QUALITATIVE CHANGES IN SERUM LIPID FRACTIONS FROM THE ISOLATED PERFUSED BOVINE LIVER

by

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STATEMENT BY AUTHOR

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ABSTRACT

The livers from eight steers fed diets containing four different tallow levels were perfused and the serum fatty acids extracted, fractionated, and analyzed. The extraction was done by the chloroform-methanol procedure. The whole serum fatty acids were fractioned into phospholipids, free fatty acids, triglycerides, and cholesterol esters by thin-layer chromatography and analyzed by gas-liquid chromatography. Time of perfusion affected an increase of C16:1 in the whole serum lipid ($P < .01$). Diet was responsible for the reduction in percent of C16:1 and C18:2 in the phospholipid fraction ($P < .05$). A highly significant time effect was observed for the percent change of C18 and C18:1 in the free fatty acid fraction. After 30 min of perfusion C18 exhibited an average 6.2 percent increase while C18:1 exhibited an average 6.2 percent decrease. This relationship suggested the activity of a saturase enzyme system. Changes in percent over time of perfusion of other fatty acids within the fractions were observed. Significant diet by time interactions were revealed for specific fatty acids in all fractions. Correlations of the change in percent were also observed among the major fatty acids of each fraction.

CHAPTER I

INTRODUCTION

Intramuscular lipid quantity of beef has been an important criterion for many years in determining grades for standardization between producers and consumers. Inherent in beef grades are factors which relate directly and indirectly to desirability and palatability. At the present time, however, much less is understood about the quality of these lipids as they affect the palatability of this product.

It has been implicated that certain fatty acid ratios and combinations can have either an advantageous or deleterious effect upon the eating quality of beef. Therefore, factors which influence and cause beef animals to deposit certain fatty acids preferentially in different combinations and ratios appear to be of significant importance to warrant investigation.

Classically, the liver has been regarded as an important organ in lipid metabolism. Currently, the major emphasis seems to be on adipose tissue in regard to the greatest percent of changes occurring in lipids. Regardless of the tissue studied, isolation of that tissue *in vitro* has been an effective method of studying changes which are encountered.

Environment and heredity both affect lipid composition in animal tissues. Heredity most likely will manifest its effect by differences in hormonal levels and balances and by the presence or absence

of specific enzymes. Nutrition is the most important environmental effect which has been studied in this area.

This experiment was designed and conducted to study the effects of different levels of tallow in the rations of beef steers on the functions of their livers. More specifically, this experiment was designed to examine the effects of diet upon liver enzyme functions regulating qualitative changes incurred in lipid fractions.

CHAPTER II

LITERATURE REVIEW

Influence of Fat Upon Palatability of Meat

The development of more sophisticated analyses has immensely improved research methods for studying palatability factors of meat since MacKintosh and Hall (1936) stated that the degree of finish of beef appeared to be associated with palatability. They reported an increasing degree of finish rendered more intense the properties of tenderness.

Hornstein and Crowe (1960) reported flavor differences that exist in pork and beef may have their origins in the fat portions of these meats. They stated that fat may not only produce different flavor compounds in different ratios, but it may also act as a storage depot for lipid-soluble foreign compounds that, on heating, also contribute to flavor.

Hornstein, Crowe, and Heimberg (1961) concluded that phospholipids did not contribute to desirable meat flavor, and in excessively lean meat they could contribute to poor flavor. Triglycerides, on the other hand, seemed to enhance the flavor of meat.

Initial increases in free fatty acids in the backfat of hogs were related to a rise in desirability of flavor of the cooked meat according to Hall, Harrison, and MacKintosh (1962). However, the

relationship of free fatty acids to flavor was not dependent upon quantity alone.

Using broths and depot fats, Hofstrand and Jacobson (1960) reported that fat did not significantly affect the taste of mutton or lamb broths. There was an indication that fat may have contributed to the aroma of the lamb and mutton broths. Wasserman and Talley (1968) reported that beef fat had little or no effect on the development of a characteristic beef aroma. However, pork and lamb fats appeared to contain some factor(s) that developed a specific aroma.

Waldman et al. (1965) reported flavor was not significantly associated with any of the individual fatty acids or ratios of fatty acids. Taste panel juiciness of the Longissimus dorsi was negatively correlated with myristic acid ($P < .05$) and palmitic acid ($P < .05$) of the same muscle. The association of juiciness with the ratio of unsaturated to saturated acids was positive ($P < .01$). Low, nonsignificant correlations were found among individual fatty acids from the neutral and phospholipid fractions of the bovine Longissimus dorsi muscle with tenderness and juiciness scores by Terrell et al. (1968).

Dryden and Marchello (1970) reported that palmitoleic acid was highly correlated with juiciness ($r = 0.78$) of the Triceps brachii. Linoleic acid of the Longissimus dorsi was negatively correlated with juiciness ($r = -.74$) and the flavor of the same muscle was enhanced ($r = 0.66$) by oleic acid.

Influence of Dietary Fat on Fat Deposition

The effect of dietary fat on fat deposition in ruminant animals has been investigated because of the questionable action of the rumen and rumen microflora upon ingested fats. It was reported by Smedley-MacLean (1943) that beef suet, mutton fat, and lard each had a definite character of its own, but that this character could be modified if exceptional quantities of any particular fatty acid were included in the diet of a beef, lamb, sheep, or pig. He stated that, in each case, a large amount of linseed oil in the diet would soften fat because a greater proportion of the liquid unsaturated fatty acids characteristic of linseed would be stored.

Contrary to this concept, Shorland (1950) reported that beef and mutton tallow were not appreciably modified by the nature of the dietary fat. However, even a poor source of fat such as barley was shown to affect the quality of pig fat by supplying unsaturated fatty acids, primarily linoleic acid, which was almost quantitatively deposited in the adipose tissue (Dahl 1958). It was reported by Dahl (1960) that linoleic acid percentages of backfats and leaf fats from pigs fed diets varying in fat levels were significantly ($P < .05$) different from each other.

Brooks (1967) fed high levels of soybean oil in diets to pigs and reported that linoleic acid was almost three times the level and significantly higher ($P < .05$) in these pigs than in those fed on other diets in the experiment. Linoleic acid was highly negatively correlated ($r = -.64, -.81, -.83, -.84$) with palmitic acid under each

treatment investigated. Koch et al. (1968) fed pigs high levels of safflower oil and tallow. Backfat and leaf fat from the pigs fed safflower oil were less saturated than those fats from control animals. These fats contained significantly ($P < .05$) more linoleic acid and significantly less oleic acid than those fats from control animals. These researchers also reported that the fatty acid composition of the intramuscular fat from the Longissimus dorsi was affected much less by diet than was leaf fat or backfat.

Hilditch and Pedelty (1941) reported that ewes fed on a "supermaintenance ration" contained higher levels of oleic acid and lower levels of stearic acid in their kidney fat than those fed on a "submaintenance ration." Palmitic acid was preferentially deposited in external fat as opposed to kidney fat. Ogilvie, McClymont, and Shorland (1961) reported that a fat emulsion of linseed oil and bile placed directly into the small intestine resulted in greatly increased levels of linoleic and linolenic acids in the kidney fat of sheep. They attributed this to the fact that hydrogenation of the unsaturated dietary acids in the rumen was not allowed, thus supporting the earlier investigations of Shorland (1950).

Tallow from sheep fed purified diets was reported to contain less stearic acid and more oleic acid than tallow from either sheep fed hay-grain diets or grazing animals (Tove and Matrone 1962). In some instances, the altered fatty acid composition was sufficient to result in tallow that was liquid at room temperature. They attributed this effect to a lesser degree of hydrogenation of the unsaturated fatty

acids by the rumen microflora of the sheep fed the purified diets. This work supported the results of Hilditch and Pedelty (1941) and Smedley-MacLean (1943), but opposed the views of Shorland (1950) and of Ogilvie et al. (1961). In further support of their views, Tove and Matrone (1962) reported that a high content of trans-acids in the dietary fat resulted in high levels of trans-acids in the sheep tallow, irrespective of the remainder of the diet, indicating the resistance of these acids to hydrogenation by the rumen microflora.

Willey et al. (1952) reported that feeding high levels of fat to steers had a markedly beneficial effect upon the efficiency of feed utilization and also significantly ($P < .05$) increased the level of fat in the blood. They reported that oleic acid percent decreased in animals fed high energy and high fat diets, but that linoleic and linolenic acid percentages exhibited little or no change. High fat diets also decreased intramuscular fat deposition but the fat present was less saturated than intramuscular fat from other treatments. Edwards, Tove, and Barrick (1958) stated that when steers were fed animal fats rich in oleic and linoleic acids in the diet, only stearic acid was found to increase in body fat.

Bowland and Hironaka (1957) stated that they found significant or highly significant correlations of plasma lipids with shoulder fat, back and loin fat, combined shoulder, back and loin fat, and area of loin muscle of pigs, while plasma lipid correlations with average daily gains approached significance at the 5 percent level. Brungardt, Bray, and Hoekstra (1963), in studying the effect of plasma lipids in

fattening cattle, reported that differences for total plasma fat, lipid phosphorus, and total, free and esterified cholesterol were found between animals fed similarly and of comparable age and weight. Between the ages of 9 and 16 months total plasma lipids increased 42.9 percent, and 76.2 percent of this increase had taken place by 13 months of age.

In further studies, Brungardt and Bray (1966) reported that high levels of cholesterol were found in steers possessing a high percent of carcass fat. Marbling score and lipid phosphorus, measured as a percent change from 9 to 16 months, were highly associated ($r = 0.52$). Data implied that the concentration of plasma lipid was positively related to amount of adipose tissue in the carcasses. This finding agreed with that of Bowland and Hironaka (1957).

Liver Enzymes Affecting Lipid Metabolism

Baldwin et al. (1966) found that when young calves were fed fat-containing diets glucose-6-phosphate dehydrogenase activity and citrate cleavage enzyme activity appeared to be lowered and the activity of α-glycerol-phosphate dehydrogenase appeared to be raised with respect to ten other enzymes whose activities exhibited no effective change in the liver.

O'hea and Leveille (1969), in a series of experiments with pigs, reported that the incorporation of acetate-1-^{14}C into fatty acids and nonsaponifiable lipids by liver slices was manyfold greater than that of glucose-6-^{14}C, the incorporation of which was insignificant. Results also revealed that the ability of adipose tissue to convert

acetate-1-^{14}C to fatty acids was ten times that of liver slices when expressed on an equal weight basis. In another experiment, a range of ^{14}C-labeled substrates was employed and their metabolism by the pig liver and adipose tissue slices evaluated. All substrates were readily incorporated into adipose tissue fatty acids, whereas incorporation into liver fatty acids was very low, especially in the case of glucose-6-^{14}C and propionate-2-^{14}C. Enzyme data revealed that the activities of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and NADP-malate dehydrogenase of backfat, abdominal fat, and kidney fat were not statistically different, but that the activity of these enzymes in the liver was extremely low. Citrate cleavage enzyme activity of the backfat was also significantly higher than the activity of this enzyme in the liver. *In vivo* studies of two experiments suggested that when acetate-1-^{14}C was used as substrate the adipose tissue accounted for 70 to 75 percent and the liver for 25 to 30 percent of the newly synthesized fatty acids.

Uchiyama, Nakagawa, and Okui (1967) reported that the rate of monoenoic acid synthesis by desaturation was markedly depressed in starvation but elevated after refeeding rats. Addition of free fatty acids to incubated supernatant solutions of liver enzymes caused a marked depression of desaturation activity, the influence of poly-unsaturated acids being greater than that of mono-unsaturated acids.

Nakagawa and Uchiyama (1969a) found that the incorporation of oleic acid into esterified lipids by the postmitochondrial supernatant of liver cells was two times faster than that of palmitoleic acid.

However, in vitro desaturation of palmitic acid by the postmitochondrial supernatant of rat liver proceeded three times faster than that of stearic acid. They attributed this discrepancy between in vitro studies and in vivo ratios to the difference of rates of incorporation of the two unsaturated acids into esterified lipids.

In further studies, Nakagawa and Uchiyama (1969b) reported that of the saturated acids studied only Δ^9-monoenes of the same chain length were formed when desaturated by liver enzymes. Myristoleic and palmitoleic acids were both formed at much faster rates than was oleic acid. However, in rats, oleic acid was incorporated into esterified lipids at a more rapid rate than palmitoleic acid, as observed earlier by Nakagawa and Uchiyama (1969a), and myristoleic acid was not found in rat tissues.

Using hen liver enzyme preparations, Allen et al. (1967) reported that desaturation of stearic acid could be inhibited by the addition of pure methyl sterculate to the preparations. There was an inverse relationship between dietary level of methyl sterculate and the desaturase activity of the liver enzyme system in converting stearic to oleic acid.

Liver Perfusion Studies

The liver is considered to be the major source of plasma lipoproteins containing triglycerides, cholesterol, and phospholipids derived endogenously (Laurell 1959; Hillyard, Cornelius, and Chaikoff 1959; Havel and Goldfien 1961; Borgström and Olivecrona 1961; Heimberg et al. 1962; Morris 1963; and Windmueller and Spaeth 1967). Triglyceride is secreted by the liver mainly as pre-β-lipoproteins (Masoro 1968).

Hillyard et al. (1959) stated that free fatty acid uptake by the liver was not markedly affected by nutritional state, variations in concentration of free fatty acids within physiological range, or by variations in availability of glucose. Mayes (1969) stated that the free fatty acids were taken up readily by the liver from the blood, but that nutritional state did influence the fate of the free fatty acids in the liver. Working with rats, he found that livers from fed rats esterified more and oxidized less of the influx of free fatty acids than did livers from starved rats.

Hillyard et al. (1959), using rat livers and palmitate-1-^{14}C bound to albumin, found that 34 to 43 percent of the ^{14}C activity in the incoming perfusate was removed in a single passage through the liver. Of the ^{14}C activity that disappeared from the perfusate, 58 percent was recovered as phospholipid or triglyceride in the liver. Heimberg et al. (1962), again using rat livers, reported that there was a net release of triglycerides from the liver of fed animals and their data suggested that both uptake and release of triglycerides by the liver occurred simultaneously. Windmueller and Spaeth (1967), working with perfused rat livers, noted that a marked acceleration in the rate of lipid release was consistently observed after four hours of perfusion, suggesting that the liver may have affected changes in the perfusate composition which permitted greater release.

Stein and Shapiro (1959), using rat livers perfused _in situ_, found that 1-^{14}C linoleic acid had a lower incorporation into the liver lipids than did 1-^{14}C palmitic acid, but for both acids maximum

incorporation was obtained before 5 min of perfusion had elapsed. Simpson-Morgan and Morris (1962) reported no significant difference in the rate of uptake or oxidation of palmitic, oleic, or linoleic acids incorporated into chylomicrons, but found significantly more linoleic acid than oleic or palmitic acid was incorporated in the phospholipids.

In an experiment using functionally hepatectomized rats, Borgström and Olivecrona (1961) reported finding more ^{14}C activity of the blood in triglycerides and a lesser extent in the phospholipids. In a similar experiment utilizing rat livers and palmitic-1-^{14}C, Morris (1963) reported that at the end of 4 hr of perfusion more than 90 percent of the ^{14}C activity in the liver was recovered as esterified fatty acids and more activity was present in phospholipids than in triglycerides. In theory, this finding seemed to support the earlier work of Borgström and Olivecrona (1961).

Kern, Eiseman, and Normell (1965), using a cottonseed oil emulsion and pig livers, found that from initial perfusion time triglyceride level increased abruptly and then decreased. The free fatty acids also increased but returned to control levels within 30 to 90 min. Total cholesterol and phospholipid levels did not change appreciably.

Brauer, Pessotti, and Pizzolato (1951) demonstrated that the isolated rat liver could form bile, and that bile flow could be maintained for extended periods of time. Conditions which were found compatible with bile formation also could be shown to result in preservation of the macroscopic and microscopic anatomy of the liver tissue, in maintenance of functional Kupffer cells and of continued detoxication

activity. Martinis et al. (1958), using calves and their livers, reported the rate of bile production in the intact animals ranged from 15 to 38 ml/hr. Values in the isolated livers varied from 1.3 to 9.6 ml/hr and the flow rates often fluctuated during the course of each individual experiment. Total blood flow rate was about 0.60 ml/min/g of liver tissue. Morris (1960) indicated that bile secretion reached a maximum when all of the hepatic sinuses were perfused, provided the oxygen supply to the liver cells was adequate.

Connolly, Head, and Williams (1964), working with goat livers, reported the importance to ruminant species of free fatty acid catabolism by the liver. This catabolism by the ruminant liver gives rise to acetate and these researchers felt that this acetate could account for the major share of endogenous acetate turnover, even though in this experiment the free fatty acid catabolism contributed a variable proportion of the acetate produced by the liver. The blood glucose levels were reported to have increased twofold during the perfusion period of 1 hr. This rise in blood glucose was paralleled by a decrease in the percentage of liver glycogen.

Whiting et al. (1968) designed and used a perfusion apparatus similar to that of Holter, McCarthy, and Kesler (1963). It was demonstrated that the isolated perfused bovine liver was capable of dehalogenating DDT to its less toxic analog, DDD, for up to 2 hr, thus supporting earlier studies by Brauer et al. (1951). The performance of the perfusion apparatus was judged to be good, and after 2 hr the livers remained natural in appearance.

CHAPTER III

MATERIALS AND METHODS

General

For this study eight half-sib Hereford steers of comparable ages were used as experimental material. Throughout their growing period all animals were subjected to similar environment with respect to nutrition and general management. After weaning, the animals were group fed alfalfa for 3 months on a maintenance basis determined by the California Net Energy System. They were then placed in separate pens at the University of Arizona Campbell Avenue Farm and started on test.

The experimental rations for this study were as follows: control (no tallow added), 5 percent tallow added, 10 percent tallow added, and 15 percent tallow added. These fat levels in the rations were maintained throughout the fattening period, but the level of concentrate was increased at 56-day intervals. Experimental rations containing 60 percent concentrate were fed from February 28 to April 24, 1969. Composition and analysis of these rations are described in Table 1. On April 25, 1969, the rations were increased to 75 percent concentrate and on June 20, 1969, the rations were increased to 90 percent concentrate. Concentrate levels were adjusted by decreasing the percentages of alfalfa and cottonseed hulls and increasing the percentage of steam-processed milo. The eight steers were divided into four groups, two steers of each group receiving the same ration.

Table 1. Composition and analysis of the starting 60 percent concentrate ration.

Item	Percent tallow			
	0	5	10	15
	%	%	%	%
Ground alfalfa hay	20.00	20.00	20.00	20.00
Cottonseed hulls	20.00	20.00	20.00	20.00
Steam-processed milo	48.80	42.65	36.20	29.70
Cottonseed pellets	4.50	5.50	7.00	8.50
Molasses	5.00	5.00	5.00	5.00
Tallow[a]	-	5.00	10.00	15.00
Dicalcium phosphate	0.60	0.65	0.60	0.60
Urea	0.60	0.70	0.70	0.70
Salt	0.50	0.50	0.50	0.50
	100.00	100.00	100.00	100.00
Vitamin A-10-P	10 g	10 g	10 g	10 g
Protein, %	12.40	12.50	12.50	12.50
Calcium, %	0.52	0.54	0.52	0.53
Phosphorus, %	0.34	0.35	0.34	0.34
Acid detergent fiber, %	20.20	20.20	20.30	20.40
Total digestible nutr., %	61.60	68.00	74.60	78.40
NE_m, megcal./100 lb.	67.70	72.60	78.10	83.60
NE_p, megcal./100 lb.	39.20	42.00	45.20	48.40

[a]Animal fat as defined by Feed Bag Redbook, 1968; antioxidant added.

Animals were not taken off feed prior to slaughter, and all animals were killed at approximately 11:00 am between August 15 and September 2, 1969, at the University of Arizona Meats Laboratory.

Perfusion Apparatus

The perfusion apparatus used in this experiment was essentially the same as that of Whiting (1968), with slight modifications. It consisted of a rectangular glass tank, 60 cm x 46 cm x 25 cm, surrounded by a stainless steel tank, 73 cm x 57 cm x 32 cm. The outer stainless steel shell was filled with water which was held at 37.5 C during perfusion. The glass tank acted as the *in vitro* body and also as the blood reservoir during perfusion. Blood flowed from the reservoir to the bottom of a glass "lung" where it encountered a steady flow of 95 percent oxygen and 5 percent carbon dioxide gas from a pressurized tank. The blood and gas then flowed up through a series of glass marbles which greatly increased the surface area for oxygenation of the blood. Blood flow from the reservoir to the top of the "lung" was accomplished by the force of gravity on the reservoir head. The gas flow was adjusted to approximately 24 ml/min and the gas was bubbled through physiological saline prior to entry into the glass "lung" to prevent dehydration of the blood.

Oxygenated blood was pumped away from the "lung" by a variable output electric Roll-Flex "heart" pump (Cole-Parmer, Chicago, Illinois). The blood was then pumped up into a series of coils submerged in the waterbath to regulate its temperature. The blood then entered the liver, which had been placed on a glass table inside the *in vitro* body,

via the cannulated portal vein and was subsequently drained back into the reservoir below the liver via the cannulated posterior abdominal vena cava.

Antifoaming agents were not used in this experiment so that it was necessary to apply a slight vacuum to the top of the "lung" to aid in foam removal. This foam was trapped in an inverted glass bottle above the reservoir and any blood obtained was returned to the reservoir every 4 min during the perfusion. Glass tubing was used as venous and arterial passages except for 110 cm of plastic tubing that served as the coil in the waterbath and 70 cm which connected the top of the "lung" with the vacuum bottle.

In Figure 1 can be seen the "lung" or oxygenation tower showing the foam accumulation at the top of the tower. This foam was drawn off by vacuum to a foam trap, as pictured in Figure 2. During perfusion, any blood collected from the foam in the trap was recycled every 4 min. The blood volume for each perfusion was 6 liters and the amount of blood recycled during any 4-min period was usually less than 0.5 percent of the total blood volume and was not believed to have affected the results to any great degree.

Sample Collection

Stunning

Stunning of the steers used in this experiment was achieved by shooting the animals in the mid-brain with a .22 caliber pistol using .22 long-caliber ammunition. This method was employed to insure proper

Figure 1. The oxygenation tower.

Foam can be observed accumulating at the top of the "lung" or oxygenation tower. Note the brighter color of the oxygenated, arterial blood, at left, as opposed to the venous blood.

Figure 2. The foam trap.

The blood collecting in the foam trap was returned to the reservoir every 4 min via the drain tube at the left in the picture.

Figure 1. The oxygenation tower.

Figure 2. The foam trap.

and instant stunning in order to reduce struggling and subsequent clotting of the blood within the liver prior to exsanguination.

Treatment of the Blood

Exansguination was accomplished while the animals were extended vertically by means of a mechanical hoist. An incision was made in the throat and the carotid arteries were severed. Blood was collected in two plastic buckets. The blood was then continually stirred with wooden paddles until clotting occurred. The larger clots were then pressed free of residual blood and discarded. The partially defibrinated blood was then filtered through a series of three filters to complete the defibrination process. The first filter consisted of one layer of Chix-Brand Cheesecloth 406 (90). The second filter consisted of two layers of the aforementioned cheesecloth. The third filter consisted of one thickness of surgical nonabsorbent cotton over one thickness of the aforementioned cheesecloth. The filtered, defibrinated blood was then collected, the volume was measured and 6 liters were placed directly into the reservoir of the perfusion apparatus. The Roll-Flex "heart" pump was started and oxygenation of the blood was begun immediately.

Liver Excision and Preparation

Immediately after the blood collection, the animal was placed on the floor on its left side. An incision was made down the mid-line and the right side of the animal was lifted exposing the liver. Care was taken to leave as much of the portal vein and abdominal vena cava attached to the liver as possible. Total elapsed time from stunning to

excision was generally 4.5 min. Once excised, the liver was washed free of extraneous matter and weighed. The portal vein was then cannulated with a glass cannula and the liver was internally rinsed with approximately 10 liters of oxygenated physiological saline at 37.5 C.

During the rinsing operation the liver was gently massaged in the direction of blood flow to aid in the removal of residual blood. Any extraneous fat was removed from the surface of the liver and the posterior abdominal vena cava and bile duct were cannulated. All cannulae were made secure with hemostats. The liver was then drained of saline solution and placed into the perfusion apparatus and connected to the blood supply via the cannula in the portal vein. Each animal's liver was perfused with its own respective defibrinated blood.

Method of Sampling

After about 2 min had been allowed for the liver to clear any residual saline solution, the initial 50-ml blood sample was obtained from the venous outflow from the cannulated posterior abdominal vena cava. Thereafter, 50-ml blood samples were taken every 30 min for 2 hr. The small amount of residual saline solution which was still present in the liver was not considered to have significantly affected the results of the perfusions. A control "perfusion," cycling blood through the perfusion apparatus with no liver present, was performed. Samples were of the same size and taken at the same time intervals as those used in the eight perfusions, but the samples were taken from the arterial blood cannula which would have been placed in the portal vein of the liver.

Bile production was measured at 60 min intervals in the eight perfusions. Small blood samples were collected at initial time and after 30, 60, and 120 min of perfusion for blood glucose determinations. Also, hematocrit readings were obtained before defibrination, at initial time, after 60 min, and after 120 min of perfusion.

At the termination of each perfusion, the liver was dissected, as in Figure 3, and given an arbitrary perfusion score. The liver in Figure 3 was a typical resulting perfused and dissected liver. The caudate lobe, directly under the shoe in the picture, was consistently the most difficult area in which to achieve satisfactory perfusion, as evidenced by the light colorings in this area.

In Figure 4 the perfusing liver can be seen with the posterior abdominal vena cava, the portal vein, and the bile duct cannulated. Note the dark appearance of the venous blood as opposed to the oxygenated blood and the natural appearance of the liver, even after 2 hr of perfusion.

Centrifugation

After the post-perfusion blood samples were collected, they were placed in a coldroom at 0 C for at least 1 hr. They were then centrifuged at 15,000 rpm in a Servall Superspeed Centrifuge for 20 min. The serum was then pipetted into glass vials and approximately 1 mg of Merthiolate was added to retard any microbial growth. The vials were stoppered with plastic tops and stored in a refrigerator at 5 C until subsequent extraction. Extractions were made 1 day after the samples were collected.

Figure 3. Post-perfusion dissected liver.

Dissection of the post-perfusion livers proved them to be well perfused except for areas of the caudate lobe, which were noticeably lighter in color

Figure 4. The perfusing liver.

The cannulated portal vein, posterior abdominal vena cava and bile duct are shown. Note the dark color of the venous blood leaving the liver via the cannula in the posterior abdominal vena cava and the natural appearance of the liver after 2 hr of perfusion.

Figure 3. Post-perfusion dissected liver.

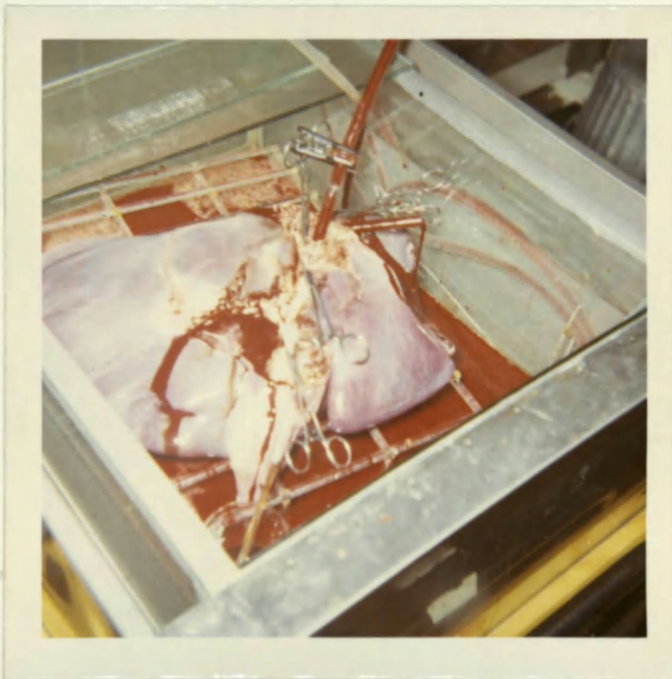

Figure 4. The perfusing liver.

Chemical Analyses

Although quantitative measurements of extracted lipid were obtained as a method of standardization of technique in thin-layer chromatography, this experiment was designed to study the qualitative effects calculated after gas-liquid chromatography. Analysis of one sample was not duplicated because of the time required to prepare one sample for gas-liquid chromatography and because previous samples had repeated well.

Lipid Extraction Procedure

Extractions were made on the day following sample collection by a modified method of that proposed by Folch, Lees, and Stanley (1957).

The extraction was carried out in the following manner:

1. A known volume of serum, usually 12 ml, was added to a one-pint Mason jar.
2. A mixture of chloroform-methanol (CM) 2:1 (v/v) was added at a ratio of 16 ml to 1 ml of serum.
3. The mixture was then blended at speed setting six for 4 min in a Sorvall Omni-Mixer.
4. The mixture was then filtered through Whatman #1 filter paper and poured into a 1000-ml separatory funnel.
5. The above steps were repeated until all of the serum from one sample was extracted. There were usually 25 ml of serum/sample.
6. The CM-lipid mixture was then washed with 5.5 ml of rinse solution/ml of serum. The rinse solution was prepared by dissolving

0.20 g of calcium chloride, 0.17 g of magnesium chloride, and 2.90 g of sodium chloride in 1000 ml of demineralized water.

7. The mixture was then allowed to stand in the separatory funnel for at least 24 hr.
8. The lower chloroform-lipid layer was then drained into a dessicated and tared 400-ml beaker.
9. The beakers were placed in a vacuum oven at 40 C and allowed to dry for at least 24 hr.
10. The beakers were dessicated for 1 hr, weighed, and the lipid was stored in glass vials in a known volume of CM 2:1.
11. The samples were placed in a freezer at -18 C until subsequent thin-layer chromatography.

Thin-Layer Chromatography of Lipid

The methods used in thin-layering the lipids were essentially those of Bowyer et al. (1963) with some modifications. All solvents used in this and following procedures were re-distilled except for chloroform. The procedure was carried out in the following manner:

1. A volume of CM-lipid mixture containing 40 mg of lipid was pipetted into a 15-ml centrifuge tube.
2. The solvent was evaporated under a stream of nitrogen and the lipid was transferred in chloroform to a 2-ml spotting tube.
3. The chloroform was evaporated under a stream of nitrogen down to a volume of about 200 ul.
4. The chloroform-lipid mixture was spotted on pre-activated thin-layer plates. The plates were 20 x 20 cm with a layer

thickness of 0.25 mm and designated Pre-Coated TLC Plates, Silica Gel F-254 (E. Merck A.G., Darmstadt, Germany). Spotting was accomplished using a Hamilton 100-ul syringe.

5. After spotting, the plates were placed in a glass tank with a solvent mixture containing light petroleum ether-diethyl ether-acetic acid (80:10:1 v/v) and allowed to develop until the solvent front reached the top of the plates.
6. The plates were then removed, the solvent on the plates allowed to evaporate, and then the plates were lightly sprayed with a 0.05 percent (w/v) solution of Rhodamine 6G in ethyl alcohol.
7. The lipid fractions were identified and marked under an ultraviolet light by the use of standards.
8. Each fraction was carefully scraped from the plate using a single-edged razor blade and placed in a 15-ml screw-top centrifuge tube for subsequent esterification.

Esterification

The lipid fractions were transesterified by a method derived from those of Bowyer et al. (1963) and Marchello, Dryden, and Ray (1968). The esterification procedure was as follows:

1. Transesterification was accomplished with the lipid fractions still on the silica gel. Two ml of benzene were added to the gel in the 15-ml centrifuge tubes. The whole serum lipid was not transesterified in the presence of gel since there was no previous thin-layering required.

2. Four ml of methylating solution were added and the tube was flushed with nitrogen gas and tightly capped.

3. The methylating solution was 5.0 percent (v/v) sulfuric acid in super-dry methanol. The super-dry methanol was prepared according to Marchello (1965).

4. The tubes were placed in a waterbath at 90 ± 5 C for 2.5 hr and stirred occasionally on a small Adams Cyclo-Mixer.

5. The tubes were then removed, cooled, and the contents washed with approximately 4 ml of distilled water. The esters were extracted with two portions (approximately 2 ml each) of light petroleum ether and removed to a 2-dram screw-top vial after subsequent centrifugation at 1500 rpm for 2 min.

6. The esters were stored at -18 C under nitrogen until they were thin-layered and subsequently analyzed by gas-liquid chromatography.

Thin-layer Chromatography of Esters

Thin-layering the esters was necessary to separate the methyl esters of the fatty acids from each fraction from any unwanted compounds that were soluble in the light petroleum ether. The most important of these unwanted compounds was cholesterol from the cholesterol ester fraction, but also important was a possible complex sugar derivative which was present in the triglyceride and free fatty acid fractions.

Essentially the same procedure was used in this thin-layer process as was used in the thin-layering of the lipids. The same solvent system was used. The band containing the esterified fatty acids

from each fraction was identified by use of a standard. The bands were scraped from the plates and placed in 15-ml centrifuge tubes and eluted once with light petroleum ether and once with diethyl ether. Centrifugation at 1500 rpm for 2 min followed each elution, and the esters in ether were stored in 2-dram vials under nitrogen at -18 C for subsequent gas-liquid chromatography.

Gas-liquid Chromatography

The esters were chromatographically separated using a Beckman GC-5 instrument equipped with a hydrogen-flame ionization detector. The instrument was fitted with a 1.83 m (3.2 mm O.D.) coiled stainless steel column packed with 100-120 mesh chromosorb W (HP) coated with diethylene glycol succinate (15 percent by weight). Instrument operating conditions were: initial column temperature, 170 C, and programmed nonlinearily to increase to 200 C over a 35-min span; detector temperature, 240 C; inlet temperature, 205 C; and carrier gas flow (nitrogen), 28 ml/min.

Identification of methyl esters was accomplished by comparison of the relative retention times with those of standard solutions of known composition. The weight percent of each ester was determined by computing its proportionate amount as measured by a disc integrator to the total area of the chromatograms.

Blood Glucose Determination

The method used for determining blood glucose levels, published in "Glucostat" (Worthington Biochemical Corp. 1968), is as follows:

1. Standard glucose solution: Five g of anhydrous dextrose was dissolved in 50 ml of 0.2 percent benzoic acid in water and diluted to 500 ml with distilled water in a volumetric flask.
2. 1:40 filtrate: Two-tenths ml of blood was added to 3.8 ml of water. This was mixed and 2.0 ml of barium hydroxide was added to the solution. Two ml of zinc sulfate was then added and mixed with the solution. The solution was then centrifuged at 1500 rpm for 2 to 3 min and filtered through Whatman #1 filter paper.
3. $Ba(OH)_2$ soltuion: Nineteen and one-tenth g of $Ba(OH)_2 \cdot 8H_2O$ was dissolved in distilled water and diluted to volume in a 2000-ml volumetric flask. This was filtered and stored in a bottle with a soda lime stopper.
4. $ZnSO_4$ solution: Twenty g of $ZnSO_4 \cdot 7H_2O$ was dissolved in distilled water and diluted to 2000 ml in a volumetric flask.
5. Procedure: To 2.0 ml of reagent (Glucostat[1] diluted to 50 ml) was added 2.0 ml of 1:40 filtrate. After exactly 10 min at 22 to 25 C, one drop of 4M HCl was added. The solution was allowed to stand for 5 min. The solution was then transferred to a cuvette and read in a Beckman Model B spectrophotometer set at 400 mu. Zero absorbance (O.D.) was set by the reagent blank.

Statistical Treatment of Data

Before applying statistical analysis, the changes in percent over time associated with the individual fatty acids were coded to

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1. Worthington Biochemical Corporation, Freehold, New Jersey.

positive values to simplify the analysis and interpretation of the results.

The changes in percent of the individual fatty acids associated with each fraction were tested for significance by means of a two-way analysis of variance by least-squares procedures (Harvey 1960). On those effects which were found to be significant ($P < .05$), the Duncan's New Multiple Range Test according to Li (1964) was applied to isolate these differences.

The statistical model for each fraction was:

$$Y_{ijk} = u + d_i + t_j + (dt)_{ij} + e_{ijk}$$

where:

u = overall mean of all observations in a fraction,

d_i = effect common to all observations in the $\underline{i}^{th}$ diet in a fraction,

t_j = effect common to all observations in the $\underline{j}^{th}$ time in a fraction,

$(dt)_{ij}$ = interaction between the $\underline{i}^{th}$ diet and the $\underline{j}^{th}$ time in a fraction,

e_{ijk} = random errors associated with livers from steers on the same diet and are assumed to be independent and normally distributed with a mean of zero and a common variance.

The form of the analysis of variance is presented in Table 2 and reveals the composition of the expected mean squares.

Table 2. Form of the analysis of variance showing composition of expected mean squares.

Source	d.f.	Composition of expected mean squares
Total	31	
Diets	3	$\sigma_e^2 + n_1 n_3 V_d$
Times	3	$\sigma_e^2 + n_1 n_2 V_t$
Diet x time	9	$\sigma_e^2 + n_1 V_{td}$
Error	16	σ_e^2

$n_1 = 2$ (livers)

$n_2 = 4$ (times)

$n_3 = 4$ (diets)

CHAPTER IV

RESULTS AND DISCUSSION

General

As mentioned in Chapter III, the steers used in this experiment were not taken off feed prior to slaughter. As the animals were fed at 7:00 am and 3:00 pm each day, the animals were all killed as near 11:00 am as possible so that standardization of the post-absorptive state at the time of death could be achieved.

The time which elapsed between stunning of the animals and initiation of perfusion for the eight perfusions in this experiment was 11.6 $\pm$ 1.6 min. As will be mentioned in later discussion, this total time of ischemia of the livers may have deleteriously affected some liver enzyme systems. Brauer et al. (1951) reported that a total of 3 min of ischemia did not seriously affect the physiology or function of rat livers. Although theoretically ischemic for about 12 min, after approximately 4.5 min had elapsed from the time of stunning, the livers in this experiment were internally rinsed with oxygenated physiological saline at 37.5 C until perfusion was initiated. This internal rinsing removed residual blood from the liver and aided in the prevention of vaso-constriction. It was believed that this rinsing also may have reduced the deleterious effects of the ischemia, provided the livers could utilize some of the oxygen in the physiological saline solution.

Bile production in the first 60 min of perfusion averaged 13 ml of bile formed, and the range was 5 to 23 ml. In the second 60 min of perfusion, the average production was 9.8 ml and the range was 9 to 10.5 ml. Martinis et al. (1958) reported that values of bile production ranged from 1.3 to 9.6 ml/hr for young calves' livers. Using liver size as a criterion for the amount of bile formed, the values for the larger livers in this experiment would have been expected to have been higher. However, Martinis et al. (1958) used blood flow rates averaging 0.60 ml/min/g of liver tissue, while the blood flow rates for this experiment averaged 0.13 ml/min/g of liver tissue. This may be one possible explanation for values which were lower, relative to liver size, than those of Martinis et al. (1958).

Differences in hematocrit values before defibrination and after 120 min of perfusion generally reflected a 9 percent reduction in the packed red blood cell volume as revealed in Table 3. A significant ($P < .05$) decrease in packed red blood cell volume due to the defibrination process was observed (Table 3) for the animals fed the 5 percent tallow diet. Nonsignificant decreases in packed red blood cell volume were evident for the animals fed the other diets. The significant decrease and the observed nonsignificant decreases were most likely due to the red blood cells discarded in the clots during the defibrination process. For each diet, significant decreases between the initial times and after 60 min of perfusion were revealed. The major source of this reduction in the packed red blood cell volume was most likely contributed by the action of the Roll-Flex "heart" pump causing the

Table 3. Mean hematocrit values.

Time of hematocrit	Means by diets (% tallow)			
	0	5	10	15
Before defibrination	$40.1^{a,xy}$ (2.9)[1]	$42.4^{a,x}$ (3.4)	$38.1^{a,y}$ (1.5)	$38.0^{a,y}$ (2.9)
0 min	$37.5^{a,x}$ (0.8)	$36.3^{b,x}$ (3.5)	$35.8^{a,x}$ (2.8)	$37.6^{a,x}$ (2.0)
60 min	$33.8^{b,x}$ (2.5)	$31.5^{c,x}$ (2.1)	$32.1^{b,x}$ (1.6)	$34.2^{b,x}$ (5.2)
120 min	$32.8^{b,x}$ (1.5)	$30.4^{c,xy}$ (0.2)	$28.1^{c,y}$ (3.0)	$32.7^{b,x}$ (2.3)

[1]Two values per mean, standard deviations are within parentheses.

[a,b,c]Means across times with different superscripts differ $P < .05$.

[x,y]Means across diets with different superscripts differ $P < .05$.

Table 4. Mean blood glucose levels.

Time	Means (mg/100 ml) by diets (% tallow)			
	0	5	10	15
0 min	$423.2^{a,x}$ (54.6)[1]	$221.7^{a,y}$ (151.7)	$204.1^{a,y}$ (39.7)	$202.2^{a,y}$ (0.8)
30 min	$564.5^{b,x}$ (13.1)	$345.0^{b,y}$ (197.8)	$418.3^{b,y}$ (77.2)	$215.8^{a,z}$ (16.7)
60 min	$634.6^{bc,x}$ (63.1)	$387.7^{b,y}$ (163.5)	$465.8^{b,y}$ (71.0)	$247.2^{a,z}$ (17.4)
120 min	$711.7^{c,x}$ (60.7)	$434.8^{b,y}$ (193.9)	$516.4^{b,y}$ (70.3)	$255.4^{a,z}$ (50.6)

[1]Two values per mean, standard deviations are within parentheses.

[a,b,c]Means across times with different superscripts differ $P < .05$.

[x,y,z]Means across diets with different superscripts differ $P < .05$.

disruption of the red blood cells and the subsequent release of the heme pigments. In Figure 5 are pictured vials of serum taken at initial time, after 30, 60, and 90 min, and at the end of 120 min of perfusion. Successive darkening of the sera caused by the rupturing of erythrocytes can be observed.

Data in Table 3 also indicated that the animals fed the 5 percent tallow diet exhibited a significantly ($P < .05$) higher initial packed red blood cell volume than the steers fed the 10 and 15 percent tallow diets. The steers fed the 0 percent tallow diet had a nonsignificantly greater percent packed red blood cell volume than those fed the 10 and 15 percent tallow diets. The animals fed the 10 percent tallow diet exhibited the lowest percent of packed red blood cell volume after 120 min of perfusion, but it was observed that the initial percent was significantly ($P < .05$) lower than that of the animals fed the 5 percent tallow diet. Diet by time interaction for the hematocrit mean values was not significant.

In Table 4 are reported the mean values of the blood glucose levels, by diet, present in the blood before and during the course of perfusion. The significant ($P < .05$) general twofold increase over time of perfusion of the blood glucose levels for the animals fed the 0, 5, and 10 percent tallow diets corresponded to the report of Connolly et al. (1964), who worked with goat livers. The fact that the livers from the steers fed the 15 percent tallow diet failed to exhibit the increase of blood glucose levels over time observed for the other diets may have been due to this diet influencing the amount of glycogen that was capable of being stored in the livers.

Figure 5. Post-perfusion serums.

Vials pictured illustrate the darkening effect of the sera caused by the rupturing of erythrocytes. Initial time serum is at the left.

Diet effects were observed for blood glucose levels (Table 4). The animals fed the 0 percent tallow diet exhibited significantly (P .05) higher levels of blood glucose over all times than those animals fed the 5, 10, and 15 percent tallow diets. The steers fed the 5 and 10 percent tallow diets contained significantly (P .05) higher levels of blood glucose than the steers fed the 15 percent tallow diet except at initial times.

The high standard deviations encountered in Table 4, particularly for the 5 percent tallow diet, can be explained. One steer fed this diet had a high initial blood glucose level (329.0 mg/100 ml) and this level increased to 571.9 mg/100 ml. The other steer had an initial blood glucose level of 114.4 mg/100 ml and increased to 297.7 mg/100 ml post-perfusion. Increases were consistent during each period of sampling. Diet by time interaction for blood glucose levels was not significant.

A control "perfusion," circulating blood through the perfusion apparatus with no liver present, was performed. Primarily, this was done to determine if any changes in fatty acid profiles of blood serum lipids would be due to handling or treatment of the blood or serum, or any effect produced by the perfusion apparatus. Secondarily, this was conducted as a check on the repeatability of extraction, esterification, and thin-layer and gas-liquid chromatography. The results of this "perfusion" are presented in Table 5. Although no statistical analysis was run on these data, the size of the standard deviations indicated that no change in the fatty acid percentages had occurred. The standard deviations were calculated from fatty acid percentages from five

Table 5. Mean percent values of fatty acids from pre-perfusion studies.

Fatty acid	Fraction				
	Whole serum	Phospholipids	Free fatty acids	Triglycerides	Cholesterol esters
C14	2.9(.29)[a]	0.5(.23)	4.3(.46)	4.9(.60)	2.3(.06)
IsoC15	0.3(.25)	0.0	0.4(.55)	0.0	1.2(.26)
C15	0.8(.15)	0.6(.22)	1.1(.61)	1.0(.37)	0.7(.07)
IsoC16	0.0	0.4(.35)	0.4(.56)	0.2(.26)	0.4(.30)
C16	18.1(.44)	17.8(.38)	22.9(1.40)	33.4(.97)	7.6(.22)
C16:1	8.0(.20)	2.5(.17)	6.0(.22)	5.2(.26)	18.1(.33)
C17	1.6(.23)	1.2(.09)	1.9(.15)	2.1(.15)	0.9(.22)
IsoC18	1.4(.09)	1.1(.09)	1.0(.24)	0.2(.23)	3.2(.79)
C18	17.0(.42)	24.7(.52)	20.6(.83)	24.8(.63)	0.7(.42)
C18:1	28.4(.52)	34.7(.78)	30.2(1.65)	26.3(.55)	20.1(.09)
C18:2	16.7(.09)	9.5(.34)	6.0(.52)	1.5(.52)	39.8(.89)
C20	0.2(.23)	0.0	0.0	0.0	1.2(.23)
C18:3	0.9(.28)	0.7(.29)	1.2(.36)	0.2(.25)	1.3(.14)
Unknown A	0.0	0.1(.22)	0.0	0.0	0.0
C22	1.2(.29)	2.7(.22)	1.4(.38)	0.0	0.0
C20:4	2.0(.22)	2.9(.27)	1.8(.58)	0.1(.22)	2.5(.51)
Unknown B	0.0	0.0	0.4(.94)	0.0	0.0
C24	0.5(.66)	0.5(.53)	0.3(.76)	0.0	0.0

[a]Five values per mean; standard deviations are within parentheses.

different samples, collected at initial time and after 30, 60, 90, and 120 min of "perfusion." Therefore, each sample was extracted, esterified, thin-layered, and gas chromatographed separately and at different times. Bowyer et al. (1963) reported standard errors of fatty acids that had been thin-layered and gas chromatographed. Conversion of the standard deviations in Table 5 to standard errors indicated the results in this experiment to be more consistent than those of Bowyer et al. (1963) except in the case of C18:1 in the free fatty acid fraction. In this case, the standard error derived from this experiment was slightly larger than that reported by those researchers.

Whole Serum Lipid

The average initial (pre-perfusion) percent of each major fatty acid of the whole serum lipid for each diet is given in Table 6. It was observed that the 15 percent tallow diet exhibited significantly ($P < .05$) more C18:1 and significantly less C18:2 in the whole serum than did the other diets. It was also evident that the 15 percent tallow diet exhibited, but not significantly, more C16 and C16:1 than did the other diets. The 5 percent tallow diet contained a nonsignificantly higher percent of C20:4 than the other diets.

As was evident from the analysis of variance of the whole serum lipid, the percent of C16:1 was highly influenced ($P < .01$) by time of perfusion (Table 17; see Appendix for Tables 17 through 21). It was evident from Table 6 that after 90 min of perfusion there was a significant ($P < .05$) increase in the percent of C16:1 and in the next 30 min there was a nonsignificant increase in the percent of this acid. A

Table 6. Least-square means indicating percent change of whole serum lipid major fatty acids over diets and time.

Fatty acid	Av. initial % of fatty acid by diets (% tallow)				Treatments: Diets (% tallow)				Treatments: Time interval				Over-all mean
	0	5	10	15	0	5	10	15	0/30[1]	30/60	60/90	90/120	
C14	1.7	2.0	1.8	2.1	-.06[2]	-.05	-.01	-.04	-.15	-.03	+.10	-.09	-.05
C15	1.3	1.3	0.8	1.0	-.05	-.05	+.01	-.04	-.06	-.34	+.06	+.21	-.03
C16	15.0	16.9	15.8	19.7	-.18	-.03	-.15	+.05	-.13	-.46	+.20	+.09	-.08
C16:1	3.7	3.7	4.1	7.3	-.11	-.04	+.01	+.04	-.05[b]	-.04[b]	+.24[a]	+.05[ab]	-.03
C17	1.7	1.6	1.6	1.5	+.01	-.06	.00	+.03	+.11	-.19	+.04	+.01	-.01
C18	18.8	12.9	14.7	16.0	+.15	-.03	-.04	+.16	+.51	-.24	-.15	+.13	+.06
C18:1	13.2[a]	16.0[a]	17.7[a]	33.9[b]	-.11	+.03	-.10	+.03	-.19	+.20	-.36	+.19	-.04
C18:2	35.4[a]	31.0[a]	35.7[a]	12.4[b]	+.24	+.36	-.26	-.14	+.51	+.13	-.23	-.21	+.05
C18:3	1.5	1.4	1.3	0.7	-.06	-.13	+.05	-.18	+.01	+.16	-.09	-.40	-.08
C22	2.2	2.4	1.8	1.5	+.09	.00	+.23	+.03	-.18	+.39	+.08	+.05	+.08
C20:4	3.7	7.3	2.2	2.6	+.19	+.18	+.20	+.10	-.18	+.69	-.16	+.31	+.17

[1] 0/30 represents percent change between initial value and value measured after 30 min of perfusion.

[2] Negative sign indicates the value measured after 30 min of perfusion was less than that at initial time.

[a,b] Means within major classes with different superscripts differ $P < .05$.

probable explanation for this increase in percent of C16:1 may have been the desaturation of C16 by the liver microsomal enzyme systems. A sizable decrease in C16 between 0 and 60 min of perfusion was noted, but this decrease was not significant. However, the correlation coefficient in Table 7 between C16 and C16:1 indicated a highly significant, but positive, relationship ($r = 0.60$) between the two acids.

Table 17 also revealed a significant ($P < .05$) diet by time interaction for C16:1. Between 0 and 30 min of perfusion the 10 and 15 percent tallow diets increased in percent of C16:1 while during the same time period the 0 and 5 percent tallow diets decreased in the percent of this acid (Table 16, see p. 59). Also contributing to this interaction was the fact that between 60 and 90 min of perfusion the 0 and 10 percent diets exhibited an increase in percent of C16:1 while the 5 and 15 percent diets showed little or no change. Between 90 and 120 min of perfusion, the 15 percent tallow diet increased in percent C16:1 whereas the other diets yielded little change in percent of this acid.

Table 7 revealed that the change in percent of C14 was highly related to C15 ($r = 0.68$) while the change in percent of C15 was negatively associated with C18:2 ($r = -.56$). The change in percent of C16 was highly correlated with C16:1 ($r = 0.60$) and C18 ($r = 0.72$) and highly negatively correlated with C22 ($r = -.82$) and C20:4 ($r = -.87$). The change in percent of C16:1 was related to C18 ($r = 0.50$), negatively related to C22 ($r = -.50$) and highly negatively associated with C20:4 ($r = -.69$). The change in percent C18 was highly correlated with

Table 7. Simple adjusted correlation coefficients between whole serum lipid major fatty acids.

Fatty acids	C15	C16	C16:1	C17	C18	C18:1	C18:2	C18:3	C22	C20:4
C14	0.68**	-.03	-.02	0.46	-.24	-.08	-.31	-.07	-.08	-.09
C15		-.04	-.17	0.26	0.02	0.38	-.56*	-.26	-.07	-.01
C16			0.60**	-.24	0.72**	0.33	0.06	-.13	-.82**	-.87**
C16:1				-.11	0.50*	0.06	0.01	0.06	-.50*	-.69**
C17					-.25	-.21	-.41	0.27	0.28	0.13
C18						0.80**	-.28	-.46	-.67**	-.59*
C18:1							-.47*	-.62**	-.44	-.26
C18:2								0.09	-.28	-.16
C18:3									0.35	0.07
C22										0.86**

* $P < .05$

** $P < .01$

C18:1 (r = 0.80), negatively related to C20:4 (r = -.59) and highly negatively associated with C22 (r = -.67). The change in C18:1 was negatively related to C18:2 (r = -.47) and highly negatively associated with C18:3 (r = -.62), whereas the change in C22 was highly correlated with C20:4 (r = 0.86).

Phospholipid Fraction

The average initial percent of each major fatty acid of the phospholipid fraction for each diet is given in Table 8. As in the whole serum lipid, the 15 percent tallow diet possessed significantly ($P < .05$) more C18:1 than the other diets and significantly less C18:2 than the 0 and 10 percent diets. The 0 percent tallow diet exhibited a nonsignificantly higher percent of C18 than the other diets and the 5 percent diet showed a higher percent of C20:4, though not significantly higher, than the other diets.

Analysis of variance for the phospholipid fraction in Table 18 revealed that the changes in percents of C16:1 and C18:2 were significantly ($P < .05$) affected by diet. From Table 8 it was evident that the 0 percent tallow diet contributed to a 5.03 percent decrease of C16:1. This result was somewhat spurious because the average initial percent of C16:1 was only 1.1 percent. This can be explained by the fact that one steer fed this diet failed to exhibit any C16:1 in this fraction while the other steer averaged 2.2 percent C16:1 in this fraction. Furthermore, the 0 percent tallow diet influenced a significant ($P < .05$) decrease in the percent of C18:2. This was interesting because the steers fed the 0 percent tallow diet exhibited the highest initial

Table 8. Least-square means indicating percent change of phospholipid fraction major fatty acids over diets and time.

Fatty acid	Av. initial % of fatty acid by diets (% tallow)				Treatments: Diets (% tallow)				Treatments: Time interval				Over-all mean
	0	5	10	15	0	5	10	15	0/30[1]	30/60	60/90	90/120	
C14	1.4	0.9	1.3	0.6	+ .05[2]	+ .06	+ .01	- .03	+ .10	- .06	- .05	+ .11	+ .03
C15	0.9	0.5	0.9	0.2	+ .08	- .05	+ .01	+ .24	- .03	+ .14	+ .03	+ .14	+ .07
C16	18.2	21.4	19.9	18.0	- .28	- .01	- .08	+ .21	+ .13	- .-6	+ .05	- .06	- .04
C16:1	1.1	1.7	2.1	2.6	-5.03[b]	-1.04[a]	+ .01[a]	- .09[a]	-1.46	-1.11	-1.24	-1.33	-1.28
C17	1.8	1.4	1.4	1.3	+ .20	- .01	+ .01	- .01	+ .09	- .05	.00	+ .15	+ .05
C18	27.00	23.9	23.0	23.3	+ .26	+ .14	+ .06	- .03	+ .41	+ .01	+ .11	- .10	+ .11
C18:1	13.4[a]	18.2[a]	22.7[a]	39.1[b]	+ .11	+ .23	+ .05	- .16	+ .16	- .16	+ .06	+ .16	+ .06
C18:2	25.1[a]	16.5[ab]	21.2[a]	7.1[b]	-1.60[b]	- .41[a]	- .43[a]	- .29[a]	- .95	- .83	- .43	- .53	- .68
C22	4.3	4.0	3.5	3.1	+ .39	+ .06	+ .09	+ .03	+ .16	+ .20	+ .06	+ .14	+ .14
C20:4	5.2	7.6	2.9	4.2	+ .74	- .01	+ .06	+ .01	+ .08	+ .41	+ .30	+ .01	+ .20

[1] 0/30 represents percent change between initial value and value measured after 30 min of perfusion.

[2] Plus sign indicates the value measured after 30 min of perfusion was greater than that measured at initial time.

[a,b] Means within major classes with different superscripts differ $P < .05$.

percent of this fatty acid in the phospholipid fraction. The preferential oxidation of C18:2 by the enzyme systems of the livers from the steers fed this diet may have been one cause for the significant decrease of C18:2, but a definite explanation for this effect was not clearly evident.

A significant ($P < .05$) diet by time interaction was observed for C18 (Table 18). From inspection of the data in Table 16 (p. 59), it was apparent that during every time interval the 0 and 5 percent tallow diets exhibited opposite effects. Between 0 and 30 min of perfusion the 5 percent tallow diet decreased in percent of C18 and the other diets increased in C18 during this time interval. Between 60 and 90 min of perfusion, the failure of the 10 and 15 percent tallow diets to exhibit a similar effect also contributed to the observed interaction.

Table 9 revealed that the change in percent of C14 was correlated with C18:1 ($r = 0.52$) and the change in percent of C16 was negatively correlated with C20:4 ($r = -.54$). The change in percent of C16:1 was related to C18:2 ($r = 0.57$) and negatively associated with C20:4 ($r = -.53$). The change in percent of C17 was negatively correlated with C18 ($r = -.55$) while that of C18:1 was negatively related to C22 ($r = -.53$). The change in percent of C18:2 was negatively associated with C22 ($r = -.55$), whereas C22 was positively correlated with C20:4 ($r = 0.57$).

Free Fatty Acid Fraction

The average initial percent of each major fatty acid of the free fatty acid fraction for each diet is given in Table 10. This

Table 9. Simple adjusted correlation coefficients between phospholipid fraction major fatty acids.

Fatty acids	C15	C16	C16:1	C17	C18	C18:1	C18:2	C22	C20:4
C14	-.24	-.05	0.12	-.15	0.12	0.52*	0.06	-.19	-.35
C15		-.25	0.17	0.17	-.42	-.41	0.25	-.10	-.13
C16			0.38	-.24	0.25	-.05	-.25	-.02	-.54*
C16:1				-.38	0.12	0.12	0.57*	-.28	-.53*
C17					-.55*	0.01	-.36	0.10	0.31
C18						0.21	-.02	-.37	-.29
C18:1							0.39	-.53*	-.35
C18:2								-.55*	-.31
C22									0.57*

* $P < .05$

Table 10. Least-square means indicating percent change of free fatty fraction major fatty acids over diets and time.

Fatty acid	Av. initial % of fatty acid by diets (% tallow)				Treatments								Over-all mean
					Diets (% tallow)				Time interval				
	0	5	10	15	0	5	10	15	0/30[1]	30/60	60/90	90/120	
C14	3.6	2.8	3.3	3.6	- .15[2]	+ .06	- .18	+ .03	- .09	- .03	+ .10	- .23	- .06
C15	1.2	1.2	1.1	1.1	.00	- .08	.00	+ .03	+ .14	- .19	+ .06	- .06	- .01
C16	22.2	23.8	22.2	22.4	- .75	- .48	- .75	- .33	-1.73	- .45	- .39	+ .26	- .58
C16:1	5.0	4.7	4.7	5.2	- .21	- .06	- .10	+ .10	- .10	- .24	- .29	+ .35	- .07
C17	1.7	2.0	1.6	1.8	+ .20	- .03	+ .03	+ .05	+ .35[a]	- .16[ab]	- .29[b]	+ .35[a]	+ .06
C18	25.4	23.1	23.7	21.1	+2.08	+1.31	+1.78	+ .64	+6.23[a]	+1.05[b]	+ .11[bc]	-1.59[c]	+1.45
C18:1	28.1	33.1	28.9	35.3	-1.76	-2.23	-1.41	-1.21	-6.20[b]	- .15[a]	+ .24[a]	- .50[a]	-1.65
C18:2	7.4	6.8	9.7	4.7	+ .64	+ .81	+ .19	+ .01	+ .44	+ .45	+1.08	- .31	+ .41
C22	1.8	0.6	1.4	1.6	+ .01	+ .16	+ .03	.00	+ .58[a]	- .59[b]	- .16[b]	+ .38[a]	+ .05
C20:4	3.0[a]	1.2[b]	1.8[b]	2.1[ab]	+ .10	+ .59	- .01	+ .30	+ .33	- .21	+ .20	+ .66	+ .24

[1] 0/30 represents percent change between initial value and value measured after 30 min of perfusion.

[2] Negative sign indicates the value measured after 30 min of perfusion was less than that at initial time.

[a,b,c] Means within major classes with different superscripts differ $P < .05$.

fraction clearly exhibited the least variation of fatty acid percentages among the diets. It can be observed, however, that the 0 percent tallow diet possessed significantly ($P < .05$) more C20:4 than did the 5 and 10 percent diets.

Time significantly affected the change in percent of C17 ($P < .05$) and C22 ($P < .01$) as shown in Table 19. However, these acids were minor in total fatty acid percentage. Furthermore, since both of these acids initially increased, then decreased, only to increase in percent again, it would seem that some extraneous error that followed no pattern may have influenced the significance encountered. It can be understood that fatty acids of low concentration were difficult to measure accurately.

By far the most highly significant change over time of perfusion in the free fatty acid fraction, and other fractions, occurred between C18 and C18:1 (Table 19). Figure 6 diagrams the relationship between these two acids during the progress of the perfusions. Between 0 and 30 min of perfusion, there was a significant ($P < .05$) increase, 6.23 percent, in C18 and between 90 and 120 min of perfusion, there was a significant ($P < .05$) decrease in the percent of C18 (Table 10). As opposed to the increase in percent of C18, the same magnitude of decrease, 6.20 percent, was observed for C18:1 between 0 and 30 min of perfusion.

It would appear from these data that a saturase enzyme system was actively saturating C18:1 to C18. This effect was in direct contrast to the reported enzyme desaturase systems in rat liver (Nakagawa and Uchiyama 1969b).

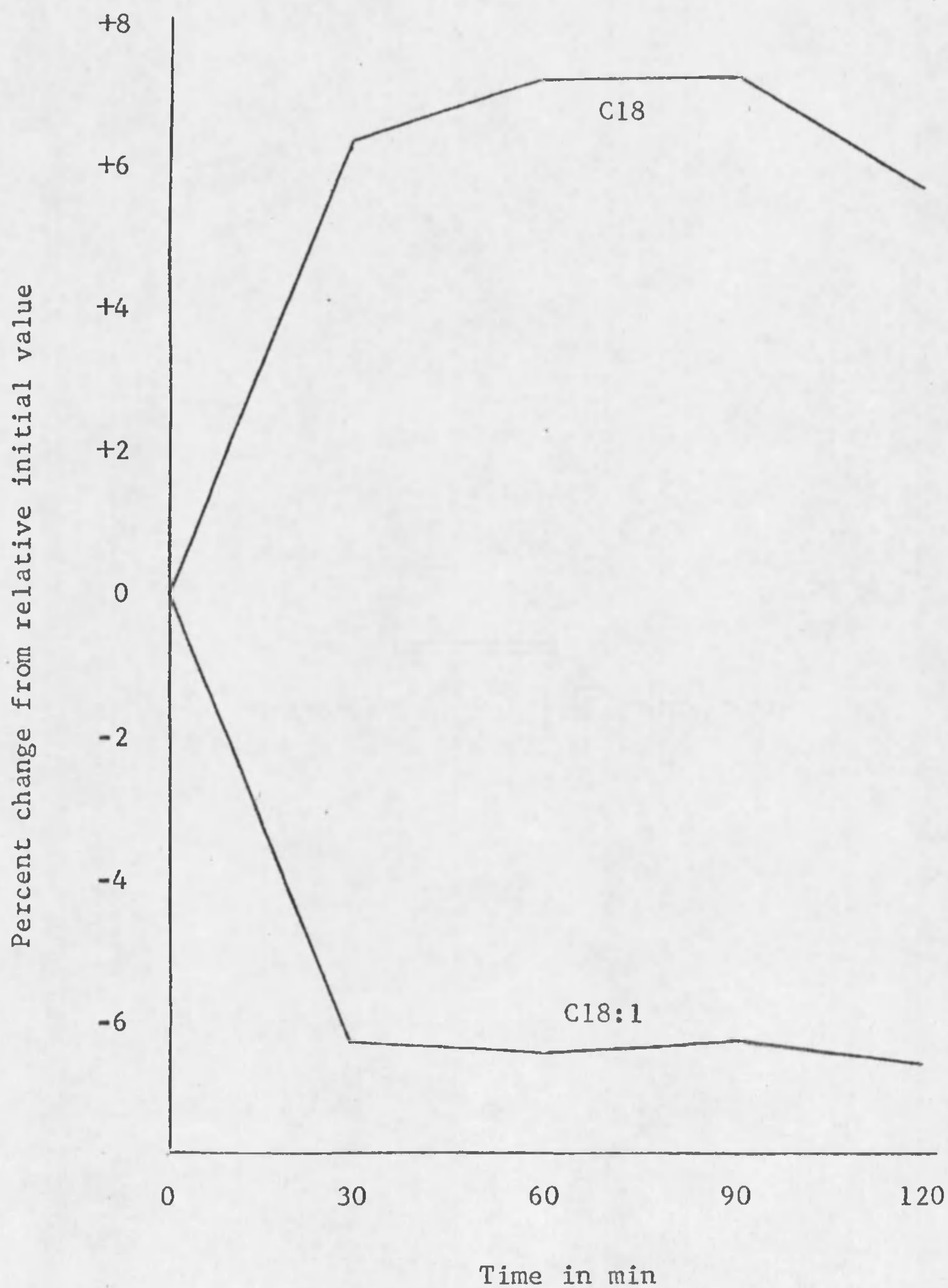

Figure 6. The free fatty acid C18 vs. C18:1 relationship.

This graphic illustration indicates that after 30 min of perfusion the decrease in C18:1 was accounted for by the increase in C18.

Table 19 revealed a significant ($P < .01$) diet by time interaction for C18. From inspection of the data in Table 16 (p. 59), it was evident that the 0 and 15 tallow diets decreased in C18 percent between 60 and 90 min of perfusion, whereas the 5 and 10 percent tallow diets exhibited an increase of C18 during this time interval. Between 90 and 120 min of perfusion the 0 and 5 percent diets revealed decreases in the percent of C18, but the other two diets exhibited little or no change in percent of this acid, thus also contributing to the observed interaction.

Data in Table 11 revealed that the change in percent of C14 was negatively associated with C18:1 ($r = -.53$) and highly negatively associated with C18 ($r = -.64$). The change in percent of C16 was highly correlated with C16:1 ($r = 0.64$), negatively related to C18 ($r = -.51$) and C18:2 ($r = -.53$) and highly negatively associated with C20:4 ($r = -.71$). The change in percent of C16:1 was negatively correlated with C18 ($r = -.50$) and C18:1 ($r = -.54$), while that of C17 was associated with C18 ($r = 0.50$) and negatively related to C18:2 ($r = -.58$).

As mentioned in the general discussion of results, hematocrit values were significantly reduced at the end of 30 min of perfusion. This factor may have accounted for the significant decrease in the percent of C18 between 90 and 120 min of perfusion due to the loss of oxygen transport ability and possible retardation effect upon the enzyme system which appeared to be saturating C18:1 to C18 in the livers. Another reason for the terminal decrease may have been due to a limiting substrate (C18:1) concentration, although this would seem not to have

Table 11. Simple adjusted correlation coefficients between free fatty acid fraction major fatty acids.

Fatty acids	C15	C16	C16:1	C17	C18	C18:1	C18:2	C22	C20:4
C14	0.23	0.45	0.33	-.24	-.64**	-.53*	-.14	0.09	-.04
C15		0.00	-.07	-.19	0.15	-.43	-.40	0.11	-.06
C16			0.64**	-.04	-.51*	-.23	-.53*	-.15	-.71**
C16:1				-.01	-.50*	-.54*	-.43	-.25	-.32
C17					0.50*	-.11	-.58*	0.27	0.12
C18						0.18	-.20	-.16	0.16
C18:1							0.32	-.20	-.17
C18:2								0.20	0.28
C22									0.46

* $P < .05$

** $P < .01$

been the case. A third possibility for the decrease may have been a feedback inhibition of C18 upon the saturase reaction.

The major changes in the free fatty acid fraction were in agreement with the observations of Mayes (1969), which had been verified by other researchers. However, saturation and not esterification rate seemed to be the cause of the C18 increase in percentage, because no time effect for C18 or C18:1 was observed in the triglyceride or phospholipid fractions.

Triglyceride Fraction

The average initial percent of each major fatty acid of the triglyceride fraction for each diet is listed in Table 12. The steers fed the control diet exhibited significantly ($P < .05$) less C16 in this fraction than those fed the other diets. The steers fed the control diet also exhibited significantly more C18 than those fed the 15 percent tallow diet. As opposed to the phospholipid fraction, nonsignificant differences were observed for the percent of C18:1.

From Table 20 the change in percent of C15 was affected by time of perfusion ($P < .05$) and also reflected a diet by time interaction ($P < .01$). Table 12 revealed that this was a minor acid and that the change over time did not follow a pattern, indicating that the change may have been due to an extraneous error, as discussed previously. This type of error could also have accounted for the significant interaction.

It was also evident from Table 20 that there was a significant ($P < .05$) diet by time interaction for C16:1. Between 0 and 30 min of

Table 12. Least-square means indicating percent change of triglyceride fraction major fatty acids over diets and time.

Fatty acid	Av. initial % of fatty acid by diets (% tallow)				Treatments								Over-all mean
					Diets (% tallow)				Time interval				
	0	5	10	15	0	5	10	15	0/30[1]	30/60	60/90	90/120	
C14	2.9	4.5	4.0	5.8	.00	- .14[2]	+ .25	- .01	- .19	- .01	+ .25	+ .05	+ .03
C15	1.7	1.7	1.1	1.4	- .04	- .06	+ .08	+ .13	+ .01[ab]	- .10[b]	+ .01[ab]	+ .18[a]	+ .03
C16	22.3[a]	29.6[b]	30.5[b]	31.9[b]	.00	- .29	+ .55	+ .44	+ .10	+ .70	+ .43	- .53	+ .18
C16:1	5.5	5.3	4.7	7.2	+ .19	+ .16	+ .28	+ .08	+ .10	.00	+ .65	- .05	+ .18
C17	1.9	2.0	1.8	2.2	+ .18	+ .18	+ .06	+ .09	+ .06	+ .01	+ .04	+ .39	+ .13
C18	37.8[a]	21.5[ab]	28.9[ab]	16.9[b]	- .01	+ .11	- .03	- .18	- .01	.00	- .18	+ .09	- .03
C18:1	20.9	30.0	23.1	30.4	-1.51	- .61	-1.10	- .40	- .75	- .61	-1.54	- .73	- .91
C18:2	5.4	4.3	3.6	2.3	+ .14	+ .05	- .46	- .01	+ .05	+ .25	- .66	+ .08	- .07

[1] 0/30 represents percent change between initial value and value measured after 30 min of perfusion.

[2] Negative sign indicates the value measured after 30 min of perfusion was less than that at initial time.

[a,b] Means within major classes with different superscripts differ $P < .05$.

perfusion, the 5 and 10 percent tallow diets exhibited an increase in the percent of C16:1 while during this time interval the 0 and 15 percent tallow diets decreased in the percent of this acid. Between 30 and 60 min of perfusion the 5 percent diet showed a decrease of this acid whereas the other diets exhibited increases in percent of C16:1. Between 90 and 120 min of perfusion, the triglyceride fraction of the 10 and 15 percent tallow diets decreased in the percent of C16:1; however, the 0 and 5 percent diets exhibited little or no change in the percent of this acid during this time interval (Table 16).

The change in percent of C16 was related to C18 ($r = 0.53$) and highly negatively associated with C18:2 ($r = -.90$) (Table 13). The change in percent of C16:1 was related to C17 ($r = 0.58$) and C18:2 ($r = 0.48$) and highly negatively correlated with C18 ($r = -.66$). Furthermore, the change in percent of C17 was negatively associated to C18 ($r = -.51$) while that of C18 was highly negatively related to C18:2 ($r = -.67$).

Although quantification of serum lipid fractions was not achieved in this experiment, the results of experiments by other researchers can be extrapolated to the data presented for this experiment. Hillyard et al. (1959), Havel and Goldfien (1961), and Mayes (1969) reported that in rat liver perfusions the amount of triglyceride in the post-perfusion blood was significantly greater than in the pre-perfusion blood. It would, therefore, seem that the triglyceride fraction fatty acids in this experiment would have tended to show higher C18 and lower C18:1 levels post-perfusion than at initial time. This

Table 13. Simple adjusted correlation coefficients between triglyceride fraction major fatty acids.

Fatty acids	C15	C16	C16:1	C17	C18	C18:1	C18:2
C14	0.26	0.18	0.05	0.08	0.10	-.33	-.29
C15		-.06	0.21	0.18	-.21	-.41	-.07
C16			-.41	-.21	0.53*	0.02	-.90**
C16:1				0.58*	-.66**	-.44	0.48*
C17					-.51*	-.32	0.27
C18						-.06	-.67**
C18:1							0.19

* $P < .05$

** $P < .01$

was not the case! The failure to observe changes in the triglyceride and phospholipid fraction fatty acids C18 and C18:1 may have been due to the fact that the time of ischemia destroyed enzymes involved in the esterification reaction.

Cholesterol Ester Fraction

The average initial percent of each major fatty acid of the cholesterol ester fraction for each diet is revealed in Table 14. The animals fed the 15 percent tallow diet exhibited significantly ($P < .05$) higher percentages of C16:1, IsoC18, and C18:1 than those fed the other diets, and significantly less C18:2 than those fed the 0 and 10 percent tallow diets.

The only significant treatment effect found in the cholesterol ester fraction fatty acids was a diet by time interaction for C18:3 ($P < .05$) (Appendix, Table 21). From the data in Table 16 it was evident that between 0 and 30 min of perfusion the 0 and 5 percent tallow diets decreased in the percent of C18:3 while the 10 and 15 percent diets contributed to an increase in the percent of this acid during this time interval. Between 30 and 60 min of perfusion, the 15 percent tallow diet exhibited a decrease in the percent of C18:3 while for the other diets increases were evident. Between 60 and 90 min of perfusion, the C18:3 content of the 0 and 10 percent tallow diets decreased, whereas the other two diets contributed to an increase in the percent of this acid. Between 90 and 120 min of perfusion the 0 and 5 percent tallow diets contributed to a decrease in C18:3 while the 15 percent tallow

Table 14. Least-square means indicating percent change of cholesterol ester fraction major fatty acids over diets and time.

Fatty acid	Av. initial % of fatty acid by diets (% tallow)				Treatments								Over-all mean
					Diets (% tallow)				Time interval				
	0	5	10	15	0	5	10	15	0/30[1]	30/60	60/90	90/120	
C14	1.3	1.6	1.8	2.1	-.05[2]	-.01	-.01	-.06	+.25	-.43	+.13	-.09	-.03
IsoC15	0.5	1.1	2.0	1.3	+.06	.00	+.04	-.04	+.14	-.16	+.10	-.01	+.02
C15	0.9	0.8	0.6	1.0	+.01	-.04	-.03	+.01	+.04	+.11	-.15	-.04	-.01
C16	7.3	9.3	6.9	10.6	-.14	+.05	-.19	-.19	-.01	-.63	.00	+.18	-.12
C16:1	4.8[a]	5.9[a]	6.6[a]	15.4[b]	-.16	-.09	-.01	+.18	+.14	-.14	-.04	-.05	-.02
IsoC18	0.5[a]	1.2[a]	0.9[a]	4.4[b]	-.01	.00	+.04	-.16	+.05	-.19	+.13	-.13	-.03
C18	0.8	0.7	0.6	0.9	-.10	-.04	-.04	-.01	+.08	-.36	+.04	+.06	-.05
C18:1	7.5[a]	10.8[a]	9.5[a]	23.8[b]	-.13	+.06	+.11	+.06	+.08	+.01	+.11	-.09	+.03
C18:2	67.7[a]	55.9[ab]	63.8[a]	33.0[b]	+.74	+.64	+.30	+.11	+.19	+.06	-.28	+.81	+.45
C20	2.5	1.8	1.8	0.9	-.03	-.04	.00	+.08	-.15	+.38	-.08	-.14	.00
C18:3	2.7	2.3	2.0	0.9	-.25	-.18	-.03	+.15	-.05	+.08	-.10	-.23	-.08
C20:4	3.2	6.1	2.8	5.0	+.19	-.15	-.23	-.11	-.21	+.08	-.05	-.11	-.08

[1] 0/30 represents percent change between initial value and value measured after 30 min of perfusion.

[2] Negative sign indicates the value measured after 30 min of perfusion was less than that at initial time.

[a,b] Means within major classes with different superscripts differ $P < .05$.

diet increased and the 10 percent tallow diet exhibited no change in the percent of C18:3.

Table 15 revealed that the change in the percent of C14 was correlated with C16 (r = 0.52) and highly correlated with C15 (r = 0.77) and C18 (r = 0.70) while being negatively related to C20 (r = -.50) and C18:3 (r = -.47). The change in percent of IsoC15 was correlated only to IsoC18 (r = 0.55) whereas that of C15 was associated with C18 (r = 0.51). The change in percent of C16 was highly related to C18 (r = 0.69), negatively related to C18:3 (r = -.47) and highly negatively correlated with C18:2 (r = -.79). The change in percent of C16:1 was negatively associated with C18:2 (r = -.55) while that of IsoC18 was correlated with C20 (r = 0.54). Furthermore, the change in percent of C18 was highly negatively related to C18:2 (r = -.71) and C18:3 (r = -.66). In general, the relationships among the saturated fatty acids which were significant in this fraction were positive, while the relationships between the saturated and unsaturated fatty acids were negative.

Conclusions

This experiment was designed to investigate the function of the liver in the qualitative changes in the major fatty acids of the serum lipid and serum lipid fractions. The steers used in this experiment were fed four levels of tallow in diets for a 170-day feeding period. It was believed that, if liver enzymes systems were responsible for any changes in the fatty acid profiles in the serum, the 170-day feeding

Table 15. Simple adjusted correlation coefficients between cholesterol ester fraction major fatty acids.

Fatty acids	IsoC15	C15	C16	C16:1	IsoC18	C18	C18:1	C18:2	C20	C18:3	C20:4
C14	-.08	0.77**	0.52*	0.12	-.44	0.70**	0.21	-.42	-.50*	-.47*	0.27
IsoC15		-.35	0.36	0.46	0.55*	0.05	0.00	-.33	-.31	-.31	-.28
C15			0.30	-.29	-.42	0.51*	-.03	-.17	-.26	-.26	0.20
C16				0.37	0.16	0.69**	0.21	-.79**	-.12	-.47*	-.05
C16:1					0.42	0.36	0.04	-.55*	0.09	-.22	-.27
IsoC18						-.09	-.37	-.41	0.54*	0.31	-.36
C18							0.37	-.71**	-.04	-.66**	0.17
C18:1								-.26	-.20	-.44	0.23
C18:2									-.16	0.27	-.14
C20										0.37	-.19
C18:3											-.06

* $P < .05$

** $P < .01$

Table 16. Significant time by diet interactions.

Time x diet		Means					
Time interval	Diet (%tallow)	WS C16:1	PL C18	FFA C18	TG C15	TG C16:1	CE C18:3
0/30	0	-.65[c][1]	+ .65[ab]	+11.65[a]	-.05[b]	- .40	- .65[b]
30/60	0	-.15[b]	- .50[bc]	+ 1.70[b]	-.15[b]	+ .65	+ .40[a]
60/90	0	+.45[a]	+1.90[a]	- .50[b]	+.35[a]	+ .10	- .20[ab]
90/120	0	-.10[b]	-1.00[c]	- 4.55[c]	-.30[b]	+ .40	- .55[b]
0/30	5	-.05	- .35[ab]	+ 4.65[a]	-.40[c]	+ .55[ab]	- .45
30/60	5	-.15	+ .55[ab]	+ .10[bc]	-.05[b]	-1.25[c]	+ .10
60/90	5	.00	- .60[b]	+ 2.20[ab]	-.30[bc]	+1.30[a]	+ .25
90/120	5	+.05	+ .95[a]	- 1.70[c]	+.50[a]	+ .05[b]	- .60
0/30	10	+.25[a]	+ .50	+ 5.95[a]	+.05	+ .55[ab]	+ .35[a]
30/60	10	-.50[b]	- .35	+ .55[b]	+.20	+ .25[ab]	+ .55[a]
60/90	10	+.45[a]	+ .20	+ .60[b]	+.05	+ .70[a]	-1.00[b]
90/120	10	-.15[b]	- .10	.00[b]	.00	- .40[b]	.00[a]
0/30	15	+.25[a]	+ .85[a]	+ 2.65[a]	+.45[a]	- .30	+ .55[a]
30/60	15	-.55[b]	+ .35[ab]	+ 1.85[a]	-.40[c]	+ .35	- .75[b]
60/90	15	+.05[a]	-1.05[b]	- 1.85[b]	-.05[b]	+ .50	+ .55[a]
90/120	15	+.40[a]	- .25[ab]	- .10[ab]	+.50[a]	- .25	+ .25[a]

[1]Means within a diet group in a column with different superscripts differ at $P < .05$.

period would be of sufficient length for differentiation of liver enzyme systems caused by the diets.

As previously discussed, the only diet effect on liver function in relation to changes in fatty acid percent was observed in the phospholipid fraction for C16:1 and C18:2. The diet effect on liver function for the change in percent of C16:1 was evident, but an explanation for the decrease in C18:2 caused by the control diet was not apparent. High levels of C18:2 in blood lipids were reported by Moore, Noble, and Steele (1968), Leat and Hall (1968), and Moore, Steele, and Noble (1969). As in this experiment, the greatest percentage of the C18:2 was esterified in the phospholipid and cholesterol ester fractions. The amount of C18:2 present in depot and intramuscular fat has been reported to be very low in total fatty acid percentage by Shorland (1953), Hornstein et al. (1961), Ostrander and Dugan (1962), O'keefe et al. (1968), and Dryden and Marchello (1970). Since no diet effects for the 5, 10, and 15 percent tallow diets were significant, it can be concluded that diet effect on liver function was minimal in relation to all of the fatty acids studied. It can also be concluded that the liver, as studied in this experiment, is not the major source or cause of the reduction of the percent of C18:2 of serum lipids before the deposition of these lipids in adipose tissue and intramuscular fat.

Data revealed that the percent of tallow in the diet was responsible for some effect which significantly reduced the percent of C18:2 in the whole serum lipid and the phospholipid and cholesterol ester fractions of the steers fed the 15 percent tallow diet. This

reduction in the percent of C18:2 was apparently accounted for at some body site other than the liver. Adipose tissue can account for a large percentage of synthesized fatty acids under experimental conditions (O'hea and Leveille 1969), and this tissue site may be very active in desaturating C18:2 prior to its deposition, if desaturation is the reason for its low concentration in storage lipids.

The fatty acid percentage of constituent lipids of microorganisms in the rumen could account for some of the serum lipid differences reported for this and other experiments. Tove and Matrone (1962) reported definite morphological differences in microorganisms in the rumen of sheep fed purified diets as opposed to those fed hay-grain or grazing diets.

Blood glucose levels were measured during the course of each perfusion. The levels at initial time were much higher than those found normally in cattle, and this fact was attributed to pre-slaughter excitation. During perfusion, significant increases in blood glucose levels were revealed, suggesting that glucose was available for catabolism for energy. It was concluded that most likely no more fatty acid catabolism for energy occurred for the *in vitro* livers in this experiment than for *in vivo* livers. The tallow levels in the diets may have affected the amount of glycogen that was capable of being stored in the livers. Also, if the steers fed the higher percent tallow diets oxidized a larger relative percentage of fatty acids for energy utilization, a larger relative percent of glycogen would have been degraded to prevent ketone formation. Both explanations could account for the diet

effect observed for the blood glucose levels at initial time and during perfusion.

The most significant time effect in the perfusions of the livers was the increase in percent of C18 and the decrease in percent of C18:1 in the free fatty acid fraction. This increase in C18 percentage appeared to have been the result of a saturase enzyme system. This saturation effect seemed to have been a true effect because it was apparent for all eight livers in this experiment.

The physical size of the steers, the nature of the excision, and the required pre-treatment of the blood made it extremely difficult to initiate perfusion in less than 12 min after stunning of the steers. The fact that the livers were ischemic during this period may have adversely affected some of the enzyme systems which could have influenced the results of this experiment. However, the effects caused by ischemia were probably reduced due to the internal rinsing procedure using oxygenated physiological saline. Although changes in fatty percentages were observed during the 2 hr which constituted each perfusion period, lengthening the perfusion times could have produced greater changes in these percents. It appears, however, that some other tissue site, most likely adipose tissue, is the source of the major qualitative changes not accounted for by liver function found between serum lipids and depot lipids.

CHAPTER V

SUMMARY

The livers from eight steers fed 0, 5, 10, and 15 percent tallow levels in diets were perfused with the defibrinated blood from the same animal from which the liver came. The perfusions of the livers and the operation of the perfusion apparatus were judged to have been good.

The percent changes of each major fatty acid of the whole serum lipid and the serum lipid fractions were coded to positive values to simplify the analysis of variance and the interpretation of the results. The only significant effect of diet was observed in the phospholipid fraction for the fatty acids C16:1 and C18:2. The 0 percent tallow diet caused a significant ($P < .05$) decrease in the percent of both acids over all time intervals. An explanation for the reduction in percent of C16:1 was evident, but an explanation for the decrease in percent of C18:2 by this diet in this fraction was not clearly evident.

The most significant change ($P < .01$) over time of perfusion occurred in the free fatty acid fraction between C18 and C18:1. During the first 30 min of perfusion a 6.2 percent increase in C18 corresponded to a 6.2 percent decrease in C18:1. The correlation coefficient for the change in percent between the two acids between 0 and 30 min of perfusion was highly significant ($r = 0.76$). A saturase enzyme system

appeared to be the most plausible explanation for the relationship of these two acids.

Since no significant changes in percent of either C18 or C18:1 in the triglyceride fraction or phospholipid fraction were observed, it was apparent that the esterification rate was not responsible for the time effect observed for these two acids in the free fatty acid fraction. The possibility of enzyme destruction by the total time of ischemia may have been one explanation for not having observed any significant C18 or C18:1 changes in the triglyceride or phospholipid fraction.

Other minor changes in the percent of specific fatty acids were observed in the lipid fractions and the whole serum lipid. Some of these changes may have been spurious because of the greater degree of error associated with minor acids caused by the lack of chromatogram peak magnitude.

Correlations between the change in percent among fatty acids in each fraction were observed. Correlations of fatty acids which revealed major changes affected by treatments were specifically discussed in relation to the changes observed.

APPENDIX

MEAN SQUARES

Table 17. Mean squares for whole serum lipid major fatty acids.

Fatty acid	Source of variation			
	Diet 3[a]	Time 3	Diet x time 9	Error 16
C14	.004	.091	.086	.106
C15	.007	.435	.128	.169
C16	.090	.679	.746	.641
C16:1	.035	.461**	.221*	.059
C17	.012	.131	.061	.045
C18	.094	.911	.337	.417
C18:1	.046	.627	1.097	.863
C18:2	.708	.971	1.505	1.816
C18:3	.075	.453	.346	.168
C22	.081	.428	.376	.331
C20:4	.016	1.380	1.200	.664

[a] Degrees of freedom

* $P < .05$

** $P < .01$

Table 18. Mean squares for phospholipid fraction major fatty acids.

Fatty acid	Source of variation			
	Diet 3[a]	Time 3	Diet x time 9	Error 16
C14	.013	.071	.087	.068
C15	.122	.054	.157	.064
C16	.323	.228	.608	.338
C16:1	49.764*	.174	.063	12.440
C17	.084	.064	.064	.121
C18	.119	.387	1.878*	.612
C18:1	.212	.188	.702	.290
C18:2	3.032*	.488	.713	.578
C22	.222	.027	.176	.158
C20:4	1.035	.283	.559	.456

[a] Degrees of freedom

* $P < .05$

Table 19. Mean squares for free fatty acid fraction major fatty acids.

Fatty acid	Source of variation			
	Diet	Time	Diet x time	Error
	3[a]	3	9	16
C14	.116	.146	.234	1.112
C15	.015	.163	.144	.174
C16	.357	5.533	4.214	3.593
C16:1	.134	.674	.982	.696
C17	.075	.903*	.353	.236
C18	3.134	90.603**	14.361**	3.450
C18:1	1.576	74.234**	4.465	3.063
C18:2	1.123	2.578	2.257	3.243
C22	.046	2.221**	.527	.341
C20:4	.554	1.045	.792	.389

[a] Degrees of freedom

* $P < .05$

** $P < .01$

Table 20. Mean squares for triglyceride fraction major fatty acids.

Fatty acid	Source of variation			
	Diet 3[a]	Time 3	Diet x time 9	Error 16
C14	.211	.261	.454	.785
C15	.064	.103*	.253**	.030
C16	1.211	2.223	1.788	1.448
C16:1	.054	.833	.895*	.299
C17	.028	.248	.415	.241
C18	1.994	1.445	2.802	2.411
C18:1	.573	1.304	3.722	1.915
C18:2	.781	1.206	1.977	2.811

[a] Degrees of freedom

* $P < .05$

** $P < .01$

Table 21. Mean squares for cholesterol ester fraction major fatty acids.

Fatty acid	Source of variation			
	Diet 3[a]	Time 3	Diet x time 9	Error 16
C14	.005	.698	.625	.288
IsoC15	.015	.145	.116	.107
C15	.005	.100	.155	.225
C16	.102	.981	.412	.373
C16:1	.168	.106	.399	.327
IsoC18	.062	.171	.174	.128
C18	.011	.356	.089	.127
C18:1	.088	.061	.547	.670
C18:2	.678	2.936	3.310	3.989
C20	.020	.500	.613	.308
C18:3	.250	.123	.792*	.239
C20:4	.263	.116	.632	.384

[a] Degrees of freedom

* $P < .05$

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