Excretion of Indolyl Acids in Phenylketonuric Monkeys

Experimental phenylketonuria has been induced in the rhesus monkey by feeding excess dietary phenylalanine[1,2]. The phenylalanine plasma level was markedly elevated, and the urinary excretion of phenylketones and phenylacetyl glutamine were comparable with that found in children with phenylketonuria. Behavioural and learning tests have demonstrated a severe degree of mental retardation in these experimental animals. This report provides data on the excretion of 5-hydroxyindolyl-acetic acid and indolyl-3-acids in these monkeys and demonstrates that the role of tryptophan is similar in both the experimental disease and in phenylketonuric infants.

Five normal monkeys and five experimental monkeys (*Macaca mulatta*) were studied over a one-year period. Each animal was placed in a metabolic chair constructed in this laboratory and modified from that previously described[2]. Only male monkeys were used and appropriate collection devices similar to those used in pediatric patients were attached to the genital area. Urine samples, taken over 24-h periods, were collected in a bottle kept in ice. Several crystals of thymol were added to each bottle as a preservative. The urine was kept frozen at $-10°$ C until ready for analysis. The animals received commercially modified milk either with or without added phenylalanine (3·0 g/kg/body-weight/day) and were fed *ad lib.* at 4-h intervals. The method of Udenfriend *et al.*[3] was used for the determination of free 5-hydroxyindolyl-acetic acid (5-HIAA), and the method of Weissbach *et al.*[4] for free indolyl-3-acids (indolyl-3-acetic, indolyl-3-lactic and indolyl-3-pyruvic acids). The control animals ranged in age from 3 to 18 months, whereas the experimental animals varied from 8 months to 24 months at the time of the urine collections.

The analysis of the 24-h urine collections is shown in Table 1. The excretion of indolyl-3-acids was not significantly different between the experimental and the control groups, but the decreased excretion of 5-HIAA in the experimental group was highly significant ($P < 0.0025$). Although not shown in Table 1 (for reasons of brevity), no differences in base line values were found for either the indolyl-3-acids or for the 5-HIAA between the younger and older animals. In addition there was no evident correlation between the total 24-h excretion of the indolyl acids and the weight of the monkey or the length of time on phenylalanine diet.

Table 1. Excretion of 5-Hydroxyindolyl-acetic Acid and Indolyl-3-Acids in Normal and Phenylketonuric Monkeys per 24 h

Group	No. of animals	No. of urine collections	5-HIAA (μg/day)	3-Indolyl-acids† (mg/day)
Control	5	15	304 ± 230*	1·48 ± 2·03*
Experimental	5	10	47 ± 26*	1·15 ± 1·45*

* Standard deviations are reported for differences between collections.
†Total of indolyl-3-acetic, indolyl-3-lactic and indolyl-3-pyruvic.

The close biochemical relationship between phenylalanine and tryptophan and tryptophan metabolites has found support in a number of observations. Work in this laboratory has shown that excess phenylalanine causes an *in vitro* inhibition of tryptophan hydroxylation in rat liver[5]. It was, therefore, not surprising that decreased serotonin was found in the brain and liver of rats fed excess phenylalanine[6]. Since 5-HIAA is the end excretory product resulting from oxidation of serotonin, any decrease in excretion represents either an inability to form the serotonin precursor (5-hydroxytryptophan) from hydroxylation of tryptophan or an inability to decarboxylate this product. Another factor which influences 5-HIAA excretion is the amount of tryptophan absorbed from the intestinal tract before it enters the blood. Akedo and Christensen[7] have reported evidence of competition between certain amino-acids in their transfer across the intestine, and it is not unlikely that excess phenylalanine may likewise interfere with the intestinal absorption of tryptophan. McKean *et al.*[8], and Guroff and Udenfriend[9] have shown that excess phenylalanine and certain other amino-acids also inhibit transport of 5-hydroxytryptophan across the blood-brain-barrier and thus effectively decrease the formation of serotonin.

An increased indolyl-3-acid excretion by human phenylketonurics has been reported by Armstrong and Robinson[10]. This increase was attributed primarily to indolyl-3-lactic acid, which is not distinguished from indolyl-3-acetic acid in the methods described here. The site of formation of the 5-HIAA is probably different from that at which indolyl-3-acids are formed.

Following a tryptophan load test, Toda and Bessman[11] found an increased excretion of indican and indolyl-3-acetic acid and a decreased excretion of kynurenic acid, xanthurenic acid and *N*-methylnicotinamide. These findings are understandable, since the former compounds arise largely from intestinal putrefaction of unabsorbed tryptophan whereas the latter products arise mainly from tissue metabolism of absorbed tryptophan. These observations would seem to offer further evidence for decreased absorption of tryptophan followed by absorption of its putrefactive products from the lower intestine[4]. Since a milk diet modifies the intestinal microflora, the effect of an excess phenylalanine milk diet on excretion of indolyl-3-acids was probably an added factor in our experimental monkeys. The effect of diet on intestinal flora could also be why all phenylketonuric children do not excrete increased amounts of indolyl-3-acids. Bessman and Toda[12], on the basis of indole tolerance tests, suggest that the increased excretion of indican and indolyl-3-acids in phenylketonuric children is due to a more general derangement of indole metabolism. However, another explanation may be that these compounds are increased mainly as a result of decreased tryptophan absorption whereas the decreased excretion of 5-HIAA is due to alterations in the activity of the hydroxylases and decarboxylases as well as to effects on the transport of amino-acids.

The decreased 5-HIAA excretion found in phenylketonuric monkeys demonstrates still another biochemical similarity of the experimental condition to that found in the human disease[13]. The return to normal of the excretion of 5-HIAA by phenylketonuric patients treated with a diet low in phenylalanine[14] is comparable with removal of the phenylalanine excess diet in the monkey.

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