Experimental Phenylketonuria in the Monkey.* (25121)

HARRY A. WAISMAN, HWA L. WANG, HARRY HARLOW AND R. R. SPONHOLZ
Depts. of Pediatrics Medical School, and of Psychology, University of Wisconsin, Madison

Our previous report(1) indicated that plasma phenylalanine and tyrosine levels in rats may be changed by supplying these amino acids to the normal diet. After 2 to 3 months on 2.5% L-tyrosine and/or 2.5% DL-phenylalanine diets, these animals showed marked retardation in temporal discrimination learning. Phenylalanine hydroxylase levels in livers were markedly reduced. It seemed worthwhile to test whether these biochemical effects and learning disability could be produced in monkeys.

Methods and materials. Six adolescent Macacus Rhesus weighing 3 kg each were tested for presence of tuberculosis and dysentery prior to experiment. After short observation, the animals were divided into 3 groups. Two animals received L-phenylalanine, 2 received L-tyrosine, and 2 received L-phenylalanine and L-tyrosine as supplement to their diet every 8 hours. The amino acids were intimately mixed with vitamin preparation,† spread on slice of bread and folded so that no dry material could be spilled when the inquisitive animal unfolded the amino acid-vitamin sandwich. Chim biscuits,‡ fresh fruit and water were also fed daily. Fasting blood samples were drawn from femoral vein into heparinized syringes at biweekly or weekly intervals. Plasma phenylalanine was determined by method of Udenfriend and Cooper (2) and plasma tyrosine analyzed by method of Udenfriend and Cooper (3).

Results. Plasma phenylalanine and tyrosine levels of these monkeys are presented in Table I. An intake of 0.5 g of L-phenylalanine 3 times daily to monkeys IA and IB did not raise the plasma phenylalanine level after 8 weeks. Only when the amino acids offered were increased to 1 g or more, 3 times daily, did the plasma phenylalanine value become significantly elevated. Plasma content gradually decreased after several weeks, but when intake was increased to 2 g or 3 g 3 times daily, markedly elevated plasma phenylalanine levels were found. These high values persisted for a longer period with higher daily intakes. The plasma tyrosine levels in these 2 monkeys paralleled the increased phenylalanine values which was to be expected on the basis of increased conversion of phenylalanine to tyrosine. During this period these 2 animals excreted up to .78 g of phenylpyruvic acid daily in the urine. This is within the range reported in phenylketonurics by other workers(4,5,6,7). This observation is also in accordance with work of Armstrong(8) who found phenylpyruvic acid excretion to occur only if blood phenylalanine value was 15 mg or more/100 ml plasma.

Phenylalanine levels were unchanged in IIA and IIB despite increasing dosages of L-tyrosine; plasma tyrosine levels were variable, with occasional very high plasma level. These animals were sacrificed after 6½ months and their tissues preserved for pathologic study. Urines from these 2 animals failed to show increased phenylpyruvic acid at any time during experiment.

The 2 monkeys IIIA and IIIB receiving both amino acids, did not show as striking elevations in plasma phenylalanine as in monkyes fed L-phenylalanine alone. Only after 3 g each were fed 3 times daily, was the high phenylalanine observed, but this effect was short-lived since near normal values were found 2 weeks later. However, tyrosine values were higher than in IA and IB because tyrosine was added in the diet and because phenylalanine was converted to tyrosine. Phenylpyruvic acid was excreted in urine when plasma levels were elevated.

* This investigation supported by Research Grants of Public Health Service.

We wish to acknowledge the help of Kenneth A. Schiltz and John M. Lockhart.

† 4 cups powdered milk, 22 g Parvo (Lederle), 1 lb. lard, Cecon (Abbott), 50 ml (1 ml = 100 mg ascorbic acid), Irrodal A. (Parke-Davis) 2¾ lb.

‡ Chim Biscuits (Kennel Food Supply, Hygrade Food Products Mfg. Co., Fairfield, Conn.).

TABLE I. Plasma Phenylalanine and Tyrosine in Monkeys Receiving Amino Acids.

	Diet + L-phenylalanine				Diet + L-tyrosine				Diet + L-phenylalanine + L-tyrosine			
	IA		IB		IIA		IIB		IIIA		IIIB	
Weeks	Phenylalanine	Tyrosine	Phenylalanine	Tyrosine	Phenylalanine	Tyrosine	Phenylalanine	Tyrosine	Phenylalanine	Tyrosine	Phenylalanine	Tyrosine
	mg/100 ml											
0	1.7	1.0	1.7	1.0	1.7	1.0	1.7	1.0	1.7	1.0	1.7	1.0
	0.5 g L-phenylalanine T.I.D.*				0.5 g L-tyrosine T.I.D.				0.5 g each amino acid T.I.D.			
4	1.5	1.8	1.3		1.4		1.6	1.6	1.8	2.8	1.8	3.1
8	1.9	2.7	1.8	1.8	1.2	1.5	1.0	1.2	.8	2.5	1.4	3.0
9	1.0 g L-phenylalanine T.I.D.				1.0 g L-tyrosine T.I.D.				1.0 g each amino acid T.I.D.			
11	15.0	11.0	9.1	7.5	.6	5.8	1.0	13.4	3.8	15.3	5.6	20.6
15	4.9	5.8	5.8	4.3	1.4	3.0	1.5	5.0	2.3	7.0	1.8	7.4
17	1.5 g L-phenylalanine T.I.D.				1.5 g L-tyrosine T.I.D.				1.5 g each amino acid T.I.D.			
20	11.7	7.3	1.8	2.7	1.5	3.2	1.4	5.5	1.6	6.0	1.8	12.6
25	3.0 g L phenylalanine T.I.D.				3.0 g L-tyrosine T.I.D.				3.0 g each amino acid T.I.D.			
27	24.7	6.4	19.2	7.2			1.2	>60	6.4	>60	5.5	
29	36.0	10.2	20.2	5.2					26.6	50	20.6	31.6
31	24.8	12.5	4.6	6.9					2.7	1.6	2.5	3.9
33	15.5		13.7									

* Three times daily.

The extraordinary high plasma phenylalanine values may be simply the result of overloading metabolic capacity of the phenylalanine hydroxylase enzyme system. In addition, the possibility exists that amount of activity of this enzyme was actually decreased by phenylalanine or by products derived from it, as has been demonstrated in rats in this laboratory (unpublished results).

Plasma tyrosine levels were highest in those monkeys which received both tyrosine and phenylalanine. In these animals both dietary tyrosine and conversion of phenylalanine to tyrosine contributed to the elevated level. When the phenylalanine decreased, it was reflected in lower tyrosine values.

The present data indicate that it is possible to produce in monkeys elevated plasma phenylalanine levels as well as urinary excretion of phenylpyruvic acid similar to that in clinical condition of phenylpyruvic oligophrenia. Psychological experiments now in progress will test intellectual performance of such animas as well as their motor development.

Summary. 1. Prolonged and sustained elevated phenylalanine plasma values have been observed in monkeys fed 3 g of L-phenylalanine 3 times daily. 2. Phenylpyruvic acid was excreted in the urine of these animals. 3. The biochemical findings are comparable to those found in phenylketonuria.

1. Auerbach, V. H., Waisman, H. A., Wyckoff, L. B., *Nature,* 1958, v182, 871.

2. Udenfriend, S., Cooper, J. R., *J. Biol. Chem.,* 1953, v203, 953.

3. ———, *ibid.,* 1952, v196, 227.

4. Penrose, L. S., Quastel, J. H., *Biochem. J.,* 1937, v31, 266.

5. Jervis, G. A., *J. Biol. Chem.,* 1938, v126, 305.

6. Fölling, A., *Z. Physiol. Chem.,* 1935, v227, 169.

7. Fölling, A., Closs, K., *ibid.,* 1938, v254, 115.

8. Armstrong, M. D., Low, N. L., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 142.

Received June 22, 1959. P.S.E.B.M., 1959, v101.