Phenylalanine Tolerance Tests in Simian Primates (35433)

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(Introduced by R. O. Greep)

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The metabolism of amino acids by nonhuman mammalian organisms is of interest in evaluating such organisms for use as animal models of human amino acid metabolic disorders (1). Of particular interest in this regard is phenylketonuria, a genetically-determined deficiency of liver phenylalanine hydroxylase activity that results in impaired metabolism of phenylalanine and has only been discovered in humans. One approach to the production of an animal model for phenylketonuria has been the feeding of large amounts of phenylalanine to rats and monkeys (2, 3). While this reproduces certain biochemical and clinical abnormalities also noted in phenylketonuria, many other characteristics, including the high tyrosine concentrations and the significant phenylalanine hydroxylase activity (3, 4) are distinctly different from human phenylketonuria. Two strains of mice have been reported to have deficient metabolism of phenylalanine (5, 6). However, the "dilute-lethal" strain (so named from its dilute hair color and early death of homozygotes) has recently been shown to metabolize phenylalanine indistinguishably from other mouse genotypes when on a normal diet (7); and in the "wabbler-lethal" strain (with defects in gait and lethality in homozygotes) the plasma phenylalanine concentrations and the plasma phenylalanine:tyrosine ratios are much lower than in human phenylketonuria. In addition the liver phenylalanine hydroxylase activity in this latter strain is much higher in relation to controls than is the case in human phenylketonuria (6). Consequently, there is still a need to develop a genetically-determined animal model for phenylketonuria.

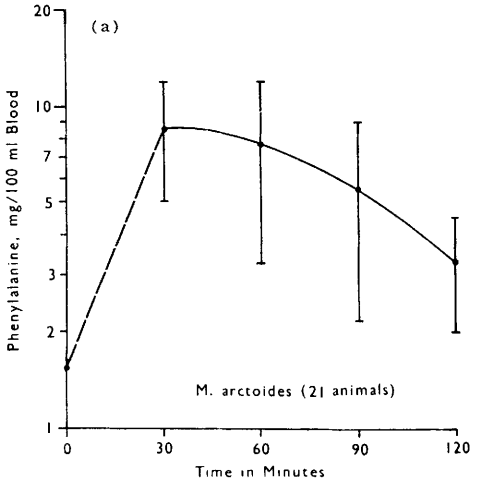
One approach to the production of inherited phenylketonuria in animals is the purposeful mating of known heterozygotes for this disorder. Certain studies in humans have indicated that intravenous phenylalanine tolerance tests are useful in differentiating heterozygotes for phenylketonuria from normal individuals (8–10). For several years, therefore, we have employed intravenous phenylalanine tolerance tests as a technique for identifying primate carriers of the mutant gene for phenylketonuria for the purposes of mating these animals. Though we have not yet found a primate with phenylketonuria, we have discovered some interesting distinctions in the tolerance to phenylalanine among individuals within two of the species studied.

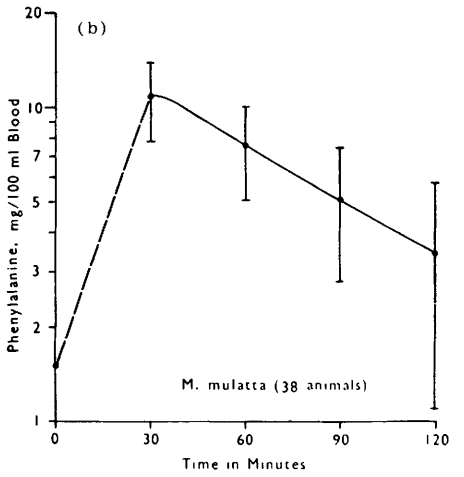
Four species of primates comprising a total of 174 animals were used in the study. These included the Taiwan macaque, *Macaca cyclopis* (81 animals), the rhesus monkey, *Macaca mulatta* (38 animals), the crab eater macaque, *Macaca fascicularis* (34 animals), and the stump-tailed macaque, *Macaca arctoides* (21 animals). Each animal was fasted overnight, then tranquilized with phencyclidine hydrochloride (1.0 mg/kg of body wt) and the arms and legs were taped to an immobilizing board for the duration of the test. In some instances, additional doses of the tranquilizer (0.5 mg/kg of body wt) were given to maintain the tranquil state. A solution of L-phenylalanine (2.5%) was prepared in a 0.4% NaCl solution of pyrogen-free water, heated to dissolve, and sterilized in an autoclave (15 min at 15 lb pressure). An amount of this L-phenylalanine solution containing 100 mg of L-phenylalanine/kg of body wt was injected

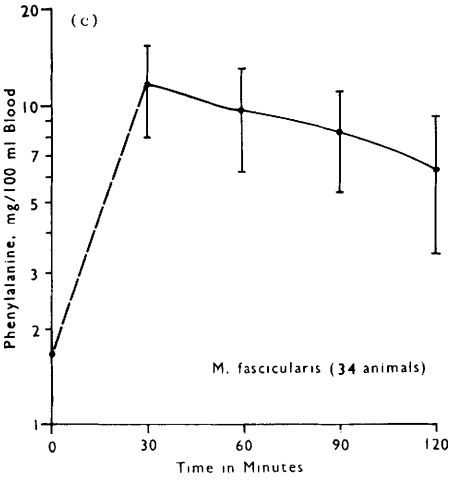
during a period of 1 min into the cephalic vein. Blood samples were obtained in 2.5-ml heparinized syringes from the femoral vein immediately prior to the injection of L-phenylalanine and at 30-min intervals thereafter. Most tests were continued for 120 min and a few for 150 min. No morbidity was encountered in any animal.

At each collection a few drops of blood were impregnated into S & S[1] No. 903 filter paper. The remainder of the blood was expressed into heparinized test tubes and stored at 4° for 2–5 days. Each filter paper blood specimen was tested for phenylalanine concentration by a standard bacterial inhibition assay (11). Plasma was separated from each heparinized whole blood specimen by centrifugation at 2000 rpm. The plasmas representing complete phenylalanine tolerance tests on 10 animals in each species were analyzed for phenylalanine and tyrosine concentrations by ion exchange column chromatography (12).

The mean phenylalanine values of the tolerance curves as determined by the bacterial inhibition assay are depicted in Fig. 1a–d. The species, *M. mulatta* and *M. arctoides*, cleared phenylalanine from the plasma, in the average, more rapidly than the other







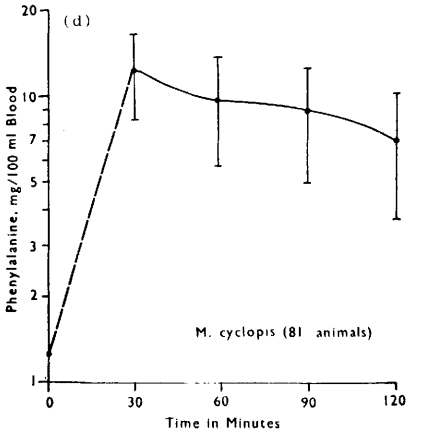


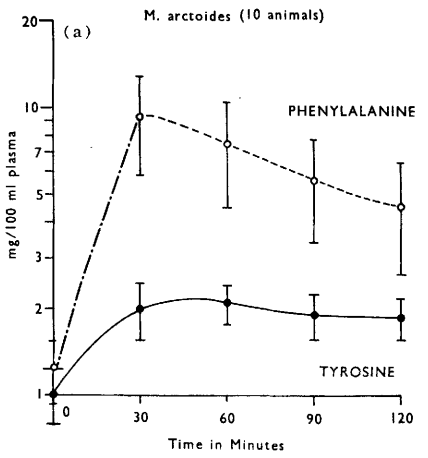
FIG. 1. Phenylalanine loading test; Phenylalanine (bacterial inhibition assay method) in plasma at intervals following intravenous injection of 100 mg of L-phenylalanine/kg of body weight: One standard deviation indicated by brackets on either side of the mean. (a) *M. arctoides*; (b) *M. mulatta*; (c) *M. fascicularis*; and (d) *M. cyclopis*.

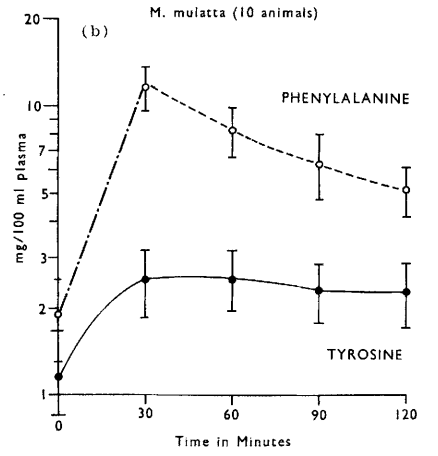
[1] Carl Schleicher & Schuell Co., Keene, New Hampshire.

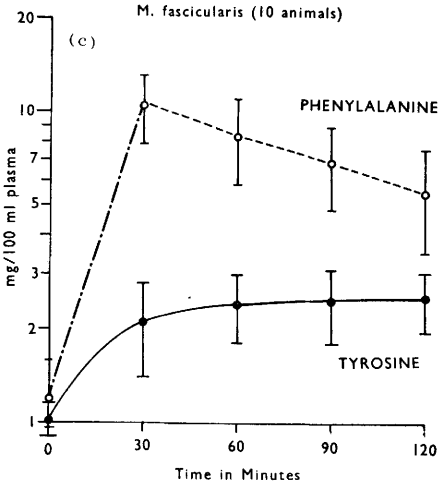
two species, *M. fascicularis* and *M. cyclopis*. The differences in the mean levels of phenylalanine in plasma at 120 min in those two groups were significant (Student's t test; $p<0.001$).

Figure 2a–d, depicts the means and standard deviations of phenylalanine and tyrosine concentrations as determined by ion exchange column chromatography in the plasma of 10 animals (selected among those with slower clearing as determined by the bacterial inhibition assay) from each species. *M. cyclopis* had a greater average phenylalanine concentration at each time interval than any of the other three species. Tyrosine concentrations, however, generally showed little variation among the species except at the 30-min interval where, in *M. cyclopis*, the average tyrosine concentration was lower than in any of the other species. It is of interest that at this time interval *M. cyclopis* also had a higher average phenylalanine concentration. This suggests that the conversion of phenylalanine to tyrosine may be less efficient in *M. cyclopis* as compared to the other species studied.

In one animal, an adult female of *M. cyclopis*, there was no increase in plasma tyrosine during the plasma phenylalanine tolerance test (Fig. 3). Moreover, in this monkey, the plasma phenylalanine concentration remained higher during the test than in







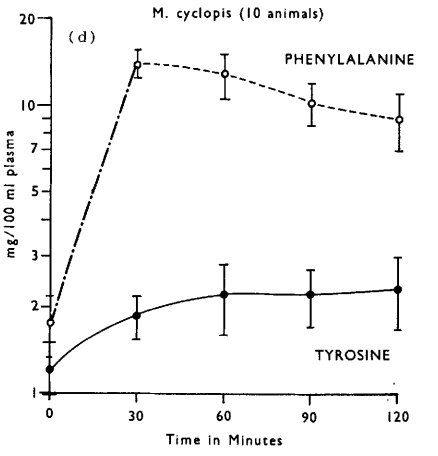


FIG. 2. Phenylalanine and tyrosine (determined by ion exhange column chromatography) in plasma at intervals following intravenous injection of 100 mg of L-phenylalanine/kg of body weight: (a) *M. arctoides*; (b) *M. mulatta*; (d) *M. fascicularis*; and (d) *M. cyclopis*.

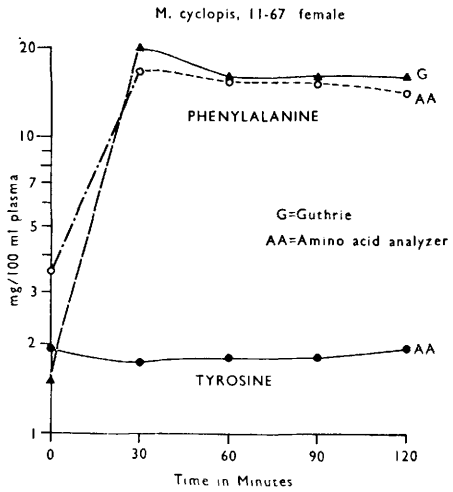


FIG. 3. Phenylalanine and tyrosine in plasma (determined by ion exhange column chromatography on plasma and bacterial inhibition assay of whole blood of a female *M. cyclopis,* following intravenous injection of 100 mg of L-phenylalanine/kg of body weight. These methods give similar values for phenylalanine in blood or plasma. Phenylalanine levels are elevated and no increase in tyrosine is evident.

other monkeys of the same species. A male offspring of this monkey and an unrelated adult male had similar biochemical characteristics determined by a phenylalanine tolerance test. The findings suggest that these animals have a relatively impaired conversion of phenylalanine to tyrosine. It seems possible therefore that they might carry a gene that in the homozygous state could produce phenylketonuria.

Several *M. facicularis* had a slower rate of phenylalanine clearance than other animals in that species. However, they appared to accumulate normal amounts of tyrosine in blood. Breeding studies are currently underway to test the inheritance of this characteristic.

The present data indicate that intravenous phenylalanine tolerance testing may be an effective method of identifying primates who have at least a partial defect in the metabolism of phenylalanine. It is hoped that specific matings of such animals will eventually reproduce a primate that is homozygous for this defect.

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1. Jones, T. C., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **28**, 162 (1969).

2. Auerbach, V. H., Waisman, H. A., and Wyckoff, L. B., Nature (London) **182**, 871 (1958).

3. Waisman, H. A., Wang, H. L., Harlow, H., and Sponholz, R. R., Proc. Soc. Exp. Biol. Med. **101**, 864 (1959).

4. Waisman, H. A., and Harlow, H. F., Science **147**, 685 (1965).

5. Coleman, D. L., Arch. Biochem. Biophys. **91**, 300 (1969).

6. Siegel, S. J., and Rauch, H., Biochem. Genet. **2**, 311 (1969).

7. Zannoni, V. G., Wever, W. W., Van Valen, P., Rubin, A., Bernstein, R., and LaDu, B. N., Genetics **54**, 1391 (1966).

8. Hsia, D. Y-Y., Nature (London) **178**, 1239 (1956).

9. Woolf, L. I., Cranston, W. I., and Goodwin, B. L., Nature (London) **213**, 882 (1967).

10. Perry, T. L. Tischler, B., Hansen, S., and MacDougall, L., Clin. Chim. Acta **15**, 47 (1967).

11. Guthrie, R., and Susi, A., Pediatrics **32**, 338 (1963).

12. Mechanic, G., Efron, M. L., and Shih, V. E., Anal. Biochem. **16**, 420 (1966).

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