# REGULATORY ASPECTS OF TRIGLYCERIDE UPTAKE BY BOVINE ADIPOSE AND MAMMARY TISSUES

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ABSTRACT

REGULATORY ASPECTS OF TRIGLYCERIDE UPTAKE
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By

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Lipoprotein lipase (LPL) and glyceride synthesis (GS) are considered essential for uptake of blood triglyceride fatty acids by extrahepatic tissues. Two experiments investigated the regulatory capability of LPL and GS in bovine adipose and mammary tissues. Initially (Experiment $T_1$) samples of adipose and mammary tissues were obtained from nine primiparous Holstein heifers 180 days (n = 6) or 260 days (n = 3) in gestation and 14 days after induced (caesarean section) lactation. In the second experiment ($T_2$) biopsy samples of mammary and shoulder subcutaneous adipose tissue were taken at 49, 21, 14, 8, and 2 days prepartum and 7, 14, 28, 60, 120, 180, 240, and 300 days postpartum from eight multiparous Holstein cows.

Mammary LPL activity (μmoles fatty acids released $hr^{-1}$ 10 mg tissue $protein^{-1}$) increased from .5 to 46.9 while adipose LPL decreased (12 to 8) with onset of lactation (Experiment $T_1$). Similarly, GS activity (μmoles palmitate incorporated $hr^{-1}$ 10 mg tissue $protein^{-1}$)

increased 8 fold in mammary tissue and decreased 6 fold in adipose tissue. In Experiment $T_2$, mammary LPL activity (μmoles glycerol released $hr^{-1}$ 100 mg tissue $protein^{-1}$) increased 6 fold between 49 and 7 days prepartum, then increased sharply (.4 to 5.1) between 7 and 2 days prepartum, reached a maximum (83 ± 12) at 120 days postpartum, and then decreased slowly until 280 days postpartum (19 ± 18). Mammary GS activity (μmoles palmitate incorporated $hr^{-1}$ 100 mg tissue $protein^{-1}$) decreased slightly between 49 and 8 days prepartum then increased 5 fold by 2 days prepartum and an additional 2 fold by 2 weeks postpartum after which it remained relatively constant until 280 days postpartum. Adipose tissue LPL and GS activities appeared to be more sensitive to energy status than to changes in lactational state (Experiment $T_2$). Although activities of the adipose enzymes were variable, two discernible peaks occurred; one 2 days prior to parturition and one during mid-lactation (120 to 180 days). Both peaks correspond to luxury grain intake.

Availability of LPL for release into plasma may represent uptake of triglyceride fatty acids by a tissue better than total tissue LPL activity since LPL catalyzes hydrolysis of blood triglyceride at the capillary membrane. Four experiments were conducted to ascertain the feasibility of using mammary venous plasma lipolytic activity (PLA), against a substrate of triolein emulsion activated with serum,

as a measure of mammary clearance of triglycerides from blood. Preheparin and peak-postheparin PLA were measured and compared to milk fat production (an overt measure of plasma triglyceride utilization by the mammary gland). In subsequent experiments, PLA was characterized with respect to: 1) LPL and GS activities in adipose and mammary tissues, 2) energy status of the cow, 3) milking stimulus, and 4) changes in lactational state. Samples were taken simultaneously from the jugular and mammary veins and the difference in PLA between the two (M-J) was assumed to be the mammary gland contribution.

Preheparin mammary PLA (M-J) was: 1) positively correlated ($r = .8$, $p < .05$) with milk fat production, mammary tissue LPL activity ($r = .7$, $p < .01$) and mammary tissue GS activity ($r = .6$, $p < .05$); 2) negatively correlated with energy status of the cow ($r = -.3$), adipose tissue LPL activity ($r = -.7$, $p < .01$) and adipose GS activity ($r = -.3$); and 3) sensitive to mammary gland emptying and prolactin injection (i.v.). Postheparin PLA in mammary venous plasma (M-J) was negatively correlated ($r = -.4$) with milk fat production and positively correlated with energy status of the cow ($r = .7$).

Preheparin PLA was not detectable in either jugular or mammary venous plasma of primiparous heifers at 17 or 14 days prepartum, but was detectable between 8 and 2 days prepartum. Mammary PLA (M-J) increased significantly ($p < .01$) on day of parturition and attained a

maximum by 2 to 4 days postpartum. Postpartum PLA (M-J) was significantly higher ($p < .01$) than PLA prepartum or PLA on the day of parturition. No difference in heparin releasable PLA between jugular and mammary venous plasma was observed before 7 days prepartum. A mean positive M-J difference in postheparin PLA occurred by 4 days prepartum. Postheparin PLA in mammary, jugular, and mammary minus jugular venous plasma increased sharply at parturition followed by an additional increase by 2 to 4 days postpartum.

Results of these experiments are consistent with the views that (1) the redistribution of lipid from adipose to mammary tissue at induction of lactation is due to a decrease in the uptake ability of adipose tissue and an increase in uptake ability of the mammary gland, (2) LPL released to mammary plasma is a more plausible regulator of triglyceride fatty acid uptake by the mammary gland than GS, (3) preheparin PLA in mammary venous plasma reflects the mammary glands ability to clear triglycerides from blood more accurately than tissue LPL activity or postheparin PLA, and (4) adipose tissue LPL and GS are more sensitive to energy status than lactational state. Results numbers 2 and 3 represent important new concepts.

# REGULATORY ASPECTS OF TRIGLYCERIDE UPTAKE BY BOVINE ADIPOSE AND MAMMARY TISSUES

By

John E. Shirley

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Dedicated:

To my parents who instilled in me a

thirst for knowledge and the value

of a smile and a funny story.

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# VITA

John Edd Shirley was born January 26, 1943 in Monroe County, Kentucky. He attended Sand Lick Elementary School and Tompkinsville High School. He served 4 years in the United States Air Force. He received the Bachelor of Science Degree in Agriculture from Western Kentucky University in August 1968, and the Master of Science Degree in Agriculture in February 1970. His Master of Science Degree was done under the supervision of Dr. L. D. Brown. The title of his thesis was "Influence of Urea on the Fermentation Pattern and Nutritive Value of Corn Silage." He will receive a Doctor of Philosophy Degree from the Department of Dairy Science and the Institute of Nutrition in 1973. He will join the faculty of the Agriculture Department at Eastern Kentucky University, Richmond, Kentucky in August 1973 as an assistant professor.

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# INTRODUCTION

The non-lactating ruminant mammary gland depends primarily on the utilization of acetate for energy. With lactogenesis, there is a sharp increase in utilization of blood sugar, ketone bodies, and triglycerides. This increased utilization of nutrients by the mammary gland shifts the animal into negative energy balance with a resultant increase in fat mobilization from body stores. This situation continues throughout early lactation, presumably because the animal is unable to consume sufficient energy to meet her needs for maintenance and milk production. The energy input:output ratio becomes more favorable as lactation advances and the animal begins to replete body energy stores.

This synopsis serves to point out the continual competition for calories between adipose tissue and the mammary gland during lactation. The mammary gland has the advantage during early lactation, a balance is achieved at midlactation, and adipose tissue is favored during late lactation. This relationship has been somewhat characterized in the literature in terms of variation in plasma lipid and milk fat at various stages of lactation. Little information is available on the enzymic changes behind this observed phenomenon.

Several metabolic alterations may contribute to this adipose-mammary relationship. However, this investigation is primarily directed toward the influence exerted by the enzymes responsible for uptake of plasma triglyceride fatty acid by adipose and mammary tissues. Fundamentally, uptake involves extracellular hydrolysis of triglyceride to free fatty acids and glycerol, and subsequent reesterification within the cell. Lipoprotein lipase (LPL) is generally accepted to be responsible for the hydrolytic step and glyceride synthesis (either $\alpha$-glycerol phosphate or 2-monoglyceride pathway or both) for reesterification within the cell. The relative influence exerted by adipose and mammary LPL and glyceride synthesis on the diversion of blood fat to the mammary gland has been investigated in nutritionally milk fat-depressed cows. These studies established that LPL and glyceride synthesis are involved in plasma glyceride fatty acid uptake by bovine adipose and mammary tissues. However, they provided little insight into the specific role each played with respect to regulation of milk fat production.

Data from other species indicating that initiation of lactation has profound effects on activities of both adipose and mammary LPL and glyceride synthesis suggest an alternative means of investigating their role with respect to milk fat production. The method suggested is the investigation of the effects of gestation, parturition,

and subsequent lactation on adipose and mammary tissue LPL and glyceride synthesis activities in the bovine.

Various workers have pointed out that LPL activity in tissue homogenates or extracts may not reflect the physiological activity of the enzymes. This conclusion is based on data suggesting that LPL exists in the cell in a state of reduced activity, is activated just prior to or during release from the cell and functions at the capillary membrane. Thus, a more accurate measure of physiological LPL activity may be that activity in the circulatory system.

The primary objectives of this study were to establish: 1) the importance of adipose and mammary triglyceride uptake ability relative to distribution of lipid between the two tissues; 2) the importance of LPL versus glyceride synthesis in regulation of triglyceride uptake; 3) the feasibility of using mammary venous plasma triglyceride lipase activity as an indicator of the mammary gland's ability to clear triglyceride fatty acids from the bloodstream.

# REVIEW OF LITERATURE

## Introduction

The intent of this review is to bring together pertinent information on the mechanism employed by extrahepatic tissues to clear lipoprotein triglycerides from plasma and possible regulators of plasma triglyceride uptake by these tissues. Emphasis will be placed on triglyceride uptake by adipose tissue and the mammary gland. A consideration of plasma lipoprotein lipase will be presented with emphasis on the origin of plasma lipoprotein lipase and the way it is cleared from the bloodstream. The contribution of plasma lipid to milk fat and effects of stage of lactation on milk fat composition will receive brief consideration.

## Uptake of Plasma Triglycerides

Long chain fatty acids serve as a readily utilizable form of energy for peripheral tissues. They are derived from the diet or from de novo synthesis in liver and adipose tissue and transported in the

blood stream as triglycerides of chylomicra and low density lipoproteins (Scow et al., 1972; Griel and McCarthy, 1969). These triglycerides are cleared from the blood stream by the liver and by extrahepatic tissues (Shapiro, 1965). The mechanism by which extrahepatic tissues, such as adipose and mammary, clear such large particles as chylomicra and low density lipoproteins from the blood stream has generated considerable interest in recent years. Adipose and mammary tissue capillaries have a continuous luminal surface which cannot accommodate the passage of intact chylomicra and other lipid particles (Scow, 1970). This section explores the mechanism used by adipose tissue and the mammary gland to clear triglycerides from the blood stream.

## Mammary Gland

The majority of investigations indicate that hydrolysis of triglycerides to free fatty acids and glycerol occurs prior to uptake by the mammocyte. Results obtained by infusing doubly labeled chylomicra into guinea pig (McBride and Korn, 1964b), goat (West et al., 1967a, 1967b), and cow (Bishop et al., 1969) are consistent with this view. Collectively, they found a decrease in the labeled glycerol:fatty acid ratio in milk fat relative to infused chylomicron triglycerides and the appearance of labeled non-esterified fatty acids in mammary venous blood. To determine where hydrolysis occurs during

uptake, Mendelson and Scow (1972) perfused the inguinal abdominal mammary glands of lactating rats in situ with doubly labeled chylomicron-triglyceride ($^{3}$H-glycerol and $^{14}$C-palmitate). The temporal relationship between infusion and appearance of $^{14}$C-palmitate in the perfusate leaving the gland was observed. They were able to conclude that hydrolysis occurs at or within the capillary membrane. Further evidence for hydrolysis at the capillary membrane was provided by electron micrographs of lactating mouse mammary tissue (Schoefl and French, 1968). These micrographs showed adhesion and engulfment of chylomicra by the inner membrane of endothelial cells of mammary capillaries with no sign of lipid globules outside the membrane.

Lipoprotein lipase has been implicated as the enzyme responsible for hydrolysis of chylomicra and low density lipoprotein triglycerides prior to uptake. Several workers observed a close correlation between mammary lipoprotein lipase activity and uptake of plasma triglycerides (McBride and Korn, 1963; McBride and Korn, 1964a; Robinson, 1963a; Hamosh et al., 1970). The non-lactating mammary gland of the rat (Hamosh et al., 1970), guinea pig (McBride and Korn, 1963 and 1964a; Robinson, 1963a), rabbit (Falconer and Fiddler, 1970), and cow (Askew et al., 1970) contain very low levels of lipoprotein lipase activity. Initiation of lactation and subsequent need for milk fat precursors is accompanied by a large increase in lipoprotein lipase

activity. Conversely, nonsuckling results in a decrease in mammary tissue lipoprotein lipase activity in guinea pigs (McBride and Korn, 1963) and rats (Hamosh et al., 1970) and an increased plasma triglyceride concentration.

## Adipose Tissue

Clearance of triglyceride fatty acids from blood by adipose tissue requires prior hydrolysis to free fatty acids and glycerol (McBride and Korn, 1964b; Robinson, 1963b; Scow et al., 1972). The extent of lipoprotein lipase involvement in blood triglyceride clearance is indicated by adipose tissue uptake of some 30 per cent of the fatty acids entering the blood of animals in positive caloric balance and in those absorbing fatty meals (Bragdon and Gordon, 1958). This uptake occurs directly from triglyceride fatty acids of chylomicra in blood (Nestel et al., 1962a, 1962b). On the other hand, in fasted animals triglyceride fatty acids in blood are not take up by adipose tissue (Bragdon and Gordon, 1958) and lipoprotein lipase activity in adipose tissue decreases (Hollenberg, 1959; Robinson, 1960; Pav and Wenkeova, 1960). Uptake of triglyceride fatty acids by adipose tissue varies with the tissue level of lipoprotein lipase activity (Bezman et al., 1962).

Adipocytes and vascular-stromal cells of adipose tissue both contain lipoprotein lipase (Rodbell and Scow, 1965; Cunningham and Robinson, 1969). Release of lipoprotein lipase into the blood stream can be accomplished by heparin infusion (Rodbell and Scow, 1965). Perfusion of adipose tissue with heparin results in a rapid release of lipoprotein lipase into the perfusate and a subsequent decrease in the rate of hydrolysis of chylomicra triglycerides (Scow et al., 1972). Since heparin presumably causes release of the lipoprotein lipase located within or near the capillary wall (Ho et al., 1967; Rodbell and Scow, 1965) it appears that the site of hydrolysis is at the capillary membrane. Blanchette-Mackie and Scow (1971), using the electron microscope and cytochemical techniques, provided convincing evidence for hydrolysis at the capillary membrane. Their findings indicate that triglycerides are hydrolyzed by lipoprotein lipase in vacuoles and microvesicles of the capillary endothelium and in the subendothelial space of adipose tissue.

## Regulation of Plasma Triglyceride Fatty Acid Uptake by Extrahepatic Tissues

Long chain fatty acids, transported through the blood stream as chylomicra and β-lipoproteins, are precursors for approximately 50 per cent of milk fat triglycerides. During lactation, the mammary

gland competes with other extrahepatic tissues for these triglycerides. Predominate among the mammary gland competitors is adipose tissue. The mechanism by which extrahepatic tissues remove chylomicra and low density lipoprotein triglycerides from circulation involves lipoprotein lipase and glyceride synthesis. Either one of these enzymic systems could directly influence uptake and consequently the final destination of circulating triglycerides. It is recognized that other factors could influence final destination of blood triglycerides but this review will be confined to possible regulation of uptake at the tissue level. Lipoprotein lipase and glyceride synthesis as possible regulatory agents will be discussed separately.

## Lipoprotein Lipase

Lipoprotein lipase (LPL) is a triglyceride lipase with preferential activity against triglycerides of lipoprotein origin. It is active against artificial triglyceride (triolein, etc.) emulsions if the emulsion is converted to an active lipoprotein substrate by addition of serum, lipoprotein fractions, or specific serum peptides from lipoproteins (Korn, 1955b; Fielding et al., 1970; Havel et al., 1970; LaRosa et al., 1970; Whayne and Felts, 1970; Miller and Smith, 1973). Contrary to early reports (Korn, 1955a), the major products of hydrolysis by LPL appear to be free fatty acids and 2-monoglyceride

(Nilsson-Ehle et al., 1971; Fielding, 1972). Although minor activity against monoglyceride substrates was observed, these contrasting results are apparently due to the state of purification of the enzyme.

The assay of LPL activity in tissue homogenates and extracts, and in plasma requires: 1) a serum activated triglyceride substrate; 2) a pH optima between 8.0 and 8.6, depending on the enzyme preparation and the nature of the substrate (Fielding, 1972; Korn, 1955a,b; Whayne and Felts, 1972); 3) a free fatty acid acceptor such as albumin (Gordon et al., 1953; Korn, 1955a); and 4) the presence of divalent cations, in particular calcium at concentrations found in serum. Addition of low concentrations of heparin to the assay medium enhances LPL activity while high concentrations inhibit activity (Korn, 1955a). A specific requirement for ammonium ions in the assay of LPL activity has been reported (Korn, 1955a) and refuted (Whayne and Felts, 1971).

It is generally accepted that LPL functions in the uptake of plasma triglyceride fatty acids by extrahepatic tissues. Prominent among these being muscle, adipose, mammary, and cardiovascular (Scow, 1970; Korn, 1959; Robinson, 1963a). LPL appears to exist in the cell in a state of reduced activity (Cunningham and Robinson, 1969; Robinson and Wing, 1970) and is activated just prior to or during release from the cell (Stewart and Schotz, 1971). The functional site of

action appears to be at the capillary membrane (West et al., 1967a,b; Robinson and Wing, 1970; Scow et al., 1972; Schoefl and French, 1968).

The importance of LPL in the removal of triglycerides from the blood is illustrated by Familial Type I hyperlipoproteinemia in which LPL is absent or present in residual levels (Fredrickson and Levy, 1972). Further, the hypertriglyceridemia associated with diabetes mellitus may be due partly to a decrease in adipose tissue LPL activity (Kessler, 1963). Hypertriglyceridemic conditions associated with oral contraceptives have been characterized partially by decreased postheparin lipolytic activity in plasma. Postheparin lipolytic activity is partially due to LPL released from extrahepatic tissues (Korn, 1955; Payza et al., 1967). LPL deficiency has also been implicated in the lipemia associated with late gestation and parturition (Otway and Robinson, 1968).

The extent of LPL involvement in blood triglyceride clearance is indicated by adipose tissue uptake of some 30 per cent of the fatty acids entering the blood in animals in positive caloric balance and in those absorbing fatty meals (Bragdon and Gordon, 1958). This uptake occurs directly from triglyceride fatty acids of chylomicra in blood (Nestel et al., 1962a,b). On the other hand, in fasted animals triglyceride fatty acids in blood are not taken up by adipose tissue (Bragdon and Gordon, 1958) and LPL activity in adipose tissue

decreases (Hollenberg, 1959; Robinson, 1960; Pav and Wenkeova, 1960). Uptake of triglyceride fatty acids by adipose tissue varies with the tissue level of LPL activity (Bezman et al., 1962). LPL activity (Hamosh et al., 1970; McBride and Korn, 1963; Robinson, 1963a) and blood glyceride fatty acid uptake (Barry et al., 1963; McBride and Korn, 1964a) are both increased in mammary tissue with initiation of lactation. Although processes other than hydrolysis by LPL are involved in glyceride fatty acid uptake (Shapiro, 1965) by adipose and, presumably, mammary tissue, it is clear that LPL plays an important role in uptake.

The parallel relationship between LPL activity and glyceride fatty acid uptake by adipose and mammary tissues suggest that the enzyme serves in a regulatory or directive capacity. Such a role is supported by the fact that lipoprotein lipase activity changes with physiological state of the animal. The direction of change is dependant on the physiological state and the tissue in question. Thus, during fasting and lactation when the need for energy mobilization is high, adipose LPL activity is decreased (Hollenberg, 1959; Robinson, 1960; Hamosh et al., 1970). On the other hand, fasting animals have elevated LPL activity in heart and skeletal muscle (Hollenberg, 1960; Borensztajn and Robinson, 1970; Borensztajn et al., 1970) and initiation of lactation results in highly elevated LPL activity in

mammary tissue (Hamosh et al., 1970; McBride and Korn, 1963; Robinson, 1963a; Askew et al., 1970). Conversely when the body returns to a positive energy balance adipose LPL activity increases.

Much of the work on regulation of LPL has involved adipose tissue as the source of enzyme and has centered around the effects of fasting and refeeding. Robinson and Wing (1970) pointed out that insulin is an effective stimulator of LPL activity when pieces of adipose tissue from fasted but not from fed rats are incubated in vitro. This effect was blocked by adrenaline, noradrenaline, and adrenocorticotrophic hormone. Glucagon and thyroid stimulating hormones have also been shown to block the stimulatory effects of insulin on LPL activity in adipose tissue from fasted rats (Nestel and Austin, 1969). That insulin stimulates formation rather than release of adipose LPL has been confirmed with the dispersed cell (adipocytes) technique (Robinson and Wing, 1970).

Investigations of mammary LPL have centered around the increase in activity noted at initiation of lactation (Hamosh et al., 1970; McBride and Korn, 1963; Robinson, 1963a). The many hormonal changes taking place at this time (Convey, 1973) suggest hormonal regulation of mammary LPL. Pursuing this possibility, Falconer and Fiddler (1970) found that prolactin administrated intraductally

induced LPL synthesis and subsequent increase in activity in the pseudopregnant rabbit mammary gland.

Several indirect observations of steroid hormone effects on LPL have been reported. Hazzard et al. (1969) and Adams et al. (1970) found postheparin lipolytic activity to be depressed in normal women taking mixed oral contraceptives. The estrogen component appeared to have the depressing effect (Hazzard et al., 1969). Patients receiving parenteral estradiol therapy have also been reported to have depressed postheparin lipolytic activity (Fabian et al., 1968). In contrast, synthetic steroids with progestational activity such as norethindione increase plasma postheparin lipolytic activity and decrease plasma triglycerides (Glueck et al., 1969; Glueck et al., 1972). Anabolic-androgenic compounds (oxandrolane) increase postheparin lipolytic activity and decrease plasma triglycerides (Glueck, 1971).

LPL exhibits certain characteristics indicative of a regulatory enzyme: 1) it is located at the first step in uptake of blood triglyceride fatty acids by extrahepatic tissues; 2) it is responsive to normal, abnormal, and induced physiological changes; 3) its activity is correlated with uptake of blood triglyceride fatty acids by extrahepatic tissues; 4) it is responsive to hormonal influences; and 5) different tissue LPL's respond differently to various stimuli

providing a means of influencing final destinations of blood triglyceride fatty acids depending on need.

## Glyceride Synthesis

A very important aspect of adipose and mammary physiology is their ability to synthesize triglycerides from free fatty acids and glycerol. This provides adipose tissue with the ability to store not only newly synthesized fatty acids but those cleared from circulation. Glyceride synthesizing capability provides the mammary gland with the ability to utilize both de novo synthesized fatty acids and those presented to the tissue via the blood stream. Fatty acid esterification in bovine adipose and mammary tissues has recently been characterized and reviewed (Storry, 1970; Benson and Emery, 1971; Askew et al., 1971; Bickerstaffe, 1971) and will receive only brief mention here.

The predominant pathway for glyceride synthesis in mammary tissue is the glycerol phosphate pathway (Storry, 1970; Bickerstaffe, 1971). Glyceride synthesis in the bovine (Kinsella, 1968; Askew et al., 1971) mammary gland occurs in the particulate fraction of the cell and is dependant upon adenosine triphosphate, coenzyme A, glycerol-3-phosphate, and magnesium. Although these results are consistent with esterification via the glycerol phosphate pathway, it has been

suggested that the monoglyceride pathway may also be operational in mammary tissue (Bickerstaffe et al., 1970; Dimick et al., 1970).

Fatty acid esterification in adipose tissue occurs predominantly, if not exclusively, via the glycerol phosphate pathway (Shapiro, 1965). Glyceride synthesis in homogenates of bovine adipose tissue requires glycerol-3-phosphate, adenosine triphosphate, coenzyme A, and magnesium chloride as cofactors (Benson and Emery, 1971). Similar requirements have been reported for fatty acid esterification by rat adipose tissue (Steinberg et al., 1961; Angel and Roncari, 1967).

Regulation of triglyceride fatty acid uptake by adipose and mammary tissues is often considered the domain of LPL. However, glyceride synthesis may function synchronously with LPL in the assimilation of circulating triglyceride fatty acids (Dole, 1961) and could regulate uptake via end product inhibition of LPL. In other words, a decrease in esterification rate could result in a local buildup of nonesterified fatty acids. There is evidence that unbound free fatty acids inhibit the lipolytic activity of LPL (Gordon et al., 1953). Nikkila and Pykalisto (1968) have suggested that the free fatty acid concentration in the tissue may be the actual effector of LPL. This observation was based on the ability of nicotinic acid to increase LPL activity via a decrease in fat mobilization. Wing and Robinson

(1968) have investigated the relationship between tissue free fatty acid concentration and LPL activity in a series of experiments using epididymal fat pads from rats. Their results include: 1) a rapid decrease in LPL activity during starvation accompanied by a rise in tissue free fatty acid concentration, 2) dibutyryl cyclic AMP (lipolytic agent) inhibition of the normal increase in LPL activity when adipose tissue from fasted rats was incubated in a complete medium with inhibition being greater at low glucose concentration, 3) caffeine plus dibutyryl cyclic AMP inhibition of the normal increase in LPL activity irrespective of glucose concentration up to 2.4 mg/ml. (A large increase in tissue and medium free fatty acids were also observed.) 4) caffeine (5mM) inhibition of the normal increase in LPL activity without any effect on concentration of free fatty acids in the tissue or medium.

These results with the possible exception of those obtained with 5 mM caffeine are all consistent with the hypothesis of end product inhibition of LPL. The effect of glucose concentration on the degree of inhibition mediated by dibutyryl cyclic AMP is of particular interest since esterification in adipose tissue proceeds predominantely through the α-glycerol phosphate pathway. Glucose availability at the cellular level could increase the α-glycerol phosphate concentration which in turn could stimulate esterification,

thus pulling the reaction in favor of increased LPL activity. Whether or not this could create an observable effect on LPL activity within the three hour incubation period used is a matter of conjecture at this point. However, glucose, fructose, and galactose have been observed to decrease free fatty acid release from adipose tissue in vitro without influencing the release of glycerol (Leboeuf et al., 1959; Buckle et al., 1961; Vaughan, 1962; Gorin and Shafrir, 1963). These results were attributed to an increase in α-glycerol phosphate and possibly an increase in energy, both of which would tend to increase esterification, thus a recycling of endogenously released free fatty acids.

There is evidence indicating that conditions conducive to high LPL activity are also conducive for high rates of fatty acid esterification. Ball and Jungas (1963) and Angel and Roncari (1967) reported that fasting decreases and refeeding increases glyceride synthesis (GS) activity in adipose tissue. Similar responses are observed in LPL activity (Hollenberg, 1959; Robinson, 1960). Addition of glucose to the incubation medium enhances fatty acid esterification by adipose tissue (Steinberg and Vaughan, 1965). Further enhancement of GS is achieved if insulin is added in the presence of glucose. Similar observations have been reported with respect to LPL (Hollenberg, 1959; Salaman and Robinson, 1966; Austin and Nestel, 1968). The feeding of restricted roughage-high grain diets stimulated the

activities of both GS and LPL in bovine adipose tissue (Benson et al., 1972). These diets tend to elevate blood glucose (Jorgensen et al., 1965; Storry and Rook, 1966).

Thus far, the evidence indicates that in adipose tissue and, presumably, mammary gland the uptake and reesterification of blood triglyceride fatty acids is closely associated with carbohydrate metabolism, that LPL and GS respond in a similar manner to physiological changes, and that either of these two systems could regulate clearance of chylomicra and low density lipoprotein triglycerides from the blood.

## Plasma Lipoprotein Lipase

In 1943, Hahn (1943) reported that injection of heparin intravenously results in rapid clearing of alimentary lipemia. Postheparin plasma was later found to contain a factor which cleared lipemia in vitro (Anderson and Fawcett, 1950). Shortly thereafter, Korn (1955a) established that the clearing factor in postheparin plasma was the enzyme lipoprotein lipase (LPL). Korn (1955a) further reported that LPL catalyzes the hydrolysis of the triglyceride moiety of chylomicra and low density lipoproteins to free fatty acids and glycerol.

These early reports provided impetus for a multiplicity of studies on plasma postheparin lipolytic activity. Normally plasma contains very small amounts of LPL (Engelberg, 1956; Korn et al., 1961). However, lipolytic activity rises markedly within minutes after intravenous injection of heparin (Korn, 1961; Porte and Williams, 1965) and then disappears exponentially. The rate of increase and level of enzyme activity achieved appeares to be dependent on the amount of heparin injected. Maximum plasma enzyme activity was observed within 10 minutes following intravenous injection of 10,000 International Units (IU) of sodium heparin into human subjects (Fielding, 1970a). LaRosa et al. (1971) found maximum activity between 30 and 240 minutes after injection of 5,000 IU of Na Heparin.

Source

LPL was first observed in plasma following intravenous injection of heparin. Subsequent studies revealed the presence of an enzyme with similar properties in various extrahepatic tissues including; heart (Korn and Quigley, 1955), mammary gland (McBride and Korn, 1963; Robinson, 1963a), adipose (Korn and Quigley, 1955; 1957), lung (Brady and Higgins, 1967), and skeletal muscle (Olivecrona and Belfrage, 1965; Wallach, 1968). The brain and pancreas apparently lack LPL activity (Swank and Levy, 1952; Miller, 1969).

The similarity between plasma clearing factor lipase and tissue LPL led to the hypothesis that the source of the plasma enzyme was the various extrahepatic tissues. This hypothesis was substantiated by employing in vitro and in situ organ perfusion techniques using heparin in the perfusate. Heart, skeletal muscle, adipose tissue, and the mammary gland have all been shown to contribute LPL to the circulation (Lequire et al., 1963; Robinson et al., 1963; Robinson and Jennings, 1965; Rodbell and Scow, 1965; Enser et al., 1967; Ho et al., 1967; Nestel, 1970).

## Clearance from the Circulatory System

Liver (Spitzer and Spitzer, 1956; Connor and Eckstein, 1959) and kidney (Constantinides et al., 1959) have been implicated as being the primary sites of removal of LPL from circulation. Clearance of plasma postheparin lipolytic activity is markedly slowed by hepatectomy (Yoshitashi et al., 1963), fatty livers (dePury and Collins, 1972), carbon tetrachloride cirrhotic livers, and nephrectomy (Constantinides et al., 1959). Homogenates of liver are inhibitory to plasma postheparin LPL activity (Yoshitashi et al., 1963). Acetone powder preparations of rat liver have been shown to inhibit LPL activity from heart muscle (Mayes and Felts, 1968). LPL activity has also been reported to be inhibited by kidney extracts (Klein et al., 1958).

These data indicate that the liver and kidney are important in clearance of LPL from circulation. Since extracts of these tissues inhibit LPL activity from other sources, it would appear that this is accomplished by a secreted factor. A well designed investigation by Naito and Felts (1970) further established the role of the liver in plasma LPL clearance and provided insight into the mechanism employed. They proposed a two-step inactivation system for LPL by liver. The first step involves the dissociation of a heparin-apoenzyme complex followed by destruction of heparin. The second step involves the removal of the apoenzyme of LPL. Evidence for the second step was weak but the first step was indicated by several points. When heparin was added to the perfusate prior to passage through the liver it partially blocked clearance suggesting that the heparin removal mechanism became saturated. Heparin added to the liver effluent stimulated LPL activity more than normal but not to preperfusion levels. This indicates heparin removal from the enzyme during passage through the liver. Heparinase could well be the agent involved in this step since its presence in liver has long been known (Jacques, 1940). Heparinase could also be the inhibitory factor associated with liver and kidney homogenates and extracts as mentioned earlier.

Pertinent to a discussion of the ability of an organ to clear a substance from circulation is its effectiveness. The effectiveness

of the liver to clear LPL has been studied by Whayne et al. (1969) and Naito and Felts (1970). Whayne et al. (1969) reported the extraction ratios across the livers of two dogs were .68 and .42 (extraction ratio being the input:output ratio). Similar values (.70) were obtained by perfusion studies with rat livers (Naito and Felts, 1970).

## Lipases Other than Lipoprotein Lipase in Postheparin Plasma

The multiplicity of hydrolytic activities in postheparin plasma (Shore and Shore, 1961; Fielding, 1970a; Greten et al., 1970; Vogel and Bierman, 1970; Nilsson-Ehle et al., 1971; Smith, 1972; Jansen and Hulsmann, 1973) suggests the presence of more than one enzyme. Differential studies of postheparin plasma lipolytic activity have indicated the presence of not only LPL activity but activities of: 1) long-chain acyl-CoA hydrolase (Jansen and Hulsmann, 1973), 2) a monoglyceride hydrolase (Shore and Shore, 1961; Greten et al., 1969; Nilsson-Ehle and Belfrage, 1972), 3) a diglyceride lipase (Greten et al., 1970), and 4) a hepatic triglyceride lipase (LaRosa et al., 1972).

## Contribution of Plasma Lipid to Milk Fat

A positive relationship exists during the lactation cycle between the level of blood lipid and the amount of milk produced. Blood lipids rise to a high level following parturition and remain high during the period of greatest milk production. The level of blood lipids gradually declines as milk production diminishes, reaching a low level as the dry period approaches. The effect of lactation on the level of blood lipids was found to be independent of effects of season and dietary fat.

These early observations by Maynard et al. (1931) initiated a renewed interest in the contribution of plasma lipids to milk fat in the bovine. Earlier studies by Meigs et al. (1919) had suggested that blood phospholipids were the source of milk fat. They (Meigs et al., 1919) reported that mammary venous blood contained less phospholipid and more inorganic phosphorus than was present in the general circulation. In some cases they were able to account for the total milk fat secreted by the decrease in phospholipids in blood as it passed through the mammary gland. McCay and Maynard (1935) reinvestigated the uptake of phospholipid by the mammary gland and found no significant difference between the phospholipid content of jugular and arterial blood and that of the mammary venous blood. This observation was confirmed the following year by Graham et al. (1936). Further investigations of

arterio-venous levels of blood lipids across the mammary gland caused Shaw and Petersen (1940a,b) and Voris et al. (1940) to conclude that blood neutral fat and glycerides are absorbed but probably no other fraction with the possible exception of cholesterol esters. Shaw and Petersen (1938) concluded that the mammary gland removed enough fat from blood to account for the milk fat plus an additional supply to be oxidized for energy. However, followup studies (Shaw and Petersen, 1940a,b; Shaw and Knodt, 1941) with improved techniques failed to confirm mammary gland removal of fat from the blood in excess of that excreted in the milk.

More recently, Riis (Ph.D. thesis as quoted by Storry, 1970) reported a positive correlation between the concentration of plasma phospholipids and milk fat production. Thus, resubmitting the suggestion that plasma phospholipids were contributors to milk fat. However, subsequent studies (Hartmann and Lascelles, 1964; Annison et al., 1967; West et al., 1967a; Varman and Schultz, 1968a; Bishop et al., 1969) have shown that plasma phospholipids and sterol esters are not utilized by the mammary gland.

Techniques, including the feeding of radioactive triglycerides to cows (Glascock et al., 1966; Bishop et al., 1969), arterio-venous difference studies in goats (Barry et al., 1963) and cows (Emery et al., 1965), and perfusion of isolated goat mammary gland (Lascelles et al., 1964; Linzell et al., 1967) have clearly demonstrated the importance

of chylomicra and low density lipoprotein triglycerides and nonesterified fatty acids of plasma as milk fat precursors. Further credence is added to these reports by the fact that Robinson et al. (1963) found no significant arterio-venous difference across the non-lactating mammary gland in plasma triglyceride fatty acids; while during lactation large arterio-venous differences were found in the combined triglycerides of chylomicra and low density lipoportein ($d < 1.019$) in plasma.

From a quantitative standpoint, the triglyceride fraction of chylomicra and low density lipoporteins have been reported to contribute 50 to 60 per cent of the milk fat triglycerides in the goat (Linzell, 1968). In agreement with this, Bickerstaffe (1971) found that the respective contributions of chylomicra and low density lipoprotein triglycerides to milk fat were 14.7 and 35.7 per cent. He (Bickerstaffe, 1971) also reported that additional contributions of triglycerides were made by high density (9.8%) and very high density (15.5%) lipoportein fractions.

## Effect of Stage of Lactation on the Amount and Composition of Milk Fat

Total daily milk production exhibits a characteristic increase for three to six weeks postpartum, followed by a gradual decline until

near the end of lactation (Brody et al., 1923; Turner et al., 1923; Drakeley and White, 1928). Fat content (per cent) varies inversely but not necessarily in direct proportion to milk production (Brown et al., 1962; Rook and Campling, 1965).

The most marked changes in milk fat occur during the early postpartum period. In general, the proportion of $C_{6-14}$ acids increase while the proportion of $C_{18}$ acids decrease with time postpartum. Little or no change occurs in the porportion of $C_{16}$ acids (Boatman et al., 1965; Stull and Brown, 1965). More specific information on proportional changes of individual fatty acids in milk fat during lactation has been reported by Stull et al. (1966) using linear, quadratic, and cubic equations. The percentage of 6:0, 8:0, and 16:1 in milk fat during lactation was best described by a linear equation with a positive slope, and 16:0 by one with a negative slope. The percentage of 10:0 and 18:2 increased to mid-lactation then leveled off while 18:0 and 18:1 exhibited a negative initial slope with a slight upturn during the last 15 to 20 weeks of lactation. The percentage of 10:1, 12:1, 14:0, 14:1, 15:0, and 18:3 could all be defined by a cubic equation; however, 10:1, 12:1, and 14:1 initially declined while the others increased. In terms of total yield, the $C_{6-14}$ acids increase during the first six weeks of lactation after which there is a steady decline.

The $C_{16-18}$ acids appear to decline at a decreasing rate throughout lactation (Storry, 1970).

These data reflect two major events: 1) a gradual increase in de novo synthesis of milk fatty acids as indicated by the increase in $C_{6-14}$ acids during early lactation and 2) a decrease in fat mobilization from body stores with advancing lactation as evidenced by the decline in $C_{18}$ acids. These conclusions are indicated since only shorter chain acids ($C_{4-10}$) are derived entirely by mammary lipogenesis while longer-chain acids ($C_{18}$) of milk fat are derived entirely from plasma. The intermediate chain acids ($C_{12-16}$) would be expected to fluctuate since they are derived from both sources (Garton, 1963; Barry, 1964; Linzell, 1968).

The conclusion that degree of fat mobilization varies with stage of lactation and, thus, accounts for observed changes in proportions of long-chain acids in milk fat must be tempered with the effects of diet (Van Soest, 1963) and season (Storry, 1970) on milk fat composition.

# METHODS AND MATERIALS

## Studies on Adipose and Mammary Tissues

### Sample Collection Techniques

Adipose tissue biopsy: Subcutaneous adipose tissue samples were collected aseptically from an area posterior to the scapula near the dorsal midline. The area was clipped and scrubbed with an iodine solution (Betadine, Purdue Frederick Co., Norwalk, Connecticut). Ten mℓ of a 2.5% procaine (Procaine Hydrochloride Solution, Bio-ceutic Laboratories, St. Joseph, Missouri) solution was injected intradermally to anesthetize a circular area approximately 15 cm in diameter. After final preparation, the area was draped and a scalpel used to make a transverse 8 cm incision in the skin and subcutaneous tissue within the anesthetized area. Approximately 5 g of subcutaneous fat was removed. The skin was closed with interrupted 0.6 mm Vetafil (Vetafil Bengen, Haver-Lockhart, Kansas City, Missouri) sutures and sprayed with furazolidone (Topazone, Eaton Laboratories, Norwich, New York).

Mammary tissue biopsy: Mammary tissue samples were collected aseptically at intervals not closer than one week. Milk from all

quarters was cultured and all quarters were free of pathogenic organisms prior to collecting the tissue. The cow was milked and the surgical field prepared as described for collecting samples of adipose tissue; however, the mammary gland was not anesthetized. The cow was placed on a hydraulic operating table in lateral recumbency. After final preparation, the area of the mammary gland was draped. A scalpel was used to make a 5 cm incision on the lateral surface of the mammary gland through the skin, subcutaneous tissue, and lateral suspensory ligament. A curved scissors was used to remove 5 g of mammary tissue. Hemostasis was established by ligating small blood vessels with 0 chromic catgut (Ethicon, Somerville, New Jersey). An absorbable gelatin sponge (Gelfoam, Upjohn Company, Kalamazoo, Michigan) was placed in the site of the excised tissue. Special care was taken in closing the lateral suspensory ligament with interrupted sutures of 0 chromic catgut (Ethicon, Somerville, New Jersey) to prevent subcutaneous hematomas. The skin incision was closed with 0.3 mm Vetafil (Vetafil Bengen, Haver-Lockhart, Kansas City, Missouri), sprayed with furazolidone (Topazone, Eaton Laboratories, Norwich, New York), and the cow was removed from the operating table.

For three consecutive days postsurgery, prophylactic treatment consisted of procaine penicillin G (Procaine Penicillin G., W. A. Butler Co., Columbus, Ohio) (10,000 units/kg daily) injected

intramuscularly and an intramammary infusion of 15 ml of Procaine Penicillin G-Furaltadone in oil (Altapen, Eaton Laboratories, Norwich, New York) into the operated quarter following the evening milking. Skin sutures were removed in seven days.

Slaughter samples: Samples of adipose and mammary tissues were obtained in some cases following slaughter. In these instances, the animal(s) would be transported to the local slaughter house (Van Alstine) between 5:30 and 6:00 A.M. on the day of slaughter. Lactating cows were routinely milked prior to transport. The animals were routinely sacrificed between 6:00 and 7:00 A.M. and representative samples of shoulder subcutaneous adipose and mammary tissues were obtained within 30 min after sacrifice.

Tissue handling procedures: Samples of adipose tissue and tissue from mammary glands of non-lactating cows were rinsed in ice cold .15 M KCl to remove surface blood, blotted on paper towels, and placed in plastic bags in an ice bath for transport to the laboratory. All samples were frozen (within 1 hr after collection) at -40°C prior to measurement of lipoprotein lipase and glyceride synthesizing activities. Freezing at -40°C for up to six months has no significant effect on LPL activity (Askew et al., 1970). Mammary tissue samples from lactating cows were treated as previously described except they were initially rinsed in ice cold .15 M KCl containing 10 IU of

oxytocin (Syntocin, Sandoz pharmaceuticals, Hanover, New Jersey) to remove residual milk. Rinsing tissues from lactating cows with oxytocin decreased variation without significantly affecting LPL activity relative to KCl rinse only (4.2 ± .3 vs 3.4 ± .6 μmoles fatty acids released $hr^{-1}$ mg $protein^{-1}$, N = 5).

Preparation of tissue for enzyme assays: Frozen mammary or adipose tissue was homogenized in .15 M KCl (pH 8.6) with a Polytron (Brinkman Instruments Inc., Des Plaines, Illinois) for 1 min and then centrifuged 10 min at 900 x g at 4°C. The supernatant was strained through cheese cloth and the filtrate used in the assays.

## Enzyme Assays

Experiment T1: Lipoprotein lipase was assayed as outlined by Askew et al. (1970) except fatty acids were measured colorimetrically (Mackenzie et al., 1967). Glyceride synthesis was measured by incorporation of $^{14}C$-labeled palmitate and oleate into glycerides as described by Benson and Emery (1971) with two modifications. An ATP generating system was added and the substrate concentration changed. Cofactor concentrations were as follows: ATP, 5 mM; DTT, 2mM; COA, .25 mM; $MgCl_2$, 2.25 mM; $KPO_4^{=}$, .05 mM; PEP, 5 mM; NaF, 15.6 mM; α-GP, 20 mM; bovine serum albumin, 2.5 mg/mℓ, $^{12}C$-palmitic acid,

$^{14}$C-palmitic acid, and $^{14}$C-oleic acid were added to give a final concentration of .1 μmoles/mℓ of each.

Experiments $T_2$ and $P_2$: Glyceride synthesis was measured by incorporation of $^{14}$C-labeled palmitate and oleate as in Experiment $T_1$.

Lipoprotein lipase activity in tissue samples taken in these experiments was determined by the method of Schotz et al. (1970) as modified herein. Blood obtained from the jugular vein of a lactating cow was allowed to clot for 2 hr at room temperature then refrigerated at 4°C overnight. Serum was separated by centrifugation and stored at 4°C until used (within 10 days). A mixture of 80 μCi of $^{3}$H-glyceryl trioleate and .133 g of unlabeled triolein was pipetted into plastic scintillation vials and dried in a sand bath under a stream of nitrogen to remove residual benzene. A mixture of .9 mℓ of 1% triton X-100 plus 8.1 mℓ of buffer (pH 8.6) containing 8% bovine serum albumin, .24 M tris-HCl, and .15 M NaCl was added to the triolein. The mixture was placed in an ice bath and sonicated 1 min using a microtip on heat system-ultrasonics model W 1850 operating at maximum power output (75 watts). The sonication step was repeated three times allowing one min for premix and sonic probe to cool between sonications. The resultant mixture was sufficient for the assay of 15 samples. When more than 15 samples were assayed, multiple batches of the substrate premix were prepared and the batches mixed by sonication before use.

Two 10 μℓ aliquots of the substrate premix were transferred to scintillation vials to which .5 mℓ of an extracted water blank was added along with 10 mℓ of scintillation fluid (Appendix A). This served as a standard for computation of specific activity of substrate.

The incubation mixture consisted of .6 mℓ of substrate premix, variable amounts of homogenate (.0-.2 mℓ), and .15 mℓ lactating serum. The mixture was made up to a total volume of 1.0 mℓ with .025 N $NH_4OH$ (pH 8.6). Serum blanks containing .15 mℓ of 3% bovine serum albumin (BSA Fraction V, Sigma Chemical Co., St. Louis, Mo.) in .15 M NaCl (pH 7.4) instead of serum were routinely included in duplicate. Tissue samples were routinely assayed with and without heparin (.05 IU per assay); however, values obtained in absence of heparin are reported due to the large variation in heparin effect between tissues (Appendix B). Assays were always done in duplicate. The incubation mixture used in standard assays for lipoprotein lipase activity is shown in Table 1.

The reaction was terminated by adding 5 mℓ of chloroform: methanol (2:1) directly into the assay flask and mixing 20 sec with a vortex. After a 10 min equilibration period, .4 mℓ of .5 N $H_2SO_4$ was added and the contents mixed twice more at 10 min intervals. The flask contents were transferred to a test tube, capped and allowed to stand overnight at 4°C. The tubes were centrifuged for 10 min at 2000 r.p.m. in a clinical centrifuge and the top layer (2 mℓ)

transferred to a second tube (using disposable pasteur pipettes) without disturbing the interface layer. The top layer was re-extracted by adding 3 mℓ of chloroform, mixing 20 sec, and equilibrating 10 min. The tubes were centrifuged for 10 min at 900 x g and .5 mℓ of the top layer mixed with 10 mℓ of scintillation fluid (Appendix A) for counting in a liquid scintillation counter (Nuclear Chicago).

TABLE 1.--IN VITRO ASSAY SYSTEM FOR BOVINE ADIPOSE AND MAMMARY LIPOPROTEIN LIPASE[a]

| Component | Quantity |
|---|---|
| | mℓ |
| Substrate Premix | 0.6 |
| Serum or 3% BSA-Saline | 0.15 |
| Heparin or .025 N $NH_4OH$ | 0.05 |
| .025 N $NH_4OH$ | 0.10 |
| Homogenate[b] | 0.10 |

[a]Components added to 25 mℓ glass-stoppered flasks, incubated at 37°C for 60 min under air in a Dubnoff Metabolic Shaker.

[b]Homogenate added after other components had been preincubated for 20 min at 37°C.

Appropriate serum blanks (3% BSA-Saline instead of serum) were run with each assay. Blank values were subtracted from each estimate

of enzyme activity to remove residual lipase effects. Recovery of $^{14}$C-glycerol added to this system was 22.99%, if allowed to set overnight, yielding a recovery factor of 4.35. A sample calculation is shown below:

a) CPM/serum flask minus CPM/saline blank = net CPM/flask.

b) Net CPM/flask ÷ CPM/μMole* = μmoles/flask.

*CPM/μmole was calculated from CPM/10 μℓ premix minus background CPM divided by μmoles in premix. 0.6 mℓ of premix contains 10 μmoles of triolein plus a few hundredths μmoles depending upon the specific activity of $^{3}$H-glyceryl trioleate. Therefore, 10 μℓ contains 0.01667 μmoles.

c) μmoles/flask times recovery factor times dilution factor times time factor = μmoles glycerol released hr$^{-1}$ g tissue$^{-1}$.

Lipolytic activity toward a serum activated triglyceride substrate, triolein, is referred to as lipoprotein lipase activity. The assay was linear with respect to tissue concentration. Linearity with time through 60 min was observed with adipose tissue LPL activity (Figure 1) but not with mammary tissue LPL activity (Figure 2). Mammary tissue LPL activity showed an early lag phase with a subsequent increase in the rate of glycerol release. This type of curve is suggestive of an initial buildup of monoglycerides with subsequent release of free glycerol. One of the major products of hydrolysis by LPL purified from postheparin plasma is apparently 2-monoglyceride (Fielding, 1972). Miller and Smith (1973) have observed an initial lag phase and attributed it to interaction of protein(s) with the surface film of a lipid

Fig. 1.--Lipoprotein lipase activity in homogenates of adipose tissue at different incubation times. Conditions of assay were as described in Table 1 except incubation time was varied as indicated. Similar results were obtained in a similar trial with tissue from two other cows.

Fig. 2.--Lipoprotein lipase activity in homogenates of mammary tissue at different incubation times. Conditions of assay were as described in Table 1 except incubation time was varied as indicated. Similar results were obtained in a similar trial with tissue from three other cows. Changing the substrate preincubation time from 20 min to 30 min gave similar results.

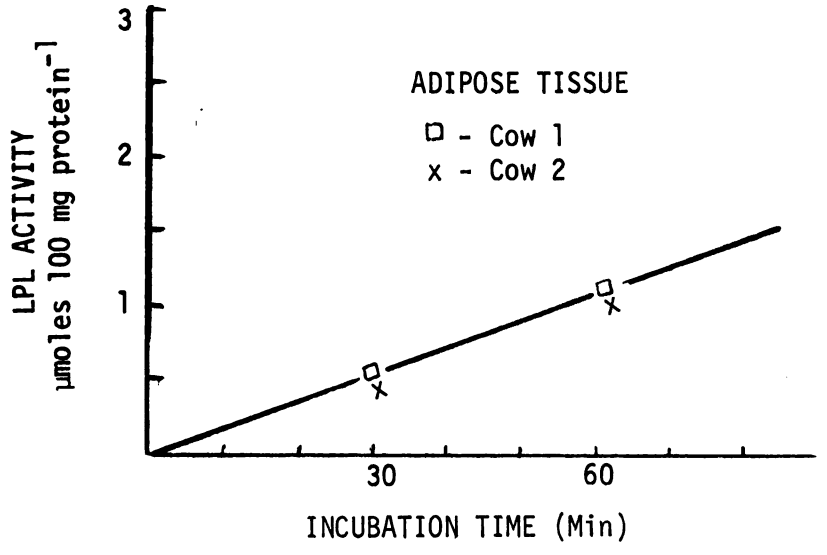



Figure 1

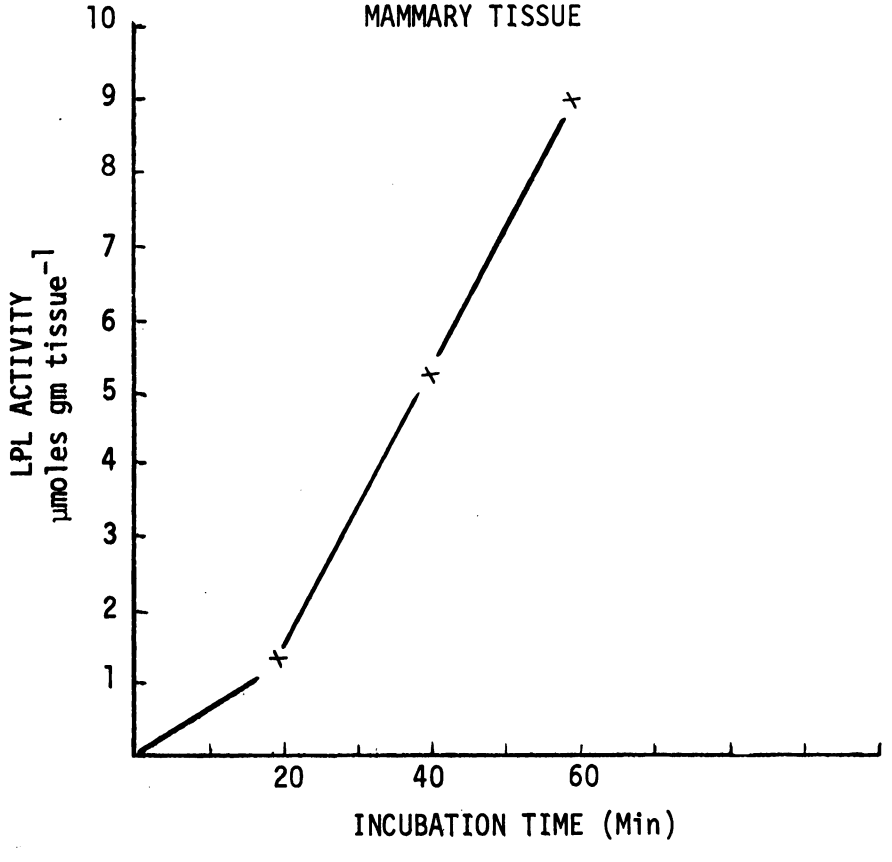



Figure 2

monolayer. Thus, the lag phase in our system could be due to insufficient emulsification. However, this seems unlikely since the lag phase was not corrected by changing preincubation time from 20 to 30 min. In order to reduce the influence of the initial lag phase (< 10 min) all assays were run for 60 min. It is realized that this violates one of the rules for a valid assay; however, it is believed that this assay is quite suitable for detection of biological differences as reported herein. This view is supported by the fact that our findings with respect to LPL activity during various physiological states is in line with other reports (Hamosh et al., 1970; Askew et al., 1970; Robinson and Wing, 1970).

## Experimental Procedures

Experiment $T_1$: Effect of Induced Lactation on Enzymic Activity in Primiparous Bovines: Fetuses were removed by caesarean section in nine primiparous Holstein heifers at 180 days (six heifers) or 260 days (three heifers) gestation. Stage of gestation was determined from breeding dates and verified by rectal palpation. Mesenteric adipose tissue was sampled immediately after the fetus was removed (Shirley et al., 1973). Mammary tissue was sampled by biopsy (Oxender et al., 1971) approximately one hour after the fetus was removed. Following a 14-day lactation period, animals were sacrificed and samples of

mesenteric adipose and mammary tissue taken. Daily milk production was monitored during the lactation period. Live body weights and mammary gland weights were taken on the day of slaughter (Shirley et al., 1973).

The ration consisted of 2.5 kg of a 16% protein concentrate, 7 kg of alfalfa hay and corn silage ad libitum before surgery. After surgery the ration was 7 kg of 16% protein concentrate plus alfalfa hay and corn silage ad libitum.

Experiment T2: Enzymic Activity during various lactational states in multiparous bovines: Biopsy samples of mammary tissue and shoulder subcutaneous adipose tissue were taken at 49, 21, 14, 8, and 2 days prepartum and 7, 14, 28, 60, 120, 180, 240, and 300 days postpartum from eight multiparous Holstein cows. Each cow was biopsied four or five times. The quarter of the udder sampled has a negligible effect on enzyme activity determination in the mammary gland (Askew et al., 1970). A complete outline of sampling dates relative to parturition, number of samples per cow and how and where they were obtained is contained in Table 2.

The animals were fed a normal herd ration consisting of a 16% protein concentrate, hay, and corn silage in amounts required for maintenance and milk production during lactation. During the nonlactating period they were maintained on pasture with some additional

TABLE 2.--EXPERIMENT $T_2$ SAMPLING DATES[a]

| Samples | Cows | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 10W | 51 | 1072 | 832 | 827 | 2095 | 2221 | 2520 |
| Days Postpartum | | | | | | | | |
| -49 | RF | | | | | | | |
| -21 | | RF | | | | | | |
| -14 | RR | | | | | | | |
| - 7 | | | RF | | | | | |
| - 2 | | | | RF | RF | | | |
| 7 | LF | RR | RR | RR | RR | | | |
| 14 | LR | LF | LF | LF | LF | | | |
| 28 | RF | LR | LR | LR | | | | |
| 60 | | | | | | RF | RF | RF |
| 120 | | | | | | RR | RR | RR |
| 180 | | | | | | LF | LF | LF |
| 240 | | | | | | LR | LR | LR |
| 300 | | | | | | RF | RF | RF |

[a]Adipose tissue and mammary gland were sampled simultaneously. Mammary samples are identified as to the quarter they were taken from i.e. RF = Right front quarter, RR = Right rear, LF = Left front, LR = left rear, SL = Sampled immediately following slaughter.

hay or silage. Two weeks prior to parturition, they were challenge fed grain at the rate of .5 kg increments per day until they reached an intake of 7 to 8 kg/day at parturition. After parturition, the amount of grain was increased until the individual cow reached maximum production or maximum intake. During lactation, adjustments in the amount of grain were made in accordance with declining milk production.

## Studies on Plasma Lipolytic Activity

### Plasma Sample Collection and Treatment

Blood samples were collected from the jugular and mammary veins with a 10 mℓ disposable syringe equipped with a 20 gauge needle. Immediately after collection, the needle was removed and the whole blood injected into a test tube containing potassium oxalate (2 mg/mℓ whole blood). Samples were transported to the laboratory in an ice bath, immediately centrifuged at 900 x g for 20 min at 4°C. Plasma was removed with a pasteur pipette, placed in a plastic vial and stored at -40°C until assayed. Under these conditions, plasma lipolytic activity is stable for at least 2 months in our hands and up to 6 months as reported by others (Fielding, 1970).

## Postheparin Plasma Samples

Sodium heparin (5000 IU/cow) was injected into the jugular vein and blood samples collected at 5, 10, and 15 minute post injection. In some studies, samples were taken only 5 and 10 min or 10 and 15 min postinjection in lactating and non-lactating cows. Maximum postheparin plasma lipolytic activity was reached at approximately 10 min in prepartum and 5 min in postpartum cows (Appendix C). A standard dose of 5000 IU of heparin was used even though dose response may be dependent on body weight.

## Mammary Arterio-Venous Technique

To estimate that portion of plasma lipolytic activity contributed by the mammary gland, samples of both jugular and subcutaneous abdominal (mammary) vein plasma were obtained. The contribution by the mammary gland was determined by subtracting the lipolytic activity of jugular venous plasma from that of mammary venous plasma. This method assumes that jugular activity is representative of systemic contribution prior to entry into the mammary gland circulatory system. The mean lipolytic activity in six paired samples of jugular vein plasma and plasma from the external pudic artery just prior to entry into the mammary gland agreed within 10 per cent.

Plasma Lipolytic Activity Assay

Plasma lipolytic activity was assayed as described for tissue LPL activity with the following exceptions: 1) 2.5 mℓ of serum from a lactating cow was added to the substrate premix prior to the sonication step, 2) the 20 min preincubation step prior to addition of enzyme was eliminated, 3) two concentrations of undiluted plasma (.2 and .3 mℓ) in duplicate were routinely included in each assay, 4) heparin was not routinely added to the assay mixture, and 5) an enzyme blank (3% BSA vice plasma) was included in duplicate in all assays to increase accuracy of estimates of specific enzyme activity. A sample assay is outlined in Table 3. This assay was linear with plasma concentrations

TABLE 3.--ASSAY FOR PLASMA TRIGLYCERIDE LIPASE[a]

| Component | Quantity |
|---|---|
| | mℓ |
| Substrate Premix | .5 |
| Plasma Sample | .0, .2, or .3 |
| 3% bovine serum albumin-Saline | .5, .3, or .2 |

[a]Components added to 25 ml flasks and incubated at 37°C for 60 min under air in a Dubnoff Metabolic Shaker.

used herein. The ratio of the activity obtained with .2 mℓ plasma and the activity obtained with .3 mℓ of plasma was .99 ± .05 (n = 43 pairs). The assay was essentially linear with time through 60 min for both preheparin lipolytic activity (Figure 3) and postheparin lipolytic activity (Figure 4).

Experimental Procedures

Experiment P1: Plasma Lipolytic Activity vs Milk Fat Production: Six Holstein cows between 60 and 120 days in lactation ranging in milk production from approximately 11 kg/day to 40 kg/day were used. Individual milk and milk fat production were determined the evening and morning immediately prior to treatment and the evening immediately following treatment. Blood samples from the jugular and mammary veins were collected prior to and 5, 10, and 15 min after heparin (5000 units/cow) injection into the jugular vein. Samples were collected between 9 and 10:30 A.M. which was 4 to 6 hr after milking. Samples were handled as previously outlined. Feed consumption data was obtained from all cows and approximate "net energy" intake calculated (Appendix D). Live body weights were measured on two consecutive days and the average used in calculating estimated net energy requirements (Appendix D).

Fig. 3.--Plasma lipolytic activity at different incubation times. Conditions of assay were described in Table 3 except incubation time was varied as indicated. Similar results were obtained with plasma from two other cows. Similar results were also obtained when the substrate premix was preincubated for 20 min prior to enzyme addition.

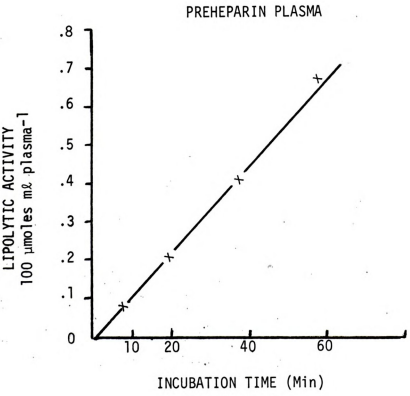

PREHEPARIN PLASMA
LIPOLYTIC ACTIVITY
100 µMoles mℓ plasma⁻¹
.8
.7
.6
.5
.4
.3
.2
.1
0
10
20
40
60
INCUBATION TIME (Min)

Figure 3

Fig. 4.--Postheparin plasma lipolytic activity at different incubation times. Conditions of assay were as described in Table 3 except incubation time was varied as indicated. Similar results were obtained with postheparin plasma from two other cows. Similar results were also obtained when the substrate premix was preincubated for 20 min prior to enzyme addition.

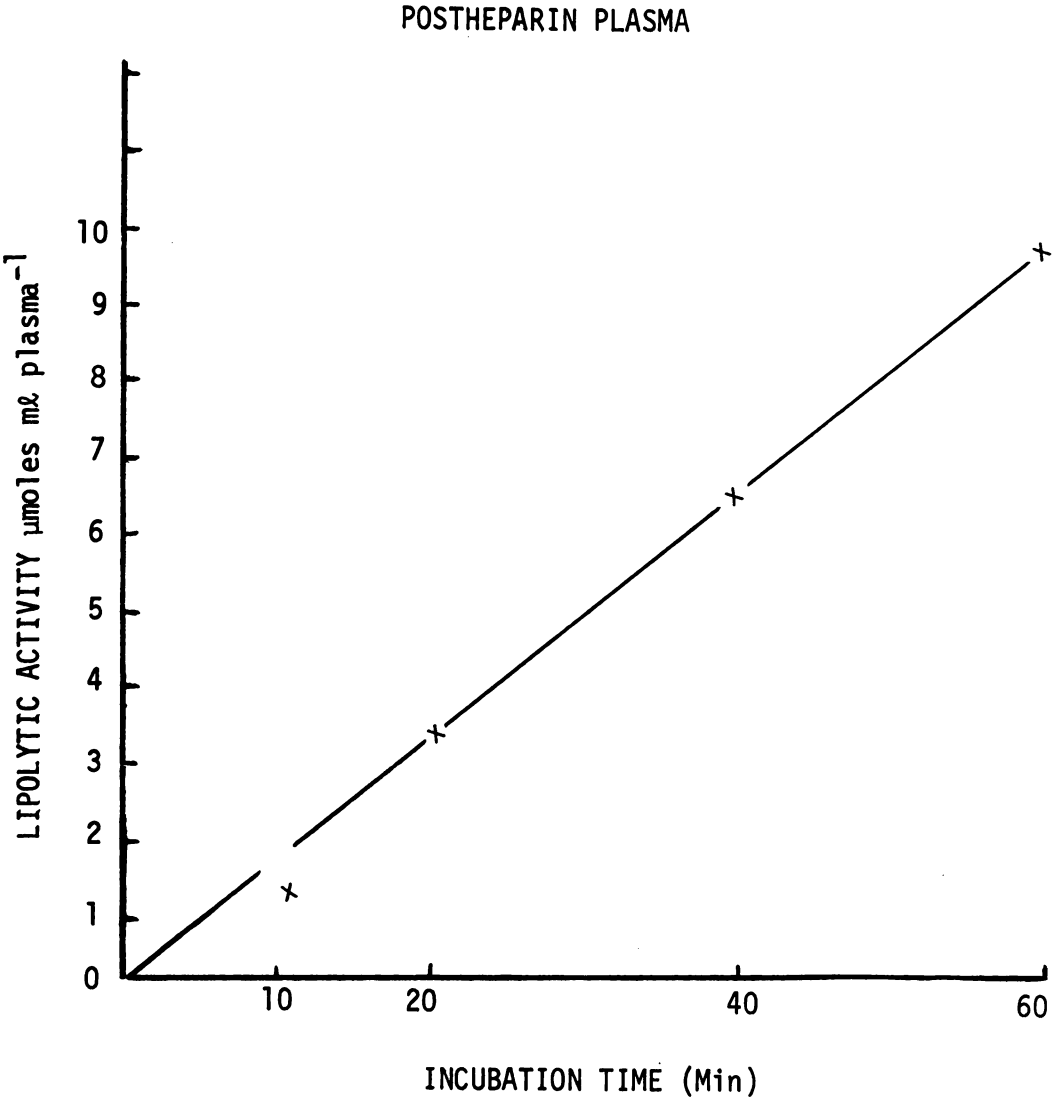

POSTHEPARIN PLASMA
LIPOLYTIC ACTIVITY µmoles mℓ plasma$^{-1}$
0
1
2
3
4
5
6
7
8
9
10
10
20
40
60
INCUBATION TIME (Min)

Figure 4

Experiment $P_2$: Plasma Lipolytic Activity vs Adipose and Mammary Tissue Enzymic Activities: Samples of mammary tissue were obtained from eight Holstein cows in various lactational states. Tissue samples were taken either by surgical biopsy or following slaughter. In some cases, as many as four biopsy samples were taken from the mammary gland of an individual cow. When this was done, a different quarter was used each time. Lipoprotein lipase activity varies less than 10% among quarters (Askew et al., 1971). Animals were always hand milked and jugular and mammary venous plasma samples taken within 1 hr prior to biopsy or slaughter. Milk and milk fat production data were obtained for the evening and morning milkings immediately prior to biopsy or slaughter.

Experiment $P_3$: Effect of Initiation of Lactation on Plasma Lipolytic Activity: Jugular and mammary venous blood was sampled from each of six Holstein cows on days seven and three prepartum (based on predicted calving date) and days three and seven postpartum. Following initial sampling, heparin was injected into the jugular vein and a second and third sample of jugular and mammary venous blood taken. Since predicted calving date and actual calving date did not always coincide, seven additional cows were used to fill in the prepartum sampling dates and provide additional postpartum samples.

Experiment P4: Effect of Time after Milking on Plasma Lipolytic Activity: Four cows between 50 and 80 days in lactation receiving the same diet were initially used to determine if milking affected lipolytic activity in mammary venous plasma. The animals were housed and milked separately from the herd. Mammary veing blood samples (10 mℓ per sample) were taken prior to the evening milking (3 P.M.), within one minute and at 10, 20, 30, 60, and 240 minutes after milking. Jugular vein plasma samples were taken prior to and within one minute after milking to determine prolactin response. Milk and milk fat production data were obtained for the morning and evening milkings on the day of the experiment. Plasma triglyceride lipase activity was measured in mammary vein samples. Prolactin was measured in jugular vein plasma as outlined by Koprowski and Tucker (1971).

Data from the initial four cows was insufficient to draw a conclusion and the milking procedure used resulted in decreased milk production during the experiment. Thus a second group of four cows was obtained for further observations. The second experiment was conducted as previously outlined with the following exceptions: a) the cows were milked in the parlor with the rest of the herd; b) jugular vein cannulas were installed three days prior to the experiment to facilitate sample collection and decrease the degree of animal excitation; and c) one minute and 20 minute post-milking samples of mammary

venous plasma were not obtained since the initial experiment indicated that samples taken at 10, 30, 60, and 240 minutes postmilking would provide ample observations.

## Other Methods

Protein was determined by the method of Lowry et al. (1951). Milk samples were tested for butterfat by the Babcock method. Plasma prolactin was assayed as outlined by Koprowski and Tucker (1971). Dry matter content of mammary tissue was determined by drying to a constant weight in a forced air oven at 85°C. Scheffe's test for significant differences between means with heterogeneous variance and unequal numbers was used for statistical analysis (Gill, 1971).

# RESULTS AND DISCUSSION

Results of these studies are divided into two categories: 1) an investigation of enzyme activities in bovine adipose and mammary tissues during various lactational states, and 2) an investigation of plasma lipolytic activity with particular emphasis on its desirability as a measure of the mammary glands ability to clear triglyceride fatty acids from the blood. Results of both studies will be related to some extent in this section.

## Tissue Studies

### Introduction

Induction of lactation is accompanied by a redistribution of lipid from adipose stores to the mammary gland. This redistribution phenomenon could be accomplished by differential regulation of blood fat uptake by the two tissues. The mechanism of blood fat uptake by adipose and mammary tissue is quite similar; fatty acids are hydrolyzed from blood triglycerides at the capillary endothelium and approximately

two-thirds of the fatty acids are reesterified into triglycerides within the cell (Scow et al., 1972). Both lipoprotein lipase (LPL) (Robinson and Wing, 1970) and glyceride synthesis (GS) (Shapiro, 1965) have been implicated as regulators of blood triglyceride fatty acid uptake by extrahepatic tissues. Investigation of the effect of lactation on these two enzyme systems in both adipose and mammary tissue should provide insight into their relative ability to perform a regulatory function and their involvement in the lipid redistribution phenomenon. In other words, does redistribution occur due to an increase in the mammary glands ability to clear blood triglycerides; a decrease in the ability of adipose tissue to remove triglycerides from the blood; or a combination thereof.

Two major experiments were undertaken to investigate these suppositions. In the initial experiment (Experiment $T_1$), samples of adipose and mammary tissues were obtained from primiparous heifers to ascertain the effect of change in lactational state on adipose and mammary tissue LPL and GS activities. In a followup experiment (Experiment $T_2$) biopsy samples of mammary and shoulder subcutaneous adipose tissue were taken at prepartum and postpartum from multiparous cows. The purpose here was to study the regulatory capability of LPL and GS with respect to both adipose and mammary tissue.

Experiment $T_1$

General: Daily milk production during the 5 days before slaughter ranged from 3.5 to 11.5 kg. Average milk production was 5.9 kg and 10.4 kg for the 180- and 260-day heifers. Body weights before slaughter ranged from 342 kg to 399 kg, averaging 370 kg. The average mammary gland weights were 9.1 kg and 10.9 kg for the 180- and 260-day heifers. There were no significant correlations between enzyme activity and milk production, gland weight, or live body weight (Shirley et al., 1973). Enzyme data essentially were alike for both groups of heifers; thus, they were grouped and discussed together.

Protein and dry matter: Dry matter content decreased from 20 per cent in prepartum tissues to 18 per cent in postpartum tissue to which oxytocin had been added to aid in removal of residual milk. Protein concentration followed the same pattern, decreasing from 130 ± 13 mg/g tissue to 62 ± 3 mg/g tissue. Approximately 56 per cent of this difference in protein can be attributed to the difference in dry matter of the tissues. Mean protein concentration of adipose tissue was 8 and 7 mg/g tissue at surgery and 14 days after caesarean section.

Lipoprotein lipase and glyceride synthesis: LPL, which serves to deliver triglyceride fatty acids from blood to mammary cells, and GS, the process by which incoming fatty acids are incorporated into

triglycerides within the cell, increased 94 and 6-fold with onset of lactation (Table 4). Mammary LPL activity was augmented further by addition of heparin to the assay system (Table 5). Heparin stimulated LPL activity in both nonlactating and lactating tissue; however, lipoprotein lipase from nonlactating tissue showed a greater response ($P = .15$) to heparin than did LPL from lactating tissue. A similar response was noted in tissue from multiparous cows although the response to heparin prepartum appeared to be somewhat related to time relative to parturition (Appendix C).

TABLE 4.--LIPID METABOLISM IN PRIMIPAROUS BOVINE MAMMARY AND ADIPOSE TISSUE.

| | Lipoprotein Lipase | Glyceride Synthesis |
|---|---|---|
| | -------μmoles $hr^{-1}$ 10 mg tissue $protein^{-1}$------- | |
| Mammary | | |
| Pregnant[a] | .5 ± .2[c] | .02 ± < .01 |
| Lactating[b] | 46.9 ± 5.5 | .12 ± < .01 |
| Adipose | | |
| Pregnant[a] | 12.0 ± 3.0 | 1.2 ± < .4 |
| Lactating[b] | 8.0 ± 1.0 | .2 ± < .01 |

[a]Primiparous heifers 180 or 260 days pregnant.

[b]Fourteen days after caesarean section.

[c]Values are mean ± standard error of mean, N = 9.

TABLE 5.--MAMMARY LPL RESPONSE TO HEPARIN.[a]

| Heparin Added | Pregnant | Lactating |
|---|---|---|
| units/mℓ Homog. | ----------% of normal[b]---------- | |
| .05 | 218[c] | 112[d] |
| .125 | 243[e] | 122[f] |

[a]Heparin obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio.

[b]No added heparin.

[c,d,e,f]Means of a given variable with different letters are significantly different. c > d (P = .08), e > f (P = .15).

Activities of LPL and GS in adipose tissue decreased with onset of lactation (Table 4). Activity (μmoles FFA $hr^{-1}$ 10 mg $protein^{-1}$) of LPL decreased (P > .05) from 12 to 8 while GS decreased (P < .05) from 1.2 to .2 in adipose tissue from pregnant and lactating heifers.

These results support the contention that an increase in mammary triglyceride uptake ability and a decrease in adipose tissue uptake ability are both involved in the redistribution of lipid at induction of lactation. The large increase in activity of mammary LPL relative to GS is suggestive of a regulatory role for LPL. With respect to adipose tissue, GS appears to be more sensitive to a change in lactational state than does LPL.

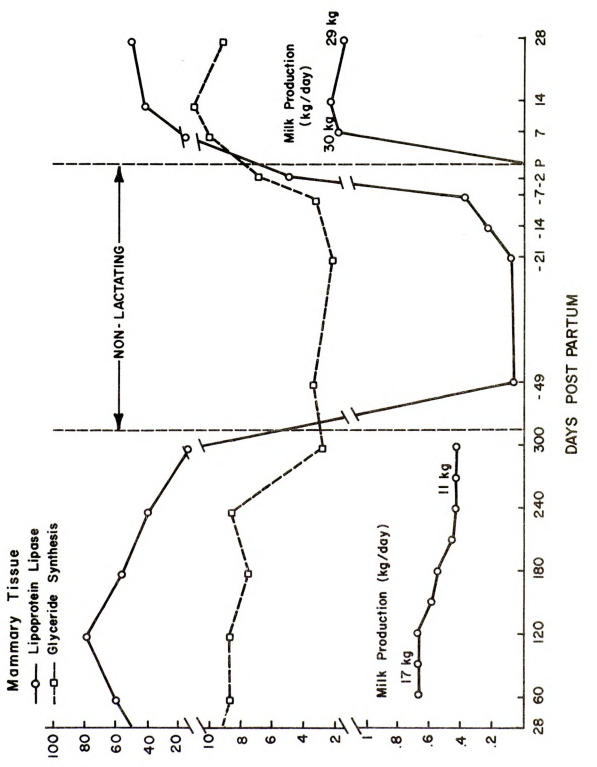
Experiment $T_2$

Mammary gland: The capability of the mammary gland to synthesize milk components is dependent on several factors, one of which is the enzymic complement of the gland. One category of enzymes appears to be constitutive in that their activity is relatively independent of lactational state of the gland; the other category appears to be inducible since their activity is drastically affected by change in lactational state (Baldwin et al., 1966; Mellenberger et al., 1973). The inducible category apparently contains the regulatory enzymes such as lactose synthetase (Brew, 1969) and acetyl-CoA carboxylase (Smith et al., 1966). To be considered regulatory to milk production an enzyme should be sensitive to changes in lactational intensity and its activity should change prior to overt changes in mammary physiology.

To study regulatory capability of LPL and GS, biopsy samples of adipose and mammary tissues were taken at 49, 21, 14, 7, and 2 days prepartum and 7, 14, 28, 60, 120, 180, 240, and 300 days postpartum from eight multiparous cows. The results of this study are graphed in Figure 5.

Mammary LPL activity (μmoles glycerol released $hr^{-1}$ mg $protein^{-1}$ ± S.E.) increased 6 fold between 49 and 7 days prepartum then increased sharply (.4 to 5.1) between 7 and 2 days prepartum. LPL activity then increased at a decreasing rate, reaching a maximum

Fig. 5.--Activities of mammary tissue lipoprotein lipase and glyceride synthesis during a complete lactational cycle. Experimental procedure was as described in Methods and Materials (Experiment $T_2$). Number of observations at each point and the quarter of udder sampled are outlined in Table 2. Lipoprotein lipase and glyceride synthesis activities were assayed as described in Methods and Materials. Standard errors for LPL means on the graph identified by days from parturition are: 28, ± 20; 120, ± 13; 180, ± 16; 240, ± 9; 300, ± 18; -2, ± 1; 7, ± 7; 14, ± 15. Standard errors for GS means on the graph identified by days from parturition are: 28, ± 2.1; 60, ± 1.3; 120, ± 1.4; 180, ± 1.5; 240, ± 2.3; 300, ± 2.8; -2, ± 2.2; +7, ± 1.7; 14, ± 3.0. Days -49, -21, -14, and -7 are represented by one observation.


Mammary Tissue
Lipoprotein Lipase
Glyceride Synthesis
NON-LACTATING
Milk Production (kg/day)
30 kg
29 kg
Milk Production (kg/day)
17 kg
11 kg
P
ENZYME ACTIVITY-μmoles hr⁻¹ 100mg protein⁻¹
DAYS POST PARTUM
28 60 120 180 240 300 -49 -21 -14 -7 -2 P 7 14 28

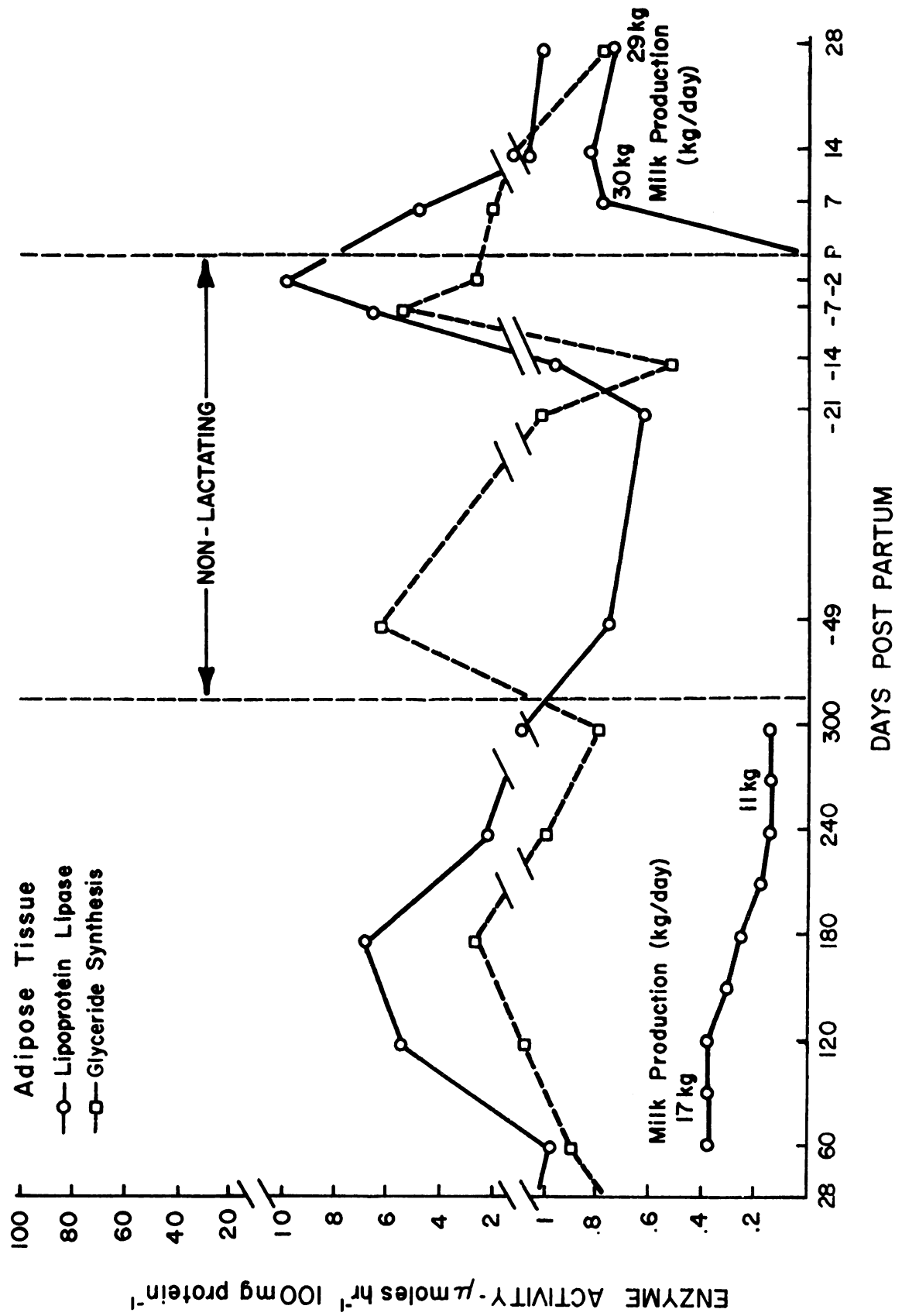
(83 ± 12) at 120 days postpartum thereafter, decreasing slowly until 280 days postpartum (19 ± 18). LPL activity across lactation described the milk production curve. The critical point in terms of regulatory capability is that the increase in tissue LPL activity precedes initiation of overt lactation.

Mammary GS activity (μmoles palmitate incorporated $hr^{-1}$ 100 mg $protein^{-1}$) decreased slightly between 49 and 7 days prepartum then increased 5 fold by 2 days prepartum and an additional 2 fold by 14 days postpartum after which it remained relatively constant until 280 days postpartum.

These data are in agreement with those in Experiment 1, in that LPL shows a larger increase in activity across parturition than does GS. On the other hand, the primiparous heifers in Experiment 1, show lower prepartum tissue GS activity than the multiparous cow tissues in this experiment (.02 ± .01 vs 3.5 ± 1.3).

Adipose tissue: The relationship between adipose tissue LPL and GS activity and lactational state is depicted graphically in Figure 6. LPL activity showed two discernible peaks, one just prior to parturition (2 days prepartum) and another between 120 and 180 days postpartum. The most apparent reason behind these peaks is luxury feed intake. The prepartum peak followed initiation of grain feeding in preparation for lactation. The midlactation peak occurred at the

Fig. 6.--Activities of adipose tissue lipoprotein lipase and glyceride synthesis during a complete lactation cycle. Experimental procedure was as described in Methods and Materials (Experiment $T_2$). Number of observations at each point and the quarter of udder sampled are outlined in Table 2. Lipoprotein lipase and glyceride synthesis activities were assayed as described in Methods and Materials. Standard errors for LPL means on the graph identified by days from parturition are: 28, ± .6; 60, ± 1; 120, ± 3; 180, ± 3; 240, ± 1; 300, ± .4; -2, ± 7; 7, ± 2; 14, ± .4. Standard errors for GS means on the graph identified by days from parturition are: 28, ± .4; 60, ± 1.2; 120, ± .2; 180, ± 1.0; 240, ± .3; 300, ± .6; -2, ± .8; 7, ± 1.0; 14, ± .7. Days -49, -21, -14, and -7 are represented by one observation.


Adipose Tissue
Lipoprotein Lipase
Glyceride Synthesis
NON-LACTATING
Milk Production (kg/day)
17 kg
11 kg
30 kg
29 kg
Milk Production (kg/day)
ENZYME ACTIVITY - μmoles hr⁻¹ 100mg protein⁻¹
DAYS POST PARTUM
100
80
60
40
20
10
8
6
4
2
1
.8
.6
.4
.2
28
60
120
180
240
300
-49
-21
-14
-7
-2
P
7
14
28

time milk production began its decline but prior to reduction in grain intake. Thus, it appears that adipose LPL activity is quite sensitive to energy status as affected by dietary grain intake. Interestingly, the lowest LPL activity observed was during the latter part of the dry period when the cows were on all roughage diets. The decreased activity immediately after parturition could be due to hormonal influences associated with parturition, to the negative energy balance status of early lactation, or a combination of the two.

Adipose GS activity (Figure 6) showed the same trends in activity as LPL with one notable exception. GS activity was highest during the early part of the dry period (49 days prepartum). This occurred at a time when LPL activity was lowest. By 14 days prepartum, GS activity had decreased some 10 fold. The other two peaks in GS activity were associated with what appears to be prior increases in LPL activity. The reason for the early dry period peak in GS activity alone is not readily apparent. It may be that adipose GS is more sensitive than LPL to hormones associated with lactation. Thus, the removal of negative influences at cessation of milking resulted in a burst of activity with the subsequent decline due to low energy intake during the dry period.

## Plasma Studies

### Plasma Lipolytic Activity vs Milk Fat Production

LPL catalyzes the hydrolysis of blood triglyceride fatty acids at the capillary membrane (Scow et al., 1972). The ability of a tissue to clear triglyceride fatty acids from blood apparently depends upon the number of enzyme (LPL) molecules bound to the capillary membrane since heparin perfusion results in the release of LPL into the perfusate with a subsequent decrease in the hydrolytic ability of the tissue toward a lipoportein substrate (Rodbell and Scow, 1965). Thus, it has been suggested that the physiologically active LPL is that releasable by heparin (Borensztajn and Robinson, 1970).

To test this supposition, simultaneous jugular and mammary venous plasma samples were obtained immediately prior to and after heparin injection in the jugular vein. Mammary gland contribution to plasma lipolytic activity is taken to be the difference between mammary venous plasma activity and jugular venous plasma activity. Milk and milk fat production data were obtained for the P.M. and A.M. prior to sampling and the P.M. following sampling. Thus, we were able to observe the relationship between pre- and post-heparin plasma lipolytic activities and daily milk fat production as well as the effect of heparin administration on subsequent milk fat output.

Results (Table 6) of this study were unexpected in view of the supposition that ability of a tissue to clear triglyceride fatty acids from the blood is best depicted by heparin releasable lipolytic activity. Milk fat production prior to sampling was best correlated ($r = .81$, $P < .05$) with preheparin lipolytic activity in mammary venous plasma ($M_0$-$J_0$). A negative correlation ($r = -.36$, $P > .05$) was observed between milk fat production and peak postheparin lipolytic activity in mammary venous plasma ($M_5$-$J_5$). These results suggest that mammary gland LPL is normally released in proportion to the amount of blood triglyceride fatty acids taken up. Thus, as the amount of milk fat produced increases so does the release of LPL into the blood stream which leaves less LPL bound to the capillary membrane at a given time resulting in an inverse relationship between heparin releasable LPL and normal milk fat output by the gland. This interpretation necessarily implies that lipolytic activity in mammary venous plasma is a suitable measure of LPL or LPL-like activity. Further evidence in support of this interpretation was provided by the observation that milk fat production the P.M. subsequent to sampling was affected by heparin injection. All cows showed an increase in milk fat output the P.M. following heparin administration. However, degree of response (post P.M.-pre P.M.) was negatively correlated ($r = -.64$, $P > .05$) with lipolytic activity in preheparin mammary venous plasma and positively

TABLE 6.--CORRELATION OF PLASMA LIPOLYTIC ACTIVITIES[1] WITH MILK AND MILK FAT PRODUCTION[2]

| Parameter | Plasma Sample[3] | | | | | |
|---|---|---|---|---|---|---|
| | $M_0$ | $J_0$ | $M_0-J_0$ | $M_5$ | $J_5$ | $M_5-J_5$ |
| | ----------Correlation Coefficients---------- | | | | | |
| Milk Production (kg) | | | | | | |
| pre A.M. | .54 | .40 | .54 | .23 | .36 | -.54 |
| day | .52 | .27 | .54 | .24 | .33 | -.39 |
| Milk Fat Production (kg) | | | | | | |
| pre A.M. | .78 | .46 | .80 | .42 | .47 | -.30 |
| day | .78 | .40 | .81 | .22 | .31 | -.36 |
| post P.M.-pre P.M. | -.64 | -.41 | -.65 | .09 | -.03 | .41 |
| Milk Fat Concentration (%) | | | | | | |
| pre A.M. | .40 | .28 | .41 | .01 | -.03 | .12 |
| day | .49 | .15 | .52 | -.20 | -.19 | .02 |
| post P.M.-pre P.M. | -.15 | .20 | -.08 | .45 | .42 | -.04 |

[1]Activity against a serum activated lipoprotein triglyceride substrate expressed as μmoles glycerol released $hr^{-1}$ 100 mℓ $plasma^{-1}$.

[2]Milk and milk fat production were measured the P.M. and A.M. prior to blood sampling and the P.M. after sampling for 6 cows. Pre A.M. indicates values obtained at the A.M. milking before sampling. Day indicates summation of pre P.M. and A.M. milk and milk fat production.

[3]Origin of lipolytic activity values. $M_0$ = total preheparin lipolytic activity in mammary venous plasma; $J_0$ = total preheparin lipolytic activity in jugular venous plasma; $M_0-J_0$ = preheparin lipolytic activity in mammary venous plasma minus that in jugular venous plasma (an indication of mammary gland contribution). $M_5$, $J_5$, $M_5-J_5$ represents same source as $M_0$, $J_0$, and $M_0-J_0$ except activity is peak postheparin lipolytic activity.

correlated ($r = .41$, $P > .05$) with peak postheparin lipolytic activity in mammary venous plasma. These results would be expected since postheparin plasma lipolytic activity was higher in cows producing smaller as compared to those producing larger amounts of milk fat as indicated by the inverse relationship between postheparin lipolytic activity and milk fat production. This would result in an increase in intra-blood triglyceride hydrolysis and subsequent increase in fatty acids available for uptake by the gland without the need for prior hydrolysis at the capillary membrane by LPL. Collectively these data strongly suggest that the physiologically active LPL is that normally released into circulation and not that available for release by heparin.

### Effect of Mammary Gland Emptying on Lipolytic Activity in Mammary Venous Plasma

The relationship between mammary venous lipolytic activity and milk fat production was further characterized by observing the effect of milk removal on lipolytic activity. Samples of mammary venous plasma were obtained from eight cows prior to and 10, 30, 60, and 240 min after milk removal. Milk and milk fat production were determined the P.M. and A.M. prior to sampling.

Results (Table 7) indicate that the level of lipolytic activity and presumably the amount of LPL released from mammary tissue is not

TABLE 7.--EFFECT OF MAMMARY GLAND EMPTYING ON LIPOLYTIC ACTIVITY IN MAMMARY VENOUS PLASMA.[1]

| Parameter | Pre-Milking | Time Post Milking (min) | | | | | |
|---|---|---|---|---|---|---|---|
| | | +1 | +10 | +20 | +30 | +60 | +240 |
| Lipolytic Activity ($M_0$)[2] | 5.7 | 4.4 | 5.9 | 5.8 | 5.6 | 6.3 | 6.3 |
| Correlation Coefficients[3] (r) | .43 | --- | .28 | --- | .37 | .49 | .71 |

[1]Samples of mammary venous plasma were taken from 8 cows prior to milking and 10, 30, 60, and 240 min after milking. Additional samples were taken at 1 and 20 min after milking from 4 of the cows.

[2]Total activity in mammary venous plasma expressed as μmoles glycerol released $hr^{-1}$ 100 mℓ $plasma^{-1}$.

[3]Correlates were $M_0$ plasma lipolytic activity and milk fat production (kg/day) the P.M. and A.M. prior to sampling. N = 8, r = .71 for significance at $P < .05$.

uniform during the milking interval. The level of activity increased with time after milking to 60 min, leveling off until at least 240 min post milking, and then apparently decreased as the gland filled with milk. Time of sampling relative to time of gland emptying dramatically affects the relationship between mammary venous plasma lipolytic activity and milk fat output (Table 7). During our initial study, sampling was done between 9 and 10:30 A.M. which was between 4 and 6 hr after milk removal. Insignificant ($P > .05$) correlation coefficients

were obtained (mammary venous plasma lipolytic activity vs milk fat production) immediately prior to and 10, 30, and 60 min after milking in the present study. The low correlation coefficients observed within the first hour after milking is primarily due to the inconsistency in plasma lipolytic activity response to milk removal. Lipolytic activity in mammary venous plasma increased post milking in four cows while it decreased or did not change in the other four cows. However, by 240 min post milking activity had returned to a level equal to or higher than that observed immediately pre-milking. The reason for these post milking fluctuations in activity is possibly a result of hormone(s) released due to milking. Since prolactin is released during milking (Koprowski and Tucker, 1971), it was thought that it might be involved. However, all cows showed a positive prolactin response to milking (Table 8), thus should have responded in the same direction unless an antagonistic hormone, possibly norepinephrine, was released in greater than normal amounts due to stress of bleeding. Results of studies with two cows in which the jugular vein, external pudic artery, and mammary vein were equipped with indwelling cannulas indicated that infusion of prolactin into either the jugular vein or external pudic artery depressed mammary venous plasma lipolytic activity (Figure 7). Little or no effect was observed on lipolytic activity in plasma from the jugular vein or external pudic artery. Collectively these data

TABLE 8.--PROLACTIN RESPONSE TO MILKING STIMULUS[a]

| Sampling Time | Cow | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1065 | 1072 | 1074 | 1128 | 1140 | 1189 | 1195 | 1196 |
| | ----------Serum Prolactin-Nonograms $m\ell^{-1}$---------- | | | | | | | |
| Pre Milking | 44 | 36 | 40 | 41 | 25 | 17 | 28 | 31 |
| Post Milking | 46 | 48 | 64 | 75 | 63 | 116 | 117 | 158 |

[a]Radioimmunoreactive prolactin assayed in duplicate in serum samples taken from the jugular vein prior to preparation of the cow for milking and within one min after milking machine was removed from the cow.

show that lipolytic activity in mammary venous plasma is affected by gland emptying and that this effect may be due to prolactin under non-stress conditions. A consistent decrease in lipolytic activity level would be in line with reports of decreased glyceride uptake by the mammary gland during the early post milking period (Shaw and Petersen, 1940).

## Relationship between Plasma Lipolytic Activity and Energy Status

Postheparin plasma lipolytic activity is depressed during fasting and returns to prefasting levels following refeeding. To test the effect of energy status on mammary contribution to plasma

Fig. 7.--Temporal response of plasma lipolytic activity to prolactin injection. Samples of plasma were obtained from the jugular and mammary veins and the external pudic artery before and after prolactin (3 mg) injection. Plasma lipolytic activity was assayed as described in Methods and Materials. Cow 1089--prolactin injected into the jugular vein. Cow 51--prolactin injected into the external pudic artery. Source of lipolytic activity: ——O—— activity in mammary venous plasma; --- --- activity in mammary venous plasma minus activity in external pudic arterial plasma; ——Δ—— activity in external pudic arterial plasma; ---•--- activity in jugular venous plasma.

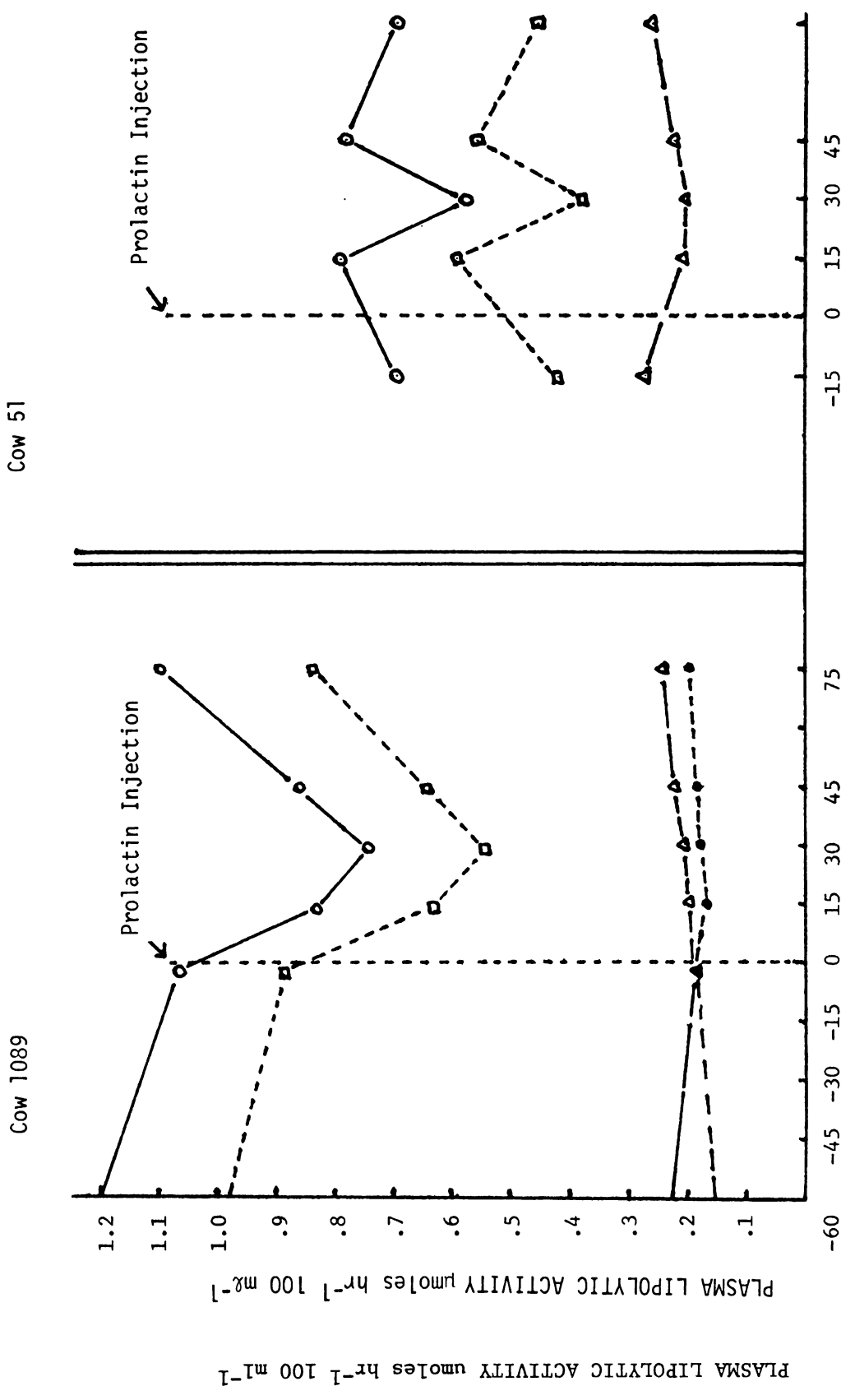

Cow 1089
Cow 51
Prolactin Injection
Prolactin Injection
PLASMA LIPOLYTIC ACTIVITY μmoles hr⁻¹ 100 ml⁻¹
PLASMA LIPOLYTIC ACTIVITY μmoles hr⁻¹ 100 mℓ⁻¹
1.2
1.1
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
-60
-45
-30
-15
0
15
30
45
75
-15
0
15
30
45
TIME FROM INJECTION (MIN)

Figure 7

lipolytic activity net energy (NE) intake (based on feed consumption data) and net energy required for body maintenance and milk production were calculated for six individual cows. The difference between energy intake and energy required was used as an index of energy status (Appendix D).

Coefficients of correlation are given in Table 9. The negative correlation ($r = -.32$) between mammary venous plasma lipolytic activity ($M_0$-$J_0$) and energy status suggest that high energy diets might have an adverse effect on the contribution of blood fat to milk fat. This relationship may partially explain the depressed milk fat production associated with high grain-restricted roughage diets (Van Soest, 1963) which provides the animal excess energy, particularly in the form of glucose (Jorgensen et al., 1965; Storry and Rook, 1966). The slight positive correlation ($r = .17$) observed between milk fat output (kg/day) and energy status does not negate the above supposition but merely indicates that milk fat production is not entirely dependent on fatty acids from plasma and that an increase in de novo synthesis within the mammary gland may occur during luxury energy intake.

Peak postheparin lipolytic activity in mammary venous plasma ($M_5$-$J_5$) was positively correlated ($r = .67$) with energy status. This supports the negative correlation observed between $M_0$-$J_0$ lipolytic activity and energy status in that a decreased normal release of

TABLE 9.--CORRELATION OF ENERGY STATUS[a] WITH MILK FAT PRODUCTION AND PLASMA LIPOLYTIC ACTIVITIES

| Correlates[b] | $M_0$-$J_0$[c] | $M_5$-$J_5$[c] | Milk fat (kg/day) | % increase in $M_5$ over $M_0$ |
|---|---|---|---|---|
| NE intake | .06 | .39 | — | — |
| $NE_i$ - $NE_r$[bd] | -.32 | .67 | .17 | .61 |

[a]Energy status is defined here as the difference between net energy consumed and net energy required.

[b]$r$ = .8 for significance at $P < .05$.

[c]$M_0$-$J_0$ indicates mammary contribution to preheparin plasma lipolytic activity. $M_5$-$J_5$ indicates mammary contribution to postheparin plasma lipolytic activity.

[d]$NE_i$ = net energy intake, $NE_r$ = net energy required.

mammary LPL into circulation results in an increase in the amount available for release by heparin. Another way to approach the amount of lipolytic activity released by heparin is in terms of the per cent increase relative to the $M_0$ level. This approach aids in decreasing variability due to individual cow differences. The use of this value instead of $M_5$-$J_5$ values had little effect on the correlation between postheparin lipolytic activity and energy status (Table 9).

## Effect of Induction of Lactation on Plasma Lipolytic Activity

Mammary tissue LPL activity increases several fold just prior to parturition in the bovine and continues to increase at a decreasing rate until near peak lactation. A working hypothesis is that the physiologically active LPL is bound to the luminal side of the capillary endothelium and is slowly released into the blood stream. In agreement with this hypothesis, we have found LPL activity in mammary venous plasma to be positively correlated with milk fat production ($r = 0.8$, $P < .05$). Thus, the availability of LPL for release into the plasma at induction of lactation may be more meaningful in terms of uptake of blood triglyceride fatty acids than total tissue activity.

If the lipolytic activity in mammary venous plasma is due to release of LPL from the mammary gland, then appearance of plasma lipolytic activity should be preceded by an increase in tissue activity of LPL. A corollary to this would be the inability of heparin to cause an increase in lipolytic activity in mammary venous plasma prior to an increase in tissue LPL activity. A second corollary is that heparin releasable lipolytic activity should appear before an increase in pre-heparin plasma lipolytic activity.

To test this supposition, jugular and mammary venous blood samples were taken from six primiparous heifers at various times

during the interval from 17 days prepartum to 8 days postpartum. After initial sampling, heparin (10 IU/kg body wt) was injected into the jugular vein and a second and third sample of jugular and venous blood taken. Preheparin samples only were obtained from an additional 7 heifers.

Preheparin lipolytic activity was not detectable in either jugular or mammary venous plasma at 17 to 14 days prepartum (Table 10).

TABLE 10.--PLASMA LIPOLYTIC ACTIVITY ACROSS PARTURITION IN PRIMIPAROUS HEIFERS.

| Days Postpartum[1] | Venous Plasma Source | | |
|---|---|---|---|
| | Mammary | Jugular | Difference |
| ----------Lipolytic activity - μmoles $hr^{-1}$ 100 mℓ $plasma^{-1}$---------- | | | |
| -17 to -14 | 0(2)[2] | 0(2) | 0(2) |
| -8 to -5 | .08 ± .04(5) | .06 ± .03(5) | .03 ± .03(5) |
| -4 to -2 | .22 ± .07(10) | .16 ± .05(10) | .06 ± .06(10) |
| Parturition | .80 ± .39(3) | .22 ± .18(3) | .59 ± .22(3) |
| +2 to +4 | 4.70 ± .64(5) | .61 ± .16(5) | 4.09 ± .60(5) |
| +5 to +8 | 4.59 ± .36(9) | .62 ± .10(9) | 4.01 ± .27(9) |

[1]Days with similar values are grouped for conciseness.

[2]Values are given as mean ± standard error. Numbers in parentheses are the number of observations. Mean of postpartum values > parturition values > prepartum values, $P < .01$.

Between 8 and 2 days prepartum, activity was detectable in small amounts and $M_0$-$J_0$ activity fluctuated between negative and positive values depending on the animal. The $M_0$-$J_0$ activity increased significantly ($P < .01$) relative to prepartum levels on day of parturition and peaked out by 2 to 4 days postpartum. Postpartum values were significantly higher ($P < .01$) than values prepartum or values on day of parturition. Mammary tissue LPL showed a major increase by 2 days prepartum (Figure 5). These data are in agreement with the stated supposition that prior increase in mammary tissue LPL activity is required for appearance of lipolytic activity in mammary venous plasma. In further support of this supposition are results obtained after heparin injection (Table 11). Peak postheparin lipolytic activity in mammary venous plasma (M-J) was negative between 14 and 7 days prepartum, indicating that no appreciable contribution was made by the mammary gland. Heparin releasable lipolytic activity in mammary venous plasma between 4 and 2 days prepartum was quite variable between animals; ranging from a positive 53 to a negative 9 units of activity with a mean and standard error of 17 ± 20. A large increase in postheparin lipolytic activity in mammary, jugular, and mammary minus jugular venous plasma was observed on the day of parturition relative to prepartum activity. The ability of heparin to increase plasma lipolytic activity was further enhanced by 2 to 4 days

TABLE 11.--PEAK POSTHEPARIN PLASMA LIPOLYTIC ACTIVITY[1] ACROSS PARTURITION IN PRIMIPAROUS HEIFERS.

| Days Postpartum | Venous Plasma Source | | |
|---|---|---|---|
| | Mammary | Jugular | Difference |
| ----------Lipolytic activity - μmoles $hr^{-1}$ 100 mℓ $plasma^{-1}$---------- | | | |
| -14 to -7 | 87 ± 9(3)[3] | 88 ± 13(3) | -0.3 ± 4(3) |
| -4 to -2 | 99 ± 29(6) | 82 ± 21(6) | 17 ± 20(6) |
| Parturition | 375 ± 60(2) | 190 ± 36(2) | 175 ± 14(2) |
| +2 to +4 | 836 ± 28(5) | 692 ± 30(5) | 145 ± 55(5) |
| +5 to +8 | 806 ± 99(8) | 648 ± 84(8) | 159 ± 31(8) |

[1]Activity present after heparin (5000 IU/animal) injection in jugular vein. Samples taken 10 min postheparin in prepartum heifers and 5 min postheparin in postpartum heifers.

[2]Days with similar values grouped for conciseness.

[3]Values represent mean ± std error. Numbers in parenthesis represent number of observations.

postpartum; however, the mammary-jugular difference tended to decrease after parturition. This decline in mammary minus jugular activity was not significant and was possibly due to increased carry over of activity from mammary to jugular vein as a result of an increased blood flow rate postpartum (Kjaersgaard, 1968).

## Plasma Lipolytic Activity vs Adipose and Mammary Tissue LPL and GS Activity

Mammary tissue LPL and GS activities are positively correlated with each other and with milk fat production (Askew et al., 1971). Similar relationships are reported elsewhere in this paper. Milk fat production was significantly correlated with mammary venous plasma lipolytic activity in this investigation. Thus, its relationship to mammary tissue LPL and GS activity was of considerable interest in determining its (plasma lipolytic activity) overall relevancy to blood triglyceride clearance by the mammary gland.

Sampling dates relative to parturition and corresponding activities of plasma triglyceride lipase and mammary and adipose tissue LPL and GS are shown in Appendix E. Correlation coefficients between the various parameters are in Tables 12 and 13. There appears to be little relationship between mammary tissue LPL activity and mammary venous plasma lipolytic activity if viewed prepartum or early postpartum (Table 12). The reason for the low correlation coefficients when the pre- and early post-partum states are considered alone is best explained by Figure 8. Mammary tissue LPL begins to increase around 7 days prepartum then increases at a decreasing rate to 60 days postpartum; while plasma lipolytic activity ($M_0$-$J_0$) does not increase

TABLE 12.--RELATIONSHIP BETWEEN PLASMA LIPOLYTIC ACTIVITY AND MAMMARY TISSUE ENZYME ACTIVITIES AT VARIOUS LACTATIONAL STATES

| Mammary Tissue Characteristic Correlated with | Plasma Sample | | |
|---|---|---|---|
| | $M_0$ | $J_0$ | $M_0$-$J_0$ |
| **Prepartum (-30 to -2 days)** | | | |
| LPL Activity | .18(4) | -.36(4) | .25(4) |
| GS Activity | -.24(4) | -.50(4) | -.19(4) |
| **First Month of Lactation (7, 14, 28 days Post)** | | | |
| LPL Activity | -.12(6) | -.70(6) | .20(6) |
| GS Activity | -.46(6) | .46(6) | -.71(6) |
| **Complete Lactational Cycle (-30 to +300 days)** | | | |
| LPL Activity | .69[a](13) | .37(13) | .69[a](13) |
| GS Activity | .63[b](13) | .70[a](13) | .59[b](13) |

[a]Significant r value ($P < .01$), N = 13.

[b]Significant r value ($P < .05$), N = 13.

TABLE 13.--RELATIONSHIP BETWEEN PLASMA LIPOLYTIC ACTIVITY AND ADIPOSE TISSUE ENZYMIC ACTIVITIES AT VARIOUS LACTATIONAL STATES

| Adipose Tissue Characteristic | Plasma Sample | | | Mammary Tissue |
|---|---|---|---|---|
| Correlated with | $M_0$ | $J_0$ | $M_0$-$J_0$ | LPL |
| Prepartum (-30 to -2 days) | | | | |
| LPL Activity | -.90(4) | -.95(4) | -.86(4) | .27(4) |
| GS Activity | -.44(4) | -.07(4) | -.48(4) | ---- |
| First Month of Lactation | | | | |
| LPL Activity | -.39(6) | -.14(6) | -.36(6) | -.60(6) |
| GS Activity | -.31(6) | .57(6) | -.60(6) | ---- |
| Complete Lactation Cycle (-30 to +300 days) | | | | |
| LPL Activity | -.73[a](13) | -.50(13) | -.73[a](13) | -.50(13) |
| GS Activity | -.28(13) | .10(13) | -.33(13) | ---- |

[a]Significant r value ($P < .01$), $n = 13$.

Fig. 8.--Pre- and postheparin lipolytic activity in mammary venous plasma and mammary tissue lipoprotein lipase activity across parturition. Preheparin plasma samples and mammary tissue samples were from the same cows (Appendix E). Postheparin samples were from primiparous heifers (Table 11). Experimental procedure was as described in Methods and Materials. Enzymic activities were assayed against a serum activated artificial triglyceride (triolein) emulsion. Plasma lipolytic activity is the activity in mammary venous plasma minus activity in jugular venous plasma.

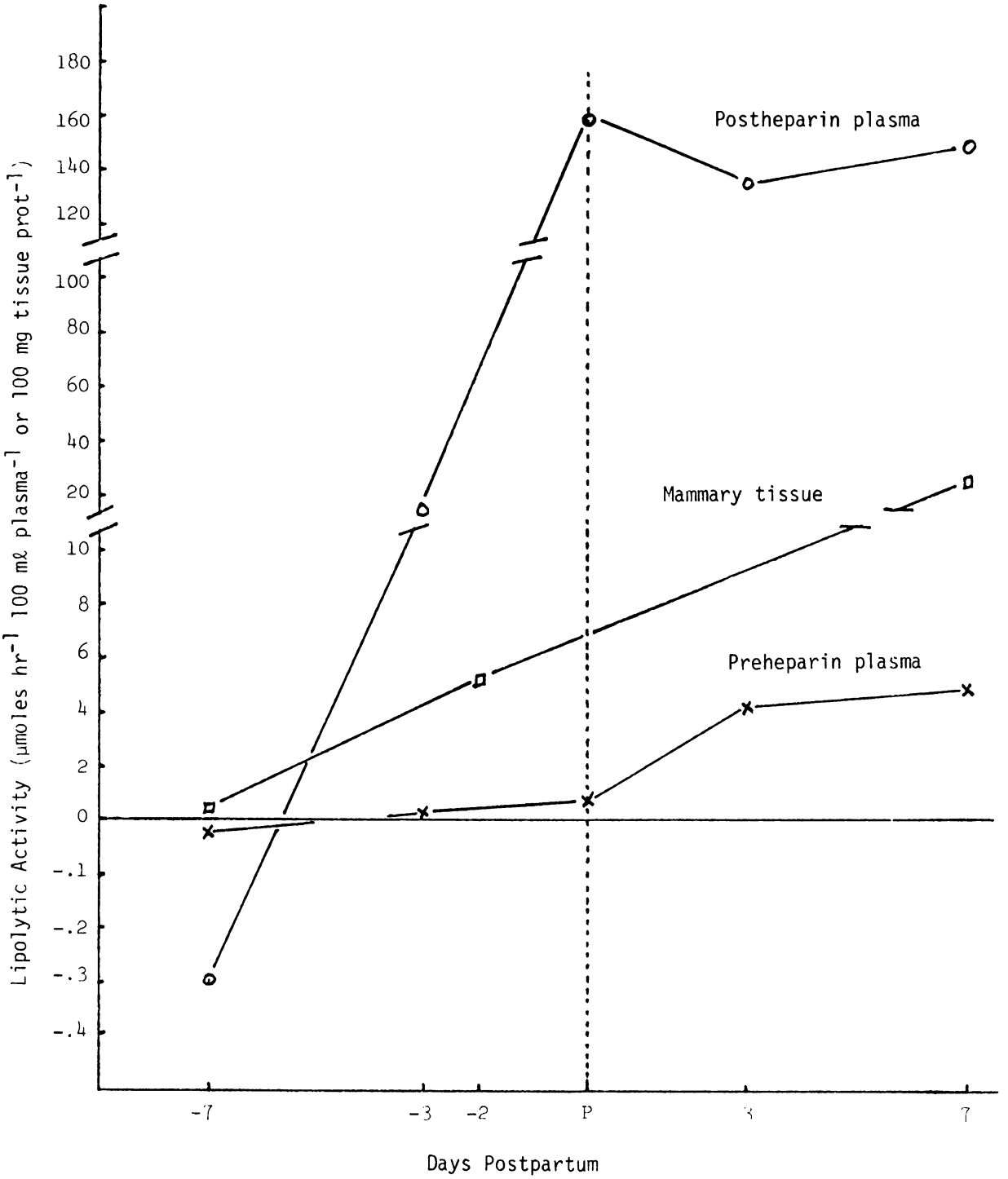

Postheparin plasma
Mammary tissue
Preheparin plasma
Lipolytic Activity (μmoles hr-1 100 mℓ plasma-1 or 100 mg tissue prot-1)
180
160
140
120
100
80
60
40
20
10
8
6
4
2
0
-.1
-.2
-.3
-.4
-7
-3
-2
P
3
7
Days Postpartum

Figure 8

significantly until the day of parturition and attains maximum levels within 3 days after induction of lactation. Therefore, over the short intervals of time included in the pre- and early postpartum period the relationship between the two is more like a precursor-product relationship than a direct in-phase relationship. In other words, it appears that the level of lipolytic activity in mammary venous plasma is dependent on a prior increase in LPL activity in mammary tissue. A precursor-product relationship is further indicated by the inability of heparin to increase M-J plasma lipolytic activity prior to the prepartum increase in activity of mammary tissue LPL.

When prepartum and early lactation data are viewed as a unit in conjunction with data obtained during late lactation (Table 12), a significant correlation ($r = .69$, $P < .01$) exist between $M_0$-$J_0$ lipolytic activity and LPL activity in the mammary gland. Mammary tissue GS and LPL activities were not significantly correlated in this study over a complete lactation. However, GS activity is significantly correlated with $M_0$-$J_0$ lipolytic activity. Thus, it appears that lipolytic activity in mammary venous plasma is in closer contact with tissue re-esterification of incoming fatty acids than is the total tissue LPL; adding credence to the supposition that $M_0$-$J_0$ lipolytic activity more adequately defines physiological LPL activity than does total tissue activity. The reason for the high positive correlation ($r = .70$,

$P < .01$) between jugular venous plasma lipolytic activity and mammary tissue glyceride synthesis activity is not readily apparent. This relationship might be due to a combination of factors: 1) $J_0$ lipolytic activity is normally quite low thus small real changes would appear large and 2) changes in $J_0$ activity often reflect changes in $M_0$ activity on a smaller scale.

Relationships between plasma lipolytic activity and adipose tissue enzymic activities are depicted in Table 13. Primary consideration is directed toward the significantly negative correlation ($r = -.73$, $P < .01$) between $M_0$-$J_0$ lipolytic activity and LPL activity of adipose tissue. Noteworthy also is the negative but nonsignificant correlation ($r = -.50$, $P > .05$) between LPL activity of mammary and adipose tissues. Taken together, these observations concur with the reciprocal relationship concept between the two tissues; they also suggest that plasma lipolytic activity ($M_0$-$J_0$) may be a more sensitive indicator of this reciprocal relationship than mammary tissue LPL activity.

# GENERAL DISCUSSION

The destination of plasma triglyceride fatty acids is altered by physiological state and is apparently a function of specific tissue need (Robbell and Scow, 1965). Diversion of plasma triglycerides from adipose tissue to the mammary gland accompanies induction of lactation (Storry, 1970). The mechanism by which this phenomenon is effected is not clearly understood; however, differential regulation of triglyceride uptake by the two tissues appears to be a plausible effector.

Results of this investigation indicate that the ability of adipose tissue and the mammary gland to clear triglycerides from the blood decreases and increases, respectively, with induction of lactation. Similar results have been observed in the rat (Homosh et al., 1970; Emery et al., 1971). The increase in mammary tissue lipoprotein lipase activity has been attributed to the observed increase in prolactin at parturition (Scow, 1970). Evidence for prolactin stimulation of mammary tissue LPL is available for the rabbit (Falconer and Fiddler, 1970) and rat (Shirley et al., 1972) but its effect on GS activity has not been observed. The reason for the decrease in adipose LPL and GS activities is not clear, although it is quite possibly

due to hormonal changes associated with parturition (Convey, 1973). These changes in enzymic activity of adipose and mammary tissues are covert changes which could account for the redistribution of lipid from adipose to mammary tissue at induction of lactation. However, it appears that the negative effects of lactation on adipose enzymic activity can be overcome by luxury grain intake which is indicative of their previously reported sensitivity to energy status (Robinson and Wing, 1970) and insulin or glucose infusion (Rao and Hawkins, 1972). These results differ from those of Hamosh et al. (1970) in that they found adipose LPL to be low throughout lactation in the rat. This suggests the possibility that regulation of triglyceride uptake by adipose tissue in the rat and cow is mediated via different effectors. The low LPL and GS activities in bovine adipose tissue during early lactation could be due to the associated negative energy balance. This would not be true for rats since the pups are too small during early lactation to provoke an energy insult.

Lipoprotein lipase (LPL) is present in both adipocytes and vascular-stromal cells of adipose tissue (Rodbell and Scow, 1965; Cunningham and Robinson, 1969) and presumably mammary tissue. Partial release of LPL from tissue can be accomplished by heparin perfusion (Rodbell and Scow, 1965), with a subsequent reduction in the tissues ability to hydrodyze a lipoprotein triglyceride substrate (Scow et al.,

1972). Since heparin releasable LPL is presumably that LPL associated with or bound to the capillary membrane (Ho et al., 1967) it appears that the functional site of LPL activity is at the capillary membrane. Strong evidence in favor of this view has been reported by Blanchette-Mackie and Scow (1971). Thus, the availability of LPL for release into the plasma may be more meaningful in terms of uptake of triglyceride fatty acids by a tissue than total tissue activity.

Results of the experiments reported herein support the concept that the LPL available for release into the blood stream more accurately reflects the triglyceride uptake ability of the tissue. In addition, they carry the concept one step further in that they indicate that there are at least three pools of LPL. The functional pool is most accurately reflected by the LPL normally released into the blood. The three pools referred to include the intracellular LPL, the heparin releasable LPL, and the functionally released LPL. The relationship between these LPL pools appear to be of the precursor-product type since the appearance of LPL-like activity in pre- or postheparin plasma is apparently dependent on a prior increase in tissue LPL activity (Figure 8). This type of relationship is also indicated by the low positive correlation between preheparin plasma lipase and mammary tissue LPL just prior to and immediately following parturition. The two lipase activities are closely related only if the entire lactation

cycle is considered. Changes in preheparin plasma lipase activity are associated with opposite changes in postheparin plasma lipase activity. Preheparin lipolytic activity in mammary venous plasma is positively correlated with milk fat production and negatively correlated with energy status while postheparin lipolytic activity is negatively correlated with milk fat production and positively correlated with energy status. In other words, an increase in the normal release rate of LPL in response to some physiological stimuli results in a decrease in the LPL available for release by heparin while a decrease in the normal release rate of LPL results in an increase in the LPL available for release by heparin.

In accord with this conceptual realtionship between pre- and postheparin lipolytic activity in mammary venous plasma is the relationship between the two plasma lipolytic activities and the increase in milk fat output following heparin injection. Heparin injection in the jugular vein in the a.m. was followed by an increase in milk fat production at the p.m. milking relative to milk fat output the p.m. prior to heparin injection. The degree of response in milk fat output to heparin injection was negatively correlated with lipolytic activity in preheparin mammary venous plasma but positively correlated with peak postheparin lipolytic activity in mammary venous plasma. The release of LPL into the blood by heparin is associated with a decrease

in the concentration of plasma triglyceride and an increase in the concentration of plasma non-esterified fatty acids (NEFA) (LaRosa et al., 1971). An increase in plasma NEFA could result in a net uptake of NEFA by the mammary gland since net uptake is quite dependent on arterial concentration (Kronfeld, 1965). Postheparin plasma lipolytic activity was higher in those cows producing less fat; therefore, in theory, plasma NEFA's were higher. Thus, milk fat output in low fat cows could have been stimulated by an increase in the net uptake of NEFA by the mammary gland. This interpretation is subject to criticism since only five to six hours elapsed between heparin injection and p.m. milking. This might not allow sufficient time for such an effect on milk fat output to occur. However, an increase in fatty acid availability for milk fat synthesis could possibly influence secretory rate. Another possibility is that heparin may exert some effect on mammary gland metabolism that is not apparent at this time.

Collectively, these results lead to the hypothesis that the functional LPL is reflected by the lipolytic activity normally present in mammary venous plasma, that heparin releasable LPL is the immediate precursor of functional LPL, and that cellular LPL is the parent material for both pre- and postheparin lipolytic activity in mammary venous plasma. This concept of functional LPL is supported by data indicating the presence of both intra and extracellular LPL activity

in adipose tissue (Cunningham and Robinson, 1969). Additional support is provided by the data of Schotz and Garfinkel (1972) indicating that two species of LPL are present in plasma. The plasma species appears to be identical to one of the tissue species. The suggestion that the physiologically active LPL is that LPL released by heparin (Borensztajn and Robinson, 1972) is an apprent conflict with the above hypothesis. However, this suggestion was based on the decrease observed in tissue LPL activity following heparin perfusion and not on actual comparisons between pre- and postheparin plasma LPL activity and triglyceride uptake ability of the tissue. Thus, in essence there is no actual conflict since the proposed hypothesis would predict a decrease in tissue lipolytic activity against a lipoportein triglyceride substrate following exposure to heparin.

Uptake of plasma triglycerides by extrahepatic tissue involves prior hydrolysis by LPL (Scow, 1972) and subsequent reesterification within the cell (Shapiro, 1965). Since non-esterified fatty acids are negative modulators of LPL activity (Gordon et al., 1953), a decrease in esterification rate could conceivably regulate triglyceride uptake by a tissue via end-product inhibition of LPL.

GS activity in mammary tissue homogenates was found to be positively correlated with milk fat production in accordance with the results of others (Askew et al., 1971). However, it appeared to be

less sensitive to change in lactational state than either mammary tissue LPL activity or lipolytic activity in mammary venous plasma. The observation that GS activity was lower than tissue LPL during lactation could be taken to mean that GS was rate limiting with respect to triglyceride fatty acid uptake. However, this interpretation is weakened by the observed sensitivity of plasma lipolytic activity to lactational state, correlation with milk fat production, and low activity with respect to tissue GS activity.

LPL (like) activity normally released into mammary venous plasma is a sensitive indicator of the mammary glands ability to clear triglycerides from the blood. One of the major unsolved problems is the factor(s) controlling the transfer of LPL from the site of synthesis to the active cite at the capillary membrane. Another interesting investigation would be to determine if LPL activity in the venous plasma associated with other extrahepatic tissues is closely related to their triglyceride uptake ability.

# SUMMARY

Lipoprotein lipase (LPL) and glyceride synthesis (GS) are considered essential for uptake of blood triglyceride fatty acids by extrahepatic tissues. Two experiments investigated the regulatory capability of LPL and GS in bovine adipose and mammary tissues. Initially (Experiment $T_1$) samples of adipose and mammary tissues were obtained from nine primiparous Holstein heifers 180 days (n = 6) or 260 days (n = 3) in gestation and 14 days after induced (caesarean section) lactation. In the second experiment ($T_2$) biopsy samples of mammary and shoulder subcutaneous adipose tissue were taken at 49, 21, 14, 8, and 2 days prepartum and 7, 14, 28, 60, 120, 180, 240, and 300 days postpartum from eight multiparous Holstein cows.

Mammary LPL activity (μmoles fatty acids release $hr^{-1}$ 10 mg tissue $protein^{-1}$) increased from .5 to 46.9 while adipose LPL decreased (12 to 8) with onset of lactation (Experiment $T_1$). Similarly, GS activity (μmoles palmitate incorporated $hr^{-1}$ 10 mg tissue $protein^{-1}$) increased 8 fold in mammary tissue and decreased 6 fold in adipose tissue. In Experiment $T_2$, mammary LPL activity (μmoles glycerol released $hr^{-1}$ 100 mg tissue $protein^{-1}$) increased 6 fold between

49 and 7 days prepartum, then increased sharply (.4 to 5.1) between 7 and 2 days prepartum, reached a maximum (83 ± 12) at 120 days postpartum, and then decreased slowly until 280 days, postpartum (19 ± 18). Mammary GS activity (μmoles palmitate incorporated $hr^{-1}$ 100 mg tissue $protein^{-1}$) decreased slightly between 49 and 8 days prepartum then increased 5 fold by 2 days prepartum and an additional 2 fold by 2 weeks postpartum after which it remained relatively constant until 280 days postpartum. Adipose tissue LPL and GS activities appeared to be more sensitive to energy status than to changes in lactational state (Experiment $T_2$). Although activities of the adipose enzymes were variable, two discernible peaks occurred; one 2 days prior to parturition and one during mid-lactation (120 to 180 days). Both peaks correspond to luxury grain intake.

Availability of LPL for release into plasma may represent uptake of triglyceride fatty acids by a tissue better than total tissue LPL activity since LPL catalyzes hydrolysis of blood triglycerides at the capillary membrane. Four experiments were conducted to ascertain the feasibility of using mammary venous plasma lipolytic activity (PLA), against a substrate of triolein emulsion activated with serum as a measure of mammary clearance of triglycerides from blood. Preheparin and peak-postheparin PLA were measured and compared to milk fat production (an overt measure of plasma triglyceride utilization

by the mammary gland). In subsequent experiments, PLA was characterized with respect to: 1) LPL and GS activities in adipose and mammary tissues, 2) energy status of the cow, 3) milking stimulus, and 4) changes in lactational state. Samples were taken simultaneously from the jugular and mammary veins and the difference in PLA between the two (M-J) was assumed to be the mammary gland contribution.

Preheparin mammary PLA (M-J) was: 1) positively correlated ($r = .8$, $p < .05$) with milk fat production, mammary tissue LPL activity ($r = .7$, $p < .01$) and mammary tissue GS activity ($r = .6$, $p < .05$); 2) negatively correlated with energy status of the cow ($r = -.3$), adipose tissue LPL activity ($r = -.7$, $p < .01$) and adipose GS activity ($r = -.3$); and 3) sensitive to mammary gland emptying and prolactin injection (i.v.). Postheparin PLA in mammary venous plasma (M-J) was negatively correlated ($r = -.4$) with milk fat production and positively correlated with energy status of the cow ($r = .7$).

Preheparin PLA was not detectable in either jugular or mammary venous plasma of primiparous heifers at 17 or 14 days prepartum, but was detectable between 8 and 2 days prepartum. Mammary PLA (M-J) increased significantly ($p < .01$) on day of parturition and attained a maximum by 2 to 4 days postpartum. Postpartum PLA (M-J) was significantly higher ($p < .01$) than PLA prepartum or PLA on the day of parturition. No difference in heparin releasable PLA between jugular

and mammary venous plasma was observed before 7 days prepartum. A mean positive M-J difference in postheparin PLA occurred by 4 days prepartum. Postheparin PLA in mammary, jugular, and mammary minus jugular venous plasma increased sharply at parturition followed by an additional increase by 2 to 4 days postpartum.

Results of these experiments are consistent with the views that (1) the redistribution of lipid from adipose to mammary tissue at induction of lactation is due to a decrease in the uptake ability of adipose tissue and an increase in uptake ability of the mammary gland, (2) LPL released to mammary plasma is a more plausible regulator of triglyceride fatty acid uptake by the mammary gland than GS, (3) preheparin PLA in mammary venous plasma reflects the mammary glands ability to clear triglycerides from blood more accurately than tissue LPL activity or postheparin PLA, and (4) adipose tissue LPL and GA are more sensitive to energy status than lactational state. Results numbers 2 and 3 represent important new concepts.

# APPENDICES

# APPENDIX A

## COMPOSITION OF SCINTILLATION FLUID

| Component | Quantity |
|---|---|
| Paradioxane | 770 mℓ |
| Xylene | 770 mℓ |
| Absolute Ethanol | 460 mℓ |
| Napthalene | 160 g |
| PPO | 10 g |
| Dimethyl POPOP | 0.1 g |

APPENDIX B

ADIPOSE AND MAMMARY LPL RESPONSE TO HEPARIN[a] (EXPERIMENT $T_2$)

| Lactational[b] State | Mammary | | | Adipose | | |
|---|---|---|---|---|---|---|
| | -Heparin | +Heparin | Dif | -Heparin | +Heparin | Dif |
| | -----LPL Activity - μmoles $hr^{-1}$ 100 mg protein------ | | | | | |
| Days Prepartum | | | | | | |
| -30 to -14 (3)[c] | .17[d] | .14 | -0.03 | 6.08 | 7.03 | +0.95 |
| -7 (1) | .40 | 4.30 | +3.90 | 11.80 | 13.60 | +1.80 |
| -2 (2) | 3.86 | 3.99 | +0.10 | 9.60 | 12.77 | +3.17 |
| Days Postpartum | | | | | | |
| 7 to 28 (5) | 45.86 | 44.04 | -1.82 | 2.20 | 2.16 | -0.04 |
| 60 to 180 (6) | 75.13 | 80.43 | +5.30 | 5.16 | 6.30 | +1.14 |
| 240 to 300 (6) | 31.20 | 36.30 | +5.10 | 1.75 | 2.10 | +0.35 |

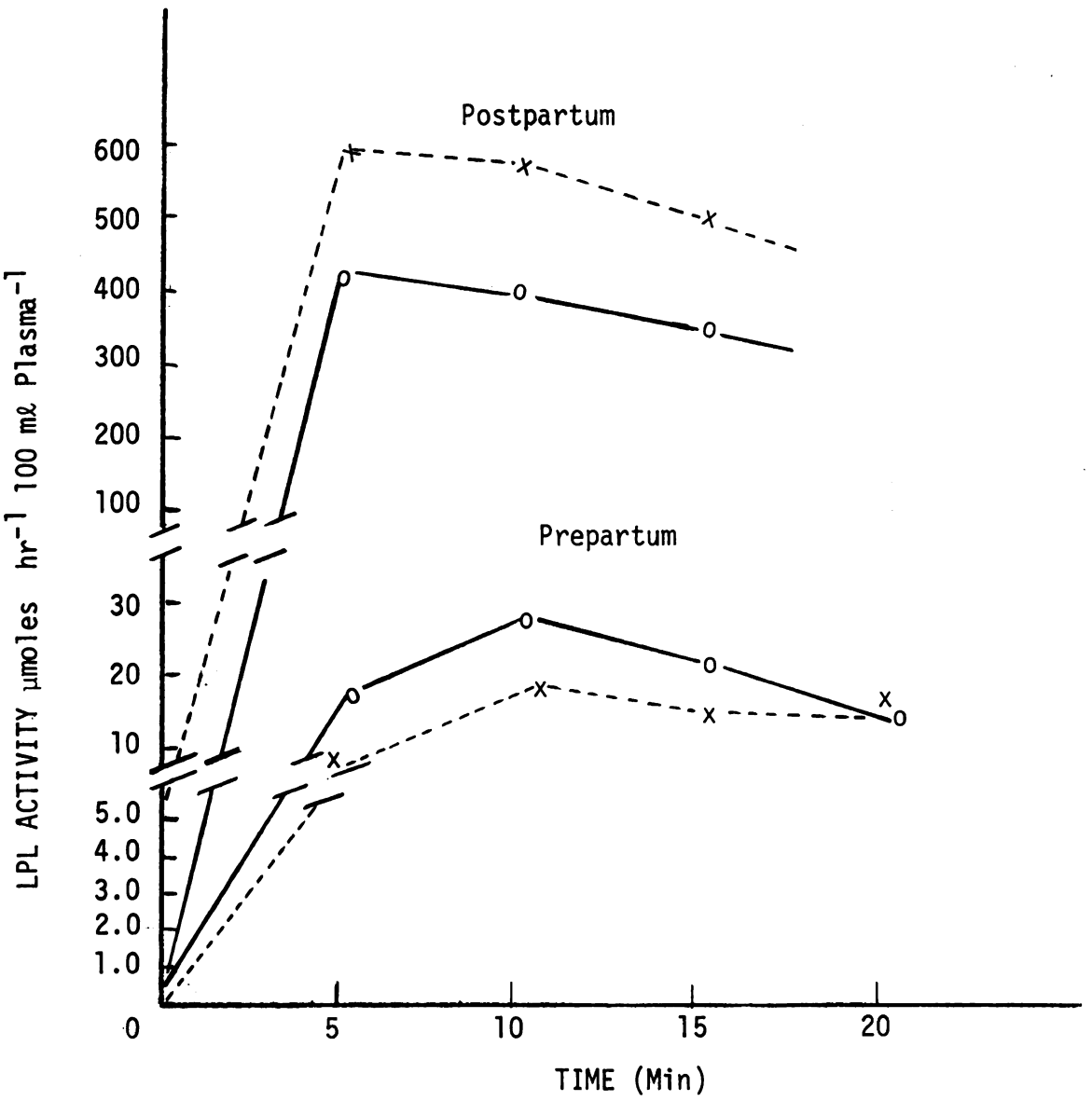
[a].05 units of heparin added to the assay mixture prior to preincubation.

[b]Days with similar values are grouped for conciseness.

[c]Number of observations included in the mean.

[d]Heterogeneous variance between lactational states distracts from the usefulness of standard errors; therefore, they are not included.

Appendix C.--Temporal response of plasma lipolytic activity to intravenous injection of heparin. Heparin (5000 IU) was injected into the jugular vein and samples of plasma withdrawn simultaneously from the jugular and mammary vein at the time indicated. Plasma lipolytic activity was assayed as described in Methods and Materials. Prepartum samples were obtained three days before actual parturition. Postpartum samples were obtained 10 days after actual parturition. Source of lipolytic activity: ——o—— jugular venous plasma; ---x--- mammary venous plasma. Similar results were obtained with another cow.


Postpartum
Prepartum
600
500
400
300
200
100
30
20
10
5.0
4.0
3.0
2.0
1.0
0
5
10
15
20
TIME (Min)
LPL ACTIVITY µmoles hr-1 100 mℓ Plasma-1

Appendix C

APPENDIX D

INDIVIDUAL COW DATA FROM EXPERIMENT $P_1$

| Parameter | Cows | | | | | |
|---|---|---|---|---|---|---|
| | 923 | 824 | 950 | 1133 | 826 | 827 |
| Body Weight (kg)[a] | 591 | 756 | 751 | 486 | 652 | 751 |
| NE intake (Mcal) | 15.0 | 46.3 | 44.2 | 16.9 | 34.1 | 22.4 |
| NE required (Mcal) | 25.5 | 32.9 | 35.9 | 27.2 | 23.1 | 19.7 |
| $NE_i - NE_r$[d] | -10.5 | +13.4 | +8.3 | -10.3 | +11.0 | +2.7 |
| Milk Production (kg) | | | | | | |
| Pre P.M. | 10.4 | 16.0 | 16.1 | 12.1 | 6.4 | 5.4 |
| Pre A.M. | 14.1 | 21.0 | 22.7 | 17.7 | 12.7 | 5.6 |
| Post P.M. | 12.5 | 21.4 | 13.6 | 11.8 | 11.4 | 5.9 |
| Milk Fat (kg) | | | | | | |
| Pre P.M. | .26 | .37 | .71 | .36 | .20 | .20 |
| Pre A.M. | .42 | .46 | .70 | .46 | .37 | .17 |
| Post P.M. | .45 | .66 | .61 | .39 | .40 | .21 |

[a]Mean of two consecutive day weights.

[b]Average caloric value of all dietary constituents consumed over a 5-day period prior to sampling. Caloric value (K cal) of feedstuff based on National Research council report (1971).

[c]Determined by calculating energy required for maintenance of body weight and milk production, based on actual body weight, milk production, and fat test as prescribed by the National Research Council (1971).

[d]$NE_i$ = net energy intake, $NE_r$ = net energy required.

## APPENDIX E

### PLASMA LIPOLYTIC ACTIVITY AND ACTIVITIES OF ADIPOSE AND MAMMARY TISSUE LPL AND GS (EXPERIMENT $P_2$)

| Days Post-partum | Sample Source | | | | |
|---|---|---|---|---|---|
| | $M_0$-$J_0$ Plasma Lipolytic Activity[a] | Mammary Tissue | | Adipose Tissue | |
| | | LPL[b] | GS[b] | LPL[b] | GS[b] |
| -30 | -.10(1)[c] | .09(1) | .50(1) | | .54(1) |
| -7 | -.16(1) | .40(1) | 3.80(1) | 11.8(1) | 6.00(1) |
| -2 | .26 ± .3(2) | 3.86 ± 1(2) | 4.70 ± 2.2(2) | 9.60 ± 7.0(2) | 1.96 ± .8(2) |
| +7 | 3.34 ± .3(3) | 66.00 ± 34(3) | 11.04 ± 1.7(3) | 3.10 ± 1.4(3) | 1.87 ± .7(3) |
| +28 | 3.42 ± .6(3) | 81.70 ± 20(3) | 9.44 ± 2.1(3) | 1.21 ± .6(3) | .78 ± .4(3) |
| +300 | 2.50 ± .9(3) | 19.30 ± 18.4(3) | 4.50 ± 2.8(3) | 1.5 ± 0.4(3) | 4.5 ± 2.8(3) |

[a] μmoles glycerol released $hr^{-1}$ 100 mℓ $plasma^{-1}$

[b] μmoles glycerol released $hr^{-1}$ 100 mg tissue $protein^{-1}$.

[c] Mean ± standard error of mean when applicable. Number of observations included in the mean are in parentheses.

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