme were low in both tissues prior to weaning. Following weaning, the activity of both enzymes increased in adipose tissue whereas in liver, citrate cleavage enzyme activity increased while malic enzyme activity remained low throughout the experiment. The activity of malic enzyme in liver (Table V) was not significantly affected by the level of dietary fat in the present experiment.

O'Hea and Leveille (119) measured the incorporation of carbon from

TABLE IV

EFFECT OF DIETARY FAT LEVEL ON ENZYME ACTIVITY IN ADIPOSE TISSUE[a,b]

	2% Fat	17% Fat	32% Fat
Fatty Acid Synthetase[c]	22.34 ± 5.34	7.75 ± 3.26	5.28 ± 1.74
Citrate Cleavage Enzyme[d]	181.17 ± 39.09	54.19 ± 22.68	31.01 ± 13.80
Malic Enzyme[c]	221.35 ± 66.29	53.85 ± 16.71	35.55 ± 9.98

[a]Values are mean ± SEM.

[b]Activity expressed as nmoles substrate utilized/min/mg supernatant protein.

[c]Significant Linear Effect ($P < 0.05$).

[d]Significant Linear Effect ($P < 0.01$).

TABLE V

EFFECT OF DIETARY FAT LEVEL ON ENZYME ACTIVITY IN LIVER[a,b]

	2% Fat	17% Fat	32% Fat
Fatty Acid Synthetase[c]	7.25 ± 1.01	3.93 ± 0.39	4.04 ± 1.13
Citrate Cleavage Enzyme[c]	9.23 ± 1.67	5.85 ± 1.32	5.13 ± 0.84
Malic Enzyme	5.62 ± 1.39	3.92 ± 1.20	4.01 ± 1.11

[a]Values are mean ± SEM.

[b]Activity expressed as nmoles substrate utilized/min/mg supernatant protein.

[c]Significant Linear Effect ($P < 0.05$).

acetate and glucose into fatty acids by adipose tissue and liver in pigs weighing approximately 12 or 30 Kg. In vitro, the liver contributed appreciably to overall lipogenesis when [1-^{14}C]acetate was the substrate for fatty acid synthesis but in vitro and in vivo, when [U-^{14}C]glucose was used as substrate, virtually all the newly synthesized fatty acids were formed in adipose tissue. Since hexoses are the predominant precursors of synthesized fatty acids in the monogastic animal, the results indicated that adipose tissue plays a nearly exclusive role in fatty acid synthesis in the growing pig. Fatty acid synthetase activity was 3-fold higher in homogenates of adipose tissue (Table IV) as compared to liver (Table V) from pigs consuming the 2% fat diet indicating that under conditions of low fat intake, adipose tissue plays the major role in the synthesis of fatty acids in the neonatal pig as well. This conclusion is supported by the observation that the activities of citrate cleavage enzyme and malic enzyme were 20- and 40-fold higher, respectively, in adipose tissue (Table IV) than in liver (Table V). The importance of these enzymes in the biosynthesis of fatty acids is discussed in Chapter I (also see references 120-122).

Mersmann et al. (105) observed that the rate of incorporation of [U-^{14}C]glucose into lipid in vitro was considerably greater in adipose tissue than the liver of the suckling pig. However, our results indicated only a small difference in fatty acid synthetase activity in liver and adipose tissue obtained from pigs reared on a high fat diet. This discrepancy can be partially explained by noting that in Mersmann's experiment, 50% of the glucose carbon incorporated into lipid by adipose tissue slices was in the glyceride-glycerol fraction. Incorporation of glyceride-glycerol was not measured in

liver. This is probably not an important point for discussion since, under conditions of high fat intake, the activity of fatty acid synthetase is low in both tissues and the majority of fatty acids deposited are most likely dietary in origin.

The close association between the activity of fatty acid synthetase and the activities of citrate cleavage enzyme and malic enzyme observed in this experiment and previous experiments (45-47, 102, 105) is not always observed. A significant time lag in the increase in citrate cleavage enzyme activity as compared to the increase in the lipogenic response induced by refeeding after fasting has been demonstrated in rat liver (123), chick liver (124), and in pig adipose tissue (125). Leveille (126) has shown that lipogenic and enzymatic adaptations to meal feeding in rat adipose tissue and in rat and chick liver are characterized by a delayed increase in the pentose pathway dehydrogenases and malic enzyme when compared to the increase in lipogenic activity. These observations suggest that rather than lipogenesis being controlled by citrate cleavage enzyme, malic enzyme, and the hexose monophosphate shunt dehydrogenases, the activities of these enzymes are controlled by a change in substrate flux associated with a change in the rate of fatty acid biosynthesis.

The decrease in liver and adipose tissue fatty acid synthetase activity in the neonatal pig (Table IV and V, respectively) upon substitution of dietary fat for carbohydrate may be a result of increased fat consumption rather than decreased carbohydrate consumption. Evidence in support of the concept that dietary fat has specific effects on lipogenesis unrelated to the amount of carbohydrate fed has been provided by Wiley and Leveille (127). Increased fat consumption has

been shown to be related to an increase in liver concentrations of long-chain fatty acyl-CoA (128-130) suggesting the possibility that these fatty acid derivatives play a role in the control of fatty acid synthesis. Goodridge (131) has shown that 100 μM palmitoyl-CoA, in the presence of albumin (24 mg/ml), inhibited the incorporation of ^{14}C-labeled citrate into fatty acids in a cytosol fraction of chick liver and specifically and reversibly inhibited the activity of acetyl-CoA carboxylase purified from chick liver. More recently, Goodridge (132) demonstrated that the inhibition of fatty acid synthesis in neonatal chick liver hepatocytes by medium free fatty acids was accompanied by an increase in the fatty acyl-CoA level. It was concluded that fatty acyl-CoA may inhibit fatty acid synthesis by directly inhibiting acetyl-CoA carboxylase or by inhibiting the mitochondrial citrate carrier and thereby reducing the activation of acetyl-CoA carboxylase caused by citrate.

Effect of Dietary Fat Level On The Fatty Acid Composition of Backfat and On Body Fat and Protein

The six primary (greater than 90% of total) fatty acids measured in backfat samples are shown in Table VI along with their relative percentages for each treatment. An increase in dietary fat level resulted in a significant increase in the relative percentage of myristic acid and a significant decrease in the relative percentages of palmitoleic and oleic acid with nonsignificant changes occurring in the remaining fatty acids. This decrease in the relative percentages of palmitoleic and oleic acid with increasing levels of dietary fat

TABLE VI

EFFECT OF DIETARY FAT LEVEL ON RELATIVE PERCENTAGE OF SIX FATTY ACIDS IN ADIPOSE TISSUE[a,c]

	2% Fat	17% Fat	32% Fat
Lauric (12:0)	2.11 ± 0.42	3.11 ± 0.68	3.34 ± 0.60
Myristic (14:0)[b]	3.62 ± 0.23	8.96 ± 0.66	9.83 ± 0.45
Palmitic (16:0)	32.20 ± 1.58	32.82 ± 0.84	33.77 ± 0.63
Palmitoleic (16:1)[b]	8.00 ± 0.26	6.86 ± 0.55	6.02 ± 0.29
Stearic (18:0)	10.28 ± 0.48	9.98 ± 0.51	10.26 ± 0.28
Oleic (18:1)	43.80 ± 0.93	39.39 ± 1.14	36.71 0.79

[a]Values represent mean ± SEM.

[b]Significant Linear Effect ($P < 0.01$).

[c]The relative percentage of these six fatty acids in the dietary butterfat used in this experiment are: lauric, 3.0; myristic, 13.4; palmitic, 34.0; palmitoleic, 5.5; stearic, 11.1; oleic, 32.1.

indicates that the rate of utilization of these two fatty acids for purposes other than fat deposition may be high relative to lauric, myristic, palmitic and stearic acid. As the level of fat in the diet increased from 2 to 32%, the relative percentage of the six predominant fatty acids in dietary butterfat was more closely reflected by the relative percentage of these fatty acids in backfat. Mason and Sewell (133), Koch et al. (134), and Jurgens et al. (135) have demonstrated that the fatty acid composition of backfat in the pig reflects the composition of dietary fat. Our results suggest that in treatments in which the same fat source is added to diets at different levels, the fatty acid pattern in backfat may reflect the level of dietary fat.

Body fat and protein, when expressed as a percentage of carcass dry matter (Table VII), was not significantly influenced by the level of dietary fat although a slight increase in the value of both of these parameters was observed as the fat content of the diet increased. Thus, with the exception of a sacrifice in weight gain which can be partially explained by decreased diet consumption, the colostrum-deprived neonatal pig appears capable of successfully substituting carbohydrate calories for butterfat calories in terms of body fat and protein deposition.

Summary to Chapter II

Thirty male neonatal pigs were obtained by cesarean section and randomly allotted from littermate groups to three diets containing 2, 17 and 32% fat as percent dry matter. Butterfat was used to replace glucose in the isocaloric, liquid, semipurified diets. Each pig was placed in a sterile isolator and fed five times daily according to

TABLE VII

EFFECT OF DIETARY FAT LEVEL ON CARCASS FAT AND PROTEIN CONTENT[a,b]

	2% Fat	17% Fat	32% Fat
Body Fat	23.2 ± 1.6	24.8 ± 1.3	26.7 ± 2.4
Body Protein	55.6 ± 1.4	57.4 ± 1.0	57.3 ± 1.7

[a]Values are mean ± SEM.

[b]Fat and Protein are expressed as a percentage of carcass dry matter.

consumption. After 14 days, pigs were weighed, sacrificed, and samples of liver and backfat obtained. The carcass of each pig was ground and samples obtained for determinations of total body fat and protein.

An increase in the level of dietary fat from 2 to 32% resulted in a significant increase in average 14 day weight gain and a tendency for increased feed efficiency. These results demonstrate that not only can the neonatal pig utilize semipurified liquid diets high in butterfat content, it tends to utilize butterfat calories more efficiently than glucose calories for growth purposes.

Increasing dietary fat level resulted in a decrease in the activity of fatty acid synthetase and citrate cleavage enzyme in adipose tissue and liver and a decrease in the activity of malic enzyme in adipose tissue. The activities of these three enzymes were higher in adipose tissue than in liver indicating that adipose tissue is the primary site of fatty acid synthesis in the neonatal pig.

Dietary fat level did not significantly affect the percentage of body fat implying that palmitic acid deposited in adipose tissue may originate either in the diet or from fatty acid synthesis in vivo. The percentage of body protein was not significantly affected by fat level in the diet.

CHAPTER III

EFFECT OF AGE AND DIETARY FAT LEVEL ON FATTY ACID OXIDATION IN THE NEONATAL PIG

Introduction

Increasing the level of fat in the diet of the colostrum-deprived neonatal pig results in a decrease in the activities of fatty acid synthetase, citrate cleavage enzyme and malic enzyme (Tables IV and V, Chapter II). Fat feeding results in a similar change in the activities of these enzymes in the growing pig (45-47, 102) and in other species (39-44, 48-50). In addition, the accumulation of body fat in the neonatal pig from birth through 14 days of age was shown to be independent of dietary fat level (Table VII, Chapter II) indicating that adipose tissue fatty acids may be either dietary in origin or a result of fatty acid synthesis. While these results imply a physiological maturity in the neonatal pig relative to the biosynthesis of fatty acids, several types of physiological immaturity of lipid metabolism have been demonstrated in the baby pig.

Circulating free fatty acid levels are low (100 μEq/100 ml) in the immediate newborn pig and increase only slightly during starvation (136). Release of FFA from adipose tissue as a response to certain stimuli is subnormal in the newborn pig. The 12 hr. pig, in contrast

to the 60 hr. pig, appears unable to mobilize FFA during short-term exposure to a temperature of 5°C (137) while longer exposure (2 hr.) of 3 hr. piglets yielded mobilization of FFA and glycerol (138). Newborn swine do not mobilize FFA in response to injected, exogenous catecholamines whereas in older pigs (older than 50 hr.), the catecholamine induced mobilization of lipid has been reported (137, 139, 140). Mersmann et al. (141) have demonstrated a decreased hepatic mitochondrial number in newborn swine suggesting that the utilization of fatty acids as a substrate for oxidation might be limited even though fat represents greater than 30% of the initial dietary dry matter intake in the suckling pig (15). The availability of free fatty acids as substrate for oxidation may be limited due to low total body fat (1-22%) at birth (104).

The rate of fatty acid oxidation and the activity of certain enzymes related to this catabolic pathway have been studied in several species at various stages of development. The rate of oxidation of palmitate is low in homogenates of liver (142-144) and heart (144) from fetal rats and in homogenates of liver and heart from the chick embryo (144). The rate of CO_2 production from palmitate oxidation in rat liver homogenate was observed to be maximum at either 2 (142) or 5 (143) days of age and declined as adult age was reached. Changes in the activity of carnitine palmitoyltransferase with development were closely associated to changes in the rate of palmitate oxidation in rat liver (142-144). Homogenates of swine liver have been reported to exhibit low rates of palmitate oxidation at day 0 with a four-fold increase by day 7 in the suckling pig whereas the rate of fatty acid oxidation in swine heart homogenates did not change postnatally (145).

After 24 hr. of age, carnitine palmitoyltransferase levels in pig liver mitochondria were more than double the level at birth and about equal to the level at 24 days (146). Significant differences in the rate of oxidation of intramuscularly injected fatty acids were not demonstrated when 1- and 7-day old pigs were compared (147).

Although existing evidence suggests that the baby pig has a lessened ability to utilize fat as an energy substrate, results from previous studies conducted in this laboratory (Table III, Chapter II) show that pigs fed isocaloric liquid diets from 0 to 14 days of age gain weight at a significantly faster rate when the butterfat content of the diet was 32% as opposed to 2% (dry matter basis).

The main objectives of this experiment were to study the effect of age and diet on the oxidation of fatty acids *in vitro* and the activity of carnitine palmitoyltransferase in the liver, kidney, heart, and skeletal muscle of the neonatal pig.

Materials and Methods

Materials

ATP (disodium salt, Sigma grade), Coenzyme A (lithium salt, grade 1-L), DL-carnitine HCl, BSA (essentially fatty acid-free, fraction V), GSH, β-NAD (grade III), L-Malic acid, Trizma Base (reagent grade), and INT(p-Iodonitrotetrazolium violet, grade 1) were purchased from Sigma Chemical Company (Saint Louis, Missouri). DL-[methyl-^{14}C]carnitine HCl, [U-^{14}C]palmitic acid, and [U-^{14}C]stearic acid were purchased from Amersham/Searle Corporation (Arlington Heights, Illinois). Myristic acid, palmitic acid, stearic acid, and [U-^{14}C]myristic acid were

purchased from Applied Science Laboratories, Inc. (Inglewood, California). DL-palmitylcarnitine chloride was purchased from Supelco, Inc. (Bellefonte, Pennsylvania). All other materials were of reagent grade.

Methods

Experimental Design, Animals, and Diets. Thirty-five colostrum-deprived pigs, obtained via cesarean section, were weighed, placed in individual sterile isolators, and randomly allotted from littermate groups to seven treatment groups. Pigs in treatment group 1 were allowed a 2 hr. post-delivery recovery period and were then sacrificed. Treatment groups 2-7 were arranged in a 2 X 3 factorial design consisting of animals fed diets containing either 2% (L) or 32% (H) butterfat on a dry matter basis (Table I, Chapter II) for 1, 7 or 21 days. Surgical technique, isolator construction, feeding procedure, and general animal care were as described by Coalson et al. (13). Pigs were fed according to appetite at 4 hr. intervals between 6:00 am and 10:00 pm and were given 50 mls of distilled H_2O daily at 12:00 noon. The energy, protein, calcium and phosphorus content of the diets is shown in Table II, Chapter II. The diets were made isocaloric by substituting glucose for butterfat.

Preparation of Tissue Homogenates. Pigs were sacrificed by a blow to the head followed by exsanguination. The heart, liver, and kidneys were removed and chilled in 0.25 M sucrose (pH 7.0) at 0°C. A muscle sample (biceps femoris) was removed from the hind leg and placed in 0.15 M KCl at 0°C. Samples (1-2 g.) of liver, heart, and kidney were

finely minced and suspended in 4 volumes of 0.25 M sucrose (pH 7.0) at 0°C. A 1-2 g. sample of leg muscle was finely minced and placed in 4 volumes of Chappell-Perry medium (148) at 0°C (pH 7.4). Homogenization of all tissues was carried out with a relatively loose fitting (approximate difference between I.D. of glass tube and O.D. of teflon pestle = 0.25 millimeters) Potter Elvehjem homogenizer for 1-2 min. at 600 rpm. The resulting homogenates were centrifuged at 600 X g for 5 minutes to yield supernates free of nuclei, whole cells, and cellular debris. The resulting supernates were used in assaying for the oxidation of fatty acids to CO_2 and acid soluble products.

The mitochondrial suspensions were prepared from liver, heart and kidney as described above except in 10% (w/v) sucrose containing 10 mM Tris-HCl buffer, pH 7.5. Skeletal muscle was homogenized in Chappell-Perry medium. The homogenates were centrifuged at 600 X g for 5 minutes and the resulting supernate was centrifuged at 12,000 X g for an additional 15 min. The resulting mitochondrial pellet was suspended in a volume of 10% (w/v) sucrose containing 10 mM Tris-HCl buffer (pH 7.5) suitable to give approximately 8 mg of mitochondrial protein per ml of suspension. The suspensions were then frozen at -20°C for subsequent determination of carnitine palmitoyltransferase activity, succinate dehydrogenase activity and concentration of mitochondrial protein per gram of wet tissue.

Fatty Acid Oxidation Assay. The oxidation of [U-^{14}C]fatty acids was assayed by the method of Lockwood and Bailey (143) with minor modification. Each tissue homogenate (0.5 ml) was incubated for 60 min. at 37°C in a 25 ml erlenmeyer flask equipped with a removable center well and a single side arm (sealed with a serum stopper, Scientific

Products, Grand Prairie, Texas), containing the following reaction mixture: sucrose (100 mM), EDTA (0.4 mM), L-malate (0.01 mM), KCl (80 mM), sodium phosphate buffer (pH 6.5) (8.0 mM), ATP (1.0 mM), CoA (0.04 mM), NAD^+ (2.5 mM), $MgCl_2$ (5.0 mM), fat free bovine serum albumin (0.17 mM), DL-carnitine (when present) (1.0 mM), and 0.25 μCi of potassium [U-^{14}C] fatty acid (1 mM). The reaction was started by the addition of 0.5 ml of albumin bound [U-^{14}C]fatty acid. Potassium salts of fatty acids were prepared according to Bjorntorp (149) and complexes of BSA with fatty acids were prepared according to Chen (150) by adding free fatty acid in a slight access of aqueous KOH to solutions of fat free BSA. The final reaction volume was 2.5 ml and the flasks were incubated in a shaking water bath (Eberbach Corporation, Ann Arbor, Michigan) at 60 cycles/min. The reaction was stopped by placing the incubation flasks in ice water (0°C) and 20 μl of 1 M $NaHCO_3$ was added to each flask to provide a carrier for $^{14}CO_2$. $^{14}CO_2$ was released by injection of 0.3 ml of 50% (v/v) $HClO_4$ through the stoppered side arm. The flasks were allowed to shake for an additional 60 minutes and the $^{14}CO_2$ released was trapped in the center wells which contained a small strip of filter paper and 0.3 ml of ethanolamine:ethylene glycol monomethyl ether (1:2, v/v). The center well contents were rinsed out with 10 ml of scintillation solution and radioactivity was counted. Incubation flask contents, containing radioactive acid soluble products, were washed out into centrifuge tubes using 2.0 ml of distilled H_2O and the precipitated protein was sedimented by centrifuging of 1000 X g for 10 min. The supernate was neutralized with 10% (w/v) KOH and the insoluble $KClO_4$ was removed by centrifugation at 1000 X g for 10 min. A 0.2-0.3 ml aliquot of the neutralized supernate was added to 10 ml of scintillation solu-

tion and counted. During a preliminary trial using pig liver homogenate, the conversion of palmitate to CO_2 and acid soluble products was found to be maximum when the reaction mixture was at a final pH of 6.5. Under these assay conditions, the reaction remained linear for 60 min. and a final fatty acid concentration of 1 mM resulted in maximum activity. The oxidation of [U-^{14}C]palmitate by homogenates of liver, kidney, heart and leg muscle was measured in all animals and the relative rate of oxidation of [U-^{14}C]myristate, [U-^{14}C]palmitate, and [U-^{14}C]stearate by liver homogenates was measured in 7 day old animals on the high and low fat diets. The radiochemical purity of all [U-^{14}C] fatty acids used in this experiment was checked by autoradiography and gas-liquid chromatography. Labeled fatty acids having greater than 95% chemical and radiochemical purity were accepted for use. Several samples were found to contain greater than 5% contamination and were discarded.

Carnitine Palmitoyltransferase Assay. Carnitine palmitoyltransferase activity was measured in all mitochondrial suspensions by the isotope exchange method of Norum (151). Mitochondrial suspensions (0.1 ml) were incubated for 7 min at 30°C in the following reaction mixture: DL-palmitoylcarnitine (0.5 mM), CoA (0.12 mM), DL-^{14}C carnitine (200,000 DPM)(0.50 mM), GSH (5 mM), and Tris buffer (pH 7.5) (10 mM) in a final volume of 1.0 ml. The reaction was stopped by the addition of 0.1 ml concentrated HCl and palmitoyl-[^{14}C]-carnitine was extracted with butanol according to Bremer (152) and counted.

Succinate Dehydrogenase Assay. Succinate dehydrogenase activity was measured in all mitochondrial suspensions by the method of

Pennington (153) in which 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium (INT) is used as the electron acceptor. Mitochondrial suspensions (0.025 ml) were incubated for 15 min at 37°C in the following reaction mixture: Potassium phosphate buffer (pH 7.4) (50 mM), INT (0.1%), sodium succinate (50 mM), and sucrose (25 mM) in a final volume of 1.0 ml. The reaction was stopped by the addition of 1 ml of 10% TCA and the formazan was extracted with 4 ml of ethyl acetate and its extinction measured at 490 mμ.

The scintillation solution used in measuring radioactivity contained the following ingredients: Permablend I (containing 91% 2,5-diphenyloxazole and 9% 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene, Packard Instrument Company, Inc., Downers Grove, Illinois), 5.5 g.; naphthalene, 120 g; and dioxane to a final volume of 1000 ml. Radioactivity was counted on a Packard Model 3320 Liquid Scintillation Spectrometer (Packard Instrument Company, Inc., Downers Grove, Illinois). External standardization was used in correcting for sample quench.

Protein determinations on mitochondrial suspensions were assayed according to Lowry et al. (112).

Statistical Analysis. Data were analyzed by analysis of variance and calculations were made of standard error of treatment means and least significant difference values for comparison of treatment means according to Snedecor and Cochran (117). Palmitate oxidation data were analyzed as a 3 X 2 X 2 factorial arrangement of treatments in a split plot design. In this design, each animal was a main plot with the main plot treatments being age (1, 7 and 21 days) and dietary fat

level (2 and 32%). The subplot treatment was the presence or absence of added carnitine in the palmitate oxidation assay mixture. Data on the relative oxidation rates of myristate, palmitate, and stearate by liver homogenates from 7 day old animals were analyzed as a 2 X 3 X 2 factorial arrangement of treatments in a split-split plot design. Dietary fat level was the main plot treatment (2 and 32%), fatty acid oxidized was the sub-plot treatment (myristate, palmitate and stearate) and carnitine level was the sub-sub-plot treatment (presence or absence of added carnitine in the fatty acid oxidation assay mixture). Tissue level of mitochondrial protein, CPT activity, and SDH activity were analyzed as a 3 X 2 factorial arrangement of treatments in a completely randomized design. The two treatment factors were age (1, 7 and 21 days) and dietary fat level (2 and 32%). In each of the three experimental designs discussed above, data collected from pigs at birth were excluded from the analysis since these pigs were not allotted to an age X diet treatment group.

Least significant difference values used in testing the difference between specific pairs of means for statistical significance are located in Tables IX, X and XI in the Appendix.

Results and Discussion

Weight Gain, Diet Consumption and Feed Efficiency

The pigs on both diets lost an average of 98.5 g. of body weight by 24 hr. of age (Table VIII) even though diet was being consumed. Twenty-one day old pigs consuming the high fat diet gained 250 g. more weight and consumed 706 ml more diet on the average than pigs of the

TABLE VIII

EFFECT OF DIETARY FAT LEVEL ON WEIGHT GAIN, DIET CONSUMPTION AND FEED EFFICIENCY IN THE NEONATAL PIG[a]

Days of Age	1		7		21	
Dietary Fat Level	2%	32%	2%	32%	2%	32%
Weight gain, g.	-94 ± 28	-103 ± 19	353 ± 41	323 ± 58	1489 ± 141	1739 ± 182
Diet Consumption, ml.	191 ± 9	192 ± 9	2131 ± 176	2011 ± 99	7178 ± 189	7884 ± 351
Feed Efficiency (g. live-weight gain/ Kcal energy consumed)	-.43 ± .13	-.45 ± .09	.14 ± .01	.13 ± .02	.17 ± .01	.18 ± .01

[a]Values represent mean ± SEM.

same age consuming the low fat diet (Table VIII). Although weight gain, diet consumption and feed efficiency were not significantly affected by dietary fat level (Table VIII), the tendency for both increased weight gain and diet consumption with an increase in dietary fat level from 2 to 32% are consistent with previous observations (Table III, Chapter II). Weight gain of pigs on both diets was lower than previously observed (Table VIII, Chapter III vs. Table III, Chapter II). The reduction in weight gain may be attributed to lower diet consumption since the efficiency of weight gain in 21 day pigs in the present experiment and 14 day pigs in the previous experiment is similar.

Several reports on the efficiency of fat utilization by the young pig were discussed in Chapter II. Although the results of those reports are variable, colostrum-deprived neonatal pigs reared in this laboratory have consistently gained more weight on the average when consuming high fat as compared to low fat diets. Results from this experiment support the conclusion in Chapter II that the young pig can effectively utilize high levels of butterfat as a dietary energy source.

Degradation of [U-^{14}C]Fatty Acids by Liver, Kidney, Heart, and Leg Muscle Homogenates

The oxidation of fatty acids to acid soluble products by tissue homogenates proceeded at a considerably faster rate than their oxidation to CO_2. This observation is illustrated in figures 1-5 by comparing parts A and B within each figure. In the oxidation of ^{14}C-labeled fatty acids by liver homogenates of mitochondria, radioactivity in the acid-soluble phase is indicative of the formation of

Figure 1. Palmitate oxidation by neonatal swine liver. Data are expressed as μg palmitate converted to acid soluble products (ASP) (A) or CO_2 (B) per 60 minutes per gram of wet tissue. The number of animals per treatment group = 5 for a total of 35 animals. The following treatment effects and interactions were significant: palmitate → ASP [age ($P < 0.01$), carnitine ($P < 0.01$), carnitine X age ($P < 0.01$), carnitine X diet ($P < 0.05$)]; palmitate → CO_2 [age ($P < 0.01$), carnitine ($P < 0.01$), carnitine X age ($P < 0.01$), carnitine X diet ($P < 0.01$), carnitine X age X diet ($P < 0.05$)].

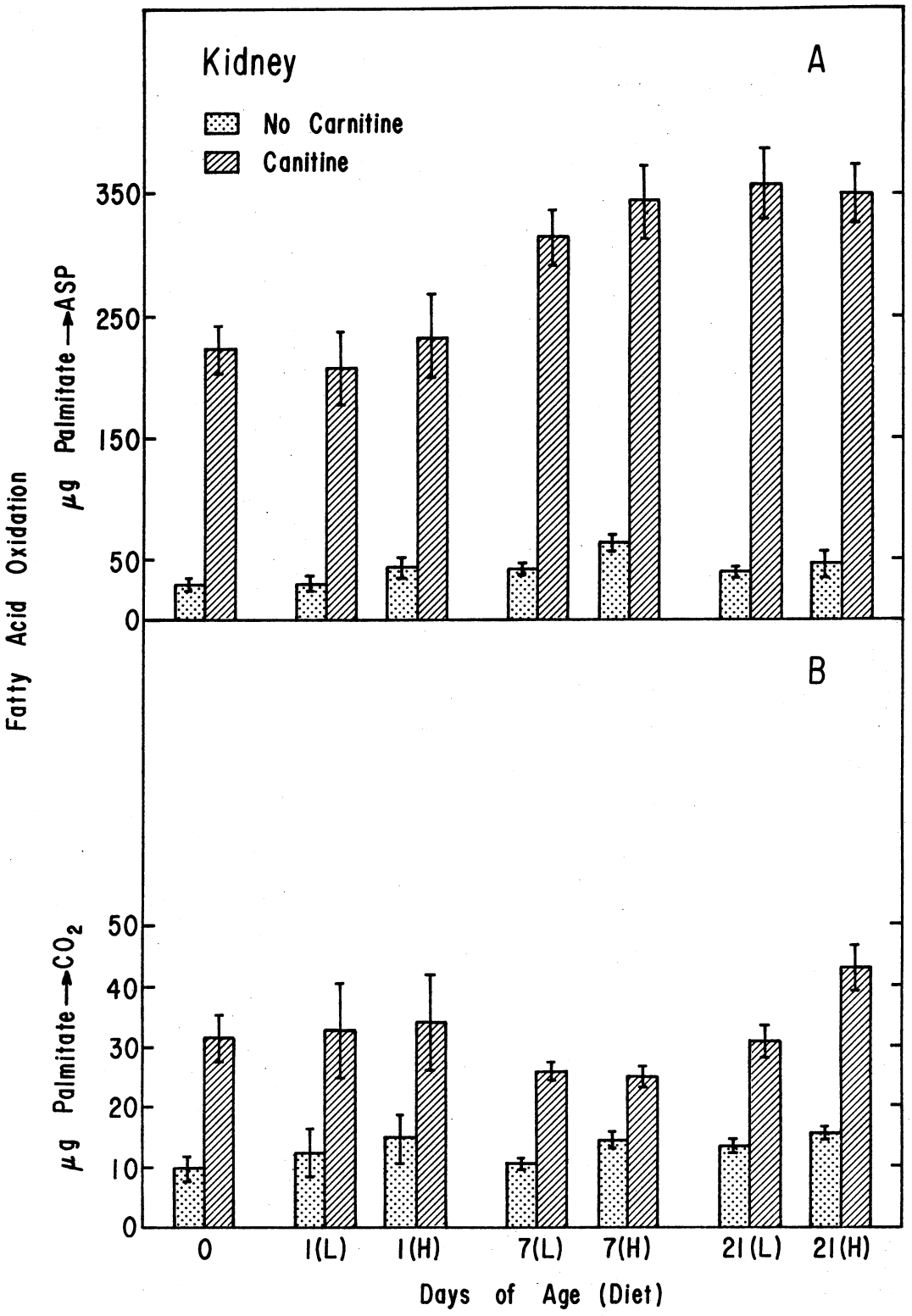

Kidney
A
No Carnitine
Canitine
Fatty Acid Oxidation
μg Palmitate→ASP
350
250
150
50
0
B
μg Palmitate→CO2
50
40
30
20
10
0
0
1(L)
1(H)
7(L)
7(H)
21(L)
21(H)
Days of Age (Diet)

Figure 2. Palmitate oxidation by neonatal swine kidney. Data are expressed as μg palmitate converted to acid soluble products (ASP) (A) or CO_2 (B) per 60 minutes per gram of wet tissue. The number of animals per treatment group = 5 for a total of 35 animals. The following treatment effects and interactions were significant: palmitate → ASP [age ($P < 0.01$), carnitine ($P < 0.01$), carnitine X diet ($P < 0.01$)]; palmitate → CO_2 [carnitine ($P < 0.01$), carnitine X age ($P < 0.05$)].

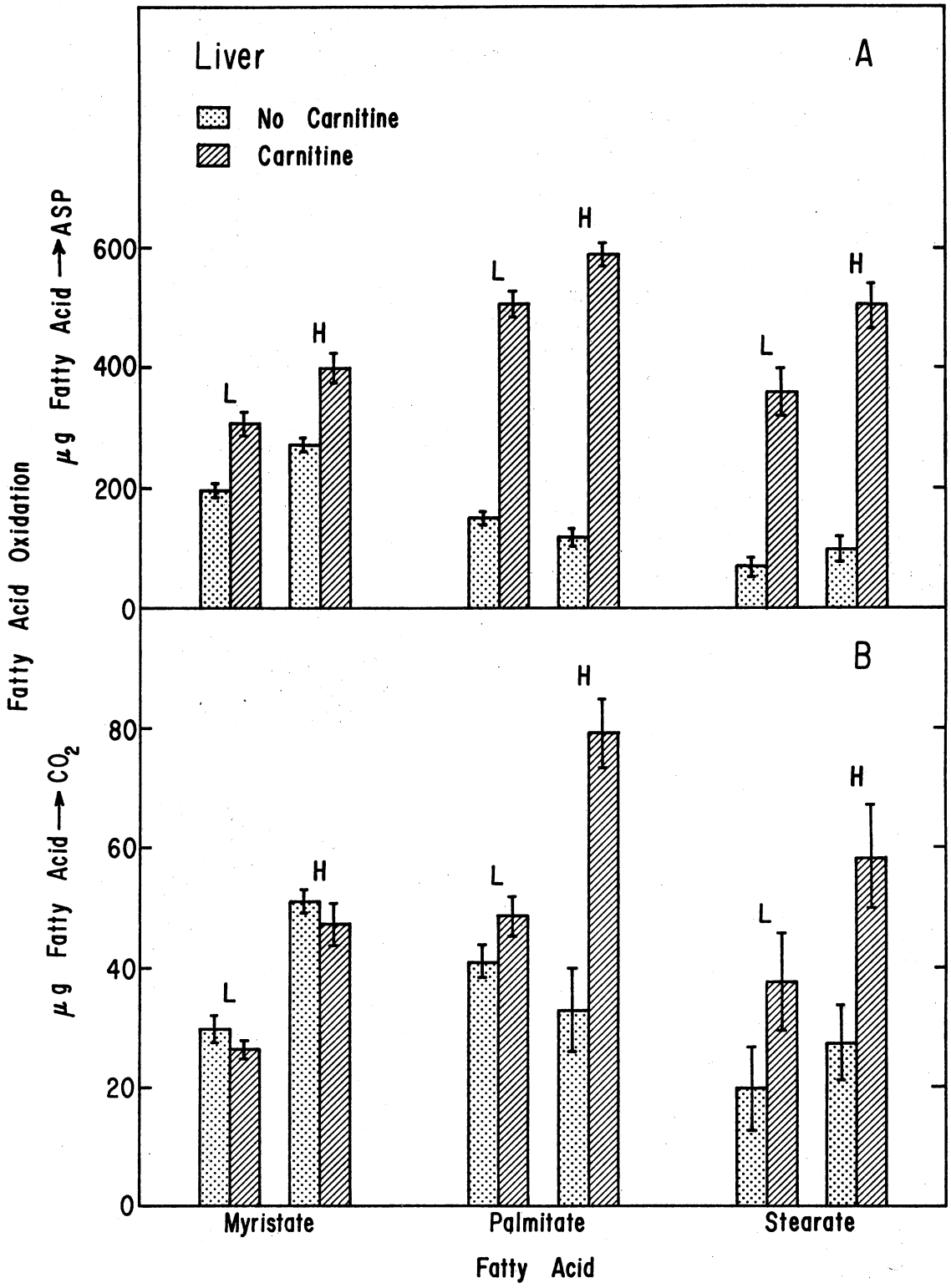

Liver
A
No Carnitine
Carnitine
Fatty Acid Oxidation
μg Fatty Acid → ASP
600
400
200
0
L
H
B
μg Fatty Acid → CO2
80
60
40
20
0
Myristate
Palmitate
Stearate
Fatty Acid

Figure 3. Palmitate oxidation by neonatal swine heart. Data are expressed as μg palmitate converted to acid soluble products (ASP) (A) or CO_2 (B) per 60 minutes per gram of wet tissue. The number of animals per treatment group = 5 for a total of 35 animals. The following Treatment effects and interactions were significant: palmitate → ASP [age ($P < 0.01$), diet ($P < 0.05$), carnitine ($P < 0.01$), carnitine X age ($P < 0.01$), carnitine X diet ($P < 0.01$)]; palmitate → CO_2 [carnitine ($P < 0.01$)].

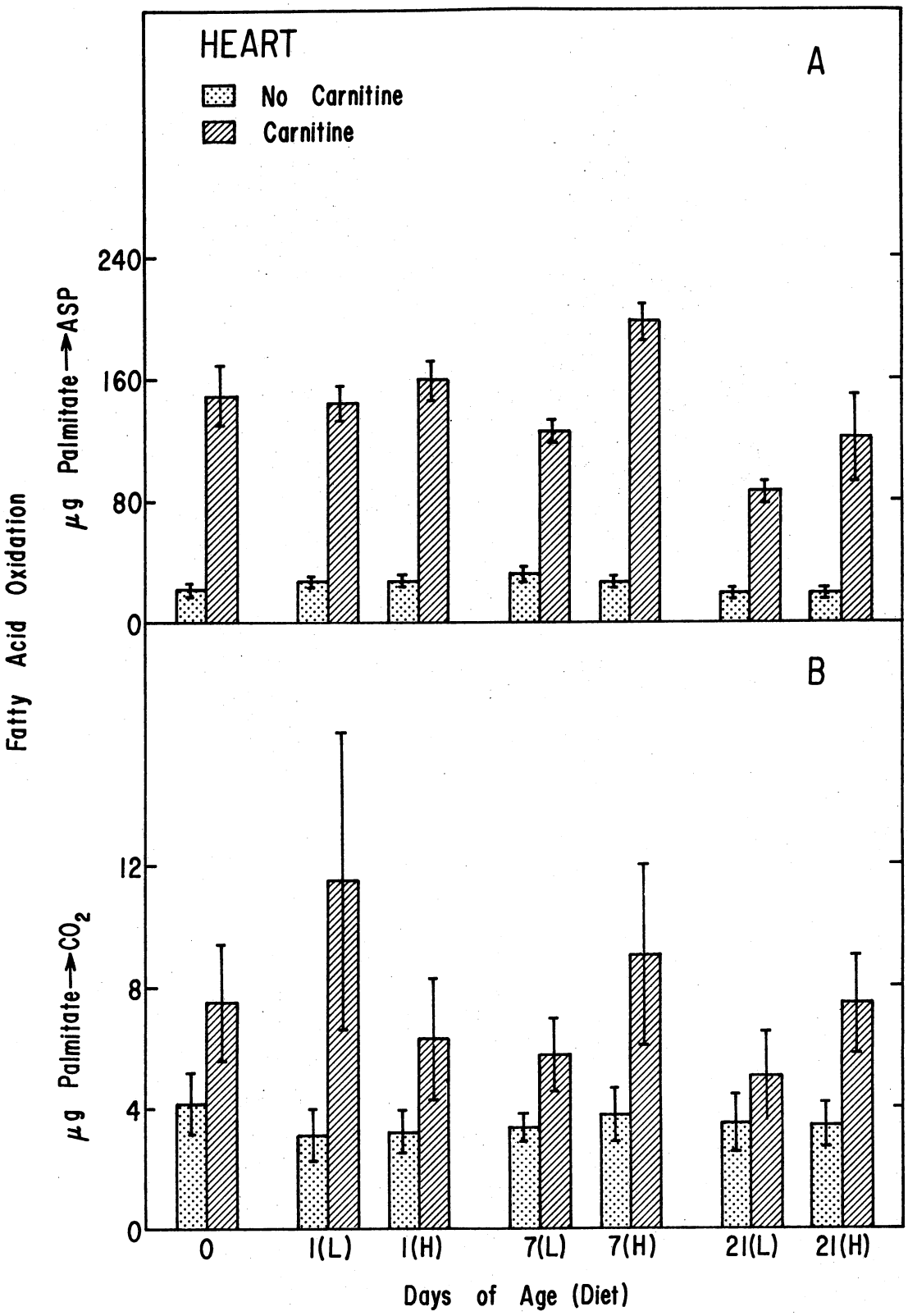

HEART
A
No Carnitine
Carnitine
240
160
80
0
μg Palmitate→ASP
B
12
8
4
0
μg Palmitate→CO2
Fatty Acid Oxidation
0
1(L)
1(H)
7(L)
7(H)
21(L)
21(H)
Days of Age (Diet)

Figure 4. Palmitate oxidation by neonatal swine leg muscle. Data are expressed as μg palmitate converted to acid soluble products (ASP) (A) or CO_2 (B) per 60 minutes per gram of wet tissue (biceps femoris). The number of animals per treatment group = 5 for a total of 35 animals. The following treatment effects and interactions were significant: palmitate → ASP [age ($P < 0.05$), carnitine ($P < 0.01$), carnitine X age ($P < 0.05$)]; palmitate → CO [age ($P < 0.05$), carnitine ($P < 0.01$)].

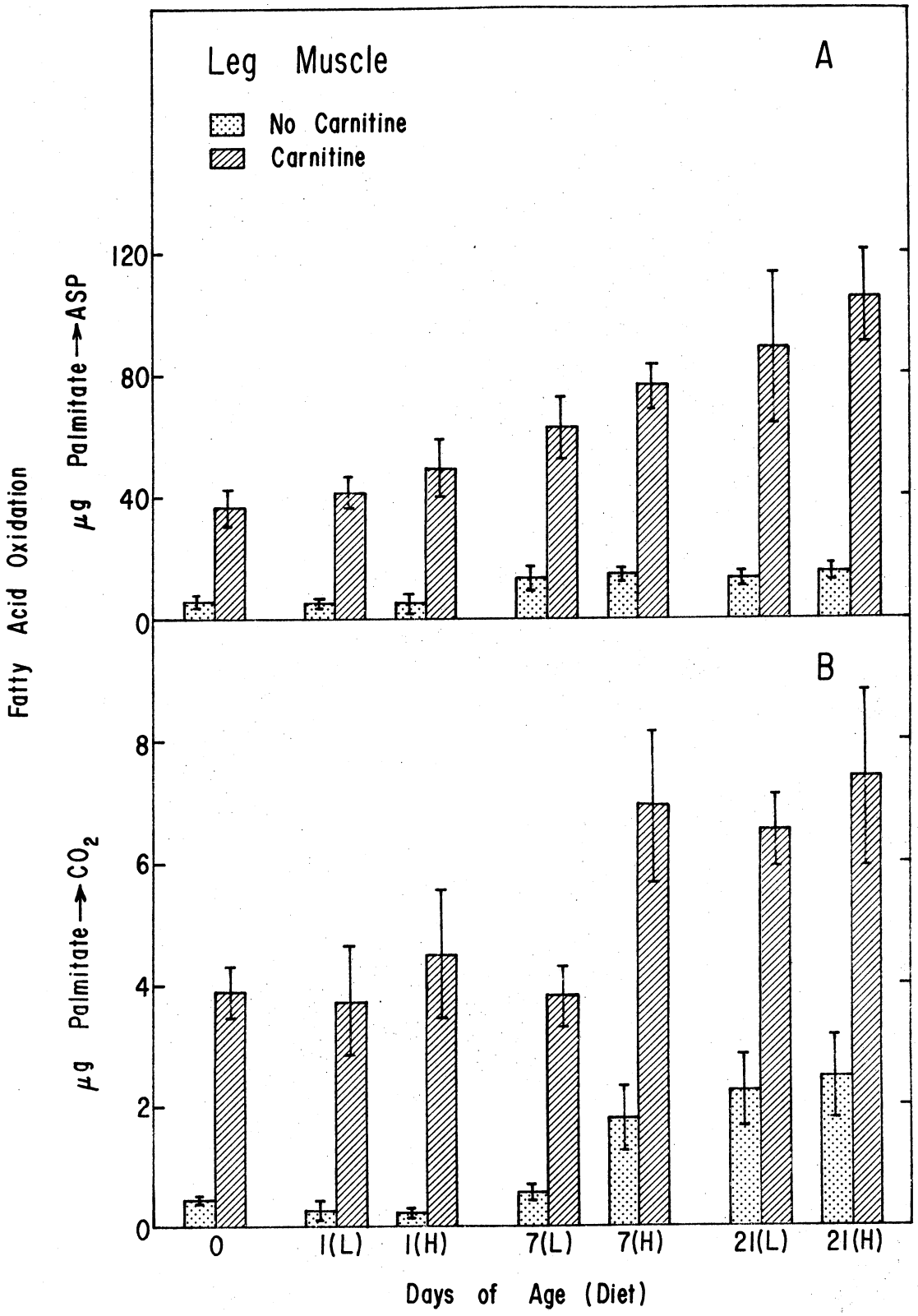
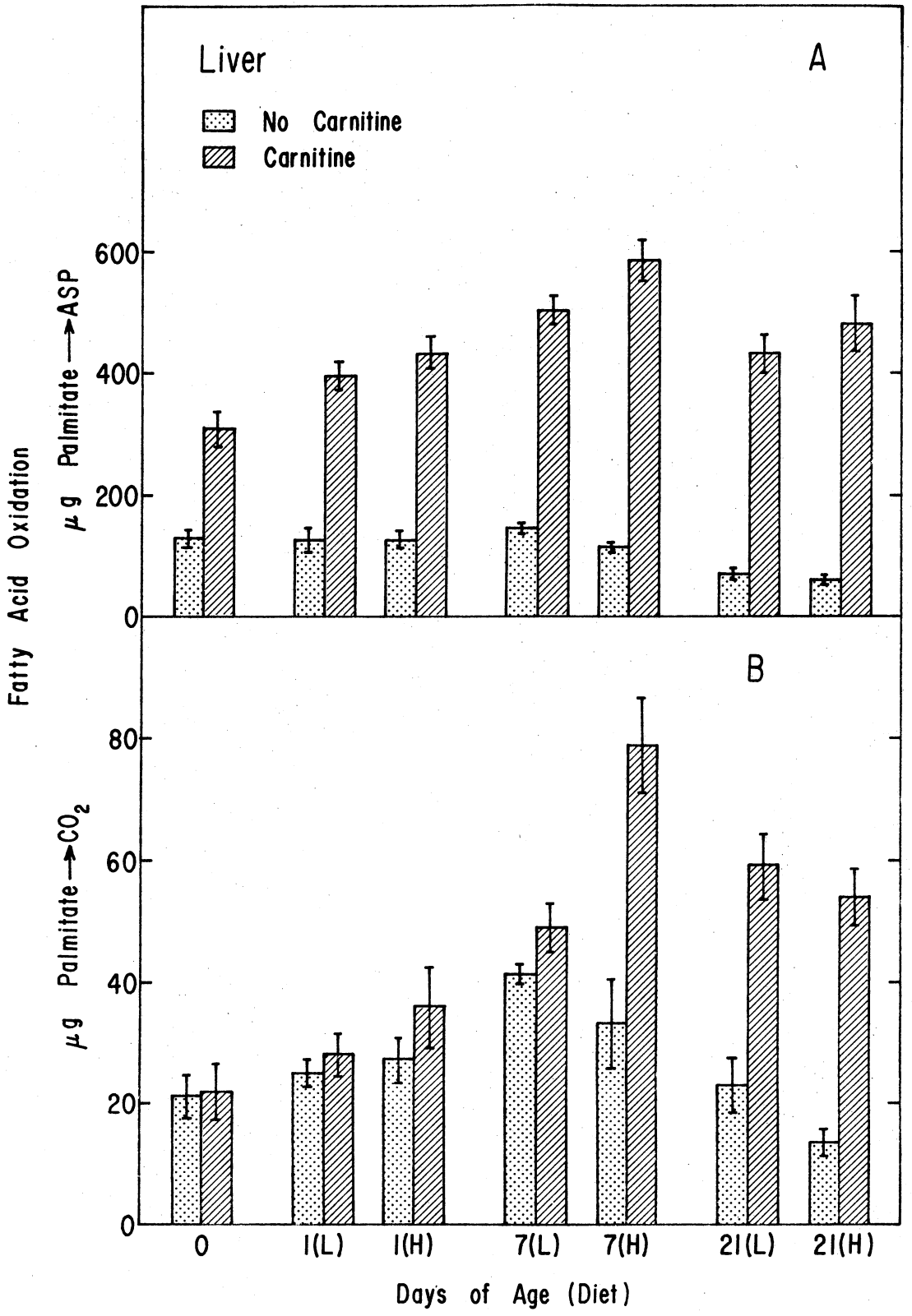

Leg Muscle
A
No Carnitine
Carnitine
Fatty Acid Oxidation
μg Palmitate→ASP
120
80
40
0
B
μg Palmitate→CO2
8
6
4
2
0
0
1(L)
1(H)
7(L)
7(H)
21(L)
21(H)
Days of Age (Diet)

Figure 5. Myristate, palmitate, and stearate oxidation by neonatal swine liver. Data are expressed as μg fatty acid converted to acid soluble products (ASP) (A) or CO_2 (B) per 60 minutes per gram of wet tissue. L = low (2%) fat diet and H = high (32%) fat diet. Pigs were 7 days of age and there were 5 animals per dietary treatment for a total of 10 animals. The following treatment effects and interactions were significant: palmitate → ASP [diet ($P < 0.05$), fatty acid ($P < 0.01$), diet X fatty acid ($P < 0.05$), carnitine ($P < 0.01$), carnitine X diet ($P < 0.01$), carnitine X fatty acid ($P < 0.01$)]; palmitate → CO_2 [diet ($P < 0.05$), fatty acid ($P < 0.01$), carnitine ($P < 0.01$), carnitine X diet ($P < 0.01$), carnitine X fatty acid ($P < 0.01$), carnitine X diet X fatty acid ($P < 0.01$)].


Liver
A
No Carnitine
Carnitine
Fatty Acid Oxidation
μg Palmitate → ASP
600
400
200
0
B
μg Palmitate → CO2
80
60
40
20
0
0
1(L)
1(H)
7(L)
7(H)
21(L)
21(H)
Days of Age (Diet)

the labeled ketone bodies acetoacetate and β-hydroxybutyrate (143, 149) and Kreb's Cycle intermediates (149). Extrahepatic tissues such as heart, kidney, and skeletal muscle are not associated with ketone body production thus the radioactive acid-soluble products formed during the oxidation of ^{14}C-labeled fatty acids by homogenates of these tissues are most likely acetyl-CoA and Kreb's Cycle intermediates. Stewart et al. (154) demonstrated that intact rat liver mitochondria, in contrast to aqueous extracts of acetone-dried ox liver and pig kidney mitochondria, contained much larger quantities of octanoyl-CoA than any other intermediate when oxidizing [8-^{14}C]octanoate. Therefore, it is unlikely that a significant portion of the radioactivity in the acid-soluble phase resulting from the oxidation of ^{14}C-labeled long-chain fatty acids by intact mitochondria in this experiment is due to the release of β-oxidation intermediates.

When assayed in the presence of added carnitine, an increase in dietary fat level increased the oxidation of palmitate to acid-soluble products in heart (Fig. 3A), liver (Fig. 1A) and leg muscle (Fig. 4A) and increased the oxidation of palmitate to CO_2 in leg muscle (Fig. 4B). However, the increased rate of oxidation was significant ($P < 0.05$) only in heart (Fig. 3A). The carnitine-stimulated rate of oxidation of palmitate to CO_2 (Fig. 5B) and stearate to acid-soluble products (Fig. 5A) was higher in liver homogenates from 7 day old pigs consuming the high fat diet ($P < 0.05$). Although the differences were not statistically significant ($P > 0.05$), the carnitine-stimulated rate of oxidation of myristate and stearate to CO_2 (Fig. 5B), and myristate and palmitate to acid-soluble products (Fig. 5A) tended to be higher in livers from animals consuming the high fat diet as compared to the

low fat diet. An increase in dietary fat level from 2 to 32% significantly ($P < 0.01$) increased the stimulatory effect of carnitine on palmitate oxidation to acid-soluble products in liver (Fig. 1A), kidney (Fig. 2A) and heart (Fig. 3A) and on palmitate oxidation to CO_2 in liver (Fig. 1B). Since the formation of fatty acyl carnitine, catalyzed by carnitine palmitoyltransferase (81, 82), is necessary for the translocation of the fatty acyl group of long-chain fatty acids across the mitochondrial membrane (77, 83), the tendency for a higher rate of fatty acid oxidation in certain tissues from pigs consuming the high fat diet may be due to an increased capacity for fatty acid uptake by mitochondria in these tissues.

Age had a significant effect on the oxidation of palmitate to CO_2 in liver ($P < 0.01$, Fig. 1B) and leg muscle ($P < 0.05$, Fig. 4B) and on the oxidation of palmitate to acid soluble products in liver ($P < 0.01$, Fig. 1A), kidney ($P < 0.01$, Fig. 2A), heart ($P < 0.01$, Fig. 3A) and leg muscle ($P < 0.05$, Fig. 4A). Mersmann and Phinney (145) reported that homogenates of swine liver exhibited low rates of palmitate oxidation at birth with a 4-fold increase by day 7 whereas the rate of fatty acid oxidation in swine heart homogenates did not change postnatally. In the present experiment, the carnitine-stimulated rate of palmitate oxidation to CO_2 by liver homogenates (Fig. 1B) increased 3.6-fold by 7 days of age in pigs consuming the 32% fat diet and increased 2.7-fold by 21 days of age in pigs consuming the 2% fat diet. The carnitine-stimulated oxidation of palmitate to acid-soluble products in liver homogenates (Fig. 1A) increased 1.6-fold and 1.9-fold from birth to 7 days of age in animals consuming the 2% and 32% fat diets, respectively. While the rate of carnitine-stimulated oxidation

of palmitate to acid-soluble products by heart homogenates (Fig. 3A) decreased 42% from birth to day 21 in animals consuming the 2% fat diet, the rate increased slightly from birth to day 7 and then decreased 23% from day 7 to day 21 in animals consuming the 32% fat diet. The carnitine-stimulated rate of palmitate oxidation to acid-soluble products by homogenates of kidney (Fig. 2A) and leg muscle (Fig. 4A) increased ($P < 0.05$) from 1 through 21 days of age. An increase ($P < 0.05$) in the rate of oxidation of palmitate to CO_2 by homogenates of leg muscle was observed between day 1 and day 21.

Changes in the carnitine-stimulated oxidation of palmitate by liver homogenates during development of the rat follow a different pattern than that observed in the neonatal pig. Augenfeld and Fritz (142) observed little change in rate of oxidation of palmitate to CO_2 in liver homogenates when comparing 1-, 2-, and 3-day old rats to adult rats while the rate of oxidation of palmitate to acetoacetate reached a maximum value by day 2 and then decreased in the adult rat to 2/3 the rate observed in the 2 day old rat. In the presence of added carnitine, Lockwood and Bailey (143) observed a decrease in the rate of oxidation of palmitate to CO_2 by rat liver homogenates from birth to 5 days of age followed by a gradual increase as the rat reached maturity whereas, the rate of oxidation of palmitate to acid-soluble products was maximum by 15 days of age and decreased considerably as the rat matured. A gradual decrease in the rate of oxidation of palmitate to CO_2 and acid-soluble products in heart homogenates was also observed from 5 days of age to maturity in the rat (143). In contrast to our data and data previously discussed, Tayler et al. (155) reported that the rate of palmitate oxidation to CO_2 and acid-

soluble products in rat liver homogenates was maximum at 1 day of age and decreased as the rat aged to maturity.

Age had a significant effect on the degree to which carnitine stimulated the oxidation of palmitate to acid-soluble products in liver ($P < 0.01$), kidney ($P < 0.05$), heart ($P < 0.01$), and leg muscle ($P < 0.05$) and to CO_2 in liver ($P < 0.01$). Carnitine stimulation of the oxidation of palmitate to both CO_2 and acid-soluble products in liver (Fig. 1) and to acid-soluble products in leg muscle (Fig. 4A) increased from birth to 21 days of age regardless of dietary fat level. Carnitine stimulation of palmitate degradation to acid-soluble products in heart (Fig. 3A) was lowest at 7 days of age in animals consuming the low fat diet and highest at 7 days of age in animals consuming the high fat diet. This resulted in a carnitine X age X diet interaction which approached significance ($P < 0.10$). The stimulation of palmitate oxidation to acid-soluble products by carnitine in kidney (Fig. 2A) was maximum at 21 days of age.

Carnitine stimulated ($P < 0.01$) the degradation of palmitate to CO_2 and acid-soluble products in homogenates of all four tissues (Fig. 1-4). The stimulating effect of carnitine on the oxidation of long-chain fatty acids was first reported by Fritz (156) in rat liver and has since been demonstrated in the liver (142, 143), heart (143), skeletal muscle (157), and adrenal gland (158) of the rat and in liver and heart of the neonatal pig (145). Addition of carnitine to liver homogenates was observed to stimulate the oxidation of palmitate to acid-soluble products throughout development of the rat (142, 143) whereas, the effect of added carnitine on CO_2 production varied with age. Lockwood and Bailey (143) observed a carnitine stimulation of

CO_2 production from palmitate in the fetal and postweaning age groups and an inhibition during the suckling period. Augenfeld and Fritz (142) observed that carnitine stimulated the oxidation of palmitate to CO_2 in homogenates of liver from fetal rats but not from newborn or adult rats. Carnitine consistently stimulated the oxidation of palmitate to both CO_2 and acid-soluble products by heart homogenates during development of the rat (143). Wittels and Bressler (159) also reported that the oxidation of palmitate to CO_2 by homogenates of heart from newborn and adult rats was stimulated by carnitine. In contrast to the results of the present experiment, Mersmann and Phinney (145) reported a marginal carnitine stimulation of palmitate oxidation to CO_2 and acid-soluble products in liver and heart homogenates during development of the pig. The carnitine stimulation of palmitate oxidation observed in homogenates of liver, kidney, heart and leg muscle in the present experiment may be a result of lower levels of tissue carnitine in pigs fed semipurified diets as opposed to suckling pigs. Also, Mersmann and Phinney (145) used a Polytron homogenizer, as compared to the loose fitting Potter Elvehjem homogenizer used in the present experiment, to prepare tissue homogenates which may have disrupted the inner mitochondrial membrane which forms the barrier to extramitochondrial long-chain fatty acids (77, 83).

Addition of carnitine to the assay medium significantly ($P < 0.01$) increased the rate of oxidation of myristate, palmitate, and stearate to acid-soluble products in homogenates of liver from 7 day old pigs (Fig. 5A). The degree to which carnitine stimulated the production of acid-soluble products was dependent upon the chain length of the fatty acid oxidized ($P < 0.01$). The average increase in the rate of

fatty acid oxidation to acid-soluble products upon the addition of carnitine to the assay medium was 5.1-fold for stearate, 4.2-fold for palmitate and 1.5-fold for myristate. Addition of carnitine to the assay medium significantly increased the rate of oxidation of stearate to CO_2 and palmitate to CO_2 in liver from animals consuming the high fat diet ($P < 0.01$) but had little effect on the rate of oxidation of myristate to CO_2 (Fig 5B). The reason for the increase in carnitine stimulation with increased fatty acid chain length is not known. Norum (151) has shown that the activity of carnitine palmitoyltransferase activity in calf-liver mitochondria increases with increasing fatty acid chain length through palmitate although there was little difference between the rate of conversion of myristylcarnitine and palmitylcarnitine to carnitine and the fatty acyl-CoA derivatives.

When measured by CO_2 production in the presence of added carnitine (Fig. 5B), palmitate was oxidized at a faster rate than myristate ($P < 0.05$) and stearate ($P < 0.10$) in liver from animals consuming the high fat diet, and at a faster rate than myristate ($P < 0.10$) in liver from animals consuming the low fat diet. The rate of CO_2 production in the absence of added carnitine showed a tendency to decrease with increasing fatty acid chain length ($P < 0.10$). In the presence of added carnitine, the rate of production of acid-soluble products in liver from animals consuming the low fat diet was higher for palmitate than myristate ($P < 0.01$) or stearate ($P < 0.01$). The carnitine-stimulated rate of fatty acid degradation to acid-soluble products (Fig. 5A) in liver from animals consuming the high fat diet was higher for palmitate and stearate than for myristate ($P < 0.05$) and tended to be

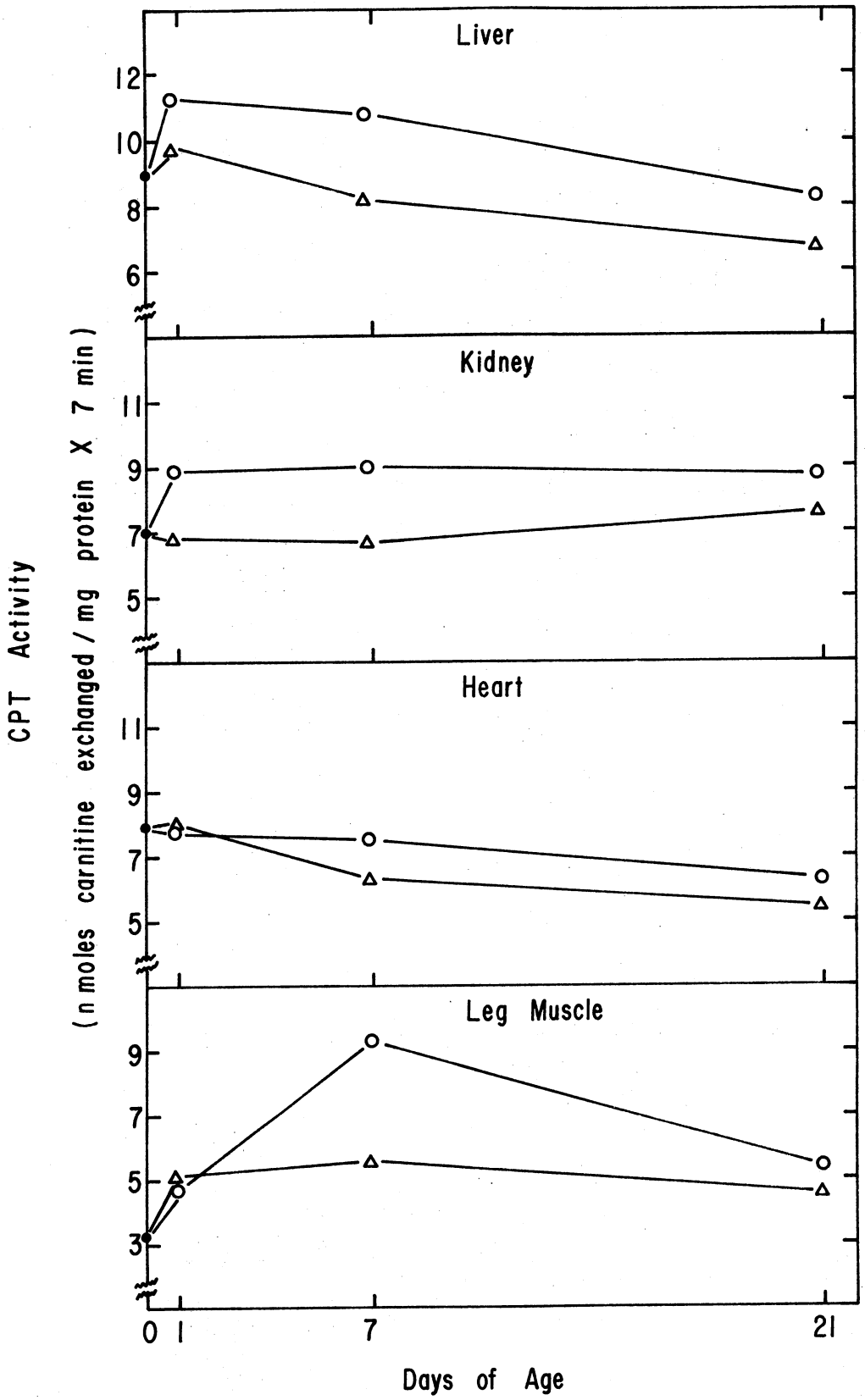
higher for palmitate than myristate ($P < 0.10$). When assayed in the absence of added carnitine, the rate of production of acid-soluble products (Fig. 5A) decreased with increasing fatty acid chain length with significant differences ($P < 0.05$) between myristate and stearate in liver from pigs consuming both the low and high fat diets and between myristate and palmitate in liver from pigs consuming the high fat diet. Mathias et al. (160) reported that the rate of [U-^{14}C]-fatty acid oxidation to CO_2 in homogenates of liver from rats 6 and 18 months of age was higher for linoleate than for oleate and palmitate with small differences between the latter two fatty acids. Miller et al. (147) compared the relative rates of oxidation of intramuscularly injected [1-^{14}C]fatty acids in pigs 1 and 7 days of age and reported that lauric acid was oxidized faster than palmitic, oleic, and linoleic acid with small differences between the latter 3 fatty acids. Similar results were reported in the experiment of Solar-Argilaga (161) in which the fractional uptake of free fatty acids by perfused liver was shown to be inversely related to the chain length and directly related to the number of double bonds in the molecule with the affinity of the liver for long-chain free fatty acids being myristic>palmitoleic> linoleic>oleic≃palmitic>stearic. Although results from the present experiment show that homogenates of liver from 7 day old pigs tend to oxidize palmitate at a faster rate than myristate and stearate when carnitine is added to the assay mixture, differences in the rate of tissue absorption of these and other fatty acids <u>in vivo</u> could result in an entirely different picture in terms of the relative rate of oxidation of fatty acids by the intact animal.

Carnitine Palmitoyltransferase Activity

Changes in the activity of carnitine palmitoyltransferase during early development of the neonatal pig are shown in Figure 6. No significant change in CPT activity due to age or dietary fat level was observed in liver, kidney, or heart. Within the same dietary treatment, CPT activity declined in liver and heart but remained essentially constant in kidney as the animals developed from 1 through 21 days of age. In leg muscle from animals consuming the high fat diet, CPT activity was significantly higher ($P < 0.05$) at 7 days of age than at 1 or 21 days of age. CPT activity in leg muscle from animals consuming the low fat diet remained essentially constant from 1 through 21 days of age. Although CPT activity (Fig. 6) in liver, kidney, and heart did not vary significantly with age, and did not parallel observed changes in palmitate oxidation, it should be noted, when comparing animals of the same age, that CPT activity was generally higher in tissue from animals consuming the high fat diet as compared to the low fat diet.

In contrast to results of the present experiment, closely paralleled changes in the activity of carnitine palmitoyltransferase and the rate of palmitate oxidation in rat liver have been reported (142, 144). Augenfeld and Fritz (142) reported a 5-fold increase in CPT activity in rat liver by 24 hr. postpartum. Wood (162) reported a 2.5-fold and 4-fold increase in CPT activity in rat liver and heart, respectively, by 2 days of age. Bieber et al. (12) reported that CPT activity in pig liver mitochondria after 24 hr. of age was more than double the activity at birth and was about equal to the activity

Figure 6. Carnitine palmitoyltransferase (CPT) activity in neonatal swine consuming a low (Δ) or high (o) fat diet. Activity expressed on a per mg mitochondrial protein basis. Each point represents the mean of 5 animals. SEM values for the mean are: liver (1.34), kidney (1.51), heart (1.13), leg muscle (1.20).


Liver
Kidney
Heart
Leg Muscle
CPT Activity
(n moles carnitine exchanged / mg protein X 7 min)
Days of Age
0
1
7
21

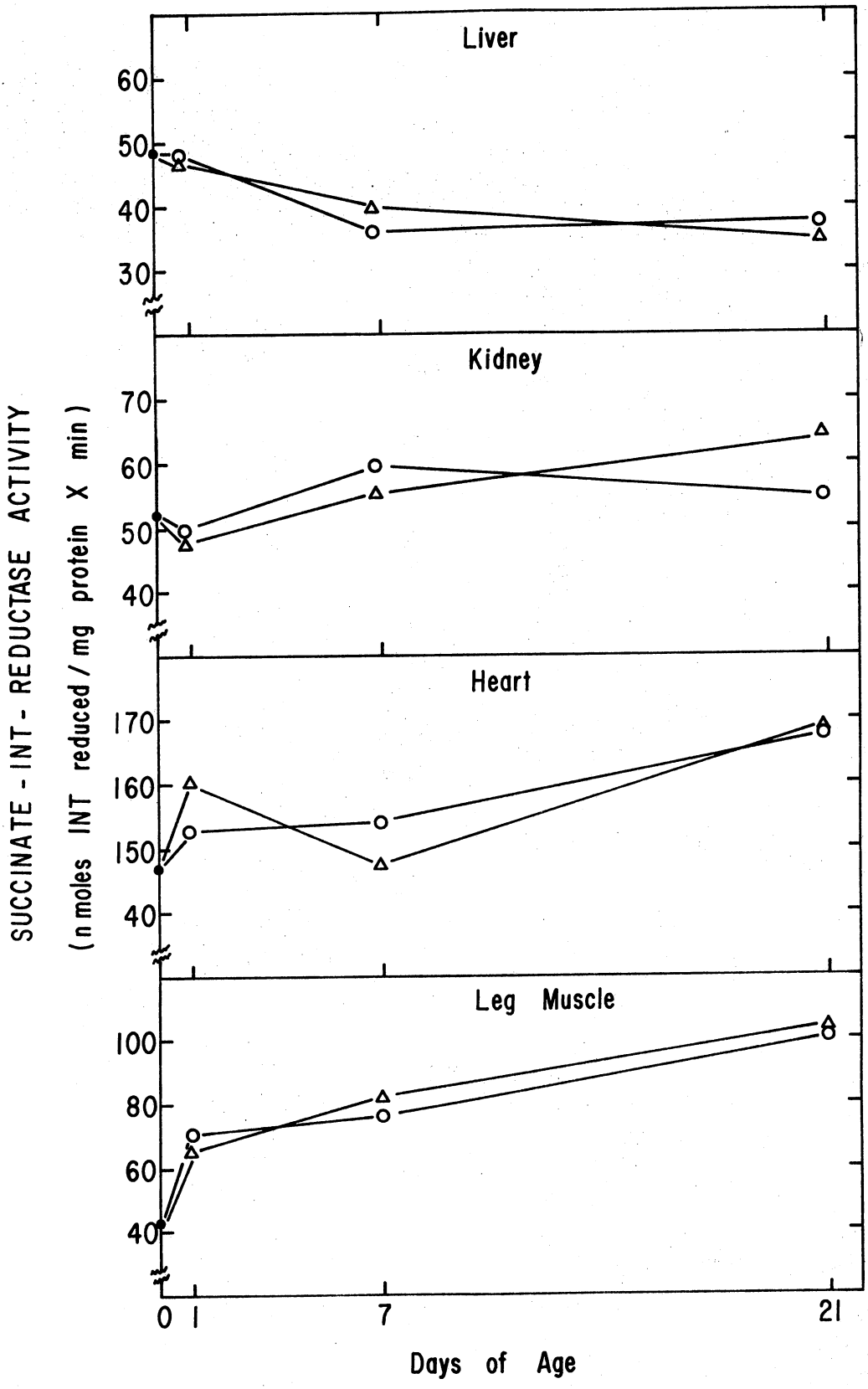
observed at 24 days.

Succinate Dehydrogenase Activity

Succinate dehydrogenase (SDH) activity was assayed in mitochondria isolated from liver, kidney, heart, and skeletal muscle (Fig. 7) to reflect the overall activity of the TCA cycle. The work of Pette et al. (163) indicates that the ratio of activities of Kreb's cycle enzymes is remarkably constant among a wide variety of cell types which indicates that the relative amounts of each are constant. The level of dietary fat had no significant effect on the activity of this enzyme when expressed on a per milligram of mitochondrial protein basis. SDH activity was significantly affected by the age of the animal in liver ($P < 0.01$) and leg muscle ($P < 0.05$) but not in kidney and heart. In liver, the enzyme activity decreased with increasing age of the animal to 7 days and then remained essentially constant from 7 through 21 days of age. In leg muscle, SDH activity increased considerably from birth to 1 day of age with a significant but less dramatic increase from 1 through 21 days of age. The 1.7-fold increase in the rate of carnitine-stimulated palmitate oxidation to CO_2 from 1 to 21 days of age (Fig. 4B) in leg muscle is closely paralleled by a 1.5-fold increase in SDH activity ($P < 0.05$, Fig. 7) during the same period. However, SDH activity increased 1.6-fold from birth through 1 day of age while CO_2 production remained essentially constant. Although an increase in the activity of this enzyme was observed from birth through 21 days of age in heart muscle, the differences were not statistically significant.

With the exception of leg muscle, changes in the rate of carnitine-

Figure 7. Succinate-INT-reductase activity in neonatal swine consuming a low (Δ) or high (o) fat diet. Activity expressed on a per mg of mitochondrial protein basis. Each point represents the mean of 5 animals. SEM values for the means are: liver (2.66), kidney (5.73), heart (12.92), leg muscle (10.15). Activity was significantly affected by age in liver ($P < 0.01$) and leg muscle ($P < 0.05$).


Liver
Kidney
Heart
Leg Muscle
60
50
40
30
70
60
50
40
170
160
150
40
100
80
60
40
0
1
7
21
Days of Age
SUCCINATE - INT - REDUCTASE ACTIVITY
(n moles INT reduced / mg protein X min)

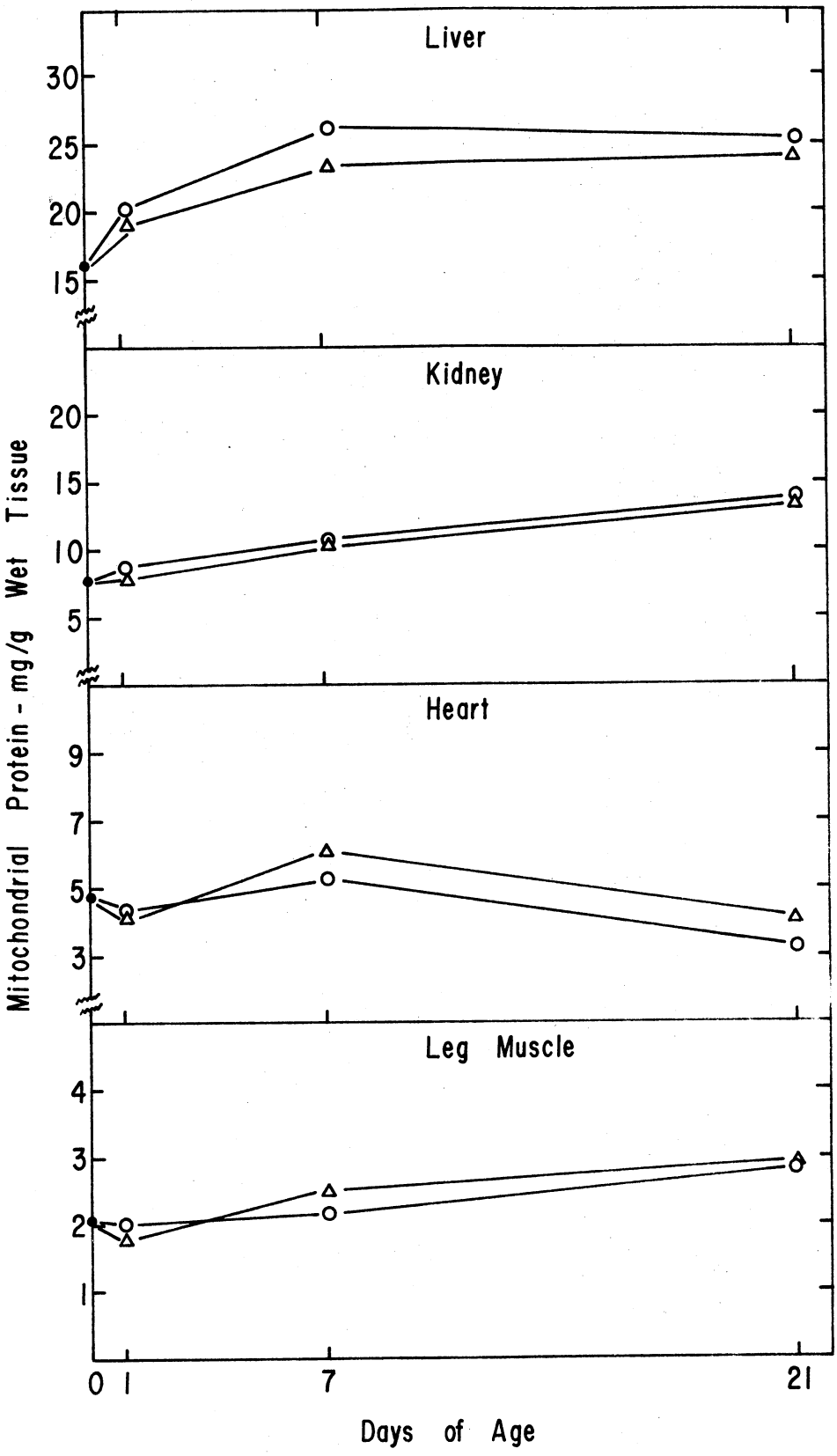
stimulated oxidation of palmitate to CO_2 did not follow the observed changes in SDH activity during early development of the neonatal pig. The lack of association between these two variables in liver, kidney and heart may be due to the fact that SDH activity was measured under conditions of maximum velocity whereas CO_2 production was measured in relatively intact mitochondria where the activity of one or more of the regulatory enzymes in the TCA cycle could be modified by metabolic effectors. Therefore, CO_2 production may not reflect the maximum catalytic activity of the TCA cycle.

Tissue Levels of Mitochondrial Protein

Changes in the level of mitochondrial protein in liver, kidney, heart, and leg muscle during early development of the neonatal pig are shown in Figure 8. The level of mitochondrial protein increased significantly ($P < 0.01$) from birth through 21 days of age in kidney and leg muscle and from birth through 7 days of age in heart muscle where it decreased by 21 days of age to a level lower than that observed in the newborn pig. The mitochondrial protein content of liver was observed to increase from birth through 7 days of age ($P < 0.05$) to essentially the same level observed in the 21 day old pig. Dietary fat level had no significant effect on mitochondrial protein content of these four tissues from animals of the same age.

Mersmann (141) previously demonstrated an increase in hepatic mitochondria between birth and day 2 in the neonatal pig whereas the results of the present experiment indicate an increase in liver mitochondrial protein from birth through 7 days of age (Fig. 8). The increase in mitochondrial protein in liver through day 7 and in leg

Figure 8. Tissue mitochondrial protein levels in neonatal swine consuming low (Δ) or high (o) fat diets. Each point represents the mean of 5 animals. SEM values for the means are: liver (2.41), kidney (1.31), heart (0.53), leg muscle (0.30). The level of mitochondrial protein was significantly affected by age in kidney ($P < 0.01$), heart ($P < 0.01$), and leg muscle ($P < 0.01$).


Liver
Kidney
Heart
Leg Muscle
Mitochondrial Protein - mg/g Wet Tissue
Days of Age
0
1
7
21

muscle through day 21 (Fig. 8) tends to parallel the observed changes in the carnitine-stimulated rate of oxidation of palmitate to both CO_2 and acid-soluble products (Fig. 1 and 4, respectively). In leg muscle, however, the 1.8- and 2.7-fold increase in the carnitine-stimulated rate of oxidation of palmitate to CO_2 and acid-soluble products respectively from birth through 21 days of age was accompanied only by a 1.5-fold increase in the level of mitochondrial protein per gram of wet tissue during the same period. The level of mitochondrial protein per gram of wet tissue in kidney increased 1.8-fold from birth through 21 days of age yet the carnitine-stimulated rate of palmitate oxidation to acid-soluble products increased only 1.5-fold between birth and 7 days of age with little increase between 7 and 21 days of age. Little similarity between changes in the level of heart mitochondrial protein (Fig. 8) and the carnitine-stimulated rate of palmitate oxidation by heart homogenates (Fig. 3) was noted between birth and day 7. However, a 34% decrease in the level of mitochondrial protein between 7 and 21 days of age was accompanied by a 31 and 38% decrease in the carnitine-stimulated oxidation of palmitate to acid-soluble products by homogenates of heart from pigs consuming the 2 and 32% fat diets respectively during the same time period.

In the present experiment, changes in the rate of palmitate oxidation in homogenates of liver, kidney, heart and leg muscle from the neonatal pig tended to parallel changes in the level of mitochondrial protein in these tissues.

Summary for Chapter III

A total of 35 pigs were obtained by cesarean section, placed in

individual sterile isolators, and randomly allotted to treatment groups. Thirty pigs received semipurified, isocaloric diets containing 2 or 32% butterfat (dry matter basis) and were sacrificed at 1, 7 or 21 days of age. Five pigs were sacrificed at 2 hr. post delivery and received no diet.

Twenty-one day old pigs showed a tendency for higher weight gain and feed consumption when comsuning the 32% fat diet although the differences were not statistically significant.

The rate of oxidation of [U-^{14}C]palmitate to CO_2 and acid-soluble products was measured in homogenates of liver, kidney, heart, and leg muscle (biceps femoris) from pigs 0, 1, 7, and 21 days of age. The relative rates of oxidation of [U-^{14}C]myristate, [U-^{14}C]palmitate and [U-^{14}C]stearate were measured in homogenates of liver from 7 day old pigs. Palmitate oxidation was stimulated by carnitine in all four tissues and, with few exceptions, the rate of carnitine-stimulated palmitate oxidation was higher in tissues from pigs consuming the 32% fat diet. The rate of palmitate oxidation increased with age in liver, kidney and leg muscle and was maximum at 21 days in kidney and leg muscle and at 7 days in liver. The rate of palmitate oxidation in heart tended to decrease with increasing age. In homogenates of liver from 7 day old animals, palmitate was oxidized at a faster rate than stearate or myristate.

The activities of carnitine palmitoyltransferase (CPT) and succinate dehydrogenase in mitochondria isolated from liver, kidney, heart and leg muscle did not vary considerably with age although CPT activity tended to be higher in those tissues from pigs consuming the high fat diet. Changes in the rate of palmitate oxidation with

age tended to parallel changes in the level of mitochondrial protein per gram of wet tissue during early development of the neonatal pig.

CHAPTER IV

CONCLUSION

Colostrum-deprived neonatal pigs were fed isocaloric liquid diets containing varying levels of butterfat to determine the effect of dietary fat level on weight gain, feed efficiency, fatty acid biosynthesis and fatty acid oxidation.

An increase in dietary fat level from 2 to 32% resulted in a significant improvement in weight gain and a tendency for increased feed efficiency in pigs at 14 days of age (Chapter II). A tendency toward increased weight gain with an increase in dietary fat level from 2 to 32% was observed in 21 day old pigs (Chapter III). However, efficient utilization of dietary fat by neonatal pigs reared in this laboratory has not always been observed in the young pig. Explanation of this conflict is beyond the scope of the present work. Efficiency of dietary fat utilization may depend on a number of factors including the type of fat (source, fatty acid composition and physical characteristics), the level of fat in the diet, the source and level of dietary protein and the ratio of dietary energy to essential nutrients. Experiments should be conducted to determine the efficiency of utilization of calories from fat as a dietary energy source and the digestibility of composite fatty acids in different fats under various dietary conditions.

Results reported in Chapter II show that, in the neonatal pig,

increasing the dietary fat level from 2 to 32% results in a decrease in the activity of fatty acid synthetase and citrate cleavage enzyme in adipose tissue and liver and a decrease in the activity of malic enzyme in adipose tissue. In pigs consuming the 2% fat diet, the activities of fatty acid synthetase, citrate cleavage enzyme and malic enzyme were 3-, 20- and 40-fold higher respectively in adipose than in liver. These results indicate that adipose tissue plays the major role in the biosynthesis of fatty acids under conditions of low fat, high carbohydrate intake.

Body fat, when expressed on a percent live weight basis, has been reported to increase from 1% in the newborn pig to as much as 15% by 14 days of age. In the present experiment (Chapter II) the percent carcass fat in the 14 day old pig was independent of dietary fat level. Therefore, the rapid deposition of body fat in the suckling pig is not a result of the high level of fat in sow's milk.

The relative percentages of lauric, myristic, palmitic, palmitoleic, stearic and oleic acids were measured in samples of backfat from all pigs (Chapter II). An increase in the relative percentage of myristic acid and a decrease in the relative percentages of palmitoleic and oleic acids was observed as the dietary fat level increased from 2 to 32%. The decrease in the relative percentage of palmitoleic and oleic acid in backfat accompanied by an increase in the level of dietary fat indicates that the rate of utilization of these two fatty acids for purposes other than fat deposition is higher than that of lauric, myristic, palmitic and stearic acid.

The effects of age and level of dietary butterfat on rate of oxidation of fatty acids, carnitine palmitoyltransferase (CPT)

activity, succinate dehydrogenase (SDH) activity and on the level of mitochondrial protein in liver, kidney, heart and leg muscle from the neonatal pig were reported in Chapter III. The rate of palmitate oxidation increased with age in liver, kidney and leg muscle. The low rate of palmitate oxidation in these tissues at birth, relative to 7 days in liver and 21 days in kidney and leg muscle, does not imply poor utilization of dietary fat as an energy source since a tendency for improved rate of gain on the 32% fat diet was observed in the neonatal pig (Chapter III).

Tissues from pigs consuming the high fat diet tended to exhibit a higher rate of palmitate oxidation and a higher CPT activity. This observation supports the theory that the CPT catalyzed formation of acylcarnitine is necessary for the translocation of long-chain fatty acids across the inner mitochondrial membrane. However, changes in the oxidation rate of palmitate and the activity of CPT with age did not parallel. Tissue levels of mitochondrial protein tended to parallel the rate of palmitate oxidation during development of the neonatal pig through day 21 (Chapter III). Since dietary fat level did not affect tissue levels of mitochondrial protein, these results indicate that the rate of palmitate oxidation in homogenates of liver, kidney, heart and leg muscle is dependent on both the level of mitochondrial protein and CPT activity.

The relative oxidation rates of myristate, palmitate and stearate were measured in homogenates of liver from the 7 day old pig. The oxidation rate of these fatty acids was higher in liver from pigs consuming the 32% fat diet. This effect was also associated with a tendency for a higher CPT activity in liver mitochondria from these

pigs. Palmitate was oxidized at a faster rate than myristate or stearate, suggesting that differences may exist in the rate of utilization of different fatty acids *in vivo* as energy substrates. Experiments to determine the relative rate of fatty acid oxidation *in vivo* are needed. These experiments should be conducted under conditions such that differences in the rate of fatty acid oxidation due to gut absorption are eliminated.

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APPENDIX

TABLE IX

LEAST SIGNIFICANT DIFFERENCE (LSD) VALUES FOR COMPARING TREATMENT MEANS (PALMITATE OXIDATION IN LIVER, KIDNEY, HEART AND LEG MUSCLE[a])

Tissue	Oxidation Product	LSD[b]		LSD[c]	
		$P < 0.05$	$P < 0.01$	$P < 0.05$	$P < 0.01$
Liver	CO_2	11.95	16.20	14.53	19.69
	ASP	69.60	94.32	74.98	101.62
Kidney	CO_2	6.79	9.20	12.29	16.67
	ASP	47.58	64.47	62.94	85.31
Heart	CO_2	4.87	6.60	5.85	7.94
	ASP	28.94	39.21	33.89	45.93
Leg Muscle	CO_2	1.88	2.55	2.66	3.61
	ASP	27.16	36.81	29.35	39.77

[a]Figures 1, 2, 3 and 4 respectively, Chapter III.

[b]Values used in comparing means representing the rate of oxidation of palmitate in the presence and absence of added carnitine within the same age and dietary fat level treatment groups.

[c]Values used in comparing means not within the same age and dietary fat level treatment groups.

TABLE X

LEAST SIGNIFICANT DIFFERENCE (LSD) VALUES FOR COMPARING TREATMENT MEANS (MYRISTATE, PALMITATE AND STEARATE OXIDATION IN LIVER FROM 7 DAY PIGS[a])

Oxidation Product	LSD[b]		LSD[c]	
	$P < 0.05$	$P < 0.01$	$P < 0.05$	$P < 0.01$
CO_2	11.13	15.18	25.24	34.43
ASP	47.25	64.45	99.95	136.33

[a]Figure 5, Chapter III.

[b]Values used in comparing means representing the rate of oxidation of fatty acid in the presence and absence of added carnitine within the same dietary fat level and fatty acid treatment group.

[c]Values used in comparing means not within the same dietary fat level and fatty acid treatment group.

TABLE XI

LEAST SIGNIFICANT DIFFERENCE (LSD) VALUES FOR COMPARING TREATMENT MEANS (CPT ACTIVITY[a], SDH ACTIVITY[b], AND TISSUE LEVELS OF MITOCHONDRIAL PROTEIN[c])

	LSD					
	CPT Activity		SDH Activity		Tissue Levels of Mitochondrial Protein	
Tissue	P<0.05	P<0.01	P<0.05	P<0.01	P<0.05	P<0.01
Liver	3.96	5.41	7.87	10.76	7.13	10.09
Kidney	4.46	6.10	16.97	23.20	3.87	5.29
Heart	3.35	5.36	38.24	52.27	1.57	2.15
Leg Muscle	3.55	4.85	30.06	41.09	0.89	1.22

[a]Figure 6, Chapter III.

[b]Figure 7, Chapter III.

[c]Figure 8, Chapter III.

VITA

Ronald Gene Wolfe

Candidate for the Degree of

Doctor of Philosophy

Thesis: LIPID METABOLISM IN THE NEONATAL PIG

Major Fields: Animal Nutrition and Biochemistry

Biological:

Personal Data: Born in Southwest City, Missouri, August 23, 1945, the son of Mr. and Mrs. Wilburn R. Wolfe

Education: Graduated from Southwest City High School, Southwest City, Missouri in May, 1963; received the Bachelor of Science degree in Animal Science from Oklahoma State University in 1970; completed requirements for the Doctor of Philosophy degree at Oklahoma State University in May, 1976.

Professional Experience: Served as a research assistant at Oklahoma State University from 1971 to 1975.

Professional Organizations: Member of the American Society of Animal Science and the American Chemical Society.