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## Urinary Excretion of Amines in Phenylketonuria and Mongolism


*Abstract.* Children with phenylketonuria excrete considerably less serotonin and tryptamine and somewhat less normetanephrine and *p*-tyramine than normal children. The excretion of these amines was not decreased in mongolism. Even during monoamine oxidase blockade, *o*-tyramine could not be detected in the urine of phenylketonurics. These findings are discussed in relation to the mental defect of phenylketonuria.


Increasing attempts have recently been made to elucidate the mechanism of the mental defect in phenylketonuria. One theory suggests that a toxic substance is produced in the brain. Mitoma *et al.* (*1*) found that *o*-tyrosine enters rat brain in vivo and is decarboxylated to *o*-tyramine, and they suggested that overproduction of this amine might cause the mental defect in phenylketonuria. Jepson *et al.* (*2*) reported that phenylketonurics treated with monoamine oxidase inhibitors excrete large amounts of phenylethylamine in the urine and lesser amounts of *o*-tyramine. Both these amines might damage brain tissue, especially during its period of active growth.

A second possible mechanism for the mental defect of phenylketonuria is failure of production of certain neurohumoral agents essential for normal brain function. Several metabolites of phenylalanine present in increased concentrations in the tissues of phenylketonurics inhibit the in vitro decarboxylation of 5-hydroxytryptophan to serotonin (*3*), and phenylpyruvate inhibits the in vitro decarboxylation of 3,4-dihydroxyphenylalanine to dopamine (*4*). Pare *et al.* (*5*) reported that serum concentrations of serotonin, and urinary excretion of its terminal metabolite, 5-hydroxyindoleacetic acid, are decreased in phenylketonurics, and they suggested that underproduction of serotonin might contribute to the mental defect in these patients. Nadler and Hsia (*6*) recently showed that plasma concentrations of norepinephrine and epinephrine, and urinary excretion of these two amines as well as of dopamine, are low in phenylketonurics.

A third possible explanation for the mental defect in phenylketonuria is based on the discovery of Chirigos *et al.* (*7*) that uptake of tyrosine by rat brain in vivo is inhibited by high concentrations of certain amino acids. If a high plasma concentration of phenylalanine competitively inhibits the transport of aromatic amino acids across the blood-brain barrier in man, the phenylketonuric brain might well suffer a deficiency of serotonin and catecholamines, as well as other consequences.

Recently the discoverers of the trisomy of the 21st chromosome in mongolism reported (*8*) diminished urinary excretion of 5-hydroxyindoleacetic acid in mongoloid children. This suggests that diminished production of serotonin might also contribute to the mental defect in mongolism. The investigation reported here attempts to clarify these possibilities by measuring the urinary excretion of biologically active amines in children having phenylketonuria and mongolism and in suitable controls.

Urine was collected for 24 hours, both before and during administration of a monoamine oxidase inhibitor, from six normal subjects, five untreated phenylketonurics, five mongoloids, and five unclassified mental defectives. All were children aged 8 to 16 years and were in good physical health and well nourished. The 15 mentally defective patients lived under uniform ward conditions and ate a standard hospital diet, while the six normal children lived at home. Subjects received no drugs except pheniprazine (12 mg/day) or nialamide (100 mg/day) as a monoamine oxidase inhibitor.

Amines were separated from other urinary constituents and were measured semiquantitatively by two-dimensional paper chromatography (*9*). Briefly, the amines in each urine specimen were adsorbed before and after acid hydrolysis onto the resin Amberlite CG-50 $H^+$ and were then eluted with 4*N* acetic acid. After the eluates had been lyophilized, the amines were separated from salts and basic amino acids by extraction with ethanol and acetone. Two-dimensional paper chromatograms were prepared from the final extracts, and the amines were developed with ninhydrin, diazotized *p*-nitroaniline, dimethylaminocinnamaldehyde, and dichloroquinonechloroimide. Amines were identified and quantitated by comparison with appropriate amounts of the authentic compounds similarly chromatographed on paper. Since accurate 24-hour urine collections are unreliable in mentally defective children, the excretion of amines was calculated in relation to urinary creatinine.

The results (Table 1) indicate that

Table 1. Urinary excretion of amines before and during monoamine oxidase (MAO) inhibition, in micrograms of free base per 100 mg of creatinine. C, control; MAO, after 5 to 7 days administration of MAO inhibitor.

| Subject | Serotonin | | Tryptamine | | Normetanephrine | | *p*-Tyramine | | Phenylethylamine | |
|---|---|---|---|---|---|---|---|---|---|---|
| | C | MAO | C | MAO | C | MAO | C | MAO | C | MAO |
| | | | | | *Normal children* | | | | | |
| 1 | 1.3 | 4.6 | 5.1 | 50 | 2.4 | 13.2 | 28 | 77 | 0 | 0 |
| 2 | 1.9 | 5.2 | 10.7 | 91 | 0.8 | 6.3 | 16 | 60 | 0 | 0 |
| 3 | 4.4 | 9.1 | 10.4 | 102 | 2.2 | 10.8 | 29 | 85 | 0 | 0 |
| 4 | 7.7 | 10.6 | 10.4 | 55 | 2.2 | 9.2 | 52 | 81 | 0 | 0 |
| 5 | 4.4 | 7.7 | 11.6 | 88 | 3.3 | 13.8 | 49 | 115 | 0 | 0 |
| 6 | 4.2 | 6.9 | 8.1 | 62 | 3.0 | 9.2 | 27 | 59 | 0 | 0 |
| | | | | | *Phenylketonurics* | | | | | |
| 7 | 0.2 | 0.9 | 1.3 | 42 | 2.5 | 10.4 | 17 | 40 | 46 | 51 |
| 8 | 0 | 0.9 | 0.3 | 8 | 0.5 | 5.7 | 6 | 26 | 3 | 6 |
| 9 | 0.1 | 1.8 | 1.0 | 54 | 0.5 | 6.8 | 11 | 59 | 5 | 77 |
| 10 | 0.2 | 1.4 | 2.1 | 25 | 2.2 | 4.4 | 16 | 28 | 0 | 11 |
| 11 | 0.5 | 1.8 | 3.9 | 38 | 0.6 | 3.1 | 37 | 42 | 0 | 15 |
| | | | | | *Mongoloids* | | | | | |
| 12 | 4.3 | 12.0 | 7.0 | 41 | 2.2 | 5.7 | 85 | 31 | 0 | 0 |
| 13 | 6.8 | 11.2 | 8.7 | 47 | 3.7 | 7.8 | 25 | 44 | 0 | 0 |
| 14 | 4.1 | 8.7 | 11.2 | 47 | 4.4 | 6.3 | 29 | 51 | 0 | 0 |
| 15 | 10.2 | 15.5 | 15.6 | 65 | 6.1 | 13.1 | 67 | 62 | 0 | 0 |
| 16 | 6.2 | 9.2 | 11.1 | 54 | 7.2 | 11.5 | 63 | 75 | 0 | 0 |
| | | | | | *Undifferentiated mental defectives* | | | | | |
| 17 | 7.7 | 13.3 | 18.0 | 130 | 4.2 | 7.3 | 37 | 95 | 0 | 0 |
| 18 | 6.4 | 7.6 | 15.8 | 53 | 2.7 | 10.5 | 52 | 47 | 0 | 0 |
| 19 | 5.3 | 6.9 | 18.3 | 73 | 2.4 | 4.2 | 61 | 62 | 0 | 0 |
| 20 | 6.2 | 7.5 | 11.9 | 73 | 3.8 | 4.5 | 69 | 53 | 0 | 0 |
| 21 | 5.7 | 8.7 | 8.2 | 62 | 3.1 | 8.3 | 40 | 59 | 0 | 0 |

urinary excretion of serotonin and tryptamine is considerably diminished in untreated phenylketonurics. The excretion of normetanephrine (the major metabolite of norepinephrine) and of *p*-tyramine tends to be lower in phenylketonurics, but there is overlapping with the values obtained in normal children and control mental defectives. Children with mongolism excreted as much serotonin as normal subjects. Phenylethylamine was found only in the urine of phenylketonurics, and in smaller amount than previously reported (*2*), while *o*-tyramine could not be detected in the urine of any subject even during monoamine oxidase blockade. Experiments in which authentic amines were added to urine indicated that approximately 30 percent of *p*-tyramine and normetanephrine, 40 percent of *o*-tyramine and phenylethylamine, 50 percent of serotonin, and 70 percent of tryptamine were recovered by the technique used. The sensitivity of the method was sufficient to detect as little as 1.5 $\mu$g of *o*-tyramine and 3 $\mu$g of phenylethylamine per 100 mg of creatinine in the original urine.

Failure to find *o*-tyramine in the urine of phenylketonurics during monoamine oxidase blockade and the low excretion of phenylethylamine, together with an earlier finding that neither amine is detectable in cerebrospinal fluid of phenylketonurics (*10*), make it appear unlikely that either of these substances produces mental defect by a direct neurotoxic action on brain. On the other hand, the results support the possibility that the mental defect of phenylketonuria is due to underproduction of serotonin and catecholamines in brain as a result of competitive inhibition by phenylalanine of aromatic L-amino acid decarboxylase (*11*). The results do not indicate any defect in serotonin production in mongolism (*12*).

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### References and Notes

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## Chemical Regulation of Flower Sex Expression and Vegetative Growth in Cucumis sativus L.

*Abstract.* Pistillate flower formation in monoecious cucumber plants is increased when allyl trimethylammonium bromide is added to aerated solution cultures used as the root medium. By contrast, gibberellin induces staminate flower formation on gynoecious cucumber plants. The two chemicals also have diametrically opposite effects in tendril formation and vegetative extension.

During the development of a monoecious cucumber plant there is a changing nodal array of flowers of the two sex types—staminate (male) and pistillate (female). The usual spatial pattern is staminate flower formation exclusively at the basal nodes with a gradual shift in flower sex expression to ultimately only pistillate flowers in the upper nodes. Thus, staminate, monoecious, and pistillate phases in sex expression progressively occur. These phases may be identified by the nodes at which the first pistillate and last staminate flowers appear. It has been shown that the nodal location of the first pistillate flower can be a constant varietal characteristic (*1*), but this position may be modified by nutrition, light, and temperature (*2*). Treatment of monoecious cucumber plants with a variety of auxins and other growth substances may shorten the duration of the staminate phase and hasten the pistillate phase of flowering (*3*). Conversely, gibberellin enhances staminate flower production and delays the appearance of pistillate flowers (*4*). Gibberellin has been used to induce staminate flowers on gynoecious cucumbers; this has resulted in an inbred line (MSU 713-5) that, independent of natural photoperiods, temperature, and nutrient status, forms only pistillate flowers (*5*). In the meantime, antigibberellin properties of 2-chloroethyl trimethylammonium chloride and related compounds on the gross morphology of certain plants have been emphasized (*6*). This report (*7*) summarizes the effects of one of these derivatives, allyl trimethylammonium bromide (AMAB), and gibberellin $A_3$ ($GA_3$) on flower sex expression, tendril formation, and internode elongation when added to aerated solution cultures (*8*) used as the root growth medium for a monoecious (Marketer) and gynoecious (MSU 713-5) cucumber (*9*).

Germinated seedlings, immediately following cotyledon expansion, were transferred to solution cultures. AMAB and $GA_3$ were added to the solution cultures in a logarithmic series of molar concentrations, each of which was replicated (Table 1). There were two plants of each variety in each replicate. The evaporated and transpired solutions were replaced periodically with nutrient solution only. Thus, the roots were in constant contact with a diminishing pool of the chemical stimuli. Vegetative and flowering responses were recorded over an interval of 50 and 60 days for the $GA_3$- and AMAB-treated plants, respectively. Plants were grown in a greenhouse maintained at 70° to 75°F night temperature and at a seasonal photoperiod of 9 to 11 hours with no supplemental lighting.

The two chemicals in the root media induced opposite responses in flower sex expression, tendril formation, and vegetative extension (Table 1). In the control monoecious cucumber plants, staminate flowers were produced exclusively on the first 17 nodes. Only one pistillate flower occurred among the first 20 nodes, and this was at the 18th. Similar plants subjected to $5 \times 10^{-4}M$ AMAB formed pistillate flowers at 9 of the first 20 nodes with the first at the second node above the cotyledons. Twelve of the 20 nodes on such plants produced staminate flowers in contrast to 19 of the 20 for the controls. Some plants treated with $5 \times 10^{-4}M$ AMAB produced exclusively pistillate flowers after the ninth node, and there was an abrupt change from the staminate to the pistillate phase without the transitory monoecious phase. Tendril formation was delayed to the 11th node. Tendril emergence also occurred at a higher node when the plants were exposed to high temperatures and long photoperiods (*10*).

Gibberellin $A_3$, at $10^{-3}M$, caused reversion from pistillate to staminate