The in Vivo Utilization of Acetoacetate, d(-)-3-Hydroxybutyrate, and Glucose for Lipid Synthesis in Brain in the 18-Day-old Rat

EVIDENCE FOR AN ACETYL-CoA BYPASS FOR STEROL SYNTHESIS*

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Previously, we have shown the preferential incorporation of label from [3-14C]octanoate; the specific activity of acetoacetate and d(-)-3-hydroxybutyrate was equivalent for about 6 min after the injection. 5) Once in the circulation, acetoacetate and d(-)-3-hydroxybutyrate are not in rapid equilibrium. 6) The half-lives for [3-14C]acetoacetate, d(-)-3-hydroxy[3-14C]butyrate, and [2-14C]glucose in blood were 4.7, 7.4, and 31 min, respectively. 7) The combined rates of utilization from blood of the ketone bodies (0.148 μmol/min/ml) are approximately the same as the rate of utilization of glucose (0.164 μmol/min/ml) in the 18-day-old rat. 8) After the injection of 3H2O proportional to body weight in 6- to 30-day-old rats, the greatest amount of tritium was found in sterols and in fatty acids in whole brain at 18 days after birth. 9) In 18-day-old rats, the ratio of disintegrations per minute (DPM) of 14C in sterols per g of brain to DPM of 14C in fatty acids per g of brain is distinctive for acetoacetate and d(-)-3-hydroxybutyrate (0.5) as compared to this ratio for glucose, octanoate, or acetate (0.23 to 0.30). 10) We propose that the difference in the ratio of DPM in sterols per g of brain to DPM in fatty acids per g of brain after the injection of the ketone bodies as compared to the ratio after the injection of the other precursors results from the cytoplasmic activation of acetoacetate. We postulate that acetoacyl coenzyme A synthetase activates acetoacetate to acetoacetyl-CoA which can be used directly for the synthesis of 3-hydroxy-3-methylglutaryl-CoA without conversion to acetyl-CoA.

In 1967, Owen et al. (1) demonstrated that the ketone bodies, acetoacetate and d(-)-3-hydroxybutyrate, were important energy sources for brain metabolism in obese adult humans who were fasting for long periods. Starting in 1971 with the work of Hawkins et al. (2) who compared the concentration of metabolites in the blood of young and adult rats and with the work of Page et al. (3) who measured the activity of the ketone body utilizing enzymes in rat brain as a function of age, it became apparent that blood ketone bodies were important for brain metabolism during the developmental period. They found that the concentration of glucose in blood was approximately the same in young and adult rats, but the concentration of the ketone bodies in blood was about 10 times greater in the young rat as compared to the adult. Hawkins et al. (2) also measured the arteriovenous difference across the brain for these and other metabolites in the blood and found a substantial influx of the ketone bodies into the brain of young rats. They demonstrated that the arteriovenous difference across the brain for the ketone bodies was about 17 times greater in young rats than in adult rats. They also found...
a substantial influx of glucose into the brain; however, a large
eflux of both pyruvate and lactate from the brain was ob-
served and was calculated to account for as much as 76% of
the glucose which had been taken up by the brain.

Edmond (4) has shown in vivo the preferential utilization of
14C-labeled ketone bodies by organs of ectodermal origin
for lipid synthesis in 10-day-old rats. Webber and Edmond (5)
have shown in vivo the preferential utilization of
14C-labeled ketone bodies and glucose by organs of ectodermal origin for
lipid synthesis in the 18-day-old rat. They also found that by
3 h after the injection of equal amounts of
14C-labeled ketone bodies and glucose, much more label was incorporated from
the ketone bodies than from glucose into sterols and fatty
acids in brain. Patel and Owen (6) have demonstrated with
cerebral cortex slices that both acetoacetate and 3-hydroxy-
butyrate are better precursors than glucose for lipid synthesis
by the brain of 7-day-old rats. Yeh et al. (7) have shown inrain homogenates that the ketone bodies are preferred over
glucose as precursors for energy production and lipid synthesis
in rats from 1 to 22 days of age.

In our previous experiments (4, 5), labeled 3-hydroxybuty-
rate, acetoacetate, glucose, and acetate were injected into rats
on the assumption that they would be used from the blood in
the same form as injected. However, during metabolic proc-
esses, a labeled metabolite which is present in the blood may
be converted to another metabolite which is more readily
utilized. The possibility of this interconversion, or of “cross-
labeling,” after the injection of a labeled compound before
incorporation into end products has become one of our major
concerns. If this interconversion of the labeled metabolite
does occur before utilization for lipid synthesis in the brain,
it would make the interpretation of our data difficult.

In addition to correlating the utilization of circulating me-
tabolites for the synthesis of lipids in 18-day-old rat brain, we
wanted to know if the age we had chosen to study, 18 days
after birth, was the period at which lipid synthesis was greater.
We chose to study brain lipid synthesis in rats 18 days
after birth since this has been reported by Klee and Sokoloff
(8), Page et al. (9), and Middleton (10) to be an accurate method for assessing fatty acid
synthesis. In the present study, we have used radioactivity
as an index of the amount of lipid synthesized by the brain
of rats of different ages.

In this study, we report on the incorporation in vivo of
1H from
2H2O into sterols and fatty acids in brain in rats 6 to 30
days of age. We also report on the search in the blood of the
18-day-old rat for evidence of cross-labeling between acetato-
cetate, d(-)-3-hydroxybutyrate, and glucose after the injec-
tion of [3-14C]acetate, d(-)-3-hydroxy[3-14C]butyrate,
[2-14C]glucose, or [1-14C]octanoate, which has been shown to
be a model precursor for the ketone bodies (13, 14). This was
necessary since evidence was found in the blood of the adult
rat for a rapid equilibration between acetoacetate and d(-)-
3-hydroxybutyrate after a pulse of either 14C-labeled acetato-
cetate or d(-)-3-hydroxybutyrate (15, 16). We could not
assume that this rapid equilibration occurred in the develop-
ing rat since it would be in conflict with the data obtained in
our previous work (5). Additionally, we report on the utiliza-
tion of these labeled metabolites as well as [2-14C]acetate
for the synthesis of sterols and fatty acids in the brain of 18-day-
old rats. We found that the synthesis of sterols and fatty acids
in the brain is at a maximum at about 18 days after birth as
judged by the incorporation of 1H from
2H2O into lipids in whole brain. We found little or no interconversion in the blood
of acetoacetate, d(-)-3-hydroxybutyrate, and glucose in the
18 day old rat. In addition, we found that [3-14C]acetato-
cetate and d(-)-3-hydroxy[3-14C]butyrate gave a distinctive ratio of
DPM1 of 14C in sterols to DPM of 14C in fatty acids as compared to this ratio found with [2-14C]glucose, [1-14C]octa-
noate, or [2-14C]acetate.

MATERIALS AND METHODS

Sprague-Dawley rats were purchased 14 days pregnant (Simonson
Laboratory, Gilroy, Calif.). The litters were undisturbed until day 10
after birth when the litter was trimmed to 10 pups by removing the
light or heavy pups, or both.

Incorporation of 1H into Brain Lipids as a Function of Age—
Paired litter mates were injected subcutaneously between the
scapulae at 6, 12, 18, 24, and 30 days after birth with
1H2O proportional to body weight (15 mCi/35 g) in 0.9% NaCl in less than 0.05 ml total
volume. Forty minutes after the injection, the rats were killed by
decapitation and the heads were dropped into liquid N2. The brains
were dissected while still frozen, weighed, saponified in 40% NaOH,
and analyzed for the incorporation of
1H into sterols and fatty acids
(5). The method of analysis was as previously described (5) except
that additional washings of both the nonsaponifiable lipid extract
with 0.2 N NaOH and of the saponifiable lipid extract with 1 N HzO
were found to be necessary to eliminate contamination from
"HzO present in the light petroleum extracts.

Studies on Metabolites in Blood for Evidence of Cross-labeling—
Ten microcuries of either d(-)-3-hydroxy[3-14C]butyrate (96 Ci/
mol), [3-14C]acetate (115 Ci/mmol), [2-14C]glucose (4.7 Ci/mol),
or [1-14C]octanoate (32 Ci/mol) in less than 0.025 ml of 0.9% NaCl
were injected subcutaneously between the scapulae in 18 day old
pups. At the appropriate time after the injection (see individual
experiments), each rat was killed by decapitation, the head was
disseected, and the liquid N2 was collected from the neck stump into
1.0 ml of isotonic NaCl containing 10 units of heparin and 25
muM of EDTA. The blood was transferred to a graduated conical 2.5-
ml centrifuge tube and centrifuged at top speed in a desk top clinical
centrifuge for 5 min. The plasma was transferred to an Amicon
centrifil cone (CF 25) and centrifuged 45 min at 800 x g at 4°C to
remove plasma proteins; the plasma filtrate was stored at -20°C until
assayed. The head was removed from the liquid N2 and stored at
-20°C until dissected and analyzed for the 14C content in brain lipids
(see following section).

Determination of the Specific Activity of d(-)-3-Hydroxybutyrate,
Acetooacetate, and Glucose in Plasma Filtrates—Plasma filtrates
were assayed for their concentration of d(-)-3-hydroxybutyrate and
acetooacetate by a modification (17) of the method of Williamson et
al. (18) and Persson (19). Plasma filtrates were assayed for their
concentration of glucose by a modification (17) of the method of
Huget and Nixon (20). The assay for the 14C content in d(-)-3-
hydroxybutyrate and in acetooacetate is a modification (17) of the
procedure of McGarry et al. (15) and for the 14C content in glucose is a
modification (17) of the procedure of Gibson et al. (21).

Incorporation of 14C into Lipids in Brain as a Function of Time—
The nonsaponifiable lipids (sterols) and the saponifiable lipids (fatty
acids) were isolated from the brains of the animals injected with
[3-14C]acetate, d(-)-3-hydroxy[3-14C]butyrate, [2-14C]glucose,
or [1-14C]octanoate as described above and from those injected with 10.0
muCl of [2-14C]acetate (6.3 Ci/mol). The saponifiable fraction obtained
from brain after the injection of [1-14C]octanoate was analyzed as
above and by gas-liquid radiochromatography of the fatty acid methyl
ester. This was necessary since the saponifiable lipid fraction would
contain "C-labeled octanoate. The 14C content of the fatty acid chains
longer than octanoate was determined by gas-liquid radiochromatog-
raphy.

The fatty acids from 5.0 ml of the light petroleum extracts obtained
from the brain of animals injected with [1-14C]octanoate were meth-
ylated with diazomethane (17, 22). The fatty acid methyl esters
were fractionated by gas-liquid radiochromatography (23) on a 2-mm column
containing 15% FFAP on Chromosorb W HP 80 to 100 mesh (Varian).
The column and column temperature previously had been standard-

1 The abbreviation used is: DPM, disintegrations per minute.
ized for the chromatography of the methyl esters of C6 to C4 fatty acids (Applied Science Labs., Standard kit No. L206). The initial column temperature was 140°C and immediately after injection was programmed to increase 3°C/min to 210°C. The mixture was resolved into its components, but only two fractions were collected. One fraction contained the methyl esters of short chain fatty acids (through C6). After the methyl ester of octanoate had been collected in the Econofluor (New England Nuclear) in the counting chamber (23), the Econofluor was drained from the chamber, the chamber was washed with 10 ml of scintillation grade xylene, and the wash was added to the Econofluor. Immediately after the wash, the chamber was filled with fresh Econofluor and the second fraction containing the methyl esters of the long chain fatty acids, C12 to C20, was collected. The chamber was washed with 10 ml of xylene and the wash was added to the Econofluor. The amount of 14C contained in each fraction was determined as described earlier. The 14C content of the fatty acid chains longer than octanoate is in the saponifiable lipid fraction of the octanoate-injected rats was calculated from the amount of 14C in the two fractions.

The radiolabeled compounds were purchased from Amersham/Searle. All enzymes, coenzymes, and buffers were purchased from the Sigma Chemical Co., and all chemicals and solvents were purchased from the J. T. Baker Chemical Co.

RESULTS

The Incorporation of 3H into Sterols and Fatty Acids in Brain—After the injection of 3H2O proportional to body weight in rats 6 to 30 days of age, sterol in brain (Fig. 1A) and fatty acids in brain (Fig. 1B) are labeled by 40 min. Since the 3H2O was injected proportional to body weight, the body water in all animals had the same specific radioactivity. Additionally, it has been shown (17) that the incorporation of 3H into sterols and fatty acids in brain is linear for 60 min in the 18-day-old rat after the injection of 3H2O proportional to body weight. At all ages, the fatty acids contained more label than did the sterols. The greatest amount of 3H was incorporated from body water into sterols and into fatty acids in the whole brain by the 18-day-old rat. The data showed that on a wet weight basis, the greatest amount of 3H was incorporated into fatty acids in the brain of 18-day-old rats (Fig. 1B), but on a dry weight basis, the greatest amount of 3H was incorporated into sterols in the brain of 12-day-old rats (Fig. 1A). This difference between the total amount of 3H incorporated into fatty acids in the whole brain and the amount of 3H incorporated per gram of brain for sterols is a reflection of a 40% increase in brain weight but only a 20% increase in 3H incorporation in the 18-day-old rats as compared to the 12-day-old rats. This difference is not observed for the synthesis of fatty acids because the amount of 3H incorporated by the whole brain is twice as great in the 18-day-old as compared to the 12-day-old rat.

14C in Acetoacetate, n-(−)-3-Hydroxybutyrate, and Glucose after the Injection of 14C-labeled Metabolites—After the injection of [2,14C]glucose (Fig. 2), an exponential decrease in the specific radioactivity of glucose in blood was observed. Neither acetoacetate nor n-(−)-3-hydroxybutyrate in the blood contained label by 150 min. This result indicates that carbon from glucose does not enter the pool from which the ketone bodies are produced in the 18-day-old rat. After the injection of [3-14C]acetacetate (Fig. 3A), an exponential decrease in its specific radioactivity in blood is observed. Almost immediately after the injection of labeled acetoacetate, label was present in n-(−)-3-hydroxybutyrate, and after 15 min, label appeared in glucose. However, the amount of label found in n-(−)-3-hydroxybutyrate was a small fraction of the amount of label present as [3-14C]acetacetate, and the amount found in glucose was exceedingly small (Fig. 3A). Although 14C was detected in both n-(−)-3-hydroxybutyrate and in glucose, the amount of 14C that would be incorporated into lipids in brain from these would be insignificant in comparison to the amount of 14C which would be incorporated from the injected acetacetate (3). Therefore, the incorporation of label into lipids in brain could be attributed primarily to [3-14C]acetacetate. After the injection of n-(−)-3-hydroxy[3-14C]butyrate (Fig. 3B), an exponential decrease in the specific radioactivity of n-(−)-3-hydroxybutyrate in blood is observed. Almost immediately after the injection of labeled n-(−)-3-hydroxybutyrate, both acetoacetate and glucose contained label. However, the

Fig. 1. The incorporation of 3H from 3H2O into brain lipids as a function of age. Pairs of rats from a single litter were injected with 3H2O proportional to body weight (15 mCi/35 g) at 6, 12, 18, 24, and 30 days of age and killed 40 min after the injection. The 3H content in brain lipids was determined ("Materials and Methods"). The average weight of whole brain for the rats was 0.78, 0.94, 1.34, 1.42, and 1.52 g at 6, 12, 18, 24, and 30 days of age, respectively. A, the incorporation of 3H into sterol; O—O, Total DPM of 3H incorporated into whole brain; ■—■, DPM of 3H incorporated per g of brain. B, the incorporation of 3H into brain fatty acids; O—O, Total DPM of 3H incorporated into whole brain; ■—■, DPM of 3H incorporated per g of brain.

Fig. 2. The specific activity of glucose (△), acetoacetate (□); and n-(−)-3-hydroxybutyrate (●) in plasma filtrates after the injection of [2,14C]glucose. Ten microcuries of [2,14C]glucose was injected into paired 18-day-old rats, and the rats were killed at 10, 30, 50, 90, and 150 min after the injection. The specific activity of glucose, acetoacetate, and n-(−)-3-hydroxybutyrate in plasma filtrate was measured ("Materials and Methods") and is expressed as curies (Ci) x 107/mol.
The Utilization of Ketone Bodies and Glucose by Brain

amount of \(^{14}C\) in acetoacetate was only a small fraction of the amount of label present as \(\text{D-}(-)-3\text{-hydroxy[3-}^{14}\text{C}]\text{butyrate}\), and the amount of \(^{14}C\) in glucose was very small [Fig. 3B]. Although \(^{14}C\) was detected in acetoacetate and in glucose in blood, the amount of label that would be incorporated into lipids in brain from these would be insignificant in comparison to the amount of \(^{14}C\) that would be incorporated from the circulating labeled \(\text{D-}(-)-3\text{-hydroxybutyrate}\) (5). Therefore, the amount of label incorporated into the lipids in brain could be attributed primarily to \(\text{D-}(-)-3\text{-hydroxybutyrate}\). For comparison, the appearance of \(^{14}C\) in acetoacetate, \(\text{D-}(-)-3\text{-hydroxybutyrate}\), and glucose was investigated after the injection of \([1\text{-}^{14}\text{C}]\text{octanoate}\) (Fig. 4), a known ketone body precursor (13, 14). After the injection of labeled octanoate, both acetoacetate and \(\text{D-}(-)-3\text{-hydroxybutyrate}\) contained \(^{14}C\) almost immediately, and the specific radioactivity of acetoacetate and \(\text{D-}(-)-3\text{-hydroxybutyrate}\) in blood was equal for the first few minutes after the injection. Blood glucose also contained \(^{14}C\) after the injection of \([1\text{-}^{14}\text{C}]\text{octanoate}\). This is probably a reflection of the high gluconeogenic activity of the liver and kidney in the young rat (24) and indicates that the carbon from octanoate enters the carbon pool, tricarboxylic acid cycle intermediates such as oxaloacetic acid and malic acid, from which glucose is produced via gluconeogenesis.

The ratio of the specific radioactivity of acetoacetate to the specific radioactivity of \(\text{D-}(-)-3\text{-hydroxybutyrate}\) in blood as a function of time after the injection of either \(\text{D-}(-)-3\text{-hydroxy[3-}^{14}\text{C}]\text{butyrate}\) or \([3\text{-}^{14}\text{C}]\text{acetoacetate}\) was calculated from the data shown in Fig. 3 and is shown in Table I. The ratios were not constant during the time that label in acetoacetate or \(\text{D-}(-)-3\text{-hydroxybutyrate}\) was available. Five minutes after the injection of \(\text{D-}(-)-3\text{-hydroxy[3-}^{14}\text{C}]\text{butyrate}\), the ratio increased from 0.14 to 1.8 over the next 15 min, and after the injection of \([3\text{-}^{14}\text{C}]\text{acetoacetate}\), the ratio decreased from 15.3 at 2.5 min to 0.8 by 20 min.

The half-life \((t_{\frac{1}{2}})\) in blood for \([3\text{-}^{14}\text{C}]\text{acetoacetate}\), \(\text{D-}(-)-3\text{-hydroxy[3-}^{14}\text{C}]\text{butyrate}\), and \([2\text{-}^{14}\text{C}]\text{glucose}\) was calculated from semilog plots of the data shown in Figs. 2 and 3 and is shown in Table II. Both ketone bodies have relatively short half-lives, 4.7 and 7.4 min for acetoacetate and \(\text{D-}(-)-3\text{-hydroxybutyrate}\), respectively. However, glucose has a much longer half-life of about 31 min. From the \(t_{\frac{1}{2}}\) and the blood concentration of these metabolites, the disposal rates for each from the blood was calculated (Table II). The rate of disposal from the blood of \(\text{D-}(-)-3\text{-hydroxybutyrate}\) (0.101 \(\mu\text{mol/min}/\text{ml}\) of plasma filtrate) was considerably greater than the rate of disposal of acetoacetate (0.045 \(\mu\text{mol/min}/\text{ml}\) of plasma filtrate) during the 30 min after injection, as shown in Fig. 4. The specific activity of acetoacetate \((\AA)\) and \(\text{D-}(-)-3\text{-hydroxy[3-}^{14}\text{C}]\text{butyrate}\) \((\text{3-HB})\) and glucose \((\Delta)\) in plasma filtrates after the injection of \([1\text{-}^{14}\text{C}]\text{octanoate}\). Ten microcuries of \([1\text{-}^{14}\text{C}]\text{octanoate}\) was injected into paired 18-day-old rats, and the rats were killed at 1, 3, 7, 15, and 25 min after the injection. The specific activity of acetoacetate, \(\text{D-}(-)-3\text{-hydroxybutyrate}\), and glucose in plasma filtrates was measured ("Materials and Methods"). The specific activity of acetoacetate and \(\text{D-}(-)-3\text{-hydroxybutyrate}\) is expressed as curies \(\times 10^{4}/\text{mol}\) (left ordinate) and the specific activity of glucose is expressed as curies \(\times 10^{7}/\text{mol}\) (right ordinate).

![Figure 3](http://www.jbc.org/)

**Fig. 3.** The specific activity of acetoacetate (AA) \((\square)\), \(\text{D-}(-)-3\text{-hydroxy[3-}^{14}\text{C}]\text{butyrate}\) \((\text{3-HB})\) \((\bigcirc)\), and glucose \((\Delta)\) in plasma filtrates after the injection of \([3\text{-}^{14}\text{C}]\text{acetoacetate}\) and \(\text{D-}(-)-3\text{-hydroxy[3-}^{14}\text{C}]\text{butyrate}\). A, ten microcuries of \([3\text{-}^{14}\text{C}]\text{acetoacetate}\) was injected into paired 18-day-old rats, and the rats were killed at 2.5, 5, 10, 15, 20, 30, and 45 min after the injection. B, ten microcuries of \(\text{D-}(-)-3\text{-hydroxy[3-}^{14}\text{C}]\text{butyrate}\) was injected into paired 18-day-old rats, and the rats were killed at 2.5, 5, 10, 15, 20, and 30 min after the injection. The specific activity of acetoacetate, \(\text{D-}(-)-3\text{-hydroxybutyrate}\), and glucose in plasma filtrate was measured ("Materials and Methods"). The specific activity of acetoacetate and \(\text{D-}(-)-3\text{-hydroxybutyrate}\) is expressed as curies/mol (left ordinate) and the specific activity of glucose is expressed as curies \(\times 10^{7}/\text{mol}\) (right ordinate).

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>(\text{D-}(-)-3\text{-Hydroxy[3-}^{14}\text{C}]\text{butyrate})</th>
<th>([3\text{-}^{14}\text{C}]\text{Acetoacetate})</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>0.28</td>
<td>15.3</td>
</tr>
<tr>
<td>5.0</td>
<td>0.14</td>
<td>5.9</td>
</tr>
<tr>
<td>10.0</td>
<td>0.21</td>
<td>2.9</td>
</tr>
<tr>
<td>15.0</td>
<td>0.53</td>
<td>1.6</td>
</tr>
<tr>
<td>20.0</td>
<td>1.8</td>
<td>0.8</td>
</tr>
</tbody>
</table>

The ratio of the specific activity of acetoacetate to the specific activity of \(\text{D-}(-)-3\text{-hydroxybutyrate}\).

**Table I**

**Ratio" after the injection of**

\(\text{D-}(-)-3\text{-Hydroxy[3-}^{14}\text{C}]\text{butyrate}\)

-2 \(\mu\text{mol/min}/\text{ml}\) of plasma filtrate.

**Table II**

Half-lives and disposal rates of metabolites in plasma filtrates from blood of 18-day-old rats

The data were obtained from the analysis of the findings presented in Figs. 2, 3, and 4. The values are the means with standard error of the mean for animals and are expressed as micromoles/ml of unvillified plasma filtrate.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Concentration ± S.E.</th>
<th>(t_{\frac{1}{2}})</th>
<th>Disposal rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>10.15 ± 0.32</td>
<td>31.0</td>
<td>0.164</td>
</tr>
<tr>
<td>(\text{D-}(-)-3\text{-Hydroxybutyrate})</td>
<td>1.49 ± 0.12</td>
<td>7.4</td>
<td>0.101</td>
</tr>
<tr>
<td>Acetoacetate</td>
<td>0.45 ± 0.03</td>
<td>4.7</td>
<td>0.047</td>
</tr>
</tbody>
</table>
The Utilization of Ketone Bodies and Glucose by Brain

The incorporation of $^{14}$C into sterols and fatty acids in brain—Immediately after the injection of either [3-$^{14}$C]acetoacetate or $\beta$-(-)-3-hydroxy[3-$^{14}$C]butyrate, sterols and fatty acids in brain are labeled (Fig. 5, A and B). The fatty acids contain more $^{14}$C than do the sterols at all time points, and the amount of $^{14}$C found in the sterols is a constant fraction of the amount of $^{14}$C found in the fatty acids. After the injection of [3-$^{14}$C]acetoacetate or $\beta$-(-)-3-hydroxy[3-$^{14}$C]butyrate, the sterols in brain contain one-half as much $^{14}$C as do the fatty acids. After the injection of [2-$^{14}$C]glucose (Fig. 5C), fatty acids and sterols in brain are labeled and again the amount of $^{14}$C found in the sterols is less than the amount of $^{14}$C found in the fatty acids. However, at each time point, the sterols contain only one-quarter the amount of $^{14}$C as compared to the fatty acids. Because of the slow appearance of label in lipids in brain after the injection of [2-$^{14}$C]glucose, the length of time before decapitation had to be greatly increased as compared to the length of time before decapitation after the injection of either [3-$^{14}$C]acetoacetate or $\beta$-(-)-3-hydroxy[3-$^{14}$C]butyrate. The incorporation of $^{14}$C from glucose into fatty acids and particularly into sterols in brain was negligible for the first 10 min (Fig. 5C) and increased slowly over the next 80 min. By contrast, after the injection of [3-$^{14}$C]acetoacetate or $\beta$-(-)-3-hydroxy[3-$^{14}$C]butyrate, label appeared immediately in the sterols and fatty acids in brain, and the incorporation of $^{14}$C was rapid for the first 20 min following the injection. These results on the incorporation of $^{14}$C into lipids in brain as a function of time from [2-$^{14}$C]glucose, [3-$^{14}$C]acetoacetate, and $\beta$-(-)-3-hydroxy[3-$^{14}$C]butyrate are compatible with the observed decrease in the specific activity of these labeled precursors in blood (Figs. 2 and 3) and are compatible with the length of time carbon from each of these labeled precursors persists in metabolic pools (5). Immediately after the injection of either [1-$^{14}$C]octanoate or [2-$^{14}$C]acetate, sterols and fatty acids in brain are labeled (Fig. 5, D and E).

![Graph](http://www.jbc.org/)

**TABLE III**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Ratio* ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$-(-)-3-Hydroxybutyrate</td>
<td>0.475 ± 0.015</td>
</tr>
<tr>
<td>Acetoacetate</td>
<td>0.494 ± 0.015</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.295 ± 0.014</td>
</tr>
<tr>
<td>Acetate</td>
<td>0.296 ± 0.012</td>
</tr>
<tr>
<td>Octanoate</td>
<td>0.289 ± 0.016</td>
</tr>
</tbody>
</table>

* The ratio of DPM in sterols per g of brain to DPM in fatty acids per g of brain.

The ratios were obtained from the analysis of the data presented in Fig. 5. For experimental conditions, see “Materials and Methods” and the legends to Figs. 2, 3, and 4. The values were calculated from all the animals, except those killed before 2.5 min, which had very low $^{14}$C content in the sterols, and are expressed as the mean with standard error of the mean.

**FIG. 5.** The incorporation of $^{14}$C into sterols in brain (open symbols) and into fatty acids in brain (closed symbols) from $^{14}$C-labeled precursors as a function of time. A, the incorporation of $^{14}$C from [3-$^{14}$C]acetoacetate into sterols in brain (□—□) and fatty acids in brain (■—■). B, the incorporation of $^{14}$C from $\beta$-(-)-3-hydroxy[3-$^{14}$C]butyrate into sterols in brain (○—○) and fatty acids in brain (■—■). C, the incorporation of $^{14}$C from [2-$^{14}$C]glucose into sterols in brain (△—△) and fatty acids in brain (●—●). D, the incorporation of $^{14}$C from [1-$^{14}$C]octanoate into sterols in brain (□—□) and fatty acids in brain (■—■). E, the incorporation of $^{14}$C from [2-$^{14}$C]acetate into sterols in brain (○—○) and fatty acids in brain (●—●). The data are expressed as DPM of $^{14}$C × 10$^{-6}$/g of brain and were obtained from the analysis of the brains of animals killed at various times after the injection of 10 $\mu$Ci of [2-$^{14}$C]glucose, [3-$^{14}$C]acetoacetate, $\beta$-(-)-3-hydroxy[3-$^{14}$C]butyrate, [1-$^{14}$C]octanoate, or [2-$^{14}$C]acetate. For the experimental conditions for panels A, B, C, and D see “Materials and Methods” and the legends to Figs. 2, 3, and 4. For E, 10 $\mu$Ci of [2-$^{14}$C]acetate was injected into paired 18-day-old rats and the rats were killed at 1, 3, 5, 7, 10, and 18 min after the injection. For the experimental conditions see “Materials and Methods.”
The $^{14}$C found in sterols is about one-quarter of the amount of $^{14}$C found in fatty acids.

The quantitative difference in the incorporation of label into sterols as compared to fatty acids in the brain from these various $^{14}$C-labeled precursors is expressed as the ratio of DPM of $^{14}$C in sterols/g of brain to DPM of $^{14}$C in fatty acids/g of brain (Table III). This ratio is distinctive for the ketone bodies, acetoacetate and D-(-)-3-hydroxybutyrate, at 0.49 and 0.48, respectively. The ratio of DPM of $^{14}$C in sterols/g of brain to DPM of $^{14}$C in fatty acids/g of brain for glucose, octanoate, and acetate is 0.23 to 0.30. The ratio observed with each of the $^{14}$Clabeled precursors was constant over the time that they were used for lipid synthesis in brain (Fig. 5). This verifies that the ratio as a function of time is distinctive for acetoacetate and D-(-)-3-hydroxybutyrate as compared to the ratio observed with glucose, octanoate, and acetate.

**DISCUSSION**

We chose to study the 18-day-old rat as a model for the synthesis of sterols and fatty acids in brain during the developmental period for a number of reasons. The rat brain at this age has been shown to contain the highest level of the major enzymes of ketone body utilization (3, 8, 9) and rat liver at this age has been shown to contain the highest level of ketone body and glucose-producing enzymes (24, 25). Whereas the concentration of glucose in blood in young rats is the same as the concentration in blood in adults, the concentration of ketone bodies in blood has been shown by Page et al. (3) and other investigators (26-28) to increase during suckling and to reach a maximum concentration at about 7 days of age, to maintain this relatively high concentration throughout the suckling period, and then to decrease to the normal adult concentration after weaning. Recently, it has been shown that the utilization of D-(-)-3-hydroxybutyrate was mainly governed by its permeability from the blood into the brain at all ages, and that 18 to 20 days after birth was the age of greatest permeability (29). This coincides with the observations of Hawkins et al. (2) that the arteriovenous difference across the brain for the ketone bodies was greatest at 18 days after birth. The activity of the ketone body-producing enzymes in the liver, the permeability of the brain to the ketone bodies in the blood, and the activity of the major ketone body-utilizing enzymes in the brain follow similar developmental profiles and are at a maximum between 16 and 20 days after birth (3, 8, 9, 25-29). Additionally, the period of 12 to 24 days after birth has been shown to be the age of most rapid myelination in the rat (30). Therefore, we wanted to compare rats during the developmental period for the ability to produce the two basic types of lipids, sterols and fatty acids. The amount of synthesis at 6 to 30 days of age was measured by the incorporation of $^3$H from body water after the injection of $^2$H$_2$O proportional to body weight. By injecting $^2$H$_2$O proportional to body weight, the specific radioactivity of the body water was equal at all ages. We found that the greatest amount of $^3$H was incorporated into sterols and fatty acids in whole brain at 18 days of age as compared to the other ages tested (Fig. 1). This is in agreement with the findings of Wells and Dittmer (31) and Cuzner and Davison (32) who studied the lipid composition of developing rat brain and with the findings of Norton and Poduslo (30) who studied the lipid composition of myelin as a function of age. However, these results do not agree with the data reported by Patel and Owen (6). They reported that brain slices incorporated $^{14}$C from D.L-3-hydroxy[3-$^{14}$C]butyrate into sterols and fatty acids maximally at 7 days of age, and that by day 18 after birth, the amount incorporated into sterols and fatty acids by brain slices was only about 30% of the amount found at 7 days of age (6). We conclude that this difference is due to the selection procedure employed by Patel and Owen (6) and results from the exclusive use of the first 0.4-mm slice from the cerebral cortex. Wells and Dittmer (31) have concluded that the change in lipids in the cerebral cortex at about 7 days after birth is associated with the outgrowth of axons and dendrites and is primarily a function of the growth of gray matter. The first cerebral slice selected by Patel and Owen (6) is predominantly gray matter which contains no myelin structure. However, _in vivo_, the intact brain contains all neural structures; thus, no selection bias would enter into our data. Additionally, it would seem improbable that the greatest amount of incorporation of $^{14}$C from labeled 3-hydroxybutyrate would occur at 1 day of age when the level of the ketone body-utilizing enzymes in brain is low and when the permeability of the brain to 3-hydroxybutyrate is 3 to 4 times lower (Fig. 3, Ref. 29) as compared to a later stage of development, 16 to 20 days of age, when the enzyme levels and permeability are much higher. We, therefore, conclude that the highest level of activity for brain lipid synthesis occurs at about 18 days after birth (Fig. 1) and corresponds to the period when the brain permeability to the ketone bodies in the blood and the major enzymes of ketone body utilization in the brain are at a maximum. Before we could assess the utilization of metabolites in blood for lipid synthesis in the brain, evidence of interconversion at 18 days of age between the injected labeled metabolites and other circulating metabolites had to be investigated.

Evidence for the interconversion of acetoacetate, D-(-)-3-hydroxybutyrate, and glucose was made by measuring the specific radioactivity of each in blood after the injection of either D-(-)-3-[1-14C]acetocacetate, D-(-)-3-[1-14C]hydroxy[3-14C]butyrate, [2-14C]glucose, or [2-14C]octanoate, which has been reported to be a model precursor of the ketone bodies (13, 14). We found that, after the injection of [2-14C]glucose, neither acetoacetate nor D-(-)-3-hydroxybutyrate contained label by 150 min. After the injection of labeled acetoacetate, D-(-)-3-hydroxybutyrate was labeled, and after the injection of labeled D-(-)-3-hydroxybutyrate, acetoacetate was labeled (Fig. 3). However, the ratios of their specific activities were not constant while the label in acetoacetate or D-(-)-3-hydroxybutyrate was maximally available. This showed that they did not equilibrate rapidly (Table I) and that equilibrium was not reached until the label had almost disappeared from the blood. Recently, estimates of the rates of utilization of glucose and of D-(-)-3-hydroxybutyrate in brain of 18-day-old rats have been made by Cremer and Heath (33). The rates of acetyl coenzyme A formation from these substrates were determined _in vivo_ from the label in intermediary metabolites after the injection of labeled glucose or labeled D-(-)-3-hydroxybutyrate. To calculate the rate of utilization of the ketone bodies and of glucose, a number of assumptions were made by these authors. One of these assumptions, that the specific radioactivity of acetoacetate is a constant fraction of the specific radioactivity of D-(-)-3-hydroxybutyrate, was taken from the findings of Barton (16). Barton found that after the injection of D-(-)-3-hydroxy[3-$^{14}$C]butyrate into adult rats, the ratio of the specific radioactivity of acetoacetate to the specific radioactivity of D-(-)-3-hydroxybutyrate fell from 0.32 at 0.81 min to 0.18 by 8 min after the injection. Our data (Table I) show that after the injection of either labeled acetoacetate or labeled D-(-)-3-hydroxybutyrate into 18-day-old rats, the ratio of the specific radioactivity of acetoacetate to the specific radioactivity of D-(-)-3-hydroxybutyrate in blood is not constant with time. Cremer and Heath (33) also assumed that the rate constant for the utilization of acetoacetate and of D-(-)-3-hydroxybutyrate is the same and is based
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upon the data of Barton (16). However, his data on the disposal rate of acetoacetate and d-(-)-3-hydroxybutyrate from blood in adult rats show that the utilization of acetoacetate is 4 times greater than d-(-)-3-hydroxybutyrate (cf. Table IV, Ref. 16). We calculated the rate of removal, disposal rate, from the blood of the 18-day-old rat for each of these metabolites (Table II), and in contrast to the findings of Barton (16), we found that d-(-)-3-hydroxybutyrate is removed from the circulation twice as fast as acetoacetate. Another assumption made by Cremer and Heath (33), that acetoacetate and d-(-)-3-hydroxybutyrate rapidly equilibrate after their injection which implies that their utilization would be indistinguishable from each other, seemed inconsistent with our previous report (5) that each gave a distinctive profile for the evolution of CO\textsubscript{2} with time from the intact rat. This assumption is also inconsistent with the findings reported here (Fig. 3, A and B), which show that the specific radioactivity of acetoacetate is neither equal to nor a constant fraction of the specific radioactivity of d-(-)-3-hydroxybutyrate while label is maximally available. To assess the validity of the measurement of the specific activity of acetoacetate and d-(-)-3-hydroxybutyrate in blood, we tested labeled octanoate for its ability to label both acetoacetate and d-(-)-3-hydroxybutyrate in the blood of 18-day-old rats. Octanoate has been reported (13, 14) to serve as a model precursor for the synthesis of the ketone bodies in the adult rat. By 1 min after the injection of [1-\textsuperscript{14}C]octanoate, both acetoacetate and d-(-)-3-hydroxybutyrate in blood contained label and the ratio of the specific radioactivity of acetoacetate to the specific radioactivity of d-(-)-3-hydroxybutyrate was near unity for the first 6 min but fell to 0.6 by 15 min after the injection. This decrease suggests a more rapid turnover of acetoacetate than d-(-)-3-hydroxybutyrate as has previously been suggested (5). Only after the injection of a known ketone body precursor, octanoate, did we observe that the ketone bodies in the blood had equivalent specific activities (Fig. 4). From our data we conclude that some of the assumptions made by Cremer and Heath (33) are not valid for the 18-day-old rat.

We found that after the injection of either [3,\textsuperscript{14}C]acetoacetate, d-(-)-3-hydroxy[3-\textsuperscript{14}C]butyrate, or [1-\textsuperscript{14}C]octanoate, the glucose in blood contained label (Figs. 3, A and B and 4). DeVivo et al. (34) have shown that glucose was labeled at 20 min after the injection of d-(-)-3-hydroxy[3-\textsuperscript{14}C]butyrate into 1-day-old rats, but they considered it insignificant. Pushpenderan and Eapen (35) have studied the incorporation of \textsuperscript{14}C from [1-\textsuperscript{14}C]palmitate into brain glucose in suckling mice. They found that the incorporation of \textsuperscript{14}C into blood glucose from [1-\textsuperscript{14}C]palmitate was 15 times greater in 5-day-old mice than in adult mice, and they found that the ability to incorporate label from palmitate into glucose was high throughout the suckling period of the mouse. They concluded (35) that their data indicated the possible utilization of palmitate for glucose synthesis in the suckling mouse. We concur with DeVivo et al. (34) that the amount of label diverted into glucose from any of these labeled metabolites is minimal. However, this observation is of consequence in that it emphasizes the high gluconeogenic activity of the liver or the kidneys, or both, in the 18-day-old rat. Gluconeogenesis in the young rat has been reviewed recently by Snell and Walker (24), who concluded that the liver or the kidneys, or both, in the neonatal rat are highly active in the synthesis of glucose via the gluconeogenic pathway from tricarboxylic acid cycle intermediates such as oxaloacetic acid and malic acid.

From studies on the disposal of the ketone bodies from the blood, we find that on a molar basis d-(-)-3-hydroxybutyrate is removed twice as fast as acetoacetate (Table II). However, after a pulse of labeled acetoacetate or labeled d-(-)-3-hydroxybutyrate, we found that the turnover time (t\textsubscript{1/2}) of acetoacetate is shorter than d-(-)-3-hydroxybutyrate (Table II). The apparent anomaly between these two observations is due to the difference in the concentration (the circulating pool size) of these metabolites in the blood; their rate of disposal is calculated from their concentration in the blood and their t\textsubscript{1/2}.

These studies to search for evidence of interconversion of blood metabolites were performed so we could relate our findings on in vivo lipid synthesis in the brain to the utilization of a particular labeled metabolite from blood. From these results on the interconversion of the plasma metabolites, we have concluded that the minor amount of label that would enter the sterols and fatty acids in brain from any of these metabolites other than the one which was injected would have no significant consequence to our overall analysis.

With the confidence that the label found in lipids in brain would reflect only the utilization of the injected metabolite, we analyzed the brains for their \textsuperscript{14}C content in sterols and fatty acids from the same animals which were used for the metabolite interconversion experiments. [3-\textsuperscript{14}C]Acetoacetate, d-(-)-3-hydroxy[3-\textsuperscript{14}C]butyrate, [2-\textsuperscript{14}C]glucose, [1-\textsuperscript{14}C]octanoate, and [2-\textsuperscript{14}C]acetate labeled the brain lipids, and all of these substrates gave a higher amount of label at each time point in the fatty acids than in the sterols (Fig. 5). The striking feature of these observations is that the labeled metabolites seem to fall into two classes with respect to the amount of label found in the sterols as compared to the amount of label found in the fatty acids in brain. The ratio of the DPM of \textsuperscript{14}C in sterols per g of brain to DPM of \textsuperscript{14}C in fatty acids per g of brain is distinctive for the ketone bodies (Table III). This ratio is constant with time for all metabolites tested. The distinguishing feature of this ratio found after the injection of labeled ketone bodies as compared to that found after the injection of the other labeled precursors (Table III) must indicate a difference in the ability of the various labeled metabolites to label the carbon precursor pool for sterol synthesis distinctively from the carbon precursor pool for fatty acid synthesis in brain. This observation does not agree with the conclusion reached by Cremer and Heath (33) that glucose and the ketone bodies were in every way alternative substrates for metabolism in the brain of the 18-day-old rat. If glucose and the ketone bodies were metabolically equivalent as suggested by Cremer and Heath (33), it would be expected that identical pools would be labeled and that carbon for sterol and fatty acid synthesis would be equivalent. Thus, the ratio of DPM of \textsuperscript{14}C in sterols per g of brain to DPM of \textsuperscript{14}C in fatty acids per g of brain would be the same after the injection of labeled glucose or labeled ketone bodies. This was not found.

What mechanism could account for the characteristic ratio found after the injection of labeled ketone bodies as compared to that found after the injection of labeled glucose, octanoate, or acetate? To alter the ratio, either the DPM of \textsuperscript{14}C in fatty acids per g of brain must be increased for the metabolites that give a low ratio (glucose, octanoate, and acetate) or the DPM of \textsuperscript{14}C in sterols per g of brain must be increased for the metabolites that give a high ratio (acetoacetate and d-(-)-3-hydroxybutyrate). We have not been able to visualize a mechanism by which the carbon from glucose or acetate could be used preferentially for the synthesis of fatty acids. Alternatively, we can visualize a mechanism by which the carbon from acetoacetate or from d-(-)-3-hydroxybutyrate could be used preferentially for the synthesis of sterols (Scheme 1).

A number of models recently have been proposed for the utilization of ketone bodies by the brain of neonatal rats (6, 7, 33, 36). However, in each of these models, the prevailing idea has been that carbon from acetoacetate and d-(-)-3-hydroxy-
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The preferential incorporation of acetocetate and d-(-)-3-hydroxybutyrate into sterols in brain of the developing rat: an acetyl-CoA bypass for sterol synthesis. Acetocetate is activated to acetoacetyl-CoA by acetoacetyl-CoA synthetase (1) in the cytosol. Acetoacetyl-CoA is incorporated into 3-hydroxy-3-methylglutaryl-CoA by HMG-CoA synthetase (2) for sterol synthesis without conversion to acetyl-CoA. D-(-)-3-hydroxybutyrate can either be converted in the mitochondria by d-(-)-3-hydroxybutyrate dehydrogenase (3) to acetocetate which must rapidly equilibrate with acetocetate in the cytosol to be utilized for lipid synthesis, or, it can be activated by a d-(-)-3-hydroxybutyryl-CoA synthetase (4) to d-(-)-3-hydroxybutyryl-CoA which can be oxidized by a d-(-)-3-hydroxybutyryl-CoA dehydrogenase (5) to acetoacetyl-CoA for lipid synthesis. These routes would permit a 4-carbon unit, acetocetate or d-(-)-3-hydroxybutyrate, to be used for sterol synthesis without cleavage to a 2-carbon intermediate (acytetyl-CoA). However, carbon from other precursors, for example glucose, must flow through a 2-carbon intermediate before incorporation into lipids. This scheme is based on the observation that labeled acetocetate and labeled d-(-)-3-hydroxybutyrate gave distinctive ratios for DPM of 14C in sterols/g of brain as a function of time as compared to this ratio found with labeled glucose, octanoate, and acetate (see "Results" and "Discussion"). The abbreviations are: Glc, glucose; AA, acetocetate; 3HB, d-(-)-3-hydroxybutyrate; HMG, 3-hydroxy-3-methylglutarate; TCA, tricarboxylic acid.

Acetocetate must pass through acetyl-CoA either in the mitochondrial or in the cytosol. The extramitochondrial activation of acetocetate to acetoacetyl-CoA by acetoacetyl-CoA synthetase and its conversion by acetoacetyl-CoA thiolase to acetyl-CoA in the cytosol does not explain our data. [2-14C]-Acetate which could be activated to acetyl-CoA in the cytosol by acetyl-CoA synthetase (36, 37) gave a ratio of 0.29, the same ratio as was found for glucose which must be metabolized to acetyl-CoA in the mitochondrion.

If acetocetate had been utilized by either the mitochondrial or cytoplasmic route in which it is cleaved to acetyl-CoA (6, 7, 33, 36) it would have given a ratio of about 0.25, as was found for the metabolites, glucose and acetate, that must be metabolized to acetyl-CoA before utilization. This was not observed. These proposed models (6, 7, 33, 36) do not explain the characteristic difference in the ratio of DPM of 14C in sterols per g of brain to DPM of 14C in fatty acids per g of brain that we observed from the labeled metabolites studied in our experiments. We believe the difference observed for the ratio of DPM in sterols to DPM in fatty acids can be explained by cytosolic activation of acetocetate to acetoacetyl-CoA followed by its direct incorporation into sterol intermediates.

We propose (Scheme 1) that acetocetate can be activated to acetoacetyl-CoA by cytoplasmic acetoacetyl-CoA synthetase, which has been reported by Buckley and Williamson (36) to be highly active in the neonatal rat. We postulate that acetoacetyl-CoA can be directly incorporated into 3-hydroxy-3-methylglutaryl-CoA by 3-hydroxy-3-methylglutaryl-CoA synthetase in the cytoplasm for sterol synthesis without conversion to acetyl-CoA. However, cytoplasmic acetoacetyl-CoA thiolase must produce acetyl-CoA that could be used for fatty acid synthesis (Scheme 1). Furthermore, we postulate that d-(-)-3-hydroxybutyrate has to rapidly equilibrate in the cell with acetocetate (mitochondrion), and that this acetocetate must be in rapid equilibrium with the acetoacetyl-CoA which is used extramitochondrially. Alternatively, d-(-)-3-hydroxybutyrate could be activated extramitochondrially to d-(-)-3-hydroxybutyryl-CoA which could be converted in turn to acetoacetyl-CoA (Scheme 1). These alternatives are suggested by the finding that after the injection of d-(-)-3-hydroxy[3-14C]butyrate, the ratio of DPM of 14C in sterols per g of brain to DPM of 14C in fatty acids per g of brain was equivalent to this ratio after the injection of [3-14C]acetocetate. This proposal represents a path by which both d-(-)-3-hydroxybutyrate and acetocetate could be preferentially incorporated into sterols without mixing with the cytosolic acetyl-CoA pool. This path would allow a preferential increase in the amount of label incorporated from the ketone bodies into the sterols in brain as compared to the fatty acids, which must obtain their carbon from the cytosolic acetyl-CoA pool. The enzymes required for the proposed utilization of acetocetate are known and are active in the brain of neonatal rats. Buckley and Williamson (36) have measured acetoacetyl-CoA synthetase in the brain of young and adult rats. They found acetoacetyl-CoA synthetase activity was 3 to 4 times higher at birth than in the adult rat, and its activity slowly declined over the developmental period to the adult level. This coincides with the high rate of sterol synthesis which is required for the growth of membranes and for myelinization during the developmental period. Experiments are in progress to test this proposal.

The utilization of label from [1-14C]octanoate deserves special attention. Our data seem to indicate that the brain of the 18-day-old rat can directly utilize octanoate. This proposal is supported by previous reports (38-40) on the direct oxidation of long chain fatty acids, palmitate and linoleate, to two carbon units by the brain of the developing rat. After the injection of [1-14C]octanoate, the specific activity of acetoacetate and d-(-)-3-hydroxybutyrate in blood (Fig. 4) is about 10-fold lower than the specific activity of acetoacetate or d-(-)-3-hydroxybutyrate after the injection of either labeled acetocetate or labeled 3-hydroxybutyrate, respectively (Fig. 3). However, the amount of label incorporated into sterols and fatty acids in brain after the injection of [1-14C]octanoate (Fig. 5D) is nearly the same as that found after the injection of either [3-14C]acetocetate or d-(-)-3-hydroxy[3-14C]butyrate (Fig. 5, A and B). Together these observations suggest that octanoate was utilized in the brain for the synthesis of sterols and fatty acids. Since octanoate cannot be incorporated directly into sterols in the brain without being metabolized to acetyl-CoA, our data indicate that octanoate can be oxidized in the brain and then utilized for sterol synthesis. The ratio of DPM of 14C in sterols to DPM of 14C in fatty acids after the injection of labeled octanoate is 0.29 which is approximately the same as this ratio after the injection of labeled glucose or labeled acetate (Table III); therefore, we believe that very little of the octanoate could have been used directly for elongation to longer chain fatty acids. If the labeled octanoate had been significantly utilized by elongation processes, then the amount of 14C found in the fatty acid fractions would have been very much greater than the amount of 14C found in the sterol fractions, and the ratios would have been very much lower than that obtained with either labeled glucose or labeled...
The Utilization of Ketone Bodies and Glucose by Brain acetate. Therefore, we conclude that labeled octanoate was taken up by the brain, predominately processed by $\beta$-oxidation to acetyl-CoA and then utilized for the synthesis of sterols and fatty acids.

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R J Webber and J Edmond