Lipogenesis in the Brain of Suckling Rats

STUDIES ON THE MECHANISM OF MITOCHONDRIAL–CYTOSOLIC CARBON TRANSFER

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1. Studies on the incorporation of [3-¹⁴C]pyruvate and D-3-hydroxy[3-¹⁴C]butyrate into the brain lipid fraction by brain homogenates of the suckling (7-day-old) rat have been carried out. 2. Whereas approximately twice as much CO₂ was evolved from pyruvate compared with 3-hydroxybutyrate metabolism, similar amounts of the radioactivity of these two precursors were incorporated into the lipid fraction. Furthermore, in both cases the incorporation into lipid was almost tripled when glucose (10 mM) or NADPH (2.5 mM) was added to the incubation media. 3. If 5 mM (–)-hydroxycitrate, an ATP–citrate lyase inhibitor, was added to the incubation the incorporation of carbon from pyruvate was inhibited to 39% of the control and from 3-hydroxybutyrate to 73% of the control, whereas CO₂ production from both precursors was not affected. 4. The incorporation from pyruvate or 3-hydroxybutyrate into lipids was not affected by the presence of 10 mM-glutamate in the medium (to encourage *N*-acetylaspargate production). However, incorporation from pyruvate was inhibited by 21% in the presence of 5 mM-amino-oxyacetate (a transaminase inhibitor) and by 83% in the presence of both hydroxycitrate (5 mM) and amino-oxyacetate. 5. Incorporation from 3-hydroxybutyrate into brain lipids was inhibited by 20% by amino-oxyacetate alone, but by 55% in the presence of both hydroxycitrate and amino-oxyacetate. 6. It is concluded that the mechanism of carbon transfer from pyruvate into lipids across the mitochondrial membrane in the suckling rat brain is mainly via citrate and *N*-acetylaspargate. 3-Hydroxybutyrate, in addition to using these routes, may also be incorporated via acetoacetate formation and transport to the cytosol.

The brain of the suckling rat is actively engaged in lipogenesis, particularly with respect to myelination and studies on both whole rats (Dhopeshwarker *et al.*, 1969) and rat brain slices (Patel & Owen, 1977) have indicated that lipogenesis is maximal around 7–10 days after birth. At this stage of development the major fuels available to the brain for both synthetic and energy purposes are ketone bodies and glucose (Itoh & Quastel, 1970; Hawkins *et al.*, 1971; Cremer & Heath, 1974) and considerable interest has been shown in assessing which of these substrates, if any, is the more important (Cremer & Heath, 1974; Patel & Owen, 1977).

Since the enzymes of lipogenesis are cytosolically located (Buckley & Williamson, 1973; Baquer *et al.*, 1975), whereas the principal site for acetyl-CoA

production from both glucose and ketone bodies for lipogenesis is mitochondrial, another equally important question is the mechanisms of transfer of the carbon from the mitochondria to the cytosol. Recent evidence suggests that in the brain of suckling rats the major (65%) portion of the carbon from either glucose or ketone bodies is transported across the mitochondrial membrane as citrate (Patel & Owen, 1976) and then cleaved in the cytosol by the ATP-dependent citrate lyase to provide acetyl-CoA, a mechanism originally proposed for liver by Srere & Bhaduri (1962). What is of interest is in what form the rest of the carbon is incorporated into lipids and does it involve transport across the mitochondrial membrane, since there have been proposals for the incorporation of the carbon from ketone bodies and glucose into lipids by purely cytosolic pathways (Buckley & Williamson, 1973; Lefresne *et al.*, 1977, 1978).

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Several additional possibilities exist and have been proposed for the transport of carbon across the mitochondrial membrane: (a) as acetate (Spencer & Lowenstein, 1962); (b) as acetylcarnitine (Fritz & Yue, 1962); (c) as *N*-acetylaspargate (D'Adamo & Yatzu, 1966; D'Adamo *et al.*, 1968; Patel & Clark, 1979). The first two of these possibilities have been shown to be relatively unimportant in brain (Tucek & Cheng, 1974), but studies with brain mitochondria (Patel & Clark, 1979) have shown that under the appropriate conditions they may synthesize and export considerable quantities of *N*-acetylaspargate. *N*-Acetylaspargate is synthesized intramitochondrially by the enzyme *L*-aspartate *N*-acetyltransferase (EC 2.3.1.17) (Benuck & D'Adamo, 1968), from aspartate and acetyl-CoA (derived from either pyruvate or 3-hydroxybutyrate). It is then transported across the mitochondrial membrane, possibly by the dicarboxylate translocator (Patel & Clark, 1979), and in the cytosol is cleaved to acetate and aspartate by the cytosolic enzyme *N*-acetyl-*L*-aspartate amidohydrolase (EC 3.5.1.15) (D'Adamo *et al.*, 1973). The acetate thus produced may then be converted into acetyl-CoA by the brain acetate thiokinase (Tucek, 1967).

In this paper experiments are reported in which the relative contributions of citrate and *N*-acetylaspargate involvement in mitochondrial-cytosolic carbon transfer in the rat brain are assessed.

Materials and Methods

Chemicals

DL-3-Hydroxybutyric and amino-oxyacetic acid were obtained from Sigma Chemical Co. Koch-Light Laboratories supplied pyruvic acid, which was purified by distilling twice under vacuum and was stored at -20°C . [$3\text{-}^{14}\text{C}$]Pyruvic acid, D-($-$)-3-hydroxy[$3\text{-}^{14}\text{C}$]butyric acid and [9,10(n)- ^3H]oleic acid were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. ($-$)-Hydroxycitric acid was a gift from Dr. A. Sullivan of Hoffman-La Roche.

All other reagents used were of the highest quality commercially available and all solutions were made up in double-glass-distilled water.

Animals

Rats used were of the Wistar strain. The birth dates of all litters were carefully recorded after daily inspection and litters were culled to 8–10 pups. Pups (1 week-old) of either sex were used for experimentation.

Lipid-synthesis studies

Incubations to study lipid synthesis in the rat brain in the presence or absence of inhibitors were carried out with homogenates (1:10, w/v) from

brains from 1-week-old rats homogenized in medium containing 0.25 M-sucrose, 0.5 mM-dipotassium EDTA and 10 mM-Tris/HCl, pH 7.4. The homogenate (equivalent to 150 mg of brain) was incubated at 25°C in a Marie flask in a final volume of 5 ml of aerated (O_2/CO_2 , 19:1) high-potassium Krebs-Henseleit-Ringer bicarbonate buffer, pH 7.4. This buffer contains 115 mM-NaCl, 25 mM- KHCO_3 , 5.9 mM-KCl, 1.18 mM- MgCl_2 , 1.2 mM- KH_2PO_4 , 1.195 mM- K_2SO_4 and 2.5 mM- CaCl_2 .

To measure the $^{14}\text{CO}_2$ production from various radioactive substrates, a microcentrifuge tube containing Hyamine hydroxide (0.5 ml) was placed in the centre well at the start of the incubation and the flasks were sealed with a rubber serum stopper. At the end of the incubation period, 0.5 ml of 36% HClO_4 was injected into the medium to stop the reaction and ensure the complete release of CO_2 . After additional shaking for 40 min the microcentrifuge tube containing the Hyamine hydroxide was transferred into scintillation fluid (Bray, 1960) and radioactivity in $^{14}\text{CO}_2$ was counted on a Packard 2425 liquid-scintillation counter.

The acidified medium was subjected to lipid-extraction procedure after having first added a known amount of [^3H]oleic acid as internal standard.

Lipid extractions

Lipids were extracted essentially by the method of Folch *et al.* (1957).

The medium containing the tissue was mixed with chloroform/methanol (1:2, v/v) to a final volume 20 times the volume of the sample. This one-phase mixture was allowed to stand at room temperature for at least 3 h to ensure complete extraction of lipids. The mixture was then filtered through a fat-free paper into a glass-stoppered vessel. At this stage, an equal volume of 0.9% (w/v) NaCl solution was added to the filtered extract, splitting it into a lower chloroform and an upper aqueous phase.

The lower chloroform layer was washed 8–9 times with three times its volume of saline in each wash. The top aqueous phase was frequently checked for any residual radioactivity from the precursors (i.e. [$3\text{-}^{14}\text{C}$]pyruvate or D-3-hydroxy-[$3\text{-}^{14}\text{C}$]butyrate). Only when the radioactivity in the top aqueous phase equalled the background activity was washing discontinued.

Since only the radioactivity incorporated into the lipids was studied, the washed chloroform layer was evaporated to dryness under N_2 and then counted for ^{14}C and ^3H radioactivities in Bray's scintillation fluid (Bray, 1960) on a Packard 2425 liquid-scintillation counter.

Losses of lipids during extraction and washing were corrected from recovery of the internal stan-

dard [9,10(n)-³H]oleic acid, which was added to each sample.

Results and Discussion

The rate of lipid synthesis in rat brain is maximal in 6–8-day-old animals (Patel & Owen, 1977). For this reason all studies on lipid synthesis were carried out on brains of 7-day-old rat pups. Also, since the effect of several inhibitors on the rates of lipogenesis were to be studied, compartmentation was kept to a minimum by use of brain homogenates. The use of brain homogenates also meant that the 'impedance' afforded by brain cell membranes to transport of inhibitors into the cells was minimized. L-Malate (2.5 mM) was a standard inclusion in all incubations to provide intramitochondrial oxaloacetate and ensure maximal rate of turnover of carbon, from either pyruvate or 3-hydroxybutyrate, in the citric acid cycle. In all experiments on lipid synthesis, blanks containing labelled pyruvate or 3-hydroxybutyrate but without homogenate were carried out to correct for the background count. Furthermore control incubations with acid-denatured homogenates were also carried out. Invariably the background count was less than 5% of that due to precursor incorporation into lipids.

Table 1 shows the incorporation of either [3-¹⁴C]pyruvate or 3-hydroxy[3-¹⁴C]butyrate in the presence of malate into lipids and also the formation of CO₂ from both these substrates. Although there was no significant difference in the amount of each precursor converted into lipids, the amount of CO₂ produced from pyruvate was approximately double that produced by 3-hydroxybutyrate. If 10 mM-glucose is additionally included in the incubation medium with either [3-¹⁴C]pyruvate or 3-hydroxy[3-¹⁴C]butyrate, there is no effect on the CO₂ production, but a marked (3-fold) increase in the amount of precursor incorporated into lipids. A similar increase in precursor incorporation into lipids although not quite so extensive (about 2.5-fold) can be generated by replacing the glucose with 2.5 mM-NADPH, suggesting that a possible explanation for this effect by glucose is a stimulation of the production of NADPH by the pentose phosphate pathway, thus allowing increased reducing potential for lipogenesis (cf. Patel & Owen, 1977). The fact that the rate of CO₂ production from pyruvate is twice that from 3-hydroxybutyrate suggests that a greater proportion of the pyruvate is being completely oxidized in the citric acid cycle than 3-hydroxybutyrate. The fraction of 3-hydroxybutyrate that is not being completely oxidized may be metabolized to acetoacetate and exported on the monocarboxylate translocase (Land & Clark, 1974; Halestrap *et al.*, 1974; Patel *et al.*, 1977) to the cytosol where it may be incorporated into lipids via

Table 1. [3-¹⁴C]Pyruvate + D-3-hydroxy[3-¹⁴C]butyrate incorporation into lipids of brains of 7-day-old rats

Incubations with the various additions were carried out using homogenates of 7-day-old rat brains. Lipid extractions and radioactivity counting were carried out by the procedure described in the Materials and Methods section. Each value is a mean $\pm$ S.D. for three determinations in each of three different experiments. Significant differences between various incubations were compared with the controls using Student's unpaired *t* test analysis and are represented as: **P* < 0.001.

Labelled substrates and other additions	Labelled substrates converted into products (nmol/h per g wet wt. of brain)	
	¹⁴ CO ₂	Lipids
[3- ¹⁴ C]Pyruvate (5 mM)	9507 $\pm$ 450	130 $\pm$ 8
+ malate (2.5 mM)		
[3- ¹⁴ C]Pyruvate (5 mM) + malate (2.5 mM) + glucose (10 mM)	9708 $\pm$ 380	360 $\pm$ 20*
[3- ¹⁴ C]Pyruvate (5 mM) + malate (2.5 mM) + NADPH (2.5 mM)	9463 $\pm$ 523	311 $\pm$ 16*
D-3-Hydroxy[3- ¹⁴ C]butyrate (10 mM) + malate (2.5 mM)	4500 $\pm$ 250	127 $\pm$ 7
D-3-Hydroxy[3- ¹⁴ C]butyrate (10 mM) + malate (2.5 mM) + glucose (10 mM)	4772 $\pm$ 222	390 $\pm$ 18*
D-3-Hydroxy[3- ¹⁴ C]butyrate (10 mM) + malate (2.5 mM) + NADPH (2.5 mM)	4492 $\pm$ 190	321 $\pm$ 22*

the pathway described by Buckley & Williamson (1973) and Patel & Clark (1978).

The ratio of amount of lipid synthesized to amount of ¹⁴CO₂ produced from the oxidation of [3-¹⁴C]pyruvate is 0.04 compared with a value of 0.08 obtained when 3-hydroxy[3-¹⁴C]butyrate was the substrate (ratios calculated from Table 1). This suggests that 3-hydroxybutyrate is a better precursor than pyruvate of cerebral lipids (cf. Patel & Owen, 1976).

Table 2 shows the effect of (–)-hydroxycitrate on the rate of lipogenesis in brain homogenates of 7-day-old rats. (–)-Hydroxycitrate is a specific inhibitor of ATP–citrate lyase, which is the enzyme responsible for cleaving citrate to oxaloacetate and acetyl-CoA in the cytosol after the passage of citrate across the mitochondrial membrane. Hydroxycitrate has a *K_i* for citrate lyase of the order 0.15–0.57 μ M (Watson *et al.*, 1969) and hence at a concentration of 5 mM, as used in the experiments of Table 2, it is likely to cause almost complete inhibition of this enzyme. Table 2 shows that whereas the presence of hydroxycitrate does not affect the rate of ¹⁴CO₂ production from either

Table 2. *Effect of (–)-hydroxycitrate on incorporation of [3-¹⁴C]pyruvate and D-3-hydroxy[3-¹⁴C]butyrate into lipids of brains of 7-day-old rats*

The method for studying the effects of various substances in 7-day-old rat brain homogenates was the same as described in the legend to Table 1. Each value is a mean $\pm$ S.D. for three determinations in each of three different experiments. Significant differences from control values were compared using Student's unpaired *t* test analysis and are represented as: **P* < 0.001.

Labelled substrates and other additions	Labelled substrates converted into products (nmol/h per g wet wt. of brain)	
	CO ₂	Lipids
[3- ¹⁴ C]Pyruvate (5 mM) + malate (2.5 mM) + glucose (10 mM)	9825 $\pm$ 430	353 $\pm$ 18
[3- ¹⁴ C]Pyruvate (5 mM) + malate (2.5 mM) + glucose (10 mM) + (–)-hydroxycitrate (5 mM)	9714 $\pm$ 521	137 $\pm$ 9*
D-3-Hydroxy[3- ¹⁴ C]butyrate (10 mM) + malate (2.5 mM) + glucose (10 mM)	4721 $\pm$ 321	373 $\pm$ 17
D-3-Hydroxy[3- ¹⁴ C]butyrate (10 mM) + malate (2.5 mM) + glucose (10 mM) + (–)-hydroxycitrate (5 mM)	4672 $\pm$ 215	270 $\pm$ 21*

[3-¹⁴C]pyruvate or 3-hydroxy[3-¹⁴C]butyrate, the rate of incorporation of [3-¹⁴C]pyruvate into lipids was inhibited to 39% of the control rate and that of 3-hydroxy[3-¹⁴C]butyrate to 73% of the control rate. These results suggest that, whereas the movement of carbon across the mitochondrial membrane from pyruvate to lipids is to a large extent (60%) via citrate, this route is only responsible for a limited incorporation of 3-hydroxybutyrate carbon into lipids (cf. Patel & Owen, 1976).

In the light of the studies of D'Adamo and his colleagues (D'Adamo & Yatzu, 1966; D'Adamo *et al.*, 1968) demonstrating the incorporation of ¹⁴C-labelled *N*-acetylaspartate into brain lipids, it has been proposed that *N*-acetylaspartate may be an additional or alternative mechanism for exporting carbon from the mitochondrial matrix to the cytosol for biosynthetic purposes (Patel & Clark, 1979). Tables 3 and 4 show the results of experiments in which this hypothesis has been tested. In these experiments brain homogenates from 7-day-old rats have been incubated in the presence of glutamate and amino-oxyacetate. The latter compound was used to inhibit the transamination route of glutamate metabolism (Hopper & Segal, 1964), thus inhibiting intramitochondrial aspartate production. At the concentration of amino-oxyacetate used (5 mM) it has been previously demonstrated that aspartate production in rat brain mitochondria is

Table 3. *Effect of amino-oxyacetate and (–)-hydroxycitrate on [3-¹⁴C]pyruvate incorporation into lipids of brains of 7-day-old rats*

For methods of studying lipid synthesis in 7-day-old rat brain homogenates see the legend to Table 1. Each value is a mean $\pm$ S.D. for three determinations in each of three different experiments. Significant differences between control and experimental incubations were compared using Student's *t* test analysis and are represented as: **P* < 0.02; ***P* < 0.001.

Labelled substrates and other activities	Labelled substrates converted into products (nmol/h per g wet wt. of brain)	
	CO ₂	Lipids
[3- ¹⁴ C]Pyruvate (5 mM) + malate (2.5 mM) + glucose (10 mM)	9679 $\pm$ 34	368 $\pm$ 22
[3- ¹⁴ C]Pyruvate (5 mM) + malate (2.5 mM) + glucose (10 mM) + glutamate (10 mM)	9705 $\pm$ 250	362 $\pm$ 29
[3- ¹⁴ C]Pyruvate (5 mM) + malate (2.5 mM) + glucose (10 mM) + amino-oxyacetate (5 mM)	9805 $\pm$ 497	292 $\pm$ 23*
[3- ¹⁴ C]Pyruvate (5 mM) + malate (2.5 mM) + glucose (10 mM) + (–)-hydroxycitrate (5 mM) + amino-oxyacetate (5 mM)	9692 $\pm$ 435	65 $\pm$ 5**

Table 4. *Effect of amino-oxyacetate and hydroxycitrate on D-3-hydroxy[3-¹⁴C]butyrate incorporation into lipids of brains of 7-day-old rats*

For experimental conditions see legend to Table 3. Each value is a mean $\pm$ S.D. for three determinations in each of three different experiments. Significant differences between control and experimental incubations were compared using Student's *t* test analysis and are represented as: **P* < 0.02; ***P* < 0.001.

Labelled substrates and other additions	Labelled substrates converted into products (nmol/h per g wet wt. of brain)	
	CO ₂	Lipids
D-3-Hydroxy[3- ¹⁴ C]butyrate (10 mM) + malate (2.5 mM) + glucose (10 mM)	4613 $\pm$ 215	381 $\pm$ 20
D-3-Hydroxy[3- ¹⁴ C]butyrate (10 mM) + malate (2.5 mM) + glucose (10 mM) + glutamate (10 mM)	4329 $\pm$ 251	373 $\pm$ 19
D-3-Hydroxy[3- ¹⁴ C]butyrate (10 mM) + malate (2.5 mM) + glucose (10 mM) + amino-oxyacetate (5 mM)	4609 $\pm$ 257	305 $\pm$ 21*
D-3-Hydroxy[3- ¹⁴ C]butyrate (10 mM) + malate (2.5 mM) + glucose (10 mM) + amino-oxyacetate (5 mM) + (–)-hydroxycitrate (5 mM)	4608 $\pm$ 236	173 $\pm$ 12**

completely inhibited (Dennis, 1976), whereas other mitochondrial activities, e.g. CO_2 production (Tables 3 and 4) and citrate efflux (T. B. Patel & J. B. Clark, unpublished work) appear to be unaffected. However, it should be noted that the mechanism of inhibition by amino-oxyacetate is by interaction with pyridoxal phosphate (Hopper & Segal, 1964) and hence the possibility that some other pyridoxal phosphate-dependent system might be affected cannot be excluded.

Table 3 shows that the addition of either exogenous glutamate or amino-oxyacetate did not significantly affect the rate of $^{14}\text{CO}_2$ production from $[3\text{-}^{14}\text{C}]\text{pyruvate}$. Furthermore, the addition of 20 mM exogenous glutamate did not affect the rate of lipogenesis from pyruvate, in contrast with the situation in rat brain mitochondria, where exogenous glutamate was necessary for *N*-acetyl-aspartate production (Patel & Clark, 1979). This may reflect the fact that brain homogenates contain sufficient endogenous glutamate for *N*-acetyl-aspartate production, whereas purified brain mitochondrial preparations are depleted. However, the addition of 5 mM-amino-oxyacetate decreased the rate of lipogenesis from $[3\text{-}^{14}\text{C}]\text{pyruvate}$ to 79% of the control rate. If hydroxycitrate was added in addition to the amino-oxyacetate then the rate of lipogenesis from $[3\text{-}^{14}\text{C}]\text{pyruvate}$ was drastically decreased to a value that was only 17% of the control rate. This suggests that as far as pyruvate carbon incorporation into lipids is concerned, the citrate and *N*-acetyl-aspartate transport mechanisms account for almost all of the carbon incorporated.

Table 4 shows the results of experiments with 3-hydroxy $[3\text{-}^{14}\text{C}]\text{butyrate}$ as a precursor of lipid synthesis and the effects of added glutamate or amino-oxyacetate. In a similar way to results in Table 3, the addition of either glutamate or amino-oxyacetate did not affect the rate of $^{14}\text{CO}_2$ production from 3-hydroxy $[3\text{-}^{14}\text{C}]\text{butyrate}$. Furthermore, addition of exogenous glutamate alone to the incubation did not affect the rate of lipogenesis (Table 4). The addition of amino-oxyacetate (5 mM) to incubations caused a 20% inhibition in rate of 3-hydroxy $[3\text{-}^{14}\text{C}]\text{butyrate}$ incorporation into lipids (Table 4), which is similar to that with $[3\text{-}^{14}\text{C}]\text{pyruvate}$ as lipid precursor (cf. Tables 3 and 4). With 3-hydroxy $[3\text{-}^{14}\text{C}]\text{butyrate}$ as lipid precursor, the inclusion of both amino-oxyacetate and (–)-hydroxycitrate in the incubation caused an additive inhibition, as seen with $[3\text{-}^{14}\text{C}]\text{pyruvate}$ in Table 3, and the residual rate of lipid synthesis was 45% of the control value (Table 4).

The effect on lipogenesis from either $[3\text{-}^{14}\text{C}]\text{pyruvate}$ or 3-hydroxy $[3\text{-}^{14}\text{C}]\text{butyrate}$ due to simultaneous inhibition of the two acetyl transport mechanisms studied, citrate and *N*-acetyl-aspartate, by including both (–)-hydroxycitrate and amino-

oxyacetate together in incubations, shows that, whereas the incorporation into lipids of $[3\text{-}^{14}\text{C}]\text{pyruvate}$ is inhibited by 82% (Table 3), that of 3-hydroxy $[3\text{-}^{14}\text{C}]\text{butyrate}$ is inhibited by 55% (Table 4). The higher inhibition of lipogenesis from $[3\text{-}^{14}\text{C}]\text{pyruvate}$ compared with 3-hydroxy $[3\text{-}^{14}\text{C}]\text{butyrate}$ produced by (–)-hydroxycitrate and amino-oxyacetate together (Tables 3 and 4) is probably due to the following reasons. First, a larger proportion of carbon from pyruvate is incorporated into lipids via citrate compared with 3-hydroxybutyrate. This is shown by the (–)-hydroxycitrate inhibition of lipogenesis produced from the respective substrates (Table 2). Secondly, 3-hydroxybutyrate can produce intramitochondrial acetoacetate, which may be transported out of the mitochondria by the monocarboxylate translocase (Patel *et al.*, 1977). This acetoacetate may then be incorporated into brain lipids by the acetoacetyl-CoA synthetase and cytosolic acetoacetyl-CoA thiolase pathway described by Buckley & Williamson (1973). The residual (approx. 20%) lipogenesis from $[3\text{-}^{14}\text{C}]\text{pyruvate}$ in the presence of both (–)-hydroxycitrate and amino-oxyacetate may be a consequence of (i) incomplete inhibition of the citrate and *N*-acetyl-aspartate pathways and (ii) other minor acetyl-group-supplying mechanisms, for example acetylcarnitine (Fritz & Yue, 1962). In addition Lefresne *et al.* (1977, 1978) have recently postulated the existence of an enzyme in the cytosol with pyruvate dehydrogenase-like activity.

Thus overall it appears that, although pyruvate is capable of contributing its carbon for lipogenesis via at least two mechanisms, citrate and *N*-acetyl-aspartate, the carbon of 3-hydroxybutyrate metabolized intramitochondrially can be transported across the mitochondrial membrane by at least three processes, citrate, *N*-acetyl-aspartate and acetoacetate. This may explain the observation that in the 7-day-old rat brain ketone bodies may be better precursors of lipid than glucose (Patel & Owen, 1977; the present paper).

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