Iowa State University
Digital Repository

Retrospective Theses and Dissertations

Iowa State University Capstones, Theses and Dissertations

1979

Diurnal variations in the activity of enzymes regulating lipid metabolism and in tissue lipid levels of adult rats fed different fat sources and cholesterol

Laura Rypstat Richards
Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/rtd

Part of the Animal Sciences Commons, Biochemical Phenomena, Metabolism, and Nutrition Commons, and the Nutrition Commons

Recommended Citation
Richards, Laura Rypstat, "Diurnal variations in the activity of enzymes regulating lipid metabolism and in tissue lipid levels of adult rats fed different fat sources and cholesterol" (1979). *Retrospective Theses and Dissertations*. 7306.
https://lib.dr.iastate.edu/rtd/7306

This Dissertation is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.

المنارة للاستشارات

www.manaraa.com

INFORMATION TO USERS

This was produced from a copy of a document sent to us for microfilming. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help you understand markings or notations which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure you of complete continuity.

2. When an image on the film is obliterated with a round black mark it is an indication that the film inspector noticed either blurred copy because of movement during exposure, or duplicate copy. Unless we meant to delete copyrighted materials that should not have been filmed, you will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., is part of the material being photographed the photographer has followed a definite method in "sectioning" the material. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again—beginning below the first row and continuing on until complete.

4. For any illustrations that cannot be reproduced satisfactorily by xerography, photographic prints can be purchased at additional cost and tipped into your xerographic copy. Requests can be made to our Dissertations Customer Services Department.

5. Some pages in any document may have indistinct print. In all cases we have filmed the best available copy.

University Microfilms International

300 N. ZEEB ROAD, ANN ARBOR, MI 48106
18 BEDFORD ROW, LONDON WC1R 4EJ, ENGLAND

8010257

RICHARDS, LAURA RYPSTAT

DIURNAL VARIATIONS IN THE ACTIVITY OF ENZYMES REGULATING LIPID METABOLISM AND IN TISSUE LIPID LEVELS OF ADULT RATS FED DIFFERENT FAT SOURCES AND CHOLESTEROL

Iowa State University PH.D. 1979

University
Microfilms
International 300 N. Zeeb Road, Ann Arbor, MI 48106 18 Bedford Row, London WC1R 4EJ, England

Diurnal variations in the activity of enzymes regulating lipid metabolism and in tissue lipid levels of adult rats fed different fat sources and cholesterol

by

Laura Rypstat Richards

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

Major: Food and Nutrition

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1979

TABLE OF CONTENTS

	Page
GLOSSARY	vii
INTRODUCTION	1
REVIEW OF LITERATURE	5
Regulation of the Activity of Acetyl-CoA Carboxylase and Fatty Acid Synthetase	6
Regulation of the Activity of HMG-CoA Reductase	23
Regulation of the Activity of Cholesterol 7α-hydroxylase	30
The Effect of Fat Saturation on Lipogenesis and Cholesterogenesis	37
Acetyl-CoA Precursors	39
Serum Lipoprotein Composition	41
Diurnal Variations in Parameters of Lipid Metabolism	42
METHODS AND MATERIALS	45
Animal Treatment	45
Dietary Treatments	48
Enzyme Assays	52
Tissue Analyses	59
Statistical Analysis	65
RESULTS	67
Experiment 1	68
Experiment 2	82
Experiment 3	98

	Page
DISCUSSION	110
Acetyl-CoA Carboxylase and Fatty Acid Synthetase	110
HMG-CoA Reductase	119
Cholesterol 7α-hydroxylase	123
Serum Lipids	126
Summary	134
SUMMARY AND CONCLUSIONS	137
BIBLIOGRAPHY	143
ACKNOWLEDGMENTS	159
APPENDIX	160

LIST OF FIGURES

		Page
Figure 1.	Pathway enzymes examined in this study that affect the utilization of acetyl-CoA	3b
Figure 2.	Experimental design	46
Figure 3.	Activity of acetyl-CoA carboxylase (ACX:ACT) at consecutive times. Values are means of 6 rats	70
Figure 4.	Serum triglyceride (TG) levels at consecutive times. Values are means of 3-6 rats	79
Figure 5.	Activity of fatty acid synthetase (FAS:ACT) at consecutive times. Values are means of 4-6 rats	102
Figure 6.	Serum triglyceride (TG) levels at consecutive times. Values are means of 6 rats	107

LIST OF TABLES

		Page
Table 1.	Stock ration for male rats: modified Steenbock XVII (1976-1978)	47
Table 2.	Composition of experimental diets based on weights and calories	49
Table 3.	Composition of water-soluble vitamin mixture	50
Table 4.	Cholesterol protocol	60
Table 5.	Reagents for total and free cholesterol determinations	61
Table 6.	Effect of dietary fat on selected body parameters (experiments 1, 2 and 3)	69
Table 7.	ACX:ACT at consecutive times or photoperiods (experiment 1)	71
Table 8.	Effect of dietary fat on ACX:ACT and FAS:ACT (experiment 1)	74
Table 9.	FAS:ACT at consecutive times or photoperiods (experiment 1)	74
Table 10.	Plasma total cholesterol levels at consecutive times or photoperiods (experiment 1)	75
Table 11.	Effect of dietary fat on plasma cholesterol, triglyceride and free fatty acid levels (experiment 1)	77
Table 12.	Plasma triglyceride levels at consecutive times or photoperiods (experiment 1)	78
Table 13.	Plasma free fatty acid levels at consecutive times or photoperiods (experiment 1)	81
Table 14.	Hepatic cholesterol concentrations at consecutive times or photoperiods (experiment 1)	83
Table 15.	Effect of dietary fat on hepatic total cholesterol concentrations (experiment 1)	84

		Page
Table 16.	Effect of time and diet on hepatic soluble protein concentrations (experiments 1 and 3)	85
Table 17.	Effect of time on ACX:ACT (experiment 2)	87
Table 18.	Effect of dietary fat on ACX:ACT (experiment 2)	88
Table 19.	Effect of time on HMG:ACT (experiment 2)	90
Table 20.	Effect of diet on HMG:ACT and C7α:ACT (experiment 2)	91
Table 21.	Effect of time on C7α:ACT (experiment 2)	93
Table 22.	Effect of time on serum cholesterol, serum triglycerides and hepatic microsomal free cholesterol (experiment 2)	95
Table 23.	Effect of diet on serum cholesterol, serum triglycerides and hepatic microsomal cholesterol (experiment 2)	96
Table 24.	ACX:ACT at consecutive time periods or photoperiods (experiment 3)	99
Table 25.	Effect of fat level on hepatic ACX:ACT and FAS:ACT and on serum cholesterol and triglyceride levels (experiment 3)	101
Table 26.	FAS:ACT at consecutive time periods or photoperiods (experiment 3)	103
Table 27.	Serum cholesterol levels at consecutive times (experiment 3)	105
Table 28.	Serum triglyceride levels at consecutive times (experiment 3)	106
Table 29.	Correlation coefficients, r, between measurements on rats fed various diets (experiments 1, 2 and 3)	108

GLOSSARY

CO	coconut oil
SO	safflower oil
10CO	10% of kcal as coconut oil
40CO	40% of kcal as coconut oil
40CC	39% of kcal as coconut oil, 0.5% by weight as cholesterol
40SO	40% of kcal as safflower oil
40SC	39% of kcal as safflower oil, 0.5% by weight as cholesterol
FFA	free fatty acids
TG	triglycerides
ACX	acetyl-CoA carboxylase
ACX:ACT	acetyl-CoA carboxylase activity, nmoles HCO_3^--fixed/mg soluble protein/min
FAS	fatty acid synthetase
FAS:ACT	fatty acid synthetase activity, nmoles fatty acids formed/mg soluble protein/min
HMG-CoA	3-hydroxy-3-methylglutaryl-CoA reductase
HMG:ACT	HMG-CoA reductase activity, pmoles mevalonate formed/mg microsomal protein/min
C7α	cholesterol 7α-hydroxylase
C7α:ACT	cholesterol 7α-hydroxylase activity, pmoles 7α-hydroxycholesterol formed/mg microsomal protein/min
SEM	standard error of the mean
MDV	mean daily values, average of values taken at 6 hour intervals over 24 hours (experiments 1 and 3)
MCV	mean combined values, average of values taken at 12 hour intervals over 24 hours (experiment 2)

INTRODUCTION

Cardiovascular disease (CVD) is recognized as a major public health problem in the United States. A multiplicity of factors has been associated with the development of CVD including hyperlipidemias, both hypercholesterolemia and hypertriglyceridemia. In some people serum cholesterol and triglyceride levels may be reduced by dietary manipulation including decreased caloric intake from fat, reduced cholesterol intake and the substitution of polyunsaturated for saturated fats. These results indicate that to understand the relationship between hyperlipidemias and CVD, it is necessary to understand the effect of dietary fat and cholesterol on the metabolic pathways of lipogenesis, cholesterogenesis and cholesterol degradation.

Important in the functioning of each of these pathways are regulatory, or rate limiting, enzymes. The de novo synthesis of long chain fatty acids from acetyl-coenzyme A (acetyl-CoA) is catalyzed by two enzyme systems acting sequentially. The enzymes, considered to be short or long term regulators of lipogenesis, are acetyl-coenzyme A carboxylase (ACX, EC 6.4.1.2) and fatty acid synthetase (FAS), respectively. The regulatory step in cholesterol synthesis is controlled by 3-hydroxy-3-methylglutaryl-coenzyme A reductase (EC 1.1.1.34; HMG-CoA reductase). The rate of

cholesterol catabolism which involves formation of primary bile acids is controlled at the step regulated by cholesterol 7α-hydroxylase (EC 1.14).

Each of these enzymes has been investigated in detail by biochemical and biophysical techniques. Changes in the activity of the enzymes are caused by a variety of factors; nutritional, hormonal, developmental, genetic, neoplastic and pharmacologic. These factors act by changing the catalytic efficiency of the enzyme or changing enzyme protein concentration via synthetic or degradative processes.

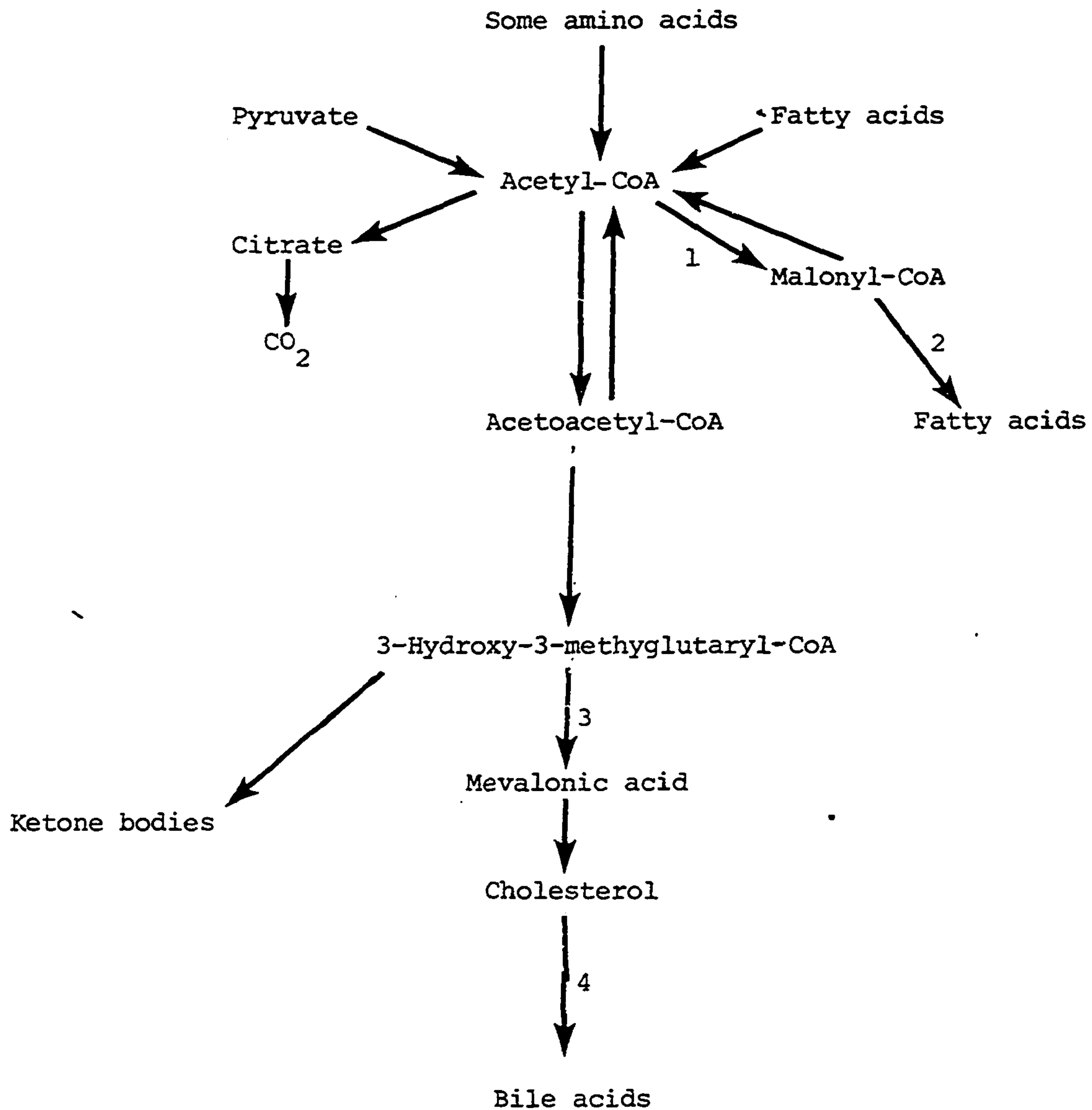
Ten years ago it was recognized that in rats the activity of hepatic HMG-CoA reductase (Back et al., 1969) and cholesterol 7α-hydroxylase (Gielen et al., 1969) as well as cholesterol synthesis vary significantly during a 24 hour period divided into 12 hour photoperiods of light and dark. This was recognized as circadian or diurnal rhythm because enzyme activity varied about a mean with a period of approximately 24 hours.

Biological periodicity has been demonstrated in virtually all plant and animal species (Black and Axelrod, 1970). Biorhythms serve to synchronize activities of a species with the periodically varying environment as well as to synchronize individuals of a species with each other, of obvious survival value. Biological rhythms may be endogenous, or due to environmental cues, such as lighting, temperature,

or feeding, the "Zeitgeber" factors. The endogenous rhythms in vertebrates, such as the circadian variation in urinary and plasma corticoids, appear to be regulated by the central nervous system through mediation of the cyclic secretion of adrenocorticotropic hormone, ACTH, by the pituitary. The circadian rhythm in some hepatic enzymes that oxidize drugs seems to be dependent on the daily corticoid rhythm ultimately controlled by hypothalamic factors.

The metabolic pathways synthesizing fatty acids or cholesterol are dependent on a supply of acetyl-CoA produced by catabolic cellular processes including β-oxidation of fatty acids, glycolysis of simple sugars and degradation of certain amino acids. Once produced, acetyl-CoA can be utilized in the citric acid cycle with subsequent production of CO_2 and H_2O or act as a substrate during the synthesis of long chain fatty acids, cholesterol or ketone bodies (Figure 1).

Regulation of the dispersal of acetyl-CoA along various catabolic and anabolic routes is not completely understood. Identification of synchrony or asynchrony among metabolic parameters related to fatty acid and cholesterol synthesis would yield insight into the control of these processes. Therefore, one of the objectives of this study was to determine whether the activities of ACX and FAS vary diurnally and



1. Acetyl-CoA carboxylase
2. Fatty acid synthetase
3. HMG-CoA reductase
4. Cholesterol 7α-hydroxylase

Figure 1. Pathway enzymes examined in this study that affect the utilization of acetyl-CoA

whether the activities of enzymes controlling lipogenesis, cholesterogenesis and cholesterol degradation are in or out of phase in utilizing acetyl-CoA.

The diurnal changes in selected serum and hepatic lipid levels were also measured to determine whether these lipid levels are correlated with enzyme activity and could, thereby, be controllers of that activity. Dietary manipulations were carried out so that the effects of fat saturation, the percent of calories from fat and the addition of exogenous cholesterol on the activity of ACX, FAS, HMG-CoA reductase and cholesterol 7α-hydroxylase and on concentration of tissue lipids could be determined.

REVIEW OF LITERATURE

Among the key enzymes of mammalian lipid metabolism are: 1) acetyl-coenzyme A carboxylase (ACX, EC 6.4.1.2) which catalyzes the biotin and ATP dependent carboxylation of acetyl-coenzyme A to produce malonyl-coenzyme A; 2) fatty acid synthetase (FAS) which catalyzes the synthesis of long chain fatty acids, primarily palmitic acid, from malonyl-CoA; 3) 3-hydroxy-3-methylglutaryl-coenzyme A reductase (EC 1.1.1.34) necessary for the reductive deacylation of 3-hydroxy-3-methylglutaryl-coenzyme A, a regulatory step in the formation of cholesterol; 4) cholesterol 7α-hydroxylase (EC 1.14) which controls the rate of formation of the first intermediate in the synthesis of primary bile acids from cholesterol.

The importance and complexity of the roles which these four enzymes play in lipid metabolism is reflected by the frequency with which they are discussed in the current literature. In a review of mammalian lipid metabolism, Van Golde and Van den Bergh (1977) discussed regulation of each of these enzymes in liver. Block and Vance (1977) and Volpe and Vagelos (1973) reviewed the enzymatic processes of fatty acid biosynthesis. Rodwell et al. (1976) and Dugan and Porter (1977) discussed regulation of HMG-CoA reductase activity by metabolic and hormonal effects. Myant and Mitropoulous (1977) dealt with

cholesterol 7α-hydroxylase and its effectors. Romsos and Leveille (1974) have reviewed the effects of diet on activity of enzymes involved in fatty acid and cholesterol synthesis.

This review will integrate information from the sources just cited with more recent publications to present an overview of hepatic lipid metabolism with emphasis on the regulatory enzymes and their effectors. A brief section on serum lipoproteins has been included. Unless noted, the data reviewed have been obtained from studies with rats.

Regulation of the Activity of Acetyl-CoA Carboxylase and Fatty Acid Synthetase

The de novo synthesis of long chain fatty acids is catalyzed by cytosolic enzymes including acetyl-CoA carboxylase (ACX) and fatty acid synthetase (FAS). These enzymes function sequentially and usually in a coordinated manner, although ACX has been generally considered rate limiting (Gangaly, 1960).

Acetyl-CoA carboxylase has equilibrium forms, an active polymer and an inactive protomer. The polymeric form is favored by the presence of di- and tricarboxylic acids, especially citrate, acetyl-CoA, high protein concentrations and pH 6.5 to 7.0. The protomer is favored by low

protein concentrations, chloride ions, and acyl-CoA derivatives (Gregolin et al., 1968). When hepatic ACX was purified to homogeneity, the polymeric form was estimated to have a molecular weight of 4,000,000 to 8,000,000 daltons while the protomeric molecular weight was estimated to be 200,000 daltons. The protomer was further resolved into two subunits (Inoue and Lowenstein, 1972). However, Mackall and Lane (1977) have questioned the existence of protomeric subunits by showing that these "subunits" were proteolytic fragments, artifacts of the isolation procedures.

When viewed by electron microscopy, inactive protomer appears as particles with maximum dimensions of 100-300 Å while the active polymer appears as a network of filaments 70-100 Å in width and up to 4000 Å in length. Kleinschmidt and coworkers (1969) speculated that the network of filaments might provide a matrix for organization of other enzymes involved in fatty acid biosynthesis.

Hepatic FAS, purified to homogeneity, is a tightly associated multienzyme complex with a reported molecular weight of 540,000 daltons (Burton et al., 1968). So far, fatty acid synthetase complexes from animals or yeast have not been dissociated into individual active enzymatic subunits although seven separate peptides and their functions have been described as components of FAS

from *E. coli*.

Short term regulation of lipogenesis

The rate of fatty acid synthesis can change quickly in response to several factors. For example, synthesis decreases rapidly when animals are fasted and increases rapidly and significantly when fasted animals are refed a fat-free diet (Nishikari et al., 1973). Changes in catalytic efficiency of existing enzyme play an important role when the rate of fatty acid synthesis is adjusted rapidly. Therefore, over the short term, enzyme activity may be regulated by allosteric effectors which bind to the enzyme to increase or decrease its efficiency or by covalent modification of the enzyme by phosphorylation-dephosphorylation.

Citrate, long chain acyl-CoA compounds and free fatty acids are among the allosteric regulators considered to be of potential physiological importance in the short term regulation of lipogenesis. For example, changes in hepatic citrate and long chain acyl-CoA thioester levels as well as ACX activity were followed after rats were fasted and refed (Nishikari et al., 1973). Following realimentation, the rate of fatty acid synthesis rose steadily for eight hours. However, the amount of enzyme protein did not change. Citrate content increased during this time while long chain acyl-CoA content decreased sharply. The data reflect a change in

catalytic efficiency due to changes in metabolite rather than enzyme concentrations during the initial period of refeeding.

In a similar study by Goodridge (1973) the citrate content in isolated hepatocytes from refed chicks was positively correlated with fatty acid synthesis under all incubation conditions. Fatty acid synthesis was, however, inhibited by free fatty acids and this inhibition was accompanied by an increase in the fatty acyl-CoA level. It is possible that citrate activated ACX while fatty acyl-CoA derivatives acted either to inhibit ACX directly or to inhibit mitochondrial citrate carrier. In either case the net result would be a reduction in the activation of ACX by citrate.

There are other reports in the literature which indicate that fatty acid synthesis may not be directly regulated by tissue citrate levels. Jacobs and Majerus (1973) working with skin fibroblasts showed that addition of albumin-bound fatty acids to fibroblasts resulted in a 26-67% inhibition in the rate of acetate incorporation into fatty acids. At the same time levels of long chain acyl-CoA derivatives and of citrate remained constant. The authors speculated that the uptake of free fatty acids resulted in a shift of citrate from cytoplasm to the mitochondria, accompanied

by a translocation of the long chain acyl-CoA derivatives into the cytoplasm. The modulation of the enzyme activity could be explained as a consequence of these shifts.

Enzymes, depending on their state of phosphorylation, will respond differently to substrate or allosteric effectors. Phosphorylation is accomplished by protein kinases which, after activation by cyclic AMP (c-AMP), catalyze the transfer of the γ-phosphate group from ATP to enzyme protein. Lee and Kim (1977) used antibodies produced against a peptide isolated from ACX to demonstrate that the γ-phosphate group from ATP was incorporated into ACX during inactivation. This inactivation was reversed by incubation with a phosphoprotein phosphatase.

The effect of c-AMP and citrate on the rate and degree of ACX phosphorylation was investigated by Lent et al. (1978). In the presence of c-AMP, ACX was inactivated due to phosphorylation. High citrate concentrations inhibited phosphorylation and increased ACX activity while reduced citrate concentrations increased rate and degree of phosphorylation.

A fatty acid binding protein (FABP) in hepatic cytosol has also been implicated in the short term control of ACX (Lunzer et al., 1977). At 8-10 µM concentrations of palmitoyl-CoA, but in the absence of FABP, ACX was inhibited by 50%; this could be reversed by the addition of increasing

concentrations of FABP. Oleate also produced an inhibition of enzyme which was reversed by FABP. It was suggested that FABP participates in short term regulation of lipogenesis by acting as the major cytosolic binder of inhibitors, such as acyl-CoA derivatives. The data did not support a direct effect of FABP on the enzyme.

A physiological role has not been defined for fatty acid synthetase in the short term regulation of lipogenesis. Although a stimulatory effect of phosphorylated sugars on FAS activity was reported (Plate et al., 1968), this finding could not be confirmed (Porter et al., 1971). Nor does hepatic FAS undergo competitive, reversible inhibition by long chain acyl-CoA derivatives as is the case for ACX.

Recently two forms, an apo- and haloenzyme, of the FAS complex have been separated from pigeon liver. They differ by the presence or absence of the prosthetic group, 4'-phosphopantetheine (Kim et al., 1977). The prosthetic group can be transferred from coenzyme A to apo-FAS to produce halo-FAS. Pigeons were fasted and refed to determine whether prosthetic group transfer was regulatory in the activity of FAS. Shortly after refeeding, high concentrations of apoenzyme were present. Following this period, halo-FAS increased and apo-FAS decreased. After refeeding for 48 hours, the pigeons were refasted and the amount of apoenzyme again

increased while the haloenzyme decreased. These responses suggest that FAS may have physiological significance in the short term regulation of fatty acid synthesis.

Long term regulation of lipogenesis

The rate of fatty acid synthesis also can be regulated by changes in the content of ACX and FAS. Majerus and Kilburn (1969) examined changes in ACX activity after fasting and fat-free refeeding. Immunological analysis of liver homogenates indicated that changes in ACX activity resulted from changes in enzyme content rather than from activation or inhibition of existing enzyme. The precipitation of enzyme by antibody, after pulse labeling with [^{3}H]leucine, indicated that the rate of enzyme synthesis increased 5- to 10-fold after fasted rats were refed a fat-free diet. The rate of degradation or half-life of ACX, measured by the loss of label, was approximately 48 hours in rats fed the fat-free diet and 18 hours in fasted rats. Therefore, the increase in hepatic ACX content associated with fat-free refeeding was due to an increased rate of enzyme synthesis and a stabilization of enzyme turnover.

In a similar study based on isotopic leucine incorporation, fasting reduced the rate of hepatic ACX synthesis to half the control value while fat-free refeeding

increased the rate almost 4-fold (Nakanishi and Numa, 1970). The half-life of ACX was 55 hours in the re-fed animals but accelerated to 31 hours in the fasted group. The increase in ACX activity in refed animals, assumed to be in a steady state nutritionally, was ascribed to a rise in ACX synthesis. The decrease in ACX activity in the fasted, or nonsteady state, was ascribed to decreased enzyme synthesis and accelerated enzyme degradation. Both of these studies indicated that hepatic ACX has a half-life of 1 to 3 days. Thus changes in the rate of enzyme degradation may play an important role over the long term as animals move from one nutritional steady state into another.

Alberts et al. (1975) have shown that differences in the rates of FAS synthesis are reflected in the amount of labeled antibody bound to polysomes. Rats were starved and refed a fat-free diet. The specific activity of FAS and iodinated antibody binding to polysomes was measured as a function of time. After a lag of 4 hours, there was a rapid rise in FAS activity which continued throughout the 44-hour experiment. Enhanced binding of antibody to polysomes began 5 hours after refeeding and continued throughout the experiment. The data demonstrate that FAS peptide precursors are associated with polysomes during times of

increased FAS activity in the liver.

Nutritional effects

This section of the review will deal with the effects of dietary fats, triglycerides and fatty acids on ACX, FAS and lipogenesis over the long term.

Hepatic lipogenesis is reduced in response to diets high in fat. Hill et al. (1958) demonstrated this by feeding rats diets containing graded amounts of fat, 0-15% by weight, for three days. Fats used were lard, corn oil, vegetable oils and hydrogenated vegetable oils. Livers from animals fed fat-free diets had the greatest capacity for converting labelled acetate to fatty acids. Adding as little as 3% of any fat to the diet caused a measurable depression in lipogenesis. When dietary fat was increased to 15%, hepatic conversion of acetate to fatty acids was reduced 90% compared to fat-free controls. Although Hill reported that the various fats used were equally effective in depressing hepatic lipogenesis, later work has shown that the composition of the dietary fat plays an important role in the regulation of lipogenesis. For example, Reiser et al. (1963) fed diets essentially fat-free or containing 30% by weight of several simple triglycerides or natural fats. Rats were fed for two weeks. One hour prior to sacrifice, [1-^{14}C]acetate was injected. The amount of labelled, therefore, recently

synthesized, fatty acids was determined in various tissues. Seven percent of the acetate was incorporated into fatty acids in rats on the low-fat diet. Diets containing the simple triglycerides depressed lipogenesis progressively as fatty acid chain length increased from four to eighteen carbon atoms. Increasing amounts of unsaturation in the fats also depressed lipogenesis. Triolein and safflower oil were most effective.

Although many workers have found polyunsaturated fatty acids (PUFA) more effective than monoenoic or saturated fatty acids (SFA) in reducing the activity of hepatic lipogenic enzymes, there is no consensus in explaining this effect.

For example, Muto and Gibson (1970) demonstrated that the induction of lipogenic enzymes by fat-free refeeding after a fast was accompanied by an increase in hepatic synthesis of saturated and monounsaturated fatty acids. Supplementation of the fat-free diet with methyl linoleate or arachidonate resulted in a decrease in ACX and FAS. Possibly, linoleate and archidonate had a specific effect in reducing the activity of lipogenic enzymes.

The role of tissue levels of polyunsaturated fatty acids (PUFA) in rats fasted and refed diets high in linoleate (safflower oil), oleate or palmitate was evaluated by

Bartley and Abraham (1972). Hepatic lipogenesis was decreased significantly by linoleate in comparison to the other fats, however, the tissue levels of linoleate did not increase. Apparently, decreases in lipogenesis observed when PUFA are fed are not related to tissue levels of PUFA.

Musch et al. (1974) pair-fed rats to exclude any effect of caloric intake on enzyme activity. Equal amounts of fat-free diets with a methyl ester supplement of oleate or α-linoleate were refed to previously fasted rats. The activities of lipogenic enzymes including FAS were significantly lower in rats fed α-linoleate compared to those fed oleate. The authors speculated that the reduction in lipogenic activity could result from: 1) inhibition of other lipogenic enzymes such as ACX, 2) conversion of fatty acids to prostaglandins and their interaction with c-AMP in regulation of synthesis or activity of lipogenic enzymes, 3) alterations in membrane lipid properties which may affect membrane-hormone interactions or 4) formation of a lipid containing repressor for genes of lipogenic enzymes.

To determine whether prostaglandin (PG) synthesis was involved in the decreased FAS activity when 15% SO compared to CO was fed, indomethacin, a drug which inhibits PG synthesis, was administered to rats on the SO diets. Administration of the drug failed to restore fatty acid

synthesis to control levels. Apparently PG synthesis is not required for the linoleate induced decrease in FAS activity (Flick et al., 1977).

The differential effect of methyl esters of saturated and unsaturated fatty acids on lipogenesis could be due to differences in ester absorption. To eliminate this possibility, Clarke et al. (1977) compared methyl ester absorption in the rat. The esters of $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ were very similar in absorption. When these esters were used in feeding studies, low levels of $C_{18:2}$ and $C_{18:3}$ effectively inhibited the activity of FAS and other lipogenic enzymes. In contrast equivalent amounts of $C_{18:1}$ had little effect on lipogenesis. The consumption of a high carbohydrate diet containing as little as 3% by weight safflower oil also caused a decrease in lipogenic enzyme activity.

Although changes in activity or content of ACX and FAS are usually coordinated when rates of fatty acid synthesis change, there is evidence that FAS may be critical in long term regulation of fatty acid synthesis. Guynn et al. (1972) demonstrated that malonyl-CoA levels were not directly related to fatty acid synthesis when high carbohydrate diets were fed. In rats trained to consume their daily ration in 3 hours, malonyl-CoA increased 2-fold while fatty acid synthesis increased only 40%. Apparently the rate

of fatty acid synthesis was limited by the ability of hepatic FAS to utilize malonyl-CoA.

Long term effects of dietary fats on hepatic ACX and FAS in chicks have also been studied (Liou and Donaldson, 1973). Activity of both enzymes was depressed equally by the addition of 4% fat to a fat-free basal diet. However, when 8% or more fat was fed, FAS activity was depressed more than the activity of ACX. Therefore, the ratio of synthetase to carboxylase activity was decreased as fat addition was increased. The data suggest that FAS rather than ACX is the rate limiting enzyme with high-fat diets.

In contrast, the enteral administration of corn oil to animals adapted to a fat-free, high-carbohydrate diet, caused a decrease within two hours of the hepatic conversion of labelled acetate to fatty acids (Bortz et al., 1963). Fatty acid synthesis from malonyl-CoA was not inhibited, while synthesis from acetyl-CoA was depressed. These findings suggest that the block in lipogenesis occurs at the step involving ACX.

Additional differences in the response of lipogenic enzymes and fatty acid synthesis to 0 to 25% of calories from soybean oil have been reported (Carrozza et al., 1979). While FAS activity was inhibited with as little as 3% fat, rate of lipogenesis and ACX activity were depressed only when

the level of dietary fat was 12% or greater. These results indicate that fatty acid synthesis is better correlated with ACX than with FAS activity.

Wiegand et al. (1973) investigated the response of FAS and glycerophosphate acyl-transferase (GPAT), active during the synthesis of glycerides, to differences in the quantity of dietary fat. The addition of 2.5 or 5% safflower oil to a fat-free diet reduced FAS activity by 25 and 40%, respectively. The activity of GPAT was not significantly different with the fat-free or fat-containing diets. It would appear that fatty acid synthesis rather than fatty acid esterification is affected by fat quantity during triglyceride production.

The rate of fatty acid synthesis was not significantly decreased when rats were fed diets containing 1.5% cholesterol and 0.5% cholic acid for 2 to 4 weeks only, despite marked decreases in the activities of ACX and FAS (Tsai and Dyer, 1973). However, the rate of hepatic fatty acid synthesis decreased when cholesterol feeding was continued for longer than five weeks.

Hormonal regulation

Insulin, glucagon, glucocorticoids, adrenaline and triiodothyronine are among the hormones known to affect lipogenic enzymes. The exact role of these hormones in allosteric or covalent modification is not completely understood. The changes can affect enzyme activity or synthetic or degradative processes which regulate enzyme content.

Insulin and glucagon are important regulators of FAS activity when previously fasted rats are refed a fat-free diet (Lakshmanan et al., 1973). The normal increase in FAS activity under these circumstances was abolished in diabetic animals. But, diabetic rats receiving insulin during refeeding had increased FAS activity which was indistinguishable from the response of nondiabetic rats. When nondiabetic animals were injected with glucagon, then fasted and refed, a reduction in FAS activity followed in proportion to the dose of glucagon.

Klain and Weiser (1973) observed a selective effect of glucagon on ACX activity and fatty acid synthesis. Within 15 minutes of glucagon injection, synthesis of fatty acids from [U-^{14}C]glucose was markedly decreased. The activity of ACX measured in liver slices collected within minutes of glucagon injection was also decreased.

The role of glucagon in the regulation of ACX activity

via phosphorylation-dephosphorylation was recently clarified (Witters, et al., 1979). Hepatocytes from fasted, refed rats were incubated with $^{32}P_i$. One group of cells was then treated with glucagon. The incorporation of label into ACX as well as ACX activity were compared in these and control cells. Cellular exposure to glucagon resulted in increased incorporation of $^{32}P_i$ into ACX and diminished the production of fatty acids from $^{3}H_2O$ thereby linking the hormone to enzymatic regulation of lipogenesis.

Halestrap and Denton (1974), who measured ACX activity in rat epididymal fat pads, found that the addition of insulin to pads with exogenous glucose led to an increased proportion of polymeric enzyme. In contrast pad exposure to adrenaline in the presence of glucose and insulin decreased enzyme activity and increased the proportion of ACX in the protomeric form. The authors were unable to find any evidence of ACX phosphorylation-dephosphorylation during hormonal manipulation. However, changes did occur in the concentration of citrate, which promoted, or fatty acid thioesters, which inhibited enzyme polymerization.

In hepatocytes from fed rats, glucagon changed fatty acid metabolism from synthesis to oxidation. Changes in cellular citrate and malonyl-CoA levels indicated that glucagon inhibited glycolysis and probably the action of acetyl-CoA carboxylase. Evidently in fasting or diabetes during which

the ratio of glucagon to insulin is high, fatty acid synthesis is inhibited but fatty acid oxidation and ketone body production are increased (McGarry et al., 1978).

The effect of interperitoneal injections of triiodothyronine on hepatic enzymes regulating fatty acid synthesis and oxidation was examined by Diamant et al. (1972). Hormone treatment increased the activity of both ACX and FAS in fed and fasted rats. Fatty acid oxidation in liver slices from the same animals was also increased. There were no net changes in plasma or liver triglyceride levels. Apparently, increased enzyme activity was due to an increased rate of fatty acid turnover.

The importance of different hormones in fatty acid synthesis may be tissue dependent. For example, glucocorticoids appear to regulate fatty acid synthesis in adipose but not liver tissue (Volpe and Marasa, 1975). A marked decrease in the amount of ACX and FAS or fatty acid synthesis in adipose tissue followed administration of hydrocortisone. In contrast, no changes in enzyme content or fatty acid synthesis occurred in liver. Apparently, the normal stimulatory effect of insulin on hepatic ACX and FAS counteracts any inhibitory effect of hydrocortisone, but in adipose tissue the stimulatory effect of insulin is not as great allowing measureable inhibition of fatty acid synthesis

by hydrocortisone.

Regulation of the Activity of HMG-CoA Reductase

Under most physiological conditions, HMG-CoA reductase (mevalonate:NADP oxidoreductase EC 1.1.1.34) is considered the rate limiting enzyme in the biosynthesis of cholesterol (Siperstein and Fagan, 1964; Shapiro and Rodwell, 1971).

Diurnal rhythm

Ten years ago a circadian or diurnal rhythm was identified in reductase activity when rats were fed ad libitum and conditioned to two twelve hour photoperiods of light and dark. Under these conditions, hepatic reductase varied from 5- to 10-fold every 24 hours. Maximum activity occurred approximately 6 hours into the dark period and minimum activity, 6 hours into the light period (Back et al., 1969; Edwards et al., 1972). Shapiro and Rodwell (1972) reported the existence in the dark cycle of 2 activity peaks separated by approximately 2 hours which were blocked by cycloheximide. Periodicity in the rate of cholesterol synthesis from acetate has been correlated with reductase activity (Dugan et al., 1972).

The diurnal rise in the level of HMG-CoA reductase is associated with refeeding and can be relocated by changing the

time of feeding (Dugan et al., 1972). When discrete meals were fed during the light photoperiod, peak reductase activity occurred 2 hours after the meal in the light period. The rate of enzyme formation increased 7- to 10-fold during this sharp rise and could be abolished by the administration of cycloheximide. Enzyme degradation, however, was constant throughout the diurnal changes in enzyme activity.

Mevalonolactone administration suppressed reductase activity more rapidly than cycloheximide administration suggesting that the catalytic efficiency of the enzyme may have been altered (Edwards et al., 1977). Additionally, reductase activity has been modified in vitro by activator-inactivator proteins. The enzyme has been inactivated in the presence of [$\gamma^{32}P$] ATP but reactivated with an activator protein (Nordstrom et al., 1977).

The diurnal rhythm of the reductase responds differently to dietary cholesterol or to feeding. Reductase activity decreased about 20-fold when rats were fasted for 36 hours but cyclic activity persisted (Shapiro and Rodwell, 1972). However, the usual rise in reductase activity was almost abolished by 10 hours of cholesterol feeding. Cholesterol synthesis was also reduced greatly. The data suggest that cholesterol feeding or fasting may regulate reductase activity and perhaps cholesterol synthesis by different mechanisms. Dietary cholesterol has been shown to increase microsomal cholesterol

ester concentrations. It has not been clear whether an increase in these esters must precede the observed inhibition of the reductase (Edwards and Gould, 1974).

Regulation by metabolites

There are three major sources of cholesterol available to the hepatocyte: hydrolysis of stored cholesterol esters, uptake of lipoproteins into the cell followed by hydrolysis of cholesterol esters and synthesis de novo from acetyl-CoA. In general, the cellular rate of cholesterol synthesis is determined by the balance of cholesterol input to the cell relative to cellular needs for structural or secretory processes.

Nervi and Dietschy (1978) examined the effect on cholesterol synthesis of the size of the bile acid pool and the amount of cholesterol reaching the liver. The rate of cholesterol synthesis varied inversely with the size of the bile acid pool which was varied by feeding taurocholate. The rate of cholesterol synthesis varied directly with the rate of bile acid synthesis which was manipulated via biliary drainage.

When intestinal lipoproteins were injected into rats, net cholesterol uptake took place only in the liver where cholesterol synthesis was inhibited. When rats were injected with lipoproteins or fed cholesterol, there was generally a correlation between inhibition of cholesterol

synthesis and cholesterol ester content of the liver (Nervi et al., 1974).

The effects of dietary fat

The effects of saturated and unsaturated fatty acids on the secretion of very low density lipoproteins (VLDL) have been investigated during liver perfusion. Approximately 50% more triglyceride (TG) was secreted from the liver when oleate rather than palmitate was perfused. The less dense VLDL from oleate contained half as much cholesterol and phospholipid per μmole TG than did the VLDL from palmitate (Heimberg and Wilcox, 1972). The infusion of oleic acid stimulated cholesterol synthesis from ^3H_2O and cholesterol output from the liver, suggesting that cholesterol synthesis is obligatory for the secretion of TG by the liver (Goh and Heimberg, 1973).

The output of TG, cholesterol and activity of HMG-CoA reductase were measured following perfusion of liver with equimolar quantities of palmitic (16:0), oleic (18:1) or linoleic acid (18:2). The activity of the enzyme decreased in the order 18:1>18:2 = 16.0. Triglyceride output decreased in the order 18:1 = 18:2>16:0. Output of free and esterified cholesterol was in the order 18:1>18:2>16:0. Apparently the degree of saturation of the fatty acids entering the liver affected the TG and cholesterol profile

of the VLDL output which in turn affected cholesterol synthesis.

The protein to which cholesterol is attached in lipoproteins can also affect the ability of cholesterol to regulate cellular HMG-CoA reductase activity. In human fibroblasts, reductase activity was suppressed by delivery of cholesterol in low density lipoprotein (LDL) or VLDL but not by serum from a patient with abeta-lipoproteinemia. This serum contained protein bound cholesterol primarily as high density lipoprotein (HDL) but lacked apoprotein B, common to LDL and VLDL (Brown et al., 1974).

The diurnal cycle in reductase activity was evident when 0, 5 or 20% corn oil was fed to rats although changes in activity differed. For example, activity varied 2-fold on 0% oil, 6-fold on 5% oil and 4-fold when 20% was fed, but the peak always occurred during the feeding cycle. Goldfarb and Pitot (1972) noted that cholesterol 7α-hydroxylase and HMG-CoA reductase activity fluctuated in a closely parallel manner. The authors suggested, therefore, that peak reductase activity reflected a diurnal fluctuation in bile acid synthesis whereby bile acids are synthesized in preparation for intake of diets containing lipid.

A study by Ide et al. (1978) indicated that hepatic reductase activity depends on the composition of the dietary fat, especially fatty acid chain length and degree of satu-

ration. Activity of HMG-CoA reductase increased significantly as fatty acid chain length increased from trioctanoin through trilaurin, tripalmitin and tristearin. Significant decreases in activity were associated with increased unsaturation. The feeding of tristearin caused the highest reductase activity while feeding safflower oil with 74% linoleate led to the lowest activity.

Dietary fats can ultimately affect several parameters of cholesterol metabolism. For example, the addition of 10% tripalmitin or safflower oil to chow diets did not affect cholesterol synthesis, the activity of HMG-CoA reductase, or fecal steroid or bile acid output. However, with the addition of cholesterol to these diets, PUFA, compared to tripalmitin, inhibited cholesterol synthesis but increased cholesterol stores and bile acid secretion (Bochenek and Rodgers, 1978).

Hormonal regulation

The rhythm in HMG-CoA reductase activity persists despite changes in lighting or feeding schedules, although there may be alterations in the time or amplitude associated with peak activity. Since hormones markedly affect cholesterol biosynthesis, it is possible that rhythmic changes in the levels of certain hormones are responsible for the diurnal variations associated with HMG-CoA reductase and

cholesterogenesis.

The involvement of insulin in the regulation of reductase activity was illustrated by the work of Lakshmanan et al. (1973). Animals made diabetic by injections of streptozotocin had greatly reduced reductase activity. However, daily protamine zinc insulin therapy restored the reductase activity to near normal and increased cholesterol synthesis from acetate.

Glucagon prevented the increase in reductase activity and cholesterol synthesis seen with stimulatory agents including insulin or thyroxine (Lakshmanan et al., 1973). Additionally glucagon inhibited the diurnal rise in hepatic activity associated with ad libitum feeding (Nepokroeff et al., 1974).

The effects of glucagon and insulin may be associated with the regulation of the level of hepatic c-AMP by these hormones (Exton et al., 1971). Glucagon increased the level of c-AMP in the liver while insulin reduced this level. Apparently, insulin and glucagon acted by controlling levels of the "second messenger", c-AMP, and increased or decreased, respectively, hepatic cholesterogenesis.

The addition of physiological doses of insulin to hepatocytes, in a study by Edwards et al. (1979), did not increase reductase activity or sterol efflux from these cells.

Possibly increased activity, reported earlier, was due to the use of pharmacological doses of the hormone. Also in this later study, glucagon and dibutyryl c-AMP inhibited induction of HMG-CoA reductase while norepinephrine caused an increase in reductase activity. Cholesterol efflux from hepatocytes was inhibited by addition of any of the three.

Regulation of the Activity of Cholesterol 7α-hydroxylase

Cholic acid and chenic (chenodeoxycholic) acid are the two primary bile acids formed from cholesterol in mammalian liver. During biosynthesis of these acids, changes in the cholesterol ring system precede side chain oxidation. Microsomal cholesterol 7α-hydroxylase catalyzes the first of these changes, the hydroxylation of cholesterol to form 7α-hydroxycholesterol. Cholesterol 7α-hydroxylase is a mixed function oxidase, an enzyme which catalyzes the introduction of one atom of molecular oxygen into a substrate while the other atom is reduced to water.

This step is considered rate limiting in bile acid synthesis based on data from numerous studies. For example, bile acid synthesis was stimulated as much as ten-fold in rats with bile fistulas. This increase in synthesis was accompanied by an increase in the activity of cholesterol 7α-hydroxylase but not in the activity of enzymes catalyzing

two subsequent steps in the synthetic pathway (Danielsson, 1972). Similar results were obtained by Shefer et al. (1973) who found essentially no rate limiting enzymatic conversions beyond the formation of 7α-hydroxycholesterol in the pathway from labelled acetate to bile acids.

Bile acid synthesis

The concentration of bile acids in the enterohepatic circulation appears to be a regulator of bile acid biosynthesis and therefore, cholesterol 7α-hydroxylase activity. Bile acid formation increased not only in response to bile fistula but also to the administration of bile acid binding resins. In contrast, normal or increased rates of bile acid synthesis were depressed by duodenal infusion of bile acids (Shefer et al., 1969). Shefer et al. (1973) fed taurocholate, taurodeoxycholate and taurochenodeoxycholate as 1% of the diet for one week. All three bile acids reduced the activity of HMG-CoA reductase but only taurocholate and taurodeoxycholate inhibited cholesterol 7α-hydroxylase. In all groups biliary secretion of bile acids was nearly double and composition of the bile acid pool was shifted and reflected the administered bile acids. The authors concluded that the composition, magnitude and enterohepatic circulation rate of the bile acid pool influence the hepatic concentration of cholesterol 7α-hydroxylase.

The mechanisms by which bile acids regulate enzyme activity at the molecular level are unknown. However, the rise in cholesterol 7α-hydroxylase activity after interruption of enterohepatic circulation of bile was prevented by treatment with actinomycin D, an inhibitor of protein synthesis. Einarsson and Johansson (1968) concluded that increased enzyme synthesis, rather than activation of pre-existing enzyme, caused the increase in hydroxylase activity. In this study the half-life of the hydroxylase was estimated to be 2 to 3 hours so that rapid changes in enzyme activity were possible.

Diurnal regulation

Several investigators have confirmed the initial observation by Gielen et al. (1969) that cholesterol 7α-hydroxylase activity varies diurnally with equal photoperiod of light and dark when rats are fed ad libitum. Under these conditions, maximum activity is generally 2 to 4 times higher than minimum activity and occurs 6 hours after the beginning of the dark period. In contrast, minimum activity occurs 6 hours into the light photoperiod.

Increased enzyme synthesis appears to be necessary for the diurnal rise in hydroxylase activity. If rats were injected with either solutions of actinomycin D or only carrier prior to the expected peak in hydroxylase activity, the rise

in activity occurred only in the carrier injected rats (Mitropoulous et al., 1972).

Lighting has little effect on enzyme rhythm. Rats were maintained under three different lighting schedules; complete darkness, continuous light or equal daily photoperiods of light and dark (Gielen et al., 1975). The extent of peak activity and the time the peak occurred were similar in all groups.

When animal groups were either fed or fasted for 24 hours, diurnal variations persisted in hydroxylase activity during fasting. Peak activity, however, was not as great and occurred earlier in the fasted group compared to that fed ad libitum (Mitropoulous et al., 1972).

Related activities of HMG-CoA reductase and cholesterol 7α-hydroxylase

Various factors affect the relationship between cholesterol synthesis and degradation and, therefore, the rate limiting enzymes of these processes. The activities of cholesterol 7α-hydroxylase and HMG-CoA reductase respond in a coordinated way to a number of regulators including time, dietary factors and some hormones.

Danielsson (1972) found that the incorporation of labelled acetate into cholesterol and the activity of cholesterol 7α-hydroxylase followed the same rhythmic patterns of diurnal variation. The author interpreted these data

to indicate that changes in the rates of bile acid and cholesterol synthesis occurred almost concomitantly. He noted that this interpretation did not agree with that of Myant and Eder (1961) who had claimed that during biliary drainage, changes in cholesterol synthesis preceded changes in bile acid synthesis.

Later work dealing with the regulation of cholesterol 7α-hydroxylase by cholesterol synthesis (Takeuchi et al., 1974) supported the findings of Myant and Edar. Rates of cholesterol synthesis and degradation were followed after oral administration of labelled glucose to fasted rats. One hour after glucose administration, hepatic cholesterol synthesis increased but hydroxylase activity did not change. However, two hours after treatment, hydroxylase activity increased, indicative of a lag phase in cholesterol degradation following the induction of cholesterol synthesis.

The effect of fat type

Conclusions differ concerning the effect of dietary fat saturation on the activity of cholesterol 7α-hydroxylase. Mayer and Mayer (1974) found after two weeks of feeding that hydroxylase activity was lower in rats fed coconut oil compared to corn oil at 40% of calories. Bile acid secretion was 3 times greater on the corn oil rather than coconut oil diet probably leading to an increase in bile acid excretion

and hydroxylase activity.

In contrast, hydroxylase activity, expressed as pmoles/ mg protein/min, did not vary when coconut, corn or peanut oils or butter were fed for five weeks (Kritchevsky et al., 1977).

Recently the activity of cholesterol 7α-hydroxylase was determined after rats were fed 0, 6 or 20% by weight of synthetic triglycerides varying in chain length or degree of saturation (Björkhem et al., 1978). The addition of tripalmitin and trierucin increased 7α-hydroxylase activity compared to the fat-free stock diet while trilinolein or triolein caused significantly lower activity. Addition of trilaurin had little effect on 7α-hydroxylation of cholesterol. The lowest level of activity was obtained with fatty acids that were readily absorbed from the intestine i.e., linoleic and oleic acids. Therefore, the authors suggested that the reduced absorption of some fatty acids is coupled with a reduced absorption of bile acids. The resulting loss of bile acids in the feces may then cause an increase in 7α-hydroxylation.

Cholesterol feeding caused markedly different responses in the activities of HMG-CoA reductase and cholesterol 7α-hydroxylase (Raicht et al., 1975). The absorption of dietary cholesterol was accompanied by inhibition of cholesterol

synthesis but enhanced synthesis of bile acids. Despite these adaptations in rates of synthesis, liver cholesterol concentration increased four-fold.

Hormonal regulation

Studies dealing with hormonal regulation of the hydroxylase are not extensive but there is evidence that hormones can regulate enzyme activity. For example, hydroxylase activity first appears at weaning which coincides with maturation of the hypothalamo-hypophysis-adrenal axis (Van Canfort, 1973). In older animals, the diurnal rhythm can be abolished by hypophysectomy or adrenalectomy (Gielen et al., 1975). Various glucocorticoids including cortisol can increase hydroxylase activity significantly. Gielen et al. (1975) observed that cholesterol 7α-hydroxylase activity and plasma corticosterone levels changed in parallel and were modified similarly by alterations in lighting or feeding schedules.

The influence of variations in thyroid activity on cholesterol synthesis and degradation has also been investigated (Takeuchi et al., 1975). Rats made either hyperthyroid by treatment with thyroid powder or hypothyroid by treatment with thiouracil were additionally given oral labelled doses of glucose or cholesterol. Cholesterol 7α-hydroxylase activity was increased by small doses of thyroid powder while

much higher doses were necessary to increase cholesterol synthesis. Suppression of cholesterol 7α-hydroxylase activity was brought about by much lower doses of thiouracil compared to those required to decrease cholesterol synthesis. Apparently the activity of cholesterol 7α-hydroxylase is more sensitive to variations in thyroid function than are the activities of the cholesterol synthesizing enzymes.

The Effect of Fat Saturation on Lipogenesis and Cholesterogenesis

Bortz (1967) investigated the effect of a corn oil meal given by stomach tube on rates of lipid and cholesterol synthesis as rats were sacrificed periodically for 24 hours after intubation. Rapid production of acyl-CoA derivatives and acetyl-CoA was accompanied by an increase in ketone body synthesis and a decrease in lipogenesis. Cholesterol synthesis was altered later. Since cholesterol and acetoacetate share the common precursor, acetyl-CoA, increases in their rates of synthesis should coincide if increased cholesterol synthesis depended only on surplus substrate. This was not observed. Therefore, cholesterol synthesis may have been increased by induction of HMG-CoA reductase via increased availability of ketone bodies, substrates for cholesterol formation, or by removal of feedback control on reductase due to release of intrahepatic bile acids in

response to dietary fat.

Cholesterol and fat may influence the pathways synthesizing cholesterol or fatty acids by different mechanisms. Feeding a fat-free diet for six days after fasting or a low-fat regimen increased the activity of ACX and FAS. However, reductase activity and cholesterol synthesis were depressed (Craig et al., 1972). With the addition of 2% cholesterol, reductase activity and cholesterol synthesis were markedly depressed while ACX and FAS activities were unaffected.

Recently, Ide et al. (1979) examined the effect of various fats on the activity of HMG-CoA reductase and other lipogenic parameters in fasted-refed rats. After 1 day of refeeding, the activity of HMG-CoA reductase or the incorporation of [1-^{14}C] acetate into fatty acids or sterols was not fat dependent. After 3 days of refeeding, effects of dietary fats on reductase activity were apparent. In contrast, rates of fatty acid synthesis did not differ due to type of fat until at least 7 days of refeeding. This may indicate that the mechanisms underlying the regulation of lipogenesis or cholesterogenesis differ over the short versus the long term.

In an extended study, incorporation of [1-^{14}C]acetate into liver cholesterol was stimulated by corn oil but liver

fatty acid synthesis was depressed by either corn oil or butter (Serdarevich and Carroll, 1971). Increased synthesis of hepatic cholesterol with corn oil may have been due to the increase in fecal steroid excretion noted with that fat.

After 10 weeks, serum cholesterol concentrations were similar with either safflower oil or lard while triglyceride levels were depressed by safflower oil (O'Brien et al., 1977). When cholesterol was added to lard or SO, serum cholesterol increased 5- or 3-fold, respectively. These fats had little effect on hepatic cholesterol but hepatic triglyceride concentrations were higher with lard compared to SO. When cholesterol was added to either diet, hepatic triglycerides were not affected but hepatic cholesterol was greatly elevated.

Acetyl-CoA Precursors

Circulating or tissue precursors of active C_2 residues enter the acetyl-CoA pool and are utilized in different metabolic pathways including lipogenesis and cholesterogenesis. The nature of these precursors and their effective contributions to the synthesis of lipids or cholesterol has been investigated by several techniques. For example, rats were injected with [^{3}H]acetate or [U-^{14}C]palmitate, oleate or linoleate after saturated or polyunsaturated fat

feeding. Tritiated acetate was incorporated into total lipid and cholesterol but ^{14}C incorporation into cholesterol was negligible (Dupont, 1970). Apparently, exogenous acetate rather than fatty acids was a more readily available cholesterol precursor.

However, in hepatocytes exogenously added pyruvate or lactate was more available as a source of sterol carbon than was acetate. Perhaps this effect was due to differences in cell membrane permeability to these precursors. It is also possible that acetate to sterol conversion was restricted by low activity of a pathway enzyme, such as acetyl-CoA synthetase. The rate of sterol synthesis was unchanged even when acetate, pyruvate or lactate concentrations were increased. Apparently acetyl-CoA concentrations are not rate limiting for cholesterogenesis (Gibbons and Pullinger, 1979).

Serum Lipoprotein Composition

The effects of dietary lipids on serum lipid concentrations as well as lipoprotein synthesis, clearance and degradation in both humans and experimental animals have been reviewed recently (Grundy, 1979; Truswell, 1978).

Lipids, except for fatty acid-albumin combinations,

are transported in plasma as soluble lipoproteins. They are classified conveniently into four classes on the basis of density, flotation rate or electrophoretic mobility: chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL).

Derivation and function of these four classes and alterations that occur in their properties due to dietary manipulation are not completely understood. However, these alterations may be important since low plasma HDL concentrations seem as characteristic of patients with heart disease as are elevated concentrations of VLDL and LDL (Lewis, 1977). Strong negative correlations have been reported between HDL cholesterol concentrations and the masses of both rapidly and slowly exchangeable cholesterol pools. Evidently, cellular concentrations of cholesterol and its storage in various tissues are related to lipoprotein composition (Miller et al., 1975).

In rats (Frnka and Reiser, 1974), gerbils (Nicolosi et al., 1976) and dogs (Lindall et al., 1971), dietary saturated but not polyunsaturated fats caused an increase in VLDL or LDL fractions and usually in cholesterol, triglyceride and phospholipid concentrations.

Diurnal Variations in Parameters of Lipid Metabolism

Diurnal variations in the activities of HMG-CoA reductase and cholesterol 7α-hydroxylase and cholesterol synthesis have been reviewed. Other parameters associated with lipid metabolism have also been measured during a 24 hour period, usually to identify changes which occur between the 12 hour dark, or food intake period, and the light period.

In both lean and obese Zucker rats, the rate of hepatic fatty acid synthesis from labelled pyruvate was increased during the dark and decreased during the light period when low-fat diets were fed. Fatty acid synthetase activity, however, did not change with photoperiod (Martin et al., 1979). This study is typical of others in which changes (diurnal or not) in the rate of a metabolic process were not accompanied by changes in the activity of the pathway enzymes.

Lipogenesis and cholesterogenesis were also significantly higher during the food intake rather than light period in a study by Kimura et al. (1970). Differences in rates of synthesis between photoperiods were more marked in younger, 90 g, rats than in older rats, perhaps because the younger rats consumed more food during the dark

period.

Lipoprotein lipase activity peaked in adipose during a dark cycle and decreased to a minimum in the light (De Gasquet et al., 1977). Variations in plasma free fatty acids, triglycerides and ketone bodies were more evident over 24 hours when fat supplied 9% rather than 73% of total calorie intake. Maxima occurred in all three parameters during the dark cycle with the low fat diet.

A diurnal rhythm was observed in the concentration of plasma triglycerides, free fatty acids, corticosterone and, to a lesser extent, insulin when rats were fed diets containing various carbohydrates with 2% fat. There was, however, no significant diurnal variation in the activity of fatty acid synthetase (Bruckdorfer et al., 1974).

Cyclic changes in the fatty acid patterns of liver lipids were identified in rats fed a high corn oil diet (Wadhwa et al., 1973). The composition of the liver triglycerides was affected by cycles associated with the individual fatty acids. In another study, hepatic protein concentrations varied over 24 hours as did the activity of certain lipogenic enzymes (Peret et al., 1976).

With fat-free diets, rhythmic variations occurred in cholesterol and fatty acid synthesis, in hepatic cholesterol and bile acid content as well as in plasma free fatty acid

levels (Bortz and Steele, 1973). Fatty acid synthesis, determined by [1-^{14}C] acetate incorporation followed the onset of the feeding period with a subsequent fall in free fatty acids. Bile acid and cholesterol content peaked in the dark although no reciprocal relationship was noted between cholesterol synthesis and either hepatic bile acid or cholesterol content.

Rhythms in some lipid parameters appear to be linked to food intake. Possibly specific metabolites resulting from alimentation are the active regulatory agents (Bortz and Steele, 1973; Fuller and Diller, 1970). However, hormones also affect the rate of many processes of lipid metabolism. Insulin and glucagon secretion are apparently controlled by food intake and vary diurnally (Bruckdorfer et al., 1974; Hellman and Hellerstrom, 1959). These and other hormones may have regulatory roles in the diurnal rhythms of lipid metabolism.

METHODS AND MATERIALS

Three experiments were designed to explore whether diurnal variations exist in the activities of selected lipogenic enzymes and in concentrations of tissue lipids. The effect of certain variables on these parameters and on the activities of HMG-CoA reductase and cholesterol 7α-hydroxylase was also examined. Variables included degree of saturation and level of dietary fat, exogenous cholesterol and length of feeding. The design used for this study is shown in Figure 2.

Animal Treatment

Male Wistar rats,[1] 3 to 5 months old, and weighing 440 to 465 g, were used in this study. Prior to the experimental period, rats were fed a modified Steenbock XVII ration (Table 1). Additionally, each rat received an oral dose of 165 μg retinyl acetate and 1.25 μg vitamin D_3 in 50 mg corn oil weekly.

During the experimental period, rats were housed individually in screen bottom cages in a room maintained at 24 ± 1°C with relative humidity approximately 45%. Cages were changed weekly. Reversed photoperiods were used in the animal room with 12 hours light (3 p.m. to 3 a.m.) or dark (3 a.m. to 3 p.m.).

---

[1]Stock colony, Department of Food and Nutrition, Iowa State University.

Experiment	Diet	Initial age of rats (months)	Feeding period (weeks)	Time of sacrifice	Analyses: Liver Enzymes Fractions: Soluble	Analyses: Liver Enzymes Fractions: Microsomal	Analyses: Lipids: Blood	Analyses: Lipids: Liver
1	40CO			3 a.m. End light	ACX		Cholesterol	
	40SO	4	12				Free	
				9 a.m. Middark	FAS		Total	Cholesterol
				3 p.m. End dark	Protein		FFA	Total
							TG	
				9 p.m. Midlight				
2	40CO	5	12	9 a.m.	ACX	HMG-CoA	Cholesterol	Cholesterol
	40CC			9 p.m.	Protein	reductase	Total	Microsomal
	40SO					Cholesterol		Free
	40SC					7α-hydroxylase		
						Protein		
3	40CO	3	4	3 a.m.	ACS		Cholesterol	
	10CO			9 a.m.	FAS		Total	
				3 p.m.	Protein		TG	
				9 p.m.				

Figure 2. Experimental design

Table 1. Stock ration for male rats: modified Steenbock XVII (1976-1978)

Dietary Component	Percent by weight
Corn meal[a]	45.6
Skim milk[b]	10.3
$CaCO_3$ + trace elements[c]	0.4
NaCl (iodized salt)[d]	0.4
Brewers yeast[a]	8.6
Casein, high protein[a]	7.0
Alfalfa meal[e]	1.7
Wheat germ[f]	10.8
Linseed meal[g]	11.6
Corn oil[h]	3.5
Corn oil + vitamin D_3[i]	0.1

[a]Teklad, Madison, Wisconsin.

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

[c]Matheson Coleman and Bell Division, Matheson Company, Inc., Norwood, Ohio (contains KI, 0.2 g; $MnSO_4$, 0.79 g; $K_2AL_2(SO_4)_4$ 0.245 g; $CuSO_4$, 1.018 g; and $CaCO_3$ to make 500 g total).

[d]Morton salt.

[e]National Alfalfa, Lexington, Nebraska.

[f]General Mills, Inc., Minneapolis, Minn.

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

[h]Mazola, Best Foods Division Corn Products Co., New York.

[i]Crystalline vitamin D_3 (cholecalciferol) diluted to 2,000 IU (50 mcg/kg diet) with corn oil.

Dietary Treatments

Five diets were used in this study (Table 2). Fat calories were supplied by coconut oil (CO) or safflower oil (SO). The CO diets were supplemented with SO which served as a source of essential fatty acids. When the level of CO was reduced (experiment 3), corn starch was substituted for coconut oil on a weight basis. A 4.3% increase in calories from protein in this ration was not considered significant since the rats were past the stage of rapid growth.

Eight (experiments 1 and 3) or 16 (experiment 2) treatment groups (diet and time) consisted of 6 rats each. A pair of littermates was assigned to diets which differed in fat type (experiment 1), fat level (experiment 3) or contained the same fat with or without cholesterol (experiment 2). Animal feeding was begun on a schedule so that each animal was fed a total of 12 weeks (experiment 1 or 2) or 4 weeks (experiment 3) before sacrifice.

Diet and water were fed ad libitum. Additionally, water- and fat-soluble vitamin supplements were given in small cups each day. The composition of the water-soluble vitamin mixture is presented in Table 3. Vitamin E was diluted with corn oil so that 50 mg daily supplied 0.75 mg DL-α-tocopherol acetate. A calibrated dropper which delivered in 2 drops approximately 50 mg of cod liver oil was used to present

Table 2. Composition of experimental diets based on weights and calories

Dietary components	Diets 40CO	40SO	40CC	40SC	10CO
			(% weight)		
Coconut oil[a]	20.5	0	20.0	0	4.0
Safflower oil[b]	0.6	21.1	0.6	20.6	0.5
Cholesterol[c]	0	0	0.5	0.5	0
Casein, vitamin free[d]	22.0	22.0	22.0	22.0	22.0
Nonnutritive fiber (cellulose)[a]	3.0	3.0	3.0	3.0	3.0
DL-methionine[f]	0.3	0.3	0.3	0.3	0.3
Corn starch[e]	50.1	50.1	50.1	50.1	66.7
Salt mix[a] (William & Briggs)	3.5	3.5	3.5	3.5	3.5
			Kcal/100 Kcal		
Fat	40.3	40.3	40.3	40.3	10.2
Casein	18.2	18.2	18.2	18.2	22.5
Corn starch	41.5	41.5	41.5	41.5	67.3

[a]Teklad Test Diets, Madison, Wisconsin.

[b]Pacific Vegetable Oil Corp., Richmond, California (DL-α-tocopherol added as antioxidant at 0.1% by weight).

[c]J. T. Baker Chemical Co., Phillipsburg, New Jersey.

[d]91% protein, Teklad Test Diets, Madison, Wisconsin.

[e]Clinton Corn Products, Clinton, Iowa.

[f]1.5 g/100 g casein, Teklad Test Diets, Madison, Wisconsin.

Table 3. Composition of water-soluble vitamin mixture

Vitamin	dose/rat/day
Thiamin[a]	40 mcg
Riboflavin	60 mcg
Pyridoxine HCl	40 mcg
Ca-pantothenate	100 mcg
Nicotinic acid	500 mcg
Folic acid	8 mcg
Biotin[b]	1 mcg
Vitamin B_{12}[c]	0.72 mcg
L-ascorbic acid	1 mg
Meso-inositol	10 mg
Para-aminobenzoic acid (USP XIV, PABA)	10 mg
Choline Cl	5 mg
Dextrin to make 500 mg[d]	-

[a]All vitamins obtained from General Biochemicals, Inc., Chagrin Falls, Ohio (known as Teklad, Madison, Wisconsin, since 1975).

[b]Biotin mixture prepared by mixing 1 mg biotin with 99 mg dextrin.

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

[d]Teklad, Madison, Wisconsin.

85 IU vitamin A and 8.5 IU vitamin D daily. Vitamin preparations were stored in amber bottles at 4° or -20° depending on length of storage.

Diets were prepared in 15 kg batches. Coconut oil was melted (23-28°) and cooled before addition to dietary dry ingredients. In experiment 2, cholesterol was dissolved in SO or liquid CO prior to addition. Diets were stored at 4°.

Termination of experiments

Groups of rats were killed by cervical dislocation[1] on a schedule which called for sacrifice of six animals per day for eight days at the termination of each experiment. Three rats on two different diets were sacrificed during a one hour period beginning 30 minutes before the recorded time of sacrifice.

Blood was obtained from the unconscious animal by heart puncture. To obtain serum, blood was spun after clotting in a clinical centrifuge at 4° and 3000 rpm. To obtain plasma for experiment 1, blood was collected in heparinized tubes (140 IU Na heparin/ml) and treated like the serum samples. Serum or plasma was removed with a Pasteur pipette and stored in covered vials at -20° until analysis.

---

[1]Cervical Dislocators, Inc., Wausau, Wisconsin.

Liver was excised immediately, rinsed in cold distilled water, blotted and weighed. Portions were removed for enzyme analyses and the remainder wrapped in Al foil and frozen in liquid nitrogen prior to storage at -70°.

Enzyme Assays

Assay linearity

Each enzyme assay was checked for linearity over a range of protein concentration and time. Quantities of soluble or microsomal protein and the incubation times used in each assay are based on the results of these preliminary experiments.

Centrifugation of liver homogenates

During preparation of homogenates, samples were centrifuged at low speeds in a Sorvall, Model RC2-B centrifuge.[1] Final centrifugation at 100,000 x g was in a Beckman Model L3-50 ultracentrifuge.[2]

---

[1] Sorvall, Inc., Norwalk, Conn.

[2] Beckman Instruments, Fullerton, Calif.

Counting of radioactivity

Assays for radioactivity were carried out in a Packard Tri Carb Model C 2425 scintillation counter[1]. Samples were counted to 1% accuracy. For double label counting, preset ^{14}C and ^{3}H channels were used. Spillover of ^{14}C into the ^{3}H channel was less than 0.1%. External and internal standardization, using ^{3}H- or ^{14}C-toluene, were used to correct for decreased efficiency due to quenching.

Acetyl-coenzyme A carboxylase

The activity of ACX was assayed at the time of sacrifice. A 4 g portion of liver was minced on a watch glass in ice. This material was homogenized in 0.1 M potassium phosphate buffer at pH 7.4, containing 0.004 M $MgCl_2$, 0.001 M EDTA and 0.01 M 2-mercaptoethanol with seven strokes of a loose-fitting Potter-Elvehjem homogenizer in an ice bath. The volume of buffer was 2 ml per gram of liver. The crude homogenate was centrifuged for 10 min at 5000 x g. The supernatant was removed and recentrifuged for 15 min at 15,000 x g. The resulting supernatant suspension was centrifuged for 1 hour at 100,000 x g. After final centrifugation an upper lipid layer floated above the soluble protein. Inclusion of lipid in

---

[1]Packard Instrument Co., Downer's Grove, Illinois.

the soluble protein was avoided by careful removal of the latter by Pasteur pipette. A portion of the final supernatant was diluted with homogenizing buffer (1→5) and the protein concentration determined. Fifty to 80 µl of the dilution which contained approximately 100 µg protein was used for each ACX assay.

The assay for ACX was carried out in triplicate as described by Craig et al. (1972) and Inoue and Lowenstein (1975). An aliquot of the 100,000 x g supernatant solution was preincubated at 37° for 30 min in 900 µl preincubation solution. This solution contained: Tris-chloride, 50 µmoles; dithiothreitol, 1 µmole; $MgCl_2$, 19 µmoles; potassium citrate, 20 µmoles, and bovine serum albumin, "fatty acid free", 0.5 mg/ml.

The final solution was at pH 7.5. The carboxylation reaction was initiated by adding to the preincubation mixture the following: ATP, 2 µmoles; acetyl-CoA, 0.2 µmoles and $[^{14}C]$ $NaHCO_3$[1], 10 µmoles ($5x10^6$ dpm). Vials were tightly capped after bicarbonate addition.

Incubations were carried out in a shaking water bath at 37° for 2 min and terminated in an ice bath by the addition with mixing of 200 µl 6N HCl. One-half ml of the acidified

---

[1]New England Nuclear, Boston, Mass.

mixture was added to a scintillation vial and evaporated at 70-80° under N_2. Three-tenths ml water and 10 ml Bray's solution[1] were added to the vial and the sample assayed for radioactivity.

Fatty acid synthetase

The FAS procedure was essentially that described by Craig et al. (1972). On the day of sacrifice a portion of soluble protein isolated from the high speed centrifugation of the liver homogenate was immediately stored under N_2 in a screw capped vial at -70°. To assay for FAS activity, the protein was removed from storage (within 1 month of animal sacrifice), thawed and diluted (1→10) with homogenizing buffer. An aliquot containing approximately 100 μg soluble protein was used for each assay and samples were run in triplicate. One ml of the incubation mixture contained: [1-^{14}C]acetyl-CoA[1], 0.05 μmole (40.5×10^4 dpm); malonyl-CoA 0.1 μmole; NADPH, 0.1 μmole; 2-mercaptoethanol, 5 μmoles; EDTA, 3 μmoles; potassium phosphate buffer, 100 μmoles at pH 6.8. Samples were incubated for 3 min at 37° in a shaking water bath. One ml absolute ethanol and 0.2 M 6N HCl were added with mixing to terminate each reaction. Product was

[1]New England Nuclear, Boston, Mass.

extracted with 2 separate 5 ml portions of heptane. The extraction solvent was transferred to a scintillation vial, and the heptane evaporated under N_2. This procedure extracted more than 90% of the isotope in the product layer. After evaporation of heptane, 10 ml Bray's solution was added and the sample assayed for radioactivity.

Cholesterol 7α-hydroxylase

The procedure used in this assay was reported by Carlson et al. (1978a). Animals were sacrificed by cervical dislocation and a 5 g portion of liver was minced on a watch glass in ice. This sample was homogenized with 9 strokes of a Potter-Elvehjem homogenizer held in an ice bath. The homogenizing buffer contained: 100 mM phosphate buffer at pH 7.4; 0.25 M sucrose; 0.075 M nicotinamide; 1 mM dithiothreitol; 2.5 mM EDTA. The volume of buffer was 4 ml per g of liver. After homogenization, samples were spun at 20,000 x g for 15 min. A 10 ml aliquot of the supernatant was spun at 100,000 x g for 60 min. The upper layer was removed by Pasteur pipette and discarded. The microsomal pellet was resuspended in 5 ml homogenizing buffer with 4 strokes of a hand held homogenizer. This suspension was assayed immediately for hydroxylase activity and a portion stored in liquid N_2[1] after being layered with N_2.

[1]Physics Department, Iowa State University.

Assays in duplicate contained a final volume of 1.05 ml including approximately 700 μg microsomal protein, 57 μmoles potassium phosphate, 6.8 μmoles cysteamine, 4.5 μmoles $MgCl_2$, 0.64 μmoles $NADP^+$, 2.6 μmoles glucose-6-phosphate and 0.2 IU glucose-6-phosphate dehydrogenase. One hundred μmoles cholesterol were added to each assay including [4-^{14}C] cholesterol[1] purified by thin layer chromatography on activated silica gel plates. The substrate containing solution included 38 μg cholesterol and [4-^{14}C] cholesterol (25×10^4 dpm), solubilized with 1.1 mg Tween 80. The assay tubes at pH 7.4 excluding the NADPH generating system were incubated for 10 min at 0°. The NADPH generating system was added. Samples were incubated at 37° for 30 min in a shaker bath. The reaction tubes were transferred to an ice bath and the reaction stopped by the addition of 10 ml 95% ethanol. This mixture was extracted twice with 20 ml portions of pet ether (B.P. 30-65°).

The samples were evaporated to dryness under N_2 and re-suspended in 100 μl benzene and 25 μl methanol. This suspension and 7α-hydroxycholesterol and cholesterol solutions were spotted under N_2 on separate channels of activated silica gel plates. The chromatograms were developed in benzene/ethyl acetate (2:3 v/v). Product was identified by

---

[1]New England Nuclear, Boston, Mass.

spraying the plates with 50% H_2SO_4 and portions containing product or substrate were scraped into scintillation vials. After the addition of 10 ml Scintisol[1] each portion was assayed for radioactivity.

HMG-CoA reductase

This procedure was carried out according to Shapiro et al. (1974) and Nordstrom et al. (1977). Microsomal suspensions were used within 6 months of termination of experiment 2. Samples were obtained from storage in liquid nitrogen, thawed and each sample assayed in duplicate. Twenty-five µl, approximately 100 µg protein, was added to 75 µl buffer containing 50 mM potassium phosphate at pH 7.5, 1 mM EDTA, 5 mM dithiothreitol (added just before use) and 0.3 M KCl. The reaction was initiated by adding 35 µl of cofactor solution which contained: 3.6 µmoles EDTA; 4.5 µmoles glucose-6-phosphate; 0.45 µmoles $NADP^+$; 0.3 IU glucose-6-phosphate dehydrogenase; 50 nmoles R,S-[^{14}3-C] HMG-CoA (1.38 Ci/mole).[2] Incubation was at 37° in a shaking water bath. Control incubations contained no added reductase. After 20 min, reaction was stopped by the addition of 15 µl 6N HCl to tubes in ice. Ten µl [3R, 4R-4-^{3}H + 3S, 4S-4^{3}H]

[1]Isolab, Inc., Akron, Ohio.
[2]New England Nuclear, Boston, Mass.

mevalonic acid lactone[1] ($2.0x10^4$ dpm and 1.8 Ci/mmole) was added as an internal standard to each tube and lactonization proceeded for 30 min. The 1/2 dram reaction vials were centrifuged for 5 min at 2500 x g in the cold to sediment protein. One hundred µl supernatant was spotted on activated silica gel thin-layer chromatography sheets[2] ruled vertically into 3 to 4 channels. Chromatographs were developed in benzene-acetone (1:1, v/v) and air dried. The region R_f 0.6 to 0.9 for each sample was removed by razor blade and added to a scintilation vial. Ten ml Bray's solution was added. The samples were counted for both ^{14}C and ^{3}H. Raw ^{14}C counts were corrected based on the recovery of [^{3}H] mevalonic acid lactone used as an internal standard.

Tissue Analyses

Plasma was used in experiment 1, serum in experiments 2 and 3.

Cholesterol

Total serum cholesterol was determined by the method of Roeschlau et al. (1974). Serum, standards and blanks were treated according to the protocol given in Table 4.

---

[1]Amersham Corp., Arlington Heights, Ill.

[2]Eastman Kodak Co., Rochester, N.Y.

Table 4. Cholesterol protocol

Sample	Serum (μl)	H_2O (μl)	Standards[a] (μl)	Enzyme reagent[b] (μl)
Serum	10	-	-	500
Serum blank	10	500	-	-
Standards	-	-	10	500
Reagent blank	-	10	-	500

[a]5, 10 or 15 μg cholesterol, recrystallized from ethanol in 10 μl isopropanol.

[b]Centrifichem, Union Carbide Corp., Rye, N.Y.

Each sample was mixed for 15 sec, then incubated for 15 min at 37° in a shaking water bath. Samples were cooled in ice and absorbance read at 520 nm within 90 min[1].

Total and free cholesterol in plasma were determined by the method of Allain et al. (1974). The protocol in Table 4 was followed. The samples were 20 or 50 μl plasma for total and free cholesterol, respectively. Standards of 5, 10, 15 or 20 μg recrystallized cholesterol in 20 or 50 μl isopropanol were used for total or free cholesterol, respectively. Plasma and reagent blanks were run. The enzyme reagents for total cholesterol determinations are given in Table 5.

[1]Gilford Spectrophotometer 240, Gilford Instrument Laboratories, Inc., Oberlin, Ohio.

Table 5. Reagents for total and free cholesterol determinations (Allain et al., 1974)

Reagent	Concentration mmoles/liter
Sodium cholate	3.0
4-Aminoantipyrine	0.82
Phenol	14.0
Na_2HPO_4	50.0
NaH_2PO_4	50.0
Carbowax-6000	0.17
Cholesterol ester hydrolase	33 U/liter
Cholesterol oxidase	117 U/liter
Horseradish peroxidase	67000 U/liter
$pH_{25°}$	6.70 $\pm$ 0.10

Cholesterol ester hydrolase was omitted from the enzyme reagent for free cholesterol determinations.

Samples and appropriate enzyme reagents were treated as described under the total serum cholesterol procedure.

Cholesterol esters were calculated as the difference between total and free plasma cholesterol concentrations.

Free microsomal cholesterol was determined by modification of the method of Carlson and Goldfarb (1977). Two hundred and fifty μl microsomal homogenate (see section on animal treatment) were added to 5 ml $CHCl_3:CH_3OH$ (2:1 by

volume) and mixed for 30 sec. One ml 0.05 M NaCl was added and the suspension mixed an additional 30 sec. The suspension was centrifuged for 5 min at 1200 x g and the top layer removed by suction. Two hundred and fifty μl of the lower layer or 250 μl of a chloroform blank and 10, 15 and 20 μg recrystallized cholesterol in chloroform were added to 50 μl Tween 80 reagent (1.0 g Tween 80 in 20 ml acetone). Solvents were evaporated under N_2 and 500 μl of the enzymatic reagent for free cholesterol were added to each tube. The procedure was completed using the directions given for total serum cholesterol.

Total liver cholesterol was determined by the method of Carlson and Goldfarb (1977). Two hundred mg liver were introduced into 5 1/2" screw top tubes; 1 ml 30% KOH in 95% ethanol was added. The mixture was saponified at 75° for 60 min, cooled and 1.5 ml 95% CH_3OH added, followed by 20 ml pet ether (B.P. 30-60°). The tubes were capped and centrifuged at 1200 x g for 5 min. Three hundred μl of extract or 150 μl of standards containing 0, 5, 10, or 15 μg recrystallized cholesterol in pet ether (B.P. 30-60°) were added to tubes containing 2.5 μg Tween in 50 μl acetone. The mixture was mixed for 15 sec. The solvents were evaporated under N_2 and 500 μl of cholesterol reagent[1] were added to each

[1]Centrifichem, Union Carbide Corp., Rye, N.Y.

tube. The tubes were vortexed for 15 sec and incubated for 30 min at 37°. The assay was completed using the procedure described for total serum cholesterol.

Triglycerides

Triglycerides were determined by the manual method of Giegel et al. (1975). One hundred μl plasma or serum and standards containing 50 to 200 μg triolein, dissolved in $CHCl_3$, were added to 3 ml extraction solvent and mixed for 15 sec. Six tenths ml 40 mM H_2SO_4 was added and the tubes again mixed for 15 sec. All tubes were centrifuged at 1000 x g at 0-5° for 5 min. One-half ml of the top layer was added to a 5 1/2" screw cap tube. The blank was set up by adding 0.5 ml extraction solvent to an empty tube. One-half ml 100 mM NaOH in isopropanol was added to each tube. The tubes were mixed for 15 sec and held 5 min at room temperature before 0.5 ml sodium periodate reagent was added. The tubes were again mixed for 15 sec and held for 2 min. Six ml acetylacetone in ammonium acetate buffer were added, the tubes capped, mixed and incubated at 56-58° for 10 min. The samples were cooled to room temperature and read within 1 hour at 415 nm.[1]

[1]Beckman DU, Beckman Instruments, Fullerton, Calif. Modified by a digital readout from Update Instruments, Madison, Wisconsin.

Free fatty acids

Plasma free fatty acids were determined by a modification of the methods of Saloni and Sardenia (1973) and Louwerys (1969). Two hundred μl plasma or 25-50 μg palmitic acid standard were added to 5 ml extraction solvent and mixed for 30 sec. An additional 2 ml H_2O and 3 ml heptane were added and samples again mixed. The tubes were centrifuged at 1200 x g for 10 min. After centrifugation, 3 ml of the heptane layer from each tube and 3 ml heptane for a blank were transferred to clean tubes. One ml 0.01% thymol blue in 70% methanol was added, followed by dropwise addition of 1N NaOH until there was a color change to blue. The tubes were mixed for 20 sec and centrifuged for 5 min at 1200 x g. The heptane layer was suctioned off and 1N HCl added until the solution became yellow. Three ml heptane were added; the tubes were mixed for 20 sec and centrifuged at 1200 x g for 5 min. Two ml of the heptane layer, 2 ml of $CHCl_3$, and 1 ml of the copper color reagent were added to clean test tubes. The mixture was mixed for 20 sec and centrifuged at 1200 x g for 5 min. Three ml of the upper phase were added to a tube containing 0.25 ml diethyldithiocarbamate dye. Absorbance was read at 440 nm within 1 hour.

Protein

Protein concentrations were determined by the method of Gornall et al. (1949) in the supernatant and in microsomal liver fractions obtained after ultracentrifugation by a modified biuret reaction. To remove interference from -SH containing buffers, aliquots containing 0.5-2.5 g liver protein or 0.5-2.5 g bovine serum albumin (standards) in buffer were precipitated with 5% TCA (Goodwin and Margolis, 1976). After centrifugation for 3 min at 1200 x g, TCA was removed by suction. One half ml 1N NaOH was added to all tubes which were then mixed to dissolve the protein. One hundred μl 5% deoxycholate were added to tubes containing microsomal protein to dissolve the lipid in that fraction. (It was necessary occasionally to add 10-50 μl additional deoxycholate to solubilize the lipid).

Four ml biuret reagent was added to the solubilized supernatant or microsomal protein and the samples read within 1 hour at 540 nm.

Statistical Analysis

Data were treated statistically by Duncan's multiple range test at the 0.05 probability level and the student t-test. Duncan's multiple range test was used to compare 2, 4 or 8 groups of diet and/or time data. For that analysis

SEM values were obtained from a pooled mean square error. Occasionally data from two individual treatment groups were compared by the student t-test.[1] Differences between treatments from this analysis have been reported in instances when significance occurred at $P \leq .1$, indicating trends.

[1]Canala calculator, Canon Inc., Tokyo, Japan.

RESULTS

Results have been organized to illustrate the effect of time and diet on ACX, FAS, HMG-CoA reductase, cholesterol 7α-hydroxylase and on selected lipid concentrations. Data have also been presented to illustrate the effect of 12 hour photoperiods on these parameters. In order to analyze for diet effect, mean daily values (MDV) have been calculated for various experimental parameters by combining values obtained over a 24 hour period in experiments 1 and 3. In experiment 2, mean combined values (MCV) were obtained by combining assay values from 9 a.m. and 9 p.m. An outline of diets, feeding periods and sacrifice times as well as the assays performed is presented in Figure 2.

Group means for initial body weights of adult rats used in each study were statistically similar by design and were similar between studies with an overall range of means from 442 g to 465 g. In general, rats fed coconut oil consistently gained more weight than those fed safflower oil although the difference in weight gain was not always significant.

Experiment 1

The objectives of experiment 1 were to determine: 1) whether the activity of ACX and FAS and selected lipid parameters vary diurnally, 2) whether these variations can be correlated, and 3) what effect degree of fat saturation has on enzyme activity and tissue lipid concentrations.

Body and liver weights

Rats on the CO diet gained an average of 30 g while those fed SO lost an average of 14 g ($p < 0.05$). These differences were reflected in the average final body weights, 497 g and 451 g for rats on the CO and SO diets, respectively ($p < 0.05$, Table 6). Weight losses occurred during the last half of the 12 week feeding period so that 50% of the animals fed SO and 25% of the animals fed CO eventually lost weight. Mean liver weights, 13.3 g and 11.0 g with CO and SO feeding, respectively, were also different ($p < 0.05$, Table 6).

Acetyl-CoA carboxylase

Time Mean ACX activity (ACX:ACT) was determined at 6 hour intervals (Figure 3). Differences in mean activity between three of those intervals, 9 p.m., 3 a.m., or 9 a.m., were not significant. With both diets, however, ACX:ACT rose from 3 p.m. to 9 p.m. ($p < 0.05$, Table 7). The

Table 6. Effect of dietary fat on selected body parameters (experiments 1, 2 and 3)

	Diet	Final body weight (g)	Weight gain (g)	Liver weight (g)	Liver wt. / Body wt. (%)
Experiment 1	40CO	497 ± 8 (24)[a,1]	30 ± 7 (24)[a]	13.3 ± 0.3 (24)[a]	2.7[a]
	40SO	451 ± 8 (24)[b]	-14 ± 7 (24)[b]	11.0 ± 0.3 (24)[b]	2.4[b]
Experiment 2	40CO	469 ± 11 (11)[a]	10 ± 11 (11)[a]	14.6 ± 0.6 (14)[a]	3.1[b]
	40CC	452 ± 10 (14)[a]	10 ± 9 (14)[a]	12.5 ± 0.6 (11)[b]	2.8[c]
	40SO	468 ± 11 (11)[a]	7 ± 11 (11)[a]	16.2 ± 0.6 (14)[a]	3.5[a]
	40SC	451 ± 10 (14)[a]	6 ± 9 (14)[a]	11.6 ± 0.6 (11)[b]	2.6[c]
Experiment 3	10CO	490 ± 7 (24)[a]	48 ± 4 (24)[a]	14.4 ± 0.3 (24)[a]	2.9[a]
	40CO	494 ± 7 (24)[a]	35 ± 4 (24)[b]	14.2 ± 0.3 (24)[a]	2.9[a]

[1]Mean ± SEM (number of rats), means not followed by the same superscript are significantly different ($p < 0.05$) by Duncan's multiple range test.

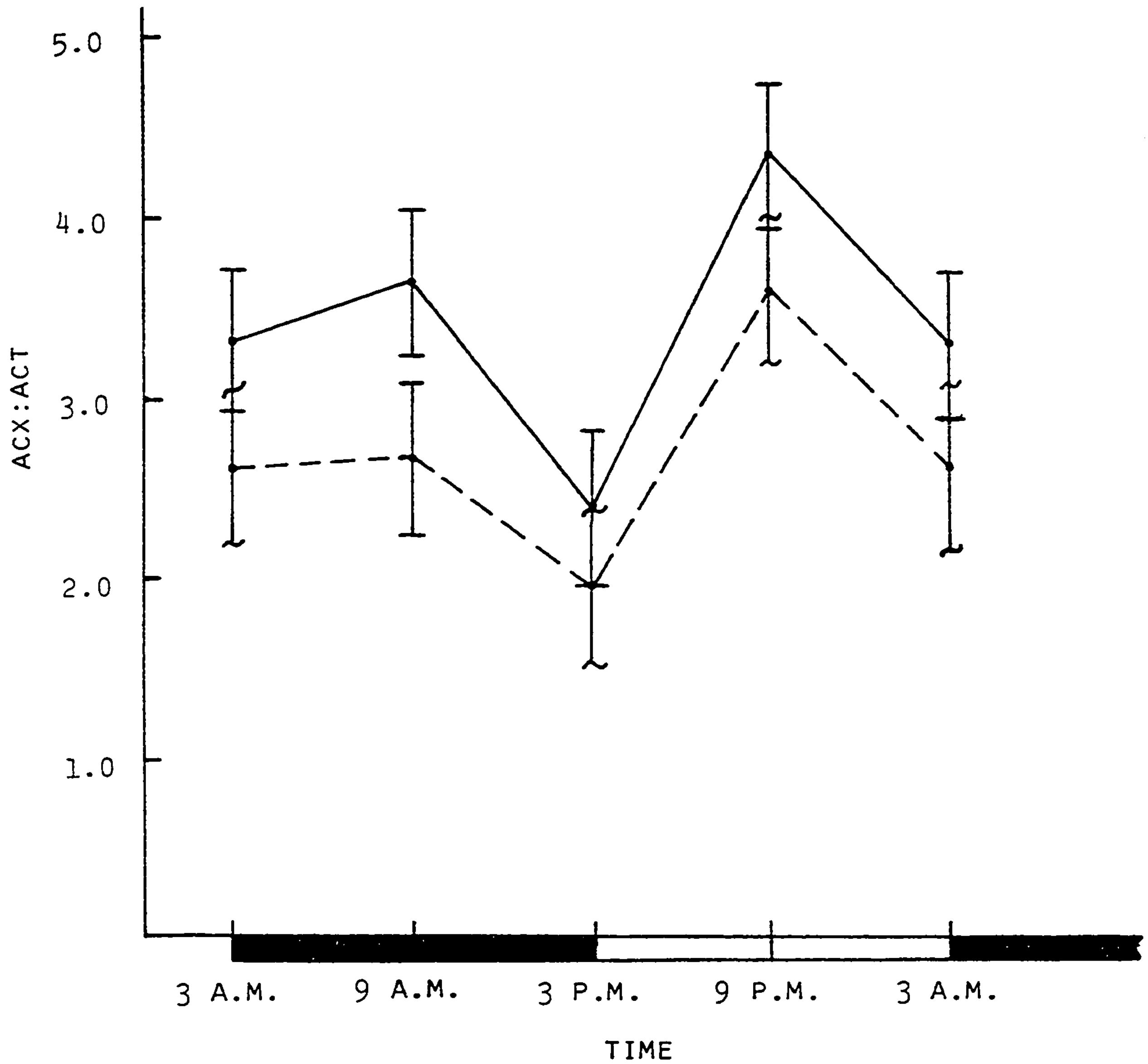


Figure 3. Activity of acetyl-CoA carboxylase (ACX:ACT) at consecutive times. Values are means of 6 rats. 40CO (——); 40SO (----). Horizontal dark bar indicates duration of dark period from 3 A.M. to 3 P.M. (experiment 1)

Table 7. ACX:ACT[1] at consecutive times or photoperiods (experiment 1)

Time	Diet	ACX:ACT
TIME		
3 a.m. End light	40CO	3.34 ± 0.39 (6)[a,b,c,2]
9 a.m. Middark	40CO	3.66 ± 0.39 (6)[a,b]
3 p.m. End dark	40CO	2.36 ± 0.39 (6)[d,c]
9 p.m. Midlight	40CO	4.38 ± 0.39 (6)[a]
3 a.m. End light	40SO	2.62 ± 0.39 (6)[b,d,c]
9 a.m. Middark	40SO	2.66 ± 0.39 (6)[b,d,c]
3 p.m. End dark	40SO	1.96 ± 0.39 (6)[d]
9 p.m. Midlight	40SO	3.62 ± 0.39 (6)[a,b]
PHOTOPERIOD		
9 a.m.; 3 p.m. Dark	40CO	3.01 ± 0.31 (12)[a,b]
9 p.m.; 3 a.m. Light	40CO	3.86 ± 0.31 (12)[a]
9 a.m.; 3 p.m. Dark	40SO	2.31 ± 0.31 (12)[a,b]
9 p.m.; 3 a.m. Light	40SO	3.12 ± 0.31 (12)[b]

[1]ACX:ACT nmoles HCO_3^- fixed/mg protein/min.

[2]Mean ± SEM (number of rats), means not followed by the same superscript are significantly different ($p < 0.05$) by Duncan's multiple range test.

increase in enzyme activity in the light period (9 p.m.) was comparable on both diets, 86% or 85% for 40CO or 40SO, respectively. Similar results were obtained when ACX:ACT was based on protein content of whole liver, protein content of liver per 100 g body weight or mg protein per ml liver homogenate.

Mean values for ACX:ACT ranged from 2.36 to 4.38 with 40CO and 1.96 to 3.62 for 40SO. Examination of the data over 24 hours precludes the existence of a diurnal rhythm in ACX activity similar to the activity evident in HMG-CoA reductase (Shefer et al., 1973) or cholesterol 7α-hydroxylase (Van Cantfort and Gielen, 1975). With the photoperiods used in this study synchronous (asynchronous) activity between ACX and the reductase or hydroxylase would have required several fold increases (decreases) in ACX:ACT between 9 a.m. and 9 p.m. or 9 p.m. and 9 a.m. Although no diurnal variations appeared in the activity of this enzyme, the enzyme was definitely stimulated during the light period.

Diet At each 6 hour interval, ACX:ACT was increased by CO versus SO feeding but differences in activity due to diet did not reach statistical significance at any one time (Table 7). However, when mean daily values (MDV) for ACX:ACT were compared, 3.44 or 2.72 with CO or SO, respectively, the difference due to diet was significant

($p < 0.05$) with an increase of 24% due to CO versus SO (Table 8).

Fatty acid synthetase

Time The range of FAS activities (FAS:ACT) over time was narrow (Table 9). Mean values for FAS:ACT at the four intervals measured ranged from 0.90 to 1.25 or 0.66 to 0.96 in the 40CO or 40SO groups, respectively. Activity did not vary over 24 hours so that the concept of a diurnal rhythm in FAS activity similar to that established for the regulatory enzymes of cholesterol synthesis and degradation was not supported.

Diet Mean FAS activity was consistently increased with CO compared to SO feeding at each time interval (Table 9). Again, these differences were statistically significant only when MDV were compared (Table 8). Values for activity were 1.08 or 0.78 for 40CO or 40SO, respectively ($p < 0.05$, Table 8).

Serum cholesterol

Time Serum mean total, free or esterified cholesterol did not vary with time (Table 10). Concentration ranges were narrow. For example, total cholesterol values ranged from 69 to 77 mg/dl or 61 to 70 mg/dl for 40CO or 40SO, respectively ly.

Table 8. Effect of dietary fat on ACX:ACT[1] and FAS:ACT[2] (experiment 1)

Diet	ACX:ACT	FAS:ACT
40CO	3.44 ± 0.19 (24)[a,3]	1.08 ± 0.09 (24)[a]
40SO	2.72 ± 0.19 (24)[b]	0.78 ± 0.09 (24)[b]

[1]ACX:ACT, nmole HCO_3^- fixed/mg protein/min.

[2]FAS:ACT, nmoles fatty acids formed/mg protein/min.

[3]Mean ± SEM (number of rats), means not followed by the same superscript are significantly different ($p < 0.05$) by Duncan's multiple range test.

Table 9. FAS:ACT[1] at consecutive times or photoperiods (experiment 1)

		Diet	FAS:ACT
TIME			
3 a.m.	End light	40CO	1.12 ± 0.18 (6)[a,b,2]
9 a.m.	Middark	40CO	1.07 ± 0.18 (6)[a,b]
3 p.m.	End dark	40CO	0.90 ± 0.18 (6)[a,b]
9 p.m.	Midlight	40CO	1.25 ± 0.18 (6)[a]
3 a.m.	End light	40SO	0.66 ± 0.18 (6)[b]
9 a.m.	Middark	40SO	0.96 ± 0.18 (6)[a,b]
3 p.m.	End dark	40SO	0.72 ± 0.18 (6)[a,b]
9 p.m.	Midlight	40SO	0.77 ± 0.18 (6)[a,b]
PHOTOPERIOD			
9 a.m.; 3 p.m.	Dark	40CO	0.98 ± 0.12 (12)[a]
9 p.m.; 3 a.m.	Light	40CO	1.18 ± 0.12 (12)[a,b]
9 a.m.; 3 p.m.	Dark	40SO	0.84 ± 0.12 (12)[a,b]
9 p.m.; 3 a.m.	Light	40SO	0.72 ± 0.12 (12)[b]

[1]FAS:ACT, nmoles fatty acid formed/mg protein/min.

[2]Mean ± SEM (number of rats), means not followed by the same superscript are significantly different ($p < 0.05$) by Duncan's multiple range test.

Table 10. Plasma total cholesterol levels at consecutive times or photoperiods (experiment 1)

		Diet	Total cholesterol (mg/dl)	Cholesterol esters (mg/dl)	Free cholesterol (mg/dl)
TIME					
3 a.m.	End light	40CO	69 ± 5 (6)[a,1]	56 ± 5 (6)[a]	13 ± 1 (6)[a]
9 a.m.	Middark	40CO	77 ± 5 (6)[a]	63 ± 5 (6)[a]	14 ± 1 (6)[a]
3 p.m.	End dark	40CO	74 ± 6 (5)[a]	60 ± 5 (5)[a]	14 ± 1 (5)[a]
9 p.m.	Midlight	40CO	73 ± 5 (6)[a]	61 ± 5 (6)[a]	12 ± 1 (6)[a]
3 a.m.	End light	40SO	62 ± 5 (6)[a]	49 ± 5 (6)[a]	12 ± 1 (6)[a]
9 a.m.	Middark	40SO	70 ± 5 (6)[a]	55 ± 5 (6)[a]	15 ± 1 (6)[a]
3 p.m.	End dark	40SO	62 ± 5 (6)[a]	50 ± 5 (6)[a]	12 ± 1 (6)[a]
9 p.m.	Midlight	40SO	61 ± 5 (6)[a]	50 ± 5 (6)[a]	11 ± 1 (6)[a]
PHOTOPERIOD					
9 a.m.; 3 p.m.	Dark	40CO	75 ± 4 (11)[a]	62 ± 3 (11)[a]	14 ± 1 (11)[a]
9 p.m.; 3 a.m.	Light	40CO	71 ± 4 (12)[a,b]	58 ± 3 (12)[a,b]	13 ± 1 (12)[a]
9 a.m.; 3 p.m.	Dark	40SO	66 ± 4 (12)[a,b]	53 ± 3 (12)[a,b]	14 ± 1 (12)[a]
9 p.m.; 3 a.m.	Light	40SO	61 ± 4 (12)[b]	50 ± 3 (12)[b]	12 ± 1 (12)[a]

[1] Mean ± SEM (number of rats), means not followed by the same superscript are significantly different ($p \leq 0.05$) by Duncan's multiple range test.

Diet When MVD were compared for CO versus SO feeding, total or esterified cholesterol concentrations were both significantly increased with the saturated fat (Table 11). Mean concentrations over 24 hours were for total cholesterol 73 or 64 mg/dl and for esterified cholesterol 60 or 51 mg/dl for 40CO or 40SO, respectively ($p < 0.05$). Average ester concentrations were 82% or 80% of total cholesterol with CO or SO, respectively. Free cholesterol concentrations averaged 13 mg/dl and were not affected by diet.

Serum triglycerides

Time Initially diets were compared over time by use of Duncan's multiple range test which used a pooled mean square error term (Table 12). Triglyceride concentrations were elevated with 40CO from the start (3 a.m.) to the mid-dark (9 a.m.) and decreased from the midlight (9 p.m.) to the start of the dark period (3 a.m.). The significance of changes during these time intervals also became apparent when the student t-test was applied to data from 40SO feeding (Table 12, Figure 4).

Diet Triglyceride MDV were higher with CO compared to SO with concentrations of 96 or 57 mg/dl, respectively ($p < 0.05$, Table 11).

Table 11. Effect of dietary fat on plasma cholesterol, triglyceride and free fatty acid levels (experiment 1)

Diet	Cholesterol Total	Cholesterol Esters (mg/dl)	Cholesterol Free	Free fatty acids (μeq/dl[1])	Triglycerides (mg/dl)
40CO	73 ± 3 (23)[a,2]	60 ± 2 (23)[a]	13 ± 0.7 (23)[a]	50 ± 14 (19)[a]	96 ± 7 (22)[a]
40SO	64 ± 3 (24)[b]	51 ± 2 (24)[b]	13 ± 0.6 (24)[a]	89 ± 13 (24)[b]	57 ± 7 (21)[b]

[1]μeq of palmitic acid.

[2]Mean ± SEM (number of rats), means not followed by the same superscript are significantly different ($p < 0.05$) by Duncan's multiple range test.

Table 12. Plasma triglyceride levels at consecutive times or photoperiods (experiment 1)

Duncan's multiple range test

		Diet	Triglycerides (mg/dl)
TIME			
3 a.m.	End light	40CO	60 ± 13 (6)[d,c,1]
9 a.m.	Middark	40CO	121 ± 13 (6)[a]
3 p.m.	End dark	40CO	96 ± 17 (4)[a,b,c]
9 p.m.	Midlight	40CO	106 ± 13 (6)[a,b]
3 a.m.	End light	40SO	34 ± 13 (6)[d]
9 a.m.	Middark	40SO	74 ± 13 (6)[b,c,d]
3 p.m.	End dark	40SO	51 ± 13 (6)[c,d]
9 p.m.	Midlight	40SO	85 ± 19 (3)[a,b,c,d]
PHOTOPERIOD			
9 a.m.; 3 p.m.	Dark	40CO	111 ± 12 (10)[a]
9 p.m.; 3 a.m.	Light	40CO	83 ± 11 (12)[a,b]
9 a.m.; 3 p.m.	Dark	40SO	62 ± 11 (12)[a,b]
9 p.m.; 3 a.m.	Light	40SO	51 ± 12 (9)[b]

Student's t-test

		Diet	Triglycerides (mg/dl)
TIME			
3 a.m.	End light	40CO	60 ± 12 (6)[2] ***
9 a.m.	Middark	40CO	121 ± 11 (6)
9 a.m.	Middark	40CO	121 ± 11 (6)
3 p.m.	End dark	40CO	96 ± 17 (4)
3 p.m.	End dark	40CO	96 ± 17 (4)
9 p.m.	Midlight	40CO	106 ± 24 (6)
9 p.m.	Midlight	40CO	106 ± 24 (6)
3 a.m.	End light	40CO	60 ± 12 (4)*
3 a.m.	End light	40SO	34 ± 6 (6)
9 a.m.	Middark	40SO	74 ± 14 (6)**
9 a.m.	Middark	40SO	74 ± 14 (6)
3 p.m.	End dark	40SO	51 ± 9 (6)
3 p.m.	End dark	40SO	51 ± 9 (6)
9 p.m.	Midlight	40SO	85 ± 22 (3)
9 p.m.	Midlight	40SO	85 ± 22 (3)
3 a.m.	End light	40SO	34 ± 6 (6)**

[1]Mean ± SEM (number of rats), means not followed by the same superscript are significantly different ($p < 0.05$) by Duncan's multiple range test.

[2]Student t-test; mean ± SEM (number of rats); *$p<0.1$; **$p<0.05$; ***$p<0.01$.

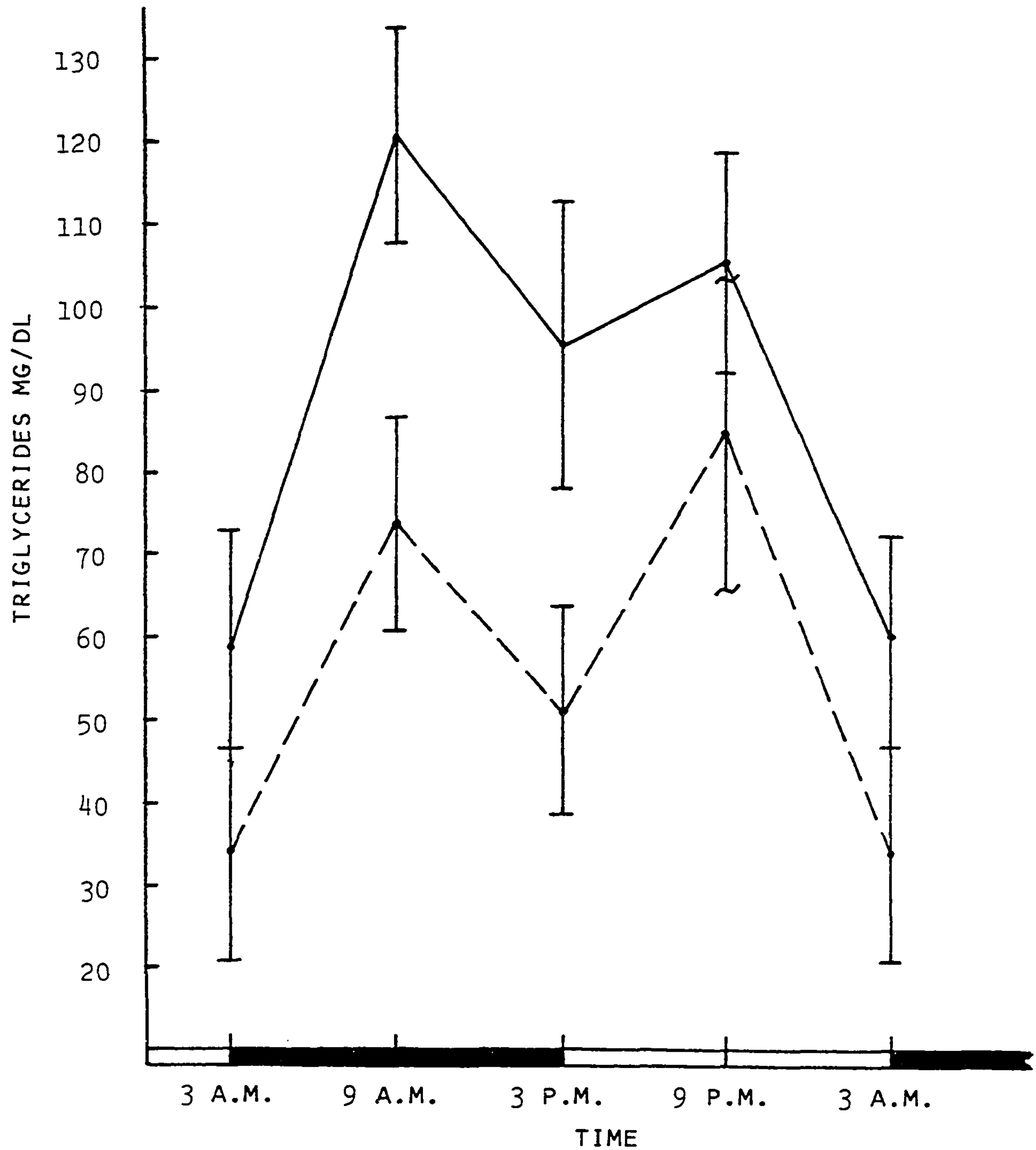


Figure 4. Serum triglyceride (TG) levels at consecutive times. Values are means of 3-6 rats. 40CO (——); 40SO (----). Horizontal dark bar indicates duration of dark period from 3 A.M. to 3 P.M. (experiment 1)

Free fatty acids

Values for SEM were large in this assay. It is not possible from the available data to differentiate between several factors which could have contributed to the variability within treatments. The relative size of the SEM term may reflect variability in animals, sampling techniques which could not control hydrolysis of fatty acid esters, or the assay procedure which required a large number of sample manipulations.

Time Mean free fatty acid (FFA) concentrations were elevated with 40SO at the beginning (3 p.m.) and six hours into the light period (9 p.m.) compared to the beginning (3 a.m.) or midpoint of the dark period (9 a.m.). These differences were significant (Table 13). Over 24 hours, ranges in mean concentrations were, in μeq palmitic acid/dl, 34 to 67 with CO and 33 to 149 with SO (Table 13).

Diet With CO feeding compared to SO, MDV for FFA were 50 and 89 μeq palmitic acid/dl ($p < 0.05$, Table 11). Free fatty acids were the only serum component which increased in concentration by the substitution of SO for CO.

Table 13. Plasma free fatty acid levels at consecutive times or photoperiods (experiment 1)

		Diet	Free fatty acids (μeq/dl[1])
TIME			
3 a.m.	End light	40CO	34 ± 27 (5)[c,2]
9 a.m.	Middark	40CO	49 ± 25 (6)[c]
3 p.m.	End dark	40CO	67 ± 36 (3)[a,b,c]
9 p.m.	Midlight	40CO	56 ± 27 (5)[b,c]
3 a.m.	End light	40SO	33 ± 27 (6)[c]
9 a.m.	Middark	40SO	42 ± 25 (6)[c]
3 p.m.	End dark	40SO	149 ± 25 (6)[a]
9 p.m.	Midlight	40SO	132 ± 25 (6)[a,b]
PHOTOPERIOD			
9 a.m.; 3 p.m.	Dark	40CO	55 ± 24 (9)[a]
9 p.m.; 3 a.m.	Light	40CO	45 ± 23 (10)[a]
9 a.m.; 3 p.m.	Dark	40SO	95 ± 21 (12)[a]
9 p.m.; 3 a.m.	Light	40SO	83 ± 21 (12)[a]

[1]μeq palmitic acid/dl.

[2]Mean ± SEM (number of rats), means not followed by the same superscript are significantly different ($p < 0.05$) by Duncan's multiple range test.

Total hepatic cholesterol

Time   Mean hepatic cholesterol concentrations did not vary significantly over 24 hours when expressed as mg total cholesterol per g wet liver (Table 14).

Diet   Safflower oil fed rats had increased mean hepatic cholesterol concentrations compared to rats fed CO at each time interval. This dietary difference became statistically significant when the groups were compared over 24 hours. Mean cholesterol concentrations (MDV) were 2.2 or 2.9 mg/g liver with CO and SO, respectively ($p < 0.05$) (Table 15).

Soluble protein

Soluble protein concentrations over 24 hours did not differ due to time or diet. The average value was 70.1 mg/g liver (Table 16).

Experiment 2

Diet was the major variable in experiment 2. Four diets were fed: 40CO, 40SO and those diets with exogenous cholesterol (0.5% by weight), 40CC and 40SC. Sacrifice time was an additional variable. Animals were killed at 2 times during a 24 hour period, 6 hours after the beginning of the dark (9:00 a.m.) or light cycle (9:00 p.m.). These times are known to coincide with maxima and minima in the

Table 14. Hepatic cholesterol concentrations at consecutive times or photoperiods (experiment 1)

		Diet	Hepatic cholesterol (mg/g liver)
TIME			
3 a.m.	End light	40CO	2.2 ± 0.3 (6)[b,1]
9 a.m.	Middark	40CO	2.3 ± 0.3 (6)[b]
3 p.m.	End dark	40CO	2.2 ± 0.3 (6)[b]
9 p.m.	Midlight	40CO	2.2 ± 0.3 (6)[b]
3 a.m.	End light	40SO	2.8 ± 0.3 (4)[a,b]
9 a.m.	Middark	40SO	2.5 ± 0.3 (6)[a,b]
3 p.m.	End dark	40SO	2.8 ± 0.3 (5)[a,b]
9 p.m.	Midlight	40SO	3.3 ± 0.3 (6)[a]
PHOTOPERIOD			
9 a.m.; 3 p.m.	Dark	40CO	2.2 ± 0.2 (12)[b]
9 p.m.; 3 a.m.	Light	40CO	2.2 ± 0.2 (12)[b]
9 a.m.; 3 p.m.	Dark	40SO	2.7 ± 0.2 (11)[a,b]
9 p.m.; 3 a.m.	Light	40SO	3.1 ± 0.2 (10)[a]

[1]Mean ± SEM (number of rats), means not followed by the same superscript are significantly different ($p < 0.05$) by Duncan's multiple range test.

Table 15. Effect of dietary fat on hepatic total cholesterol concentrations (experiment 1)

	Total cholesterol (mg/g liver)	Total cholesterol (mg/liver)
40CO	2.2 ± 0.1 (24)[a,1]	29 ± 2 (24)[a]
40SO	2.9 ± 0.2 (21)[b]	33 ± 2 (21)[a]

[1]Mean ± SEM (number of rats), means not followed by the same superscript are significantly different ($p < 0.05$) by Duncan's multiple range test.

Table 16. Effect of time and diet on hepatic soluble protein concentrations (experiments 1 and 3)

		Experiment 1				Experiment 3	
		Diet	Protein (mg/g liver)			Diet	Protein (mg/g liver)
TIME				TIME			
3 a.m.	End light	40CO	72 ± 2 (6)[a,b,1]	3 a.m.	End light	10CO	80 ± 2 (6)[a]
9 a.m.	Middark	40CO	69 ± 2 (6)[a,b,c]	9 a.m.	Middark	10CO	80 ± 2 (6)[a]
3 p.m.	End dark	40CO	66 ± 2 (6)[b,c]	3 p.m.	End dark	10CO	80 ± 2 (6)[a]
9 p.m.	Midlight	40CO	72 ± 2 (6)[a,b]	9 p.m.	Midlight	10CO	77 ± 2 (6)[a]
3 a.m.	End light	40SO	75 ± 2 (6)[a]	3 a.m.	End light	40CO	78 ± 2 (6)[a]
9 a.m.	Middark	40SO	72 ± 2 (6)[a,b]	9 a.m.	Middark	40CO	81 ± 2 (6)[a]
3 p.m.	End dark	40SO	63 ± 2 (6)[c]	3 p.m.	End dark	40CO	80 ± 2 (6)[a]
9 p.m.	Midlight	40SO	72 ± 2 (6)[a,b]	9 p.m.	Midlight	40CO	77 ± 2 (6)[a]
DIET							
		40CO	70 ± 1 (24)[a]			10CO	79 ± 1 (24)[a]
		40SO	70 ± 1 (24)[a]			40CO	79 ± 1 (24)[a]

[1]Mean ± SEM (number of rats), means not followed by the same superscript are significantly different ($p < 0.05$) by Duncan's multiple range test.

activity of HMG-CoA reductase and cholesterol 7α-hydroxylase.

Body and liver weights

Rats fed CO or SO with and without cholesterol, gained on the average 10 or 6 g, respectively. The addition of cholesterol to either dietary fat caused significant increases in average liver weights ($p < 0.05$, Table 6). During the final half of this experiment, 25 rats on the two SO containing diets, died. At the end of the 12 week feeding period, rats from each group had lost weight: 43 to 45% of the animals fed CO and 27 to 29% of those surviving on the SO diets. Although 50% of the 40SO and 25% of the 40CO rats had lost weight in experiment 1, no deaths had occurred (see Appendix).

Acetyl-CoA carboxylase

Time   The activity of ACX within diet groups did not vary significantly between the middark and midlight periods, although with 40CO increased activity at midlight approached significance ($0.1 > p > 0.05$). This trend was not apparent in experiment 1 (Table 17, Table 7).

Diet   The increase in MCV for ACX:ACT (Table 18) when 40CO was compared to 40SO, 2.55 or 2.02, respectively, approached significance ($t = 1.8$; $0.1 > p > 0.05$).

Table 17. Effect of time on ACX:ACT[1] (experiment 2)

TIME		Diet	ACX:ACT
9 a.m.	Middark	40CO	2.19 ± 0.25 (6)[a,b,2]
9 p.m.	Midlight	40CO	2.90 ± 0.28 (5)[a]
9 a.m.	Middark	40CC	1.58 ± 0.25 (6)[b,c]
9 p.m.	Midlight	40CC	2.17 ± 0.23 (7)[a,b]
9 a.m.	Middark	40SO	1.99 ± 0.25 (6)[b,c]
9 p.m.	Midlight	40SO	2.04 ± 0.28 (5)[b,c]
9 a.m.	Middark	40SC	1.39 ± 0.23 (7)[c]
9 p.m.	Midlight	40SC	1.35 ± 0.23 (7)[c]

[1]ACX:ACT, nmoles HCO_3^- fixed/mg protein/min.

[2]Mean ± SEM (number of rats), means not followed by the same superscript are significantly different ($p < 0.05$) by Duncan's multiple range test.

Table 18. Effect of dietary fat on ACX:ACT[1] (experiment 2)

Diet	ACX:ACT
40CO	2.55 $\pm$ 0.19 (11)[a,2]
40CC	1.88 $\pm$ 0.17 (13)[b]
40SO	2.02 $\pm$ 0.19 (11)[a,b]
40SC	1.37 $\pm$ 0.17 (14)[c]

[1]ACX:ACT, nmoles HCO_3^- fixed/mg protein/min.

[2]Mean $\pm$ SEM (number of rats), means not followed by the same superscript are significantly different ($p < 0.05$) by Duncan's multiple range test.

Combined values for activity over 24 hours had been significantly higher with CO compared to SO in experiment 1. However, the sample size averaged 24 per treatment versus 12 in experiment 2.

The addition of cholesterol to either diet caused reductions in MCV of 26 or 32% for CO or SO, respectively ($p < 0.05$, Table 18). Additionally, MCV for ACX:ACT were 1.88 or 1.37 for 40CC or 40SC, respectively. This represented a significant difference due to fat type ($p < 0.05$, Table 18).

HMG-CoA reductase

Time Mean reductase activity was significantly increased in the middark compared to the midlight period when CO or SO were fed (Table 19). The differences in values between minima and maxima were 9-fold, 151 versus 17, with CO but less than double with SO ($p < 0.05$).

The time effect was abolished with the addition of cholesterol to either fat due to the extensive depression of HMG:ACT by cholesterol (Table 19).

Diet Fat saturation affected reductase activity, averaged over 24 hours, significantly (Table 20). The substitution of 40SO for 40CO caused a decrease in these values to 29 from 68, respectively ($p < 0.05$). Again, differences in reductase activity due to fat disappeared when cholesterol was added to the diet. Combined reductase

Table 19. Effect of time on HMG:ACT[1] (experiment 2)

TIME		Diet	HMG:ACT
9 a.m.	Middark	40CO	151 ± 3 (3)[a,2]
9 p.m.	Midlight	40CO	17 ± 2 (5)[c]
9 a.m.	Middark	40CC	10 ± 2 (6)[c]
9 p.m.	Midlight	40CC	7 ± 2 (5)[c]
9 a.m.	Middark	40SO	35 ± 2 (5)[b]
9 p.m.	Midlight	40SO	21 ± 3 (4)[c]
9 a.m.	Middark	40SC	10 ± 2 (6)[c]
9 p.m.	Midlight	40SC	8 ± 2 (5)[c]
9 a.m.	Middark	All	38 ± 1 (20)[a]
9 p.m.	Midlight	diets	13 ± 1 (19)[b]

[1]HMG:ACT, pmoles mevalonate/mg protein/min.

[2]Mean ± SEM (number of rats), means not followed by the same superscript are significantly different ($p < 0.05$) by Duncan's multiple range test.

Table 20. Effect of diet on HMG:ACT[1] and C7α:ACT[2] (experiment 2)

Diet	HMG:ACT	C7α:ACT
40CO	68 ± 2 (8)[a,3]	30 ± 4 (11)[b,c]
40CC	9 ± 2 (11)[c]	49 ± 4 (13)[a]
40SO	29 ± 2 (9)[b]	21 ± 4 (11)[c]
40SC	8 ± 2 (11)[c]	37 ± 3 (14)[b]

[1]HMG:ACT, pmoles mevalonate/mg protein/min.

[2]C7α:ACT, pmoles 7-OH cholesterol/mg protein/min.

[3]Mean ± SEM (number of rats), means not followed by the same superscript are significantly different ($p < 0.05$) by Duncan's multiple range test.

activities were depressed with exogenous cholesterol from 68 to 9 or from 28 to 9 for CO or SO, respectively, ($p < 0.05$, Table 20).

Cholesterol 7α-hydroxylase

Seventy percent of the microsomal bound cholesterol has been estimated as accessible to cholesterol 7α-hydroxylase by in vitro assay (Van Cantfort and Gielen, 1975). Therefore, estimates of cholesterol 7α-hydroxylase activity (C7α:ACT) in this work are based on the participation of 70% endogenous cholesterol in the enzyme reaction.

Time

When analyzed by Duncan's multiple range test with a pooled mean square error term, C7α:ACT was increased in one instance during the dark compared to the light photoperiod (Table 21). However, differences due to time were more apparent when paired sets of data were analyzed by a student t-test (Table 21). Maximum C7α:ACT was then significantly greater than minimum activity in the case of diet 40CO, 37 versus 21 ($p < 0.01$), and diet 40SC,50 versus 25 ($p < 0.001$). When all diets were combined, values for C7α:ACT were 42 or 28 for the dark and light photoperiods, respectively ($p < 0.01$, Table 21).

Table 21. Effect of time on C7α:ACT[1] (experiment 2)

	Diet	C7α:ACT[2]	C7α:ACT[3]
TIME			
9 a.m.; Middark	40CO	37 ± 5 (6)[b,c]	37 ± 4 (6)*
9 p.m.; Midlight	40CO	21 ± 6 (5)[c]	21 ± 1 (5)
9 a.m.; Middark	40CC	55 ± 7 (7)[a]	55 ± 7 (7)
9 p.m.; Midlight	40CC	42 ± 5 (6)[a,b]	42 ± 6 (6)
9 a.m.; Middark	40SO	21 ± 5 (6)[b,c]	21 ± 3 (6)
9 p.m.; Midlight	40SO	20 ± 6 (5)[c]	20 ± 3 (5)
9 a.m.; Middark	40SC	50 ± 5 (7)[a,b]	50 ± 7 (7)**
9 p.m.; Midlight	40SC	25 ± 5 (7)[c]	25 ± 4 (7)
9 a.m.; Middark	All	42 ± 3 (26)[a]	42 ± 4 (26)**
9 p.m.; Midlight	diets	28 ± 3 (23)[b]	28 ± 3 (23)

[1]C7α:ACT, pmoles 7-OH cholesterol/mg protein/min.

[2]Mean ± SEM (number of rats), means not followed by the same superscript are significantly different ($p < 0.05$) by Duncan's multiple range test.

[3]Student t-test, mean ± SEM (number of rats); * $p < 0.01$; ** $p < 0.001$.

Diet   Enzyme activity was not affected by type of dietary fat (Table 20). The addition of cholesterol, however, to either fat caused increases in MCV of 65% or 70% for CO and SO, respectively ($p < 0.05$, Table 20). With cholesterol addition, C7α:ACT was greater with 40CC than 40SC, 49 versus 37 ($p < 0.05$, Table 20).

Plasma cholesterol

Time   Mean cholesterol concentrations did not change with photoperiod on any diet (Table 22). Similar results had been obtained in experiment 1 when the same time periods were considered (Table 10).

Diet   The mean combined cholesterol concentrations for CO and SO obtained at 9 a.m. and 9 p.m. failed to indicate any differences in serum concentrations due to fat type. This was expected because combined concentrations obtained at 9 a.m. and 9 p.m. in experiment 1 also had not been different due to fat type. As expected, the addition of cholesterol to either dietary fat resulted in significant increases in plasma MCV (Table 23). Increases were from 67 to 84 mg/dl with CO or from 55 to 77 mg/dl with SO ($p < 0.05$). Serum cholesterol concentrations were not different when cholesterol supplemented fats were compared (Table 23).

Table 22. Effect of time on serum cholesterol, serum triglycerides and hepatic microsomal free cholesterol (experiment 2)

	Diet	Serum: Total cholesterol (mg/dl)	Serum: Triglyceride (mg/dl)	Hepatic free cholesterol (μg/mg)[1]
TIME				
9 a.m. Middark	40CO	69 ± 8 (6)[a,b,c,2]	140 ± 18 (6)[a]	23 ± 2 (6)[a]
9 p.m. Midlight	40CO	64 ± 7 (7)[a,b,c]	101 ± 20 (5)[a,b]	21 ± 2 (5)[a,b]
9 a.m. Middark	40CC	84 ± 8 (6)[a]	144 ± 18 (6)[a]	17 ± 1 (7)[b]
9 p.m. Midlight	40CC	83 ± 7 (7)[a]	98 ± 17 (7)[a,b]	21 ± 1 (7)[a,b]
9 a.m. Middark	40SO	58 ± 8 (6)[b,c]	109 ± 18 (6)[a,b]	21 ± 2 (6)[a,b]
9 p.m. Midlight	40SO	50 ± 8 (5)[c]	91 ± 20 (5)[a,b]	26 ± 2 (5)[a]
9 a.m. Middark	40SC	79 ± 8 (6)[a,b]	112 ± 18 (6)[a,b]	22 ± 1 (7)[a]
9 p.m. Midlight	40SC	75 ± 8 (5)[a,b]	64 ± 17 (7)[b]	26 ± 1 (7)[a]

[1]μg free cholesterol/mg microsome protein.

[2]Mean ± SEM (number of rats), means not followed by the same superscript are significantly different ($p < 0.05$) by Duncan's multiple range test.

Table 23. Effect of diet on serum cholesterol, serum triglycerides and hepatic microsomal cholesterol (experiment 2)

Diet	Serum: Total cholesterol (mg/dl)	Serum: Triglyceride (mg/dl)	Hepatic free cholesterol (μg/mg)[1]
40CO	67 ± 6 (11)[b,c,2]	122 ± 13 (11)[a]	22 ± 1 (11)[a,b]
40CC	84 ± 5 (13)[a]	119 ± 12 (13)[a]	19 ± 1 (14)[b]
40SO	55 ± 6 (11)[c]	101 ± 13 (11)[a]	23 ± 1 (11)[a]
40SC	77 ± 5 (13)[a,b]	86 ± 12 (13)[a]	24 ± 1 (14)[a]

[1]μg free cholesterol/mg microsomal protein.

[2]Mean ± SEM (number of rats), means not followed by the same superscript are significantly different ($p < 0.05$) by Duncan's multiple range test.

Serum triglycerides

Time   Photoperiod had no effect on serum triglyceride levels (Table 22). These results confirm observations made at 9 a.m. and 9 p.m. with 40CO or 40SO feeding in experiment 1 (Table 12).

Diet   Degree of fat saturation or addition of cholesterol to either diet had no influence on MCV which in mg/dl were: 40CO, 122; 40SO, 101; 40CC, 119; 40SC, 86 (Table 23). When MCV were calculated for 40CO and 40SO at 9 a.m. and 9 p.m. in experiment 1, the means also had not been affected by type of fat.

Microsomal cholesterol (free)

Time   Mean free cholesterol concentrations expressed as μg cholesterol/mg microsomal protein did not change with photoperiod on the unsupplemented diets (Table 22). However, with the addition of cholesterol to either fat, higher microsomal concentrations in the light period approached significance.

Diet   Fat type had no influence on microsomal cholesterol concentrations which were 22 or 23 μg cholesterol per mg protein for 40CO or 40SO, respectively (Table 23). With addition of cholesterol to either fat, MCV for 40SC was greater than that for 40CC ($p < 0.05$, Table 23).

Experiment 3

The third experiment was designed to determine the effect of feeding the same fat (CO) at 10 or 40% of calories for 4 weeks. The major objective was to determine whether variations in the diurnal activity of ACX or FAS would be apparent, if enzyme activity would increase, as expected, with a low-fat, high-carbohydrate diet. A reduced feeding period of 4 weeks might also affect ACX or FAS activity over 24 hours since changes in the activity of lipogenic enzymes had been reported when the feeding period was varied (Tsai et al., 1975). The effect of decreased fat and increased carbohydrate on serum cholesterol and triglyceride levels was also determined.

During this experiment, weight losses in individual rats did not occur. Weight gains with the high-fat diet were 48 g versus 35 g on the low-fat diet ($p < 0.05$, Table 6). Final mean liver weights were not different due to diet (Table 6).

Acetyl-CoA carboxylase

Time Differences in mean acetyl-CoA carboxylase activity were not significant over time with either diet (Table 24). In contrast, differences in mean activity between the start of the light and midlight period had been significant in experiment 1 with either 40CO or

Table 24. ACX:ACT[1] at consecutive time periods or photoperiods (experiment 3)

		Diet	ACX:ACT
TIME			
3 a.m.	End light	10CO	2.22 ± 0.27 (6)[a,b,2]
9 a.m.	Middark	10CO	1.94 ± 0.27 (6)[a,b,c]
3 p.m.	End dark	10CO	2.50 ± 0.27 (6)[a]
9 p.m.	Midlight	10CO	1.86 ± 0.27 (6)[a,b,c]
3 a.m.	End light	40CO	1.66 ± 0.27 (6)[a,b,c]
9 a.m.	Middark	40CO	1.36 ± 0.27 (6)[b,c]
3 p.m.	End dark	40CO	1.67 ± 0.27 (6)[a,b,c]
9 p.m.	Midlight	40CO	1.10 ± 0.27 (6)[c]
PHOTOPERIOD			
9 a.m.; 3 p.m.	Dark	10CO	2.23 ± 0.20 (12)[a]
9 p.m.; 3 a.m.	Light	10CO	2.04 ± 0.20 (12)[a,b]
9 a.m.; 3 p.m.	Dark	40CO	1.52 ± 0.20 (12)[b,c]
9 p.m.; 3 a.m.	Light	40CO	1.38 ± 0.20 (12)[c]

[1]ACX:ACT, nmole HCO_3^- fixed/mg protein/min.

[2]Mean ± SEM (number of rats), means not followed by the same superscript are significantly different ($p < 0.05$) by Duncan's multiple range test.

40SO. With 40CO, absolute values for ACX activities were about one-half those obtained in experiment 1 with the same diet. Data from either experiments 1 or 3 do not support the existence of a diurnal rhythm in ACX:ACT similar to those established for HMG-CoA reductase or cholesterol 7α-hydroxylase.

Diet Mean ACX:ACT was elevated at each 6 hour interval with 10CO compared to 40CO. Differences due to diet, however, were significant only when MDV were compared (Table 25). With decreased fat but increased carbohydrate intake ACX:ACT increased by 50% ($p < 0.05$).

Fatty acid synthetase

Time Mean FAS activity in group 40CO did not vary over time in this experiment, however, with the low-fat, high-carbohydrate diet maximum activity occurred midway through the light period (Figure 5, $p < 0.05$). The activity of FAS had been stable on either high-fat, low-carbohydrate diet in experiment 1. The activity of FAS fell within a narrow range with 40CO feeding. The value was 70% of that observed with the same diet in experiment 1 (Table 26, Table 8). With 10CO, FAS:ACT increased 90% over 24 hours.

Table 25. Effect of fat level on hepatic ACX:ACT[1] and FAS:ACT[2] and on serum cholesterol and triglyceride levels (experiment 3)

	Enzymes		Serum	
	ACX:ACT	FAS:ACT	Cholesterol (mg/dl)	Triglyceride (mg/dl)
10CO	2.13 ± 0.14 (24)[a,3]	1.21 ± 0.08 (22)[a]	79 ± 4 (24)[a]	157 ± 9 (24)[a]
40CO	1.45 ± 0.14 (24)[b]	0.75 ± 0.08 (22)[b]	75 ± 4 (24)[a]	138 ± 9 (24)[a]

[1]ACX:ACT, nmoles HCO_3^- fixed/mg protein/min.

[2]FAS:ACT, nmoles fatty acids formed/mg protein/min.

[3]Mean ± SEM (number of rats), means not followed by the same superscript are significantly different ($p < 0.05$) by Duncan's multiple range test.

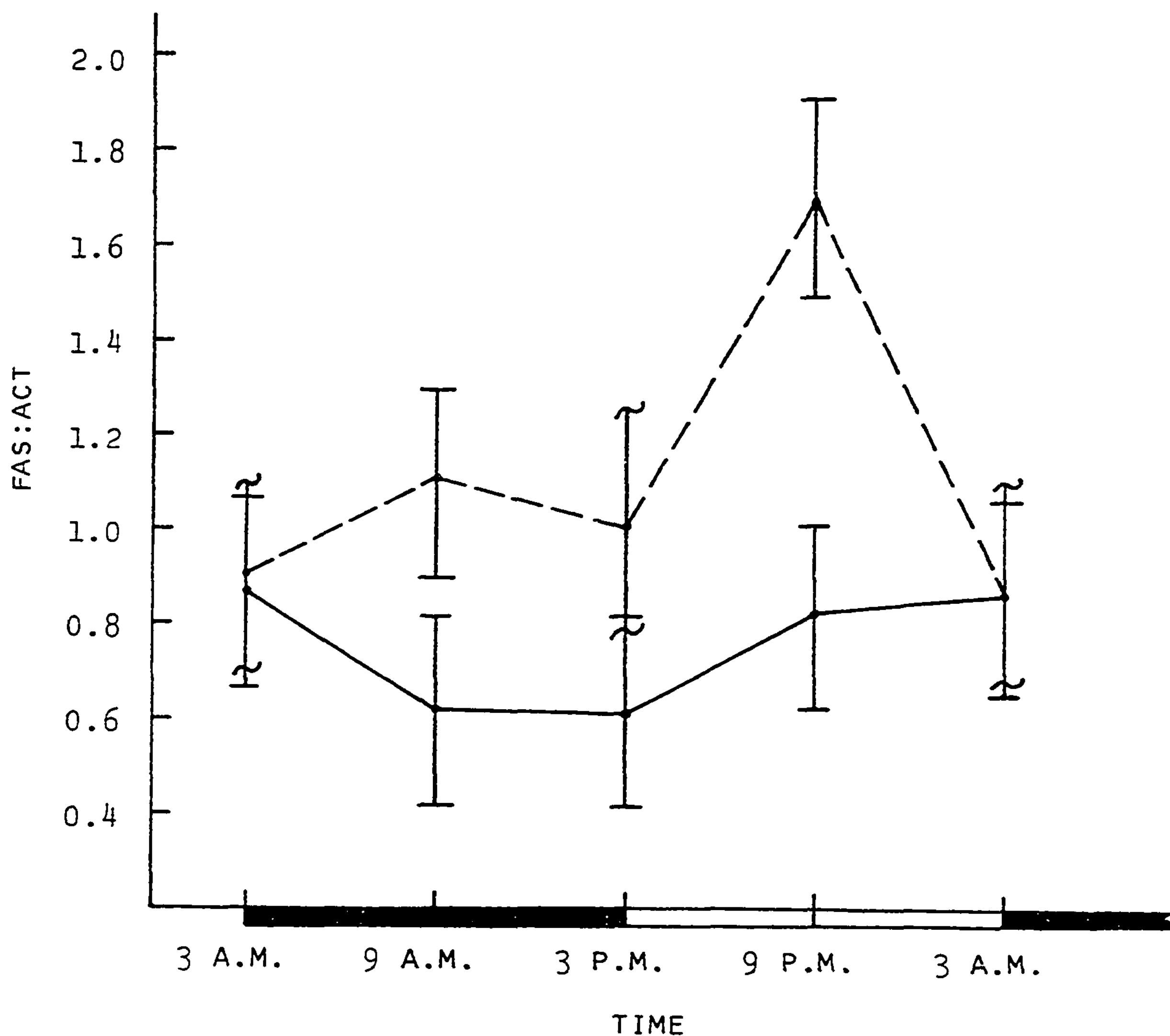


Figure 5. Activity of fatty acid synthetase (FAS:ACT) at consecutive times. Values are means of 4-6 rats. 40CO (——); 10CO(----). Horizontal dark bar indicates duration of dark period from 3 A.M. to 3 P.M. (experiment 3).

Table 26. FAS:ACT[1] at consecutive time periods or photoperiods (experiment 3)

	Diet	FAS:ACT[a]
TIME		
3 a.m. End light	10CO	0.90 $\pm$ 0.16 (6)[b,2]
9 a.m. Middark	10CO	1.11 $\pm$ 0.20 (4)[b]
3 p.m. End light	10CO	1.06 $\pm$ 0.16 (6)[b]
9 p.m. Midlight	10CO	1.72 $\pm$ 0.16 (6)[a]
3 a.m. End light	40CO	0.87 $\pm$ 0.16 (6)[b]
9 a.m. Middark	40CO	0.62 $\pm$ 0.20 (4)[b]
3 p.m. End dark	40CO	0.62 $\pm$ 0.16 (6)[b]
9 p.m. Midlight	40CO	0.83 $\pm$ 0.16 (6)[b]
PHOTOPERIODS		
9 a.m.; 3 p.m. Dark	10CO	1.31 $\pm$ 0.13 (12)[a]
9 p.m.; 3 a.m. Light	10CO	1.09 $\pm$ 0.14 (10)[a,b]
9 a.m.; 3 p.m. Dark	40CO	0.85 $\pm$ 0.13 (12)[b,c]
9 p.m.; 3 a.m. Light	40CO	0.62 $\pm$ 0.14 (10)[c]

[1]FAS:ACT, nmoles fatty acids formed/mg protein/min.

[2]Mean $\pm$ SEM (number of rats), means not followed by the same superscript are significantly different ($p < 0.05$) by Duncan's multiple range test.

Diet

Mean FAS activity was elevated at each time period with 10CO compared to 40CO. However, only mean daily values for FAS:ACT, 1.21 or 0.75, were significantly different ($p < 0.05$, Table 25) and reflected 62% more activity with 10CO, or decreased fat and increased dietary carbohydrate, compared to the 40CO diet.

Serum cholesterol

Time Serum cholesterol concentrations did not vary due to time in this experiment or in experiment 1 (Table 27, Table 10). Concentration ranges were narrow, 65 to 79 mg/dl or 71 to 90 mg/dl with 40CO or 10CO, respectively.

Diet Differences in serum cholesterol concentrations did not reach statistical significance due to diet. Values were 79 or 75 mg/dl for 10CO or 40CO, respectively (Table 25).

Serum triglycerides

Time Serum triglyceride concentrations at the start of the dark period (3 a.m.) represented minima for each diet ($p < 0.05$, Table 28). The lowest mean triglyceride value also had appeared at 3 a.m. in experiment 1 with either diet (Table 17). In experiment 3, triglyceride ranges were 102 to 185 mg/dl for 40CO and 71 to 187 mg/dl for 10CO (Figure 6).

Table 27. Serum cholesterol levels at consecutive times (experiment 3)

	Diet	Total cholesterol (mg/dl)
TIME		
3 a.m. End light	10CO	80 ± 8 (6)[a,1]
9 a.m. Middark	10CO	71 ± 8 (6)[a]
3 p.m. End dark	10CO	77 ± 8 (6)[a]
9 p.m. Midlight	10CO	90 ± 8 (6)[a]
3 a.m. End light	40CO	75 ± 8 (6)[a]
9 a.m. Middark	40CO	65 ± 8 (6)[a]
3 p.m. End dark	40CO	76 ± 8 (6)[a]
3 p.m. Midlight	40CO	79 ± 8 (6)[a]

[1]Mean ± SEM (number of rats), means not followed by the same superscript are significantly different ($p < 0.05$) by Duncan's multiple range test.

Table 28. Serum triglyceride levels at consecutive times (experiment 3)

TIME	Diet	Triglycerides (mg/dl)
3 a.m. End light	10CO	71 ± 18 (6)[c,1]
9 a.m. Middark	10CO	148 ± 18 (6)[a,b]
8 p.m. End dark	10CO	145 ± 18 (6)[a,b]
9 p.m. Midlight	10CO	187 ± 18 (6)[a]
3 a.m. End light	40CO	102 ± 18 (6)[b,c]
9 a.m. Middark	40CO	185 ± 18 (6)[a]
3 p.m. End dark	40CO	171 ± 18 (6)[a]
9 p.m. Midlight	40CO	169 ± 18 (6)[a]

[1]Mean ± SEM (number of rats), means not followed by the same superscript are significantly different ($p < 0.05$) by Duncan's multiple range test.

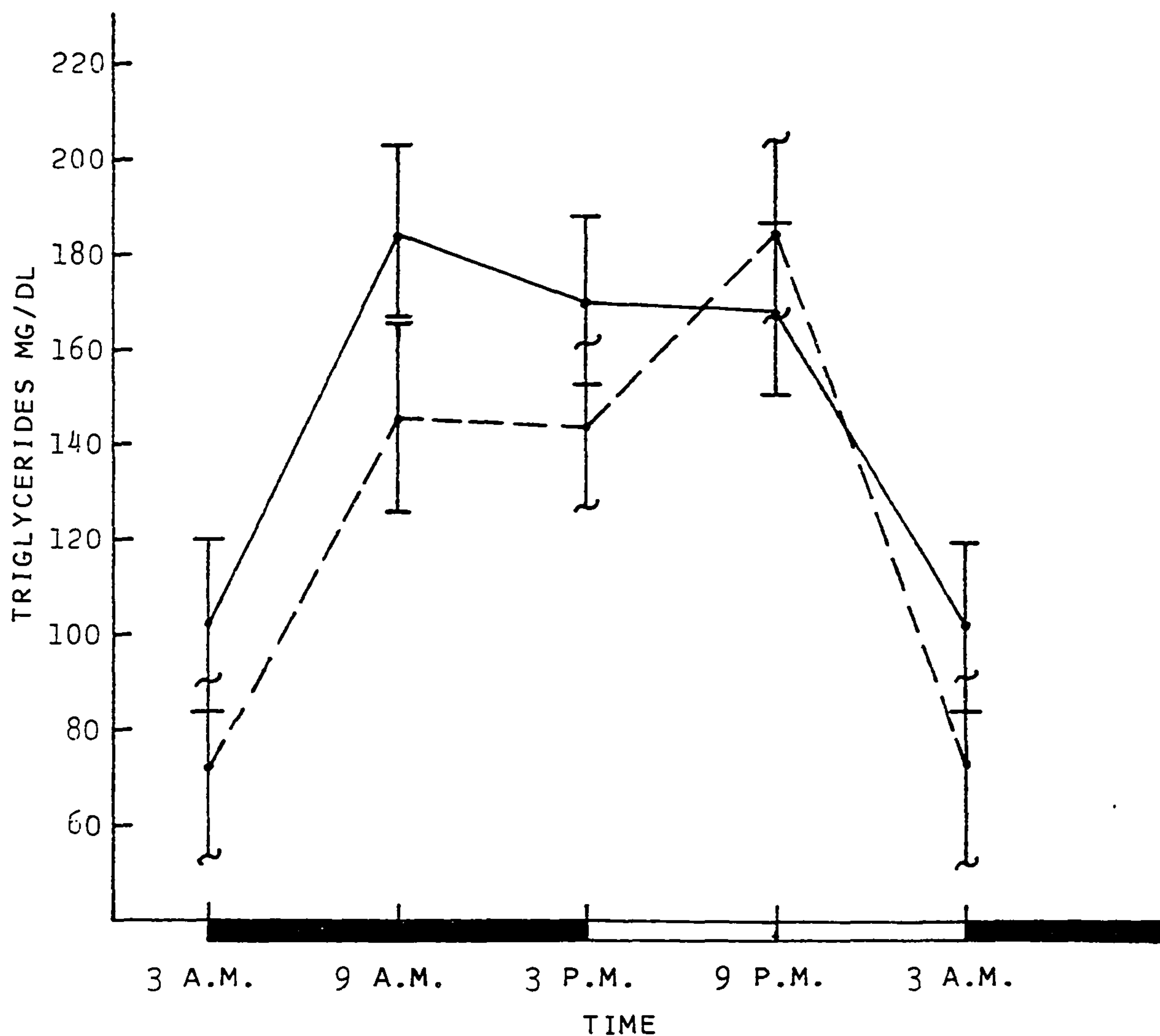


Figure 6. Serum triglyceride (TG) levels at consecutive times. Values are means of 6 rats. 40CO (——); 10CO(----). Horizontal dark bar indicates duration of dark period from 3 A.M. to 3 P.M. (experiment 3)

Diet   Mean combined triglyceride values were similar when 10CO was substituted for 40CO (Table 25). These means were 157 and 138 mg/dl for 10CO or 40CO, respectively.

Soluble protein

Soluble protein concentrations did not vary due to time or diet. Mean daily values were 79 mg/g liver for both diets (Table 16).

Correlation coefficients

Correlation coefficients from experiments 1, 2 and 3 are given in Table 29. These data will be used in the discussion.

Table 29. Correlation coefficients, r, between measurements on rats fed various diets (experiments 1, 2 and 3)

Experiment	Diet	Correlation	r[a]	p[b]	n[c]
		FAS-ACX			
1	40CO		0.53	<0.01	24
1	40SO		0.40	<0.05	24
1	40CO + 40SO		0.53	<0.001	48
3	40CO		0.57	<0.01	22
3	10CO + 40CO		0.50	<0.001	44

[a]r; correlation coefficient.
[b]p; level of significance.
[c]n; number of rats.

Table 29 (Continued)

Experiment	Diet	Correlation	r[a]	p[b]	n[c]
		FAS-TOTAL CHOLESTEROL (SERUM)			
1	40CO		0.53	<0.01	22
1	40SO		0.41	<0.04	24
1	40CO + 40SO		0.34	<0.02	47
3	10CO 40CO +		0.35	<0.02	48
		FAS-CHOLESTEROL ESTERS			
1	40CO 40SO +		0.31	<0.04	47
		FAS-FREE FATTY ACIDS			
1	40CO 40SO +		-0.35	<0.02	45
		FAS TRIGLYCERIDES			
1	40CO 40SO +		0.65	<0.001	43
3	10CO		0.61	<0.03	20
		ACX-TRIGLYCERIDES			
1	40CO 40SO +		0.46	<0.01	43
1	40SO		0.52	<0.01	21
		HMG-CoA REDUCTASE-CHOLESTEROL 7α-HYDROXYLASE			
2	40CC		0.58	<0.06	12
		HMG-CoA REDUCTASE-TRIGLYCERIDES			
2	40CC		0.62	<0.05	10
		HMG-CoA REDUCTASE-ACX			
2	All diets		0.30	<0.06	40
		CHOLESTEROL 7α-HYDROXYLASE-SERUM CHOLESTEROL			
2	All diets		0.27	<0.07	47
		CHOLESTEROL 7α-HYDROXYLASE-HMG-CoA			
2	40SC		0.58	<0.05	14

DISCUSSION

This study was designed to explore whether diurnal variations exist in the activities of selected lipogenic enzymes and in the concentrations of tissue lipids. These parameters and HMG-CoA reductase and cholesterol 7α-hydroxylase activities were also determined as dietary fat, cholesterol, feeding periods or age of rats were changed. Objectives were to identify any recognizable patterns or correlations which would clarify interrelationships in lipid metabolism.

Acetyl-CoA Carboxylase and Fatty Acid Synthetase

Diurnal variation

The metabolic pathways for synthesis of fatty acids, cholesterol, ketone bodies or ultimately CO_2 with release of energy, depend on a supply of precursor acetyl-CoA. Regulation of the supply and dispersal of this precursor is not completely understood (Bortz and Steele, 1973; Mayes and Topping, 1974; Gibbons and Pullinger, 1979). It was recognized ten years ago that the activities of HMG-CoA reductase and cholesterol 7α-hydroxylase vary diurnally (Back et al., 1969; Gielen et al., 1969). The possibility of synchronous or asynchronous rhythms in the activity of these enzymes and the enzymes controlling lipid synthesis due to the

dispersal of their shared precursor, acetyl-CoA, was explored in this study. Mean acetyl-CoA carboxylase and fatty acid synthetase activities were compared at 6 hour intervals (experiments 1 and 3) to determine whether changes would occur smoothly between minima and maxima as in HMG-CoA reductase or cholesterol 7α-hydroxylase activity.

The activities of FAS and ACX were highly correlated in experiment 1 ($r = +0.53$; $N = 48$; $p < 0.001$) or experiment 3 ($r = +0.35$; $N = 44$; $p < 0.02$) when considered over 24 hours. Additionally, the results from the present study indicate that the activities of FAS or ACX are generally stable when measured at six hour intervals over a 24 hour period (Tables 7, 9, 24, 26). The activity of these enzymes also did not differ when observations were divided into equal photoperiods consisting of a dark, or feeding period, and a light period. Martin et al. (1979) and Bruckdorfer et al. (1974) have also observed that FAS activity did not change between light and dark photoperiods.

However, an exception to the general stability of FAS on high fat diets appeared when a low fat diet was fed for one month (experiment 3). A significant elevation in activity occurred between the beginning and midpoint of the light period with 10CO (Figure 6). Synthetase activity was elevated throughout the 24 hour period with the low-fat diet

in the experiment. Evidently in conjunction with elevated activity, an apparent stimulation of FAS could be detected during the period when food is normally not eaten.

In contrast to the stability of ACX when low or high fat diets containing coconut oil were fed for one month (experiment 3), ACX activity was elevated between the start of light and midway into the light period when high fat diets were fed for 3 months (experiment 1).

In view of the general stability of ACX on high-fat diets, selective stimulation during this interval was surprising. Data from this study cannot identify changes due to high-fat intake in the activity of enzymes in other pathways utilizing acetyl-CoA. However, De Gasquet et al. (1977) observed elevated activity during the middark period, with high-versus low-fat diets, of an enzyme concerned with ketone body production. It is conceivable that changes in ketogenesis or rates of fatty acid oxidation due to high fat diets could affect ACX activity. Differences in age of rats used in experiments 1 and 3 may also have affected the activity of ACX. At the termination of experiments 1 and 3, rats were 7 months or 4 months old, respectively. Such differences in age have affected the outcome of other studies in lipid metabolism (Kimura et al., 1970; Dupont et al., 1972).

Several investigators using labelled precursors have shown that fatty acid synthesis in mice or rats can vary diurnally or is elevated significantly during the period of food intake (dark cycle). Initially [1-^{14}C]acetate was used to demonstrate this rhythm (Kimura et al., 1970 Edwards et al., 1972; Bortz and Steele, 1973). Recently the rate of incorporation of ^{3}H (from $^{3}H_2O$) into fatty acids has been used to free the analysis of the effect of endogenous carbon sources and similar results were found over time (Hems et al., 1975; Cornish and Cawthorne, 1978). However, the rates of certain lipogenic processes have been dissociated from the observed activity of enzymes in those processes (Tepperman et al., 1968). For example, the rate of fatty acid synthesis was reduced by $C_{18:1}$ but this acid did not inhibit hepatic FAS activity (Clarke et al., 1977). Additionally, FAS activity was stable during a 24 hour period while the rate of fatty acid synthesis increased during the dark or food intake period (Martin et al., 1979).

The absence of a well-defined cycle in ACX and FAS activity over 24 hours observed in this and other work may reflect the estimated half-lives of those enzymes, 55-59 hours (Nakanishi and Numa, 1970) and about 70 hours (Tweto and Larrabee, 1972) for ACX and FAS, respectively. These half-lives seem to preclude any sudden changes in enzyme quantity although acute alterations of ACX activity by metabolic

effectors are possible (Inoue and Lowenstein, 1972; Lee and Kim, 1977).

Effects of fat composition

Degree of saturation and chain length of fatty acids derived from dietary triglyceride are known to modify activity or synthesis of lipogenic enzymes. Generally, effects of fat composition on hepatic lipogenesis have been investigated by measuring the rate of fatty acid synthesis from labeled precursors or the activity of lipogenic enzymes during a variety of nutritional manipulations. In the present study the effect of diets containing CO with 3% polyunsaturated fat (PUFA) or SO with 75% PUFA was determined on the activity of ACX and FAS.

These oils were chosen, not only because of differences in polyunsaturation, but because the coefficient of digestibility of CO is very similar to that of other vegetable oils (Deuel, 1955, p. 222). Had fully saturated safflower oil been used as a representative saturated fat, the experiments would have been confounded by incomplete absorption. When these fats were fed for 3 months as 40% of calories, and SO substituted for CO, mean daily activity of both enzymes decreased (Table 8). Reduced ACX activity due to SO feeding was also evident in experiment 2 but failed to be statistically significant ($t = 1.8$; $0.05 < p < 0.1$) (Table 18).

The carboxylation of acetyl-CoA to malonyl-CoA via the action of acetyl-CoA carboxylase is of central importance in control of long chain fatty acid synthesis. The inhibitory effect of free fatty acids or their CoA derivatives on this step has been traced to the competition of these substances with acetyl-CoA for active enzyme sites (Bortz and Lynen, 1963). Thus, fatty acids, free or bound to albumin, or their acyl-CoA derivatives have inhibited the activity of ACX or FAS (Pande and Mead, 1968). In addition, this competitive inhibition was increased with increasing fatty acid chain length (Nilsson et al., 1974). Goodridge (1973) has proposed that these derivatives may inhibit acetyl-CoA carboxylase directly or they may inhibit mitochondrial citrate carrier and so reduce the activation of acetyl-CoA carboxylase caused by citrate. Free fatty acid concentrations in the present study were negatively correlated with FAS activity ($r=-0.35$; $N=45$; $p<0.02$), suggesting that fatty acid inhibition may have been operative during lipogenesis.

The degree of fat saturation can also affect regulation of fatty acid synthesis. For example, saturated or monounsaturated fatty acids or their methyl esters have been ineffective in reducing hepatic FAS or ACX activity, although, archidonate, linolenate and linoleate progressively decreased the activity of these enzymes (Bartley and Abraham, 1972, Musch et al., 1974). The effect of SO after a fat-free, high-

carbohydrate diet was to reduce the induction of hepatic FAS activity compared to CO or methyl oleate (Flick et al., 1977). Under these conditions, the rate of enzyme synthesis was reduced while the rate of enzyme degradation was increased by SO but not by the other fats. It was proposed that SO or a metabolite may regulate the transcription or translation of FAS messenger RNA.

The depression in enzyme activity found with 40SO compared to 40CO in our study might be traced to differences in fatty acid saturation and/or chain length. Safflower oil contains predominantly polyunsaturated as well as long chain fatty acids while fatty acids in CO are predominantly saturated and medium chain length. It is recognized that the use of CO and SO containing fatty acids of varying chain length added another variable to our study. However, the significance of this discrepancy in chain length is not clear since investigators have not agreed on the effect of this variable on lipogenesis. Reiser et al. (1963) noted a depression due to feeding medium chain triglycerides while Leveille et al. (1967) failed to find differences in selected lipid parameters when coconut or corn oil was fed.

The response of lipogenic enzymes to different fatty acids may depend on the experimental approach. In vitro addition of either saturated or unsaturated fatty acids to isolated hepatocytes resulted in a decrease in ACX quantity and

activity. Phospholipids increased, compared to nonesterified fatty acids or triglycerides, during the procedure and may have regulated ACX synthesis (Kitajima et al., 1975).

In experiment 2 the addition of cholesterol to either CO or SO resulted in decreases in ACX activity of 26 or 32%, respectively. However, 38% more activity was observed when cholesterol was added to CO compared to SO (Table 18). In other studies, fatty acid synthesis and ACX activity have both been depressed when cholesterol and cholic acid were fed with 20% corn oil diets (Tsai and Dyer, 1973; Tasi et al., 1975). In contrast, activity of ACX has been unaffected by the addition of 2% cholesterol to a 4% fat diet (Craig et al., 1972).

Apparently, the addition of cholesterol to the diets in the present study did not channel more acetyl-CoA through the fatty acid synthesizing pathways even though this addition reduced the activity of HMG-CoA reductase (Table 20).

Amount of fat

As an adjunct to the study of diurnal variations in ACX and FAS activities, 10 or 40% of calories were fed from CO as part of a month long experiment (experiment 3). Mean daily ACX or FAS activities decreased by 32% and 38%, respectively, when CO was raised from 10 to 40% of calories (Table 25). Similarly, incorporation of labelled acetate into fatty acids

had been depressed almost 50% when 40 rather than 10% of calories were supplied by SO (Diller and Harvey, 1964). Fatty acid synthesis has also been inhibited by increasing amounts of corn oil or soybean oil, while the latter depressed the activities of FAS and ACX (Carrozza et al., 1979, Triscari et al., 1978).

The compensatory changes in the pathways utilizing acetyl-CoA as fat is decreased and carbohydrate increased are not clear. Insulin secretion may be increased under these circumstances leading to a decrease in the glucagon to insulin ratio. Such a decrease is known to alter fatty acid metabolism so that synthesis rather than oxidation of fatty acids is favored (McGarry et al., 1978). Additionally, lipogenesis decreased as fatty acid concentrations were increased during liver perfusion although rates of VLDL secretion, cholesterogenesis or ketogenesis were not altered (Mayes and Topping, 1974). A study such as this, including measurement of the rates of fatty acid oxidation or insulin concentrations as fat and carbohydrate are manipulated, would aid in an interpretation of enzyme data similar to those obtained in our study.

It is probable that long-term regulation of lipogenesis by amounts of dietary fat occurs through changes in hepatic enzyme content rather than through activation or inhibition of preformed enzyme (Majerus and Kilburn, 1969). This question

cannot be resolved with the data from this study. But as an extension of the present experiment, the effect of changing dietary fat levels on enzyme content could be followed by quantitative precipitation of the enzyme by an appropriate antibody preparation.

HMG-CoA Reductase

Diurnal rhythm

HMG-CoA reductase, the rate limiting enzyme in cholesterogenesis, shows a circadian rhythm with peak activity approximately six hours into the dark cycle and a minimum about 12 hours later. In rats, fed ad libitum, the time at which peak activity occurs during 24 hours may be shifted by adjusting the timing of the light-dark period. However, presentation of food or lighting is not the primary stimulus for this activity, since the rhythm persists during fasting or total darkness (Rodwell et al., 1976). Synthesis of the reductase appears to take place continuously with the possible exception of the period of rapidly decreasing activity after the peak. Up to a ten-fold increase in activity has been reported between minima and maxima when rats were fed a low-fat diet (Shapiro and Rodwell, 1971).

Evidently stimulation of enzyme activity is partially dependent on fat type and amount. Ide et al. (1978) found a 5-fold increase in reductase activity with coconut oil and a

3-fold increase with safflower oil when either fat was fed as 20% of calories. In the present work, reductase activities increased between minima and maxima almost 9-fold with 40CO but only 2-fold with 40SO feeding.

However, when cholesterol was added to either fat in experiment 2, diurnal rhythm in reductase activity disappeared (Table 19). Higgins and Rudney (1973) made similar observations after short-term cholesterol feeding and noted that although diurnal reductase activity was no longer detectable, activity may have been depressed to the limits of their assay. Cholesterol feeding seemed to have two effects: an immediate inhibition of reductase activity independent of protein synthesis, followed by inhibition of protein synthesis. However, the mechanism of these responses is not clear.

Amount and type of fat

Amount and degree of saturation of dietary fats are known to influence the activity of HMG-CoA reductase (Goldfarb and Pitot, 1972, Ide et al., 1978). Results from the present study indicate that the substitution of 40SO for 40CO over a 12 week period inhibited the activity of the reductase 51% (Table 20). However, Ide et al. (1978) who fed similar diets for 4 or 3 weeks only, failed to obtain differences in mean reductase activities. Results were also

not affected by fat type when 20% of calories were supplied by SO or tripalmitin (Bochenek and Rodgers, 1978).

Other investigators have examined the effect of fat saturation on in vitro rates of hepatic cholesterogenesis by measuring the incorporation of radioactive substrates into cholesterol. Generally, feeding PUFA rather than saturated fats has resulted in increased cholesterogenesis (Reiser et al., 1963; Dupont, 1966). But, in meal-fed rats these results were reversed (Triscari et al., 1978).

The rate of cellular cholesterol synthesis is determined by the balance of cholesterol entering the cell to the needs of the cell for cholesterol for specialized products. In the hepatocyte cholesterogenesis is related to entry of chylomicrons containing cholesterol (Nervi et al., 1974) and to loss of sterol during lipoprotein synthesis (Goh and Heimberg, 1976), bile acid synthesis (Nervi and Dietschy, 1978) or cholesterol secretion into the bile.

Perfusion of isolated rat liver with free fatty acids (FFA) has shown that the output of triglycerides in VLDL was proportional to the number of carbon atoms in saturated FFA, but decreased as the number of double bonds increased in FFA. Additionally, the rate of cholesterogenesis, estimated by HMG-CoA reductase activity, was dependent on the rate of VLDL secretion because of the obligatory requirement for cholesterol in VLDL (Kohout et al., 1971; Goh and Heimberg, 1976). Based

on these data, the inhibition of HMG-CoA reductase by PUFA found in the present study might be attributable indirectly to depression of VLDL synthesis by PUFA derived from dietary triglycerides.

Regulation of reductase activity occurs through alterations in the amount of enzyme protein and possibly by modification of its catalytic efficiency (Nordstrom et al., 1977). Effects of fat saturation on these parameters have not been determined.

However, an increase in the concentration of microsomal cholesterol esters has been negatively correlated with the activity of HMG-CoA reductase (Harry et al., 1973; Edwards and Gould, 1974; Ide et al., 1978). Apparently, increased ester formation suppressed cholesterogenesis by end product inhibition of the reductase (Edwards and Gould, 1974). Polyunsaturated, rather than saturated, fats have increased hepatic ester accumulation (Kellogg, 1974). It is possible that ester concentrations also increase in the microsomes with PUFA feeding. A determination of microsomal ester concentrations would have been helpful in explaining reductase inhibition by SO in experiment 2.

In experiment 2, consumption of cholesterol with CO or SO for 12 weeks inhibited reductase activity by 87 or 72%, respectively (Table 20). Values for activity were so small that differences due to fat saturation, apparent before cholesterol feeding, had disappeared. Reduction of similar

magnitudes have been reported with 1% cholesterol in corn oil, safflower oil or tripalmitin diets (Raicht et al., 1975). However, Bochenek and Rodgers (1978) found not only an inhibition of reductase activity but significantly more activity remaining when cholesterol was added to a saturated rather than unsaturated fat. Rats in that study compared to our animals received 1/2 the calories from fat, were rapidly growing and were fed for only 4 weeks. These factors may have caused elevated reductase activity with the saturated fat.

Cholesterol 7α-hydroxylase

Diurnal rhythm

Hydroxylation in the 7α position of cholesterol is the major rate limiting step in the biosynthesis of bile acids and is catalyzed by cholesterol 7α-hydroxylase.

The diurnal rhythm, a two to four-fold increase during 24 hours, of cholesterol 7α-hydroxylase is controlled at the level of enzyme synthesis and degradation (Mitropoulous et al., 1972). The mechanisms by which external or internal stimuli act in maintaining this rhythm are largely unknown. As in HMG-CoA reductase the rhythm persists during fasting or total darkness (Myant and Mitropoulous, 1977). However, if light and dark photoperiods are reversed for at least three weeks, the diurnal rhythm in hydroxylase activity and cholesterol synthesis can be reversed (Danielsson, 1972).

Thus, assay of this activity is made more convenient and such a procedure was followed in our study.

Mean hydroxylase activities were higher in the dark compared to the light photoperiods in each dietary group (Table 21). Differences between maxima and minima were significant when CO was fed ($t = 3.1$; $p < 0.01$) or when SO was fed with cholesterol ($t = 3.8$; $p < 0.001$). However, diurnal variation when CO was fed with cholesterol only approached significance ($t = 1.5$; $0.2 > p > 0.1$). Variability in enzyme activity within an experimental group has been estimated at 25-32% (Mitropoulous et al., 1973; Björkhem et al., 1978). Activity differences were not observed due to photoperiod in 50% of the hydroxylase determinations in another study (Mayer and Mayer, 1974). Such variability within treatments may reflect difficulty in fully activating the enzyme under various assay conditions.

Amount and type of fat

The rate of bile acid synthesis is influenced by the magnitude and circulation rate of the bile acid pool (Shefer et al., 1973). Although it is difficult to measure magnitude and rate of bile circulation, sterol balance studies have been used to indicate the effect of dietary changes in this system (Kellogg, 1974; Raicht et al., 1975). Results concerning the effect of fat saturation on sterol balance are

contradictory. Fecal excretion of bile acids or bile acid synthesis have been increased by polyunsaturated fat feeding in some studies (McGovern and Quackenbush, 1973a; Carlson et al., 1978b) but were unaffected in others (Kellogg, 1974; Bochenek and Rodgers, 1978).

In the present study, the activity of cholesterol 7α-hydroxylase was not different due to type of fat (Table 20). Similar conclusions can be drawn from data published by O'Brien et al. (1977) and Kritchevsky et al. (1977). Conversely, hydroxylase activity was inhibited by corn rather than coconut oil while bile acid elimination was decreased in another short-term study (Mayer and Mayer, 1974). However, increased hydroxylase activity was observed when saturated or monounsaturated fats, tripalmitin and trierucin, rather than triolein or trilinolein, were fed at either 6 or 20% fat levels (Björkhem et al., 1978). Variability in the effect of fat type on hydroxylase activity may reflect differences in the assay procedures used by different laboratories.

Depending on the level in the diet or length of feeding, dietary cholesterol has not affected or has enhanced cholesterol 7α-hydroxylase activity (Mitropoulos et al., 1973; Shefer et al., 1973). In the present study, the addition of 0.5% cholesterol to CO or SO increased hydroxylase

activity considerably. Additionally, activity was 31% greater with the saturated compared to unsaturated fat (Table 20). In contrast, when tallow, rather than CO, was used with or without cholesterol and feeding terminated after 4 rather than 12 weeks, hydroxylase activities did not differ (O'Brien et al., 1977).

In an extensive study of sterol balance, cholesterol feeding: 1) increased cholesterol absorption, 2) inhibited cholesterol synthesis and reductase actitivity, 3) enhanced conversion of cholesterol to bile acids and hydroxylase activity, 4) slightly increased excretion of endogenous neutral steroids, and 5) increased liver cholesterol levels (Raicht et al., 1975). Our data with cholesterol feeding are limited but do confirm a reduction in the activity of HMG-CoA reductase, as an estimate of reduced hepatic cholesterogenesis, as well as an increase in cholesterol 7α-hydroxylase activity, as an estimate of increased bile acid synthesis.

Serum Lipids

Diurnal variations

There is evidence that some plasma lipid concentrations vary considerably over 24 hours and that these variations are related to amount of dietary fat. For example, triglyceride (TG) concentrations were lowest in the light period when 9 or 70% of calories were fed from lard (De Gasquet et al., 1977). But with 2% fat, concentrations were lowest at the start of the

dark photoperiod (Bruckdorfer et al., 1974). In the present study TG concentrations were low at the beginning of the dark but elevated at the middark period with each diet (experiments 1 and 3). In ad libitum fed animals, the latter should be a period of alimentation and consequently of increased serum TG concentrations from dietary fat. Additionally, a decrease was noted from the midlight to the beginning of the dark period with high-fat diets in experiment 1. In this period prior to the resumption of feeding, reduced serum TG concentrations would be expected.

Free fatty acid (FFA) concentrations peaked at the end of the light period on fat free diets (Bortz and Steele, 1973) or 6 hours into the light or dark photoperiods with lard supplying 70 or 9% of calories, respectively (De Gasquet et al., 1977). In the present study FFA concentrations were highest at the beginning and 6 hours into the light period with SO but did not vary over time with CO (Table 13). The increase in the light period may reflect increased TG breakdown and release from the adipose while feeding ceases.

Concentrations of total or free cholesterol did not vary with time nor did hepatic cholesterol (Table 10). Although hepatic cholesterogenesis varies diurnally (Edwards et al., 1972), serum and liver cholesterol concentrations do not appear to respond to this rhythm. Possibly, bile acid synthesis, synchronous with cholesterol synthesis, utilizes the

newly synthesized cholesterol.

Individual serum or tissue lipid concentrations from each dietary treatment in experiment 1 and 3 were combined depending on the photoperiod of collection. Presumably, these combined data reflected concentrations of metabolites available to or synthesized by the animal during either a period of feeding or inactivity. Results from this type of analysis indicated that lipid concentrations did not change with time. No attempt was made to identify lipoprotein composition in this study. Possibly, shifts in lipid concentrations occurred in lipoproteins in response to feeding patterns.

Type of fat

Relatively low serum cholesterol concentrations are typical for the rat, in contrast to some species including man. Feeding studies with cholesterol-free diets conducted in this laboratory have demonstrated that cholesterol concentrations in the Wistar rat are resistant to change by dietary manipulation (Heng, 1977, Carlson et al., 1978b). Yet serum cholesterol concentration may be increased in the rat by the administration of cholesterol or bile acids (Tsai et al., 1975).

In experiment 1, feeding SO instead of CO lowered cholesterol and triglyceride but elevated free fatty acid concentrations in the serum (Table 11). The change in serum

cholesterol due to type of fat was small, amounting to about 9 mg/dl and could be verified statistically only by combining data from all animals fed the same diet. This may explain why, with a reduced number of observation periods in experiment 2, serum cholesterol and TG levels were statistically not different.

Degree of dietary fat saturation can modify serum lipid concentrations by one of several mechanisms. These include changes in: 1) the rate of hepatic lipogenesis or cholesterogenesis, 2) the rate of secretion or clearance of lipoproteins, 3) the synthesis of bile acids or excretion of neutral sterols or, 4) the redistribution of lipids from serum to other body pools.

It has already been noted that the activities of the lipogenic enzymes, ACX and FAS, were significantly depressed by feeding SO in place of CO. Since the control of triglyceride synthesis appears to be at the level of fatty acid synthesis rather than esterification (Wiegand et al., 1973), decreased TG concentrations, associated with SO compared to CO, could be due, in part, to decreased lipogenic enzyme activity. Support for this association comes from the fact that FAS activity and TG concentrations were strongly correlated in experiment 1 ($r=+0.65$; $N=43$; $p<.001$) as were ACX activity and TG concentrations ($r=+0.46$; $N=43$; $p<0.01$).

The secretion rate of lipoproteins from the liver may

also be dependent on dietary fat saturation. Morris et al. (1975) found that the rate of hepatic VLDL-TG secretion was greater in rats fed CO compared to SO and correlated with TG concentrations in the serum. However, these results may be species dependent. In a similar study with gerbils, the relative secretion rates of these two fats were reversed (Nicolosi et al., 1976).

In the present study, levels of serum FFA were elevated while levels of TG were decreased by SO compared to CO (Table 11). These results could reflect increased levels of lipoprotein lipase activity associated with PUFA feeding (Bagdade et al., 1970; Pawar and Tedwell, 1968). Additionally, the activity of this enzyme has been negatively correlated with serum TG levels (Persson et al., 1966).

Spritz and Mishkel (1969) proposed that ingestion of polyunsaturated fats leads to lower serum lipid levels because PUFA in lipoproteins occupy a greater volume than do saturated fats. According to this theory, spatial configurations of TG derived from dietary PUFA, compared to saturated fats, are altered so that fewer lipid molecules can be accommodated by the apoprotein of VLDL. Support for this hypothesis has come from work with primates in which TG concentrations were correlated with VLDL protein (Howard, 1979). Very low density lipoprotein particles were significantly larger and less densely packed and, therefore, TG concentrations

lower when primates were fed SO compared to CO.

Differences in the utilization of linoleate or saturated fats may contribute further to the decrease in plasma TC concentrations when fats such as SO, containing primarily linoleate are fed in place of saturated fats. Results from both in vivo and in vitro experiments have shown that linoleate was preferentially incorporated into phospholipids while saturated fats were diverted into TG (Nestel and Steinberg, 1963; Nicolosi et al., 1976). Linoleate also appears to be oxidized at a higher rate than saturated fats (Dupont, 1970) which can again be reflected in decreased hepatic TG synthesis (Nichman et al., 1967).

Serum cholesterol concentrations could be modified by dietary fat through changes in hepatic cholesterol synthesis or degradation. Although reductase activity was depressed almost 60% when SO was substituted for CO in experiment 2, cholesterol 7α-hydroxylase activity and serum cholesterol levels were not affected by fat saturation (Tables 20 and 23).

Liver cholesterol content, expressed as mg cholesterol/g liver, was increased by SO compared to CO in experiment 1 (Table 15). This increase was accompanied by a decrease in serum cholesterol and appears to indicate a shift of cholesterol between body pools due to diet. However, without detailed studies of the kinetics of cholesterol movements between various body pools, the validity of this assumption

cannot be ascertained.

The effect of dietary cholesterol in elevating levels of serum cholesterol in humans and rats is well-documented. Therefore, the significant increase in serum cholesterol obtained when 0.5% cholesterol was added to either CO or SO was expected (experiment 2, Table 23).

However, in experiment 2 the type of fat fed with cholesterol did not cause a difference in serum cholesterol or TG concentrations (Table 23). Similarly the addition of cholesterol to SO or lard was without effect on levels of either of these serum lipids (Frnka and Reiser, 1974).

Cholesterol is readily absorbed from fat containing diets (Raicht, et al., 1975) but degree of fat saturation does not appear to affect absorption (Reiser et al., 1963; McGovern and Quackenbush, 1973b). This may partially explain why in experiment 2 serum cholesterol levels, though increased by the inclusion of cholesterol with fat, did not respond to changes in degree of fat saturation.

Feeding cholesterol is known to decrease hepatic HMG-CoA reductase activity and cholesterogenesis (Shapiro and Rodwell, 1972; Higgins and Rudney, 1973). Reductase activity was also decreased in the present study when cholesterol was added to CO or SO. However, the decrease in endogenous cholesterol

synthesis was not reflected in serum levels of cholesterol.

In experiment 2, a significant increase in hydroxylase activity resulted when saturated rather than polyunsaturated fats were fed with cholesterol. However, this increased hydroxylase activity was not associated with reduced serum cholesterol levels. Perhaps the addition of cholesterol to CO, compared to SO, resulted in decreased stores of hepatic cholesterol. This problem could be explored in future work.

Amount of fat

Serum lipid concentrations may be affected by quantity of dietary fat (Howard, 1979), chemical composition of the dietary carbohydrate (Corey et al., 1974) and length of feeding period (Narayan et al., 1974).

In this study, when 10 or 40% of calories were fed as CO for one month, serum TG concentrations did not respond (Table 25). Data relating TG concentrations to increased amounts of dietary fat are contradictory. For example, when dietary lard was increased, TG concentrations increased likewise (De Gasquet et al., 1977). But TG levels fell when the amount of dietary corn oil was increased (Narayan et al., 1976). These experiments differed from the present study in that lower fat levels were used as well as different carbohydrates, both of which could influence TG levels.

Serum cholesterol concentrations were also similar at the two fat levels (Table 25) which confirms a report that different levels of dietary corn oil did not change serum cholesterol concentrations (Narayan et al., 1974; Narayan et al., 1976).

Summary

Variations in activities of the lipogenic enzymes and in concentration of serum lipids during alternating photoperiods were not well-defined. Although a diurnal rhythm has been confirmed in the activities of HMG-CoA reductase and cholesterol 7α-hydroxylase, the activities of the enzymes regulating lipogenesis, ACX and FAS did not exhibit similar variations. The activity of FAS was stable on high-fat diets following 1 or 3 month feeding periods.

However, when dietary fat was reduced with a concomitant increase in carbohydrate, FAS activity was stimulated during the postprandial or light period. The activity of ACX was stable after a 1 month feeding period in young rats with either low- or high-fat diets but was stimulated during the nonfeeding period after high-fat diets were fed for 3 months to adult rats. In contrast, when ACX or FAS activities from any experiment were considered, in either the dark or light photoperiod, feeding states did not affect enzyme

activities. A final integration of these data will be possible when additional information is available concerning diurnal variations in other parameters which can modify lipogenesis. Little is known about the periodicity of such related metabolic processes as fatty acid oxidation, ketogenesis, lipoprotein synthesis or secretion and changes in hormone levels.

Variations in dietary fat unsaturation affected enzyme activity. The lipogenic enzymes were depressed by feeding polyunsaturated compared to saturated fats and this effect was reflected in the reduced concentrations of serum triglycerides with PUFA. Similarly HMG-CoA reductase activity was reduced by polyunsaturated compared to saturated fats. With polyunsaturated fat, serum cholesterol levels were reduced. Though this was not at the level of statistical significance in experiment 2, it suggests that reductase activity measured in the same animal may be related to serum cholesterol levels. Even though reductase activity was severely depressed with cholesterol feeding, serum cholesterol levels were significantly elevated when cholesterol was fed with either fat. Evidently exogenous cholesterol absorption more than compensated for reduced reductase activity, based on cholesterol concentrations. Cholesterol 7α-hydroxylase activity was not affected by variations in fat saturation. This observation can be related to the similarities in serum cholesterol levels with either dietary fat but the addition of cholesterol

to either diet stimulated cholesterol 7α-hydroxylase activity significantly. Again, serum cholesterol levels were not affected, indicating the importance of absorption of dietary cholesterol in cholesterol homeostasis.

When the amount of fat supplied by CO was decreased from 40 to 10% of calories with a corresponding increase in dietary carbohydrate, activities of both ACX and FAS increased. However, this change was not reflected in increased levels of serum TG or total cholesterol.

SUMMARY AND CONCLUSIONS

This study was designed to explore the activity of enzymes regulatory in fatty acid synthesis, acetyl-CoA carboxylase and fatty acid synthetase, at intervals over a 24 hour period. Another objective was to determine synchronony or asychronony in the activities of the lipogenic enzymes to those controlling cholesterol synthesis, HMG-CoA reductase, and bile acid synthesis, cholesterol 7α-hydroxylase. A third objective was to measure concentrations of selected plasma or tissue lipids to determine whether these lipid levels were correlated with enzyme activity and could thereby regulate that activity. Additionally, these parameters were examined to determine the effect of food intake, i.e., photoperiod. A final objective was to determine the consequences of manipulating amount or degree of saturation of dietary fat, cholesterol addition or length of feeding period on enzyme activities and lipid concentrations.

Male Wistar rats, 3, 4 or 5 months old, were fed semi-purified diets in each experiment in this study. High-fat diets, 40% of calories from CO or SO, with and without cholesterol or a low-fat diet, 10% calories from CO, were fed for 1 or 3 months. Photoperiods were maintained so that alimentation during the dark period would occur beginning at 3 a.m. and end at 3 p.m. The light or

postprandial period was set from 3 p.m. to 3 a.m. Animals were sacrificed at 6 or 12 hour intervals. Parameters measured at these times included activities of ACX, FAS, HMG-CoA reductase and cholesterol 7α-hydroxylase. Concentrations of total and free cholesterol, TG and FFA were measured in the serum while total and free microsomal cholesterol were measured in the liver. Not all measurements were made in all experiments.

The activities of FAS and ACX were strongly correlated when the dietary component was ignored. At 6-hour time periods during 24 hours, the activity of FAS was relatively stable. The activity of ACX, however, was enhanced midway through the light period with either high-fat diet. This stimulation may reflect adaptations in other pathways utilizing acetyl-CoA when high-fat diets are fed. Activities of either ACX or FAS did not differ between 12 hour periods of light or dark. Apparently, enzyme activity did not respond to alimentation during the dark period or inactivity during the light. Our observations confirm data in the literature based on measurements of FAS during similar photoperiods. However, increased lipogenesis from labelled precursors during alimentation has been reported. In general, in our work there was an absence of a well-defined cycle of ACX or FAS activity over 24 hours similar to that for HMG-CoA reductase or cholesterol 7α-hydroxylase.

The activity of either lipogenic enzyme was depressed by feeding polyunsaturated compared to saturated fat throughout 24 hours. Inhibition of enzyme activity by PUFA was significant when mean daily values for enzyme activity were compared. This observation is supported by reports in the literature. Inhibition of activity by PUFA may be related to differences in degree of unsaturation and/or chain length of the fatty acids in SO and CO.

Serum concentrations of total or esterified cholesterol were elevated with saturated fat feeding compared to PUFA in the first experiment. Although serum levels of cholesterol in rats are relatively resistant to change by dietary manipulation, the extended feeding period for these high-fat diets probably led to concentration differences. Serum TG levels were also elevated by feeding saturated fats compared to PUFA. Serum TG may have been lowered in response to the decreased activity of the lipogenic enzymes observed in this experiment with PUFA feeding. In the second experiment with fewer animals per treatment, this effect of PUFA on the activity of the lipogenic enzymes or serum cholesterol and TG concentrations could not be verified statistically.
Free fatty acid serum concentrations were increased with PUFA versus saturated fat feeding. This may have been the result of higher lipoprotein lipase activities reported by other workers with similar feeding regimens. Liver cholesterol

concentrations were higher when SO was fed in the place of CO, indicative of an inverse relationship between serum and liver cholesterol levels. Kinetic data are needed to ascertain whether this constitutes a shift of cholesterol between serum and liver pools.

Although photoperiod did not affect enzyme activities, some serum lipid concentrations were affected by that parameter. For example, concentration of FFA were elevated during the light period when SO was fed possibly reflecting an increased rate of adipose catabolism of stored TG during the postprandial period. Additionally, TG concentrations were depressed prior to alimentation and increased during alimentation depending on the influx of dietary fats.

When high-fat diets were fed with or without cholesterol in the second experiment, lipid parameters were examined at two points assumed to be extremes in the activity of HMG-CoA reductase and choelsterol 7α-hydroxylase. Although the activity of the reductase was increased by feeding CO versus SO at each time, this effect was not reflected in serum cholesterol concentrations. When cholesterol was added to either diet, however, reductase activity was so severely depressed that neither a differential effect of fat on reductase activity nor the diurnal variation was evident. In contrast, the type of fat did not affect cholesterol 7α-

hydroxylase activity. But the addition of cholesterol to either fat stimulated the activity of that enzyme. Furthermore, activity was significantly elevated with the CO compared to the SO-containing diet. Apparently, the effectiveness of cholesterol absorption with either dietary fat more than compensates for the enzymatic adaptations under these conditions.

The activity of ACX was depressed by the addition of cholesterol to either PUFA or CO although the enzyme was more active when cholesterol was fed with CO. The decreased utilization of acetyl-CoA in this pathway did not stimulate reductase activity.

In experiment 3, diets were fed in which calories from CO were reduced to give a low-fat, high-carbohydrate diet. When fat was fed at 10% rather than 40% of calories, the activity of both lipogenic enzymes increased. But serum cholesterol and TG concentrations were unaffected by dietary fat level. Under these conditions the activity of ACX was relatively stable at the 4 periods during 24 hours. In contrast, this activity had been enhanced in experiment 1 on the high-fat diets. Experiment 3 differed from experiment 1 in that rats were younger and fed for 1 month only. Under these conditions, FAS activity responded to decreased fat intake and was enhanced at the midlight period. Increased lipogenesis would be expected with an increase in

dietary carbohydrate. Evidently, the increase was sufficient to make detectable an apparent stimulation of the enzyme at this point.

BIBLIOGRAPHY

Alberts, A., A. Strauss, S. Hennessy and P. Vagelos. 1975. Regulation of the synthesis of hepatic fatty acid synthetase: Binding of fatty acid synthetase antibodies to polysomes. Proc. Natl. Acad. Sci. U.S. 72:3956-3960.

Allain, C., L. S. Poon, S. Cicely, W. Richmond and P. C. Fu. 1974. Enzymatic determination of total serum cholesterol. Clin. Chim. 20:470-475.

Back, P., B. Hamprecht and F. Lynen. 1969. Regulation of cholesterol biosynthesis in rat liver: Diurnal changes of activity and influence of bile acids. Arch. Biochem. Biophys. 133:11.

Bagdade, J., W. Hazzard, and J. Carlin. 1970. Effect of unsaturated dietary fat on plasma lipoprotein lipase activity in normal and hyperlipidemic states. Metab. Clin. Exp. 19(12):1020-4.

Bartley, J. C. and S. Abraham. 1972. Hepatic lipogenesis in fasted, re-fed rats and mice: Response to dietary fats of differing fatty acid composition. Biochim. Biophys. Acta 280:258-266.

Björkhem, I., R. Blomstrand and L. Svensson. 1978. Effect of different dietary triglycerides on 7α-hydroxylation of cholesterol and other mixed-function oxidations. J. Lipid Res. 19:359-368.

Black, I. and J. Axelrod. 1970. Regulation of biochemical circadian rhythms. Pages 135-155 in G. Litwack, ed. Biochemical actions of hormones. Vol. 2. Academic Press, New York.

Block, K. and D. Vance. 1977. Control mechanisms in the synthesis of saturated fatty acids. Ann. Rev. Biochem. 46:263-298.

Bochenek, W., and J. Rodgers. 1978. Effects of saturated and unsaturated fats given with and without dietary cholesterol on hepatic cholesterol synthesis and hepatic lipid metabolism. Biochim. Biophys. Acta 528:1-16.

Bortz, W. M. 1967. Fat feeding and cholesterol synthesis. Biochim. Biophys. Acta 137:533-539.

Bortz, W. and F. Lynen. 1963. The inhibition of acetyl-CoA carboxylase by long chain acyl-CoA derivatives. Biochim. Z. 337:505-509.

Bortz, W. M. and L. A. Steele. 1973. Synchronization of hepatic cholesterol synthesis, cholesterol and bile acid content, fatty acid synthesis and plasma free fatty acid levels in the fed and fasted rat. Biochim. Biophys. Acta 306:85-94.

Bortz, W., S. Abraham, and I. L. Chaikoff. 1963. Localization of the block in lipogenesis resulting from feeding fat. J. Biol. Chem. 228:1266-1272.

Brown, M. S., S. E. Dana and J. L. Goldstein. 1974. Regulation of 3-hydroxy-3-methylglutaryl Coenzyme A reductase activity in cultured human fibroblasts. J. Biol. Chem. 250:789-796.

Bruckdorfer, K. R., S. S. Kang, I. H. Kahn, A. R. Bourne and J. Yudkin. 1974. Diurnal changes in the concentrations of plasma lipids, sugars, insulin and corticosterone in rats fed diets containing various carbohydrates. Horm. Metab. Res. 6:99-106.

Burton, D. N., A. G. Haavik and J. W. Porter. 1968. Comparative studies of the rat and pigeon liver fatty acid synthetases. Arch. Biochem. Biophys. 126:141-154.

Carlson, S. and S. Goldfarb. 1977. A sensitive enzymatic method for the determination of free and esterified tissue cholesterol. Clin. Chim. Acta 79:575-582.

Carlson, S., A. Mitchell and S. Goldfarb. 1978a. Sex-related differences of hepatic microsomal 3-hydroxy-3-methylglutaryl Coenzyme A reductase and cholesterol 7α-hydroxylase. Biochim. Biophys. Acta 531:115-124.

Carlson, S., C. Shriver and L. Arnrich. 1978b. Dietary fat and cholesterol metabolism in adult rats undergoing rapid tissue repletion. J. Nutr. 108:1170-1179.

Carrozza, G., G. Livrea, R. Caponetti and L. Manasseri. 1979. Response of rat hepatic fatty acid synthesis and activities of related enzymes to changes in level of dietary fat. J. Nutr. 109:162-170.

Clarke, S. D., D. R. Romsos and G. A. Leveille. 1977. Differential effects of dietary methyl esters of long-chain saturated and polyunsaturated fatty acids on rat liver and adipose tissue lipogenesis. J. Nutr. 107: 1170-1189.

Corey, J., K. Hayes, B. Dorr and M. Hegsted. 1974. Comparative lipid response of four primate species to dietary changes in fat and carbohydrate. Atherosclerosis 19:119-134.

Cornish, S. and M. Cawthorne. 1978. Fatty acid synthesis in mice during 24-hr cycle and during meal feeding. Horm. and Metab. Res. 10:286-289.

Craig, M. C., R. E. Dugan, R. A. Muesing, L. L. Slakey and J. W. Porter. 1972. Comparative effects of dietary regimens on the level of enzymes regulating the synthesis of fatty acids and cholesterol in rat liver. Arch. Biochem. Biophys. 151:128-136.

Danielsson, H. 1972. Relationship between diurnal variations in biosynthesis of cholesterol and bile acids. Steroids 19:63-71.

De Gasquet, P., S. Griglio, E. Pequignot-Planche and M. I. Malewiak. 1977. Diurnal change in plasma and liver lipids and lipoprotein lipase activity in heart and adipose tissue in rats fed a high and low fat diet. J. Nutr. 107:199-212.

Deuel, H. 1955. Biochemistry. Vol. 2. Interscience Publishers, New York.

Diamant, S., E. Gorin and E. Shafrir. 1972. Enzyme activities related to fatty-acid synthesis in liver and adipose tissue of rats treated with triiodothyronine. Eur. J. Biochem. 26:553-559.

Diller, E. and O. Harvey. 1964. Interrelationship of sterol and fatty acid biosynthesis in rat liver slices as related to dietary lipids. Biochemistry 3:2004-2007.

Dugan, R. E. and J. W. Porter. 1977. Hormonal regulation of cholesterol synthesis. Pages 198-242 in G. Litwack, ed. Biochemical actions of hormones. Vol. 4. Academic Press, New York.

Dugan, R., L. Slakey, A. Briedis, and J. Porter. 1972. Factors affecting the diurnal variation in the level of β-hydroxy-β-methylglutaryl Coenzyme A reductase and cholesterol-synthesizing activity in rat liver. Arch. Biochem. Biophys. 152:21-27.

Dupont, J. 1966. Synthesis of cholesterol and total lipid by male and female rats fed beef tallow or corn oil. Lipids 1:409-414.

Dupont, J. 1970. Dietary lipid, fatty acid oxidation and incorporation of carbon into cholesterol. Lipids 5: 908-914.

Dupont, J., M. Mathias and N. Cabacungan. 1972. Dietary lipid, fatty acid synthesis and cholesterol metabolism in aging rats. Lipids 7:576-589.

Edwards, P. A. and R. G. Gould. 1974. Dependence of the circadian rhythm of hepatic β-hydroxy-β-methylglutaryl Coenzyme A on ribonucleic acid synthesis. J. Biol. Chem. 249:2891-2896.

Edwards, P. A., H. Muroya and R. G. Gould. 1972. In vivo demonstration of the circadian rhythm of cholesterol biosynthesis in the liver and intestine of the rat. J. Lipid Res. 13:396-401.

Edwards, P., G. Popjak, A. Fogelman and J. Edmond. 1977. Regulation of 3-hydroxy-3-methylglutaryl Coenzyme A reductase by endogenously synthesized sterols in vitro and in vivo. J. Biol. Chem. 252:1057-1063.

Edwards, P. A., D. Lemongella and A. M. Fogelman. 1979. The effect of glucagon, norepinephrine, and dibutyryl cyclic AMP on cholesterol efflux and on the activity of 3-hydroxy-3-methylglutaryl CoA reductase in rat hepatocytes. J. Lipid. Res. 20:2-7.

Einarsson, K. and G. Johansson. 1968. Effect of actinomycin D and puromycin on the conversion of cholesterol into bile acids in bile-fistula rats. FEBS Lett. 1:219-222.

Exton, J., S. Lewis, R. J. Ho, G. Robinson and C. R. Park. 1971. The role of cyclic AMP in the interaction of glucagon and insulin in the control of liver metabolism. Ann. N.Y. Acad. Sci. 185:85-100.

Flick, P. K., J. Chen, and P. R. Vagelos. 1977. Effect of dietary linoleate on synthesis and degradation of fatty acid synthesis from rat liver. J. Biol. Chem. 252: 4242-4249.

Frnka, J. and R. Reiser. 1974. The effects of diet cholesterol on the synthesis of rat serum apolysoproteins. Biochim. Biophys. Acta 360:322-338.

Fuller, R. and E. Diller. 1970. Diurnal variation of liver glycogen and plasma free fatty acids in rats fed ad libitum or single daily meal. Metabolism 19:226-229.

Gangaly, J. 1960. Studies on the mechanism of fatty acid synthesis. VII. Biosynthesis of fatty acids from malonyl-CoA. Biochim. Biophys. Acta 40:110-118.

Gibbons, G. F. and C. Pullinger. 1979. Utilization of endogenous and exogenous sources of substrate for cholesterol biosynthesis by isolated hepatocytes. Biochem. J. 177:255-263.

Giegel, J., A. Ham, L. Bell and C. William. 1975. Manual and semi-automated procedures for measurement of triglycerides in serum. Clin. Chem. 21:1575-1581.

Gielen, J., J. Van Cantfort, B. Robaye, and J. Renson. 1969. Rythme circadien de la cholesterol-7α-hydroxylase chez la rat. C. R. Acad. Sci. Paris 269:731-732.

Gielen, J., J. Van Cantfort, B. Robaye and J. Renson. 1975. Rat-liver cholesterol 7 α-hydroxylase. Eur. J. Biochem. 55:41-48.

Goh, E. H. and M. Heimberg. 1973. Stimulation of hepatic cholesterol biosynthesis by oleic acid. Biochem. Biophys. Res. Comm. 55:382-388.

Goh, E. and M. Heimberg. 1976. Effects of free fatty acids on activity of hepatic microsomal 3-hydroxy-3-methylglutaryl Coenzyme A reductase and on secretion of triglyceride and cholesterol by liver. J. Biol. Chem. 252: 2822-2826.

Goldfarb, S. and H. C. Pitot. 1972. Stimulatory effect of dietary lipid and cholestyramine on hepatic HMG CoA reductase. J. Lipid Res. 13:797-801.

Goodridge, A. G. 1973. Regulation of fatty acid synthesis in isolated hepatocytes. Evidence for a physiological role for long chain fatty acyl Coenzyme A and citrate. J. Biol. Chem. 248:4318-4326.

Goodwin, C. D. and S. Margolis. 1976. Improved methods for the study of hepatic HMG CoA reductase: one-step isolation of mevalonolactone and rapid preparation of endoplasmic reticulum. J. Lipid Res. 17:297-303.

Gornall, A. G., C. S. Bardawill, and N. N. David. 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 177:751-766.

Gregolin, C., E. Ryder, R. C. Warner, A. K. Kleinschmidt, H. Chang and M. D. Lane. 1968. Liver acetyl Coenzyme A carboxylase. II. Further molecular characterization. J. Biol. Chem. 243:4236-4245.

Grundy, S. 1979. Dietary fat and sterols. Pages 89-118 in R. Levy, B. Refkind, B. Dennis and N. Ernst, eds. Nutrition, lipids and coronary heart disease. Vol. 1. Raven Press, New York.

Guynn, R., D. Veloso and R. Veech. 1972. The concentration of malonyl-CoA and the control of FA synthesis in vivo. J. Biol. Chem. 247:7325-7331.

Halestrap, A. P. and R. M. Denton. 1974. Hormonal regulation of adipose-tissue acetyl-Coenzyme A carboxylase by changes in the polymeric state of the enzyme. Biochem. J. 142:365-377.

Harry, D., M. Dini and N. McIntyre. 1973. Effect of cholesterol feeding and biliary obstruction on hepatic cholesterol biosynthesis in the rat. Biochim. Biophys. Acta 296:209-220.

Heimberg, M. and H. G. Wilcox. 1972. The effect of palmitic and oleic acids on the properties and composition of the very low density lipoprotein secreted by the liver. J. Biol. Chem. 247:875-880.

Hellman, B., and C. Hellerstrom. 1959. Diurnal changes in the function of the pancreatic islets of rats as indicated by nuclear size in the islet cells. Acta Endocrinol. 31:267-281.

Hems, D. A., E. A. Rath and T. R. Verrinder. 1975. Fatty acid synthesis in liver and adipose tissue of normal and genetically obese (ob/ob) mice during the 24-hour cycle. Biochem. J. 150:167-173.

Heng, C. S. 1977. Influence of dietary fat, meal pattern and length of feeding on cholesterol metabolism, cardiac stress-strain response and morphology in maturing male rats. Unpublished Ph.D. Thesis. Iowa State University, Ames, Iowa.

Higgins, M. and H. Rudney. 1973. Regulation of rat liver β-hydroxyl-β-methylglutaryl CoA reductase activity by cholesterol. Nature (London), New Biology 246:60-61.

Hill, R., J. Lingzusoro, F. Chevallier and I. Charkoff. 1958. Regulation of hepatic lipogenesis: The influence of dietary fats. J. Biol. Chem. 233:305-310.

Hornstra, G. 1971. The influence of dietary sunflower seed oil and hardened coconut oil on intra-arterial occlusive thrombosis in rats. Nutr. Metabol. 13: 140-149.

Howard, C. F. Jr. 1979. Effects of quantity and unsaturation of dietary fat on serum components in normal and diabetic macaca nigra. J. Nutr. 109:892-903.

Hwang, D., M. M. Mathias, J. Dupont and D. Meyer. 1975. Linoleate enrichment of diet and prostaglandin metabolism in rats. J. Nutr. 8:995-1002.

Ide, T., H. Okamatsu and M. Sugano. 1978. Regulation by dietary fats of 3-hydroxy-3-methylglutaryl-Coenzyme A reductase in rat liver. J. Nutr. 108:601-612.

Ide, T., T. Tanaka and M. Sugano. 1979. Dietary fat-dependent changes in hepatic cholesterogenesis and the activity of 3-hydroxy-3-methylglutaryl-CoA reductase in fasted-refed rats. J. Nutr. 109:807-818.

Inoue, H. and J. M. Lowenstein. 1972. Acetyl Coenzyme A carboxylase from rat liver. J. Biol. Chem. 247:4825-4832.

Inoue, H. and J. M. Lowenstein. 1975. Acetyl Coenzyme A carboxylase from rat liver. Pages 3-5 in J. Lowenstein, ed. Methods of Enzymology. Vol. 35. Academic Press, New York.

Jacobs, R. and P. Majerus. 1973. The regulation of fatty acid synthesis in human skin fibroblasts. J. Biol. Chem. 248:8392-8401.

Kellogg, T. F. 1974. Steroid balance and tissue cholesterol accumulation in germfree and conventional rats fed diets containing saturated and polyunsaturated fats. J. Lipid Res. 15:574-578.

Kim, M., A. A. Qureshi, R. A. Jenik, F. A. Lornitzo and J. W. Porter. 1977. Apo- and halofatty acid synthetases from pigeon liver. Arch. Biochem. Biophys. 181:580-590.

Kimura, T., T. Maji and K. Ashida. 1970. Periodicity of food intake and lipogenesis in rats subjected to two different feeding plans. J. Nutr. 100:691-697.

Kitajima, K., S. Tashiro and S. Numa. 1975. Acetyl-Co-enzyme-A carboxylase in cultured hepatocytes. Eur. J. Biochem. 54:373-383.

Klain, G. J. and P. C. Weiser. 1973. Changes in hepatic fatty acid synthesis following glucagon injections in vivo. Biochem. Biophys. Res. Commun. 55:76-83.

Kleinschmidt, A. K., J. Moss and M. D. Lane. 1969. Acetyl Coenzyme A carboxylase: Filamentous nature of the animal enzyme. Science 166:1276-1278.

Kohout, M., B. Kohowtova and M. Heimberg. 1971. The regulation of hepatic triglyceride metabolism by free fatty acids. JBC 246:5067-5074.

Krause, M. and L. Mahan. 1979. Food, Nutrition and Diet Therapy, 6th edition. W. B. Saunders, Philadelphia, p. 460.

Kritchevsky, D., S. A. Tepper and J. A. Story. 1977. Influence of dietary fats on lipid metabolism in rats fed a semipurified diet. Nutr. Reports Int. 15:65-70.

Lakshmanan, M., C. Nepokroeff and J. Porter. 1973. Control of the synthesis of rat liver fatty acid synthetase by insulin, glucagon and cyclic AMP. Proc. Nat. Acad. Sci. USA 69:3516.

Lee, K. H. and K. H. Kim. 1977. Regulation of rat liver acetyl Coenzyme A carboxylase. Evidence for interconversion between active and inactive forms of enzyme by phosphorylation and dephosphorylation. J. Biol. Chem. 252:1748-1751.

Lent, B. A., K. H. Lee and K. H. Kim. 1978. Regulation of rat liver acetyl-CoA carboxylase. J. Biol. Chem. 253:8149-8156.

Leveille, G., R. Pardini, and J. Tillotson. 1967. Influence of medium-chain triglycerides on lipid metabolism in the rat. Lipids 2:287-294.

Lewis, B. 1977. Plasma-lipoprotein interrelationships. Biochem. Soc. Trans. 5:589-599.

Liou, G. I. and W. E. Donaldson. 1973. Relative activities of acetyl-CoA carboxylase and fatty acid synthetase in chick liver: effects of dietary fat. Can. J. Biochem. 51:1029-1033.

Lindall, A. W., F. Grande and A. Schultz. 1971. The effect of dietary fats on the serum lipoproteins of normal dogs. Proc. Soc. Exp. Biol. Med. 136:1032-1037.

Louwerys, R. R. 1969. Colorimetric determination of free fatty acids. Anal. Biochem. 32:331-333.

Lunzer, M. A., J. A. Manning and R. K. Ockner. 1977. Inhibition of rat liver acetyl-Coenzyme A carboxylase by long chain acyl Coenzyme A and fatty acid. J. Biol. Chem. 252:5483-5487.

McGarry, J., Y. Takabayashi and D. Foster. 1978. The role of malonyl-CoA in coordination of fatty acid synthesis and oxidation in isolated rat hepatocytes. J. Biol. Chem. 253:8294-8300.

McGovern, R. and F. Quackenbush. 1973a. Turnover of bile acids in the hypercholesterolemic rat as affected by saturation of dietary fat. Lipids 8:466-469.

McGovern, R. and F. Quackenbush. 1973b. Effect of dietary fat saturation on absorption and intestinal secretion of cholesterol by the hypercholesterolemic rat. Lipids 8:470-472.

McGregor, L. and S. Renaud. 1977. Influence of dietary linoleic acid level on coagulation, aggregation and fatty acid composition of rats. Nutr. Metab. 21 (Suppl 1): 192-196.

Mackall, J. and M. Lane. 1977. Changes in mammary gland acetyl Coenzyme A associated with lactogenic differentiation. Biochem. J. 162:635-642.

Majerus, P. W. and E. Kilburn. 1969. Acetyl-Coenzyme A carboxylase, the roles of synthesis and degradation in regulation of enzyme levels in rat liver. J. Biol. Chem. 244:6254-6262.

Martin, R. J., D. J. Stoltz and D. C. Buck. 1979. Diurnal changes in adipose and liver tissue metabolism of lean and obese zucker rats. J. Nutr. 109:412-417.

Mayer, G. and D. Mayer. 1974. Secretion of bile acids and cholesterol-7 α-hydroxylase activity in dependence of the fatty acid composition in rats on a fat-rich diet. pp. 443-448 in S. Matern, J. Hackenschmidt, and W. Gerak, eds. Advances in bile acid research. F.K. Schattauer Verlag, Stuttgart.

Mayes, P. and D. Topping. 1974. Regulation of hepatic lipogenesis by plasma free fatty acids: Simultaneous studies on lipoprotein secretion, cholesterol synthesis, ketogenesis and gluconeogenesis. Biochem. J. 140:111-114.

Miller, J., A. Chait and B. Lewis. 1975. The relationship between dietary fat composition and plasma cholesterol esterification in man. Clin. Sci. Mol. Med. 49:617-620.

Mitropoulos, K., S. Balasubramaniam and N. Myant. 1973. The effects of interruption of enterohepatic circulation, etc. and cholesterol feeding on cholesterol 7α-hydroxylase. Biochim. Biophys. Acta 326:428-438.

Mitropoulos, K. A., S. Balasubramaniam, G. F. Gibbons and B. E. A. Reeves. 1972. Diurnal variation in the activity of cholesterol 7α-hydroxylase in the livers of fed and fasted rats. FEBS Letters 27:203-238.

Morris, D., A. Chait, R. Cohen and M. France. 1975. Modification of the rate of secretion of triglyceride into plasma by dietary polyunsaturated fat. Clin. Sci. Mol. Med. 49:4P.

Musch, K., M. A. Ojakian and M. A. Williams. 1974. Comparison of α-linolenate and oleate in lowering activity of lipogenic enzymes in rat liver: Evidence for a greater effect of dietary linolenate independent of food and carbohydrate intake. Biochim. Biophys. Acta 337:343-348.

Muto, Y. and D. M. Gibson. 1970. Selective dampening of lipogenic enzymes of liver by exogenous polyunsaturated fatty acids. Biochem. Biophys. Res. Comm. 38:9-15.

Myant, N. B. and H. A. Eder. 1961. The effect of biliary drainage upon synthesis of cholesterol in the liver. J. Lipid Res. 2:363-368.

Myant, N. and K. Mitropoulous. 1977. Cholesterol 7α-hydroxylase. J. Lipid Res. 18:135-153.

Nakanishi, S., and S. Numa. 1970. Purification of rat liver acetyl coenzyme A carboxylase and immunochemical studies of its synthesis and degradation. Eur. J. Biochem. 16:161-173.

Narayan, A., J. McMullen, D. Butler, T. Wakefield and W. Calhoun. 1974. The influence of a high level of dietary corn oil on rat serum and liver lipids. Nutr. Rep. Int. 10:25-33.

Narayan, K. A., J. J. McMullen, D. P. Butler, T. Wakefield, and W. K. Calhoun. 1976. The influence of a high level of corn oil on rat serum lipoproteins. Atherosclerosis 23:1-17.

Nepokroeff, C. M., M. R. Lakshmanan, G. C. Ness, R. E. Dugan and J. W. Porter. 1974. Regulation of the diurnal rhythm of rat liver β-hydroxy-β-methylglutaryl coenzyme A reductase activity by insulin, glucagon, cyclic AMP and hydrocortisone. Arch. Biochem. Biophys. 160:387-393.

Nervi, F. O. and J. M. Dietschy. 1978. The mechanisms of and the interrelationship between bile acid and chylomicron-mediated regulation of hepatic cholesterol synthesis in the liver of the rat. J. Clin. Inves. 61:895-909.

Nervi, F. O., H. J. Weis and J. M. Dietschy. 1974. The kinetic characteristics of inhibition of hepatic cholesterogenesis by lipoproteins of intestinal origin. J. Biol. Chem. 250:4145-4151.

Nestel, P. and D. Steinberg. 1963. Fate of palmitate and of linoleate perfused through the isolated rat liver at high concentrations. J. Lipid Res. 4:461-469.

Nichman, M., R. Olson and C. Sweeley. 1967. Metabolism of linoleic acid-1-^{14}C in normolipemic and hyperlipemic humans fed linoleate diets. Amer. J. Clin. Nutr. 20: 1070-1083.

Nicolosi, R. J., M. G. Herrera, M. el Lozy and K. C. Hayes. 1976. Effect of dietary fat on hepatic metabolism of ^{14}C-oleic acid and very low density lipoprotein triglyceride in the gerbil. J. Nutr. 106:1279-1285.

Nilsson, A., R. Sundler and B. A. Kesson. 1974. Effect of different albumin-bound fatty acids on fatty acid and cholesterol biosynthesis in rat hepatocytes. FEBS Letters 45:282-285.

Nishikari, K., N. Iritani and S. Numa. 1973. Levels of acetyl coenzyme A carboxylase and its effectors in rat liver after short term fat-free refeeding. FEBS Letters 32:19-21.

Nordstrom, J. L., V. W. Rodwell and J. J. Mitschelen. 1977. Interconversion of active and inactive forms of rat liver 3-hydroxy-3-methylglutaryl-CoA reductase. J. Biol. Chem. 252:8924-8934.

O'Brien, B., C. Skutches, G. Henderson and R. Reiser. 1977. Interrelated effects of food lipids on steroid metabolism in rats. J. Nutr. 107:1444-1454.

Pande, S. and J. Mead. 1968. Inhibition of enzyme activities by free fatty acids. J. Biol. Chem. 243: 6180-6185.

Pawar, S. S. and H. C. Tedwell. 1968. Effect of ingestion of unsaturated fat on lipolytic activity of rat tissues. J. Lipid Res. 9:334-336.

Peret, J., M. Chanez and G. Pascal. 1976. Taux de protéines dans la ration et variations circadiennes des activités enzymatiques impliquées dans le métabolisme des glucides et dans la lipogenése chez le rat mâle. J. Physiol. 72:683-696.

Persson, B., P. Bjorntarp and B. Hood. 1966. Lipoprotein lipase activity in human adipose tissue. Metabolism 15:730-741.

Plate, C. A., V. C. Joshi, B. Sedgwick and S. J. Wakil. 1968. Studies on the mechanism of fatty acid synthesis. J. Biol. Chem. 243:5439-5445.

Porter, J. W., S. Kumar and R. E. Dugan. 1971. Synthesis of fatty acids by enzymes of avian and mammalian species. Progr. Biochem. Pharmacol. 6:1-101.

Raicht, R., B. Cohen, S. Shafer and E. Mosbach. 1975. Sterol balance studies in the rat. Effects of dietary cholesterol and β-sitosterol on sterol balance and rate-limiting enzymes of sterol metabolism. Biochim. Biophys. Acta 388:374-384.

Reiser, R., M. C. Williams, M. F. Sorresl and N. L. Murtz. 1963. Biosynthesis of fatty acids and cholesterol as related to dietary fat. Arch. Biochem. Biophys. 102:276-285.

Rodwell, V., J. Nordstrom and J. Mitschelen. 1976. Regulation of HMG-CoA reductase. Adv. Lipid Res. 14:1-74.

Romsos, D. and G. Leveille. 1974. Effect of diet on the activity of enzymes involved in fatty acid and cholesterol synthesis. Adv. Lipid Res. 12:97-116.

Roseschlau, P., E. Bernt and W. Gruber. 1974. Enzymatische Bestimmung des Gesamt-cholesterins im Serum. Z. f. Klin. Chem. u. Klin. Biochem. 12:226.

Saloni, F. and L. Sardenia. 1973. Colorimetric microdetermination of free fatty acids. Clin. Chem. 19:419-424.

Serdarevich, B. and K. K. Carroll. 1971. In-vivo incorporation of labelled acetate into liver and serum lipids of rats on different dietary regimens. Can. J. Biochem. 50:557-562.

Shapiro, D. J. and V. W. Rodwell. 1971. Regulation of hepatic 3-hydroxy-3-methylglutaryl Coenzyme A reductase and cholesterol synthesis. J. Biol. Chem. 246:3210-3216.

Shapiro, D. J. and V. W. Rodwell. 1972. Fine structure of the cyclic rhythm of 3-hydroxy-3-methylglutaryl Coenzyme A reductase. Biochemistry 11:1042-1045.

Shapiro, D., J. Nordstrom, J. Mitschelen, V. Rodwell and R. Schimke. 1974. Micro assay for 3-hydroxy-3-methylglutaryl-CoA reductase in rat liver and in L-cell fibroblasts. Biochim. Biophys. Acta 370:369-377.

Shefer, S., S. Hauser, I. Bekersky, and E. H. Mosbach. 1969. Feedback regulation of bile acid biosynthesis in the rat. J. Lipid Res. 10:646-655.

Shefer, S., S. Hauser, V. Lapar and E. Mosbach. 1973. Regulatory effects of sterols and bile acids on hepatic 3-hydroxy-3-methylglutaryl CoA reductase and cholesterol 7α-hydroxylase in the rat. J. Lipid Res. 14:573-580.

Siperstein, M. D. and V. M. Fagan. 1964. Studies on the feed-back regulation of cholesterol synthesis. Adv. Enzyme Regul. 2:249-264.

Spritz, N. and M. A. Mishkel. 1969. Effects of dietary fats on plasma lipids and lipoproteins: An hypothesis for the lipid-lowering effect of unsaturated fatty acids. J. Clin. Invest. 48:78-86.

Takeuchi, N., M. Ito and Y. Yamamura. 1974. Regulation of cholesterol 7α-hydroxylation by cholesterol synthesis in rat liver. Atherosclerosis 20:481-494.

Takeuchi, N., M. Ito, K. Uchida and Y. Yamamura. 1975. Effect of modification of thyroid function on cholesterol 7α-hydroxylation in rat liver. Biochem. J. 148:499-503.

Tarladgis, B., B. Watts, and M. Younathan. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Amer. Oil Chem. Soc. 37:44-48.

Tepperman, H., S. LaGarya and J. Tepperman. 1968. Effects of dehydroepiandrosterone and diet protein on liver enzymes and lipogenesis. Am. J. Physiol. 214:1126-1132.

Triscari, J., J. Hamilton and A. Sullivan. 1978. Comparative effects of saturated and unsaturated lipids on hepatic lipogenesis and cholesterogenesis in vivo in the meal fed rat. J. Nutr. 105:815-825.

Truswell, A. S. 1978. Diet and plasma lipids - a reappraisal. Am. J. Clin. Nutr. 31:977-989.

Tsai, A. and I. Dyer. 1973. Liver cholesterol concentration in relation to hepatic cholesterogenesis and activity of certain liver enzymes in rats. J. Nutr. 103:1119-1125.

Tsai, A. C., D. R. Romsos and G. A. Leveille. 1975. Effect of dietary cholesterol on hepatic lipogenesis and plasma insulin and free fatty acid levels in rats. J. Nutr. 105:939-945.

Tweto, J. and A. Larrabee. 1972. The effect of fasting on synthesis and 4'-phosphopantetheine exchange in rat liver fatty acid synthetase. J. Biol. Chem. 247:4900-4904.

Van Canfort, J. 1973. Controle par les glucocorticosteroides de l'activite circadienne de la cholesterol-7α-hydroxylase. Biochimie. 55:1171-1173.

Van Cantfort, J. and J. Gielen. 1975. Cholesterol 7α-hydroxylase. Eur. J. Biochem. 55:33-40.

Van Golde, L. M. G. and S. G. Van den Bergh. 1977. Liver. pp. 35-150 in F. Synder, ed. Lipid Metabolism in Mammals. Vol. 2. Plenum Press, New York.

Volpe, J. J. and P. R. Marasa. 1975. Hormonal regulation of FA synthesis, acetyl-CoA carboxylase in adipose and liver. Biochim. Biophys. Acta 380:454-472.

Volpe, J. J. and P. R. Vagelos. 1973. Saturated fatty acid biosynthesis and its regulation. Ann. Rev. Biochem. 42:21-60.

Wadhwa, P. S., C. E. Elson and D. J. Pringle. 1973. Diurnal changes in the fatty acid patterns of rat liver lipids. J. Nutr. 103:899-903.

Wiegand, R. D., G. A. Rao and R. Reiser. 1973. Dietary regulation of fatty acid synthetase and microsomal glycerophosphate acyltransferase activities in rat liver. J. Nutr. 103:1414-1424.

Witters, L. A., E. M. Kowaloff and J. Avruch. 1979. Glucagon regulation of protein phosphorylation. J. Biol. Chem. 254:245-248.

ACKNOWLEDGMENTS

The author wishes to thank Dr. Lotte Arnrich for her support and guidance throughout the course of this study. Her patience and thoroughness during the completion of the dissertation were especially appreciated.

The author also thanks Drs. Donald Beitz, Jacqueline Dupont, Jane Love and Dare McGilliard for their participation in the course of her graduate work.

The author is grateful to Shirley Chen and Cynthia Shriver for their interest and reassurances, whenever possible, that progress was being made in this study. Thanks also go to Mary Higgins for offering a hand, an ear or a reminder, whichever was most appropriate during this time, to Veronica Arthur, who assumed the many duties asked of an officemate without a protest and to Mirza Baig and Stephen Mma for excellent assistance during the sacrifice periods.

Additionally, the author thanks Barney Cook for his help and encouragement throughout her graduate work. She is also grateful that her sister, Susan, and her children, Anne, Paul and Steven supported her efforts during this often demanding period.

APPENDIX

One hundred rats were assigned to 4 diets in experiment 2. Respiratory difficulties were noted in some of these animals as the study progressed. After approximately 7 weeks, animals from all diet groups had severe diarrhea and weight loss. Similar effects were noted simultaneously in the stock colony. Rats were given tetracycline (6g/ liter distilled H_2O) via their drinking water for 2 weeks. Most animals regained weight during this treatment which was discontinued 3 weeks before sacrifice.

However, during the final 5 week period of experiment 2, 25 rats fed either the 40SO or 40SC diets died in similar circumstances. First, the ears and eyes of the rats became pale, there was blood loss and the animals did not eat. Death followed 1 to 4 days after the initial symptoms. On autopsy, massive blood clots were found in or around the kidneys, epididymides, spleen, anal area, chin or in the limbs. Blood clotting time[1] was approximately 3 times longer in one of these animals compared to a control (stock colony) rat.

When the surviving rats were sacrificed, no internal hemorrhaging was evident, although lungs of rats on all

[1]Laboratory Animal Resources, School of Veterinary Medicine, Iowa State University.

diets often had lesions. Evidently, animals on all diets survived the respiratory difficulties so that lung infections were probably not the cause of blood loss or internal clot formation.

Diet and/or tetracycline administration may be implicated in the early deaths. All diets were checked for oxidative products from unsaturated fatty acids using a standard 2-thiobarbituric acid (TBA) test (Tarladgis et al., 1960).[1] No measurable amounts of TBA reactive materials were found in the samples. However, in rats fed high PUFA compared to saturated fats, an increase was reported in the time necessary for formation of a platelet thrombus and a decrease in the area of that aggregation (Hornstra, 1971; McGregor and Renaud, 1977). Prostaglandin E_1 (PGE_1) is a highly potent inhibitor of thrombocyte adhesion and aggregation. The synthesis of PGE_1 was increased in rats fed corn oil rather than beef tallow (Hwang et al., 1975) and may have been increased in the present study due to the highly unsaturated nature of safflower oil.

One of the effects of tetracycline is to decrease synthesis of vitamin K by intestinal bacteria (Krause and Mahan, 1979). Possibly a synergistic effect of tetracycline and the high PUFA diet led to the prolonged clotting time

---

[1]Dr. Mark Love, Food and Nutrition Dept., Iowa State University.

and internal clotting which were apparently responsible for the early deaths noted in this study. Measurement of PGE_1 in the serum of these animals would have been useful in interpreting these data.