Utilization of $\text{L}(+)-3$-Hydroxybutyrate, $\text{D}(-)-3$-Hydroxybutyrate, Acetoacetate, and Glucose for Respiration and Lipid Synthesis in the 18-Day-old Rat*

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A comparison has been made in vivo between $\text{L}(+)-3$-hydroxy$[3-^{14}\text{C}]$butyrate, $\text{n}(-)-3$-hydroxy$[3-^{14}\text{C}]$butyrate, $[3-^{14}\text{C}]$acetoacetate, and $\text{n}(-)[2-^{14}\text{C}]$glucose for sterol and fatty acid synthesis and respiration in the 18-day-old suckling rat. (a) Sterols and fatty acids in spinal cord, brain, and skin were preferentially labeled by these metabolites over sterols and fatty acids in the liver and kidneys. (b) More label was incorporated into sterols and fatty acids in spinal cord, brain, and kidneys from $\text{L}(+)-3$-hydroxy$[3-^{14}\text{C}]$butyrate than from $\text{n}(-)-3$-hydroxy$[3-^{14}\text{C}]$butyrate. (c) More label was incorporated into sterols and fatty acids in spinal cord, brain, and skin from $\text{n}(-)-3$-hydroxy$[3-^{14}\text{C}]$butyrate than from $[3-^{14}\text{C}]$acetoacetate. (d) In all organs less label was incorporated into sterols and fatty acids from $\text{n}(-)[2-^{14}\text{C}]$glucose than from other metabolites; unexpectedly poor were the liver and kidneys which contained substantially less label. (e) The retention of label from $\text{n}(-)-3$-hydroxy$[3-^{14}\text{C}]$butyrate in the sterols and fatty acids from spinal cord and brain was investigated. (f) The time course of evolution of $^{14}\text{CO}_{2}$ over 2½ h from each of these metabolites revealed a more rapid utilization of $[3-^{14}\text{C}]$acetoacetate maximum at 10 min than $\text{n}(-)-3$-hydroxy$[3-^{14}\text{C}]$butyrate maximum at 30 min; by contrast, label from $\text{n}(-)[2-^{14}\text{C}]$glucose and $\text{L}(+)-3$-hydroxy$[3-^{14}\text{C}]$butyrate was retained maximally in metabolic pools over a 2-h period, indicating a much slower utilization. (g) The evidence that the $\text{L}(+)-3$-hydroxy$[3-^{14}\text{C}]$butyrate is a favored substrate for the synthesis of sterols and fatty acids but less favored for oxidation, while $\text{n}(-)-3$-hydroxy$[3-^{14}\text{C}]$butyrate is a favored substrate for oxidation but less favored for the synthesis of sterols and fatty acids, suggests that these isomers are preferentially metabolized in different compartments.

The ketone bodies, acetoacetate and 3-hydroxybutyrate, have been shown to be efficient precursors for the biosynthesis of sterols and fatty acids in the central nervous system at early stages in the development of the rat (1-3). It was shown that acetoacetate and 3-hydroxybutyrate were preferentially utilized by the organs of ectodermal origin, the brain, spinal cord, and skin, over the lung, kidney, and liver for sterol and fatty acid synthesis in developing rats at 9 to 11 days after birth (3). The data indicated that $\text{L}(+)-3$-hydroxy$[3-^{14}\text{C}]$butyrate labeled fatty acids and sterols more efficiently than did $[3-^{14}\text{C}]$acetoacetate, suggesting that the former substrate was the preferred precursor for the biosynthesis of lipids. Although the comparison was made by injection of equal amounts of mass and of label of each substrate, this was an unexpected finding (3). It was thought that only 50% of the $\text{L}(+)-3$-hydroxybutyrate, the physiological $\text{D}(-)$ isomer, would be utilized by the $\text{D}(-)$-specific $\beta$-hydroxybutyrate dehydrogenase (EC 1.1.1.30) (4). In addition, it has been shown that the concentration of 3-hydroxybutyrate in the circulation of the young rat was 2 to 5 times greater than the concentration of acetoacetate (5) and therefore the specific activity of the circulating acetoacetate should have been 4 to 10 times higher than that of 3-hydroxybutyrate after the injection. Contrary to expectation, the cholesterol labeled from $[3-^{14}\text{C}]$acetoacetate had a lower specific activity than the cholesterol labeled from 3-hydroxy$[3-^{14}\text{C}]$butyrate (3). Additionally, the rate of uptake of acetoacetate by the brain at a given concentration is twice the rate of uptake of 3-hydroxybutyrate (5). It was expected from these three observations that acetoacetate should be the favored substrate, but this was not found (3). Two of the possibilities that could explain this apparent anomaly were investigated in this study. (a) Acetoacetate, as compared to 3-hydroxybutyrate, may have been preferentially utilized for respiration, an alternative metabolic fate, and therefore less would have been available for lipid synthesis. (b) Since the racemic mixture of 3-hydroxybutyrate was used in the previous study (3), the $\text{L}(+)\text{~isomer may have made an unanticipated contribution to the synthesis of lipids.}$

In this study we report on a comparison of $\text{L}(+)-3$-hydroxy$[3-^{14}\text{C}]$butyrate, $\text{n}(-)-3$-hydroxy$[3-^{14}\text{C}]$butyrate, $[3-^{14}\text{C}]$acetoacetate, and $\text{n}(-)[2-^{14}\text{C}]$glucose as a source of carbon for respiration and as precursors of sterols and fatty acids in various organs in the suckling rat at 18 days after birth. It is known that at this age the ketone body concentration in the circulation, as well as the level of the various enzymes needed for ketone body utilization in the brain, are at a maximum (5-7). We have found that although $\text{L}(+)-3$-hydroxybutyrate was oxidized to $\text{CO}_{2}$ much less efficiently than the $\text{n}(-)$ isomer, it was superior to the other metabolites as a substrate for sterol and fatty acid synthesis in the brain, spinal cord, and kidney.

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Materials and Methods

Unlabeled (+)-3-hydroxybutyrate was prepared from the DL mixture by the method of McCann (8). The purity of the final product was examined by three methods. Polarimetric measurements on the free acid gave [α]D24 -24°, which is within experimental error of the published value of -24.9° (9). The enzymic assay by the method of Williamson et al. (10) with the N(-)-3-hydroxybutyrate specific dehydrogenase (EC 1.1.1.30) showed there was less than 1.0% contamination by N(-)-3-hydroxybutyrate. The methyl ester of the L(+)-3-hydroxybutyrate co-chromatographed with the methyl ester of authentic L(+)-3-hydroxybutyrate on gas-liquid chromatography on a 2-m column containing 15% FFAP on Chromosorb W (Varian) at 100°C.

L(+)-3-Hydroxy[3,4-14C]butyrate was prepared from L(-)-3-hydroxy[3,4-14C]butyrate by enzymic conversion of the N(-)-3-hydroxy[3,4-14C]butyrate to [3,4-14C]acetoacetate. The acetoacetate was deacylated by the addition of 1 N HCl to pH 1.0, and the acetoacetate was removed by rotary evaporation after the sample was taken to pH 7.0. L(+)-3-Hydroxy[3,4-14C]butyrate was separated from the other reaction components by thin layer chromatography on Silica Gel G with water-saturated dichethyl ether-acetic acid-water (85:15:4 by vol.) as solvent system (11). The purified L(+)-3-hydroxy[3,4-14C]butyrate was eluted from the silica gel with water, and the volume was reduced by rotary evaporation with absolute ethanol. The complete purification procedure beginning with the enzymic reaction was repeated twice. The enzymic deacylation of the L(+)-3-hydroxybutyrate was determined with the N(-)-3-hydroxybutyrate-specific dehydrogenase in the Deniges precipitation assay as described by McGarry et al. (12). Experiments showed that even a large excess of L(+)-3-hydroxybutyrate did not interfere with the quantitative conversion of the N(-)-isomer to acetoacetate as judged either by the Deniges precipitation assay (12) or by the spectrophotometric assay of Williamson et al. (10). The L(+)-3-hydroxy[3,4-14C]butyrate was contaminated by less than 0.6% of the N(-) isomer, and the yield was 41% of the original L(+)-isomer in the racemic mixture.

The radio-labeled compounds were purchased from Amersham/International. (13). Experiments showed that even a large excess of L(+)-3-hydroxy[3,4-14C]butyrate, L(+)-3-hydroxy[3-14C]acetoacetate, or D(+)-2-14C]glucose and killed 3 h after injection (cf. "Materials and Methods"). The 14C content in each organ is expressed as disintegrations x 10^-3 per min per g of tissue. The values given are the means, with deviation from the mean, for each pair of animals.

Table I. For experimental conditions see "Materials and Methods" in the legend to Table I. The 14C content in each organ is expressed as disintegrations x 10^-3 per min per g of tissue. The values given are the means, with deviation from the mean, for each pair of animals.

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<td>Disintegrations x 10^-3/min/g tissue</td>
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<td>Spinal cord</td>
<td>294.3 ± 24.6</td>
<td>141.5 ± 20.6</td>
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<td>4.6 ± 0.5</td>
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<tr>
<td>Spinal cord</td>
<td>20.0 ± 0.6</td>
<td>1.7 ± 0.4</td>
<td>1.3 ± 0.1</td>
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1 Weight of organs = standard error in grams: spinal cord, 0.135 ± 0.006; brain, 1.28 ± 0.02; portion of the skin from the abdomen, 0.49 ± 0.03; pair of kidneys, 0.54 ± 0.01; liver, 1.14 ± 0.03. 3-P-Hydroxy-steroid content of the tissues was measured as in Ref. 3 and is expressed as milligrams of cholesterol per g wet weight of tissue ± S.E. calculated from the weight of digitonin precipitable steroid: spinal cord, 20.0 ± 2.6; brain, 4.2 ± 0.6; portion of the skin from the abdomen, 3.3 ± 0.5; pair of kidneys, 5.0 ± 0.9; liver, 3.9 ± 0.7.

Table II. For experimental conditions see "Materials and Methods" in the legend to Table I. The 14C content in each organ is expressed as disintegrations x 10^-3 per min per g of tissue. The values given are the means, with deviation from the mean, for each pair of animals.

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<td>Disintegrations x 10^-3/min/g tissue</td>
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<tr>
<td>Spinal cord</td>
<td>264.0 ± 3.7</td>
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<td>34.0 ± 6.9</td>
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<td>Kidney</td>
<td>20.7 ± 1.3</td>
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<tr>
<td>Liver</td>
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<td>7.2 ± 1.6</td>
<td>5.4 ± 0.5</td>
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1 From the inbred colony of Dr. Sidney Roberts in the Department of Biological Chemistry, UCLA School of Medicine.
in kidney and liver by the ketone bodies, acetoacetate and \( \text{D(-)-3-hydroxybutyrate} \), by \( \text{n-glucose} \), and by \( \text{L(+)-3-hydroxybutyrate} \) in the 18-day-old rat. This labeling pattern is similar to that previously reported for \( \text{[3-}^{14}\text{C]acetoacetate and [3-}^{14}\text{C]butyrate} \) in the 10-day-old rat \( (1, 2) \), and results from the differences in the ability of these organs to produce or utilize acetoacetate and \( \text{D(-)-3-hydroxybutyrate} \), a feature determined by their enzymic complement during the developmental period \( (1) \). To our surprise we found that \( \text{L(+)-3-hydroxy[3-}^{14}\text{C]butyrate} \) not only labeled the sterols and fatty acids in the various organs in a pattern qualitatively similar to \( \text{D(-)-3-hydroxy[3-}^{14}\text{C]butyrate} \), but also that twice as much label was incorporated from it into the sterol fraction in spinal cord, brain, and kidney as from \( \text{D(-)-3-hydroxy[3-}^{14}\text{C]butyrate} \). These findings explain the observation made previously that label from \( \text{[3-}^{14}\text{C]acetate} \) was incorporated more efficiently into the sterols and fatty acids in the central nervous system in neonatal rats than label from \( \text{[3-}^{14}\text{C]acetoacetate} \). Since \( \text{D(-)-3-hydroxybutyrate} \) is converted to acetoacetate by the \( \text{D(-)-specific 3-hydroxybutyrate dehydrogenase in the mitochondrion} \), our findings led us to believe that label from \( \text{[3-}^{14}\text{C]acetate} \) may exist an alternative route for the metabolism of \( \text{3-hydroxybutyrate} \). However, this seems unlikely since a comparison of \( \text{[3-}^{14}\text{C]butyrate} \) and \( \text{[3-}^{14}\text{C]acetate} \) showed that sterols and fatty acids in tissues of the developing rat were poorly labeled by the latter, less than 10% of that observed from an equivalent dose of \( \text{[3-}^{14}\text{C]butyrate} \). Since the label from \( \text{[3-}^{14}\text{C]butyrate} \) was not distributed preferentially to either fatty acids or sterols and 90% of the tritium was lost during metabolism, it was concluded that the utilization was mediated via acetoacetate or acetoacetyl-CoA \( (14) \).

A comparison of the incorporation of label in sterols and fatty acids from \( \text{L(+)-3-hydroxy[3-}^{14}\text{C]butyrate} \) and from \( \text{[1-}^{14}\text{C]glucose} \)-labeled ketone bodies and glucose shows that the incorporation of label from glucose is very poor \( (\text{Tables I and II}) \). The amount of label in the fractions at the various times is shown as a percentage of the label in the equivalent fraction at 4 h after injection. The total \( \text{14C} \) content in organs was determined \( (\text{Materials and Methods}) \). The amount of label in the fractions at the various times is shown as a percentage of the label in the equivalent fraction at 4 h after injection. The total \( \text{14C} \) content in the fractions at 30, 60, 90, and 120 days after injection is plotted as a percentage of the \( \text{14C} \) content in these fractions 4 h after the injection.

The amount of label retained in organs in spinal cord over 120 days after the injection of \( \text{V(-)-3-hydroxy[3-}^{14}\text{C]butyrate} \) was significantly different than the amount retained in brain \( \text{(Fig. 1)} \). In spinal cord only 10% of the label in sterols at 4 h after the injection was lost by the first time point \( (30 \text{ days}) \). After this small decrease the \( \text{14C} \) content in the sterols in the spinal cord remained relatively stable. In brain 10% of the label present at 4 h was also lost by the first time point; however, by the 60th day an additional 30% was lost. Thereafter the level remained relatively constant. These findings are in agreement with the studies reported by Smith and Eng \( (22) \) and Hajra and Radin \( (23) \). In contrast to the small decrease in the \( \text{14C} \) content of the sterols, 70 to 80% of the label in the fatty acids in spinal cord and brain disappeared by 30 days after the injection of \( \text{V(-)-3-hydroxy[3-}^{14}\text{C]butyrate} \), but thereafter the \( \text{14C} \) content of the fatty acids remained relatively constant for the next 90 days. This is also in agreement with the studies reported by Hajra and Radin \( (23) \). In these previous studies \( (22, 23) \) \( \text{[1-}^{14}\text{C]acetate} \) was used as the precursor for lipid synthesis. However, acetate is not a circulating metabolite in the rat. For this reason the retention of label by the sterols and fatty acids in the spinal cord and brain from a natural metabolite, \( \text{V(-)-3-hydroxy[3-}^{14}\text{C]butyrate} \), was investigated. It has been shown that label from \( \text{[2-}^{14}\text{C]acetate} \), \( \text{[3-}^{14}\text{C]acetoacetate} \), and \( \text{[3-}^{14}\text{C]butyrate} \) was preferentially incorporated into the sterols and fatty acids in spinal cord, brain, and skin in the 10-day-old rat \( (3) \). It has also been shown that within minutes after the injection of \( \text{[2-}^{14}\text{C]acetate} \) the ketone bodies in the circulation of the 18-day-old rat contain \( \text{14C} \) \( (14) \). This may explain the similarity in the preferential distribution of label from acetate and ketone bodies into sterols and fatty acids in the organs of ectodermal origin \( (3) \).

A comparison of the amount of \( \text{14C} \) incorporated into the

![Fig. 1. Ten 18-day-old rats from a single litter (mean body weight, 31.9 ± 0.8 g) were injected with 10 \mu Ci of \( \text{V(-)-3-hydroxy[3-}^{14}\text{C]butyrate} \) (26 Ci/mol) between the scapulae and two were killed at 4 h and at 30, 60, 90, and 120 days after injection. The \text{14C} content in organs was determined \( (\text{Materials and Methods}) \). The amount of label in the fractions at the various times is shown as a percentage of the label in the equivalent fraction at 4 h after injection. The total \( \text{14C} \) content in sterol and fatty acid fractions in brain was 36,700 and 44,800 dpm and in spinal cord was 25,200 and 18,800 dpm, respectively, at 4 h after the injection. Sterol fractions: brain, ○; spinal cord, ●. Fatty acid fractions: brain, □; spinal cord, △.]}
lips in spinal cord and brain shows that spinal cord has a greater capacity per unit weight than the brain to utilize D(-)-3-hydroxy[3-14C]butyrate for the synthesis of sterols and fatty acids, Tables I and II. This suggests that myelination in the spinal cord is more active than in the brain at 18 days after birth. Also, a larger fraction of the label initially incorporated is retained in the sterol fraction in spinal cord as compared to brain as a function of time after the injection of D(-)-3-hydroxy[3-14C]butyrate in the 18-day-old rat (Fig. 1). This should be expected since the spinal cord and its myelin lamellae are more mature than the brain and its myelin lamellae at this time (24).

Respiration—The time course of the oxidation of each substrate as monitored by the expiration of 14CO2 from the intact rat gave an indication of how long the metabolic pools were maximally labeled from each substrate (Fig. 2). There was a very rapid evolution of 14CO2 from [3-14C]acetate which had a maximum 10 min after injection. However, the evolution of 14CO2 from D(-)-3-hydroxy[3-14C]butyrate did not reach a maximum until 30 min after the injection. This comparison suggests that during the longer period in which the metabolic pools were labeled by D(-)-3-hydroxy[3-14C]butyrate, labeled precursors were available for lipid synthesis and may have accounted for the heavier labeling of the sterols and fatty acids as compared to the labeling from acetate (Tables I and II). Since the profiles of 14CO2 evolution from [3-14C]acetate and D(-)-3-hydroxy[3-14C]butyrate by the 18-day-old rat are quite distinct (Fig. 2), it would appear that once acetate and D(-)-3-hydroxybutyrate are present in the circulation there is minimal interconversion. This observation is currently under investigation as it has been assumed that there was rapid equilibration between acetate and 3-hydroxybutyrate in the circulation of the developing rat (25).

In the preparation of the data in Fig. 2, corrections have not been made for the different specific activities of the substrates in the circulation; thus the amplitude of each curve is relative. However, the profile of each curve is an indicator of the time carbon from each labeled precursor persisted in the metabolic pools. In contrast to acetate and D(-)-3-hydroxybutyrate which were oxidized rapidly, D-[2-14C]glucose and L(+)-3-hydroxy[3-14C]butyrate must have been utilized relatively slowly since label from both of these metabolites persisted maximally in the metabolic pool longer than that from either D(-)-3-hydroxybutyrate or acetate. By 150 min 68% of the label injected as either [3-14C]acetate or D(-)-3-hydroxy[3-14C]butyrate was expired as 14CO2. However, by 150 min only 38% of the label injected as L(+)-3-hydroxybutyrate was expired as 14CO2. Although label from D-[2-14C]glucose persisted maximally in the metabolic pool longer than that from D(-)-3-hydroxybutyrate or acetate, the sterol and fatty acid fractions were not as extensively labeled from glucose as from the other substrates. Hawkins et al. (5) have shown that the concentrations of 3-hydroxybutyrate and glucose in the circulation of 16- to 18-day-old rats were about 2 and 6 mM, respectively. Even if one were to consider that the specific activity of circulating 3-hydroxybutyrate must have been about 3 times greater than that of circulating glucose during the early parts of the experiment, the amount of label found in sterols and fatty acids from D(-)-3-hydroxy[3-14C]butyrate was much greater than expected (cf. Tables I and II). Current experiments seek an explanation for the apparent slow utilization of label from glucose and the reason why label from glucose appears to remain maximally in metabolic pools for extended periods.

Utilization of L(+)-3-Hydroxybutyrate—The time course of 14CO2 expiration from L(+)-3-hydroxy[3-14C]butyrate closely parallels that from D-[2-14C]glucose. However, L(+)-3-hydroxy[3-14C]butyrate proved to be far superior than D-[2-14C]glucose as a source of label by 3 h for sterol and fatty acid synthesis in all organs investigated (Tables I and II). The amount of label expired as 14CO2 from L(+)-3-hydroxy[3-14C]butyrate over the 2½-h period was one-third less than the amount of label expired from either D(-)-3-hydroxy[3-14C]butyrate or [3-14C]acetate. However, by 3 h more label from L(+)-3-hydroxy[3-14C]butyrate was incorporated into sterols and fatty acids in all the organs studied than from either D(-)-3-hydroxy[3-14C]butyrate or [3-14C]acetate. The poorer oxidation of the L(+)-3-hydroxy[3-14C]butyrate to CO2 may reflect in part its more extensive incorporation into sterols and fatty acids. This suggests that the L(+) isomer of 3-hydroxybutyrate is a favored substrate for lipid synthesis but less favored for respiration, while the D(-) isomer is the favored substrate for respiration but less favored for lipid synthesis, suggests that these isomers are preferentially metabolized in different compartments.

In the liver and kidney the amount of label incorporated by 3 h from L(+)-3-hydroxy[3-14C]butyrate is 20 to 40 and 5 to 10 times greater in sterols and fatty acids, respectively, than the amount of label incorporated from [2-14C]glucose. Although label from both [2-14C]glucose and L(+)-3-hydroxy[3-14C]butyrate persisted in metabolic pools for over 2 h as suggested by the time course of evolution of expired 14CO2, it was surprising that the sterols and fatty acids in liver and particularly kidney should be more efficiently labeled from L(+)-3-hydroxy[3-14C]butyrate than from [2-14C]glucose. This is indeed striking since L(+)-3-hydroxybutyrate has yet to be reported as a circulating metabolite.
Early studies by McKenzie (26) and Lehninger and Greville (27) suggested that mammalian tissue had the capacity to metabolize L(+)-3-hydroxybutyrate in vivo and in vitro. It was thought, however, that the metabolism of the L(+) isomer was physiologically unimportant (4, 27, 28). McKenzie, as part of his studies on the resolution of the isomers of 3-hydroxybutyrate, showed that the urine of a dog, which had been fed gram quantities of nL-3-hydroxybutyrate, contained ketone bodies enriched in the levorotatory d(-)-3-hydroxybutyrate (26). He concluded that the dog had the capacity to utilize preferentially the L(+) isomer. Early in vitro studies on the metabolism of 3-hydroxybutyrate by Lehninger and Greville (27) showed that L(+)3-hydroxybutyrate was metabolized but to a lesser extent than the d(-) isomer. However, it was established that the 3-hydroxybutyrate dehydrogenase associated with the mitochondrion had an absolute stereospecificity for d(-)-3-hydroxybutyrate (29, 30), and Klee and Sokoloff demonstrated that the mitochondria from the brain of developing rats did not metabolize L(+)-3-hydroxybutyrate to acetoacetate (4). It is worthy of note that 3-hydroxybutyrate is unique in that both isomers are found in intermediary metabolism; d(-)-3-hydroxybutyrate is found as a circulating metabolite and as d(-)-3-hydroxybutyryl-S-fatty acid synthetase during fatty acid synthesis, while L(+)-3-hydroxybutyrate is found as L(+)-3-hydroxybutyryl-coenzyme A in the β-oxidation of fatty acids. The suckling rat, especially at 18 days after birth, has serum ketone body concentration approaching 2 mM (5) from very active ketogenesis which is fueled by the β oxidation of fatty acids derived from dietary triglycerides. L(+)-3-Hydroxybutyryl-coenzyme A may be an intermediate of greater importance during the suckling period than previously thought, should L(+)-3-hydroxybutyrate prove to be a circulating metabolite generated by active β oxidation. The physiological significance of the metabolism of L(+)-3-hydroxybutyrate during the suckling period in the rat remains to be determined.

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REFERENCES