Reports

Effects of Phenylalanine Diet on Brain Serotonin in the Rat

Abstract. Hooded rats fed diets rich in phenylalanine showed poorer performance on the Hebb-Williams maze than controls, as well as decreased brain serotonin levels. There did not appear to be a causal relationship between these findings, and the findings do not appear to be attributable either to a generalized behavioral defect or to inadequate dietary intake.

Phenylketonuria is characterized by a block in the conversion of dietary phenylalanine to tyrosine with the concommitant production of abnormal keto acids. The relationship between this metabolic block and the mental deficiency accompanying this genetic disease, however, is quite unclear. A number of hypotheses have been advanced, including that of aberrant tryptophan metabolism. This hypothesis stems from clinical studies showing that phenylketonurics have lower levels of serum serotonin and 5-hydroxyindoleacetic acid excretion than do normal controls (*1*), and that serum serotonin levels are increased when patients are put on a therapeutically efficacious low-phenylalanine diet (*2*). In addition, the phenylalanine metabolites—phenylpyruvic, phenyllactic, and phenylacetic acids, which are produced in abnormal quantities—have been shown to inhibit 5-hydroxytryptophan decarboxylase in vitro (*3*). A similar postulation has been made for abnormal catecholamine production on the basis of decreased concentrations of "epinephrine-like" material in blood platelets of phenylketonurics (*4*) and of the inhibition of 3,4-dihydroxyphenylalanine decarboxylase by these same keto acids (*5*).

The present study was undertaken, in part, to determine the effect of phenylalanine feeding on brain serotonin. These results constitute part of a larger study being conducted by one of us (*6*).

Eighteen hooded rats from the department of psychology's animal colony at the University of Michigan were weaned at 17 days of age, divided randomly by split litters into control and experimental groups, housed in individual cages, and fed daily an amount of food which would normally be consumed in 24 hours. This amount of food began at 4 g/day and was gradually increased to 12 g/day at 48 days of age. The basic diet consisted of a mixture of ground Rockland rat diet, powdered milk, and corn oil. The diet of the experimental group was supplemented with L-phenylalanine (*7*) at a level of 5 g/kg of body weight. Animals were run on an open field test for emotionality at 46 days of age. At 49 days of age, the animals were placed on a 23-hour food deprivation schedule and remained on this schedule for the 21 days of testing on the Hebb-Williams test of rat "intelligence" and on a black-white successive discrimination problem, both of which employ hunger as the motivating drive. While on the deprivation schedule the animals were allowed access to food for 1 hour a day, all other food intake being limited to that amount briefly available after they had successfully solved the maze problem. In all cases, the food reward in the goal box consisted of the basic control diet and sugar (*8*).

Between 71 and 74 days of age, each animal was killed by decapitation. The brain was removed immediately, sectioned between medulla and spinal cord, washed, blotted dry, and analyzed for serotonin content (*9*).

The mean brain serotonin concentration for control animals was 0.584 μg/g of tissue, with a standard deviation of 0.014. Phenylalanine animals had a mean serotonin level of 0.468 μg/g, with a standard deviation of 0.005. This difference is statistically significant ($P < .05$).

Results of the behavioral tests are in the direction of greater errors on the Hebb-Williams maze and fewer trials to meet a criterion on the successive discrimination problem by animals fed phenylalanine. No differences between groups appeared in measures taken on the open field test. The effect of phenylalanine feeding on serotonin levels is *not* thought to be the casual factor in the production of the behavioral results, both because the correlations between serotonin level and each of the behavioral tasks are low, and because animals fed isocarboxazid, having a significant *increase* in the brain serotonin ($P < 0.001$), showed similar behavioral deficits.

Although there were no differences between groups in body weight or food intake before the deprivation schedule, the mean food intake during the hour of food accessibility on the deprivation schedule was 5.2 g ($\sigma = 1.5$) for controls and 3.3 g ($\sigma = 0.9$) for experimental animals. This difference was significant ($P < .01$). The mean daily phenylalanine dosage was thereby reduced to 2.8 g/kg of body weight ($\sigma = 0.4$). However, there was no difference in body weight between groups either during or at the end of the deprivation schedule, indicating that experimental animals ate more from the goal box than did controls and were probably more strongly motivated to perform the maze task correctly. Further, no significant correlation or consistent trend could be found between food intake or body weight and brain serotonin levels. It seems unlikely, therefore, that the observed decrease in amine levels could be due to decreased ingestion of other amino acids (*10*) or that the behavioral differences could be attributed to inadequate motivation of the experimental group.

All animals throughout the experiment seemed healthy and active, and members of different groups were indistinguishable by their general behavior.

Phenylalanine has been reported to inhibit uptake of serotonin's precursor, 5-hydroxytryptophan, into brain (*11*), and its metabolites are reported to inhibit both glutamic acid and 5-HTP-

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Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to contributors" [*Science* **125**, 16 (1957)].

DOPA decarboxylase. It seems likely, therefore, that phenylalanine loading leads to alterations of a number of metabolic processes and that the decrease in central serotonin is an auxiliary phenomenon (*12*).

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8. A detailed report on the behavioral studies is in preparation.
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10. A similar effect of phenylalanine diet on brain serotonin has recently been reported by H. L. Wang, V. R. Harwalker, H. A. Waisman, *Federation Proc.* **20**, 6 (1961).
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Calendar of Gametogenic Development in the Prepuberal Male Mouse

Abstract. The timing of the first meiotic cycle in the young male mouse was established by serial sacrifices and subsequent paraffin sectioning of testis and epididymis. Meiosis commences in mice 8 to 10 days old. The highest frequency of any one phase of meiosis was found at days 17 to 19, when 47 percent of the tubules were found to contain late pachytene.

In the course of an electron microscope study of meiosis in the mouse (*1*), it appeared desirable to obtain certain stages of meiosis in higher frequencies than those obtainable in the adult mouse (60 to 100 days). The tubular stages of the adult mouse show a random distribution, depending on the time required for their respective completion (*2*).

Hermann (*3*) and Clermont and Perey (*4*) have studied the development of the seminiferous epithelium of the mouse and rat, respectively. However, it appeared desirable to observe and record gametogenesis in the prepuberal mouse in order to establish a timetable of progressive development of the germinal cells similar to that established by Clermont and Perey in their studies of the rat. An experiment (*5*) was therefore undertaken for the specific purpose of determining the optimal prepuberal ages for obtaining the desired meiotic stages in high frequencies.

Approximately 30 newborn male CF No. 1 mice were randomly selected from different litters. These were then placed in groups of five, with a lactating female, and allowed to grow until the time of sacrifice. Mice were killed at birth and at 3, 5, 8 to 10, 10 to 12, 15 to 17, 17 to 19, 22 to 24, 29 to 31, 36 to 38, and 43 to 45 days. The testes and epididymides were immediately excised and fixed in Carnoy or in Zenker-formol. After embedding in paraffin, the tissues were sectioned at 7 μ and stained with Feulgen-fast-green or periodic acid Schiff.

We are in agreement with the results stated by Clermont and Perey (*4*) that in the prepuberal state, the supporting cells give rise to Sertoli cells and the gonocytes constitute the germinal stem line.

In newborn (0 to 1 day) mice cross sections of the testis measure about 1 mm. The tubules are widely spaced; they are nonconvoluted and do not possess lumina. The tubular cross sections had an average diameter of 40 to 50 μ. The testis cross sections contained from 20 to 30 tubules. The tubular content corresponds to that described for the newborn rat by Clermont and Perey (*4*). Throughout the development of meiotic stages the mouse is approximately 2 days ahead of the rat.

At 3 days some gonocytes are positioned on the basement membrane and about 10 percent of them are in some phase of mitosis. As the number of supporting cells seems to be diminishing, we expected to find occasional ones in the epididymis. We did. The epididymis also contained those gonocytes that did not become aligned on the basement membrane.

At 5 days there is an increase in the number of tubular cross sections, approaching 200. Within each tubule the spermatogenic descendants of the gonocytes number up to a dozen; 50 percent of these are in active mitosis, and the remainder appear to be resting.

At 8 to 10 days the beginning of meiosis is apparent for the first time. Thirteen percent of the tubules counted contained spermatocytes in leptotene. The spermatogonia are approaching the adult pattern of the A, Intermediate, and B types. It appears that the earliest transition from spermatogonia to spermatocytes is abrupt, thus shortening those stages known in the adult as type B spermatogonia and resting spermatocytes. Thus the intermediate spermatogonial cells appear to progress directly into leptotene soon after telophase.

Tables 1 and 2 show the observations on the mice from 10 to 12 days through 6 weeks. The tables show that at 10

Table 1. Age of latest cell types observed in testicular tubules expressed as percent of the tubules counted. The average number of tubules counted for each age group is 108. Meiosis does not begin until day 8. Abbreviations: Lept., Leptotene; Zyg., zygotene; M-I, first meiotic division; M-II, second meiotic division.

Age of mice (days)	Gonia	Resting cytes	Lept.	Zyg.	Pachytene			M-I and M-II	Spermatids (1–8)*
					Early	Mid	Late		
8–10	80	7	13						
10–12	11.3	24.5	11.3	37.7	9.4	5.6	0		
15–17			3.9	13.7	22.9	28.5	30.8		
17–19		1	0	7	14.5	22.0	47.0	3.5	
22–24						2	30.0	12.0	36.6

* These numbers refer to the spermiogenic stage (*2, 4*).

Table 2. Percentage of tubular sections in the various stages of the cycle. The number of tubules in each case is 100.

Age of mice (days)	Percentage in					
	I*	II–III	IV–VI	VII–VIII	IX	X–XII
29–31	12	18	36	9	8	13
36–38	6	19	21	26	10	18
43–45	14	15	10	29	16	16
100	18	7	4	23	23	25

* These numbers refer to the spermatogenic stage (*2, 4*).