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Effect of Hyperphenylalaninaemia on Lipid Synthesis from Ketone Bodies by Rat Brain

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The effect of hyperphenylalaninaemia on the metabolism of ketone bodies *in vivo* and *in vitro* by developing rat brain was investigated. The incorporation *in vivo* of [^{14}C]acetoacetate into cerebral lipids was decreased by both chronic (for 3 days) and acute (for 6h) hyperphenylalaninaemia induced by injecting phenylalanine into 1-week-old rats. In studies *in vitro* it was observed that the incorporation of the radioactivity from [^{14}C]-acetoacetate and 3-hydroxy[^{14}C]butyrate into cerebral lipids was inhibited by phenylpyruvate, but not by phenylalanine. Phenylpyruvate also inhibited the incorporation of ^{3}H from $^{3}H_2O$ into lipids by brain slices metabolizing either 3-hydroxybutyrate or acetoacetate in the presence of glucose. These findings suggest that the decrease in the incorporation *in vivo* of [^{14}C]acetoacetate into cerebral lipids in hyperphenylalaninaemic rats is most likely caused by phenylpyruvate and not by phenylalanine. Phenylpyruvate as well as phenylalanine had no inhibitory effects on ketone-body-catabolizing enzymes, namely 3-hydroxybutyrate dehydrogenase, 3-oxo acid CoA-transferase and acetoacetyl-CoA thiolase, in rat brain. Phenylpyruvate but not phenylalanine inhibited the activity of the 2-oxoglutarate dehydrogenase complex from rat and human brain. These findings suggest that the metabolism of ketone bodies is impaired in brains of untreated phenylketonuric patients, and in turn may contribute to the diminution of mental development and function associated with phenylketonuria.

The chemical analysis of brains from untreated phenylketonuric patients at autopsy has revealed a marked decrease in the lipid content (Crome *et al.*, 1962; Menkes, 1968; Menkes & Aeberhard, 1969). Hyperphenylalaninaemia induced in laboratory animals by dietary means has also been shown to cause a decrease in cerebral lipids (Chase & O'Brien, 1970; Clarke & Lowden, 1969). The incorporation *in vivo* of labelled acetate and glucose into brain lipids is decreased in hyperphenylalaninaemic rabbits (Barbato *et al.*, 1968) and rats (Shah *et al.*, 1970). The synthesis of cholesterol from [^{14}C]mevalonate is diminished by brains from hyperphenylalaninaemic rats (Shah *et al.*, 1969). The decrease in cerebral lipids in hyperphenylalaninaemic rats is largely attributed to their low myelin content (Shah *et al.*, 1972; Johnson & Shah, 1973), which in turn reflects a decrease in lipid synthesis.

During the postnatal period the developing rat brain is able to metabolize glucose and ketone bodies for energy production (for a review see Krebs *et al.*, 1971). Edmond (1974) demonstrated that ketone bodies were readily incorporated *in vivo* into cerebral lipids in 1-week-old rats. In the present paper we describe the effect of phenylalanine and phenylpyruvate on the biosynthesis of cerebral lipids from ketone bodies. The effect of phenylalanine and phenylpyruvate on ketone-body-utilizing enzymes in rat brain and on the 2-oxoglutarate dehydrogenase complex in rat and human brain is also reported.

Materials and Methods

Materials

Chemicals. DL-3-Hydroxy[3-^{14}C]butyrate (sp. radioactivity 1–5mCi/mmol), 2-oxo[1-^{14}C]glutarate (sp. radioactivity 10–15mCi/mmol), [1-^{14}C]acetate (sp. radioactivity 1–3mCi/mmol), [U-^{14}C]glucose (sp. radioactivity 1–5mCi/mmol) and $^{3}H_2O$ (sp. radioactivity 25mCi/g) were purchased from New England Nuclear Corp., Boston, Mass., U.S.A. [3-^{14}C]Acetoacetate was prepared from ethyl[3-^{14}C]-acetoacetate (Krebs *et al.*, 1966; Owen *et al.*, 1973), and was stored at −20°C until used. L-Phenylalanine, phenylpyruvate and thiamin pyrophosphate were from Sigma Chemical Co., St. Louis, Mo., U.S.A. NAD^+, CoA and acetyl-CoA were purchased from P-L Biochemicals, Milwaukee, Wis., U.S.A. 3-Hydroxybutyrate dehydrogenase was obtained from Boehringer Mannheim Corp., New York, N.Y., U.S.A. All other reagents were of the highest purity commercially available.

Animals. Rat pups (5 days old) with their nursing mother (10–12 pups per litter) (Charles River Breeding Laboratories, Wilmington, Mass., U.S.A.) were raised on Wayne Lab-Blok (Allied Mills, Chicago, Ill., U.S.A.) and water *ad libitum*.

Studies in vivo

Experimental hyperphenylalaninaemia. (*a*) Chronic. On the fifth postnatal day each litter was divided into two groups of six pups each. Hyperphenylalaninaemia was induced in one group by injecting intraperitoneally (three times a day for 3 days) 0.5ml of 2.5% phenylalanine in sterile 0.9% NaCl solution. The other group (control) received 0.5ml of 0.9% NaCl three times a day. On the day of the experiment, the last (ninth) injection of phenylalanine was given 1h before the administration of [3-^{14}C]acetoacetate (75×10^5 d.p.m. in 50μl of water per animal) between the scapulae. Animals were killed after 2h. During the last 2h, pups were separated from the mother. (*b*) Acute. Litters (7 days old) were randomly divided into two groups. Acute hyperphenylalaninaemia was induced in one group by injecting intraperitoneally 0.5ml of 2.5% phenylalanine in 0.9% NaCl three times each 2h apart. The control group received 0.5ml of 0.9% NaCl three times by the same route. Immediately after the last injection of phenylalanine or NaCl solution, 50μl of [3-^{14}C]acetoacetate (5×10^5 d.p.m.) was injected into each pup as described above. Pups were then separated from the mother and killed 2h later.

After the pups were killed brains were rapidly removed and divided into two halves. One portion was weighed, homogenized in 5ml of 50mM-potassium phosphate buffer, pH7.4, and centrifuged at 100000*g* for 1h. To a sample (0.5ml) of this supernatant was added 0.1ml of 36% (w/v) $HClO_4$ to remove any $^{14}CO_2$. The acid-stable radioactivity was counted in Diotol scintillation fluid (Herberg, 1960). The other half of the brain was weighed, flattened with a spatula and transferred into 15ml of chloroform/methanol (2:1, v/v) and left overnight. The tissue was shaken vigorously for 1min, and the extracted lipids were separated into non-saponifiable (mainly sterols) and saponifiable (fatty acids) fractions (Folch *et al.*, 1957), and the radioactivity was determined. The results are expressed as d.p.m. of [3-^{14}C]acetoacetate incorporated into the products/2h per g of tissue.

Studies in vitro

Preparation and incubation of brain slices. Animals were decapitated and slices of rat cerebral cortex of about 0.4mm in thickness (average wt. 75mg) were prepared by using a hand-microtome at 25°C (Manjo & Bunker, 1957). Only the first slice was used and was immediately transferred to a 25ml Erlenmeyer flask containing 3ml of oxygenated high-K^+ Krebs–Ringer bicarbonate buffer, pH7.4 (a fivefold increase in KCl replacing a corresponding number of milliequivalents of NaCl) (Berl *et al.*, 1968). The buffer contained labelled substrates and other compounds as indicated in Tables 2–4. The flask was gassed with O_2+CO_2 (95:5) and sealed with a rubber serum stopper equipped with a hanging polyethylene centre well. The flask was placed in a shaking water bath at 37°C for 1h. At the end of the incubation period, 0.3ml of hydroxide of Hyamine-10X was injected into the centre well and 0.5ml of 36% (w/v) $HClO_4$ was injected into the medium to stop the reaction and to ensure complete release of CO_2. After additional shaking for 40min, the contents of the cup were transferred into toluene scintillation fluid (Patel, 1974*b*), and the radioactivity in $^{14}CO_2$ was determined. The tissue was removed and rinsed three times in water and placed in a tube containing 10ml of chloroform/methanol (2:1, v/v). The lipids were separated into non-saponifiable and saponifiable fractions and the radioactivity was determined as described above. In experiments in which ^3H_2O was used, the chloroform/methanol extracts were washed with $CaCl_2$ solution (0.05%) for six to eight times until the aqueous phase became free of radioactivity by contaminating ^3H_2O. The results are expressed as the amount of radioactive substrate incorporated into the products investigated.

Preparation of brain extracts and determination of enzyme activities. Brain homogenates (20%, w/v) were prepared in ice-cold sucrose buffer (0.25M-sucrose / 10mM-Tris / HCl / 1mM-2-mercaptoethanol, pH7.4) by homogenizing at about 500rev./min for 1min in a glass homogenizer fitted with a Teflon pestle. The homogenate was sonicated for two 30s periods at about 60W (model W185, Heat Systems-Ultrasonics, Plainview, N.Y., U.S.A.) and was then centrifuged for 20min at 30000*g* (Williamson *et al.*, 1971). The supernatant fluid was used to determine enzyme activities. The activity of 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30) was measured spectrophotometrically (Smith *et al.*, 1969) and colorimetrically (Williamson *et al.*, 1971; Walker, 1954). An appropriately diluted solution of commercially available preparation of 3-hydroxybutyrate dehydrogenase was also tested to determine the effect of phenylpyruvate on this enzyme (Smith *et al.*, 1969). Activities of acetoacetyl-CoA thiolase (EC 2.3.1.9) and 3-oxo acid CoA-transferase (EC 2.8.3.5.) were determined by measuring the decrease in E_{303} of acetoacetyl-CoA in the presence of CoA or succinate respectively (Williamson *et al.*, 1971).

Mitochondria from rat brain were isolated by the method of Clark & Nicklas (1970). The mitochondria isolated from rat brain by this procedure have been shown to have functional integrity, as judged by the P/O and the respiratory-control ratios (Patel, 1972).

Assay of 2-oxoglutarate dehydrogenase complex. The activity of the 2-oxoglutarate dehydrogenase

complex was measured in brain preparation as described (Patel, 1974*b*). Briefly, the washed mitochondria from rat brain were suspended in a phosphate buffer, pH7.4 (10mM-potassium phosphate/1mM-dithiothreitol/1mM-EDTA), and were subjected to three cycles of freezing and thawing immediately before the activity was assayed. The composition of the reaction mixture is described in the legend of Fig. 1. Incubations were carried out in duplicates in sealed flasks and $^{14}CO_2$ was collected as described above. Adult human brain cortex was obtained at autopsy (within 16h after death) and placed in ice-cold 0.3M-sucrose. Homogenates of brain (20%, w/v) in 0.3M-sucrose were prepared and centrifuged at 100000*g* for 30min. The supernatant was discarded and the pellet was resuspended in the phosphate buffer, pH7.4. The suspension was subjected to three cycles of freezing and thawing immediately before the activity of 2-oxoglutarate dehydrogenase complex was assayed as described above. A munit of activity is defined as 1nmol of 2-oxo[1-^{14}C]glutarate decarboxylated to $^{14}CO_2$/min at 37°C. The specific activity is expressed either per mg of mitochondrial protein or per g wet wt. of tissue. Mitochondrial protein was measured as described by Lowry *et al.* (1951), with crystalline bovine serum albumin as a standard protein.

Results

In experiments *in vivo* chronic hyperphenylalaninaemia caused a significant decrease in the incorporation of [3-^{14}C]acetoacetate into non-saponifiable lipids and fatty acids in the brain (Table 1). This decrease cannot be attributed to an alteration in the availability of labelled substrate in the brain because the availability of radioactivity in the soluble fraction of the brain between the two groups was similar (Table 1). The decrease in the incorporation of labelled acetoacetate into cerebral lipids would be even more marked if the results were expressed on the basis of actual weight. Hyperphenylalaninaemia is known to decrease the growth of the brain (Table 1; Chase & O'Brien, 1970). Acute hyperphenylalaninaemic treatment had no effect, as expected, on the brain weight of animals. This treatment also significantly decreased the incorporation of [3-^{14}C]-acetoacetate into cerebral lipids without altering the amount of radioactivity in the soluble fraction of the brain. An apparent discrepancy in the rate of incorporation of labelled substrate (d.p.m./2h per g) in the two different experiments is simply due to the two different doses of [^{14}C]acetoacetate injected into the pups.

The oxidation of 3-hydroxy[3-^{14}C]butyrate to CO_2 by cerebral-cortex slices from suckling rats was higher than that from adult rats (Table 2). This observation is consistent with the higher activities of ketone-body-utilizing enzymes in brains from suckling rats as compared with adults (Page *et al.*, 1971). The incorporation *in vitro* of [3-^{14}C]acetoacetate and 3-hydroxy[3-^{14}C]butyrate into cerebral lipids by 1-week-old rats was markedly stimulated in the presence of glucose (compare the results for 7-day-old rats in Table 2 with those reported in Table 3). This marked stimulation of lipid synthesis may be attributed to the generation of NADPH via the pentose phosphate pathway. Also the rate of incorporation of labelled ketone bodies into cerebral lipids was the highest at about 7 days postnatally and decreased markedly at the time of weaning (Patel, 1975); the results for adult

Table 1. *Effect of chronic and acute hyperphenylalaninaemia on the biosynthesis of cerebral lipids in vivo from* [3-^{14}C]*acetoacetate by developing rat brain*

Chronic (for 3 days) and acute (for 6h) hyperphenylalaninaemia was induced in 7- or 8-day-old rats by injecting phenylalanine in 0.9% NaCl solution as described in the Materials and Methods section. In chronic and acute experiments about 75×10^5 and 5×10^5 d.p.m. of [3-^{14}C]acetoacetate in 50μl respectively was injected between the scapulae of each animal. After 2h animals were killed; the radioactivity in the cytosol and lipid fractions was determined as described in the Materials and Methods section. The results are the means±S.E.M. for five to eight animals. N.S., Not significant ($P>0.05$).

Treatment	Body wt. (g)	Total brain wt. (mg)	$10^{-2}\times$Radioactivity in the soluble fraction of the brain (d.p.m./g)	$10^{-2}\times$Radioactivity in cerebral lipids (d.p.m./2h per g)	
				Non-saponifiable	Fatty acids
Chronic hyperphenylalaninaemia					
NaCl	15.6±0.3	835±9	292±20	176±10	160±9
Phenylalanine	15.3±0.6	728±8	249±15	115±13	111±9
P	N.S.	<0.001	N.S.	<0.01	<0.005
Acute hyperphenylalaninaemia					
NaCl	16.4±0.2	699±5	6.17±0.45	9.55±0.82	17.38±2.13
Phenylalanine	16.3±0.2	707±7	6.62±0.27	7.12±0.22	10.30±0.29
P	N.S.	N.S.	N.S.	<0.025	<0.025

Table 2. *Effect of phenylalanine and phenylpyruvate on the metabolism of* [3-^{14}C]*acetoacetate and* DL-3-*hydroxy*[3-^{14}C]*butyrate in the absence of glucose to* $^{14}CO_2$ *and lipids by brain slices from rats during postnatal period*

Rat brain slices were prepared and incubated with an appropriate labelled substrate plus other additions for 1 h at 37°C. The collection of $^{14}CO_2$ and the separation of non-saponifiable lipids and fatty acids were carried out as described in the Materials and Methods section. The results are the means ± S.E.M. of five or six animals. *P* values: * <0.025; † <0.005; ‡ <0.001.

Age (days)	Additions: Labelled substrate	Additions: Other compounds (5 mM)	Labelled substrate converted into product (nmol/h per g): CO_2	Non-saponifiable lipids	Fatty acids
7	[3-^{14}C]Acetoacetate (5 mM)	None	4310 ± 243	58 ± 2	79 ± 7
		Phenylalanine	4345 ± 172	57 ± 5	83 ± 6
		Phenylpyruvate	2816 ± 252†	41 ± 2‡	56 ± 4*
7	DL-3-Hydroxy[3-^{14}C]butyrate (10 mM)	None	6717 ± 217	99 ± 8	98 ± 8
		Phenylalanine	6888 ± 270	120 ± 13	93 ± 7
		Phenylpyruvate	5380 ± 144‡	70 ± 12	62 ± 2†
14	DL-3-Hydroxy[3-^{14}C]butyrate (10 mM)	None	8512 ± 509	15.5 ± 1.3	56 ± 3
		Phenylalanine	8604 ± 140	14.3 ± 0.8	64 ± 10
		Phenylpyruvate	5353 ± 475†	9.6 ± 0.6†	36 ± 3†
70 (Adult)	DL-3-Hydroxy[3-^{14}C]butyrate (10 mM)	None	4156 ± 173	—	—
		Phenylalanine	4175 ± 172	—	—
		Phenylpyruvate	3081 ± 185†	—	—

Table 3. *Effect of phenylalanine and phenylpyruvate on the metabolism of labelled acetoacetate, 3-hydroxybutyrate, glucose and acetate to* $^{14}CO_2$ *and lipids by brain slices from 1-week-old rats*

Rat brain slices were prepared and incubated with an appropriate labelled substrate plus other additions for 1 h at 37°C. The collection of CO_2 and the separation of non-saponifiable lipids and fatty acids were carried out as described in the Materials and Methods section. The results are the means ± S.E.M. of five or six animals. *P* values: * <0.05; † <0.01; ‡ <0.005 and § <0.001.

Labelled substrate + other compound	Addition (5 mM)	Labelled substrate converted into product (nmol/h per g): CO_2	Non-saponifiable lipids	Fatty acids
[3-^{14}C]Acetoacetate (2.5 mM) + glucose (5 mM)	None	5373 ± 93	160 ± 6	383 ± 13
	Phenylalanine	5555 ± 260	176 ± 4	364 ± 17
	Phenylpyruvate	3805 ± 72§	131 ± 7†	294 ± 15§
DL-3-Hydroxy[3-^{14}C]butyrate (5 mM) + glucose (5 mM)	None	4193 ± 222	129 ± 5	360 ± 18
	Phenylalanine	3726 ± 274	119 ± 9	319 ± 16
	Phenylpyruvate	2849 ± 263‡	71 ± 5§	270 ± 17†
[U-^{14}C]Glucose (5 mM)	None	5278 ± 165	146 ± 4	286 ± 5
	Phenylalanine	5292 ± 74	142 ± 8	223 ± 10§
	Phenylpyruvate	5019 ± 176	105 ± 6§	233 ± 13‡
[1-^{14}C]Acetate (5 mM) + glucose (5 mM)	None	1018 ± 34	90 ± 8	381 ± 15
	Phenylalanine	999 ± 46	70 ± 5	341 ± 17
	Phenylpyruvate	974 ± 38	58 ± 3‡	335 ± 14*

rats are not shown, because of negligible rates of incorporation (Table 2). Table 3 shows that both ketone bodies are better precursors for cerebral lipogenesis in developing rat brain than is glucose.

Phenylpyruvate at a concentration of 5 mM in the incubation medium significantly decreased the oxidation to CO_2 and the incorporation into cerebral lipids of labelled ketone bodies in both the absence (Table 2) and the presence (Table 3) of glucose, and at all ages tested (Table 2). On the contrary, phenylalanine at the same concentration had no effect on the metabolism of ketone bodies by rat brain slices. The oxidation of [U-^{14}C]glucose and [1-^{14}C]acetate to CO_2 was not affected by phenylpyruvate, whereas their incorpora-

Table 4. *Effect of phenylalanine and phenylpyruvate on the incorporation of ^3H from ^3H_2O into lipids by brain slices from 1-week-old rats*

Rat brain slices were prepared and incubated with appropriate unlabelled substrates plus other additions for 1h at 37°C. Each flask contained about 0.6mCi of ^3H_2O. The results are reported for the total lipids (non-saponifiable lipids plus fatty acids) and are the means±S.E.M. of five to seven animals. *P* values: * <0.05; † <0.01.

Unlabelled substrates	Addition (5mM)	^3H incorporated into the total lipids (ng-atoms/h per g)
Acetoacetate (2.5mM)+glucose (5mM)	None	3346± 76
	Phenylalanine	3500±202
	Phenylpyruvate	2924±133*
DL-3-Hydroxybutyrate (5mM)+glucose (5mM)	None	3404±182
	Phenylalanine	3236± 64
	Phenylpyruvate	2748± 77†

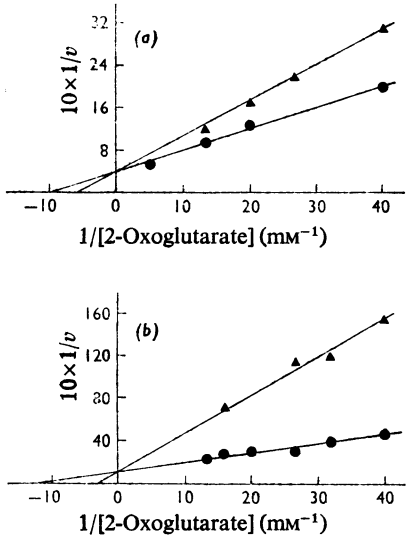

Fig. 1. *Lineweaver–Burk plots of the effect of phenylpyruvate on the activity of 2-oxoglutarate dehydrogenase complex from adult rat (a) and human (b) brains*

The reaction mixture contained, in a final volume of 1 ml: 11mM-potassium phosphate buffer, pH7.4; 1.1mM-dithiothreitol; 1.1mM-EDTA; 3mM-$MgCl_2$; 0.1mM-thiamin pyrophosphate; 0.2mM-CoA; 2mM-NAD^+ and about 0.3mg of mitochondrial protein. 2-Oxo[1-^{14}C]-glutarate (sp. radioactivity 50d.p.m./nmol) was varied as indicated. Other additions were: ●, none; ▲, phenylpyruvate (5mM for human brain and 1mM for rat brain). The activity of 2-oxoglutarate dehydrogenase complex in mitochondria from adult rat brain and in crude particulate preparation from adult human brain (autopsy specimen) was assayed as described. A munit of activity is defined as 1nmol of 2-oxo[1-^{14}C]glutarate decarboxylated to $^{14}CO_2$/min per mg of mitochondrial protein for rat brain and g wet wt. for human brain at 37°C.

tion into cerebral lipids was markedly inhibited by this oxo acid (Table 3). Again, phenylalanine had no effect on the metabolism of labelled glucose and acetate.

Previous studies (Foster & Katz, 1966; Jungas, 1968) have shown that the incorporation of ^3H from ^3H_2O is proportional to the total rate of lipogenesis by rat adipose tissue. We have used this approach to measure the total rate of lipid synthesis from ketone bodies in the presence of either phenylalanine or phenylpyruvate. Phenylpyruvate, but not phenylalanine, inhibited the incorporation of ^3H from ^3H_2O into lipids by brain slices metabolizing either acetoacetate or 3-hydroxybutyrate in the presence of glucose (Table 4). These findings suggest that the availability of the acetyl moieties for lipogenesis may be limiting in the presence of phenylpyruvate.

In preliminary experiments we observed that both phenylalanine and phenylpyruvate had no inhibitory effect on the activities of 3-hydroxybutyrate dehydrogenase, 3-oxo acid CoA-transferase and acetoacetyl-CoA thiolase (soluble plus mitochondrial) in adult rat brain (results not shown). Because phenylpyruvate interfered with the assay of activity of 3-hydroxybutyrate dehydrogenase by the colorimetric assay of Walker (1954), a commercially available 3-hydroxybutyrate dehydrogenase preparation was assayed spectrophotometrically by the method of Smith *et al.* (1969). With this approach, no effect of phenylpyruvate was noted on this enzyme.

As shown in Fig. 1, K_m values for 2-oxoglutarate of the 2-oxoglutarate dehydrogenase complex from human and rat brain preparations were 85 and 100μM respectively. These findings are similar to those reported by Patel (1974*b*). Phenylpyruvate competitively inhibited the 2-oxoglutarate dehydrogenase complexes from adult human and rat brains, with an apparent K_i of about 1.6mM. Phenylpyruvate also

inhibited in a similar manner the activity of this enzyme from human foetal brain and 2-week-old rat brain (results not shown).

Discussion

During the postnatal period the developing mammalian brain rapidly metabolizes both glucose and ketone bodies (for a review see Krebs *et al.*, 1971). In fact, the rate of metabolism of ketone bodies at a given concentration is greater in the developing rat brain than in that of the adult (Hawkins *et al.*, 1971). This is presumably due to the higher activities of ketone-body-utilizing enzymes in brains from developing rats (Page *et al.*, 1971). Edmond (1974) reported that ketone bodies are incorporated into cerebral lipids *in vivo* in 1-week-old rats. Our data extend (Tables 1 and 3) this observation and show that the biosynthesis of lipids in developing brains is greater with ketone bodies than with glucose.

Previous studies have shown that hyperphenylalaninaemia induced either *in vitro* (Weber *et al.*, 1970) or *in vivo* (Miller *et al.*, 1973) in rats diminished cerebral glycolysis by inhibiting hexokinase and pyruvate kinase. The transport of pyruvate across the inner mitochondrial membrane in rat brain mitochondria (Land & Clark, 1974; Halestrap *et al.*, 1974) and its metabolism via pyruvate carboxylase in rat and human brain preparations (Patel, 1972; Patel *et al.*, 1973) have been shown to be inhibited by phenylpyruvate (for a review see Patel & Arinze, 1975). These and other findings (Shah *et al.*, 1970) clearly show that the metabolism of glucose is impaired in brains of hyperphenylalaninaemic rat. A decrease in energy metabolism due to this impairment may be overcome in the developing brain by an increase in ketone-body oxidation. However, the findings reported in the present paper show that the metabolism of ketone bodies *in vivo* is also impaired in brains of hyperphenylalaninaemic rats (Table 1). Further, in studies *in vitro* we have demonstrated that it is phenylpyruvate, not phenylalanine, that exerts this inhibition (Tables 2–4). A decrease in the incorporation of [3-^{14}C]acetoacetate into cerebral lipids in chronically induced hyperphenylalaninaemic rats may have resulted from (i) a decrease in the activity of one or more key enzymes on the pathway of lipogenesis owing to diminished cerebral protein synthesis (Agrawal *et al.*, 1970; Swaiman *et al.*, 1968), and/or (ii) an inhibition of one or more enzyme activities, as discussed below. However, in acute hyperphenylalaninaemic rats (Table 1) and in experiments *in vitro* (Tables 2 and 3) the inhibition of cerebral lipid synthesis from labelled ketone bodies was mostly due to the inhibition of the activity of the enzyme(s) by phenylpyruvate.

Previous studies (Patel, 1972; Patel *et al.*, 1973; Land & Clark, 1973) have shown that several enzymes involved in the biosynthesis of fatty acids from pyruvate in the brain are inhibited by phenylpyruvate. This includes the mitochondrial enzymes, namely pyruvate carboxylase and citrate synthase, and the cytosolic enzyme, fatty acid synthetase. These enzymes are also involved in the incorporation of the acetyl moiety formed in the mitochondria from ketone bodies into long-chain fatty acids synthesized *de novo* in the cytosol. The inhibition by phenylpyruvate of the 2-oxoglutarate dehydrogenase complex from rat and human brains as reported here (Fig. 1) is of some interest. In the mitochondria, acetoacetate is converted into acetoacetyl-CoA by the action of 2-oxo acid CoA-transferase in the presence of succinyl-CoA. The latter is formed from 2-oxoglutarate by the 2-oxoglutarate dehydrogenase complex, and hence the inhibition of this enzyme by phenylpyruvate may decrease the metabolism of ketone bodies.

Shah *et al.* (1968, 1969) observed that the biosynthesis of sterols from [^{14}C]mevalonate is inhibited by phenylpyruvate. Our present data (Tables 1–3) and those of Edmond (1974) demonstrated that ketone bodies are suitable precursors for the synthesis of sterols in the developing rat brain. Because phenylpyruvate markedly inhibits the incorporation of ^{14}C-labelled ketone bodies into cerebral fatty acids and sterols, it is suggested that phenylpyruvate exerts its inhibitory effects at several points on these pathways. The common pathway for the flow of ketone-body carbon into these two pathways in the cytosol is up to the point at which the generation of the acetyl moiety occurs. An inhibition by phenylpyruvate up to this portion of the pathway should have an adverse effect on both pathways. This is consistent with the inhibitory effect of phenylpyruvate on the transport of 3-hydroxybutyrate across the inner mitochondrial membrane in rat brain mitochondria (Land & Clark, 1974) and on one or more mitochondrial enzymes as mentioned above (Fig. 1; Patel, 1972; Land & Clark, 1973). In addition to this, phenylpyruvate can also exert separate inhibitory effects on the remainder of the pathways distal to the branching point, for example, the inhibition of the fatty acid synthetase complex by phenylpyruvate (Land & Clark, 1973). It is possible that phenylpyruvate decreases the formation of a common precursor(s) needed for the biosynthesis of both fatty acids and sterols. One such likely compound is NADPH. The pentose phosphate pathway and the cytosolic $NADP^+$–malate dehydrogenase supply the reducing equivalents (NADPH) required for the reductive biosynthesis of these lipids (Flatt & Ball, 1966; Hanson & Ballard, 1968; Patel, 1974*a*). Phenylpyruvate inhibits brain 6-phosphogluconate dehydrogenase (EC 1.1.1.44) (Weber *et al.*, 1970) and both the cytoplasmic and mitochondrial $NADP^+$–malate dehydrogenases (EC 1.1.1.40) (M. S. Patel, unpublished work). A decrease by phenylpyruvate in the generation and the incorpora-

tion of acetyl-CoA from ketone bodies and/or in NADPH in the cytosol is consistent with findings reported here.

Our findings show that hyperphenylalaninaemia impairs the metabolism of ketone bodies for energy generation and lipid biosynthesis in developing rat brain. It is suggested that similar impairment in cerebral ketone-body metabolism in untreated phenylketonuric patients may, in part, contribute to mental retardation associated with phenylketonuria.

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