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BRAIN UPTAKE AND PROTEIN INCORPORATION OF AMINO ACIDS STUDIED IN RATS SUBJECTED TO PROLONGED HYPERPHENYLALANINAEMIA

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Abstract—The uptake into brain and the incorporation into brain protein of intraperitoneally administered, labelled amino acids has been studied in myelinating rats during prolonged hyperphenylalaninaemia maintained by administration of *p*-chlorophenylalanine. Compared with controls, there was a 50% reduction in both uptake and incorporation into protein of leucine and a parallel reduction in the acid-soluble leucine pool. With glycine and lysine no such changes were observed. On the other hand, when each of the three amino acids was injected directly into the brain, the only significant differences observed between controls and hyperphenylalaninaemic animals were again with leucine, which showed an increased incorporation into protein and an increased specific activity in the otherwise reduced acid-soluble pool.

It is concluded that hyperphenylalaninaemia reduces the rate of transport of leucine into the brain and hence reduces the brain pool of leucine, but that any effects on protein synthesis are small. The validity of the model, and the implications of the findings, in relation to phenylketonuria, are discussed.

IN CLASSICAL phenylketonuria, the biochemical defect has been identified as the diminished activity of the liver enzyme, phenylalanine hydroxylase (EC 1.14.16.1) and most of the biochemical manifestations of the disease can be accounted for by this deficiency. Although the irreversible brain damage, which has been associated with a defect of myelination, can be moderated by dietary control of blood levels of phenylalanine during infancy, the mechanism which links the high phenylalanine level to this brain damage is still unresolved. One of the difficulties in studying this problem has been the lack of a suitable model to simulate phenylketonuria. Phenylalanine or metabolites known to be elevated in phenylketonuria have been shown to inhibit brain enzymes *in vitro*. However, the concentrations used have usually been much higher than their probable brain concentration *in vivo*.

AGRAWAL *et al.* (1970) showed that animals treated with a single load of phenylalanine had impaired incorporation of the amino acids leucine and methionine into brain protein. This finding could account for the reduced myelination if the production of enzymes responsible for synthesis of the lipids of myelin was reduced, or the synthesis of the structural proteins of myelin was inhibited. However, the short-term response of a normal animal to a single acute dose of phenylalanine may be very different from the situation which prevails in phenylketonuria, where the patient is subjected to prolonged exposure to hyperphenylalaninaemia and so may have an adapted response. Furthermore, simple phenylalanine loading leads to elevated tyrosine levels and to production of metabolites of tyrosine in excess of normal, which is the opposite of the phenylketonuric situation.

We have been concerned to produce an animal showing a sustained high level of blood phenylalanine and with a reduced tolerance to a phenylalanine load similar to phenylketonuric patients, rather than showing the rapid clearance observed in normals. An additional criterion is that the animal should have a near normal blood tyrosine level. In a recently described model (ANTONAS *et al.*, 1974; COPENHAVER *et al.*, 1973), rats were treated for several days with a regime of *p*-chlorophenylalanine and phenylalanine. The animals so obtained showed prolonged hyperphenylalaninaemia with low tyrosine levels when challenged with a phenylalanine load. As we show in this present work, they also have elevated brain phenylalanine levels with near normal tyrosine levels. We also report results on the incorporation of various amino acids into the brain proteins of our experimentally induced phenylketonuric rats.

The results of AGRAWAL *et al.* (1970) did not show conclusively whether the raised phenylalanine level impaired the uptake of amino acids into the brain or interfered directly with protein synthesis. However, MCKEAN *et al.* (1968) have shown that for a period of 30–60 min after injection with high doses of phenylalanine the concentration of a number of the large hydrophobic amino acids, including leucine, in the brain is much reduced, while the phenylalanine is elevated. Furthermore, OLDENDORF (1973), observing the effect of a single passage through the brain circulation, showed that the rate of uptake of the large neutral amino acids, including leucine, into brain is reduced by the presence of high levels of added phenylalanine in the circulating blood, an effect which was also elicited by passage of human phenylketonuric serum. This suggests a direct effect on amino

acid transport. In this present study, we have investigated the incorporation of amino acids administered both directly into the brain and also intraperitoneally, and from our results we conclude that the impaired incorporation of amino acids into brain protein results from the impaired uptake of amino acids into the brain, and not from any direct interference with protein synthesis.

MATERIALS AND METHODS

Experimental designs. Wistar rats of either sex, age 14 days at the start of the experiment, were used. Each litter, together with the dam was kept in a separate cage and allowed food and water *ad lib.* until killed. Experimentally induced phenylketonuric rats were produced by the method previously described (ANTONAS *et al.*, 1974) i.e. 4 days treatment with 300 mg/kg *p*-chlorophenylalanine + 200 mg/kg L-phenylalanine. Phenylalanine loads administered 1 h prior to the radioactive amino acid were 500 mg/kg L-phenylalanine. In all cases, control groups received injections of equal volumes of saline.

In the following experiments, the effects of the injection of radioactive amino acids into 4 groups of animals were compared:

Group (*a*) Control rats which had had only saline injections on the 4 preceding days and which were given only saline instead of a phenylalanine load.
Group (*b*) Control rats treated as in (*a*) but given a phenylalanine load 1 h before administration of the radioactive amino acid.
Group (*c*) Rats treated for 4 preceding days with *p*-chlorophenylalanine and phenylalanine and then on the day of the experiment given only saline instead of phenylalanine load.
Group (*d*) Rats treated as in (*c*) but given a phenylalanine load 1 h before administration of the radioactive amino acid.

In the first series of experiments, the radioactive amino acids were injected intraperitoneally and the animals killed *three hours* later. In the second series, the radioactive amino acids were injected directly into the lateral ventricle of the brain (by the method of NOBLE *et al.*, 1967) and the animals killed after only *1 hour*. For the intraperitoneal administration, in one such set of 4 groups of rats, each animal was given 1·125 μCi of 318 μCi/μM L[U-^{14}C]leucine in 250 μl. In another set, each rat was given 1·125 μCi of 311 μCi/μM L[U-^{14}C]lysine in 250 μl, and in a third set each rat received 1·125 μCi of 114 μCi/μM of [U^{14}C]-glycine in 250 μl. For the intraventricular administration, the doses were reduced to 0·5 μCi for each amino acid at the same specific activity as above, dissolved in 10 μl. These latter doses represent less than 2·5% of the brain pool of the amino acid concerned (HIMWICH & AGRAWAL, 1969).

At the appropriate time the animals were killed (see above) and the total counts in the homogenate, the counts in the protein, and the total brain DNA determined. In the second series, the brains were halved along the median plane and the right and left halves processed separately, to verify that the injection had entered the ventricle and consequently been equally distributed. The figures for the two halves were added to give the value for the whole brain. Three animals each from groups (*b*) and (*d*) which had not been treated with radioactive amino acid were killed before and at 1, 2, 3 and 4 h after loading with phenylalanine. Their brain homogenates were pooled and their phenylalanine and tyrosine content determined.

Analyses of brain. To determine the amount of radioactive amino acid incorporated, animals were killed by fracturing the neck and the brains were rapidly removed and weighed. The brains were homogenized in 3 ml of 5% (w/v) ice-cold trichloracetic acid in a Potter glass homogenizer with a Teflon pestle. Samples of the homogenate were taken for their radioactive content to be determined. The precipitate was washed twice with 2 ml of 5% (w/v) trichloracetic acid and the supernatant fluids were pooled. The pooled supernatant fraction was then extracted 3 times with 2 vol of ether and the residual aqueous layer was then evaporated to dryness. The amino acids were extracted from the residue with 3 portions of 2 ml of acetone containing 5% (v/v) 5 M-HCl. The pooled acetone extracts were then carefully evaporated down to approx 0·1 ml, and the solution made up to 1·0 ml in a volumetric flask with acetone. A portion of this solution was used for the separation of the amino acids.

Recovery of radioactive leucine added to homogenates and carried through this procedure to this point was nearly 100%. Leucine was separated by 2-dimensional paper chromatography in the following solvent systems: *n*-butanol: acetic acid:water (12:3:5, by vol), then methanol:water: pyridine (4:1:2, by vol). Lysine was separated with *n*-butanol:pyridine:water (1:1:1 by vol) followed by a phenol solvent (500 g phenol:125 ml water having 1 ml of 0·88 ammonia added per 200 ml). Glycine was separated by high voltage electrophoresis in 8·5% formic acid, pH 1·8, followed by chromatography in butanol:acetic acid:water (12:3:5 by vol).

The amino acids were located on the chromatograms by spraying with fluorescamine as described by FELIX & JIMENEZ (1974). The spots of the appropriate amino acid were excised and eluted into 2·0 ml of sodium borate buffer (0·2 M at pH 9·0) and a sample used for the determination of the amino acid with fluorescamine (UDENFRIEND *et al.*, 1972). The remainder of the eluate was transferred to filter paper collars in counter vials, dried, and their radioactive content determined in a Packard Tri-Carb Counter, after the addition of liquid scintillator. Counting efficiences were determined by the method of BUSH (1963) and from the above data specific activities were then calculated.

The trichloracetic acid-insoluble precipitate was extracted for 20 min at 90°C with 3·0 ml of 5% (w/v) trichloracetic acid (further extraction with hot trichloracetic failed to extract any further significant counts). After centrifugation, the DNA content of this supernatant fluid was determined by the method of BURTON (1956). The hot trichloracetic acid-insoluble protein pellet was dissolved in 3·0 ml of M-NaOH, and a sample removed for the determination of radioactive content. To determine the specific activity of the incorporated amino acid, concentrated hydrochloric acid was added to 1·0 ml of this alkaline protein solution in a pyrex ampoule to a final concentration of 6·0 M with respect to HCl. The ampoule was sealed and heated at 105°C for 24 h. The contents were then evaporated to dryness and 1 ml of 80% (v/v) ethanol/water added.

The specific activities of the amino acids in these protein hydrolysates were then determined in the same way as for the soluble amino acids described above. The phenylalanine content of the first trichloracetic acid supernatant of brain was determined as follows. Phenylalanine and *p*-chlorophenylalanine were separated by TLC on cellulose plates (the solvent used was the upper phase of amyl alcohol:acetic acid:water 180:15:60, w/v/v). The cellulose from

TABLE 1. EFFECT OF PHENYLALANINE LOADING ON BRAIN AMINO ACID UPTAKE AND INCORPORATION INTO PROTEIN. INTRAPERITONEAL INJECTION: RESULTS EXPRESSED AS d.p.m./μg DNA. MEAN VALUES ± S.D. (NO. OF ANIMALS)

Precursor [U-^{14}C]Amino acid injected	Incorporated into	Saline pretreated		PCPA pretreated	
		Saline controls	Phenylalanine loaded	Saline controls	Phenylalanine loaded
Leucine	Homogenate	22·8 ± 8·3(7)	16·3 ± 4·7(9)*	13·8 ± 5·1(9)	8·9 ± 2·0(8)*
	Protein	15·0 ± 4·6(7)	11·0 ± 3·3(9)*	9·6 ± 2·5(9)	6·5 ± 1·8(8)†
Lysine	Homogenate	12·4 ± 1·5(9)	12·1 ± 2·4(9)	12·0 ± 2·5(5)	11·7 ± 3·7(9)
	Protein	7·8 ± 1·8(9)	7·9 ± 2·1(9)	8·3 ± 1·5(5)	7·7 ± 2·0(9)
Glycine	Homogenate	4·0 ± 0·9(9)	4·8 ± 2·2(8)	3·6 ± 0·6(9)	3·5 ± 0·8(8)
	Protein	1·9 ± 0·5(9)	2·0 ± 0·6(8)	1·5 ± 0·2(9)	1·5 ± 0·4(8)

P values: * <0·05, † <0·01.

TABLE 2. EFFECT OF PHENYLALANINE LOADING ON CONTENT AND SPECIFIC ACTIVITY OF LABELLED AMINO ACID IN ACID SOLUBLE FRACTION OF BRAIN. INTRAPERITONEAL ADMINISTRATION: RESULTS REPORTED ARE MEANS OF TRIPLICATE DETERMINATION ON POOLED SAMPLES

Precursor [U-^{14}C]Amino acid injected	Saline pretreated		PCPA pretreated	
	Saline controls	Phenylalanine loaded	Saline controls	Phenylalanine loaded
Leucine				
Content (μM/g Brain)	0·492	0·338	0·417	0·294
Specific Activity (d.p.m./nM)	94·4	40·5	23·0	21·3
Lysine				
Content (μM/g Brain)	0·208	0·187	0·211	0·271
Specific Activity (d.p.m./nM)	20·0	17·8	14·8	15·5
Glycine				
Content (μM/g Brain)	3·40	3·68	3·37	3·50
Specific Activity (d.p.m./nM)	30·6	27·78	29·3	33·3

TABLE 3. SPECIFIC RADIOACTIVITY OF AMINO ACIDS IN BRAIN PROTEIN INTRAPERITONEAL INJECTION: RESULTS EXPRESSED AS d.p.m./nM AMINO ACID. MEAN VALUES ± S.D. (n = 3)

Precursor [U-^{14}C]Amino acid injected	Saline pretreated		PCPA pretreated	
	Saline control	Phenylalanine loaded	Saline control	Phenylalanine loaded
Leucine	115·9 ± 22·1	94·6 ± 6·8†	88·5 ± 18·3	53·4 ± 9·9*
Lysine	15·7 ± 3·4	14·2 ± 4·6	15·0 ± 6·1	17·1 ± 3·4
Glycine	6·7 ± 1·5	6·4 ± 0·8	5·8 ± 1·2	6·0 ± 2·13

P values: * <0·05, † <0·01.

the appropriate area of the plate was recovered and eluted, and the phenylalanine content determined fluorimetrically by the method of MCCAMAN & ROBINS (1962). Tyrosine in these extracts was determined by the method of WONG *et al.* (1964).

RESULTS

In the case of intraperitoneally administered leucine there was a highly significant fall in the uptake of radioactivity into the brain, and also in the incorporation into brain protein (Table 1), in the animals treated with *p*-chlorophenylalanine and loaded with phenylalanine (group *d*), compared with the untreated controls (group *a*) or with the *p*-chlorophenylalanine treatment alone (group *c*). The *p*-chlorophenylalanine treatment also produced lowered uptake and incorporation, compared with controls (group *a*). From Tables 2 and 3, it can be seen that the reduced uptake and incorporation of leucine in the treated animals was parallelled by a similar reduction in both the size of the free pool of leucine and the specific activity of leucine in both the free pool and the brain protein.

These results also show that adaption to the hyperphenylalanine has not occurred; indeed prolonged pretreatment with *p*-chlorophenylalanine has enhanced the effect of a phenylalanine load as compared with control animals given a phenylalanine load (group *b*). With [^{14}C]lysine there was no difference between any of the groups of animals; and although the mean counts were 10% lower in group *d* when [^{14}C]glycine was administered, this was not statistically significant. One striking feature in these results is the close parallel which exists between the amount of radioactive label (d.p.m.) in the total homogenate and the label incorporated into protein

TABLE 4. EFFECT OF PHENYLALANINE LOADING ON BRAIN ACID UPTAKE AND INCORPORATION INTO PROTEIN: INTRAVENTRICULAR INJECTION: RESULTS EXPRESSED AS d.p.m./μg DNA. MEAN VALUES ± S.D. (NO. OF ANIMALS)

Precursor [U-^{14}C]Amino acid injected	Incorporated into	Saline pretreated		PCPA pretreated	
		Saline controls	Phenylalanine loaded	Saline controls	Phenylalanine loaded
Leucine	Homogenate	81·7 ± 20·6(8)	110·4 ± 11·0(8)*	100·0 ± 29·9(7)	131·1 ± 37·0(6)
	Protein	43·3 ± 15·0(8)	59·9 ± 8·9(8)	50·0 ± 14·3(7)	69·5 ± 16·3(6)*
Lysine	Homogenate	116·8 ± 18·9(8)	118·4 ± 23·8(9)	122·2 ± 7·4(6)	115·0 ± 26·6(7)
	Protein	68·6 ± 7·8(8)	68·6 ± 14·0(9)	66·2 ± 10·0(6)	60·7 ± 15·5(7)
Glycine	Homogenate	301·7 ± 44·5(5)	327·8 ± 68·2(7)	342·9 ± 79·5(8)	332·9 ± 70·9(8)
	Protein	76·2 ± 11·9(5)	69·3 ± 7·8(7)	79·5 ± 22·5(8)	81·9 ± 15·7(8)

P value: * <0·05.

TABLE 5. EFFECT OF PHENYLALANINE LOADING ON CONTENT AND SPECIFIC ACTIVITY OF LABELLED AMINO ACID IN ACID SOLUBLE FRACTION OF BRAIN. INTRAVENTRICULAR ADMINISTRATION: RESULTS REPORTED ARE MEANS OF TRIPLICATE DETERMINATION ON POOLED SAMPLES

Precursor [U-^{14}C]Amino acid injected	Saline pretreated		PCPA pretreated	
	Saline controls	Phenylalanine loaded	Saline controls	Phenylalanine loaded
Leucine				
Content (μM/g Brain)	0·402	0·260	0·386	0·218
Specific Activity (d.p.m./nM)	194·0	279·0	280·0	439·7
Lysine				
Content (μM/g Brain)	0·218	0·195	0·21	0·244
Specific Activity (d.p.m./nM)	129·3	143·2	144·5	116·3
Glycine				
Content (μM/g Brain)	3·7	3·43	3·18	3·51
Specific Activity (d.p.m./nM)	57·81	39·3	40·6	41·8

TABLE 6. SPECIFIC RADIOACTIVITY OF AMINO ACIDS IN BRAIN PROTEIN. INTRAVENTRICULAR INJECTION: RESULTS EXPRESSED AS d.p.m./nM AMINO ACID. MEAN VALUES ± S.D. (n = 3)

Precursor [U-^{14}C]Amino acid injected	Saline pretreated		PCPA pretreated	
	Saline control	Phenylalanine loaded	Saline control	Phenylalanine loaded
Leucine	101·0 ± 55·8	140·7 ± 60·6	117·8 ± 31·1	133·8 ± 28·3
Lysine	125·8 ± 30·1	130·0 ± 43·8	127·2 ± 15·7	123·8 ± 13·8
Glycine	221·1 ± 61·3	294·4 ± 53·3	237·1 ± 63·9	287·7 ± 24·0

for each amino acid (Table 1). This suggests that the effect of hyperphenylalaninaemia is on the amino acid uptake by the brain rather than on protein synthesis. For this reason, the second series of experiments were carried out, when the amino acids were administered directly into the ventricle. These results are presented in Table 4.

It is clear that there is no change in retention of radioactivity by the brain or of incorporation into brain protein of either [^{14}C]glycine or [^{14}C]lysine, when rats are given loading doses of phenylalanine either with or without pretreatment with *p*-chlorophenylalanine. Furthermore, treatment with *p*-chlorophenylalanine alone had no effect. In the experiment with [^{14}C]leucine, there was a large increase both in the retention of radioactivity in the brain and the incorporation of radioactivity into the brain protein in the experimental animals which received the phenylalanine load. The measurements of the free pool of these amino acids confirms that, as might have been suspected from the intraperitoneal experiments, there is a reduced pool of leucine in the hyperphenylalaninaemic animals (Table 5), which presumably arises because of an impaired transport of leucine into the brain. The smaller leucine pool results in less dilution of the intraventricularly administered leucine, leading to the increase specific activity of this amino acid in the free pool. As the smaller pool would be expected on kinetic grounds to have a lower turnover, this probably accounts for the greater retention of [^{14}C]leucine in the free pool. The higher specific activity of the free leucine would lead to a greater incorporation of radioactivity into the brain protein if the rate of synthesis of protein was unchanged, or even if it was somewhat reduced, in which case an increase of specific activity of the incorporated leucine would be expected.

Such an increase in specific activity of leucine in

TABLE 7. EFFECT OF ROUTE OF ADMINISTRATION OF U-^{14}C-LABELLED AMINO ACID: RATIO d.p.m. IN PROTEIN/d.p.m. TOTAL HOMOGENATE. MEAN VALUES ± S.D. (NO. OF ANIMALS)

Precursor [U-^{14}]Amino acid injected	Injection site	Saline pretreated		PCPA pretreated	
		Saline controls	Phenylalanine loaded	Saline controls	Phenylalanine loaded
Leucine	Intraperitoneal	0·66 ± 0·07(7)	0·67 ± 0·15(9)	0·70 ± 0·13(9)	0·71 ± 0·09(8)
	Intraventricular	0·54 ± 0·05(8) ‡	0·53 ± 0·14(8) *	0·50 ± 0·04(7) ‡	0·53 ± 0·02(6) ‡
Lysine	Interperitoneal	0·63 ± 0·12(9)	0·65 ± 0·13(9)	0·70 ± 0·08(5)	0·67 ± 0·13(9)
	Intraventricular	0·59 ± 0·05(8)	0·58 ± 0·05(9)	0·54 ± 0·08(6) †	0·53 ± 0·08(7) †
Glycine	Intraperitoneal	0·46 ± 0·07(9)	0·46 ± 0·12(8)	0·42 ± 0·04(9)	0·44 ± 0·02(8)
	Intraventricular	0·25 ± 0·03(5) ‡	0·22 ± 0·06(7) ‡	0·23 ± 0·05(8) ‡	0·25 ± 0·02(8) ‡

P Values: * <0·05; † <0·01; ‡ <0·001.

the brain protein was observed (Table 6), but the S.D. of these results was large and the increase is therefore not highly significant. From Table 6 it can be seen that there was also an apparent (though not very significant) increase in the specific activity of the glycine in the brain protein in the phenylalanine loaded animals which is not consistent with the unchanged uptake or incorporation of radioactivity in these experiments or with the size or specific activity of the free pool.

DISCUSSION

The results show that the uptake and incorporation into protein of glycine and lysine is not affected by hyperphenylalaninaemia, whereas intraperitoneally-administered leucine has a lowered transport into the brain, a lower free pool within the brain, and a lower incorporation of radioactive leucine into brain protein. Intraventricular administration of leucine results in greater retention of radioactive leucine in the reduced pool, and a somewhat greater incorporation of radioactivity into brain protein in the hyperphenylalaninaemic animals. These results show that the effect of hyperphenylalanaemia is not primarily on protein synthesis in the brain but on the rate of transport of leucine (and possibly other large hydrophobic amino acids) into the brain. This is strongly reinforced by the fact that the ratio of protein d.p.m./total homogenate d.p.m. (Table 7) is remarkably constant for each amino acid for a given route of administration. There is, however, a consistently higher ratio of protein d.p.m./total homogenate d.p.m. for a given amino acid administered by the intraperitoneal route than when administered by the intraventricular route. This is probably due to the fact that in the intraventricular series only 1 h elapsed between administration and killing as against 3 h when the intraperitoneal route was used. Thus, radioactivity which has been incorporated into protein is held in the brain, whereas the excess free amino acid and its metabolites will be cleared from the system, so that the ratio of protein d.p.m./total homogenate d.p.m. would be expected to rise with time. The difference between the ratios was most marked for glycine, which probably reflects the greater metabolic utilization of this amino acid.

In rats treated with *p*-chlorophenylalanine but with no phenylalanine load (group *c*), the lowered incorporation of intraperitoneally administered leucine into brain protein was closely matched by a lowered retention in the homogenate. Also on this same treatment, when leucine was administered by the intraventricular route, there was increased retention of counts in the homogenate, though the apparent increase in incorporation into protein was not significant. Again, these results suggest that the main effect is upon transport, which is not inconsistent, as we find that rats on this treatment have a significant hyperphenylalaninaemia even 24 h after the last administration of treatment. However, the failure to observe a significant increase in incorporation into protein or specific activity of incorporated leucine on the intraventricular administration of leucine, in spite of increased retention in the homogenate, indicates that the possibility that *p*-chlorophenylalanine has some effect on protein synthesis cannot be completely ruled out. GAL *et al.* (1970) have shown that *p*-chlorophenylalanine is apparently incorporated into brain protein, and this could lead to impairment of enzyme function and so to reduction of protein synthesis. However, no comparable impairment of incorporation of either lysine or glycine into protein was observed in these animals, which would have been expected from a general effect on protein synthesis; indeed, in the case of glycine administered intraventricularly, there was an apparent increase in specific activity of protein incorporated glycine in the phenylalanine loaded animals.

The reduced pool size of leucine is in accordance with the observations of MCKEAN *et al.* (1968) and confirms that the primary effect of the raised phenylalanine level in phenylketonuria is to impair the transport of certain amino acids into the brain, especially of amino acids with large hydrophobic sidechains like leucine. Furthermore, this demonstrates that the effect still occurs in animals which have been subjected to sustained hyperphenylalaninaemia. The selective effect on transport is consistent with the suggestion that there are several transport systems for amino acids each specific for different groups of amino acids,

TABLE 8. PHENYLALANINE AND TYROSINE CONTENT OF BRAIN AFTER INTRAPERITONEAL ADMINISTRATION OF PHENYLALANINE. RESULTS EXPRESSED AS μg/g WET WEIGHT BRAIN. EACH VALUE IS THE MEAN OF DUPLICATE DETERMINATIONS DONE ON THE POOLED HOMOGENATE OF 3 BRAINS

	Saline controls		p-Chlorophenylalanine pretreated	
	Phenylalanine	Tyrosine	Phenylalanine	Tyrosine
0 h	11·6	11·3	11·3	10·0
1 h	42·8	36·1	66·8	16·0
2 h	12·5	28·6	48·3	9·9
3 h	22·0	18·5	49·1	13·8
4 h	15·0	15·0	37·5	13·5

and that there is some overlap between these groups. Our results would fit such a situation if only leucine of the three amino acids studied shared a component of a transport system with phenylalanine; this is certainly in keeping with findings in renal, intestinal and white cell transport of amino acids (SCRIVER & HECHTMAN, 1970).

These results represent changes observed in an experimental situation which closely resembles that prevailing in phenylketonuria. Our previous study (ANTONAS *et al.*, 1974) showed that the plasma levels of phenylalanine and tyrosine in our experimental animals were similar to the levels observed in patients suffering from the disease. Free phenylalanine and tyrosine levels in the brain homogenate are shown in Table 8; from them it is apparent that in the loaded animals there is a sustained, high level of phenylalanine, whereas the tyrosine level only rises above normal in the initial period of 1 h. By contrast, the phenylalanine level in the controls falls back to near normal at 2 h, and the brain tyrosine remains significantly elevated at 3 h. This suggests that studies carried out with this model in the period from 1 to 4 h after phenylalanine load may well be relevant to the situation which prevails in phenylketonuria.

We therefore conclude that, although there is markedly reduced transport of leucine into the brain of these animals and also a reduced pool of leucine in the brain (as low as 60% of controls in the intraventricular series of experiments), any consequent reduction in protein synthesis is of a much lower order. However, even a small reduction in the rate of protein synthesis in the developing brain could have serious cumulative consequences, if the effect was on structural proteins or rate-limiting enzymes.

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