Effect of Phenylalanine and Its Metabolites on the Brain Serotonin Level of the Rat.* (27819)

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(Introduced by R. C. Corley)
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Although it is well established that serotonin metabolism is altered in phenylketonuria, it has not been determined whether phenylalanine *per se* or its metabolites or both are responsible for these changes. It has been shown that blood serotonin levels are lowered in human phenylketonuria(1) and that rats fed high phenylalanine diets have lowered brain serotonin levels(2,3,4,5). In the investigation presented here phenylalanine and its important metabolites were fed to rats in an effort to determine their relative abilities to lower brain serotonin levels.

Methods and materials. Wistar rats used for these experiments were fed a natural diet with the following composition: ground yellow corn, 55.75%; middlings, 13.00%; meat and bone scraps, 13.00%; linseed oil meal, 6.00%; dried skim milk, 6.00%; liver meal, 1.50%; yeast, 2.00%; alfalfa meal, .75%; mineral mixture, 2.00%. Food and water were given *ad libitum*. Animals were on their respective diets approximately 2 weeks prior to sacrificing by decapitation. L-Phenylalanine and phenylacetic acid were obtained from Nutritional Biochemicals Corp. Phenylpyruvic acid was obtained from Sigma Chemical Co. or synthesized in our laboratory(6). Ortho-hydroxyphenylacetic acid was obtained from K and K Laboratories. L-Phenylalanine was determined by the method of McCaman and Robins(7), phenylpyruvic acid by the method of Saifer(8), serotonin by the method of Bogdanski(9), ortho-hydroxyphenylacetic acid by chromatography in n-butanol-pyridine-water (14-3-3) followed by development with nitroaniline and visual comparison of color intensity with standards, and phenylacetic acid by chromatography in n-butanol-acetic acid-water (12-3-5) followed by color development with bromcresol green and comparison of color intensity with standards of phenylacetic acid. The urine used for chromatography of phenylacetic acid was treated with boiling 6 N hydrochloric acid for 3 hours to release bound phenylacetic acid prior to chromatography.

Results and discussion. In a preliminary experiment it was found that phenylpyruvic acid was rapidly metabolized by the animals so that there was only a very slight increase in urinary phenylpyruvic acid even when fed at levels as high as 3% of the diet. However, when fed at 4.5% of the diet urinary excretion was markedly increased (Table I).

Inspection of chromatograms run on aliquots of 24-hour urines indicated that most or all of the ingested ortho-hydroxyphenylacetic acid and phenylacetic acid were excreted in the urine. The rats fed ortho-hydroxyphenylacetic acid averaged 198 mg of urinary ortho-hydroxyphenylacetic acid per 24 hours. The rats fed phenylacetic acid averaged 245 mg of urinary phenylacetic acid per 24 hours. Inasmuch as the rats excreted approximately 10 ml of urine in 24 hours, these very high urinary levels of phenylalanine metabolites represent concentrations 10-20 times higher than those observed in human phenylketonuria. Serum phenylalanine levels are not raised by feeding these 2 metabolites because their formation from phenylalanine is irreversible. Table I shows that the brain serotonin levels also are not altered when phenylacetic acid is fed. Brain serotonin levels were somewhat higher than controls when o-hydroxyphenylacetic acid was added to the diet. However, this difference was not significant at the 5% level of probability.

The effect of phenylpyruvic acid on brain serotonin levels is more difficult to determine, because this keto acid is so readily converted

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TABLE I. Comparison of Brain Serotonin Levels with Serum Phenylalanine Levels.

Supplement added to diet	Brain serotonin, μg/g	Serum phenylalanine, mg/100 cc	Urinary phenylpyruvic acid, mg/day
None (15)*	.47	2.4	.4
7% L-Phenylalanine (6)	.31†	14.6	.8
4.5% Phenylpyruvic acid (9)	.40‡	6.0	37.2
3.0% o-Hydroxyphenylacetic acid (11)	.53	1.9	.3
4.0% Phenylacetic acid (6)	.49	2.1	.3

* Numbers in parentheses indicate No. of animals in each experimental group. Serum phenylalanine and urinary phenylpyruvic acid determinations were performed on only 6 animals receiving o-hydroxyphenylacetic acid. Avg wt of animals in each group was 170-185 g at beginning of experiment. All experimental values represent means for that particular group.

† Significantly lower than control group ($P < .01$).

‡ Significantly lower than control group ($P < .05$).

to phenylalanine. However, brain serotonin levels were depressed most effectively by supplementing the diet with 7% phenylalanine, which resulted in a several-fold increase in serum phenylalanine level but only a very small increase in urinary phenylpyruvic acid (Table I). On the other hand, the diet supplemented with phenylpyruvic acid caused a massive excretion of phenylpyruvic acid and a 2- to 3-fold increase in serum phenylalanine, yet was less effective in lowering brain serotonin levels. These data indicate that the brain serotonin level is lowered by an elevated serum phenylalanine level *per se* and that the urinary level of phenylpyruvic acid is, at best, a far less important factor.

There is good evidence that DOPA decarboxylase and 5-hydroxytryptophan decarboxylase are the same enzyme(10,11,12). Fellman(13) has shown that production of adrenaline by this enzyme *in vitro* is markedly inhibited by phenylpyruvic acid, phenyllactic acid and phenylacetic acid but not by L-phenylalanine. Others(14) have shown that this enzyme is inhibited *in vitro* by phenylpyruvic acid but not by phenylalanine in the production of adrenalin. The production of serotonin by this enzyme is strongly inhibited *in vitro* by phenylacetic acid, phenylpyruvic acid and phenyllactic acid but is only slightly inhibited by phenylalanine(15). Experiments presented here suggest that the lowering of brain serotonin *in vivo* is not the result of inhibition of this enzyme by phenylalanine metabolites. In fact, the brain serotonin level was somewhat elevated by feeding o-hydroxyphenylacetic acid. The reduction in brain serotonin by phenylalanine but not by its metabolites as observed in our experiments would probably best be explained by the fact that transport of the precursor of serotonin, 5-hydroxytryptophan, into brain is inhibited by phenylalanine(4). If lowered brain serotonin is the cause of the mental defect in phenylketonuria, it is important to control the serum phenylalanine level of phenylketonuric children more closely than can be done by checking for urinary phenylpyruvic acid, since its level is not appreciably increased until the serum phenylalanine level has reached a concentration of at least 15 mg per 100 ml of serum(16). A simple method for determining serum phenylalanine is available(17).

Summary. Wistar rats were placed on diets supplemented with 7% phenylalanine, 4.5% phenylpyruvic acid, 3% ortho-hydroxyphenylacetic acid or 4% phenylacetic acid. Analysis of brain samples for serotonin after several days on these diets showed that phenylalanine feeding effectively lowered brain serotonin, phenylpyruvic acid feeding did so less effectively, and phenylacetic acid and o-hydroxyphenylacetic acid feeding caused no significant change. The implications of these findings in relation to the treatment of phenylketonuria are discussed.

1. Pare, C. M. B., Sandler, M., Stacey, R. S., *Lancet,* 1957, vI, 551.

2. Wang, H. L., Harwalker, V. R., Waisman, H. A., *Arch. Biochem.,* 1962, v97, 181.

3. Yuwiler, A., Louttit, R. L., *Science,* 1961, v134, 831.

4. McKean, C. M., Schanberg, S. M., Giarman,

N. J., *ibid.*, 1962, v137, 604.

5. Culley, W. J., Saunders, R. N., Jolly, D. H., Mertz, E. T., *Fed. Proc.*, 1962, v21, 364.

6. Herbst, R. M., Shemin, D., *Organic Synthesis*, 1943, v2, 519.

7. McCaman, M. W., Robins, E., *J. Lab. Clin. Med.*, 1962, v59, 885.

8. Saifer, A., Harris, A. F., *Clin. Chem.*, 1959, v5, 203.

9. Bogdanski, O. F., Pletscher, A., Brodie, B. B., Udenfriend, S., *J. Pharmacol. Exp. Therap.*, 1956, v117, 82.

10. Westermann, E., Balzer, H., Knell, J., *Arch. Exp. Path. Pharmak.*, 1958, v234, 194.

11. Yuwiler, A., Geller, E., Eiduson, S., *Arch. Biochem.*, 1959, v80, 162.

12. Udenfriend, S., Lovenberg, W., Weissbach, H., *Fed. Proc.*, 1960, v19, 7.

13. Fellman, J. H., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 413.

14. Boylen, J. B., Quastel, J. H., *Biochem. J.*, 1961, v80, 644.

15. Davison, A. N., Sandler, M., *Nature*, 1958, v181, 186.

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

17. Culley, W. J., Mertz, E. T., Luce, M. W., Calandro, J. M., Jolly, D. H., *Clin. Chem.*, 1962, v8, 266.

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Studies on the Effect of Tolbutamide on Insulin Secretion. (27820)

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The very extensive use of the sulfonylurea drugs, particularly tolbutamide and chlorpropamide in treatment of diabetes has prompted a great deal of research on the mode of action of these drugs(1-7), yet the physiologic mechanisms responsible for the hypoglycemic action are not understood. Levine(8), examined in detail the various hypotheses on the mode of action of these drugs and finally stated, "The many *in vivo* observations compel one to accept the conclusion that the hypoglycemic sulfonamides somehow promote the release of additional insulin from the beta cells of the islets of Langerhans, a phenomenon spoken of as *beta cytotrophism*." The data presented here do not support this hypothesis. We have measured the insulin secretion rate in dogs given tolbutamide by intravenous infusion and have found that it is not significantly different from the secretion rate in control dogs given a saline infusion. In contrast to this, glucose infusion produces a secretion rate 5 to 10 times that of the saline treated controls.

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Methods. Adult female dogs were used for this study. Average body weight for the group was 30 lb, but body weights varied from 20 to 50 lb. The general state of nutrition also varied to the same extent. Fifteen dogs were treated with sodium tolbutamide, 1.0 g in 500 ml saline administered intravenously. Fourteen dogs, serving as controls, received 500 ml saline intravenously. A group of 8 dogs were infused with 500 ml of 10% glucose in saline.

Operative procedure. The animals were fasted for 24 hours before the experiment. They were anaesthetized with Nembutal and a mixture of O_2 and 5% CO_2 was administered by intra-tracheal tube throughout the entire procedure. The intravenous infusion of tolbutamide (or glucose or saline) was started at the beginning of the abdominal surgery so that the drug was present in the pancreas for about 30 minutes before collection of pancreatic venous blood was started. Rate of infusion of the 500 ml volume was so regulated that the infusion was continued throughout the entire period of blood collection.