Hyperketonemia-Ketogenesis and Ketone Body Metabolism

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Abstract

In bovine ketosis, hyperketonemia is only one biochemical disturbance that occurs among a multiplicity of metabolic changes. The changes can be grouped into two major categories and these are: 1) a reduction in available carbohydrate and 2) an increase in the quantities of fat and ketone bodies being metabolized. The two major ketone bodies, AcAc and BHB, are interconvertible and their ratio may reflect or alter the redox state of the various tissues. The ketone bodies are readily utilized, are used for milk fat production, and can account for 20 to 30% of the animal's total respiratory CO₂. There is some evidence that several tissues, notably the brain, can gradually adapt to ketone body utilization while conserving glucose. In the normal ruminant, ketone bodies are produced by the rumen epithelium from dietary fatty acids, notably butyrate, and certain ketogenic diets possibly can predispose the animal to ketosis. During active ketosis, however, most of the excess ketone bodies are produced from FFA in the liver. The excessive ketogenesis seems dependent upon two factors, both of which must operate: 1) a primary factor or FFA mobilization from the body's fat stores and 2) an hepatic factor which involves a shift in hepatic FFA utilization away from the two pathways of esterification and oxidation to CO₂ to the third pathway of partial oxidation to ketone bodies. The availability of carbohydrate seems to play a critical role in each case. Hypoglycemia will cause increased FFA mobilization from the fat stores, and insufficient carbohydrate metabolism in the liver shifts the pathways of hepatic FFA utilization from that of esterification and oxidation to CO₂ to that of ketogenesis. An insufficient mobilization or supply of precursors for the production of oxaloacetate and eventually glucose, relative to the animal's increased needs, thus may be the critical phase of the disorder.

Introduction

The purpose of this paper is to attempt to summarize the present knowledge of ketone body metabolism and ketogenesis in the cow and emphasis will be given to the whole animal and various organ systems. Sheep and other animals often will have to be referred to, however, since more research has been done on these species. The overall subject is complex, and the available research data allow for differences of opinion. While ketosis in sheep can be experimentally induced by starvation or undernutrition during pregnancy (37, 68), ketosis in cows is more difficult to induce. Recent reports, however, indicate that giving thyroid hormones along with a very high protein diet will ensure good milk production and exert the required metabolic strain for producing severe ketosis (35).

The term ketosis or hyperketonemia actually describes only one biochemical sign, and it specifically refers to an accumulation of ketone bodies in the body fluids. It can characterize many conditions. Ketosis of varying intensity, in fact, may occur in all mammalian species and can be brought forth, for example, by starvation, low carbohydrate and high fat diets, cold exposure, anesthesia, impaired liver function, hypoglycemia, and endocrine disorders such as diabetes or growth hormone and other endocrine excesses (27, 81). Females of any species are more susceptible to ketosis than are corresponding males, and this predisposition is exaggerated during lactation or pregnancy (15, 21, 27), the latter being related to the so-called "metabolic drain."7

In effect, the term bovine ketosis is not all-inclusive, and it is an inadequate name for the syndrome. Many biochemical changes occur, and the accumulation of ketone bodies is a single factor in the multiplicity of changes that occur. There also is evidence of abnormal liver function in ketotic cows (30). Table 1 summarizes the principal gross changes in metabolic con-

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2 Author's work mainly was supported by USPHS Grant AM-05976.
Table 1. Metabolite concentrations in blood and liver of normal and ketotic ruminants.a

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Cows</th>
<th>Sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (blood, mg/100 ml)</td>
<td>Ketotic (blood, mg/100 ml)</td>
</tr>
<tr>
<td>Glucose</td>
<td>50</td>
<td>&lt;40</td>
</tr>
<tr>
<td>Ketones</td>
<td>&lt;10</td>
<td>&gt;30</td>
</tr>
<tr>
<td>FFAb</td>
<td>0.3</td>
<td>0.6-2</td>
</tr>
<tr>
<td>Glycogen</td>
<td>3</td>
<td>&lt;0.8</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Fat</td>
<td>3</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

a Summarized from References 7, 29, 37, 46, 70. b Values in mM per liter.

centrations in the blood and liver of both normal and ketotic cattle. Sheep also are included for comparison. One major series of change is the marked reduction in carbohydrate, i.e. the blood glucose concentration and the liver glucose and glycogen content. A second major series of change is the increase in liver and blood fat (FFA) and the increase in ketone bodies (ketosis). The blood concentration of acetate, as well, sometimes may be increased during ketosis (1, 68), and this possibly arises from the increased free fatty acid (FFA) oxidation in the tissues. The first of these series of changes (in carbohydrate) undoubtedly influences the second, but the present author will concentrate on the ketosis and refer to the carbohydrate changes only when necessary.

Origin of Ketone Bodies

Precursors and formation of ketone bodies. The ketone bodies are a group of compounds which are acetoacetate (AcAc), B-hydroxybutyrate (BHB), and acetone. A fourth compound, isopropanol, has been detected in rumen contents, but its significance is not known at the present time (80). Figure 1 shows that these ketone bodies arise from acetoacetyl CoA which is a normal intermediate in fatty acid oxidation but which also is readily formed from acetyl CoA. During ketosis, the liver is the primary site of ketone bodies, but once they are formed, the liver cannot reconvert them to acetoacetyl CoA. This is because liver is deficient in the necessary activating enzyme system.

Acetoacetate is the parent ketone body (Fig. 1) and acetone and BHB are its daughters. Most of the AcAc is reduced to BHB by action of the enzyme B-hydroxybutyrate dehydrogenase. The reaction is reversible, and the two compounds are interconvertible (20, 60, 81). Acetoacetate, by itself is an unstable compound and forms acetone and CO₂ irreversibly and nonenzymatically at about 5% per hour at body temperatures (20). Acetone is poorly utilized, however, and is of little importance to the animal unless the ketosis is severe and of long duration (51, 53). Acetone also is volatile and its characteristic odor arises from the breath in ketotic conditions (hence, the term acetonemia).

The principal precursors of ketone bodies (Fig. 1) are fatty acids (32, 50, 56), which are mainly free fatty acids (FFA) of a 16 to 18 carbon chain length and which are mobilized from the fat stores or adipose tissue of the body (31, 55). Dietary short-chain fatty acids with an even number of carbon atoms (chain length of 4 to 10) are also precursors; butyrate (4 carbon) is the principal fatty acid in this group (36, 78, 84). Propionate, the 3 carbon dietary fatty acid, only is glucogenic. While certain amino acids (e.g. tyrosine, isoleucine, phenylalanine, and leucine) can form ketone bodies, the majority are glucogenic. The enzymatic possibilities of synthesizing ketone bodies seem widely spread among animal tissues and acetate (Fig. 1) can be metabolized partly by way of AcAc formation (34, 43, 58, 77, 82). The quantitative significance of this phenomenon is still not clear, however. Most of these experiments were performed in vitro or else by labeled acetate with the subsequent finding of the label in the ketone bodies. Much of this may have been due to exchange reactions with only little or no net total ketone body synthesis. When acetate is administered to living ruminants in large quantities, significant increases in ketone bodies do not occur (84). In addition, liver tissue does not seem to utilize much acetate (57).

Sites of ketogenesis. While some form of ketone body production occurs in a number of tissues of the body, it is the liver and ruminal
FIG. 1. Major pathways of ketone body and free fatty acid metabolism and gluconeogenesis. The reaction shown by the dotted line does not occur in liver. The participation of acetate and amino acids in ketogenic and FFA metabolic pathways is small.

(and omasal) epithelium that are the principal sites of ketogenesis in the ruminant. Table 2 summarizes some of the present information in this regard. It is of considerable interest that the mammary gland of the normal (fed or starved) cow utilizes both of the two major ketone bodies but that during ketosis, AcAc is produced. As a total, however, ketone bodies are still being utilized during ketosis since BHb utilization is greater than the AcAc being liberated. It is most likely that BHb is being converted to AcAc, and in this context the bovine mammary gland cannot be regarded as a ketogenic organ. In this same regard, sheep lung, kidney, and sometimes muscle can interconvert ketone bodies and, thus, produce one or the other of the two compounds. Their overall effect, however, is a utilization of ketones. According to our present knowledge only the digestive tract and liver are major sites of total

<table>
<thead>
<tr>
<th>Organ</th>
<th>BHB</th>
<th>AcAc</th>
<th>Total</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>38, 64</td>
</tr>
<tr>
<td>Rumen</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6, 71</td>
</tr>
<tr>
<td>Udder, normal cow</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>45, 75</td>
</tr>
<tr>
<td>Udder, ketotic cow</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>45, 72</td>
</tr>
<tr>
<td>Lung, sheep</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>49</td>
</tr>
<tr>
<td>Kidney&lt;sup&gt;b&lt;/sup&gt;, man, rat</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>61, 82</td>
</tr>
<tr>
<td>Muscle, working</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>33, 34</td>
</tr>
<tr>
<td>Muscle, resting</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>33, 34</td>
</tr>
<tr>
<td>Brain, man</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>62</td>
</tr>
</tbody>
</table>

<sup>a</sup> BHb, β-Hydroxybutyrate; AcAc, acetoacetate; Total, both BHb and AcAc.

<sup>b</sup> Not including urinary excretion.

JOURNAL OF DAIRY SCIENCE Vol. 54, No. 6
ketone body production in ruminants. These appropriately can be termed alimentary ketogenesis and hepatic ketogenesis, respectively.

Alimentary ketogenesis occurs both in isolated tissues from cattle and sheep (36, 66, 78) and in the living animal by analyses of portal and arterial blood (6, 38, 71). Dietary fatty acids with an even number of carbon atoms are the precursors, but butyrate is the principal fatty acid involved (84). Since the ketone bodies are formed during the process of absorption into the blood, this alimentary ketogenesis entirely ceases if the animal stops eating (38, 71). In this regard also, different types of rations tend to result in different types of fatty acids being produced. High-grain rations usually increase the proportion of propionate, but some high-moisture silages increase the amount of butyrate being produced (3, 73). Butyrate would be metabolized as ketone bodies and be undesirable during ketosis. Furthermore, some silages already contain as much as 2 to 4% butyric acid, and a higher incidence of ketosis has been associated with its feeding.

During ketosis, hepatic ketogenesis accounts for the bulk of ketone body production in the body particularly if the animal has a reduced feed intake. The major sources of these ketone bodies are the FFA which are mobilized from the body's fat stores. The relative roles of the digestive tract and liver to ketone body production in sheep are summarized in Table 3. In adequately fed sheep alimentary ketogenesis accounted for virtually all the ketone body forma-

Table 3. Ketone body production in sheep.a

<table>
<thead>
<tr>
<th>Source</th>
<th>Fed normal Ketotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digestive tract</td>
<td>1-2</td>
</tr>
<tr>
<td>Liver</td>
<td>Nearly 0</td>
</tr>
<tr>
<td></td>
<td>8 or more</td>
</tr>
</tbody>
</table>

a Summarized from References 13, 18, 38, 71.

are interconvertible, but they must be converted back to AcAc before being utilized by the extrahepatic tissues. The enzyme, \( \beta \)-hydroxybutyrate dehydrogenase, which catalyzes this NAD-linked reaction is widely distributed in the tissues of the body, and it is found tightly bound to the mitochondrial membrane (47). The enzyme, however, is present in only low concentrations in liver tissue of fed ruminants (60). The significance of this latter finding is unknown at the present time since the liver does produce at least some BHB in most instances. It is possible that an induction of the enzyme occurs as the animal becomes ketotic.

Table 4 summarizes some of the recent literature on the ratios of BHB to AcAc in the blood and livers of normal and ketotic cows. The sheep and three nonruminant species also are included for comparisons. Several notable features are evident from these data. Firstly, BHB is by far the most predominant ketone body in all species. Secondly, the ratio of these reduced to oxidized ketone bodies in the ruminant is not the same in blood as it is in liver, and it is usually greater in the liver. A third fact is that, in ruminants, the ratio of BHB to AcAc decreases as the animal becomes ketotic; i.e. the amount of AcAc increases more than does BHB. This is opposite to the situation in man, the rat, and the dog which are all nonruminants; in these species the ratio increases during ketosis.

Many of these changes, however, cannot be adequately explained in the light of present knowledge. The two varying sources of ketogenesis in ruminants (digestive and liver), as opposed to only one source in nonruminants (liver), could influence the situation somewhat since BHB seems to be the predominant ketone body that is produced in the rumen (38). This clearly is not the only factor involved in the change in ratios during ketosis, however, since changes in liver also occur. Furthermore, the great release of AcAc and uptake of BHB (and possibly acetate) by the mammary gland during bovine ketosis, and by other tissues as well, will affect these BHB to AcAc ratios (Table 2).

While interpretation of these wide fluctuations in the ratios of BHB to AcAc is difficult, the conversion of AcAc to BHB actually may be an advantage and play a special role in the transport of ketone bodies by the blood. This is because BHB is a stable compound but AcAc is not. The AcAc readily decomposes to acetone and CO\(_2\). Other suggestions for a useful physiological role of BHB is that the reaction serves as a "hydrogen shuttle" across the mitochondrial membrane (24, 34). This would permit extra mitochondrial formation of NAD from NADH.
Table 4. \( \beta \)-Hydroxybutyrate:acetoacetate concentration ratios in the blood and liver of various animal species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Blood</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal Ketotic</td>
<td>Normal Ketotic</td>
</tr>
<tr>
<td>Ruminants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cow</td>
<td>18-25 4.6</td>
<td>50 5.3</td>
</tr>
<tr>
<td>Cow</td>
<td>13 3.6</td>
<td>6.0 5.2</td>
</tr>
<tr>
<td>Sheep</td>
<td>6-9 2-4</td>
<td>( \infty ) ( a,b ) 3.8 ( a )</td>
</tr>
<tr>
<td>Nonruminants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Man</td>
<td>2.0 4.5</td>
<td>— 1.8 ( a )</td>
</tr>
<tr>
<td>Rat</td>
<td>1.1 3.3</td>
<td>2.6 2.1</td>
</tr>
<tr>
<td>Dog</td>
<td>1.9 2.1</td>
<td>— —</td>
</tr>
</tbody>
</table>

\( a \) Hepatic production ratio.
\( b \) Both hepatic and digestive tract production ratio.

Utilization of Ketone Bodies

Utilization in the whole body. Most tissues can utilize ketone bodies, and this is done by converting AcAc to acetoacetyl CoA (Fig. 1). The latter can then form acetyl CoA and undergo oxidation in the TCA cycle, or it can be used for fat synthesis, particularly by the mammary gland, or for the formation of other compounds. Free acetone, however, is glucogenic by forming pyruvate and as stated earlier is usually of little importance (51, 53). Since ketone bodies are produced in the liver but are utilized by other tissues, a ketosis then could theoretically be the result of a) an underutilization by the extrahepatic tissues or b) an overproduction by the liver. The underutilization theory was widely accepted at one time (27, 32) because it was held that carbohydrate was necessary to continue the breakdown, in all the body tissues, of ketone bodies formed during fat catabolism. In other words, instead of fat going completely to \( CO_2 \) and water, it stopped at the ketone stage whenever carbohydrate utilization was defective. The concept frequently was summed up by the statement that “fat burns only in the flame of carbohydrate.” This view no longer seems appropriate, however, in view of modern knowledge.

Many studies have now demonstrated an extensive extrahepatic utilization of ketone bodies. Most of the earlier studies were inconclusive, however, since they were confined to experiments on diabetic ketosis, eviscerated animals, in vitro tissue preparations, or measuring ketone body concentration differences in blood traversing particular organs (see 18 for ref.).
By the use of isotopically labeled ketone bodies, however, it has been possible to extend these studies for a more kinetic approach and to estimate their production and utilization by the whole animal and at their own endogenous rate of metabolism (18, 20, 48). None of these studies has yet been on ketotic cows, but such studies using either labeled AcAc or BHB have been reported on sheep with induced pregnancy ketosis (Fig. 2). These experiments (in this case AcAc) show that both the rate of ketone body turnover and their complete oxidation to CO₂ are proportional to their plasma concentration and this is true up to an AcAc concentration of about 10 mg per 100 ml (total ketone bodies expressed as acetone, would be about 20 mg/100 ml). It can be concluded, therefore, that ketone body concentrations, up to about 20 mg/100 ml, merely are a reflection of the rate of ketone production and that utilization in turn is regulated by blood ketone concentration. Maximal ketone body utilization evidently occurs at about 20 mg/100 ml, and after reaching this maximum only small increases in production would cause large increases in blood concentration. This would help explain the extremely high ketone concentration (e.g. 50 to 100 mg/100 ml) occasionally observed in ketotic animals.

The maximal utilization of ketone bodies in sheep seems to be about 7 to 10 g per hr (0.4 g per hr per kg³/₄, Fig. 2) and this is about the same in pregnancy ketosis as it is in artificial ketosis, i.e. normal sheep infused with large quantities of ketone bodies (20). All of these facts point out that ketone body utilization is not significantly impaired during ketosis, but rather there is an increased production of ketone bodies and that in severe ketosis they are produced faster than the body can utilize them. The maximal capacity of the tissues to utilize ketones is considerable, and Figure 2 shows that ketotic sheep can derive 20 to 30% of its respiratory CO₂ from the ketone bodies. It must be pointed out, however, that some impairment of ketone body utilization possibly could occur in the terminal stages of ketosis when the animal is near death or even in severe diabetes (11, 74). It seems clear, nevertheless, that the initial development of ketosis is due to overproduction, that is, increased ketogenesis.

Adaptation to ketosis. All species of animals gradually undergo adaptations during starvation or when their feed intake is decreased. This subject is beyond the scope of this paper, but it obviously is of importance in ruminant ketosis since most of these animals will reduce their feed consumption for one reason or another. While the subject of food deprivation has been studied extensively, the subject recently has been revived and made clearer by more modern techniques. These studies (26), which were principally on humans, point out that two metabolic adaptations are of prime importance to conserve glucose and protein for survival and so that fat can be more easily utilized. The first adaptation would be that of “glucose exclusion” from most of the tissues and the use of FFA and ketone bodies as the major metabolic fuels. This is most strikingly evident for brain, which is the major user of glucose. After a week or so of starvation the brain begins to utilize considerable amounts of ketone bodies (62), thereby sparing glucose and body protein for glucose synthesis. A second adaptation would be the Cori cycle which shuttles liver glucose to the periphery and which in turn sends lactate back to the liver for resynthesis of glucose. Less new carbohydrate needs to be formed, protein is conserved, and the energy for this shuttle is ultimately derived from fat.

The aforementioned postulated adaptations could be applicable to acute and chronic forms of ketosis in ruminants and why cattle usually manage to survive but many sheep cannot survive. Ruminants have additional glucose requirements than does normal man and these are lactose for milk synthesis and fructose for the fetuses. If a glucose shortage develops gradually, however, the brain and many other tissues could still partially adapt to a ketone and FFA utilization. If the shortage occurs rapidly, the tissues can not adapt, the animal will show acute nervous symptoms and may succumb in a short time. The cow seems to have an advantage over the sheep, however, in that it can sharply reduce its milk production, thereby conserve glucose, be less acutely afflicted, and usually survive.

In this regard, it is now known that glucose utilization (and milk production in the cow) decreases in ruminants that have been acutely fasted (12, 44) but their metabolic rate also decreases. As a result of this acute fasting, glucose does not seem to be spared by the body as much, and it always seems to account for about 10% of the respiratory CO₂ (12, 17). During a more mild and prolonged glucose shortage, however, the situation could possibly be altered and glucose could be spared as in the studies on man.

Excretion of ketone bodies. In cows, two major avenues of ketone body excretion are available and these are by way of the urine and the milk. The kidneys (82) and particularly the mammary gland, however, also utilize ketone
bodies for metabolism, the latter using BHB mainly for lipogenesis (23, 52). A third avenue of excretion is that of acetone by way of the breath. This is easily detectable but the pathway is quantitatively unimportant (51, 53). Schultz (73) states that ketones in milk are roughly one-half of the blood values but urine concentrations are more variable and usually exceed the blood levels by about 4 times. The total amount of ketone bodies that are excreted varies with the severity of ketosis and excretion in urine far exceeds that in milk. The total amount excreted, however, probably never exceeds 10% of the amount produced (67).

Toxicity of ketone bodies. Since AcAc and BHB are strong acids, excessive accumulation in the body can produce an acidosis. Their excretion in the urine if severe and if prolonged can involve losses of Na and K ions so that the alkali reserve of the plasma will be lowered. The severity of acidosis in bovine ketosis, however, is much less than in diabetes mellitus of nonruminants (37, 68, 79).

β-Hydroxybutyrate, by itself, seems to be relatively nontoxic, but high concentrations of AcAc and acetone can produce nervous symptoms and possibly contribute to clinical signs. Sollman (76) states that acetone is about as toxic as methyl alcohol. Infusion of large amounts of AcAc into sheep and both AcAc and BHB into dogs leads to hypoglycemia (20, 54). This apparently is due to stimulation of insulin secretion by the pancreas. Furthermore, the ketone bodies increase in proportion to the concentration of their precursors, the FFA, but a lag time of roughly one-half hour seems to be taken for this conversion. A number of other hormonal factors such as insulin, growth hormone, ACTH, adrenal steroids, and thyroxin also bring about

Regulation of Hepatic Ketogenesis

As previously stated, FFA form the bulk of ketone bodies that are produced during ketosis and this takes place in the liver. In severe ketosis, this ketogenesis grossly exceeds the body's needs and this is what Krebs (39) refers to as "pathological ketosis." Why does this excessive production occur? The answer seems related both to the rate of FFA mobilization from the adipose tissue, a "primary factor," and to its metabolic fate in the liver, an "hepatic factor."

Free fatty acid mobilization. The mobilization of FFA seems to be the first or primary factor that must occur for ketogenesis to be initiated, and this occurs when additional fuel is needed to support the body's metabolism (31, 55). Free fatty acids also are released when dietary fat is absorbed and metabolized (16) but this would be only of minor importance for ruminants which exist on relatively low fat diets. Additional fuel will be needed, for example, during emergency situations and FFA will be released from the fat stores by action of the sympathetic nervous system. Figure 3 exemplifies this action and shows the results in a sheep of an injection of norepinephrine. The animal can quickly change its rate of fat mobilization. This occurs within minutes and is proven by measurements of both plasma FFA and glycerol concentrations and by measurement of the rate of glycerol production (or release by adipose tissue). Furthermore, the ketone bodies increase in proportion to the concentration of their precursors, the FFA, but a lag time of roughly one-half hour seems to be taken for this conversion. A number of other hormonal factors such as insulin, growth hormone, ACTH, adrenal steroids, and thyroxin also bring about
or inhibit FFA mobilization and ketogenesis, but this is beyond the scope of this paper and has been reviewed elsewhere (15, 65).

A much greater need for FFA as an additional fuel undoubtedly occurs when feed intake is insufficient to meet energy needs. Diurnal fluctuations in plasma FFA concentrations are inversely correlated with feed intake and FFA are a sensitive index of the animal's nutritional status (68). Heavily lactating cows usually are losing weight simply because energy output exceeds energy intake, and part of the released FFA forms ketone bodies. This probably is what Kronfeld (42) refers to as a "feeding ketosis." It has also been called a "physiological ketosis" (39). Other large needs for FFA as fuel occur when glucose is deficient as in hypoglycemia, or when it cannot be utilized as in insulin lack. These factors are all potent stimulators of FFA release, and the role of glucose is now known to be of special importance. This was first described in man (31) and later shown to be true both for cows (4, 41) and for sheep (5, 69). Furthermore, intravenous glucose injections quickly depress plasma free fatty acids and eventually the ketosis (14, 19, 41).

The data adapted (Fig. 4) from Reid and Hinks (69) for pregnant sheep seem to be the most complete in this regard. The plasma concentrations of the two metabolic fuels, glucose and FFA, are inversely proportional to each other. This means that when blood glucose concentrations are normal, FFA concentrations are low and of limited importance, but as blood glucose falls to hypoglycemic levels, FFA concentrations increase in a linear or straight-line relationship. Figure 4 also shows the relationship of FFA to ketone bodies. Blood ketone bodies increase linearly or in direct proportion to FFA concentrations but only up to a certain point (here, about 1.5 mEq/liter). After this point ketone body concentrations increase rapidly and can assume almost any magnitude. It is at this point that the body probably shifts from what Krebs (39) calls a "physiological ketosis" to that of a "pathological ketosis." Before this, ketogenesis seems to be controlled merely by the rate of FFA mobilization from the adipose tis-

![Fig. 4. Relationship between plasma free fatty acids, glucose, and ketone bodies in pregnant sheep. Note the inverse and linear relationship between free fatty acids and glucose (left) and the curvilinear relationship between ketone bodies and free fatty acids (right). Adapted from Reid and Hinks (69) and reprinted from Dukes' Physiology of Domestic Animals, ed. M. J. Swenson. Copyright 1970 by Cornell Univ. Used by permission of Cornell Univ. Press (15).](image-url)
the liver. The consequent rise in ketone body concentration is relatively small, and it is balanced by increased ketone body utilization in the extrahepatic tissues.

What happens to cause the greatly increased build-up of ketone bodies in the blood at about this time? Part of the explanation simply could be that the maximal capacity of the body to utilize ketone bodies has been attained (see also Fig. 2) but this cannot be the entire explanation since the total ketone body concentration at this point (Fig. 4) was only about 5 mg/100 ml. The most likely explanation is that there is an "hepatic factor" or a shift in the pattern of FFA utilization in the liver itself.

Free fatty acid utilization in the liver. The liver is a major utilizer of FFA since it becomes fatty after long periods of fat mobilization. The extent to which FFA are removed by the liver, however, is probably greater than generally recognized. Recent experiments in dogs (9) and sheep (16) show that about 25% of the total FFA release in the body is taken up by the liver regardless of the actual quantities or conditions involved. Thus, if more FFA is being mobilized, more will be taken up.

Several recent studies show that there are three major pathways for FFA utilization within the liver (55, 56). These pathways are visualized in Figure 1 and consist of: a) esterification to triglycerides (and some phospholipids) where they can be stored in the liver or released as lipoproteins, b) complete oxidation to CO\textsubscript{2} in the TCA cycle, and c) partial oxidation to ketone bodies. Moreover, these three pathways are interrelated and could act competitively. If pathway a) or b) or both are reduced, then ketogenesis will increase all out of proportion to the concentration or influx of FFA being presented. Table 5 shows that this indeed may be the case. In these studies on fasting ketosis in the rat and diabetic ketosis in the dog, the percentage of FFA-\textsuperscript{14}C traversing both the pathways of esterification (although less so in the diabetic dog and the TCA cycle (to CO\textsubscript{2}) was reduced and a marked shift to the third or ketogenic pathway was in evidence.

Table 6 shows that this may also be true for the ruminant. In these experiments on sheep, the esterification and CO\textsubscript{2} pathways were not measured and only net FFA uptake and ketone production by the liver were estimated. Ketotic sheep had lower blood glucose and higher FFA concentrations than normal animals and their livers were taking up greater quantities of FFA and producing more ketone bodies. The results indicate, as in Table 5, that a much higher percentage of FFA taken up by the liver was accounted for as oxidation to ketone bodies in the ketogenic sheep (about 80%, Table 6) than in the normal sheep (30%). Some ketone bodies possibly could be derived from compounds such as triglycerides or amino acids but it is unlikely that this could make up this large a difference between the two groups.

This concept of an "hepatic factor" or increased oxidation of FFA to ketone bodies by the liver, along with a reduction in the two pathways of esterification and complete oxidation to CO\textsubscript{2}, is important since it would cause the rate of ketogenesis to increase over and above that merely due to the "primary factor" or increased FFA release from the adipose tissue. As pointed out by Mayes and Felts (56), the energy requirement of the liver could still be met even though the FFA were being diverted and only partially oxidized to ketone bodies. Moreover, ketone body production is still a type of respiration, and it can provide the liver with adequate amounts of energy.

The mechanisms for this shift in FFA pathways seem obscure, but they seem related to the availability of carbohydrate in the liver. A decrease in the liver's carbohydrate metabolism (glucose and glycogen, see Table 1) would most probably reduce the liver's capacity to esterify the incoming fatty acids. In a similar vein, it would also reduce its capacity for synthesis of new fatty acids (lipogenesis) from acetyl CoA. Carbohydrate in the form of glycerol is needed for esterification and factors such as citrate and NADPH are needed for lipogenesis (8, 64, 65). In addition, and as pointed out by others (2,}

<p>| Table 5. Fate of free fatty acids in normal and ketogenic (fasted or diabetic) rat and dog livers. |
|---------------------------------|-----------------|-----------------|---------|---------|
| FFA-\textsuperscript{14}C recovered (%) | Rat liver\textsuperscript{a} | Dog liver\textsuperscript{b} |
|---------------------------------|-----------------|-----------------|---------|---------|</p>
<table>
<thead>
<tr>
<th>Pathway</th>
<th>Fed</th>
<th>Fasted</th>
<th>Normal</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esterification</td>
<td>78</td>
<td>52</td>
<td>70</td>
<td>67</td>
</tr>
<tr>
<td>Resp. CO\textsubscript{2}</td>
<td>13</td>
<td>7</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Ketone bodies</td>
<td>8</td>
<td>38</td>
<td>16</td>
<td>33</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Total</td>
<td>99</td>
<td>97</td>
<td>96</td>
<td>104</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Rat livers perfused for 90 minutes with 150 mg oleate-\textsuperscript{14}C (Ref. 56).

\textsuperscript{b} Intact anesthetized dogs infused with palmitate-\textsuperscript{14}C and with samples taken of arterial, portal, and hepatic venous blood (recalculated from Ref. 9).
Table 6. Free fatty acids and ketone body metabolism by normal and ketotic sheep livers.\(^{a}\)

<table>
<thead>
<tr>
<th>Pregnant sheep</th>
<th>Arterial cone FFA</th>
<th>Ketone bodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose (mg/100 ml)</td>
<td>FFA (mM/liter)</td>
</tr>
<tr>
<td>Fed (8)</td>
<td>49</td>
<td>0.4</td>
</tr>
<tr>
<td>Ketotic (9)</td>
<td>32</td>
<td>0.9</td>
</tr>
</tbody>
</table>

\(^{a}\) Data of Katz and Bergman (38). Intact sheep were used and samples were taken of arterial, portal, and hepatic venous blood.

\(^{b}\) Production as per cent of FFA uptake × 4.25 since FFA have a mean carbon chain of 17 and ketone bodies of only 4.

A deficiency of oxaloacetate in the liver would reduce the pathways of FFA oxidation to CO\(_2\). Oxaloacetate also is a reflection of available carbohydrate and will be discussed below.

Role of oxaloacetate in the liver. The concept of an oxaloacetate deficiency playing a role in the diversion of FFA away from CO\(_2\) production to that of ketogenesis is not new but it has had further impetus in recent years. This is because an oxaloacetate deficiency had been difficult to demonstrate (8, 28), but recent studies have now indicated that this does seem to occur in both diabetic and bovine ketosis (7, 39). Furthermore, oxaloacetate is now known to play a double role between gluconeogenesis and the production of CO\(_2\) (39). Figure 1 shows that it is an intermediate and is required for both pathways. In regard to glucose formation, there are only four major precursors: propionate, lactate, amino acids, and glycerol. Oxaloacetate is an intermediate in the formation of glucose from all of these precursors except glycerol. Glycerol can form up to one-fourth of the glucose requirements of the animal (22) but, even so, at least three-fourths of the gluconeogenesis must still go through oxaloacetate.

There have been three hypotheses regarding ways in which an hepatic oxaloacetate deficiency can occur. Two of these are directly related to gluconeogenesis and are: a) an excessive utilization of oxaloacetate because of increased gluconeogenesis and b) a deficient production of oxaloacetate because of precursor shortage (propionate, lactate, or amino acids). The third hypothesis (83) is that of an altered "redox state" or equilibrium due to a change in the NADH to NAD ratio so that much of the oxaloacetate is changed to malate (Fig. 1); this hypothesis is difficult to prove, however, and recent attempts to do so have failed (8, 40).

Krebs (39) has suggested that during all forms of ketosis the first theory or a high rate of gluconeogenesis is the responsible factor for a decreased hepatic oxaloacetate concentration. He has supported this theory with the fact that PEP carboxykinase activity is greatly increased during alloxan diabetes in the rat. This enzyme specifically controls the utilization of oxaloacetate for phosphoenolpyruvate, and eventually glucose formation. Furthermore, excessive glucose formation occurs in diabetes.

In ruminants, however, a truly excessive gluconeogenesis probably does not occur. Ketotic cows do not seem to turn over more glucose than comparably lactating cows (45), their livers have not contained more PEP carboxykinase activity (7, 8, 25) and, in addition, their blood glucose concentrations are low. In pregnant ketotic sheep, glucose turnover and actual hepatic glucose production are decreased (12, 38) from that of normal pregnant sheep. The final answer may be one of semantics and involve both of the first two hypotheses. It probably can be stated that there is an insufficient production of oxaloacetate (from propionate, lactate, and amino acids) in relation to the rate of gluconeogenesis. Lactating cows obviously need more glucose if lactose is being synthesized and the end result is that gluconeogenesis needs to be high but it is still insufficient for the animal's requirements. The ability of the animal to mobilize up or supply carbohydrate precursors, therefore, seems inadequate. If adequate carbohydrate, or its precursors, is restored, the liver will reduce its ketogenesis (19). Repeated carbohydrate administration sometimes is needed for prolonged effects, however.

References


(70) Roderick, L. M., G. S. Harshfield, and W. R. Merchant. 1933. Further observations on


