Hypoglycemia in Ketotic Cows

D. S. KRONFELD
Section of Nutrition, Department of Clinical Studies, School of Veterinary Medicine
University of Pennsylvania, Kennett Square 19348

Abstract
Two types of hypoglycemia have been differentiated in ketotic cows by tracer kinetics. One is associated with fasting ketosis and is characterized by decreases in the mass of glucose in rapid exchange with plasma glucose and in the rate of glucose transport through this pool. The other occurs in cows with spontaneous ketosis and is characterized by undiminished glucose transport or mass and by changes in glucose distribution. Similar changes follow insulin administration and opposite changes follow glucocorticoid administration. These differences in glucose kinetics between fasting ketosis and spontaneous ketosis are complemented by a dozen other demonstrated differences between the two conditions, mainly concerning ruminal, hepatic and mammary metabolism. These metabolic differences suggest the desirability of distinguishing between different types of ketosis for designing and testing control measures.

Introduction
Hypoglycemia in cows with acetonemia was first reported by Sjollema (61). Its discovery depended upon improvements in glucose assay and especially the use of potassium fluoride to protect glucose in blood samples taken in the field and requiring 24 to 48 hr transport to the Utrecht laboratory (Seekles, personal communication).

It came as a surprise to some, for the full clinical syndrome of acetonemia had not been observed in cows with a carbohydrate deficit induced by fasting or fasting-plus-phloridzin (62). On the other hand, clinical signs typi-

1 Acetonemia is used specifically to mean a particular metabolic disease developing in well-fed cows (24, 35, 62) while ketosis is used more generally to mean an increase of ketone bodies in the body fluids. Acetonemia may also be described as clinical spontaneous ketosis, whether uncomplicated or complicated. The latter condition is usually difficult to differentiate upon clinical examination from undernutritional ketosis which is secondary to some other preceding disease. This is discussed briefly later in this article and in detail in a recent clinically oriented review (35).

cal of the disease were observed in cows given insulin (24), and transient relief was obtained in clinical cases by administering glucose parenterally (67). Glucose therapy was tried because the digestive syndrome of acetonemia was thought to be a deferred or chronic form of milk fever.

The theory that the fresh lactational demand for glucose exceeds the glucose supply and lowers blood glucose had been in vogue for milk fever (4, 19). The finding of hypocalcemia and hyperglycemia in this disease (22, 45), in addition to the above findings relating to acetonemia, led to the transportation bolus bolus of the glucose imbalance theory from milk fever to acetonemia.

Thirty years passed before this supply-demand concept of hypoglycemia was questioned. Tracer kinetic experiments indicated that the glucose pool size and transport rate were normal in cows during the early clinical stages of uncomplicated spontaneous ketosis (43). Similar experiments on sheep with pregnancy toxemia, and on fasted pregnant sheep or fasted lactating cows revealed the changes which had been expected in spontaneously ketogenic cows, i.e. a decreased glucose pool size and transport rate (37, 42). These findings were challenged on the grounds that the method employing a single tracer injection yields invalid results and because the results in cows with spontaneous ketosis conflicted with those in ketotic pregnant sheep (9, 10).

I shall try to convince you that such criticisms are unwarranted and that the hypoglycemia which develops in cows with spontaneous ketosis is associated initially with changes in glucose distribution rather than a decrease in glucose transport. The latter change is typical of fasting. I will then attempt to relate these two forms of hypoglycemia to two of the four types of ketosis which I believe occur in cows. These separate conditions should be differentiated for the purpose of prevention, treatment, and investigation.

Glucose Kinetics
Any functional system may be studied by observing its responses (outputs) to known stimuli (inputs). We may define the glucose system in the cow as the set of variables which determine
the plasma glucose concentration. Among these variables are the flows of glucose into, out of, and within the system, together with dietary, endocrine, and other factors which control these flow rates.

Glucose tolerance tests. The response of the plasma glucose concentration to a single intravenous injection of glucose is much the same in normal and (spontaneously) ketotic cows; the plasma glucose falls to the preinjection level in about 2 hr (23, 59). A longer time interval would be expected in underfed or diabetic animals. The glucose tolerance tests reported by Shaw in 1943 appear in retrospect to be the turning point where he began to distinguish between (under) nutritional and metabolic (spontaneous) ketosis (59, 60).

Interpretations of glucose tolerance tests are complicated by the fact that the glucose load elicits regulatory responses, e.g. insulin release. Such perturbations usually make the responses of the system nonlinear (Fig. 1). The utilization rate of the added glucose is likely to vary with the dose and to be different from that of the plasma glucose prior to the injection. This can be circumvented by tracer glucose.

Tracer kinetics. By definition, the tracer may be differentiated from the tracee by the observer but not by the system. It does not perturb the system, and the responses are linear and amenable to relatively simple yet rigorous mathematical analysis (12, 15, 52). Therein lies the advantage of using a tracer to study kinetics.

In a typical tracer experiment, the dose (input) and response (output) data are analysed mathematically to obtain physiologically meaningful information. This implies the use of a model, i.e. a mathematical or physical construct or both which represents the system and fits the data. The more extensive and precise the data, the better the model may represent the system.

In the first tracer glucose kinetic experiments on spontaneously ketotic cows, $^{14}$C-U-glucose was injected in a few seconds, and 5 assays of plasma glucose specific activity were obtained 30 to 180 min postinjection. A semilog plot of these data formed a straight line so the tracer dilution was analysed as a first order process (43). This implied that the glucose pool comprises a single compartment. Glucose transport through this pool was about 1.0 g/min in four cows when ketotic and following recovery (Table 1). The compartment mass divided by the plasma glucose concentration

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2 When the responses of a system are linear, according to the superposition principle, if one input and the corresponding output are known, then the output may be calculated for any other known input, or the input may be calculated for any other output (59). For example, if the response of plasma glucose specific activity to an instantaneous tracer input is observed, then the response to a constant tracer input or the combination of an instantaneous input followed by a continuous constant input may be calculated, e.g. by convolution (15, 52).

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Fig. 1. A cow weighing 472 kg was injected intravenously with 95 g glucose as a 50% solution then the next day 190 g glucose (doses of 0.2 and 0.4 g/kg body weight, respectively). The changes in plasma glucose concentration are shown above. The increments in plasma glucose concentration, expressed as a fraction of the dose, are shown below. Note that these responses are clearly not superimposable. Linear responses, e.g. like those to various tracer inputs would be superimposable.2

2 Ibid.
Table 1. Glucose kinetics in normal, fasted, and spontaneously ketotic cows.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Plasma cone (mg/100 ml)</th>
<th>Transport (g/min)</th>
<th>Space (% weight)</th>
<th>Method</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (4)</td>
<td>58^c</td>
<td>1.10</td>
<td>25</td>
<td>p3</td>
<td>41</td>
</tr>
<tr>
<td>Normal (8)</td>
<td>58^c</td>
<td>1.05</td>
<td>24</td>
<td>S 3</td>
<td>41</td>
</tr>
<tr>
<td>(same)</td>
<td>58^c</td>
<td>1.05</td>
<td>24</td>
<td>S 1</td>
<td>41</td>
</tr>
<tr>
<td>Fasted (4)</td>
<td>48^c</td>
<td>.45</td>
<td>21</td>
<td>S 3</td>
<td>41</td>
</tr>
<tr>
<td>(same)</td>
<td>48^c</td>
<td>.45</td>
<td>21</td>
<td>S 1</td>
<td>41</td>
</tr>
<tr>
<td>Spontaneous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--1</td>
<td>38</td>
<td>.96</td>
<td>80</td>
<td>S 1</td>
<td>43</td>
</tr>
<tr>
<td>--2</td>
<td>36</td>
<td>.95</td>
<td>55</td>
<td>S 1</td>
<td>43</td>
</tr>
<tr>
<td>--3</td>
<td>38</td>
<td>1.06</td>
<td>55</td>
<td>S 1</td>
<td>43</td>
</tr>
<tr>
<td>--4</td>
<td>32</td>
<td>1.05</td>
<td>72</td>
<td>S 1</td>
<td>43</td>
</tr>
<tr>
<td>--5</td>
<td>27</td>
<td>1.37</td>
<td>72</td>
<td>S 1</td>
<td>43</td>
</tr>
<tr>
<td>--6</td>
<td>36</td>
<td>.71</td>
<td>38</td>
<td>S 1</td>
<td>43</td>
</tr>
<tr>
<td>--7</td>
<td>37</td>
<td>.92</td>
<td>33</td>
<td>P 3</td>
<td>41</td>
</tr>
<tr>
<td>--8</td>
<td>40</td>
<td>.89</td>
<td>36</td>
<td>P 3</td>
<td>41</td>
</tr>
</tbody>
</table>

^a Uncomplicated cases; 1–5 were studied within 24 hr of the first observed nervous signs; 6–8 had been hypophagic for 2–4 days.

^b p, primed infusion of tracer; S, single injection; 1, monoexponential analysis; 3, 3-compartment analysis (Fig. 3).

^c Mean.

^d Range.

^e Unpublished preliminary results.

gave an apparent volume of distribution (or space) of 26% body weight in the normal cows, approximating the extracellular volume. The mass was slightly larger during hypoglycemia so that the space was 65% body weight, approaching total body water. The supply-demand hypothesis of hypoglycemia would predict a smaller glucose pool size (mass) and decreased transport rate so the above results ran contrary to expectations.

The weakness in these experiments lay in the limited period of observation of the response and the paucity of the data. Surprisingly, it was the mode of tracer administration which received adverse criticism.2 This came mainly from protagonists of a method using tracer administration by primed infusions, a limited observational period of 30 to 180 minutes, and monoexponential analysis (9, 63). This criticism is tantamount to declaring that the tracer perturbs the system and that the perturbation varies with the mode of tracer administration! Moreover, it diverted attention from the real deficiencies in observational period and rigorous analysis which have plagued most studies of glucose kinetics irrespective of the mode of tracer administration.

An ideal tracer experiment would define the input and determine the entire response from zero to infinity or, at least, from the first to the last discernible change. If tracer glucose is injected into the vein of a cow, the semilog plot of plasma glucose specific activity against time usually has two points of inflection, one about 30 to 45 min, the other 150 to 180 min postinjection (Fig. 2). The “final slope” remains unchanged until the specific activity falls so low that duplicate assays vary by a factor of 2; this occurs at about 24 hr following a dose of 2 mCi 14C-U-glucose. Three exponential terms are necessary and sufficient to fit the curve (Fig. 2). Integration of this equation from zero to infinity, which assumes that the observed “final slope” is indeed final, measures the magnitude of the response to the tracer.3 the magnitude of the response is the area subtended by the curve of plasma glucose specific activity plotted against time following an instantaneous injection of tracer glucose, or the asymptotic value of the plasma glucose specific activity obtained during a constant infusion, with or without a prime. These time-integrals are mathematically equivalent (49). They both require extrapolation to infinity so are poorly determined if the data are few or inconsistent or the observational period is limited. The common procedures of using the mean specificity of a “plateau” during a tracer infusion, or extrapolating the observed “final slope” following an injection, usually underestimate the magnitude of response, hence, overestimate the net transport rate of tracer through the system.
We have performed single injection, double tracer experiments on two cows with spontaneous ketosis, and some preliminary results are in Figure 3. The glucose pool space, comprising the plasma-equivalent spaces of compartments 1 and 2, was enlarged greatly in one cow, slightly in the other. Glucose transport was diminished in one cow which had been hypophagic for 3 or 4 days.

Six years ago we gave primed infusions of $^{14}$C-U-glucose for 240 min to two cows with spontaneous ketosis which had been hypophagic for at least 2 days. Blood samples were taken every 15 min during the infusion and 75 min thereafter. We first tried to analyse the data according to Steele (63). His equation combines a monoexponential term, representing the response to the prime, with a monoexponential rising to an asymptote, which represents the infusion; both terms have one and the same rate constant (63). This equation is equivalent to that in our early studies with single injections; the model is the same. The monoexponential analysis is applied to a limited observational period 60 to 180 min postprime. The prime :infusion “ratio” is chosen with the objective of obtaining a constant specific activity during this period. If this is successful, the specific activity may be assumed constant from zero to infinity, and the calculations become very simple. (The mean specific activity is divided into the prime for the glucose pool size and into the infusion rate for the glucose transport rate.) In our experiments, the plasma glucose specific activity was rising slightly from 60 to 180 min so we determined the best (monoexponential) fit by an iterative procedure. The estimates were 53 and 67% body weight for the glucose and 0.55 and 0.46 g/min transport, respectively. Unfortunately, the monoexponential equation best-fitting the 60 to 180 min data did not predict the data obtained before or after this interval. The calculated results were, therefore, erroneous or, to be more explicit, not as fully representative of the system as possible in the light of all the data available.

The data from either experiment were insufficient to provide a good test for three compartment models so attempts were made to fit both sets of data simultaneously. The best fit was obtained when the rate constants were constrained to be the same for both experiments while the proportionality constant (hypothetical specific activity at zero time) was allowed to differ between experiments. The solution for the model in Figure 3 indicates that the glucose space was slightly enlarged while glucose transport was slightly depressed (Table 1).
Fig. 3. This 3-compartment model was used to analyze averaged data 5 to 720 min following single injections of $^{14}$C-U-glucose into 8 normal cows (Nav) or data 1 to 720 min after $^{14}$C-U-3H-2-glucose was injected into two cows with spontaneous ketosis. One of the latter had shown clinical signs for less than a day (SK1), the other had been hypophagic (SK2). The first two compartments are taken to represent physical mixing processes only, i.e. they form the glucose pool (31, 41). The third compartment partitions glucose transport out of the pool into a recycling flow and irreversible disposal, i.e. net transport of glucose through the system.

To summarize all 8 tracer kinetics studies on spontaneously ketotic cows: the glucose space was greatly enlarged, and the glucose transport was normal in the five cows which had only recently shown clinical signs; the glucose space was increased only slightly, and the glucose transport rate was tending to diminish in the three cows which had been hypophagic for 2 to 4 days (Table 1).

Further understanding of these changes has been sought by studying changes in glucose kinetics induced by fasting or insulin administration (31, 37, 41). Glucose transport is diminished by fasting but not insulin. The glucose space is enlarged by insulin but not fasting. Comparison of the changes found in these conditions suggests that the hypoglycemia developing in cows with spontaneous ketosis is due initially to changes in glucose distribution like those induced by insulin, with the possible subsequent addition of changes in glucose transport like those induced by fasting. The insulin induced changes probably reflect increased permeability in the membranes of some cells, e.g. muscle and, perhaps, capillaries (41). The fasting changes probably reflect the diminishing availability of exogenous glucose precursors.

At this stage I will presage the idea that conditions which exist in feeding cows with subclinical ketosis may induce insulin release. The ensuing hypoglycemia causes its characteristic clinical signs, including hypophagia (5, 24, 36). This in turn will inhibit further insulin release and bring on other changes characteristic of fasting, some of which are conducive to recovery (35). Before developing this concept further, I will comment on ketosis and its possible relationships with hypoglycemia.

**Fasting Ketosis and Hypoglycemia**

Fasting induces ketosis in ruminants which is dependent upon fat mobilization and hepatic ketogenesis (Fig. 4). This situation may be studied readily in fasted pregnant sheep; hypoglycemia develops progressively along with hyperketonemia, and both deviations become severe (51). The hypoglycemia is often assumed to contribute to the development of the ketosis, but it is not an essential condition. For example, if glucocorticoids are administered throughout the period of starvation, neither hypoglycemia nor clinical signs develop, but the plasma levels of free fatty acids and ketone bodies increase slightly more than with fasting alone (38). In fasted lactating cows, blood glucose falls for 24 to 48 hr, then returns usually to the lower part of the normal range, even though the hyperketonemia continues to increase (54). The upswing in blood glucose concentration occurs whenever milk production subsides abruptly; this was at 24 hr in the first report of this phenomenon (54), but it has occurred more often at 48 to 72 hr in our experiments.

**Spontaneous Ketosis and Hypoglycemia**

"A disease which is called acetonemia—is especially observed in cows that give high milk yields and that are in excellent nutritional condition, i.e. more or less fat" (62). "The incidence of ketosis is relatively high in many herds . . . fed rations which are nutritionally adequate in every respect" (60). Six examples of spontaneous ketosis developing in well-fed or excessively fed cows, together with some discussion of the underlying mechanisms, were presented recently (33). A working definition is that spontaneous ketosis develops initially in
Undernutritional ketosis is mainly attributable to fat mobilization and increased hepatic ketogenesis. Alimentary ketogenesis subsides (55) and mammary acetoacetate release does not occur (39). The fat mobilization is probably stimulated by nerves which release norepinephrine in the adipose tissue. It may be facilitated by hypoglycemia, but this is not essential. When lactating cows are fasted, a mild hypoglycemia develops initially but then regresses while hyperketonemia increases (54). This form of ketosis may be prompted simply by underfeeding (primary undernutritional ketosis) or by anorexia developing in the course of some disease (secondary ketosis). In a sense, underfeeding occurs in all highly productive cows during the first two months of lactation, for they are in negative energy balance. Most adapt successfully to this homeostatic challenge, however, and it does not appear to determine whether a cow remains healthy or develops clinical disease.

cows whose food consumption is equal to or greater than that of the majority of cows which adapt successfully to the strain of lactation.

Subclinical ketosis. Cows showing no physical signs of disease often have ketosis during the early stages of lactation. I propose that we should recognize three metabolic types of subclinical ketosis which may be differentiated from each other and from fasting ketosis in terms of sites of ketogenesis with the aid of blood analysis (Table 2). This differentiation depends on a knowledge of the specific precursors and products of ketogenesis at each site (Table 3), and the proposition that the

Table 2. Three types of subclinical ketosis in feeding cows may be differentiated by the sites of ketogenesis and by blood analysis from each other and from fasting ketosis.

<table>
<thead>
<tr>
<th>Type</th>
<th>Ali</th>
<th>Hep</th>
<th>Mam</th>
<th>3-OH-B</th>
<th>AcAc</th>
<th>FFA</th>
<th>AC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fasting</td>
<td>−</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

* Ketogenesis: Ali, alimentary; Hep, hepatic; Mam, mammary.

Plasma concentrations: 3-OH-B, hydroxybutyrate; AcAc, acetoacetate; FFA, free fatty acids; and AC, acetate.

Change from normal: 0 none; + increased; − decreased.
rate of ketogenesis at each site is mainly dependent upon the availability of substrates (32).

Type 1 is dependent primarily upon alimentary ketogenesis, hence, the availability of butyrate. It may be induced by feeding silage with a high butyric acid content or some silages high in lactic acid (13). As an example, we once found plasma acetoacetate and beta-hydroxybutyrate concentrations of 3.2 and 32 mg/100 ml, respectively, in a cow 3 hr after feeding; her blood free fatty acid and glucose, together with 31 variables measured in her liver, were all normal (8).

Type 2 involves hepatic ketogenesis superimposed on alimentary ketogenesis. This combination reflects the paradoxical situation of a cow feeding to her limit yet being in negative energy balance during peak lactation. The rate of hepatic ketogenesis may be inferred from the plasma free fatty acid level; Adler has emphasized that this should be used to distinguish between ketosis of alimentary or hepatic origin (2).

Some degree of fat mobilization, and incidentally hepatic ketogenesis, seems essential for maximal milk production. The mobilized fatty acids and their derivatives (acetate and beta-hydroxybutyrate) are used for mammary fat synthesis. The glycerol becomes available for hepatic gluconeogenesis. Moreover, oxidation of these nutrients spares glucose which then becomes available to the udder. The upper limit of milk production seems to be limited by the availability of glucose (39, 44). The homeostatic priorities in adaptation to lactational demands appear to restrain milk secretion to the supply of glucose but to mobilize body fat freely at the risk of ketosis. So I doubt that it is possible to eliminate subclinical ketosis Type 2 when striving for high production, especially for milk fat (4).

Type 3 has mammary acetoacetate production superimposed on alimentary and hepatic ketogenesis. The mammary arteriovenous difference of acetoacetate is negligible in normal or fasted cows. It is negative in some cows with subclinical ketosis, those I would class in Type 3, reaching about 1.5 mg/100 ml (58; unpublished data). It is always negative in clinical ketosis developing in apparently well-fed cows, averaging about 2 mg/100 ml, range 1 to 5 mg/100 ml (39).

Mammary acetoacetate production seems to be a sign of extensive metabolic disorder in the udder (32, 39). It bears a significant linear relationship to mammary acetate uptake (but not the beta-hydroxybutyrate uptake). Hyper-acetemia appears to be a crucial factor; I think it reflects the superimposition of endogenous (hepatic) acetate production upon the exogenous (ruminal) production. The surest way to lower the plasma acetate is to starve the cow; ruminal acetate production and mammary acetoacetate production then subside. (So does milk production!) On the other hand, endogenous acetate production depends to a large extent upon the plasma free fatty acid level (3), which might be depressed by a higher energy intake. This reasoning suggests that to combat subclinical ketosis Type 3, the cow should be underfed or overfed—a paradox (6).

4 Breeding and feeding cows for low-fat high-protein milk would probably diminish the mobilization of body reserves and eliminate the ketosis problem.

Mammary acetoacetate production may be detected conveniently by taking blood samples from the tail artery and the mammary vein. Micro-hematocrit tubes are filled then centrifuged for three min. The tubes are broken and the plasma from each is blown on to nitroprusside powder or tablets. A slightly denser purple color development with the venous plasma than with the arterial plasma indicates a difference of 1 mg/100 ml; a 1+ color difference indicates 2 to 4 mg/100 ml acetoacetate difference.

6 In practice, extremism is hazardous. Very high energy intakes do not eliminate ketosis. Indeed they often tend to increase its incidence. This has been my experience with problem herds in the field (33), and it has been observed in three recent trials of high grain intakes under controlled conditions (18, 21, 64). High energy intakes may lead also to milk fat depression and increase the incidence of abomasal displacement, indigestion (anorexia), mastitis and milk fever (18, 53, 64, 65, 66). (In addition to their published reports, I have received further details on health records, which support these statements, from Doctors Swanson, Thomas and Gardner.)
Clinical ketosis. Ketosis gives rise to clinically observable manifestations indirectly by way of acidosis which commonly becomes severe in diabetes and pregnancy toxemia but is rare in lactating cows, or by inducing hypoglycemia. Hyperketonemia may provoke hypoglycemia by releasing insulin, a phenomenon which varies from species to species and has not been studied in the cow (25, 47).

The metabolic disorder in cows during the early stages of clinical spontaneous ketosis appears to be just like that of cows with subclinical ketosis Type 3—borderline ketosis (57)—except for the added development of hypoglycemia (Fig. 5). The rate of onset and severity of clinical signs is better correlated with the degree of hypoglycemia than the hyperketonemia (16). Moreover, the typical signs of "acetonemia" develop in cows given insulin and are ameliorated promptly by glucose infusion (24, 36). In general, the clinical signs of hypoglycemia depend not only on its degree but also on its duration and the rate of fall. The relationships of the development of hypoglycemia and its clinical signs to changes in other relevant variables in ketotic cows have been difficult to establish precisely because a series of venipunctures once or twice a day seem to obstruct the pathogenetic process—the clinical syndrome becomes delayed, softened, or suppressed altogether. Our observations on a few cows which have developed clinical signs despite frequent venipunctures (unpublished data), plus our experience with insulin-induced hypoglycemia (36, 37, 41), suggest that a slow decline of plasma glucose from 60 to 70 mg/100 ml to 25 to 30 mg/100 ml over 24 to 48 hr, then the persistence of this degree of hypoglycemia for another day or two, will usher in the clinical syndromes typical of acetonemia.

Detailed accounts of the clinical syndromes of acetonemia are presented elsewhere (16, 24, 35). It is important to emphasize here that hypophagia develops after ketosis per se has existed for several days. The first clinical signs are slight behavioral changes which may escape notice. They are followed by depression with intermittent nervousness and various signs of dissociation and neural hypertonia. The severity of these neurologic signs varies, but when the
opportunity has existed, through daily testing for ketonuria and physical examination of the cows, we have observed them invariably 2 to 5 days prior to the development of hypophagia and hypolaetia. They are also seen prior to hypophagia in cows given small repeated doses of insulin, which do not develop ketosis but do develop clinical signs typical of acetonemia.1

Under common circumstances, virtually all affected cows have an altered appetite and nearly 90% have hypophagia at the time of physical examination (16). Hypophagia brings on certain signs (wasting, staggering, weakness) and metabolic changes, such as mild hypocalcemia and variable liver damage. The latter may become severe and persistent, with the cow showing chronic intermittent ketosis and never regaining her former production (20, 35, 56). On the other hand, hypophagia would be conducive to remission in several respects, e.g.: inhibition of insulin release, further diminution of milk production, diminished ruminal production of butyrate and acetate, which would arrest alimentary ketogenesis and mammary acetacetate production (Fig. 5).

Insulin. The insulin status of ketotic cows deserves further attention. In general, insulin operates in two feedback arcs, one regulating plasma glucose, the other fat mobilization. It usually depresses plasma free fatty acids and ketone bodies more powerfully than plasma glucose (48, 68). I postulate that in spontaneous ketosis Type 3, the prevailing metabolic and endocrine conditions reverse that homeostatic priority so that insulin becomes less able to suppress the ketosis (which is not entirely dependent upon fat mobilization), becomes secreted in excess, and induces hypoglycemia. This engenders hypophagia which in turn inhibits further insulin release.

Three very high plasma concentrations of immunoreactive insulin were found in ketotic cows within a few hours of the first clinical signs (27), but subsequent assays have revealed only depressed plasma insulin, albeit in cows further advanced in the clinical syndrome (R. B. Wilson and Kronfeld, unpublished data). The hypothesis calls for a transient rise then depression; this has been difficult to demonstrate, mainly because of the predilection of cows with subclinical ketosis to remit when subjected to serial venipunctures.

The changes in glucose kinetics found in spontaneously ketotic cows are similar to those induced by insulin, but they may be induced by some other factor acting like insulin rather than by insulin itself. They are the reverse of the changes induced by glucocorticoid administration (40) so are consistent with the relative adrenocortical insufficiency hypothesis (60). Another possibility was gentisic acid, a hypoglycemic agent produced in the rumen from aromatic amino acids, but gentisate administration failed to enlarge the glucose space (34). Another is methionine deficiency which may influence the permeability of lipoprotein membranes (46).

Many controlling factors are involved in the regulation of the blood glucose concentration, glucose transport, and glucose distribution. Insulin appears to play a central role under most circumstances, but its effects may be greatly influenced by other factors, e.g.: the plasma levels of glucocorticoids, growth hormone, and glucagon, or fatty acids and ketone bodies. All of these variables should be studied systematically and in concert to describe the conditions which collectively determine the development of hypoglycemia in ketotic but feeding cows.

Diagnostic problem. The presence of some concurrent disease (or "complication") in a ketotic cow has usually been taken to indicate that the ketosis is secondary, i.e. undernutritional (Fig. 4). This assumption is now questionable in the light of studies involving the routine testing for ketolactia. In one study, diseases in general occurred about twice as frequently in cows which had tested positive for ketolactia than in those which tested negative (17). We have studied ketogenic cows with abomasal displacement which have had very high plasma free fatty acid levels but no mammary acetacetate production, i.e. fasting or secondary ketosis, and others which have had only slightly elevated plasma free fatty acids, high plasma acetate and mammary acetacetate production, i.e. spontaneous ketosis (39; unpublished data).

For the purpose of clinical management, I try to differentiate clinical ketosis into three categories: a) primary spontaneous ketosis, b) primary undernutritional ketosis, which has been exceedingly rare in my experience, and c) secondary undernutritional ketosis. Primary undernutritional ketosis is caused by frank underfeeding, which can be indicated by simple thumb rules, e.g. the 3-rule or the ABC (35). Undernutritional and spontaneous ketosis may be differentiated by blood analysis (Table 2), the most convenient test being for the negative mammary arteriovenous difference of acetacetate.5

For the purpose of clinical investigation, I have usually selected cows with uncomplicated spontaneous ketosis (to avoid possible confusion
Table 4. Comparison of fasting ketosis with spontaneous ketosis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fasting</th>
<th>Initially</th>
<th>Later</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruminal concentrations of acetate, propionate and butyrate</td>
<td>Decreases</td>
<td>Normal(^a)</td>
<td>Decreases</td>
<td>14, 50</td>
</tr>
<tr>
<td>Plasma acetate concentration</td>
<td>Decreases</td>
<td>Raised or normal(^a)</td>
<td></td>
<td>1, 39</td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>Mild, often transient</td>
<td>Severe usually</td>
<td>Variable</td>
<td>16, 54</td>
</tr>
<tr>
<td>Glucose transport</td>
<td>Decreased</td>
<td>Normal(^a)</td>
<td>May decrease</td>
<td>Table 1</td>
</tr>
<tr>
<td>Glucose space</td>
<td>Normal</td>
<td>Enlarged greatly(^a)</td>
<td>Enlarged slightly</td>
<td>Table 1</td>
</tr>
<tr>
<td>Plasma free fatty acids</td>
<td>Raised greatly</td>
<td>Raised variably(^a)</td>
<td></td>
<td>28</td>
</tr>
<tr>
<td>Liver fat content</td>
<td>Increases</td>
<td>Normal(^a)</td>
<td>Increases</td>
<td>56</td>
</tr>
<tr>
<td>Liver acetoacetate concentration</td>
<td>Doubled</td>
<td>Quadrupled(^a)</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Liver 3-hydroxybutyrate concentration</td>
<td>Raised by 50%</td>
<td>Quadrupled(^a)</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Liver citrate concentration</td>
<td>Decreased greatly</td>
<td>Decreased(^a)</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Liver pyruvate carboxylase (mitochondria)</td>
<td>Increased</td>
<td>Normal(^a)</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Liver NAD-malate dehydrogenase</td>
<td>Normal</td>
<td>Raised</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Liver NADH concentration</td>
<td>Increased</td>
<td>Decreased(^a)</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Milk production depression</td>
<td>Severe</td>
<td>Moderate or mild</td>
<td>Moderate or severe</td>
<td>16, 39, 54</td>
</tr>
<tr>
<td>Mammary uptake—glucose</td>
<td>Decreases</td>
<td>Normal(^a)</td>
<td></td>
<td>39</td>
</tr>
<tr>
<td>Mammary uptake—acetate</td>
<td>Decreases</td>
<td>Increases(^a)</td>
<td></td>
<td>39</td>
</tr>
<tr>
<td>Mammary acetoacetate release</td>
<td>Negligible</td>
<td>Appreciable(^a)</td>
<td></td>
<td>39</td>
</tr>
</tbody>
</table>

\(^a\) Significantly different from fasting ketosis.
between complicated spontaneous ketosis and secondary undernutritional ketosis), and compared these with cows fasted experimentally. The latter should simulate the metabolic conditions which occur in secondary or primary undernutritional ketosis and some of the changes found in spontaneous ketosis after hypophagia supervenes. Much confusion has arisen regarding ketosis in cows because the sick cow is usually examined after hypophagia had developed, at which time it is easy to think that the diminished food intake has caused the hypoglycemia and ketosis, an order of events much studied and relatively well understood. Studies of subclinical ketosis and the initial stages of uncomplicated clinical ketosis have brought out many differences from fasting ketosis (Table 4) and suggested the following sequence of events, ketosis — hypoglycemia — hypophagia, rather than the reverse.

Summary
The general carbohydrate insufficiency theory of ketosis and the simple supply-demand concept of hypoglycemia have been supported by numerous experiments in experimentally fasted cows but not by comparable studies on cows with spontaneous ketosis. The picture which has emerged is that many apparently healthy, highly productive, well-fed and feeding cows develop ketosis—subclinical or borderline ketosis. This involves a combination of alimentary ketogenesis and hepatic ketogenesis, the latter being dependent upon the negative energy balance and varying with lactational demands. There is no indication that the lactational demand is greater for carbohydrate than for fat or protein. Indeed, several types of experiments have indicated that milk secretion is curtailed by the availability of glucose rather than outstripping the supply. In contrast, the lactational demand does appear to be readily conducive to excessive fat mobilization so contributes to the development of ketosis.

Borderline ketosis may persist or regress without adverse effects, or it may proceed into a clinical condition associated with mammary acetoacetate release and hypoglycemia. The clinical signs include hypophagia, which sets up another crucial state, leading either to chronic ketosis and poor production associated with persistent liver damage, or more commonly to recovery.

The particular form of hypoglycemia found in cows with spontaneous ketosis is not like that induced by undernutrition. Glucose transport through the blood remains normal, at least in the early stages of the clinical syndrome; this finding does not support suggestions that hepatic glucose production (or gluconeogenesis) is either decreased or increased (26). Instead, an enlarged volume of distribution, i.e. glucose space, appears directly responsible for the hypoglycemia.

I suggest that this form of hypoglycemia is the untoward consequence of an exaggerated homeostatic response to subclinical ketosis in the feeding cow, which in the normal course of events would tend predominantly to suppress fat mobilization and hepatic ketogenesis. These are not the only causes of this form of ketosis so the anti-lipolytic anti-ketogenic forces are relatively ineffective, become exaggerated in vain, and misdirectedly provoke hypoglycemia. The chief malefactor, I suspect, will turn out to be insulin, with its action modified by several other hormones and metabolites, so that its usually predominant anti-ketogenic effect becomes weaker than its effect on blood glucose. This particular hypothesis may be proven wrong without diminishing the need for a thorough study of the control system which regulates blood glucose concentration and transport in the lactating cow, not only because of its significance in disease but also because of its important role in health and milk production.

References


