Effect of Infusion of Carnitine and Glucose on Blood Glucose, Ketones, and Free Fatty Acids of Ketotic Cows

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Abstract

Carnitine was infused into control (mid-lactation), feed-restricted ketotic and spontaneously ketotic cows. Blood glucose, acetoacetate, β-hydroxybutyrate, and plasma free fatty acids were determined. DL-Carnitine infusion into control animals had no effect upon blood glucose or ketones nor did it affect milk yield or composition. DL-Carnitine infusion into spontaneously ketotic cows gave variable results. Infusion at 20 micromoles (3.9 mg) DL-carnitine per kilogram per hour caused blood glucose to rise and ketones to fall in three cases; in a fourth case blood ketones rose and glucose fell. A single case treated with 145 μmoles L-carnitine (28.6 mg) per hour per kilogram increased acetoacetate accompanied by decreased plasma free fatty acids, whereas glucose showed only a transient elevation. L-Carnitine infusion into feed-restricted and spontaneously ketotic cows was consistent only in lowering plasma free fatty acids. Glucose infusion into feed-restricted ketotic cows increased blood glucose and decreased blood acetoacetate; however, plasma free fatty acids were unaffected. Glucose infusion into a spontaneously ketotic cow immediately decreased plasma free fatty acids. Fatty acid metabolism in the ketotic cow did not function at peak efficiency and effects of carnitine infusion were the result of increased β-oxidation of long-chain fatty acids.

Introduction

Ketosis in all species studied so far is characterized by increased rates of hepatic ketogenesis, resulting in higher steady state ketone body concentrations in blood. This point was underscored in recent elegant studies of Katz and Bergman (14), who showed that while in fed sheep the primary site of ketone body synthesis resides in the portal bed (0.6 to 0.8 mmoles per hr per kg $^{0.75}$), in fasted ketotic sheep the primary site of ketone body synthesis shifts to the liver (1.34 to 1.93 mmoles per hr per kg $^{0.75}$).

The carbon source for hepatic ketone body synthesis is derived from circulating FFA. Thus, increased ketogenesis is invariably related to enhanced fatty acid oxidation by the liver (11) (14). Plasma free fatty acids have been shown to increase in SK cows by Adler et al. (1) and Kronfeld (17). Dole (7) observed similar increased plasma FFA in non-ruminants as a result of starvation, and Masoro (19) increased intracellular free fatty acids in muscle during fasting. While a clear correlation has been established between hepatic FFA oxidation and ketogenesis, effects of enhanced fatty acid oxidation in hepatic gluconeogenesis are still controversial (24). Clearly, fatty acid oxidation stimulates gluconeogenesis in rat liver homogenates (6), rat liver slices (4) and perfused rat liver (26). Free fatty acid stimulated gluconeogenesis, however, is not observed in guinea pig liver (22); and although Krebs claimed such an effect for the fasted ruminant (16), this was not true with in vivo experiments in fasted sheep (14). If the interaction of fatty acid oxidation and gluconeogenesis is still to be established, the importance of fatty acid oxidation as a source of energy in the high producing dairy cow is unquestioned.

The role of carnitine in fatty acid oxidation has been well established (Fritz (12) for review). Brockhuysen et al. (5) demonstrated that fat emulsions infused into starved dogs elevated blood ketones, but adding carnitine to the infusion decreased blood ketones to pre-infusion levels of approximately zero. The absolute requirement of carnitine for fatty acid oxidation suggested to us that carnitine infusion into SK cows might be beneficial in enhancing fatty acid oxidation by both liver and muscle, thereby exerting a sparing effect on available carbohydrate stores. Our observation that total daily carnitine secretion in the milk of ketotic cows was significantly increased

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(8) supported the possibility that a carnitine deficit might develop in tissues of these animals. Such a deficiency would have a deleterious effect on the efficiency of fatty acid oxidation. Pertinent to this was our finding (9) that in ketotic guinea pigs free carnitine was decreased below the controls in muscle (and liver) and indirect evidence was obtained (9) that fatty acid oxidation rates were impaired in the skeletal muscle of these animals.

The experiments described in this paper were to evaluate effects of carnitine on blood ketone, blood glucose and plasma free fatty acids in spontaneously and feed-restricted ketotic cows. Although not measured directly, steady state concentrations of blood glucose in the fed or fasted ruminant will approximate rates of gluconeogenesis to the extent that glucose utilization remains constant, because this species has little or no intestinal absorption of dietary carbohydrate. Similarly, blood free fatty acids are resultant of rates of mobilization from fat depots and rates of oxidation. Since agents such as carnitine probably do not affect fat mobilization, any changes in blood free fatty acids will, to a large extent, reflect their rates of oxidation.

Experimental Procedure

Animals. Spontaneous ketosis. The studies on SK cows began in the fall of 1968. During the first fall and winter carnitine was infused into two control animals (one Ayrshire and one Holstein) both in mid-lactation, and two ketotic Ayrshires and two ketotic Holsteins. In the fall and winter of 1969–70 only two ketotic Ayrshires were treated.

Ketotic animals were initially detected by decreased milk production and by reduced feed intake. These animals were tested for ketonuria and if the test was positive, a blood sample was tested for acetoacetate. When acetoacetate was above 5 mg per 100 ml blood, animals were catheterized intravenously for subsequent treatment. In practice, most of the animals had blood acetoacetate between 10 and 20 mg per 100 ml prior to treatment. Table 1 indicates the principal clinical signs shown by these animals prior to experiment.

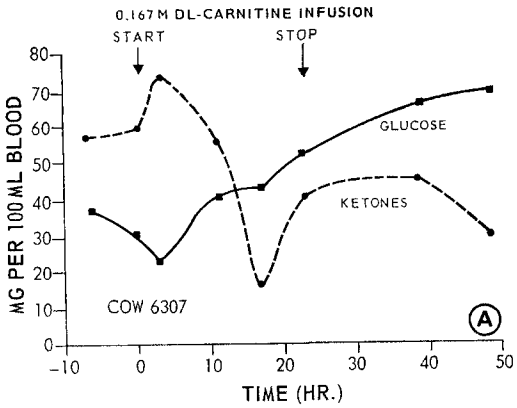
Induced ketosis. A group of 7 cows was fed a maximum intake of corn based concentrate and low quality chopped hay (50% digestibility) for 14 days prepartum to 14 days postpartum. On Day 15, concentrate intake was decreased by equal daily amounts until the 21st day postpartum when only low quality hay was fed. Animals remained on that diet for about one week while infusion experiments were done. From Day 14 to 21 postpartum, the calculated net energy balance declined from +0.43 to −16.56 Mcalories per day, milk yield decreased from an average 26.0 to 15.6 kg per day, and total average blood ketone concentrations increased from 10.4 to 35.5 mg per

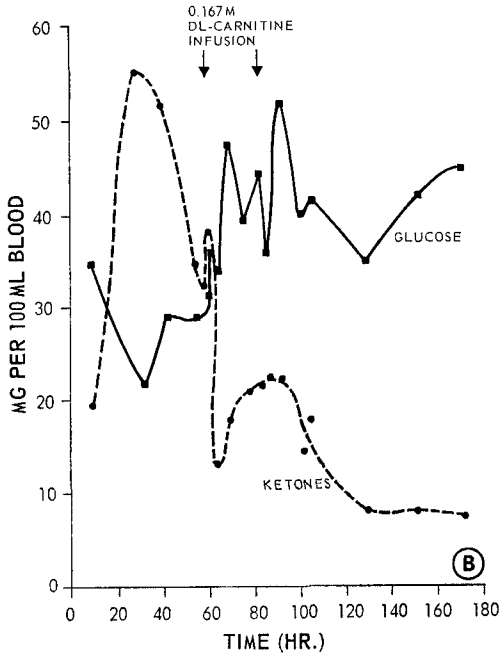
TABLE 1. Clinical signs in spontaneously ketotic cows at beginning of experiment.

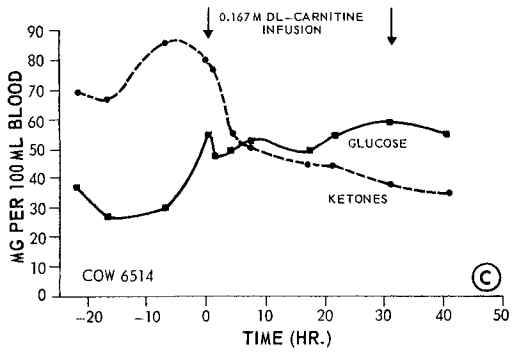
Cow	Feed consumption	Milk production	Ketonuria	Body temp	Digestive system
6307	Decreased roughage No concentrate	Decreased (> 25%)	Present	Normal	Apparently normal
6137	Decreased roughage No concentrate	Marked decrease (> 50%)	Present	Normal	Feces hard Mucus covered Rumen hypomotile
6514	Normal	Slight decrease (< 25%)	Present	Normal	Normal
6210	Decreased roughage Decreased concentrate	Decrease (> 25%)	Present	Normal	Dry mucus Covered feces Rumen motility normal
6208	Slight decrease in concentrate Roughage normal	Decrease (> 25%)	Present	Normal	Slight diarrhea
6319	Roughage normal Slight decrease in concentrate	Slight decrease (< 25%)	Present	Normal	Apparently normal

100 ml (10). Of the 7 animals, detailed infusion results of 3 are reported; a full report has been prepared for publication elsewhere (10).

Infusions were with a Harvard continuous infusion pump through a 1.15 mm polyethylene catheter inserted into the jugular vein and taped into position. Blood samples, from the noncatheterized jugular vein, were taken into heparin-containing evacuated tubes and immediately cooled in ice. Acetoacetate was determined by the method of Walker (23) within 2 hr after sampling or stored as the neutralized trichloracetic acid supernatant at −15 C until the assay was completed. β-Hydroxybutyrate was determined on neutralized perchloric acid supernatant according to Williamson et al. (25).







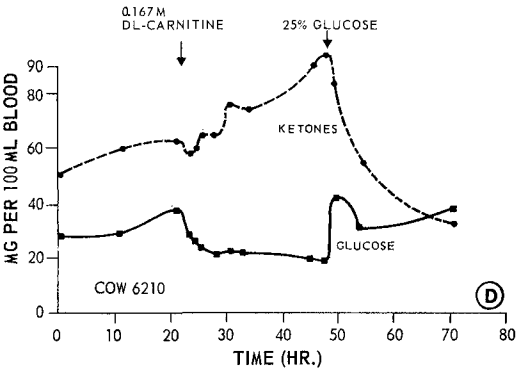


FIG. 1. Effect of DL-carnitine infusion on the blood glucose and ketones of spontaneously ketotic cows. Infusion was 20 μmoles (3.9 mg) per hour per kilogram.

A. Ayrshire (6307) infused with carnitine 10 weeks postpartum. This animal was also treated for ketosis by a veterinarian (500 ml 50% glucose) at the 3rd and 8th week postpartum.

B. Holstein (6137) infused with carnitine 4 weeks postpartum.

C. Holstein (6514) infused with carnitine 8 weeks postpartum. This animal was treated for ketosis by a veterinarian twice in the 7th week postpartum.

D. Ayrshire (6210) infused with carnitine 4 weeks postpartum.

The same supernatant was used for glucose according to the method of Slein (20). Free fatty acids in plasma were extracted by the procedure of Albrink (2).

Glucose was purchased from Fisher Scientific; DL-carnitine HCl was purchased from General Biochemicals Incorporated. The gift of L-carnitine HCl from Dr. Y. Kawashima of the Otsuka Pharmaceutical Factory, Tokushima, Japan is gratefully acknowledged.

DL-Carnitine HCl was neutralized with NaOH prior to infusion. L-Carnitine HCl was infused without neutralization. Initial infusion rate for DL-carnitine of 20 μmoles (3.9 mg) DL-carnitine per kilogram body weight per hour

into ketotic cows was based on the studies of Broekhuysen et al. (5). The feed-restricted animals received only 23.8 g L-carnitine during a 24-hr infusion. One of the SK cows received 8.0 g of L-carnitine in 5.5 hr.

Results

In a preliminary study, DL-carnitine was infused into two normal cows during midlactation. These animals showed no significant response to this treatment either in changes in blood ketones, blood glucose, milk production, or milk composition.

The effect of DL-carnitine infusion into cows with clinical ketosis is seen in Figure 1A to 1D. The animals depicted in Figure 1A and 1B both demonstrated an initial elevation of total ketones followed by a rapid decrease to near normal ketones, succeeded by a second transient rise and fall to normal. Blood glucose in both cases gave the anticipated increase as ketone bodies decreased. However, glucose remained high and was not affected by the transient elevation in blood ketones. The cow shown in Figure 1C responded to DL-carnitine infusion with only a moderate decline in blood ketones and only a moderate increase in blood glucose. However, blood glucose for this animal was in the range of a normal lactating cow during the period of carnitine infusion. The animal shown in Figure 1D responded to DL-carnitine infusion with increased blood ketone bodies and decreased blood glucose. Glucose was infused subsequently and ketones returned to normal.

DL-Carnitine infusion into SK cows has elicited variable responses in blood glucose and ketones (Fig. 1A to 1D). It was recognized that the presence of the unnatural D-isomer might affect the responses of animals to L-carnitine infusion. The results in Figure 2 are from an SK cow which received 80 g of L-carnitine during infusion. This high rate of infusion resulted in an initial transient rise in glucose and then a decrease which was associated with a concomitant elevation in acetoacetate. Plasma free fatty acids decreased when acetoacetate was maximum. The rapid decline in plasma free fatty acids is an indication of the stimulation of fatty acid metabolism by carnitine.

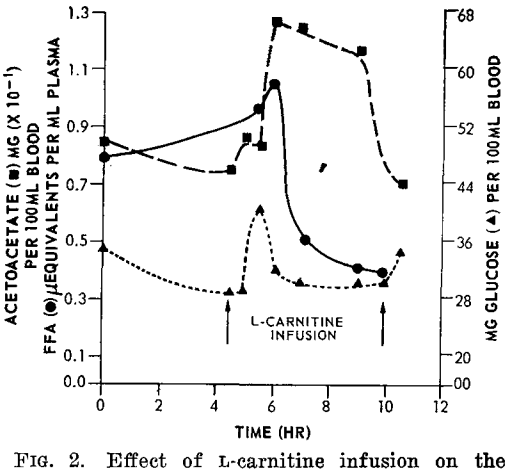


FIG. 2. Effect of L-carnitine infusion on the blood glucose and acetoacetate and plasma free fatty acids of a spontaneously ketotic Ayrshire cow (6208) at the 5th week postpartum. Infusion was 145 μmoles (28.6 mg) per hour per kilogram.

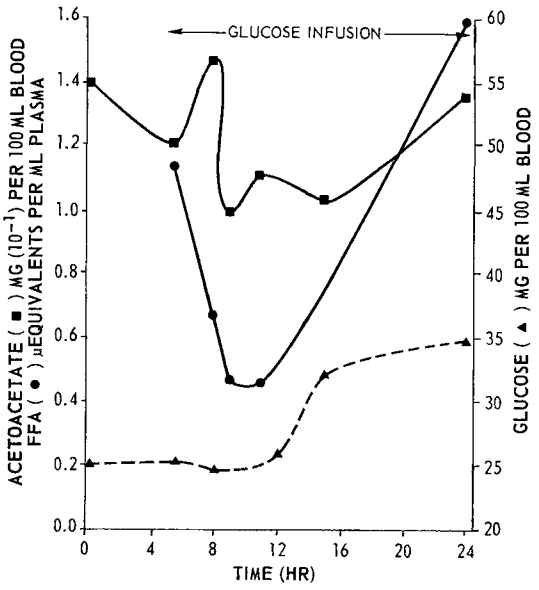


FIG. 3. Effects of glucose infusion on components of blood of a spontaneously ketotic Ayrshire cow (6319) 4 weeks postpartum. Glucose was infused at 6.2 g per hour.

The infusion of glucose into a spontaneously ketotic animal is demonstrated in Figure 3. Under these conditions, although blood glucose rose from 25 to 35 mg per 100 ml, acetoacetate remained relatively high in agreement with the observations of Kronfeld (17). Plasma free fatty acids decreased initially, but by the end of glucose infusion, they had risen beyond preinfusion values.

Decreased free fatty acids after glucose administration have been reported for the ketotic cow (17) and for humans (7, 13).

Since the supply of SK cows is usually unreliable and the duration of symptoms variable, an attempt was made to induce ketosis by feed restriction. For the three cows the following average changes in blood components occurred during the six or seven days when

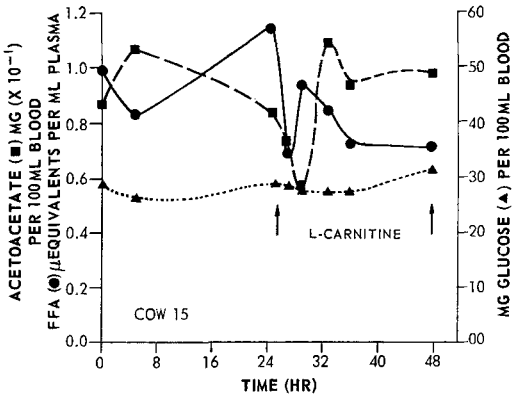


FIG. 4. Effect of L-carnitine infusion on blood components of a concentrate restricted Holstein cow. Infusion was 23.8 g per 24 hours. This animal received no concentrate during the restriction period.

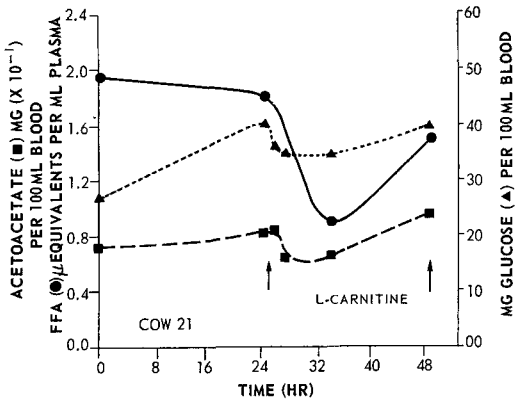


FIG. 6. Effect of L-carnitine infusion on blood components of a concentrate restricted Holstein cow. Infusion was 23.8 g per 24 hours. Concentrate intake for this animal was 3 kg per day.

feed was gradually withdrawn: glucose decreased from 37.8 to 30.0 mg per 100 ml blood, acetoacetate increased from 1.5 to 6.8 mg per 100 ml blood, and β-hydroxybutyrate increased from 9.2 to 23.9 mg per 100 ml blood. Plasma free fatty acids were not determined for all animals. However, in Cow 15 free fatty acids increased from 0.50 to 1.15 μeq per milliliter plasma during the restrictive period, and the three animals averaged 1.55 μeq per milliliter plasma on the first day of treatment. Free fatty acids of these animals averaged 0.25 μeq per milliliter plasma at 15 weeks postpartum. These results are in reasonable agreement with the free fatty acids reported for normal, spontaneously ketotic, and starved lactating cows (1, 17).

The effects of L-carnitine infusion into concentrate restricted animals is demonstrated in Figures 4, 5 and 6. As with the SK animals, the effects of carnitine infusion were variable. In one case, acetoacetate decreased when L-carnitine was infused (Fig. 5), whereas in another case, it remained almost unchanged (Fig. 6) or was increased (Fig. 4). Feed-restricted ketotic cows infused with carnitine revealed only slight inconsistent changes in blood glucose. L-Carnitine infusion, as with the SK cow (Fig. 2), consistently decreased plasma free fatty acids. The variability in response in FRK cows may in part have been due to the low rates of carnitine infusion. There is no evidence that a different response was obtained with DL- than with L-carnitine.

The responses of FRK cows to glucose infusion are illustrated in Figures 7, 8, and 9. A consistent elevation of blood glucose and decreased acetoacetate was observed. However, unlike in SK cows, plasma free fatty acids

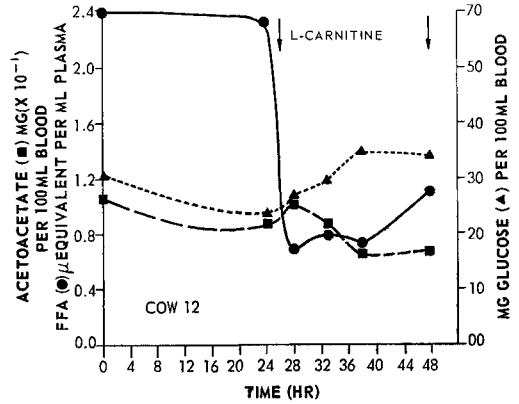


FIG. 5. Effects of L-carnitine infusion on blood components of a concentrate restricted Holstein cow. Infusion was 23.8 g per 24 hours. Concentrate intake for this animal was 1 kg per day.

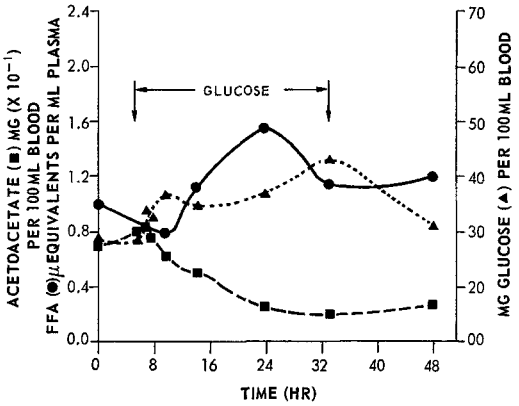


FIG. 7. Effect of glucose infusion on blood components of a concentrate restricted Holstein cow. Infusion was 200 g per 24 hours. Other details as in Figure 4.

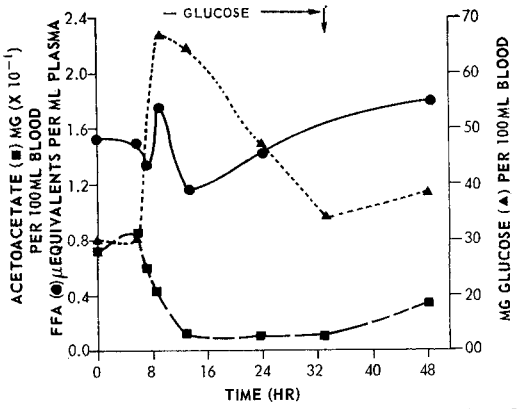


FIG. 8. Effect of glucose infusion on blood components of a concentrate restricted Holstein cow. Infusion as in Figure 7. Other details as in Figure 5.

were not decreased below preinfusion by glucose administration.

The failure of glucose infusion to decrease free fatty acids cannot be explained readily since glucose through its stimulation of Krebs cycle activity should also enhance fatty acid oxidation. While glucose per se may have some sparing effect on fatty acid oxidation, this cannot be the sole explanation. While there is much available data on the effects of FFA oxidation on rates of gluconeogenesis, relatively little is known about the effects of glucose oxidation on rates of lipolysis, fatty acid oxidation and esterification.

Discussion

Hyperketonemia and hypoglycemia were induced in cows during peak lactation by gradual restriction of concentrate until the diet consisted solely of low digestibility (50%) chopped hay. There are, of course, certain obvious differences between FRK cows and SK cows. The feed-restricted ketotic cows showed none of the common symptoms of ketosis and, most significantly, completely returned to normal immediately upon restoration of a properly balanced diet. Some specific metabolic differences between SK and FRK cows have recently been documented by Kronfeld et al. (18). Nevertheless, FRK cows are suitable for evaluating antiketogenic metabolites and are, of course, easier to obtain than the SK animals.

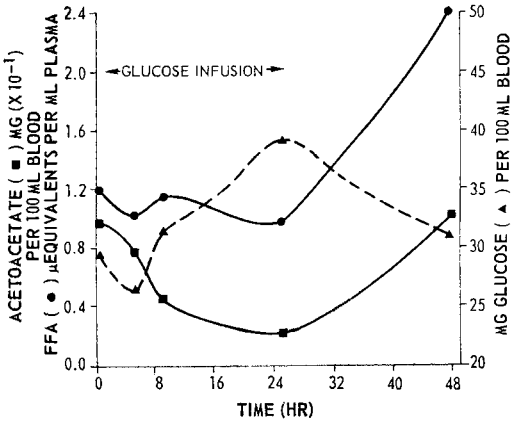


FIG. 9. Effects of glucose infusion on blood components of a concentrate restricted Holstein cow. Infusion rate as in Figure 7. Other details as in Figure 6.

Our results show that carnitine infusion into ketotic animals can affect lipid oxidation and utilization, providing further evidence that fatty acid oxidation may not be functioning at maximum efficiency during ketosis. The oxidation of fatty acids by the high producing dairy cow is vital to meet the total energy requirement for milk production. When maximum energy from fat oxidation is realized by the animal, then precursors such as alanine, pyruvate, or lactate can be utilized predominantly for gluconeogenesis or for maintenance of Krebs cycle intermediates. If fatty acid oxidation is slowed, gluconeogenic precursors are diverted to a greater extent to energy metabolism and glucose synthesis will be depressed. In the lactating cow efficient oxidation of fatty acids must be extremely critical since conservation of glucose for lactose synthesis and maintenance of Krebs cycle function are of paramount importance; thus, there will be a glucose sparing effect to the extent that energy needs are met by free fatty acids.

Increased ketone bodies in the blood can be ascribed to an excess of production over utilization (3). Changes which occur in the blood ketone concentrations may thus reflect changes in either production or utilization. Our experiments did not determine whether carnitine infusion enhances ketone body formation in the liver, resulted in a sparing effect on ketone body oxidation in muscle by a stimulation of β-oxidation of long-chain fatty acids, or a combination of both. The studies of Söling and Appels (21) with eviscerated, narcotized rats demonstrated that carnitine infusion decreased both acetoacetate and glucose utilization by extrahepatic tissue. This effect may help explain the results in Figure 1A and 1B. In both animals glucose and ketones increased simultaneously. This result is more easily explained by decreased utilization of both glucose and ketones by peripheral tissue than by increased synthesis in liver since an increase in blood glucose has generally been

associated with a decrease in blood ketones (Fig. 7, 8 and 9).

Carnitine infusion into spontaneous and feed-restricted ketotic cows increased or decreased blood acetoacetate unpredictably, but consistently decreased blood free fatty acids. This is probably related to the stage of ketosis when carnitine infusions are started. From our knowledge of carnitine action we can predict that it acts through enhancing FFA oxidation in muscle, in liver, or in both. In early ketosis when the carbohydrate, (i.e., oxalacetate precursor) supply is still adequate for the Krebs cycle, the primary carnitine effect may be one of carbohydrate sparing by increasing FFA oxidation to CO_2 in both muscle and liver. In advanced ketosis on the other hand, with hepatic oxalacetate severely depleted, the primary action of carnitine may be one of enhanced FFA oxidation in liver where, because of impaired Krebs cycle function, this results in excessively high rates of ketogenesis which in turn may result in high blood acetoacetate.

The effects of carnitine infusion into ketotic animals lend support to the possibility that fatty acid metabolism may not be at peak efficiency. From earlier studies with the ketotic guinea pig we concluded that an impairment of fat oxidation existed in the muscle (9). In addition, carnitine in muscle was also decreased. If a similar situation exists in the ketotic cow for muscle carnitine and fatty acid oxidation, our observation of increased carnitine secretion by ketotic cows (8) may suggest that these animals are indeed being subjected to a shortage of tissue carnitine. In addition, carnitine turnover increases during starvation (15). The area of carnitine metabolism in particular and methyl group metabolism in general requires further investigation, particularly for the dairy cow.

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