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

(Received for publication, February 7, 1977)

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A comparison has been made in vivo between \( \text{L}(+)\)-3-hydroxy[3-\text{14C}]butyrate, \( \text{n}(-)\)-3-hydroxy[3-\text{14C}]butyrate, \( \text{L}(+)\)-acetoacetate, and \( \text{n}(-)\)-2-[\text{14C}]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-\text{14C}]butyrate than from \( \text{n}(-)\)-3-hydroxy[3-\text{14C}]butyrate. (c) More label was incorporated into sterols and fatty acids in spinal cord, brain, and skin from \( \text{n}(-)\)-3-hydroxy[3-\text{14C}]butyrate than from [3-\text{14C}]acetoacetate. (d) In all organs less label was incorporated into sterols and fatty acids from \( \text{n}(-)\)-2-[\text{14C}]glucose than from the 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-\text{14C}]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 2\%h from each of these metabolites revealed a more rapid utilization of [3-\text{14C}]acetoacetate maximum at 10 min than \( \text{n}(-)\)-3-hydroxy[3-\text{14C}]butyrate maximum at 20 min; by contrast, label from \( \text{n}(-)\)-2-[\text{14C}]glucose and \( \text{L}(+)\)-3-hydroxy[3-\text{14C}]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-\text{14C}]butyrate is a favored substrate for the synthesis of sterols and fatty acids but less favored for oxidation, while \( \text{n}(-)\)-3-hydroxy[3-\text{14C}]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 ecdysozal 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 \( \text{D}(+)\)-3-hydroxy[3-\text{14C}]butyrate labeled fatty acids and sterols more efficiently than did [3-\text{14C}]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 \( \text{D}(+)\)-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-\text{14C}]acetoacetate had a lower specific activity than the cholesterol labeled from 3-hydroxy[3-\text{14C}]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 \( \text{L}(+)\) 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-\text{14C}]butyrate, \( \text{n}(-)\)-3-hydroxy[3-\text{14C}]butyrate, [3-\text{14C}]acetoacetate, and \( \text{n}(-)\)-2-[\text{14C}]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.

* This research was supported by United States Public Health Service Research Grants HD-06576, HD-04612, and GM-00364 from the National Institutes of Health.
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Utilization of \( \alpha \)-3-Hydroxybutyrate and Ketone Bodies

**MATERIALS AND METHODS**

Unlabeled \((\pm)-3\)-Hydroxybutyrate was prepared from the NL 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 \( [\alpha]_D^25 = +4.4^\circ \), which was within experimental error of the published value of \( +24.8^\circ \) (9). The enzymic assay by the method of Williamson et al. (10) with the \((\pm)-3\)-hydroxybutyrate specific dehydrogenase (EC 1.1.1.30) showed there was less than 1.0% contamination by \( \alpha \)-3-hydroxybutyrate. The methyl ester of the \((\pm)-3\)-hydroxybutyrate co-chromatographed with the methyl ester of authentic \( \alpha \)-3-hydroxybutyrate on gas-liquid chromatography on a 2-m column containing 15% FFAP on Chromosorb W (Varian) at 100°C.

\((\pm)-3\)-Hydroxy[\(3-\)\(^14\)C]butyrate was prepared from NL-3-hydroxy[\(3-\)\(^14\)C]butyrate by enzymic conversion of the \( \beta \)-3-hydroxy-3-[\(3-\)\(^14\)C]acetate, or \( \beta \)-3-[\(2-\)\(^14\)C]glucose, \( \alpha \)-3-hydroxy[\(3-\)\(^14\)C]butyrate was separated from the other reaction components by thin layer chromatography on Silica Gel G with water-saturated diethyl ether:88% formic acid (71, v/v) as solvent system (11). The purified \((\pm)-3\)-hydroxy[\(3-\)\(^14\)C]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 \((\pm)-3\)-hydroxybutyrate was determined with the \( \alpha \)-3-hydroxybutyrate-specific dehydrogenase in the Deniges precipitation assay as described by McGarry et al. (12). Experiments showed that even a large excess of \((\pm)-3\)-hydroxybutyrate did not interfere with the quantitative conversion of the \( \beta \)-isomer to acetoacetate as judged either by the Deniges precipitation (12) or by the spectrophotometric assay of Williamson et al. (10). The \((\pm)-3\)-hydroxy[\(3-\)\(^14\)C]butyrate was contaminated by less than 0.6% of the \( \alpha \)-isomer, and the yield was 41.4% of the original \( \alpha \)-isomer in the racemic mixture.

The radioactive compounds were purchased from Amersham/Scarle, \( \alpha \)-3-Hydroxybutyrate dehydrogenase, NL-3-hydroxybutyrate, buffers, and coenzymes were purchased from the Sigma Chemical Co.

**Short Term Studies**

Eighteen-day-old Sprague-Dawley rats from an inbred colony were injected subcutaneously between the scapulae with 10.0 \( \mu \)Ci of either \((\pm)-3\)-hydroxy[\(3-\)\(^14\)C]butyrate, \( \beta \)-3-hydroxy[\(3-\)\(^14\)C]butyrate, \( \beta \)-3-[\(2-\)\(^14\)C]glucose or \( \beta \)-3-[\(2-\)\(^14\)C]glucose in less than 0.025 ml of 0.9% NaCl. The labeled precursors were given by intravenous injection rather than by subcutaneous injection to allow the injected labeled \( \alpha \)-3-hydroxybutyrate to diffuse into the circulation, thereby minimizing the increase in the size of the circulating pool of \( \beta \)-3-hydroxybutyrate, acetoacetate, or glucose. Immediately after the injection each rat was placed in a respiration chamber of 165 ml total volume through which the air flow was regulated at 200 f 10 ml/min (13). The \( ^{14} \)C0\(_2\), in the expired air was collected at defined intervals by passing the air through gas washing cylinders (Kimax) in parallel; each contained 60 ml of 0.1 M HCl and 100 ml of 0.1 M HCO\(_3\). The \( ^{14} \)C content in stero2 fraction in tissues of 18-day-old rats 3 h after injection of \( ^{14} \)C-labeled precursors

**RESULTS AND DISCUSSION**

**Lipid Synthesis by Three Hours**

The amount of label incorporated into each of the lipid fractions in organs after the administration of equivalent amounts of \((\pm)-3\)-hydroxy[\(3-\)\(^14\)C]butyrate, \( \beta \)-3-hydroxy[\(3-\)\(^14\)C]butyrate, \( \beta \)-3-[\(2-\)\(^14\)C]glucose, or \( \beta \)-3-[\(2-\)\(^14\)C]glucose is shown in Tables I and II. The \(^{14} \)C content in each organ is expressed as disintegrations \( \times 10^{-3} \) per min per g of tissue. The values given are the means, with deviation from mean, for each pair of animals. The mean body weight of the eight rats was 34.0 \( \pm \) 0.4 (S.E.) g.

<table>
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<tr>
<th>Organ</th>
<th>Disintegrations ( \times 10^{-3} ) per min per g tissue</th>
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<tbody>
<tr>
<td>Spinal cord</td>
<td>Brain</td>
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<td></td>
<td>( 294.3 \times 10^{-3} )</td>
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<tr>
<td>264.0 ( \pm ) 3.7</td>
<td>115.4 ( \pm ) 16.8</td>
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**Table I**

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<tr>
<th>Substance</th>
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**Table II**

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<thead>
<tr>
<th>Substrate</th>
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<tbody>
<tr>
<td>Spinal cord</td>
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<td></td>
<td>( 264.0 \times 10^{-3} )</td>
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<tr>
<td>264.0 ( \pm ) 3.7</td>
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**From the inbred colony of Dr. Sidney Roberts in the Department of Biological Chemistry, UCLA School of Medicine.
Retention of Label in Lipids from $\alpha$-(-)-3-Hydroxy[3-$\mu$C]butyrate. The retention of label in the sterol and fatty acid fractions in 18-day-old rats was measured at intervals up to 120 days after the injection. By 30 days after the injection the $\mu$C content in the lipids of skin, kidney, and liver was negligible. A comparison of the amounts of label retained in the sterol and fatty acid fractions in spinal cord and brain is presented in Fig. 1. The $\mu$C content in the fractions at 30, 60, 90, and 120 days after injection is plotted as a percentage of the $\mu$C content in these fractions 4 h after the injection.

The amount of label retained in spinal cords in spinal cord over 120 days after the injection of $\alpha$-(-)-3-hydroxy[3-$\mu$C]butyrate was significantly different than the amount retained in brain (Fig. 1). In spinal cord only 10% of the label in the fatty acids in spinal cord was lost by the first time point (30 days). After this small decrease the $\mu$C 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. Therefore 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 $\mu$C 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 $\alpha$-(-)-3-hydroxy[3-$\mu$C]butyrate, but thereafter the $\mu$C 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) [1-$\mu$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 spinal cord and brain from a natural metabolite, $\alpha$-(-)-3-hydroxy[3-$\mu$C]butyrate, was investigated. It has been shown that label from [2-$\mu$C]acetate, [3-$\mu$C]acetate, and $\alpha$-(-)-3-hydroxy[3-$\mu$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 with minutes after the injection of [2-$\mu$C]acetate the ketone bodies in the circulation of the 18-day-old rat contain $\mu$C (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 $\mu$C incorporated into the

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**Fig. 1.** Ten 18-day-old rats from a single litter (mean body weight, 31.9 ± 0.8 g) were injected with $\alpha$-(-)-3-hydroxy[3-$\mu$C]butyrate (20 Ci/mol) between the scapulae and two were killed at 4 h and at 30, 60, 90, and 120 days after injection. The $\mu$C content in organs was determined ("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 $\mu$C content in the fractions at 30, 60, 90, and 120 days after injection is plotted as a percentage of the $\mu$C content in the fractions 4 h after the injection.
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]acetacetate 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 acetacetate (Tables I and II). Since the profiles of 14CO2 evolution from [3-14C]acetacetate and D(-)-3-hydroxy[3-14C]butyrate by the 18-day-old rat are quite distinct (Fig. 2), it would appear that once acetacetate 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 equilibrium between acetacetate 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 acetacetate 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 acetacetate. By 150 min 68% of the label injected as either [3-14C]acetacetate 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 acetacetate, 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 D-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 21/2-h period was one-third less than the amount of label expired from either D(-)-3-hydroxy[3-14C]butyrate or [3-14C]acetacetate. 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]acetacetate. 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. The evidence 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 D-[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 D-[2-14C]glucose. This is indeed striking since L(+)-3-hydroxybutyrate has yet to be reported as a circulating metabolite.

**Fig. 2.** Eight 18-day-old rats were injected between the scapulae with 10.0 μCi of L(+)-3-hydroxy[3-14C]butyrate, D(-)-3-hydroxy[3-14C]butyrate, [1-14C]acetacetate, or D-[2-14C]glucose. The body weights are presented in Table I. Expired 14CO2 was determined as described under "Materials and Methods." 14CO2 was collected over 5-min intervals until 1 h, then at 15-min intervals until 2 h after the injection. A final collection was made at 21/2 h. The data are plotted in disintegrations per min × 10−2 in 14CO2 expired per min at the end point of each interval after the injection. All the data points are not included since they would only crowd the curves without improving their definition. 14CO2 from L(+)-3-hydroxy[3-14C]butyrate, ○—○; D(-)-3-hydroxy[3-14C]butyrate, ×—×; [1-14C]acetacetate, △—△; and D-[2-14C]glucose, A—A.
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 \( \alpha \)-3-hydroxybutyrate, contained ketone bodies enriched in the levorotatory \( \delta(-) \)-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 \( \delta(-) \) isomer. However, it was established that the 3-hydroxybutyrate dehydrogenase associated with the mitochondrion had an absolute stereospecificity for \( \delta(-) \)-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; \( \delta(-) \)-3-hydroxybutyrate is found as a circulating metabolite and as \( \delta(-) \)-3-hydroxybutyryl-S-fatty acid synthetase during fatty acid synthesis, while \( L(+) \)-3-hydroxybutyrate is found as \( L(+) \)-3-hydroxybutyryl-coenzyme A in the \( \beta \) 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 \( \beta \) 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 \( \beta \) oxidation. The physiological significance of the metabolism of \( L(+) \)-3-hydroxybutyrate during the suckling period in the rat remains to be determined.

Acknowledgment—We thank Professor G. Poppjak for his critical reading of this manuscript.

REFERENCES
Utilization of L(+) -3-hydroxybutyrate, D(-)-3-hydroxybutyrate, acetoacetate, and glucose for respiration and lipid synthesis in the 18-day-old rat.

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