The Effect of Phenylpyruvate on Pyruvate Metabolism in Rat Brain

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1. The effect of phenylalanine and phenylpyruvate on the metabolism of pyruvate by isolated mitochondria from rat brain was investigated. 2. Phenylpyruvate inhibited the fixation of $\text{H}^{14}\text{CO}_3^-$ in the presence of pyruvate by intact rat brain mitochondria, whereas phenylalanine and other metabolites of this amino acid had no inhibitory effect on this process. 3. Pyruvate carboxylase activity in freeze-dried rat brain mitochondrial preparations was also inhibited only by phenylpyruvate, and a 'mixed type' inhibition was observed. 4. The K_m for pyruvate of rat brain pyruvate carboxylase was about 0.2 mM. 5. The concentration of phenylpyruvate required for a 50% inhibition of $\text{H}^{14}\text{CO}_3^-$ fixation by the intact mitochondria and of pyruvate carboxylase activity was dependent on the concentration of pyruvate used in the incubation medium. 6. The possible significance of inhibition of pyruvate carboxylase activity by phenylpyruvate in the brains of phenylketonuric patients is discussed.

The pathogenesis of brain malfunction in phenylketonuria, an inborn error of phenylalanine metabolism in humans, is still not well understood. As a result of an impairment in phenylalanine metabolism in liver, the concentrations in plasma of this amino acid, and of its metabolites phenylpyruvate and *o*-hydroxyphenylacetate are markedly elevated in patients with phenylketonuria (Jervis & Drejza, 1966). Hyperphenylalaninaemia induced in laboratory animals by feeding diets high in phenylalanine has been shown to decrease the synthesis of cerebral lipids (Barbato *et al.*, 1968; Geison *et al.*, 1968; Shah *et al.*, 1970). High concentrations of phenylalanine and phenylpyruvate have been shown to inhibit glycolysis and particularly pyruvate kinase and hexokinase activities in brain (Weber *et al.*, 1969). Further, it is known that the deaminated metabolites of phenylalanine, namely phenyl-lactate and phenylpyruvate, exert greater inhibitory effects on fatty acid synthesis at lower concentrations than phenylalanine (Shah *et al.*, 1970). These observations are supported by studies in which a decrease in O_2 consumption by rat brain preparations was observed in the presence of phenylpyruvate (Howell & Lee, 1963; Itoh, 1965). Inhibition was observed only when pyruvate was used as a substrate (Gallagher, 1969).

In brain, pyruvate is mainly metabolized via the pyruvate dehydrogenase complex and pyruvate carboxylase in the mitochondria. Pyruvate carboxylase, an anaplerotic enzyme, replenishes the C_4 compound that is required in many biosynthetic processes. A possible effect of phenylpyruvate on the metabolism of pyruvate via pyruvate carboxylase in rat brain mitochondria is the subject of this paper. Preliminary

reports of this work have been published (Patel, 1971; Patel & Grover, 1971).

Materials and Methods

Chemicals

$\text{NaH}^{14}\text{CO}_3$ (sp. radioactivity 10–20 mCi/mM) was purchased from New England Nuclear Corp., Boston, Mass., U.S.A. NADH, potassium ATP and acetyl-CoA were from P-L Biochemicals, Milwaukee, Wis., U.S.A.; phenylpyruvate, *p*-hydroxyphenylpyruvate, *o*-, *m*- and *p*-hydroxy derivatives of phenylacetic acid, potassium pyruvate, bovine serum albumin and Ficoll were from Sigma Chemical Co., St. Louis, Mo., U.S.A. L-Phenylalanine was obtained from General Biochemical Inc., Chagrin Falls, Ohio, U.S.A., and phenylacetic acid was from Mann Research Laboratories, New York, N.Y., U.S.A. Lactate dehydrogenase (EC 1.1.1.27), malate dehydrogenase (EC 1.1.1.37) and citrate lyase (EC 4.1.3.6) were purchased from Boehringer (Mannheim) Corp., New York, N.Y., U.S.A. All other reagents were of the highest purity commercially available.

Methods

Preparation of mitochondria. Mitochondria from rat brain were isolated essentially as described by Clark & Nicklas (1970). Male Sprague-Dawley rats (weight approx. 200 g) fed *ad lib.* on Purina laboratory chow were decapitated and the cerebral hemispheres were rapidly removed into ice-cold buffered sucrose medium (0.25 M-sucrose–0.5 mM-EDTA–10 mM-tris–HCl buffer, pH 7.4). The tissue was chopped with

scissors and was washed twice with ice-cold buffered sucrose medium. The material from three rats was homogenized with 20ml of buffered sucrose medium and was centrifuged at 5°C for 3min at 2000g. The supernatant was transferred to a clean tube and was centrifuged for 8min at 12500g. The crude mitochondrial pellet was resuspended in 6ml of a 3% (w/v) Ficoll medium (3% Ficoll–0.12M-mannitol–0.03M-sucrose–25 μ M-EDTA, pH7.4). This suspension was carefully layered on to 14ml of a 6% (w/v) Ficoll medium (6% Ficoll–0.24M-mannitol–0.06M-sucrose–50 μ M-EDTA, pH7.4) and centrifuged at 11500g for 30min. The supernatant was then removed by suction and the slight fluffy layer removed from the pellet. The mitochondrial pellet was resuspended in 20ml of buffered sucrose medium and re-centrifuged at 11500g for 10min. The mitochondria were finally resuspended in 0.3M-sucrose solution.

Integrity of mitochondria. Oxidative phosphorylation and the respiratory-control ratio of rat brain mitochondria were measured polarographically with an electrode in a 2ml closed chamber at 28°C. The reaction mixture used contained 230mM-mannitol, 70mM-sucrose, 20mM-tris–HCl buffer, 20 μ M-EDTA and 5mM-potassium phosphate buffer, pH7.2, and about 2mg of mitochondrial protein was added. As substrates 5mM-pyruvate and 1mM-malate were used and 0.25mM-ADP was added to stimulate respiration. The P:O ratio and the respiratory-control ratio with pyruvate+malate were measured in several preparations and were 2.72 ± 0.04 and 8.4 ± 0.3 (mean $\pm$ S.E.M. of five experiments) respectively.

Carboxylation of pyruvate (fixation of $H^{14}CO_3^-$) by mitochondria. The composition of the reaction mixtures and the additions are described in the legends of the appropriate tables and figures. Incubations were carried out in small tubes (12cm $\times$ 1.8cm), which were shaken in a water bath at 37°C. The reaction was started by adding the mitochondrial suspension after a temperature-equilibration period of about 5min. After appropriate incubation periods, the reactions were stopped by adding 1ml of 12% (v/v) $HClO_4$. The tubes were then gassed with CO_2 for 4min to remove residual $H^{14}CO_3^-$. Radioactivity in a sample of the mixture was determined in Ditol scintillation solution (Herberg, 1960) with a Packard liquid-scintillation spectrometer.

Analysis of intermediates. Mitochondrial protein precipitated with $HClO_4$ was removed by centrifugation at 650g for 5min and a portion of the supernatant was neutralized with 2M- K_2CO_3 . Pyruvate (Bucher *et al.*, 1965), malate (Hohorst, 1965) and citrate (Moellering & Gruber, 1966) were measured enzymically in the neutralized extracts. In an attempt to measure quantitatively the utilization of pyruvate by brain mitochondria, it was observed that phenylpyruvate added in the incubation medium was slowly

acted on by lactate dehydrogenase in the presence of NADH. This was confirmed by the addition of a known quantity of phenylpyruvate in both the presence and the absence of pyruvate in the assay system. It took less than 5min to determine 0.1 μ mol of pyruvate after the addition of lactate dehydrogenase in the assay medium, whereas it took about 1h to assay the same quantity of phenylpyruvate in both the presence or the absence of an equimolar quantity of pyruvate (M. Sutnick & M. S. Patel, unpublished work). Owing to this non-specificity of lactate dehydrogenase towards phenylpyruvate, the utilization of pyruvate by brain mitochondria was not determined.

Separation of labelled intermediates. By using high-voltage electrophoresis (model L24 apparatus; Shandon Scintific Co., Sewickley, Pa., U.S.A.), labelled compounds were separated as described previously (Patel & Hanson, 1970). A portion of the neutralized sample was spotted on a strip (4cm $\times$ 61cm) of Whatman chromatography paper no. 3MM. Labelled organic acids were separated on strips by using 2M-acetic acid buffer adjusted to pH2.6 with pyridine, for 3.5h with a current of 10mA per strip. The strips were dried and cut into sections (4cm $\times$ 1.2cm). The radioactivity was measured in 15ml of toluene, containing 230ml of ethanol, 4g of 2,5-diphenyloxazole and 15mg of 1,4-bis-(5-phenyloxazol-2-yl)benzene/l, with a Packard liquid-scintillation spectrometer. The radioactive spots were identified by comparing their migration with those of known radioactive organic acids (Patel & Hanson, 1970).

Pyruvate carboxylase assay. Samples of brain mitochondria suspended in 0.3M-sucrose were freeze-dried and then resuspended in water. Pyruvate carboxylase (EC 6.4.1.1) activity was measured by a modification (Ballard & Hanson, 1967) of the method of Utter & Keech (1963). One unit of activity represents 1 μ mol of HCO_3^- fixed/min at 37°C.

Protein measurements. Mitochondrial protein was measured as described by Lowry *et al.* (1951), with dry bovine serum albumin as standard protein.

Results

The composition of the reaction mixture used to study carboxylation of pyruvate via pyruvate carboxylase by brain mitochondria under optimum conditions is shown in Table 1 legend. Addition of phenylalanine, phenylacetate and *o*-, *m*- or *p*-hydroxyphenylacetate at the concentration indicated had no effect on the fixation of $H^{14}CO_3^-$ by the mitochondria in the presence of pyruvate. However, phenylpyruvate, the deaminated metabolite of phenylalanine, inhibited this reaction by about 60% (Table 1).

The results presented in Fig. 1 show that the rate

Table 1. *Effect of phenylalanine and its metabolites on pyruvate carboxylation by rat brain mitochondria*

The reaction mixture contained, in a final volume of 1 ml, 6.6mM-potassium phosphate buffer, pH 7.4, 6.6mM-triethanolamine buffer, pH 7.4, 5mM-potassium pyruvate, 4mM ATP (potassium salt), 10mM-MgCl₂, 20mM KH¹⁴CO₃ (2 μ Ci) and 157mM-sucrose. Various metabolites of phenylalanine were added to the final concentrations indicated. The reaction was started by adding mitochondria, which contained about 0.4mg of protein. Incubation was carried out for 15min at 37°C. The results are the means $\pm$ S.E.M. of four experiments.

Addition	Pyruvate carboxylation	
	(nmol of HCO ₃ ⁻ fixed/ mg of protein)	(% of control)
None	123 $\pm$ 7	100
L-Phenylalanine (10mM)	131 $\pm$ 6	107
Phenylpyruvate (10mM)	49 $\pm$ 6	40
Phenylacetate (10mM)	133 $\pm$ 5	108
<i>o</i> -Hydroxyphenylacetate (10mM)	119 $\pm$ 10	97
<i>m</i> -Hydroxyphenylacetate (5mM)	133 $\pm$ 8	108
<i>p</i> -Hydroxyphenylacetate (10mM)	112 $\pm$ 7	91

of pyruvate carboxylation by brain mitochondria was linear for incubation times of up to at least 30 min in the present studies. Phenylalanine did not affect the fixation of H¹⁴CO₃⁻, whereas the rate of phenylpyruvate-induced inhibition of this process was linear with time (Fig. 1). The effect of various concentrations of phenylpyruvate and phenylalanine (0–10mM) on the fixation of H¹⁴CO₃⁻ in the presence of 5mM-pyruvate are shown in Fig. 2. Increasing the concentration of phenylpyruvate from 0 to 10mM resulted in an increased inhibition of pyruvate carboxylation, whereas phenylalanine at several concentrations had no effect on this process. Phenylpyruvate at 5mM inhibited pyruvate carboxylation in brain mitochondria by about 50%. In these experiments pyruvate was added at a concentration of 5mM in the incubation medium. Although 5mM-pyruvate ensures the maximum carboxylation of pyruvate under the experimental conditions used in these studies, it is far above the physiological concentration of pyruvate in the cell. In view of this, the effect of various concentrations of pyruvate on the fixation of H¹⁴CO⁻ by brain mitochondria was investigated. Fig. 3 shows that the fixation of H¹⁴CO₃⁻ was dependent on pyruvate concentration, and about 80% of the total carboxylation was observed at about 1mM-pyruvate. The 'K_m' for pyruvate for carboxylation of pyruvate by brain mitochondria was about 0.5mM. In the light of this observation, the effect of various concentrations of phenylpyruvate on the fixation of H¹⁴CO₃⁻ in the presence of 0.5mM-pyruvate by the mitochondria was further investigated. As the concentration of pyruvate was lowered to 0.5mM, the incubation time was also decreased to

Fig. 1. *Effect of phenylalanine and phenylpyruvate on the fixation of H¹⁴CO₃⁻ by rat brain mitochondria*

The reaction mixture is described in Table 1. The additions were: none (○), 10mM-phenylalanine (●), or 10mM-phenylpyruvate (△). The incubation was carried out at 37°C and reactions were terminated at the times indicated. The results are the means $\pm$ S.E.M. of four experiments.

15 min to ensure that there was sufficient pyruvate in the medium during incubation. Fig. 2 shows that, in contrast with phenylalanine, an increase in the concentration of phenylpyruvate resulted in an increased inhibition of the carboxylation process; however, a greater inhibition was observed at a lower concentration of phenylpyruvate than that required to produce the same degree of inhibition in the presence of 5 mM-pyruvate. Phenylpyruvate at a concentration of 0.5 mM inhibited this reaction by about 50% (Fig. 2).

As phenylpyruvate was found to decrease the carboxylation of pyruvate by brain mitochondria (Table 1; Figs. 1 and 2), the effect of this inhibitor on the formation of the tricarboxylic acid-cycle

intermediates from pyruvate and $\text{H}^{14}\text{CO}_3^-$ by the mitochondria was investigated. The results in Table 2 show that citrate and malate were the main products and about threefold more citrate than malate was formed. Phenylalanine did not affect the formation of citrate, whereas the formation of malate was decreased in the presence of this amino acid. It is suggested that in the presence of phenylalanine some labelled oxaloacetate formed from pyruvate was transaminated to aspartate. Although we did not determine the formation of aspartate enzymically, it was observed from the high-voltage electrophoresis separation that a corresponding amount of labelled aspartate was formed when phenylalanine was added in the incubation medium (results not shown). Addition of 5 mM-phenylpyruvate significantly decreased the formation of both citrate and malate from pyruvate by brain mitochondria (Table 2, Expt. 2). The separation of labelled intermediates on high-voltage electrophoresis also supported these findings (Table 2, Expt. 1).

The observed inhibitory effect of phenylpyruvate on the carboxylation of pyruvate by rat brain mitochondria (Table 1, Figs. 1 and 2) could be due to (a) the effect of the inhibitor on the transport of pyruvate across the inner mitochondrial membrane, (b) its effect on the metabolism of pyruvate via pyruvate carboxylase, or (c) both. To clarify this point, the effect of phenylpyruvate on pyruvate carboxylase activity was examined. As shown in Table 3, phenylalanine, phenylacetate and its three hydroxy-derivatives had no appreciable effect on pyruvate carboxyl-

Fig. 2. Effect of various concentrations of phenylalanine and phenylpyruvate on the fixation of $\text{H}^{14}\text{CO}_3^-$ by rat brain mitochondria

The reaction mixture is described in Table 1, except that pyruvate was added at either 5 mM (—) or 0.5 mM (---). Phenylalanine (○) or phenylpyruvate (●) was also added at the concentrations indicated. Incubations were carried out at 37°C for 15 and 30 min with 0.5 and 5.0 mM-pyruvate in the incubation mixture respectively. The results are the means $\pm$ S.E.M. of four to six experiments.

Fig. 3. Effect of pyruvate concentration on the fixation of $\text{H}^{14}\text{CO}_3^-$ by rat brain mitochondria

The reaction mixture is described in Table 1, except that the concentration of pyruvate was varied as indicated. The incubation was carried out for 30 min at 37°C.

Table 2. *Effect of phenylpyruvate on the formation of the tricarboxylic acid-cycle intermediates from pyruvate by rat brain mitochondria*

Rat brain mitochondria were incubated in the reaction mixture containing 5mM-pyruvate and $\text{KH}^{14}\text{CO}_3$ as described in Table 1. Incubation was carried out for 30min at 37°C. The results are means $\pm$ S.E.M. of five experiments.

Expt. no.	Addition	Intermediates formed			
		Citrate (nmol/mg of protein)	Malate (nmol/mg of protein)	$10^{-2} \times [^{14}\text{C}]$ Citrate (d.p.m./mg of protein)	$10^{-2} \times [^{14}\text{C}]$ Malate (d.p.m./mg of protein)
1	None	120 $\pm$ 6	35 $\pm$ 4	139 $\pm$ 14	12.0 $\pm$ 1.3
	L-Phenylalanine (5mM)	—	—	127 $\pm$ 8	7.3 $\pm$ 0.6
	Phenylpyruvate (5mM)	—	—	56 $\pm$ 6	3.4 $\pm$ 0.4
2	None	94 $\pm$ 9	39 $\pm$ 4	—	—
	L-Phenylalanine (5mM)	94 $\pm$ 7	23 $\pm$ 3	—	—
	Phenylpyruvate (5mM)	36 $\pm$ 5	16 $\pm$ 2	—	—

Table 3. *Effect of phenylalanine and its metabolites on pyruvate carboxylase activity in rat brain*

Pyruvate carboxylase activity in rat brain mitochondria was assayed as described in the Materials and Methods section. Various metabolites of phenylalanine were added to the final concentrations indicated. The results are the means $\pm$ S.E.M. of four experiments.

Addition	Pyruvate carboxylase activity	
	(munits/mg of protein)	(% of control)
None	65 $\pm$ 1.3	100
L-Phenylalanine (10mM)	66 $\pm$ 2.1	102
Phenylpyruvate (10mM)	34 $\pm$ 2.1	52
Phenylacetate (10mM)	62 $\pm$ 1.7	95
<i>o</i> -Hydroxyphenylacetate (10mM)	58 $\pm$ 1.6	89
<i>m</i> -Hydroxyphenylacetate (5mM)	63 $\pm$ 1.4	97
<i>p</i> -Hydroxyphenylacetate (10mM)	61 $\pm$ 1.2	94

ase activity, whereas phenylpyruvate at 10mM inhibited this enzyme by about 50%. An increase in the inhibition of pyruvate carboxylase activity was observed with an increase in phenylpyruvate concentration from 0 to 10mM, whereas phenylalanine had no effect on pyruvate carboxylase activity (Fig. 4). The concentration of phenylpyruvate required to inhibit this enzyme was likely to be dependent on the concentration of pyruvate used in the assay. When the pyruvate concentration was lowered from 10 to 0.5mM in the assay medium, phenylpyruvate exerted a greater inhibitory effect on this enzyme. It should be noted that at 0.5mM-pyruvate 50% inhibition was exerted by less than 1mM-phenylpyruvate (Fig. 4). The double-reciprocal plot of pyruvate carboxylase for pyruvate is shown in Fig. 5. The K_m for pyruvate of rat brain pyruvate carboxylase was about 0.2mM, in good agreement with that for highly purified pyruvate carboxylase from chicken liver (Scrutton *et al.*, 1969) and rat liver (Wimhurst & Manchester, 1970). Addition of 5mM-phenylpyruvate

caused a 'mixed type' inhibition of rat brain pyruvate carboxylase.

Discussion

Hyperphenylalaninaemia observed in the untreated phenylketonuric patient is the result of an inborn error in the *p*-hydroxylation of phenylalanine to tyrosine by hepatic phenylalanine hydroxylase (Jervis, 1953; Wallace *et al.*, 1957; Mitoma *et al.*, 1957). As phenylalanine is metabolized via an alternate pathway in this disorder, the concentrations of several intermediates such as phenylpyruvate, phenyl-lactate, phenylacetate and phenylacetylglutamine are elevated in blood, and are also excreted in large quantities in urine (Jervis & Drejza, 1966). Swaiman *et al.* (1968) reported that hyperphenylalaninaemia inhibited protein synthesis in the developing rabbit brain. Agrawal *et al.* (1970) concluded, however, from their experiments with rats that the decrease in cerebral protein synthesis was secondary

to the inhibition of the transport of several amino acids by high concentrations of phenylalanine. In addition to the inhibitory effect of phenylalanine, the toxic effects of its metabolites have been reported. Phenylpyruvate has been shown to inhibit 5-hydroxytryptophan decarboxylase (Davison & Sandler, 1958), dihydroxyphenylalanine decarboxylase (Hartman *et al.*, 1955) and glutamate decarboxylase (Hanson, 1959). Although phenylalanine did not inhibit these decarboxylases, Yuwiler & Geller (1969) observed a decrease in brain serotonin concentrations in phenylalanine-fed rats.

Previous studies have demonstrated that phenylpyruvate, a deaminated metabolite of phenylalanine, diminished O_2 consumption by rat brain preparations (Howell & Lee, 1963; Itoh, 1965; Gallagher, 1969). Further, Gallagher (1969) observed

that O_2 uptake was diminished by rat brain mitochondria in the presence of phenylpyruvate only when pyruvate was used as substrate. These observations support a previous study (Kini, 1965), in which phenylpyruvate was shown to inhibit a purified pig brain pyruvate dehydrogenase complex.

The results in the present paper clearly show the inhibition of pyruvate metabolism via another pyruvate-utilizing reaction, namely pyruvate carboxylase, in rat brain mitochondria. Phenylpyruvate, in contrast with phenylalanine, phenylacetate and *o*-hydroxyphenylacetate, decreased the carboxylation of pyruvate by intact rat brain mitochondria (Figs. 1 and 2). Also, rat brain pyruvate carboxylase was inhibited in the presence of phenylpyruvate (Fig. 5). A distinct relationship between the concentration of pyruvate and phenylpyruvate is observed both with the intact mitochondria and for pyruvate carboxylase activity. Scrutton *et al.* (1969) have observed a similar inhibition by phenylpyruvate of a highly purified chicken liver pyruvate carboxylase and that the inhibition occurred at the transcarboxylation step of the pyruvate carboxylase reaction. Further, the K_m and K_i for pyruvate and phenylpyruvate of chicken liver pyruvate carboxylase were 0.44 and 0.48 mM, respectively. These values indicate that a build up of phenylpyruvate in the brain would inhibit the metabolism of pyruvate via pyruvate carboxylase. Although the concentration of phenylpyruvate in brain is not known, it is known that the concentration of phenylpyruvate in blood of fasting untreated phenylketonuric patients is about 0.1 mM and is increased by about fourfold after the ingestion of phenylalanine (Jervis, 1952). The presence of phenylalanine aminotransferase in the brain is known (Benuck *et al.*, 1971), although the rate of phenylpyruvate formation *in vivo* in the brain remains to be investigated. If phenylpyruvate is injected into rats, it is inferred from the seizure-threshold studies that this compound is readily taken up by the brain (Gallagher, 1971; also see Agrawal *et al.*, 1970).

Pyruvate carboxylase synthesizes the C_4 intermediate, oxaloacetate, which can be used for the synthesis of aspartate, glutamate and glutamine *de novo* in the brain. Carver (1965) and Carver *et al.* (1965) observed that the injection of phenylalanine into adult and pregnant rats lowered the concentrations of aspartate, glutamate and glutamine in adult and foetal brains. An inhibitory action of phenylpyruvate on both pyruvate carboxylase and the pyruvate dehydrogenase complex could account for the diminished incorporation of ^{14}C from labelled glucose into amino acids as observed by Itoh (1965).

Several studies have documented that the lipid content is decreased in brains of untreated phenylketonuric patients (Crome *et al.*, 1962; Menkes, 1966; Gerstl *et al.*, 1967). Hyperphenylalaninaemia induced by dietary means has been shown to be

Fig. 4. Effect of phenylalanine and phenylpyruvate on rat brain mitochondrial pyruvate carboxylase activity

The enzyme was assayed as described in the Materials and Methods section except that pyruvate was added at either 10 mM (—) or 0.5 mM (---). Phenylalanine (○) or phenylpyruvate (●) was also added at the concentrations indicated. The results are the means $\pm$ S.E.M. of four to six experiments.

Fig. 5. Effect of phenylpyruvate on the double-reciprocal plot for pyruvate of pyruvate carboxylase

The concentration of pyruvate was varied as indicated. ●, No phenylpyruvate added; o, 5 mM-phenylpyruvate added. Initial velocity (v) was expressed as nmol of $\text{H}^{14}\text{CO}_3^-$ fixed/min per mg of mitochondrial protein at 37°C.

associated with a decreased concentration of lipids in the infant monkey (O'Brien & Ibbott, 1966) and rats (Chase & O'Brien, 1970). Further, hyperphenylalaninaemia decreased the incorporation of [^{14}C]-acetate and [^{14}C]-glucose into brain lipids *in vivo* in rabbits (Barbato *et al.*, 1968) and rats (Shah *et al.*, 1970) respectively. The inhibition by phenylpyruvate of pyruvate carboxylase activity is consistent with a diminished fatty acid synthesis. Large amounts of lipids are deposited in the developing brain. Therefore in untreated phenylketonuric patients, a decrease in biosynthesis of fatty acids and cholesterol *de novo* from pyruvate could play a significant role in a retardation of the cerebral development as well as in the functioning of the brain.

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