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Metabolic characteristics of the ketotic state in the bovine

Scott E. Mills
Iowa State University

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Metabolic characteristics of the ketotic state in the bovine

by

Scott E Mills

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

Department: Animal Science
Major: Nutritional Physiology

Approved:

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For the Major Department

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For the Graduate College

Iowa State University
Ames, Iowa

1982
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INTRODUCTION

The ketotic state refers to a condition common to dairy cows during early lactation, characterized by high plasma concentrations of the ketone bodies, β-hydroxybutyrate and acetoacetate. Development of the ketotic state results from a negative energy balance, established due to major nutrient requirements by the lactating mammary gland, and in particular, requirements for glucose. Associated hypoglycemia indirectly increases adipose fatty acid mobilization and hepatic fatty acid oxidation resulting in hepatic ketone body production.

Ketosis refers to a state where measurable consequences of the ketotic state are taking place. Ketosis may be divided into two main types. First, clinical ketosis, where the consequences of the ketotic state are readily apparent, is characterized by decreased appetite, decreased milk production, and a generally sick cow. Second, subclinical ketosis, which is not as well-defined as clinical ketosis, may be characterized by fewer obvious signs and the cow may not become ill, but overall productivity may be negatively influenced.

Economic losses from both types of ketosis are difficult to estimate due to the uncertainty of losses in milk production with either type. In addition to losses in milk production, ketosis imposes financial losses because of increased veterinary services and decreased productive life spans of cows. The incidence of ketosis in the U.S. is about 5 percent. If a similar number of cows are subclinical, which may be an underestimate, nearly 1 million cows every year would be affected. Dollar losses would probably be in the hundreds of millions.
Good management can reduce the incidence of ketosis but elimination is unlikely. Milk production in the U.S. has increased roughly 70 percent since 1960. Selection for milk production likely will continue to increase the potential for milk production in years to come. Consequently, there is a need to understand how ketosis develops and what effects the ketotic state has on metabolism.

The present studies were undertaken to characterize, in a sequential manner, the development of the ketotic state through clinical ketosis in lactating cows, and to develop a ketosis model in steers in order to study metabolic effects of ketosis. Specific objectives were:

1. To enhance the ketotic state in lactating dairy cows through ration manipulation until clinical ketosis developed.

2. To measure capacities of hepatic gluconeogenesis and ketogenesis, adipose lipolysis and lipogenesis in tissue slices from dairy cows.

3. To measure plasma and hepatic metabolite concentrations from dairy cows.

4. To attempt to induce a ketotic state in steers with phlorizin and/or 1,3-butanol.

5. To measure hepatic gluconeogenic and ketogenic capacities and hepatic metabolite concentrations in steers.
The purpose of this review is to acquaint the reader with three areas:

1. the challenge facing the dairy cow at the initiation of lactation.
2. how this challenge is met by reviewing and summarizing the importance and regulation of three key metabolic processes: a. adipose tissue metabolism, b. hepatic fatty acid metabolism, and c. gluconeogenesis.
3. the current status of the understanding of ketosis.

Postparturient Adaptations of Dairy Cows

To truly appreciate the remarkable synthesizing capacity of the dairy cow, it is appropriate to examine the adaptive changes that occur during the periparturient period. The last two months of gestation impose a substantial increase in nutrient demand as indicated by the guidelines published by the NRC (1978). Fetal growth curves (Bauman and Currie, 1980) show that over 50% of fetal development occurs during these last months. The additional nutrients are directed toward fetal membranes, the gravid uterus, and the mammary gland (Bauman and Currie, 1980). Recent reviews (Bauman and Currie, 1980; Thatcher et al., 1980) conclude that the adaptations necessary to meet these nutrient demands and to assure the concurrence of a functional mammary gland with parturition are regulated by the combined signals from endocrine systems of the dam and conceptus (fetus and membranes).
Metabolic demands of pregnancy and the alterations the dam must incur are miniscule compared to those imposed by lactation. The lactating mammary gland is one of the most metabolically active tissues of the body with nutrient requirements often increasing three to five fold over maintenance (NRC, 1978). The increased nutrient demands are of such magnitude that the cow has been depicted as an appendage to an udder rather than the reverse (Brown, 1969).

As is the case with fetal and mammary development, hormonal signals from both the dam and conceptus are believed responsible for coordinating parturition and the onset of lactogenesis, as well as influencing post-partum production and reproduction (Eley et al., 1981a; 1981b). Understanding of events responsible for initiation of lactation is increasing as evidenced by reports of lactation induction in nonpregnant cows (Chakriyarat et al., 1978; Collier et al., 1975; Collier et al., 1977; Keller et al., 1977; Kensinger et al., 1979). Unfortunately, the proportion of successful inductions and the milk yields obtained indicate that there is much more to be learned. Although species variation exists, minimal requirements for lactation induction seem to include increases in prolactin and adrenal corticoids, with a concomitant decline in progesterone (Tucker, 1974).

Shirley et al. (1973b) attempted to correlate changes in hormones around the time of parturition with changes in enzyme activity of the mammary gland. When compared to prepartum, lipoprotein lipase (LPL) activity during early lactation increased approximately 100 fold while glyceride synthetic rates were increased six fold. Correlations of
enzyme changes with hormones were generally low with the exception of progesterone. Heparin activation of LPL was greater during pregnancy than during lactation, suggesting that during lactation LPL exists in a more active state. A similar study (Shirley et al., 1973a) with more frequent mammary biopsies indicated a rapid induction of LPL activity beginning at eight days prepartum, with maximum activity attained at 16 weeks postpartum. The eighth day prepartum corresponds to initiation of a fall in progesterone and a rise in prolactin (Thatcher et al., 1980). Although prolactin concentration declines within one week after parturition, it is believed that different signals exist for initiating and maintaining lactation (Tucker, 1974).

Besides the ability to extract preformed fatty acids from blood, lactating bovine mammary tissue shows increased activity of enzymes concerned with de novo synthesis of fatty acids. These include acetyl-CoA carboxylase and fatty acid synthetase (Mellenberger et al., 1972) plus NADPH generating dehydrogenases (i.e. isocitrate, glucose-6-phosphate, and 6-phosphogluconate dehydrogenases) (Mellenberger et al., 1972; Shirley et al., 1973b). In mammary tissue from lactating cows, in vitro fatty acid synthesis from acetate was increased 20 fold over that in pregnant cows (Mellenberger et al., 1972). Lactose synthesizing capacity also has been studied in pregnant and lactating cows. In vitro rates of lactose synthesis from glucose correlated well with enzyme activities between 30 days prepartum and 40 days postpartum (Mellenberger et al., 1972).

Hormonal and enzymatic changes are not the only adaptations occurring with preparation for milk synthesis. Increased blood flow and
nutrient supply to mammary tissue have been reported (Baldwin and Yang, 1974). The signal(s) that are responsible for initiation of lactogenesis are unknown. It is apparent however, that after parturition marked alterations in nutrient partitioning and general metabolism of the animal must occur to meet the demands of the mammary gland. Bauman and Currie (1980) refer to such a total alteration as homeorhesis or "orchestrated changes for the priorities of a physiological state."

The changes that occur involve increased nutrient intake; increased lipolysis and decreased lipogenesis in adipose; increased gluconeogenesis, glycogenolysis, and ketogenesis in liver; increased protein mobilization and noncarbohydrate oxidation in muscle; and increased absorption and mobilization of minerals. If all systems are coordinated, milk production proceeds without incident whereas a lack of coordination enhances susceptibility to metabolic problems.

Adipose Tissue Metabolism in Ruminants

The two basic functions of adipose tissue are synthesis and mobilization of lipids. To only say that adipose tissue serves as an energy depot for mammals is to minimize its utility. Adipose is a metabolically active tissue through which 10 to 80 percent of the daily energy flux passes, depending on intake and physiological state (Emery, 1979). Mobilized fat not only serves as a source of energy and as a precursor for other components (i.e. membranes, prostaglandins) but in the lactating cow it is a major contributor to milk fat (Dimick et al., 1970; Moore and Christie, 1980). These roles only partly explain,
however, the importance of adipose tissue to the lactating cow. Bauman (1976) illustrated the immense capacity cows have for fat mobilization in his summary of an energy balance study on one cow for an entire lactation. Over the first 62 days of lactation, the average net energy loss was 20.6 Mcal/day. If 85 percent of this loss was met by body fat, mobilization of approximately 2 kg/day of body triglyceride would be required.

More extensive energy balance studies (Flatt, 1966) indicate that during peak lactation most cows are utilizing body stores and have the demonstrated potential to utilize body fat for half their energy needs (Hemken, 1971). Moreover, mobilization can occur without noticeable changes in body weight due to changes in water and feed intake and in water retention (Flatt et al., 1965; Moe et al., 1971). During late lactation, as the need for energy subsides, energy balance becomes positive with cows depositing 10 to 15 Mcal/day in adipose tissue while still producing milk (Bauman, 1976; Flatt, 1966). The cycle of body fat mobilization to sustain high production and to replace it when demands for energy are low is essential if maximum milk production is to be realized.

Fatty acid deposition in adipose tissue

Fatty acids for deposition into adipose originate from two sources. First, fatty acids that are transferred in blood are esterified to glycerol and complexed into lipoproteins. Their origin may be directly from the diet (chylomicrons) or following previous metabolism in the
liver (low density lipoproteins) (Emery, 1980; Vernon, 1980). Second, fatty acids can be synthesized directly in adipose tissue from various precursors (Prior and Jacobson, 1979b; Whitehurst et al., 1978). Numerous studies, both in vivo (Broad and Ulyatt, 1980; Ingle et al., 1972b; Prior, 1978) and in vitro (Ballard et al., 1969; 1972; Bauman and Davis, 1975; Ingle et al., 1972a; Liepa et al., 1978) indicate, that adipose tissue is the major site of fatty acid synthesis in nonlactating ruminants, with a variable but minor contribution from the liver.

Identifying the precursors for de novo fatty acid synthesis has produced some surprising and confusing data. After initial reports of Hanson and Ballard (1967; 1968), numerous studies (Baldwin et al., 1973; Ballard et al., 1972; Hood et al., 1972; Ingle et al., 1972a) have indicated that acetate is the principal precursor for fatty acid synthesis in adipose tissue of ruminants. Comparative rates of glucose and acetate incorporation into fatty acid have shown acetate is utilized 10 to 100 fold more rapidly than is glucose, although only if glucose also was present in the incubation media (Ballard et al., 1969; Hanson and Ballard, 1967; Liepa et al., 1978; Whitehurst et al., 1978; Yang and Baldwin, 1973a). Glucose is thought to be both the source of glycerol and of reducing equivalents (Whitehurst et al., 1978). Preferential metabolism of acetate is in line with basic concepts of ruminant physiology, indicating that adaptative mechanisms to spare glucose operate when possible.

Recently, however, both lactate and to a lesser extent pyruvate have been shown to be incorporated into adipose fatty acids (Prior and
Jacobson, 1979a; 1979b; Whitehurst et al., 1978) at rates that should allow glucose to be utilized. Failure to show fatty acid synthesis from glucose is peculiar, particularly considering the extent to which lactate is formed from glucose in adipose tissue (Yang and Baldwin, 1973a). Although the lactate concentration found necessary for maximum lipogenesis leaves some doubt about its physiological role (Vernon, 1980), the data of Whitehurst et al. (1978) and Prior and Jacobson (1979a) leave some pressing questions. First, why should lactate and pyruvate, but not glucose, serve as precursors for fatty acid synthesis? It is logical that if glucose is metabolized to lactate and lactate to fatty acids, then glucose should be utilizable for fatty acid synthesis. The problem does not seem to lie in label dilution, because approximately 50 percent of labeled glucose ends up in lactate and pyruvate in incubated bovine adipose cells (Yang and Baldwin, 1973a), although the relative specific activities of glucose and lactate were not given.

The answer to the first question may lie partly in a second question generated by the lactate studies (Prior and Jacobson, 1979a; Whitehurst et al., 1978). Why is fatty acid synthesis from acetate stimulated by glucose but not by lactate? As stated already, glucose is thought to generate glycerol-3-phosphate and NADPH for fatty acid synthesis and esterification (Whitehurst et al., 1978). Lactate incorporation into fatty acids in the absence of glucose indicates ample glycerol-3-phosphate and NADPH availability, yet lactate does not increase acetate incorporation into fatty acids to the extent that glucose does (Prior and Jacobson, 1979a; Whitehurst et al., 1978). The interactions of acetate,
lactate, and glucose remain to be elucidated fully. In vivo work by Prior (1978) indicates that in ad lib fed sheep, lactate is incorporated into carcass adipose fatty acids at 38 percent the rate for acetate. Despite some obvious contradictions, ruminants still seem to conserve glucose for necessities and utilize acetate as the major precursor of fatty acids.

Ruminants differ from nonruminants in that rates of passage and digestion of conventional diets is slower, resulting in a more continual flow of absorbed nutrients to body tissues. It is this slow passage that has been given as the reason for the much depressed response in ruminants to lipogenic and lipolytic stimuli (Baldwin et al., 1976; Bauman, 1976). This is not to say that bovine adipose metabolism is not adaptive. Lipogenic rates have been shown to respond to diet (Baldwin and Smith, 1971; Ballard et al., 1972; Pothoven et al., 1975; Prior and Jacobson, 1979b) fasting (Pothoven and Beitz, 1973; Prior and Jacobson, 1979b), age (Baldwin et al., 1973; Ingle et al., 1972a; Pothoven and Beitz, 1973), pregnancy (Grichting et al., 1977b), and lactation (Baldwin and Smith, 1971; Emery, 1979; Emery, 1980, Grichting et al., 1977a).

Rates of fat accumulation generally have been correlated with activities of lipoprotein lipase (Baldwin and Smith, 1971; Shirley et al., 1973a), acetyl-CoA carboxylase (Ingle et al., 1973; Pothoven and Beitz, 1975) and the system of glyceride synthesis (Emery, 1979; Emery, 1980).
Triglyceride mobilization from adipose tissue

The amount of fatty acid released from adipose tissue is a function of two processes: the rate of triacylglycerol hydrolysis and the rate of fatty-acyl-glyceride synthesis. Rates of both these processes are regulated and have been shown to fluctuate with diet (Ballard et al., 1972; Prior and Jacobson, 1979b; Yang and Baldwin, 1973b), fasting (Baldwin et al., 1973; Jackson et al., 1968; Prior and Jacobson, 1979b; Pothoven and Beitz, 1973; Pothoven and Beitz, 1975), hormone stimulation (Yang and Baldwin, 1973a, 1973b), and lactation (Grichting et al., 1977b; Ingle et al., 1972a; Metz and Van den Bergh, 1977; Pike and Roberts, 1980; Shirley et al., 1973b; Yang and Baldwin, 1973b). Regulation can be both acute, involving changes in enzyme activity, or chronic, which is slower to respond yet generally longer lasting. Chronic regulation may involve changes in metabolic capacity and enzyme concentration (Baldwin et al., 1976).

Lipolysis in ruminants is thought to occur by a mechanism similar to that in nonruminants. The initial step involves hydrolysis of triacylglycerol to diacylglycerol by the hormone-sensitive lipase (HSL) that is activated by cyclic AMP (Bauman, 1976; Vernon, 1980). The diacylglycerol is hydrolyzed by nonregulated lipases to free fatty acids and glycerol (Vernon, 1980), although some evidence exists for incomplete hydrolysis resulting in accumulation of mono- and diglycerides (Etherton et al., 1977; Yang and Baldwin, 1973b). Incomplete hydrolysis may be particularly evident during times of maximum lipolytic activity (Scow and Chernick, 1970; Vernon, 1980).
Glycerol kinase activity is very low in ruminant adipose tissue and, therefore, rates of release of glycerol from adipose have been taken to be indicative of lipolytic rates, excepting incomplete hydrolysis as stated in the previous paragraph (Baldwin et al., 1976; Emery, 1980). Free fatty acids, on the other hand, either may be released from the cell or re-esterified to glycerol-3-phosphate to reform triglycerides (Benson and Emery, 1971; Emery, 1980). Esterification by the monoglyceride pathway is generally thought to be minimal in bovine adipose (Emery, 1980). Re-esterification and tissue accumulation of free fatty acids (FFA) are primary reasons given for in vitro ratios of FFA: glycerol differing from the theoretical 3:1.

Changes in FFA concentrations in blood are correlated to changes in fatty acid entry rates and utilization rates (Baldwin and Smith, 1971; Bell, 1980; Jackson et al., 1968; Katz and Bergman, 1969; Leng and Annison, 1964; Robinson, 1963). However, entry rate of fatty acids into blood is the sum of entry rates from intestinal absorption, adipose lipolysis, and circulating triglyceride (lipoprotein) hydrolysis due to the action of lipoprotein lipase (Emery, 1979). Consequently, plasma entry rates of FFA do not necessarily reflect rates of lipolysis. Often, however, changes in plasma FFA concentration, such as occur with fasting or ketosis, are explained best by changes in rates of lipolysis and are taken to be indicative of such rates.

The ratio of oleic:stearic acid in all lipid fractions of plasma also has been used as an indicator of increased lipolysis (Wallenius and Witchurch, 1976; Yamdagni and Schultz, 1970). Increased ratios have
been noted during fasting (Brumby et al., 1975), lactation (Wallenius and Witchurch, 1976), and ketosis (Waterman and Schultz, 1972; Yamdagni and Schultz, 1970). Almost 50 percent of the fatty acids of adipose is oleic (Pothoven et al., 1974). Presumably the increased ratio of oleic:stearic reflects the relative contribution of diet and lipolysis, although differential metabolism of free fatty acids has been proposed (Vernon, 1980).

**Regulators of metabolism in adipose tissue**

Adaptations imposed by differing physiological states (fast, lactation) and ration are, at least in part, attributable to hormone and substrate changes. The list of effectors discussed subsequently is far from exhaustive but they are the ones best documented to have definite effects.

**Catecholamines**  
Additions of epinephrine or norepinephrine to adipose tissue preparations (Bartos et al., 1975; Etherton et al., 1977; Pothoven et al., 1975; Yang and Baldwin, 1973b) or by intravenous injection in vivo (Metz, 1974; Sidhu and Emery, 1973) have resulted in increased release of both FFA and glycerol. Increases of lipolysis in response to catecholamines have been in the range of one to six fold. Similar responses have been shown with direct additions to dibutyryl-cyclic AMP to adipose preparations (Bartos et al., 1975; Metz, 1974), indicating that actions of catecholamines are similar in ruminants and nonruminants, that is, via adenylyl cyclase activation.
HSL, the enzyme activated by cAMP, exists in active and inactive states (Vernon, 1980). Nonstimulated (basal) rates of fatty acid and glycerol release from adipose tissue in vitro have been used to reflect amounts of active HSL, while epinephrine stimulated rates reflect total HSL activity. However, Metz (1974) and Etherton et al. (1977) indicate that complete activation of HSL does not occur after either epinephrine or cAMP administration. Theophylline, which increases cAMP concentrations via inhibition of phosphodiesterase, increased in vitro glycerol release two fold in steers (Etherton et al., 1977) and nine fold in lactating cows (Metz, 1974). Effects of theophylline in combination with epinephrine were additive (Etherton et al., 1977). Additive effects may indicate a major regulatory function for phosphodiesterase in attenuating catecholamine response.

The increased lipolysis after theophylline in lactating cows is similar to responses from epinephrine in rats (Bauman and Davis, 1975). Differences in responses between lactating cows and dairy steers cannot be explained by differences in theophylline concentrations, because they were similar (Etherton et al., 1977; Metz, 1974). Different responses may reflect either a greater proportion of inactive enzyme or increased phosphodiesterase activity during lactation. Increased proportions of inactive enzyme are contrary to other reports (Sidhu and Emery, 1972; Yang and Baldwin, 1973b), while phosphodiesterase activity data are lacking.

Effects of catecholamines on lipogenesis are less well documented. Yang and Baldwin (1973a) and Bartos et al. (1975) found decreased fatty
acid synthesis from acetate when epinephrine was added in vitro to
adipose preparations. The decrease probably is mediated by increased
fatty acid concentration which would inhibit acetyl-CoA carboxylase.
Similar responses were observed if long-chain fatty acids were substituted
for epinephrine (Vernon, 1977; Yang and Baldwin, 1973b) or high-fat
rations were fed to sheep (Vernon, 1976). Yang and Baldwin (1973b)
showed glucose oxidation to be depressed by epinephrine; this was
preferential for [1-\textsuperscript{14}C]glucose indicating decreased pentose phosphate
pathway activity. Glucose incorporation into glyceride-glycerol was
stimulated, however, indicating re-esterification (Yang and Baldwin,
1973b).

**Growth hormone** The role of growth hormone (GH) in regulating
bovine metabolism, and in particular adipose metabolism, is understood
poorly. Studies with goats (Radloff and Schultz, 1966) and lactating
cows (Williams et al., 1963) indicate between two- and four-fold
increases in circulating FFA when dosed with GH. Such increases seem
to be due to increased production rather than decreased utilization of
FFA (Johnson et al., 1969; Williams et al., 1963). Further evidence of
a lipolytic role for GH is the finding of increased GH concentrations in
high-yielding, lactating cows as opposed to low yielders and the signifi-
cant correlations between circulating amounts of GH and FFA (Hart et al.,
summarized in vitro data for GH and concluded that no direct effect of
GH on adipose metabolism has been found.
Recent reviews on stress and hormonal fluctuations indicate that GH is responsive to many stimuli, including increased nutrient demand (Johnson and Vanjonack, 1976; Moberg, 1976; Trenkle, 1978). Hart et al. (1979) concluded GH or GH in concert with decreased insulin results in fat mobilization and increased availability of energy yielding metabolites, which in turn may regulate GH secretion. This conclusion agrees with data presented by Emery (1980) and Reynaert and Peeters (1977).

The action of GH and catecholamines seems to be entirely different. Whereas the effects of epinephrine are immediate, GH takes a few hours to be manifested (Radloff and Schultz, 1966). There are some indications in rats that this manifestation involves protein synthesis (Shapiro, 1977). GH also seems to be unique in its overall effects of not only increasing lipolytic rates but being correlated positively with production of milk (Machlin, 1973; Peel et al., 1981) and lean body mass (Machlin, 1972).

Insulin and glucose These two effectors will be considered together primarily because data are lacking on the extent of direct insulin effects on adipose metabolism and, while glucose may have effects without insulin, the effects seem to be enhanced by insulin. Yang and Baldwin (1973b), using nonlactating cows, showed decreased FFA release from epinephrine stimulated adipocytes in the presence of either glucose, insulin, or glucose plus insulin. The effects were not additive. Data of Metz and van den Bergh (1977) indicate that either glucose or glucose plus insulin decreases FFA release from both basal and stimulated adipose preparations. The small antilipolytic effects of glucose and insulin that were exhibited in nonlactating or late lactation cows were not
exhibited in cows during early lactation (Metz and Van den Bergh, 1972; 1977).

Responses of cows in mid-lactation to excess energy intakes (high grain) or insulin injections revealed 50 percent reductions in basal and stimulated FFA and glycerol release compared to normal fed cows (Yang and Baldwin, 1973b). Reduced lipolytic rates were equivalent to those observed in nonlactating cows. Bergman (1968) substantiated these results in vivo by showing a decreased concentration and entry rate for glycerol in sheep given insulin injections.

Antilipolytic effects are not the only consequence of glucose and insulin. Insulin, in the presence of glucose, stimulated the in vitro incorporation of acetate into fatty acids and glucose into glycerol (Yang and Baldwin, 1973a). In most studies, it is not possible to separate the effects of glucose and insulin. Baldwin and Smith (1971), however, showed that insulin increased both the incorporation of acetate into fatty acids and glucose into glycerol by 50 percent when glucose was present in all incubations.

**Glucagon**

The effect of glucagon on adipose metabolism in ruminants is equivocal. Radloff and Schultz (1966) injected glucagon into goats and found initial declines in plasma FFA followed by increases after 4 to 8 hours. These effects are probably indirect because glucose showed initial increases but was below pre-injection concentrations at 4 to 8 hours. Increases in plasma FFA (Bassett, 1971) and glycerol (Brockman et al., 1975a; Brockman, 1976) in response to glucagon have shown but only if the insulin increase due to the glucagon injection is
blocked. These results led Brockman (1978) to conclude that any physiological effects of glucagon on adipose metabolism are slight, if existent, and that the main effects are hepatic. In vitro effects of glucagon on adipose lipolysis also have been absent (Etherton et al., 1977).

**Free fatty acids and beta-hydroxybutyrate**

Ten mM beta-hydroxybutyrate (BHBA) (Bartos et al., 1975; Metz and van den Bergh, 1972) or butyrate (Metz and van den Bergh, 1972) decreased both basal and stimulated lipolysis 50 percent in adipose tissue incubated in vitro but were ineffective if dibutyryl cAMP was included also. Metz and van den Bergh (1972) also showed acetate, propionate, and acetoacetate to be ineffective in decreasing either basal or stimulated lipolysis. A subsequent study showed 2 mM BHBA to be as effective as 10 mM in decreasing both FFA and glycerol release from stimulated adipose tissue while butyrate at 2 mM was only slightly effective (Metz, 1974). These results not only suggest a physiological role for BHBA in regulating lipolysis but points to a direct effect upon the activity of HSL. Yang and Baldwin (1973b) were unable to show an antilipolytic effect for BHBA but the 150 mM concentration used may be responsible.

Further examination by Metz (1974) revealed that although BHBA had no effect on dibutyryl cAMP stimulated lipolysis, it completely negated the nine-fold increase in glycerol release due to theophylline. This negative suggests that BHBA either increases phosphodiesterase activity or decreases cAMP production. Evidence in support of the latter was presented (Metz, 1974).
In vivo results have given support to the in vitro data. Infusion of BHBA into fasted, fasted plus phlorizin, or diabetic goats reduced FFA concentrations (Menahan et al., 1966b). Furthermore, prolonged fasting of goats resulted in a negative correlation between plasma FFA and BHBA (Menahan et al., 1966a).

Direct effects of BHBA on adipose metabolism during high BHBA production are well-documented but the interaction with insulin, if any, is less clear. In nonruminants, the antilipolytic effects of BHBA are thought to be both direct and by stimulation of insulin secretion (Robinson and Williamson, 1980). Menahan et al. (1966b) found the effects of BHBA on FFA concentrations to be reduced slightly in diabetic goats. Similar BHBA concentrations in BHBA infused (fasted + phlorizin) and in diabetic goats gave dissimilar FFA concentrations suggesting that insulin may aid regulation of adipose metabolism (Menahan et al., 1966b). Insulin treatments were not given but tolbutamide, a stimulator of insulin secretion, decreased FFA concentrations when injected into fed or fasted goats but had no effect in diabetic goats. Schwalm and Schultz (1976b) found diabetic goats to have elevated FFA and ketones over fasted goats, suggesting that insulin deficiency not only may have negated some of the BHBA effects but impaired ketone metabolism as well. Horino et al. (1968) failed to show an insulin response to BHBA injection but blood concentrations of BHBA were not examined and may have been too low to produce a response.

Vernon (1980) recently published an extensive review of adipose metabolism in ruminants. His conclusion that the various factors shown
to regulate adipose tissue metabolism in ruminants have similar roles in nonruminants is consistent with the data presented.

Responses of adipose tissue to lactation

Some of the unique adaptations that occur at the time of parturition have been discussed already and duplication will be kept to a minimum. Lactation in many, if not all species brings rapid changes in adipose metabolism resulting in decreased lipogenesis and increased lipolysis, which increases nutrient supply for milk production.

Studies with rats show that the initiation of lactation is accompanied by dramatic decreases in adipose fatty acid synthesis, lipoprotein lipase activity, glyceride synthesis, and the number of insulin receptors per cell (Flint et al., 1979; 1980; 1981; Hamosh et al., 1970; Robinson and Wing, 1963; Smith, 1973; Smith and Walsh, 1976). Prolactin is increased and insulin generally is decreased during this time (Flint et al., 1981). Synthetic rate changes that occur in rat adipose and mammary tissue are no less dramatic than changes that occur in the lactating cow, indicating nutrient demands of these tissues are just as heavy or the regulatory mechanisms governing synthetic rates are more dependent on physiological state than on energy status.

For the rat, prolactin has been implicated in initiating changes of lactation. A recent paper by Flint et al. (1981) suggests however, that prolactin in the rat may have only indirect effects on adipose metabolism. In rats two days after removal of pups, fatty acid and glyceride synthesis, and the number of insulin receptors increased five to eight fold while prolactin decreased 60 percent. Similar effects were found in suckling
rats given bromocriptine (a prolactin secretion inhibitor) but not if simultaneous injections of bromocriptine and prolactin were given. These data suggest prolactin is maintaining depressed synthetic rates. If pups are removed and prolactin is given to maintain lactation, reversal to a nonlactating state results however, implicating other factors, perhaps from the mammary gland, in maintaining low rates of lipogenesis and high rates of lipolysis in adipose tissue.

Control mechanisms in ruminants are even less clear than in the rat but the tenaciousness of the regulation early in lactation is evidenced by the susceptibility of lactating cows to ketosis and the inability of glucose and insulin to revert adipose tissue from a lipolytic to a lipogenic state (Metz and van den Bergh, 1972; 1977). As in the rat, adaptations of bovine adipose tissue to lactation include increased basal and stimulated lipolysis (Jaster and Wegner, 1981; Metz and van den Bergh, 1977; Pike and Roberts, 1980), decreased fatty acid synthesis (Grichting et al., 1977a; Vernon et al., 1980) and decreased glyceride synthesis (Emery, 1979; Pike and Roberts). Baldwin et al. (1973) found increased rates of fatty acid and glyceride synthesis in lactating cows but from the production records it seems that cows were near the end of lactation and in positive energy balance.

Jaster and Wegner (1981) showed increased concentrations of beta-adrenergic receptors on adipocytes from early lactation cows compared to nonlactating cows and correlated these increases with increased lipolytic rates during lactation. Increased rates of basal or stimulated lipolysis have not always been observed (Grichting et al., 1977a; Sidhu and Emery,
1972) and the increased FFA:glycerol ratio during lactation indicates decreased re-esterification (Metz and van den Bergh, 1977; Sidhu and Emery, 1972; 1973; Yang and Baldwin, 1973b).

Emery (1979) recently reviewed the mechanisms of fatty acid release from adipose. From comparisons of HSL activity during varying physiological states, he concluded that HSL activity was regulated by minute to minute changes and that evidence for long term control is lacking. On the other hand, rates of glyceride synthesis correlate very well with total lipolytic and lipogenic capacity, suggesting the rate of glyceride synthesis determines the rate of FFA release. Increased HSL activity at parturition is generally small relative to the decrease in glyceride synthesis (Metz and van den Bergh, 1977; Shirley et al., 1973b).

Hove and Halse (1978) and Lomax et al. (1979) reported decreased concentrations and secretory responses of insulin in cows during early lactation. Decreased insulin probably is partly responsible for some of the chronic changes occurring in adipose tissue. Baldwin et al. (1976) suggest that insulin directly or indirectly increases synthesis of lipogenic enzymes. Consequently, low insulin would decrease the capacity for lipid synthesis and encourage lipolysis by leaving the catabolic actions of lipolytic hormones unopposed.

Hepatic Fatty Acid Metabolism

Within physiological ranges, fatty acid uptake by the liver changes with the concentration of FFA in the plasma (Heimberg et al., 1974).
Adaptations within the liver govern the fate of fatty acids depending on physiological state. The scheme that follows outlines what is believed to be the primary end products of hepatic fatty acid metabolism.

**FREE-FATTY ACIDS**

- Oxidation
- Esterification
- ACETYL-CoA
- TRIGLYCERIDES and PHOSPHOLIPIDS
- KETONE BODIES
- CO₂
- ACETATE

The processes of esterification and acetate formation now will be discussed briefly whereas production and utilization of ketones will be dealt with in 'Ketone Metabolism in Animal Tissues'.

**Esterification of fatty acids**

The enzymes required for acylation of glycerol-3-phosphate are located in both the microsomal and mitochondrial liver cell fractions (Bremer et al., 1974). Although differences exist between the systems, the presence of an acylating system on the outer mitochondrial membrane allows for direct competition for FFA between oxidation and triglyceride formation. It is not surprising then that a major control point for the fate of fatty acids exists at the acylation step (see 'Ketone Metabolism in Animal Tissues'-'Regulation of Hepatic ketogenesis').

Numerous studies with perfused rat livers, indicate that output of triglyceride is proportional to the concentration of FFA in the medium (Heimberg et al., 1974; Mayes, 1976). The rate of output as triglyceride
secreted per mole of available FFA is less, however, during fasting than in the fed state, resulting in a reciprocal relationship between esterification and oxidation (Mayes, 1976). These results are with less than saturating concentrations of FFA. Decreased esterification with fasting does not seem to result from decreased availability of glycerol-3-phosphate, because correlations between rates of esterification and concentrations of glycerol-3-phosphate have been poor for rats (McGarry and Foster, 1971b). Also, McGarry et al. (1973) showed that inhibition of fatty acid oxidation during fasting of rats resulted in virtually all the fatty acids being esterified, demonstrating that capacity for esterification is not limiting. The present belief is that the fate of fatty acids is regulated by their uptake into mitochondria, not by the capacity of liver for esterification. Further evidence in support of this will be presented in the section 'Ketone Metabolism in Animal Tissues'.

Heimberg et al. (1974) state that quantity of VLDL secreted by the liver in normal fed rats is determined by the necessity to secrete triglycerides. This statement suggests the pathway of triglyceride synthesis acts as an overflow valve for the liver, secreting esterified fatty acids when the need for oxidation is low or when oxidation capacity is saturated.

Besides fasting, decreased hepatic triglyceride output has been noted with experimental diabetes, and administration of glucagon, dibutyryl cAMP, or catecholamines (Heimberg et al., 1974; Nikkila, 1974; Woodside and Heimberg, 1976) while glucose and insulin are stimulatory (Nikkila, 1974; Woodside and Heimberg, 1976). Heimberg et al. (1974)
noted that the decreased triglyceride output of perfused rat liver due to dibutyryl cAMP was accompanied by an increase in both glycogenolysis and ketone production. The similar responses noted for glucagon, experimental diabetes, norepinephrine, and fasting suggest a common mechanism, perhaps elicited via cAMP.

Mayes (1976) proposed a model to explain the effects of insulin and cAMP on hepatic FFA esterification. Lipoproteins draw triglyceride from a small pool that is in equilibrium with two other pools: stored triglyceride and FFA. As in adipose tissue, the rate of triglyceride mobilization to FFA in liver is regulated by cAMP-dependent HSL. Consequently, increased cAMP from glucagon and catecholamines would result in increased fatty acid availability for oxidation and decreased triglyceride availability for lipoprotein secretion. Insulin, on the other hand, decreases cAMP concentration and increases VLDL secretion. Mayes' (1976) proposal has much support (Nikkila, 1974).

Nikkila (1974) found in rats infused with [14C]palmitic acid that triglyceride production was unaffected by glucose infusion and associated hyperinsulinemia, but increased 50 percent if glucose was given in drinking water for five days. Woodside and Heimberg (1976) found a delayed response to insulin in diabetic rats infused with fatty acid and suggested that insulin may be doing more than regulating the rapid flux of cAMP, perhaps by regulating the activity of certain hepatic enzymes.

Heimberg et al. (1974) looked at the relationships between hepatic triglyceride output, accumulation, and ketogenesis as a function of FFA uptake in fed, normal, and diabetic rats. Their results may explain much
of the variation observed in seemingly similar experiments. In normal fed rats, the primary fate of FFA was triglyceride secretion, which increased linearly as uptake of FFA increased. Upon saturation of the triglyceride pathway, liver triglyceride accumulation and ketone body production increased in direct proportion to the increased uptake of FFA.

In diabetic animals, fatty acid oxidation to ketones is the primary route of metabolism with production increasing in direct proportion to uptake of FFA (Heimberg et al., 1974). Triglyceride secretion also increased with increasing FFA uptake but due to siphoning by the oxidative pathway, maximum rates required a three-fold increase in FFA concentration. Their data indicate that at any given FFA concentration, normal animals secreted more and oxidize less triglyceride than diabetic animals. Triglyceride accumulation in diabetic rats occurred after the capacity of the other two pathways was met, again requiring 2 to 3 fold greater concentrations of FFA than normal animals. Similarly, Woodside and Heimberg (1976) found oleic acid perfusions had no effect on fat accumulation in anti-insulin serum treated rats but increased hepatic triglyceride 3.5 fold in eight hours in normal fed rats.

**Acetate production from long-chain fatty acids**

Lindsay (1975), in reviewing previous work on fatty acid metabolism in sheep, concluded that a major portion of long-chain fatty acids oxidized were released as free acetate and that more than one tissue (liver, muscle, and mammary) may be responsible. This conclusion is supplemented by findings that only 50 to 60 percent of total acetate
entry rate could be accounted for by ruminal production (Annison and Armstrong, 1974; Annison et al., 1974; Kronfeld, 1968). Recently however, Pethick et al. (1981) calculated that acetate entry may have been over estimated by approximately 10 percent in the earlier experiments, which would lessen but not eliminate the contribution of endogenous acetate.

Baird et al. (1975) and Costa et al. (1976), using cannulated cattle to measure liver metabolism, found a variable, yet net production of acetate in lactating cows. Costa et al. (1976) proposed that the acetate was derived from oxidation of free fatty acids that were shown to be extracted by the liver in sufficient quantities. Annison and Armstrong (1970) stated that endogenous acetate production closely parallels the mobilization and metabolism of FFA in response to nutritional status. Palmquist (1972) showed that plasma acetate could be derived from palmitate and that the percentage of acetate derived from palmitate increased upon fasting. However, it was not clear whether endogenous acetate production actually increased with an increased supply of fatty acids.

Soling et al. (1974), using perfused rat liver, found livers from diabetic animals released three to four times more acetate than livers from fed or starved rats. In response to a hexanoate load, all three groups showed significant increases in acetate production but the diabetic animal showed a greater absolute increase. Differential response could be accounted for by a greater ketone production in the normal and starved rats due to the hexanoate load. Differences in
acetate production between starved and diabetic rats cannot be explained by enzyme activities because both groups showed equivalent increases in acetyl-CoA hydrolase and decreases in acetate thiokinase activity (Soling et al., 1974). Thus, acetate production may be particularly important when high rates of fatty acid oxidation are occurring.

Pethick et al. (1981) looked at endogenous acetate production in normal and fasted diabetic sheep. Although acetate production in liver accounted for an increased percentage of acetate entry in fasted sheep (44 vs 23), this increase could all be accounted for by decreased rumen acetate production. The reason for the absence of increased acetate production during fasting (increased FFA available) is not known. A five fold increase in plasma ketone body concentration in fasted sheep suggests increased fatty acid oxidation, but only a 36 hour fast may not have been adequate to increase FFA concentration to high enough levels.

The role of carnitine in fatty acid transport in ruminant tissues is not well understood. Although free acetate can diffuse through the mitochondrial membrane, the high concentrations of total acid soluble carnitine (short-chain fatty acid - carnitine complex) and the high activity of carnitine acetyltransferase in ruminant muscle and liver suggests a role for carnitine in the transport of acetate (Snoswell and Henderson, 1980). Snoswell and Henderson (1980) reported that total acid soluble carnitine increases significantly in muscle and liver as the severity of a ketotic state increases. Secretion of acid-soluble carnitine into milk by ketotic cows also has been reported (Erfle et al., 1970). The relationship between acetyl carnitine production and hepatic acetate production needs further study.
Ketone Metabolism in Animal Tissues

A number of excellent reviews have appeared recently dealing with the regulation of production and utilization of ketone bodies (KB) and their role in metabolism (McGarry and Foster, 1972; McGarry and Foster, 1980; Robinson and Williamson, 1980; Williamson, 1981). The majority of this review material deals with monogastric animals but until more is known about intermediary metabolism in ruminants, the basic assumption of similar metabolism will be made. Known discrepancies will be discussed.

The term ketone bodies collectively refers to three compounds: acetoacetate (AcAc), beta-hydroxybutyrate (BHBA), and acetone. KB should not be regarded as waste products or simply as end products of incomplete metabolism, for they serve important roles in animal tissues. Ketone bodies act as lipid precursors, oxidative fuels, and regulators of metabolism. The present review will deal primarily with regulation of production of KB and potential effects of those KB on metabolism.

Pathways of ketone production and utilization

The primary substrates for hepatic ketogenesis are FFA. Consequently, a prerequisite for ketone synthesis is a flux of FFA across the liver. This flux occurs from increased adipose mobilization brought about by the interplay of regulatory hormones as discussed already. After activation to the acyl-CoA derivative in the cytosol, the fatty acid may cross the mitochondrial membrane and enter the oxidative pathway (see 'Regulation of Hepatic Ketogenesis'). Transport across the inner
mitochondrial membrane is achieved by the enzyme carnitine acyltransferase I (CAT I) (Fig. 1 p. 46) converting the fatty acyl-CoA to the carnitine ester. On the inner surface of the inner mitochondrial membrane, carnitine acyltransferase II (CAT II) reforms fatty acyl-CoA and liberates free carnitine. Oxidation of the fatty acid yields acetyl-CoA that enters the mitochondrial pool. Obviously, any substrate that can form acetyl-CoA can form ketone bodies. But it is generally believed that the size of the acetyl-CoA pool dictates the flow to ketone bodies, which increases during rapid acetyl-CoA generation such as with fatty acid oxidation (McGarry and Foster, 1980). It should be mentioned that medium- and short-chain fatty acids do not require the carnitine carrier system and can be quite ketogenic.

Ketone synthesis within the liver is thought to proceed from acetyl-CoA via the hydroxymethylglutaryl-CoA (HMG-CoA) cycle as shown below:

1. $2 \text{Acetyl-CoA} \rightleftharpoons \text{Acetoacetyl-CoA} + \text{CoA}$
2. $\text{Acetoacetyl-CoA} + \text{Acetyl-CoA} \rightleftharpoons \text{Hydroxymethylglutaryl-CoA} + \text{CoA}$
3. $\text{HMG-CoA} + \text{Acetoacetate} + \text{Acetyl-CoA}$
4. $\text{Acetoacetate} + \text{NADH} + H \rightleftharpoons \text{beta-hydroxybutyrate} + \text{NAD}$

Enzymes for reactions 1 to 4 are: acetoacetyl-CoA thiolase, HMG-CoA synthase, HMG-CoA lyase, and D-beta-hydroxybutyrate dehydrogenase (HBDH) (McGarry and Foster, 1980). These enzymes are not limited to the liver, but HMG-CoA synthase is found in large quantities only in liver, which limits ketone production to this organ (McGarry and Foster, 1980). Direct deacylation of acetoacetyl-CoA is catalyzed by acetoacetyl-CoA deacylase;
5. Acetoacetyl-CoA ⇆ Acetoacetate + CoA
This reaction is thought to contribute little to ketone production due to the low activity of the enzyme and its high Km for acetoacetyl-CoA (Newsholme and Start, 1973b).

Ketone bodies diffuse readily into the blood and are transported to extrahepatic tissues where, before oxidation, they are re-converted to acetyl-CoA as follows.

6. Beta-hydroxybutyrate + NAD ⇆ Acetoacetate + NADH + H
7. Acetoacetate + Succinyl-CoA ⇆ Acetoacetyl-CoA + Succinate
8. Acetoacetyl-CoA + CoA ⇆ 2 Acetyl-CoA
These reactions are mitochondrial and the enzymes of reactions 6 and 8 are common to the ones for ketone formation. Reaction 7 is catalyzed by 3-oxoacid-CoA transferase and is found in many tissues, the liver being a notable exception (Newsholme and Start, 1973b). The very low activity of this transferase is believed to be responsible for the lack of ketone oxidation by liver.

Acetoacetyl-CoA may also be synthesized directly, catalyzed by acetoacetyl-CoA synthetase:

9. acetoacetate + ATP + CoA ⇆ Acetoacetyl-CoA + AMP + PP
Acetoacetyl-CoA synthetase is found in the cytosol with only about 10 percent as much activity as 3-oxoacid-CoA transferase (Robinson and Williamson, 1980). Coupled with a cytosolic thiolase, this enzyme may provide acetyl-CoA for synthetic purposes.

Utilization of ketone bodies is controlled largely by blood concentrations rather than by changes in enzyme activities (Bates et al.,
1968; Robinson and Williamson, 1980). This control seems to be true for both ruminants and nonruminants (Baird et al., 1970), however, it is not absolute. In rat brain, starvation increases the activity of 3-oxoacid transferase (Robinson and Williamson, 1980). Such an increase may not be true for ruminants where utilization of ketones by brain is not detectable (Leng and Annison, 1964) although the enzymes are present (Watson and Lindsay, 1972).

Decreased ketone utilization has been found during prolonged starvation (Robinson and Williamson, 1980) and in diabetic animals (Ballasse and Havel, 1971; Keller et al., 1978; Sherwin et al., 1976). Decreased utilization has been accompanied by decreased 3-oxoacid transferase activity in skeletal muscle from diabetic rats (Fenselau and Wallis, 1976). Kaufman and Bergman (1971) found ketone utilization by the kidney to be reduced in induced acidotic sheep compared to fasted. Impaired KB catabolism suggests that some of the severe ketoacidosis may be due to underutilization rather than over-production of KB. Exaggerated increases in KB accumulation relative to production also may result from saturation of extra-hepatic KB metabolism (Robinson and Williamson, 1980). It is not clear whether the effects of tissue saturation or underutilization have been differentiated.

Ballasse and Havel (1971) have suggested that insulin is necessary for tissue utilization of ketones, because utilization seem to decrease with diabetes and is increased by insulin administration. Bates et al. (1968), on the other hand, were not able to detect impaired ketone utilization in 48 hour alloxan diabetic rats. Leng (1966) found
differing effects of glucose and insulin on ketone metabolism in ketotic sheep. Glucose seemed to decrease ketone entry rates while insulin increased utilization.

Changes in rates of ketone utilization also may originate within the cell. The reactions involved in ketone utilization are thought to be near equilibrium in the cell. Thus, reversibility may occur if the acetyl-CoA pool is large (Robinson and Williamson, 1980). Increased fatty acid oxidation by extrahepatic tissues may lead to a reversal but it seems unlikely because increased ketone body concentrations decrease fatty acid mobilization and actually would make the contribution of ketones to the acetyl-CoA pool larger.

During lactation, a large portion of the ketone pool is directed toward the mammary gland. This direction is accomplished by both an increase in mammary gland weight and an increase in blood flow. BHBA can be incorporated into milk fat of cows (Smith and McCarthy, 1969) and goats (Linzell et al., 1967). Parallel changes of acetoacetyl-CoA synthetase with mammary lipogenic rates suggest a path of ketone utilization that by-passes mitochondrial conversion to acetyl-CoA (Robinson and Williamson, 1980).

Peculiarities of ketone body metabolism by ruminants

Ruminants are unique in that high rates of ketone production may occur in epithelial tissue of the rumen as well as in the liver. This uniqueness was dramatically illustrated by Baird et al. (1979) using catheterized lactating cows to measure both gut and liver metabolite fluxes. Fed and starved cows had similar plasma concentrations and total splanchnic
productions of ketones. But, while the fed cows had equivalent KB contributions from the gut and liver, KB in starved cows originated only from the liver. In fed ruminants in positive energy balance, the primary precursor of KB is butyrate from rumen fermentation (Katz and Bergman, 1969; Leng and West, 1969; Roe et al., 1966). Conversion of butyrate to BHBA occurs in both rumen epithelium and liver. During negative energy balance, free fatty acid oxidation in the liver assumes a major role in ketone production (Katz and Bergman, 1969).

Rumen epithelium has sufficient activity of all the enzymes necessary to produce acetoacetate and beta-hydroxybutyrate by the HMG-CoA pathway (Baird and Hibbitt, 1969; Baird et al., 1970). Butyrate and long-chain fatty acids have been shown to be effective substrates for these enzymes (Cook et al., 1968; Giesecke et al., 1979; Taylor and Jackson, 1968). Thus, Baird et al. (1970) suggested that the HMG-CoA pathway is the primary path of ketone body production.

Hird and Symons (1961), studying differential labelling patterns of BHBA formed from butyrate, concluded that up to 26 percent of the KB from rumen epithelium were produced by acetoacetyl-CoA deacylase. Low activity of this enzyme has been found in rumen epithelium (Baird et al., 1970). Bush and Milligan (1971b) proposed that a significant amount of ketones could be formed by reversal of 3-oxoacid-CoA transferase. Additions of 15 mM succinate increased acetoacetyl-CoA disappearance three fold. How functional this reverse pathway is in the intact animal is not known. The ratio of BHBA:AcAc present in portal blood from gut origin is high (4:1) (Baird et al., 1979) despite the apparent low
activity of beta-hydroxybutyrate dehydrogenase (HBDH) in rumen epithelium (Baird et al., 1970; Watson and Lindsay, 1972).

Pathways of ketone production in the liver and utilization by extra-hepatic tissues are thought to be similar in ruminants and nonruminants (Baird et al., 1970; Koundakjian and Snoswell, 1970). Synthesis of KB by the HMG-CoA pathway is considered to predominate and the lack of HMB-CoA synthase in all extra-hepatic tissues, except rumen, excludes these tissues from any ketogenic capacity (Baird and Hibbitt, 1969; Baird et al., 1970). The presence of acetoacetyl-CoA thiolase and HBDH in many extra-hepatic tissues (mammary, kidney, striated muscle, heart, and brain) suggests that a variety of tissues are capable of ketone body utilization (Baird and Hibbitt, 1969; Baird et al., 1970; Koundakjian and Snoswell, 1970). HBHA oxidation to carbon dioxide has been shown for kidney, heart, spleen, brain, and striated muscle with little or no oxidation found in liver or rumen tissue (Leng and Annison, 1964).

Although the pathways of ketone body production and utilization seem similar in ruminants and nonruminants, the cellular distribution of enzymes may differ. HMG-CoA synthase and HMG-CoA lyase seem to be confined to the mitochondria much as in nonruminants (Baird et al., 1970; Hannah Research Report, 1978). But curiously, HBDH appears predominantly in the cytosolic fraction. Nielsen and Fleischer (1969) isolated mitochondria from bovine heart and kidney and found HBDH activity to be equivalent to that in the rat. On the other hand, while rat liver showed increased HBDH activity over other tissues, no HBDH was detected in liver from bovine or ovine.
Subsequent studies indicate the majority of HBDH activity from liver, kidney, and rumen tissue is in the cytosol with kidney having the greatest activity (Koundakjian and Snoswell, 1970; 1972; Watson and Lindsay, 1972). Williamson and Kuenzel (1971) studied the stereospecificity of kidney HBDH and found the preferred substrate to be L-BHBA (the D isomer is the product of ketone metabolism). Furthermore, no D-BHBA was formed if AcAc was a substrate. They concluded that the sheep kidney enzyme was unlikely to play a major role in ketone body interconversions.

Koundakjian and Snoswell (1972) also found HBDH from rumen and liver to have equal activity if the substrate was either the L or DL mixture of BHBA, concluding that these enzymes were similar to the kidney enzyme. Watson and Lindsay (1972) however, found D-BHBA to be an equally effective substrate for liver and rumen but not for kidney HBDH.

While impressive, failure to isolate mitochondrial HBDH may be an artifact of mitochondrial damage during isolation. Koundakjian and Snoswell (1970) studied the rate of ketone production from intact isolated mitochondria. Acetoacetate production from palmitoylcarnitine was equivalent in sheep and rats, however, no BHBA was detected from sheep mitochondria. Attempts to increase BHBA production by lowering the NAD:NADH ratio or by inhibiting the Krebs cycle were quite effective in rats but totally ineffective in sheep. Thus, it would seem that a major difference exists between ruminant and nonruminant tissues in hepatic ketone production.
The consequences of cytoplasmic HBDH are only speculative. The BHBA:AcAc ratio declines during starvation in ruminants while it increases in rats (Baird et al., 1979; Koundakjian and Snoswell, 1970). The suggestion has been made that the low activity of HBDH in ruminant liver coupled with the unfavorable redox state in the cytoplasm allows for a predominant acetoacetate production (Koundakjian and Snoswell, 1970). The explanation is probably not as simple as this suggestion because the liver in the fed state produces BHBA while extracting AcAc, whereas fasting (nonlactating) resulted in increased ketone production but no change in the BHBA:AcAc ratio. In contrast, fasted lactating cows showed a three-fold decrease in the hepatic BHBA:AcAc ratio (Baird et al., 1979). Baird (1965) found a 10-fold increase in AcAc production but detected no change in BHBA production in liver slices from ketotic cows as compared to normal. Baird's results suggest not only a shift in the source of ketones but in the ratio of their output as well during ketosis.

A number of suggestions have been made for the role of HBDH in non-ruminants. One possibility is that it serves to shuttle electrons into the mitochondria; another is that the ratio of BHBA:AcAc reflects the mitochondrial redox state of the liver and in turn coordinates the redox state in extra-hepatic tissues (Nielsen and Fleischer, 1969). Recently, Zammit et al. (1979) proposed that mitochondrial redox state not only dictates the BHBA:AcAc ratio, but, by so doing, regulates total ketone body production by modulation of a futile cycle. Why the cellular distribution of HBDH differs between species and how this variation influences ruminant metabolism remains obscure.
Metabolic effects of ketone bodies

Increased ketone body concentrations are regulatory in that they signal a carbohydrate deficiency and alter metabolism in an attempt to correct the deficiency. A prolonged deficiency, however, can lead to exaggerated ketone body concentrations and accompanying pathological consequences. Thus, it seems that ketone bodies act to buffer an energy deficient state but if the severity overwhelms their capacity, metabolism is altered to assure maximum probability of survival.

As already discussed, ketone bodies are oxidized readily by many tissues and thus conserve substrates that may be needed in other capacities. This conservation seems to be particularly true for glucose although the antilipolytic effect of ketones has been mentioned already. Robinson and Williamson (1980) reviewed the effects of ketones on carbohydrate metabolism and showed that, in all tissues tested, increased ketone availability was accompanied by either decreased glucose uptake, decreased pyruvate oxidation, or both. All tissues were capable of ketone oxidation and exhibited high rates of glycolysis. Subsequent studies reported by Robinson and Williamson (1980) indicate that the inhibition is via an increased acetyl-CoA pool. Acetyl-CoA would directly inhibit pyruvate dehydrogenase (PDH) and, through increased citrate, to inhibit phosphofructokinase. Direct inhibitory effects of ketones on PDH activity in bovine liver mitochondria have been shown (Rifkin, 1963).

The effects of ketone bodies on the mammary gland deserve special note because of its high demands for glucose. Both starvation and
administration of acetoacetate were found to decrease the glucose arterial-venous difference across the mammary gland of lactating rats (Robinson and Williamson, 1977a; 1977b). Glucose utilization in vitro was decreased 33 percent by acetoacetate and was accompanied by increased concentrations of citrate and pyruvate (Williamson et al., 1974). Similar metabolite increases were reported in starved lactating rats (Robinson and Williamson, 1977a). Thus, the mammary gland of the rat seems to respond much like other ketone utilizing tissues. Starved lactating goats showed a four-fold decline in glucose extraction by the mammary gland with decreased incorporation into lactose, glycerol, and carbon dioxide (Chaiyabutr et al., 1980). A three-fold decline in mammary blood flow was the probable cause of the decreased glucose extraction rather than any change in acetoacetate concentration. Whether changes similar to those in rats occur in ruminants remains to be shown.

It seems unlikely that glucose-sparing mechanisms would be as beneficial to ruminants as to rats, considering the relatively low oxidation of glucose and its minor contribution to fatty acid synthesis. In other words, mechanisms to conserve glucose are active even in fed ruminants. Robertson et al. (1980) found PDH activity to be low in ruminant adipose tissue and found a high correlation (.99) between activity of PDH and incorporation of glucose into fatty acids. This strong correlation may be one reason for low glucose oxidation and high glucose conversion to lactate in adipose tissue. PDH activity was much greater in tissues that do not synthesize fatty acids, suggesting a potential role for KB in limiting glucose utilization (Robertson et al., 1980).
Much attention has been focused recently on the interrelations of ketone bodies, muscle metabolism, and hepatic gluconeogenesis and ketogenesis in nonruminants. A recurrent finding during prolonged starvation or diabetic ketonemia is decreased blood concentrations of both alanine and glucose (Binkiewicz et al., 1974; Sherwin et al., 1975; Sherwin et al., 1976; Wolfsdor, 1980).

The decline in blood glucose can be accounted for by decreased hepatic glucose output (Owen et al., 1969) while that of alanine by the five-fold drop in V-A differences across the forearm (Felig and Wahren, 1974). Felig and Wahren (1974) also found fractional extraction of alanine by the splanchnic bed to be decreased by 50 percent during prolonged starvation. These decreases occur despite reduced plasma insulin and elevated glucagon, changes that would be expected to increase hepatic amino acid extraction and gluconeogenesis (Wahren et al., 1972). Dimunition of muscle proteolysis and hepatic amino acid extraction, which are occurring at high rates in the postabsorptive state, are envisioned as protective mechanisms against severe loss of body protein (Felig and Wahren, 1974; Robinson and Williamson, 1980). Indeed, death will occur after loss of 30 to 50 percent of total body protein (Robinson and Williamson, 1980). As may be presumed, ketone bodies are thought to be responsible for depressing amino acid availability and subsequent metabolism.

Sherwin et al. (1975) found a six-hour continuous infusion of BHBA to give similar declines in plasma alanine (40 percent) as a three to five week fast. Urinary nitrogen also declined by 30 percent, indicating
decreased muscle catabolism. BHBA infusions into three to five week fasted humans resulted in further declines in plasma alanine suggesting a concentration dependent response (Sherwin et al., 1975). Greater reductions in plasma alanine in fasted diabetic (Sherwin et al., 1976) and ketotic hypoglycemic children (Pagliara et al., 1972) compared to normal children support the role of KB on muscle proteolysis.

Mebane and Madison (1964) infused either D-BHBA or acetoacetate into catheterized dogs and noted a 50 percent reduction in hepatic glucose production. Acetate infusions had no effect while L-BHBA was just as effective as D-BHBA. Mebane and Madison (1964) suggest the decrease in glucose production is not due to changes in the acetyl-CoA pool or in the redox state of the liver. The fact that alanine therapy reversed the depression in glucose output suggests that decreased glucose production is the result of decreased substrate supply rather than altered hepatic metabolism (Ozand et al., 1978; Pagliara et al., 1972). Dependence of hepatic glucose production on substrate supply is supported by the prophylactic effect of glucocorticoids (Pagliara et al., 1972).

Decreased glucose production in the liver has been most readily explained by the decreased availability and hepatic uptake of alanine (Felig and Wahren, 1974; Pagliara et al., 1972). Effects of KB on decreasing amino acid availability and utilization for glucose synthesis have been shown to be independent of fluctuations in insulin or glucagon (Miles et al., 1981; Sherwin et al., 1976). Recently however, Miles et al. (1981) suggested that increased insulin in response to ketone
bodies may decrease hepatic glucose production. While no increase in peripheral plasma insulin was found in response to a ketone body load, increased concentrations of C-peptide suggest increased insulin secretion and probable sequestration by the liver. Miles et al. (1981) contend that the 25 to 50 percent reduction in glucose production cannot be accounted for by decreased alanine because only 5 to 10 percent of glucose production in the postabsorptive state is from alanine. Pagliara et al. (1972) indicate that more than 30 percent of the net glucose production after a fast of 24 to 48 hours can be contributed by alanine, but this increased contribution is probably due to the depletion of glycogen and decreased glucose output.

Ozand et al. (1978) infused alanine into starved rats to increase blood concentrations to those found in fed rats. Not only was glucose synthesis increased from alanine but a two-fold increase occurred from lactate as well. Ketogenesis from oleate was abolished and concentrations of circulating ketones were reduced drastically. Their results suggest alanine may influence rates of gluconeogenesis and ketogenesis by means other than substrate supply. Furthermore, lactate concentrations may increase as much as 30 percent leaving doubt as to an absolute substrate deficiency (Miles et al., 1981). Little is known about effectors of hepatic gluconeogenic capacity during long periods of energy deficiency, but ketone bodies seem to be likely candidates.

Metabolic acidosis can occur in uncontrolled diabetes (McGarry and Foster, 1972). Although acidosis is an extreme situation and not always noted, even during ketone body infusions (Mebane and Madison, 1964), the
possibility of intracellular acidosis due to ketone body accumulation seems likely. Iles et al. (1977) looked at the effects of pH on glucose production in perfused rat liver. Lowering the pH of the perfusion medium from 7.4 to 6.8 resulted in a 4 fold decline of hepatic lactate extraction while glucose output was diminished 3 fold. Results with pyruvate in the infusion media, showed that lowering the pH reduced pyruvate extraction 2 fold and glucose output by the liver 1.5 fold. Hepatic metabolite data, including decreased total oxaloacetate concentrations, were suggestive of a limitation prior to phosphoenolpyruvate formation with decreased cycling through the Krebs cycle.

Nosadini et al. (1980) proposed that the mechanism of the antiketogenic response of alanine was an increase in the concentration of oxaloacetate and a concomitant increase in acetyl-CoA oxidation. Additionally, ketone bodies have been shown to decrease the activity of Krebs cycle enzymes from bovine mitochondria (Rifkin, 1963), while bovine ketosis therapy results in increased concentrations of Krebs cycle metabolites and a proposed increase in Krebs cycle activity (Baird and Heitzman, 1970). Thus, it seems that rates of gluconeogenesis and ketogenesis are both dependent on adequate availability of oxaloacetate and that outside influences that alter Krebs cycle activity will influence both processes. Furthermore, the ability of alanine to decrease ketogenesis and increase gluconeogenesis simultaneously, suggests that normal Krebs cycle activity produces ample oxaloacetate for both ketone oxidation and gluconeogenesis. The role of ketones in regulating hepatic metabolism needs further work.
Proposed regulation of hepatic gluconeogenesis by ketone bodies, deserves a comment as to its potential importance in ruminants. Gluconeogenesis in monogastrics can be considered a catabolic process because the substrates (amino acids, glycerol, and lactate) are derived ultimately from body tissue. Short-term carbohydrate deficiencies of less than 24 hours are met by increased gluconeogenic rates. Long-term carbohydrate deficiencies cannot be alleviated by muscle catabolism, however, and a need for alternate fuel supplies other than glucose is presented. Consequently, the protein-sparing effect and concomitant decrease in gluconeogenesis, as ketone bodies increase, are not just beneficial but mandatory for survival.

In ruminants, gluconeogenesis is an anabolic process with the majority of precursors being supplied from the diet, especially in the fed state. Thus, any endogenous signal that might limit the rate of gluconeogenesis could have catastrophic results. At first glance, the unique role of gluconeogenesis in ruminants seems to down-play the potential importance of ketone bodies on hepatic gluconeogenesis. However, during early lactation it is likely that an increased proportion of glucose originates from endogenous tissue sources and it is probable that preservation of these tissues is as important in ruminants as in nonruminants. It is possible, thus, that as the ketotic state develops, limitations of endogenous substrates may occur. This possible limitation and the effects it may have on gluconeogenesis have not been studied in ruminants.
Regulation of hepatic ketogenesis

The integration of fatty acid metabolism and glucose production in the liver is illustrated in Figure 1. The transfer of citrate from the mitochondria to the cytosol is of minor importance in ruminants but is a major pathway for generation of acetyl-CoA for fatty acid synthesis in many nonruminants. Assuming the pathway of triglyceride synthesis (Fig. 1) processes long-chain fatty acids the mitochondrion does not use (McGarry et al., 1973), then the potential mechanisms for regulating ketone production are:

1. concentration of fatty acyl-CoA,
2. rate of fatty acyl-CoA entry into mitochondria,
3. acetyl-CoA concentration, in part based on the rate of acetyl-CoA oxidation via Krebs cycle.

The third point is based on the lack of evidence for altered activity of ketogenic enzymes during increased ketone body production.

Free fatty acids are primary substrates for ketone synthesis during energy deficiency and therefore an adequate supply is a prerequisite for KB synthesis. This prerequisite was confirmed in diabetic rats where the ketotic state was negated by depletion of adipose (McGarry and Foster, 1980). However, they cite numerous publications indicating that an increase in free fatty acids is not always associated with increased ketogenesis. Furthermore, ketone body production can be inhibited despite maintained concentrations of free fatty acids (McGarry and Foster, 1980). Thus, two levels of control exist; one being the supply of fatty acids to the liver and the other being hepatic adjustments directing the fate of fatty acids (McGarry and Foster, 1972; 1977; 1980).
Figure 1. Hepatic fatty acid and glucose metabolism
A similar dual control seems likely in ruminants. Bergman (1971) showed that ketone body concentrations in sheep blood increased exponentially as plasma free fatty acids increased. Although impaired KB catabolism may be partly responsible, the magnitude of response to only slight increases in FFA suggests a major shift in FFA metabolism. A study with lactating and nonlactating cows being starved revealed similar increases and concentrations of FFA as starvation progressed (Baird et al., 1979). However, fasted lactating cows showed immediate and drastic increases in ketone bodies reaching values almost ten fold higher than fasted nonlactating cows.

Katz and Bergman (1969), using catheterized sheep to measure trans-liver balances, calculated that nearly 80 percent of the FFA taken up by the liver of ketotic sheep would have to be oxidized to account for ketone body production. In nonketotic sheep, less than 30 percent oxidation of FFA extracted would account for the ketones produced, prompting the authors to conclude that a shift in fatty acid metabolism within the liver may be a factor in ketosis development. Jarrett et al. (1976) compared muscle preference for energy substrates in fasted and exercised sheep. Exercise resulted in greater concentrations of FFA but three-fold lower concentrations of KB compared to starved sheep. Furthermore, the hind-limb of fasted sheep extracted 400 percent more FFA but extracted only 30 percent the amount of KB in the exercised sheep. Thus, it seems that as in humans, FFA concentration alone is not sufficient to increase KB production and that hepatic control also exists.
Much early work focused upon decreased disposal of acetyl-CoA via the Krebs cycle as the reason for increased ketogenesis. Because oxaloacetate (OAA) is necessary for acetyl-CoA condensation, a deficiency of OAA would limit Krebs cycle activity (Fig. 1). Deficiencies of OAA could result if a major portion was shuttled to gluconeogenesis (Krebs, 1966), when there is an insufficient supply of precursors (Bergman, 1973; Hibbitt, 1980), or when the redox state of the mitochondria is reduced (Bergman, 1973). Problems with assaying mitochondrial OAA have hampered the generation of equivocal evidence of a deficiency. Other workers (Newsholme and Start, 1973b), have debated the absolute OAA deficiency, instead implicating a direct inhibition of citrate synthase.

McGarry and Foster (1971a) suggested that decreased Krebs cycle activity may result from increased ketogenesis but in most cases is not the cause of the increase. Using free octanoate, which is not utilized in TG synthesis and does not require carnitine to enter the mitochondria, they showed that ketogenesis occurred with no depression of carbon dioxide production until high rates of ketone production were achieved. McGarry and Foster (1972) give much support for their conclusion that a decrease in Krebs cycle activity is not a prerequisite to obtaining rapid ketogenesis. Furthermore, the decreased Krebs cycle activity during active ketogenesis may have been the result of decreased lipogenesis rather than a deficiency within the Krebs cycle (McGarry and Foster, 1980).

Role of malonyl-CoA in regulating fatty acid oxidation Because ketone bodies act as an alternative to glucose for oxidation, it would
be logical to assume that the signal for increased ketogenesis would adequately reflect the carbohydrate status of the liver and thus the need for KB. This assumption was taken by McGarry and colleagues (McGarry et al., 1978a; 1978b; McGarry and Foster, 1979a) in looking for potential regulators of fatty acid transfer into mitochondria.

Malonyl-CoA was found to inhibit fatty acid oxidation (McGarry et al., 1978c) in liver homogenates. Positive correlations between hepatic lipogenesis and malonyl-CoA concentrations (McGarry and Foster, 1979a) while findings of decreased malonyl-CoA concentrations with increased ketogenesis (McGarry et al., 1978b; 1978d) suggest malonyl-CoA fluctuates in concert with carbohydrate availability. Further evidence in support of malonyl-CoA is the reciprocal relation found between rates of hepatic lipogenesis and ketogenesis (Benito and Williamson, 1978). McGarry et al. (1978a) identified carnitine palmitoyltransferase I (CAT I) as the site of inhibition by malonyl-CoA with no change in activity noted for carnitine palmitoyltransferase II (CAT II) (Fig. 1).

Recent evidence suggests that malonyl-CoA may be more important in preventing oxidation of newly synthesized fatty acids than in directing fatty acids in fasted rats (Cook et al., 1980; Ontiko and Johns, 1980). Benito and Williamson (1978) found no increase in fatty acid oxidation in liver hepatocytes from starved rats when fatty acid synthesis was inhibited in vitro (decreased malonyl-CoA). This lack of an increase however, was no doubt due to the high concentrations of fatty acid used, which are known to inhibit acetyl-CoA carboxylase (Sugden et al., 1980). Inhibition of fatty acid synthesis by high levels of FFA may not be an
adequate explanation for the attenuated response in fasted rats to malonyl-CoA inhibition of ketogenesis (Ontiko and Johns, 1980). Thus, regulation by malonyl-CoA may be only part of the total regulation.

The role of malonyl-CoA in the regulation of fatty acid oxidation in ruminants is unknown. It seems unlikely that malonyl-CoA would be a major physiological regulator, considering that little fatty acid synthesis occurs in ruminant liver and thus malonyl-CoA concentrations should fluctuate little.

**Hormonal effects** Hormonal regulation of ketogenesis has centered on the relative concentrations of insulin and glucagon. The ketotic state, as we know it to exist in all species, is characterized by a deficiency of insulin. Insulin deficiency results in increased fatty acid mobilization from adipose tissue with additional effects within the liver. Perfusions of livers from insulin-treated diabetic rats, showed increased rates of TG secretion and decreased ketone productions compared to nontreated livers (Woodside and Heimberg, 1976). The insulin effect was time dependent, taking a minimum of 24 hours to restore synthetic rates to those observed before the diabetic state was induced. This time dependency suggests a role for insulin in regulating fatty acid metabolism that may involve enzyme synthesis.

Whether an insulin deficiency is enough to create a ketotic state is contested highly. Sherwin et al. (1978) infused somatostatin into human subjects to decrease both insulin and glucagon. Blood BHBA concentrations increased five fold. A similar study in dogs showed no effect of somatostatin alone but addition of glucagon increased palmitate
oxidation to ketone bodies and carbon dioxide two fold (Keller and Shulman (1979). The concentrations of FFA also can influence directly their hepatic fate, making definitive conclusions about hormones difficult. Insulin withdrawal from insulin-dependent diabetics results in increased hepatic production of ketones and glucose (Miles et al., 1980). Accompanying these changes are dramatic increases in plasma glucagon (Miles et al., 1980).

Numerous studies have shown positive ketogenic responses to glucagon; both in vivo (Keller and Shulman, 1979) and in vitro (McGarry and Foster, 1979a; McGarry et al., 1978b; Siess et al., 1978). The responses are thought to be mediated via cyclic-AMP. Ketogenic rates from medium- and short-chain fatty acids are not responsive to dibutyryl cAMP, suggesting that one role of glucagon is to direct FFA between oxidation and esterification (Williamson, 1979).

Glucagon probably has numerous effects on the liver. Decreased lipogenesis and malonyl-CoA concentrations (McGarry and Foster, 1979a) could result from inhibition of acetyl-CoA carboxylase and pyruvate kinase by phosphorylation (Williamson, 1979). This inhibition would relieve the inhibition on carnitine acyltransferase and increase FFA oxidation rates. Increased glycogenolysis (McGarry and Foster, 1978) and perhaps direct effects on rates of esterification (Williamson, 1979) and carnitine turnover (Zammit, 1981) also have been implicated as increasing the ketogenic response.

Glucagon and insulin have been suggested to have similar effects for ruminants as for nonruminants. Brockman (1979c) found increased
glucagon secretion with increased carbohydrate demand in sheep, suggesting an elevation during ketosis-prone periods. Glucagon infusions into alloxan-diabetic sheep (insulin concentrations maintained at normal) increased hepatic acetoacetate production (Brockman, 1976). In fasted alloxan-diabetic sheep, injections of somatostatin to depress glucagon, decreased plasma BHBA concentrations (Brockman and Johnson, 1977). This decrease suggests direct hepatic effects of glucagon much like effects in nonruminants.

Plasma insulin concentrations generally are decreased during early lactation (Hart et al., 1979; Smith et al., 1976) and perhaps to a greater extent during ketosis (Hove, 1978; Schwalm and Schultz, 1976a). Total glucagon concentrations may increase after parturition (Manns, 1972) but variations in gut and pancreatic responses make interpretation difficult (Berzins and Manns, 1979). It seems unequivocal that a lowered insulin:glucagon ratio results in increased ketogenesis and this lowered ratio may be particularly important during ketosis development. The role of these hormones, particularly glucagon, in maintaining a ketogenic state may be of little importance as high concentrations of FFA have similar effects on ketogenesis as glucagon.

Bovine Lactation Ketosis

Lactation ketosis may occur in healthy cows during early lactation with the greatest incidence being between 10 to 40 days postpartum. As indicated already under 'Postparturient Adaptations of Dairy Cows', most high-producing cows during the first 4 to 8 weeks of lactation are in
negative energy balance. Energy deficiency results both from the lag in energy intake as opposed to energy output in milk and from the limitations of intake imposed by forage rations.

Attempts to locate specific defects in metabolism have been futile. This failure has resulted in the general belief that ketosis is simply a matter of supply and demand (Baird, 1981a; Bergman, 1971). The high demand and priority allotted to the mammary gland for nutrients, in particular glucose, exceeds the cow's ability to supply adequate quantities of nutrients for all tissues (Hibbitt, 1980). Rapid mobilization of fatty acids from adipose tissue results, with concomitant increases in ketone production.

It would seem from the preceding comments that ketosis is contingent on the mammary gland playing a primary role of dictating nutrient needs. That is, the mammary gland extracts the glucose it needs to meet lactation requirements. During early lactation, glucose demand is high creating a deficiency in peripheral tissues. If the demand for glucose far exceeds the supply, the fractional availability to peripheral tissue would be less, resulting in ketosis. Lactose content of milk is constant and is synthesized primarily (70-85 percent) from glucose (Lindsay, 1979). Thus milk volume would be dependent on the quantities of glucose extracted (Chaiyabutr et al., 1980), while ketosis on glucose availability to extra-mammary tissues. It is not known how mammary extraction of glucose varies during lactation but extraction may not be a simple function of supply (Faulkner et al., 1980). The theory of a central role for the mammary gland in ketosis, however, is far from being universally accepted.
Theories of ketosis development generally have been based on inference. That is, results obtained after ketosis develops are taken to reflect changes occurring before ketosis develops. Consequently, little is known about precipitating causes of ketosis. This lack of knowledge results from two primary limitations. First, the nation-wide incidence of spontaneous ketosis is only about 5 percent, making collection of adequate numbers of samples difficult. Second, by the time ketosis is diagnosed, animals are generally off feed, hampering definitive explanations as to cause or effect. These limitations have led to the need for animal models of ketosis.

One model that has been used extensively and that lends support for the central role of the mammary gland in ketosis development is the fasted lactating cow. Under fasting conditions, the supply of gluconeogenic precursors is limited resulting in decreased glucose availability, increased fractional extraction of glucose by the mammary gland, and increased stress on extra-hepatic tissues to oxidize KB and to supply gluconeogenic precursors. A deficiency of adequate substrate also may increase the ketogenic response by decreasing Krebs cycle activity (Hibbitt, 1980).

While many similarities exist between this fasting model and spontaneous ketotic cows (Baird et al., 1972), differences in glucose production (Hibbitt, 1980; Kronfeld, 1971) and distribution (Kronfeld, 1971) cause doubts as to its validity. These points of doubt will be elaborated on subsequently, but are mentioned now to illustrate problems encountered in ketosis research and to indicate areas of research that
would most supplement our understanding. One, is the need to study the lactating cow prior to clinical ketosis in an attempt to outline developmental changes. Secondly, animal models are necessary but they must be evaluated in light of what is known about spontaneous ketosis so that the models accurately reflect the syndrome.

Metabolic changes during bovine ketosis

**Blood metabolites**

Concentrations of blood metabolites and hormones typically observed in ketotic and nonketotic cows are summarized in Table 1. Hypoglycemia and the associated hypoinsulinemia during ketosis may be the major contributors initiating the ketotic state. Glucose entry rates are purported to be normal during the initial stages of ketosis (Kronfeld, 1971), adding support for a central role of the mammary gland rather than impaired metabolism. Decreased insulin concentrations may well be a response to the decreased glucose concentrations. Not only are insulin concentrations depressed, but insulin secretory response to feeding (Hove and Halse, 1978) or glucose infusion (Hove, 1978; Lomax et al., 1979) is attenuated also, suggesting pancreatic alterations.

Low insulin concentrations would have a number of consequences. First, maximum rates of adipose lipolysis would be achieved causing release of FFA and glycerol. Second, without the stimulatory effects of insulin on protein synthesis in muscle greater quantities of amino acids would be available for gluconeogenesis and mammary protein synthesis (Brockman, 1978). Third, increased availability of endogenous ketogenic (FFA) and glucogenic (glycerol and amino acids) precursors would promote
Table 1. Blood metabolite and hormone levels in ketotic and nonketotic lactating cows

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Nonketotic</th>
<th>Ketotic</th>
<th>Referencesa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>50</td>
<td>28-39</td>
<td>A,B,C,D</td>
</tr>
<tr>
<td>Ketone bodies (mg/dl)</td>
<td>5</td>
<td>11-40</td>
<td>A,B,C,D</td>
</tr>
<tr>
<td>Acetate (mg/dl)</td>
<td>10</td>
<td>20</td>
<td>D</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>14-20</td>
<td>8-15</td>
<td>A,B,C,E</td>
</tr>
<tr>
<td>FFA (mM)</td>
<td>.14</td>
<td>.4-1.2</td>
<td>A,B,C,D</td>
</tr>
<tr>
<td>Glycerol (mM)</td>
<td>.08</td>
<td>.15</td>
<td>F</td>
</tr>
<tr>
<td>BHBA:AcAc</td>
<td>13-25</td>
<td>3.5-4.5</td>
<td>B,I</td>
</tr>
<tr>
<td>Lactate:pyruvate</td>
<td>17</td>
<td>23</td>
<td>F</td>
</tr>
</tbody>
</table>

Hormones

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Nonketotic</th>
<th>Ketotic</th>
<th>Referencesa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (ng/ml)</td>
<td>.75-1.5</td>
<td>.4-.5</td>
<td>G,K</td>
</tr>
<tr>
<td>(uU/ml)</td>
<td>10-30</td>
<td>5</td>
<td>D,H</td>
</tr>
<tr>
<td>Growth hormone (ng/ml)</td>
<td>6.5</td>
<td>5.8</td>
<td>J</td>
</tr>
</tbody>
</table>

a(A) Radloff and Schultz, 1967; (B) Bergman, 1971; (C) Schultz, 1974; (D) Schwalm and Schultz, 1976a; (E) Yamdagni and Schultz, 1970; (F) Baird et al., 1968; (G) Hove and Halse, 1978; (H) Manns, 1972; (I) Hibbitt and Baird, 1967; (J) Reynaert and Peeters, 1977; (K) Hove, 1978.

maximum rates of these hepatic processes. Fourth, any direct inhibitory effects by insulin on rates of gluconeogenesis or ketogenesis would be decreased. The increased concentration of acetate (Table 2) may reflect low insulin although the possibility of increased endogenous production cannot be ruled out (Schwalm and Schultz, 1976a). A finding of decreased acetate entry rates during ketosis is support for decreased acetate utilization during ketosis (Kronfeld, 1968).
As already discussed, increased rates of lipolysis and ketogenesis can be means of conserving glucose by providing competing oxidative substrates. Increased concentrations of both FFA and ketone bodies reflect the hypoglycemic state and the need for alternative fuel sources. It is curious that glycerol concentrations do not change to the same extent as FFA concentrations. Bergman (1968) had found glycerol turnover and concentration to be linearly related in sheep. A FFA:glycerol ratio greater than three indicates either differential metabolism for FFA and glycerol or incomplete hydrolysis within the adipose.

Changes in the ratios of BHBA:AcAc and Lact:Pyr in ketotic cows are reflected in both plasma (Table 1) and liver (Table 2). The characteristic decrease in BHBA:AcAc in ruminants is clearly evident. The fact that both liver and plasma ratios change concurrently suggests that the primary shift is in the liver which is the site of production. The mammary gland has been suggested to convert BHBA to AcAc resulting in a ratio shift across the gland (Kronfeld, 1971).

Hepatic metabolites As discussed already, carbohydrate status somehow dictates the need for increased ketogenesis. In rat studies, rapid increases in KB concentrations occur 15 to 20 hours after initiation of a fast, which correlates well with depletion of glycogen (Williamson, 1979). It is not too surprising then to find a major reduction in hepatic glycogen during ketosis (Table 2). This reduction seems to be a prerequisite for the ketotic state to be manifested, but the signal that initiates the reduction in ruminants is unknown.
Table 2. Hepatic metabolites in ketotic and nonketotic cows

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Condition of cow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonketotic</td>
</tr>
<tr>
<td>Glycogen</td>
<td>3-4</td>
</tr>
<tr>
<td>Fat</td>
<td>3</td>
</tr>
<tr>
<td>BHBA</td>
<td>.5</td>
</tr>
<tr>
<td>AcAc</td>
<td>.01</td>
</tr>
<tr>
<td>BHBA:AcAc</td>
<td>40-50</td>
</tr>
<tr>
<td>Lactate:pyruvate</td>
<td>13-30</td>
</tr>
<tr>
<td>Alanine</td>
<td>1.6</td>
</tr>
<tr>
<td>Glutamine</td>
<td>2.5-3.5</td>
</tr>
<tr>
<td>Oxaloacetate</td>
<td>2x10^-3</td>
</tr>
<tr>
<td>Glutamate</td>
<td>3.2</td>
</tr>
<tr>
<td>α-Ketoglutarate</td>
<td>.11</td>
</tr>
<tr>
<td>Citrate</td>
<td>.34</td>
</tr>
<tr>
<td>Phosphoenolpyruvate</td>
<td>.12</td>
</tr>
<tr>
<td>2-Phosphoglycerate</td>
<td>.05</td>
</tr>
<tr>
<td>3-Phosphoglycerate</td>
<td>.31</td>
</tr>
</tbody>
</table>

^Glycogen and fat are percent wet weight tissue; other metabolites are umoles/g wet weight tissue.

^(A) Bergman, 1971; (B) Baird, 1967; (C) Hibbitt and Baird, 1967; (D) Baird et al., 1968; (E) Baird, 1977; (F) Baird and Heitzman, 1970; (G) Baird and Heitzman, 1971; Treacher et al., 1976.

Fat accretion in the liver occurs in most cows during the initial stages of lactation (Collins and Reid, 1980). This accumulation can be accounted totally for by increased TG (Brumby et al., 1975; Collins and Reid, 1980) and probably is due to the rapid mobilization of adipose lipid as negative energy balance develops. Reid et al. (1979a) found livers from fed cows to extract FFA and secrete TG while fasting resulted in net extraction of each. Decreased TG secretion also has been shown when perfused livers from starved rats were compared to those
from fed rats (Mayes, 1976). The fact that nonketotic cows accumulate less TG (Table 2), and return to normal sooner than ketotic cows suggests potential hepatic impairment by lipid (Reid et al., 1979a; 1979b). One consequence of proposed impaired hepatic function attributed to fatty liver may be reduced fertility (Reid et al., 1979b; 1979c). How fatty liver affects liver function and the extent of involvement of fatty liver in ketosis development is not known.

Hepatic glycogen concentrations seem to reflect, with reasonable accuracy, the carbohydrate status of cows. It is possible that glycogen concentrations influence the direction of metabolism, directing fatty acids toward oxidation when carbohydrate demand exceeds supply. It is possible also that an intermediate of glycolysis or the Krebs cycle has a regulatory role much like malonyl-CoA in humans and rats. Some of the metabolite changes occurring in ketosis are listed in Table 2. All Krebs cycle intermediates tested, with the exception of malate, plus the glucogenic amino acids, were found to be decreased significantly in ketotic livers (Baird et al., 1968; Baird, 1977). These findings do not aid in pin-pointing a particular regulatory metabolite, instead suggesting that many metabolites fluctuate with carbohydrate availability and are potential regulators of fatty acid oxidation.

Oxaloacetate has dual roles between glucose and carbon dioxide production. These roles are particularly important in ruminants where nearly all glucose must be synthesized and all glucogenic precursors except glycerol must enter the oxaloacetate pool. The decline in hepatic oxaloacetate concentration has prompted the suggestion that a high rate
of gluconeogenesis is the predisposing factor to ketosis (Baird et al., 1968; Krebs, 1966). While OAA depletion may be the case for non-ruminants, such as rats (Krebs, 1966), there are no indications of increased gluconeogenic rates (Kronfeld, 1971) or increased enzyme activities (Bergman, 1971; Hibbitt and Baird, 1967) in ruminants. The question then remains as to why the intermediate concentrations are depressed during ketosis.

Baird and his colleagues (Baird, 1977; Baird et al., 1972; Baird et al., 1979) have shown that almost all the changes indicated for spontaneous ketotic cows also occur in lactating cows that are fasted or anorexic after ketosis was induced with thyroxine plus a high protein diet (Hibbitt and Baird, 1967). A notable exception is plasma acetate, which declines during fasting (Baird et al., 1972). Significant decreases in activities of phosphoenolpyruvate carboxykinase (PEPCK) and pyruvate kinase also occur after a six-day fast (Baird et al., 1972), while similar trends are apparent during ketosis (Baird and Heitzman, 1971). The similarity of results in hepatic changes with fasted and ketotic cows suggest a common origin for the two situations. It seems apparent the common factor would be a shortage of glucose precursors during starvation, but how this shortage would result in the fed animal is not as apparent.

Baird et al. (1974), Bergman (1971), and Hibbitt (1980) maintain that it is the inability of cows to supply glucogenic precursors (amino acids, propionate, lactate, and glycerol) relative to milk output and glucose production that results in a net deficiency of glucose. Along
this same line, an increased Krebs cycle activity due to an increased acetyl-CoA pool would drain off some of the OAA. Hibbitt and Baird (1967), however, noted a reduction in oxygen uptake by ketotic liver compared to liver from normal lactating cows. One point needs to be reiterated, samples from spontaneous ketotic cows almost always are taken after the ketotic stage has been reached and clinical signs are apparent. Thus, these samples may reflect results of ketosis rather than point to causes. Kronfeld (1971,1972) has been insistent upon specifying the type of ketosis encountered, suggesting that the ketosis of fasting, and that observed following inappetence in primary ketosis, may not reflect precipitative changes. In other words, the anorexic state may be causing characteristic blood and liver changes but these changes may not reflect the state of the cow prior to reduction in feed intake. While anorexia will limit glucogenic precursor availability, another possibility, as yet unexplored in ruminants, is that the hyperketonemia may restrict endogenous supply of gluconeogenic precursors much like is seen in nonruminants.

Baird (1981b) recently examined data of hepatic metabolite concentrations and trans-liver balances in cows during different physiological states. He was able to identify three different states which were equated with the level of carbohydrate sufficiency. The nonketotic lactating cow is intermediate between nonlactating and fasting or ketotic. Greater carbohydrate deficiency (ketotic > nonketotic lactating > nonlactating) is associated with decreasing glycolytic and Krebs cycle intermediates. Table 2 shows two of these states (ketotic
and nonketotic lactating) and the basis for division of physiological state. Differences in physiological state are evident also in percentage hepatic extraction of lactate, being lowest in nonlactating, intermediate in lactating, and highest during fasting (Baird et al., 1980). Subsequent studies revealed these physiological states to be interconvertible as will be discussed in following section.

Effects of therapeutic agents The therapeutic agents most often used for the treatment of lactation ketosis are geared to increasing glucose supply relative to glucose demand. These agents may be in the form of glucose precursors (propionate, propylene glycol, glycerol, and lactate), hormones that affect carbohydrate availability (glucocorticoids, ACTH), or glucose itself (Burtis et al., 1968; Emery et al., 1964; Schultz, 1958; Schultz, 1974).

Baird et al. (1980) found hepatic glucose production to increase after parturition and to correlate directly, on a molar basis, with hepatic extraction of propionate. Propionate infusions into nonketotic lactating cows increased plasma glucose concentration only 15 percent while hepatic glucose production showed initial decreases but was increasing after 24 hours of infusion. Hepatic extraction of lactate following propionate infusion into lactating cows decreased and the percentage extraction declined to levels seen in nonlactating cows. Thus, increased availability of propionate created a state of increased carbohydrate sufficiency. Propionate may also be anti-ketogenic. In vitro, propionate caused an 85 percent decrease hepatic ketogenesis from butyrate (Bush et al., 1970). This decrease probably was due to
an increased supply of OAA for oxidation, although a direct effect on activity of HMG-CoA synthetase has been proposed (Bush and Milligan, 1971a).

Glucose (Treacher et al., 1976) and glucocorticoids (Baird and Heitzman, 1970; Baird and Heitzman, 1971) also increase the level of carbohydrate sufficiency in lactating cows, causing increased concentrations of hepatic metabolites even greater than those observed in non-lactating cows. These changes, while dramatic for Krebs cycle intermediates, were much less pronounced for glycolytic metabolites. Baird (1981a) suggests that both glucose and glucocorticoids, at least initially, decrease hepatic glucose production. Decreased glucose production was evident during the initial stages of a glucose infusion, but was clearly reversed and returned to pre-infusion values by 48 hours (Baird et al., 1980). Depression in hepatic glucose output somehow may be related to glucose concentration which was inversely related to hepatic production over 48 hours of glucose infusion.

Similar to effects of a glucose load, glucocorticoids may initially depress hepatic gluconeogenesis. Baird and Heitzman (1970, 1971) found decreased activity of PEPCK in nonlactating, lactating, and ketotic cows 48 hours after dexamethasone (a synthetic glucocorticoid) administration. Reilly and Black (1973) observed a decrease in glucose production during a three-hour cortisol infusion. This rapid decrease suggests that changes in enzyme concentration are not necessarily involved in depressed rates of glucose production. Also, an increase in the fractional rate of incorporation of alanine into glucose was noted. Baird and Heitzman (1970, 1971) found the activities of aspartate and tyrosine amino-
transferases and concentrations of some glucogenic amino acids to be increased following dexamethasone treatment. Increases in amino transferase activity suggests a greater availability of glucogenic precursors. However, metabolism of precursors to glucose is regulated by the most limiting (activity) enzyme which may be PEPCK.

Heitzman et al. (1972) compared the subcellular distribution of PEPCK and citrate synthase (CS) activity in rats and cows following glucocorticoid administration. In both rats and cows, PEPCK and CS activities were altered inversely to one another. Dexamethasone increased PEPCK and decreased CS in rats while the converse was true in cows. Decreased PEPCK activity in cows was confined to the cytosol enzyme while the mitochondrial fraction was unaffected. These results suggest that the major antiketogenic action of glucocorticoids within the liver is to shuttle glucogenic substrates through oxaloacetate and oxidation. Increased OAA availability would decrease both the acetyl-CoA:CoA ratio and ketogenesis (Baird et al., 1974). Increased Krebs cycle activity may help explain the increased concentrations of lactate and pyruvate following glucocorticoids if they are less well-utilized for glucose, but it does not explain the five-fold increase in liver glycogen and the recovery from ketosis (Baird and Heitzman, 1971).

Part of the answer to the therapeutic effects of glucocorticoids may lie in their actions on extra-hepatic tissues. Braun et al. (1970) observed a two-fold increase in plasma glucose 24 hours following an intramuscular injection of dexamethasone into normal and lactating cows, and this increase coincided with a 25 percent drop in milk production.
Corticoids are known to bind to mammary tissues of the bovine (Gorewit and Tucker, 1976a; 1976b) and to decrease glucose utilization (Gorewit and Tucker, 1977; Kronfeld and Ramberg, 1981; Livingston and Lockwood, 1975; Reilly and Black, 1973). The decreased glucose utilization may account for the decreased milk production and increased glucose concentration. The suppressive effects of glucose on hepatic gluconeogenesis have been discussed already. Insulin secretion may be involved also because this would decrease lipolysis and promote glycogen synthesis. It is interesting to note in the study of Braun et al. (1970), plasma glucose responded similarly to nonketotic cows given dexamethasone, with peaks at 24 hours, but showed little depression in milk production. After two days, glucose concentration had approached a new plateau while milk production increased.

When examining metabolic effects of glucocorticoids, the time after corticoid administration may be critical. Ballard et al. (1968) found no change in PEPCK, CS, or PC in either cytosolic or particulate fractions of liver from dexamethasone treated cows. Although the time interval after administration was not given it may be inferred from their statements that more than 48 hours passed before sampling the treated cows. Thus, it seems that initially, glucocorticoids suppress hepatic glucose production but it is doubtful that the duration of the suppression is long-lasting. Hepatic citrate concentration was still elevated over nontreated ketotic cows, suggesting a maintained state of carbohydrate sufficiency. It seems there is still much to be learned about the mechanisms of glucocorticoids alleviating ketosis but their effectiveness in ketosis therapy is unequivocal.
Gluconeogenesis in the Ruminant

Ruminants are herbivorous animals with a considerable capacity for pregastric fermentation. Thus, the majority of the digestible carbohydrates (cellulose, hemicellulose, starch, and soluble sugars) are fermented to volatile fatty acids. Such fermentation leaves a very small amount of intact glucose available for absorption. The extent of fermentation and the by-pass of α-glucose polymers (starch) to the small intestine is dependent on the type of starch available (Armstrong and Smithard, 1979) and the quantity consumed (Otchere et al., 1974). Generally, as the percentage starch in the ration is increased, the greater is the intact contribution of absorbed glucose to total glucose entry rate. Direct measurements have indicated no more than 10 percent of the glucose requirements are absorbed as glucose (Otchere et al., 1974), but this percentage is no doubt subject to prevailing conditions.

In the normal fed bovine, therefore, gluconeogenesis must provide up to 90 percent of the glucose requirement. Herein lies a major difference between ruminants and nonruminants. Hepatic glucose production is a continual process in ruminants, increasing after feeding in parallel with nutrient availability (Ballard et al., 1969). In contrast, monogastric liver extracts glucose in the fed state but produces glucose in the fasted state (Ballard et al., 1969). A difference is also evident in enzymatic adaptations. Increased rates of gluconeogenesis in fasted rats are associated with increased activity of PEPCK, whereas neither lactation, fasting, nor ketosis had any effect in ruminants (Baird and Heitzman, 1970; Baird and Heitzman, 1971; Ballard et al., 1969). Young
et al. (1969) found little change in PEPCK activity in cattle during eight days of fasting or during feeding energy-dense rations. These results have been interpreted as indicating that the major limitation to gluconeogenesis in ruminants is substrate supply.

Results with pyruvate carboxylase (PC) in ruminants are more conflicting. Like PEPCK, a significant amount of PC activity is found in the cytosol (Ballard et al., 1969), which is in contrast to nonruminants where both enzymes are predominantly mitochondrial. Ballard et al. (1969) found increased PC activity, in cytosol and mitochondria, with lactating, fasting, and ketotic cows when compared to nonlactating cows. Increased activity is in contrast to the work of Baird et al. (1968) where lactation decreased PC activity compared to nonlactating and ketotic cows. Propionyl-CoA carboxylase showed a similar trend to PC (Baird et al., 1968) while fructose-1,6-diphosphatase increased three fold in lactating and ketotic cows compared to nonlactating. A lack of consistent changes in enzyme activities probably is reflective of the continual need for gluconeogenesis in ruminants with little necessity for fluctuations in capacity. Data of Mathias and Elliot (1967) lend support for a steady gluconeogenic rate finding no change in propionate conversion to Krebs cycle intermediates in liver homogenates from cows in various stages of lactation or suffering from ketosis.

Dependency on gluconeogenesis is exaggerated postpartum due to the imposition of lactation and greatly increased glucose requirement. A direct relationship seems to exist between mammary extraction of glucose, lactose synthesis, and milk volume (Rook, 1979). Young (1977) describes
a cow that daily during peak lactation is producing 89 kg of milk containing 4.43 kg of lactose. An approximation of required hepatic glucose production is obtainable from glucose kinetic data. Assuming that 75 percent of the lactose is from glucose (Lindsay, 1979; Schmidt, 1971) and that 60 to 70 percent of the mammary extraction of glucose yields lactose (Lindsay, 1971), then 10.2 kg of glucose would have to be extracted daily by the mammary gland. If this extraction represents 70 to 90 percent of the glucose entry rate (Lindsay, 1971) and approximately 90 percent of the entry rate is of hepatic origin (Young, 1977), then gluconeogenesis would be responsible for about 11 kg of glucose per day. Therefore, the liver has to synthesize roughly its own weight in glucose daily. Assuming a plasma volume of 30 liters with a glucose concentration of 50 mg/dl, the glucose pool would have to be replenished totally every 2 minutes. Baird (1971b) measured hepatic glucose production in nonlactating cows and reported a value of 1 kg per day. While not all cows will have a 10-fold increase in glucose requirement as the cow illustrated, it is apparent that lactation imposes a tremendous burden on the liver to maintain optimum rates of gluconeogenesis. It is easy to visualize how even a small impairment in hepatic metabolic activity, and in particular gluconeogenesis, may culminate in metabolic disorders. It should be mentioned that the preceding calculations are based on observations of cows milking at much lower rates than 90 kg/day, and it is possible the glucose requirement is overestimated. Lindsay (1979) recently suggested that during high glucose demands, glucose sparing mechanisms become increasingly important and may account for much
of the glucose made available for lactose synthesis. His proposal, however, does not diminish the importance of gluconeogenesis in maintaining milk production.

Sources of carbon for gluconeogenesis

Glucose entry rates vary widely among ruminants in different physiological states, increasing during pregnancy (Leng, 1970; Lindsay, 1970) and lactation (Baird, 1981b; Leng, 1970; Lindsay, 1970; 1971; Wiltrout and Satter, 1972) and decreasing during fasting (Baird, 1981b; Leng, 1970; Lindsay, 1970). Stage of lactation is also important as entry rates are highest during peak lactation but are less during early and late lactation (Lindsay, 1970). Lindsay (1970) and Leng (1970) reported positive correlations between digestible energy intakes and glucose entry rates, indicating the importance of supply of glucogenic precursors from the diet. Baile et al. (1969), added to the major role of nutrient supply in regulating glucose production, by showing that goats made hyperphagic by hypothalamic lesions had glucose entry rates twice as great as normal goats.

In fasted sheep, glucose production declined by 33 percent at 24 hours and by 50 percent at six days (Lindsay, 1970). Lactating and non-lactating cows had nearly equal hepatic glucose productions after 4 days of fasting but the percentage drop was much more dramatic in the lactating group due to the higher initial glucose production rates (Baird, 1981b). These results indicate that endogenous precursors can supply some glucogenic precursors but not at rates necessary to mimic the fed state.
A rough estimate of the capacity of ruminants to supply endogenous precursors can be obtained from comparing glucose entry rates in the fed and fasted state. Assuming kidney glucose production accounts for 10 percent of the total glucose entry, a value of 10.5 g/(day x kg$^{-0.75}$) is obtained for total glucose production from fed nonlactating cows (Baird, 1981b; Wiltrout and Satter, 1972). Leng (1970) reported 2 to 5 day fasted cows had glucose entry rates averaging 7.2 g/(day x kg$^{-0.75}$). Similar values with cows have been reported after only 24 hours fasting (Lindsay, 1970). Twenty-four hours fast is probably not adequate for ruminants to be in a nonabsorbing state. For sheep, nutrient absorption is near negligible after 3 to 4 days fasting as glucose entry rates decrease to a plateau. Consequently, glucose production rates from cows fasted less than about 4 days may represent synthesis from substrates of both dietary and endogenous origin. Glucose entry rates reported by Leng, of 7.2 g/(day x kg$^{-0.75}$) or nearly 70 percent of the fed state, may be an overestimate of endogenous production. Baird (1981b) found hepatic glucose production of 3.1 g/(day x kg$^{-0.75}$) after 6 days fasting of cows. For total glucose production, correction is necessary for kidney glucose production, which probably increases during fasting (Krebs et al., 1965; Newsholme and Start, 1973a). Assuming kidney glucose production doubled to about 2 g/(day x kg$^{-0.75}$), total production would be 5.1 g/(day x kg$^{-0.75}$). Thus, endogenous sources can account for nearly 50 percent of the glucose entry rate found in fed nonlactating cows. Similarly, fasting in sheep decreases glucose entry rates approximately 50 percent (Leng, 1970).

Bergman (1973) states that only propionate, glycerol, amino acids, lactate, and pyruvate serve as significant precursors for gluconeogenesis.
in ruminants. Measuring their individual contributions to glucose production has presented some problems. As a consequence, a clear picture does not exist as to their relative importance. Two techniques are in vogue to estimate precursor-product relations. First, transfer quotients can be calculated from constant specific radioactivities of glucose and one of its precursors following infusion of radioactive precursor. A transfer quotient gives a minimal value of precursor utilization due to exchange of metabolites within metabolic pathways (Lindsay, 1978; Wiltrout and Satter, 1972). Second, metabolite balances across organs, particularly the liver, yield net extraction and thus the maximum contribution a precursor can make to a product. The trans-organ technique is also subject to metabolite exchange as well as to errors associated with measuring blood flow and small concentration differences. Where both of these techniques are used and results compared, a more accurate picture of precursor-product relations evolves.

Propionate Propionate is the only volatile fatty acid produced in the rumen that is capable of supporting net glucose synthesis. Rates of propionate production in the rumen (Herbein et al., 1978), and, more directly, hepatic extractions of propionate (Baird, 1981b; Bergman et al., 1966) are related positively to glucose production. Wiltrout and Satter (1972) continuously infused [2-14C]propionate into the rumen, sampled blood glucose, and concluded that 32 and 45 percent of the glucose was derived from propionate in dry and lactating cows, respectively. The correction made for metabolite exchange increased the transfer in the lactating group to 61 percent. Their results suggest that propionate is
utilized more efficiently for glucose synthesis during lactation. Similar responses to increased glucose demand have been observed when comparing pregnant and nonpregnant sheep (Leng, 1970). Leng (1970) suggests there is generally more glucogenic substrate absorbed than is used for glucose synthesis, citing the observation of increased glucose entry rates but constant feed intakes during pregnancy.

Propionate production could account for nearly all the glucose production in sheep (Leng, 1970) while values of 75 to 85 percent have been indicated for cows (Wiltrout and Satter, 1972). Some propionate may undergo metabolism during absorption (Leng, 1970; Lindsay, 1970), but the extent of conversion into lactate seems to be less than 5 percent (Weigand et al., 1972). Of the propionate absorbed, over 90 percent is extracted by the liver (Baird et al., 1980; Bergman et al., 1966), suggesting that liver is the sole site of gluconeogenesis from propionate.

Baird (1981b), using trans-liver balances, found that propionate could account for no more than 55 percent of the glucose output in lactating and nonlactating cows. Lindsay (1978) noted good agreement between estimates from isotope dilution and hepatic uptake suggesting little need for correction of metabolite exchange. His values of 42 percent agree reasonably well with the uncorrected values of Wiltrout and Satter (1972). Thus, it would seem that propionate contributes 35 to 50 percent of the carbon for glucose synthesis in the fed state.

Glycerol Most of the glycerol in the body is as a component of TG in lipid stores. Turnover of these stores in the fed state is low, thus glycerol availability is low, accounting for the 5 percent contribution of glycerol to glucose entry (Bergman, 1973; Leng, 1970; Lindsay,
Glycerol may be quite important, however, during periods of energy deficiency because it can be metabolized to glucose in both liver and kidney (Bergman, 1973; Lindsay, 1978). Fasting increased glycerol turnover two fold in normal sheep and 4.5 fold in ketotic pregnant ewes (Bergman et al., 1968). Over 50 percent of the available glycerol was converted to glucose, which resulted in a mean of 28 percent of the glucose entry rate being derived from glycerol in the fasted pregnant ewes and 23 percent in the nonpregnant fasted ewes (Bergman et al., 1968). Ketosis was induced by fasting, which would decrease propionate availability and glucose entry. However, entry rates were still equivalent to those in nonpregnant fed sheep where glycerol accounted for only 5 percent of the glucose. Glycerol infusion into fed sheep, at rates equivalent to glycerol entry rates in ketotic sheep, resulted in a 28 percent contribution to glucose entry (Bergman et al., 1968). The literature thus indicates that glycerol is gluconeogenic and if all available glycerol were converted to glucose, nearly 50 percent of the glucose needs for maintenance could be met.

**Amino acids** Trenkle (1980) points out that a positive correlation exists between the requirement for amino acids and glucose, suggesting that some of the amino acids may be used to supply substrates for gluconeogenesis. Studies looking at potential availability of amino acids, either by quantitating the amount of protein passing through the abomasum (Leng, 1970) or by quantitating urea nitrogen excretion (Trenkle, 1980), indicate protein could contribute over 50 percent of the glucose entry. Gut protein and urea nitrogen excretion may overestimate amino
acid availability for metabolism due to incomplete digestibility and absorption of protein, urea recycling, and ammonia absorption from the rumen (Bergman, 1973).

Wolff and Bergman (1972) using five amino acids, measured both incorporation into glucose (\(^{14}\text{C}-\text{amino acids}\)) and extraction by the liver. Between 11 and 29 percent of the glucose entry could have come from these five amino acids. Alanine and glutamine accounted for 80 percent of the conversion to glucose and 40 percent of these five amino acids extracted by the liver. Exchange of labeled amino acids with nonlabeled intermediates within the liver makes the 11 percent figure potentially low. Lindsay (1978), however, found similar values (11 percent) with both isotope dilution and hepatic extraction. Glutamic acid in the former study (Wolff and Bergman, 1972) was not extracted by the liver but made a substantial contribution to glucose synthesis. This discrepancy was explained by the glutamine-glutamate couple which together showed a net extraction. Glycine and serine were not gluconeogenic even though considerable extraction occurred (Wolff and Bergman, 1972).

Alanine and glutamine have been proposed to be involved in a nitrogen and carbon shuttle between muscle and liver (Bergman, 1973; Newsholme and Start, 1973a). This proposal suggests that deaminantion within the muscle results in amination of pyruvate and glutamate, with a net release of alanine and glutamine. The source of carbon for alanine and glutamine synthesis is unknown, but Trenkle (1980) suggests that only glutamine provides a net synthesis of glucose. Balance studies across the kidney indicate that glutamine is released in the fed state and extracted during ketosis where it may supply ammonia for acid
neutralization and carbon for gluconeogenesis. One question raised from these previous comments, if alanine does not account for the carbon of protein catabolism, where is it accounted for?

Alanine and glutamine extraction by lactating cow liver could account for a maximum of 18 percent of the glucose output (Baird, 1977). Black et al. (1968), using lactating cows and goats, injected individual amino acids and determined their incorporation into glucose. Aspartic acid was found to be the most glucogenic because it appeared earliest and with the highest specific radioactivity in plasma glucose. From the incorporation of amino acids into lactose, Black et al. (1968) calculated that approximately 30 percent of glucose entry could be derived from alanine, aspartate, serine, glutamate, and tyrosine. Their calculation assumed the radioactivity in lactose was all derived from glucose, which probably is not the case and direct conversion of amino acids into lactose within the mammary gland is likely. Consequently, the 30 percent is probably an overestimate.

As with other gluconeogenic precursors, the potential for amino acids seems greater than their incorporations indicate (Bergman, 1973). Many of the studies were with maintenance fed sheep and it would seem that more investigations of amino acid metabolism with lactating cows is warranted. Lindsay (1979) discussed the role of glycogenic amino acids during lactation suggesting amino acids may aid in increasing glucose availability without actually increasing glucose synthesis. He suggests that a major role of amino acids during lactation is to spare glucose metabolism in peripheral tissue thus increasing glucose availability for absolute needs such as lactose synthesis.
Lactate and pyruvate  Numerous publications indicate that lactate may supply approximately 20 percent of glucose entry (Lindsay, 1970; Lindsay, 1978; Prior, 1978). Baird (1981b) found that lactate extraction by the liver could account for 11 and 23 percent of the glucose output in nonlactating and lactating cows, respectively. The increase during lactation was accounted for by a three-fold increase in percentage of lactate extraction. However, as Young (1977) has indicated, it is important to differentiate between lactate derived via Cori cycle activity, which would not result in net glucose synthesis, and lactate derived from other sources.

Giesecke and Stangassinger (1980) recently reviewed lactate metabolism of sheep and indicated that up to 50 percent of the lactate is derived from glucose. Baird (1981b) showed portal lactate production in nonlactating cows to be 0.85 mmoles/(h x kg^{0.75}). Using lactate entry rate data from sheep of 1.8 mmoles/(h x kg^{0.75}) (Giesecke and Stangassinger, 1980), nearly 50 percent of lactate entry rate may thus be accounted for by portal production. However, not all portal lactate production is exogenous because some may result from glycolysis within the rumen epithelium. It also may be misleading to extrapolate entry rates across species, but these data indicate that a potentially large portion of lactate entry is not derived from glucose.

It is possible to quantitate the extent of Cori cycle activity using specifically labelled glucose (Young, 1977). Tritiated glucose labeled in either the 2 or 6 position will be lost prior to lactate formation and consequently will not be recycled. Comparing glucose
irreversible loss from 2- or 6-labeled glucose to that from \([U^{14}C]\)glucose will give an indication of the magnitude of chemical recycling. Bergman (1973) and Brockman et al. (1975b) cite data indicating recycling may account for 10 to 33 percent of glucose turnover in monogastrics. Results with sheep (Brockman et al., 1975b) and steers (Russell, unpublished; Dr. Rich Russell, Department of Animal Science, ISU, Ames, IA.) indicate very little chemical recycling, probably less than 5 percent of glucose entry. Low recycling indicates that in order for lactate to contribute 20 percent of glucose entry, a large portion of the lactate must come from sources other than glucose.

In addition to uptake by the liver, uptake of lactate by the kidney is significant. Lindsay (1978) reports that up to 65 percent of renal gluconeogenesis may be from lactate and pyruvate. They calculate that from liver and kidney combined, lactate may contribute up to 28 percent of the glucose synthesized. It is apparent that more work is necessary to define the sources of lactate and subsequent metabolism.

Regulation of gluconeogenesis in ruminants

Gluconeogenesis is not as much an episodic event in ruminants as in nonruminants. The consequences of a more constant rate on enzymatic adaptations have been discussed already. Also, the close correlations between digestible feed intake, propionate production, and glucose production have been mentioned. Correlations also have been noted between the irreversible loss of glycerol and propionate, and the production of glucose from each substrate (Lindsay, 1978). Results with lactate versus glucose production, however, were not as apparent (Lindsay, 1978). Judson and Leng (1973b) infused a casein hydrolysate and noted an
increased glucose output, which was related to the infusion rate. All these examples point to a major role of substrate supply in regulating glucose output. Substrate supply is not the sole regulator, however, because metabolite and hormonal signals are superimposed on substrate supply. As in nonruminants, these signals may alter the fate of various substrates, the availability of substrates, or both.

**Regulation by metabolites**

Butyrate increases the gluconeogenic rate in nonruminants, supposedly by increasing the activity of PC and decreasing pyruvate dehydrogenase and phosphofructokinase (Newsholme and Start, 1973a). The mechanism of action in ruminants is unknown, but increased rates of gluconeogenesis in lamb liver cells have been shown after butyrate supplementation (Clark et al., 1976). The physiological significance of butyrate regulation is questionable for two reasons. First, the response to butyrate in the study of Clark et al. was much more apparent when the substrate was galactose than propionate. Second, Judson and Leng (1973b) were unable to show an increased gluconeogenic rate from propionate during an intraportal infusion of butyrate. A lack of response may be because the portal concentration of butyrate is normally high.

Glucose is an effective suppressor of gluconeogenesis both in vivo (Judson and Leng, 1973a) and in vitro (Seto et al., 1971). The extent of suppression is dependent on the quantity of glucose infused (Judson and Leng, 1973a). Judson and Leng (1973a) suggest that the effect of glucose is extra-hepatic because low glucokinase activity would prevent glucose equilibration between blood and liver. One suggestion is that increased
insulin in response to glucose, suppresses lipolysis and proteolysis thus decreasing substrate availability (Judson and Leng, 1973a; Lindsay, 1978).

Baird (1981b) found glucose infusions into lactating cows to suppress hepatic glucose output by 60 percent. Decreased glucose production occurred in spite of normal propionate extraction. While the quantity of glucose synthesized from propionate was not measured, these results suggest intrahepatic effects of glucose. The fact that gluconeogenesis from propionate in vitro is decreased by glucose, supports an intrahepatic effect of glucose (Seto et al., 1971). Direct hepatic effects in vivo also may result from hormonal fluctuations. Indeed, insulin concentration in portal blood was markedly increased by glucose infusions in lactating cows (Baird, 1981b).

**Regulation by hormones** The significance of hormonal modulation of metabolism in ruminants has been questioned due to characteristic attenuated fluctuations and responses to hormones. However, hormones are no doubt equally important to ruminants as nonruminants, as hormone deficiencies (diabetic) are usually just as severe in ruminants (Schwalm and Schultz, 1976b).

Indirect effects of insulin on hepatic gluconeogenesis in ruminants are similar to those in nonruminants. By promoting peripheral utilization of substrate and preventing catabolism, effective hepatic starvation may result. Decreasing endogenous substrate availability may be particularly critical when glucose demand is high. In nonproducing ruminants that probably receive ample precursor from the diet, the liver has first priority of substrate. In such ruminants, the effects of insulin on
glucose production are minimal, resulting in 10 to 15 percent decreases during the initial stage of infusion (Brockman et al., 1975a; Brockman, 1978). Brockman (1978) has suggested that insulin has much greater capacity to influence glucose disposal than glucose output. In vitro results however, indicate that while insulin had only slight effects on basal glucose formation from lactate or fructose, it completely negated the two-fold stimulatory effect of glucagon (Clark et al., 1976).

Thus, portal blood insulin:glucagon ratio may be important in regulating hepatic metabolism as has been suggested for nonruminants (Unger, 1971). Insulin:glucagon ratio is normally lower in ruminants than in nonruminants (Bassett, 1975) and shows little postprandial fluctuations (Bassett, 1975; Brockman, 1978). However, fasting causes a 2 to 3 fold decline (Bassett, 1975), which is probably important in optimizing the glucogenic response from endogenous precursors. How insulin:glucagon responds during ketosis is unknown but intuitively, a decline would be expected from decreased insulin and glucose concentrations.

As already mentioned, glucagon increases glucose production in vivo (Brockman and Bergman, 1975; Brockman and Creer, 1980) and in vitro (Clark et al., 1976). Furthermore, a glucagon deficiency results in decreased glucose production (Brockman, 1979b). Stimulatory effects of glucagon result from an increase in glycogenolysis (Brockman, 1978), increased hepatic extraction of glucogenic precursors (Brockman and Bergman, 1975; Brockman et al., 1975a) and increased incorporation of precursors into glucose (Brockman and Bergman, 1975; Brockman et al.,
Brockman and Manns (1974) noted increased PC activity following glucagon administration while PEPCK and glucose-6-phosphatase were unchanged. PC also has been implicated as a potential site for insulin action (Judson and Leng, 1973a).

Glucocorticoids are very glucogenic in monogastrics, promoting muscle catabolism and thus amino acid availability, and increasing activity of a number of hepatic enzymes (Wicks et al., 1974). Significance of glucocorticoids in ketosis therapy already has been discussed. As in nonruminants, glucocorticoids promote muscle catabolism in ruminants (Bergman, 1973). Increases in glucocorticoids have been noted during fasting (Mills and Jenny, 1979) and ketosis (Bergman, 1973). Increased glucocorticoids during times of glucose deficiency would increase endogenous precursor supply and promote gluconeogenesis.

It becomes apparent that although substrate availability in ruminants quite obviously will limit gluconeogenic rates, signals similar to nonruminants are responsible for regulating availability and metabolism glucose precursors. Increased demand for glucose results in alterations that maximize availability and utilization of glucogenic precursors from both diet and endogenous sources.

Summary of the Literature

Lactation imposes a tremendous metabolic challenge to the dairy cow and requires coordination of many systems. Anabolic systems of mammary and liver tissue are emphasized with nutrients partitioned to meet productive capacity. Nutrients are derived from both the diet and from
endogenous sources. Ketosis is thought to be a consequence of inadequate nutrient supply in the face of a greatly increased nutrient output in milk. This is clearly evident if dietary nutrients are restricted.

Feed restriction results in a greater dependency on endogenous stores to meet milk production requirements. Adipose mobilization is enhanced to meet energy requirements, but a loss of glucogenic precursors from the diet is not replaced as readily. Decreased availability of glucose enhances the ketotic state by increasing the loss of glycogen and by increasing fatty acid oxidation. True ketosis, however, is not preceded by decreased dietary intake. Supposedly, nutrient drain by the mammary gland is severe enough to create a large energy deficit.

Ketosis is accompanied by major changes in concentrations of both blood and liver metabolites. Most notable are the increases in blood and liver ketone bodies, and marked alterations in liver glycogen and tri-glycerides. Ketone bodies have vast regulatory inputs on metabolism. How the ketotic state, and changes that accompany the ketotic state, affects tissue metabolism has not been explored.
PART I. METABOLIC STUDIES WITH LACTATING COWS

Introduction

Research in bovine ketosis dates back over 50 years, but productive research in ketosis (based on number of publications) has fallen sharply over the last decade. Our knowledge of ketosis management and therapy may have reduced ketosis to a tolerable incidence and thus diminished the urgency to seek a better understanding of its cause.

Ketone bodies are recognized as important energy sources, sparing oxidation of both FFA and glucose (Newsholme and Start, 1973b). A recent review has emphasized the importance of ketone bodies as physiological regulators of metabolism (Robinson and Williamson, 1980). Depressions in rates of lipolysis (Bartos et al., 1975; Metz and van den Bergh, 1972), muscle proteolysis (Felig and Wahren, 1974; Sherwin et al., 1975) and hepatic gluconeogenesis (Owen et al., 1969) may result from direct effects of ketone bodies.

How ketone bodies affect metabolism in ruminant tissues is unknown. In vivo data suggest that impaired metabolism is not responsible for ketosis development (Kronfeld, 1971). The most prevalent theory for ketosis development in the dairy cow is that the large nutrient demand by the mammary gland, relative to nutrient absorption from the gut, results in hypoglycemia and hypoinsulinemia and excessive fatty acid mobilization and ketogenesis (Baird, 1981a; Bergman, 1971; Fisher et al., 1971; Hibbitt, 1980). Only a few studies have compared tissue metabolism in normal and ketotic ruminants (Chung et al., 1959; Gallagher, 1960),
however, and virtually no data are available comparing tissue metabolic rates within the same animal as ketosis develops.

The present study was initiated to characterize the metabolism of the dairy cow before parturition and following parturition as the ketotic state develops.

Materials and Methods

Cows and management

Five dairy cows (3 Holstein, 1 Ayrshire, and 1 Brown Swiss), with an average 305 day milk production of nearly 7800 kg from the previous lactation, were placed on experiment 30 days before expected calving date. Cows were group fed during the dry period. Corn silage and brome hay were offered ad libitum with 4 to 5 kg of cracked corn per cow top dressed on the silage. Ration intakes were not recorded but were estimated to supply twice the net energy requirement of cows during the last 30 days of gestation (NRC, 1978). Chemical composition of feed-stuffs used during the course of this study are shown in Table 3.

Following parturition, herd silage-grain mix (Table 3) was offered ad libitum, plus 5 to 8 kg of alfalfa hay daily. Approximately 10 days postpartum, intake of the silage-grain mix was reduced 15 to 20 percent from a consecutive two day average during greatest intake, while the hay allocation was not changed. Also at this time, a ketone precursor, 1,3-butanediol (BD) was mixed into the silage-grain mix of four cows. Amounts of BD were increased over a one week period and ranged, after adaptation, between 0.7 to 1.4 kg per day. 1,3-butanediol was added in
Table 3. Chemical composition of feedstuffs

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Corn silage</th>
<th>Grain mix&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Alfalfa hay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>64</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Crude protein</td>
<td>2.5</td>
<td>11.1</td>
<td>18.7</td>
</tr>
<tr>
<td>Fat</td>
<td>3.1</td>
<td>4.1</td>
<td>2.7</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>7.8</td>
<td>2.7</td>
<td>24.5</td>
</tr>
<tr>
<td>Calcium</td>
<td>.04</td>
<td>.82</td>
<td>1.07</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>.08</td>
<td>.35</td>
<td>.32</td>
</tr>
</tbody>
</table>

<sup>a</sup>For cows postpartum, approximately 60 percent silage and 40 percent grain mix were mixed before feeding.

<sup>b</sup>Grain mix is 64 percent cracked corn, 5 percent whole or crushed oats, 29 percent soybean meal (44 percent), 1 percent dicalcium phosphate, 1 percent trace mineral salt, 2 x 10<sup>6</sup> I.U. vitamin A, and 2.5 x 10<sup>5</sup> I.U. vitamin D.

...
Table 4. Milk ketone rating and amounts of 1,3-butanediol fed to cows

<table>
<thead>
<tr>
<th>Cow</th>
<th>Early post-partum</th>
<th>Preketotic</th>
<th>Ketotic</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E&lt;sub&gt;b&lt;/sub&gt;</td>
<td>L&lt;sub&gt;c&lt;/sub&gt;</td>
<td>E</td>
</tr>
<tr>
<td>6965</td>
<td></td>
<td>0</td>
<td>0(.5) 2(.9)</td>
<td>3(1.1) 3(1.4)</td>
</tr>
<tr>
<td>7272</td>
<td></td>
<td>0</td>
<td>0(.5) 2.5(.9)</td>
<td>3(.9) 3(1.1)</td>
</tr>
<tr>
<td>7409</td>
<td></td>
<td>1</td>
<td>1(.5) 3(.5)</td>
<td>3(.7) 3(.7)</td>
</tr>
<tr>
<td>7559</td>
<td></td>
<td>0</td>
<td>2(0) 3(0)</td>
<td>0</td>
</tr>
<tr>
<td>7337</td>
<td></td>
<td>0</td>
<td>0(.9) 1(.9)</td>
<td>0(.9) 2.5(1.1)</td>
</tr>
</tbody>
</table>

*aPeriod designations are outlined in text.*

*b* 3 day average early in period.

*c* 3 day average late in period.

*d* Data show milk ketone rating (0 is nondetectable to 3 which is greatest concentration) and amount of BD fed in parentheses.

To 40). Treatment consisted of a return to ad libitum intake of the silage-grain mix, a single 10 ml intramuscular injection of dexamethasone<sup>1</sup>, plus twice daily drenches with 150 g of propylene glycol.<sup>2</sup> One ketotic cow also required 500 ml dextrose<sup>3</sup>, given intravenously at the initiation of treatment.

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<sup>1</sup>Dexamethasone (2 mg/ml), ISU Veterinary Service, Ames, IA.

<sup>2</sup>Propylene glycol (13.7 M), ISU Veterinary Service, Ames, IA.

<sup>3</sup>Dextrose - 50 percent; Bio-CEUTIC laboratories, St. Joseph, MO.
Experimental protocol

Biopsy technique
Liver and adipose biopsies were taken at near two week intervals from each cow, which divided the study into five distinct periods. These are: 1. dry (D), 2. early postpartum (EPP), 3. preketotic (PK), 4. ketotic (K), and 5. treated (TRT). The feeding program associated with each period is outlined in Table 5. Following parturition, tissue biopsies marked the end of a particular period. Following tissue biopsies, treatments associated with a subsequent period were initiated.

Distinctions between periods PK and K are based on the appearance of clinical signs of ketosis in four of the five cows during period K. Attempts were made to biopsy ketotic cows before a major anorexia occurred. However, clinical signs of ketosis and anorexia occur almost simultaneously. Thus, attainment of clinical ketosis without feed restriction was not accomplished. Besides slight depressions in feed intake, other characteristic signs of ketosis were evident including: gradual depressions in milk production over the last few days prior to treatment, milk ketones giving a +3 rating (range of 0-3), and behavioral changes. Discussion of behavioral signs will follow in 'Results and Discussion'. One cow (7337) did not show clinical signs of ketosis but was given the ketosis treatment just as for the ketotic cows.

Lidocane\(^4\) was used for local cutaneous anesthesia. For liver sampling, 3 to 4 ml of lidocane were injected from a central point in a

\(^4\)Lidocane hydrochloride-2 percent; Med Tech, Inc, Elkwood, KS.
Table 5. Feeding program for experimental animals

<table>
<thead>
<tr>
<th>Period</th>
<th>Feeding program</th>
</tr>
</thead>
<tbody>
<tr>
<td>D - dry</td>
<td>Ad libitum intake - approximately twice NE requirement for gestation</td>
</tr>
<tr>
<td>EPP - early postpartum</td>
<td>Ad libitum intake of silage-grain mix plus 5 to 8 kg alfalfa hay</td>
</tr>
<tr>
<td>PK - preketotic</td>
<td>15 to 20 percent restriction in mix. 5 to 8 kg alfalfa hay continued</td>
</tr>
<tr>
<td>K - ketotic</td>
<td></td>
</tr>
<tr>
<td>TRT - treated</td>
<td>Ad libitum silage-grain mix plus 5 to 8 kg alfalfa hay</td>
</tr>
</tbody>
</table>

pin-wheel design with a radius of 1 to 2 cm. Three to four grams of liver could be taken per biopsy if performed properly. As a rule, no more than three biopsies were performed on a cow in one day to keep hemorrhage to a minimum. Six to eight grams of liver tissue were taken by needle biopsy (Hughes, 1962) after a 12 to 15 mm incision was made either at the 11th or 12th intercostal space. A 1 gram liver section was rinsed in saline and immediately plunged into a thermos of liquid nitrogen. Time from taking the section to immersion was always less than thirty seconds. These samples were stored under liquid nitrogen 8 to 10 months until assayed for metabolites. The remainder of an individual liver sample was immersed in ice cold buffered saline, pH 7.4, and transported to the laboratory.
Obtaining liver samples by biopsy is not difficult but a few suggestions may be helpful. Guiding the cannula, once penetration of the peritoneum was achieved, varied depending on state of the animal and the site of incision. Generally, in fed cattle, the liver will be compressed against the right thoracic wall. Entering the body cavity at the 12th intercostal space, the cannula should be directed as cranially as possible causing a shallow penetration of the liver. If the 11th intercostal space is used, directing the cannula towards the left shoulder will avoid both the diaphragm and the large hepatic vessels. In cases where the liver has sagged away from the right thoracic wall (cattle not on full feed or shortly after calving), liver biopsies are more difficult. The liver changes position, moving both cranially and medially. In such cases, the 12th intercostal space should be avoided.

Adipose biopsies were made on the day following liver biopsies. Measures were taken with the adipose biopsies to prevent contamination of adipose tissue with the anesthetic; two 10 to 12 cm perpendicular lines were drawn on the animal surrounding, but about 2 cm away from, the area to be incised. Intersection of the lines was most dorsal-cranial. Lidocane injection along these lines formed a subcutaneous blockade of nerve transmission. Four to eight grams of subcutaneous adipose were removed from a 10 to 12 cm vertical incision along the back between the shoulder and the 13th rib. Incisions were begun about 15 cm from the tip of the spinous processes of the thoracic vertebra. Tissue samples were maintained at 37° in buffered saline, pH 7.4 until incubated. Subsequent biopsies of liver and adipose on the same animal were accomplished by varying the surgical site slightly.
Incubation procedure  Thin slices of liver (80 to 150 mg) were
made with a Stadie-Riggs microtome (Stadie and Riggs, 1944) within 45
minutes after biopsy and incubated in 25 ml flasks containing 3 ml of
Ca^{++} free, pH 7.4, Krebs-Hensleit bicarbonate buffer (Lasser, 1961).
Substrates were added to the flasks, depending on the metabolic activity
to be measured. For measuring gluconeogenic capacity, 30 micromoles
plus approximately 1 microcurie of the following substrates were added
to different flasks: L-[U-^{14}C]aspartic acid, L-[U-^{14}C]alanine,
Ketogenic capacity of liver was determined by the total accumulation of
L-beta-hydroxybutyrate in the 3 ml of media after incubation with 30
micromoles Na-[1-^{14}C]butyrate, or 3 micromoles [1-^{14}C]stearate bound to
albumin (3 percent final albumin concentration). For radioactive carbon
dioxide collection from all liver incubations, a hanging well containing
folded filter paper was suspended above the media and the flask stoppered.

Adipose tissue was sectioned into 80 to 150 mg portions and incu­
bated in a manner similar to liver with the following exceptions. All
media contained 5 mM glucose and 3 percent bovine serum albumin (BSA).
Fatty acid synthetic rates from 10 mM Na-[2-^{14}C]acetate and Na-L(+)[U-
^{14}C]lactate were measured in the presence of 0.3 units bovine insulin
while endogenous lipolytic rates were measured with and without 1.5
micrograms epinephrine. Also, the hanging well was omitted from incuba­
tion flasks.

All liver and adipose incubations were performed in triplicate with
background contamination (either no tissue or tissue killed immediately
with 0.5 ml 1.5N H₂SO₄) from each substrate also incubated in triplicate. All incubations were continued for 2 hours at 37° with constant shaking using Dubnoff metabolic shakers (90 strokes per minute) under an atmosphere of 95 percent O₂ and 5 percent CO₂.

At the end of the incubation period with liver tissue, 0.10 ml of 25 percent KOH was injected with a syringe onto the filter paper. Tissue was killed by injecting 0.5 ml 1.5N H₂SO₄ into the media. Flasks were shaken for an additional 1 hour to collect ¹⁴CO₂. Adipose tissue incubations containing radioactive substrates for fatty acid synthetic rate determinations, were stopped by injecting 0.5 ml of 1.5N H₂SO₄ into the media. Adipose tissue incubations for lipolytic rates contained no radioactive substrates and were stopped by removing the adipose tissue at the end of the incubation period and placing the flask on ice.

Preparation for incubation Substrates, with the exception of stearate, were made to a concentration of 60 micromoles per ml in Krebs-Hensleit salt solution (bicarbonate free) containing 10 mM phosphate buffer, pH 7.4. Radioactive isotopes of substrates were diluted in the same salt solution to a concentration of 1 microcurie per 0.1 ml. Prepared substrates and isotopes were divided into aliquots and stored frozen until needed for incubations. On the evening prior to incubations, 0.5 ml of each substrate and 0.1 ml of appropriate isotope were added to each flask, which were capped and stored refrigerated over-night. Krebs-Hensleit bicarbonate buffer was made the morning of an incubation, the pH adjusted to 7.4 at 37° with a continual stream of 95 percent O₂ and 5 percent CO₂, and maintained at these conditions until used. Just prior
to initiation of tissue incubation, 2.4 ml of continually gassed Krebs-Hensleit bicarbonate buffer was added to individual flask containing 0.6 ml of substrate, tissue added, re-gassed, and the incubation begun.

The stearate-albumin complex was made in the following manner. BSA was freed of fatty acid by extracting twice for 12 hours with 95 percent ethanol (40g albumin/l). Stearic acid was dissolved in ethanol and kept refrigerated. Thirty micromoles of stock stearic acid was added to a small flask, 10 microcurries of [l-\(^{14}\)C]stearate added and dried under air. A 15 percent molar excess of a KOH solution were added and stirred slowly (magnetic stirrer) in a warm water bath until stearate dissolved. Once dissolved, the pH was adjusted slowly to pH 7.4 by drop-wise addition of HCl. The mixture should be cloudy but large flakes of stearic acid should be avoided. Such flakes indicate too rapid a decline in pH or that the final pH is too low. Working quickly, 5 ml of 20 percent BSA in water was added and stirring continued until a clear solution was obtained. This solution was maintained in the refrigerator overnight but also may be frozen. Krebs-Hensleit salt solutions (bicarbonate free), concentrated six fold, were stored frozen. On the morning of an incubation, an aliquot of concentrated salt solution was gassed with 95 percent \(O_2\) and 5 percent \(CO_2\) after addition of the recommended amount of bicarbonate. Dilution with bicarbonate reduced the concentration to three times recommended strength. Ten ml of this gassed salt solution was added to the stearate-albumin complex, diluted to 30 ml, and 3 ml portions were pipetted into individual flasks for incubation.
Analytical procedures

Liver and adipose incubations All metabolic rates were corrected for background radioactive contamination. Radioactivity was quantified with a Beckman LS-8000 scintillation counter using Beckman EP Ready-solv scintillation cocktail. Counting efficiency was determined using the programmed external standard method with automatic quench correction. Output by this method yields an 'H-number' which is compared to H-numbers from quenched standards for efficiency value.

Filter papers from suspended wells in incubation flasks, containing trapped \(^{14}\)CO\(_2\), were counted by liquid scintillation for determination of rates of substrate oxidation. Counting of these samples was delayed 1 week to avoid errors due to chemiluminescence.

Media containing synthesized glucose for isolation was spiked with 5000 dpm of \([6-^3\text{H}]\)glucose, transferred to 50 ml centrifuge tubes, neutralized with Ba(OH)\(_2\), decanted, and frozen. Glucose isolation and specific radioactivity determination was by the method of Mills et al. (1981). Recovery of \([^{14}\text{C}]\)glucose was corrected for \([^3\text{H}]\)glucose recovery.

Media from adipose incubations for glycerol determinations and from liver incubations for BHBA determinations were deproteinized by the procedure of Somogyi (1945). Glycerol was assayed as described by Eggstein and Kuhlmann (1974). Beta-hydroxybutyrate was assayed according to Williamson and Mellanby (1974). Adipose tissue samples incubated for

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5 Beckman Inst. Inc., Fullerton, CA.
Fatty acid synthetic rate determinations were treated as described by Pothoven and Beitz (1973).

Liver and blood Liver samples kept frozen in liquid nitrogen were ground to a fine powder under liquid nitrogen in a mortar. The powder was funneled quickly into a preweighed Potter-Elvehjem homogenization tube containing 4 to 5 ml of ice-cold 10 percent HC104 while vortexing. After weighing rapidly to determine tissue weight, homogenization completed the deproteinization. Extra HC104 was added to a final ratio of HC104:tissue (V:W) of approximately 5:1. An aliquot of homogenate was removed for glycogen determination (Keppler and Decker, 1974) and the remainder was placed in a centrifuge tube and centrifuged in the cold at 10,000 x g for 5 minutes. A known volume of supernatant was neutralized with KOH (using universal indicator7), centrifuged, and the supernatant was used for analyses. All assays on the tissue extract were conducted that same day. BHBA was assayed as already described.

Other methods used for determination of metabolites were: acetoacetate (Williamson and Mellanby, 1974), lactate (Gutmann and Wahlefeld, 1974), pyruvate, phosphoenolpyruvate, and 2-phosphoglycerate (Czok and Lamprecht, 1974), and citrate (Dagley, 1974). Care was taken with lactate determinations to avoid contamination by the presence of lactate on the skin. Objects contacting reaction media were not handled directly because this was found to lead to erroneously high results.

7Fischer Scientific Co., St. Louis, MO.
Powdered liver tissue, as just described, was used for determinations of triglyceride (Eggstein and Kuhlmann, 1974) and DNA (Munro and Fleck, 1969). For triglyceride analysis, hydrolysis of ester bonds yields free glycerol, which is analyzed enzymatically. Alcoholic-KOH is used for hydrolysis and is an unsuitable media for direct glycerol analysis. Therefore, an aliquot of this solution was dried, re-suspended in assay buffer and filtered. Subsequent work with the same filters (and others from the same company) showed a small glycerol contamination of the filters. Glycerol contamination was fairly constant and corrections were made.

Blood samples were collected into heparinized tubes at 3 day intervals just before the morning feeding. Plasma was assayed for free fatty acids (Nixon and Chan, 1979), and insulin by RIA. Neutralized filtrates of plasma were prepared (Somogyi, 1945) and assayed for glucose, and glycerol and BHBA as already described.

Instrumentation

Enzymatic analyses using spectrophotometric detection of metabolites of incubation media, liver tissue, and blood (with the exception of glucose) were conducted using a Gilford 2600 single-beam spectrophotometer. Spectrophotometric detection from glucose analyses was conducted

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8 Millipore Corp. (filter HAW), Bedford, MA. Glycerol free filters are available.

9 Antibody kindly supplied by A. Trenkle, ISU, Ames, IA.

10 Glucostat, Worthington Biochemicals, Freehold, NJ.

11 Gilford Instruments, Oberlin, OH.
using a Beckman 24\textsuperscript{12} spectrophotometer.

Chemicals

Enzymes, substrates, and cofactors needed for metabolite determinations were purchased from Sigma\textsuperscript{13}. Radioactive substrates were purchased from the following sources: L-[U-\textsuperscript{14}C]amino acids (alanine, aspartic acid, and glutamic acid) and Na-[2-\textsuperscript{14}C]propionate from ICN\textsuperscript{14}, Na[2-\textsuperscript{14}C]acetate, Na-[l-\textsuperscript{14}C]butyrate and [1-\textsuperscript{14}C]stearic acid were from Amersham\textsuperscript{15}, and Na-[U-\textsuperscript{14}C]lactate was from NEN\textsuperscript{16}.

Presentation of results

Liver Metabolite concentrations are based on wet weight of the fresh tissue. Metabolic rates are expressed as the micromoles of substrate incorporated into glucose or converted to CO\textsubscript{2} over 2 hours per mg DNA. Calculations are based on the total DPM \textsuperscript{14}C incorporated into glucose or CO\textsubscript{2} divided by the specific radioactivity of the substrate added. BHBA production is expressed as BHBA accumulation per mg DNA over 2 hours.

Adipose Fatty acid synthetic rates are expressed as nmol substrate incorporated into tissue fatty acid per 100 mg wet tissue over

\textsuperscript{12}Beckman Instruments, St. Louis, MO.

\textsuperscript{13}Sigma Chemical Co., St. Louis, MO.

\textsuperscript{14}ICN Pharmaceuticals, Inc., Irvine, CA.

\textsuperscript{15}Amersham/Searle Corp., Arlington Heights, IL.

\textsuperscript{16}New England Nuclear, Boston, MA.
2 hours. Glycerol release is in nmoles glycerol per 100 mg wet tissue over 2 hours.

**Statistical analysis**

Data were analyzed by GLM (Goodnight, 1979) and period differences determined by orthogonal comparisons. Of the four cows that developed clinical ketosis, one cow died from hemorrhage after a liver biopsy and did not complete the treated period, while another cow developed ketosis within 2 weeks after restricting intake, thus missing period PK. Data were generated for missing values in period PK and TRT by GLM (Goodnight, 1979).

**Results and Discussion**

The five cows reported in this text completed at least four of the five biopsy periods. Nine cows were initiated on study but for various reasons four did not complete the study. Reasons for incompletion varied from excessive fattness at calving resulting in poor feed consumption and milk production, to illness of unknown origin, and external teat damage. Fat cows no doubt reflect the prepartum feeding program while other problems were more likely random occurrences characteristic of animal studies.

Four cows developed clinical signs of ketosis including anorexia, decreased milk production, high concentrations of milk ketones, and modified behavior. Behavioral signs were variable among cows. Two of the four cows exhibiting ketosis showed a hyperexcitability. One of these two cows was excitable to the point of being almost unmanageable
and making biopsy of liver and adipose tissue difficult. The two remaining ketotic cows exhibited depression and lethargy. Data from these four cows were grouped for statistical and summary purposes and are designated 'ketotic'. One cow (7337), despite being given large amounts of 1,3-butanediol, showed no unnatural behavioral signs and only an occasional positive milk ketone test; i.e. two days prior to treatment she gave a strongly positive test for ketones (Table 6). Data from this cow are presented and discussed as a negative control but were not used for statistical analysis.

**Milk production, feed intake, and body weight changes**

Although data on a number of variables (milk production, feed intake, and blood analysis) were collected on a continuous basis, in order to summarize the data, period and subperiod means will be presented. Milk production and feed intake data are in Table 6. The ketotic group produced more milk on less feed than did the control cow. Limiting silage-grain mix intake by 15 to 20 percent decreased milk output by about 12 percent in the ketotic group but not in the control cow, suggesting that intake was still adequate to meet nutrient needs for milk synthesis in the control cow. The quantities of silage-grain mix offered and consumed are in Table 6 also. Silage-grain mix was offered ad libitum the first few days during PK to allow normal feeding patterns following biopsies during EPP. Silage-grain mix was then restricted to quantities indicated in PK-L. Neither ketotic cows nor the control cow consumed the quantity of silage-grain mix offered after restriction (PK and K).
Table 6. Milk production and silage-grain mix intake by ketotic cows and the control cow

<table>
<thead>
<tr>
<th>Period</th>
<th>Early post-partum</th>
<th>Preketotic</th>
<th>Ketotic</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E^a L^b</td>
<td>E  L</td>
<td>E  L</td>
<td>E  L</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Milk (kg /day)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketotic</td>
<td>25 35</td>
<td>39 34</td>
<td>34 30</td>
<td>24 31</td>
</tr>
<tr>
<td>Control</td>
<td>22 26</td>
<td>27 29</td>
<td>29 28</td>
<td>23 32</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Complete silage (kg DM /day)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketotic-I^c</td>
<td>11.1 13.5</td>
<td>14.7 10.5</td>
<td>10.2 6.9</td>
<td>11.8 10.5</td>
</tr>
<tr>
<td>-O^d</td>
<td>--- ad libitum</td>
<td>--- 11.5</td>
<td>11.5 11.5</td>
<td>ad libitum</td>
</tr>
<tr>
<td>Control-I^c</td>
<td>11.2 16.0</td>
<td>13.7 11.8</td>
<td>11.8 8.3</td>
<td>11.2 12.5</td>
</tr>
<tr>
<td>-O^d</td>
<td>--- ad libitum</td>
<td>--- 13.7</td>
<td>13.7 8.3</td>
<td>ad libitum</td>
</tr>
</tbody>
</table>

^a Three day average early within the period.
^b Three day average late within the period.
^c Intake.
^d Offered.

BD was mixed with silage-grain mix during periods PK and K. BD may cause feed refusal when 10 to 20 percent of rations fed to rats is replaced by BD. Substituting 6 to 8 percent of the total ration with BD for growing cattle, caused increases in plasma KB and increased ration intake (Bonner et al., 1975). Hess and Young (1972) and Bonner et al. (1976) fed BD to lactating cows at the rate of 4 to 6 percent of the
ration and found no effects on feed intake. Actual quantities of BD fed in the studies with growing cattle and lactating cows were not given but are estimated to be between 0.5 and 1.2 kg/day. Quantities of BD offered to cows in the present study were between 0 to 1.4 kg/day (Table 4), suggesting that BD was not responsible for feed intake restriction.

Intake of alfalfa hay was not measured, so total feed consumption is not known. It is possible that increased consumption of hay compensated for restriction of silage-grain mix. Increased consumption of hay does not seem to be an adequate explanation however, for further silage-grain mix restriction during the late stages of K for the ketotic cows (Table 6). Voluntary feed restriction reached 40 percent of the silage offered in the ketotic group (K) and no doubt added to the ketotic state. Restriction of silage-grain mix during late K is no doubt related to the ketosis because anorexia is a characteristic of the syndrome (Schultz, 1974). Decreased silage-grain mix consumption by the control cow during late K was not voluntary, but was invoked in an attempt to induce ketosis. All silage-grain mix offered, was consumed despite high levels of BD, adding further support to the suggestion that BD was not limiting intake.

Treatment depressed milk production for the first few days (TRT-E) but production was approaching pre-ketotic amounts by 7 to 10 days (TRT-L). Depressed milk production may result from glucocorticoid therapy. Gluco-corticoids decrease glucose utilization in bovine mammary tissue (Gorewit and Tucker, 1977), and have been implicated in decreasing milk output (Braun et al., 1970). Intake of complete silage increased after treatment
of ketotic cows. One ketotic cow died during the fourth liver biopsy leaving only three cows for the treated period. One of these three cows showed a 30 percent reduction in silage-grain mix intake the day before the last biopsy (TRT-L). Intake reduction by this cow accounts for the slight decline in silage-grain mix intake noted during TRT-L. Both increased intake and decreased milk production would aid in alleviating energy deficiency and signs of ketosis.

Greater stress due to greater nutrient demand by the ketotic group is also evident in loss of body weight (Table 7). Ketotic cows lost an average of 3.1 kg per day for 36 days while the control cow lost at half this rate. As a percentage of the initial postpartum body weight (taken

<table>
<thead>
<tr>
<th>Cow</th>
<th>Body weight loss (kg)</th>
<th>Interval after parturition (days)</th>
<th>Average weight loss (kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketotic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6965</td>
<td>114</td>
<td>33</td>
<td>3.45</td>
</tr>
<tr>
<td>7275</td>
<td>110</td>
<td>44</td>
<td>2.45</td>
</tr>
<tr>
<td>7409</td>
<td>105</td>
<td>40</td>
<td>2.61</td>
</tr>
<tr>
<td>7559</td>
<td>101</td>
<td>27</td>
<td>3.74</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>36</td>
<td>3.10</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7337</td>
<td>77</td>
<td>47</td>
<td>1.63</td>
</tr>
</tbody>
</table>
3 days postpartum) ketotic cows lost 7.7 percent (range 7.4 to 8.2) and the control cow lost 5.1 percent of their body weight through period K. Total body weight loss was equivalent for the four ketotic cows and was 40 percent greater than the control. The remarkable similarity between the four ketotic cows in total weight loss is curious, but the significance is not known.

**Plasma metabolite changes**

Concentrations of plasma metabolites in ketotic cows are presented in Table 8. Insulin and glucose were significantly (P<.05) decreased following parturition and remained low through ketosis development. BHBA, FFA, and glycerol concentrations increased (P<.05) during early lactation. Whereas BHBA concentrations continued to increase until treatment was given, FFA and glycerol seemed to reach maximum concentrations prior to ketosis development.

Metabolite concentrations were much more exaggerated in ketotic cows than in the control cow (Table 9). Concentrations of all plasma metabolites assayed were nearly equivalent between the control cow and the ketotic cows during the dry period. However, following parturition until ketosis treatment was given, glucose and insulin were 30 percent greater, glycerol 35 percent less, and FFA and BHBA 60 percent less in the control cow.

Plasma metabolite data further indicate a major dependency on endogenous nutrients by the ketotic cows to meet requirements for lactation. Depressed concentrations of glucose and insulin in ketotic cows
Table 8. Plasma metabolite concentrations in ketotic cows

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Dry</th>
<th>Early post-partum</th>
<th>Pre-ketotic</th>
<th>Ketotic</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E</td>
<td>L b</td>
<td>E a</td>
<td>L b</td>
<td>E</td>
</tr>
<tr>
<td>Insulin</td>
<td>.63</td>
<td>.37</td>
<td>.42</td>
<td>.43</td>
<td>.40</td>
</tr>
<tr>
<td>Glucose</td>
<td>69</td>
<td>51</td>
<td>43</td>
<td>40</td>
<td>125</td>
</tr>
<tr>
<td>BHBA</td>
<td>.47</td>
<td>1.8</td>
<td>3.1</td>
<td>3.7</td>
<td>4.5</td>
</tr>
<tr>
<td>FFA</td>
<td>.24</td>
<td>.66</td>
<td>1.1</td>
<td>.75</td>
<td>.97</td>
</tr>
<tr>
<td>Glycerol</td>
<td>.03</td>
<td>.07</td>
<td>.08</td>
<td>.08</td>
<td>.08</td>
</tr>
<tr>
<td>SGOT</td>
<td>53</td>
<td>81</td>
<td>87</td>
<td>75</td>
<td>75</td>
</tr>
</tbody>
</table>

* Early within period.
** Late within period.
Mid-period.
^ Standard error on the mean.

Concentrations of metabolites are: insulin (ng/ml); glucose (mg/dl); BHBA, FFA, and glycerol (mM); and SGOT (IU/l).

* P<.01 for period effects.
** P<.001 for period effects.

would increase fatty acid mobilization from adipose tissue as discussed already in 'Review of Literature; Metabolic changes during bovine ketosis'.

The apparent decline in FFA concentration during K may result from inhibition of adipose tissue lipolysis with increasing KB (BHBA) concentration (Metz and van den Bergh, 1972). KB continue to increase during
Table 9. Plasma metabolite concentrations in the control cow

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Dry</th>
<th>Early postpartum</th>
<th>Pre-ketotic</th>
<th>Ketotic</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (ng/ml)</td>
<td>.37</td>
<td>.68</td>
<td>.46</td>
<td>.55</td>
<td>.44</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>64</td>
<td>65</td>
<td>53</td>
<td>56</td>
<td>48</td>
</tr>
<tr>
<td>BHBA (mM)</td>
<td>.45</td>
<td>.48</td>
<td>1.77</td>
<td>1.2</td>
<td>1.9</td>
</tr>
<tr>
<td>FFA (mM)</td>
<td>.12</td>
<td>.31</td>
<td>.50</td>
<td>.21</td>
<td>.48</td>
</tr>
<tr>
<td>Glycerol (mM)</td>
<td>.03</td>
<td>.05</td>
<td>.05</td>
<td>.05</td>
<td>.04</td>
</tr>
<tr>
<td>SGOT (IU/L)</td>
<td>61</td>
<td>74</td>
<td>65</td>
<td>63</td>
<td>59</td>
</tr>
</tbody>
</table>

^Early within period.

b Late within period.

K despite an apparent decline in availability of FFA, which suggests a greater incomplete oxidation of available FFA. Glycerol, which is released from adipose tissue upon hydrolysis of TG, increased in concentration following parturition to a much lesser extent than FFA in both the ketotic cows and the control cow. Previous work has indicated similar changes in FFA and glycerol concentrations when comparing ketotic and nonketotic cows (Table 1). The FFA/glycerol ratio was always greater than three, suggesting that production and/or utilization rates of either FFA or glycerol are not reflected in plasma concentrations.

Treatment for ketosis depressed concentrations of both BHBA and FFA but had little effect on plasma glycerol. Treatment for ketosis thus
seems to reverse the lipolytic and ketogenic state characteristic of ketosis, but this reversal is not reflected in glycerol concentrations. Thus, BHBA and FFA seem to reflect more accurately the energy status of lactating cows. Decreased concentrations of BHBA and FFA following treatment may well result from the rapid and sustained increases in glucose and insulin concentrations.

Effects of ketosis treatment on plasma glucose and insulin concentrations were not confounded due to one ketotic cow receiving a glucose load as part of her treatment for ketosis (Materials and Methods). Blood samples in the glucose treated cow, were taken twice between 24 and 48 hours after treatment and averaged 165 mg/dl, not greatly different from the average plasma glucose of other treated ketotic cows (TRT-E). Plasma insulin during the first 48 hours after treatment averaged 1.3 ng/ml, again, not greatly different from other treated cows. All cows had initial post-treatment plasma insulin concentrations greater than 1 ng/ml and plasma glucose was less than 90 mg/dl in only one cow. A more likely explanation for the rise in average glucose concentration is the combined effects of decreased milk production, increased feed intake, intake of supplemental propylene glycol, and injection of glucocorticoids. Glucocorticoids would not only be responsible for decreased milk production as discussed already, but for increasing glucose availability by:

1. decreasing glucose utilization in peripheral tissues
   (Livingston and Lockwood, 1975), and
2. increasing muscle catabolism and gluconeogenic precursor availability (Bergman, 1973).
Glucocorticoids also increase hepatic PEPCK activity in rats (Wicks et al., 1974), but this does not seem to be the case in ruminants (Baird and Heitzman, 1970; 1971; Heitzman et al., 1972).

**Hepatic metabolites**

Hepatic glycogen is an unlikely source of plasma glucose following treatment for ketosis because glycogen concentrations were essentially depleted during K in the ketotic group (Table 10). Glycogen declined by 75 percent and triglyceride increased over 600 percent early postpartum in the ketotic group.

Restricted feeding caused a further depletion of glycogen and accumulation of triglyceride, reaching values 10 percent and 1000 percent, respectively, of those found prior to parturition. Hepatic triglyceride

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Dry</th>
<th>Early postpartum</th>
<th>Pre-ketotic</th>
<th>Ketotic</th>
<th>Treated</th>
<th>SE^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ketotic</td>
<td>6.7</td>
<td>1.7</td>
<td>1.7</td>
<td>0.55</td>
<td>3.3</td>
<td>0.65*</td>
</tr>
<tr>
<td>control</td>
<td>6.1</td>
<td>4.6</td>
<td>3.3</td>
<td>1.6</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ketotic</td>
<td>1.12</td>
<td>7.0</td>
<td>11.3</td>
<td>11.0</td>
<td>6.8</td>
<td>0.16*</td>
</tr>
<tr>
<td>control</td>
<td>1.15</td>
<td>2.1</td>
<td>1.3</td>
<td>1.9</td>
<td>1.3</td>
<td></td>
</tr>
</tbody>
</table>

^a Standard error of the mean.

*p < .01 for period effect.
concentration paralleled plasma concentrations of FFA, with both showing peak concentrations during PK and prior to appearance of clinical signs of ketosis. Depletion of hepatic glycogen and accumulation of triglyceride are characteristic of a negative energy balance and the ketotic state (see 'Review of Literature; Bovine Lactation Ketosis'). The greatest changes in hepatic glycogen and TG occurred from transition from period D to EPP. Negative energy balance was, thus, established very soon following parturition even when cows were fed ad libitum. In contrast to the ketotic group, the less stressed control cow was better able to maintain liver glycogen while liver triglycerides showed little change. Glycogen in the control cow declined just prior to treatment and coincided with the imposed restriction in silage-grain mix fed (Table 6).

Hepatic concentrations of ketone bodies, and glycolytic and Krebs cycle intermediates are listed in Table 11 and 12. BHBA and AcAc concentrations in ketotic cows (Table 11) increased following parturition and reached maximum values during K. Compared to period D, BHBA increased 10 fold and AcAc 15 fold resulting in a decline in the BHBA/AcAc ratio as ketosis developed. This is in contrast to results with the control cow (Table 12); hepatic BHBA concentration, although elevated during PK to levels observed in the ketotic cows, showed no further increase during K and was less than 50 percent as concentrated as in livers from the ketotic group during K. AcAc in the control cow, showed less fluctuation than ketotic cows following parturition resulting in an increase in the BHBA/AcAc ratio. Decreased BHBA/AcAc is characteristic of the ketotic state (Bergman, 1971; Hibbitt and Baird, 1967). Koundakjian and Snoswell
Table 11. Hepatic metabolite concentrations in ketotic cows

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Dry</th>
<th>Early postpartum</th>
<th>Pre-ketotic</th>
<th>Ketotic</th>
<th>Treated</th>
<th>SEa</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHBA</td>
<td>270</td>
<td>1097</td>
<td>1796</td>
<td>2652</td>
<td>454</td>
<td>193**</td>
</tr>
<tr>
<td>AcAc</td>
<td>33</td>
<td>83</td>
<td>290</td>
<td>613</td>
<td>18</td>
<td>57**</td>
</tr>
<tr>
<td>BHBA/AcAc</td>
<td>8.2</td>
<td>13</td>
<td>6.2</td>
<td>5.2</td>
<td>25.2</td>
<td>9</td>
</tr>
<tr>
<td>Lactate</td>
<td>829</td>
<td>558</td>
<td>588</td>
<td>860</td>
<td>413</td>
<td>173</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>29</td>
<td>38</td>
<td>27</td>
<td>26</td>
<td>28</td>
<td>7</td>
</tr>
<tr>
<td>Lact/Pyr</td>
<td>29</td>
<td>15</td>
<td>22</td>
<td>33</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>Citrate</td>
<td>301</td>
<td>105</td>
<td>86</td>
<td>64</td>
<td>203</td>
<td>61*</td>
</tr>
<tr>
<td>PEPc</td>
<td>44</td>
<td>59</td>
<td>48</td>
<td>79</td>
<td>77</td>
<td>23</td>
</tr>
<tr>
<td>2PGA</td>
<td>31</td>
<td>49</td>
<td>30</td>
<td>35</td>
<td>41</td>
<td>9</td>
</tr>
</tbody>
</table>

 Period Concentrationb or ratio

---

aStandard error of the mean.

b nmoles/g wet weight.

cPhosphoenolpyruvate.

d2-Phosphoglycerate.

* P<.1, PK+K vs D+EPP+TRT.

** P<.001, for period effect.

(1970) suggested that the increase in AcAc may depress the central nervous system, causing the characteristic altered behavior of ketosis.

HBDH, the nucleotide-linked enzyme catalyzing the inter-conversion of AcAc and BHBA, is thought to be of sufficient activity to catalyze a
Table 12. Hepatic metabolite concentrations in the control cow

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Dry</th>
<th>Early postpartum</th>
<th>Pre-ketotic</th>
<th>Ketotic</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHBA</td>
<td>140</td>
<td>364</td>
<td>1640</td>
<td>1045</td>
<td>239</td>
</tr>
<tr>
<td>AcAc</td>
<td>35</td>
<td>44</td>
<td>15</td>
<td>93</td>
<td>15</td>
</tr>
<tr>
<td>BHBA/AcAc</td>
<td>4</td>
<td>8.3</td>
<td>109</td>
<td>11.2</td>
<td>16</td>
</tr>
<tr>
<td>Lactate</td>
<td>626</td>
<td>395</td>
<td>496</td>
<td>544</td>
<td>1190</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>25</td>
<td>67</td>
<td>25</td>
<td>34</td>
<td>39</td>
</tr>
<tr>
<td>Lact/Pyr</td>
<td>25</td>
<td>5.9</td>
<td>19.8</td>
<td>16</td>
<td>31</td>
</tr>
<tr>
<td>Citrate</td>
<td>310</td>
<td>250</td>
<td>98</td>
<td>55</td>
<td>878</td>
</tr>
<tr>
<td>PEP(^b)</td>
<td>25</td>
<td>62</td>
<td>110</td>
<td>55</td>
<td>64</td>
</tr>
<tr>
<td>2PGA(^c)</td>
<td>41</td>
<td>25</td>
<td>55</td>
<td>25</td>
<td>102</td>
</tr>
</tbody>
</table>

\(^a\) nmoles/g wet weight.  
\(^b\) Phosphoenolpyruvate.  
\(^c\) 2-Phosphoglycerate.

near equilibrium reaction in rats (Newsholme and Start, 1973b). The BHBA/AcAc ratio in freeze-clamped rat liver has been used to assess the [NAD]/[NADH] ratio within the mitochondrial compartment where HBDH is located (Williamson et al., 1967). Likewise, changes in BHBA/AcAc have been attributed to changes in redox state. In ruminants, hepatic HBDH is in cytosol where the redox state is oxidized normally far more
(greater NAD/NADH) than in the mitochondria (Koundakjian and Snoswell, 1970). Koundakjian and Snoswell (1970) suggested that the redox state in hepatic cytosol is unfavorable to BHBA production, which causes a lowered BHBA/AcAc ratio with increasing FFA oxidation. The large BHBA/AcAc ratio in the fed state is suggested to be the result of BHBA production from rumen epithelium. As discussed already in 'Ketone Metabolism in Animal Tissues; Peculiarities of ketone body metabolism by ruminants', the liver does not produce predominately AcAc, and alterations within the liver may be primarily responsible for the shift in BHBA/AcAc during ketosis development.

Because HBDH is cytosolic, the ratio of BHBA/AcAc should reflect the redox state of the cytosol in an equivalent manner to lact/pyr, which also is catalyzed by a near equilibrium cytosolic reaction. Ketotic cows during K showed an increased lact/pyr ratio compared to other periods following parturition. Data similar to those in the present study, showing decreased BHBA/AcAc and increased lact/pyr during ketosis or energy deficiency, have been published (Baird et al., 1968; 1972; Bergman, 1971; Hibbitt and Baird, 1967; Treacher et al., 1976). Thus, changes in the ratio of reactants and products of two cytosolic enzymes during ketosis development, indicate opposite trends in redox state. One possibility for the discrepancy is that HBDH may not be of sufficient activity to approach equilibrium with reactants and products (Baird et al., 1968; Koundakjian and Snoswell, 1970), or the discrepancy may reflect different nucleotide pools within the cytoplasm. If the BHBA/AcAc ratio is not dependent on redox state, the question remains as to how an
increased AcAc production results. Obviously more research is needed to resolve the discrepancy.

Citrate concentrations decreased in liver after parturition in both the ketotic group and the control cow, and were increased by treatment of ketosis. Baird (1977) showed similar depressions in citrate concentration in spontaneous and fasted ketosis. Treatment with glucocorticoids in normal (Baird and Heitzman, 1970) and ketotic cows (Baird, 1977; Baird and Heitzman, 1971) increased hepatic citrate two to four fold. Citrate concentrations fluctuate inversely to concentrations of KB, suggesting a potential regulatory role in hepatic FFA metabolism for citrate or a product of this metabolite much like for malonyl-CoA in nonruminants (McGarry et al., 1978b).

Data presented thus far verify much of what is known to occur during ketosis. Ketotic cows are more dependent on endogenous sources of nutrients than are nonketotic cows resulting in most of the observed plasma and liver changes. Ketotic cows:

1. Lose more body weight during early lactation,
2. accumulate more plasma FFA and KB, and more hepatic TG and KB,
3. have less plasma glucose and insulin, and less hepatic glycogen.

Adipose metabolism

Data for adipose tissue metabolism are given in Tables 13 and 14. Reduction in triglyceride content of adipose following parturition increases the difficulty in obtaining adequate quantities of tissue for study. Adipose samples were not always obtainable necessitating the elimination of one ketotic cow and period PK from analyses. No differences
Table 13. Adipose lipolytic rates before and after parturition

<table>
<thead>
<tr>
<th>Incubation condition</th>
<th>Dry</th>
<th>Early postpartum</th>
<th>Ketotic</th>
<th>Treated</th>
<th>SE&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mean&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>242</td>
<td>223</td>
<td>360</td>
<td>342</td>
<td>58*</td>
<td>292</td>
</tr>
<tr>
<td>Epinephrine stimulated</td>
<td>367</td>
<td>377</td>
<td>584</td>
<td>507</td>
<td>62**</td>
<td>459</td>
</tr>
</tbody>
</table>

<sup>a</sup>Standard error of mean.

<sup>b</sup>Mean for condition.

*P<.10, D+EPP vs K+TRT.

**P<.05, D+EPP vs K.

Table 14. Fatty acid synthesis in adipose before and after parturition

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Dry</th>
<th>Early postpartum</th>
<th>Ketotic</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>196 ± 26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Lactate</td>
<td>80 ± 19</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean ± standard error of mean.
were evident between the ketotic group and the control cow, so data were combined for adipose tissue metabolism. Epinephrine stimulation caused a 54±2 percent average increase in glycerol release from incubated adipose tissue (Table 13). The small standard error for this estimate reflects the uniformity across periods. The constant epinephrine effect across periods suggests that the relative proportion of hormone sensitive lipase (HSL) in the active state did not vary. The 40 to 50 percent increase in basal and epinephrine stimulated lipolytic rates during K compared to D, suggests an increase in total HSL concentration.

Emery (1979) reviewed the regulation of adipose lipolysis and concluded that glyceride synthetic mechanisms were more apt to respond to long-term control than was HSL. Some of the increased rate of adipose lipolysis with lactation may be related to how the data are presented. Based on tissue weight, loss of fat during early postpartum would increase the number of cells per gram and confound any cellular effects. Metz and van den Bergh (1977), also using adipose sections from cows, found increased basal and epinephrine stimulated FFA and glycerol release following parturition compared to prepartum. They concluded that increased fatty acid mobilization after parturition was due to both decreased re-esterification of hydrolyzed fatty acids and increased lipolytic rates. Increased basal and stimulated FFA and glycerol release from adipose tissue slices also has been demonstrated in rats. Metz and van den Bergh (1977) suggest that increased adenylate cyclase activity may be responsible for increased lipolytic activity. Studies with tissue slices, however, all are plagued with the problem of comparing the data to a cellular basis,
but measuring on a tissue basis. Jaster and Wegner (1981) and Pike and Roberts (1980), using isolated adipocytes, found greater basal and stimulated glycerol release in early lactating as compared to dry cows. Their data suggest that increased lipolysis may involve increased concentrations of HSL.

Fatty acid synthesis from acetate during the dry period was two-fold greater than that from lactate (Table 14). Lactate incorporation into adipose fatty acids in steers (Prior and Jacobson, 1979a; 1979b; Whitehurst et al., 1978) and sheep (Prior, 1978) has been shown. The present data agree with Prior and Jacobson (1979a) that acetate is the primary lipogenic precursor. Fatty acid synthesis was nondetectable following parturition. Pike and Roberts (1980) found glucose incorporation into fatty acids and CO₂ to be decreased 65 and 97 percent, respectively, in lactating compared to dry cows. A subsequent study with acetate (Pike and Roberts, 1981) did not detect fatty acid synthesis following parturition, while Grichting et al. (1977a) found acetate utilization for fatty acid synthesis to be 10 percent of that found in nonlactating cows. Elimination of adipose fatty acid synthetic capacity with initiation of lactation seems to be true for rats as well, suggesting an adaptive response to lactation rather than being necessitated by energy deficiency (Smith, 1973).

Insulin is both lipogenic (Baldwin and Smith, 1971; Yang and Baldwin, 1973a) and antilipolytic (Metz and van den Bergh, 1977; Yang and Baldwin, 1973b). Consequently, depressed insulin during early lactation would spare not only glucose and acetate from fatty acid and TG synthesis, but
would leave the effects of lipolytic hormones unopposed. Depressed insulin may be partly responsible for decreased fatty acid synthesis and esterification in adipose tissue following parturition (Emery, 1979; Metz and van den Bergh, 1977). It is doubtful, however, that depressed insulin is the total answer for the altered metabolism because rates of fatty acid synthesis were not restored after treatment for ketosis, yet insulin concentrations were increased. Prolactin and/or signals from the mammary gland may prove to be primary regulators of adipose metabolism with the initiation of lactation (Flint et al., 1981). Decreased fatty acid synthesis and increased lipolytic activity would increase substrate availability for synthesis of milk components.

Ketone production and fatty acid metabolism in liver

Hyperketonemia associated with the ketotic state results primarily from increased hepatic ketone production (Baird, 1977; Baird et al., 1975; Katz and Bergman, 1969; McGarry et al., 1970). KB catabolism, however, may reach a maximum or even decline with high concentrations of plasma KB, which may intensify the hyperketonemia (Ballasse and Havel, 1971; Keller et al., 1978). Table 15 shows the total BHBA accumulation from liver slices when incubated with butyrate or stearate, and reports the oxidation of these substrates to carbon dioxide.

Butyrate is not utilized for triglyceride synthesis nor does it require the carnitine acyl transferase (CAT I,II - Fig. 1) system for oxidation. Consequently, comparing rates of butyrate and stearate oxidation should be indicative of regulation prior to beta-oxidation. Butyrate was a better precursor of BHBA than was stearate, averaging four fold
Table 15. Fatty acid oxidation by ketotic cow liver

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Period</th>
<th>Dry</th>
<th>Early postpartum</th>
<th>Pre-ketotic</th>
<th>Ketotic</th>
<th>Treated</th>
<th>SE&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyrate</td>
<td>BHBA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.73</td>
<td>1.64</td>
<td>1.41</td>
<td>0.45</td>
<td>2.24</td>
<td>0.24***</td>
</tr>
<tr>
<td></td>
<td>CO&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.2</td>
<td>7.1</td>
<td>5.2</td>
<td>4.2</td>
<td>6.7</td>
<td>0.72**</td>
</tr>
<tr>
<td>Stearate</td>
<td>BHBA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.38</td>
<td>0.63</td>
<td>0.50</td>
<td>0.20</td>
<td>0.46</td>
<td>0.08*</td>
</tr>
<tr>
<td></td>
<td>CO&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.06</td>
<td>.07</td>
<td>.06</td>
<td>.18</td>
<td>.19</td>
<td>0.03*</td>
</tr>
</tbody>
</table>

|                     | Ratio<sup>d</sup> | 1:7   | 1:2.6            | 1:2.8       | 1:2.3   | 1:5     |

<sup>a</sup>Standard error on mean.

<sup>b</sup>BHBA (umoles/(mg DNA x 2h)).

<sup>c</sup>CO<sub>2</sub> (umoles carbon/(mg DNA x 2h)).

<sup>d</sup>Ratio of BHBA accumulation from stearate and butyrate.

* P<.05, for period effect.

** P<.025, PK+K vs D+EPP+TRT.

*** P<.01 for period effect.

greater production (Table 15). Greater butyrate oxidation is not surprising in light of the regulatory controls established for transport of long-chain fatty acids into mitochondria (McGarry et al., 1978a). Carbon dioxide production from butyrate was also greater than from stearate, but the over 50 fold production differential between substrates was much
greater than for BHBA production. The actual amount of butyrate and stearate converted to BHBA is unknown, but if all BHBA is assumed to come from added substrate, butyrate, across all periods, was partitioned approximately equally towards BHBA and CO$_2$ while only 6.5 percent of stearate formed CO$_2$. Preferential oxidation of oleate to ketone bodies was observed in rat liver homogenates (McGarry et al., 1978c), while octanoate gave approximately equal productions of ketones and carbon dioxide (McGarry and Foster, 1971a). The reason and, consequently, significance of this differential metabolism of long-chain and short-chain fatty acids are unknown.

Following parturition, BHBA accumulation from butyrate progressively declined until cows were treated for ketosis (Table 15). After treatment, rates of production were equivalent to the dry period (D). Conversely, stearate oxidation to BHBA increased following parturition but was depressed during period K. Differential oxidation of butyrate and stearate is reflected in the decreased stearate:butyrate ratio of BHBA accumulation following parturition (Table 15).

Carbon dioxide production from butyrate was depressed during PK and K (Table 15). Again, assuming all BHBA is derived from butyrate, then total butyrate oxidation to carbon dioxide and BHBA declined following parturition, reaching a maximum of 65 percent during period K compared to D. Production of CO$_2$ from stearate increased three-fold during K but total stearate oxidized to butyrate plus CO$_2$ was depressed nearly 40 percent compared to D and 55 percent compared to EPP and PK. In the control cow (Table 16), BHBA production was generally lower and more constant, showing neither postpartum depression nor response to treatment.
Table 16. Fatty acid oxidation in control cow liver

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Period</th>
<th>Dry</th>
<th>Early postpartum</th>
<th>Pre-ketotic</th>
<th>Ketotic</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyrate</td>
<td>BHBA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.04</td>
<td>1.15</td>
<td>1.06</td>
<td>1.02</td>
<td>1.10</td>
</tr>
<tr>
<td></td>
<td>CO&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.42</td>
<td>3.0</td>
<td>9.18</td>
<td>5.76</td>
<td>3.36</td>
</tr>
<tr>
<td>Stearate</td>
<td>BHBA</td>
<td>0.19</td>
<td>0.20</td>
<td>0.49</td>
<td>0.45</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>CO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.02</td>
<td>0.05</td>
<td>0.03</td>
<td>0.04</td>
<td>0.10</td>
</tr>
</tbody>
</table>

<sup>a</sup>BHBA (umoles/(mg DNA x 2h)).

<sup>b</sup>CO<sub>2</sub> (umoles carbon/mg DNA x 2h)).

Thus, lactation in the ketotic group seems to result in a decreased capacity for beta-oxidation and acetyl-CoA formation when given overwhelming quantities of substrate (butyrate). Clinical ketosis resulted in depressions in both butyrate and stearate oxidation, indicating a more severe hepatic impairment. Treatment increased butyrate and stearate oxidation to rates noted during early lactation, suggesting that hepatic impairment is associated with the ketotic state and is not permanent.

Baird (1965) found ketone production from liver slices without added substrate (endogenous production) to be increased five-fold in ketotic cows over normal lactating cows. Acetoacetate accounted for 90 percent of the increase in total ketones produced. Endogenous production was not measured in the present study, nor was production of AcAc measured from
added substrate, making direct comparisons difficult. Although in the study by Baird (1965), it seems the pathways of ketone body formation are at least as active in ketotic cows, the significance of endogenous production has to be questioned. Rates of endogenous BHBA production reported by Baird, averaged between 5 and 17 percent of the rates in the present study from stearate and were as low as 1.2 percent of rates from butyrate. Equivalent endogenous production of BHBA in the present study would be below reliable detectability by the methods employed. Thus, endogenous BHBA production, even in the ketotic cow, is probably a minor component to total ketone production. Capacities of metabolic pathways can be measured only with saturating concentrations of substrate, obviously a limitation when measuring endogenous production.

Endogenous ketone production is not always a minor component of total KB production. Harken et al. (1969) and McGarry and Foster (1971a) found total endogenous ketone production increased in fasted and diabetic rats over normal perfused rat livers. In the case of the diabetic, rates were maximal and could not be increased by added substrate. Such was not the case in the present study as evidenced by the difference between butyrate and stearate.

Hibbitt and Baird (1967) found a two-fold increase in endogenous ketone production in ketotic cow liver but respiration rates ($O_2$ uptake) were depressed 35 percent. This response is in line with depressed $CO_2$ production noted for butyrate during period K (Table 15). Chung et al. (1959) noted a 70 percent decrease in BHBA production from butyrate in ketotic cow liver, while palmitate and octanoate, but not butyrate,
oxidation were depressed 50 percent in mitochondria from ewes with pregnancy toxemia (Gallagher, 1960).

Thus, in vitro data suggest that ketosis results in, or may be preceded by, depressed liver function as indicated by fatty acid oxidation. While this depression is not in keeping with increased ketogenic rates, in vivo production was not measured. Capacity during EPP or PK is approximately 5 mmoles per minute, which is in line with total ketone production noted for lactating cows (Baird et al., 1975).

Enzymatic capacity for ketogenesis is thought to be fairly constant over a wide range of physiological states (Williamson et al., 1968). Ketone body production is, thus, dependent on the size of the acetyl-CoA pool, which is a function of both rate of generation and utilization of acetyl-CoA. McGarry and Foster (1969) and McGarry et al. (1978c) using liver homogenates or slices from fed, fasted, and diabetic rats, found similar rates of ketone body production from acetyl-CoA, octanoate, and oleate. When results are expressed on a total liver basis, AcAc production in diabetic or starved rats was only 50 percent that of normal rats as a result of liver shrinkage (McGarry and Foster, 1969). Constant rates of KB production in rats in different physiological states are neither the case in vivo (McGarry and Foster, 1971a; McGarry et al., 1978d) nor in liver perfusions (Harken et al., 1969; McGarry and Foster, 1971a; 1971b; McGarry et al., 1973). Rates of ketone body production from either oleate or octanoate increase from normal to starved to diabetic rats. Changes in ketogenic rates from oleate are greater than from octanoate (McGarry and Foster, 1971a; 1971b). Differences in
ketogenic rates from octanoate have been attributed to utilization of acetyl-CoA. Depressed lipogenic rates during starvation and diabetes could account for most of the increased rates of KB formation, although a smaller depression of CO₂ production also was noted (McGarry and Foster, 1971a).

A reciprocal relationship between fatty acid synthesis and ketogenesis was noted in rats (Benito and Williamson, 1978). This reciprocal relationship is also important in the regulation of acetyl-CoA production. An intermediate in fatty acid oxidation, malonyl-CoA, is a potent inhibitor of carnitine acyl transferase I, and thus long-chain fatty acid oxidation. During energy deficiency and depressed lipogenesis, increased rates of oxidation of long-chain fatty acids would increase the acetyl-CoA pool and thus rates of ketogenesis. Differences in ketogenic rates between starved and diabetic rats are attributed to the rate of acetyl-CoA production, with diabetics oxidizing more endogenous fatty acids (McGarry and Foster, 1971a). McGarry et al. (1978b) suggested that the lack of response in liver homogenates to physiological states may be dilution of regulators (malonyl-CoA) controlling the fate of ketogenic substrates. Whether the same argument would apply for tissue slices is not known. Krebs et al. (1969) questioned the reliability of tissue slices for studying ketogenic rates, particularly from long-chain fatty acids. While this is always subject to doubt, rates of BHBA production from butyrate suggest the ketogenic machinery are intact and functional.

Because fatty acid synthesis in ruminant liver is a minor route of acetyl-CoA disposal, increased fatty acid oxidation to BHBA would not be
expected to be due to decreased fatty acid synthesis. Stearate oxidation to BHBA did increase following parturition and may well be due to a greater fractional oxidation rate. Depressed butyrate oxidation (Table 15) following parturition in ketosis-prone cows suggests hepatic impairment and reduced acetyl-CoA production. Other possibilities exist, however; depressed BHBA may reflect the shift toward greater AcAc formation as evidenced by hepatic metabolites (Table 11). Also acetyl-CoA can form acetyl-carnitine, which was not measured.

Peak lactation in rats is accompanied by a significant depression in ketogenesis from butyrate and oleate (Whitelaw and Williamson, 1977). Reduced ketogenesis is much more pronounced for oleate and is due to an increased partitioning of long chain fatty acids toward triglyceride formation. Fatty acid synthesis and malonyl-CoA are increased causing an inhibition of fatty acid oxidation and increased disposal of acetyl-CoA through malonyl-CoA. Thus, the rat liver, which is the center of fatty acid synthesis and secretion, seems to respond quite differently to lactation than does the ruminant liver.

As already discussed in 'Review of Literature - Regulation of hepatic ketogenesis', malonyl-CoA is an important regulator of hepatic fatty acid oxidation in nonruminants, but is unlikely to be important in ruminants due to low rates of fatty acid synthesis in the liver. However, the importance of malonyl-CoA in ruminant liver has yet to be explored. Citrate concentrations in the liver (Table 11) correlate well with hepatic ketone concentrations and suggests that citrate, or a metabolite of citrate may play a regulatory role in FFA disposal.
Mitochondrial citrate equilibrates with the cytosol, and the finding of large fluctuations in plasma citrate with changes in physiological state, suggest a potential direct effect of citrate without subsequent metabolism (Baird, 1977).

**Metabolism of gluconeogenic substrates in liver**

Individual gluconeogenic substrates were incubated with liver slices in triplicate. The total micromoles of substrate, within a period, incorporated into glucose (Fig. 2) and CO$_2$ (Fig. 3), and the contribution of each substrate to the total are presented. Summing the incorporation of all substrates to yield a total is not intended to indicate total glucose production potential, instead it is merely a convenient way of presenting the data.

Caution must always be used when interpreting data from tissues studied outside the physiological environment. Not only are substrate concentrations and ratios varied, but potential insufficiencies of cofactors and other required components for metabolism leave a perpetual question as to the validity of in vitro results. Results should be viewed in light of what is known physiologically and what can be substantiated by in vivo data. This is not to say that in vitro rates are not meaningful. Barring changes in cofactor availability, rates of substrate utilization are dependent on substrate availability, uptake by the tissue, dilution within the tissue, and capacity of the metabolic pathways. Assuming only the latter varies appreciably, in vitro results can yield valuable clues about changes in metabolic patterns, and have the advantage of being able to isolate a tissue or subcellular fraction.
Figure 2. Hepatic gluconeogenesis from various substrates by ketotic cows

[D refers to dry period; EPP, early lactation; PK, preketotic, TRT, treatment; ALA, alanine; ASP, aspartate; GLU, glutamate; LAC, lactate; PRO, propionate; K, ketotic]
Partial verification of an in vitro system comes from comparing metabolic rates, which should be equivalent to or greater than those observed in vivo. Taking the maximum incorporation of propionate into glucose (1.25 umoles/(mg)DNA x 2 hr), this calculates to roughly 0.6 moles of propionate metabolized to glucose daily for a 10 kg liver. This would yield 0.3 moles of glucose per day. Glucose entry rates in mature Holstein dairy cows have been estimated to be between 7 to 14 moles/day (Baird et al., 1975; Wiltrout and Satter, 1972). If 40 percent of this is met by propionate, then glucose production from propionate would range from 2.8 to 5.6 moles/day. Thus, in vitro rates are about 10 percent of the in vivo rates. It must be kept in mind that not all cells of a tissue slice are exposed to incubation media on an equivalent basis. The Stadie-Riggs microtome is designed to cut 500 micron slices, which, if the hepatocytes are 15 to 25 microns in diameter (Bloom and Fawcett, 1968; Reid and Collins, 1980), would be over 20 cell layers thick. Also, peripheral cell damage from slicing would diminish the number of viable cells for a given weight of tissue. Considering these points, the value of 10 percent may be quite reasonable.

Bergman (1973) suggests that only propionate, glycerol, amino acids, lactate, and pyruvate serve as significant precursors for glucose synthesis. Substrates were chosen for the current study based on both their proposed importance as physiological gluconeogenic substrates and their variable entry points into metabolic pathways. Propionate and lactate, averaged across all periods, contributed each about 40 percent of the total glucose synthesized from the five substrates, with the
three amino acids contributing slightly less than 10 percent each (Fig. 2). Similar ratios of substrate conversion to glucose have been shown with isolated sheep liver cells, with the exception of lactate, which was not measured (Morton and Buttery, 1977). Clark et al. (1976), using isolated lamb liver cells, showed propionate and lactate to be utilized to an equal extent while glutamate and aspartate were metabolized at 50 percent and alanine at 25 percent that of propionate and lactate.

It is difficult to rate the importance of glucose precursors based on in vitro data due to the unphysiological availability of substrates. Morton and Buttery (1977) showed only small variations in utilization by sheep liver cells of 20 amino acids measured in vitro. However, in vivo, threonine carbon conversion to glucose, which was chosen to reflect essential amino acid metabolism, accounted for less than 5 percent of threonine and 1 percent of glucose irreversible loss (Egan, 1978). Further evidence that not all amino acids are equally gluconeogenic was presented by Heitmann and Bergman (1980), who studied in vivo fluxes of 25 amino acids across four vascular beds in sheep. Only the amino acids alanine, glutamine-glutamate, glycine-serine, and arginine-ornithine were found to serve as important carbon and nitrogen transport sources for liver synthetic purposes. Thus, the relative contribution of each substrate will not necessarily reflect its importance in vivo, even if substrate were sufficiently available.

Following parturition, capacity for glucose synthesis (Fig. 2) was depressed, though not significantly. Depressed synthesis could be totally accounted for by decreased propionate utilization. Mathias and
Elliot (1967) found propionate utilization by bovine liver tissue to be decreased during early lactation as opposed to late lactation. Additions of vitamin B\textsubscript{12} to incubation media, in the study of Mathias and Elliot, was without effect, suggesting that B\textsubscript{12} was not limiting. Kenna et al. (1981) found in vitro gluconeogenic rates from [2-\textsuperscript{14}C]propionate to be greater 30 days postpartum compared to 60, 90, or 120 days postpartum. However, gluconeogenic rates were not measured during the dry period.

Decreases in substrate oxidation to CO\textsubscript{2} following parturition (EPP, PK), were not evident (Fig. 3). However, propionate made only a small contribution to CO\textsubscript{2} production and thus, small changes across periods may have been difficult to detect. The apparent small oxidation of propionate is a direct consequence of the isotope used. Comparisons of [1-\textsuperscript{14}C]propionate and [2-\textsuperscript{14}C]propionate metabolism in vivo (Annison et al., 1963) and in vitro (Leng and Annison, 1963; Seto et al., 1971) indicate the 1-carbon is lost readily and makes a large contribution to CO\textsubscript{2} while the 2- and 3-carbons are incorporated to a greater extent into glucose. Propionate carbon lost as \textsuperscript{14}CO\textsubscript{2} represents 24 percent of the total labeled carbon of propionate metabolized to glucose and CO\textsubscript{2}.

Wiltrout and Satter (1972) show that, in theory, 25 percent of labeled carbon from [2-\textsuperscript{14}C]propionate is lost per turn of the Krebs cycle. Thus, on an average, propionate cycled once within the Krebs cycle before entering the gluconeogenic pathway.

Total propionate utilization combined for glucose and CO\textsubscript{2}, indicates a decrease following parturition. It is not known whether this reflects decreased incorporation into Krebs cycle intermediates or dilution within
Figure 3. Hepatic oxidation of gluconeogenic substrate by ketotic cows

[D refers to dry period; EPP, early lactation; PK, preketotic; K, ketotic; TRT, treatment; ALA, alanine; ASP, aspartate; GLU, glutamate; LAC, lactate; PRO, propionate]
pathways by endogenous substrate. The former seems likely considering the reduction was specific for propionate. Baird et al. (1968) found a 25 percent reduction in propionyl-CoA carboxylase activity in lactating compared to nonlactating cows.

Preferential metabolism of aspartate to CO\(_2\) compared to lactate and alanine (Fig. 3), can be partly explained by an isotope effect. Upon metabolism of aspartate, lactate, and alanine to OAA, both carboxyl groups of OAA (carbon one and four) from aspartate would be \(^{14}\)C labeled, while only one carboxyl (carbon one) group of OAA from lactate and alanine would contain \(^{14}\)C. Both carbons one and four would be lost as \(^{14}\)CO\(_2\) if OAA were oxidized via citrate, while only carbon four would be lost as \(^{14}\)CO\(_2\) if OAA equilibrated quickly with malate and was metabolized via gluconeogenesis. Aspartate forming more \(^{14}\)CO\(_2\) than glutamate suggests that much of the aspartate was metabolized via the Krebs cycle.

Absence of a significant lactation effect (D vs EPF) on gluconeogenic capacity is not surprising considering the continual need for glucose synthesis in ruminants. Glucose entry rates increase during lactation (Baird, 1981b; Bergman, 1973; Bergman and Hogue, 1967; Lindsay, 1970; 1971; Wiltrout and Satter, 1972) and decrease with fasting (Baird, 1981b; Lindsay, 1970), but these changes occur without consistent changes in gluconeogenic enzyme activities (Baird and Heitzman, 1970; 1971; Baird et al., 1972; Ballard et al., 1969; Young et al., 1969). Consequently, gluconeogenic rate is thought to be related more to nutrient supply than to changes in efficiency of nutrient utilization (Lindsay, 1971). High correlations between digestible energy intake and glucose production
(Leng, 1970), no doubt reflect the relation of energy intake to volatile fatty acid production and microbial protein synthesis. Propionate and amino acids would account for a majority of glucose production in the fed state. Heitmann and Bergman (1980) found total amino acid extraction by the liver to be equivalent to the amount added to blood by the portal-drained viscera.

Substrate supply, however, is not the only factor regulating hepatic gluconeogenesis. Heitmann and Bergman (1980) noted a 70 percent increase in alanine extraction by the liver during fasting with no change in arterial concentration and a substantial decrease in quantity absorbed from the gut. Baird (1981b) showed that infusions of propionate or glucose into lactating cows reduced lactate extraction by the liver 50 percent. These results suggest that a hepatic effect on substrate utilization exists. Changes in substrate utilization by the liver may well be related to the hormonal environment, because glucagon increases hepatic extraction of many gluconeogenic amino acids (Brockman and Bergman, 1975; Brockman et al., 1975a), while enhancing their conversion into glucose (Brockman and Bergman, 1975). Propionate metabolism to glucose was increased also by glucagon, but this may have been more of a general hepatic effect as opposed to being substrate specific (Brockman and Greer, 1980). Glucagon is thought to increase hepatic concentrations of cAMP, which has been shown to increase conversion of pyruvate to glucose, and to decrease pyruvate oxidation in beef liver slices (Atwal and Sauer, 1973).
Figures 2 and 3 are somewhat superimposable, suggesting that variation in hepatic utilization of gluconeogenic substrates is not due to preferential metabolism toward glucose synthesis or toward oxidation. During period K, total substrate transfer into both glucose (Fig. 2) and CO$_2$ (Fig. 3) was significantly (P<.05) depressed. Visual inspection indicates the depression occurred across all substrates. This is indeed the case when variation of individual substrates during K is compared to the mean of periods D, EPP, and PK. Averaged across all substrates, substrate utilization by liver showed depressions of (mean ± SE) 66±6 percent for glucose and 47±6 percent for CO$_2$. The low standard errors are reflective of the uniformity across substrates.

Few studies have dealt with liver metabolism in ketotic ruminants. Hibbitt and Baird (1967), using liver slices, found liver from ketotic cows had a 35 percent lower respiration rate. Rumen epithelium from the same ketotic cows exhibited slightly greater rates of respiration compared to nonlactating cows, suggesting that the depression observed for liver may have been tissue specific. Gallagher (1960), using mitochondrial preparations from normal and pregnancy-toxemic ewes, noted equivalent oxidation rates from various Krebs cycle intermediates, but oxidation of palmitate, octanoate, and propionate was depressed in pregnancy-toxemic ewes. Chung et al. (1959) measured short-chain aliphatic acid incorporation into metabolic intermediates in normal and ketotic cow liver slices. Ketotic cows exhibited depressed CO$_2$ production from acetate and propionate and depressed BHBA formation from acetate and butyrate. Glucose production from propionate was observed
but was unexplainably low in both normal and ketotic cows. Mathias and Elliot (1967) also studied labelling patterns of Krebs cycle intermediates from $^{14}C$propionate. No differences were observed between ketotic, post-ketotic, or early lactation cows, but all data indicated decreased propionate utilization compared to cows in late lactation.

Although variable, the published results just discussed agree with the present results, which indicate impaired hepatic metabolism during ketosis. Similar conclusions have been made from in vivo studies using sulfobromophthalein (BSP) clearance in ketotic cows (Robertson et al., 1959). Glutamate-oxaloacetate transaminase (GOT) is used frequently as a diagnostic indicator of liver degeneration although, it is not specific to liver tissue (Kidder and McCullagh, 1980). Serum GOT concentrations in the current study (Table 8), were lowest in the dry period (D), showed no postpartum differences, and were well-within the normal range. Thus, liver impairment may occur independently of liver degeneration. Furthermore, treatment for ketosis re-established metabolism of gluconeogenic substrates (Figures 2 and 3), suggesting that the impairment of liver function is not permanent. Serum enzyme activities in cows with fatty cow syndrome (increased liver TG) are frequently increased (Morrow, 1976; Morrow et al., 1979), and BSP clearance rates are slower than rates reported for ketotic cows (Robertson et al., 1959). That liver degeneration seems to occur in fatty cow syndrome, but not in ketosis may suggest

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17SGOT assays performed and interpreted by ISU Veterinary Diagnostic laboratory, Ames, IA.
a difference in the etiology of the associated hepatic metabolic impairments. The relationships, however, between liver fat, hepatic impairment, and ketosis are far from clear.

Metabolic rates are based on DNA content to avoid potential confounding by changes in labile constituents such as fat, protein, glycogen, and water. Decreases in both oxidation and conversion to glucose, of all gluconeogenic substrates, suggest an overall depression in metabolic rate. Smith and Osborne-White (1965) noted a six-fold increase in propionate removal by homogenates of sheep liver if cytochrome C was added to the media. They suggested that an alteration in the rate of oxidative phosphorylation influences propionate metabolism. It is attractive to suggest for the present study, that the decrease in substrate utilization could be limited to the Krebs cycle because all substrates must mix with Krebs cycle intermediates. Unfortunately, no substrate, such as glycerol, was tested that is metabolized independently of the Krebs cycle.

Concluding Remarks

Obtaining adequate numbers of cases of lactation ketosis has hampered progress in understanding the etiology of ketosis development. Kronfeld (1971) has been insistent upon distinguishing between ketosis caused by feed deprivation and that created spontaneously (adequate feed available). However, a fine line must exist between adequate and inadequate feed availability as variation in digestibility and energy density of rations can create deficiencies of nutrients even if adequate
dry matter is available. Ketosis seems to develop in lactating cows when the supply of nutrients from the ration is limiting and dependency on endogenous sources is increased. Because many cows are normally borderline ketotic, slight feed restriction seems to be an appropriate tool to increase the number of clinical cases.

It is realized that ketosis usually develops slowly over 4 to 6 weeks of lactation, however, and drastic feed restriction may lead to a ketosis that is uncharacteristic of the true state. For these reasons, feed restriction was kept minimal, with only enough changes to tax an already delicate balance of metabolism. BD, a ketone precursor, also was included in the ration; BD was added to enhance any effects of ketone bodies on metabolism. Overall effects of BD were probably minimal because similar amounts (0.6-1.2 kg/day) have been fed to lactating cows (Bonner et al., 1976; Hess and Young, 1972) with only small changes in plasma ketone body concentration.

A prerequisite for an increased ketogenic state is a supply of free fatty acids from adipose tissue. To insure adequate adipose lipids for mobilization, cows were fed excess energy during the last 4 weeks of the dry period. Fronk et al. (1980) fed cows a high-energy ration for 8 weeks prepartum and showed that cows over conditioned in the dry period have slightly increased plasma FFA concentrations, increased hepatic triglyceride, and an increased incidence of ketosis. Care must be taken, however, not to get cows too fat during the dry period. Although the incidence of ketosis was related to the level of feeding, liver TG in over conditioned cows at 2 days postpartum were nearly 7 percent of
wet tissue weight. Also, at least three of the five reported cases of ketosis occurred within the first week postpartum, suggesting the cows were affected adversely by the feeding program. In the present study, the control cow showed little variation in hepatic TG, while the increases in the ketotic group seem to be related to greater milk production and a greater adipose fatty acid mobilization. Also, time postpartum until clinical ketosis developed averaged 36 days, indicating cows were normal during early postpartum. Thus, the prepartum program employed was probably beneficial in increasing body weight but was not severe enough to create early postprandial problems.

Our goal was to create a situation for dairy cows that would result in a high incidence of uncomplicated ketosis. The manipulations outlined seem to be an adequate means of meeting this goal as evidenced by postpartum cow responses:

1. Feed intake and milk production increased during the first 2 weeks postpartum.
2. Plasma and liver metabolite concentrations indicated progressive development of ketosis.
3. Ketosis developed slowly averaging 5 weeks postpartum and 3 weeks after feed restriction and 1,3-butane diol supplementation.
4. Voluntary feed restriction is indicative of a natural, non-induced ketosis.

Cows destined to become ketotic, voluntarily ate less silage-grain mix and milked more than did the control cow. These two factors were probably important in ketosis development, or lack of development, as
the ketotic group relied much more on endogenous sources to meet nutrient requirements. A greater dependency on endogenous nutrients is evident in the greater body weight loss, lower plasma glucose and insulin, and greater plasma FFA and BHBA of the ketotic cows.

It has been established that one result of ketosis is a depression in feed intake. It seems from the present results that voluntary feed intake limitation may proceed and add to ketosis development. Hay consumption was not measured so caution must be emphasized. Baile (1971) reviewed the complicated regulatory controls of feed intake to the extent they are known. Besides the effects of stressors (disease, heat, etc), a number of energy metabolites, including acetate, propionate, and lactate can act on ruminal, hepatic, and hypothalamic receptors to limit feed intake. Recent evidence (Leek et al., 1978a; 1978b; Ryan, 1981; Upton et al., 1977) suggests that ketone bodies, or a metabolite of them, depress reticulo-ruminal activity in sheep. Ryan (1981) suggests that receptors deep within the mucosa of the rumen wall are accessible from either the rumen or blood, and thus the nutritional status of the animal (changes in KB concentration) may modulate rumen activity. While it cannot be stated conclusively that 1,3-butanediol had no palatability effect, a potential effector of feed intake may be the concentration of ketone bodies.

It seems likely that direct consequences of the increased dependence on endogenous reserves are the reduction in hepatic glycogen and the increase in hepatic triglycerides (Table 10). Hepatic extraction of FFA by the liver is related directly to the concentration in blood (Heimberg
et al., 1974). Partitioning of FFA toward oxidation or triglyceride formation is regulated in nonruminants (McGarry et al., 1978a; 1978b) and, much evidence suggests regulation in ruminants as well (Baird et al., 1979; Bergman, 1971; Jarrett et al., 1976). Glycogen concentration reflects this partitioning in rats, elevated (energy sufficient) levels being associated with low oxidation, while depressed glycogen (energy deficient) results in an increase in fatty acid oxidation and decreased triglyceride formation (Heimberg et al., 1974; McGarry and Foster, 1978; McGarry et al., 1973). Increased fatty acid oxidation and KB production likely is related to hepatic glycogen (Table 10) and perhaps to citrate (Tables 11 and 12), which were depressed sooner and to a greater extent following parturition in the ketotic group. Maintenance of glycogen may be critical to attenuating fatty acid oxidation and ketone production and is indicative of the energy imbalance associated with high production. Glycogen depletion occurred during the first 2 weeks postpartum even when cows were fed ad libitum. Thus, the ketotic cows were geared toward fatty acid oxidation prior to any attempts to enhance fatty acid mobilization and oxidation.

Hepatic triglyceride also increased during EPF suggesting that either more FFA were available than could be adequately metabolized or that a depression in lipoprotein secretion is occurring also. Reid et al. (1979a) found fasted cows had a net hepatic extraction of both FFA and TG, which resulted in liver lipid accumulation. Subsequent work by Reid et al. (1981), associated a decreased hepatic content of rough endoplasmic reticulum in underfed lactating cows with decreased ability
to secrete lipoprotein. While lipoprotein flux rates were not measured in the present study, it would seem that a depression in lipoprotein secretion might explain some of the increase in hepatic TG in the ketotic cows.

Hepatic TG accumulation in high producing cows is common but levels rarely exceed 5 percent wet weight (Collins and Reid, 1980; Reid et al., 1979b). Values of 11 percent reported in the present study are equivalent to levels noted in ketogenic cows (Baird et al., 1968; Bergman, 1971; Hibbitt and Baird, 1967). Persistence of hepatic TG may present problems as the increase noted in normal cows at 1 week postpartum was down to prepartum levels by 4 weeks (Reid et al., 1981). Cows maintained at 60 percent of requirements, maintained increased concentrations of liver TG and showed decreased numbers of mitochondria per cell (Reid et al., 1981). Similar mitochondrial changes have been reported for fasted nonlactating cows (Reid et al., 1977) and fasted lactating cows (Reid, 1973). Reid and Collins (1980) examined 20 cows postpartum, grouped them according to mild or severe fatty liver, and examined morphological changes. Severe fatty liver was accompanied by decreased rough endoplasmic reticulum and mitochondria, and evidence of mitochondrial damage. Hepatic TG content of severe fatty livers was back to prepartum levels by 8 weeks postpartum indicating that cows were able to adapt. To what extent a continually elevated TG content influences hepatic morphology and ketosis development is not known.

Fatty liver in chicks is associated with decreased gluconeogenic rates (Bannister, 1976a) and significant depressions of pyruvate
carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK), and glucose-6-phosphatase (Bannister, 1976b). Supplementation with biotin restored activity of PC but not PEPCK, prompting the suggestion that a biotin deficiency may not be the only complication of the syndrome (Bannister, 1976b). Thus, fatty liver, or changes accompanying fatty liver, may have detrimental effects on hepatic metabolism in cows. Liver TG in the present study paralleled plasma FFA concentrations, plateauing during PK and prior to any detectable changes in metabolic rate. Consequently, the concentration per se may not be as important as the duration of the increased TG concentration. It is also possible that changes occurring simultaneously with increased liver TG may be influencing hepatic metabolism to a greater extent than TG concentration.

One change that may be important is the hepatic concentrations of ketone bodies (Table 11). Rifkin (1963) studied bovine mitochondrial metabolism, and showed a depression in the activity of the enzyme complexes pyruvate dehydrogenase and α-ketoglutarate dehydrogenase by AcAc. Iles et al. (1977), using perfused rat liver, found a 30 percent reduction in glucose output if media pH was lowered from 7.4 to 6.8. Thus, it is possible that accumulation of AcAc or AcAc plus BHBA have a poisoning effect on hepatic metabolism resulting in depressions of metabolic rate. A poisoning effect would fit the data presented herein indicating a general metabolic depression during ketosis. Direct effects of KB on gluconeogenesis have not been explored.
PART II. METABOLIC STUDIES WITH STEERS

Introduction

Results presented already from lactating cows indicate there is an impairment of hepatic metabolism during ketosis. As suggested, ketone body accumulation within the liver may have direct effects on limiting hepatic metabolism. If KB influence hepatic metabolism in cows, similar effects also may be apparent in steers. Previous work has indicated that 1,3-butanediol (BD) when fed to young cattle at high concentrations, increases ketone body levels in blood and causes nervous symptoms characteristic of ketosis (Bonner et al., 1975). The purpose of the present experiment was to:

1. Test effects of increased blood KB, by feeding high levels of 1,3-butanediol, on hepatic gluconeogenic and ketogenic rates,
2. Create a model ketosis in steers using phlorizin and BD separately or in combination, and to monitor hepatic metabolism as before,
3. Compare gluconeogenic and ketogenic capacities in vitro in steers on full feed and, subsequently, on restricted feed.

The third objective was included to help answer a question raised from results with the lactating cows. The relationship between feed intake, glucose precursor availability, and in vivo glucose production has been discussed already. To what extent feed restriction influenced hepatic metabolism in the study with lactating cows, and hepatic metabolism in general is not known.
Materials and Methods

Experimental animals and design

One Shorthorn and three Holstein steers with mean initial body weights of 190 kg (range 150-230), were put on the primary study using a 4x4 Latin square design. Due to limitations in feeding equipment and housing, two steers completed the experimental program before the remaining two were started.

Within 48 hours of completing the Latin square experiment, three of the four steers were restricted in ration intake for 6 days (one steer died following its third liver biopsy). Two steers were fed half the basal ration, while the third was fasted. Data from these three steers will be combined.

All four steers received the same basal ration of 3140 g chopped alfalfa hay and 1476 g concentrate\(^1\) throughout the Latin square experiment. Rations were aliquoted in 12 equal portions fed at 2-hour intervals using automatic feeders. The two steers fed half the basal ration following the Latin square experiment, were given six equal portions at 4-hour intervals. Steers were penned individually and chained to the feed bunk, allowing access to feed and water with ample freedom to lie down.

The four treatments applied to the four steers in the Latin square are listed in Table 17. Two weeks were allowed between experiments for

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\(^1\)Concentrate was 92 percent cracked corn, 5 percent anhydrous Na\(_2\)H\(_2\)PO\(_4\), and 3.2 percent trace mineral salt.
Table 17. Summary of experimental treatments used in the Latin square

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experimental protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control - C</td>
<td>basal ration fed every 2 hours in 12 equal portions</td>
</tr>
<tr>
<td>1,3-butanediol - BD</td>
<td>as in Control, with 1,3-butanediol mixed with concentrate and fed to tolerance</td>
</tr>
<tr>
<td>Phlorizin - P</td>
<td>as in Control, with twice daily subcutaneous injections of 1 g phlorizin</td>
</tr>
<tr>
<td>Phlorizin + butanediol - PBD</td>
<td>as in BD, with twice daily subcutaneous injections of 1 g phlorizin</td>
</tr>
</tbody>
</table>

Acclimation to the subsequent treatment. BD was added to the basal ration as a supplement, not as a replacement, and quantities added were increased over a 7-day period. BD was offered to steers to maximize BD intake (and thus blood KB), and to approach a nervous condition noted by previous authors (Bonner et al., 1975), without decreasing feed intake. Steers vary in their ability to tolerate BD as indicated by the maximum intakes obtainable (Table 18). Total ration consumption during BD feeding was not always achieved. Steer 8241 refused 30 percent of its ration the last 2 days prior to liver biopsy during treatment BD. The same steer, during treatment PBD, was off feed 2 days prior to liver biopsy, but ate 80 percent of the ration the day before liver biopsy.

Phlorizin, dissolved in propylene glycol (1g/4ml), was injected subcutaneously twice daily for 7 days prior to liver biopsy. Steers received 1.5 g phlorizin per day on the first day and 2 g per day
Table 18. Quantities of 1,3-butanediol fed to steers

<table>
<thead>
<tr>
<th>Steer</th>
<th>Butanediol</th>
<th>Phlorizin + butanediol</th>
</tr>
</thead>
<tbody>
<tr>
<td>8241</td>
<td>600</td>
<td>600</td>
</tr>
<tr>
<td>8263</td>
<td>850</td>
<td>820</td>
</tr>
<tr>
<td>8446</td>
<td>450</td>
<td>450</td>
</tr>
<tr>
<td>8466</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>550</td>
</tr>
</tbody>
</table>

<sup>a</sup>Three day average prior to liver biopsy.

<sup>b</sup>8466 did not complete BD experimental period.

thereafter. Phlorizin (phloretin-2'-glucoside) is a naturally occurring compound that binds to the glucose receptor and prevents glucose transport. Acting on the kidney glucose receptor, phlorizin results in a major excretion of glucose in urine. Rates of glucose excretion with 2 g phlorizin per day are maximum at about 6 hours after dosing, after which excretion rates decline (Young et al., 1974). Injecting phlorizin every 12 hours, minimizes the quantity of phlorizin required while maximizing glucose excretion. Glucose excretion averaged 216 g per day for treatment P and 288 g per day for treatment PBD. Glucose irreversible loss in a 200 kg steer is roughly 600 g per day (Van Maanen et al., 1978). Thus, glucose excretion in the urine may reduce by one-third to one-half the amount of glucose available for body metabolism. Phlorizin had no apparent effect on the ability of steers to tolerate BD (Table 18).
Experimental protocol

The experiments to be described herein are only a part of the total sampling and analyses acquired from steers during the Latin square trial. In vivo glucose kinetics as well as profiles of blood metabolites and hormones were obtained by Randy Lyle and Gerry de Boer.

Liver biopsy Following the two week adaptation period to treatments, liver biopsies were conducted as discussed already. Biopsy of steer pairs were performed on the same day with 5 to 6 grams of liver tissue taken. Approximately 0.5 g was immersed in liquid nitrogen and the remainder was placed in ice cold buffered saline (pH 7.4). The liver sample in liquid nitrogen was assayed for metabolites within 48 hours, while liver tissue in saline was returned immediately to the lab for in vitro incubations.

Incubation procedure Liver tissue was sectioned as described already. Incubation media for gluconeogenic and ketogenic rate determinations was Krebs-Hensleit bicarbonate buffer (pH 7.4) with 1 percent BSA (with the exception of stearate). Media was 3 percent BSA and was free of Ca$^{++}$ when stearate was the substrate.

Gluconeogenic capacities were determined from 10 mM; Na-L-[$^{14}$C]-aspartate, Na-L(+)[U-$^{14}$C]lactate, or Na-[2-$^{14}$C]propionate. Ketogenic rates (BHBA production) were determined from; 10 mM Na-butyrate, and 10 mM 1,3-butanediol, or 1 mM [1-$^{14}$C]stearate bound to fatty acid free BSA. Hanging wells containing filter paper for trapping CO$_2$ were placed in all flasks containing radioactive substrates.
All incubations of liver slices were performed in triplicate with background determinations, also in triplicate, conducted as follows. Contaminations of CO$_2$ and glucose from radioactive gluconeogenic substrates were measured by incubating without liver and otherwise conducting all procedures as for flasks with tissue. Endogenous BHBA production (no substrate added) was measured and subtracted from flasks incubated with butyrate, BD, or stearate. Endogenous rates were low and fairly constant across treatments. Contamination of CO$_2$ and tissue triglycerides (TG) from $^{14}$C-stearate was measured from tissues killed with 0.5 ml of 10 percent HClO$_4$ at the initiation of incubations.

After a 1 hour incubation in a Dubnoff metabolic shaker operating at 90 strokes per minute, reactions were stopped by injecting 0.5 ml of 10 percent HClO$_4$. In flasks containing a hanging well, 0.1 ml of 25 percent NaOH was injected onto the filter paper and the incubation continued an additional hour.

Preparation for incubations Substrates for incubations, with the exception of stearate, were made up, at a concentration of 60 micromoles per ml, in Krebs-Hensleit salt solution (bicarbonate free) plus 10 mM KH$_2$PO$_4$ (pH 7.4). Appropriate radioactive substrates were added to the solutions, aliquots removed, and stored frozen until needed for an incubation. Preparation of stearate-albumin and the set-up for incubations were as discussed already in 'Metabolic Studies with Lactating Cows'.
Analytical procedures

Liver incubations Media from incubations for gluconeogenic rate determinations were spiked with measured amounts of [6-\(^3\)H]glucose (usually 5000 DPM), transferred to a 50 ml centrifuge tube, and the protein precipitated by centrifuging in the cold after addition of 0.5 ml of 10 percent HClO\(_4\). The supernatant was transferred to a 15 ml conical centrifuge tube, mixed with 0.05 ml universal indicator\(^2\), and neutralized with KOH. KClO\(_4\) was precipitated by centrifugation and the resulting supernatant was used for glucose specific radioactivity determinations (Mills et al., 1981). Procedural losses were corrected for by [6-\(^3\)H]glucose recovery. Two milliliters of media from incubations with butyrate, BD, or stearate was added to a 15 ml conical centrifuge tube, 0.5 ml of 10 percent HClO\(_4\) was added, and the protein was precipitated by centrifugation. One milliliter of supernatant was transferred to 12x75 mm plastic test tubes, mixed with 0.01 ml universal indicator, and neutralized with KOH. Tubes were capped and frozen until assayed for BHBA.

Tissues from stearate incubations were rinsed in saline, blotted dry, and transferred to 30 ml screw-capped tubes. Lipids were extracted according to Bligh and Dyer (1959) after homogenizing tissue samples in extracting solvents using a blade homogenizer\(^3\). The chloroform layer from the extraction was passed through glass wool and evaporated to

\(^2\)Fisher Scientific Co., St. Louis, MO.

\(^3\)Brinkman polytron, Kinematica, Luzern, Switzerland.
dryness. Ten milliliters of hexanes were added, vortexed, and the solvent poured through small glass columns containing 0.5 g Florisil, which had been deactivated previously by equilibration with 7 percent water (Carroll, 1961). Triglycerides were separated from FFA by eluting TG into scintillation vials with 10 ml of 15 percent ether in hexane. Solvent was evaporated and scintillation cocktail added. All quantitation of radioactivity was as described in 'Metabolic Studies with Lactating Cows'.

**Liver tissue** Preparation of tissue homogenates and deproteinized extracts from liver stored in liquid nitrogen for glycogen and BHBA determinations was as described already in 'Metabolic Studies with Lactating Cows'. Excess liver not used in incubations was stored frozen (-20°) and used for determination of triglycerides and DNA. DNA was determined by the method of Munro and Fleck (1969).

For determination of triglycerides, 100 to 200 mg of liver were added to a 4 dram vial. Water (1.5 ml) was added and the tissue sonicated until homogeneous. Lipids were extracted according to Bligh and Dyer (1959) and the chloroform extract used for triglyceride determinations as described by Eggstein and Kuhlmann (1974). As with triglyceride analysis in 'Metabolic Studies with Lactating Cows', the alcoholic-KOH triglyceride-hydrolyzing media was dried, resuspended in assay buffer, and filtered. Glycerol-free filters were purchased from Millipore.

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4 Fisher Scientific Co., St. Louis, MO.

5 Millipore Corp., Bedford, MA.
Presentation of results DNA determinations were from small slices of liver tissue stored frozen. Duplication of results across slices from the same tissue proved difficult, whereas variation was low for triplicate analysis on the same slice. It is not known if individual slices were not homogeneous with respect to the whole tissue or if perhaps the -20° was not cold enough to preserve DNA. Metabolite concentrations and metabolic rates will be presented on a tissue weight basis because of the variability in DNA determinations and because only minor changes occurred in tissue glycogen and triglyceride.

Miscellaneous

All instrumentation was the same as that described already in 'Metabolic Studies with Lactating Cows'. All chemicals and radioisotopes also were purchased as already outlined. Purchase of 1,3-butanediol for incubation was from Aldrich. Steer 8466 died after three treatments were complete, thus one square was absent from the 16 of the 4x4 Latin square. Predicted values for missing data were generated and data were analyzed by GLM for a Latin square (Goodnight, 1979). Predicting missing data falsely increases the degrees of freedom (df) for error. Consequently, the df for error were reduced by one and all tests of significance were corrected. Differences between treatments were tested using orthogonal comparisons (Goodnight, 1979). For testing differences between fasted and control steers, a t-test analysis was used (Goodnight, 1979).

6Aldrich Chemical Co., Milwaukee, WI.
Results and Discussion

**BD feeding and occurrence of nervous signs**

Bonner et al. (1975) found that calves given over 0.8 kg of BD daily exhibited hyperactivity, nervousness, profuse urination, and muscular tremors. Total blood ketones in their study were elevated 3 to 10 fold with acetone plus acetoacetate accounting for nearly all the increase. If KB concentrations are responsible for the nervous condition then acetone plus acetoacetate, rather than BHBA, may be the causative agents, however, total KB concentration (AcAc + BHBA) cannot be discounted. Romsos et al. (1974) found that rats given 48 percent of their dietary energy as BD were extremely hyperactive and often exhibited bizarre behavior. Total plasma ketones increased nearly 20 fold with BHBA accounting for 70 percent of the total. AcAc, although increased 10 fold over non-BD fed rats, showed little fluctuation over low-BD fed rats where no nervous symptoms were observed. The results of Romsos et al. suggest that either total KB concentrations are responsible for nervous displays or that other factors are involved.

Total plasma KB concentrations increased 3 and 7 fold for treatments BD and PBD, respectively (Table 19). Hyperexcitability accompanied by muscular tremors occurred in steers during four of the seven periods BD was fed. Although treatment PBD had, on the average, 2 fold greater concentrations of total KB, appearance of nervous signs were not restricted to the PBD treatment. Considerable animal variation may result for the appearance of nervous signs, as steer 8446 had the
Table 19. Plasma concentrations of ketones in steers treated with 1,3-butanediol and phlorizin

<table>
<thead>
<tr>
<th>Ketone</th>
<th>Treatment</th>
<th>Control</th>
<th>Phlorizin</th>
<th>Butanediol</th>
<th>Phlorizin + butanediol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (mM) or ratio</td>
<td>---------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-hydroxybutyrate</td>
<td>.25±.03b</td>
<td>.39±.04</td>
<td>.64±.04</td>
<td>1.58±.49</td>
<td></td>
</tr>
<tr>
<td>Acetoacetate</td>
<td>0</td>
<td>.02±.004</td>
<td>.06±.01</td>
<td>.15±.05</td>
<td></td>
</tr>
<tr>
<td>β-hydroxybutyrate + acetoacetate</td>
<td>.25</td>
<td>.41</td>
<td>.70</td>
<td>1.73</td>
<td></td>
</tr>
<tr>
<td>β-hydroxybutyrate: acetoacetate</td>
<td>---</td>
<td>19.5</td>
<td>10.7</td>
<td>10.5</td>
<td></td>
</tr>
</tbody>
</table>

aKetone concentration represents an average of the three days before liver biopsy for all steers.

bMean ± standard error of the mean.

highest KB concentrations (30 mM BHBA and 3 mM AcAc with PBD) but no indication of nervous abnormalities. Curiously, steer 8446 was fed the least amount of BD, which suggests that nervous signs are more related to the amount of BD fed rather than to the concentration of KB.

Whether KB can cause nervous signs is unknown to the author. Miles et al. (1981) and Sherwin et al. (1975) infused BHBA into fed or fasted humans. Total KB increased from the normal of about 0.2 mM to 2.5 mM in fed and to 10 mM in fasted subjects. Data on behavioral changes were not reported but it is assumed none were present or they would have been mentioned. Schwalm and Schultz (1976b) found that
diabetic goats increased total KB 10 fold to nearly 3 mM; they did not mention behavioral changes. Ketosis in dairy cattle is occasionally accompanied by nervous signs (Schultz, 1974). Whether the nervous signs of ketosis and the nervous signs accompanying BD feeding are related remains unresolved.

**Plasma and liver metabolite concentrations**

Feeding BD (BD or PBD) increased plasma KB concentrations over control and seems to increase BHBA and AcAc similarly (Table 19). Increased BHBA concentrations also are evident in the liver (Table 20). Close agreement between trends of changes in hepatic and plasma BHBA suggests that the liver may be responsible for the conversion of BD to BHBA. Metabolism of BD by liver may be at least partly responsible for BHBA production as shown by in vitro data (Table 21). BHBA production from BD occurred at about 50 percent the rate of butyrate oxidation to BHBA, and about 10 fold greater than rates from stearate.

BD is thought to be metabolized after absorption from the gut in ruminants because of the failure to find significant changes in volatile fatty acid ratios in the rumen with BD feeding (Bonner et al., 1975; Hess and Young, 1972). In rats, Tate et al. (1971) presented evidence that BD is oxidized to BHBA within liver cytosol. Electrophoretic analysis of the enzyme initiating BD metabolism was similar, if not identical, to alcohol dehydrogenase. If BD is oxidized to BHBA in the cytoplasm and BHBA subsequently is oxidized to AcAc within the mitochondria, then for every mole of BD oxidized to AcAc, the cytosol
Table 20. Hepatic metabolite concentrations in steers treated with 1,3-butanediol and phlorizin

<table>
<thead>
<tr>
<th>Hepatic metabolite</th>
<th>Control</th>
<th>Phlorizin</th>
<th>Butanediol</th>
<th>Phlorizin + butanediol</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen b</td>
<td>3.8</td>
<td>3.2</td>
<td>3.7</td>
<td>3.1</td>
<td>.31</td>
</tr>
<tr>
<td>Triglyceride b</td>
<td>.26</td>
<td>.54</td>
<td>.25</td>
<td>.83</td>
<td>.21</td>
</tr>
<tr>
<td>β-hydroxybutyrate d</td>
<td>.26</td>
<td>.30</td>
<td>.66</td>
<td>1.58</td>
<td>.41</td>
</tr>
</tbody>
</table>

aFour steers/treatment for C, P, and PBD; three steers for BD.

bConcentrations in percent wet weight.

cP<.1 for C+BD vs P+PBD.

dConcentrations in μmoles/g wet weight.

eP<.1 for C+P vs BD+PBD.

would gain two moles of NADH and the mitochondria one mole of NADH. Mehlman et al. (1971b, 1972) in examining metabolite concentrations in freeze-clamped livers from rats fed BD, found that both the cytosol and the mitochondria were more reduced than in rats not fed BD. Thus, BD oxidation to BHBA and AcAc may not be restricted to tissues normally producing KB (liver and rumen epithelium).

BD, as the sole treatment, had no effect on concentrations of hepatic glycogen or triglyceride (Table 20). Steers given BD alone were not stressed energetically and had little need for glycogen or adipose fatty acid mobilization. BD is reported to have a metabolizable energy
value of 6.5 kcal/g and glucose has about 4 kcal/g in rats (Romsos et al., 1974). Corn, on the other hand, has a ME value of about 3.4 kcal/g in cattle (NRC, 1978). Rations without supplemental BD were adequate for small rates of growth in the size steers used in the present study. Thus, BD supplementation added to a positive energy balance.

Mehlman et al. (1966) and Stoewsand et al. (1965) fed high amounts of BD to rats and noted increased plasma FFA and hepatic triglyceride concentrations. Both Mehlman et al. and Stoewsand et al. replaced carbohydrates in the diet with BD on a calorie basis, whereas in the present study BD was supplemented without replacement. Consequently, in the rat studies, animals were deficient in carbohydrate and responded with increased adipose mobilization.

Phlorizin alone had little effect on plasma BHBA (Table 19) or on hepatic BHBA and glycogen (Table 20). When combined with BD, phlorizin increased plasma BHBA over BD alone, which suggest an increased fatty acid mobilization from adipose and subsequent oxidation to KB. Increased adipose lipolysis with phlorizin also is suggested in hepatic metabolites (Table 20). Hepatic triglycerides increased slightly, but significantly (P<.1) with phlorizin administration. Glycogen concentrations also were least with phlorizin-treated steers, indicating a greater dependency on endogenous nutrient sources.

Phlorizin has been used in steers (Staubus et al., 1960), ewes (Burtis et al., 1968), lactating cows (Weik and Zander, 1975), and goats (Menahan et al., 1966b) to create a glucose stress (increased glucose requirement) and increase the animals dependency on endogenous nutrients.
Increased glucose irreversible loss in sheep (Egan, 1978) and steers (Young et al., 1974) given phlorizin reflects the added glucose stress. Phlorizin effects were similar to removing carbohydrate from the diet as in the rat studies (Mehlman et al., 1966; Stoewsand et al., 1965). Steers were carbohydrate (glucose) stressed and responded with increased adipose tissue mobilization.

One goal of the present study was to mimic in steers metabolite changes found in blood and liver of lactating cows during ketosis. Characteristic signs include increased plasma and hepatic KB, increased hepatic triglycerides and decreased hepatic glycogen. The combination of phlorizin and butanediol came the closest to mimicking lactation ketosis, with increased plasma and hepatic BHBA and increased hepatic TG. However, the change in hepatic TG, by all treatments, was negligible compared to lactating cows and probably reflects a positive energy balance across all treatments. Positive energy balance is also suggested by the lack of response in hepatic glycogen. Thus, a true ketotic state, similar to that observed in lactating cows, was not achieved by the experimental design.

Metabolism of liver slices

Table 21 shows the effects of treatments on in vitro hepatic oxidation of butyrate, BD, or stearate to BHBA. Feeding BD alone (period BD) depressed the capacity of liver to synthesize BHBA from all substrates. Phlorizin, either alone or with BD, showed a trend for increased capacity over controls, but effects were not significant (P<.1). Mehlman et al.
Table 21. Hepatic metabolism of fatty acids and 1,3-butanediol to β-hydroxybutyrate in steers treated with 1,3-butanediol and phlorizin

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Treatmenta</th>
<th>Control</th>
<th>Phlorizin</th>
<th>Butanediol</th>
<th>Phlorizin + butanediol</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyrate</td>
<td></td>
<td>1100</td>
<td>1247</td>
<td>688**</td>
<td>1172</td>
<td>105</td>
</tr>
<tr>
<td>Stearate</td>
<td></td>
<td>43</td>
<td>42</td>
<td>32</td>
<td>90</td>
<td>22</td>
</tr>
<tr>
<td>Butanediol</td>
<td></td>
<td>554</td>
<td>610</td>
<td>322*</td>
<td>640</td>
<td>124</td>
</tr>
</tbody>
</table>

*a Four steers/treatment for C, P, and PBD; three steers for BD.

*P<.1, BD vs C+P+PBD.

**P<.01, BD vs C+P+PBD.

Table 22. Stearate oxidation and esterification in liver of steers treated with 1,3-butanediol and phlorizin

<table>
<thead>
<tr>
<th>Productb</th>
<th>Treatmenta</th>
<th>Control</th>
<th>Phlorizin</th>
<th>Butanediol</th>
<th>Phlorizin + butanediol</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon dioxide</td>
<td></td>
<td>4.0</td>
<td>3.6</td>
<td>3.2</td>
<td>4.1</td>
<td>.64</td>
</tr>
<tr>
<td>Triglyceridec</td>
<td></td>
<td>5.7</td>
<td>6.8</td>
<td>3.8</td>
<td>6.0</td>
<td>2.1</td>
</tr>
</tbody>
</table>

*a Four steers/treatment for C, P, and PBD; three steers for BD.
b*nmoles stearate converted to product per 100 mg per hour.
cLiver triglyceride.
(1971a), using rat liver slices, noted a 54 percent reduction in liver capacity to synthesize BHBA from glucose and a 22 percent reduction in BD oxidation to AcAc, but no change in BHBA production, in rats fed BD for 7 weeks. Stearate oxidation to carbon dioxide and esterification into triglyceride (Table 22) tended to be lower with BD feeding alone compared to the other three treatments. BD thus may have a suppressive effect on hepatic fatty acid metabolism. If BD is metabolized solely in the cytosol and is not metabolized via the HMG-CoA pathway, then effects of BD seem to be quite broad, depressing both mitochondrial and cytoplasmic reactions.

Rates of oxidation of gluconeogenic substrates to carbon dioxide are shown in Table 23. Feeding BD as the sole treatment, depressed oxidation of lactate and propionate, but not of aspartate, when compared to the other treatments. No differences in substrate oxidation were evident between treatments C, P, and PBD. As was seen with butyrate and BD oxidation (Table 21), phlorizin treatment prevented the depressive effects of BD.

Compared to treatment C, lactate and propionate utilization for glucose synthesis (Table 24) was depressed during treatment BD while phlorizin (P and PBD) increased gluconeogenic rates from all substrates. As was seen with aspartate oxidation (Table 23), BD had no effect on aspartate incorporation into glucose. Phlorizin increased substrate utilization for glucose synthesis by liver tissue. Increased rates of gluconeogenesis are suggestive of an increase in activity or concentration of a rate limiting enzyme. That substrate oxidation was not increased concomitant with increased glucose synthesis, suggests that
Table 23. Effects of 1,3-butanediol and phlorizin on hepatic oxidation of gluconeogenic substrates to carbon dioxide in vitro

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Control</th>
<th>Phlorizin</th>
<th>Butanediol</th>
<th>Phlorizin + Butanediol</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate</td>
<td>189</td>
<td>207</td>
<td>186</td>
<td>187</td>
<td>19</td>
</tr>
<tr>
<td>Lactate</td>
<td>225</td>
<td>243</td>
<td>165**</td>
<td>241</td>
<td>16</td>
</tr>
<tr>
<td>Propionate</td>
<td>87</td>
<td>82</td>
<td>67*</td>
<td>79</td>
<td>7</td>
</tr>
</tbody>
</table>

^Four steers/treatment for C, P, and PBD; three steers for BD.

^bNmoles of substrate oxidized to carbon dioxide per 100 mg per hour.

*P<.05, BD vs C+P+PBD.

**P<.001, BD vs C+P+PBD.

Table 24. Effects of 1,3-butanediol and phlorizin on hepatic gluconeogenesis measured in vitro

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Control</th>
<th>Phlorizin</th>
<th>Butanediol</th>
<th>Phlorizin + Butanediol</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate</td>
<td>101</td>
<td>143</td>
<td>95</td>
<td>133</td>
<td>7</td>
</tr>
<tr>
<td>Lactate</td>
<td>334</td>
<td>497</td>
<td>220</td>
<td>537</td>
<td>38</td>
</tr>
<tr>
<td>Propionate</td>
<td>974</td>
<td>1181</td>
<td>760</td>
<td>1397</td>
<td>103</td>
</tr>
</tbody>
</table>

^aFour steers/treatment for C, P, and PBD; three steers for BD.

^bNmoles substrate incorporated into glucose per 100 mg liver per hour.

^cP<.01, P.PBD vs C+BD.

^dP<.025, BD vs C.

^eP<.1, BD vs C.
the increased capacity may be restricted to the cytoplasm (such as PEPCK or fructose 1,6-diphosphatase). However, gluconeogenic rates from aspartate and propionate were increased approximately 35 percent while lactate utilization increased nearly 55 percent. Increased activity of pyruvate carboxylase potentially could account for the increased lactate utilization.

Young et al. (1964) found PEPCK activity to double in rats fasted for 24 hours, while the increased activity due to fasting could be reversed by administration of carbohydrate (glucose or glycerol) but not by amino acids. Increased PEPCK activity during carbohydrate deficiency is likely a result of plasma hormonal fluctuations as both glucocorticoids and cAMP, which are increased by glucagon, epinephrine, and other hormones, cause increased activity of PEPCK in rat hepatocytes (Wicks et al., 1974). It is possible that the glucose drain caused by phlorizin may signal a carbohydrate deficit and a need for a greater capacity for glucose synthesis, much like in fasted rats.

Glucocorticoid concentrations have been shown to increase in plasma of fasting cattle (Mills and Jenny, 1979). Effects of glucocorticoids in cattle may differ from those in rats, however, because decreased PEPCK activity has been demonstrated for cows given dexamethasone (Baird and Heitzman, 1970; 1971). It is not known how the glucose drain caused by phlorizin affected glucocorticoids in the present study. Plasma insulin tended to be lower and glucagon tended to be higher with phlorizin treatments (P and PBD), but data were variable and not statistically significant (data will be summarized by others). Furthermore, Young
et al. (1969) found no increase, and perhaps a decrease, in liver PEPCK activity in heifers fasted for 8 days. While evidence exists in ruminants for a lack of gluconeogenic enzyme adaptations to physiological states, more work is required to examine the relation between phlorizin-induced increases in gluconeogenic rates and enzyme capacities.

Glucose is a potent inhibitor of gluconeogenesis both in vivo (Judson and Leng, 1973a) and in vitro (Seto et al., 1971). Thus, differences between treatments in glucose accumulation with liver slice incubations could create differences in gluconeogenic rates. Table 25 shows total glucose accumulations during incubations. While steers fed the control ration tended to accumulate more glucose, differences were small, and not statistically significant (P>.1). Glucose accumulations were not corrected for tissue weight added to the incubation. Total glucose accumulation of about 6000 nmoles far exceeds the quantity synthesized from substrate (8 to 120 fold) and probably reflects mobilization of glycogen within liver slices. Seto et al. (1971) found a 20 percent reduction in glucose synthesis from [2-14C]propionate when 100 micromoles of glucose, 15 fold greater quantities than total accumulations in the present study, were added to liver slice incubations. Thus, it seems unlikely that glucose, and more especially, differences in glucose accumulation could account for the present differences in gluconeogenic rates.

Phlorizin not only increased hepatic gluconeogenic rates, but negated the suppressive effects of BD (Tables 23 and 24). BD feeding to rats has led to decreased plasma glucose concentrations, but increased
Table 25. Effects of 1,3-butanediol and phlorizin on total glucose accumulation from liver slices

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Control</th>
<th>Phlorizin</th>
<th>Butanediol</th>
<th>Phlorizin + butanediol</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate</td>
<td>7890</td>
<td>6170</td>
<td>6110</td>
<td>6610</td>
<td>600</td>
</tr>
<tr>
<td>Lactate</td>
<td>7780</td>
<td>6500</td>
<td>6170</td>
<td>6280</td>
<td>890</td>
</tr>
<tr>
<td>Propionate</td>
<td>6500</td>
<td>6170</td>
<td>6280</td>
<td>5170</td>
<td>600</td>
</tr>
</tbody>
</table>

*Four steers/treatment for C, P, and PBD; three steers for BD.*

PEPCK and PC activity in kidney and liver (Mehlman et al., 1970; 1971b; 1972). Mehlman et al. (1972) incubated kidney cortex slices and found rats fed BD had an increased capacity for gluconeogenesis. However, Mehlman et al. (1971b) infused rat livers with and without 20 mM BD and noted a 62 percent depression in glucose production from lactate when BD was included. BD had no effect if fructose was the substrate indicating that the inhibition was occurring below the level of the triose-phosphates. Based upon liver metabolite concentrations, Mehlman et al. (1971b) suggested that BD inhibits the conversion of OAA to PEP.

Effects of BD seem to be much broader than on any particular enzyme reaction because BD depressed BHBA production, and gluconeogenic substrate oxidation and conversion to glucose. It is not known whether effects of BD are direct or via metabolites such as ketone bodies.
Butyrate, which is metabolized readily to KB in liver, caused a transient in vivo increase in glucose production in sheep, but the increase may have resulted from glycogenolysis (Judson and Leng, 1973b). In vitro, 5 mM butyrate decreased pyruvate oxidation and conversion to glucose from beef liver slices (Atwal and Sauer, 1973). Short-chain fatty acids and KB may increase rates of gluconeogenesis in rat kidney (Newsholme and Start, 1973a) and lamb hepatocytes (Clark et al., 1976). Results from experiments examining effects of butyrate and/or KB on gluconeogenesis are conflicting and there is evidence of species variation (Arinze and Rowley, 1975). Too little work has been done with cattle with respect to BD, fatty acids, and KB on rates of gluconeogenesis to do any more than speculate on the mode of action of BD. Effects of BD are reminiscent of the impaired hepatic metabolism noted during ketosis with the lactating cows as discussed already in 'Metabolic Studies with Lactating Cows'. Elucidating the nature of the relation between effects of BD and ketosis on hepatic metabolism awaits further research.

**Metabolism in livers of feed-restricted steers**

One question addressed at the onset of this section, was what are the effects of feed restriction on gluconeogenic rates. The three steers surviving the Latin square study were restricted in intake, and data are compared to data accumulated during feeding of the control ration. Table 26 shows how feed restriction affected liver metabolite concentrations. When feed is restricted, steers are more dependent on endogenous nutrients to meet glucose and energy requirements as hepatic glycogen
Table 26. Hepatic metabolite concentrations in fed (Control) and feed restricted steers

<table>
<thead>
<tr>
<th>Treatment^a</th>
<th>Control</th>
<th>Feed restricted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen (% wet weight)</td>
<td>4.0±.6</td>
<td>2.1±1.0</td>
</tr>
<tr>
<td>Triglyceride (% wet weight)</td>
<td>.14±.16</td>
<td>.69±.35</td>
</tr>
<tr>
<td>ß-hydroxybutyrate (µmoles/g wet weight)</td>
<td>.28±.04</td>
<td>.32±.2</td>
</tr>
</tbody>
</table>

^a Three steers per treatment and one sample per steer.

concentration decreased by 50 percent and hepatic triglycerides increased almost 5 fold. FFA mobilized from adipose seem to have been esterified preferentially rather than being oxidized to KB. Partitioning of FFA between oxidation and esterification is regulated and rates of oxidation are negatively correlated with glycogen concentrations (McGarry and Foster, 1978; McGarry et al., 1978a; 1978b).

That glycogen concentrations declined only 50 percent, indicates that feed restricted steers were able to partly compensate for energy restriction. Staubus et al. (1960) found that in steers fasted for 72 hours and simultaneously given phlorizin, plasma total-reducing substances did not change, prompting the suggestion that liver glycogen was maintaining plasma glucose concentrations. It is doubtful that glycogen can have long-lasting effects on maintaining glucose homeostasis,
therefore other adaptive mechanisms likely are involved. Only one of the three steers in the present study was fasted. The other two steers were given half of the control ration, which, at their body weight of 250 kg at the start of the fast, calculates to approximately 61 percent of the maintenance requirements (NRC, 1978). The relatively large standard errors on means of hepatic metabolites are reflective of the difference in response between the feed restricted steers and the fasted steer. The fasted steer had the lowest glycogen (0.24 percent) and the highest hepatic triglyceride (1.4 percent). Thus, steers fed sub-maintenance amounts of energy were able to adapt without drastic changes in hepatic metabolites.

Feed restriction had essentially no effects on either butyrate or BD oxidation to BHBA, or stearate conversion to carbon dioxide and hepatic triglyceride (Table 27). Stearate oxidation to BHBA was slightly depressed during fasting, which is contrary to what happens in rats (McGarry and Foster, 1969) and ruminants in vivo (Leng and West, 1969). Stearate oxidation to BHBA was very low compared to butyrate and does not seem to have been well-extracted by the liver as evidenced by the low rates of oxidation to carbon dioxide and esterification to triglyceride. Consequently, the biological significance of decreased BHBA production from stearate during feed restriction is doubtful. That BHBA production from butyrate did not change with fasting is consistent with a lack of adaptation of ketogenic enzymes (Williamson et al., 1968).
Table 27. Effects of feed restriction on hepatic metabolism of 1,3-butanediol and fatty acids to \( \beta \)-hydroxybutyrate in vitro

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Treatment</th>
<th>Control</th>
<th>Feed restricted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>607±194</td>
<td>742±82</td>
</tr>
<tr>
<td>1,3-butanediol</td>
<td></td>
<td>1188±190</td>
<td>1071±141</td>
</tr>
<tr>
<td>Butyrate</td>
<td></td>
<td>58±7</td>
<td>28±3*</td>
</tr>
<tr>
<td>Stearate</td>
<td></td>
<td>4.2±.3</td>
<td>3.9±.5</td>
</tr>
<tr>
<td>Stearate</td>
<td></td>
<td>6.5±1.5</td>
<td>6.0±1.1</td>
</tr>
</tbody>
</table>

\( ^a \)Three steers per treatment and one sample per steer.
\( ^b \)nmoles of \( \beta \)-hydroxybutyrate accumulated during incubation with substrate per 100 mg liver per hour.
\( ^c \)nmoles of stearate oxidized to carbon dioxide per 100 mg liver per hour.
\( ^d \)nmoles of stearate metabolized to tissue triglyceride per 100 mg liver per hour.

*\( P<.05 \).

Gluconeogenic substrate oxidation to carbon dioxide increased slightly with feed restriction and approached significance (\( P<.15 \)) for lactate (Table 28). More dramatic is the increased incorporation of substrate into glucose during feed restriction (Table 29). Lactate utilization for gluconeogenesis was variable, being increased in all
Table 28. Effects of feed restriction on hepatic oxidation of gluconeogenic substrates to carbon dioxide in vitro

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Control</th>
<th>Feed restricted</th>
<th>p&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate</td>
<td>182±10</td>
<td>200±27</td>
<td>&gt;.5</td>
</tr>
<tr>
<td>Lactate</td>
<td>256±40</td>
<td>399±48</td>
<td>&lt;.15</td>
</tr>
<tr>
<td>Propionate</td>
<td>88±4</td>
<td>100±20</td>
<td>&gt;.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Three steers per treatment and one sample per steer.

<sup>b</sup> Significance level of t-test.

<sup>c</sup> Nmoles of substrate oxidized to carbon dioxide per 100 mg liver per hour.

---

Table 29. Effects of feed restriction on hepatic gluconeogenesis measured in vitro

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Control</th>
<th>Feed restricted</th>
<th>p&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate</td>
<td>97±11</td>
<td>130±9</td>
<td>&lt;.1</td>
</tr>
<tr>
<td>Lactate</td>
<td>362±82</td>
<td>942±347</td>
<td>&lt;.18</td>
</tr>
<tr>
<td>Propionate</td>
<td>919±125</td>
<td>1602±114</td>
<td>&lt;.025</td>
</tr>
</tbody>
</table>

<sup>a</sup> Three steers per treatment and one sample per steer.

<sup>b</sup> Significance level for t-test.

<sup>c</sup> Nmoles of substrate metabolized to glucose per 100 mg liver per hour.
three steers upon feed restriction but to a much greater extent in the one fasted steer.

Increased substrate utilization and preferential metabolism to glucose during Feed Restriction is equivalent to results from the same steers when given phlorizin (Tables 23 and 24). Thus, the origin of the signal indicating a need for increased gluconeogenesis is probably the same. That signal must be, directly or indirectly, a deficiency in glucose availability. As discussed already, in contrast to monogastrics, gluconeogenic enzyme capacities of ruminants are thought to not adapt to an energy deficiency. The data presented herein suggest that the rate limiting enzyme for gluconeogenesis in ruminants has not been found or that other changes are occurring. Total glucose accumulations during the course of tissue incubations were less when the steers were feed restricted, but as discussed already, this is unlikely to be a source of the difference in gluconeogenic rates (Table 30). The standard errors on means of substrate utilization for glucose synthesis were larger during feed restriction than during control. Variability can be accounted for by less glucose accumulation by the fasted steer, which probably reflects the low liver glycogen concentrations.

Metabolism of liver slices with supplemental ketone bodies

To further understand the effects of KB and FFA on hepatic metabolism, liver slices from steer 8446 during the control feeding period and during fasting had supplemental KB (or KB + stearate) added to incubation media. Triplicate determinations of gluconeogenic substrate oxidation
Table 30. Effects of feed restriction on total glucose accumulation in vitro

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Control</th>
<th>Feed restricted</th>
<th>p^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate</td>
<td>8055±500</td>
<td>5000±1280</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Lactate</td>
<td>7170±170</td>
<td>6780±1833</td>
<td>&gt;0.8</td>
</tr>
<tr>
<td>Propionate</td>
<td>6110±330</td>
<td>5000±1055</td>
<td>&gt;0.3</td>
</tr>
</tbody>
</table>

^aThree steers per treatment and one sample per steer.

^bSignificance level based on t-test.

and conversion to glucose, with and without supplemental KB, are shown in Tables 31 and 32. Statistical analysis was not performed on these data because only one steer was used. KB (5 mM) or KB plus stearate seem to increase substrate oxidation (Table 31) in both the control and fasted states with the exception of lactate, which was depressed during fasting. The depression in lactate oxidation may be explainable by a change in redox state. Starvation in rats (Newsholme and Start, 1973b) and cattle (Baird et al., 1972) results in a more reduced cytosol, which would favor lactate formation from pyruvate. However, redox states were not determined in the present study, and in vivo data support a greater hepatic lactate utilization during energy deficiency (Baird, 1981b).
Table 31. Effects of ketone bodies or ketone bodies plus stearate on hepatic oxidation of gluconeogenic substrates to carbon dioxide in steer 8446 during feeding and fasting

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Control&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Control + KB&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Fast</th>
<th>Fast + KB&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate</td>
<td>167±7&lt;sup&gt;e&lt;/sup&gt;</td>
<td>188±12</td>
<td>225±23</td>
<td>270±10</td>
</tr>
<tr>
<td>Lactate</td>
<td>161±14</td>
<td>180±16</td>
<td>470±14</td>
<td>256±27</td>
</tr>
<tr>
<td>Propionate</td>
<td>79±10</td>
<td>110±7</td>
<td>93±15</td>
<td>142±28</td>
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</tbody>
</table>

<sup>a</sup>Control period from latin square experiment.

<sup>b</sup>5 mM each acetoacetate and β-hydroxybutyrate added to incubation media.

<sup>c</sup>5 mM each acetoacetate and β-hydroxybutyrate plus 1 mM stearate-albumin added to incubation media.

<sup>d</sup>nmoles of substrate oxidized to carbon dioxide per 100 mg liver per hour.

<sup>e</sup>Mean ± SE for triplicate determinations.

Fasting increased substrate oxidation over the fed state, which was less apparent when combined with data from steers restricted in intake (compare Tables 28 and 31). Effects of fasting were most apparent from lactate (Table 31). Chan and Freedland (1972) suggest that lactate is normally not utilized by the liver due to inhibition of pyruvate carboxylase by methylmalonyl-CoA and pyruvate dehydrogenase (PDH) by propionyl-CoA. During starvation, propionate availability would be decreased and perhaps, also, the inhibitory effects on PC and PDH.
A decreased availability of propionate during fasting and activation of PC would explain the large increase in lactate incorporation into glucose (Table 32). Fasting increased gluconeogenic rates from lactate to a greater extent than did restricted feeding, while rates from aspartate and propionate were similar from the restricted feeding and fasting (Gluconeogenic rates from the two restricted fed steers are: aspartate 128±4, lactate 594±7, and propionate 1504±72; compare with

<table>
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<th>Substrate</th>
<th>Control\textsuperscript{a}</th>
<th>Control \textsuperscript{b}</th>
<th>Fast</th>
<th>Fast \textsuperscript{c}</th>
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<td>688±65</td>
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<td>1799±111</td>
<td>836±196</td>
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</tbody>
</table>

\textsuperscript{a}Control treatment from latin square.

\textsuperscript{b}5 mM each acetoacetate and \textbeta-hydroxybutyrate added to incubation media.

\textsuperscript{c}5 mM each acetoacetate and \textbeta-hydroxybutyrate plus 1 mM stearate-albumin added to incubation media.

\textsuperscript{d}nmole of substrate incorporated into glucose per 100 mg liver per hour.

\textsuperscript{e}Mean ± SE for triplicate determinations.
Table 32). Thus, increased gluconeogenic rates may involve more than one reaction common to all substrates.

KB had no significant effects on gluconeogenic rates during the control state (Table 32). However, KB plus stearate depressed gluconeogenic rates during the fasted state. Depressed gluconeogenic rates with added KB plus stearate were nearly equivalent to rates seen during the control period. Thus, the increased rates noted with fasting were completely negated by KB plus stearate. That KB plus stearate had such immediate effects suggests that increased rates with fasting involved enzyme activation and not enzyme synthesis.

Because KB and stearate were added simultaneously, it is impossible to conclusively attribute the inhibition of gluconeogenesis to either fraction. Clark et al. (1976), using lamb liver cells, found that butyrate and many long-chain fatty acids up to oleate, increased gluconeogenic rates from propionate. In monogastrics, stimulation of gluconeogenesis by FFA during fasting is thought to be an important regulatory mechanism, although in vitro effects of FFA have not always been shown (Schimmel and Knobil, 1969). Stearate uptake by liver in the present study was low as evidenced by the low production of BHBA, and low conversion of stearate to carbon dioxide and tissue triglyceride. As a consequence, it is unlikely that stearate had any major impact on hepatic metabolism and KB are the more logical effectors.
Concluding Remarks

Creating a valid ketosis model is a worthy endeavor and would make many opportunities available for studying ketosis that are not available in lactating cows. Creating a complete ketosis model does not seem to have been accomplished in the present study. A greater energy deficiency and glucose stress, and thus perhaps a better model for ketosis, may be obtained if:

1. a maintenance or slightly submaintenance ration is fed, with BD and phlorizin given as in the present study, or
2. a maintenance ration is fed that includes starch, with BD replacing, or a caloric basis, starch from the ration and phlorizin used as in the present study.

Also, if BD intake is limited, so is the potential for increasing KB concentrations; thus, supplementing rations with octanoic acid plus BD may increase the potential for generating KB.

Adaptations of gluconeogenic rates in steers to physiological states seem to parallel responses in rats. That is, a carbohydrate deficiency (caused by phlorizin or feed restriction) is translated into an increased capacity for glucose synthesis. In rats, increased gluconeogenesis involves a complex pattern of hormonal and substrate changes resulting in increased availability of amino acids and glycerol plus increased capacities of PEPCK and PC (Newsholme and Start, 1973a). The ruminant liver is geared toward gluconeogenesis in the fed state and starvation does not increase PEPCK activity (Young et al., 1969). It seems that
full capacity for gluconeogenesis is not realized in growing steers and increased rates are possible when glucose demand increases. The signals that are responsible for the increased capacity and the hepatic targets that are affected remains obscure.

Ketone bodies are recognized to be potent regulators of metabolism in monogastrics (Felig and Wahren, 1974; Owen et al., 1969; Robinson and Williamson, 1980; Williamson et al., 1974). Direct effects of KB on hepatic metabolism have not been defined. Rifkin (1963), using bovine mitochondria, found that acetoacetate could inhibit Krebs cycle activity. Feeding BD to steers to increase KB concentration, resulted in impaired hepatic activity that involved both cytoplasmic and mitochondrial reactions. Inhibitory effects of BD were negated if phlorizin was given simultaneously.

It is possible that BD or KB are suppressing the release of a gluconeogenic activator (such as glucagon), which is released upon phlorizin administration. Glucagon can increase ketone body production in ruminants (Brockman, 1976) and nonruminants (Keller and Shulman, 1979; McGarry et al., 1978b) and has been shown to increase in concentration in sheep plasma during energy deficiency (Brockman, 1979a). Glucagon is also a potent stimulator of ruminant gluconeogenesis both in vivo (Brockman and Bergman, 1975) and in vitro (Clark et al., 1976). Decreased insulin also may increase the hepatic response to glucagon as insulin, in vitro, blocked the stimulatory action of glucagon on gluconeogenic rate (Clark et al., 1976). In the present study, during phlorizin administration (periods P and PBD), average plasma concentrations
of insulin were lowest (0.27 vs 0.33 ng/ml) and glucagon highest (83 vs 68 pg/ml) compared to the average of periods C and BD, respectively. Analyses and presentation of hormonal responses in steers during the Latin square trial will be presented elsewhere. It is likely that portal concentrations of hormones would be better indicators of hormonal availability to the liver and that possibly, liver availability is not reflected totally in peripheral plasma concentrations. Such actions of BD and/or KB are purely speculative and further experimentation is necessary.

Direct effects of KB on hepatic oxidation and glucose synthesis from gluconeogenic substrates are also evident. KB had minimal effects on substrate oxidation and incorporation into glucose in energy sufficient steers, but negated and increased gluconeogenic capacity during energy deficiency. KB effects were much less apparent on substrate oxidation than on gluconeogenesis suggesting inhibition by KB is restricted to gluconeogenic enzymes. The nature of this proposed inhibition is not known.
GENERAL SUMMARY

Major emphasis during the first study was on the adaptation of hepatic metabolism in cows following parturition. Impairments of ketogenic and gluconeogenic capacities in lactating cows during ketosis are proposed to result from an overproduction and accumulation of acetoacetate and β-hydroxybutyrate. There is little evidence in the literature to support such a proposition and, thus, a second study was initiated to help answer two questions that emerged from the first study. First, because feed intake declined prior to ketosis in the dairy cows, one question was whether depressed intake could account for impaired hepatic metabolism. Second, are ketone bodies physiological regulators of hepatic metabolism?

Restricted feed intake or a complete fast in steers increased gluconeogenic capacity of liver slices. It seems unlikely that a similar effect would result in lactating cows, because the lactating cow should be utilizing the full capacity of her liver for glucose synthesis. Considering there was no change in gluconeogenic capacity from cow liver during energy sufficiency (dry period), and energy deficit (early postpartum), it may well be that the mechanisms creating the increased capacity during energy deficiency in steers, are continually in progress in the lactating cow. Gluconeogenic capacities may be quite different in nonpregnant, nonlactating cows compared to lactating cows. Thus, starved or phlorizin-treated steers may more closely resemble the hepatic metabolism of lactating cows. A certain amount of evidence supports this proposal.
The two substrates best utilized for glucose synthesis were propionate and lactate. In the cow study, propionate and lactate were utilized approximately on an equivalent basis. In the steer study from the latin square project, propionate utilization was 3 fold greater than lactate. However, in the three steers restricted in intake, the difference between lactate and propionate utilization is decreased to a factor of 1.7. In the one steer that was fasted, no differences existed between lactate and propionate utilization, just as in the cows. Thus, during energy deficit, lactate may become an increasingly important gluconeogenic substrate. Baird (1981b) found lactating cows increased by three-fold the percentage extraction of lactate by the liver compared to nonlactating cows. If lactating cows are utilizing their maximum liver capacity for gluconeogenesis, then little change in capacity would be expected from restriction of feed. It seems reasonable to assume then, that the impaired hepatic metabolism noted in the ketotic cows was not a result of depressed feed intake.

The other question was whether KB could account for depressed hepatic metabolic activity. BD, which increased plasma and hepatic KB, depressed both hepatic ketogenic and gluconeogenic capacity. Similar depressions were noted during ketosis in the lactating cows. While the similarity of results between BD-fed steers and ketosis in lactating cows is close, the concentrations of KB in steer plasma and liver during period 1,3-BD were only about 25 percent of the concentrations found in ketotic cows during K. It is possible that steers are more susceptible to inhibition of gluconeogenesis by KB. It is possible also that the
effects of BD and the effects observed during ketosis in lactating cows are mediated by an intermediate not monitored during these studies. The effects of BD and KB deserve further investigation.

KB may well have direct inhibitory effects on hepatic gluconeogenesis. A total of 10 mM KB were added to incubation flasks of liver slices from fasted steers. Ten millimolar KB are high even for a lactating cow, but are within physiological ranges. In the lactating cows in this study, plasma BHBA averaged 4.5 mM during ketosis. AcAc was not measured but from hepatic BHBA/AcAc, it can be calculated that AcAc would be roughly 1 mM. The concentration of KB used in vitro, were about 1.5 fold greater than the concentrations found in plasma of cows in the present study. Thus, KB, at physiological concentrations, can inhibit hepatic gluconeogenesis. Direct inhibition of gluconeogenesis in fasted steers by KB averaged 50 percent, which is approximately equivalent to the depression noted in lactating cows during ketosis.

Robinson and Williamson (1980) reviewed what is known about the role of KB as regulators of metabolism. The best documented effects of KB are an inhibition of muscle proteolysis (Felig and Wahren, 1974; Sherwin et al., 1975) and decreased adipose lipolysis (Menahan et al., 1966a; 1966b; Metz and van den Bergh, 1972). Depressed rates of hepatic glucose production in rats due to KB also have been shown, but have been attributed to decreased availability of amino acids for glucose synthesis (Mebane and Madison, 1964; Owen et al., 1969). Data presented in the present study supplement what is currently known about the actions of KB and suggest that direct hepatic effects are likely.
Development of ketosis in lactating cows has been presented as being a consequence of a large energy deficit resulting from an increase in nutrient output in milk that was greater than the increase in feed intake. An energy deficit results in a greater dependency on endogenous nutrients to meet requirements for precursors for synthesis of glucose and milk components. Adipose triglycerides are mobilized and partly oxidized in the liver to KB, which accumulate and result in a clinical ketosis.

An energy deficit is no doubt responsible for initiating the ketogenic response in cows during early lactation, but, in view of what has been presented, KB may have a role in clinical ketosis development in addition to the production of symptoms. During early postpartum, the major glucose precursors are probably propionate and amino acids from the diet, glycerol from the adipose tissue, and amino acids from endogenous protein reserves. Milk production thus is dependent on glucose synthesized from a steady supply of both dietary and endogenous glucose precursors. Both endogenous glucose precursors, glycerol from adipose tissue and amino acids from muscle, are subject to restricted availability by KB. Increased KB, superimposed on a continual demand for milk production, may limit endogenous glucose precursor availability. Furthermore, Ryan (1981) suggests that KB may depress reticulo-rumen activity and may limit intake. The effects of feed restriction on ketosis development are well documented (Baird et al., 1972; Fisher et al., 1971). Decreased nutrient availability imposed by increasing concentrations of KB may well cause results similar to restricted feeding. A decreased availability of
glucose precursors in combination with impaired hepatic metabolism, as shown in the present study, may accentuate the ketogenic state and culminate in clinical ketosis.

Treatment of ketosis resulted in a return to normal feed intake, an initial depression in milk production, and a restoration of plasma and hepatic metabolites to concentrations observed during early postpartum. Milk production increased within a week following treatment to amounts observed during preketosis without concomitant increases in plasma and hepatic KB. Treatment seems to have resulted in an adaptation to a more efficient utilization of available nutrients. Part of this adaptation may be the increased hepatic gluconeogenic capacity following treatment. Whether glucocorticoids play a role in the adaptation of hepatic gluconeogenic capacity is not known. Glucocorticoids do increase muscle proteolysis in ruminants (Bergman, 1973). If amino acid availability had been a limitation to glucose production, part of the effect of glucocorticoids may be to correct this limitation.

Cows destined to become ketogenic were secreting more energy in the milk than they were consuming during the early postpartum period when they were fed ad libitum. A large energy deficit within the first week of lactation may signify the inability of cows to adequately adapt to lactation demands for stress. Future work should emphasize the transition into lactation and look for stimulants of feed intake and gluconeogenic capacity. Also, how the development of fatty liver and the potential limitations imposed by fatty liver affects hepatic metabolism should be investigated. Hepatic capacity for propionate metabolism
indicated a depression following parturition. The significance of this depression and how it may relate to negative energy balance and ketosis development deserves examination. As already discussed, treatment of ketosis seems to give not only immediate aid to cows, but helps them adapt to lactation. What such an adaptation involves is only speculative, but it seems reasonable that an understanding of the adaptive mechanisms may allow adjustments in metabolism to be initiated prior to ketosis development. Future work may involve:

1. hormonal adaptations to lactation and ketosis with particular reference to hormones concerned with energy metabolism and balance (growth hormone, glucagon, glucocorticoids, insulin, and thyroxine).

2. how the above mentioned hormones affect both in vivo and in vitro hepatic metabolism. Examination should involve both acute and chronic effects and encompass substrate and enzyme kinetics.

Adaptive responses to hormone therapy also should be examined in nonlactating ruminants such as steers. Steers are hardy and tolerate strenuous experimentation. Work with steers may include:

1. effects of ketone bodies on hepatic and muscle metabolism.
   This may involve in vivo substrate kinetics and trans-organ and trans-limb balances.

2. effects of ketone bodies on hormone production with particular reference to hormones that may affect hepatic metabolism.

3. regulation of the partitioning of fatty acids between oxidation and esterification in the liver.
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biotin on gluconeogenesis in biotin-deficiency and fatty liver and 

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Dr. Allen Trenkle and Dr. Jim Thomas for service as members of my graduate committee.

A very special thanks to my wife, Jan, and son, Christopher for allowing me freedom with minimal resistance.
APPENDIX
Table Al. Means of in vitro substrate incorporation into glucose in ketotic cows

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<th>Period</th>
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nmoles incorporated into glucose/(mg DNA x 2 h))

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nmoles incorporated into carbon dioxide/(mg DNA x 2 h))
Table A2. In vitro incorporation of substrate into glucose in dairy cows

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Table A3. In vitro incorporation of substrate into carbon dioxide in dairy cows

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Table A4. In vitro incorporation of gluconeogenic substrate into glucose in steers

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Table A5. In vitro of gluconeogenic substrates into carbon dioxide in steers

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Figure Al. Regression of total in vitro glucose accumulation on liver glycogen concentration for cows

[Data are from periods D, EPP, and PK for control and ketotic cows and averaged across all substrates]
Figure A2. Regression of average gluconeogenic substrate incorporated into glucose on liver glycogen concentration for cows
[Data are from periods D, EPP, and PK for control and ketotic cows and averaged across all substrates]
Figure A3. Regression of average gluconeogenic substrate incorporated into glucose on total in vitro glucose accumulation for cows [Data are from periods D, EPP, and PK for control and ketotic cows and averaged across all substrates]

\[ y = 0.131x + 204 \]

\[ r^2 = 0.33 \]

\[ P < 0.1 \]
Figure A4. Regression of the average ratio of glucose specific activity to substrate specific activity on liver glycogen concentration in cows

[Data are from periods D, EPP, and PK for control and ketotic cows and averaged across all substrates]
Figure A5. Regression of the average ratio of glucose specific activity to substrate specific activity on total in vitro glucose accumulation in cows

[Data are from periods D, EFP, and PK for control and ketotic cows and averaged across all substrates]