Relationship Between $\beta$-Hydroxybutyrate and Acetoacetate Plus Acetone Contents of Blood and Urine of the Ruminant

L. A. MENAHAN, W. B. HOLTSMALL, L. H. SCHULTZ, and W. G. HOEKSTRA
Departments of Dairy Science and Biochemistry
University of Wisconsin, Madison

Abstract

Separation of the blood plasma, whole blood, and urine ketones was made under normal conditions and in various states of hyperketonemia and ketonuria. The relationship of blood level of a given ketone fraction to urine concentration and total excretion of that ketone fraction was best expressed as a log-log equation for both the acetoacetic acid plus acetone fraction and the $\beta$-hydroxybutyric acid fraction. At low total blood or urine ketone body concentrations, $\beta$-hydroxybutyrate was by far the major constituent, but at increasing ketone body concentrations the ratio of $\beta$-hydroxybutyrate to acetoacetate plus acetone decreased from approximately 7 to values between 1 and 2.5. Relationships for blood plasma ketone fractions were similar to those for whole blood.

The ruminant, even under normal conditions, has a measurable level of ketone bodies in blood and urine. Hyperketonemia and ketonuria characterize certain pathologic conditions found in ruminant species during late pregnancy and early lactation.

Although Reid (8) has investigated the partitioning of the ketone bodies in blood and urine, detailed information concerning the ketone body fractions in the normal concentration ranges was not reported.

Since differences between the metabolism of $\beta$-hydroxybutyrate and acetone fractions have been reported (2), it was deemed desirable to confirm and extend the findings of Reid (8) relating the $\beta$-hydroxybutyrate and the acetoacetate plus acetone fractions of whole blood, blood plasma, and urine ketone bodies.

Experimental Procedure

Mature female Saanen goats were used as experimental animals throughout the studies. To create states of hyperketonemia, goats were infused with butyric acid during metabolic states characterized by hypoglycemia. The hypoglycemia was created by phlorizin treatment or by fasting the goats in late pregnancy. Details of the experimental methods have been published (6, 7).

Blood was collected by venous puncture of the external jugular. Potassium oxalate was used as anticoagulant. In the studies on plasma ketone bodies, the blood was centrifuged at 4,000 g for 20 min in a refrigerated centrifuge and the plasma removed. During collection, urine was preserved with toluene.

In the studies with whole blood, tungstic acid protein-free filtrates (4) were prepared immediately. Acetoacetate plus acetone and $\beta$-hydroxybutyrate fractions were converted separately to acetone, which was distilled by the method of Behre (1). The acetone was determined by Block and Bolling's method (3). Blood ketone bodies were expressed as mg acetone equivalent/100 ml whole blood. Recovery of sodium $\beta$-hydroxybutyrate added to blood was 91 ± 6.0%.

In studies with blood plasma, a Ba(OH)$_2$·ZnSO$_4$ filtrate was prepared as described previously (6). The separate ketone body fractions were prepared as for the tungstic acid filtrates of whole blood.

For comparison, some plasma samples also were assayed for $\beta$-hydroxybutyrate and acetoacetate by an enzymatic procedure (16). The procedure utilized a reaction volume of 1.1 ml. The $\beta$-hydroxybutyrate dehydrogenase used in the assay was obtained from C. F. Boehringer (Mannheim, Germany). All enzymatic assays were made with a single-beam spectrophotometer (PMQ II, Carl Zeiss, Germany).

Urine ketone bodies were separated like whole blood and plasma ketone bodies after treatment with copper sulfate and calcium hydroxide (13).

Methods used for determining the regression
and correlation coefficients were as described by Steel and Torrie (11).

**Results**

Comparison of enzymatic and colorimetric procedures. A comparison of the values for the total acetoacetate plus acetone fraction determined colorimetrically, with values for the acetoacetate fraction determined by the enzymatic method, indicated that approximately 30% of the acetoacetate plus acetone fraction was free acetone (Table 1). There was close agreement between the β-hydroxybutyrate values determined by the two methods. The higher β-hydroxybutyrate:acetoacetate ratio as determined enzymatically resulted because this method does not measure free acetone.

Total ketone bodies vs. separate ketone body fractions. Good agreement was obtained for determination of ketones either as a total or combined from individual fractions in blood or urine (Table 2). It might be expected that splitting the ketone bodies into the respective fractions might give consistently higher values because of exposing the ketone bodies (especially β-hydroxybutyrate) and other acetone-yielding substances to longer reaction times; this was not the case.

Blood and urinary acetoacetate plus acetone fraction. At whole blood or blood plasma concentrations of acetoacetate plus acetone fraction below 2 mg acetone/100 ml, there was a reasonably linear relationship between the whole blood or plasma concentration and the urine concentration of this fraction (Fig. 1). Above this blood or plasma concentration of acetoacetate plus acetone, a given increase in plasma or whole blood concentration of this fraction resulted in a disproportionately large increase in urine concentration of this fraction. This probably is the result of a kidney threshold phenomenon. Thus, over the full range of concentration, a log-log equation expressed best the relationship between whole blood or plasma concentration and urine concentration of acetoacetate plus acetone.

It is also apparent from Fig. 2 that above a plasma or whole blood concentration of acetoacetate plus acetone of 2 mg acetone/100 ml, rather small increments of the blood ketones resulted in large increases in total urinary excretion of acetoacetate plus acetone.

**Blood and urinary β-hydroxybutyrate fraction.** The pattern of change in urine β-hydroxybutyrate concentration with increasing blood or plasma concentration with increasing blood or plasma β-hydroxybutyrate concentration was similar to that found for the acetoacetate and acetone fraction (Fig. 3). The log-log

**TABLE 1**
Comparison of an enzymatic method\textsuperscript{a} with a colorimetric procedure\textsuperscript{b} for determination of plasma ketones\textsuperscript{c}.

<table>
<thead>
<tr>
<th>Plasma</th>
<th>β-hydroxybutyrate (mg acetone/100 ml)</th>
<th>Acetoacetate (plus acetone)\textsuperscript{d} (mg acetone/100 ml)</th>
<th>β-hydroxybutyrate:acetoacetate (plus acetone)\textsuperscript{d} ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of comparisons</td>
<td>22</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Colorimetric method</td>
<td>13.55 ± 1.22</td>
<td>8.18 ± 0.99</td>
<td>1.70 ± 0.11</td>
</tr>
<tr>
<td>Enzymatic method</td>
<td>14.39 ± 1.39</td>
<td>5.73 ± 0.64</td>
<td>3.18 ± 0.33</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Williamson, Mellanby, and Krebs (16).
\textsuperscript{b} Behre and Benedict (1).
\textsuperscript{c} Values represent the means ± SEM.
\textsuperscript{d} Colorimetric method includes acetone; enzymatic method does not.

**TABLE 2**
Comparison between determination of total ketones as a single fraction and combining the β-hydroxybutyrate and acetoacetate plus acetone fractions determined individually\textsuperscript{a}.

<table>
<thead>
<tr>
<th>No. of comparisons</th>
<th>Total</th>
<th>Combined</th>
<th>Total/combined × 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood (mg acetone/100 ml)</td>
<td>66</td>
<td>13.7 ± 1.7</td>
<td>11.8 ± 1.4</td>
</tr>
<tr>
<td>Urine (mg acetone/day)</td>
<td>20</td>
<td>1,360 ± 408</td>
<td>1,360 ± 414</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Ketones determined by the method of Behre and Benedict (1). Values represent the means ± SEM.
equations relating blood and urine concentrations of the ketone fraction were similar for the \( \beta \)-hydroxybutyrate and the acetoacetate plus acetone fractions.

Likewise, the pattern of increase in total urinary excretion of \( \beta \)-hydroxybutyrate and of acetoacetate plus acetone with increasing blood or plasma concentrations of the respective fraction was similar (Fig. 4).

**Ratio of \( \beta \)-hydroxybutyrate to acetoacetate and acetone.** The ratio of \( \beta \)-hydroxybutyrate to acetoacetate plus acetone in plasma, whole blood, or urine was between 1 and 2.5 at whole blood or plasma total ketone concentrations greater than 5 mg acetone/100 ml but, at lower total ketone concentrations, this ratio increased to as high as 7 (Fig. 5, 6).

Although considerable variability was apparent, there was a significant association \((r = .55)\) between the ratio of \( \beta \)-hydroxybutyrate to acetoacetate and acetone fractions in whole blood or plasma and the ratio in urine (Fig. 7).

**Discussion**

In the present study, acetone comprised approximately 30% of the acetoacetate plus acetone fraction. This agrees well with the
value of 20% reported by Reid (8), when one considers that different methods of analysis were used in determining this fraction.

A comparison between a conventional colorimetric ketone body determination method (1) and a newer enzymatic procedure (16) was made in the present study. The specific determination of acetoacetate achievable in the enzymatic method would seem to be desirable, especially in studies concerning the mechanism of ketogenesis. The ratio of $\beta$-hydroxybutyrate to acetoacetate in venous blood plasma of goats, as determined enzymatically in the present study, was 3.18. Using the same enzymatic method and venous blood, values for the $\beta$-hydroxybutyrate:acetoacetate ratio have been reported to range from 2.7 to 3.7 for man (9, 16), and from 2.2 to 3.7 for the rat (17).

Although some differences in the absolute values describing the log-log relationship between blood $\beta$-hydroxybutyrate concentration and urine $\beta$-hydroxybutyrate concentration and excretion were noted, our findings are in general agreement with those of Reid (8).

In the present study, a log-log relationship expressed best the change in urine acetoacetate plus acetone concentration and excretion with increasing blood acetoacetate and acetone concentration. Reid (8) found that both the urine acetoacetate plus acetone concentration and
Fig. 3. Relationship between whole blood or plasma concentration and urine concentration of \( \beta \) hydroxybutyrate. • Whole blood; x plasma.

Fig. 4. Relationship between average daily whole blood or plasma \( \beta \)-hydroxybutyrate concentration and daily urinary excretion. • Whole blood; x plasma.
Fig. 5. Changes in the ratio of β-hydroxybutyrate to acetoacetate plus acetone in whole blood or plasma with increasing total blood or plasma ketone concentration. N = 111; • whole blood; x plasma.

Fig. 6. Changes in the ratio of β-hydroxybutyrate to acetoacetate plus acetone in urine with increasing total blood or plasma ketone concentration. N = 42; • whole blood; x plasma.
FIG. 7. Relationship between blood or plasma and urine ratio of \( \beta \)-hydroxybutyrate to acetoacetate plus acetone. • Whole blood; x plasma.

**References**


